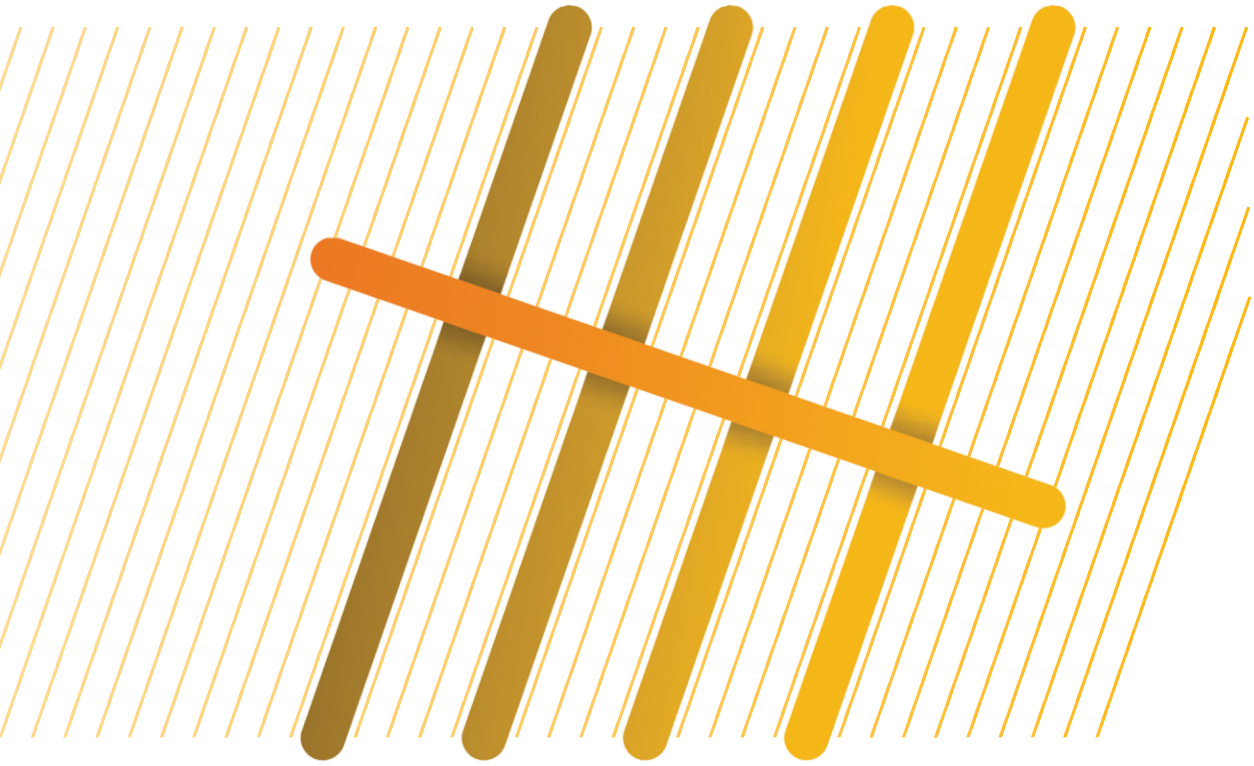

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BOOK OF ABSTRACTS

CANDIDA RUGOSA LIPASE IMMOBILIZED ONTO TITANIA: IMPROVED THERMAL STABILITY AND REUSE POTENTIAL

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Enzyme catalyzed reactions have been extensively exploited for a wide range of applications in biotechnology. In spite of a broad implementation of enzymes in different fields, some constraints referred to their cost and process stability still exists. To overcome a limit related to short catalytic lifetime of enzymes in process conditions, a spectrum of immobilization methods have been extensively studied to increase stability and enhance reuse, offer easier separation, making production economically viable.

Lipase from *C. rugosa* is a globular glycoprotein with molecular mass of 57 kDa. With 31 acidic and 18 basic amino acids exposed on the surface, its IEP is located at pH 4.65. Titania (Degussa P25) was selected as support material for its well defined morphology: nanometer-sized solid particles of spherical shape. Being amphoteric, titania particles develop positive and negative charge below and above IEP (pH 6.7), respectively.

Tris-HCl buffer at pH 7.6 was used for immobilization. Although the overall charge of lipase is negative, localized electrostatic attractions together with hydrophobic interactions govern the adsorption of enzyme onto the support. Owing to the patchwork surface charge of lipase, positively charged Lys are accessible for the electrostatic interactions with the negatively charged support.

The adsorption of lipase onto titania was fast, after 1h 65% of lipase was adsorbed.

One of advantages of enzyme immobilization is improvement in thermal stability. Therefore, the stability of lipase immobilized onto titania was determined at 50 and 60 ^{o}C. In terms of half-life, at 50 ^{o}C, $t_{1/2}$ of free lipase was 55 min, while after immobilization, $t_{1/2}$ increased to 180 min. The thermostability of lipase was increased more than 3-fold after immobilization. After 1 h of incubation at 60 ^{o}C, the free lipase was inactive, while the remaining activity of immobilized enzyme was close to 60%. Even after 2h the activity remained 40%, or in terms of half-life, increased more than 7-fold.

A significant improvement in thermal stability of immobilized lipase seems to be result of restrict movements of protein after adsorption, preventing conformational changes and unfolding.

The reusability of immobilized enzyme is one of the most important advantages for application. Adsorption, as an immobilization method, is usually considered as a method with poor reuse potential, as the linkages established are usually weak and the enzyme could be easily desorbed. Reuse stability in water was tested. Remaining lipase activity was about 90% after nine reuses. As strength of interaction between enzyme and support can be judged from the ability of enzyme to resist removal – leaching, the result implies a strong interaction between lipase and titania as support. The result points to the significant potential for reuse of lipase in water environment after immobilization onto titania.

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