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Encapsulation of lipase within metal-organic framework (MOF) with enhanced activity intensified under ultrasound

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ABSTRACT

The enzyme under lower-intensity ultrasonic irradiation leads to favorable conformational changes, thereby enhancing its activity. In this study, lipase activity was augmented upto 1.6-folds after ultrasonic treatment at 22 kHz and 11.38 W cm^{-2} for 25 min. This highly activated lipase was encapsulated within zeolite imidazolate framework-8 (ZIF-8) as a metal-organic framework (MOF) material *via* facile one-step biomineralization method by simply mixing aqueous solution of 2-methylimidazole (13.3 mmol) and zinc acetate (1.33 mmol) along with sonicated lipase within 10 min at room temperature (28 ± 2 °C). The prepared lipase-MOF was characterized by using FT-IR, FT-Raman, XRD, BET, confocal scanning laser microscopy, TGA and SEM. Further, the thermal stability of lipase embedded MOF was evaluated in the range of 55–75 °C on the basis of half-life which showed 3.2 folds increment as against free lipase. In Michaelis–Menten kinetics studies, sonicated lipase entrapped MOF showed nearly same K_m and V_{max} values as that of sonicated free lipase. Moreover, the immobilized lipase exhibited up to 54% of residual activity after seven successive cycles of reuse, whereas it retained 90% of residual activity till twenty-five days of storage. Finally, the conformational changes occurred in lipase after sonication treatment and encapsulation within MOF were analyzed by using FT-IR data analysis tools and fluorescent spectroscopy.

1. Introduction

Nowadays, enzymes are reaching high levels of implementation as a green catalyst in various fields as diverse as food processing, energy production, fine and pharmaceutical chemistry. However, low stability (thermal and narrow pH range), difficulty in recovery and reusability of enzyme hampers their implementation in industries. Enzyme immobilization is a requisite for their use as industrial purpose in most of these instances, since it permits possible increase in stability, convenience in handling, ease of separation from the reaction mixture and reuse [1–4]. Thus, many efforts have been put to develop a different immobilization techniques to greatly improve the performance of enzyme [5,6].

Recent advancement in nanotechnology has provided a wealth of diverse nano-scaffolds that could be considered as potential support for enzyme immobilization [7,8]. Porous structured nanomaterials have been adapted as a promising alternative carrier for enzyme immobilization by using a broad variety of physical and chemical methods [9,10]. Among various types of host porous nanostructured materials, mesoporous silica has widely been investigated due to its versatile composition, large surface area, tailored pore-size distribution and controllable pore geometry. However, they often suffer from leaching of the immobilized enzyme during the reaction process due to the lack of specific interaction between enzyme molecules and silica material, which results in a loss of activity upon reuse [11]. Additionally, hierarchical porous mesoporous silica material synthesis is complicated in many aspects [12]. Moreover, catalytic performance is affected probably due to enzyme deactivation and diffusion restrictions during immobilization procedure [13,14].

In recent years, a new interesting class of hybrid porous material called metal-organic framework (MOF) has generated a lot of research interests. It encompasses metal ions and organic ligands linked together by strong coordination bonds. This has gained considerable attention due to its superior properties such as high surface area, giant porosity, easy tenability of pore size and modifiable surfaces [15–17]. Hence, the use of MOFs as immobilization platform for enzymes has recently intrigued extensive attention. It has large hierarchical surface area with remarkable porosity for high loading capacity along with strong affinity which prevents enzyme leaching. Enzyme–MOF biocomposite is usually synthesized by adsorption, covalent binding or cage inclusion [18,19]. For the first time, microperoxidase-11 has been successfully immobilized within a mesoporous MOF (Tb-mesoMOF) which

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demonstrates superior enzymatic catalysis performance as compared to its mesoporous silica material MCM-41 [20]. A significant breakthrough in synthesis of protein-embedded MOF was achieved by Lyu et al. In his work, cytochrome c (Cyt c) was directly embedded in zeolitic imidazolate framework (ZIF)-8 *via* co-precipitation method by mixing methanol solution of enzyme, zinc nitrate and 2-methylimidazole in the presence of polyvinylpyrrolidone (PVP), which stabilizes protein in methanol and maintains dispersion [21]. Further, similar method was employed to immobilize enzymes such as catalase, horseradish peroxidase (HRP) and lipase into different MOF supports like ZIF-90, ZIF-8 and ZIF-10 [22–24]. More recently, glucoamylase was successfully immobilized within ZIF-8 by using water as a solvent to avoid denaturation of enzyme during MOF preparation and the resultant enzyme-encapsulated MOF showed high thermal as well as operational stability and long-term storage stability [25].

In most of immobilization techniques, the activity of immobilized enzymes is usually lower than that of native form which is mainly due to the unfavorable conformational transition of enzyme after immobilization procedure [26]. Recently, low frequency ultrasound was employed for activation of enzymes to improve the catalytical activities by altering its 3D structure perturbing loop and domain regions of an enzyme and thus enhancement in activity. However, there are reports indicating gradual decrease of activity of sonicated enzyme when kept at room temperature for nearly 50–60 min due to the reconfiguration of the enzyme and de-emulsification [27]. To get highly active enzyme in immobilized form, in this work, we have enhanced the enzyme activity by using sonication treatment and successively entrapped them (in 10 min) within the MOF *via* one pot biomineralization strategies in aqueous solution. We used lipases as a model enzyme which has high world-wide enzyme market due to their potential and widespread applications in different biotransformation reactions such as flavours, cosmetics etc [28]. This is the first report on encapsulation of highly activated lipase within MOF without altering superior enzyme conformation. After fabrication of enzyme molecules, frame works lock enzyme molecules in active conformation and create a stabilizing microenvironment. This confinement also prevent the structural changes that lead to unfolding of sonicated lipase caused by reconfiguration and de-emulsification which is determined by structural data of enzyme calculated by FT-IR data analysis tools and fluorescent spectroscopy. Also, hierarchical porosity and structures provide large surface area to improve the enzyme encapsulation yield of MOF. Additionally, it makes them mechanically robust and thermally stable which would greatly expand the applications of biocatalysis [29]. Moreover, enzyme entrapment also reduces leaching from the support, which can be regarded as one of the main shortcomings of numerous other immobilization methods. Further, the prepared lipase-MOF was characterized by powdered X-ray diffraction (XRD), Brunauer, Emmett, and Teller (BET) surface area, thermo gravimetric analysis (TGA), confocal scanning laser microscopy, Fourier Transform Raman (FT-Raman) spectroscopy and Fourier transform infrared (FT-IR). The size and morphology of lipase capped MOF was analyzed by scanning electron microscopy (SEM). Also, the kinetic parameters (V_{max} and K_m) and thermal stability of free lipase and MOF of lipase were determined in terms of thermal deactivation constant (k_d), half-life ($t_{1/2}$) and deactivation energy (E_d). 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

Lipase (from *Aspergillus niger* source) was obtained from Sigma Aldrich, Bengaluru, India. Gum arabic, *p*-nitrophenyl acetate, 2-methylimidazole and zinc acetate were purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, India. Chloroform and isoamyl alcohol were purchased from S.D. Fine Chemicals, Mumbai, India. All other chemicals were of AR grade and procured from reliable sources and used without any further purification.

2.2. Experimental

2.2.1. Lipase activity assay

The lipase activity was assayed by hydrolysis of *p*-nitrophenyl acetate as substrate according to Palacios et al. [30] The substrate (1.9 mL buffer 100 mM sodium phosphate pH 7.0 mixed with 0.1 mL of 100 mM *p*-nitrophenyl acetate) was mixed with enzyme solution and kept in a water bath at 40 °C. After 5 min of incubation, the reaction was terminated by adding Marmur solution (2.0 mL, chloroform: isoamyl alcohol, 24:1) followed by centrifugation at 6000 rpm for 5 min at 4 °C. The clear yellowish aqueous phase supernatant (*p*-nitrophenol) was taken off to measure enzyme activity at 405 nm in UV/Vis spectrophotometer (Jasco V-730 UV–vis Spectrophotometer, USA). One enzyme unit is defined as the amount of the enzyme that liberates one μmole of *p*-nitrophenol per min at optimized conditions.

The protein concentration was measured by using Bradford protein assay by using BSA as standard [31].

2.2.2. Preparation of sonicated lipase

The lipase activity was intensified by using probe sonicator (3.5 cm radius and 22 kHz frequency, Dakshin India Ltd., Mumbai, India) [32]. Briefly, lipase (80 mL containing 10.29 U mL^{-1} of lipase and protein content of 5 mg mL^{-1}) was taken into a specially designed narrow necked round bottom glass vessel (100 mL) and kept into the ice bath (4 °C) to avoid denaturation of enzyme during ultrasound intensification. The enzyme was irradiated at different ultrasonic intensity (2–21 W cm^{-2}) and duty cycle (33–88% that is 2–32 s on and 4 s off) with respect to time (0–60 min) and further assayed for lipase activity immediately after treatments using spectrophotometric method as described in the subsequent section.

2.2.3. Synthesis of the lipase MOF

The enzyme embedded MOF was prepared by method reported by Liang *et al. [*29] Experimentally, sonicated lipase (1 mL, protein content 5 mg mL^{-1}) was mixed in an aqueous solution of zinc acetate (1.33 mmol, 1 mL). The separate solution of 2-methylimidazole (Hmim, 13.3 mmol, 4 mL) was prepared and mixed together with enzyme solution under stirring at room temperature (28 ± 2 °C). After stirring for about 10 min, the white precipitation was collected by centrifuging at 6000 rpm for 10 min and washed with sodium sulphate buffer (100 mM, pH 7.0) for five times. The product was re-dispersed in same buffer for lyophilization and used for further experiments. For comparison, pure ZIF-8 MOF was synthesized by the same procedure as mention above but in the absence of enzyme solution.

2.2.4. Characterization of prepared lipase MOF

The prepared ultrasound assisted intensified lipase embedded MOF was confirmed by Fourier transform infrared (FT-IR) spectroscopy (Shimadzu IRAffinity-1 FT-IR spectrophotometer, scan range: 400–4000 cm^{-1}) and Fourier Transform Raman (FT-Raman) spectroscopy (BRUKER RFS 27: stand-alone FT-Raman Spectrometer, scan range: 50–4000 cm^{-1}). Powder X-ray diffraction (XRD, Philips PW 1830 X-ray Diffraction) was used to investigate crystal structures of the lipase-MOF. A thermo gravimetric analysis (TGA) STA 449 F3 Jupiter® − NETZSCH instrument was used to calculate the percentage weight loss of lipase-MOF over 25–500 °C in nitrogen atmosphere with a heating rate of 10 °C min^{-1}. The surface area of MOF before and after immobilization were determined by Barrett-Emmett-Teller (BET). Nitrogen adsorption measurements were performed, using a Porous Material Inc. (BET −201A), to determine BET surface area. Confocal scanning laser microscopy was used to investigate the presence of fluorescein isothiocyanate (FITC) tagged lipase within the ZIF-8 MOF.