Biodiesel production in supercritical fluid condition by enzymatic process using stepwise reaction method

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In this study, transesterification of soybean oil was investigated in supercritical fluid condition by enzymatic process using *Candida antartica, Candida rugosa* and *Rhizopus oryzae* lipases. Moreover, to increase reaction rate and enzyme stability, stepwise reaction was carried out. Among various lipases, *R. oryzae* lipase was a suitable enzyme for biodiesel production. The reaction factors, such as pressure, temperature, agitation speed, enzyme concentration, enzyme ratio of *C. rugosa* and *R. oryzae* lipases and water contents were considered to increase biodiesel production in mixed enzyme system. Optimal conditions were as follows: pressure 130 bar, temperature 50 °C, agitation speed 200 rpm, enzyme concentration 20% and ratio of *C. rugosa* and *R. oryzae* lipases 1:1. Moreover, In stepwise reaction, when initial concentration of methanol was 90 mmol in reaction medium and then 90 mmol of methanol were added every 30 min during biodiesel production, conversion yield of biodiesel was over 99 % at 2 h. Finally, in a repeated biodiesel production process, biodiesel conversion was maintained at over 90 % after 10 reuses.