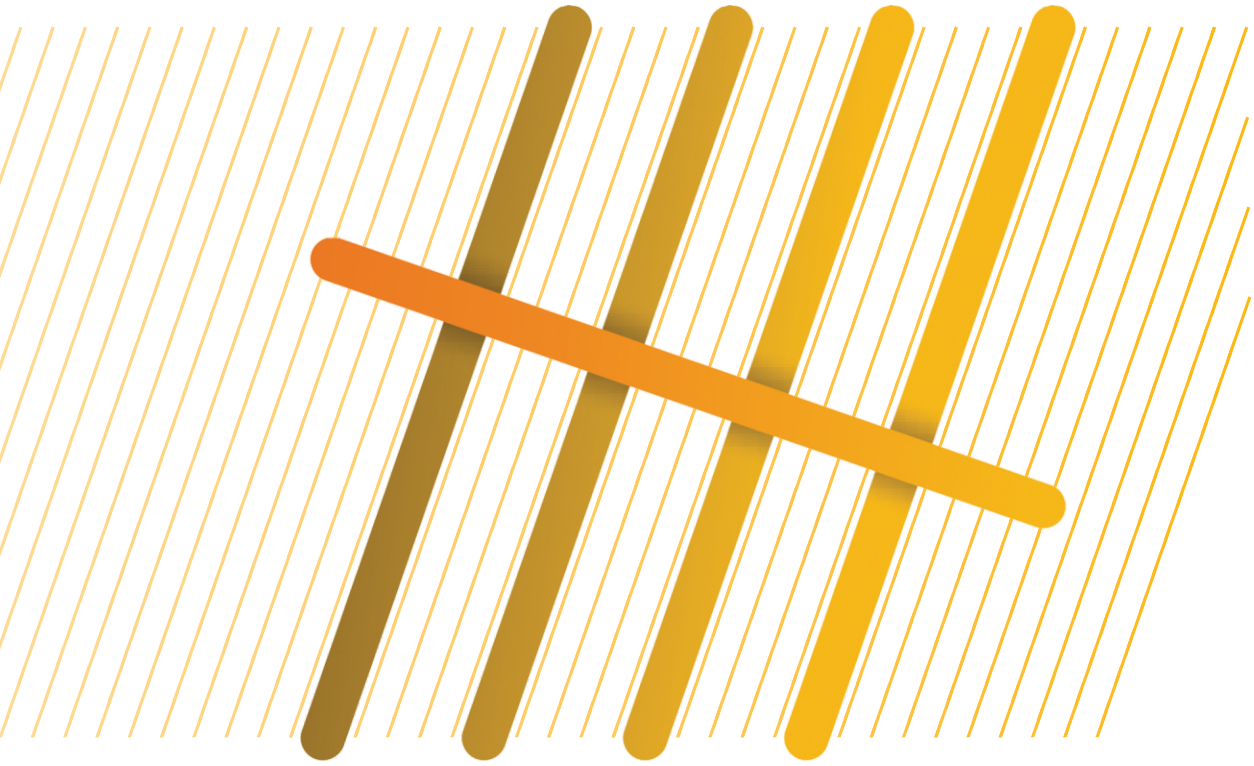

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BOOK OF ABSTRACTS

CANDIDA RUGOSA LIPASE IMMOBILIZED ONTO TITANIA AS NANOBIOCATALYST IN ORGANIC SOLVENT

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Kinetic measurements can be used to predict the optimum kinetic behaviour of a particular biocatalyst. Based on those predictions, optimisation of biocatalytic reactions, as well as process design to improve productivity and reduce the cost of various processes can be performed.

The kinetic parameters of lipase immobilized onto titania were determined in cyclohexane using solutions of amyl alcohol and octanoic acid in a range of concentrations: 0.05 to 0.6 M for alcohol and 0.05 to 1 M for acid. at 40 ^{o}C with mechanical stirring at 150 rpm on rotary shaker. One unit (1 U) is defined as that quantity of enzyme which under test conditions synthesizes 1 mmol of amyl-octanoate per min.

The rate of esterification was determine as the residual acid content by titration with sodium hydroxide, The quantity of ester formed was calculated as being equivalent to acid consumed. This was confirmed by determination of ester concentration using gas chromatography–mass spectrometry (GC-MS) performed with a GCMS (QP2010 Ultra, Shimadzu, Kyoto, Japan). Spectrum analysis was performed using NIST11 and Wiley8 database libraries, and relative ratios of components were calculated from the corresponding peak areas.

Most of the kinetic studies of lipase catalyzed esterification reaction in organic solvent assume ping-pong model with inhibition by alcohol. In order to find kinetic model of amyl caprylate synthesis, catalyzed with *C. rugosa* lipase immobilized onto titania, series of experiments were performed at determined conditions. The experimentally obtained data were fitted with model for bisubstrate ping-pong bi-bi model with alcohol inhibition using Matlab software. The rate equation describing this model is given by:

$$\upsilon=(V_{max}, [Ac] [Al])/([Al][Ac] + K_{Al}[Ac] + K_{Ac}[Al] + (K_{Ac}/ K_{i,al}) [Al]^2)$$

where υ is the initial reaction rate, V_{max} is the maximum reaction rate, [Ac] and [Al] are the concentrations of octanoic acid and amyl alcohol, K_{Al} and K_{Ac} are Michaelis constants for amyl alcohol and octanoic acid, and $K_{i,al}$ is the amyl alcohol inhibition constant.

Kinetic constants (V_{max}, K_{Al}, K_{Ac}, $K_{i,al}$) were calculated using a non-linear regression fit of the 48 experimental points. Parameters were optimized using MatLab software.

Values of kinetic constants from ping-pong bi-bi model with alcohol inhibition are: V_{max} = 26.36 μmol min^{-1}g^{-1}; K_{Al} = 0.200 moldm^{-3}; K_{Ac} = 0.522 moldm^{-3}; $K_{i,al}$ =0.644 moldm^{-3}

The increase of concentration of octanoic acid increases the rate of esterification achieving the maximum rate of 26.36 μmol min^{-1} at 1 mol/L of acid and 0.3 mol/L of alcohol. On the other hand, the increase of concentration of amyl alcohol confirmed hypothesis of substrate inhibition.

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