Original Article

Biofpr Biofuels Bioproducts & Biorefining

Optimization of the enzymatic butanolysis of jatropha oil for biodiesel production using Eversa

Hassan Acherki, Abderrahim Bouaid, Universidad Complutense de Madrid, Madrid, Spain
Jorge Mario Marchetti, Norwegian University of Life Sciences, Drøbakveien, Norway

Received July 20 2021; Revised October 05 2021; Accepted October 18 2021;
View online November 15, 2021 at Wiley Online Library (wileyonlinelibrary.com);
DOI: 10.1002/bbb.2309; *Biofuels, Bioprod. Bioref*. 16:219–227 (2022)

Abstract: The use of non-edible oils for energy production is attracting increasing attention, with a particular focus on those oils that can be produced from plants growing in non-arable areas. Among the possible alternatives, oils with high free fatty acid content, such as jatropha, have shown great potential. The optimization of jatropha oil in the presence of a bio-based alcohol (butanol) was investigated for the production of biodiesel using a commercial enzyme (Eversa from Novozymes). The optimization was conducted by changing the reaction temperature and the amount of catalyst, with a 6:1 butanol/oil molar ratio, while the reaction time was set to 60 min. The optimal result was found at the highest reaction temperature (42 °C) and the highest catalyst amount (9.79%), with a biodiesel yield of 83%. A second optimization was conducted, allowing the time to run for up to 8 h. Here the final biodiesel produced was in line with the EU14214 standards, fulfilling all the requirements with the exception of the requirement for oxidation stability. In addition to fulfilling most of the EU14214 standards, the cost of this new enzyme is lower than that of other commercial alternatives. Results show that biodiesel produced using butanol as alcohol in the transesterification process improved cold flow properties in terms of cloud point (CP), pour point (PP), and cold filter plugging point (CFPP). © 2021 Society of Chemical Industry and John Wiley & Sons, Ltd

Key words: biodiesel; DOE; Eversa; enzymatic transesterification; free fatty acids

Introduction

Increasing importance is being attributed to the fulfillment of the United Nations (UN) sustainable goals. Among those goals is UN goal 7, affordable and clean energy, which has led to an increase in research to find a solution to produce this. Among all the possible different clean energy sources, biodiesel has increased its importance due to political and societal change and due to its environmental benefits, moving away from fossil fuels and towards renewable sources.[1,2] Among some of the environmental benefits, biodiesel contributes less to global warming and produces less contaminant in its emission in comparison with fossil fuels.[3]

In general terms, biodiesel is defined as the mono alkyl esters of long-chain fatty acids derived from lipids such as

Correspondence to: Jorge Mario Marchetti, Faculty of Sciences and Technology, Norwegian University of Life Sciences, Drøbakveien 31, Ås 1432, Norway. E-mail: jorge.mario.marchetti@nmbu.no

vegetable oils, animal fats and microalgae oils. Its production is generally done with the esterification reaction of fatty acids and/or the transesterification reaction of triglycerides, in the presence of an alcohol and, most of the time, in the presence of a catalyst.[4]

Different catalysts have been evaluated for this reaction, including basic, acid, and enzymatic. Supercritical process have also been considered.[5–9] For biodiesel to be competitive against fossil fuel, cheaper raw materials need to be used so that the processing cost can be reduced from 70–80% of the overall biodiesel productions cost.[10,11] To achieve this goal, the reactants are have more impurities. Non-edible oil has a lower price and can be used for biodiesel production without giving rise to food-versus-fuel issues.[12–14] Oils from avocado stones,[15] jatropha seeds,[16] coffee grounds,[17] and jojoba,[18] among others, have been used successfully for biofuel production.

Among the different catalytic possibilities for production, solid heterogenous catalysts have gained a significant place in today's research arena, due to their high efficiency for the production of biofuel but also because they create the possibility of treating less pure reactants and can be reused a few times before regeneration is required.[19]

Another catalytic route to treat less pure reactants under milder conditions is by using enzymes. They are being considered increasingly, mainly because they are highly selective, can treat impurities (fatty acid and water for example), and their operational requirements are quite moderate (low temperature <40 °C and no need for pressure).[20] Some enzymes have already been tested technically for the production of biodiesel with promising results. However, one of the main restrictions on their use on an industrial scale has been their elevated cost.

Several different enzymes have been tested and evaluated for biodiesel production.[21] Among them, Noureddini *et al.*[22] produced biodiesel using *Pseudomonas cepacian* for the transformation of soybean oil in the presence of methanol. The process was carried out at a low 35 °C temperature but with a molar ratio of 15:1; the yield achieved was 65% after 1 h. Similarly, Lopresto *et al.*[23] studied the production of biodiesel using the same catalysts The authors found that, under the conditions of 37 °C, and 200 RPM, a stoichiometric molar ratio for ethanol, and a mass ratio of biocatalyst to oil of 3%, the yield obtained was 46%. Shah *et al.*[24] used the same enzymes but for the ethanolysis of jatropha oil. The authors obtained a yield of 98% at a reaction temperature of 50 °C in 8 h with a molar ration of 4:1.

Some commercial enzymes can also be used for biodiesel production. Zhou *et al.*[25] studied the use of Lipozyme TL100L for the conversion of waste seed oil into biodiesel. The authors carried out the experimental work with an enzyme load around 10%, a reaction temperature of 31 °C, and a reaction time of almost 7 h, with a final yield of 93%. The use of Novozymes 435 for biodiesel production has been studied widely, Nguyen *et al*[26] tested this catalyst for the production of biodiesel from animal fat. The reaction time was 12 h, but it reached a yield of over 96% for a molar ratio of 14:1 and a temperature of around 40 °C.

In 2015, Novozymes released a new enzyme, Eversa Transform 2.0.[27] This enzyme, as far as we are aware, has only been tested by Chang *et al.*[28] and Sun *et al.*[29] for the production of biodiesel from refined palm oil and Semen Abutili seed oil respectively. Chang *et al.*[28] carried out a reaction with a methanol oil molar ratio of 8:1, at a reaction temperature of 40 °C for 24 h, achieving a 97% yield. Sun *et al.*[29] studied the ethanolysis reaction, which lead to a biodiesel yield of 94% when running a higher lipase concentration of 6%, at a reaction temperature of 37 °C and for an 11 h reaction. Both of these studies showed promising results for this new catalyst.

The objective of the present study is to investigate the catalytic butanolysis of a high free fatty acid oil obtained from the jatropha plant using enzyme Eversa Transform 2.0 as a catalyst due to its promising results and the limited knowledge on its use with butanol. A design of experiments and response surface methodology was carried out. Variations on the reaction temperature and catalysts amount were studied. Their effect over the biodiesel production yield was quantified.

Materials and methods

Reagents and materials

Jatropha oil was obtained from Gracomsa Alimentaria (Valencia, Spain). The physic-chemical properties of the oil, as well as its fatty acid content, were determined and are summarized in Table 1. The catalyst employed was Eversa 2.0 from Novozymes. The alcohol used for the reactions was 1-butanol, of 99.8% purity, supplied by Panreac (Madrid, Spain). All reactants and catalysts were used as received.

Setup of the experiments

Experiments were carried out in a stirred batch reactor of 250 cm^3 volume. This reactor was provided with temperature and speed control and was immersed into a thermostatic bath capable of maintaining the reaction temperature to within ±0.1 °C of the set value by means of an electrical device connected to a proportional–integral–derivative controller. The impeller speed was tested

Table 1. Properties and fatty acid composition of jatropha oil.

Property	Units	Value
Kinematic viscosity (40 °C)	$mm^2 \cdot s^{-1}$	38.76
Density (30 °C)	$kg \cdot m^{3-1}$	0.92
Acidity index	mg (KOH)·g^{-1}	18.78
Iodine index	I_2*(100 g)$^{-1}$	115
Fatty acid composition		
Palmitic (C16:0)	wt%	13.72
Stearic (C18:0)	wt%	18.52
Oleic (C18:1)	wt%	45.61
Linoleic (C18:2)	wt%	21.18
Linolenic (C18:3)	wt%	0.2
Others	wt%	0.77

between 300 and 600 rpm and a value of 350 rpm was found appropriate to avoid external mass transfer limitation. The alcohol molar ratio used was 6:1. An excess of butanol is necessary to drive the equilibrium towards butyl ester formation. The 6:1 butanol to oil molar ratio (MR) was selected on the basis of preliminary and other studies.[30,31] There was a slight increase when the MR was raised from 4.5:1 to 8:1; the 6:1 ratio appears to give the best results. On the other hand, economic factors must also be taken into account; in this sense, the use of a relatively moderate molar ratio of 6:1 is most desirable. Thus, the initial alcohol/oil molar ratio was fixed at 6:1. Biobutanol was used as an alcohol in order to meet the challenging requirement of low temperature properties compared with those obtained using methanol or ethanol as alcohols – in addition to the fact that methanol is highly toxic, can be absorbed through the skin and is 100% miscible with water, so any kind of spill presents a serious problem.

Transesterification reactions

The oil was first added to the reactor. When the reaction temperature was reached a mix of alcohol and enzyme was added to the reactor and the reaction time was started. Samples were withdrawn at predetermined times. After 1 h the reaction was stopped, and the alcohol and enzyme were removed by separation and filtration. Figure 1 shows the separation of the phases; the biodiesel, the enzyme, and the glycerol can be seen.

A second round of experiments was carried out with a final time of 8 h. This second study was conducted to produce biodiesel to EU standards, in compliance with EN 14214.

The free fatty acids present in the oil were also converted into biodiesel when using this enzyme. Although the esterification reaction took place, it was not studied individually. It was the objective of this work to study whether the enzyme, Eversa, could be used for high free fatty acid oil reactants without the need for a prepurification step.

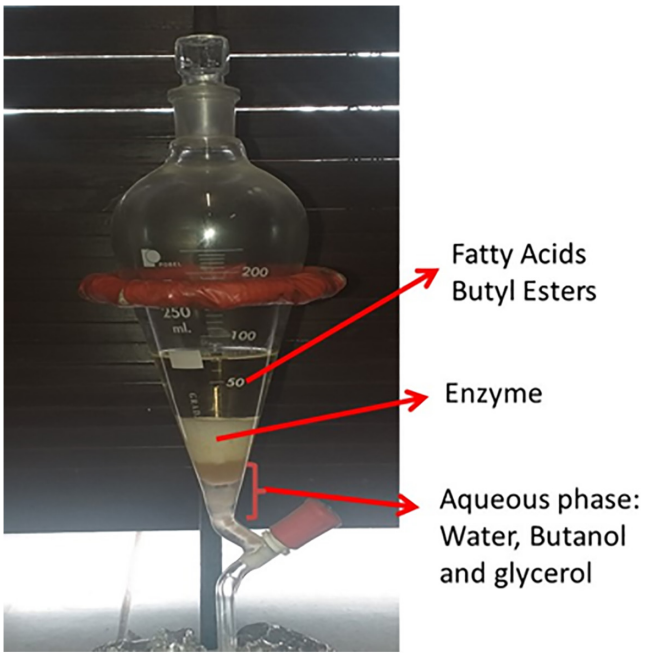


Figure 1. Phases after 4 h of reaction.

Analysis of the samples

Reaction products were monitored by capillary column gas chromatography, using a Hewlett–Packard 5890 series II equipped with a flame ionization detector (FID). The injection system was split–splitless. The carrier gas was helium at a flow rate of 1 mL·min^{-1}. The separation program consisted of an initial oven temperature of 120 °C was increased at 5 °C·min^{-1} to 160 °C, then at 20 °C·min^{-1} to 320 °C, and maintained for 20 min to complete the program. The internal standard technique has been used in order to quantify the amount of the chemical species.

Samples were analyzed according to the following procedures: acid value AV (AOCS Ca 5a-40), moisture content (Karl Fischer method), and viscosity ν (ISO 3104). The oxidation stability of butyl esters was analyzed using the Rancimat method, with Metrohm 743 (Rancimat, Herisau, Switzerland). The cloud point (CP), pour point (PP), and cold filter plugaging point (CFPP) of butyl esters were measured by an automatic analyzer (cloud and pour point measurements CPP 97-2), following the ASTM D97 and ASTM D2500 methods.

The reaction yield was calculated in accordance with Eqn (1):

$$Y_{FABE} = \frac{FABE(\%)}{TG(\%) + DG(\%) + MG(\%) + FABE(\%) + FFA(\%)} \quad (1)$$

where *FABE* is fatty acid butyl ester; *TG* is triglycerides; *DG* is diglyceride; *MG* is monoglycerides; *FFA* is free fatty acids.

Design of the experiment and analysis of data

Response surface methodology

Response surface methodology (RSM) involves of a group of statistical and mathematical tools that are useful for the development of models, and the improvement and optimization of a designed process and products.[32–34] This methodology will allow us to understand better the effect of the different variables involved and their joint effect on the reaction yield. The main purpose for using it is optimization.[32,33]

Design of the experiment

The transesterification reaction of jatropha oil with butanol in the presence of an enzyme was carried out to determine the effects of two selected factors with two levels, reaction temperature (T) and amount of catalyst (C), on jatropha oil conversion in the reaction with butanol (X_{JOB}). A central composite design (CCD) was used, which was full factorial and involved two factors and two levels. The design of the experiment contained 4 central points, 4 linear and 4 non linear stages. The center point was replicated 4 times for accuracy. In addition, the center point repeatability is used for the statistical analysis of the experiments and for the determination of the significance of the curvature. The values of the selected factors and their associated levels for the reactions are presented in Table 2.

Selection of the levels was carried out on the basis of results obtained in a preliminary study, considering the experimental installation limits and the working condition limits for each chemical species. These considerations made it possible to fix the upper temperature level at 42 °C, to avoid the loss of enzymatic activity caused by temperature. Temperatures less than 28 °C do not lead to effective enzymatic activity[35] and therefore yields obtained under these conditions are too low for industrial purposes. For this reason, the minimum temperature level was fixed at 28 °C. On the basis of preliminary experiments, the levels used for the catalysts concentration were chosen, usually between 4 and 10 wt%.[36] The levels chosen were 4.2 and 9.8 wt% of the whole mass reaction.

Statistical analysis

The statistical analysis was conducted using Statgraphics Centurion, version 17 (Statgraphics Technologies, Inc., The Plains, VA, USA). This approach allows us to determine the effect of each of the factors as well as their mutual interaction over the yield. Eight experiments, in total, were performed, 4 for the central point and 4 for the linear stage that were then analyzed using the statistical tools to determinate the significance of the curvature in the response surface plot and therefore the model that is required to be used.

Results and discussion

Experiments of different stages and their responses

As previously mentioned, the purpose of the butanolysis of jatropha oil using the enzyme Eversa Transform 2.0 was to study the effects of the reaction temperature as well as the amount of the catalyst on the reaction yields after 60 min, 4 h, and 8 h. The factorial CCD with two factors and two levels, which consisted of three stages, was used to optimize the process. Accordingly, a total of 12 experiments, four experiments for the linear stage, four for the center points, and four for the non-linear stage, respectively, were performed randomly, and the responses were recorded. The natural factors for the reaction temperature (T) and catalyst amount (C), and the coded factors for different levels of temperature (X_T) and catalyst amount (X_C), as well as the reported yield after 60 min, can be seen in Table 3.

As is evident from Table 3, the maximum experimental yield is 72% for the highest value of temperature and catalysts amount. From Table 3, it can also be seen that both of the variables that were studied had an impact on the reaction

Table 2. Values of the factors for different levels in the experimental design.

Factors	Levels				
	−α	−1	0	1	α
Temperature (°C)	28	30	35	40	42
Catalyst amount (wt%)	4.2	5	7	9	9.8

Table 3. Yields value for the CCD design.

Types of experiments	Run	Temp (°C)	Cat. (wt%)	X_T	X_M	Y_{FABEs} (%)
Linear stage	1	40	9	1	1	72
	2	40	5	1	–1	64
	3	30	9	–1	1	67
	4	30	5	–1	–1	68
Center point stage	5	35	7	0	0	58
	6	35	7	0	0	60
	7	35	7	0	0	63
	8	35	7	0	0	59
Non-linear stage	9	42	7	1.41	0	70
	10	28	7	–1.41	0	69
	11	35	9.8	0	1.41	68
	12	35	4.2	0	–1.41	64

yield. The reaction temperature had a lower impact on the yield when approaching low values, which can be related to not having enough energy to overcome the activation energy of the reaction under consideration.

Results of statistical analysis and response optimization

Statistical analysis of the responses

The analysis was performed to determine the influences of the reaction temperature (T) and catalyst amount (C) and their interactions (TC) on the responses of the butanolysis process. For this analysis we used Statgraphics Centurion software. The presence and significance of curvature ($P \leq 0.05$) in the response surface plot were also tested and the results can be seen in Table 4, where the different parameters obtained from the statistical analysis are presented.

As can be seen from Tables 3 and 4, the amount of catalyst, and its interaction with temperature, have the greatest effect at this reaction time, while temperature alone has a smaller effect. The values of R^2, and R^2 adjusted for the yield, were found to be 93.74 and 88.53, respectively (Table 4). This presents a good agreement between the model and the data.

From Table 4 it can be seen that the curvature is significant and therefore needs to be taken into consideration. A quadratic model was fitted with the data from the central, linear and non linear experimental information.

Table 4. Results from the statistical analysis for different parameters.

Parameters and types of tests	Responses Y_{FABEs}(%) $\bar{y}$ = 67.75
Main effects and interactions	I_T = 0.5
	I_C = 7.0
	I_{TC} = 4.5
R^2 and R^2 adjusted (%)	
R^2 (%)	93.74
R^2 adjusted (%)	88.53
Significance test:c confidence level:	95%
Mean responses (%)	60.00
Standard deviation	t = 3.18; s = 2.16
Confidence interval	±3.434
Significance of curvature	
Curvature	7.75
Confidence curvature interval	4.85
Significance	Yes

Regression models and response optimization

The natural values were used for the regression model. As presented in Table 4, the curvature is significant, therefore a quadratic model was used. Equation (2) shows this regression:

$$Y_{FABE} = 376.974 - 14.814T - 17.584C + 0.189T^2 + 0.225TC + 0.750C^2 \quad (2)$$

where T is temperature and C is catalysts amount.

To show the effects of the interaction of the temperature and the amount of catalysts on the yield of biodiesel production, three-dimensional (3D) response surface plots were produced based on the regression model presented above. The response surface plot for the effects of the interaction of the two factors

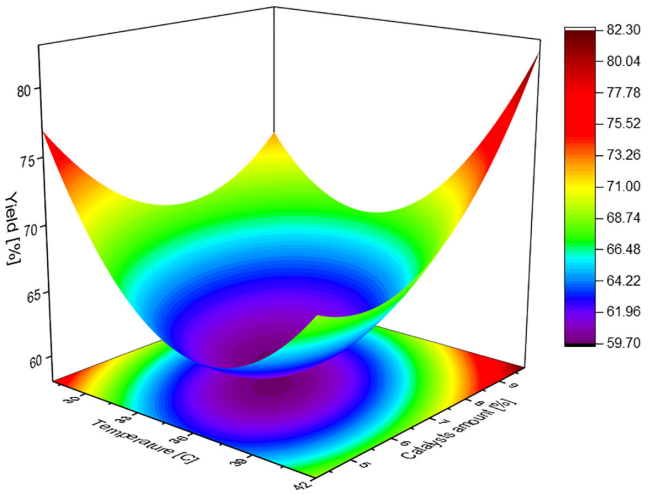


Figure 2. Response surface plot for the effects of reaction temperature and catalyst amount on the yield.

Table 5. Optimum conditions predicted for maximum yield at the optimum values.

Factors	Yield	
	Optimum factors	Optimum response (%)
Temperature (°C)	42.07	
Catalyst amount (%)	9.82	82.66

on Y_{FABEs} is shown in Fig. 2. As can be seen in Fig. 2, the plot shape is that of a bowl with a minimum in the middle and a maximum on one of the edges. This maximum is achieved when temperature and the amount of catalyst is as high as possible.

This behavior could be due to the fact that when there are lower levels of catalyst we have a relatively high yield, perhaps because the free fatty acids of the oil are esterified and some oil is transesterified, which will produce water in the medium (as a result of the esterification reactions) and glycerol molecules (as a result of the transesterification reactions); in this case the catalyst could be losing activity, perhaps due to the glycerol becoming more soluble at the water-enzyme interface, which deactivates the enzyme as it is an amphiphilic molecule working on the interface between the water and oil, as mentioned by Neilsen *et al.*[37]

When we further increase the amount of catalyst, the transesterification reaction predominates because the starting FFA present in the medium has already been consumed and the fatty acid butyl esters increase due to the transesterification reaction, which may be a possible explanation for the behavior observed in the graph. Similar results were found by Nordblad *et al.*[38]

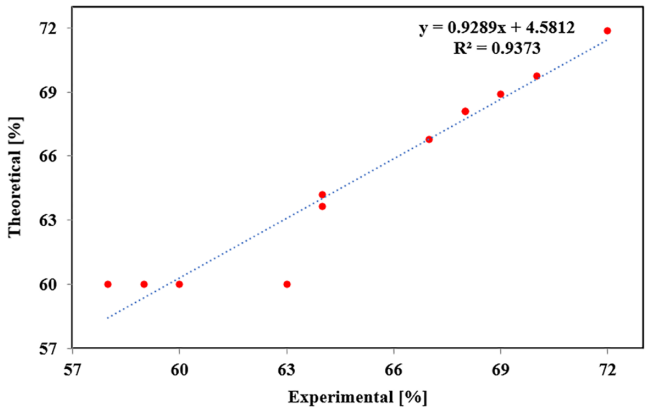


Figure 3. Fitting of predicted and experimental values of YFABEs.

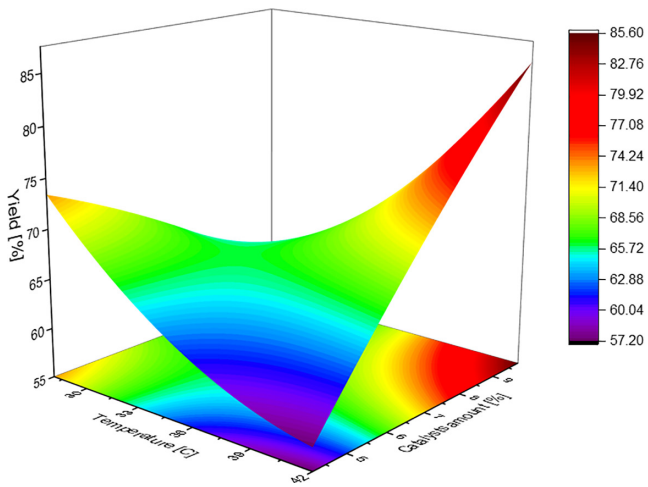


Figure 4. Surface plot for reaction time of 4 h.

By using the quadratic model equations produced and the RSM, it was estimated that the maximum Y_{FABEs} of 82.66% could be found at the optimum temperature of 42.07 °C and a catalyst amount of 9.82%. Table 5 shows this result.

The maximum Y_{FABEs} (82.66%) found in the current study was in line with the fatty acid butyl ester yield that was obtained by Navas *et al.*[39] in a reaction of castor oil with butanol using MgO/γ-Al_2O_3 as catalyst, applying 5% catalyst, an alcohol:oil molar ratio of 6:1, a reaction temperature of 80 °C and a reaction time of 6 h. The authors also obtained a yield of 85% when using ZnO/γ-Al_2O_3 as catalyst under the previously mentioned reaction conditions. These results are promising and show that this catalyst has strong potential to produce biodiesel from high free fatty acid-based oils. These results are comparable to those obtained by Avhad *et al.*[40] and Keneni and Marchetti,[41] where the use of jatropha with different catalysts was studied and the yields obtained

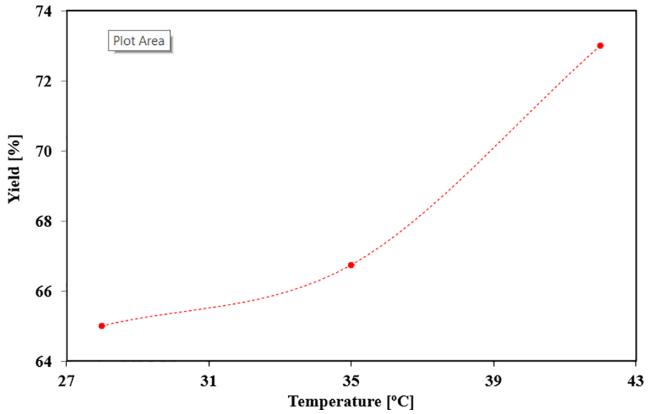


Figure 5. Effect of temperature changes on biodiesel yield for reaction time of 4 h.

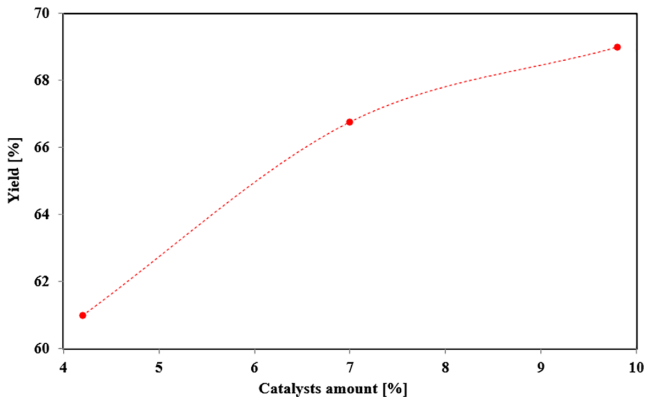


Figure 6. Effect of catalyst amount changes on biodiesel yield for reaction time of 4 h.

were 96% in both cases. It is important to point out that the catalysts employed were different and therefore the operational conditions were also different.

Figure 3 shows the plot of the predicted values and experimental results for the biodiesel yield. As can be seen from Fig. 3, the agreement between the data and the model is satisfactory, with a R^2 of 0.93.

In addition to the previous mentioned Design of Experiments (DOE) and optimization methodology, a RSM was also conducted for a 4 h reaction time. This was carried out with the idea of evaluating the evolution of the RSM surface and the curvature. As can be seen in Fig. 4, the curvature is less and less relevant and this is due to the fact that the equilibrium conversion is closer and closer for all tested operational conditions, making the final answer similar in all cases. Figures 5 and 6 show the variation in the yield when the temperature and the amount of catalyst change, respectively. It can be seen that, in both cases, there is a positive effect, meaning higher operational conditions (higher values of temperature and catalysts concentration) gives higher yield of biodiesel.

Table 6. Quality control o butyl ester compared to EN 14214.

Properties	Biodiesel	EU standard EN 14214
Viscosity (40 °C)	4.87	Max. 5.00 $mm^2 \cdot s^{-1}$
Acid value (mg KOH·g^{-1})	0.15	Max. 0.50 mg KOH·g^{-1}
Water content	230	Max. 500 mg·kg^{-1}
Ester content (wt.%)	98.5	Min. 96.5% (m·m^{-1})
Monoglyceride content (wt.%)	0.2	Max. 0.80% (m·m^{-1})
Diglyceride content (wt.%)	0.15	Max. 0.20% (m·m^{-1})
Triglyceride content (wt.%)	0.20	Max. 0.20% (m·m^{-1})
Free glycerol (wt.%)	0.01	Max. 0.02% (m·m^{-1})
Total glycerol (wt.%)	0.12	Max. 0.25% (m·m^{-1})
Cloud point (°C)	–6.00	*
Pour point (°C)	–7.50	*
Cold filter plugging point (CFPP)	–3.5	*
Oxidative stability (h)	3	Min. 8 h

*Not specified. EN 14214 uses time and location dependent values for the cold filter plugging point (CFPP) instead.

Optimization and fuel properties

Beside the experimenal work done for the DOE and RSM, used for the optimization of the process; a new set of experiments with a longer reaction time were carried out in order to produce biodiesel that was in line with the EU standards 14214. The reaction was carried out under the optimal operational conditions but for a reaction time of 8 h. The final yield was 98.5%, whereas EU standards required 96.5%. Table 6 shows the result of testing for some of the physicochemical properties of the butyl ester produced and its comparison with EU standards. As can be seen, the biodiesel produced fulfills all of the requirements with the exception of the requirements for oxidative stability, which is considerably under the limit. The use of commercial synthetic or natural antioxidants could improve the oxidation stability of the biodiesel fuel obtained.

Conclusion

The present study was carried out to investigate the catalytic butanolysis of jatropha oil using Eversa 2.0, and to study the influence of the reaction temperature and the amount of catalyst used on the biodiesel production yield. A full

factorial CCD that involved two factors and two levels was used to determine the effects of the factors and to optimize the process. It was found that both factors had some influence on the process and the curvature was significant when the process was studied after 1 h of reaction; a quadratic regression model was therefore required. The optimal yield was estimated at 82.66% when the temperature was 42 °C and the amount of catalyst was 9.8%. The comparison of the experimental data and the model was very accurate, with a regression factor of 0.93.

Longer reaction times of 4 h and 8 h were also tested, which allow us to see that the variation of the RSM as well as to reach a biofuel that satisfied the EU14214 standards. When the reaction is close to the equilibrium, the final yield for almost all the tested operational conditions is quite similar. When doing a Response Surface Methodology analysis, the data presents a linear tendency, making the quadratic model not required any longer in this analysis. The final biofuel produced satisfied the EU standards in all aspect except for oxidative stability, which was under the limit.

This approach has been proven effective for the treatment of high free fatty acid oils, such as frying oil, or waste oils, in the presence of a renewable alcohol and a bio-based catalyst, making it a greener and more sustainable process for the production of biofuels from non-edible oils.

Acknowledgements

Financial support from Norwegian University of Life Sciences (Ås, Norway) (NMBU) and Universidad Complutense de Madrid (Madrid, Spain) (UCM) is gratefully appreciated. We want to thank Novozymes for their donation of the enzyme.

References

1. Mishra VK and Goswami R, A review of production, properties and advantages of biodiesel. *Biofuels.* **9**:273–289 (2018).
2. Avhad MR and Marchetti JM, A review on recent advancement in catalytic materials for biodiesel production. *Renew Sustain Energy Rev* **50**:696–718 (2015).
3. Chattopadhyay S and Sen R, Fuel properties, engine performance and environmental benefits of biodiesel produced by a green process. *Appl Energy* **105**:319–326 (2013).
4. Mohiddin MNB, Tan YH, Seow YX, Kansedo J, Mubarak NM, Abdullah MO *et al*., Evaluation on feedstock, technologies, catalysts and reactor for sustainable biodiesel production: A review. *J Ind Eng Chem* **98**:60–81 (2021).
5. Avhad MR and Marchetti JM, Innovation in solid heterogeneous catalysis for the generation of economically viable and ecofriendly biodiesel: A review. *Catal Rev* **58**(2):157–208 (2016).
6. Changmai B, Vanlalveni C, Ingle AP, Bhagat R and Rokhum L, Widely used catalysts in biodiesel production: A review. *RSC Adv* **10**:41625–41679 (2020).
7. Santos S, Puna J and Gomes J, A review on bio-based catalysts (immobilized enzymes) used for biodiesel production. *Energies* **13**:3013 (2020).
8. Bohlouli A and Mahdavian L, Catalysts used in biodiesel production: a review. *Biofuels* **12**:885–898 (2021). https://doi.org/10.1080/17597269.2018.1558836.
9. Jayakumar M, Karmegam N, Gundupalli MP, Gebeyehu KB, Asfaw BT, Chang SW *et al*., Heterogeneous base catalysts: Synthesis and application for biodiesel production - A review. *Bioresour Technol* **331**:125054 (2021).
10. Rezania S, Oryano B, Park J, Hashemi B, Yadav KK, Kwon EE *et al*., Review on transesterification of non-edible sources for biodiesel production with a focus on economic aspects, fuel properties and by-product applications. *Energ Conver Manage* **201**:112155 (2019).
11. Gebremariam SN and Marchetti JM, Economics of biodiesel production. *Energ Conver Manage* **168**:74–84 (2018).
12. Gui MM, Lee KT and Bhatia S, Feasibility of edible oil vs. non-edible oil vs. waste edible oi as biodiesel feedstock. *Energy* **33**:1646–1653 (2008).
13. Shahzad K, Nizami AS, Sagir M, Rehan M, Maier S, Khan MZ *et al*., Biodiesel production potential from fat fractions of municipal waste in Makkah. *PLoS One* **12**(2):0171297 (2017).
14. Fonseca JM, Teleken JG, de Cinque Almeida V and da Silva C, Biodiesel from waste frying oil: methods of production and purification. *Energ Conver Manage* **184**:205–218 (2019).
15. Avhad MR, Sanchez M, Bouaid A, Martinez M, Aracil J and Marchetti JM, Modeling chemical kinetics of avocado oil ethanolysis catalyzed b solid glycerol enriched calcium oxide. *Energ Conver Manage* **126**:1168–1177 (2016).
16. Keneni YG, Hvoslef-Eide AK and Marchetti JM, Optimization of the production of biofuel from Jatropha oil using a recyclable anion exchange resin. *Fuel* **278**:118253 (2020).
17. Caetano NS, Silva VFM, Melo AC, Martins AA and Mata TM, Spent coffee grounds for biodiesel production and other applications. *Clean Technol Environ Policy* **16**:1423–1430 (2014).
18. Sanchez M, Avhad M, Marchetti JM, Martinez M and Aracil J, Enhancement of the jojobyl alcohols and biodiesel production using a renewable catalyst in a pressurized reactor. *Energ Conver Manage* **126**:1047–1053 (2016).
19. Zabeti M, Daud WMAW and Aroua MK, Activity of solid catalysts for biodiesel production: A review. *Fuel Process Technol* **90**(6):770–777 (2009).
20. Cavalcante FTT, Neto FS, Falcao IRA, Souza JES, Junior LSM, Sousa PS *et al*., Opportunities for improving biodiesel production via lipase catalysis. *Fuel* **288**:119577 (2021).
21. Tan T, Lu J, Nie K, Deng L and Wang F, Biodiesel production with immobilized lipase: A review. *Biotechnol Adv* **28**:628–634 (2010).
22. Noureddini H, Gao X and Philkana RS, Immobilized pseudomonas cepacian lipase for biodiesel fuel production from soybean oil. *Bioresour Technol* **96**:769–777 (2005).
23. Lopresto CG, Naccarato S, Albo L, de Paola MG, Chakraborty S, Curcio S *et al*., Enzymatic transesterification of waste vegetable oil to produce biodiesel. *Ecotoxicol Environ Saf* **121**:229–235 (2015).
24. Shah S and Gupta MN, Lipase catalyzed preparation of biodiesel from Hatropha oil in a solvent free system. *Process Biochem* **42**(3):409–414 (2007).
25. Zhou Y, Li K and Sun S, Simultaneous esterification and transesterification of waste phoenix seed oil with a high free fatty acid content using a free lipase catalyst to prepare biodiesel. *Biomass Bioenergy* **144**:105930 (2021).
26. Nguyen HC, Liang SH, Chen SS, Su CH, Lin JH and Chien CC, Enzymatic production of biodiesel from insect fat using methyl

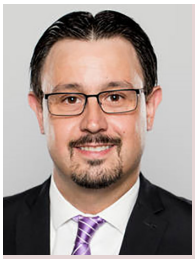
acetate as an acyl acceptor: Optimization by using response surface methodology. *Energ Conver Manage* **158**:168–175 (2018).
27. Novozymes releases Eversa enzymes. *Ethanol Prod Mag* **21**(2):25 (2015).
28. Chang MY, Chan ES and Song CP, Biodiesel production catalyzed by low-cost liquid enzyme Eversa Transform 2.0: Effect of free fatty acids content on lipase methanol tolerance and kinetic model. *Fuel* **283**:119266 (2021).
29. Sun S, Guo J and Chen X, Biodiesel preparation from Semen Abutili (Abutilon theophrasti medic) seed oil using low-cost liquid lipase Eversa transform 2.0 as catalyst. *Ind Crops Prod* **169**:113643 (2021).
30. Bouaid A, Acherki H, Garcia A, Martínez M and Aracil J, Enzymatic butanolysis of oil. Biorefinery approach. *Fuel* **2091**:141–149 (2017).
31. Bouaid A, Martínez M, El Boulifi N, Hahati K and Aracil J, Biodiesel production from biobutanol. Improvement of cold flow properties. *Chem Eng J* **238**:234–241 (2014).
32. Muriithi DK, Koske JKA and Gathungu GK, Application of central composite design based response surface methodology in parameter optimization of watermelon fruit weight using organic manure. *Am J Theor Appl Stat* **6**(2):108–116 (2017).
33. Montgomery DC, *Design and Analysis of Experiments*, 8th edn. John Wiley & Sons, IC, New York, NY (2013).
34. Myers RH, Montgomery DC, Vining GG, Borror CN and Kowalski SM, Response surface methodology: A retrospective and literature survey. *J Qual Technol* **36**(1):53–77 (2004).
35. Andrade TA, Errico M and Christensen KV, Transesterification of castor oil catalyzed by liquid enzymes: Optimization of reaction conditions. *Comput Aided Chem Eng* **40**:2863–2868 (2017).
36. Andrade TA, Errico M and Christensen KV, Influence of the reaction conditions on the enzyme catalyzed transesterification of castor oil: a possible step in biodiesel production. *Bioresour Technol* **243**:366–374 (2017).
37. Neilsen PM, Rancke-Madsen A, Holm HC and Burton R, Production of biodiesel using liquid phase formulations. *J Am Oil Chem Soc* **93**:905–910 (2016).
38. Nordblad M, Silva VTL, Woodley JM and Nielsen PM, Identification of critical parameters in liquid enzyme-catalyzed biodiesel production. *Biotechnol Bioeng* **111**(12):2446–2453 (2014).
39. Navas MB, Lick ID, Bolla PA, Casella ML and Ruggera JF, Transesterification of soybean and castor oil with methanol and butanol using heterogeneous basic catalysts to obtain biodiesel. *Chem Eng Sci* **187**:444–454 (2018).
40. Avhad MR, Gangurde LS, Sanchez M, Bouaid A, Aracil J, Martinez M *et al*., Enhancing biodiesel production using green glycerol-enriched calcium oxide catalyst: An optimization study. *Catal Lett* **148**:1169–1180 (2018).
41. Keneni YG and Marchetti JM, Butanolysis of Jatropha oil using glycerol enriched non-calcined calcium oxide: Optimization of the process. *Fuel* **289**:119908 (2021).

Hassan Acherki

Dr Acherki is a researcher at the Laboratory of Design, Optimization and Scale-up of Integrated Processes in the Complutense University of Madrid, Spain. His current work is focused on the conversion of sustainable oils into high value-added products and studying the use of renewable raw materials processed in biorefineries for the design of industrial products.

Abderrahim Bouaid

Dr Bouaid is a chemical engineering professor in the Complutense University of Madrid and a member of the Laboratory of Design, Optimization and Scale-up of Integrated Processes. He is an expert in chemical engineering, biodiesel production, renewable energy, optimization of chemical processes, and approaches to the transformation of sustainable oils into high value-added products and biorefineries. He has published several papers in international peer-reviewed journals and has contributed several book chapters over his 20 years of university experience. He is also a reviewer of several reputable international journals.



Jorge Mario Marchetti

Dr Jorge Marchetti is a professor and the leader of the Reaction Engineering and Catalysis Group at the Norwegian University of Life Sciences in Ås, Norway. He is an expert on renewable energy, biofuels, green chemistry, and on processing system engineering. His work focuses on the valorization of waste biomass and its transformation into biochemicals, biofuels, and bioplastics following a biorefinery concept approach. His work is also related to the development and application of catalysts, kinetics, processing modeling, and techno-economical assessments.