Chinese Journal of Catalysis 37 (2016) 389–397

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/chnjc

Article

Immobilized cellulase on Fe_3O_4 nanoparticles as a magnetically recoverable biocatalyst for the decomposition of corncob

Qikun Zhang *, Junqing Kang, Bing Yang, Leizhen Zhao, Zhaosheng Hou, Bo Tang

College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, Shandong, China

ARTICLE INFO

Article history:
Received 2 December 2015
Accepted 8 December 2015
Published 5 March 2016

Keywords:
Magnetic nanoparticle
Cellulase
Enzyme immobilization
Corncob
Glutaraldehyde

ABSTRACT

A magnetically recoverable biocatalyst was successfully prepared through the immobilization of cellulase onto Fe_3O_4 nanoparticles. The magnetic nanoparticles were synthesized by a hydrothermal method in an aqueous system. The support (Fe_3O_4 nanoparticles) was modified with (3-aminopropyl)triethoxysilane, and glutaraldehyde was used as the cross-linker to immobilize the cellulase onto the modified support. Different factors that influence the activity of the immobilized enzyme were investigated. The experimental results indicated that the suitable immobilization temperature and pH are 40 °C and 6.0, respectively. The optimal glutaraldehyde concentration is ~2.0 wt%, and the appropriate immobilization time is 4 h. Under these optimal conditions, the activity of the immobilized enzyme could be maintained at 99.1% of that of the free enzyme. Moreover, after 15 cyclic runs, the activity of the immobilized enzyme was maintained at ~91.1%. The prepared biocatalyst was used to decompose corncobs, and the maximum decomposition rate achieved was 61.94%.

1. Introduction

The limited reserves of coal, crude oil, and natural gas have expedited the recycling of natural biological resources [1,2]. Cellulose, as one of the main types of bioresources, is a renewable raw material and potential feedstock employed in the industry [3]. Therefore, reusing agricultural byproducts that mainly consist of cellulosic material is particularly important [4–7]. However, most of the cellulose resources have not been efficiently used. For instance, corncobs are only used at ~0.5% of the total output [8,9].

To efficiently use corncobs, research scientists have attempted to convert the main components (~40% cellulose and 30% hemicellulose) of corncobs into glucose, xylose, furfural, and ethanol via many decomposition methods [10,11]. One of the most promising strategies is decomposing cellulose with cellulase [12].

However, the potential of such a process is limited as hydrocellulose enzymes cannot be efficiently reused. Specifically, they lack long-term stability under processing conditions, and it is very difficult to recycle and reuse the catalyst from the reaction system [13]. Immobilizing enzymes on a solid support offers an attractive protocol to improve the reusability of conventional biocatalyst systems. Immobilized cellulase has more advantages than free cellulase for batch treatment or continuous processes. The immobilized cellulase can be easily re-

* Corresponding author. Tel: +86-18663770029; Fax: +86-531-86180589; E-mail: zhangqk@sdnu.edu.cn
This work was supported by the National Basic Research Program of China (973 Program, 2013CB933800), the Natural Science Foundation of Shandong Province (ZR2013EMM004), the Jinan University Independent Innovation Program (201401245), and the College Students' Innovation Training Program of Ministry of Education (201410445074).
DOI: 10.1016/S1872-2067(15)61028-2 | http://www.sciencedirect.com/science/journal/18722067 | Chin. J. Catal., Vol. 37, No. 3, March 2016

moved from the reaction mixture and displays adaptability to varying engineering designs [14,15].

Many supports can potentially be used to immobilize cellulase such as pumice [16], electro-spun polyacrylonitrile, nanofibrous membranes [17], methacrylate copolymer [18], and graphene nanoparticles [19]. Generally, any solid support with surface functional groups that can provide strong chemical interactions with the enzyme can be used [20,21]. However, employing nanoparticle supports is of particular interest. Nanoscale particles offer larger specific areas and afford a reasonably high enzyme loading capacity toward potentially achieving higher enzyme activity. Furthermore, using nanoparticles may offer a better solution for diffusion problems that are often encountered in conventional reaction systems. Currently, nano-sized magnetic particles are widely used for the immobilization of proteins, peptides, and enzymes [22]. Of particular interest, using magnetic nanoparticles as supports for enzyme immobilization offers several advantages as follows: (1) higher specific surface area that favors higher binding efficiencies; (2) lower mass transfer resistance and reduced fouling; (3) the immobilized enzymes can be selectively separated using an applied magnetic field, thereby affording reduced operation costs; and (4) the application of a continuous biocatalysis system [23].

Popular immobilization strategies include chemical and physical (ionic exchange, hydrophobic adsorption, affinity adsorption) binding to porous supports, non-porous nanomaterials, and the immobilization of enzymes in the absence of a support. Particularly, covalent coupling methods have been used to immobilize enzymes onto different supports [24]. Accordingly, enzymes with amino acid residues can be site-directly immobilized via the formation of covalent bonds between the amino acid residue and an active group on the support. Comparatively, the covalent immobilization method can eliminate or significantly reduce enzyme leakage through increased bond strength.

Accordingly, in this paper, a biocatalyst was prepared, whereby the immobilized enzyme was prepared via the covalent immobilization strategy [25]. In a typical process, magnetic Fe_3O_4 nanoparticles coated with (3-aminopropyl)triethoxysilane were activated via glutaraldehyde, and the activated magnetic supports were effectively coupled with cellulase with high enzymatic activity via covalent bonding [26–28]. The immobilization of cellulase onto the Fe_3O_4 nanoparticles was investigated by Fourier transform infrared spectroscopy (FT-IR). The size and surface morphology of the particles before and after binding cellulase were characterized by transmission electron microscopy (TEM), and the thermal stability of the immobilized enzyme was evaluated by thermal gravimetric analysis (TGA). Furthermore, several interdependent parameters, i.e., temperature, pH, glutaraldehyde concentration, and immobilization time, that affect the immobilization efficiency and enzymatic activity of the biocatalyst were investigated. Subsequently, the activity of the immobilized cellulase toward the decomposition of corncob to glucose was examined. The effect of several parameters on the efficiency of the decomposition reaction was also evaluated.

2. Experimental

2.1. Raw materials

All chemicals employed in this paper were of pure grade and used as received without further purification. Solutions were prepared with deionized water. Cellulase was purchased from Novozymes (Denmark). The corncobs were collected from the nearby countryside. Glutaraldehyde (50%) and ammonia ($NH_3{\cdot}H_2O$) were obtained from Sinopharm Chemical Reagent Co., Ltd., China. (3-Aminopropyl)triethoxysilane (APTS; 96%) was purchased from Shandong Qufu Wanda Chemicals Co., Ltd., China.

2.2. Preparation of Fe_3O_4 magnetic nanoparticles

Magnetic Fe_3O_4 nanoparticles were prepared using a hydrothermal method. In a typical process, 4.48 g (0.112 mol) NaOH was dissolved in 50 mL distilled water at room temperature, after which 1.02 g $Na_2S_2O_3{\cdot}5H_2O$ was added. The resulting solution was placed into a 250-mL round-bottom flask to form a homogeneous mixture by vigorous stirring. The solution was purged with N_2 for 30 min. Subsequently, 11.12 g (0.03 mol) $FeSO_4{\cdot}7H_2O$ was dissolved in 100 mL distilled water and then immediately poured into the aforementioned solution. After stirring for 2 h, the solution was transferred to a hydrothermal reactor that was heated at 160 °C for 16 h. After completion of the reaction, the upper mixture was washed with distilled water by magnetic decantation, and thorough dialysis was performed until solution neutrality was achieved [29]. A black precipitate was obtained. After separation with a magnet, the precipitate was redispersed in distilled water. The resulting Fe_3O_4 nanoparticle aqueous solution (diluted to 10 mg/mL) was stored in a sealed container.

2.3. Preparation of APTS-coated Fe_3O_4 (APTS@Fe_3O_4) magnetic nanoparticles

The prepared Fe_3O_4 magnetic nanoparticles were diluted to a solution consisting of 182 mL ethanol, 10 mL distilled water, and 8 mL APTS. The solution was transferred to a 500-mL three-neck round-bottom flask and stirred at 40 °C for 2 h in N_2. The resulting nanoparticles were washed thrice with distilled water and twice with ethanol, and then dried into a powder at room temperature under vacuum.

2.4. Immobilization of cellulase on APTS@Fe_3O_4 magnetic nanoparticles

Approximately 0.05 g APTS@Fe_3O_4 nanoparticles were dispersed in 25 mL buffer solution prepared with acetic acid and sodium acetate. Then, 0.5 mL glutaraldehyde and 0.5 mL cellulose were added to the buffer solution in a conical flask and shaken at 40 °C for 3 h. The immobilized cellulase nanoparticles were then washed with distilled water and separated with a magnet. The washing process was repeated several times until no free cellulase was detected in the rinsing solution (wa-

ter). The residual concentration of cellulase in the rinsing solution were determined by the dinitrosalicylic acid (DNS) method. The amount of immobilized cellulase was calculated by thermal gravimetric analysis [30].

2.5. Determination of cellulase activity

To evaluate the enzymatic activity of the free and immobilized enzyme, cellulase activity units (U) are defined as the required amount of enzyme to produce 1 μg glucose from the hydrolysis of sodium carboxymethylcellulose within 1 min at 40 °C and pH = 4.6. In a typical procedure, 4 mL buffer solution (pH = 4.6) was introduced into a conical flask containing 0.05 g immobilized cellulase. Then, 1 mL sodium carboxymethylcellulose solution (1.0 g sodium carboxymethylcellulose dissolved in 100 mL buffer solution) was introduced to the flask, and the mixture was shaken at 40 °C for 30 min. Cellulase molecules catalytically degraded sodium carboxymethylcellulose molecules to glucose molecules. Subsequently, the magnetic immobilized cellulase was separated and recovered with an external magnet. The upper solution without biocatalyst was introduced into the colorimetric tube. Then, 1.5 mL DNS reagent solution was added to the upper solution and heated in boiling water for 5 min to give a colored product, which was evaluated by measuring the solution absorbance at 540 nm. (The DNS reagent solution was prepared by dissolving 182.0 g sodium potassium tartrate tetrahydrate, 21.0 g sodium hydroxide, 6.3 g 3,5-dinitrosalicylic acid, 5.0 g phenol, and 5.0 g sodium sulfate in 1000 mL distilled water and kept in the dark for 7–10 d before use.) A parallel sodium carboxymethylcellulose solution sample without enzyme was prepared as a blank control. The amount of hydrolyzed glucose was calculated from the normalization equation of the standard curve. The enzymatic activity of the free and immobilized enzymes was calculated as follows:

$$X = \frac{W \times n \times 1000}{T \times M} \quad (1)$$

where X, W, n, T, and M are the cellulase activity units (U), mass of glucose in solution calculated from the normalization equation of the standard curve (g), dilution factor of the enzyme solution, reaction time (min), and mass of the enzyme sample (g), respectively [13].

2.6. Pretreatment of corncob

Prior to use, the corncobs obtained from the nearby countryside were air-dried, ground into a powder, and sieved with 200-mesh sieve. Then, 1.5 mol/L ammonia was added to the corncob powder at a mass ratio of 10:1. The mixture was pretreated at 80 °C for 3 h, after which the corncobs were separated from the mixture. The pretreated power was washed with water to pH = 7.0 and dried at room temperature [31,32].

2.7. Decomposition of corncob

For the decomposition studies, 0.20 g pretreated corncobs were transferred to a 100-mL conical flask. Then, 25.0 mL sodium acetate buffer solution (pH = 6.0) and 50.0 mg immobilized cellulase were introduced into the flask. The flask was shaken at 40 °C for a given time. Then, the glucose released into solution was evaluated according to the method described in Section 2.5 [33]. The decomposition rate was calculated as follows:

$$D = \frac{m \times 25}{M \times n \times 1000} \times 100\% \quad (2)$$

where D, m, n, and M are the decomposition rate (%), mass of glucose in solution calculated from the normalization equation of the standard curve (mg), content of cellulose in the corncob sample (%), and mass of the corncob sample (mg), respectively.

2.8. Characterization

The size and morphology of the nanoparticles were determined by TEM (Hitachi H600) at an acceleration voltage of 200 kV. Mass loss measurements were conducted via TGA (Netzsch TG-20) in N_2 atmosphere from 50 to 600 °C at a heating rate of 20 °C/min. To confirm the immobilization of cellulase on the surface of the Fe_3O_4 nanoparticle support, FT-IR (Bruker Tensor 27) analysis was conducted within a wavenumber range of 4000–500 cm^{-1}. Powder XRD (Bruker D8 Advance) was used to investigate the crystal structure of the obtained nanoparticles. The magnetic properties of the nanoparticles before and after immobilization were analyzed by vibrating sample magnetometry (VSM; JDM-13E, Digital Measurement System, Inc.).

The enzymatic activity of the magnetic biocatalysts was determined by direct measurement of the absorbance of the glucose solution at 540 nm using a UV-visible spectrophotometer (TU 1900, Shimadzu Corporation, assembled in China).

3. Results and discussion

3.1. TEM analysis of the magnetic nanoparticles

The size and surface morphologies of the bare and coated Fe_3O_4 nanoparticles and biocatalysts were examined by TEM. The average size of each layer was calculated (Table 1) to assess the change in particle size after each process.

Additionally, as observed from Fig. 1, nearly monodisperse Fe_3O_4 nanospheres were successfully prepared by the hydrothermal method. Compared with the bare particles, the surface of the magnetic biocatalyst was rougher owing to the membranous layer covering the particle support.

3.2. FT-IR analysis

Fig. 2 shows the FT-IR spectra of cellulase, bare and coated Fe_3O_4 nanoparticles, and representative biocatalyst. The band

Table 1
Diameter of the bare and APTS-coated Fe_3O_4 nanoparticles and representative biocatalyst.

Sample	Diameter (nm)
Bare Fe_3O_4	15 ± 3
APTS-coated Fe_3O_4	50 ± 10
Biocatalyst	110 ± 20

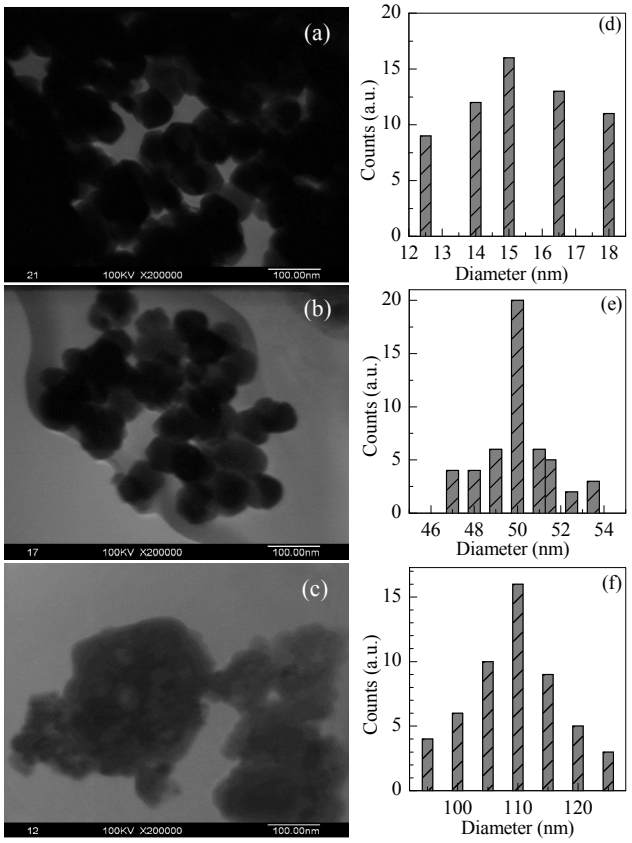


Fig. 1. TEM images and mean diameters of bare Fe_3O_4 (a, d), APTS@Fe_3O_4 (b, e), and representative biocatalyst (c, f).

at 567 cm^{-1} was attributed to the vibration of Fe–O bonds in the crystalline lattice of bare Fe_3O_4. In contrast, the biocatalyst sample featured a weaker absorption band (567 cm^{-1}). The absorption peak at 3450 cm^{-1} was attributed to O–H in the bare and coated Fe_3O_4 samples. In the biocatalyst sample, the absorption peak at 1047 cm^{-1} was attributed to the stretching vibrations of C–O of carboxyl. The band at 3400 cm^{-1} could be associated with N–H stretch, which overlapped with the O–H stretch absorption peak at 3100 cm^{-1}. The absorption peak at 1650 cm^{-1} was attributed to the C=O stretch of amide. These results confirmed that cellulase was successfully immobilized onto the Fe_3O_4 nanoparticles.

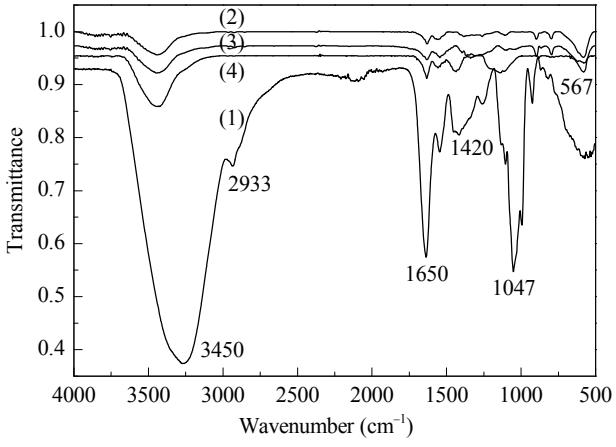


Fig. 2. FT-IR spectra of cellulase (1), bare Fe_3O_4 (2), APTS@Fe_3O_4 (3), and representative biocatalyst (4).

3.3. XRD crystal analysis

The change in the crystallinity of the Fe_3O_4 nanoparticles before and after coating with APTS and after loading with cellulase was investigated by XRD (Fig. 3). According to the Joint Committee on Powder Diffraction Standards (JCPDS) database, the XRD pattern of a standard Fe_3O_4 crystal with a spinel structure has six characteristic peaks at 2θ = 30.1°, 35.5°, 43.1°, 53.4°, 57.0°, and 62.6° that correspond to the (220), (311), (400), (422), (511), and (440) planes of Fe_3O_4, respectively. As observed in Fig. 3, the bare Fe_3O_4 nanoparticles featured distinct crystalline peaks at 2θ = 30.40°, 35.80°, 43.60°, 53.70°, 57.20°, and 62.70°. The XRD patterns of the starting material (Fe_3O_4), APTS@Fe_3O_4, and biocatalysts were consistent with the pattern exhibited by standard magnetite. Therefore, it could be concluded that the modification treatment did not cause a phase change in Fe_3O_4, and the magnetite nanoparticles modified with APTS and cellulase also featured the spinel structure. Furthermore, as the APTS and cellulase coatings are amorphous, new peaks attributable to these groups were not detected in the XRD patterns. However, the crystallinity of the samples decreased after the coating process. This phenomenon was likely due to the partial destruction or hindering of the regularity of the Fe_3O_4 structure after coating with APTS and cellulase. The most probable structure of the prepared biocatalysts is shown in Fig. 4.

3.4. Magnetic properties

The magnetic properties of the bare and coated Fe_3O_4 and biocatalyst nanoparticles were determined by VSM. Fig. 5 shows the corresponding magnetization curves. The curve of bare Fe_3O_4 displayed no coercivity features. The mass saturation magnetization (M_s) of the bare Fe_3O_4 nanoparticles was 71.96 emu/g, thereby indicating that the prepared particles exhibit superparamagnetic behavior. Furthermore, changes in the M_s of the particles after coating with APTS and enzyme were observed. M_s decreased from 71.96 to 54.74 emu/g (for

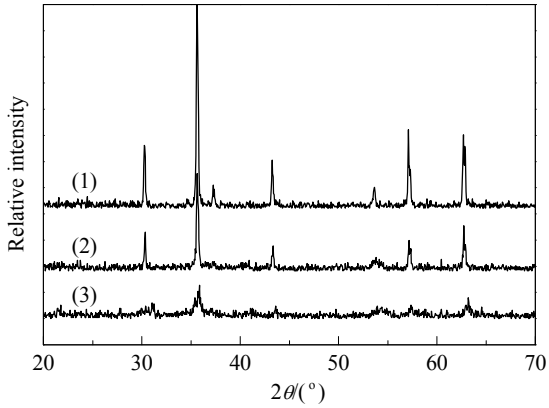


Fig. 3. XRD patterns of the bare Fe_3O_4 (1), APTS@Fe_3O_4 (2), and representative biocatalyst (3) nanoparticles.

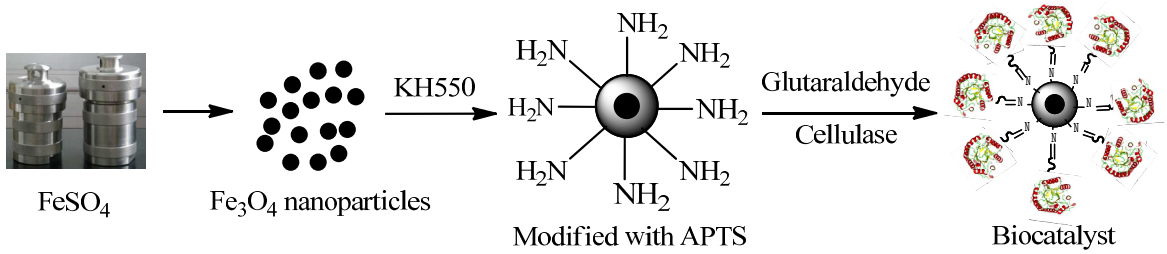


Fig. 4. Schematic representation of the preparation of the magnetic biocatalyst.

APTS@Fe_3O_4) and to 48.81 emu/g (for biocatalyst). This decrease was attributed to the contribution of the non-magnetic APTS shell to the total mass of the particles, which apparently weakened the magnetic properties.

3.5. Enzyme activity

Stability and activity are essential attributes of a highly efficient immobilized enzyme. The activities of free cellulase and the prepared biocatalysts were determined by the DNS method. In a typical process, a 1.0 mg/mL glucose standard solution was prepared by dissolving 0.2 g glucose in 200 mL distilled water. A series of glucose solution concentrations were prepared by dilution of the glucose standard solution. The DNS reagent was then used as mentioned above to treat the glucose solutions. The absorbance of the treated solution (measured at 540 nm) increased linearly with increasing glucose concentrations in the range of 0–50 mg/L. Using this method, the concentration of glucose produced upon decomposition of sodium carboxymethycellulose by the free cellulase and prepared biocatalysts can be detected and calculated. And the enzymatic activity of the free and immobilized cellulase was obtained accordingly. The results are reported in Table 2, which shows that the obtained immobilization yield was as high as 99.1%.

3.6. Cellulase immobilization

To investigate the optimal conditions of cellulase immobilization, several interdependent parameters that influence the cellulase activity and immobilization efficiency were investigated. Specifically, the immobilization temperature, buffer solution pH, concentration of glutaraldehyde, and immobilization time were considered.

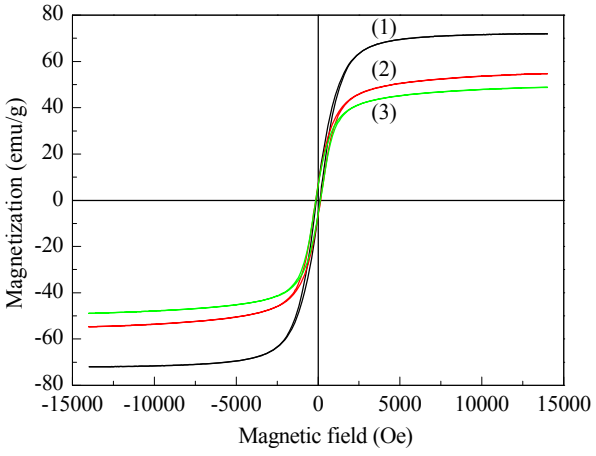


Fig. 5. Magnetization curves of the bare Fe_3O_4 (1), APTS@Fe_3O_4 (2), and representative biocatalyst (3) nanoparticles.

3.6.1. Immobilization temperature

Cellulase can be covalently immobilized onto the Fe_3O_4 magnetic nanoparticles by forming a Schiff base linkage between the aldehyde group of glutaraldehyde and the terminal amino group of modified Fe_3O_4 magnetic nanoparticles and cellulase. Various factors including the coupling temperature, coupling time, and glutaraldehyde concentration can affect the interactions.

The immobilized enzyme activities at different immobilization temperatures were evaluated, and the results are shown in Fig. 6(a). As observed, the immobilization temperature influenced the activity of the immobilized enzyme. The activity of the immobilized cellulase increased with increasing temperatures in the range of 25–40 °C, and then decreased abruptly. Generally, higher temperatures benefit the immobilization of cellulase onto the nanoparticles, leading to higher enzyme loadings. However, excessively high temperatures can accelerate the deactivation of the enzyme. Therefore, at excessively high temperatures, the activity of the immobilized cellulase decreased. Thus, the optimal immobilization temperature was determined as 40 °C, corresponding to a maximum relative enzyme activity of 84.56%.

3.6.2. Buffer solution pH

The variation in the biocatalyst activity in buffer solution at different pHs was determined, and the result is shown in Fig. 6(b). The immobilized biocatalysts displayed maximum activity at pH = 6 (~99.1%). This result agreed with the product specification of cellulose, which was purchased from Novozymes (Denmark). Excessively high or low buffer solution pHs accelerated the deactivation of the enzyme. Therefore, the optimum buffer solution pH was determined as 6.0.

Table 2
Enzymatic activity results.

Sample	W/mg	n	T/min	M/g	X/(U/g)	Immobilization yield (%)
Free enzyme	2.92	5	30	0.048	10234.4 ± 100	100.00
Biocatalyst	2.86	5	30	0.050	9521.3 ± 95	93.03
Biocatalyst	2.87	5	30	0.048	10142.3 ± 100	99.10
Biocatalyst	2.86	5	30	0.055	8654.5 ± 90	84.56

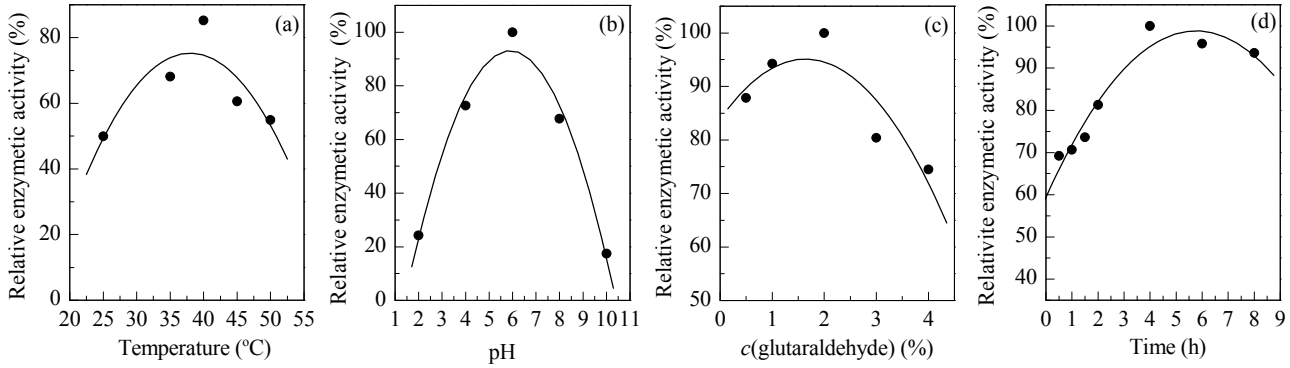


Fig. 6. Variations in the activity of immobilized cellulase as a function of enzyme immobilization temperature (a), buffer pH (b), glutaraldehyde concentration (c), and immobilization time (d). Immobilization conditions: cellulase amount = 0.5 mL; temperature = 40 °C (except (a)); pH = 6.0 (except (b)); glutaraldehyde concentration = 1.0% (except (c)); reaction time = 2 h (except (d)); the free cellulase was defined as the 100% activity.

3.6.3. Glutaraldehyde concentration

Glutaraldehyde plays a significant role in the immobilization process. Considerable changes in the immobilized enzyme activity were observed at varying glutaraldehyde concentrations (Fig. 6(c)). The activity of immobilized cellulase increased with increasing glutaraldehyde concentrations in the range of (0.5–2.0) wt%, and then decreased abruptly. In a typical procedure, the amino group on the surface of APTS@Fe_3O_4 and enzyme react with the aldehyde group on the glutaraldehyde. Glutaraldehyde connects the enzyme to the aminated support as a coupling agent. In general, the immobilization degree increases with increasing glutaraldehyde concentrations. However, excessively high glutaraldehyde concentrations accelerate the deactivation of the enzyme, thereby accelerating the self-cross-linking of the enzyme or the aminated support. Therefore, at an excessively high glutaraldehyde concentration, the activity of immobilized cellulase showed a decreasing tendency. Thus, the optimum glutaraldehyde concentration was 2.0%.

3.6.4. Immobilization time

The immobilization time greatly influences the activity of immobilized cellulase. The variation in the enzymatic activity with immobilization time is presented in Fig. 6(d). As observed, the activity of immobilized cellulase increased with immobilization time until equilibrium was reached within 6 h of reaction. The result indicated that with increasing coupling time from 0 to 4 h, the enzymatic activity increased with the amount of immobilized cellulase. The optimal immobilization time was determined at 4 h.

3.7. Thermal stability of the prepared biocatalysts

Fig. 7 shows the TGA curves of the bare and coated Fe_3O_4 nanoparticles, cellulase, and representative biocatalyst. The bare and coated Fe_3O_4 nanoparticles displayed excellent thermal stability at increasing temperatures up to 600 °C, with a mass loss of 1% and 6%, respectively. In contrast, the TGA curve of the biocatalyst displayed two distinct mass loss stages. The first mass loss (3.2%) was observed within 0–100 °C. Thereafter, the biocatalyst was stable up to 240.5 °C. However, between 240.5 and 500 °C, a rapid and continuous mass loss occurred, and by 500–600 °C, no further significant changes were observed. The first mass loss was attributed to the evaporation of water or small amounts of free APTS adsorbed by the particles. The subsequent change was attributed to the desorption and thermal degradation of cellulase molecules immobilized on parent Fe_3O_4.

To determine whether the grafting treatment can stabilize cellulase, thermal analysis of pure cellulase was also conducted. Within the temperature range of 0–240.5 °C, the relative mass of pure cellulase changed from 100% to 36.24%, thus indicating that pure cellulase has poor thermal stability and immobilization on Fe_3O_4 nanoparticles can stabilize the cellulase.

Additionally, the Fe_3O_4 content of the prepared magnetic biocatalyst, calculated from the TGA data, was 78.0%, and the immobilization loading of cellulase was 161 mg/g. These results confirmed that the magnetic biocatalyst possesses high enzyme content and is expected to display excellent magnetic properties.

3.8. Decomposition of corncob

Prior to use, the corncobs were pretreated using the method described in Section 2.6 and subsequently decomposed using the method described in Section 2.7. The results are presented

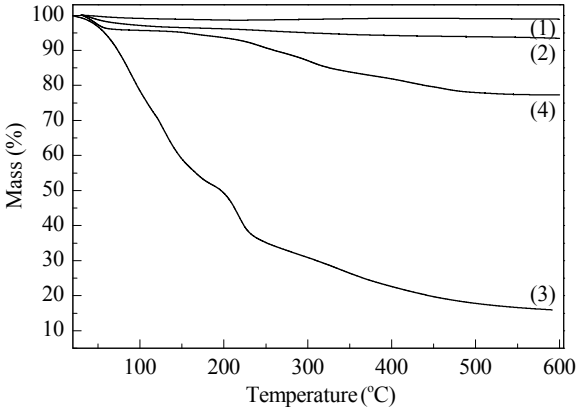


Fig. 7. TGA curves of the bare Fe_3O_4 (1), APTS@Fe_3O_4 nanoparticles (2), cellulase (3), and representative biocatalyst (4).

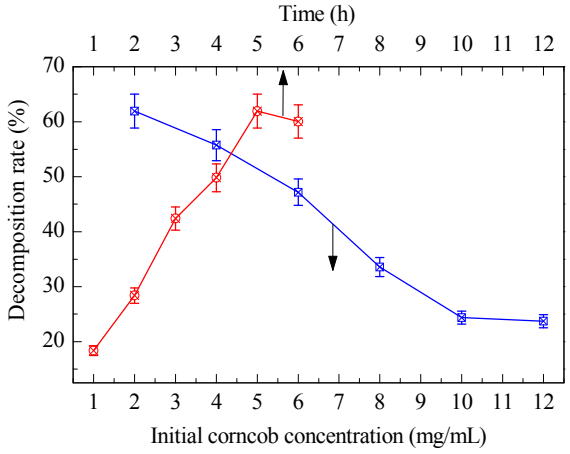


Fig. 8. Variations in the decomposition rate of corncob as a function of reaction time and initial corncob concentration. Reaction conditions: temperature = 40 °C; pH = 6.0.

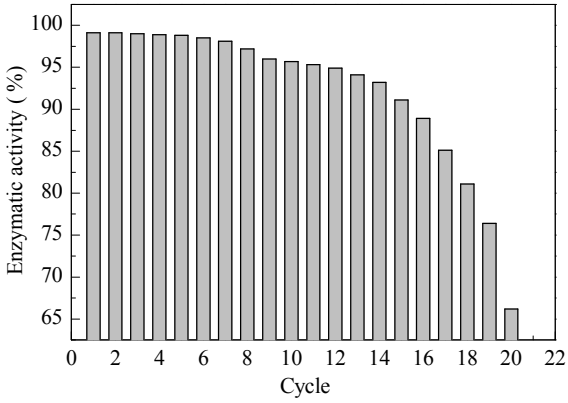


Fig. 9. Recovery and recycling stability of the immobilized cellulase. Reaction conditions: temperature = 40 °C; pH = 6.0; initial corncob concentration = 2.0 mg/mL; reaction time = 5 h; the free cellulase was defined as the 100% activity.

in Fig. 8. The decomposition rate changed with reaction time and initial corncob concentration. At a given initial corncob concentration (2.0 mg/mL), the glucose content in the reaction solution increased from 73.4 to 247.8 mg/L as the reaction time increased from 0 h to 6 h, and the decomposition rate of corncob decreased from 18.36% to 61.94%. When the initial corncob concentration increased from 2.0 to 16.0 mg/mL and the reaction time was set to 5 h, the glucose content in the reaction solution increased from 247.8 to 568.8 mg/L. However, the total decomposition rate of corncob decreased from 61.94% to 23.72%.

Cellulase is a complex mixture that consists of a variety of hydrolytic enzymes. Cellulase can be divided into three categories: C1 enzyme, Cx enzyme, and β-glucoside enzyme. C1 enzymes are the catalyst that initiates the decomposition of cellulose. They can destroy the crystalline structure of cellulose. Cx enzymes are typically involved in the subsequent decompositions stages of cellulose, specifically, the β-1,4 glycosidic bonds of cellulose. β-Glucoside enzyme can decompose cellobiose, cellotriose, and other low molecular dextrin to glucose. Reaction conditions are expected to influence the decomposition rate of corncob. Accordingly, studying the effects of several interdependent parameters such as reaction temperature, reaction time, pH, the influence of pretreatment and initial corncob concentration on the total decomposition rate of corncob deserves further investigations.

3.9. Recovery and recycling of immobilized cellulase

The recovery and recycling stability of the immobilized cellulase was studied under the same conditions as those employed in the activity assays. After each cycle, the cellulase-containing magnetic nanoparticles were magnetically separated and washed with distilled water to remove residual substrate and product before the subsequent experiment.

Fig. 9 shows the residual activity of the immobilized cellulase after each cycle. The results showed that the activity of the immobilized enzyme on Fe_3O_4 nanoparticles gradually declined within the first 15 cycles from 99.1% to 91.1%. After 15 cycles, the activity of the immobilized catalyst decreased further, however, remained relatively high. After 20 cycles, the enzymatic activity was 66.2%. Hence, the prepared biocatalyst is more suitable for practical use than free cellulase.

4. Conclusions

A magnetically recoverable biocatalyst was successfully prepared through the covalent immobilization of cellulase onto aminated Fe_3O_4 nanoparticles. The mass saturation magnetization of the bare Fe_3O_4 nanoparticles and representative prepared biocatalyst were 71.96 and 48.81 emu/g, respectively. The enzymatic activity of the immobilized cellulase was 10142.3 U/g, which was 99.1% of the free enzyme activity. The immobilized enzyme exhibited significant thermal stability (up to 500 °C) and good durability. After 15 cycles, its activity remained at 91.1%. The prepared biocatalyst was used to decompose corncob, and the maximum decomposition rate attained was 61.94%.

Acknowledgments

We like to thank Yiling Bei and Guohui Huang for their experimental contribution.

References

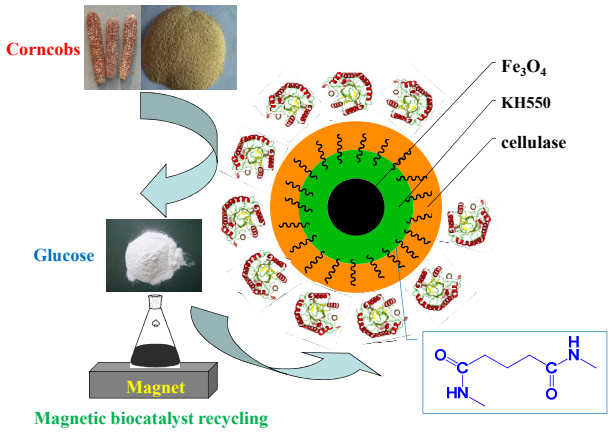
[1] W. L. Xie, N. Ma, *Energy Fuels*, **2009**, 23, 1347–1353.
[2] W. J. Goh, V. S. Makam, J. Hu, L. F. Kang, M. R. Zheng, S. L. Yoong, C. N. B. Udalagama, G. Pastorin, *Langmuir*, **2012**, 28, 16864–16873.
[3] L. Wang, X. G. Fan, P. Tang, Q. P. Yuan, *J. Chem. Technol. Biotechnol.*, **2013**, 88, 2067–2074.
[4] S. J. Bao, X. G. Zhang, X. M. Liu, L. Y. Xin, J. P. Qu, *Chin. J. Catal.*, **2003**, 24, 909–913.
[5] W. S. Lim, J. W. Lee, *Bioresour. Technol.*, **2013**, 130, 97–101.
[6] A. Karlsson, A. Aspegren, *J. Chromatogr. A*, **2000**, 866, 15–23.
[7] E. Cherian, M. Dharmendirakumar, G. Baska, *Chin. J. Catal.*, **2015**, 36, 1223–1229.
[8] L. Wang, H. Z. Chen. *Process Biochem.*, **2011**, 46, 604–607.

Graphical Abstract

Chin. J. Catal., 2016, 37: 389–397 doi: 10.1016/S1872-2067(15)61028-2

Immobilized cellulase on Fe_3O_4 nanoparticles as a magnetically recoverable biocatalyst for the decomposition of corncob

Qikun Zhang *, Junqing Kang, Bing Yang, Leizhen Zhao, Zhaosheng Hou, Bo Tang
Shandong Normal University



A biocatalyst was successfully prepared through the immobilization of cellulase onto Fe_3O_4 nanoparticles and employed as a magnetically recoverable catalyst for the decomposition of corncob.

[9] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple, M. Ladisch, *Bioresour. Technol.*, **2005**, 96, 673–686.
[10] Y. Ping, H. Z. Ling, G. Song, J. P. Ge, *Biochem. Eng. J.*, **2013**, 75, 86–91.
[11] L. L. Ding, B. Zou, H. Q. Liu, Y. N. Li, Z. C. Wang, Y. Su, Y. P. Guo, X. F. Wang, *Chem. Eng. J.*, **2013**, 225, 300–305.
[12] P. Obama, G. Ricochon, L. Muniglia, N. Brosse, *Bioresour. Technol.*, **2012**, 112, 156–163.
[13] Q. K. Zhang, X. T. Han, B. Tang, *RSC Adv.*, **2013**, 3, 9924–9931.
[14] J. S. Lupoi, E. A. Smith, *Biotechnol. Bioeng.*, **2011**, 108, 2835–2843.
[15] J. L. Rahikainen, J. D. Evans, S. Mikander, A. Kalliola, T. Puranen, T. Tamminen, K. Marjamaa, K. Kruus, *Enzyme Microb. Technol.*, **2013**, 53, 315–321.
[16] N. K. Pazarlioğlu, M. Sariişik, A. Telefoncu, *Process Biochem.*, **2005**, 40, 767–771.
[17] T. C. Hung, C. C. Fu, C. H. Su, J. Y. Chen, W. T. Wu, Y. S. Lin, *Enzyme Microb. Technol.*, **2011**, 49, 30–37.
[18] Y. Y. Yu, J. G. Yuan, Q. Wang, X. R. Fan, X. Y. Ni, P. Wang, L. Cui, *Carbohyd. Polym.*, **2013**, 95, 675–680.
[19] A. A. Gokhale, J. Lu, I. Lee, *J. Mol. Catal. B*, **2013**, 90, 76–86.
[20] L. Y. Wang, H. X. Wang, A. J. Wang, M. Liu, *Chin. J. Catal.*, **2009**, 30, 939–944.
[21] F. Liguori, C. Moreno-Marrodan, P. Barbaro, *Chin. J. Catal.*, **2015**, 36, 1157–1169.
[22] S. H. Huang, M. H. Liao, D. H. Chen, *Biotechnol. Prog.*, **2003**, 19, 1095–1100.
[23] G. Zheng, S. Yan. *Biotechnol. Prog.*, **2004**, 20, 500–506.
[24] F. Lopez-Gallego, L. Betancor, C. Mateo, A. Hidalgo, N. Alonso-Morales, G. Dellamora-Ortiz, J. M. Gaisan, R. Fernandez-Lafuente, *J. Biotechnol.*, **2005**, 119, 70–75.
[25] O. Barbosa, R. Torres, C. Ortiz, R. Fernandez-Lafuente, *Process Biochem.*, **2012**, 47, 1220–1227.
[26] Y. H. Dong, Y. Cai, Z. K. Sun, J. Liu, C. Liu, J. Wei, W. Li, C. Liu, Y. Wang, D. Y. Zhao, *J. Am. Chem. Soc.*, **2010**, 132, 8466–8473.
[27] S. Laurent, D. Forge, M. Port, A. Roch, C. Robio, L. V. Elst, R. N. Muller, *Chem. Rev.*, **2008**, 108, 2064–2110.
[28] G. H. Zhao, J. Z. Wang, Y. F. Li, X. Chen, Y. P. Liu, *J. Phys. Chem. C*, **2011**, 115, 6350–6359.
[29] R. G. Chaudhuri, S. Paria, *Chem. Rev.*, **2012**, 112, 2373–2433.
[30] M. Dashtban, M. Maki, K. T. Leung, C. Q. Mao, W. S. Qin, *Crit. Rev. Biotechnol.*, **2010**, 30, 302–309.
[31] M. Chen, L. M. Xia, P. J. Xue, *Int. Biodeter. Biodegr.*, **2007**, 59, 85–89.
[32] E. Viola, F. Zimbardi, V. Valerio, F. Nanna, A. Battafarano, *Appl. Energy*, **2013**, 102, 198–203.
[33] E. Bahcegul, E. Tatli, N. I. Haykir, S. Apaydin, U. Bakir, *Bioresour. Technol.*, **2011**, 102, 9646–9652.

Page numbers refer to the contents in the print version, which include both the English version and extended Chinese abstract of the paper. The online version only has the English version. The pages with the extended Chinese abstract are only available in the print version.