

**Enzymatic alcoholysis catalyzed by lipases –nonaqueous  
environment modification**

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## Abstract

Enzymology is rapidly growing branch of biochemistry, which is focused on the study of catalytic proteins. One of its subfields is microaqueous enzymology, which focuses on processes taking place in environments other than water, for example, organic solvent, ionic liquid or supercritical fluid.

The aim of this work was to develop a method for controlling the enzymatic alcoholysis of vegetable oil with primary alcohols. Method focus on maximizing the yield of esters and improving the stability of the biocatalyst during the process. The dissertation was divided into 3 parts. The first part examined the effect of the addition of water to substrate mixtures on the yield of the transesterification reaction and the stability of the biocatalyst. In the second part was studied the effect of the addition of small amounts of diethylamine (DEA) on the esters yield and the enzymatic reaction progress in time. During the last part was considered modification of the reaction environment with additions of both substances simultaneously (i.e. water and DEA). Moreover were made attempts to develop a mathematical model for a deeper understanding of the investigated topics.

As a result of the research with addition of water to reaction of sunflower oil with 2-methylbutyl alcohol, catalyzed by an enzyme preparation in the form of powdered *Mucor circinelloides* - M.C. mycelium, was possibility to increase the amount of esters produced by up to 6%. In the same reaction system by addition of DEA in the amount of 15 mM, esters production was risen by about 4%. During studying the simultaneous addition of both substances, the synergistic effect of water and amine was proven by obtaining almost 15% more esters compared to the reaction carried out without these additives. The transesterification process with the same substrates was run in a column reactor using M.C. lipase embedded on polyurethane foams. By adjusting the amount of water in the reaction medium, the time of efficient operation of the biocatalyst was increased from 6 to 100 days.

Mathematical model based on Gompertz function was built. This model allows an approximate prediction of the studied phenomena ( $R^2=0.97$ ). Also was possible the evaluation of the effects of individual variables (water addition and DEA) on the effect of the enzymatic transesterification. During this research it was proved that DEA as well as water significantly affect the kinetics of the studied phenomena.