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Two Products one catalyst: Emulsifiers and biodiesel production combining enzymology, nanostructured materials engineering and simulation models.

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Abstract

Crises are the driving force of the inventions that have led man to overcome himself. The challenge that concerns us today is to maintain our lifestyle with the energy expenditure involved. In the present work, it's proposed the production of emulsifiers such as monoglycerides (MG) and diglycerides (DG) and biodiesel (FAEE) from renewable sources using *Pseudomonas fluorescens* lipase immobilized over Ca modified mesoporous SBA-15 supports (L_{PF}/Ca/SBA-15) as biocatalyst. Using a packed bed reactor thermostated at 37ºC, the catalyst was capable to convert sunflower oil and commercial ethanol (96 wt%) into FAEE (35.0 wt%), MG (12.2 wt%) and DG (43.2 wt%). From these results, a separation by vacuum distillation was simulated. A good separation of the biodiesel in the top of the column at approx. 325°C and the emulsifiers in the bottom of the column at 484ºC, was obtained.

Thus, from interdisciplinary technologies such as enzymology, nanostructured materials design and computer simulation, a project for the production and purification of two products, emulsifiers and biodiesel, with one catalyst is offered.

Keywords: Biocatalysis, Biodiesel, Emulsifiers, SBA-15, Commercial ethanol, Vacuum distillation simulation.

1. Introduction

Since man established himself and founded the big cities, he has carried out an extractive exploitation of the planet natural resources to maintain his lifestyle [1,2].

After evaluating social, economic and environmental impact of non-renewable resources extraction, it is concluded that this kind of activities cannot be sustained over time. For that reason, all the efforts in the development of technology are focused toward a green chemistry [3].

For a long time, biodiesel derived from vegetable oil has been accepted as a promising alternative and a renewable fuel for its substitution or blending with petrodiesel [4].

Due to its physical and chemical properties, similar to petrodiesel, biodiesel or biodiesel blends can be used in diesel engines without any change [3,5]. However, the actual production in batch reactors, using homogeneous catalyst, has many disadvantages such as: required oil quality (as free fatty acids or water content), soap formation, water contamination, and high purification costs [6,7]. Moreover, the main by–product of biodiesel production is glycerol, which represents about 10 wt% of vegetable oil used as raw material. This can be used in food, drugs, cosmetics and tobacco industries, but a high purity is necessary.

Crude glycerol derived from biodiesel production using homogeneous catalysis, possesses very low value because of its low purity [8,9]. Therefore, it cannot improve the biodiesel production cost and, in some cases, is a residue. Heterogeneous catalysts appear as an appropriate option to replace homogeneous catalyst for transesterification reaction of vegetable oils to produce biodiesel, without the homogeneous disadvantages. Specifically, biodiesel production using an immobilized enzyme on a nanostructured material (a biocatalyst) eliminates the difficulties of the reactions catalyzed under acid or alkali conditions. Also, the products has a very high purity and can be easily separated from reaction mixture, the catalyst can be reused for many times, the process is environmentally friendly and could be mount in continuous [10–12]. In previous work, we describe a very active biocatalyst where the lipase from *Pseudomonas Fluorescens* was immobilized on the SBA-15 material modified with calcium (L_{PF}/Ca/SBA-15). It could transesterify different kind of oils (used frying waste oil, sunflower oil and soybean oil) and even esterify free fatty acids (FFA) present in the oils, using commercial ethanol [13].

In this study, a L_{PF}/Ca/SBA-15 biocatalyst able to control the transesterification reaction of sunflower oil and commercial ethanol (both renewable raw materials) to obtain MG and DG besides of biodiesel (FAEE), was developed and used in a packed bed reactor [13]. Then, a vacuum distillation process was simulated to separate the

products of the homogeneous mixture, obtaining a high purity of biodiesel, MG and DG. These last two products are often used as emulsifiers in food, cosmetic and pharmacy industry [14–18].

Finally, two products can be obtained with one catalyst. It leads to a value added to this catalyst because emulsifiers can be obtained in soft conditions compared with the actual process where high energy consume is necessary [19].

2. Experimental Section

2.1. Chemicals

Pseudomonas fluorescens (PFL, ≥20,000 IU/g at 55 °C, pH 8.0) lipase was acquired from Sigma-Aldrich Co. (St. Louis, USA). This enzyme, produced by "Amano Labs", has a high lipolytic activity, and was characterized by their researchers. It has a molecular weight of about 33kD, an isoelectric point of pI=4, a pH stability range 4<pH<10, and an optimum pH of activity in the range 8<pH<10 [20].

Sunflower oil (‘‘Vicentin’’ brand) was acquired at a local store. This oil is in agreement with the Argentinean specifications for food oil. The reagents employed were KH_2PO_4, K_2HPO_4 (Anedra), $Ca(NO_3)_2$ (Cicarelli), absolute ethanol 99.8% (analytical grade, Taurus), commercial bioethanol 96% v/v (Porta Hnos.), hydrochloric acid (HCl) (analytical grade, Cicarelli), n-Hexane (analytical grade, Merck), isopropanol (analytical standard, FLUKA), acetonitrile (analytical grade, Merck) and miliQ water. Syringe filters (polypropylene, 25mm diameter and with 0.2 µm of pore size) were supplied by VWR.

2.2. Synthesis and modification of SBA-15

The mesoporous material SBA-15 was synthesized dissolving 4.0 g of Pluronic P123 in 30 g of water. 120 g of 2M HCl solution were added under stirring at 40°C. Then, 8.50 g of Tetraethyl orthosilicate (TEOS) were incorporated into this solution with stirring at 40°C for 20 h, according to Zhao et al. (1998). The mixture was aged at 100°C overnight without stirring. The solid product was filtered, washed, and air-dried at 60°C overnight. Calcination was carried out at 500 °C for 8 h, with a heat rate of 1°C /min [13,21–23].

The support was modified by conventional impregnation method, to reach theoretical metal loadings of 2.5 wt%. The SBA-15 (0.75 g) was dispersed at room

temperature in the precursor solution of $Ca(NO_3)_2$ and then, the solvent (distilled water) was removed slowly in a rotary evaporator, under vacuum at 50°C for 30 min. The resulting powder was dried at 60°C and calcined for 8 h at 500°C to obtain the modified material [24]. The sample was named as Ca/SBA-15.

2.3. Material Characterization

The physic-chemical properties of the synthesized material Ca/SBA-15 were characterized elsewhere [13] by Small-angle X-ray Scattering (SAXS), Transmission Electron Microscopy (TEM), Scanning Electron Microscope (SEM), Infrared Spectroscopy (IR), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP) and N_2 adsorption isotherms.

2.4. Pseudomonas fluorescens lipase immobilization on Ca/SBA-15

In a typical procedure, Ca/SBA-15 (125 mg) was suspended in 10 ml of enzyme solution (5 mg/ml, in 25 mM phosphate buffer, pH 8). The suspension was kept under moderate stirring, for 24 h at room temperature, and then centrifuged and washed two times with 10 ml of 25 mM phosphate buffer (pH 8).

Determination of protein content was carried out according to the Bradford assay [25]. The first centrifugation supernatant (10 µL) was mixed with a solution of 50 µL Bradford reagent in 200 µL of water. After exactly 6 min, the absorbance at λ=595 nm was determined using a Cary 50 spectrophotometer. Protein content was estimated through a calibration curve, using concentrations of 0.05, 0.1,0.25, 0.5 and 1 mg/ml of BSA (98%) as protein standard [26]. The resulting biocatalyst was named as L_{PF}/Ca/SBA-15 [13].

2.5. Oils biocatalytic ethanolysis

2.5.1. Batch reactor

Reactions were carried out in vials shaken at 180 oscillations/min through a horizontal shaker at 37°C. These were performed by mixing oil and ethanol in an alcohol/oil molar ratio = 4/1 and started by adding L_{PF}/Ca/SBA-15 (175 mg/g oil) to the substrates mixture. Water content in the reaction mixture varied from 0 to 77 wt% respect to the biocatalyst mass.

2.5.2. Packet bed reactor

A thermostated column (24 cm of length by 0.6 cm of diameter) was used to package the catalyst L_{PF}/Ca/SBA-15 (9.1 wt%) mixed with glass beads, in order to avoid the excessive compaction. In the reactor bottom, a glass wool stopper was placed. Then, the catalyst mixture was added, placing another stopper of glass wool in the reactor top. Finally, pieces of glass were added until complete the reactor length, to maintain a homogeneous mixed flux. The column and the mixing tank were thermostatized at 37ºC, the raw materials were fed by a peristaltic pump (0.5 mL/min) as showed in Scheme 1. The mixture contained 50 g oil and 10 mL absolute ethanol with 3.2% V/V of water. The estimated residence time in the reactor was 20 min.

In both reactors, samples of 20 µL were collected at different times, diluted to a volume of 1 mL with acetonitrile, filtered with a 0.45 µm pore size syringe filter, and analyzed by HPLC.

All reactions were performed at least in duplicate. The results were expressed as mean values, with relative percentage differences between them always less than 5% of the mean.

2.6. Chromatographic analysis (HPLC)

The analyses were performed with a Perkin Elmer Series 200 HPLC, equipped with an UV-vis detector, a solvent delivery unit for binary gradient elution, an Agilent Eclipse Plus 18 column (C18 with a diameter of 4.6 mm x 25 cm length and a particle size of 5µm) and TotalChrom software for remote management and quantification.

The UV-vis detector wavelength was set at 210 nm, the column temperature was maintained at 30°C during the assays and the flow rate was 1 mL/min. For the quantification, methyl-heptadecanoate was used as an internal standard, with a final concentration of 10 mg/mL in acetonitrile. For the chromatographic runs, the following gradient elution was used: 6 min of 30%/70% water/acetonitrile, 10 min of 100% acetonitrile, 15 min of 80%/20% acetonitrile/isopropanol-n-hexane (5/4), 29 min in gradient up 30%/70% acetonitrile/isopropanol-n-hexane (5/4).

2.7. Simulation Analysis

Distillation process simulation was carried out using Honeywell's UniSim Design R440 software. The thermodynamic properties of the pure compounds (critical properties and acentric factor) were calculated using the group contribution method.

Peing Robinson equation of state was used as the thermodynamic model for the calculation of vapor phase properties, while UNIQUAC model was used for the liquid phase.

Initially, a short cut distillation calculation was performed to obtain the initial values necessary to carry out the rigorous calculation. Linoleic acid methyl ester (Me-L) was chosen as the light key component (LK) and 1-Monostearin (1-S) was used as a heavy key component. The key components composition restriction for both, top and bottom streams, was 0.0001 in molar fraction. The pressure in the reboiler was maintained at 0.8 atm, while in the condenser it was reduced to 0.6 atm. The feed flow was 100 mol/h.

A perforate trays distillation column was preferred as it is shown in the Scheme 3. The column itself consisted in 26 theoretical trays with feed inlet at stage 12. A total condenser was chosen for the upper stream (stage 0), and a Kettle reboiler for the bottom (stage 27). The optimum external reflux and the distillate molar flow were used as restriction values, assigning them those values obtained in the short cut calculation.

3. Results and discussion

3.1. Biocatalyst activity in batch reactor

In previous work, L_{PF}/Ca/SBA-15 was evaluated and described as the better biocatalyst to produce biodiesel using sunflower oil and ethanol [13]. As it was mentioned there, one of the key requirement to obtain a good catalytic performance is the water content in the reaction mixture [27,28]. This is necessary to produce changes in the lipase architecture that allow the subtract access to the active sites. For this reason, in order to obtain the optimal conditions where high quantity triglycerides (TG) are converted into MG, DG and biodiesel (FAEE) the water content in the reaction medium was evaluated. As shown in the Figure 1, when the reaction occurs in water absence, the TG conversion is minimal. As expected, the catalyst activity increases when water is added. The best activity was achieved with 32 wt% of water respect to the catalyst mass. Thus, a 79% of TG conversion was achieved, versus a 23% without added water.

Under this condition, the TG conversion was evaluated as function of reaction time. As shown in Figure 2, MG and DG mass fractions decrease from 14 wt% and 32 wt% at 2 h of reaction to 4 wt% and 5 wt% at 24 h of reaction, respectively. An 88 wt%

of biodiesel was obtained at this time. Thus, to maximize MG and DG production, the reaction time has not to be superior to 2 h. From these results, it is observed that the transesterification reaction can be controlled by the reaction time to favor MG and DG production.

3.2. Biocatalyst activity in packed bed reactor

An advantage that heterogeneous catalysts offer is the capacity to set up a continuous production process, which has an economical benefit in the industrial area. For this reason, a packed bed reactor was employed as described in Scheme 1. A priori, in order to verify the enzymatic activity, a test reaction was carried out, filling the column with the support Ca/SBA-15 without the immobilized lipase. As it can be observed in Figure 3, only a 25% of TG was converted. The detected residual activity is produced by calcium species supported on mesoporous silica, in concordance with Albuquerque *et al.* (2008) and Samart *et al.* (2010). However, when it is compared with recycle 1 (Figure 3), where the enzyme was immobilized on the support (L_{PF}/Ca/SBA-15), an 85% of TG conversion is achieved after all of the reaction mixture passed through the catalyst bed (120 min). These conditions allowed reaching a products distribution similar to the one reached in the batch reactor after 2 h.

To evaluate if the catalyst performance can be improved, recycles of the reaction mixture were carried out. A 92% of TG conversion was achieved in recycle 3. While the FAEE mass fraction also increased at this recycle, the concentrations of MG and DG were not modified roundly.

In order to evaluate the possibility of replacing absolute ethanol by commercial ethanol, the reaction was carried out using it. It should be clarified that commercial ethanol has a similar water contain (4% V/V) than the optimized absolute ethanol and water mixture (3.2% V/V) found in section 2.1. Then, no difference were found when absolute ethanol mixed with water or commercial ethanol were used (Figure 4 I), which leads to the advantage of decreasing costs using a renewable raw material.

Considering again that water is an important factor to biocatalyst activity, in other experiment, the reaction was carried out by addition of water (6% V/V) to the commercial ethanol. As it can be observed in the Figure 4 II, conversion of TG and mass fraction of MG decrease, indicating that absolute ethanol is appropriately replaced by commercial ethanol without water addition.

On the other hand, inhibition of the transesterificación reaction by glycerol has been already mentioned by other authors. Glycerol interacts with the catalyst surface and difficulties the mass transfer process between the reactive mixture and it [29,30]. In the experiment corresponding to Figure 4 III, the catalyst packed in the reactor was pretreated with 1.25 g of glycerol, which represents a 25% of glycerol produced when 50 g of oil are used. As it can be seen in this Figure, only a 36 wt% of TG was converted, indicating that glycerol inhibited the transesterification reaction according to Guldhe *et. al.* (2015).

Salis *et. al.* (2009) mention that the hydrophobic character of the support improves the biocatalyst activity. Nevertheless, as it was determined by us [13], when the SBA-15 surface is modified with calcium, it increases its basicity and the biocatalyst improves its activity [13,31]. This apparent discordance could be explained taking into account the polar character of the glycerol produced during the reaction, which would have a low tendency to interact with the hydrophobic support and therefore to inhibit the transesterification. In the case of the Ca/SBA-15, the calcium species on the surface favor the biocatalyst activity at short reaction times when the production of glycerol is still incipient. However, when glycerol begins to produce, it would interact with the polar surface of Ca/SBA-15, inhibiting the mass transfer and decreasing the activity.

3.3. Catalytic performance in continuous regime

An important aspect to be studied in a catalytic process is the stability of the catalyst over the time on stream (TOS). The effect of TOS on the TG, DG, MG and FAEE mass fraction, using L_{PF}/Ca/SBA-15 at 37ºC is shown in Figure 5. As it can be appreciated, beyond a TOS 60 min, the steady state seems to be reached and mass fractions of TG, DG, MG and biodiesel remained almost constant.

This result can be explained considering the three steps of the transesterification reaction of TG (Scheme 2). In the first step, DG and biodiesel are obtained from TG and ethanol; in the second step, MG and biodiesel are obtained using as subtract the DG produced in the first step. Finally, in the third step, biodiesel and glycerol are produced from second step's MG.

Step 1) TG + A ⟶ BD + DG

Step 2) DG + A ⟶ BD + MG

Step 3) MG + A ⟶ BD + GL

Scheme 2: Steps of transesterification reaction: TG: triglycerides, DG: diglycerides, MG: monoglycerides, BD: Biodiesel, GL: Glycerin, A: Ethanol.

Then, taking into account that glycerol production inhibits the biocatalyst activity (section 2.2.), the results obtained indicate that a non-glycerol production regimen was achieved because the activity was not altered on the time of stream. A partial transesterification activity of TG is maintained (steps 1 and 2) and two products are obtained, biofuels and emulsifiers. Using this system, a good performance of the L_{PF}/Ca/SBA-15 in a packed bed reactor, without catalyst in the product mixture, could be accomplished. Now, the question is: how to separate MG and DG from biodiesel?

3.4. Simulation of vacuum distillation to separate emulsifiers from biodiesel

There are many forms to separate and purify MG and DG from biodiesel. However, a high grade of purity is necessary to use the emulsifiers in food or cosmetic industry. For this reason, vacuum distillation process was selected as the appropriate technique to carry out this separation [17,18]. It was performed by using Honeywell's UniSim Design R440 simulation software. The thermodynamic properties of the pure compounds, calculated by the group contribution method, are shown as the supplementary material, S1.

As it was mentioned in section 2.7., the scheme 3 shows the configuration of vacuum distillation column. The temperature profile obtained after the simulation can be observed in the Figure 6 and the fluxes in Table 1. It should be highlighted here that the optimum external reflux and the distillate molar flow, used as restriction values in the calculations, converged with an error in the order of 10^{-6} for these parameters.

Flux 1 is the feed composed by the mixture of emulsifiers and biodiesel produced in the reactor. In Flux 2, biodiesel is the main component with a 97.4 wt%, collected at 0.6 atm and 325.7°C. Meanwhile, in Flux 3, MG and DG are the principal components whit almost 90 wt%, at 0.8 atm and 484.3ºC.

Table 1: Vacuum distillation conditions and simulation results.

Flux	Mass flow [kg/h]	Temperature [ºC]	Pressure [atm]	Composition [wt%]			
				MG	FAEE	DG	Triglyceride
Flux 1	41930	100	1	12.2	35.0	43.2	9.6

Flux 2	15240	325.7	0.6	0.0	97.4	0.0	2.6
Flux 3	25610	484.3	0.8	28.8	4.0	60.6	6.6

The variation of the liquid composition throughout the 28 stages of separation (from 0 to 27) that forms the distillation process is shown in Figure 7.

As it can be seen, the FAEE predominates in the output stream of stage 0, with a mass fraction greater than 90%. This flux corresponds to the product of top or distillate, which is obtained as a liquid stream that leaves the total condenser. Meanwhile, the emulsifiers are in greater proportion in the bottom stream, that is, the output flux of stage 27 or bottom product.

The graph also permits to distinguish how in the upper part of the column, the liquid stream is enriched in FAEEs as it ascends from stage 12 to 0; meanwhile in the lower column part (stages 12 to 27), the descending liquid is rich in mono and diglycerides.

Furthermore, the input of the feed in tray number 12 does not generate a disturbance in the profile of compositions, and also it does not alter the temperature and pressure profiles (see Supplementary material, S1). This verifies that stage 12 is indicated to introduce said stream.

Despite that a process of vacuum distillation may seem expensive, with the technology presented in this paper the neutralization of homogeneous catalysts, the biodiesel washing for its purification from catalyst, the soaps and glycerol produced, the water contamination with soaps, the energy consumed for biodiesel drying and costs for glycerol purification could be eliminated.

With these results, we conclude the study of a new and efficient technology to obtain two products (emulsifiers and biodiesel) of great importance for the actual society. A sustainable process was proposed using renewable raw materials (vegetal oil and commercial ethanol) as substrates using a biocatalyst in a continuous system and vacuum distillation to produce and purify the products.

4. Conclusions

In this work, a continuous packed bed reactor was employed, using the LPF/Ca/SBA-15 biocatalyst. Biodiesel (35.0 wt%) and emulsifiers (55.4 wt%) were produced from renewable materials as commercial ethanol and sunflower oil . Then,

using a vacuum distillation simulation, the product mixture can be separated obtaining a purity 94.7 wt% of FAEE and 89.4 wt% of emulsifiers.

The advantages offered by this system are: sunflower oil and commercial ethanol used are renewable and non-toxics substrates, stability of biocatalyst on time on stream, non-glycerol production, non-water resources contamination or drying biodiesel cost and no more disputes between the edible oil use for biofuel or for food.

Finally, in this paper, technologies such as enzymology, nanostructured materials engineering and simulation models were combined for the obtaining of two products with one catalyst: emulsifiers and biodiesel, and their subsequent purification. This is our contribution to rethink tomorrow having as principal goal a sustainable concept.

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Figure Captions

Figure 1: Effect of water content on transesterification activity of L_{PS}/Ca/SBA-15 (400 $mg_{protein}/g_{support}$). Reaction in batch conditions: ethanol/oil molar ratio = 4/1; 87,5 mg of catalyst/g of oil; sunflower oil and absolute ethanol as reactives; 37 °C, reaction time = 2 h and constant shaking (180 oscillations/min).

Figure 2: Triglycerides conversion in function of time using of L_{PS}/Ca/SBA-15 (400 $mg_{protein}/g_{support}$) catalysts. Reaction conditions: ethanol/oil molar ratio = 4/1; 175 mg of catalyst/g of oil; 32 wt% of water with respect to catalysts; sunflower oil and absolute ethanol as reactives; 37 ºC and constant shaking (180 oscillations/min).

Figure 3: Triglycerides conversion in function of recycle in a column reactor using L_{PS}/Ca/SBA-15 (400 $mg_{protein}/g_{support}$) as catalyst. Reaction conditions: oil mass = 50g; ethanol/oil molar ratio = 4/1; 20 mg of catalyst/g of oil; 32 wt% of water with respect to catalysts; sunflower oil and absolute ethanol as reactive and 37 ºC.

Figure 4: Triglycerides conversion in a packed bed reactor using L_{PS}/Ca/SBA-15 (400 $mg_{protein}/g_{support}$) as biocatalyst. Reaction conditions: ethanol/oil molar ratio = 4/1; 20 mg of catalyst/g of oil and 37 ºC. Reactives: sunflower oil and I) commercial ethanol (4% V/V of water), II) commercial ethanol with water (6% V/V of water), III) commercial ethanol and packed bed pretreated with 1.25 g of glycerol.

Figure 5: Triglycerides conversion in function of TOS in a packed bed reactor. L_{PS}/Ca/SBA-15 (400 $mg_{protein}/g_{support}$) as catalyst. Reaction conditions: ethanol/oil molar ratio = 4/1; 20 mg of catalyst/g of oil; 32 wt% of water with respect to catalysts; sunflower oil and commercial ethanol as reactives and 37 ºC.

Figure 6: Temperature range respect the theorical trays.

Figure 7: Variation of the liquid composition throughout the separation process.

Scheme 1: Vacuum distillation column. Flux 1: reactor feeding, Flux 2: top distilled, Flux 3: residual bottom. a) Column, b) Total condenser, c) Reboiler.

Scheme 2: Steps of transesterification reaction: TG: triglycerides, DG: diglycerides, MG: monoglycerides, BD: Biodiesel, GL: Glycerin, A: Ethanol.

Scheme 3: Continuous reactor: 1) Heating plate, 2) Mixing tank of reactive, 3) Peristaltic pump, 4) Thermostatized column, 5) Temperature Control.

S1: Thermodynamic properties of the pure compounds.

S2: Pressure profile along the 28 distillation steps.

Figure 1

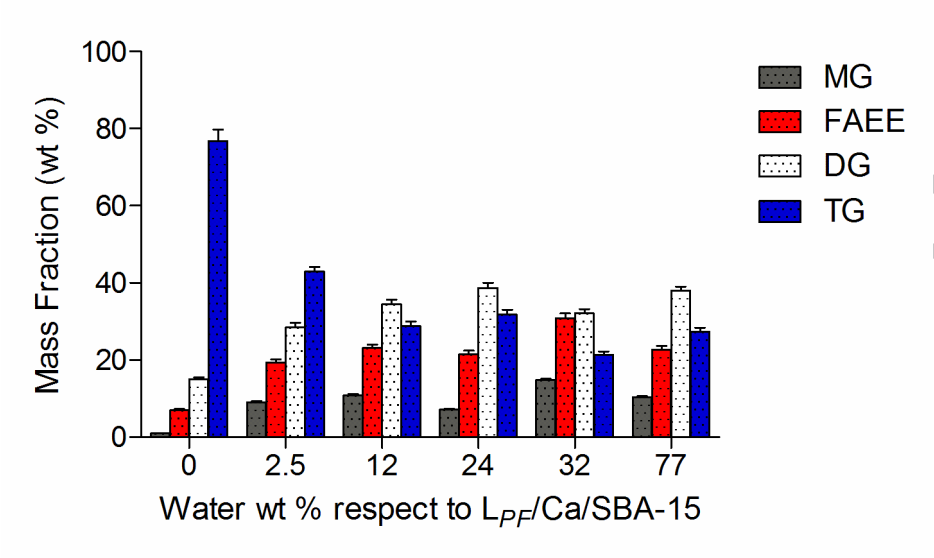


Figure 2

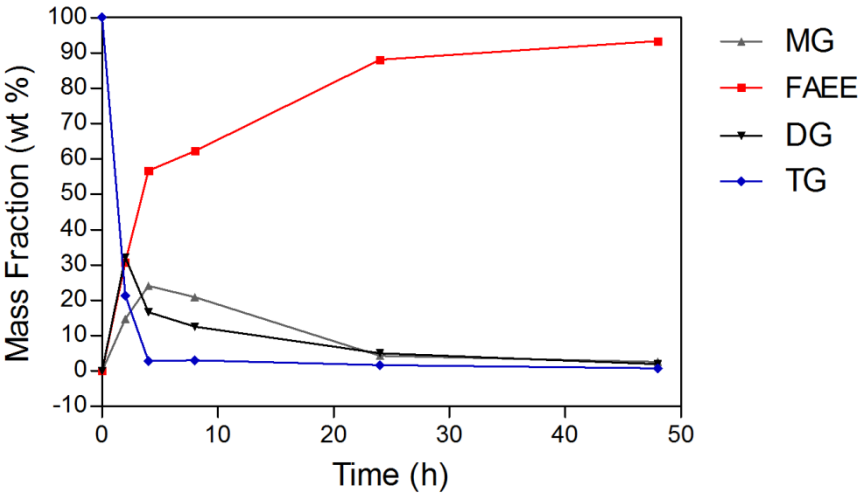


Figure 3

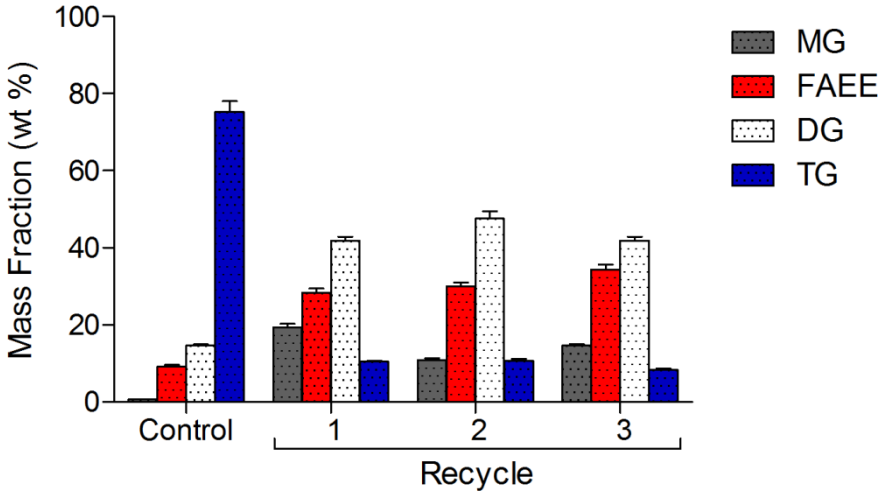


Figure 4

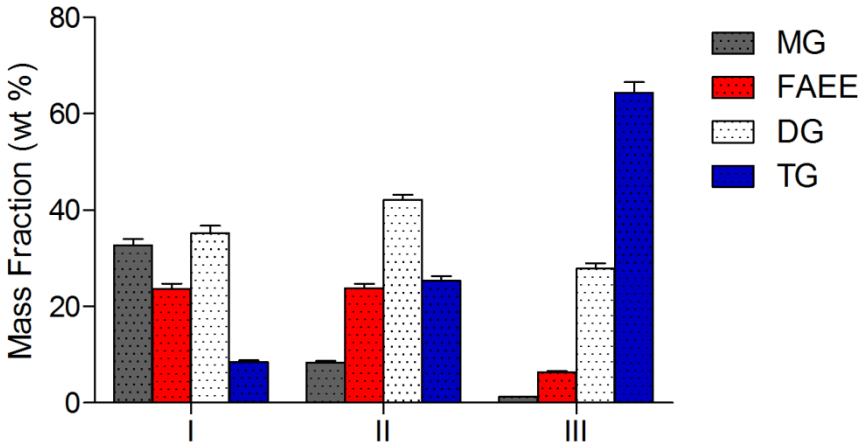


Figure 5

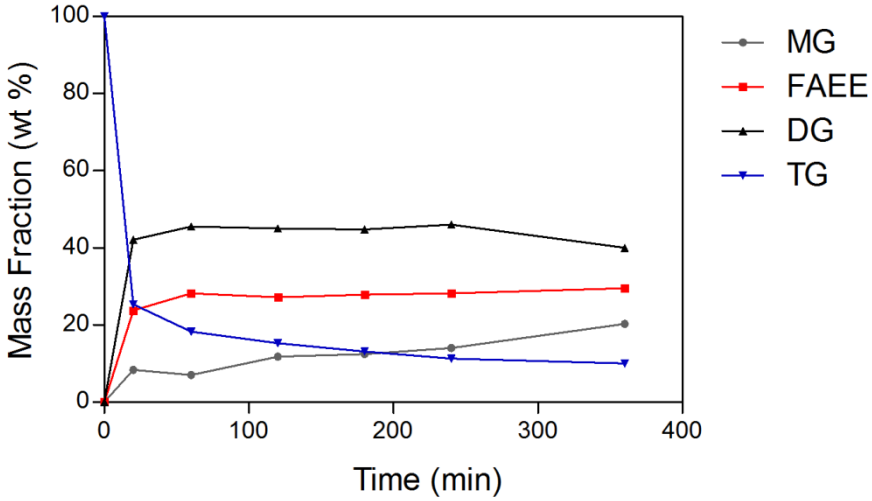


Figure 6

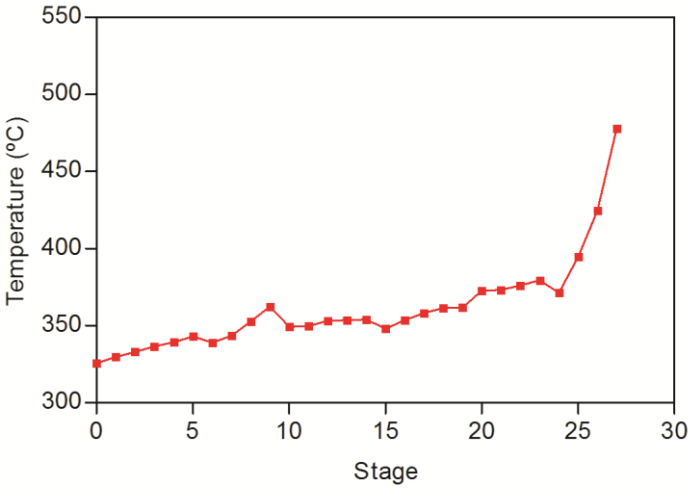
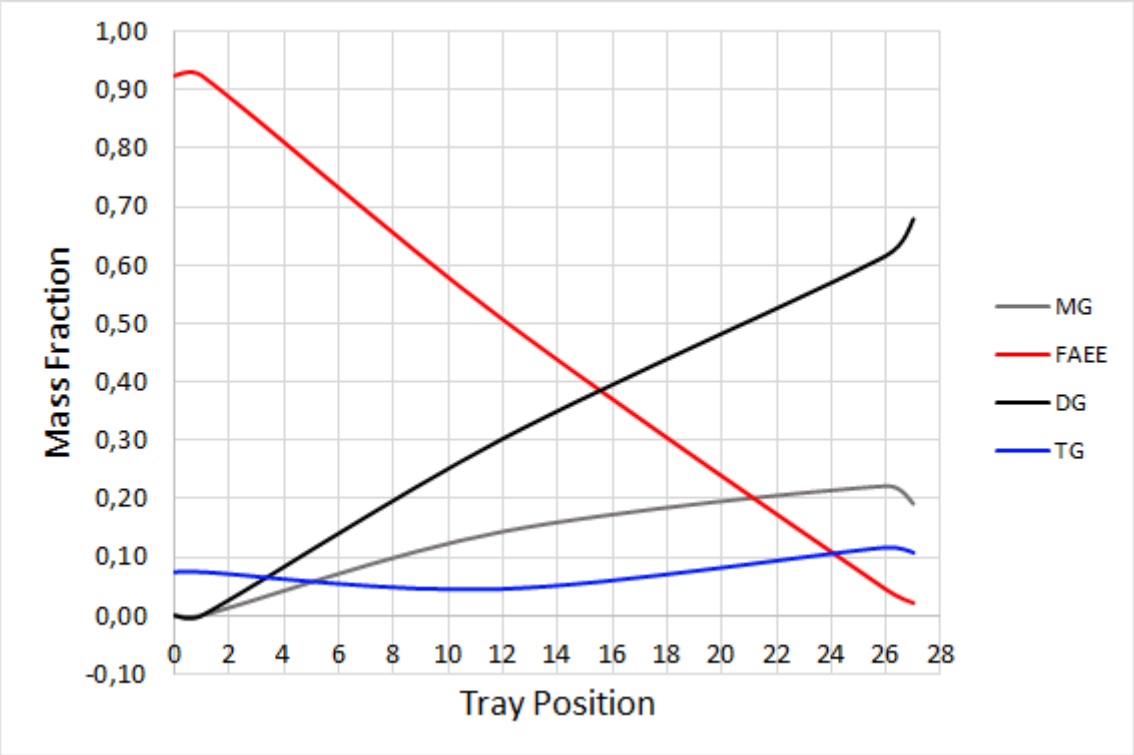
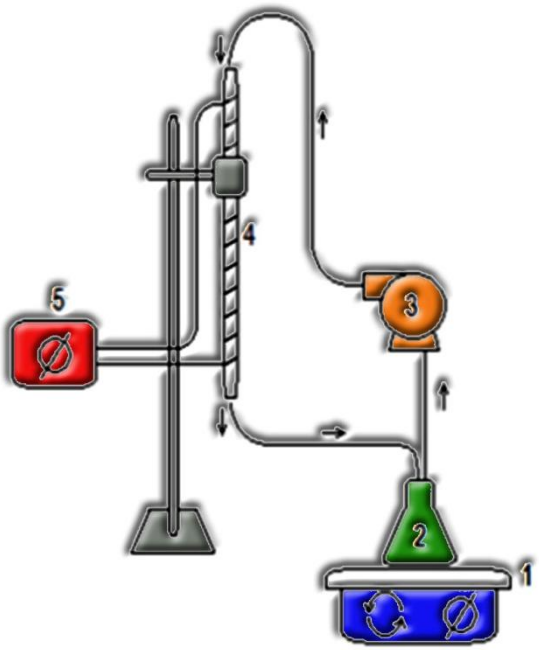


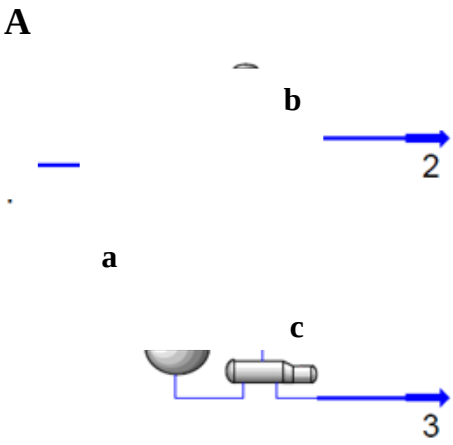
Figure 7



Scheme 1



Scheme 3



Higlights:

Biodiesel and emulsifiers were produced in a continuous system using L_{PF}/Ca/SBA-15.

Sunflower oil and commercial ethanol were used as raw material.

The biocatalyst was stable on the time of stream.

Partial TG transesterification activity is maintained under a non-glycerol production.

Emulsifiers and biofuel can be separated by vacuum distillation.