Journal of Molecular Catalysis B: Enzymatic 110 (2014) 32–38

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Lipase immobilized on novel ceramic supporter with Ni activation for efficient cinnamyl acetate synthesis

Zhongjie Wu[a], Wei Qi[a,b,c,d,*], Mengfan Wang[a,c], Rongxin Su[a,b,c,d], Zhimin He[a,b,d]

[a] Chemical Engineering Research Center, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China
[b] State Key Laboratory of Chemical Engineering, Tianjin University, Tianjin 300072, PR China
[c] Tianjin Key Laboratory of Membrane Science and Desalination Technology, Tianjin University, Tianjin 300072, PR China
[d] The Co-Innovation Center of Chemistry and Chemical Engineering of Tianjin, Tianjin 300072, PR China

ARTICLE INFO

Article history:
Received 2 March 2014
Received in revised form
14 September 2014
Accepted 15 September 2014
Available online 28 September 2014

Keywords:
Lipase
Immobilization
Metal ceramic
Activation
Cinnamyl acetate

ABSTRACT

The metal ceramic powder (MCP) was used for lipase immobilization. The MCP containing Ni^{2+} (Ni-MCP) exhibited the best affinity to lipase. The effects of heating rate, lipase concentration and immobilization time on immobilized lipase (Ni-MCP-lipase) activity were measured. Under the optimal preparation conditions (heating rate: 1 °C/min, lipase concentration: 9 mg/mL, immobilization time: 8 h), Ni-MCP-lipase could obtain 1.4 U/g and 216% of activity yield. The Ni-MCP-lipase, with improved thermal stability and storage stability, had optimal pH value of 6.0 and optimal temperature of 40 °C. The characterizations clarified the effect of heating rate on Ni-MCP-lipase activity, and confirmed that lipase had been efficiently immobilized on Ni-MCP surface. Finally, cinnamyl acetate synthesis demonstrated that Ni-MCP-lipase had improved efficiency compared with free lipase. Under the optimal reaction conditions (Ni-MCP-lipase loading: 3 g; reaction temperature: 35 °C; acetic acid/cinnamyl alcohol: 2:1 in 15.0 mL reaction system), the cinnamyl acetate yield would reach 62.56%.

1. Introduction

Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) has aroused significant attention in the fields of biotechnology, food industry, pharmaceutical chemistry due to its application in organic solvent and broad specific catalysis function [1,2]. Just as other enzymes, immobilized lipase would obtain improved stability and reusability. The solid carriers with appropriate hardness, density and specific surface area would make the immobilized lipase appropriate for the application. Many solid materials, such as silicon particle [3], smectite [4] and Fe_3O_4-chitosan particles [5] have been used as carriers for lipase immobilization. Physical adsorption and covalent cross-linking are the main methods for lipase immobilization. However, the physical adsorption only provided weak affinity between the carrier and lipase molecular, which could not supply excellent reusability. The cross-linking agents, such as glutaraldehyde might result in enzyme activity loss. In another way, enzymes modified by some small molecules could obtain higher activity, better catalytic properties [6], such as amino acid, anhydride and polyalcohol. However, the modified enzyme needed to be immobilized for further practical application.

Recently, due to the high immobilization efficiency, simple immobilization process and improved catalytic properties, enzyme immobilization by metal affinity method has attracted significant attention [7]. Moreover, some kinds of metal ion could improve enzyme activity and catalytic properties [8,9]. However, the carriers for enzyme immobilization through metal affinity, usually lacked mechanical strength, density or hardness and leaded to some disadvantages in practical application. Fortunately, metal ceramic could be used as the ideal material, not only the significant mechanical strength, excellent hardness and abrasive resistance, but also mass of metal active sites on the surface for enzyme immobilization. However, the study on metal ceramic applied for enzyme immobilization has been barely reported.

Therefore, in this paper, the metal ceramic powder was used as carrier for lipase immobilization integrated the advantages of metal affinity immobilization method, metal activation effects and metal ceramic. Lipase immobilized on the metal ceramic powder would obtain high immobilization efficiency, better catalytic properties and excellent reusability. In addition, the disadvantages of physical adsorption and covalent cross-linking method would be overcome. Firstly, metal ceramic powder (MCP) would be prepared and used

* Corresponding author at: Chemical Engineering Research Center, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China. Tel.: +86 22 27407799; fax: +86 22 27407599.
E-mail address: qiwei@tju.edu.cn (W. Qi).

http://dx.doi.org/10.1016/j.molcatb.2014.09.010
1381-1177/

as carrier. Secondly, lipase was immobilized on MCP through a simple process. Then, the optimal conditions were investigated to obtain the MCP-lipase with the highest activity. At the same time, the catalytic property and stability of MCP-lipase were measured. Furthermore, FT-IR spectra, SEM and BET-N_2 method were used for characterization. At last, to measure the practical application of MCP-lipase, synthesis of cinnamyl acetate, a kind of important spice widely used in daily detergents [10], was selected as model reaction to evaluate the efficiency of enzyme. Considering the improved properties, the MCP-lipase would have wide prospects for hydrolysis, degradation and synthesis applications.

2. Materials and methods

2.1. Material

Lipase from procine pancreas, Type II (PPL) (CAS 9001-62-1) was purchased from Sigma Chemical Company (USA); cinnamyl alcohol (E1226034) was purchased from Aladdin Company; glyceryl triacetate, ethyl acetate, silica, calcium oxide, magnesium oxide were purchased from Guangfu Company (Tianjin, China). Other chemicals were analytical grade and obtained from common commercial sources without further purification.

2.2. Carrier preparation

The carrier preparation process had been mentioned in our previous work [11], briefly, metal hydroxide precipitation was prepared by dissolution of metal chloride in deionized water and precipitated by NaOH solution. The precipitation was filtered and washed for 3 times. Then, the obtained metal hydroxide precipitation was dried at 70 °C for 24 h.

Subsequently, metal ceramic powder (MCP) was prepared by mixing of 22 g matrix (containing 10 g SiO_2, 5 g Al_2O_3, 5 g Na_2SiO_3, 1 g MgO, 1 g CaO), 5 g metal hydroxide precipitation and 10 g deionized water. Then the MCP was obtained by drying in muffle at 150 °C for 2 h and calcining through 1 °C/min, 3 °C/min, 5 °C/min, 7 °C/min to 850 °C, respectively, and the temperature was preserved for 4 h. Meanwhile, the ceramic powder (CP) was also prepared with the same process as the control without mixing metal hydroxide precipitation.

2.3. Lipase immobilization

Surface activation: MCP surface was 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 for 6 times.

Immobilization process: 5.0 g of activated MCP was added into 10.0 mL lipase solution with certain concentration and stirred at 4 °C for different time, then the immobilized lipase (MCP-lipase) was washed by deionized water for 3 times to remove the unimmobilized lipase.

2.4. Enzyme activity assay

The activities of free lipase and MCP-lipase were determined by hydrolysis of glyceryl triacetate [12]. The enzyme samples were added into 30.0 mL saturated glyceryl triacetate solution (pH 6.3) and the NaOH solution (0.05 mol/L) was added into the mixture to maintain the solution with pH 6.3. Meanwhile, the reaction time was set as 30 min. One unit activity (U) of free lipase or MCP-lipase was defined as the amount of enzyme needed to liberate 1.0 μmol of acetic acid in 1 min at 35 °C and pH 6.3.

The activity and activity yield of lipase were calculated based on the following equations:

$$\text{Lipase activity (U)} = \frac{(V_{sample} - V_{blank}) \times 50}{M_e \times 30} \quad (1)$$

$$\text{Activity yield (\%)} = \frac{E_{immobilized}}{E_{free}} \times 100 \quad (2)$$

where V_{sample} (mL) is the volume of NaOH (0.05 mol/L) solution used to neutralize the acetic acid liberated by lipase hydrolysis; V_{blank} (mL) is the volume of NaOH (0.05 mol/L) solution consumed by glyceryl triacetate solution, while in this situation, the lipase was heated at 90 °C for 24 h. M_e is the lipase mass added into the glyceryl triacetate solution. 50 is the conversion factor of NaOH to acetic acid. 30 (min) is the reaction time. $E_{immobilized}$ is the activity of all MCP-lipase obtained from the original lipase solution after immobilization, and E_{free} is the activity of all free lipase before immobilization.

2.5. Catalytic properties of lipase or MCP-lipase

Reaction temperature: The activities of free lipase and Ni-MCP-lipase were determined by adding the enzyme samples (30 mg of free lipase or 1 g of Ni-MCP-lipase) into 30.0 mL substrate solution (saturated glyceryl triacetate solution) at different temperatures (25–55 °C) for 30 min, and pH value was maintained 6.3.

Reaction pH: The activities of free lipase and Ni-MCP-lipase were determined by adding the enzyme samples (30 mg of free lipase or 1 g of Ni-MCP-lipase) into 30.0 mL substrate solution (saturated glyceryl triacetate solution) under different pH (5–10) for 30 min, and the temperature was maintained 35 °C.

2.6. Stabilities of lipase or MCP-lipase

The thermal stabilities of free lipase (1 mg/mL) and Ni-MCP-lipase (50 mg/mL) were determined by measuring the residual activities of enzyme samples incubated in phosphate buffer at pH 6.3 and 70 °C. The incubating time was from 1 h to 5 h and the time interval was 1 h. The storage stabilities of free lipase (1 mg/mL) and Ni-MCP-lipase (50 mg/mL) were determined by measuring the residual activities of enzyme samples in phosphate buffer at pH 6.3 and 4 °C. The storage time was set from 1 to 7 days.

2.7. Characterizations

The XRD (X'Pert Pro, 3.0 kV, Cobalt bomb), SEM (S4800, 5 kV, high power mode), BET-N_2 adsorption (F-sorp 2400, liquid nitrogen temperature), EDS (S-4800, 15 kV, 15,000× magnification) and IR spectrum (Bio-Rad FTS 6000, FTIR, KBr disk method) were performed to characterize the properties of Ni-MCP and Ni-MCP-lipase.

2.8. Enzymatic synthesis of cinnamyl acetate

Synthesis reactions were carried out in 50 mL shaking flask at 35 °C under 150 rpm for 10 h. The reaction mixture contained 0.12 g acetic acid, 0.268 g cinnamyl alcohol, 15.0 mL *n*-hexane and certain amount of free lipase or Ni-MCP-lipase.

The cinnamyl acetate (CA) yield was determined by measuring the acetic acid content in the reaction mixture through titration with NaOH solution (0.1 mol/L) and calculated based on the following equation:

$$\text{CA yield (\%)} = \frac{C_0 - C_s}{C_c} \times 100 \quad (3)$$

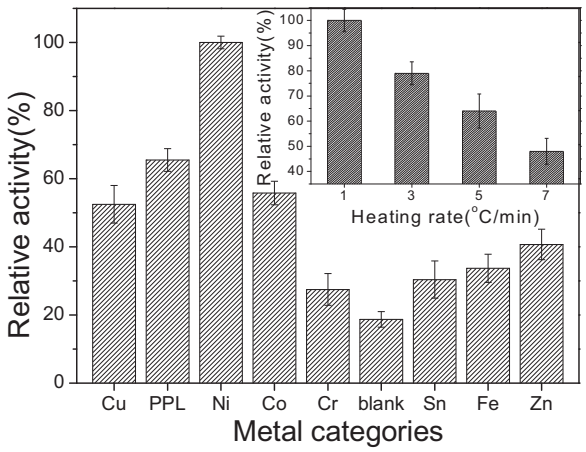

Fig. 1. The MCP-lipase activity changed with the categories of MCP. Calcining conditions. Metal ceramic ingredient: 22 g matrix and 5 g metal precipitation; ceramic ingredient: 22 g matrix; drying process: 150 °C for 2 h; heating process: heated to 850 °C through 1 °C/min and preserved at 850 °C for 4 h. Immobilization conditions. Lipase concentration 10 mg/mL; lipase solution 10.0 mL, MCP 5.0 g, immobilization time 8 h, in ice-water bath. MCP-lipase loading was 1 g; free lipase loading was 20 mg. The activity of Ni-MCP-lipase was regarded as 100%. The inset was the effect of heating rate on Ni-MCP-lipase activity with the heating rate from 1 to 7 °C/min.

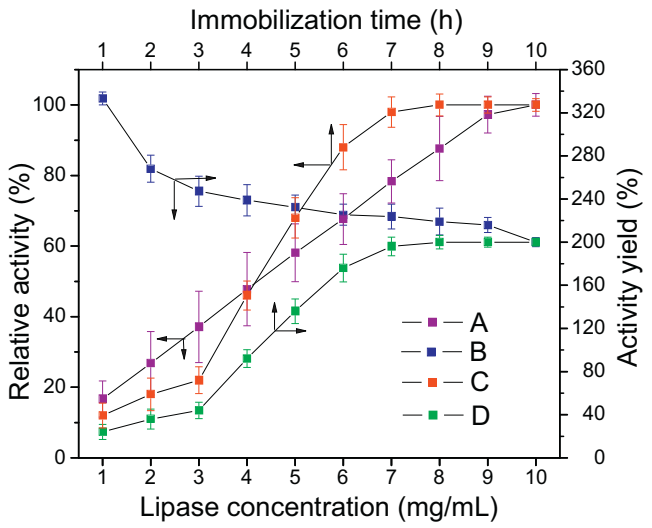

Fig. 2. Ni-MCP-lipase activity changed with lipase concentration and immobilization time. (A) Ni-MCP-lipase relative activity – lipase concentration; (B) activity yield – lipase concentration; (C) Ni-MCP-lipase relative activity – immobilization time; (D) activity yield – immobilization time. Immobilization conditions: lipase solution 10.0 mL, Ni-MCP 5.0 g, in ice-water bath. The maximum activity of Ni-MCP-lipase was assumed as 100%.

where C_0 (mmol/mL) is the initial concentration of acetate acid; C_s (mmol/mL) is the terminal concentration of acetate acid, and C_c (mmol/mL) is the initial concentration of cinnamyl alcohol.

3. Results and discussion

3.1. Immobilization process

3.1.1. Calcining

MCP contained different categories of metal hydroxide precipitation exhibited different affinity to lipase. Therefore, seven kinds of MCP (Cu-MCP, Ni-MCP, Co-MCP, Cr-MCP, Sn-MCP, Fe-MCP, Zn-MCP) were prepared to measure their affinities to lipase. The result of 1 g Ni-MCP loading 15.5 mg lipase was obtained via Coomassie brilliant blue method [13]. It could be known from Fig. 1 that Ni-MCP-lipase showed the highest activity and 1 g Ni-MCP-lipase had higher activity than 20 mg free lipase, which indicated the strong affinity and activation of Ni-MCP to lipase; while lipase immobilized on the ceramic powder (CP-lipase) without any metal hydroxide precipitation showed the lowest activity. This might be caused by the specific chelation between Ni^{2+} on MCP and certain active amino acid residues of lipase molecules [14] for Ni-MCP-lipase; while CP-lipase had only physical adsorption.

Subsequently, influence of heating rate on Ni-MCP-lipase activity was performed. As shown in the inset of Fig. 1, Ni-MCP calcined under different heating rate showed different affinity to lipase. As the heating rate turned slower, the Ni-MCP-lipase showed higher activity and the optimal heating rate was 1 °C/min. This might be that Ni-MCP calcined under different heating rate gained different specific surface areas. Thus, it was confirmed that Ni-MCP under 1 °C/min heating rate could be the excellent matrix for lipase immobilization.

3.1.2. Lipase immobilization

Different concentrations of lipase solution were investigated for lipase immobilization on Ni-MCP. In Fig. 2(A), during the range of 1–10 mg/mL, the Ni-MCP-lipase activity increased with lipase solution concentration. As the lipase concentration reached 9 mg/mL, the Ni-MCP-lipase activity would not improve obviously, which indicated that almost all active Ni^{2+} sites on surface were occupied by lipase. It could also found in Fig. 2(B) that although activity yields decreased as the lipase concentration increased, all activity yields were beyond 200%, which indicated that Ni^{2+} on Ni-MCP surface had the activation effect on lipase. Meanwhile, immobilization time would influence the amount of lipase immobilized on Ni-MCP and would finally determine the activity of Ni-MCP-lipase. In Fig. 2(C) and (D), the Ni-MCP-lipase activity and immobilization efficiency improved with the extension of immobilization time. As the immobilization time reached 8 h, the Ni-MCP-lipase activity reached the highest value and would not increase anymore. The result indicated that lipase had occupied almost all active sites on Ni-MCP at this time. The immobilization results demonstrated that Ni-MCP could serve as carrier for lipase immobilization and had activation effect on lipase molecule. This might be that, just as other Ni^{2+} loaded carriers [14,15], Ni^{2+} on MCP surface would combine some amino acid of the lipase molecule and make the immobilized lipase obtain better catalytic properties. Therefore, it could be concluded that lipase immobilized on Ni-MCP obtained the advantages of immobilization and activation, and the Ni-MCP was a suitable and excellent solid material for lipase immobilization.

3.2. Ni-MCP-lipase properties

As usual, the optimal temperature and pH of free enzyme would be changed after immobilization. As shown in Fig. 3(a), the optimal pH of Ni-MCP-lipase and free lipase were 6 and 6.5, respectively. In addition, Ni-MCP-lipase gained wider application range compared with free lipase. As many lipase catalytic reactions were conducted in acidic conditions, Ni-MCP-lipase would obtain further practical application. In Fig. 3(b), the free lipase had the optimal temperature of 50 °C, while the Ni-MCP lipase had the optimal temperature of 40 °C and exhibited higher activity in the range of 20–40 °C, which favored the application of lipase at milder conditions and protected enzymatic product from being decomposed at higher temperature [16].

Besides, the enzyme after immobilization would gain an improvement in storage stability and thermal stability. As shown in Fig. 3(c), the Ni-MCP-lipase could maintain more than 80% of the initial activity after 12 days, while the free lipase just could maintain 40% of the initial activity after 8 days. Fig. 3(d) was the thermal stability of Ni-MCP-lipase, it showed that Ni-MCP-lipase

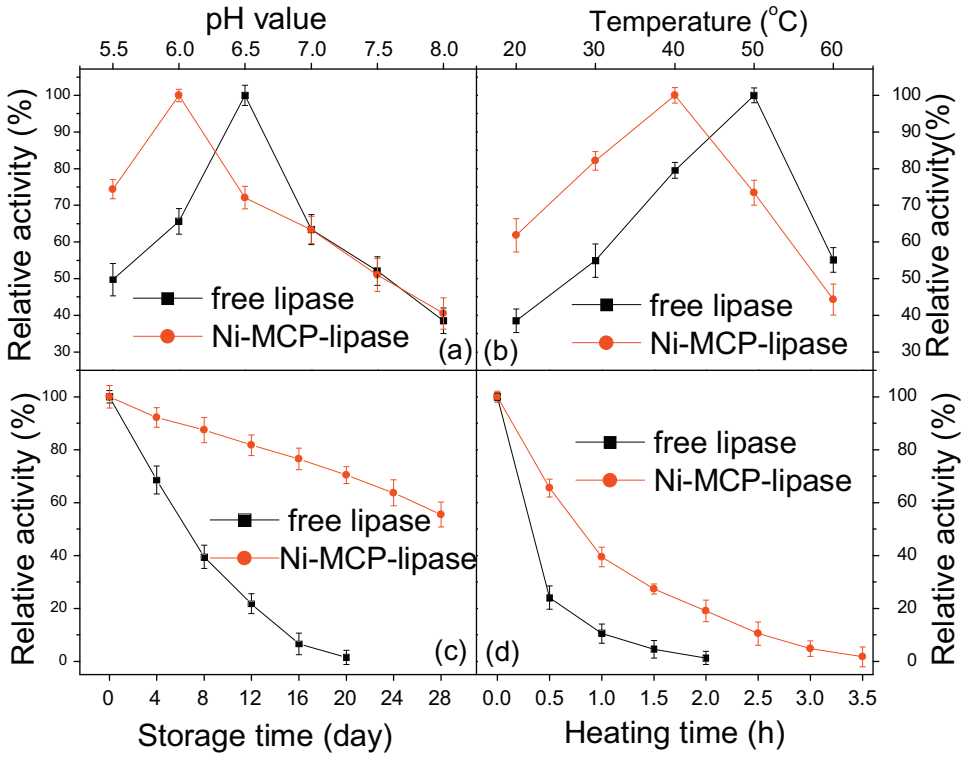

Fig. 3. The optimal pH (a), temperature (b), storage stability (c) and thermal stability (d) of free lipase and Ni-MCP-lipase. Assuming the highest relative activity of free lipase and Ni-MCP-lipase as 100%, respectively. (a) Enzyme loading: 30 mg free lipase or 1 g Ni-MCP-lipase; the temperature was maintained at 35 °C. (b) Enzyme loading: 30 mg free lipase or 1 g Ni-MCP-lipase; the pH value was maintained 6.3. (c) Storage conditions: 4 °C, pH 6.3 for 1 mg/mL of free lipase and 50 mg/mL of Ni-MCP-lipase. (d) Incubation conditions: 70 °C, pH 6.3 for 1 mg/mL of free lipase and 50 mg/mL of Ni-MCP-lipase.

still retained more than 40% of the initial activity after heated at 70 °C for 1 h, while the free lipase had lost most activity and remained only 15% of the initial activity under the same conditions. Furthermore, the Ni-MCP-lipase could retain more than 50% of its initial activity after 4 cycles for hydrolysis of glyceryl triacetate (Fig. 6). It was well known that the flexibility of enzyme molecule would be limited and the microenvironment around lipase would change after immobilization [17], which was the main reason for the change of catalytic properties after immobilization. Therefore, the Ni-MCP-lipase showed excellent properties and industrial application prospect.

3.3. Characterizations

The XRD patterns of CP and Ni-MCP were shown in Fig. 4. All samples exhibited a strong diffraction peak at $2\theta = 32°$; other low intensities appeared at 24.5° and 35°. The results indicated that CP structure was retained in Ni-MCP after NiO was introduced into the framework [18]. The introduction of NiO in Ni-MCP led to the decrease of some diffraction peak intensities, as the contents of MgO and CaO decreased after NiO mixed, their diffraction intensities decreased and even disappeared. While the SiO_2 and Al_2O_3 were main components in CP, and their corresponding main peaks could still be detected in Ni-MCP. Thus, CP and Ni-MCP showed similar structure, and NiO would not destroy the initial structure after introduced into the matrix.

The EDS pattern of CP and Ni-MCP were shown in Fig. 5. It exhibited that all elements mixed in CP or Ni-MCP could be detected. The elements contents of CP and Ni-MCP were shown in Table 1. It can be seen that the EDS experimental data showed almost consistent results with the calculated values, thus it indicated that all ingredients were calcined into the CP or Ni-MCP, which supplied identical and stable environment for lipase immobilization.

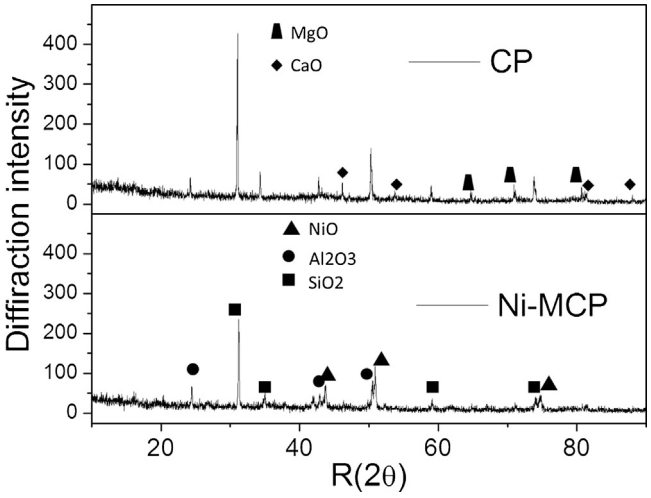

Fig. 4. The XRD diagram for CP and Ni-MCP. Scanning range was from 10° to 90° and scanning rate was 4°/min.

Table 1
Element contents in CP and Ni-MCP.

	Si	Al	Mg	Ca	Ni
CP (calculated)	26.42%	12.03%	2.73%	3.25%	
CP (measured)	25.54%	13.46%	4.4%	3.75%	
Ni-MCP (calculated)	21.53%	9.8%	2.22%	2.65%	14.57%
Ni-MCP (measured)	20.43%	10.44%	3.77%	3.08%	16.04%

FTIR could reveal the formation of new chemical bonds during the immobilization process. As shown in Fig. 6, the adsorption bands at 1090 cm^{-1} were the Si—O stretching vibration, which was assigned to antisymmetric vibration of Si—O—Si [19]. The

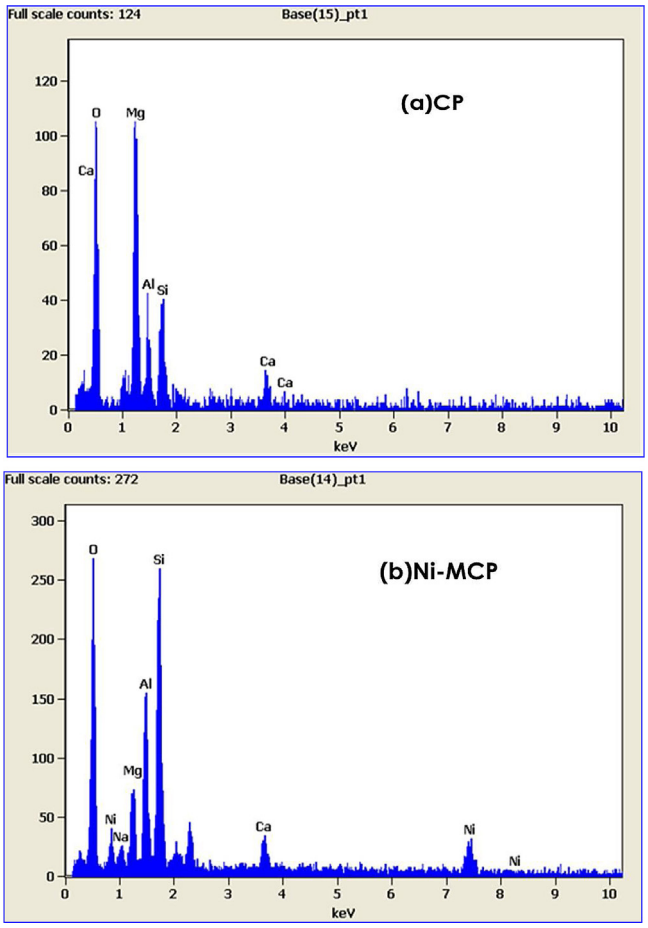

Fig. 5. The EDS diagram of CP and Ni-MCP. Accelerating voltage: 15.0 kV, magnification: 15,000×

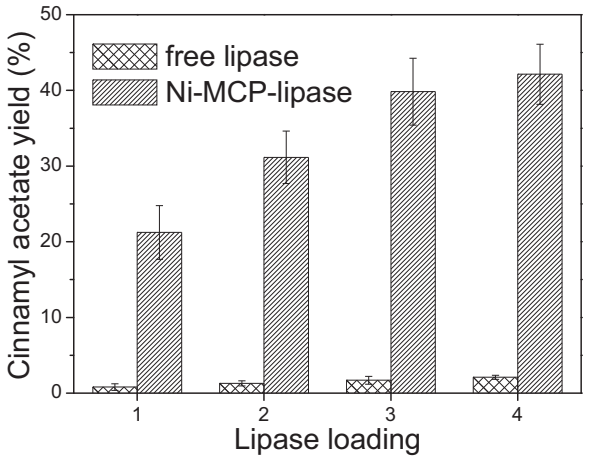

Fig. 7. Cinnamyl acetate yield by free lipase and Ni-MCP-lipase. Lipase loading was 1.0 g, 2.0 g, 3.0 g. 4.0 g for Ni-MCP-lipase and 10.0 mg, 20.0 mg, 30.0 mg, 40.0 mg for free lipase; acetic acid 0.12 g, cinnamyl alcohol 0.268 g, *n*-hexane 15.0 mL, reaction temperature 35 °C, reaction time 10 h, shaking rate 150 rpm.

adsorption bands at 600 cm^{-1} and 460 cm^{-1} were assigned to the Ni—O stretching vibration and antisymmetic vibration. Compared with Ni-MCP, the adsorption bands at 3511 cm^{-1} were assigned to N—H and O—H stretching vibration, respectively [20]. While the adsorption bands at 1727 cm^{-1} and 1644 cm^{-1} were assigned to C=O of carbonyl group stretching vibration [20]. Thus, the FTIR confirmed that the lipase was immobilized on Ni-MCP.

The morphology of Ni-MCP-lipase and Ni-MCP were shown in Fig. 6(a) and (b). According to the SEM images, Fig. 6(b) showed that the Ni-MCP under 1 °C/min heating rate had many pores and channels on the surface with specific surface area of 40.0 m^2/g demonstrated by BET-N_2. Fig. 6(a) showed the surface morphology of Ni-MCP-lipase. Compared with Fig. 6(b), many pores on Ni-MCP surface had been filled, leading to the decrease in specific surface area to 30.8 m^2/g demonstrated by BET-N_2, which confirmed that lipase have been immobilized on Ni-MCP surface [21]. Moreover, the Ni-MCP under 3 °C/min, 5 °C/min, 7 °C/min heating rate had the specific surface area of 28.4 m^2/g, 17.9 m^2/g, and 10.4 m^2/g, respectively. Thus Ni-MCP under 1 °C/min heating rate supplied the largest area for lipase immobilization. This might be that as the heating rate increased, the liquidity of Ni-MCP in preparation process improved, which leaded to the Ni-MCP surface turning smooth. Meanwhile, the formation of pore structure would be restricted. Thus Ni-MCP through 1 °C heating rate showed the best affinity to lipase.

3.4. Cinnamyl acetate synthesis

3.4.1. Immobilization improvement

The synthesis of cinnamyl acetate was used to investigate the catalytic properties of Ni-MCP-lipase. As shown in Fig. 7, cinnamyl acetate yield improved with Ni-MCP-lipase loading, while free lipase almost had no obvious effect on cinnamyl acetate synthesis.

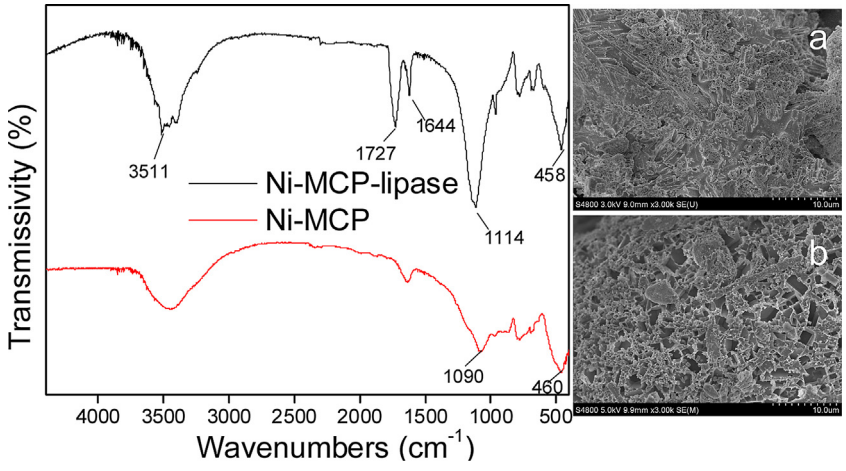

Fig. 6. The FTIR spectra and SEM morphology of Ni-MCP-lipase (a) and Ni-MCP (b).

Table 2
Optimization of reaction conditions for cinnamyl acetate synthesis.

Entry	Enzyme loading (g)	Temperature (°C)	Acid/alcohol ratio	Yield (%)
Influence of enzyme loading				
1	1	35	1:1	21.23
2	2	35	1:1	31.15
3	3	35	1:1	39.84
4	4	35	1:1	41.13
Influence of temperature				
5	3	35	1:1	39.84
6	3	45	1:1	41.81
7	3	55	1:1	44.15
Influence of acid/alcohol ratio				
8	3	35	1:2	20.58
9	3	35	3:4	32.72
10	3	35	1:1	39.84
11	3	35	3:2	50.87
12	3	35	2:1	62.56
13	3	35	3:1	64.46

Biocatalyst: Ni-MCP-lipase; solvent: 15.0 mL *n*-hexane; reaction time: 10 h; shaking rate: 150 rpm.

There was a significant improvement for Ni-MCP-lipase on cinnamyl acetate synthesis. This might be that immobilized lipase (Ni-MCP-lipase) obtained the suitable conformation which was beneficial to combine substrate and promote cinnamyl acetate synthesis [22]. Thus, compared with free lipase, Ni-MCP-lipase exhibited improved efficiency in biocatalysis reaction.

3.4.2. Synthesis conditions

Ni-MCP-lipase was used for cinnamyl acetate synthesis. The reaction was optimized with respect to enzyme loading, reaction temperature and molar ratio (acetic acid/cinnamyl alcohol). As shown in Table 2 (entry 1–4), enzyme loading showed a significant effect on cinnamyl acetate synthesis, i.e. cinnamyl acetate yield increased as enzyme loading. When the enzyme loading reached 3 g, no significant improvement on cinnamyl acetate yield was observed as enzyme loading further increased. Thus, the enzyme loading of 3 g was employed.

Undoubtedly, reaction temperature was an important factor for the synthesis reaction. In order to measure the effect of temperature on reaction, reaction temperature was set from 35 to 55 °C for each batch. As shown in Table 2 (entry 5–7), the yield of cinnamyl acetate increased with reaction temperature. However, the cinnamyl acetate yield did not increase obviously with temperature. Considering the energy consumption and cinnamyl acetate stability, the reaction temperature was set 35 °C. Usually, the molar ratio of substrates exhibited a significant effect on many kinds of acid/alcohol synthesis reactions [23]. In order to obtain the maximum yield of the desired product, molar ratio was set from 1:2 to 3:1. As shown in Table 2 (entry 8–13), the cinnamyl acetate yield increased with molar ratio, and excess acetic acid would promote cinnamyl alcohol to be turned into cinnamyl acetate. Since the further increase of acetic acid had no significant effect on cinnamyl acetate synthesis and simultaneously led to the loss of lipase activity [24], the molar ratio was set 2:1.

Hence, the optimized reaction conditions for cinnamyl acetate synthesis were enzyme loading of 3 g, reaction temperature of 35 °C, and molar ratio of 2:1. Under the optimized conditions, the cinnamyl acetate yield would reach 62.56%. Moreover, the yield of cinnamyl acetate could reach more than 40% after 4 cycles by utilization of Ni-MCP-lipase (Fig. 8).

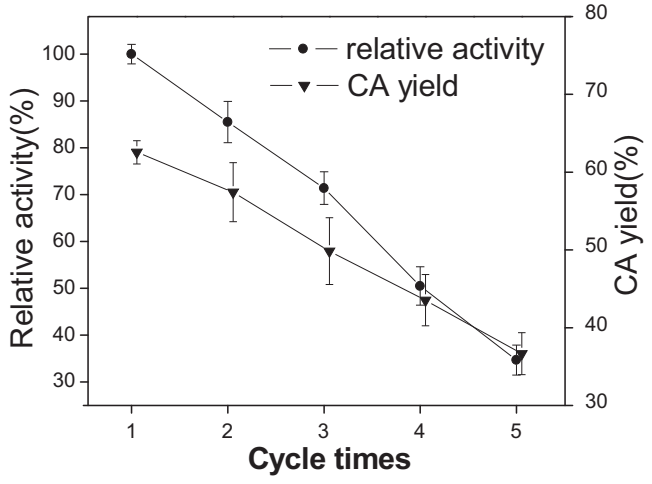

Fig. 8. The recycle stability of Ni-MCP-lipase in glyceryl triacetate hydrolysis and cinnamyl acetate synthesis. Glyceryl triacetate hydrolysis: Ni-MCP-lipase 0.5 g; saturated glyceryl triacetate solution 4.0 mL; reaction time 30 min. Cinnamyl acetate synthesis: Ni-MCP-lipase 3.0 g; solvent 15.0 mL *n*-hexane; acid/alcohol ratio 3:1; reaction time 10 h; reaction temperature 35 °C; shaking rate 150 rpm.

4. Conclusion

Immobilization of lipase on Ni-MCP proved to be a simple and efficient method. Lipase was immobilized on Ni-MCP through affinity between Ni^{2+} and MCP surface. Meanwhile, the Ni^{2+} on Ni-MCP had an activation effect on lipase. Ni-MCP-lipase showed improved catalytic properties under the optimal immobilization conditions, i.e. Ni-MCP-lipase activity reached 1.4 U/g and activity yield retained 216%, which indicated the activation of Ni-MCP on lipase. Furthermore, Ni-MCP-lipase was used for cinnamyl acetate synthesis. Remarkably improved cinnamyl acetate yield also indicated the activation of Ni-MCP. Thus, Ni-MCP not only immobilized lipase efficiently, but also showed the activation effect on lipase.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (51173128, 31071509, 20976125, 21206113), the 863 Program of China (2012AA06A303, 2013AA102204), the Ministry of Science and Technology of China (2012YQ090194), the Beiyang Young Scholar of Tianjin University (2012) and the Program of Introducing Talents of Discipline to Universities of China (No. B06006).

References

[1] T. Liu, Y. Zhao, X. Wang, X. Li, Y. Yan, Bioresour. Technol. 132 (2013) 99–102.
[2] K. Liu, G. Zhao, B. He, L. Chen, L. Huang, Bioresour. Technol. 123 (2012) 616–619.
[3] M.M. Zheng, Y. Lu, L. Dong, P.M. Guo, Q.C. Deng, W.L. Li, Y.Q. Feng, F.H. Huang, Bioresour. Technol. 115 (2012) 141–146.
[4] A.A. Tzialla, I.V. Pavlidis, M.P. Felicissimo, P. Rudolf, D. Gournis, H. Stamatis, Bioresour. Technol. 101 (2010) 1587–1594.
[5] Y. Wu, Y. Wang, G. Luo, Y. Dai, Bioresour. Technol. 100 (2009) 3459–3464.
[6] J.-Z. Liu, T.-L. Wang, L.-N. Ji, J. Mol. Catal. B: Enzym. 41 (2006) 81–86.
[7] M. Saleemuddin, Thermal Biosensors, Bioactivity, Bioaffinitty, Springer Berlin Heidelberg, Berlin, 1999, pp. 203–226.
[8] N. Carvajal, C. Torres, E. Uribe, M. Salas, Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 112 (1995) 153–159.
[9] Q.-X. Chen, W.-Z. Zheng, J.-Y. Lin, Y. Shi, W.-Z. Xie, H.-M. Zhou, Int. J. Biochem. Cell Biol. 32 (2000) 879–885.
[10] S. Bhatia, G. Wellington, J. Cocchiara, J. Lalko, C. Letizia, A. Api, Food Chem. Toxicol. 45 (2007) S53–S57.
[11] Z. Wu, W. Qi, M. Wang, Y. Wang, R. Su, Z. He, Biochem. Eng. J. 77 (2013) 190–197.
[12] S. Gao, Y. Wang, X. Diao, G. Luo, Y. Dai, Bioresour. Technol. 101 (2010) 3830–3837.
[13] R. Du, R. Su, X. Li, X. Tantai, Z. Liu, J. Yang, W. Qi, Z. He, Cellulose 19 (2012) 371–380.
[14] A.d. Vasconcellos, A.S. Paula, R.A. Luizon Filho, L.A. Farias, E. Gomes, D.A. Aranda, J.G. Nery, Microporous Mesoporous Mater. 163 (2012) 343–355.
[15] H. Horchani, S. Ouertani, Y. Gargouri, A. Sayari, J. Mol. Catal. B: Enzym. 61 (2009) 194–201.
[16] B. Joseph, P.W. Ramteke, G. Thomas, Biotechnol. Adv. 26 (2008) 457–470.

[17] D.A. Cowan, R. Fernandez-Lafuente, Enzyme Microb. Technol. 49 (2011) 326–346.
[18] J. Bing, L. Li, B. Lan, G. Liao, J. Zeng, Q. Zhang, X. Li, Appl. Catal. B: Environ. 115–116 (2012) 16–24.
[19] G.A. Petkova, K. Záruba, V. Král, Biochim. Biophys. Acta 1824 (2012) 792–801.
[20] H. Shen, S. Pan, Y. Zhang, X. Huang, H. Gong, Chem. Eng. J. 183 (2012) 180–191.
[21] R. Reshmi, G. Sanjay, S. Sugunan, Catal. Commun. 8 (2007) 393–399.
[22] G.D. Yadav, S. Devendran, Process Biochem. 47 (2012) 496–502.
[23] K.P. Dhake, P.J. Tambade, Z.S. Qureshi, R.S. Singhal, B.M. Bhanage, ACS Catal. 1 (2011) 316–322.
[24] M. Petkar, A. Lali, P. Caimi, M. Daminati, J. Mol. Catal. B: Enzym. 39 (2006) 83–90.