

## Stage II

### Optimization of the laboratory technology

Critical factors affecting the productivity of enzymatic biodiesel production process are: lipase type, raw materials used (vegetable fat / animal, type of alcohol, molar ratio), reaction medium, the reaction temperature, water concentration in the reaction medium, lipase microenvironment, the amount of resulted glycerol.

Lipases most commonly used and which gave the best results for enzymatic synthesis of biodiesel are those in immobilized form that have vast applications in terms of substrate selectivity and regioselectivity.

Raw materials for biodiesel production include all vegetable oils available (refined oil, crude oils, inedible oils), waste oils and fats, lard, tallow, etc. Biodiesel production processes using waste fats and oils have proven to be economically viable.

For each type of alcohol - fat - lipase studied system is necessary to determine the optimal molar ratio of substrates used; if the reactions performed in organic solvent medium requires a slightly excess of alcohol to achieve a satisfactory performance in organic solvent-free systems, alcohol should be introduced in small successive portions, in order to avoid enzyme inactivation.

Optimum temperature for the enzymatic transesterification process is the result of interaction between the operational stability of lipase and the transesterification rate, and depends on: the molar ratio of alcohol: oil, organic solvent nature, thermal stability of the enzymatic preparation.

The main alcohols used until present for biodiesel production include lower aliphatic alcohols: methanol, ethanol, propanol, iso-propanol, 2-propanol, n-butanol, and izobutanol; methanol and ethanol being produced in industry and being the cheapest, were generally used for biodiesel production.

An optimum concentration of water in the enzymatic transesterification system must lead to an increase of esters synthesis rate while maintaining the concentration of free fatty acids to a minimum level in the reaction mixture. change over time as the total methyl esters. A change in their different mode of variation does not change the total content of methyl esters.