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Facile synthesis of glucoamylase embedded metal-organic frameworks (glucoamylase-MOF) with enhanced stability

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ABSTRACT

The self-assembled glucoamylase metal-organic framework (glucoamylase-MOF) was synthesized by facile one-step method within 20 min by simply mixing aqueous solution of 2-methylimidazole (160 mM), glucoamylase (5 mg/mL) and zinc acetate (40 mM) at room temperature (28 ± 2 °C). The prepared glucoamylase-MOF was characterized by using FT-IR, confocal scanning laser microscopy, XRD and SEM. The robustness and thermal stability of glucoamylase embedded MOF was evaluated in terms of half-life (in the range of 60–80 °C) which showed 6 folds increment as against free form. Further, in Michaelis–Menten kinetics studies, glucoamylase entrapped MOF exhibited higher K_m value and lower V_{max} value as compared to native enzyme. Moreover, the immobilized glucoamylase exhibited up to 57% of residual activity after six consecutive cycles of reuse, whereas it retained 91% of residual activity till 25 days of storage. Finally, the conformational changes occurred after the encapsulation of glucoamylase in the interior of MOF, which was analyzed by using FT-IR data analysis tools.

1. Introduction

From many decades, enzymes are extensively practised catalyst in sectors as diverse as chemical, pharmaceutical and food due to their unique properties such as, highly selective, highly efficient, biodegradable, sustainable and greener in nature [1–3]. Apart from all these desirable features, their industrial applications are often handicapped due to insufficient stability in operational conditions, difficulties in recovery from reaction mixture and reusability for multiple cycles [4,5]. These hurdles can be prevailed by the enzyme immobilization tools. The immobilization of enzyme greatly enhances the properties of enzymes in terms of thermal stability, tolerance to extremely high pH and organic solvents, selectivity and activity to meet the demands of practical uses. It also facilitates the efficient recovery and re-use of the enzyme, thus enabling its cost-effective use and thereby making their industrial applications economically feasible [6,7].

Porous structured nanomaterials have been adapted as a promising alternative carrier for enzyme immobilization by using a broad variety of physical and chemical methods [8,9]. Among available porous nanomaterials, mesoporous silicate is generally employed as carrier for enzyme immobilization due to large surface area, tailored pore-size distribution, controllable pore geometry and versatile composition, their thermal and mechanical stability and toxicological safety [10,11]. Nevertheless, the lack of specific interaction between enzyme molecules and mesoporous silica material resulted into leaching during reaction process which has significantly affected the recovery and reduced the reusability [12]. Additionally, synthesis of hierarchical porous mesoporous silica materials is complicated in many aspects [13]. Moreover, catalytic performance is affected probably due to deactivation of enzymes and diffusion restrictions [14,15]. This two steps immobilization process limits their applications in catalysis due to aforementioned difficulties, hence there is need of searching host matrix material to immobilize enzymes with the improved catalytic properties.

Recently, metal-organic frameworks (MOF) has emerged as potential hybrid organic-inorganic crystalline material in the vast field of catalysis, separation, drug/enzyme carriers, energy conversion/storage for their characteristics such as large pore volumes, tunable pore sizes with well-defined cavities, high surface areas, crystallographic structure and designable organic ligands [16,17]. Hence, enzyme fabricated MOF has recently intrigued extensive attention due to large hierarchical surface area with remarkable porosity for high loading capacity and strong affinity for enzymes to prevent leaching [18,19]. Lykourinou et al. have successfully immobilized the microperoxidase-11 into a mesoporous MOF which showed superior catalytic activity together with recyclability as compared to mesoporous silica material MCM-41 [20]. Lyu et al.

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have prepared Cyt c @ZIF-8 (zeolitic imidazolate frameworks) crystals by simply mixing Cyt c, zinc nitrate and 2-methylimidazole in methanol in the presence of polyvinylpyrrolidone (PVP), to maintain dispersion and stabilization of the protein in methanol. Further, similar method was employed to immobilize enzymes like horseradish peroxidase (HRP) and lipase into MOF supports like ZIF-8 and ZIF-10 [21]. Chia-Kuang Tsung et al. have used the same one pot synthesis strategy to immobilize catalase enzyme into the ZIF-90 MOF crystals [22]. More recently, the multi-enzymes (glucose oxidase and HRP)/ZIF-8 composite was synthesized by using water as a solvent to avoid the rigid structures of MOF and to preserve activities of embedded enzymes [23].

Glucoamylase, a macromolecular substrate acting enzyme, which is usually employed in the food and beverage industries for saccharification of oligosaccharides and starch at higher temperature [24]. Hence, to enhance robustness, thermal stability and extended shelf-life time for industrial applications, in this work, we have synthesized glucoamylase embedded MOF by rapid, low cost one-pot biomimetic mineralization (in the absence of capping agents) strategies to encapsulate the enzyme within a protective shields in aqueous solution at ambient conditions. The frame work around the enzymes not only stabilizes the catalytic conformation of enzyme to improve catalytic performance but also makes them mechanically robust and thermally stable. Also, hierarchical porosity and structures provide large surface area to improve the enzyme encapsulation yield of MOF [25]. Further, the prepared glucoamylase-MOF was characterized by powdered X-ray diffraction (XRD), Fourier transform infrared (FT-IR) and confocal scanning laser microscopy. The size and morphology of glucoamylase capped MOF was analyzed by scanning electron microscopy (SEM). Also, the kinetic parameters (V_{max} and K_m) and thermal stability of free glucoamylase and glucoamylase-MOF were determined in terms of thermal deactivation constant (k_d), half-life ($t_{1/2}$) and deactivation energy (E_d). In addition, conformational changes occurring after immobilization were estimated by FT-IR data analysis tools. Lastly, reusability and storage stability of enzymes embedded MOF were studied to check its durability and industrial feasibility.

2. Materials and methods

2.1. Materials

Glucoamylase (138 U activity per mg) was a gift from Riddhi Siddhi GlocoBiols Ltd. Gokak, India. Maltodextrin, 2-methylimidazole and zinc acetate were purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, India. Glucose oxidase-peroxidase (GOD-POD) kit was purchased from Accurex Biomedical Pvt. Ltd. Mumbai, India. All other chemicals were of AR grade and procured from reliable sources and used without any further purification.

2.2. Glucoamylase activity assay

Glucoamylase activity was evaluated on the basis of maltodextrin hydrolysis and amount of glucose released after reaction was estimated by glucose oxidase-peroxidase (GOD-POD) method [26,27]. Briefly, maltodextrin (10 mg/mL in 0.1 M sodium phosphate buffer, pH 7.0) was mixed with glucoamylase and incubated for 15 min at 50 °C. The reaction was terminated by 5% tricarboxylic acid. Above mixture (25 μL) was added to GOD-POD working solution (2.5 mL) and incubated in dark for 30 min at room temperature (~28 ± 2 °C). The absorbance was measured at 505 nm by using UV–vis spectrophotometer (Hitachi U2001, Japan) to quantify the amount of glucose released. Glucoamylase activity as a unit (U) is defined as the amount of enzyme required to release one μmol of glucose per minute at optimized condition.

Protein content was estimated by Bradford protein assay using bovine serum albumin as the standard [28].

2.3. Synthesis of the glucoamylase-MOF

The enzyme embedded MOF was prepared by the method reported by Liang et al. [25]. Experimentally, glucoamylase (150 mg, 5 mg/mL considering total reaction mixture) was dissolved in an aqueous solution of zinc acetate (160 mM, 20 mL). The separate solution of 2-methylimidazole (40 mM, 10 mL) was prepared and mixed together with enzyme solution under stirring at room temperature (28 ± 2 °C). The mixture then turned milky almost immediately after mixing. After stirring for about 20 min, the white precipitation was collected by centrifuging at 7000 rpm for 8 min, and washed with phosphate buffer for five times. The product was re-dispersed in phosphate buffer for lyophilization and used for further experiments.

2.4. Synthesis of the pure MOF (ZIF-8) crystals

The synthesis of pure MOF crystals was done by the same procedure as that of glucoamylase-MOF but in the absence of enzyme solution. An aqueous solution of zinc acetate (40 mM, 10 mL) was added into 2-methylimidazole solution (160 mM, 20 mL) under stirring at room temperature (28 ± 2 °C). After stirring for about 20 min, the product was collected by centrifuging at 7000 rpm for 8 min, and washed with DI water for five times. Further, the product was dried for overnight and used for characterization.

2.5. Characterization of prepared glucoamylase-MOF

The prepared glucoamylase-MOF was confirmed by Fourier transform infrared (FT-IR) spectroscopy using Shimadzu IRAffinity-1 FT-IR spectrophotometer from 4000 to 400 cm^{-1} with sample dispersed in the KBr pellets. Powder x-ray diffraction (Philips PW 1830 X-ray Diffraction) was used to investigate crystal structures of the glucoamylase entrapped MOF. The morphology of glucoamylase-MOF was determined by scanning electron microscope (JEOL – JSM-6380) under 15.0 kV. Samples for SEM measurements were prepared by first suspending the composites in methanol and then 100 microliters of the sample solution was dropped onto a silica wafer. After complete evaporation of methanol, the silica wafer was sputter-coated with a thin layer of conductive platinum to improve the electrical conductivity. Confocal scanning laser microscopy was used to investigate the presence of fluorescein isothiocyanate (FITC) tagged glucoamylase within the ZIF-8 MOF. The tagging of glucoamylase (5 mg/mL in 10 mM, pH 9.5 carbonate buffer) was done by of FITC (8 mg, dissolved in DMSO). The solution was shaken for 4 h at 250 rpm at ambient temperature in dark. Free FITC was removed via dialysis against DI water and subsequent used for synthesis of fluorescently labelled glucoamylase-MOF composite. Laser scanning confocal microscope images were taken on Leica, Germany (DMi8 microscope and SP8 scanner).

2.6. Thermal deactivation kinetics studies

Free glucoamylase and MOF of glucoamylase were incubated at 60, 70 and 80 °C in sodium phosphate buffer (pH 7.0, 0.1 M). The samples were withdrawn after every 10 min of interval till 60 min, chilled quickly and then assayed for residual activity as mentioned in glucoamylase activity assay section. A semi-log plot of residual activity vs. time was plotted from which the inactivation rate constant (k_d) was calculated as the slope, and half life ($t_{1/2}$), the time