Preparation of carrierized cellulase Cellulase@MIL-88B(Fe) and its enzymatic properties

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ABSTRACT
In this study, cellulase was modified by immobilization technique using MIL-88B(Fe) as the carrier, and the immobilization temperature, time, cellulase concentration and pH of 30°C, 6 h, 1.2 mg/mL and 5.0, respectively, were investigated as the optimal preparation conditions. Characterization analysis using FT-IR, XRD and TGA revealed that the successful immobilization of cellulase on MIL-88B(Fe) did not disrupt the integrity of the carrier lattice structure. Compared with the free enzyme, Cellulase@MIL-88B(Fe) maintained 78% and 85.21% of its activity after 10 cycles and 49 d, respectively, and its t1/2 and Ed values were higher than those of the free enzyme, and it was able to maintain a higher activity over a wider pH and temperature range, indicating its higher thermal stability and heat-loss activity. In addition, the Km of Cellulase@MIL-88B(Fe) (1.46 mg/mL) was slightly higher than that of free cellulase (1.41 mg/mL), and Cellulase@MIL-88B(Fe) was able to be applied in the actual enzymatic digestion of corn cobs.

1. Introduction
The industrial application of cellulase is limited by its difficulty in recovery and its three-dimensional structure susceptible to activity inactivation caused by distortions in different conditions such as pH, temperature and solution type. The biocatalytic activity of cellulases can be enhanced by immobilization using suitable materials, of which inorganic materials are commonly used as carriers, but their low specific surface area limits the loading capacity of cellulase immobilization. Metal–organic backbones (MOFs) are an emerging class of inorganic–organic hybrid materials composed of metal ions and organic ligands, which have the advantages of large specific surface area, tunable pore size and surface chemistry and are therefore considered promising candidates for cellulase immobilization (1,2). MIL-88B(Fe) was reported to be a three-dimensional porous iron-based MOF consisting of trimeric octahedral clusters of terephthalic acid and iron (3). Amari et al. successfully immobilized laccase in magnetic amino functionalized Fe3O4-NH2@MIL-101(Cr) for the study of decolorization of wastewater (4). Wang et al. used a magnetic metal–organic framework to immobilize α-L-rhamnosidase and found that the conversion of rutin remained at 73.55% after 30 cycles at 60°C (5). Lin et al. loaded cellulase onto chitosan-coated Fe3O4 magnetic nanoparticles using glutaraldehyde as a cross-linking agent and found that the immobilized enzyme was able to maintain a higher activity over a wider pH and temperature range compared to free cellulase and still maintained 80% of the initial activity after five cycles (6). Poorakbar et al. combined AuNPs with PSNs to immobilize cellulase and increase its stability, and this immobilization resulted in a much higher activity than the free enzyme at higher temperatures, with 28% activity after 5 cycles (7). Zhang et al. immobilized cellulase on Zn-mIm and found that cellulase@Zn-mIm showed superior tolerance to alkaline environment, maintaining 65% activity at pH 8.0, and cellulase@Zn-mIm maintained 77% activity after four cycles (8). Qi et al. used UIO-66-NH2 immobilized cellulase and found that cellulase@Zn-mIm showed superior performance in terms of pH stability, thermal stability and catalytic efficiency after immobilization (9). Wang et al. immobilized cellulase with sodium alginate-polyethylene glycol-chitosan and used it for hydrolysis of MCC, and the total yield of reducing sugars was observed to be higher than that of the free enzyme (10). Qi et al. used UIO-66-NH2 immobilized cellulase and found that cellulase@Zn-mIm showed superior performance in terms of pH stability, thermal stability and catalytic efficiency after immobilization (9).
sugars was 22.68% higher than that of free cellulase after five cycles of repeated use (10). Wang et al. immobilized peroxidase in CAT@MIL-125-NH₂ and CAT@AminoMIL-125-NH₂ respectively and found that CAT@AminoMIL-125-NH₂ had higher activity recovery, good pH stability, stability against denaturant and thermal stability compared to CAT@MIL-125-NH₂ and free enzyme (11).

At present, domestic and foreign research scholars mainly focus on finding various carriers and methods to immobilize cellulase, expecting to enhance its stability, however, there are fewer studies on immobilized cellulase in hydrolysis of cellulose-based biomass, etc. In this paper, cellulase was immobilized with homemade MIL-88B(Fe) as the carrier, and the effects of preparation conditions such as immobilization temperature, time, cellulase concentration and solution pH on the immobilization effect of cellulase were investigated, and Cellulase@MIL-88B(Fe) by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), thermogravimetric analysis (TGA) Fe) was characterized and compared with MIL-88B(Fe) carrier to explore its structural changes, followed by systematic studies on the enzymatic properties, kinetic parameters, thermodynamic parameters, and hydrolysis of Cellulase@MIL-88B(Fe) for cellulose-based biomass applications, and comparison with free cellulase to evaluate its catalytic performance for cellulase in biomass conversion of lignocellulose provides a basis for the application of cellulases in the efficient utilization of lignocellulose in biomass conversion.

2. Materials and methods

2.1. Preparation of Cellulase@MIL-88B(Fe)

2.1.1. Preparation of MIL-88B(Fe)
The MIL-88B(Fe) material was prepared according to the method previously reported in the literature (12) with slight modifications: 1.512 g of FeCl₃·6H₂O and 0.462 g of terephthalic acid were weighed and dissolved in 120 mL of N,N-dimethylformamide. After stirring continuously for 60 min, the solution was transferred to a hydrothermal reactor and reacted in an oven at 120°C for 12 h. After the reaction was finished, it was allowed to cool naturally to room temperature, and the solid obtained by centrifugation was washed three times with N,N-dimethylformamide and methanol solutions, respectively. After that, it was dried in an oven at 80°C overnight to obtain MIL-88B(Fe) solid.

2.1.2. Preparation of Cellulase@MIL-88B(Fe)
10 mg of MIL-88B(Fe), 40 mg of 1-ethyl-(3-dimethylaminopropyl)carbodiimide (EDC) and 20 mg of N-hydroxysuccinimide (NHS) were added to 10 mL of 1.2 mg/mL cellulase solution (pH 5.0) and reacted at 30°C for 6 h. After the reaction, the mixed solution was centrifuged (5000xg, 5 min), and the resulting solid was washed three times with sodium acetate buffer (50 mM pH 5.0) to remove the unreacted cellulase to obtain immobilized cellulase (Cellulase@MIL-88B(Fe)). Afterwards, Cellulase@MIL-88B(Fe) was resuspended in sodium acetate buffer (50 mM, pH 5.0) and stored at 4°C for further analysis.

2.2. Structural characterization of Cellulase@MIL-88B(Fe)

2.2.1. FT-IR analysis of Cellulase@MIL-88B(Fe)
A small amount of cellulase mixed with KBr powder was ground and pressed into thin slices on a Fourier infrared spectrometer (Perkin-Elmer, Thermo Scientific) to obtain FT-IR spectra, and the FT-IR plots were processed with PeakFit V4.12 software, and finally the free enzyme was calculated from the area of each sub-peak and the relative content of the secondary structure of Cellulase@MIL-88B(Fe).

2.2.2. X-ray diffraction (XRD)
X-ray diffraction analysis of MIL-88B(Fe) and Cellulase@MIL-88B(Fe) was performed on an X-ray diffractometer with an angular variation of 5°C–50°C.

2.2.3. Thermogravimetric (TGA) analysis
Thermal stability analysis of MIL-88B(Fe) and Cellulase@MIL-88B(Fe), heated at a heating rate of 10°C/min in a N2 atmosphere from room temperature to 750°C, characterized by a pyrolytic weight analyzer (TGA), heat loss of MIL-88B(Fe) and Cellulase@MIL-88B(Fe) during heating was recorded and compared.

2.3. Characterization of the enzymatic properties of Cellulase@MIL-88B(Fe)

2.3.1. Optimal temperature and pH of Cellulase@MIL-88B(Fe)
The effect of temperature on Cellulase@MIL-88B(Fe) activity was investigated by hydrolyzing CMC with free cellulase and Cellulase@MIL-88B(Fe) at different temperatures (40°C–80°C) at pH 5.0. The relative activities of free cellulase and Cellulase@MIL-88B(Fe) at other temperatures were calculated using the highest activity of both enzymes in the temperature range as 100%. Similarly, the pH of Cellulase@MIL-88B(Fe) was examined by hydrolyzing CMC with free cellulase and Cellulase@MIL-88B(Fe) at different pH (1.0–13.0) at their respective optimum temperatures.
2.3.2. Reusability and storage stability of Cellulase@MIL-88B(Fe)
The reusability of Cellulase@MIL-88B(Fe) was evaluated by continuous repeated hydrolysis of CMC using Cellulase@MIL-88B(Fe), and the residual activity of Cellulase@MIL-88B(Fe) was determined at the end of each experiment with an initial activity of 100%.

Free cellulase and Cellulase@MIL-88B(Fe) were stored at 4°C for 56 days and cellulase activity was measured at 7-day intervals under their respective optimal conditions to study the storage stability of both enzymes. The initial enzyme activity was defined as 100%.

2.3.3. Organic tolerance of Cellulase@MIL-88B(Fe)
Free cellulase and Cellulase@MIL-88B (Fe) were placed in methanol, ethanol, isopropanol and tert-butanol for 2 h, after which the activities of both enzymes were measured and the relative activities of each group were calculated using the enzyme activity without the addition of organic solvents as 100%.

2.3.4. Mechanical and thermodynamic parameters of thermal inactivation of Cellulase@MIL-88B(Fe)
The half-log plot of residual cellulase activity versus time was used to determine the thermal inactivation rate constant (k_d), half-life (t_1/2) of both enzymes, and the inactivation energy (E_a) of free cellulase and Cellulase@MIL-88B(Fe) by Arrhenius. In addition, the enthalpy of thermal deactivation (ΔH^0), Gibbs free energy (ΔG^0) and entropy (ΔS^0) of both enzymes were calculated based on the Eyring transition state theory.

2.3.5. Kinetic study of Cellulase@MIL-88B(Fe)
The Mie constants (k_m) and maximum reaction rates (V_max) of free cellulase and Cellulase@MIL-88B(Fe) were determined by hydrolyzing different concentrations of CMC (0.1%–1.0% (w/v)).

2.3.6. Hydrolysis of Cellulase@MIL-88B(Fe) in CMC and corn cobs
CMC and pretreated corn cobs were immersed in sodium acetate buffer (50 mM pH 5.0), and then free cellulase or Cellulase@MIL-88B (Fe) was added for enzymatic digestion, and the supernatant was periodically taken for analysis of enzymatic efficiency.

3. Results and discussion
3.1. Preparation and condition optimization of Cellulase@MIL-88B(Fe)
In this paper, the effects of immobilization temperature, time, cellulase concentration and pH were investigated to obtain the optimal immobilization conditions.

Temperature is one of the most important factors affecting the effect of enzyme immobilization. Therefore, the effect of temperature in the range of 25°C–45°C on the immobilization of cellulase was investigated in this paper, and the results are shown in Figure 1a. As can be seen in Figure 1a, the lowest recovery of enzymatic activity of Cellulase@MIL-88B(Fe) was observed when the temperature was 25°C. This may be because the functional groups on MIL-88B(Fe) and in the cellulase molecule were not sufficiently activated due to the low temperature, resulting in low binding efficiency of both. The enzymatic activity recovery of Cellulase@MIL-88B(Fe) increased with temperature in the range of 25°C–30°C, indicating that the increase in temperature accelerated the reaction rate in this temperature range, allowing more cellulase molecules to be immobilized on MIL-88B(Fe). The enzymatic activity recovery of Cellulase@MIL-88B(Fe) reached a maximum when the immobilization temperature was 35°C. The recovery of Cellulase@MIL-88B(Fe) decreased with the increase of temperature, which was caused by the denaturation and inactivation of cellulase due to high temperature. Therefore, 35°C is chosen as the optimal fixation temperature in this paper. Miao et al. found that the optimal immobilization temperature was 30°C when using magnetic chitosan microspheres loaded with cellulase (13). Mo et al. found that 25°C was the optimal immobilization temperature when immobilizing cellulase on chitosan@magnetic porous biochar (14).

Time is also one of the main factors that substantially affects the immobilization process. In this paper, the enzymatic activity return rate of Cellulase@MIL-88B(Fe) was investigated in the time range of 2–12 h. As can be seen from Figure 1b, the activity of Cellulase@MIL-88B(Fe) was low when the immobilization time was 2 h. This was because the immobilization time was short and only a small amount of cellulase was immobilized in the carrier. With the increase of immobilization time, more and more cellulase molecules were loaded on MIL-88B(Fe), so the activity of Cellulase@MIL-88B(Fe) increased rapidly. The activity of Cellulase@MIL-88B(Fe) reached the maximum at the immobilization time of 6 h. After that, its activity started to decrease gradually with the increase of time, which was mainly due to the excessive cellulase binding to MIL-88B(Fe), which had some effects on the structure of the cellulase molecule and led to the destruction of the active site of cellulase. Besides, prolonged immobilization causes cellulase to aggregate on the carrier, and the increased spatial site resistance hinders the contact of cellulase...
with the substrate and the diffusion of the product in solution, reducing the activity of immobilized cellulase Cellulase@MIL-88B(Fe) \(^{(15)}\). Raza et al. found that the optimal immobilization time was 6 h in the preparation of multilayer magnetic hollow particles (MMHPs) for immobilization of cellulase \(^{(16)}\).

As shown in Figure 1c, the effect of cellulase concentration on the immobilization effect of cellulase was further investigated in this paper. When the cellulase concentration was in the range of 0.5–1.2 mg/mL, the activity of Cellulase@MIL-88B(Fe) increased with the increase of concentration. The optimal value of Cellulase@MIL-88B(Fe) activity was reached at a cellulase concentration of 1.2 mg/mL. After that, the activity of Cellulase@MIL-88B(Fe) started to decrease with further increase of cellulase concentration. It indicates that the number of active sites on MIL-88B(Fe) is limited, and the high concentration of cellulase causes the accumulation and agglomeration of enzyme molecules on the carrier, which leads to the increase of spatial site resistance impeding the binding of substrate to the active site of cellulase, thus leading to the decrease of Cellulase@MIL-88B(Fe) activity. When the concentration of cellulase is low, the amount of MIL-88B(Fe) is too much relative to cellulase, which leads to insufficient utilization of the carrier, so the activity of immobilized enzyme is also low. Li et al. found the optimal cellulase concentration of 2 mg/mL in the optimization of carbon nanotube-sodium alginate immobilized cellulase \(^{(17)}\).

In this paper, the effect of solution pH in the range of 3.0–7.0 on the effect of immobilized cellulase was investigated. As shown in Figure 3d, the enzyme activity of Cellulase@MIL-88B(Fe) gradually increased with increasing pH in the range of pH 3.0–5.0. The maximum enzymatic activity of Cellulase@MIL-88B(Fe) was observed at pH 5.0. At pH > 5.0, the enzymatic activity of Cellulase@MIL-88B(Fe) started to show a decreasing trend, and the electrostatic repulsion between cellulase and the residual carboxyl groups on the MIL-88B(Fe) carrier prevented the effective binding of cellulase to MIL-88B(Fe). Therefore, the optimal value of immobilization pH was 5.0. Wang et al. in the immobilization of cellulase by styrene-maleic anhydride copolymer nanoparticles also found that pH 5.0 was the optimal immobilization environment for immobilized cellulase \(^{(18)}\). Raza et al. found that c-MMHPs immobilized cellulase best when the solution pH was 5.0 \(^{(16)}\).

### 3.2. Structural characterization of Cellulase@MIL-88B(Fe)

#### 3.2.1. FT-IR analysis of Cellulase@MIL-88B(Fe)

The FT-IR spectra of MIL-88B(Fe) and Cellulase@MIL-88B(Fe) are shown in Figure 2a. The broad peaks between...
3300 and 3500 cm$^{-1}$ are characteristic absorption peaks of the -OH and -NH groups, the peak around 2985 cm$^{-1}$ belongs to the stretching vibration peak in -CH$_3$, and the absorption peaks around 552, 1396, and 1572 cm$^{-1}$ are characteristic peaks of the MIL-88B(Fe) carrier, where the peak around 552 cm$^{-1}$ is an Fe-O vibration peak, as previously reported for MIL-88B(Fe). The two peaks observed at 1572 and 1396 cm$^{-1}$ belong to the C=O asymmetric vibrational peak and symmetric vibrational peak of the carboxylic acid group, respectively, confirming the presence of dicarboxylic acid ligands on the MIL-88B(Fe) carrier (3). Compared with the FT-IR spectrum of MIL-88B(Fe), Cellulase@MIL-88(Fe) showed a new absorption peak at 1051 cm$^{-1}$, indicating the successful loading of cellulase on MIL-88B(Fe). Changes in secondary structure can directly lead to changes in enzyme activity. Therefore, in this study, the secondary structure changes between free cellulase and Cellulase@MIL-88B(Fe) were investigated based on FT-IR spectroscopy, and the results are shown in Figure 2b and c. The relative content of the secondary structure of cellulase before and after immobilization was studied by curve fitting and semi-quantitative calculations according to the previously reported method to study the area of each group of peaks in the amide III band region of the cellulase molecule as shown in Table 1. Compared with free cellulase, Cellulase@MIL-88B(Fe) increased $\alpha$-helix and $\beta$-turn by 15.81% and 1.89%, respectively, and decreased $\beta$-sheet and random coil by 10.39% and 7.31%, respectively. The presence of a large number of hydrogen bonds between $\alpha$-helix and $\beta$-sheet can maintain the natural conformation of the enzyme molecule, which is the main source of
rigidity of the enzyme molecule and can resist the influence of external environment. The conformational change of Cellulase@MIL-88B(Fe) may be a key factor affecting the changes in pH, thermal stability and thermodynamic stability. Wu et al. immobilized cellulase on Fe3O4@MtK-10 composites and found that cellulase on MtK-10 had lower α-helix and β-sheet content compared to free cellulase (19).

3.2.2. XRD analysis of Cellulase@MIL-88B(Fe)
To determine whether cellulase immobilization on MIL-88B(Fe) has an effect on the crystal structure of MIL-88B(Fe). Therefore, XRD analysis of MIL-88B(Fe) and Cellulase@MIL-88B(Fe) was carried out in this paper, and the results are shown in Figure 3. As shown in Figure 3, the intensity and position of the characteristic diffraction peaks of the prepared MIL-88B(Fe) at 2θ of 9.2° (002), 11.0° (101), 17.1° (103) and 18.4° (202) can match well with the data reported previously for MIL-88B(Fe), indicating that the prepared MIL-88B(Fe) is well crystallized. In addition, no new diffraction peaks appeared in the XRD spectra of Cellulase@MIL-88B(Fe) compared to MIL-88B(Fe), indicating that the immobilization of cellulase on MIL-88B(Fe) did not have any effect on changing the physicochemical properties of MIL-88B(Fe).

3.2.3. TGA analysis of Cellulase@MIL-88B(Fe)
The thermal stability of MIL-88B(Fe) and Cellulase@MIL-88B(Fe) was examined by analysis of thermogravimetric (TGA) as well as to determine whether cellulase was immobilized on MIL-88B(Fe), and the results are shown in Figure 4. From the results, it can be seen that MIL-88B(Fe) and Cellulase@MIL-88B(Fe) have similar curves. When the temperature was higher than 300°C, MIL-88B(Fe) showed a significant mass loss, which was caused by the collapse of the MIL-88B(Fe) structure. Compared with MIL-88B(Fe), the TG curve of Cellulase@MIL-88B(Fe) has 2 distinct weight loss steps, the 1st mass loss (<100°C) is due to the evaporation of adsorbed and bound water; the 2nd mass loss (~300°C) is due to the collapse of the MOF backbone, which is consistent with the trend of MIL-88B(Fe). When the temperature reached 750°C, the mass loss of Cellulase@MIL-88B(Fe) was about 60.25%, which was higher than that of MIL-88B(Fe), indicating that the cellulase was successfully loaded on the carrier.

3.3. Enzymatic properties of Cellulase@MIL-88B(Fe)
3.3.1. Optimal temperature and pH of Cellulase@MIL-88B(Fe)
Temperature is one of the most important factors affecting the catalytic efficiency of enzymes. The reaction rate increases with the increase of reaction temperature, but too high a temperature can affect the natural conformational structure of the enzyme leading to denaturation and inactivation. Therefore, enzymes can only perform most efficiently at the optimum temperature. In general, the immobilized enzyme is more resistant to high temperature than the free form. In this paper, in order to investigate the optimum temperature of Cellulase@MIL-88B(Fe), the activity of Cellulase@MIL-88B(Fe) was measured at 30°C–70°C, respectively, and the results are shown in Figure 5a. The optimum temperature (60°C) of Cellulase@MIL-88B(Fe) was increased by 10°C compared to that of free cellulase (50°C), and its stability at high temperature was higher than that of free enzyme. The activity of Cellulase@MIL-88B(Fe) was 2.9% higher than that of the free enzyme at 70°C. The amino acid residues in the enzyme molecule react with the active site on the carrier to change the conformational structure of the enzyme molecule and enhance the ‘rigid’ structure of the enzyme molecule, which limits the natural conformational change of the enzyme molecule and reduces the sensitivity of the enzyme molecule to temperature, thus enhancing the temperature tolerance of the enzyme molecule. Imran et al. immobilized cellulase on sodium alginate-dry gel matrix and found that the immobilized cellulase

| Enzyme               | β-sheet (%) | Random coil (%) | α-helix (%) | β-turn (%) |
|----------------------|-------------|-----------------|-------------|------------|
| Free Cellulase       | 19.22       | 19.51           | 33.76       | 27.51      |
| Cellulase@MIL-88B(Fe)| 8.83        | 12.20           | 49.57       | 29.40      |
increased its thermal stability line to 82% after 26 h at 75°C compared to free cellulase (20).

In this paper, to investigate the optimum pH of Cellulase@MIL-88B(Fe), the relative activities of Cellulase@MIL-88B(Fe) at different pHs (3.0–8.0) were examined separately and compared with free cellulase. As seen in Figure 5b, the relative activities of both free cellulase and Cellulase@MIL-88B(Fe) reached their maximum at pH 5.0, indicating that the immobilization of cellulase did not change its pH optimum. Cellulase@MIL-88B(Fe) is relatively more stable at low or high pH compared to the free enzyme. The enzyme activity recovery of Cellulase@MIL-88B(Fe) was 71.21% at pH 8.0, while that of free cellulase was only 38.95%, indicating that cellulase was immobilized to effectively resist the pH change. The activity of Cellulase@MIL-88B(Fe) was higher than that of free cellulase at most pH values, which suggests that the pH tolerance of Cellulase@MIL-88B(Fe) is much higher than that of free cellulase, and the reason for this result may be that the interaction between the enzyme molecule and the carrier can stabilize the conformation of the enzyme active site. In addition, the high resistance of immobilized enzymes to pH changes may be related to their reduced mobility after immobilization. Yasin et al. immobilized cellulase on Carr@PAMAM@GA gels using a covalent immobilization method and found no change in the optimal pH (5.0) of immobilized cellulase (21).

3.3.2. Reusability and storage stability of Cellulase@MIL-88B(Fe)
One of the most important goals of immobilized enzymes is their reusability in industrial applications. In this paper, Cellulase@MIL-88B(Fe) was used to hydrolyze CMC 10 times continuously under the same conditions to assess its reusability. Figure 6 shows the changes of residual activity of Cellulase@MIL-88B(Fe) after 10 uses. As can be seen from the figure, the Cellulase@MIL-88B(Fe) residual activity gradually decreased with the increase in the number of cycles. After 5 and 10 consecutive applications, Cellulase@MIL-88B(Fe) retained high residual activity at 90.10% and 78.37% of the initial activity, respectively. This indicates that Cellulase@MIL-88B(Fe) exhibits good operational stability, which is mainly due to the fact that cellulase is covalently immobilized on MIL-88B(Fe), so that the conformation of the enzyme molecule is restricted and not easily affected by external environmental factors. The reason for the substantial decrease in residual activity of Cellulase@MIL-88B(Fe) after eight repeated uses could be the denaturation of the cellulase molecule and degradation of the active site due to multiple repeated uses as well as the detachment of the enzyme from the carrier. Sillu et al. reported that cellulase immobilized on magnetic nanotubes retained 68.2% of its initial activity after seven uses (22). Li et al. demonstrated that immobilization of cellulase with carbon nanotubes and sodium alginate retained about 70% of the activity after the 7th application (17).
One of the important parameters to assess the biological function of an enzyme is its storage stability. In this paper, to investigate the storage stability of Cellulase@MIL-88B(Fe), free cellulase and Cellulase@MIL-88B(Fe) were stored in sodium acetate buffer solution (pH 5.0), and their stability was investigated by measuring the residual activity curves of the two enzymes at different times, and the storage stability of cellulase before and after immobilization is shown in Figure 7. The storage stability of both free cellulase and Cellulase@MIL-88B(Fe) decreased with increasing storage days, but Cellulase@MIL-88B(Fe) was much more stable than its free form. After 49 d of storage, free cellulase and Cellulase@MIL-88B(Fe) retained 76.74% and 85.21% of the initial activity, respectively, indicating that cellulase can be immobilized to reduce its denaturation rate and thus enhance the storage stability of cellulase, and similar results have been reported in other studies. Ahmeda et al. reported the immobilization of cellulase on UiO-66-NH2, which retained about 65% of its initial activity after 30 d of storage, while free cellulase lost almost all of its activity (23). Han et al. chose Fe3O4@SiO2@p(NIPAM-co-GMA) to immobilize cellulase and found that free cellulase and immobilized enzyme retained 34.7% and 59.6% of activity, respectively, after 40 d of storage at 4°C (24). The main reason for this is that cellulase is immobilized, which increases the binding between the enzyme and the carrier, thus establishing a more stable three-dimensional conformation placing the enzyme molecule in distortion causing inactivation.

3.3.3. Organic tolerance of Cellulase@MIL-88B(Fe)

In this paper, the effect of organic solvents on Cellulase@MIL-88B(Fe) activity was investigated by placing free cellulase and Cellulase@MIL-88B(Fe) in organic solvents such as methanol, ethanol, isopropanol and tert-butanol for a period of time, and the results are shown in Figure 8. Cellulase@MIL-88B(Fe) was more stable than free cellulase in four organic solvents, Cellulase@MIL-88B(Fe) had the highest activity in isopropanol solution (69.91%), followed by methanol solution (65.80%), n-butanol solution (34.72%) and ethanol solution (29.61%), while the activity of free cellulase in isopropanol, methanol, n-butanol and ethanol was 32.98%, 9.68%, 48.57% and 24.41%, respectively. The results were similar to previous reports that demonstrated that the organic solvent tolerance of the enzyme was improved to a certain extent after immobilization, mainly because the enzyme was covalently immobilized on the carrier and its conformational structure was changed to enhance the structural rigidity of the enzyme, resulting in increased stability of the enzyme (25,26).

3.3.4. Mechanical and thermodynamic parameters of thermal inactivation of Cellulase@MIL-88B(Fe)

The thermal inactivation rate constant (k_d), half-life (t_{1/2}) and thermal inactivation energy (E_d) are parameters that reflect the kinetics of enzyme thermal inactivation. To evaluate the thermal stability of free cellulase and Cellulase@MIL-88B(Fe), in this paper, free cellulase and Cellulase@MIL-88B(Fe) were placed at 55, 60 and 65°C for 300 min, and their residual activities were measured every 60 min, and the results are shown in Figure 9a. The k_d and t_{1/2} values of free cellulase and Cellulase@MIL-88B(Fe) were measured, and the results are shown in Table 2. As can be seen from Table 2, the k_d of cellulase gradually increased with the increase of temperature, and the k_d of free cellulase was all much higher than that of Cellulase@MIL-88B(Fe), and the k_d of free cellulase gradually increased with the increase of temperature.
cellulase was 1.23–2.36 times higher than that of Cellulase@MIL-88B(Fe) in the temperature range of 55°C–65°C, indicating that Cellulase@MIL-88B(Fe) has a lower inactivation rate and better inactivation resistance. This result was confirmed by the $E_d$ of free cellulase and Cellulase@MIL-88B(Fe) calculated by the Arrhenius equation (Figure 9b and Table 2), with an $E_d$ of 79.25 ± 2.46 kJ/mol for Cellulase@MIL-88B(Fe) much higher than that of free cellulase (29.70 ± 1.93 kJ/mol). The $t_{1/2}$ of the enzyme is the time for the enzyme activity to decrease to half of the initial activity under continuous assay conditions is an important parameter for assessing changes in protein structure and enzyme stability at high temperatures. As seen in Table 2, the $t_{1/2}$ of Cellulase@MIL-88B(Fe) was 1.31–3.23 times higher than that of free cellulase over the entire temperature range (55°C–65°C), indicating that cellulase loaded on MIL-88B(Fe) had higher stability. This may be due to the covalent binding of cellulase in MIL-88B(Fe), which increases the rigidity of the enzyme molecular structure.

From Table 3, we know that the $\Delta H^0$ of cellulase decreases with increasing temperature, which indicates that only less energy is needed to inactivate cellulase at high temperatures. Interestingly, the $\Delta H^0$ of Cellulase@MIL-88B(Fe) was much higher than that of free cellulase at the same temperature, indicating that Cellulase@MIL-88B(Fe) requires more energy to be inactivated compared to free cellulase. The $\Delta G^0$ variation of the thermal denaturation of free cellulase and Cellulase@MIL-88B(Fe) showed that the $\Delta G^0$ value of MIL-88B(Fe)-immobilized cellulase was higher, which further indicated that the immobilized enzyme was more resistant to inactivation than free cellulase. All these data can show that Cellulase@MIL-88B(Fe) can exhibit better thermal stability than free cellulase.

### 3.3.5. Kinetic study of Cellulase@MIL-88B(Fe)

To better investigate the binding efficiency and catalytic efficiency of free cellulase and Cellulase@MIL-88B(Fe) with the substrate, the kinetic parameters ($K_m$ and $V_{max}$) were determined.
V_{max}) of free cellulase and Cellulase@MIL-88B(Fe) were investigated in this paper, and the results are shown in Table 4. The value of $K_m$ represents the interaction between cellulase and substrate, and a lower $K_m$ indicates a high affinity of cellulase to the substrate, resulting in a higher cellulase activity. As shown in Table 4, the $K_m$ (1.46 mg/mL) value of Cellulase@MIL-88B(Fe) was slightly higher than that of free cellulase (1.41 mg/mL), while the $V_{max}$ (0.057 U/mg) value of immobilized enzyme was lower than that of free enzyme (0.062 U/mg). The analysis may be due to a slight change in the three-dimensional conformation of cellulase during immobilization, which increases the rigidity of the enzyme molecule. Secondly, the spatial site block of Cellulase@MIL-88B(Fe) inhibits the diffusion of substrate to the enzyme active site, which also leads to an increased resistance between immobilized cellulase and substrate. Other researchers have previously reported an increase in cellulase $K_m$ and a decrease in $V_{max}$ after immobilization (12).

**3.3.6. Hydrolysis of CMC and corn cob in Cellulase@MIL-88B(Fe)**

As seen from the continuous hydrolysis (72 h) of CMC as a substrate in Figure 10a, the yield of reducing sugars generated from the hydrolysis of CMC by free cellulase and Cellulase@MIL-88B(Fe) increased with time. After 48 h, the rate of CMC hydrolysis by free cellulase started to decrease because the free enzyme was susceptible to inhibition by other factors such as substrate, product, temperature, and pH in the solution, which decreased its activity. In contrast, Cellulase@MIL-88B(Fe) is relatively resistant to the external environment, so it is less affected. At 72 h, the degree of hydrolysis of CMC was 18.18%, which was 1.22% higher than that of free cellulase. This study further evaluated the potential of Cellulase@MIL-88B(Fe) for the conversion of lignocellulosic biomass to reducing sugars. Agricultural waste (corn cob) was selected as a substrate and hydrolyzed using Cellulase@MIL-88B(Fe) under the same conditions as hydrolyzed CMC. As seen in Figure 10b, the conversion of the substrate from CMC to corn cob did not affect the ability of Cellulase@MIL-88B(Fe) to biocatalyze although there was a decrease in reducing sugar yield. At 72 h, the reducing sugar yield of Cellulase@MIL-88B(Fe) hydrolyzed corn cobs could reach 15.85%, which was 2.27% higher than that of free cellulase. The low saccharification rate of free cellulase is influenced by the incomplete removal of other components such as lignin and pectin from the corn cob, in addition to the reasons mentioned above. These results all indicate that hydrolysis using Cellulase@MIL-88B(Fe) can minimize the effect of reaction conditions induced cellulase inactivation and thus improve the hydrolysis efficiency.

**4. Conclusion**

In this study, 1.2 mg/mL cellulase was dissolved in pH 5.0 buffer and reacted at 30°C for 6 h. The Cellulase@MIL-88B(Fe) had the highest activity. The cellulase was successfully immobilized in MIL-88B by FT-IR, XRD and TGA analysis. The cellulase was successfully immobilized on MIL-88B(Fe) without affecting the complete lattice structure of MIL-88B(Fe). For The enzymatic properties of Cellulase@MIL-88B(Fe) were investigated, and it was found that, compared with free cellulase, Cellulase@MIL-88B(Fe) had no effect on the lattice structure. The optimum temperature of Cellulase@MIL-88B(Fe) was increased by 10°C, and the optimum pH was not changed. pH and temperature range, and exhibited better organic solvent tolerance, reusability and storage stability. In addition, Cellulase@MIL-88B(Fe) is
promising for the hydrolysis of cellulose-based biomass. The application of Cellulase@MIL-88B(Fe) in hydrolysis of cellulotic biomass is promising.

Data availability
Data will be made available on request.

Author contributions
All authors contributed equally in the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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