Electronic Supplementary Material (ESI) for RSC Advances.

Lipase Immobilized in Microemulsion Based Organogels (MBGs) as an Efficient Catalyst for Continuous-Flow Esterification of Protected Fructose.

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Supporting Information

FFD Experiments:

Table S1. Real and coded values (+ higher level, 0 intermediate, - lower level) for the independent variables in the FFD (2^{5-1}) for esterification for both solvents used.

Variable	-1	0	+1
Temperature (^{0}C)	40	45	50
Amount of enzyme (μg)	30	105	180
Substrate concentration in heptane (mM)	5	25.5	50
Substrate concentration in toluene (mM)	5	52,5	100
Molecular sieves (%)*	10	15	20
Stirring (rpm)	50	150	250

*w/v

Once the relevant variables were selected by factorial design, a central composite rotatable design (CCRD) was employed to obtain the optimum conditions for esterification synthesis for each solvent used. This design was considered the axial points for obtaining a quadratic model. The variables, along with their coded and values, are given in Table S2 and S3, for heptane and toluene, respectively. In the CCRD, each of the three selected variables was varied at five levels.

Table S2. Real and coded values (+ higher level, 0 intermediate, - lower level) for the independent variables in the CCRD (2^3) for esterification performed in heptane.

Variables	-1,68	-1	0	+1	+1,68
Amount of enzyme (μg)	30	54.3	90	125.7	150
Substrate concentration (mM)	5	10.1	17.5	24.9	30
Stirring (rpm)	150	180.4	225	269.6	300

Conditions maintained constant: temperature (50^{0}C) and amount of molecular sieves (15%).

Table S3. Real and coded values (+ higher level, 0 intermediate, - lower level) for the independent variables in the CCRD (2^3) for esterification performed in toluene.

Variables	-1,68	-1	0	+1	+1,68
Temperature (^{0}C)	45	47	50	53	55
Substrate concentration (mM)	5	10.1	17.5	24.9	30
Molecular sieves (%)*	10	12.02	15	17.98	20

*w/v
Conditions maintained constant: amount of enzyme (105 µg) and stirring (150rpm).

The experimental designs and results analysis were carried out using the software Statistica 6.0 (Statsoft, Inc., USA) according to the significance level established to obtain the mathematical model. The significance of the regression coefficients and the associated probabilities, p(t), were determined by Student's t test; the model equation significance was determined by Fisher's F test. The variance explained by the model is given by the multiple determination coefficients, R^2

Experimental data and the matrix for the FFD for the selection of most important variables conditions for esterification reaction in a batch conditions are represented in Table S4. FFD consisted of sixteen factorial points and three replications at the central point for to calculated the experimental error.

Table S4. Matrix of fractional factorial experimental design (2^{5-1}) with coded values and conversion reactions performed in toluene and heptanes.

Entries	T (^{0}C)	E (µg)	S (mM)	M. S. (%)[a]	St (rpm)	Heptane (%)[b]	Toluene (%)[b]
1	-1	-1	-1	-1	+1	62	64,1
2	+1	-1	-1	-1	-1	64,4	54,7
3	-1	+1	-1	-1	-1	56,1	52,8
4	+1	+1	-1	-1	+1	59,4	59,9
5	-1	-1	+1	-1	-1	49,7	2,2
6	+1	-1	+1	-1	+1	58,4	30,9
7	-1	+1	+1	-1	+1	66	27,1
8	+1	+1	+1	-1	-1	25,8	42,2
9	-1	-1	-1	+1	-1	56,1	56,6
10	+1	-1	-1	+1	+1	64,8	64,1
11	-1	+1	-1	+1	+1	56,7	49,0
12	+1	+1	-1	+1	-1	62,1	50,9

13	-1	-1	+1	+1	+1	59,3	14,8
14	+1	-1	+1	+1	-1	62,6	42,9
15	-1	+1	+1	+1	-1	41	18,9
16	+1	+1	+1	+1	+1	55,9	17,9
17	0	0	0	0	0	46,2	35,9
18	0	0	0	0	0	46,2	34,9
19	0	0	0	0	0	44	33,8

[a] w/v

[b] Measured by Lowry–Tinsley method.

Pareto chart from CCRD performed in toluene and hexane:

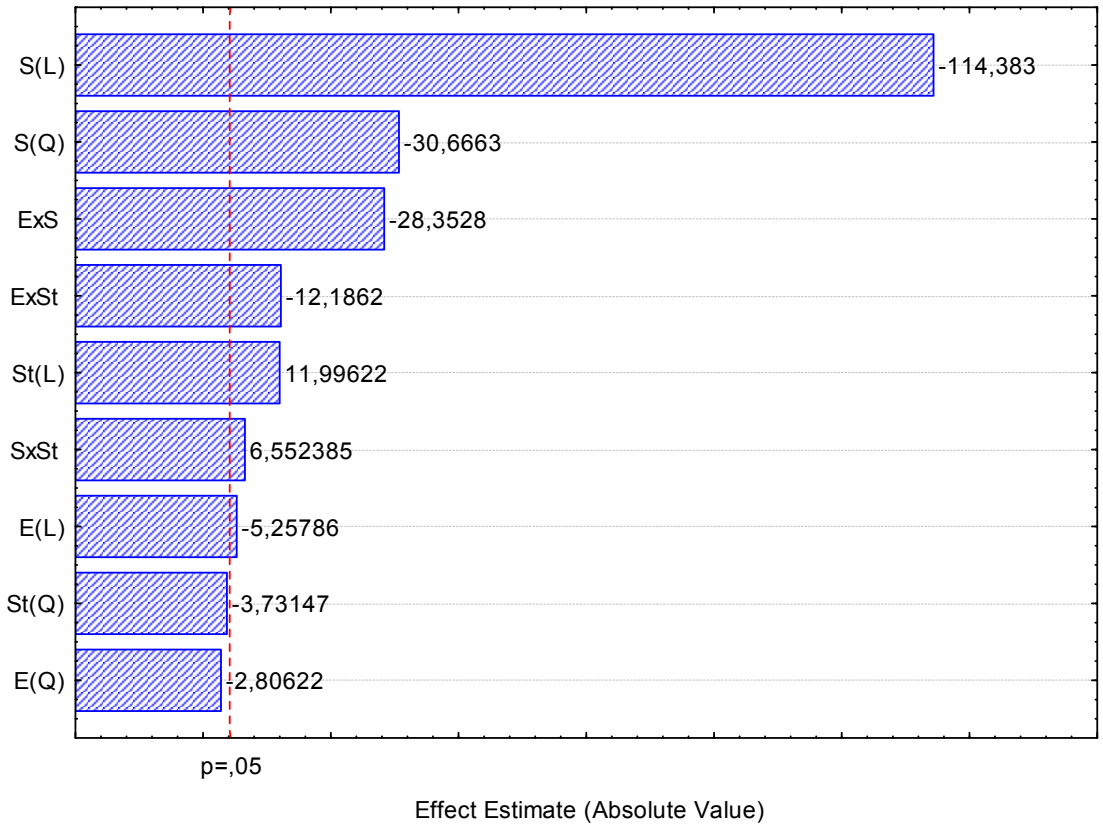

Figure S1. Pareto chart from CCRD performed in heptane showing the significance of experimental variables: T, temperature; S, substrate concentration; E, amount of enzyme; St, stirring; L, linear effect; and Q, quadratic effect

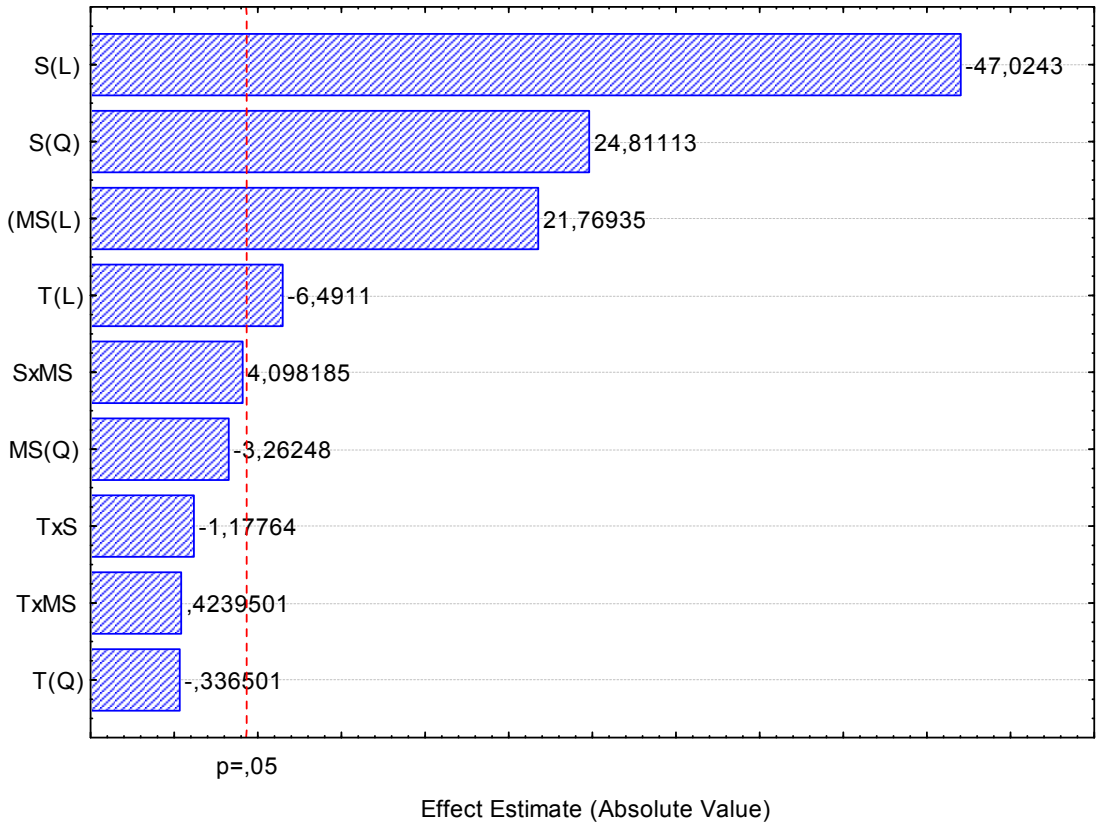

Figure S2. Pareto chart from CCRD performed in toluene showing the significance of experimental variables: T, temperature; S, substrate concentration; MS, amount of molecular sieve; L, linear effect; and Q, quadratic effect

Statistical testing of the model was done by the Fisher's statistical test for ANOVA.

Table S7. Variance analysis for validation of mathematical models (ANOVA)*

Factor	Sum of Squares		Degrees of freedom		Mean Square		F calculated		F tabulated	
	H	T	H	T	H	T	H	T	H	T
Regression	5083,16	1964,10	7	4	726,16	491,02	11,90	23,87	3,29	3,25
Residuals	549,12	246,82	9	12	61,01	20,56				
Lack of fit	548,45	245,60	7	10						
Pure error	0,66	1,21	2	2						
TOTAL	5632,28	2210,91	16	16						

Confidence level 95%.
H for reactions performed in heptane and in toluene.