Biodiesel production by enzymatic transesterification of olive oil

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Chemical processes for production of biodiesel are well known. There are three general pathways to ester production from oils and fats: a) base catalyzed transesterification of the oil with alcohol, b) direct acid catalyzed esterification of the oil with methanol, and c) conversion of the oil to fatty acids and alkyl esters through acid catalysis. However, new biochemical routes to biodiesel production, based on the use of enzymes, have recently become very attractive.

Biodiesel (fatty acid methyl ester) was produced by transesterification of triglycerides (triolein) present in olive oil with methanol. Lipase B from *Candida antarctica* adsorbed onto a macroporous resin (Novozym®435) was purchased from *Sigma-Aldrich* (St. Louis, MO). Pure olive oil (Hannaford brand) was used in all the experiments. Hexane, isopropanol and methanol were the elution solvents utilized in HPLC. Methanol and hexane were also used as reactant and solvent, respectively, in the transesterification reactions.

The concentrations of both triolein and methyl oleate were measured with a Hewlett Packard HP 1050 UV/VIS spectrophotometer. This detector was coupled to a reverse phase, C_{18}, high-performance liquid chromatography (HPLC) column. The gradient elution technique was developed indigenously after numerous trials. A wavelength of 230 nm gave the best results.

Experiments were conducted to determine the influence of a) the molar ratio of methanol to triolein, b) semi-batch (stepwise addition of methanol) versus batch operation, c) mass of catalyst, d) mixing speed and e) reaction temperature on reaction rate and conversion. Step-wise methanolysis to maintain approximately a 3:1 methanol:triolein molar ratio with an overall ratio of 8:1 proved to be the optimal set-up. The initial reaction rate increased with the amount of catalyst until it reached a maximum; for enzyme concentrations higher than 2000 U/ml olive oil, the rate was barely affected by addition of enzyme. Final conversion did not depend on the catalyst concentration.

Mixing speed did not play a significant role in the range 50 – 400 rpm and 60°C was the optimal reaction temperature. The final conversion and yield remained constant up to a temperature of 60°C.

Studies were also performed to determine the difference in performance between a) test-tube scale and lab-scale reactors and between b) used and clean oil as the raw material. For identical reaction conditions, all the data obtained at test-tube scale could be extrapolated to lab scale production of biodiesel, within 5-10%. Despite the fact that conversion of used cooking oil was somewhat lower, no major differences were observed between virgin and used oils.

Finally, the life of Novozym®435 was determined; even though the enzyme's relative activity decreased with reutilization, it still retained 95% of its activity after 5 batches and more than 70% after as many as 8 batches. Repeated use of the enzyme did not affect the overall conversion.