



Article Preparation of Ordered Macroporous ZIF-8-Derived Magnetic Carbon Materials and Its Application for Lipase Immobilization

Yongheng Shi, Hao Zhou, Lingmei Dai, Dehua Liu and Wei Du *🕩

Key Laboratory for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China; shiyh19@mails.tsinghua.edu.cn (Y.S.)

* Correspondence: duwei@tsinghua.edu.cn

Abstract: Metal-organic framework materials (MOFs) and their derivatives are considered ideal immobilization carrier materials because of their large specific surface area, high porosity and excellent structural designability. Among them, ZIF-8 has great potential for immobilization of enzymes due to mild synthesis conditions, and good biocompatibility. However, conventional ZIF-8 crystals have poor separation and recovery efficiency due to their small pore size and poor acid stability, greatly limiting their application in enzyme immobilization and further application. Although the carbonization of ZIF-8 by pyrolysis has been shown to be one of the approaches that can enhance its chemical stability, this still does not effectively solve the problem of the difficulty of recycling. Herein, we developed a strategy of pre-carbonization immersion (immersion in aqueous FeSO₄ solution before carbonization) to synthesize ordered macroporous ZIF-8-derived carbon materials with stable ferromagnetism (denoted as CZ-x-M-y, where x denotes the carbonization temperature and y denotes the concentration of the impregnated $FeSO_4$ solution) and used them to immobilize lipases for biodiesel production. XRD analysis showed that the magnetic properties in the materials came from Fe₃C species. We found that the magnetic carbon materials obtained by carbonization at 600 °C showed the best immobilization effect, where CZ-600-M-0.3 (using 0.3 mol·L⁻¹ FeSO₄ aqueous solution to soak ZIF-8 and carbonized at 600 °C) had the highest enzyme loading of 183.04 mg \cdot g⁻¹, which was 49.7% higher than that of the non-magnetic CZ-600. In addition, CZ-600-M-0.5 maintained the highest enzyme activity, which was 81.9% of the initial activity, after five batches of reuse. The stable magnetic support materials reported in this study have promising potential for the industrial application of immobilized lipase.

Keywords: biodiesel; enzyme immobilization; lipase; magnetic modification; MOF-derived carbon; zeolitic imidazolate frameworks (ZIF-8)

1. Introduction

Lipase (EC.3.1.1.3) is a highly efficient biocatalyst that has been widely used in the modification of fats and oils, synthesis of pharmaceutical intermediates, food processing, and biodiesel production [1–5]. Especially for the industrial production of biodiesel, the bio-enzyme-catalyzed method offers milder reaction conditions, higher yields, and less industrial waste than the traditional acid/base-catalyzed method [6,7]. The high cost of enzyme preparations has made the enhancement of enzyme reusability a research topic of great interest, usually achieved through enzyme immobilization [8,9]. In addition, the stability and catalytic activity of the enzyme in the reaction system can be effectively improved after immobilizing the enzyme by choosing suitable support materials and methods.

The performance of immobilized enzymes is closely related to the properties of the support materials, making the development of novel support materials highly focused. Metal–organic frameworks (MOFs) are a new class of porous crystalline materials that consist of metal nodes (metal ions or metal clusters) self-assembled with organic ligands through ligand bonds to form a three-dimensional ordered lattice structure. MOFs are



Citation: Shi, Y.; Zhou, H.; Dai, L.; Liu, D.; Du, W. Preparation of Ordered Macroporous ZIF-8-Derived Magnetic Carbon Materials and Its Application for Lipase Immobilization. *Catalysts* **2024**, *14*, 55. https:// doi.org/10.3390/catal14010055

Academic Editors: Diego Luna and Jose M. Guisan

Received: 18 December 2023 Revised: 5 January 2024 Accepted: 10 January 2024 Published: 12 January 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). regarded as a new type of immobilized enzyme support materials with great potential due to their large specific surface area, high porosity, and extreme designability of structure and function [10,11]. However, due to the small pore size of MOFs (usually less than 2 nm), their performance in the immobilization of larger-sized enzyme molecules (e.g., lipases) is not effective, hindering the diffusive mass transfer of the substrate and affecting the catalytic performance of the immobilized enzymes [12]. On the other hand, ZIF-8 (formed by coordination of zinc ions and 2-methylimidazole) is a MOF material with mild synthesis conditions and low biotoxicity, which has been regarded as a potential immobilized enzyme carrier in recent years. However, the acid stability of ZIF-8 is poor and its crystal structure can be damaged by fatty acids and other substances, which limits the application of ZIF-8 immobilized lipases [12]. The chemical stability of the materials can be effectively enhanced by obtaining their derived porous carbon materials through high-temperature pyrolytic carbonization, which is due to the fact that the structure of carbon materials mainly relies on covalent bonds [13,14]. Our group has already prepared single-crystal macroporous ZIF-8-derived carbon materials using a hard template method-pyrolytic carbonization strategy and successfully applied them to immobilized lipases [12]. This not only effectively solves the problem of mass transfer resistance caused by the small pore size of ZIF-8, but also greatly improves the chemical stability of the support materials, so that they are capable of being applied in the high-value conversion system of oils and fats.

A major constraint to the large-scale industrial application of immobilized enzymes is the high cost introduced by their separation and recovery. The low density, high dispersibility and small particle size of MOFs and their derived carbon materials immobilizing enzymes make it difficult to separate and recover from reaction systems [15]. Separation methods such as filtration and centrifugation, which are commonly used in laboratories, have high equipment and operating costs and thus are not suitable for large-scale industrial applications. The magnetic functionalization modification of the support materials allows for efficient separation and recovery from the reaction system with an external magnetic field [16–19]. This separation method has much lower equipment requirements and has great potential for industrial scale-up production.

The current studies mainly focus on the magnetic modification of MOFs, while little research has been conducted on MOF-derived carbon materials, especially macroporous carbon materials. One of the main ways to modify materials for magnetic functionalization is by loading magnetic nanoparticles onto the surface of the material, and most of the time this method is effective [20,21]. However, the limitation of this method is that the magnetic nanoparticles are connected to the surface of the material through weak interactions (electrostatic interactions, van der Waals forces, etc.), and the magnetic nanoparticles may be dislodged from the material during use, resulting in a loss of magnetism. In contrast, the strategy of allowing magnetic species to be embedded in the backbone of the material by means of covalent bonding connections may be more advantageous. Therefore, based on the previous work of our group, we prepared ZIF-8-derived magnetic carbon materials with ordered macroporous structure by soaking iron salt solution before the pyrolytic carbonization step, and explored the effect patterns of the carbonization temperatures and the concentrations of the iron salt solution soaked on the morphology structure and the performance of immobilized lipases of the materials. We achieved the immobilization of these carbon materials to TLL (the lipase Eversa® Transform 2.0, a commercialized variant of the lipase from *Thermomyces lanuginosus*) by physical adsorption and investigated the effect of immobilized lipase in the catalyzed methanolysis of soybean oil to produce a biodiesel reaction system.

The pre-carbonization immersion strategy developed in this paper can effectively prepare ZIF-8-derived porous carbon materials with stable magnetic modifications, and at the same time solves the problems of limited stability and difficult separation of ZIF-8 in immobilized lipase applications, which makes the MOF materials more promising for immobilized lipase applications.

2. Results and Discussion

2.1. Synthesis and Characterization of CZ-x-M-y and CZ-x

Figure 1 demonstrated the preparation process of ordered macroporous ZIF-8-derived carbon materials, CZ-x (x denotes the temperature of pyrolysis, x = 600, 700 and 800), and magnetic carbon materials, CZ-x-M-y (x denotes the temperature of pyrolysis, x = 600, 700 and 800; y denotes the molar concentration of aqueous FeSO₄ solution during soaking, y = 0.03, 0.1, 0.3, 0.5 and 0.9), where the synthesis process of CZ-x-M-y included two steps: soaking and pyrolytic carbonization. Figure 2a-c shows SEM images of CZ-600, CZ-700 and CZ-800, respectively. The carbon material obtained after the carbonization of SOM-ZIF-8@PS between 600 and 800 °C could be clearly seen as cuboctahedron shaped particles. And the particles had ordered and connected macroporous structures (~200 nm in diameter), which were formed by thermolysis of the PS microspheres in the temperature range described above. A further verification of this process could be obtained from the FTIR spectra (Figure 3a): the series of infrared absorption peaks belonging to PS with a wavenumber in the vicinity of 3000 cm⁻¹ disappeared when SOM-ZIF-8@PS was calcined to CZ-600. In addition, comparing the FTIR spectra of CZ-600, CZ-700, and CZ-800, it could be found that the absorption peaks near 1500 cm^{-1} gradually disappeared as the pyrolysis temperature increased from 600 to 800 °C, which mainly originated from the C=C bond of the imidazole aromatic ring in 2-methylimidazole, implying that the degree of decomposition of ZIF-8 gradually increased. The fact that there were still numerous absorption peaks below the wave number of 1300 cm⁻¹ in the FTIR spectra of CZ-600 proved that the degree of pyrolysis of ZIF-8 at 600 °C was low, resulting in a large number of groups still existing in CZ-600, which led to the generation of these absorption peaks. In contrast, the FTIR spectra of CZ-700 and CZ-800 were almost left with only a few broad absorption peaks, indicating a reduction in the number and type of groups in the framework of the materials.

The SEM images of CZ-600-M-y (y = 0.03, 0.1, 0.3, 0.5 and 0.9) are shown in Figure 2d-h, respectively. It could be observed that when the concentration of the soaking $FeSO_4$ solution was low, the ordered Microporous structures in the carbon materials were able to remain intact, as in CZ-600-M-0.03 (Figure 2d), CZ-600-M-0.1 (Figure 2e), and CZ-600-M-0.3 (Figure 2f); whereas, by increasing the concentration, the macroporous structures in the samples appeared to be deformed (as in CZ-600-M-0.5, Figure 2g) or even collapsed (as in CZ-600-M-0.9, Figure 2h). Furthermore, the characteristic diffraction peaks of Fe₃C and ZnS were observed in the XRD patterns of the CZ-x-M-y samples (Figure 3b), which indicated that the Fe and Zn elements in Fe-ZIF-8@PS existed as Fe3C and ZnS, respectively, after calcination in the range of 600 to 800 °C. We considered that it was the etching effect on the framework of the porous carbon materials during the formation of these species that led to these changes in the structure of the samples. Specifically, $FeSO_4$ entered into the pores of SOM-ZIF-8@PS during the soaking process, and was then pyrolyzed together with ZIF-8 at high temperatures ranging from 600 to 800 °C. Fe²⁺ was probably reduced by substances such as C in the framework of the porous carbon materials and penetrated into the carbon framework to form Fe₃C. The S elements in SO_4^{2-} were reduced and combined with Zn^{2+} to form ZnS. And a portion of the O elements formed volatile carbon oxides with the C elements in the framework, which was the major cause of the etching effect.

The Fe₃C species in CZ-x-M-y made the samples magnetic and able to be attracted by magnets. The magnetization curves of CZ-600-M-y (Figure 3c) indicated that the samples had remarkable ferromagnetism. The saturation magnetization of CZ-x-M-y is shown in Figure 3d. It could be concluded that the saturation magnetization of the samples tended to increase with the growth of the concentration of FeSO₄ solution used for soaking; whereas the magnitude of change in the saturation magnetization of the samples became significantly lower when the concentration continued increasing after 0.5 mol·L⁻¹. We considered that the saturation magnetization intensities of the CZ-x-M-y samples were related to the content of Fe₃C in them. The above results indicated that the diffusion concentration of FeSO₄ in the pores of the SOM-ZIF-8@PS samples became saturated

as the FeSO₄ solution concentration gradually increased to 0.5 mol·L⁻¹. Therefore, the saturation magnetization intensity of the carbonized CZ-x-M-y was basically unchanged when continuing to increase the FeSO₄ solution concentration. Based on this result, further explorations were centered around the CZ-x-M-0.5 samples.



Figure 1. Preparation process of ordered macroporous ZIF-8-derived magnetic carbon materials.



Figure 2. SEM images of (**a**) CZ-600, (**b**) CZ-700, (**c**) CZ-800, (**d**) CZ-600-M-0.03, (**e**) CZ-600-M-0.1, (**f**) CZ-600-M-0.3, (**g**) CZ-600-M-0.5 and (**h**) CZ-600-M-0.9.

The N₂ adsorption–desorption isotherms at 77 K and DFT pore size distributions of CZ-x-M-0.5 are illustrated in Figure 3e,f, respectively. It could be revealed that abundant mesoporous structures (2–50 nm) were possessed in CZ-x-M-y. The BET surface areas and DFT pore volumes of CZ-x-M-0.5 (x = 600, 700 and 800) were calculated to be 157.6 m²·g⁻¹ and 0.211 cm³·g⁻¹, 148.4 m²·g⁻¹ and 0.404 cm³·g⁻¹, and 88.1 m²·g⁻¹ and 0.256 cm³·g⁻¹, respectively (as shown in Table 1). It was noticed that the BET surface areas of CZ-x-M-y gradually decreased with the increase of pyrolysis temperature from 600 to 800 °C. Consid-

ering that the degree of decomposition of the ZIF-8 framework increased as the pyrolysis temperature raised during this temperature range, resulting in part of the microporous structure enlarging into mesopores or even collapsing. As a consequence, the total specific surface area was reduced instead.



Figure 3. (a) FTIR spectra of CZ-600, CZ-700, CZ-800, CZ-600-M-0.03, CZ-600-M-0.9, and SOM-ZIF-8@PS. (b) XRD patterns of CZ-600, CZ-600-M-0.03, CZ-600-M-0.9, CZ-700-M-0.9, CZ-800-M-0.9, and SOM-ZIF-8@PS. (c) Magnetization curves of CZ-600-M-y (y = 0.03, 0.1, 0.3, 0.5, and 0.9). (d) Saturation magnetization of CZ-600-M-y, CZ-700-M-y, and CZ-800-M-y (y = 0.03, 0.1, 0.3, 0.5 and 0.9). (e) N2 sorption-desorption isotherms of CZ-600-M-0.5, CZ-700-M-0.5, and CZ-800-M-0.5 at 77 K. (f) DFT pore size distributions of CZ-600-M-0.5, CZ-700-M-0.5, and CZ-800-M-0.5.

Table 1. The BET surface areas and DFT pore volumes of CZ-x-M-0.5 (x = 600, 700 and 800).

Material	BET Surface Areas (m ² ·g ⁻¹)	DFT Pore Volumes (cm ³ ·g ⁻¹)
CZ-600-M-0.5	157.6	0.211
CZ-700-M-0.5	148.4	0.404
CZ-800-M-0.5	88.1	0.256

2.2. Immobilization Properties of CZ-x-M-y@TLL and CZ-x@TLL

TLLs were immobilized in the pores of CZ-x and CZ-x-M-y through physical adsorption. Figure 4a,b displays the comparison of enzyme loading, specific activity and activity recovery of CZ-x-M-0.5@TLL (x = 600, 700 and 800), respectively. A gradual increase in enzyme loading of CZ-x-M-0.5@TLL (x equaled from 800 to 600) was observed, which followed the same trend as that of the BET surface areas of the support materials mentioned above. The process of physical adsorption of lipases on support materials occurred mainly on their active surfaces, thus support materials with a larger specific surface area might have a higher enzyme loading capacity. The enzyme activity recovery of immobilized lipase was co-affected by several factors, including the particle size, specific area, and pore size of the carriers [22], which led to significant differences in the enzyme activity recoveries of CZ-x-M-0.5@TLL (x = 600, 700 and 800). Considering together the magnitude of enzyme loadings, specific enzyme activities, and the enzyme activity recoveries of the samples, CZ-600-M-0.5@TLL could be considered to possess optimal immobilization performance.



Figure 4. Comparison of (**a**) enzyme loading, (**b**) specific activity and activity recovery of CZ-x-M-0.5@TLL (x = 600, 700, and 800). Comparison of (**c**) enzyme loading, (**d**) specific activity and activity recovery of CZ-600-M-y@TLL (y = 0.03, 0.1, 0.3, 0.5, and 0.9) and CZ-600@TLL (shaded in the figure). Comparison of the changes in (**e**) enzyme loading and (**f**) activity recovery of CZ-600@TLL, and CZ-600-M-0.5@TLL with increasing liquid enzyme addition volume.

In addition, all the CZ-600-M-y@TLL (y = 0.03, 0.1, 0.3, 0.5, and 0.9) had higher enzyme loading than CZ-600@TLL (Figure 4c). This was caused by the possession of more mesopores in CZ-600-M-y due to the etching effect. These mesoporous structures improved the enzyme loading of the support materials, however, at the same time, the mass transfer resistance of the reaction substrates in the mesoporous structures resulted in the decrease of the catalytic performance of the lipases immobilized in the mesopores. Therefore, all of the CZ-600-M-y@TLL showed lower enzyme activity recoveries than that of the CZ-600@TLL (Figure 4d).

The mesoporous structures in the magnetic carriers CZ-x-M-y mentioned above were the main structural differences between them and the non-magnetic carriers CZ-x. Thus, it was essential to explore the regularities of these mesoporous structures affecting the performance of immobilized lipases. We obtained a series of immobilized lipases, CZ-600@TLL and CZ-600-M-0.5@TLL, with gradually increasing enzyme loadings, respectively, by varying the concentration of enzyme proteins in the initial solution during the immobilization process (keeping the total volume constant and varying the amount of liquid enzyme added to 20, 50, 200, 500, and 700 µL, respectively) (Figure 4e). However, the patterns of change in activity recovery with enzyme loading were very different for these two kinds of immobilized enzymes (Figure 4f): there was no significant change in the activity recovery of CZ-600@TLL, whereas the activity recovery of magnetically modified CZ-600-M-0.5@TLL was first increased and then decreased. The mesoporous structures existing in CZ-600-M-0.5 could provide an explanation for this result. We considered that when the enzyme loading was low, TLL was adsorbed in the mesopores in CZ-600-M-0.5, with larger mass-transfer resistance leading to lower activity of the immobilized lipase; whereas when the enzyme loading was high, multilayer adsorption of TLL occurred in CZ-600-M-0.5, which impeded the contact of the active site of lipase with the substrate, causing a decrease in the activity. Considering the magnitude of the enzyme loading and enzyme activity recoveries of these samples, it could be concluded that the CZ-600-M-0.5@TLL sample with a liquid enzyme addition of 200 μ L had the optimal immobilization performances.

2.3. The Methanolysis Reactions of Soybean Oil Catalyzed by CZ-x-M-y@TLL and CZ-x@TLL

We used immobilized lipases CZ-600-M-y@TLL (y = 0.03, 0.1, 0.3, 0.5 and 0.9) and CZ-600@TLL with equal enzyme activities to catalyze the reaction of the methanolysis of soybean oil and measured the concentration of the biodiesel products in the reaction system at different reaction times using the gas chromatography method. The yields of the reactions were calculated from the measured product concentrations and the curves of the reaction yields versus reaction time were plotted (Figure 5a). The results showed that the magnetic CZ-600-M-y@TLL and the non-magnetic CZ-600@TLL obtained essentially identical reaction curves in the catalyzed soybean oil methanolysis reaction system. This indicated that the magnetic modification of the support materials did not cause negative effects on the catalytic performances of the immobilized lipases.

The economic cost of the separation and recovery of immobilized lipases from the reaction system was one of the most important factors for their large-scale industrial application. All of the magnetic immobilized lipases CZ-600-M-y@TLL (y = 0.03, 0.1, 0.3, 0.5, and 0.9) we obtained were able to be separated and recovered very simply and efficiently by external magnetic fields (e.g., magnets) (Figure 5b), instead of using the traditional approach of centrifugation. It was positive for the industrial application of immobilized lipases since the process of separation by an external magnetic field caused less mechanical damage to the immobilized lipases and had a lower cost compared to centrifugation.



Figure 5. (a) Comparison of CZ-600@TLL and CZ-600-M-y@TLL biocatalysts in biodiesel production. (b) Photographs of CZ-600-M-y@TLL separated and recycled from the reaction system by the magnet after finishing the reactions. (c) Comparison of the reusability of different CZ-600-M-y@TLL. (d) Comparison of the saturation magnetization of different CZ-600-M-y support materials, CZ-600-M-y@TLL immobilized lipases, and them after five cycles of reuse.

To test the reusability performances of these samples, we used all of the CZ-600-My@TLL for five consecutive reaction batches. It could be found that after five reaction batches, all of CZ-600-M-y@TLL still retained approximately 80% of the initial activity, in particular, CZ-600-M-0.5@TLL retained 81.9% of it (Figure 5c). Moreover, the saturation magnetizations of all of CZ-600-M-y@TLL showed almost unchanged intensity after five reaction batches (Figure 5d), which indicated that the stable doping of magnetic species had been achieved in this work.

3. Materials and Methods

3.1. Materials

The lipase Eversa[®] Transform 2.0 from *Thermomyces lanuginosus* was purchased from Novozymes (Bagsvaerd, Denmark). $Zn(NO_3)_2 \cdot 6H_2O$ (99%), FeSO₄ · 7H₂O (99%) and polyvinylpyrrolidone (PVP, K30) were purchased from Beijing Sinopharm Chemical Reagent Co., Ltd. (Beijing, China), China. 2-methylimidazole (98%), Styrene and Tributyrin (98%) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd., Shanghai, China. K₂S₂O₈ (99.5%) was purchased from Shanghai Meiruier Biochemical Technology Co., Ltd., China. Bicinchroninic acid (BCA) protein assay kit was purchased from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China. Soybean oil was purchased from the local market. The analytical-grade heptadecanoic acid methyl ester was purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the other chemicals used in this work were of analytical grade.

3.2. Preparation of CZ-x-M-y and CZ-x

Single-crystal ordered macroporous ZIF-8 (SOM-ZIF-8@PS) embedded with polystyrene (PS) microspheres with a diameter of about 200 nm was synthesized with reference to the method of Shen et al. [23] Then, SOM-ZIF-8@PS was soaked with the aqueous solution of FeSO₄. The mixture was degassed in vacuum for 30 min to promote sufficient penetration of the solution into the SOM-ZIF-8@PS. The solid obtained by filtration of the mixture was dried at 60 °C to obtain Fe-ZIF-8@PS (Fe-doped SOM-ZIF-8@PS). Next, Fe-ZIF-8@PS was placed in a tube furnace under N₂ atmosphere and calcined at a set temperature (600, 700 and 800 °C) for 1 h with a heating rate of 5 °C min⁻¹. After cooling, magnetic macroporous ZIF-8-derived carbon materials were obtained, denoted as CZ-x-M-y. Similarly, SOM-ZIF-8@PS was pyrolyzed under the same conditions to obtain macroporous carbon materials, denoted as CZ-x.

3.3. Immobilization of Lipase

We used the physical adsorption method to achieve the immobilization of lipase on CZ-x and CZ-x-M-y. First, 50 mg of CZ-x or CZ-x-M-y was mixed with a solution consisting of 680 μ L PBS buffer (100 mmol·L⁻¹, pH = 7.0) and 120 μ L anhydrous ethanol. The mixture is then treated with ultrasound to disperse the powder evenly. Subsequently, 200 μ L of liquid lipase Eversa[®] Transform 2.0 (the initial concentration of TLL was 8.3 mg mL⁻¹) was added. The mixture was maintained in a shaker at 40 °C and 200 rpm for 4 h for the immobilization of lipase. The immobilized lipase (CZ-x@TLL or CZ-x-M-y@TLL) was then separated by centrifugation (6000 rpm and 5 min), washed once with DI water, and lyophilized. The supernatant and immobilized lipase were stored at 4 °C for further analysis. The enzyme loading was calculated from the difference in protein concentration between the initial solution and the supernatant after immobilization (protein concentration was measured using the BCA Protein Assay Kit).

3.4. Measurement of Enzyme Activity

According to the previous report of our group [24], we used the hydrolysis reaction of triglyceride to determine the specific enzyme activity and enzyme activity recovery. One unit of enzyme activity (U) was defined as the amount of enzyme required to release 1.0 µmol of butyric acid per minute under the conditions described. Enzyme activity

recovery was the ratio of the specific enzyme activity of the immobilized enzyme to the specific enzyme activity of the free enzyme.

3.5. Lipase-Catalyzed Methanolysis of Soybean Oil

In this work, methanolysis of soybean oil was chosen to evaluate the performance of immobilized lipase in practical applications. The molar ratio of methanol to soybean oil was 4:1, and 120 U of the immobilized lipase was added per gram of soybean oil. The reaction was carried out in a shaker at 40 °C and 200 rpm. In addition, 10 wt% DI water (based on the mass of soybean oil) was added to maintain the activity of lipase through the interfacial activation effect.

The yields of biodiesel (fatty acid methyl esters, FAMEs) and fatty acids were measured by Agilent 7890A gas chromatography (GC) from Agilent Technologies (Santa Clara, CA, USA) using an ethanol solution of heptadecanoic acid methyl ester (1 mg L^{-1}) as an internal standard.

3.6. Reusability of the Immobilized Lipases

After 12 h of reaction under the same conditions as above, the immobilized lipase was recovered from the reaction system using a magnet, washed twice with tert-butanol and lyophilized, and then continued to be used for the next batch of reaction. The yield of biodiesel was determined after each reaction batch using gas chromatography. The retention of immobilized lipase activity was estimated as the ratio of biodiesel yield per batch of reaction to the first.

3.7. Characterization

The morphology and structure of the samples were characterized using a highperformance field emission scanning electron microscope (SEM, Zeiss Merlin, 5.0 kV) from Carl Zeiss (Jena, Germany). Powder X-ray diffraction (XRD) patterns were recorded by an X-ray diffractometer MiniFlex 600 from Rigaku (Tokyo, Japan) at 40 kV and 15 mA with Cu K α (λ = 0.15406 nm) as the anode. The N₂ adsorption–desorption isotherms of the samples at 77 K were measured using a fully automated specific surface area and void analyzer (Micromeritics 3Flex, Norcross, Georgia, USA). Fourier transform infrared (FTIR) patterns were measured using Nicolet FTIR 6700 from Thermo Fisher Scientific (Waltham, MA, USA). The magnetic hysteresis loops of the samples were measured using a vibrating sample magnetometer (VSM, Lake Shore Cryotronics, Inc., Westerville, OH, USA).

4. Conclusions

In summary, this work presents a simple soaking and one-step pyrolysis carbonization strategy for the preparation of ordered macroporous ZIF-8-derived magnetic carbon materials, CZ-x-M-y, which can be used for immobilization of lipase for biodiesel production. The mesoporous–macroporous structure in such novel support materials enhances their capacity to immobilize lipases. The enzyme loading of CZ-x-M-y increased with the decrease in the carbonization temperature, in which the material, CZ-600-M-0.3, possessed the largest enzyme loading of 183.04 mg \cdot g⁻¹ at the carbonization temperature of 600 °C, which was 49.7% higher compared with that of the non-magnetically modified CZ-600. At the same time, this novel support material has stable ferromagnetism, which enables it to be separated and recovered from the reaction system very conveniently under the action of an external magnetic field, showing great potential for industrial application. The immobilized lipase CZ-600-M-0.5@TLL showed the best reusability, which retained 81.9% activity after five catalytic reaction batches. The present work broadens the prospect of MOF materials in immobilizing lipase and provides a new idea.

Author Contributions: Conceptualization, Y.S. and H.Z.; methodology, Y.S., H.Z. and L.D.; investigation, Y.S.; writing—original draft preparation, Y.S.; writing—review and editing, W.D.; supervision, W.D.; project administration, D.L. and W.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Monteiro, R.R.; Arana-Peña, S.; da Rocha, T.N.; Miranda, L.P.; Berenguer-Murcia, Á.; Tardioli, P.W.; dos Santos, J.C.; Fernandez-Lafuente, R. Liquid lipase preparations designed for industrial production of biodiesel. Is it really an optimal solution? *Renew. Energy* 2021, *164*, 1566–1587. [CrossRef]
- 2. Basso, A.; Serban, S. Industrial applications of immobilized enzymes—A review. Mol. Catal. 2019, 479, 110607. [CrossRef]
- 3. Chandra, P.; Enespa; Singh, R.; Arora, P.K. Microbial lipases and their industrial applications: A comprehensive review. *Microb. Cell Fact.* **2020**, *19*, 169. [CrossRef] [PubMed]
- 4. Gotor-Fernández, V.; Brieva, R.; Gotor, V. Lipases: Useful biocatalysts for the preparation of pharmaceuticals. *J. Mol. Catal. B-Enzym.* **2006**, 40, 111–120. [CrossRef]
- Sarmah, N.; Revathi, D.; Sheelu, G.; Yamuna Rani, K.; Sridhar, S.; Mehtab, V.; Sumana, C. Recent advances on sources and industrial applications of lipases. *Biotechnol. Prog.* 2018, 34, 5–28. [CrossRef]
- Cavalcante, F.T.T.; Neto, F.S.; Rafael de Aguiar Falcão, I.; Da Erick Silva Souza, J.; de Moura Junior, L.S.; da Silva Sousa, P.; Rocha, T.G.; de Sousa, I.G.; de Lima Gomes, P.H.; de Souza, M.C.M.; et al. Opportunities for improving biodiesel production via lipase catalysis. *Fuel* 2021, 288, 119577. [CrossRef]
- Guldhe, A.; Singh, B.; Mutanda, T.; Permaul, K.; Bux, F. Advances in synthesis of biodiesel via enzyme catalysis: Novel and sustainable approaches. *Renew. Sustain. Energy Rev.* 2015, 41, 1447–1464. [CrossRef]
- Ortiz, C.; Ferreira, M.L.; Barbosa, O.; dos Santos, J.C.S.; Rodrigues, R.C.; Berenguer-Murcia, A.; Briand, L.E.; Fernandez-Lafuente, R. Novozym 435: The "perfect" lipase immobilized biocatalyst? *Catal. Sci. Technol.* 2019, *9*, 2380–2420. [CrossRef]
- 9. Remonatto, D.; Miotti, R.H., Jr.; Monti, R.; Bassan, J.C.; de Paula, A.V. Applications of immobilized lipases in enzymatic reactors: A review. *Process Biochem.* **2022**, *114*, 1–20. [CrossRef]
- 10. Lian, X.; Fang, Y.; Joseph, E.; Wang, Q.; Li, J.; Banerjee, S.; Lollar, C.; Wang, X.; Zhou, H.-C. Enzyme-MOF (metal-organic framework) composites. *Chem. Soc. Rev.* 2017, *46*, 3386–3401. [CrossRef]
- 11. Liang, W.; Wied, P.; Carraro, F.; Sumby, C.J.; Nidetzky, B.; Tsung, C.-K.; Falcaro, P.; Doonan, C.J. Metal-Organic Framework-Based Enzyme Biocomposites. *Chem. Rev.* 2021, *121*, 1077–1129. [CrossRef] [PubMed]
- Zhou, H.; Zou, Z.Q.; Dai, L.M.; Liu, D.H.; Du, W. Ordered Macro-Microporous ZIF-8 with Different Macropore Sizes and Their Stable Derivatives for Lipase Immobilization in Biodiesel Production. ACS Sustain. Chem. Eng. 2022, 10, 14503–14514. [CrossRef]
- Wang, D.; Jana, D.; Zhao, Y. Metal-Organic Framework Derived Nanozymes in Biomedicine. Acc. Chem. Res. 2020, 53, 1389–1400.
 [CrossRef]
- 14. Song, B.; Ren, D.; Wang, Z.; Huang, Y.; Zhang, X.; Zhang, S.; Gong, X.; Chen, W. Laccase immobilization on bimetallic MOFderived porous carbon materials for the removal of bisphenol A. *J. Chem. Technol. Biotechnol.* **2023**, *98*, 919–931. [CrossRef]
- 15. Nadar, S.S.; Rathod, V.K. Magnetic-metal organic framework (magnetic-MOF): A novel platform for enzyme immobilization and nanozyme applications. *Int. J. Biol. Macromol.* **2018**, *120*, 2293–2302. [CrossRef]
- 16. Gao, X.; Zhai, Q.; Hu, M.; Li, S.; Jiang, Y. Hierarchically porous magnetic Fe₃O₄ / Fe-MOF used as an effective platform for enzyme immobilization: A kinetic and thermodynamic study of structure–activity. *Catal. Sci. Technol.* **2021**, *11*, 2446–2455. [CrossRef]
- Ji, Y.; Wu, Z.Z.; Zhang, P.; Qiao, M.; Hu, Y.; Shen, B.X.; Li, B.Z.; Zhang, X. Enzyme-functionalized magnetic framework composite fabricated by one-pot encapsulation of lipase and Fe₃O₄ nanoparticle into metal-organic framework. *Biochem. Eng. J.* 2021, 169, 107962. [CrossRef]
- Li, Q.; Pan, Y.; Li, H.; Alhalhooly, L.; Li, Y.; Chen, B.; Choi, Y.; Yang, Z. Size-Tunable Metal-Organic Framework-Coated Magnetic Nanoparticles for Enzyme Encapsulation and Large-Substrate Biocatalysis. ACS Appl. Mater. Interfaces 2020, 12, 41794–41801. [CrossRef]
- 19. Suo, H.; Geng, H.; Zhang, L.; Liu, G.; Yan, H.; Cao, R.; Zhu, J.; Hu, Y.; Xu, L. Covalent immobilization of lipase on an ionic liquid-functionalized magnetic Cu-based metal-organic framework with boosted catalytic performance in flavor ester synthesis. *J. Mat. Chem. B* 2023, *11*, 1302–1311. [CrossRef]
- Fu, C.-W.; Lirio, S.; Shih, Y.-H.; Liu, W.-L.; Lin, C.-H.; Huang, H.-Y. The Cooperativity of Fe₃O₄ and Metal-Organic Framework as Multifunctional Nanocomposites for Laser Desorption Ionization Process. *Chemistry* 2018, 24, 9598–9605. [CrossRef]
- Ke, F.; Qiu, L.-G.; Yuan, Y.-P.; Jiang, X.; Zhu, J.-F. Fe₃O₄@MOF core–shell magnetic microspheres with a designable metal–organic framework shell. J. Mater. Chem. 2012, 22, 9497. [CrossRef]
- 22. Zhao, X.; Qi, F.; Yuan, C.; Du, W.; Liu, D. Lipase-catalyzed process for biodiesel production: Enzyme immobilization, process simulation and optimization. *Renew. Sustain. Energy Rev.* **2015**, *44*, 182–197. [CrossRef]

- 23. Shen, K.; Zhang, L.; Chen, X.; Liu, L.; Zhang, D.; Han, Y.; Chen, J.; Long, J.; Luque, R.; Li, Y.; et al. Ordered macro-microporous metal-organic framework single crystals. *Science* 2018, 359, 206–210. [CrossRef]
- 24. Hu, Y.; Dai, L.; Liu, D.; Du, W. Rationally designing hydrophobic UiO-66 support for the enhanced enzymatic performance of immobilized lipase. *Green Chem.* **2018**, *20*, 4500–4506. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.