Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Consecutive Lipase Immobilization and Glycerol Carbonate Production under Continuous-Flow Condition

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Support Information

1. Details on Reaction optimization

Aiming to develop a better method in terms of selectivity we decided to move forward our studies to investigate the use of surfactants on the glycerol carbonate reaction since in the beginning of our work we have identified that the reaction conditions applied were biphasic and the use of surfactants could help on producing a more homogeneous reaction media. Initially we have investigated the reactions catalyzed by Novozym 435 and after, applied the best reaction conditions to our *Candida antarctica* lipase B immobilized on Accurel MP1000 resin.

We have screened several surfactants at reaction times of 48 hours, analyzing conversion and selectivity obtained in each case. In addition to the solvent free system, we have also tested a few organic solvents such as acetonitrile, tert-butanol and tetrahydrofuran (THF). The obtained results are summarized on Table 2.

Table S1: Evaluation of surfactants and solvents on the enzymatic synthesis of GC catalyzed by Novozym 435.

Surfactants	Solvent free		Acetonitrile		*t*- Butanol		THF	
	Conv (%)	sel (%)	Conv (%)	sel (%)	Conv (%)	sel (%)	Conv (%)	sel (%)
AOT	43	47	32	0	11	18	27	45
SDS	62	57	20	68	0	0	9	59
Brij	64	67	50	55	41	74	54	53
Brij 35	57	8	24	0	38	5	0	0
Brij 52	66	35	80	22	39	25	55	24
Brij 58	66	39	51	30	27	32	23	24
Brij 76	61	55	33	43	0	0	56	41
Brij 78	62	55	33	43	9	34	49	43
Brij 98	62	39	55	26	31	26	51	29

*conversion and selectivity measured by GC-MS, following the reference [26]. Reaction Conditions: Glycerol: DMC (3 mmol: 9 mmol), 20% of immobilized enzyme, 15% of surfactants, 1 mL of solvents, 60° C, 200 rpm and 48 hours.

As can be observed by the results presented on Table S2, the solvent free system in general presented the best combination between conversion and selectivity towards glycerol carbonate production and the selectivity obtained under these conditions are much higher than the ones obtained on the system without surfactants indicating that homogeneity of reaction media can be an important factor for selective issues.

To evaluate the estimated effects of several variables in this reaction system, as well as to optimize reaction conditions, a three level factorial design was carried out to compare the performance of the four selected surfactants (SDS, Brij, Brij 76 and Brij 78) in the synthesis of glycerol carbonate under *Candida antarctica* lipase B immobilized on Accurel MP1000 resin catalysis. For each surfactant one design of experiments was employed, resulting in 29 experiments for each surfactant (Table S3) where enzyme concentration, surfactant concentration and glycerol/DMC molar ratio were evaluated.

Table S2. Real and codded values

Variable	**-1**	**0**	**+1**
Enzyme (%)	5	12,5	20
Surfactant (%)	5	10	15
Molar ratio (Gli:DMC)	1:1	1:2	1:3

Reaction Conditions: Temperature (60° C) and stirring (200 rpm) for 48 hours

Table S3: Experimental factorial design 3^3 with levels of studied variables and conversion results for each surfactant from the reaction catalyzed by *Candida antarctica* lipase B immobilized on Accurel MP100.

Entry	Enzyme (% w/v)	Surfactant (%)	Gly/DMC	Conversion (%)			
				SDS	BRIJ	BRIJ 76	BRIJ 78
1	5	5	1:1	10	50	14	58

2	12.5	5	1:1	34	55	20	43
3	20	5	1:1	9	10	22	47
4	5	10	1:1	15	44	39	54
5	12.5	10	1:1	26	53	47	55
6	20	10	1:1	25	11	19	33
7	5	15	1:1	37	37	54	28
8	12.5	15	1:1	45	51	22	32
9	20	15	1:1	43	22	20	35
10	5	5	1:2	33	49	59	53
11	12.5	5	1:2	49	53	61	25
12	20	5	1:2	54	19	62	34
13	5	10	1:2	41	49	59	51
14	12.5	10	1:2	29	40	66	58
15	12.5	10	1:2	30	37	60	59
16	12.5	10	1:2	26	36	58	62
17	20	10	1:2	51	12	65	29
18	5	15	1:2	48	51	57	37
19	12.5	15	1:2	48	50	59	30
20	20	15	1:2	50	49	63	29
21	5	5	1:3	40	52	61	49
22	12.5	5	1:3	45	51	62	36
23	20	5	1:3	57	51	64	34
24	5	10	1:3	43	48	63	48
25	12.5	10	1:3	53	48	63	28
26	20	10	1:3	53	52	69	49
27	5	15	1:3	45	45	60	37
28	12.5	15	1:3	52	46	63	31
29	20	15	1:3	48	47	70	29

*conversion and selectivity measured by GC-MS [26]. Reaction Conditions: 60° C and 200 rpm for 48 hours.

From the analysis of the estimated effects for the reaction with SDS and Brij it was observed that the molar ratio between glycerol and DMC was the most significant variable, suggesting that higher conversions can be achieved at higher molar ratios (within the range

studied), according to Pareto graphs (Figures S1, S2, S3 and S4). The difference between SDS and Brij is related to the amount of surfactant needed to achieve good conversions, where Brij is more efficient with low amounts of surfactant.

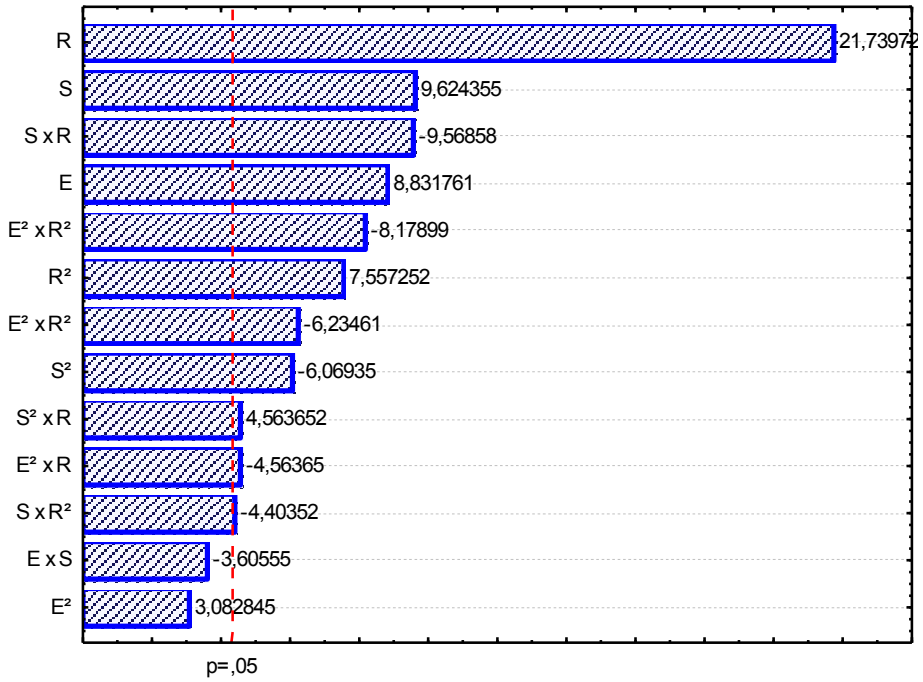

Figure S1. Pareto chart for the estimated effects on the reaction performed in the presence of SDS as surfactant.

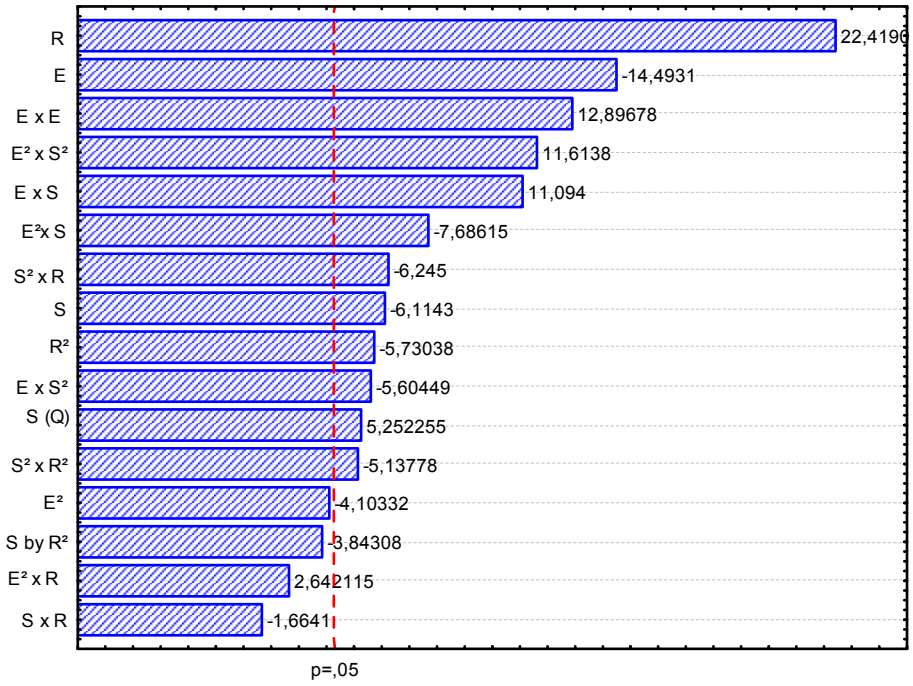

Figure S2. Pareto chart for the estimated effects on the reaction performed in the presence of Brij as surfactant.

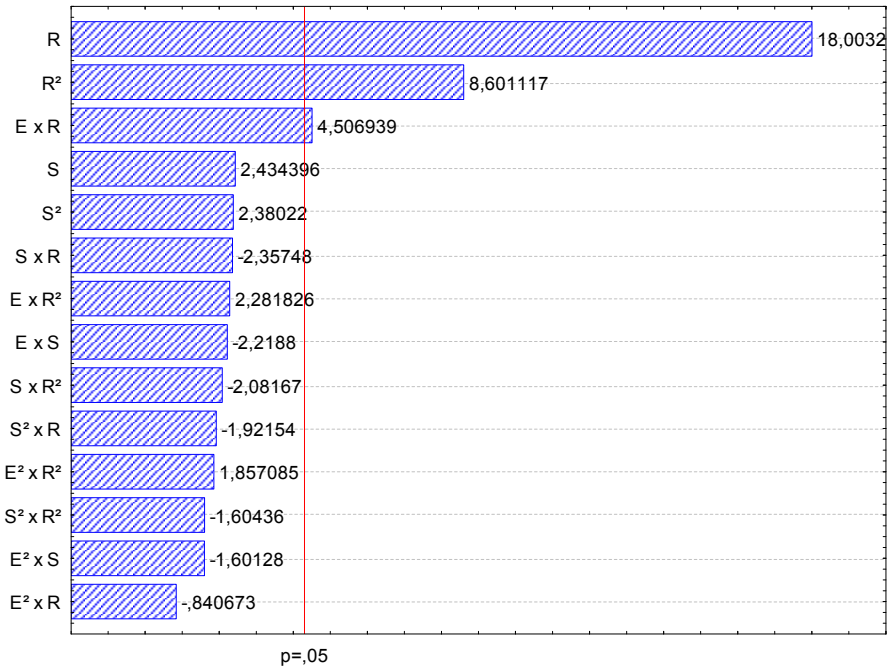

Figure S3. Pareto chart for the estimated effects on the reaction performed in the presence of Brij 76 as surfactant.

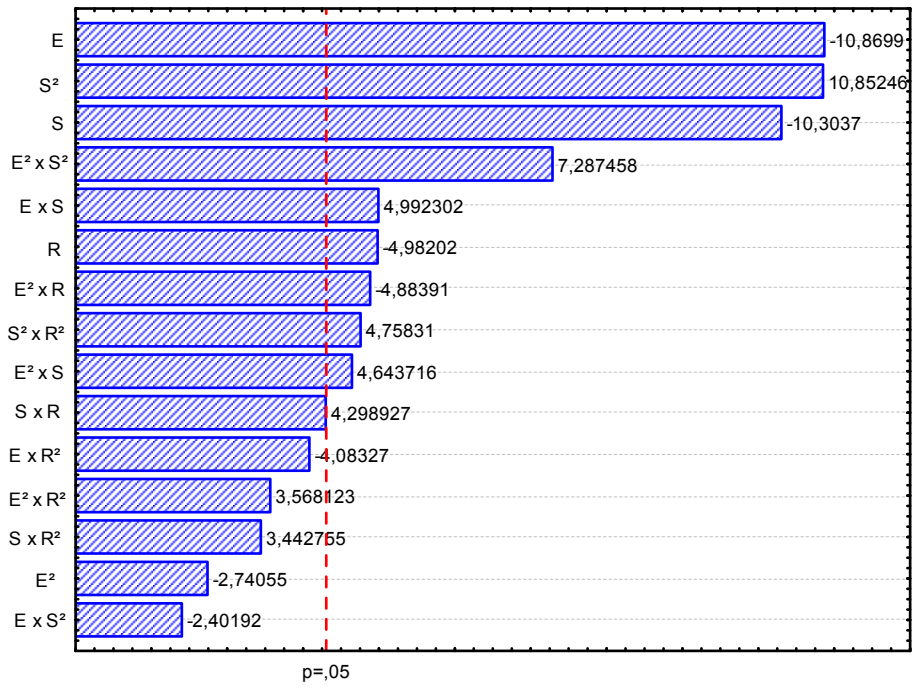

Figure S4. Pareto chart for the estimated effects on the reaction performed in the presence of Brij 78 as surfactant.

For reactions with Brij 76, the molar ratio clearly showed to be the most significant variable both, in its linear and quadratic terms. All other variables showed no significant influence. For Brij 78 the amount of enzyme was the most significant variable along with the amount of surfactant. The response surfaces of the studied variables can be observed in Figure S5.

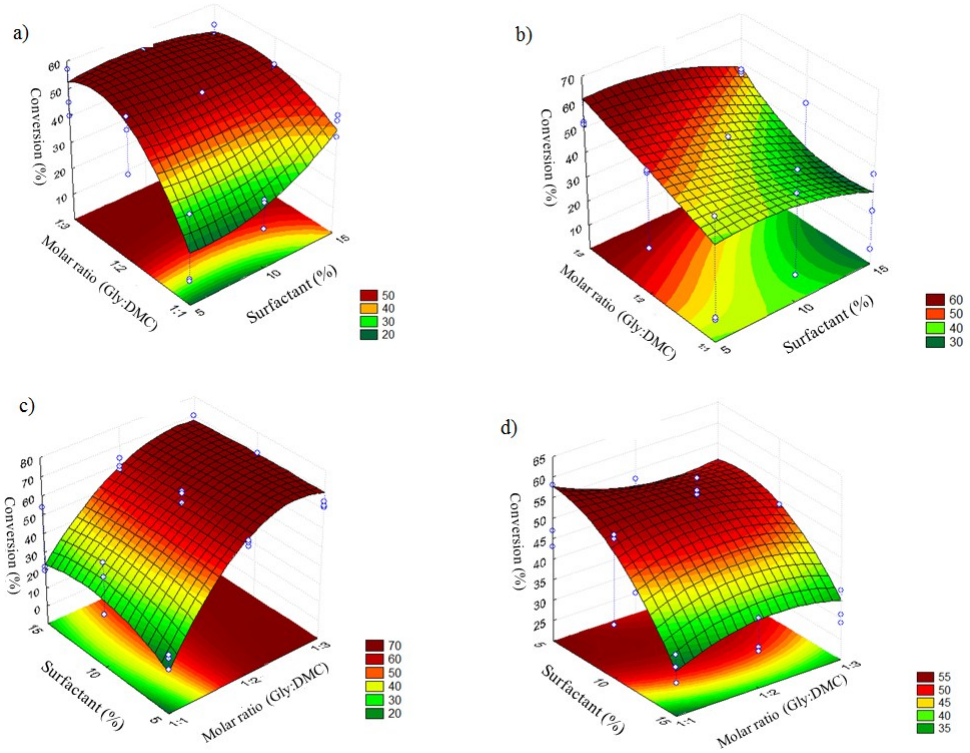

Figure S5: Response surfaces of glycerol carbonate synthesis in the presence of different surfactants: (a) SDS, (b) Brij, (c) Brij 76 and (d) Brij 78.

The validation of the mathematical model for the reactions with each surfactant was performed by analysis of variance (ANOVA) and R^2 parameter. For all surfactants studied, calculated F values were higher than F values tabulated, showing the validity of the model.

The high coefficients of determination for SDS (R2 = 0.89), Brij (R^2 = 0.79), Brij 76 (R^2 = 0.92) and Brij (R^2 = 0.78) show that the mathematical models satisfactorily explain the data obtained (Table S4, S5, S6 and S7).

Table S4. Analysis of variance (ANOVA) for the reaction carried out with SDS as surfactant.

Factor	Sum of Squares	Liberty Degrees	Mean Square	F Calculated	F tabulated	P value
Regression	4441,358	13	394,8947	9,9231	2,448	0,000039
Residue	516,435	15	36,11478			
Lack of adjust	507,768	13	40,35231			
Pure Error	8,667	2	4,333333			
TOTAL	4957,793	28				

Table S5. Analysis of variance (ANOVA) for the reaction carried out with Brij as surfactant.

Factor	Sum of Squares	Liberty Degrees	Mean Square	F Calculated	F Tabulated	P value
Regression	6093,66828	16	380,854267	2,8970	2,602	0,034119
Residue	1577,5731	12	131,464425			
Lack of adjust	1568,906	10	156,890643			
Pure Error	8,667	2	4,33333333			
TOTAL	7671,241	28				

Table S6. Analysis of variance (ANOVA) for the reaction carried out with Brij 76 as surfactant.

Fator	Sum of Squares s	Liberty Degrees	Mean Square	F Calculated	F Tabulated	P value
Regression	8188,91674	14	584,922624	11,8269	2,484	0,000020

Residue	692,393606	14	49,4566861
Lack of adjust	657,727	12	54,8105783
Pure Error	34,667	2	17,3333333
TOTAL	8881,310	28	

Table S7. Analysis of variance (ANOVA) for the reaction carried out with Brij 78 as surfactant.

Factor	Sum of Squares	Liberty Degrees	Mean Square	F Calculated	F tabulated	P value
Regression	2936,44463	15	195,762976	3,1225	2,533	0,022876
Residue	815,003643	13	62,6925879			
Lack of adjust	806,337	11	73,3033615			
Pure Error	8,667	2	4,33333333			
TOTAL	3751,448	28				