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Combining Enzymatic Esterification with Conventional Alkaline Transesterification in an Integrated Biodiesel Process

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Abstract An integrated biodiesel process that combines enzymatic esterification and alkaline transesterification is suggested. With focus on the enzymatic step, the paper provides proof of concept and suggestions for further process development. Hence, palm fatty acid distillate (PFAD) has been enzymatically converted to fatty acid methyl esters in a two-step process using the immobilized lipase Novozym 435 in packed-bed columns. With only a small excess of methanol, the first reaction stage could reduce the free fatty acid (FFA) content from 85% to 5%. After removal of water by simple phase separation, it was possible to lower the FFA content to 2.5% in a second reaction stage. Both reaction stages are relatively fast with suggested reaction times of 15 min in column 1 (productivity 10 kg/kg/h) and 30 min in column 2 (productivity 5 kg/kg/h), resulting in 15% FFA after column 1 and 5% FFA after column 2. A lifetime study indicated that approximately 3,500 kg PFAD/kg Novozym 435 can be treated in the first reaction stage before the enzyme has become fully inactivated. With further optimization, the enzymatic process could be a real alternative to today's sulfuric acid catalyzed process.

Keywords Fatty acid methyl ester (FAME) · Biodiesel · Palm fatty acid distillate (PFAD) · Enzyme · Lipase · Novozym 435 · Esterification · Packed-bed reactor (PBR)

Introduction

Vegetable oils from palm, rapeseed, and soya beans, which together account for most of the worldwide production, are under increased pressure as feedstocks for biodiesel. This is due to both economical (high prices of commodity food oils) and ethical (food versus fuel

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debate) reasons. Therefore, the use of low-value and non-edible feedstocks for biodiesel production is gaining interest. The use of Jatropha oil [1, 2], algae oil [3], and oil from other exotic sources [4] is already described in literature. Also used cooking oils and tallow are potential sources for biodiesel production [5]. All of the above oils consist mainly of triglycerides and can, after purification, be converted into fatty acid alkyl esters with the conventional alkali-catalyzed transesterification process [6].

Crude vegetable oils contain certain impurities, such as free fatty acids and phosphatides. These impurities should be removed in order to improve stability, nutritional and organoleptic quality, and/or fuel properties of the oil. Refining can be done either chemically (neutralization, bleaching, and mild deodorization) or physically (degumming, bleaching, and stripping). During chemical refining, soapstock is produced as a sidestream, which can be acidulated into acid oil (AO). During physical refining, free fatty acid (FFA) is removed in the deodorization unit to produce the fatty acid distillate (FAD), which typically contains between 80% and 95% FFA and some minor components such as tocopherols and sterols [7]. This acid-rich fraction is relatively pure and contains less phosphatides and other elements compared to AO. Further, FAD and AO are byproducts and are significantly less expensive than the corresponding virgin plant oils.

Palm oil usually contains between 2.5% and 5% of FFA, depending on the fruit ripeness at harvesting. For crude palm oil with low phosphatides, high initial FFA content (up to 5%) and high carotene content, physical refining is preferred. Over 95% of the crude palm oil in Malaysia is refined through the physical route [8]. Palm FAD (PFAD) is the most abundantly available acid feedstock, with an estimated yearly production of approximately 1.8 million tons [9]. This, in combination with the non-food status has spurred interest in the conversion of palm FAD into higher-value products, including biodiesel [10].

Acid feedstocks cannot be converted to fatty acid alkyl esters via the conventional alkaline catalyzed transesterification process. When alkali is added to these feedstocks, the FFA will react with the catalyst to form soaps; however, FFA can be converted into fatty acid methyl esters (FAME) by an acid catalyst. Sulfuric acid is most often used. The resulting FAME product does typically not meet biodiesel specifications directly, but needs further reaction or workup. Alternatively, in an integrated process, crude oil can be deodorized and the FAD stream de-acidified by converting it to FAME before it rejoins the deodorized oil stream. The combined streams are now sufficiently low in FFA to enter the alkaline catalyzed transesterification reaction (Fig. 1) [9].

Sulfuric acid is a problematic catalyst for a number of reasons, including its corrosiveness (need for high-quality steel in the construction materials) and the need to remove residual sulfuric acid to meet the low-sulfur specification of biodiesel. Lately, a number of acidic, heterogeneous catalysts have been developed, such as the Amberlyst BD20 (Rohm and Haas, Philadelphia, PA) [11] and Lewatit GF 101 (Lanxess, Leverkusen, Germany). These offer a number of advantages compared to the homogenous sulfuric acid catalysis, but still require a high methanol (MeOH) excess (typically 5 to 10 MeOH/FFA molar equivalents) and somewhat elevated temperatures (up to 90 °C) and hence pressures (more than 3 bar). The need for a high MeOH excess has a negative impact on the overall process economy, since all MeOH needs to be dried before it can be reused.

As another approach, enzymatic catalysis has been suggested. Biodiesel production from triglycerides by enzyme catalyzed transesterification has been described in a number of publications, employing a wide range of different feedstocks [12]. Enzymes, in this case lipases, operate under very mild conditions (30–50 °C, no harsh chemicals) and with

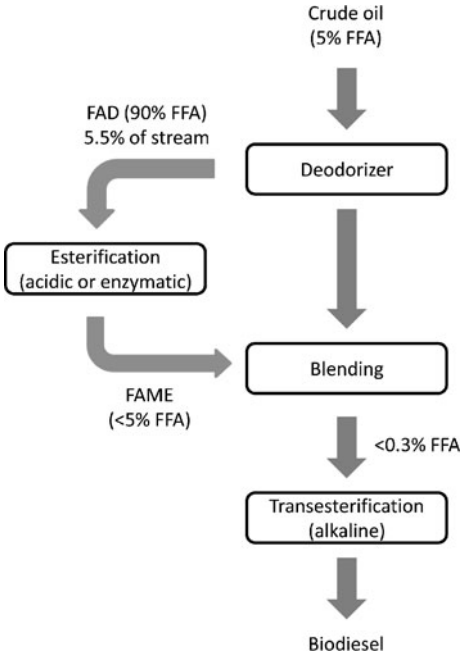

Fig. 1 Schematic flow diagram showing how PFAD is produced, esterified, and recirculated to the neutral stream during biodiesel production

approximately stoichiometric amounts of MeOH. Actually, high concentrations of MeOH can lead to enzyme inactivation. This negative effect, however, appears to be most severe with insoluble MeOH [13]. With high-FFA feedstocks, such as FAD, MeOH is much more soluble [14]. With immobilized enzymes, the process can ideally be done in packed-bed columns for continuous operation. Packed-bed columns offer a compact design with easy process control and have been successfully implemented for enzymatic interesterification of vegetable oils [15].

The Novozym 435 catalyzed conversion of FAD to FAME has recently been explored by Rahman Talukder et al. [16] and Du et al. [14]. In the former study, Novozym 435 is compared to Amberlyst 15 for the acid esterification of PFAD. The Novozym 435 enzyme is an immobilized *Candida antarctica* B-lipase [17] that is well-described in the scientific literature for catalyzing a range of biocatalytical reactions, including several experimental biodiesel applications. The authors concluded that the immobilized enzyme has 50-fold higher specific activity while end conversions were 95% and 97% for Novozym 435 and Amberlyst 15, respectively. In the paper by Du et al., soybean FAD was used as feedstock, and it is found that the enzyme is relatively stable towards MeOH dissolved in this substrate. Again, 95% end conversion is reported, in this case obtained by addition of molecular sieves. In addition, Watanabe and coworkers have, in a series of papers, described conversion of AO to biodiesel using Novozym 435 [18].

In this paper, a process for esterification of PFAD using Novozym 435 is described. The objective is to develop a process that can be used as a plug-in alternative to the sulfuric acid catalyzed esterification that is currently installed in many biodiesel plants. Hence, the enzymatic step is not a stand-alone biodiesel process, producing EN-14214/ASTM D6751 biodiesel. The target was merely to lower the FFA value to less than 5%. The resulting product can then typically be blended with the deodorized oil and continue through to alkaline transesterification with <0.3% total FFA (Fig. 1).

Experimental Procedures

Materials and Methods

The PFAD was obtained from a local palm oil refining plant. All other chemicals were purchased from Sigma-Aldrich. A dropping point cell instrument from Mettler Toledo was used to measure melting points. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury-Vx 400-MHz instrument at 25 °C. Glass columns used for packed-bed reactors when packed with immobilized enzyme were of the brand "Omnifit" and purchased through Sigma-Aldrich. The content of microelements (phorsphorus, calcium, magnesium, sodium, potassium, and iron) was determined via inductively coupled plasma (ICP) spectroscopy (iCAP 6000 series, Thermo Scientific, Zellik, Belgium).

^{1}H NMR analyses were performed on a small sample of reaction mixture (20 μL) in $CDCl_3$ (0.7 mL). Percent FAME was calculated as the ratio of the $-OCH_3$ integral (s, 3.65 ppm) to the $-CH_3$ integral (t, 0.85 ppm) and hence expresses the molar ratio of FAME out of all fatty acid. FFA titrations were conducted after evaporating a product sample (approximately 2 mL) on rotary evaporator to remove MeOH and water. The evaporated sample was then weighed into a conical flask and dissolved in 2-propanol (iPrOH, 10 mL). Indicator (0.5 mL of 1% phenolphthalein in iPrOH) was added and the solution titrated with 0.1 M aqueous NaOH to a purple color. Percent FFA (as oleic acid) was calculated as $c(\mathrm{NaOH}) \times V(\mathrm{NaOH}) \times M(\text{oleic acid})/m(\text{sample}) \times 100\%$. The gas chromatographic (GC) analysis of fatty acid composition of the PFAD was performed according to the EN14105 official method. Hence, a quantity (0.1 g) of sample was spiked with internal standards and silylated. The derivatized sample was then diluted with n-heptane (HPLC grade, 50 μL derivatized mix in 1.5 mL heptane) and separated on a 5890 Series II Plus Hewlett Packard gas chromatograph, equipped with an on-column injection system, a flame ionization detector and a DB-5HT capillary column (15 m×0.32 mm i.d., 0.10 μm film thickness) from Agilent Technologies. The calculations were done identical to the method described in EN14105.

Enzymatic Reactions

Enzymatic batch reactions were performed in 2 mL Eppendorf tubes using a Thermomixer adjusted to 45 °C, 1,200 rpm. PFAD (1 mL) was added to Novozym 435 (20 mg) followed by MeOH (1.5 eq=0.19 mL/mL; 6 eq=0.77 mL/mL). For column experiments in the "aquarium reactor", the 25×1 cm glass column was packed with Novozym 435 (5 g or 1 g), followed by addition of glass beads to fill out any void volume. The "aquarium" was thermostated at 45 °C and the substrate mixture was pumped upflow through the column. Substrate (for the first reaction stage) was made from PFAD and MeOH, using 0.2 mL MeOH per mL PFAD. Volumetric flow was determined by collection of the product mixture in a measuring cylinder. For the second reaction stage, a new column was packed with Novozym 435 (5 g). For the lifetime experiment, only 1 g Novozym 435 was used.

For data analysis, the retention time of the substrate in the column was calculated as reaction time (h)=mass of enzyme (g)/enzyme bulk density (g/mL)/volumetric flow (mL/h). Productivity (kilogram product mixture produced per kilogram enzyme per hour) was calculated as productivity (kg/kg/h)=volumetric flow (mL/h)×density of product (g/mL)/mass of enzyme (g)=density of product (g/mL)/enzyme bulk density (g/mL)/reaction time (h). Bulk density of Novozym 435 was determined to 0.34 g/mL and the product density to approximately 0.85 g/mL.

Results and Discussion

Feedstock Properties

Composition and parameters for PFAD can naturally fluctuate. Table 1 contains data obtained for the PFAD batch used in the present work. The high melting point (49 °C) initially gave some practical problems with solidification of the substrate in tubing and columns, and also raised some concerns about the enzyme lifetime. Hence, the melting point of different PFAD blends was investigated. Using a dropping point type instrument, the melting point was found to decrease from 49.1 to 39.9 °C upon addition of 1.5 eq MeOH. Methanol equivalents (eq) refer to the molar ratio of MeOH/FFA. For simplicity, it was in the calculations of equivalents assuming that the PFAD is 100% oleic acid. In this way 1 eq equals 0.13 mL/mL (MeOH/PFAD). Adding more than 1.5 eq MeOH did not significantly decrease the melting point further. On the other hand, blending with FAME was studied as a way to further lower the melting point. Even though the melting point could be reduced to 35.0 °C with 30% FAME added together with the 1.5 eq MeOH, this idea was not pursued further.

Table 1 PFAD data

Basic properties	
Melting point (°C)	49
FFA (% as C16:0)	83.9
Water by Karl Fischer titration (%)	0.87
Elements via ICP (ppm)	
P	1.14
Ca	4.63
Mg	0.99
Na	5.62
K	2.43
Composition by GC (%)	
Monoglycerides (MG)	1.03
Diglycerides (DG)	3.01
Triglycerides (TG)	5.90
Total FFA+MG+DG+TG	93.8
Fatty acid composition (%)	
C12:0	0.1
C14:0	1.0
C16:0	53.1
C18:0	4.0
C18:1c	34.0
C18:2c	7.8
Saturated fatty acids (SFA)	58.2
Monounsaturated fatty acids (MUFA)	34.0
Polyunsaturated fatty acids (PUFA)	7.8
Calculated iodine value (IV)	44.7
Oxidation parameters	
Peroxide value (PV)	0.69
p-Anisidine value (AnV)	54.5

Batch Esterification Reactions

Watanabe and coworkers have reported the somewhat surprising result that a large excess of MeOH (5–7.5 eq) has a positive effect on Novozym 435 lifetime in the conversion of acid oil to FAME [18]. Hence, to determine the optimal MeOH dosage, a series of batch experiments were conducted using 1.5 and 6 eq MeOH to convert PFAD at 45 °C. Samples were withdrawn for NMR analysis to calculate the conversion to FAME after 4- and 24-h reaction. After 24 h, the reaction mixture was removed from Novozym 435, and the enzyme was reused in the next reaction cycle. The results (Fig. 2) clearly showed that using a large excess of MeOH would lead to a significant loss of enzymatic activity already in the second reaction cycle. The decline then seems to level out. With 1.5 eq MeOH there is a much slower decline in conversion, apparently accelerating in reaction cycles 9 and 10. Interestingly, with 1.5 eq MeOH, the conversions after 4 and 24 h are almost identical. This indicates that the reaction has reached an equilibrium situation already after 4 h.

Continuous Esterification Reaction

After the initial batch trials, further experiments were conducted with a packed-bed reactor (PBR). Due to the high melting point of the substrate, the PBR was submerged in a thermostated water bath together with a substrate reservoir. The piston pump delivering the flow is also traced with hot water. The column in this "aquarium" setup was packed with Novozym 435 and the reservoir filled with substrate mixture consisting of melted PFAD and 0.2 mL MeOH per milliliter PFAD. This corresponds to 1.56 eq. The "aquarium" was thermostated at 45 °C and the substrate pumped upflow through the column. The resulting product was collected in a measuring cylinder for determination of volumetric flow rate. In the cylinder, the product quickly formed a lower watery phase (water and methanol) and an upper oily phase (FFA, glycerides, and FAME). Despite the upflow operation of the column, no signs of water accumulating in the column were experienced. Downflow operation was tested in a few trials, giving essentially identical results to those obtained with the upward flow. Using this setup, the flow rate was varied by adjusting the pump speed. The actual volumetric flow rate was measured and the composition of the (oil phase

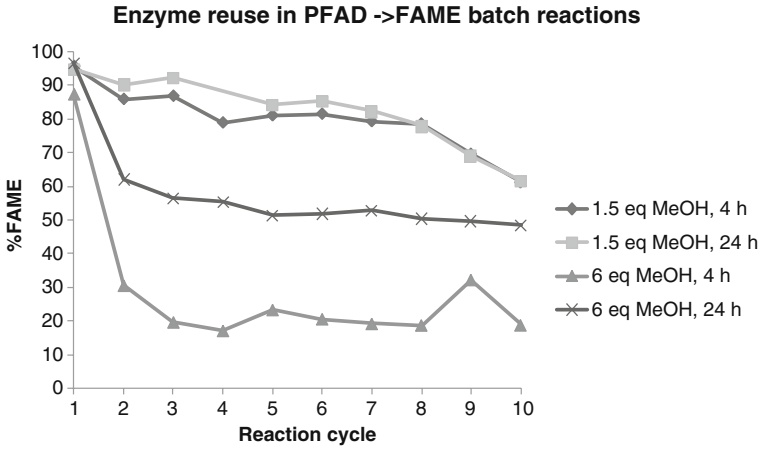

Fig. 2 FAME content by NMR in batch reactions with Novozym 435 at 45 °C

of) the product was analyzed by NMR and FFA titration. Figure 3 below shows percent FAME and percent FFA plotted against reaction time. Reaction time or retention time in the column is calculated as described in the experimental section.

These results indicate that an equilibrium state is reached at approximately 5% residual FFA. Since the target is to obtain <5% FFA, a second reaction stage seems necessary. Hence, the flow rate was fixed at a reaction time of approximately 15 min (i.e., productivity approximately 10 kg/kg/h) to produce a large batch (2 L) of product with 80% FAME and 15% residual FFA as a feedstock for a second reaction stage. The water phase was removed in a separation funnel. NMR analysis further showed that the oil phase still contained 0.22 eq dissolved MeOH, i.e., just sufficient to reach full conversion; however, to have an excess, more MeOH (0.1 mL/mL) was added, and the resulting mixture was used as substrate for a second reaction stage. Hence, at a total of 0.3 mL/mL (2.3 eq) MeOH had been added. Figure 4 below shows, similar to Fig. 3, the outcome of the second reaction stage trials.

Even though the data are more scattered here, it seems clear that the equilibrium now lies at approximately 2.5% FFA, i.e., well below our target at 5% FFA. Actually, the 5% FFA can be obtained with a reaction time of 30 min, corresponding to a productivity of 5 kg/kg/h. In other experiments, the effect of drying the product coming out of the first stage was investigated, rather than simply decanting the water layer; however, no clear benefit (higher conversion in the second stage) could be observed (data not shown). The methanol dosage in the second step reaction has not been optimized. Hence, it is possible that no further methanol addition prior to the second stage is needed (i.e., that the dissolved 0.22 eq is sufficient). Also, the temperature for the second stage could be optimized, since with a lower melting substrate, it should be possible to lower the temperature below 45 °C.

Enzyme Lifetime and Productivity

The presented data shows that it is technically possible to convert PFAD into FAME containing less than 5% FFA with Novozymes 435 in a two-stage process with PBRs; however, process economy is obviously the key question. The most critical parameter here

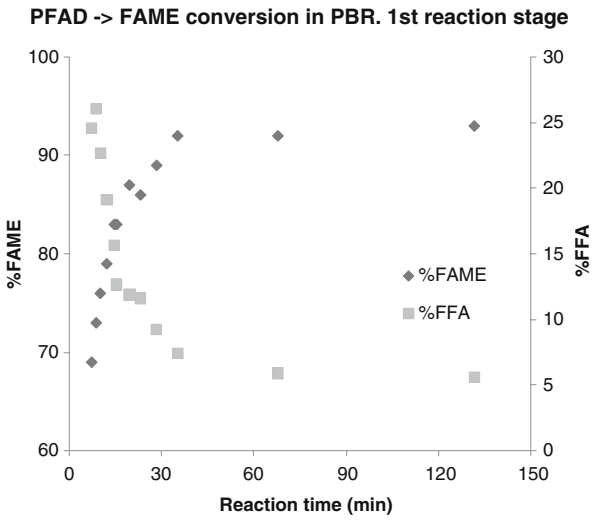

Fig. 3 FFA and FAME content of the product from a first pass of PFAD and MeOH (0.2 mL/mL) through the "aquarium reactor"

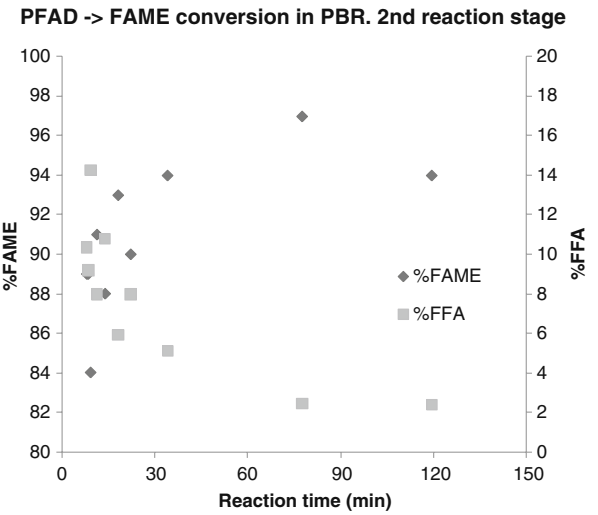

Fig. 4 FAME and FFA content of the product from the second pass through the "aquarium reactor." Substrate was product from the first stage containing 80% FAME, 15% FFA, added 0.1 mL/mL MeOH

is the enzyme lifetime. The enzyme is expected to lose activity over time. Many factors can affect this: inactivation due to methanol and temperature, inactivation due to oxidation from peroxides, etc., in the oil (notice the very high anisidine value) or leaching of enzyme from the carrier (washout by the polar water/methanol phase). To experimentally address this, a new column with Novozym 435 was placed in the aquarium reactor, and substrate for the first reaction stage (PFAD with 0.2 mL/mL MeOH) was pumped upflow at 45 °C at a constant flow rate. The chosen flow rate was the same as the one used to produce substrate for the second reaction stage (i.e., 15-min reaction time, giving approximately 80% FAME, 15% FFA). FAME and FFA was then measured daily, see Fig. 5.

At this flow rate, the reaction has not reached equilibrium. Hence, any decrease in enzyme activity should immediately be seen in the conversion (lower FAME/higher FFA content). Due to the different contributing factors, enzyme inactivation is likely to be a function of time as well as amount of converted oil. Hence, the two proportional axes are

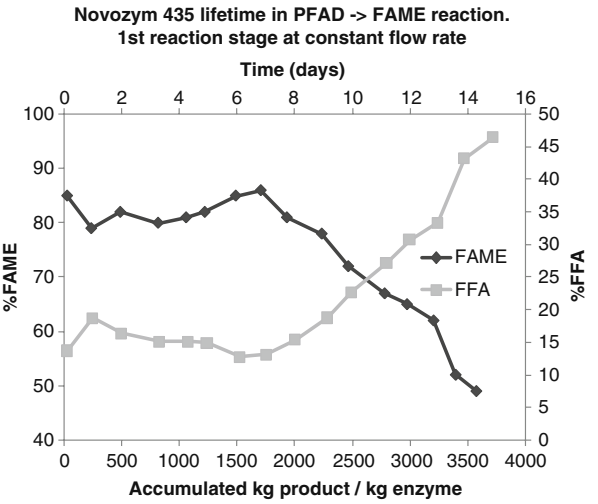

Fig. 5 Enzyme inactivation leads to lower activity over time

shown in Fig. 5. The data indeed shows a significantly declining enzyme activity over the two weeks of the experiment, going from 85% to 50% FAME, 15% to 45% FFA. The initial slow decline, followed by a faster declining conversion, is to be expected from the reaction kinetic. From Fig. 3 it appears that the reaction is initially fast, and then slows down to reach higher conversions. Therefore, it requires only little residual enzyme (catalyst) activity to reach 50% or 60% FAME within a certain reaction time. Hence, it can be assumed that most of the enzyme activity is lost after 14 days and 3,500 kg product per kilogram enzyme. Enzyme lifetime has not been evaluated for the second reaction stage, but is likely to be similar or better than the lifetime for the first reaction stage (less methanol, less water, same or lower temperature, and oil oxidation products have potentially been scavenged by the enzyme in the first stage).

It should be noted that in an industrial setup, the process would likely be designed not with only one column (per reaction stage), but with a serial connection of a handful of columns. The columns would contain enzyme of different ages and by shuffling the columns around after an enzyme reload (so that the oldest is always first), a constant conversion can be obtained at a constant flow rate, and it is possible to utilize a column with even very little residual enzyme activity. Such a setup is well-described for enzymatic interesterification [19].

Combining the Enzymatic Esterification and Conventional Transesterification Processes

The present paper focuses on the enzymatic part of the proposed integrated enzymatic–alkaline biodiesel process. A rough outline of a two-step process based on PBRs with immobilized enzyme is suggested. An obvious next step would be to take an actual crude palm oil through all the steps outlined in Fig. 1: deodorization, enzymatic treatment of the PFAD, blending of de-acidified PFAD with the deodorized oil, and alkaline transesterification of the mixture to produce in-spec FAME. These unit operations are all well-known in the industry, so the integration is likely to be successful.

Conclusion

An enzymatic process is suggested for de-acidification of the distillate stream coming out of the deodorizer installed in many biodiesel plants operating with FFA-containing oils. This would be a plug-in alternative to the standard sulfuric acid catalysis. With PFAD, the most abundantly available acid feedstock, a two-stage process is outlined. The immobilized enzyme (Novozym 435) is conveniently packed in columns, and water removal between the two columns is done by means of simple and fast decantation. The enzyme requires only a small excess of MeOH. With 0.2 mL/mL MeOH, the first reaction stage could reduce the FFA content from 85% to 5%, whereas the second stage could lower the FFA content to 2.5% after water removal and addition of 0.1 mL/mL MeOH. It is suggested to run column 1 with a reaction time of 15 min (productivity 10 kg/kg/h) and column 2 with a reaction time of 30 min (productivity 5 kg/kg/h), resulting in 15% FFA after column 1 and 5% FFA after column 2. A lifetime study indicated that the activity of Novozym 435 drops significantly (in column 1) after 2 weeks continuous production, corresponding to 3,500 kg product per kilogram enzyme; however, productivity in an actual industrial setup over both reaction stages is still very uncertain. On the other hand, improvements of enzyme lifetime, and thereby process economy, can possibly be made from optimizing the suggested process conditions. As an example, Watanabe and coworkers optimized the Novozym 435 catalyzed

esterification of AO by the addition of glycerol to allow more than 100 reaction cycles in a batch process [18]. Moreover, ongoing research is focused on the development of low-cost immobilized enzyme products to allow even more cost-competitive enzymatic processes for bulk products like those produced in the biodiesel and vegetable oil industry.

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