Proceedings of the Pakistan Academy of Sciences: A Physical and Computational Sciences 61(3): 311-316 (2024)
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ISSN (Print): 2518-4245; ISSN (Online): 2518-4253
http://doi.org/10.53560/PPASA(61-3)680

Pakistan Academy of Sciences

Research Article

Kinetics and Thermodynamic Study of Cellulase Embedded Metal Organic Frameworks

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Abstract: Cellulase is an important enzyme used for many purposes in different industrial sectors. Even though cellulase has so many applications, it easily denatures with a little change in pH and temperature, which causes low stability, usability, and activity. To enhance its activity and stability, immobilization of porous materials is the best way to enhance its activity, stability, and life span. For immobilization, a Metal-organic framework (MOF) is considered as a potential candidate. Cellulase@Zn- benzene 1-4 di-carboxylic acid (BDC) by hydrothermal method and Zn-cellulase-benzene 1-4 dicarboxylic acid (BDC) by *de novo* approach were prepared, and their activities were analyzed. Zn-cellulase-benzene 1-4 dicarboxylic acid (BDC) produced by the *de novo* approach, shows higher activity, stability, catalytic performance, and life span than the free enzyme, and cellulase@Zn- benzene 1-4 di-carboxylic acid (BDC) produced by the hydrothermal method.

Keywords: Cellulase, Immobilization, Metal-organic Framework, Kinetics, De-novo Synthesis.

1. INTRODUCTION

In many biotechnology processes, enzymes are used as highly effective catalysts. Cellulase, one of these enzymes, is essential for biotechnology applications. Cellulases are produced by a wide variety of organisms, including bacteria, fungi, protozoans, and some animals. Cellulase enzymes have shown potential in various industries, such as the production of biofuels, pulp, paper manufacturing, food processing, textiles, detergents, and food. Cellulase is commonly used to break down 1, 4 glycosidic bonds in cellulose crystals to create glucose [1]. This hydrolysis breaks down the cellulose into smaller oligosaccharides and ultimately glucose, which can be used as a source of energy by the organism. Despite the potential of cellulase enzymes in cellulose hydrolysis, their practical application is limited by their low stability, reusability, and activity [2, 3]. Enzyme immobilization is a potent and straightforward strategy for addressing several of difficulties connected with utilizing enzymes as industrial catalysts, such as enzyme inhibition, selectivity, stability, and recyclability, by chemicals in the reaction media [2, 4]. Enzymes can be more reliably preserved during storage and working settings if they are immobilized so that they can be easily isolated from the reaction medium. Immobilization facilitates the separation of enzymes from their reaction media and increases their stability during storage and operation [5].

Nanomaterials have gained significant interest as potential substrates for protein immobilization in recent times, owing to their exceptional features, including remarkably favorable mass transfer resistance, high surface area, and easy diffusibility [6]. Metal-organic frameworks (MOFs) stand out among other Nano-materials due to their astounding surface-to-volume ratio and structural variety. Since MOFs have inherent structural,

Received: September 2023; Revised: August 2024; Accepted: September 2024
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catalytic, electrical, optical, and magnetic properties that make them suitable for use in a variety of applications, including biological processes, industrial catalysis, and sensing, their potential has been expanded beyond catalysis using MOFs as immobilization platforms for enzymes. Due to their simplicity in functionalization, availability of appropriate hydrophilic/hydrophobic groups, and robust electrostatic interactions with proteins, MOF-enzyme platforms offer a variety of benefits, including high enzyme-substrate ratios, water stability, and reusability [7].

MOF is a particular kind of porous crystal that is composed of a network of metal atoms and a network of organic ligands. Since MOFs may be designed with a wide range of cavities, porosity, topology, surface area, and pore volume characteristics, they have the potential to be used in many different fields. The unique structural properties of MOFs enable them to be tailored for specific applications, making them highly versatile and attractive materials for various fields of research [8]. In addition, MOFs have become widely used as a substrate for immobilizing enzymes [9]. Cellulase-embedded MOFs are a promising avenue for the efficient conversion of cellulose into biofuels and other value-added products [10]. The *de novo* approach for the creation of cellulase-embedded MOF without the need of pre-existing structures guarantees the repeatability and comprehension of the experimental setup with specific reaction conditions. In contrast, the hydrothermal approach suggests using high temperatures and high pressures to synthesize the MOF embedded with cellulase [11].

In the present study both the hydrothermal process and the *de novo* methods are used to construct a cellulase-embedded MOF. This study aims to evaluate the catalytic efficacy, stability, and activity of the cellulase-embedded MOF made using these techniques with that of a conventional cellulase. A thorough comparison between the cellulase-embedded MOFs made by the hydrothermal method and the *de novo* approach has been carried out.

2. MATERIALS AND METHODS

Cellulase (*Aspergillus niger*) (C1184), zinc chloride (7646-85-7) and benzene 1-4 dicarboxylic acid (4612-26-4) was purchased from Sigma Aldrich, H_2O_2, aqueous ammonia, and rice husk were purchased from local market. Rice husk was dried for one day and then ground to fine powder.

2.1. Preparation Cellulase@Zn- benzene 1-4 di-carboxylic acid (BDC) MOF by Hydrothermal Method

2.1.1. Zinc-benzene 1-4 dicarboxylic acid (BDC) preparation

Zinc chloride and benzene 1-4 di-carboxylic acid were prepared separately in distilled water. 1.65 g of benzene 1-4 di-carboxylic acid (BDC) and 1.36 g of zinc chloride were separately dissolved in 100 ml of distilled water. 30 ml of zinc chloride solution was added dropwise to BDC solution with constant stirring for one hour. Mixture was transferred to a hydrothermal reactor and kept in an oven at 110°C overnight, solution's volume was reduced by heating. After reduction of volume, it was transferred into a china dish and dried to remove any moisture. Dried powdered of Zn-BDC MOF was obtained [12].

2.1.2. Preparation of Cellulase@Zn-benzene 1-4 di-carboxylic acid (BDC)

1 g of cellulase was dissolved in 100 ml of distilled water with constant stirring for 5-6 hours to dissolve the cellulase. Zn- BDC MOF was mixed with cellulase with constant stirring for 5–6 hours. Enzyme was properly embedded on the MOF. It was then filtered and dried in a desiccator for a few days.

2.1.3. Preparation of Zn-cellulase-BDC MOF by de novo method

In the *de novo* approach, preparation of MOF and embedding of enzyme was done simultaneously. 1.36 g of zinc chloride, 1.65 g of BDC, and 1 g of cellulase were dissolved separately each in 100 ml of water. Then 30 ml of cellulase solution was mixed with 30 ml of BDC solution. The 30 ml of zinc chloride was added dropwise into the above-mentioned solution with continuous stirring. After that, it was kept for 24 hours at room temperature. It was then centrifuged at 4000 rpm for 10–15 minutes, washed it 3 times with distilled water, and finally left it to dry for a few days [13].

2.2. Characterization

2.2.1. Fourier transforms infrared spectroscopy (FTIR)

FTIR JASCO 4100 was used to record the FTIR spectrum within the range of 4000cm to 400 cm.

2.2.2. Scanning electron microscopy (SEM)

Using SEM technique, size, and morphology of enzyme embedded MOF was examined.

2.3. Measurement of Cellulase Activity

2.3.1. Pre-treatment with H_2O_2 and aqueous ammonia

Initially, three beakers were taken; in each beaker, 2 g of rice husk was introduced, and then 3% of H_2O_2 was added in each beaker. A hot plate stirrer was then taken, and each sample was heated at 85°C for 5 to 6 hours at a speed of 150 rpm. Next, the slurry was filtered with a vacuum filter and washed with distilled water. Then, each sample was treated with 20% ammonia for 5 hours at 100°C on a hot plate stirrer. After that, each solution was washed with distilled water and filtered with the help of a vacuum filter.

2.4. Enzymatic Hydrolysis

2.4.1. Rice husk hydrolysis with standard cellulase

Standard cellulase, Zn-cellulase-BDC MOF and Cellulase@Zn BDC MOF were taken in three different Erlenmeyer flasks. Pre-treated rice husk was introduced in each flask. Enzyme substrate ratio was 1:10. Each flask was placed on a hot plate and stirred at 50 °C at 200 rpm for 6 hours for complete hydrolysis. Each flask was cooled and solution was filtered through a vacuum filter. During the stirring, the sample was aliquoted after every 15 minutes for two hours, and the reactivity was checked by UV spectrophotometry. The absorbance of the treated sample was measured with a UV spectrophotometer at 270 nm. The blank for the experiment was the pre-treated rice husk without enzyme treatment [14].

2.5. Standard Curve of Glucose

For the preparation of the stock solution, 200 mg of glucose was dissolved in 20 ml of distilled water, and dilutions were prepared ranging from 0.5 mg/ml to 4.5 mg/ml. The absorbance was measured on a UV spectrometer at 270 nm.

3. RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. SEM analysis

Figure 1 shows that SEM images of Zn-cellulase-BDC MOF and Cellulase@Zn BDC MOF are similar. Both have thin layers with broken and intertwined edges. Particles are slightly spherical and have uniform growth in all directions.

3.1.2. FTIR analysis

An FTIR chromatogram (shown in Figure 2) proved that both cellulase embedded MOF similar. It confirmed the functional group of the bond generated during the enzyme embedded MOF synthesis procedure. Broad absorption bands

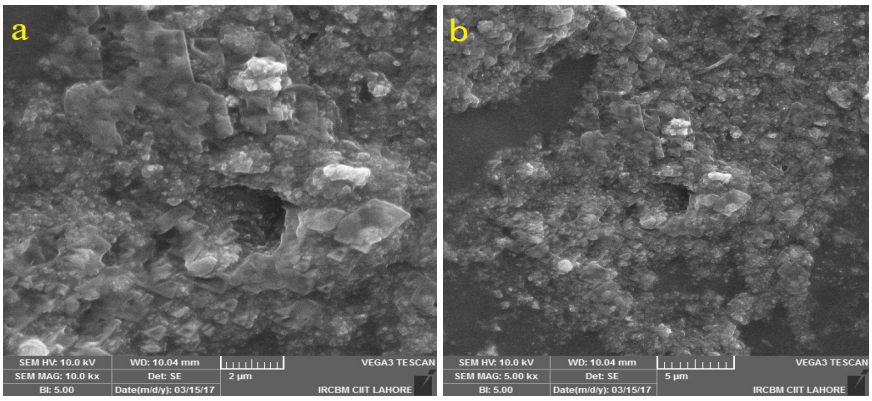

Fig. 1. SEM Images of enzyme embedded MOF, (a) Zn-cellulase-BDC MOF and (b) Cellulase@Zn BDC.

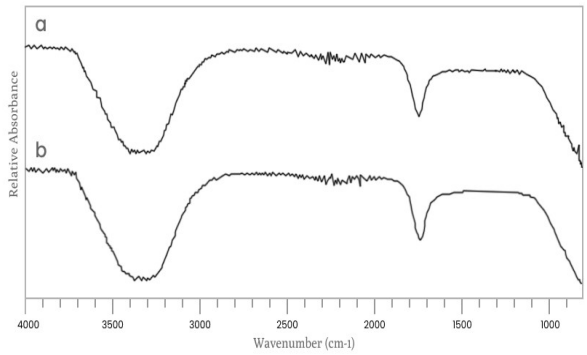

Fig. 2. FTIR of cellulase embedded MOFs, (a) Zn-cellulase-BDC MOF and (b) Cellulase@Zn BDC.

seen at approximately 3000–3500cm^{-1} generally attributed to water molecules' stretching vibrations. Strong peaks were seen in MOFs, which are associated with the C=O stretching mode at around 1710 cm^{-1}.

3.2. Absorbance Measured on UV Spectrophotometer After Enzymatic Hydrolysis

Activity of cellulase@Zn BDC MOF and Zn-cellulase-BDC MOF was measured and compared with the activity of standard cellulase. Graph was plotted against absorbance and reaction time. It has been observed that in all three experiments, enzymes active reached equilibrium after 60 min. Standard cellulase showed maximum absorbance at 0.26 nm in Figure 3. cellulase@Zn-BDC showed maximum absorbance at 0.34 nm (Figure 4), and Zn-cellulase-BDC showed maximum absorbance at 0.56 nm (Figure 5).

3.3. Amount of Glucose Released After Enzymatic Hydrolysis

Rice husk was used as substrate to check the activity of enzyme embedded on MOF. Rice husk was treated with both MOF based cellulases and results were compared with the standard cellulase. Activity of each enzyme was measured by calculating the amount of glucose released from the rice husk after treating with cellulase. Standard curve of glucose was established for calculating the amount of glucose as shown in Figure 6. Absorbance measures at 270 nm and was converted into glucose concentrations using the standard curve and graphed versus time. Standard cellulase released 0.6 mg of glucose, Cellulase@Zn-BDC MOF released 0.8 mg, and Zn-cellulase-BDC MOF released 1.2 mg of

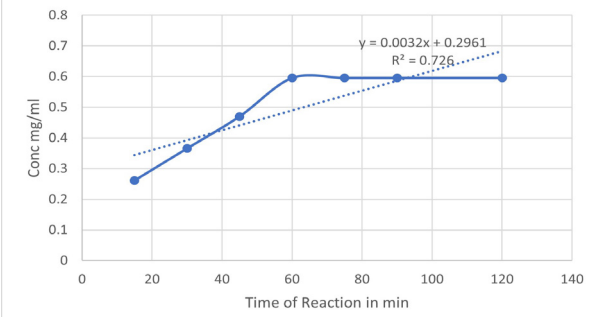

Fig. 3. Absorbance of standard cellulase with reaction time.

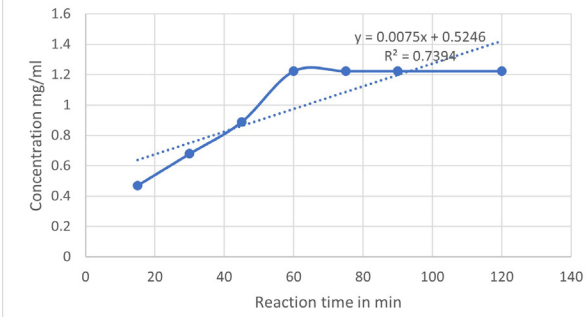

Fig. 5. Maximum absorbance of Zn-cellulase-BDC at 0.56 nm.

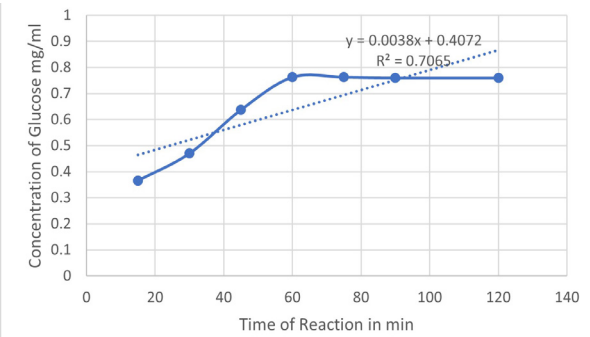

Fig. 4. Maximum absorbance of cellulase@Zn-BDC at 0.34 nm.

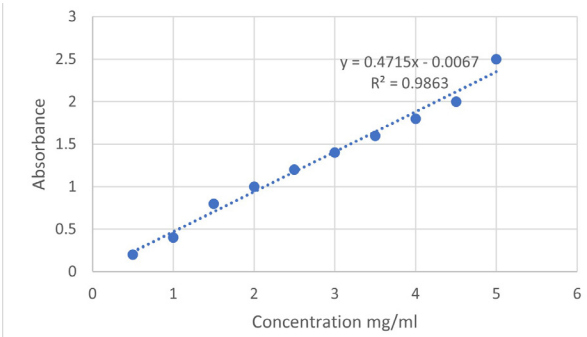

Fig. 6. Standard curve plotted by measuring the concentration of glucose at 270 nm.

glucose after enzymatic hydrolysis. The rate of the reaction is also measured in Figure 7. It indicated that Zn-cellulase-BDC *(de novo*) has high rate of reaction than standard cellulase and Cellulase@ Zn-BDC (hydrothermal). This represents, that Zn-cellulase-BDC which is synthesized by the *de novo* method, showed better activity than standard and Cellulase@Zn-BDC.

3.4. Calculated Reaction Velocity

Kinetic study of both the cellulase embedded MOF has been carried out and compared with the standard. From Figure 8 it has been observed that the kinetic activity of Zn-cellulase-BDC MOF (*de novo*) was much higher as compared to Celllase@ Zn-BDC MOF and standard cellulase. The kinetic study showed that activity of enzymes became stable over time as more product and less substrate was present in the reaction and stable after 60 minutes.

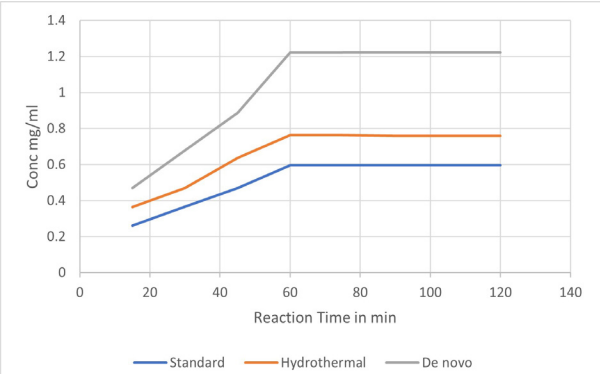

Fig. 7. Amount of glucose released by enzymatic hydrolysis of rice husk was calculated from the standard curve and plotted against time to calculate the rate of reaction.

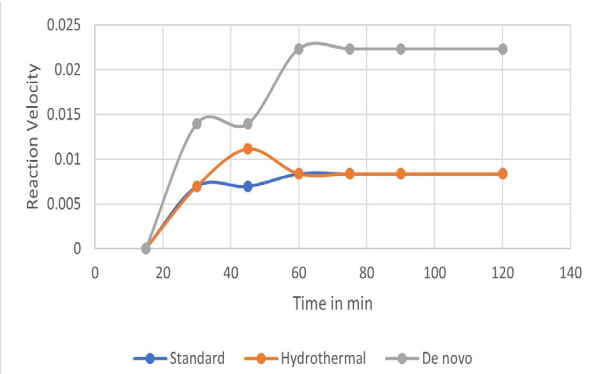

Fig. 8. Graph of reaction velocity for quantity of glucose produced vs time.

4. DISCUSSION

The essential and enhanced spatial arrangement of the enzyme within MOFs accounts for the high activity and stability of the MOF to meet the needs of industrial applications and to get a deeper understanding of the catalytic behaviour [15, 16]. To increase catalytic performance and decrease resistance to reagent mass transfer, enzymes are embedded in the MOF and the synthesis process is carefully regulated [17, 18]. When Zn-Cellulase-BDC MOF was put in comparison with Cellulase@ Zn-BDC MOF and ordinary cellulase, the reaction velocity was consistently higher. The robustness and stability of the Zn-Cellulase-BDC MOF are indicated by its long-term high activity and the stabilization of the response velocity. The distinct characteristics of the MOF structure are responsible for the Zn-Cellulase-BDC MOF's reported improved performance. Catalytic efficiency is predicted to rise because of the better and crucial spatial arrangement of the enzyme within the MOF, which improves substrate accessibility to the active sites [19, 20]. In addition, Zn-Cellulase-BDC MOF's catalytic ability appears to be much enhanced by the *de novo* synthesis technique used in its creation [21]. By using a controlled synthesis method, it is possible to minimize resistance to reagent mass transfer, maximize catalytic performance, and guarantee accurate enzyme embedding on the MOF.

5. CONCLUSIONS

In this study, cellulase was immobilize on MOF by two different methods, *de novo* and hydrothermal method. The kinetic activity of the enzymatic hydrolysis of rice husk after pre-treatment with H_2O_2 and aqueous ammonia were studied. It has been observed that enzyme embedded on MOF show increased activity and high rate of reaction. Kinetic study also proved that *de novo* method is best for the synthesis of enzyme embedded MOF.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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