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Chelate immobilization of amylase on metal ceramic powder: Preparation, characterization and application

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ABSTRACT

The ceramic powder with high metal content (MCP) was prepared as a new kind of immobilization carrier. Cu-MCP showed the best affinity to α-amylase and exhibited maximal activity. The effects of metal content, chelation time and amylase concentration on Cu-MCP-amylase activity were investigated. Under the optimal preparation conditions (the ratio of metal hydroxide precipitation to matrix (P/M): 0.23, chelation time: 8 h and amylase solution: 2.2 g/L), the activity of Cu-MCP-amylase could reach 31.5 U/g (54.5% of activity yield). Then, both Cu-MCP and Cu-MCP-amylase were characterized by XRD, SEM, BET-N_2 and FTIR. These characterizations confirmed that the Cu-MCP was a superior carrier with significant mechanical strength, considerable surface area, stable environment, homogeneous surface and low cost. Meanwhile, it also indicated that amylase had been efficiently immobilized on the external surface of Cu-MCP. Next, the properties of Cu-MCP-amylase were determined. It had optimal pH 7.0 and optimal temperature 70 °C with improved thermal stability. Finally, Cu-MCP-amylase showed significant catalytic capacity and stability in a column reactor, which could retain 80% of initial catalytic capacity after 40 recycle times.

1. Introduction

Amylase, a kind of hydrolase which can convert starch into reduced-sugars, syrups and dextrin [1], is an important biocatalyst in food industry. Like many other enzymes, immobilized amylase would gain improved stability and reusability. Usually, amylase immobilized on the solid carriers which have suitable hardness, density and porosity, seems to be the most appropriate way for the application [2]. Many solid carriers, such as glass beads [3], porous molecular sieves [4], zirconium dioxide [5], and alumina [6] have been used to immobilize amylase. Physical adsorption and covalent cross-linking are the common methods for amylase immobilization [3,5,7–12]. However, the physical adsorption only supplies weak affinity between carrier and enzyme, often leading to the leaking of enzyme from carriers [11]; while cross-linking agent, such as glutaraldehyde, usually results in the activity lose of enzyme [13].

In recent years, due to the high immobilization efficiency, simple immobilization method and improved stability, enzyme immobilized through metal affinity has attracted increasing attention [14–19]. However, metal ions were usually chelated on silica gel [20], molecular sieves [21], membranes [22] and films [16] without excellent mechanical strength and hardness, leading to many operating difficulties in the traditional reactor, such as fragmentation, abrasion and deformation. Fortunately, metal ceramic, i.e. ceramic mixed with metal or alloy, had significant mechanical strength [23], electrical conductivity [24] and abrasive resistance [25]. It was often used as electric conduction material [26], heat-resisting material [27] and antierode container [28]. But few references reported the application of metal ceramic in the field of enzyme immobilization.

Therefore, in this paper, the advantages of metal affinity and ceramic powder were combined to simply prepare the immobilized enzyme with high immobilization efficiency, low enzyme activity loss as well as the stable, cheap, hard, and abrasive resisted carriers, so as to overcome the problems caused by the physical adsorption and covalent cross-linking method for amylase immobilization. It meant that efficient immobilization of amylase could be realized through chelation between enzyme and metal ion on the metal ceramic. In detail, firstly, a novel metal ceramic powder (MCP) with high metal content, excellent hardness and abrasive resistance was used as carrier. Secondly, MCP immobilized with amylase (MCP-amylase) was prepared by a simple process of

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chelation with amylase on MCP. Then, the optimal preparation conditions were investigated to obtain the highest MCP-amylase activity. Meanwhile, the optimal catalytic temperature and pH, as well as the thermal stability of MCP-amylase were also measured. Furthermore, XRD, SEM, IR spectra and BET-N_2 method were used to characterize this novelly immobilized enzyme. At last, to investigate the practicability, MCP-amylase was applied to the batch reactor and column reactor to evaluate its efficiency and operation properties. Considering of many practical characteristics, this novel immobilization method would have widely potential applications in enzyme electrode, biosensor and biocatalysis.

2. Materials and methods

2.1. Material

α-Amylase (1,4-α-D-glucan-glucanohydrolase; A109181) was purchased from Aladdin company; starch, maltose, silica, alumina, calcium oxide, magnesium oxide were purchased from Guangfu company (Tianjin, China); 3,5-dinitrosalicylic (DNS) and other chemicals were analytical grade and obtained from common commercial sources without further purification.

2.2. Preparation of MCP

Firstly, the metal chloride ($FeCl_3$, $CuCl_2$, $NiCl_2$, $CoCl_2$, $PbCl_2$, $CrCl_3$, $TiCl_4$, $MnCl_2$, $ZnCl_2$) was respectively dissolved in deionized water until saturation, and sufficient amount of NaOH (100 g/L) was slowly added into the solution with vigorous stirring. The obtained metal hydroxide precipitation was filtered and washed for 3 times with deionized water, and then dried at 70 °C for 24 h. Secondly, the MCP was prepared by mixing 22 g of matrix (containing 10 g SiO_2, 5 g Al_2O_3, 5 g Na_2SiO_3, 1 g CaO, 1 g MgO), 10 g deionized water and metal hydroxide precipitation at a certain precipitation/matrix ratio (P/M ratio, w/w). Finally, the MCP was obtained by drying in muffle at 150 °C for 2 h and calcining at 850 °C for 10 h. Meanwhile, the ceramic powder (CP) was also prepared with the same process as the control without mixing metal hydroxide precipitation.

2.3. Immobilization of amylase on MCP

MCP surface was firstly activated by 5% (w/w) sulfuric acid for 1 h under vigorous stirring at room temperature. Then, the activated MCP was washed with deionized water at least 6 times. For immobilization of amylase, 0.4 g of activated MCP was added into 5.0 mL amylase solution of a certain concentration and stirred at 4 °C for different time, then the MCP-immobilized amylase (MCP-amylase) was washed by deionized water for 3 times to remove the un-immobilized amylase.

2.4. Enzyme activity assay

The activities of free amylase and MCP-amylase were determined according to DNS method [29]. One unit (U) of free amylase activity was defined as the amount of enzyme which produced reduced-sugar equaled to 1.0 μmol maltose in 1 min at 40 °C and pH 6.5. One unit (U) of Cu-MCP-amylase activity was defined as the amount of enzyme which produced reduced-sugar equaled to 1.0 μmol maltose in 1 min at 40 °C and pH 7.0.

The activity yield was calculated based on the following equation:

$$\text{Activity yield}(\%) = \frac{E(\text{immobilized})}{E(\text{free})} \times 100 \quad (1)$$

where E (immobilized) is the activity of all Cu-MCP-amylase obtained from the original amylase solution after immobilization and E (free) is the activity of all free amylase before immobilization.

2.5. The optimal conditions of enzyme activity

The optimal temperature of free amylase and Cu-MCP-amylase were determined by adding the enzyme samples (15 mg of free amylase or 1 g of Cu-MCP-amylase) into 15.0 mL of substrate solution (0.1 g/L) under different temperatures (20–90 °C) for 30 min, and the pH value was maintained 6.5 for free amylase and 7.0 for Cu-MCP-amylase. The optimal pH of free amylase and Cu-MCP-amylase were determined by adding the enzyme samples (15 mg of free amylase or 1 g of Cu-MCP-amylase) into 15.0 mL of substrate solution (0.1 g/L) with different pH (6–12) for 30 min, and the temperature was maintained 40 °C. Then the activities of free and immobilized enzyme samples were determined.

2.6. Thermal stability of enzyme

The thermal stabilities of free amylase and Cu-MCP-amylase were determined by measuring the residual activities of enzyme samples incubated in phosphate buffer (pH 6.5 for 1 mg/mL of free amylase, pH 7.0 for 50 mg/mL of Cu-MCP-amylase) at 80 °C. The incubating time was set from 1 h to 5 h and time interval was set as 1 h.

2.7. Characterization of CP, MCP and MCP-amylase

The XRD (D8-Focus, angle range 10–80° at a speed of 4°/min), SEM (S4800, 5 kV, high power mode), BET-N_2 adsorption (F-sorp 2400, liquid nitrogen temperature), laser particle size analysis (Mastersizersiong bed) and IR spectrum (Bio-Rad FTS 6000, FTIR, KBr disk method) were performed to characterize the properties of CP, MCP and MCP-amylase.

2.8. Enzymatic hydrolysis of starch by MCP-amylase in a batch and a column reactor

For the batch reactor, a certain amount of Cu-MCP-amylase was added into 15 mL of starch solution (0.1 g/L). After reacted for 15 min at 40 °C under 150 rpm shaking, Cu-MCP-amylase was separated by filtration, and then 3.0 mL of sample solution and 2.0 mL of DNS solution were mixed and heated in boiling water bath for 10 min to quench the reaction. After diluted to 30.0 mL, the starch conversion and the reduced-sugar production ability could be calculated based on the following equations.

$$\text{Starch conversion}(\%) = \frac{A(\text{sample}) - A(\text{blank})}{A(\text{absolute}) - A(\text{blank})} \times 100 \quad (2)$$

$$\text{Reduced-sugar production}(\text{mg}/\text{min}) = \frac{0.0276 \times V \times [A(\text{sample}) - A(\text{blank})]}{t} \quad (3)$$

where A (sample), A (absolute) and A (blank) were the absorbance of sample, absolutely hydrolyzed products, and original starch without hydrolysis at 540 nm, respectively. The starch solution hydrolyzed by amylase for 24 h was regarded as the absolute hydrolysis. t is the reaction time (min); V is the original volume of starch solution (mL); 0.0276 is the ratio of increased mass of maltose to increased absorbance of solution in unit volume (mg/mL).

For the column reactor, a certain amount of Cu-MCP-amylase was packed in a condenser pipe (i.d. 0.75 cm, height 27 cm). A thermostatic water-bath was used to control the column temperature at 50 °C. 15 mL of starch solution (0.1 g/L) was fed from the