ABSTRACT

Under micro-aqueous conditions, esterases especially lipases catalyze acyl transfer reactions to synthesize a variety of esters, thioesters and amides. Free lipases are easily denatured and have very low acylation activities in non-aqueous solvents. While on the other hand the immobilization process prevents the enzymes from their direct exposure to the unnatural non-aqueous media.

In the present work different biocatalysts, acylating agents and other parameters were investigated to catalyze the acylation of different nucleophiles to produce various valuable esters and amides.

To optimize basic parameters, acylation of octanol with octanoic acid was studied as a model process. Different lipases were immobilized by various techniques using a variety of immobilization supports. Compared to other biocatalysts, *Candida rugosa* type VII lipase immobilized on O-Propargyl dextrans was found to have maximum activity for octyl octanoate synthesis. The biocatalyst worked best at 35°C in presence of n-hexane as the reaction medium having 0.2% moisture. This suitable combination of reaction conditions was found using Response Surface Methodology. ANOVA results revealed that temperature, moisture and enzyme concentration significantly influenced the acylation process.

Studies on *O*-acylation processes include synthesis of Octyl oleate, Citronellol Palmitate, Phytyl oleate, Retinyl butyrate, Glucose laurate and maleated starch.

Octyl oleate was synthesized by acylation of octanol with oleic acid using conditions optimized for octyl octanoate. Kinetic studies show that lipase mediated acylation of octanol by oleic acid follows Ping Pong BiBi mechanism with $Km_{oleic\ acid}$ equal to 12.14 $mmol.L^{-1}$ and $Km_{octanol}$ equal to 120.2 $mmol.L^{-1}$

In case of acylation of citronellol with palmitic acid, *Candida rugosa* type VII lipase immobilized on *O*-Propargyl dextrans worked the best at 30°C in n–Hexane. Michaelis Menten constant for citronellol ($Km_{citronellol}$) was found to be 775.3 $mmol.L^{-1}$ and the Michaelis Menten constant with reference to palmitic acid ($Km_{palmitic\ acid}$) was found to be 423.5 $mmol.L^{-1}$.

Similarly Phytyl oleate was synthesized with a conversion yield of 73% in 60 hours with Km_{phytol} = 195.8 mmol.L^{-1} and $Km_{oleic\ acid}$ = 133.26 mmol.L^{-1}.

Retinyl butyrate was synthesized by acylation of retinol using butyric acid as acylating agent. Lipase of *Aspergillus niger* immobilized by adsorption on PgD was found to be the most suitable along with n-hexane as the non-aqueous solvent. K_m [Retinol] was 78.5 mmol.L^{-1} and K_m [Butyric Acid] = 111.79 mmol.L^{-1}. Apparent activation energy was calculated from the Arrhenius plot thus obtained. Its value came out to be 10.171 K cal/mole.

Acylation of glucose to produce glucose mono laurate was investigated optimizing all the reaction parameters including reaction medium, acylating agent and the biocatalyst. The mycelial lipase from *Rhizopus arrhizus* was found to acylate specifically at primary position producing *O*-acyl glucose in tertiary alcohol, 2-methyl-2-butanol as the reaction medium. Analysis of variance revealed that temperature, moisture and enzyme concentration significantly influenced the acylation of glucose with lauric acid. The soluble lipase from *Rhizopus arrhizus* supported preparation of maleated starch in solvent free system at 35°C. Acylation of starch was confirmed through FTIR-Spectroscopy. X-Ray Diffractomery showed that acylation did not change the granules structure but the surface became rougher.

Acylation of amines is important in providing intermediates for the synthesis of bioactive molecules. Enzyme catalysed acylation, followed by deacylation provides a clean procedure for resolution of optically active amines. The mycelial lipase of *Rhizopus arrhizus* gave best results for acylation of butyl amine. The Mycelial lipase proved to be more effective biocatalyst for trans-acylations using ethyl palmitate as acylating agent for the acylation of n-butyl amine.

Michaelis Constant with reference to butyl amine was found to be 304.4 µmol.L^{-1} mmole L^{-1} and the K_m [ethyl palmitate] was 494.5 µmol.L^{-1}. The activation energy of enzymic acylation of butyl amine was found to be 18.47 K.cal/mole.

N-acylation of N-methyl glucamine led to the formation of glucamides which are non-ionic surfactants. Naturally immobilized mycelial lipase of *Rhizopus arrhizus* served as the best biocatalyst. After 72 hours reaction in 2-methy-2-butanol at 35°C the reaction mixture contained 48 % N-Oleoyl-N-Methyl Glucamide.