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Research article

Magnetic immobilization and characterization of α-amylase as nanobiocatalyst for hydrolysis of sweet potato starch

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ABSTRACT

Magnetic immobilization of enzymes became emerging method for efficient recovery of biocatalyst under magnetic field. Sweet potato (*Ipomoea batatas*) root starch is abundantly produced in tropical countries. Thus the present work was focused on immobilizing α-amylase on to magnetic nanoparticles and use as biocatalyst for hydrolysis of sweet potato starch into glucose. The hydrolysis of sweet potato starch using immobilized α-amylase was investigated and the maximum glucose yield of 42.89 mg/g was obtained using 3% (w/v) of sweet potato starch concentration with an initial pH 4 at 40 °C in 80 min reaction time. The optimal concentration of immobilized α-amylase for maximum glucose yield was found as 1% (w/v). The kinetics of hydrolysis reaction was studied using Michaelis–Menten equation. The sweet potato starch was found to have more affinity towards magnetically immobilized α-amylase with substrate affinity constant (Km) of 0.16 mg/ml and the maximum reaction rate (V_{max}) of 3.63×10^{-3} μmol/ml s. The magnetically immobilized nanocomposite of α-amylase can be easily recovered and reused for maximum utilization.

1. Introduction

Fossil fuels are a finite energy resource and we can portend the depletion of these resources in a few decades. Biofuels are energy sources derived from biological materials that distinguishes them from other non-fossil fuel energy sources such as wind, hydro and wave energy [1,2]. We have been on the man-hunt for cheaper and renewable energy resources since the dawn of this century. Starch is one the best and most high yielding feedstock for ethanol production, hydrolysis is required to produce ethanol from starch by fermentation [3–5].

Starch was traditionally hydrolyzed using acids as catalyst. The major disadvantages of acid hydrolysis of starch include the possible inhibitory effect of by-products, neutralization of hydrolyzates before fermentation, and the expensive construction material for equipment. Such drawbacks have estimated enzyme based hydrolysis superior to conventional acid based hydrolysis [6,7]. The specificity of enzymes as catalyst toward the substrate, their inherent mild reaction conditions, and the absence of secondary reactions has made the amylase as preferred catalyst for starch hydrolysis in to starch hydrolysate [8–10].

In today's scenario there is an immense attraction toward nanoparticles and its application in catalysis, due to their unique large surface-to-volume ratio and quantum size effects [11]. The preparation of metal nanoparticles can be classified into physical and chemical methods. Nanoparticles prepared by chemical methods have usually a narrow size distribution when compared to physical methods [12,13]. Developing a novel method of immobilizing enzymes as biocatalyst is important for easy recovery and reuse in industrial biotechnology. Thus the present work was focused on optimization process parameters and kinetics of hydrolysis of sweet potato starch using nanobiocomposite of α-amylase immobilized on to magnetic nanoparticles. The immobilized α-amylase was recovered under magnetic field and reused.

2. Materials and methods

2.1. Chemicals and biochemicals

Fungal α-amylase of *Aspergillus oryzae* was purchased from HiMedia, Mumbai India. Dinitro salicylic acid, Gluteraldehyde, $FeSO_4$ and $FeCl_3$ were purchased from SRL Pvt., Ltd., Mumbai, India.

2.2. Sweet potato starch preparation

Sweet potato is the largest vegetable crop grown in tropical countries. The root of sweet potato was sliced into small pieces and dried. The dried slices were ground in to powder and dried at 80 °C overnight.

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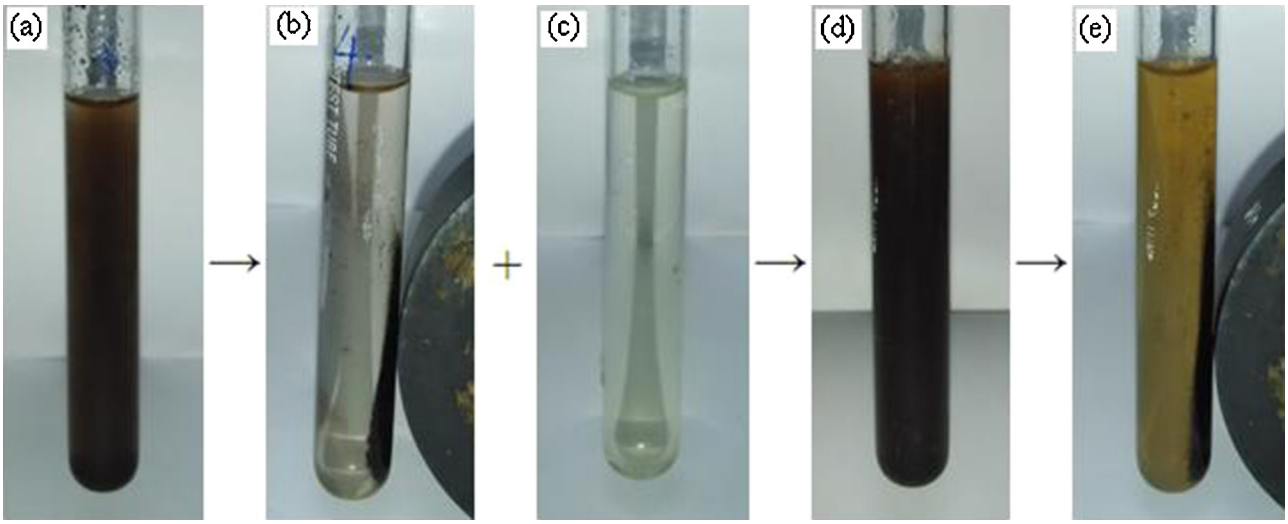

Fig. 1. (a) MNP suspension (b) MNP under magentic field (c) amylase solution (d) amylase immoblized on pretreated MNP (e) amylase immbilized MNP under magentic field.

2.3. Magnetic immobilization of α-amylase enzyme

Magnetic nanoparticle was prepared by co-precipitation of $FeSO_4$ and $FeCl_3$ solution. 50 ml of 0.2 M $FeCl_3$ and 50 ml of 0.1 M $FeSO_4$ solutions were prepared and mixed together. The solution was kept under constant mixing with drop wise addition of 50 ml of 10% NaOH to the mixture until black precipitate was obtained. The resultant precipitate was magnetically decanted and dried in oven at 80 °C. The dried magnetic nanoparticles and α-amylase were mixed in the ratio of 1:1 in phosphate buffer (pH 4) and kept in a shaker for 30 min. Gluteraldehyde (1%, v/v) was added in to the mixture of enzyme-magnetic nanoparticle and continued in the shaker for 2 h. Then the magnetically immobilized α-amylase was separated by magnetic decantation [14].

2.4. Characterization of immobilized α-amylase using FTIR, SEM with EDS

The magnetic effect of both MNP and MNP with amylase was confirmed using a strong magnet. The structure, size, shape and elemental composition of the synthesized magnetically immobilized α-amylase were analyzed using field emission scanning electron microscope (FESEM) from CARL ZEISS, Germany and energy dispersive spectroscope (EDS) from Oxford Instruments, United Kingdom. The phase structure of the nanocatalyst was studied by X-ray diffraction analysis (XRD) from Rikagu, Japan.

2.5. Batch studies on hydrolysis of sweet potato starch using immobilized α-amylase

The effect of sweet potato starch concentration on hydrolysis by magnetically immobilized α-amylase was studied under fixed other conditions. The starch concentration was varied from 2.5% to 6% (w/v) with an interval of 0.5%. The effect of initial pH (3–8) of the starch solution on hydrolysis was studied under fixed other conditions. The effect of reaction temperature (25, 30, 35, 40 and 45 °C) and magnetically immobilized α-amylase concentration (0.2, 0.4, 0.6, 0.8 and 1.0%(w/v)) on hydrolysis of sweet potato starch were studied under fixed other conditions. The reusability of magnetically immobilized α-amylase for hydrolysis of sweet potato starch was studied under constant conditions.

2.6. Estimation of glucose

The glucose concentration in sweet potato starch hydrolysate was estimated using Dinitro Salicylic Acid (DNSA) method. The samples collected from reaction mixture were mixed with DNS reagent. Then the reaction mixture was mixed well and capped in order to avoid evaporation and boiled in water bath for 10 min. Then reaction mixture was cooled to room temperature and absorbance was observed at 540 nm by using spectrophotometer [15].

3. Results and discussion

3.1. Magentic separation of immobilized α-amylase on magnetic nanoparticles

The magnetic effect of MNP and MNP with amylase was confirmed using a strong magnet as shown in Fig. 1. The MNP suspension in Fig. 1(a) was strongly attracted by a magnet as shown in Fig. 1(b). The MNP was completely accumulated nearer to the magnet. The α-amylase immobilized MNP in Fig. 1(d) was separated under the influence of magnetic field as shown in Fig. 1(e). Thus the magnetically immobilized α-amylase can be effectively separated and reused.

3.2. Activity and specific activity of immobilized enzyme

The activity and specific activity of MNP immobilized α-amylase were found to be 8.65 U/ml and 9.95 U/mg, respectively. The activity and specific activity of free enzyme were found to be 13.22 U/ml and 11.26 U/mg, respectively. The immobilized amylase has retained 88.37% of its original activity as compared to free enzyme. The amylase loading on MNP was found to be 173.91 mg/g.

3.3. Characterization of immobilized α-amylase using SEM, EDS, FTIR and XRD

The surface characteristics of magnetically immobilized α-amylase were studied using FE-SEM as shown in Fig. 2. The shape of magnetic nanocomposite of α-amylase was confirmed as spherical. The size of the nanocomposite was found to vary from 51.58 nm to 21.10 nm and the average particle size was 37.18 nm. The surface of the nanocomposite was found porous in nature which might have enhanced the catalytic activity of nanocomposite of α-amylase.