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Special Issue

Glucoamylase immobilization on bacterial cellulose using periodate oxidation method

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Abstract - Glucoamylase immobilized to bacterial cellulose beads, which were activated by the periodate oxidation method, was discussed. The immobilized glucoamylase activity increased by about 40% more than the value in previous research. The maximum immobilized activity of 133.3 U/g-BC was achieved when the loading glucoamylase concentration was 1.0%. The results of pH and thermal stability analyses indicated that the activity of immobilized glucoamylase was significantly influenced by the environment. Furthermore, about 46% of the activity remained after 14 repeated usages.

Index Terms - bacterial cellulose beads; glucoamylase; immobilization; periodate oxidation method

I. INTRODUCTION

Amylase is one of the most widely used enzyme groups in the industry since starch is the most abundant form of storage polysaccharide in plants. Starch hydrolysis that uses enzymes for the production of glucose, fructose or maltose is an important process in food industries since the cost is lower and the procedures are much simpler [1,2]. Glucoamylase is one of the key enzymes used for starch processing [3]. Recently, glucoamylase has attracted more attention since it can be used together with α-amylase to convert starch to simple sugars, which serve as a feedstock for production of bioethanol [4].

Glucoamylase hydrolyzes α-1,4- and -1,6-glycosidic linkages of starch to produce glucose [3]. The conventional enzymatic reactions were performed in the solution within the soluble enzyme and its substrate. Therefore, the enzymes were difficult to recycle for another reaction. For the sake of economic in industry, reuse technology for the enzymes, which is also known as immobilized enzyme technology, was developed.

Bacterial cellulose, which is an indigenous food of South-East Asia, was used as the immobilized carrier in this study. Bacterial cellulose has unique physical properties which differ from those of plant cellulose and has become a new functional material. During the past decade, the production of bacterial cellulose, with its unique structure and properties, such as excellent mechanical strength, ultra-fine fiber, biodegradability, and high crystallinity, has been developed [5-8]. Its widespread fields of application include foods, acoustic diaphragms, the production of unusually strong paper, and medical applications such as wound dressings and artificial skins [6]. In the present work, acetobacter was used to obtain the ultrafine cellulose fibrils (50-80 nm in width and 3-8 nm in thickness). Cellulose fibrils are three-dimensional network structures with a micrometer- to nanometer-scale [9].

Traditional production of bacterial cellulose occurs in stationary culture conditions, and a thick, gelatinous membrane is accumulated on the surface of a culture medium. Under agitated culture conditions, the fibrous suspension form of cellulose can be produced [8]. The unique pellets formed of cellulose, which differ from the membrane and fibrous types mentioned above, can be obtained by the airlift reactor [10-12]. Although the applications of bacterial cellulose rarely use the pellet type, this type has potential in enzyme immobilization since the pellet form is usually used in this field.

In a previous study, the immobilization of glucoamylase with several activated methods on bacterial cellulose beads was investigated [13]. In the present work, another activated procedure, periodate oxidation method, was tested. Periodate oxidation is a reaction converting 1,2-dihydroxyl (glycol) groups to a pair of aldehyde groups. Since it is a highly specific reaction without significant side reactions, this method has been widely used in structural analysis of carbohydrates [14].

In this work, the effects of thermal, pH, and storage stability for immobilized enzymes were studied and compared with those of free enzymes.

II. MATERIALS AND METHODS

2.1. Materials

Glucose and peptone were obtained from Merck Co. Ltd. Germany. Yeast extract was obtained from Sigma Chemical Co. Glucoamylase (exo-1,4-α-glucosidase, EC 3.2.1.3, from Aspergillus niger), soluble starch (from potato, the substrate in determining enzyme activity acc. to Zulkowsky), and 3,5-dinitro salicylic acid were purchased from Sigma Chemical Co. For the activated reagents, 1,4-butanediol diglycidyl ether was obtained from Sigma Chemical Co. The other chemicals were of analytical grade.

2.2. Microorganism and cultivation conditions

The microorganism, A. xylinum subsp. Sucrofermentans BPR2001, was purchased from Japan Collection of Microorganisms (RIKEN, Saitama, Japan). The culture medium contained (%, w/v) the following:

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mannitol, 2.5; yeast extract, 0.5; peptone, 0.3; agar, 1.5 at pH 5.5 was used for its maintenance. The Hestrin & Schramn (HS) medium, which is a general culture medium for production of bacterial cellulose, was also used in this study and consisted of 2.0% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.27% (w/v) Na2HPO4 ·12H2O, and 0.115% (w/v) citric acid monohydrate [12]. After sterilization at 121 oC, the pH value of the medium was adjusted to 5.0. The organisms were inoculated into a 500 ml flask containing 300 ml of the HS medium and then cultivated at 30 oC and 120 rpm. The cultivation time was at least 48 hr and based on the size of bacterial cellulose beads for the desired experiments. The cellulose beads were obtained by dissolving the cells with 0.1N NaOH at 90 oC for 30 min and then washed twice with deionized water.

2.3 Activation and Immobilization

The bacterial cellulose beads (1 g) were suspended in 1.5 ml of 1 M NaOH and then 0.2 ml of 1,4-butanediol diglycidyl ether was added. The mixture was stirred at 60 oC for 2 hr. After washing, the beads were suspended in 4 ml of 0.1 M NaOH containing 0.5 g of glucose and shaken at 40 °C for 24 h. After washing, the beads were suspended in 1.5 ml of 0.1 M NaIO4 and incubated at 4 °C for 1 h with shaking. After washing again, the beads were incubated in 0.1 M HCl for 30 min at room temperature. Finally, the activated bacterial cellulose beads were washed successively with distilled water.

The procedure for immobilization was carried out by adding the bacterial cellulose beads (1g) to 3mL glucoamylase (0.1%) in 5 mM sodium phosphate at pH 4.5 and 25 oC for 60 min. After that, the derivatives were washed with distilled water.

2.4 Estimation of enzyme activities

The activity of the free and the immobilized glucoamylase was determined according to the amount of glucose produced by enzyme. The hydrolysis reactions were carried out in the phosphate buffer with 1% starch as the substrate and the solution was incubated at 60 °C for 3 min. The released glucose was measured using the DNS (3,5-dinitrosalicylic acid) method. One unit of enzyme is defined as the amount of enzyme which releases reducing carbohydrates equivalent to 1 μmol glucose from soluble starch per min.

For kinetic analysis, the rates of reaction were measured at various substrate concentrations. The results can be substituted into the Hanes-Woolf equation to obtain the kinetic parameters (Michaelis constant Km and maximum rate Vmax).

2.5. pH and thermal stability of immobilized glucoamylase

The pH stability of the immobilized glucoamylase was performed by incubating the immobilized enzyme in buffers of varying pH at 25 °C for 1 hr and determining the hydrolytic activity at the optimum pH and temperature. The thermal stability was tested by incubating the immobilized enzyme at varying temperatures and then determining the activity at its optimum reaction temperature. The results were expressed as relative activities (which is the ratio of the activity of immobilized enzyme after incubation to the activity at the optimum reaction pH).

III. RESULTS AND DISCUSSIONS

3.1 The enzyme concentration of immobilization

Experiments were performed using different enzyme concentration from 0.1% to 5% and the results are shown in Fig.1. The maximum loading activity was achieved about 133.3 U/g-BC at glucoamylase concentration 1.0% since the values are similar when the concentrations are above 1.0%. On the other hand, the immobilized enzyme activity using 0.1% enzyme concentration was 67.1 U/g-BC, which is higher than the 47.6 U/g-BC in the previous research using the epoxy method with glutaraldehyde coupling [13]. The periodate oxidation method for glucoamylase immobilization on bacterial cellulose beads had a significant influence on the efficiency. The immobilized enzyme activity was improved by about 40%.

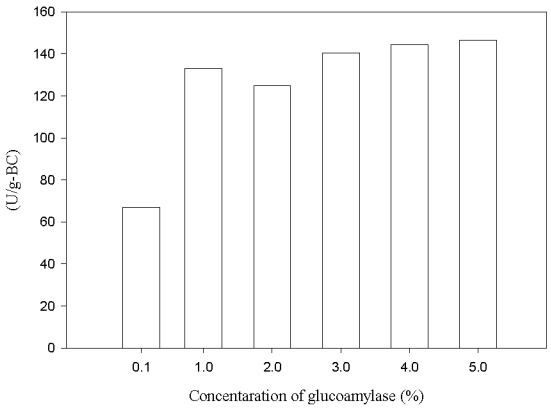

Fig.1. Effect of the enzyme concentration on the activity of immobilized glucoamylase

3.2 Effect of pH on enzyme activity

The effect of pH on the relative activity of glucoamylase immobilized on the bacterial cellulose beads was studied by varying the pH of the reaction medium from 3 to 6.5 at an interval of 0.5 and the pH profile is shown in Fig. 2.

After the immobilization, the optimum pH was similar to the value for free enzyme. This phenomenon was different from the previous study on bacterial cellulose beads in that the optimal pH shift to acid was found [13]; furthermore, this study also differed from other research studies which used acrylic carriers [15] and chitosan-clay composite [16]. The better operating range, i.e., when the relative activities of the enzyme were above 90%, was also similar to the range for free enzyme from 3.5~5.5. In other words, the characteristic of the immobilized enzyme activity was similar to the free enzyme.

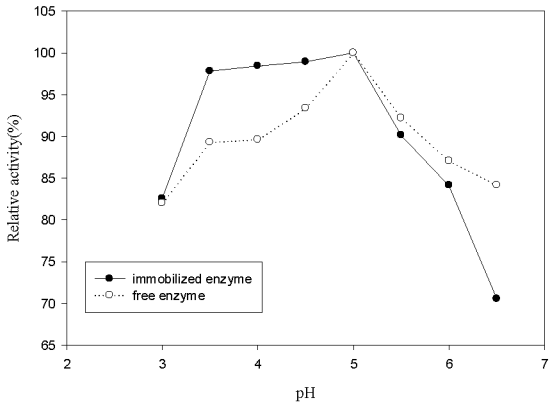

Fig.2. Effect of substrate pH on the relative activity of free and immobilized glucoamylase

3.3 Effect of temperature on enzyme activity

The temperature dependence of the hydrolytic activity of free and immobilized glucoamylase is shown in Fig. 3. The optimum reaction temperature of the glucoamylase was at 60 °C in both cases. The better operating range, i.e., when the relative activities of the enzyme were above 90%, was at 50~80 °C by immobilization, which was wider than that of the free enzyme (60~70 °C). The behavior of a 10 °C decrease in the lower optimum activity temperature was similar to the results obtained in bacterial cellulose beads [13], chitosan-clay composite [16], polyaniline polymer [17], activated charcoal [18] and montmorillonite [19].

The 30 °C expansion in the range of optimum activity temperature for the immobilized enzyme on the bacterial cellulose beads was one of the interesting findings of the present work. The industrial applications of glucoamylases no longer require temperatures around 60~70 °C to reach the optimum catalytic activity temperature. The fast decrease in the relative activities at the high temperature range was due to the thermal denaturation.

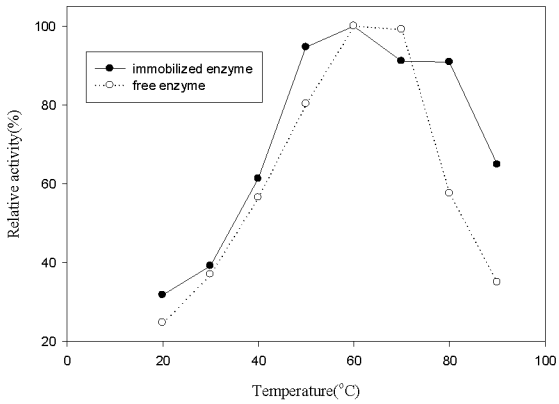

Fig.3. Effect of reaction temperature on the relative activity of free and immobilized glucoamylase

3.4 Stability of the immobilized enzyme

The stability of the relative activity of the free and the immobilized glucoamylase with pH is shown in Fig. 4. Free enzyme was stable at pH 4 and the relative activities were maintained at 90% in the pH range of 3 to 6. The immobilized glucoamylase was stable at pH 5 and the relative activity was maintained above 80% in the pH range of 4 to 5. The range of better operating pH for the immobilized enzyme was narrowed down in this activated method. The result indicated that the enzyme activity had more influence on the pH after immobilization.

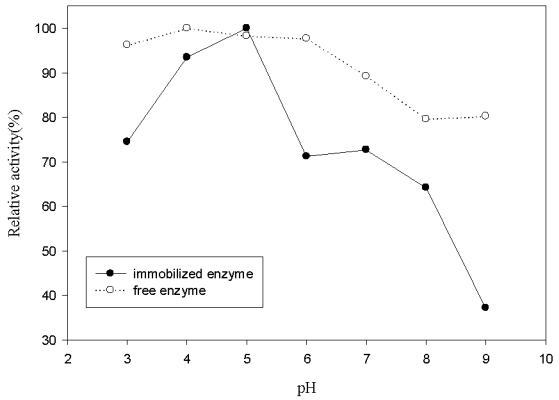

Fig.4. pH stability of free and immobilized glucoamylase.

The thermal stabilities of the free and the immobilized glucoamylase in terms of the relative activities are compared in Fig. 5. The enzyme was found to be stable up to a temperature of 50 °C for the free enzyme, and only up to 40 °C for the immobilized enzyme. Therefore, the 10 °C decreased maximum stability temperature represents the significance influence for temperature than the free enzyme. Hence, the effect of thermal deactivation for the immobilized enzyme was significant as compared to the free enzyme state.

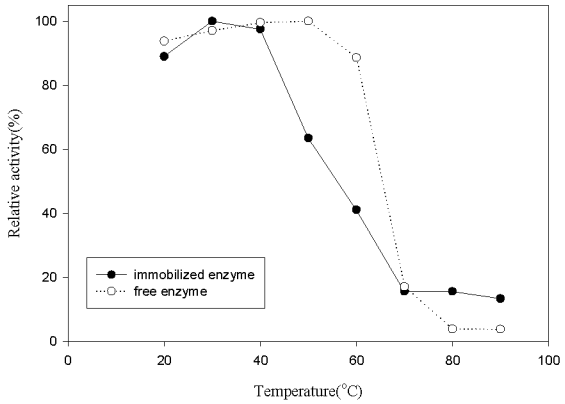

Fig.5. Thermal stability of free and immobilized glucoamylase

3.5 Reuse stability of immobilized enzymes

Glucoamylase immobilized in the bacterial cellulose beads was used repeatedly to hydrolyze starch since reusability was important for repeated applications in a batch or a continuous reactor. The repeatedly used relative activity of the immobilized glucoamylase is shown in Fig. 6. After 3 repeated usages, the activity of immobilized glucoamylase was significantly decreased to 71% and, furthermore, to about 46% at 5 to 14 repeated usages. This phenomenon can be explained with the previous discussion on stability since the immobilized glucoamylase for activation using periodate oxidation was significantly influenced by the environment.

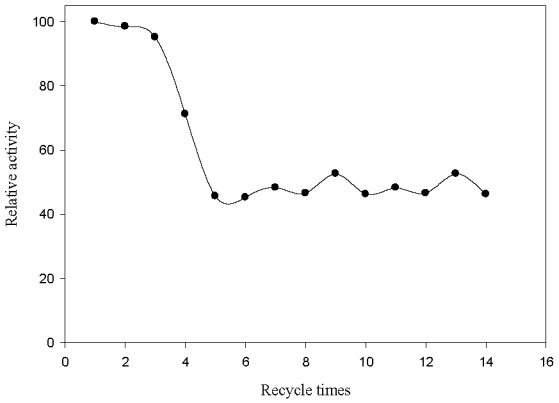

Fig.6. Effect of repeated use on the relative activity of immobilized glucoamylase

IV. CONCLUSION

Glucoamylase immobilized to bacterial cellulose beads with the activation by periodate oxidation method was discussed. The immobilized glucoamylase activity was increased by about 40% more than the value in the previous research. The maximum immobilized activity was achieved to 133.3 U/g-BC when the loading glucoamylase concentration was 1.0%. From the results of stability analysis in thermal and pH, the activity of immobilized glucoamylase was significantly influenced by the environment. Furthermore, about 46% of the activity remained after 14 repeated usages.

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BIOGRAPHY

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