Electronic Supplementary Material (ESI) for Green Chemistry

Continuous Flow Valorization of Fatty Acid Waste Using Silica-Immobilized Lipases

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SUPPORTING INFORMATION

1. Experimental Design:

A two level factorial design was adopted for a preliminary study designed to identify the best conditions on the esterification reaction between different fatty acids sources and solketal. First, we carried out a fractional factorial designs 2^{4-1} to determine the variables with higher influence on the desired reaction under catalysis of KX-2**.**

Variables with their respective levels used on the fractional factorial design (FFD) are presented on Table 2.

Table S1: Real and codified values (+ high, 0 intermediate, - lower levels) for independent variables, 2^{4-1}.

Variables	-1	0	+1
Temperature (^{0}C)	40	50	60
Amount of enzyme (%)	0.1	0.55	1
Substrate concentration (mM)	50	75	100
Stirring (rpm)	50	150	250

It is also observed in Table 4 that the curvature check was not significant, as it shows a $p > 0.05$ with no need of adding points to the quadratic mathematical model.

Table S2: Estimate effects for substrates studied in FFD.

Variable	Effect		p-value	
	Stearic acid	Acid Residue	Stearic acid	Acid Residue
Mean	77,9375	71,96250	<0,0001*	<0,0001*
Curvature check	-8,2083	-3,85833	0,0596	0,1410
Temperature (T)	8,3750	8,52500	0,0167*	0,0097*
Amount of enzyme (E)	18,1750	9,92500	0,0036*	0,0072*
Substrate concentration (S)	-16,3250	22,77500	0,0044*	0,0013*
Stirring (St)	-1,0250	-3,22500	0,4484	0,0629

The values for the significant variables had to be readjusted (ESI) due to the different values of the amount of enzyme and substrate concentration for stearic acid and acid residue, respectively.

Table S3: Real and codified values (+ high, 0 intermediate, - lower levels) for independent variables, $2^3$for each substrate (stearic acid and acid residue).

Variables	-1		0		+1	
	Stearic acid	Acid Residue	Stearic acid	Acid Residue	Stearic acid	Acid Residue
Temperature (^{0}C)	50		55		60	
Amount of enzyme (%)*	0,5	0,5	1	0,85	1,5	1,2
Substrate concentration(mM)	40	80	60	115	80	150

The experimental data have been adjusted to the propose model and the adequacy was performed by the analysis of variance and parameter R^2. The equations 1 e 2 represents the mathematical model for the esterification of solketal in function of the studied variables for reactions with stearic acid and acid residue, respectively.

Equation 1. $Y= 89.72 + 5.13E -2.04S +2.18T$

Equation 2. $Y= 92.64 + 4.08E$

Where Y is the percentage of conversion, and E, T and S are the uncoded values of amount of enzyme, temperature and substrate concentration, respectively. Statistical testing of the model was done by the Fisher's statistical test for ANOVA (see supplementary data).

The calculated F for both reactions, with stearic acid and acid residue (16.67 and 56.4 respectively) were higher than those tabulated Fs ($F_{3,7} = 4.34$ and $F_{2,8} = 4.45$ for reaction with stearic acid and acid residue, respectively) showing the experimental model validity. The goodness of the model can be checked by the determination (R^2). The determined coefficients (R^2 = 0.91 and 0.96 for reaction with stearic acid and acid residue, respectively) implies that the sample variation of 91 and 96% for ester production is attributed to the independent variables and can be accurately explained by the model.

2. XPS Analysis

XPS (aka ESCA) measurements were performed in a ultra high vacuum (UHV) multipurpose surface analysis system (Specs^{TM} model, Germany) operating at pressures $<10^{-10}$ mbar using a conventional X-Ray source (XR-50, Specs, Mg-Kα, 1253.6 eV) in a “stop-and-go” mode to reduce potential damage due to sample irradiation. The survey and detailed Fe and Cu high-resolution spectra (pass energy 25 and 10 eV, step size 1 and 0.1 eV, respectively) were recorded at room temperature with a Phoibos 150-MCD energy analyzer. Powdered samples were deposited on a sample holder using double-sided adhesive tape and subsequently evacuated under vacuum ($<10^{-6}$ Torr) overnight. Eventually, the sample holder containing the degassed sample was transferred to the analysis chamber for XPS studies. Binding energies were referenced to the C1s line at 284.6 eV from adventitious carbon. The curve deconvolution of the obtained XPS spectra were obtained using the Casa XPS program.

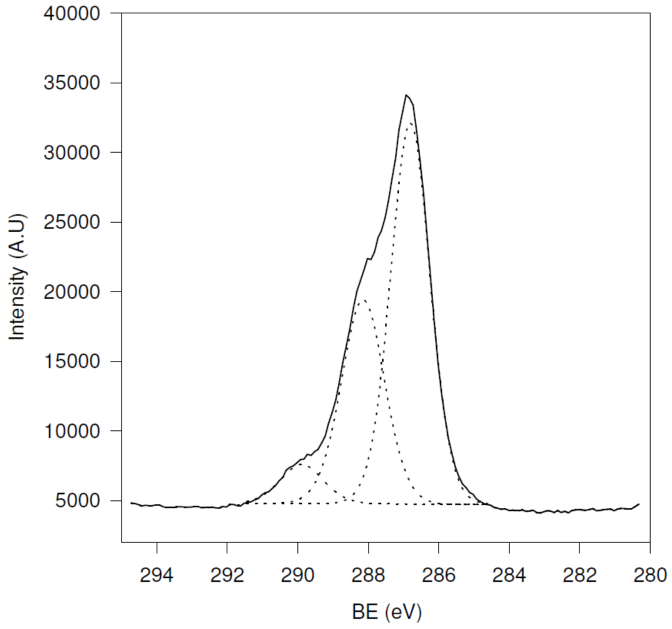

Figure S1: XPS analysis of immobilized enzyme where a proportion of 25 g acetonitrile and 75 g buffer solution of Cal-B at 293 K was used. No activity was observed.

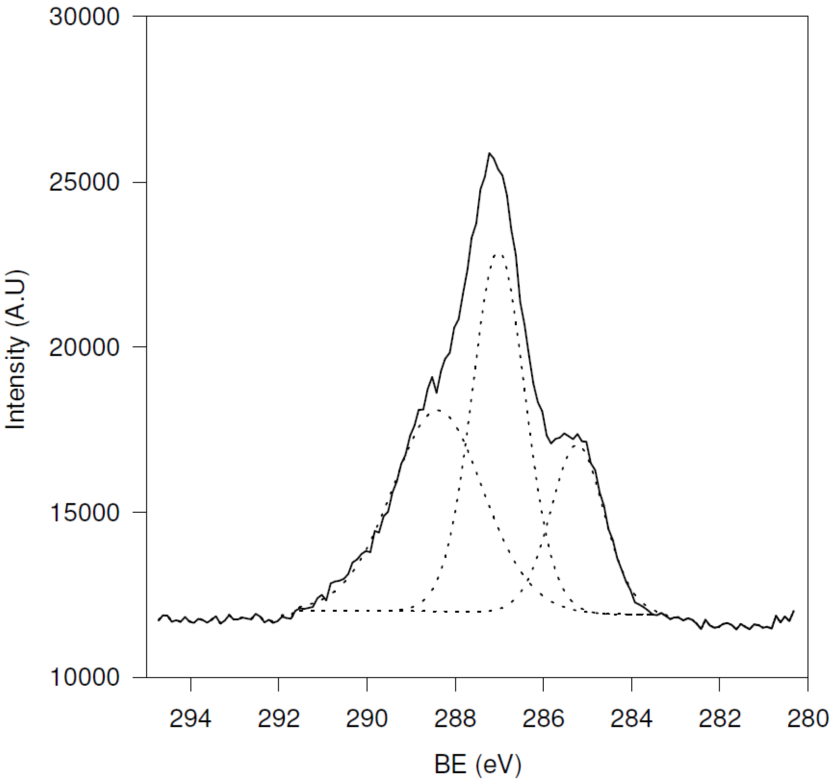

Figure S2: XPS analysis of immobilized enzyme where only buffer solution of Cal-B at 293 K was used. No activity was observed.