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# Immobilization of lipase onto mesoporous silica KIT-6 and montmorillonite K10 for enzymatic hydrolysis of tributyrin

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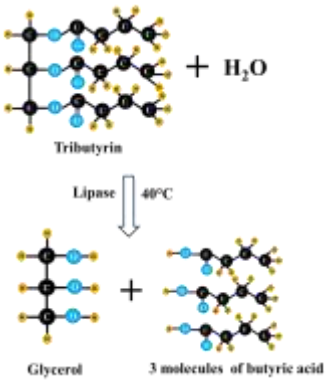
**GRAPHICAL ABSTRACT**




**ABSTRACT**

Mesoporous silica KIT-6 and montmorillonite (MMT) K-10 clay were prepared and used for immobilization of the enzyme, *Candida rugosa* lipases (CRL), aiming at their use as biocatalysts for the hydrolysis of tributyrin. Immobilization of the enzymes onto the supports was performed by physical adsorption using 0.1 M phosphate buffer solutions (pH 7) as the dispersion medium. The activity of the immobilized CRL for tributyrin hydrolysis was investigated at incubation temperature of 40 °C during 120 min and different concentration of the lipase solution for both the supports. Characterization by XRD showed that the long-range ordering in the KIT-6 and crystallinity of the MMT K-10 materials were affected slightly by the lipase immobilization. These results give an indication to the presence of lipase-support interaction in the immobilized lipase systems. Additionally, the results of FTIR spectroscopy verified the presence of silanols on the surfaces of MMT K-10 and KIT-6 materials. Nitrogen adsorption data showed the resulting immobilized enzyme catalysts were rendered porous, with the KIT-6 giving significantly higher specific surface areas than the MMT K-10. The immobilization of CRL on KIT-6 and MMT K-10 through hydrogen bonding with the silanol groups, led to an increase in the hydrolysis activity compared to that of free lipase. However, the activity of KIT-6 immobilized CRL was higher than was observed on MMT K-10 immobilized CRL. Furthermore, lipase immobilized on mesoporous silica KIT-6 was shown to be recyclable up to 5 times in aqueous medium. The high surface area and large pore diameter of the mesoporous silica KIT-6 may be the crucial factors that play an important role in retaining the enzyme in the support, and consequently, improving the lipase activity and stability.

*Keywords*: mesoporous silica KIT-6, montmorillonite K-10, immobilization, *Candida rugosa* lipase, hydrolysis.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## 1. INTRODUCTION

Candida rugosa lipase (CRL), a mesophilic lipase having high activity and broad specificity in the reaction medium, is one of the enzymes most frequently employed in biotechnology, with application in food and flavouring, fine chemicals, pharmaceutical and cosmeceutical industries [1-3]. Lipases have been widely found in biological systems, where they catalyze the hydrolysis of triacylglycerols to glycerol and fatty acids in aqueous media [4]. In the presence of enzymes as biocatalysts, the chemical reactions such as hydrolysis, esterification, transesterification, and aminolysis were possible, where the lipases showed high specificity and catalytic activity [5-7]. However, the difficulties in their recovery from the reaction systems, poor recyclability and low long-term operational stability, impose limitations on the commercial uses of lipases. Nevertheless, these inherent properties of enzymes may be overcome through immobilization of the enzymes, structure modification and the use of additives [8, 9].

Enzyme immobilization is the most commonly used strategy to impart the desirable features of conventional heterogeneous catalysts onto biological catalysts. Immobilized enzymes are often more stable and easier to recover than free enzyme in solution, enabling the reuse of the immobilized enzyme without a significant loss in biological activity [10]. Furthermore, they showed superior activity compared to the free enzyme, including greater thermal and pH stability [11-13].

The synthesis of stable, enzyme supported materials represents a significant practical fundamental challenge. There are several methods available for immobilization of enzymes on various supports, including adsorption, ionic bonding, covalent binding, cross-linking, entrapment, and encapsulation [14, 15]. Binding of the enzyme to a support through physical adsorption is the most simple

highly accessible and highly connected open pore networks.

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