Optimization of 2nd generation biodiesel production through enzymatic transesterification of low quality acid oils

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Lipase-catalyzed transesterification of waste oils is considered a promising and environment-friendly method for the production of second generation (Gen2) biodiesel compared to the conventional alkali-catalyzed transesterification. This study focuses on the enzymatic production of Gen2 biodiesel from renewable feedstocks with high acidity, (e.g. acid oils and waste frying oil) using both low-cost commercial lipases and in-house novel biocatalysts. The impact of critical transesterification conditions (lipase load, water content, methanol to oil ratio, and pH) on fatty acid methyl esters (FAME) yield was evaluated and optimized by Response Surface Methodology (RSM). Other process parameters, such as the temperature, the duration of the reaction, and the mode of methanol addition (at once or stepwise), were also assessed. The optimal conditions were 42% w/w_{oil} enzyme load and 32% w/w_{oil} water content, 3:1 molar ratio of methanol to oil (three-step addition), and pH = 7.0. Under optimal conditions, the maximum FAME yield of 71.3±0.6% was achieved using Biolipasa R10000 lipase after 48h at 30°C. To increase the FAME yield, a combination of lipases that can synergistically catalyze the reaction was also studied. The results show that Lipozyme RM combined with Biolipasa IN displayed the best performance (90.1±0.4%). Apart from commercial enzymes, the LIP2 lipase from novel recombinant *Yarrowia lipolytica* strains was used to catalyze the transesterification reaction, either as free enzyme or displayed on the yeast cell surface (whole-cell biocatalyst - WCB). It was observed that the transesterification efficiency employing in-house produced enzymes, was lower (compared to commercial one), but quite promising (> 60%). WCB recycling, to reduce the production costs for industrial applications, was also tested through repeated transesterification cycles. These results are of great importance providing new perspectives for the development of an industrial enzymatic transesterification process with economic potential.