EVALUATION OF THE OPTIMAL REACTION CONDITIONS FOR THE METHANOLYSIS AND ETHANOLYSIS OF CASTOR OIL CATALYZED BY IMMOBILIZED ENZYMES

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ABSTRACT: As an alternative to the use of chemical catalysts, immobilized enzyme Lipozyme 435 was evaluated as catalyst for biodiesel production, comparing its efficiency in the castor oil transesterification with methanol and ethanol. Different reaction conditions were assessed and optimized, including the reaction temperature (35 – 60 °C), alcohol-to-oil molar ratio (from 3:1 to 6:1), amount of catalyst (from 3 to 15 wt% by weight of oil), addition of water (0 – 15 wt%), and use of n-hexane as a solvent (0 – 75 wt%). For the transesterification with methanol, the optimal reaction conditions were 3:1 methanol-to-oil molar ratio, 5 wt% of enzymes, 7.5 wt% of water, 50 wt% n-hexane, at 50 °C. The fatty acid methyl esters content was 96.8 % and 1.0 % FFA. Regarding the reactions with ethanol, 98.0 % fatty acid ethyl ester was obtained and 1.3 % FFA, when the reaction was carried out at 60 °C, 4:1 ethanol-to-oil molar ratio, 5 wt% of enzymes, 40 wt% of n-hexane and no addition of water.
Keywords: biodiesel, enzymatic process, castor oil, transesterification.

1 INTRODUCTION

Biodiesel is a non-toxic, renewable and biodegradable alternative fuel that can reduce the energy dependence on fossil fuel sources. Biodiesel is obtained by the transesterification of triglycerides (TAG) derived from vegetable oil or animal fats with short chain alcohols, generating fatty acid alkyl esters (biodiesel) and glycerol, usually through chemical-catalyzed reaction routes, which include alkali and acid catalysts [1,2].

Despite the high conversion, short reaction time and reduced catalysts costs, have several drawbacks, such as the need of wastewater treatment, difficulties with glycerol recovery and, in the case of alkali catalysts, sensitivity to the presence of free fatty acids (FFA) and water. These disadvantages suggest the replacement of the chemical catalysts. The enzyme-catalyzed route has emerged as a competitive greener alternative [3–5]. This route has a higher compatibility with different raw materials, a low sensitivity to water and FFA content, and requires fewer process steps, providing a better separation of the products. The stability and ease of recovery of immobilized enzymes for reuse can compensate the higher costs of this catalyst [6]. In enzyme-catalyzed transesterification, it is generally recommended to use a solvent, such as n-hexane and tert-butanol, in order to reduce mass transfer limitations [7,8].

Used or virgin vegetable oils are the most attractive raw materials for biodiesel production as consequence of their environmental benefits and renewability [9]. Non-edible castor oil is an attractive option due to its low cost and ability to be cultivated under arid and diverse weather conditions. Compared to other triglycerides sources, castor oil provides a better homogeneity of the reaction medium, due to its higher polarity. This feature is a consequence of the castor oil composition. Castor oil is essentially composed of ricinoleic acid (between 80 and 90 %). This fatty acid includes a hydroxyl group in its molecular chain [10–12].

Methanol and ethanol are commonly used as acyl acceptor for the transesterification. Due to the low cost, high availability and easier production process, methanol is generally chosen for the reaction, producing fatty acid methyl esters (FAME). The use of ethanol, producing fatty acid ethyl esters (FAEE), prevails in regions where ethanol is cheaper than methanol. Besides that, in many complexes biorefineries, the production of biodiesel is associated to simultaneous production of bioethanol.

Integration of these processes is reported to save expenses with the purchase of raw materials, which promote the use of ethanol [13–15].

Many studies have already been reported evaluating the optimal reaction conditions for the enzymatic transesterification of triglycerides. Adewale et al. (2017) optimized the biodiesel production from crude tall oil with methanol in presence of the lipase Eversa Transform using the Taguchi optimization method. A predicted 97.02 % biodiesel yield was obtained for a 16 h reaction time, at 40 °C [16]. Navarro López et al. (2016) obtained an 83 % FAME conversion by transesterification of saponifiable lipids extracted from wet Nannochloropsis gaditana biomass with methanol catalyzed by Rhizopus oryzae, using n-hexane as solvent [17]. Lipases from Candida antarctica (CALB), Thermomyces lanuginosus (TLL) and Rhizomucor miehei (RML) were investigated by Babaki et al. (2015) for the methanolysis of canola oil.

They obtained a conversion of 98% FAME using enzymes from TLL after 96 h, observing a high enzyme stability and reusability [6]. Baron et al. (2014) evaluated the ethanolysis of castor oil in a solvent-free system using immobilized enzyme from Burkholderia cepacia LTEB11, reaching a 90 % conversion of FAEE in 6 hours of reaction [18]. Assessment of castor oil methanolysis in a free solvent system was done by Andrade et al. (2017) using liquid lipase Eversa Transform. 94 % FAME yield was obtained at 35 °C after 8 hours reaction [19].

In the present work, it is proposed to transesterify castor oil using immobilized Lipozyme 435 as catalyst.

The optimal reaction conditions were obtained and methanolysis and ethanolysis of castor oil were compared. The reaction temperature, alcohol-to-oil molar ratio, enzyme content, and amount of water and n-hexane were evaluated. The study further investigates the final concentrations in the biodiesel-phase, maximizing the reaction yield.

2 MATERIALS AND METHODS

2.1 Materials

Ecological castor oil was purchased from Urtegaarden ApS (Denmark). Novozymes A/S

(Denmark) kindly donated the immobilized enzyme Lipozyme 435. Methanol, ethanol, n-hexane, acetonitrile, and isopropanol of HPLC grade were purchased from Sigma-Aldrich. Ethanol 96 % (v/v) was acquired from VWR. HPLC calibration standards including methyl esters (ricinoleate, linoleate and oleate), ethyl esters (linoleate and oleate), and fatty acids (ricinoleic, linoleic and oleic acid) of a 99% purity grade were also acquired from Sigma-Aldrich. Standards of tri-, di-, and monoglycerides, as well as ethyl ricinoleate were obtained by transesterification and separation on a preparative HPLC.

2.2 Transesterification reactions

The reactions were carried out in a 100 mL round-bottom flask equipped with a water-cooled condenser system. The reactor was immersed in a thermostat oil bath and equipped with a magnetic stirrer. Castor oil, enzyme, water and n-hexane were weighed into the flask and heated up to the reaction temperature. Alcohol (methanol or ethanol) was added to the system in four stepwise additions at two-hour intervals, in order to minimize alcoholic enzyme inhibition. The reactions were carried out at 750 rpm, for 8 hours, to ensure equilibrium was reached. Figure 1 shows the set-up built for the transesterification reaction.

Figure 1: Transesterification reaction set-up

Five reaction variables selected to be optimized were: the reaction temperature (35, 50 and 60 °C); the amount of enzyme (varying from 3 to 15 wt% by weight of castor oil); the alcohol-to-oil molar ratio (between 3:1 and 6:1); the addition of water (0-15 wt% by weight of castor oil); and the addition of the solvent n-hexane (0 – 75 wt% by weight of castor oil).

The set of experiments performed for the castor oil methanolysis, at different conditions is shown in Table I.

The table includes the alcohol-to-oil molar ratio, amount of enzyme [E], water [W] and n-hexane [n-H], and reaction temperature T. Reactions from E1 to E20 made use of ethanol 96%. Reactions E21 and E22 used absolute ethanol as acyl acceptor. Table II presents the set of experimental reactions for the castor oil ethanolysis.

Table I: Experimental conditions for the enzymatic methanolysis of castor oil

Reaction	Methanol-to-Oil	[E] (wt%)	[W] (wt%)	[n-H] (wt%)	T (°C)
M1	6.0:1	3.0	5.0	0.0	50
M2	6.0:1	5.0	5.0	0.0	50
M3	4.5:1	5.0	5.0	0.0	50
M4	3.0:1	5.0	5.0	0.0	50
M5	3.0:1	5.0	7.5	0.0	50
M6	3.0:1	5.0	10.0	0.0	50
M7	3.0:1	5.0	15.0	0.0	50
M8	3.0:1	5.0	5.0	25.0	50
M9	3.0:1	5.0	0.0	25.0	50
M10	3.0:1	5.0	5.0	50.0	50
M11	3.0:1	5.0	7.5	15.0	50
M12	3.0:1	5.0	7.5	40.0	50
M13	3.0:1	5.0	7.5	50.0	50
M14	3.0:1	5.0	0.0	15.0	35
M15	3.0:1	5.0	0.0	15.0	60

Table II: Experimental conditions for the enzymatic ethanolysis of castor oil

Reaction	Ethanol-to-Oil	[E] (wt%)	[W] (wt%)	[n-H] (wt%)	T (°C)
E1	6.0:1	3.0	5.0	0.0	50
E2	6.0:1	5.0	5.0	0.0	50
E3	4.5:1	5.0	5.0	0.0	50
E4	3.0:1	5.0	5.0	0.0	50
E5	3.0:1	7.0	5.0	0.0	50
E6	3.0:1	10.0	5.0	0.0	50
E7	3.0:1	15.0	5.0	0.0	50
E8	3.0:1	10.0	7.5	0.0	50
E9	3.0:1	10.0	10.0	0.0	50
E10	3.0:1	10.0	15.0	0.0	50
E11	4.0:1	5.0	7.5	25.0	50
E12	4.0:1	5.0	7.5	50.0	50
E13	4.0:1	5.0	7.5	75.0	50
E14	4.0:1	5.0	7.5	0.0	50
E15	4.0:1	5.0	10.0	0.0	50
E16	6.0:1	5.0	10.0	0.0	50
E17	4.0:1	5.0	0.0	15.0	50
E18	4.0:1	5.0	0.0	25.0	50
E19	4.0:1	5.0	0.0	40.0	50
E20	4.0:1	5.0	0.0	40.0	35
E21	4.0:1	5.0	0.0	40.0	50
E22	4.0:1	5.0	0.0	40.0	60

2.3 Sample preparation and analysis

Reaction samples were collected after 8 hours of reaction and centrifuged at 6000 rpm for 5 min in a Spectrafuge™ Mini Laboratory Centrifuge to separate oil, glycerol and enzyme phases. Samples from the oil phase were diluted in a 4:5 w/w hexane to isopropanol solution, mixed thoroughly in a vortex mixer, and filtered through a Whatman 0.2 mm filter unit into small vials.

An Agilent 1200 Series High-Performance Liquid Chromatography (HPLC) system with UV detection at 205 nm equipped with a Phenomenex Luna C18 column (particle size 3 µm, 150 x 4.60 mm) was used to quantify the product composition. The column temperature was set at 25 °C. A three-gradient system (A - acetonitrile, B - water and C - 4:5 w/w hexane to isopropanol solution) was used as a solvent mixture at a flow rate of 0.8 mL/min, with a runtime of 35 minutes for each analysis.

The mobile-phase employed the following steps: 80% A + 20% B in 0 min, 100% A in 10 min, 70% A + 30% C in 22 min, isocratic elution until 30 min, followed by 80% A + 20% B in 35 min. The composition of fatty acid alkyl esters, tri-, di-, monoglycerides, and free fatty acids in the oil phase were calculated based on the generated calibration curves. Calibration curves were carried out in triplicate to ensure the accuracy of the measurements.

3 RESULTS AND DISCUSSION

3.1 Identification of fatty acid alkyl esters

Determination of the castor oil conversion into fatty acid alkyl esters was obtained from the HPLC analysis. Figure 2 shows the main fatty acid alkyl esters obtained from the methanolysis of castor oil. The highest peak at 8.0 minutes corresponded to methyl ricinoleate, while the peak at 14.5 minutes referees to methyl linoleate. Predominant fatty acid alkyl esters from the ethanolysis of castor oil are shown in Figure 3. The elution time of ethyl ricinoleate was around 10.0 minutes, while 15.8 minutes was the time necessary for ethyl linoleate to pass through the chromatographic column. In both Figure 2 and Figure 3, the peak at 4.2 minutes corresponded to the elution time of ricinoleic acid.

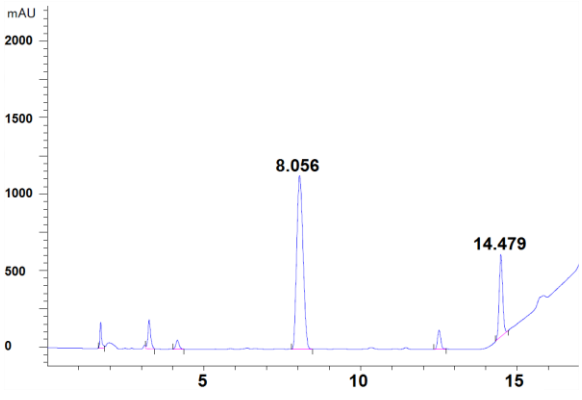

Figure 2: FAME peaks chromatogram

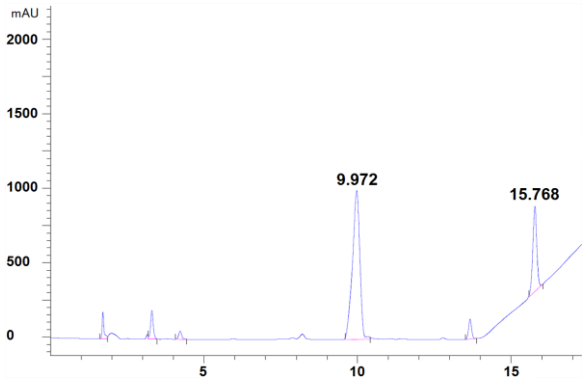

Figure 3: FAEE peaks chromatogram

3.2 Influence of the reaction conditions on the transesterification

Results for the methanolysis and ethanolysis of castor oil are presented in Table III and Table IV, respectively.

The tables show the composition of unreacted oil (TAG), fatty acids alkyl esters (FAME or FAEE) and FFA in the biodiesel phase. The remaining parts of the mixture was composed by the intermediate products di- and monoglycerides.

For the castor oil methanolysis, the highest biodiesel production was obtained when the reaction was performed at 50 °C, 3:1 methanol-to-oil molar ratio, and additions of 5.0 wt% enzyme, 7.5 wt% water and 50.0 wt% of n-hexane, by weight of castor oil (M13). For this set of conditions, 96.8 %, in mass, of the biodiesel phase corresponded to FAME, while only 1.0 % referred to FFA.

In case of the ethanolysis of castor oil, the best set of conditions corresponded to reaction E22, using absolute ethanol: 4:1 ethanol-to-oil molar ratio, additions of 5.0 wt% enzyme, 40.0 wt% of n-hexane, by weight of castor oil, at 60 °C and with no addition of water, resulting in 98.0 % FAEE and 1.3 % FFA.

Table III: Experimental results obtained for FAME production

Reaction	TAG (%)	FAME (%)	FFA (%)
M1	15.3	75.4	1.5
M2	18.2	73.0	3.7
M3	18.1	73.7	6.0
M4	17.4	72.7	8.3
M5	19.2	70.9	8.1
M6	18.3	71.7	8.8
M7	18.6	69.1	11.0
M8	19.0	71.2	7.9
M9	2.9	91.6	2.4
M10	20.3	72.9	6.2
M11	2.0	94.6	1.5
M12	1.5	95.9	1.4
M13	1.4	96.8	1.0
M14	5.7	89.6	1.2
M15	0.9	92.5	1.4

Table IV: Experimental results obtained for FAEE production

Reaction	TAG (%)	FAEE (%)	FFA (%)
E1	69.8	16.4	10.7
E2	38.4	49.2	9.6
E3	41.7	47.5	8.8
E4	40.3	52.9	6.8
E5	21.3	63.0	14.3
E6	13.1	74.3	11.6
E7	9.4	73.3	16.4
E8	15.7	66.5	16.7
E9	17.0	66.5	15.4
E10	19.7	62.2	18.1
E11	41.8	47.2	8.8
E12	39.2	50.3	8.0
E13	42.8	41.0	13.7
E14	29.9	56.1	12.0
E15	28.7	57.4	12.3
E16	33.2	53.5	11.3
E17	8.8	84.2	5.5
E18	9.2	83.9	5.1
E19	9.2	84.7	4.2
E20	19.8	73.3	4.1
E21	2.4	95.8	0.8
E22	0.7	98.0	1.3

Influence of the individual conditions on the biodiesel yield was evaluated.

3.2.1 Influence of enzyme content

The impact of the amount of immobilized enzyme added in the reaction medium was evaluated for the ethanolysis of castor oil. Figure 4 represents the behavior of the biodiesel phase composition when the other variables remained unchanged. Favorable influence on the biodiesel yield was observed when the added enzyme amount increased from 5 to 10 wt%. The content of FFA was also observed. In the presence of water, enzymes also hydrolyzed the castor oil, producing free fatty acids that were subsequently esterified to produce biodiesel.

However, because of the high cost of enzymes compared to chemical catalysts, high enzyme concentrations are not recommended. Increase in the biodiesel yield was also observed when comparing reactions E1 and E2. Increase of the enzyme content from 3 to 5 wt% tripled the FAEE content in the biodiesel phase.

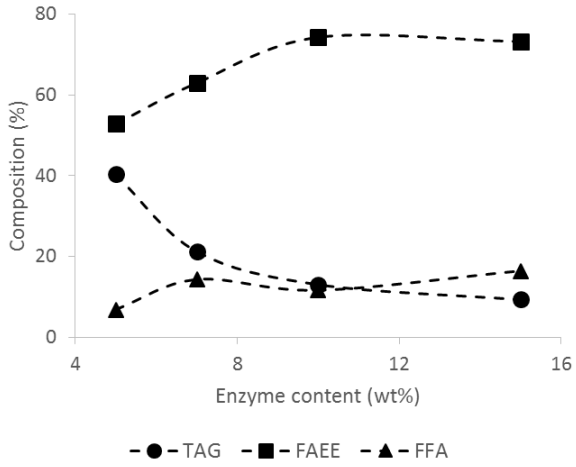

Figure 4: Influence of enzyme content on FAEE yield for enzymatic ethanolysis of castor oil for reactions at 50 °C, with addition of 5 wt% water, 3:1 alcohol-to-oil molar ratio and no presence of n-hexane

3.2.2 Influence of the temperature

Experiments were made at temperatures within the typical temperature range for Lipozyme 435 [20].

Temperatures above 60 °C may cause enzyme denaturation, which decreases the biodiesel yield.

Reactions E19 and E20 were carried out at the same reaction conditions, differing only in the reaction temperature: 50 and 35 °C, respectively. While E19 obtained a biodiesel content of 84.7 % in the biodiesel phase, this value reduced to 73.3 % when the temperature was lower. The same behavior was observed when absolute ethanol was used in reactions E21 and E22. When the temperature was raised from 50 to 60 °C, biodiesel content increased from 95.6 to 98.0 %.

3.2.3 Influence of the water content

Addition of water to the reactor provides a larger water-lipid interaction that can activate the enzyme [21]. Figure 5 represents the behavior of the biodiesel phase composition for the ethanolysis of castor oil according to the amount of water added in the reactor. Increase in the amount of water added resulted in higher hydrolysis rate of castor oil, leading to an increase in the FFA content while the biodiesel content decreased.

Reactions E11 and E18 were carried out using the same reaction conditions except for the added amount of water. When 7.5 wt% water was added into the reactor, FAEE and FFA contents were 47.2 and 8.8 %, respectively. With no addition of water into the reactor, the FAEE content increased to 83.9 % and the FFA content decreased to 5.1 %, leading to higher transesterification and lower hydrolysis yield. Even though water was not added into the system in reaction E18, ethanol 96 % contains 4% water, resulting in the formation of FFA.

Therefore, reactions E19 and E21 compared the use of ethanol 96 % and absolute ethanol. In the absence of water in the ethanol solution, the FAEE content increase from 84.7 to 95.8 %. The presence of FFA in the product from reaction E21 is probably due to the possible presence of water in the castor oil. Based on that, it is recommended not to add water for the ethanolysis of castor oil using Lipozyme 435 as catalyst.

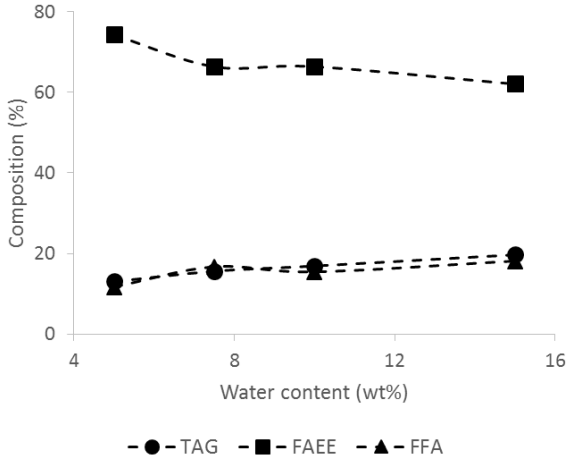

Figure 5: Influence of water content on FAEE yield for enzymatic ethanolysis of castor oil for reactions at 50 °C, with addition of 10 wt% enzymes, 3:1 alcohol-to-oil molar ratio and no presence of n-hexane

The influence of water addition on the methanolysis of castor oil is shown in Figure 6. The FAME content remained nearly constant between 69 and 71 % when the added amount of water ranged from 7.5 to 15 wt%.

However, increase in FFA formation from castor oil hydrolysis was observed. Similar behavior was observed when reactions M8 and M9 were performed, with addition of 25 wt% of n-hexane. The FAME content increased from 71.2 to 91.6 % when water addition decreased from 5 % to no addition. However, when the solvent content was set at 50 wt% (reaction M10 and M13), the FAME content increased from 72.9 to 96.8 % when the water content increased from 5 to 7.5 wt%.

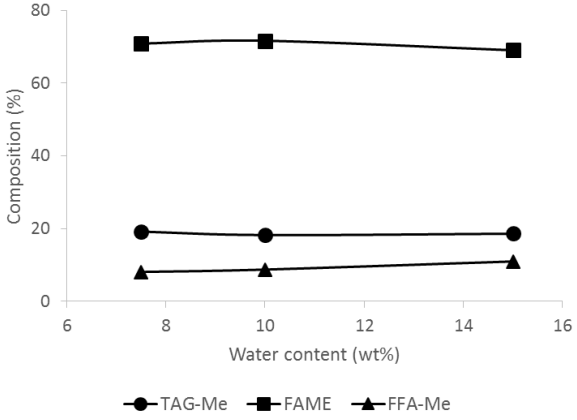

Figure 6: Influence of water content on FAME yield for enzymatic methanolysis of castor oil for reactions at 50 °C, with addition of 5 wt% enzymes, 3:1 alcohol-to-oil molar ratio and no presence of n-hexane

3.2.4 Influence of the alcohol-to-oil molar ratio

Figure 7 shows the variation in composition of the biodiesel phase after the methanolysis and ethanolysis, at different alcohol-to-oil molar ratios. Three moles of alcohol are necessary to react one mole of triglyceride. In the transesterification reactions, excess of alcohol favors the esterification of fatty acids, increasing the reaction rate.

However, methanol and ethanol at high concentrations lead to the enzyme inhibition, which decreases the production of biodiesel. According to Figure 7, in both methanolysis and ethanolysis the fatty acid alkyl esters content suffered small variations, indicating that the enzyme inhibition was compensated by the increase of the reaction rate. Reactions E15 and E16 showed that at lower ethanol-to-oil molar ratio the biodiesel conversion is higher, obtaining 57.4 and 53.5 % of FAEE when the molar ratio was 4:1 and 6:1, respectively. Lower concentrations of alcohol also results in a reduction in the manufacturing costs.

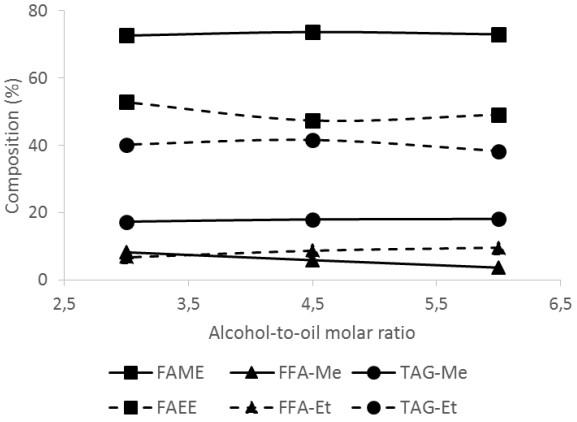

Figure 7: Influence of alcohol-to-oil molar ratio on FAME and FAEE yield for enzymatic alcoholysis of castor oil for reactions at 50 °C, with addition of 5 wt% enzymes, 5 wt% water and no presence of n-hexane

3.2.5 Influence of n-hexane content

The use of n-hexane to reduce mass transfer limitations and increase the biodiesel conversion was evaluated. Figure 8 shows the behavior of the biodiesel content when different solvent concentrations are used for the methanolysis of castor oil. A significant increase in the FAME content was observed comparing the system without n-hexane and with addition of 20 wt%. At lower ratios, a continuous increase in the FAME yield was noticed with increase in the n-hexane content in the reactor, reaching 96.8 % FAME when 50 wt% n-hexane was added.

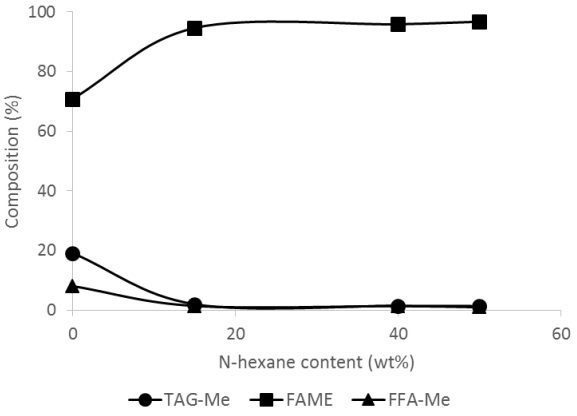

Figure 8: Influence of n-hexane concentration on FAME yield for enzymatic methanolysis of castor oil for reactions at 50 °C, with addition of 5 wt% enzymes, 7.5 wt% water and 3:1 alcohol-to-oil molar ratio

Although the reaction conditions individually affect the biodiesel yield, different combinations of these conditions can also result in different behaviors in the biodiesel yield. This feature was observed when different contents of water and n-hexane were added. In the castor oil ethanolysis, when the reactor contained 7.5 wt% water (reactions E11 to E14), presence of n-hexane resulted in a negative effect on the biodiesel conversion, with 56.1, 50.3 and 41.0% FAME content when n-hexane additions of 0, 50 and 75 wt% were respectively used. For the methanolysis of castor oil, the FAME content remained at 70 % when no h-hexane was added into the reactor and the water content varied from 5 to 15 wt% (reactions M4 to M7). Similar FAME content was observed for the reactions in which the water content remained at 5 wt% and n-hexane was increased from 0 to 50 wt% (reactions M4, M8 and M10).

4 CONCLUSIONS

Reactions conditions of the castor oil methanolysis and ethanolysis catalyzed by immobilized enzyme Lipozyme 435 were evaluated and optimized. Optimal contents of 96.8 and 98.0 % were obtained for FAME and FAEE, respectively. Both reactions performed better at low alcohol-to-oil molar ratios (3:1 in case of methanol and 4:1 in the use of ethanol), since high alcohol concentrations can result in enzyme inhibition. The optimal enzyme content was 5 wt% in both cases, with no need of water addition in the ethanolysis and 7.5 wt% water being optimal for the methanolysis. Methanolysis performed best at 50 °C, with 50 wt% n-hexane, while ethanolysis performed best at 60 °C and 40 wt% n-hexane.

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