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Investigation of Enzyme Immobilization Effects on its Characteristics

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ABSTRACT

Background: Enzymes are well known as sensitive catalysts in the laboratory and industrial scale. To improve their properties and for using their significant potential in various reactions as a useful catalyst the stability of enzymes can often require improvement. Enzymes Immobilization on solid supports such as epoxy-functionalized ferric silica nanocomposite can be effective way to improve their characteristics.
Methods: In this study silica coated magnetite nanoparticles were Functionalized with GPTSM as a linker, then immobilization reaction performed by using various amounts of lipase B from Candida Antarctica (CALB), for the next step immobilization effects on thermal stability and optimum pH were investigated in comparison with free CALB.
Results: Results illustrated enzyme was successfully immobilized on nano particles and immobilized derivative retains 100% of its activity by 55°C while free CALB loss its activity at the same condition.
Conclusion: Immobilization of CALB on Fe_3O_4@SiO_2 particles resulted in significant improvements in its characteristics such as thermal stability and methanol tolerance compared to the free CALB.

1. Introduction

In order to perform various chemical synthesis Enzyme based biocatalysts are interesting option. Enzymes are one of the high profile catalysts which are very effective and precise (bio)-catalysts that regulate processes and perform a wide range of reactions in living matter [1].

Nowadays enzymes such as lipase B from Candida Antarctica (CALB) is an interesting lipase which is applicable in many chemical reactions such as the synthesis of triglycerides, esterification of terpenic alcohols and various chemical reactions [2]. But there are some constraints in enzymatic process such as high cos tand low stability of enzymes. Therefore, finding away to reuse of lipase considering its high cost is essential which is achievable by immobilization, also immobilization could enhances lipase properties such as thermal stability and activity [3]. One of the most frequently used immobilization technique is covalent attachment, covalent binding of a lipase to a solid carrier has several advantages such as minimizing lipase leaching in aqueous media and helping it to tightly fix on carrier [4, 5].

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There are several approaches to improve operational quality of enzymes including easy recovery, reusability, which immobilization on solid carriers is one of the most common strategies that used to overcome on enzymes weaknesses [6]. As in the one report mohammadi et al have been immobilized Rhizomucor miehei lipase on Aldehyde-Functionalized silica and silica nanoparticles by covalent attachment [7]. In recent years magnetite nanoparticles have drawn great attention because of those high potential in a wide range of applications, especially for enzyme immobilization as Wang X et al immobilized Porcine pancreas lipase (lipase L1),Candida rugosalipase (lipase L2), and Pseudomonas cepacialipase (lipase L3) onto the amino-functionalized Fe_3O_4 nanoparticles [8].

However these advantages could be marginalized by unwanted interactions, which are caused by reacting agents such as erosion, but the deposition of silica layer on magnetite nanoparticles can prevent them to be eroded by environmental reactions [9]. In this work we report Simple, covalent immobilization of Candida Antarctica Lipase B on epoxy- functionalized ferric silica nanocomposite which few reports can be found explaining covalent immobilization of CALB on epoxy functionalized supports.

2. Material and Methods

Para-nitrophenyl butyrate, Candida Antarctic Lipase a Lipase B and, 3 ethylamine and glycidyloxypropyl) trimethoxysilane (GPTMS) were from Sigma. Thermogravimetry (TGA) and differential thermal analysis (DTA) were carried out from 10 °C to 800 °C at a heating rate of 20 °C/min in air atmosphere using a STA 503M system from Bahr GmbH, Germany.

2.1. Functionalizing of silica coated magnetite nanoparticles

1 gram of nanocomposite silica magnetite particles were added to 50 mL of dry toluene then 1 mL of GPTMS and 200mL Et3N were added, the mixture refluxed for 4 h under nitrogen atmosphere and vigorous stirring. The modified supports were washed with acetone for several times [7].

2.2. CALB immobilization

(100 mg) of functionalized nanocomposite silica magnetite particles were dispersed in 2ml phosphate buffer (10 mM pH 7) then 50 µl candida Antarctica were added to the suspension and the mixture was stirred gently for 24 h.

Then the amount of lipase which immobilized on supports were determined by the Bradford method [10, 11].

2.3. Enzyme activity assay

Immobilized lipase activity was analyzed spectrophotometrically by measuring the increment in absorbance at 348 nm. Briefly 10 mg of immobilized lipase was dispersed in 50 µl buffer then 20 µl of this suspension was added in the UV cell which contained 2.5 ml buffer and 20 µl p-NPB, enzymatic activity is given as 1 µmol of p-nitrophenol released per minute per mg of the enzyme (IU) under the conditions described above [7].

2.4. Thermal inactivation of CALB and its immobilized preparation

Free enzyme and immobilized preparation of CALB were incubated in 25 mM sodium phosphate at pH 7.0 at different temperatures and their activities were measured using the p-NPB assay [7, 11].

2.5. Determination of the optimum pH activity

To determine the optimum pH of activity, the free and immobilized lipase activity were measured in different range of pH from 5 to 9 at 25 °C [11].

2.6. Stability of CALB and its immobilized preparation in presence of methanol

Free enzyme and its immobilized preparation were incubated in 25 mM sodium phosphate buffer pH 7.0 and 20% or 50% of methanol at 25 °C. The activity of each sample was measured using the p-NPB assay [12].

3. Results and Discussion

3.1. Functionalizing

The successful functionalization of Fe3O4@SiO2 can also be concluded from TGA curves. As shown in Fig. 1, its main mass loss (≈10%) takes place around 300–600 °C, which can be attributed to the removal Of the functional groups from the surface of the support.

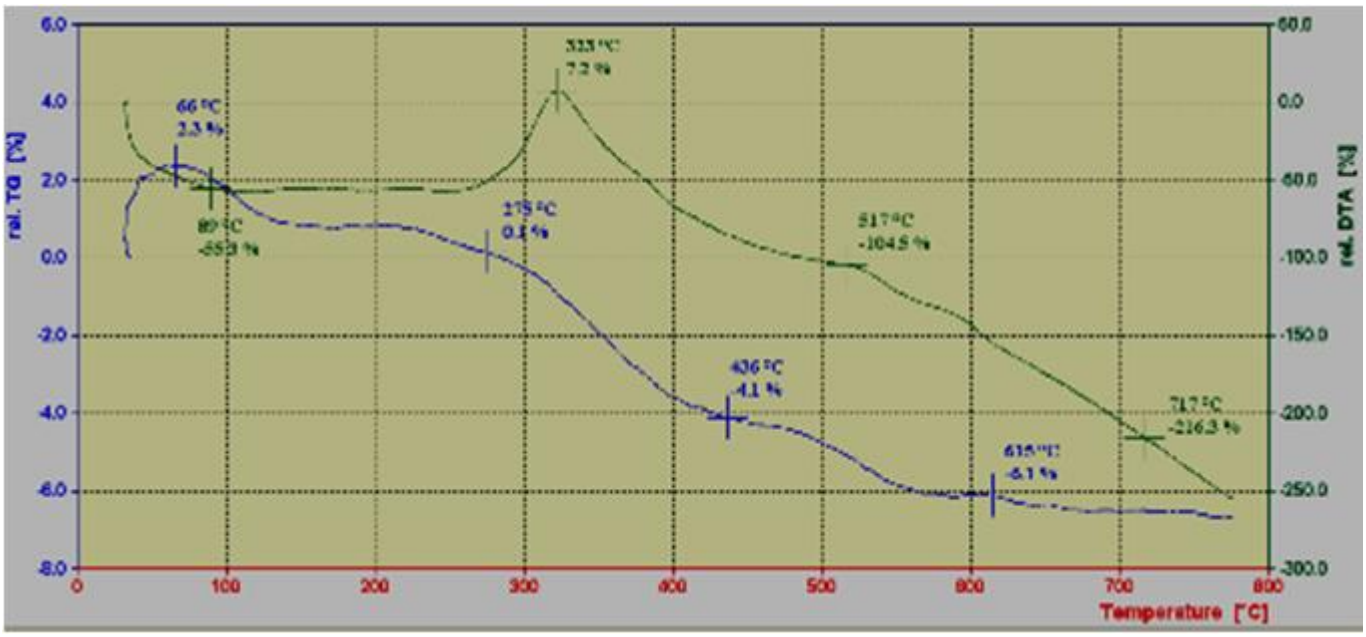

Fig. 1: TG analysis of epoxy-functionalized particles.

3.2. Immobilization

Immobilization of CALB was performed by incubation of a crude protein preparation in presence of Fe_3O_4@SiO_2–epoxy at room temperature. As can be seen in Table 1, high enzyme immobilization yield (84%) was obtained by producing 6.3 (U/mg enzyme) specific activity. Compared to the specific activity of the free enzyme (6.5 U/mg enzyme) it shows about only 3% reduced activity. Decrease in enzyme specific activity during covalent immobilization is a common phenomenon which can be attributed to denaturation of the protein caused by the coupling process and/or diffusion limitation after immobilization of enzyme [13].

Table 1: Immobilization parameters of CALB.

Enzyme derivative	Immobilization time	Immobilization Yield (%)	U/mg enzyme
Free CALB	---	---	6.5
Immobilized CALB	24h	84	6.3

3.3. Thermal stability

Free and immobilized CALB thermal inactivation were investigated in various temperatures (Fig. 2). As results show both the soluble enzyme and immobilized preparation retain almost the complete activity after 24h incubation at 45°C. Although increasing temperature to 50°C causes to lose 12% of initial activity of soluble enzyme after 2h while the immobilized derivative retains 100% of its activity at the same condition. Furthermore increasing temperature to 55 °C causes to quick inactivation of CALB while the immobilized CALB remains completely active after 2h at the same temperature.

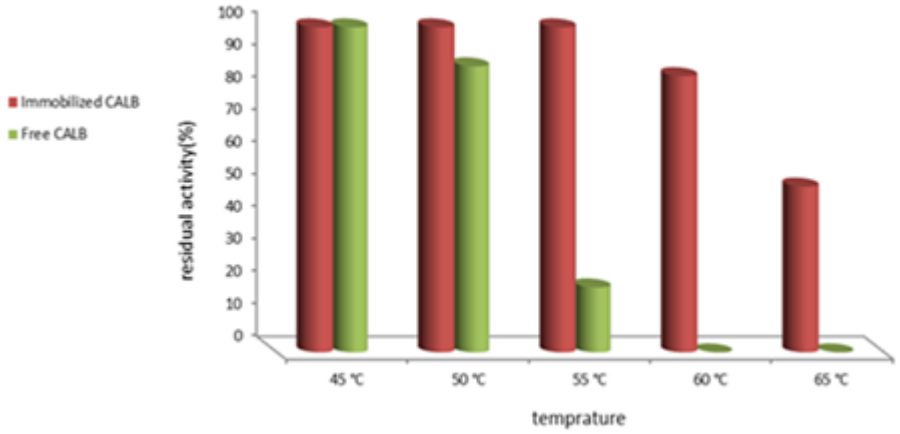

Fig. 2: Thermal stability of CALB and its immobilized preparation.

3.4. Optimum pH activity

The effect of pH on the activity of the free and immobilized enzyme was also examined at different pH values ranging from 5 to 9 at 25°C. In Fig.4, the activities of the soluble and immobilized CALB were plotted versus the pH. The results show the optimum activity for both free CALB and its immobilized form at pH 7.5. It means that covalent immobilization of CALB on Fe_3O_4@SiO_2–epoxy has no effect on its optimum pH.

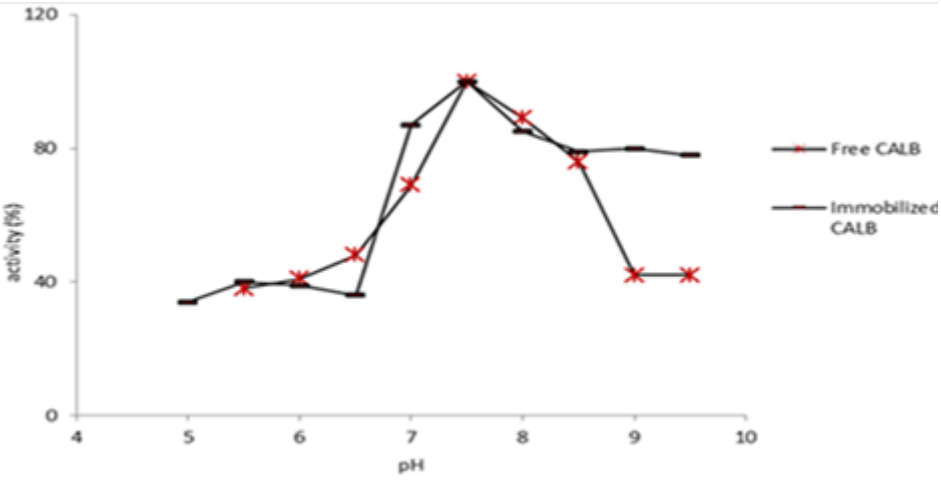

Fig. 3: Optimum pH activity of CALB and is immobilized preparation.

3.5. Effects of methanol

Several studies illustrated lipases could be inhibited by methanol because of its effects on lipase activity [12]. In present report for evaluating stabilities of free and immobilized derivative of CALB their activity were investigated in presence of 20% and 50 wt% methanol. Fig.5 shows the stability of the covalently immobilized preparation compared to the crude lipase in the presence of methanol. Liquid CALB retained 80% and 65% of residual activity in 20% and 50% of methanol, respectively, after 24 h incubation at 25°C. Even better, covalently immobilized CALB showed excellent stability with increasing concentrations of methanol.

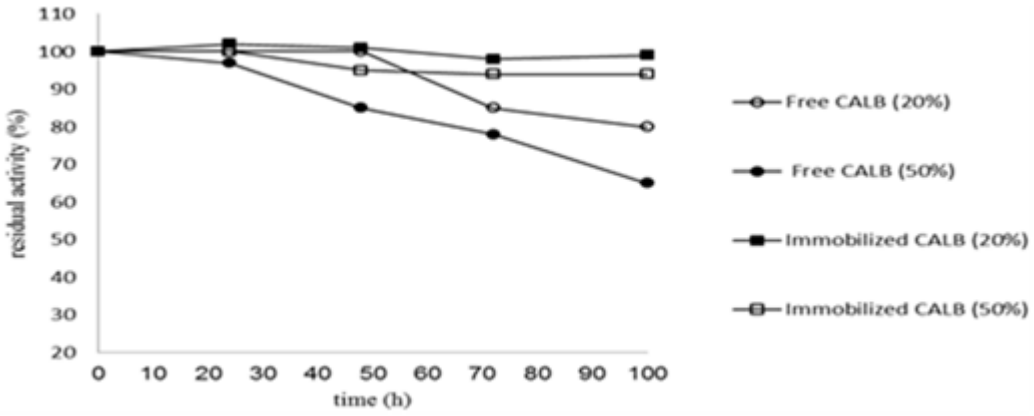

Fig. 4: Co-solvent stability of free CALB and its immobilized preparation in the presence of 20% and 50% of methanol.

4. Conclusion

Functionalizing of nanoparticles according to TG analysis were successfully performed and covalent immobilization of CALB on epoxy functionalized Fe_3O_4@SiO_2 particles resulted in significant improvements in its thermal stability and methanol tolerance compared to the free enzyme, also results proved covalent attachment of CALB do not overshadow enzyme activity.

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