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ORIGINAL ARTICLE

Preparation, Structural Characterization, and Enzymatic Properties of Alginate Lyase Immobilized on Magnetic Chitosan Microspheres

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Abstract

Alginate lyase is an enzyme that catalyses the hydrolysis of alginate into alginate oligoalginates. To enhance enzyme stability and recovery, a facile strategy for alginate lyase immobilization was developed. Novel magnetic chitosan microspheres were synthesized and used as carriers to immobilize alginate lyase. The immobilization of alginate lyase on magnetic chitosan microspheres was successful, as proven by Fourier transform infrared spectroscopy and X-ray diffraction spectra. Enzyme immobilization exhibited the best performance at an MCM dosage of 1.5 g/L, adsorption time of 2.0 h, glutaraldehyde concentration of 0.2%, and immobilization time of 2.0 h. The optimal pH of the free alginate lyase was 7.5, and this pH value was shifted to 8.0 after immobilization. No difference was observed at the optimal temperature (45 °C) for the immobilized and free enzymes. The immobilized alginate lyase displayed better thermal stability than the free alginate lyase. The K_m values of the free and immobilized enzymes were 0.05 mol/L and 0.09 mol/L, respectively. The immobilized alginate lyase retained 72% of its original activity after 10 batch reactions. This strategy was found to be a promising method for immobilizing alginate lyase.

Keywords Alginate lyase · Immobilization · Magnetic chitosan microspheres · Enzyme stability · Enzyme recovery

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Introduction

Alginate lyase is an enzyme that catalyses alginate into alginate oligoalginates. The alginate lyase acts by cleaving the 1,4-glycosidic bond of alginate via a beta-elimination mechanism [1]. Numerous alginate lyases have been isolated from *Vibrio* sp., *Pseudomonas aeruginosa*, and *Microbulbifer* sp. [2, 3]. Alginate lyases are divided into endolytic and exolytic modes based on the cleavage sites on alginate polysaccharide [4]. These enzymes have been used to degrade alginate in *Saccharina latissima, Laminaria japonica,* and other algae into alginate oligoalginates [5, 6]. Alginate lyases also have been used as pharmaceutical enzymes and enzymatic tools for biotechnology and molecular biology [7]. There are multiple advantages of using alginate lyases to produce alginate oligoalginates from alginate, including the environmental friendliness of this process, its high reaction rate under mild reaction conditions, its high specificity, its low energy consumption and side reactions [8]. However, the high cost, enzyme instability and difficulty in recovery of enzymes greatly limit their application in industry.

Immobilized enzyme technology can overcome the instability and difficulty in recovery. Enzyme immobilization is a technology that uses a carrier to restrain enzymes in a particular area and still maintains the specific catalytic activity of the enzymes, making them recoverable and reusable [9]. Methods of enzyme immobilization include physical (adsorption and entrapment) and chemical (crosslinking and covalent binding) methods [10]. Enzyme activity usually decreases upon immobilization [11]. The carrier material is particularly important for the immobilized efficiency of enzymes and enzyme stability. The carriers used for the immobilization of enzymes include natural organic materials, inorganic materials, organic polymers, and magnetic carriers [12]. Recently, the immobilization of alginate lyase has attracted great attention due to the enzyme reusability, high operational stability, and easy recovery of the product from enzymatic hydrolysates [13].

Magnetic nanomaterials are considered green materials. Unlike filtration and centrifugation, magnetic nanomaterials can be recovered by magnets, which are easy, cost effective, and efficient in preventing enzyme loss [14]. Among the magnetic nanomaterials, Fe_3O_4 nanoparticles have been used to immobilize cellulase, lipase, and amylase with good immobilization efficiency due to their small particle sizes, large specific surface areas, and strong magnetic properties [15–17]. Attaching inorganic or polymeric coatings to Fe_3O_4 nanoparticles can improve the functionality or biocompatibility of these nanostructures, which can prevent the self-aggregation of magnetic cores [18]. Natural polymer gel carriers (chitosan, calcium alginate, etc.) and organic synthetic polymers (polyacrylamide, polyethyleneimine, etc.) are often used as the base materials for carrier preparation because of the functional groups on their surfaces. Among these materials, chitosan (β-1,4–2-amino-2-deoxy-D-glucose) is an aminopolysaccharide with amino (-NH_2) and hydroxyl (-OH) groups in its molecular chain that can be easily chemically modified as active sites for biological binding [19]. Chitosan is one of the most abundant polysaccharides in nature, and it has a low cost, biodegradability, good biocompatibility, and nontoxicity, leading to its wide applicability in water treatment, enzyme immobilization, etc. [20]. There is little information on the immobilization of alginate lyases on magnetic chitosan microspheres.

The aim of this work was to synthesize novel magnetic chitosan microspheres (MCMs). Furthermore, the alginate lyase was immobilized using MCM and glutaraldehyde as the carrier and crosslinking agent, respectively. In addition, the structural characterization and enzymatic properties of the immobilized alginate lyase were investigated.

Material and Methods

Materials

Fe_3O_4 nanoparticles, alginate lyase, 3, 5-dinitrosalicylic acid, and other reagents were provided by Merck Chemicals Co., Ltd. (Shanghai City, China). The activity of alginate lyase was 20,000 U/g.

Preparation of MCM

Chitosan (1.0 g) was placed in a 250-mL glass flask. Then, 100 mL of acetic acid was added. Chitosan and acetic acid were mixed evenly. Fe_3O_4 nanoparticles (1.0 g) were placed in the above solution. The mixtures were dispersed ultrasonically (400 W ultrasonic power, 6 s interval, 30 s ultrasonic, and 30 min total working time). The dispersed mixtures were subsequently added into the Span-80/liquid paraffin system (160 mL of liquid paraffin and 10 mL of Span-80) under stirring (120 rpm and 30 min). Afterwards, 16 mL of 5% (w/v) glutaraldehyde was added. The sample was kept in a water bath at 40 °C for 1.0 h. The pH was regulated to 9.0 with 1 mol/L NaOH/HCl. Then, the mixtures were held at 70 °C for 2.0 h. The products were collected with a magnet. Next, the collected products were washed thoroughly with 100 mL of petroleum ether, 100 mL of acetone, and 200 mL of distilled water successively. Finally, the washed products were dried at 60 °C for 3.0 h. The dried products were MCM.

Immobilization of Alginate Lyase

For the MCM dosage experiment, 0.05 g (0.5 g/L MCM dosage), 0.10 g (1.0 g/L MCM dosage), 0.15 g (1.5 g/L MCM dosage), 0.20 g (2.0 g/L MCM dosage), 0.25 g (2.5 g/L MCM dosage), and 0.30 g (3.0 g/L MCM dosage) of MCM were added to 100 mL of disodium hydrogen phosphate-sodium dihydrogen phosphate buffer (DPB) (2.0 mol/L, pH 7.2) for 12.0 h. The MCM was first separated by a magnet and then dissolved in 50 mL of 0.6 g/L alginate lyase solution. The mixtures were agitated at 130 rpm in an oscillator for 1.0 h.

For the adsorption time experiment, 0.15 g of MCM (1.5 g/L MCM dosage) was added to 100 mL of DPB for 12.0 h. The MCM was first separated by the magnet and then dissolved in 50 mL of 0.6 g/L alginate lyase solution. The mixtures were agitated at 130 rpm for 0.5 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, and 3.0 h.

For the glutaraldehyde concentration experiment, 0.15 g of MCM (1.5 g/L MCM dosage) was added in 100 mL of DPB for 12.0 h. The MCM was first separated by the magnet and then dissolved in 50 mL of 0.6 g/L alginate lyase solution. The mixtures were agitated at 130 rpm for 2.0 h. Afterwards, 0.0% (0.00 mL of glutaraldehyde), 0.1% (0.05 mL of glutaraldehyde), 0.2% (0.10 mL of glutaraldehyde), 0.3% (0.15 mL of glutaraldehyde), 0.4% (0.20 mL of glutaraldehyde), and 0.5% (0.25 mL of glutaraldehyde) glutaraldehyde concentrations were carried out, and the MCM was crosslinked for 2.0 h.

For the immobilization time experiment, 0.15 g of MCM (1.5 g/L MCM dosage) was added to 100 mL of DPB for 12.0 h. The MCM was first separated by the magnet and then dissolved in 50 mL of 0.6 g/L alginate lyase solution. The mixtures were agitated

at 130 rpm for 2.0 h. Afterwards, 0.2% glutaraldehyde was used and the MCM was crosslinked for 1.0 h, 2.0 h, 3.0 h, 4.0 h, 5.0 h, and 6.0 h.

In all experiments, the alginate lyase-bonded MCM was collected by the magnet after crosslink and washed with DPB for five times. Thereafter, the immobilized alginate lyase was lyophilized in a freeze drier and stored at 4 °C before use.

Enzyme Activity Assay

Either the immobilized alginate lyase (50 mg) or free alginate lyase (5 mg) was dissolved in 20 mL of DPB (2.0 mol/L, pH 7.5). The activities of the immobilized and free alginate lyase were measured by a 3,5-dinitrosalicylic acid assay [21]. One unit of enzyme activity of alginate lyase (U/mL or U/g) was defined as the amount of alginate lyase used to produce 1 μg of reducing sugar per minute. Proteins in the enzyme solutions were determined by the Bradford's method [22]. The enzyme activity recovery (EAR, %), immobilized efficiency (IE, %), and adsorption capacity of MCM (AC, mg/g) were calculated as follows:

$$EAR = \frac{A_1}{A_0} \times 100\% \tag{1}$$

$$IE = \frac{C_0 - C_1}{C_0} \times 100\% \tag{2}$$

$$AC = \frac{(C_0 - C_1)V}{M} \times 100\% \tag{3}$$

where A_0 (U/mL or U/g) and A_1 (U/mL or U/g) are the activity of the free alginate lyase and observed activity of the immobilized alginate lyase, respectively; C_1 (mg/mL) and C_0 (mg/mL) are the protein concentrations of the buffer system after immobilization and the initial protein concentrations of the buffer system, respectively; V (mL) is the volume of the immobilized system; and M (g) is the MCM weight.

Structural Characterization of the Immobilized Alginate Lyase

The particle morphology of MCM was characterized through scanning electron microscopy (SEM) [18]. The chemical structures of chitosan, MCM and the immobilized alginate lyase were characterized by Fourier transform infrared spectroscopy (FTIR) [23]. The crystal structures of Fe_3O_4 nanoparticles, MCM and the immobilized alginate lyase were measured by using X-ray diffraction (XRD) [24]. The hysteresis loops of MCM and the immobilized alginate lyase were obtained by vibrating sample magnetometry (VSM) [25].

Characterization of the Enzymatic Properties of the Immobilized Alginate Lyase

Optimal pH

Each sample of 100 mg sodium alginate was dissolved in 20 mL of citric acid-sodium citrate buffer (pH 6.0), 20 mL of citric acid-sodium citrate buffer (pH 6.5), 20 mL of DPB

(pH 7.0), 20 mL of DPB (pH 7.5), 20 mL of Tris–HCl buffer (pH 8.0), and 20 mL of Tris–HCl buffer (pH 8.5). The immobilized alginate lyase (10 mg) or free alginate lyase (5 mg) was dissolved in the sodium alginate solution. The mixtures were held at 40 °C for 1.0 h. Then, the enzyme activity of the immobilized or free alginate lyase was measured. The maximum enzyme activity was set to 100%. Free alginate lyase was used as a control.

Optimal Temperature

Sodium alginate (100 mg) was dissolved in 20 mL of DPB (pH 7.5). The immobilized alginate lyase (10 mg) or free alginate lyase (5 mg) was dissolved in the sodium alginate solution, respectively. The mixtures were kept for 1.0 h at 35 °C, 40 °C, 45 °C, 50 °C, and 55 °C, respectively. Then, the enzyme activity of the immobilized or free alginate lyase was measured. The maximum enzyme activity was set to 100%. Free alginate lyase was used as a control.

Thermal Stability

Sodium alginate (100 mg) was dissolved in 20 mL of DPB (pH 7.5). The immobilized alginate lyase (10 mg) or free alginate lyase (5 mg) was dissolved in the sodium alginate solution, respectively. The mixtures were reacted for 120 min at 45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C, and 75 °C, respectively. Afterwards, the enzyme activity of the immobilized or free alginate lyase was measured every 20 min. The maximum enzyme activity was set to 100%. Free alginate lyase was used as a control.

Determination of K_m and V_m

The double reciprocal plot method (Lineweaver−Burk plot) was used to calculate the K_m and V_m values of the immobilized and free alginate lyase, respectively. Sodium alginate (100 mg) was dissolved in 20 mL of DPB at pH values of 7.5 and 8.0, respectively. Sodium alginate solutions (pH 7.5 or pH 8.0) of 0.00 mL, 1.00 mL, 1.25 mL, 1.67 mL, 2.50 mL, and 5.00 mL were placed in 96-well plates. DPB solutions (pH 7.5 or pH 8.0) of 10.00 mL, 9.00 mL, 8.75 mL, 8.33 mL, 7.50 mL, and 5.00 mL were added to the above sodium alginate solution sequentially. The immobilized alginate lyase (5 mg) was placed into each hole to dissolve in the sodium alginate solution (pH 8.0). The free alginate lyase (1 mg) was placed in each hole to dissolve in the sodium alginate solution (pH 7.5). The mixtures were held at 45 °C for 10 min. Then, the mixtures were evaluated by a microplate reader.

Operational Stability

In this experiment, 100 mg of sodium alginate was dissolved in 20 mL of DPB at pH 8.0 in a reaction vessel. Afterwards, 10 mg of immobilized alginate lyase was dissolved. The mixtures were kept at 45 °C for 1.0 h. The immobilized alginate lyase was collected by a magnet and washed with DPB 5 times. The immobilized alginate lyase was resuspended in a fresh substrate solution. This procedure was repeated 10 times. The enzyme activity of each batch was determined. The initial enzyme activity of this experiment was set to 100%.

Data Analysis

All experiments were carried out at random. All trials were performed in triplicate. Analysis of variance (ANOVA) of the data was carried out as described in a previous study [26].

Results and Discussion

Optimization of Immobilization Conditions

Immobilization conditions strongly affected the EAR [27]. To enhance the EAR of alginate lyase, immobilization conditions including MCM dosage, adsorption time, glutaraldehyde concentration and immobilization time were optimized. MCM was used as a carrier for enzyme immobilization. To investigate the effect of the MCM dosage on the EAR of alginate lyase, six different dosages of MCMs (0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, 2.5 g/L, and 3.0 g/L) were applied at an adsorption time of 1.0 h without glutaraldehyde. The effect of the MCM dosage on the EAR is shown in Fig. 1(a). The optimal dosage of MCM for immobilization was 1.5 g/L with the highest EAR of 35%. The EAR increased significantly ($P < 0.05$) with increasing MCM dosage from 0.5 g/L to 1.5 g/L and decreased significantly ($P < 0.05$) above 1.5 g/L. The results showed that a low MCM dosage had a low EAR. The possible reason for this phenomenon was that only a few enzyme molecules were immobilized on the carrier with a low dosage of MCM. In contrast, an excessive dosage of MCM prevented the full use of the carrier, leading to a low EAR [28].

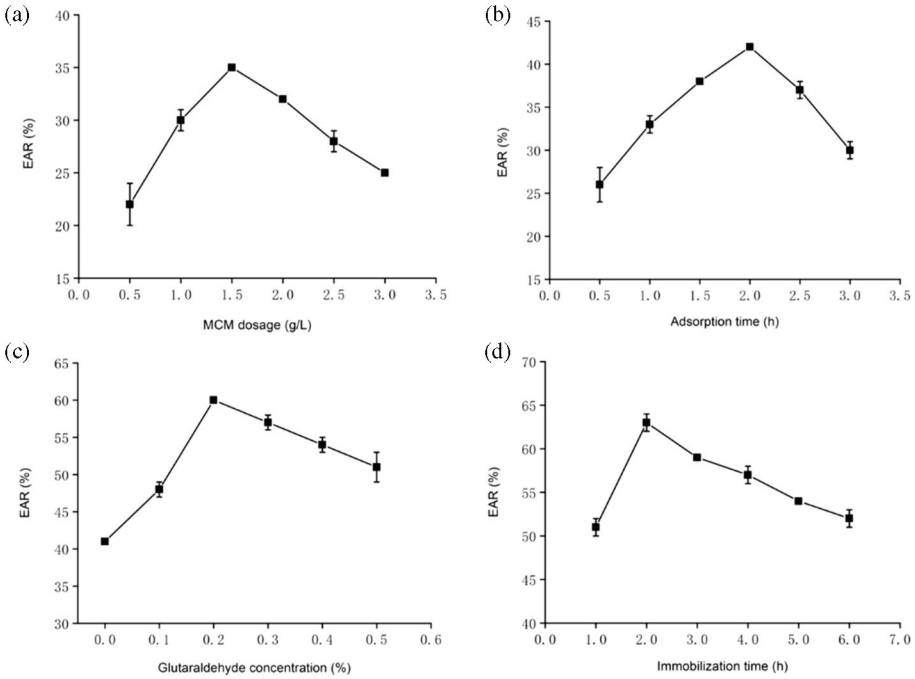

Fig. 1 The EAR of the immobilized alginate lyase under different conditions

Previous studies suggested that a proper time was necessary for better adsorption between the carriers and enzymes [29]. To investigate the effect of adsorption time on the EAR, different adsorption times (0.5 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, and 3.0 h) with 1.5 g/L of MCM without glutaraldehyde were carried out. The immobilization efficiency reached a peak value of 42% at 2.0 h (Fig. 1(b)). The EAR increased significantly ($P<0.05$) with increasing adsorption time for the first 2.0 h and decreased significantly ($P<0.05$) afterwards. The results showed that further prolonging incubation caused the mass loss of EAR. A possible reason for this phenomenon might be that shaking for a long time could cause the shedding of adsorbed enzyme molecules during the preparation process [30]. In addition, each carrier MCM was absorbed by more than one enzyme molecule on the surface, affecting the contact between the immobilized alginate lyase and the substrate and preventing the full expression of the enzyme activity [30].

Glutaraldehyde was used as a crosslinking agent for enzyme immobilization. This agent could be combined with the amino groups on the surface of enzyme molecules to form Schiff bases [31]. The firmness of binding of the enzyme molecule to the carrier depended on the glutaraldehyde concentration [32]. However, a high concentration of glutaraldehyde could lead to inactivation of the enzyme/protein, significantly reducing immobilization [33]. Different glutaraldehyde concentrations (0.0%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) at an adsorption time of 2.0 h and immobilization time of 2.0 h with 1.5 g/L MCM were tested. The EAR increased before it decreased with increasing glutaraldehyde concentration (Fig. 1(c)). The optimal glutaraldehyde concentration was 0.2% with a maximum EAR of 60%. Compared with 0.0% glutaraldehyde (EAR of 41%), the EAR of 0.2% glutaraldehyde increased by 46%. This result indicated that glutaraldehyde significantly prompted immobilization.

Immobilization time significantly affected the EAR. As shown in Fig. 1(d), the EAR of alginate lyase increased with increasing immobilization time from 1.0 h to 2.0 h at an adsorption time of 2.0 h with 1.5 g/L of MCM and 0.2% glutaraldehyde. This phenomenon could have occurred due to the increase in enzyme loading. The EAR reached a peak value of 63% at 2.0 h. This finding suggested that the monolayer adsorption of alginate lyase reached its saturation point. When the immobilization time was overly long, the overloading of alginate lyase could lead to a multilayered stacking of enzymes, blocking the substrate molecules from accessing the internal alginate lyase and thus decreasing the enzyme activity [34].

Under the optimized immobilization conditions (1.5 g/L MCM, 2.0 h adsorption time, 0.2% glutaraldehyde, and 2.0 h immobilization time), the AC of MCM was determined. The AC of MCM was 178 mg/g. Alginate lyase showed an excellent IE of 72%.

Structural Characterization of the Immobilized Alginate Lyase

The conventional and SEM images presented in Fig. 2 show the MCM particles. As shown in Fig. 2, the entrapment method could obtain a spheroidal structure of MCM with an average size of 30 ± 20 nm, and there were surface wrinkles on MCM, which favoured enzyme immobilization. MCM exhibited an evident agglomeration phenomenon, which could be mainly caused by the coercive force of magnetic particles or magnetic dipole interactions between particles [18].

FTIR spectroscopy was employed to investigate the structural changes in the immobilized alginate lyase. The prominent peak at 3445 cm^{-1} was associated with the vibrational modes of the -NH_2 and -OH functional groups, which was consistent with

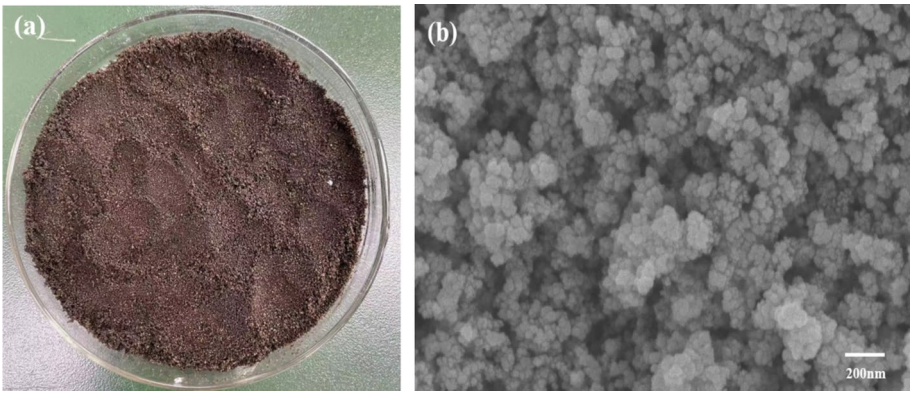

Fig. 2 Photograph (a) and SEM (×50,000) (b) of MCM

typical absorption bands at approximately 3300 cm^{-1} (Fig. 3). The absorption peaks at 2910 cm^{-1} and 2850 cm^{-1} were assigned to -CH_3 and -CH_2, respectively. In Figs. 3(b) and (c), the absorption peak at 3445 cm^{-1} was significantly reduced due to the reaction between the -NH_2 groups on chitosan and the aldehyde groups of the glutaraldehyde crosslinking agent. Adding glutaraldehyde led to an increase in the methyl and methylene groups on chitosan. Glutaraldehyde also significantly enhanced the absorption intensity in the range from 2910 cm^{-1} to 2850 cm^{-1}, indicating that magnetic Fe_3O_4 was successfully crosslinked to chitosan [35]. The peaks at 1651 cm^{-1} (1655 cm^{-1}) and 1599 cm^{-1} were assigned to amide I (C = O stretching vibration) and amide II (N–H

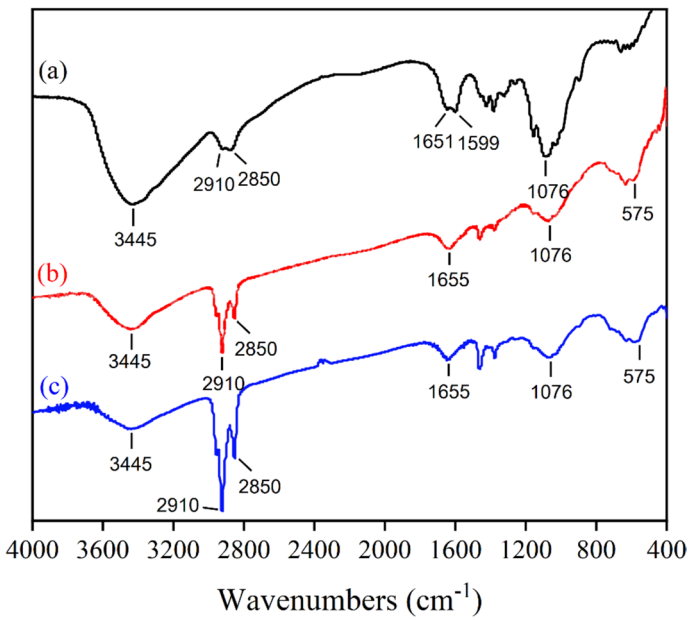

Fig. 3 FTIR spectra of chitosan (a), MCM (b) and the immobilized alginate lyase (c)

in-plane bending vibration), respectively [23]. The peak at 1076 cm^{-1} was attributed to the stretching vibration of the C–O–C bond. The absorption peaks at 1655 cm^{-1} and 1076 cm^{-1} were significantly enhanced due to the amide I and the stretching vibration of the C–O–C bond in alginate lyase (Fig. 3(c)). The results proved that the alginate lyase was immobilized successfully. The absorption peak at 575 cm^{-1} represented the Fe–O bending vibration, which was the characteristic absorption peak of magnetic Fe_3O_4 (Figs. 3(b) and (c)). The results suggested that the MCM and immobilized alginate lyase retained the structure of Fe_3O_4 [36].

XRD could reveal changes in the crystal structure of magnetic nanoparticles. Figure 4 shows the XRD patterns of Fe_3O_4 nanoparticles, MCM, and immobilized alginate lyase, which were basically the same. The diffraction peaks of the Fe_3O_4 crystals all appeared at 2θ values of 30.373° (220), 35.742° (311), 43.325° (400), 53.983° (422), 57.338° (511), and 62.911° (440). This result indicated that the crystal structures of magnetic Fe_3O_4 nanoparticles were not destroyed during the preparation of MCM and enzyme immobilization [37].

The magnetic properties of the magnetic nanoparticles were characterized by VSM (Fig. 5). Hysteresis curves of both MCM and immobilized alginate lyase were S-shaped without remanence or coercivity. This result indicated that both MCM and immobilized alginate lyase had good superparamagnetism [38]. The saturation magnetization strengths of MCM and immobilized alginate lyase were 11.34 emu/g and 7.78 emu/g, respectively. The saturation magnetization strength of the immobilized alginate lyase was lower than that of MCM. This phenomenon occurred because MCM was modified by enzymes [39]. The immobilized alginate lyase still showed good superparamagnetism. This property was beneficial for recovering the immobilized alginate lyase from enzymatic hydrolysates.

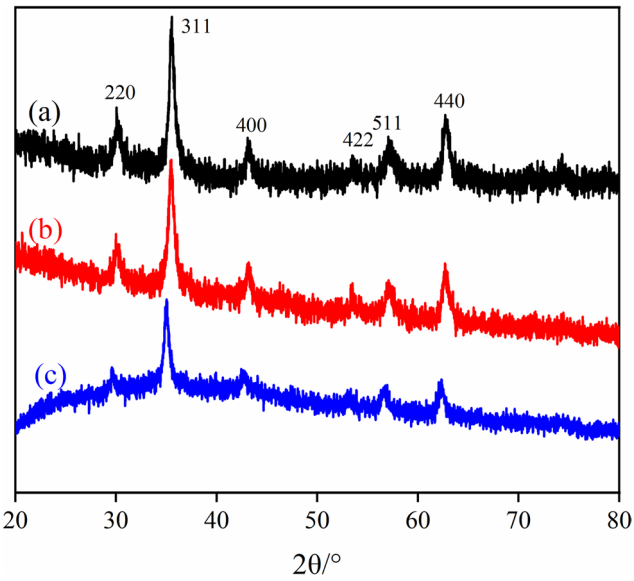

Fig. 4 XRD of Fe_3O_4 nanoparticles (a), MCM (b) and the immobilized alginate lyase (c)

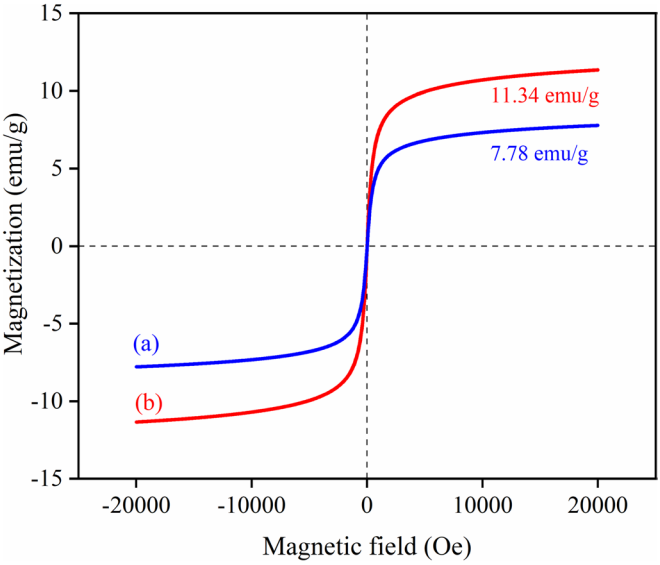

Fig. 5 VSM of MCM (a) and the immobilized alginate lyase (b)

Characterization of the Enzymatic Properties of the Immobilized Alginate Lyase

Optimal pH and Temperature

The optimal pH of alginate lyase immobilized on magnetic chitosan microspheres was investigated (Fig. 6). The free enzyme was used as a control. The optimal pH values of the free and immobilized enzymes were 7.5 and 8.0, respectively. The optimal pH of the immobilized alginate lyase was higher than that of the free lyase. This result indicated that immobilization contributed significantly to the improvement in pH tolerance. The shift

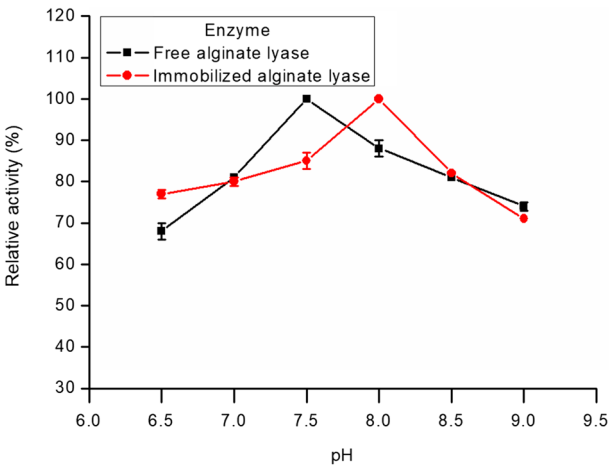

Fig. 6 The optimal pH of the immobilized and free alginate lyase

of the enzymatic optimal pH after immobilization was because the amino groups in the immobilized alginate lyase would adsorb H^+ in the solution resulting in an increase in the acidity of the internal microenvironment [40]. To offset the impact of its internal microenvironment, the optimal pH of the immobilized enzyme would shift towards alkaline.

The optimal temperature of alginate lyase immobilized on MCM was studied (Fig. 7). The free enzyme was used as a control. The optimal temperature of the enzyme did not change after immobilization. The immobilized alginate lyase had no difference in the optimal temperature (45 °C) from the free alginate lyase (Fig. 7). However, the immobilized alginate lyase showed increased thermal resistance at high temperatures. The relative activity of the immobilized alginate lyase was approximately 80%, while the free alginate lyase was less than 50% at 60 °C.

Thermal Stability

The thermal stability of the free and immobilized enzymes was investigated. The free alginate lyase lost almost all its activity at 70 °C and 75 °C. In comparison, the immobilized alginate lyase still retained approximately 80% of its activity at 70 °C for 120 min and still had approximately 40% of its activity at 75 °C for 120 min (Fig. 8). The results showed that the immobilized alginate lyase exhibited stronger tolerance to high temperatures than that of the free enzyme. This phenomenon arose because of the covalent binding of enzymes on the support [40]. The immobilized alginate lyase showed relatively good thermal stability. The good thermal stability of the immobilized alginate lyase would be a significant advantage for its industrial application. The immobilized enzymes could catalyse a reaction under high temperature conditions.

K_m of the Immobilized and Free Alginate Lyase

The K_m value of an enzyme could approximately reflect the affinity between the substrate and the enzyme molecule. The larger the K_m value was, the lower the affinity

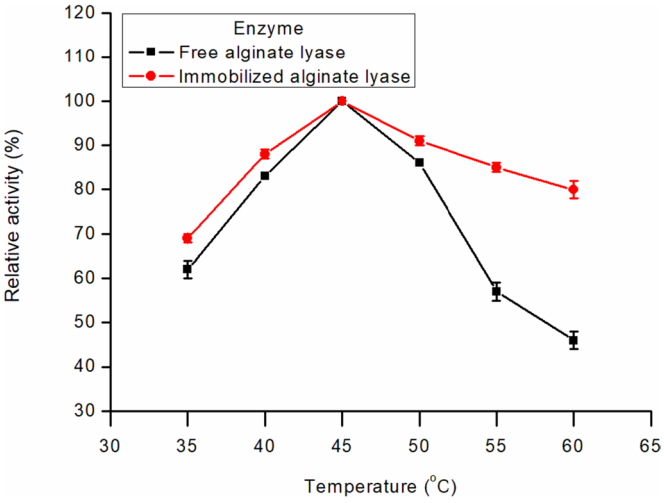

Fig. 7 The optimal temperature of the immobilized and free alginate lyase

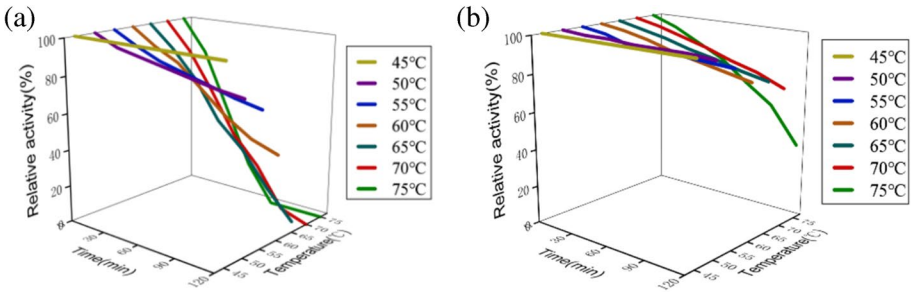

Fig. 8 The thermal stability of the free (a) and immobilized alginate lyase (b)

between the substrate and enzyme molecule was. K_m was calculated as 0.05 mol/L and 0.09 mol/L, and V_m was 0.50 mmol/(L·min) and 0.34 mmol/(L·min) for the free and immobilized forms, respectively (Table 1 and Fig. 9). The K_m value indicated that the free alginate lyase was more sensitive to the substrate than that of the immobilized alginate lyase. This phenomenon occurred because the spatial structures and active sites of the enzyme molecules were changed after immobilization [41, 42]. The immobilized alginate lyase formed a particular steric hindrance, hindering the contact between the enzyme molecules and substrate molecules.

Table 1 Michaelis constant of alginate lyase

Enzyme	K_m (mol/L)	V_m [mmol/(L·min)]
Free alginate lyase	0.05	0.50
Immobilized alginate lyase	0.09	0.34

Fig. 9 The K_m of alginate lyase

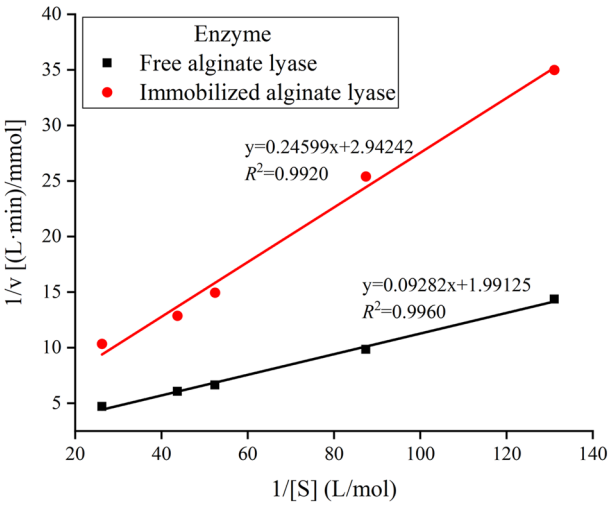

Table 2 Operational stability of the immobilized alginate lyase

Bach reaction	Relative activity (%)
1	98 ± 0
2	97 ± 0
3	94 ± 1
4	92 ± 1
5	89 ± 0
6	88 ± 1
7	84 ± 1
8	81 ± 0
9	75 ± 1
10	72 ± 2

Operational Stability

To evaluate the immobilization process, the operational stability was investigated. Retention of more than 70% of alginate lyase activity after 10 reuses was obtained (Table 2). This result indicated that the immobilized enzymes performed good operational stability. It could be regarded as the hydrophilic microenvironment that surrounded the enzymes. The enzyme activity decreased slowly during the reuse process. The loss of enzyme activity of the immobilized enzymes could result from leakage of the immobilized alginate lyase and protein denaturation during the catalytic process [43].

Conclusion

A novel method for preparing magnetite chitosan microspheres was developed. Alginate lyase was successfully immobilized on magnetic chitosan microspheres. The enzymatic properties, thermal stability, and reusability of the immobilized alginate lyase were enhanced. The results showed that the activity and stability of the immobilized enzymes were better than those of the free enzymes. The results suggested that immobilizing alginate lyase on magnetic chitosan microspheres was a promising strategy. The immobilized alginate lyase had good potential in industrial applications.

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Data Availability The data in this research article are fully presented in the manuscript.

Declarations

Ethical Approval This article does not contain any studies with human participants by any of the authors.

Consent to Participate Not applicable.

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Conflicts of Interest The authors declare no conflict of interest.

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