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# Production of biodiesel from rapeseed oil by porcine pancreatic mediated transesterification reaction in organic solvent

Mina Kia-Kojouri, Ghasem Najafpour Darzi*, Banin Rupani & Meghdad Mohammadpour
Biotechnology Research Lab., Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran
E-mail: najafpour8@gmail.com, najafpour@nit.ac.ir

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Enzymatic transesterification of rapeseed oil with methanol has been carried out in a batch process. Methyl ester is produced in the course of enzymatic reaction. The product is used as biofuel in diesel engine without any further post treatment. There is no serious emission of hazardous particulate matter, since raw material does not contain any impurities such sulfur dioxide. The reaction is carried out under various conditions and the desire operating parameters were determined. In this work the effects of enzyme concentration, methanol to oil molar ratio, water content, type of solvent, volumetric ratio of *t*-butanol and reaction temperature have been investigated. Maximum methyl ester conversion of 70.32% is obtained for enzyme 6% (w/w of oil), water 5% (v/v of oil) and methanol to oil ratio of 9:1 at 37°C for 72 h. In order to identify a suitable condition for biodiesel production, different solvents have been used**.** The highest methyl ester conversion is observed with *t*-butanol as moderate polar solvent.

**Keywords:** Biodiesel, Enzymatic transesterification, *t*-butanol, Porcine Pancreatic

Biodiesel is a clean-burning diesel fuel produced from vegetable oils, animal fats or grease. Its chemical structure is that of fatty acid alkyl esters (FAAE). Recently, depletion of petroleum resources[1,2], increase in petroleum price[3] and environmental pollution due to exhaustion of hazardous particulates from diesel engine[1] are among the factors that have encouraged researchers to find alternative for these fuels. Biodiesel as a renewable, biodegradable and nontoxic fuel is a suitable alternative diesel fuel which is produced by transesterification reaction[1,4]. The transesterification process of triglyceride can be accomplished in presence of an alcohol uses a chemical catalyst or bio catalyst for biodiesel production. In the course of the transesterification reaction, the molecules of tri-acylglycerols react with alcohol and form methyl esters of fatty acid and glycerol. The overall reaction of triglyceride transesterification with alcohol is shown in (Fig. 1)[5-8].

Chemical transesterification have some difficulties like removal of catalyst, excessive energy consumption, difficult to recover glycerol and high amount of alkaline waste water from the catalyst. Enzymatic transesterification of oil is a beneficial alternative to resolve the drawbacks of chemical transesterification[9-13] since in this process the recovery of glycerol and purification of fuel are easy[14]. In past decades, it has become more attractive for many researchers to conduct research on enzymatic route of biodiesel production[10,15-20]. The major problem for commercialization of enzymatic transesterification for the production of biodiesel is the high price of enzyme production. In order to reduce the price of this process, uses of immobilized lipases are recommended[1]. Another problem confronting the process is enzyme deactivation due to excessive methanol drops. The step wise addition of methanol to the reaction mixture in solvent-free system or use of organic solvents, such as *n*-hexane, *t*-butanol is desired to overcome this adverse effect[2].

The most important factor affecting on prices and profitability of biodiesel production is the price of raw material which accounts significant part of the total production costs. Therefore, researchers are focused on low-cost feed-stocks for the industrial production[21].Variety of feed-stocks can be used for the production of biodiesel. Vegetable oils and animal fats are the main feed-stocks for biodiesel production[22].The most commonly vegetable oils can be used as raw materials for the production of biodiesel such as soybean, sunflower, palm, rapeseed, cottonseed and jatropha. Choosing source of oil used for biodiesel production usually depends on the regional climate[23] and economic factors. In the

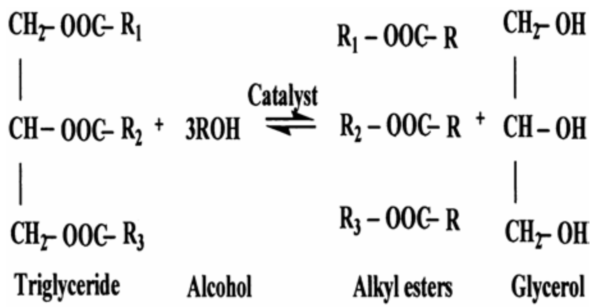

Fig. 1—The overall chemical reaction of triglyceride transesterification

Europe, the rapeseed is the most common source for the production of biodiesel[23,24] which is the third oil produced in the world. Several researchers have investigated biodiesel production from rapeseed oil using lipases[25-27].

The yield of biodiesel also depends on type of enzyme used in the process. For example, use of lipase immobilized *Pseudomonas fluorescens*, *P. cepacia*, *Mucor javanicus*, *Candida rugosa* and *Rubus niveus* by the enzymatic alcoholysis of soybean oil in similar conditions were reported in literature[10]. They have reported that immobilized *P. fluorescens* lipase showed the highest enzymatic activity among others. The use of enzyme is one the main economic factors affecting on price of production of biodiesel. Porcine pancreatic lipase is one of the low-cost free lipases which have high activity and thermo stability. Only a small number of studies on transesterification of oils by the porcine pancreatic lipase have been found in the literature[28,29]. In order to identify the optimal condition for the transesterification reaction of rapeseed oil using porcine pancreatic lipase; it was essential to perform the enzymatic reaction.

In present work, the transesterification reaction for the production of biodiesel from rapeseed oil and methanol using *t*-butanol as solvent, free porcine pancreatic lipase as catalyst in a batch system was studied. The effects of enzyme concentration, methanol to oil molar ratio, water content, type of solvent, volumetric ratio *t*-butanol to oil and reaction temperature were investigated.

The reported conversion of glyceride to methyl ester using porcine pancreatic lipase is 67% while the fatty acids released from position 1,3 are then converted to methyl ester[2]. There is an opportunity for lipase to interact with fatty acid in position 2; in that case the conversion can be enhanced. It was observed the highest methyl ester conversion exceeded the theoretical maximum value. In fact, it has been demonstrated that acyl group has migrated and formed extra methyl ester. The obtained results are validated at optimal conditions.

## Experimental Section

### Chemical

Refined rapeseed oil purchased from Behshahr Industrial Co. (Tehran, Iran). The fatty acid composition of this oil was reported by the manufacturer are as follow: palmitic acid (4.96%), stearic acid (2.23%), oleic acid (57.36%), linoleic acid (27.12%), linolenic acid (7.29%), gadoleic acid (1.04%). The average molecular weight of 880 was determined from oil content. Porcine Pancreatic lipase with 100 units/mg protein (using olive oil), palmitic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester and pentadecanoic acid methyl ester as the internal standard were obtained from Sigma Aldrich Co. (USA). Other analytical grade chemical materials were purchased from Merck Co. (Darmstadt, Germany).

### Production of biodiesel

The methanolysis reactions were carried out in a 100 mL screw-capped vessel and kept in an incubator shaker for sufficient time (72 h), at mild temperature (37°C) and gentle mixing (180 rpm). In order to avoid deactivation of lipase by methanol drops, methanol was initially mixed with *t*-butanol as a solvent then oil and free lipase solution added into the reaction mixture. Lipase solution was diluted in 0.5 M sodium phosphate buffer (*p*H 7) at 25°C. At the end of the reaction; samples were centrifuged to separate methyl ester on the top layer from by-product glycerol in lower layer. After evaporation of excess methanol and *t*-butanol in vacuum, the methyl ester conversion of the reaction mixture was analyzed using a gas chromatography (Aglient Technologies model 7890) equipped with a capillary column HP-5 (USA) (30 m × 0.25 µm × 0.32 mm) and flame ionization detector. Helium was used as a carrier gas at a flow rate of 32.12 mL/min. initially the column oven temperature was maintained at 100°C for 1min, raised to 180°C at 10°C/min, then raised to 200°C at 2°C/min, then raised to 203°C at 0.5°C/min and finally raised to 210°C at 2°C/min where it was maintained at this temperature for 2 min. The temperatures of the detector and injector were at 250 and 220°C, respectively. Pentadecanoic acid methyl ester was used as internal standard. The methyl ester conversion was determined in accordance to European standard test method of EN

1410[30,31]. The experiments were replicated and mean values were reported.

## Results and Discussion

### Effect of enzyme concentration on biodiesel conversion

The enzyme concentration is one of the most economical factors affecting on the methyl ester conversion for biodiesel production. In order to study the effect of enzyme concentration, the reaction was examined in the range of 1 to 7% (w/w of oil) enzyme concentration. Results showed the methyl ester conversion was enhanced by increasing of enzyme concentration but after that the methyl ester conversion became almost constant. This phenomenon explained with enhancement of enzyme concentration, enzyme active sites are more available to substrate. The availability of enzyme consequently increased surface mass transfer which caused high conversion of oil to methyl ester. In addition similar results were reported by other researchers[32-34]. The highest conversion was achieved with 6% enzyme which was desired enzyme concentration. From economic point of view, the highest percentage of the methyl ester (69.71%) was obtained when applying 6% (w/w of oil) enzyme concentration. This amount was used for subsequent experiments.

### Effect of water content on biodiesel conversion

The water content is an important parameter in bioconversions in non-aqueous media. The water content is an effective parameter on the lipase activity[35]. The effect of water on increasing trends of the methyl ester conversion was studied[26,32,36]. The water content in the reaction mixture depends on the oily source, the lipase and organic solvent used[37]. Figure 2 shows the effect of water content on production of methyl ester, ranging from zero to 20% (v/v of oil). It declares the reaction rate was low in absences of water molecule; as the water content increases up to 5% the methyl ester production increased up to 69.6%. When the water content from 5 to 20% increased, then conversion of methyl ester has severely decreased from 69.6 to 9.3%. This behavior declared that might be due to occurrence of some side-reactions. The esters hydrolysis reaction at high water content can lead to decrease the methyl ester conversion[37]. Therefore, the desired water content in the transesterification reaction should be defined the middle value between the minimizing hydrolysis reaction and maximizing enzyme activity for the transesterification reaction[18].

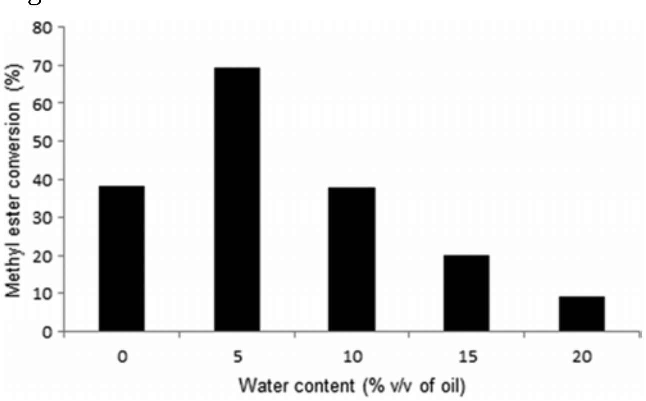


Fig. 2 — Effect of the water content on the methyl ester conversion. Reaction condition: enzyme concentration 6% (w/w of oil), methanol to oil molar ratio 9:1, *t*-butanol 0.75% (v/v of oil), reaction temperature 37°C

### Effect of molar ratio of methanol to oil on biodiesel conversion

The molar ratio of methanol to oil is one of the most important operational parameters on methyl ester conversion. Although, according to overall equilibrium of transesterification, stoichiometric ratio, the minimum molar ratio of 3 moles of methanol per mole of triglyceride are required[1]. Normally, the reaction is carried out in an excess amount of methanol in order to shift the equilibrium to get maximum conversion. However, extra methanol provides the high reaction conversion but attendance of insoluble methanol drops in the reaction mixture could decrease the enzyme activity. The stepwise addition of methanol or use of organic solvent is found to overcome this adverse effect. In this work, in order to verify the effect of molar ratios of methanol to oil reaction were experimented at 3:1 - 25:1 molar ratios. Figure 3 shows the increasing molar ratio of methanol, from 3:1 to 9:1, biodiesel production was

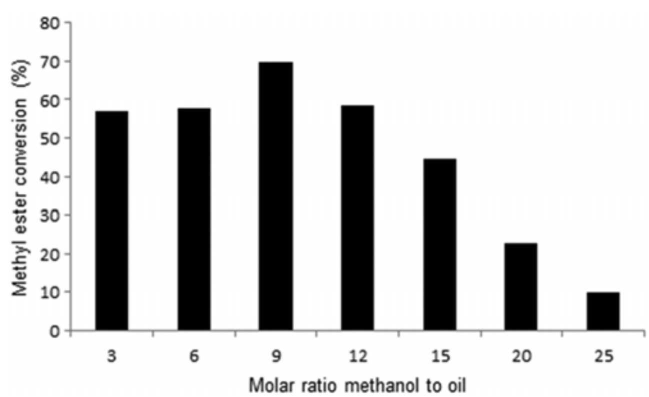


Fig. 3 — Effect of the methanol to oil molar ratio on the methyl ester conversion. Reaction condition: enzyme concentration 6% (w/w of oil), *t*-butanol 0.75% (v/v of oil), water content 5% (v/v of oil), reaction temperature 37°C

increased up to 69.84%. Further increase in molar ratio from 9:1 to 25:1, the conversion was decreased to 9.87%. It can explained that the solubility of water in oil increases at certain concentration of methanol and the reaction mixture well mixed and then it gets the reverse when the molar ratio reaches at certain value[21]. Then due to decrease in enzyme activities by excessive methanol drops in the reaction mixture caused to decrease to low values[38,39].

**Effect of temperature on biodiesel conversion**

The temperature plays a critical role in enzymatic transesterification. The rate of enzymatic transesterification reaction increases with an increase in reaction temperature but further increase in temperature beyond certain point can result in a decrease of the methyl ester conversion. Enzyme at higher temperature accelerates enzyme deactivation and also possibility of enzyme to be denatured. In this study, the effect of temperature on enzymatic transesterification at various temperatures of 30, 37, 45 and 55°C was investigated. Figure 4 shows the methyl ester conversion was increased with increasing the reaction temperature. Maximum methyl ester conversion was obtained at 37°C. As illustrated in this figure (Fig. 4), when reaction temperature was above 37°C; the methyl ester conversion has decreased due to loss of enzyme activities. This behaviour in transesterification reactions was reported by other researchers[40,41].

**Effect of solvent types on biodiesel production**

External surface of the enzyme can be coated by glycerol as by-product and inhibits production of biodiesel due to the presence of excessive methanol drops in the reaction. Organic solvents are suitable solution to overcome these adverse effects. The presence of organic solvent increases the solubility of substrates in the reaction mixture, operational stability of lipase and omits the cogency by adding methanol in several small portions[2]. So the methyl ester conversion can be affected by types of solvent used in the reaction. In this work, 3 types of solvent were used; *n*-hexane, acetone and *t*-butanol (Fig. 5a). When acetone as a solvent was used, the reaction conversion was the lowest value (1.4%); because of the polarity of the solvent that cannot solubilize the non-polar reactants like rapeseed oil. While *t*-butanol compared to other solvents resulted in maximum conversion. It showed that *t*-butanol as a moderately polar solvent can solubilize oil, methanol and glycerol in the reaction mixture. Therefore, various volumetric ratio of *t*-butanol as optimum solvent were tested. Figure 5b shows the methyl ester conversion increases

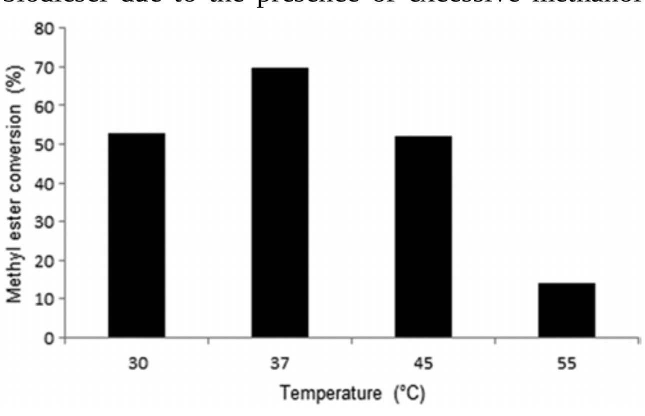


Fig. 4 — Effect of temperature on the methyl ester conversion. Reaction condition: enzyme concentration 6% (w/w of oil), water content 5% (v/v of oil), methanol to oil molar ratio 9:1 and *t*-butanol 0.75% (v/v of oil)

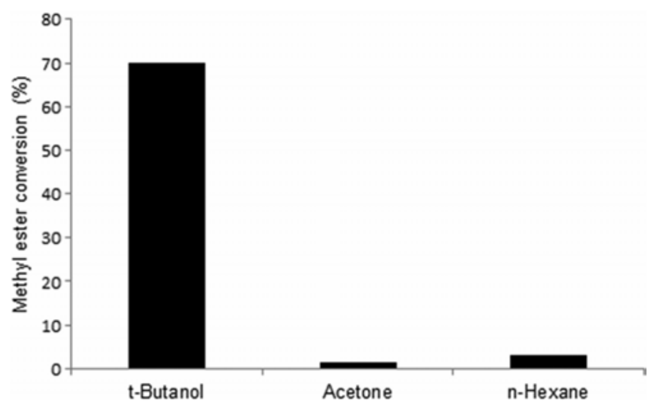


Fig. 5a — Effect of organic solvents on the methyl ester conversion. Reaction condition: enzyme concentration 6% (w/w of oil), methanol to oil molar ratio 9:1, water content 5% (v/v of oil), reaction temperature 37°C

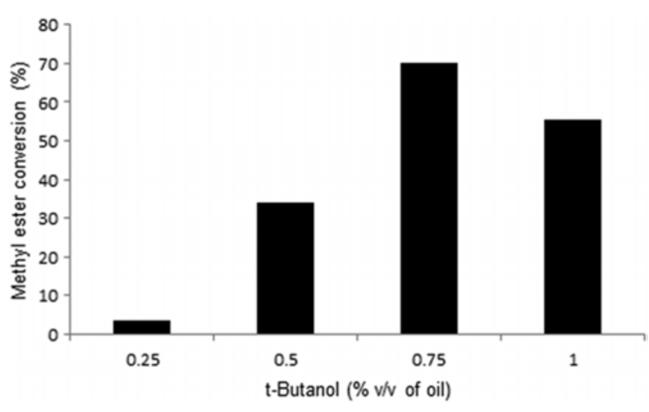


Fig. 5b — Effect of *t*-butanol to oil volumetric ratio on the methyl ester conversion. Reaction condition: enzyme concentration 6%, methanol to oil molar ratio 9:1, water content 5%, reaction temperature 37°C with increasing volumetric ratio of *t*-butanol upto 0.75% and when volumetric ratio of *t*-butanol was above 0.75%, the methyl ester conversion has decreased. The reason for this behavior might be due to reducing the substrates concentration in the reaction mixture caused by excess amount of *t*-butanol which leads to reducing interaction between substrates and enzyme[2]. The maximum conversion of methyl ester with 6% enzyme obtained was 70.32%.

#### Reaction conversion rate

The reaction conversion is an important issue in reactor performance. In order, to evaluate the rate of methyl ester production**,** the reaction was carried out at optimum conditions. The methyl ester conversion was measured at different reaction times from 12-72 h. Samples were analyzed in every 12 h. It was observed that at first 48 h, the reaction methyl ester conversion has increased then the production was slow down as the reaction progressed and finally reached to equilibrium state.

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