Effect of Operating Variables and Kinetics of the Lipase Catalyzed Transesterification of Ethylene Carbonate and Glycerol

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Abstract: Glycerol carbonate (GC) is a value-added product originating from the valorization of widely available glycerol (Gly), a side stream from the production of biodiesel. Here we approach the production of this chemical comparing two reactions based on the transesterification of Gly with dimethyl carbonate (DMC) and ethylene carbonate (EC). When using DMC, it was observed that the free enzyme CALB (lipase B from Candida antarctica) gave the best results, whereas Eversa Transform (a genetic modification of Thermomyces lanuginosus lipase) performed better than the rest if EC was the reagent. With the selected catalysts, their immobilized analogous enzymes Novozym 435 and Lipozyme TL IM, respectively, were also tested. Observing that the yields for the reaction with EC were significantly faster, other operating variables were evaluated, resulting the best performance using a closed system, tert-butanol as solvent, a concentration of enzyme Eversa Transform of 3% \( \text{w/w} \), a molar excess of EC:Gly of 9:1 and a temperature of 60 \( ^\circ \text{C} \). Finally, several runs were conducted at different temperatures and molar ratios of EC:Gly, fitting a kinetic model to all experimental data for the reaction catalyzed with Eversa Transform. This model included the bimolecular transesterification reaction of Gly and EC catalyzed by the lipase and a reversible ring-opening polymerization of EC.

Keywords: glycerol; glycerol carbonate; Novozym 435; Lipozyme TL 100 L; Eversa Transform 2.0; kinetic model

1. Introduction

Valorization of glycerol (Gly) is a blossoming topic of research given the need to make good use of the abundant surplus caused by the development of the biodiesel industry [1], which, in the end, has led to a marked decline in the retail prices in spite of its use in the food, healthcare, pharmaceutical, or tobacco industries [2]. Although the valorization of Gly for energy related purposes has been pursued [3,4], it has not proved as successful as using this compound as a platform chemical owing to its rich reactivity that can lead to a very wide array of products of interest for several industries [5,6].

One of the many products that can be obtained is glycerol carbonate (GC), which has attracted a lot of interest in the last years due to its outstanding physicochemical characteristics. It has been used mainly as a green solvent for purposes that include chemical reaction media, \( \text{CO}_2 \) separation
Several reviews have described extensively the methods of production of GC [7–10], which could be summarized in the following reaction pathways: (i) addition of CO or CO$_2$ to Gly under pressurized conditions; (ii) reaction of urea and Gly under vacuum conditions so as to remove the ammonia generated as by-product; and (iii) transesterification of Gly with organic carbonates (OC).

For the latter route, the authors of the present work have conducted extensive research using both dialkyl carbonates like dimethyl carbonate (DMC) [11,12] or cyclic carbonates like ethylene carbonate (EC) [13–16], or more recently, propylene and butylene carbonate [17]. The advantage of this reaction pathway is that alcohols or glycols can be concomitantly produced when dialkyl or cyclic carbonates, respectively, are used as co-substrates along with Gly.

Whilst the synthesis of GC from Gly by any of the methods described above has mostly been attained with homogeneous and heterogeneous catalysts, the use of enzymes for this process has received far less attention. Thus far, the reaction between Gly and DMC has been reported in the literature mainly using Novozym 435 (immobilized *Candida antarctica* Lipase B) [18–21], although some efforts have also been made with lipase from *Aspergillus niger* supported on magnetic nanoparticles [22]. In addition, enzymes have also been used successfully for the transesterification of Gly with diethyl carbonate [23] or the interesting reaction for the simultaneous production of GC and biodiesel via the reaction of DMC and different oils, thus avoiding the generation of Gly [24–27]. In all cases, the conversions attained were quantitative and with high selectivities to the desired product of almost 100%.

The free enzyme Eversa Transform (a genetic modification of *Thermomyces lanuginosus* lipase) is another product commercialized by Novozymes that has shown very promising applications in transesterification reactions, for it has been used to yield biodiesel from different oils as feedstock [28,29] or to concentrate unsaturated monoacylglycerols from fish oil [30]. Lipozyme TL 100 (*Thermomyces lanuginosus* lipase) is yet another free enzyme that has been tested for the synthesis of sucrose-6-acetate, an intermediate to the synthesis of sucralose, a sweetener; in addition, its immobilized form Lipozyme TL IM was also tried in this study [31]. The latter has also been reported in interesterification reactions to obtain trans-free fats from canola oil and palm oil oleins or hydrogenated soybean oil blends [32] or in acetylation reactions to obtain eugenyl acetate, with antimicrobial activity [33].

Given the limited miscibility between Gly and organic carbonates [13,15], many authors have tried performing the respective transesterifications in the presence of solvents to favor the mutual solubility of the compounds [34,35]. This approach has also been tried in enzymatic reactions, where the solvation of enzymes is known to affect positively on the activity of enzymes like CALB (lipase B from *Candida antarctica*) [36].

The aim of this work is to study the synthesis of GC by transesterification of Gly with EC (reaction 1) and DMC (reaction 2) with the liquid lipase preparations CALB, Lypozyme TL 100, and Eversa Transform as well as the immobilized lipase preparations Novozyme 435 and Lypozyme TL IM. A progressive optimization of the operational conditions with the best preparations is performed afterwards, selecting the most productive system to perform its kinetic study, finally.

### 2. Materials and Methods

#### 2.1. Chemicals

The reactants used for experimental work were glycerol (purity > 99.9%) from Fisher Chemical, dimethyl carbonate (purity = 99%) from Acros Organics Ltd. and ethylene carbonate (purity = 99%), supplied by Scharlau. As solvents, tert-butanol (purity = 99%) from Alfa Aesar and tetrahydrofuran (purity > 99.9%) from Fisher Chemical were used. For calibration of the HPLC method, the following were used: methanol (purity > 99.9 %) from Scharlau, ethylene glycol (anhydrous, purity = 99.8%)
and glycerol carbonate (purity 99%) from Sigma-Aldrich in addition to citric acid (purity = 99%) from Sigma Aldrich.

2.2. Enzymes

The following free enzymes were employed throughout the experimental work: CALB (Candida antarctica lipase B) and Lyposyme TL 100 L (Thermomyces lanuginosus lipase) and Eversa Transform 2.0 (lipase from a genetic modification of Thermomyces lanuginosus), which were a kind gift from Novo Nordisk Bioindustry. For stability purposes, the commercial formulation of such free enzymes contains glycerol and water; thus, considering the small amounts of the limiting reactant Gly used in the experiments, HPLC analysis of the enzymes was made so as to calculate accurately the amount of Gly to be added in each of the experiments. It was found that the Gly content was 0.37, 0.23 and 0.03 g/L for Eversa Transform, CALB and Lyposyme TL 100 L, respectively.

As for immobilized enzymes, Novozym 435 (Candida antarctica lipase B immobilized on a macroporous acrylic resin) and Lyposyme TL IM (Thermomyces lanuginosus lipase immobilized on silica gel) were tested here.

2.3. Transesterification Runs and Analytical Method

Kinetic runs were performed in 50 mL round-bottom flasks magnetically stirred and heated in glycerol baths placed on IKA heating plate RCT basic with controlled magnetic agitation and PDI temperature control, working in initial reaction volumes of 25 mL. After mixing the organic carbonate and Gly, the cosolvent (either THF or tert-butanol) was added till getting only one liquid phase. Afterwards, the addition of the biocatalyst was made after the contents of the flasks reached the desired temperature. Two different operation modes were tried using the reaction flask open to the atmosphere or closed with a lid to check for volume changes before and after the experiments. These volume changes were monitored by volumetric and gravimetric measurements, while chemical composition of the remaining liquid was determined by HPLC as explained in the next paragraph.

For runs with free enzyme, samples were withdrawn at reaction volumes of 100 µL at a time and then prepared for analysis in 900 µL of an aqueous solution of 5 g/L of citric acid. For runs using immobilized enzyme, 300 µL were withdrawn and subjected to centrifugation so that 100 µL of the supernatant could be finally sampled and prepared for analysis as described above. Subsequent analysis of samples was performed by a HPLC method following a device and method reported in previous works [14]. Briefly, for the HPLC analysis, a method based on ion-dipole interaction and molecular volume exclusion was employed, with a REZEX H+ 300 x 7.5 mm column for carboxylic acids as stationary phase, placed in an oven at 65 °C, H2SO4 5 mM in Milli-Q water flowing at 0.5 mL min⁻¹ as eluent, and a refractive index equipment set at 45 °C as detector.

2.4. Statistical Methods

Once the most reactive system was chosen, several runs changing two classical operation conditions: temperature and molar ratio of carbonate—in excess—to glycerol. A kinetic model was then proposed on the basis of the temporal evolution of the involved compounds concentrations. This model was fitted to all retrieved data at the same time by using a gradient non-linear regression algorithm (Levenberg-Marquardt) coupled to numerical integration (4th order Runge-Kutta) of the kinetic equations, algorithms that were implemented in software Aspen Custom Modeler v10. The statistical analysis led to optimal values of the kinetic parameters in the equations, together with their error intervals and a series of goodness-of-fit parameters that indicate the adequacy of the kinetic model to represent the change of the system chemical composition with time. The basic goodness-of-fit parameter is SQR: the sum of quadratic residues, which should tend to zero as the fitting improves; Other goodness-of-fit parameters are calculated from it: the residual mean squared error (RMSE) corresponds to the square root of SQR divided by the degrees of freedom, for which, again, a trend to
zero would be indicative of better fittings; and finally Fisher $F$, with a tendency to infinity for the best fit, as $SQR$ is in its denominator. They were calculated with these equations:

$$SQR = \sum_{i=1}^{N} (C_{i,\text{exp}} - C_{i,\text{calc}})^2$$  \hspace{1cm} (1)

$$\text{RMSE} = \sqrt{\frac{SQR}{N-K}}$$  \hspace{1cm} (2)

$$F = \frac{\sum_{i=1}^{N} C_{i,\text{calc}}^2 / K}{\sum SQR / (N-K)}$$  \hspace{1cm} (3)

where $C_{i,\text{exp}}$ is the molar concentration of each compound, $C_{i,\text{calc}}$ is the concentration of compound $i$ estimated by the kinetic model with the optimal values of the kinetic parameters, $N$ is the number of data, and $K$ is the number of kinetic constants in the model (3, for the proposed model).

3. Results and Discussion

3.1. Preselection of Reaction, Enzyme, and Operating Mode

3.1.1. Selection of Enzymes for Each Reaction

The three free enzymes CALB, Lypozone TL 100 L, and Eversa Transform were tested to investigate their performance at a molecular level in the transesterification of Gly with EC and DMC. The conditions selected for this screening experiments considered a molar ratio of OC to Gly of 2:1 and a temperature of 60 °C in a closed system, which have been tested previously for the same reactions catalyzed with potassium methoxide (Esteban et al., 2015a). Figures 1 and 2 show the conversion of Gly reached as well as the yields to each of the products achieved after 24 and 48 h for reactions A and B, respectively.

![Figure 1](image1.png)  \hspace{1cm} (a)

![Figure 2](image2.png)  \hspace{1cm} (b)

The results from Figure 1a show that Eversa Transform 2.0, an enzyme formulated by Novozymes A/S for biodiesel production from waste oil, is the most active free enzyme for the reaction between Gly and EC, reaching a yield to GC of 42% and 54% after 24 and 48 h, respectively, compared to 38% and 49% for CALB (lipase B from Candida antarctica) after the same periods or 31% and 33% for Lypozone TL 100 L.
TL 100, an enzyme preparation containing the lipase of *Thermomyces lanuginosus*, a thermophilic fungus normally found in compost heaps and employed in detergent formulation.

Figure 1b depicts that the performance of Eversa Transform in the case of reaction 2 is definitely much lower, practically not observing any product in the first 24 h and reaching only a conversion of 4% after 48 h, which agrees with the fact that no products were observed using the immobilized form Lipozyme TL IM when performing this reaction [18]. On the other hand, Lipozyme TL 100 showed a remarkable improvement reaching 15% of yield after 24 h and 23% after 48 h, although CALB was by far the best for this reaction, obtaining 41% and 54% of yield after the same periods. The latter fact indeed is a good indicator of why it is the enzyme of choice of previous works for this reaction, although it was used in its supported form Novozyme 435 [18–21].

For this reason, with the two best performing enzymes Eversa Transform and CALB, further experiments were conducted for up to 100 h employing this time a molar ratio of OC to Gly of 3:1.

As Figures 2 and 3 depict, yields to GC observed were 83% and 81% for reactions A and B, respectively. Bearing these results in mind, these long run experiments were extended to commercially available immobilized enzymes corresponding to TL 100 L and CALB, i.e., Lipozyme TL IM and Novozyme 435 for the two reactions. The aforementioned figures display the yields to product observed with immobilized enzymes, which are comparatively lower than those with the free enzyme, which is more than presumably ascribable to the fact that there are strong internal mass transfer limitations within the particles of the supported enzyme (it is important to state that the amount of active enzyme in the solids is very high, 10 times higher than in liquid enzyme preparations). When observing results of reaction 2, these are comparable to those obtained by Jung et al. under the same conditions of temperature, molar ratio of DMC to Gly of 3:1 and using THF as a solvent after 48 h, where they reached approximately 49% of yield compared to our 42% employing tert-butanol [18].

![Figure 2](image-url). Comparison of the use of the Eversa Transform and Lipozyme TL IM for the reaction between glycerol with ethylene carbonate. Reaction conditions: closed system; T = 60 °C; DMC:Gly = 3:1; amount of enzyme: 3% v/v; solvent: tert-butanol (30% v/v) agitation speed: 400 rpm.
Transform in reaction 1 for the open and closed systems. In Figure 5 it can be seen that after 100 h of operation, the type of system has been found to exert a slight influence on the performance of Eversa Transform.

3.1.2. Selection of Open vs. Closed System

Throughout the experiments it was observed that the initial 25 mL reacting mixture underwent certain degree of volume loss; subsequently, the two most active free enzymes were tested under the same conditions in an open (without lid) and closed (with lid) systems for comparison.

Figure 4 represents the yields to product and volume losses obtained operating with Eversa Transform in reaction 1 for the open and closed systems. In Figure 5 it can be seen that after 100 h of operation, the type of system has been found to exert a slight influence on the performance of Eversa Transform in reaction 1: the yield to GC decreased from 83% to 77% and the loss of volume increased from 4% to 10% when an open system was employed.

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**Figure 3.** Comparison of the use of the enzymes CALB (lipase B from *Candida antarctica*) and Novozyme 435 in the transesterification of glycerol with dimethyl carbonate. Reaction conditions: closed system; T = 60 °C; DMC:Gly = 3:1; amount of enzyme: 3% w/w; solvent: tert-butanol (30% v/v) agitation speed: 400 rpm.

**Figure 4.** Yield to product and volume loss in reaction 1 under an open and a closed system using Eversa Transform. Conditions: T = 60 °C; EC:Gly = 3:1; enzyme load: 3% w/w; solvent: tert-butanol (30% v/v) agitation speed: 400 rpm.

Figure 5 depicts the kinetic evolution of each of the species in the reaction in the (A) open and (B) closed systems, respectively, where it can be seen that the molar amount of carbonate that disappears is higher than that due to its reaction with Gly. This fact is more evident in the closed system, where a fast reduction in carbonate and Gly is observed at the beginning of the process. However, the
temporal evolutions of the products are faster in the open system, suggesting that a certain shift in the equilibrium towards the products is obtained in this system or, at least, that the presence of some water from the environment could result in an enhancement of the transesterification activity of the enzyme.

For reaction 2, Figure 6 shows a much more significant difference of performance as the yield to product practically doubled from 41% to 81% when using the closed system with respect to the open one, while the volume loss amounted to 45% with the latter setup in contrast to 20% with the closed system. This seems to be related to the higher volatility of DMC compared to that of EC. In fact, the reduction in the concentration of DMC, a reagent, results in a lower yield to GC. This is verified in trends observed in Figure 7: the concentration of DMC declines much more rapidly in an open system.

For reaction 2, the significant volume loss could be easily ascribable to the removal of the methanol generated during the reaction, whose boiling point is 64.7 °C with the reaction taking place at the nearby temperature of 60 °C. Additionally, tert-butanol and DMC have boiling points of 83 and 90 °C, respectively, which could lead to some depletion of this component, too.

Based on the results described throughout Section 3.1, it can be concluded that to obtain the best yields to GC without incurring in too much loss of reaction volume, the best approach is to conduct the transesterification of Gly with EC (reaction 1) catalyzed by Eversa Transform in a closed reactor, the system employed in the following sections.

Figure 6. Yield to product and volume loss in reaction 2 using an open and a closed system with CALB as catalyst. Conditions: T = 60 °C; DMC:Gly = 3:1; enzyme load: 3% w/w; solvent: tert-butanol (30% v/v) agitation speed: 400 rpm.
Again, the runs were conducted for 100 h, after which yields to GC of 60%, 83%, and 85% were attained.

Fermentation should take place mainly at the liquid-liquid interface and its surroundings.

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The reaction [22]. As turbidity is observed when adding the enzyme, an emulsion should be created. This effect has already been observed in literature for this reaction [18,37] or, and even in some cases, it appears that increasing higher amounts of enzyme can be detrimental to the performance of the reaction [22]. As turbidity is observed when adding the enzyme, an emulsion should be created and the enzymatic action should take place mainly at the liquid-liquid interface and its surroundings.

3.2. Effect of Variables on the Transesterification of Glycerol with Ethylene Carbonate

3.2.1. Effect of the Solvent

The effect of the solvent is tested for the transesterification of Gly with EC for the most active enzyme, i.e., Eversa Transform. For this, the effect of another hydrophilic solvent like tetrahydrofuran (THF) has been tested due to the promising performance shown in the past for the transesterification with DMC [18,19] and compared to tert-butanol, which had already been tested for the transesterification of soybean oil with DMC (Seong et al., 2011). The yield to GC after 100 h in tert-butanol was of 83% as described in Figure 3, whereas only 57% was obtained with THF in the medium under the same conditions. Curiously, the productivity in the first case (in the first six hours, that is, initial conditions) is 6 mmoles of GC·h−1·L−1, while, when using THF as solvent, the value of this parameter is 5 mmoles GC·h−1·L−1, so, possibly, enzyme deactivation due to THF happens.

3.2.2. Effect of the Enzyme Concentration

This effect was studied by trying three different levels of loading, namely 2, 3, and 4% w/w. Again, the runs were conducted for 100 h, after which yields to GC of 60%, 83%, and 85% were attained for the three concentrations of enzyme. As shown in Figure 8, there is a saturating behavior of the concentration of catalyst, which is observed using a concentration of enzyme higher that 3% w/w. This effect has already been observed in literature for this reaction [18,37] or, and even in some cases, it appears that increasing higher amounts of enzyme can be detrimental to the performance of the reaction [22]. As turbidity is observed when adding the enzyme, an emulsion should be created and the enzymatic action should take place mainly at the liquid-liquid interface and its surroundings.
3.2.3. Effect of the Molar Ratio of Reactants and Temperature

The transesterification of Gly with EC had been tested employing relatively low molar ratios ranging from 1.5:1 to 3:1, although in the absence of any solvents in all cases [13,14,16,38,39]. The molar ratio of reactants may affect the equilibrium conversion and the kinetics of the reaction. Considering the low rates of reaction in enzymatic reactions in contrast with chemically catalyzed reactions and also the fact that here this reaction has been performed in the presence of a solvent, thus diluting the concentration of the species, it has been decided to make use of larger molar excess of organic carbonate ranging from 3:1 to 9:1. Additionally, the reaction was evaluated at 50, 60, and 70 °C, as this is the temperature range that has been considered in kinetic studies [11,16].

Figure 9 shows that better results are obtained as the molar excess increases from 3:1 to 9:1 at all temperatures, which agrees with the fact that even larger excesses of 17:1 and 21:1 of diethyl carbonate to Gly were reported previously as a strategy to reach conversions of 84% or 97% [40,41]. On the other hand, temperature does not show a clear positive effect on performance at 100 h reaction time, but there is a sharp increase in initial reaction rate when calculating it for the first 6 h of reaction, as expected.

![Figure 9. Effect of the molar ratio of EC to Gly and temperature on the yield to GC. Conditions: enzyme load: 3% w/w; solvent: tert-butanol (30% v/v); t = 100 h; agitation speed: 400 rpm.](image)

3.3. Kinetic Modeling of Enzymatic Transesterification of Glycerol With Ethylene Carbonate

To study the kinetics of this reacting system, the evolution of each of the components has been monitored from the experiments performed at different values of temperature (50, 60, and 70 °C) and molar ratio of EC to Gly (3:1 to 9:1), at a constant enzyme concentration (3% w/w). To avoid problems due to solvent and EC evaporation (they seemed to form a low-boiling point azeotrope), runs were performed in closed reactors provided with an adequate sampling system.

It can be observed that the evolution of EC cannot be explained only on the basis of the transesterification reaction. At the same time, both products (GC and EG) are obtained in equimolar amounts, thus avoiding a possible further reaction of GC with Gly and a polymerization of EC and EG or Gly driven by the lipases (condensation polymerization). However, ring-opening polymerization of ethylene carbonate driven by Eversa Transform 2.0 is possible [42].

\[
\text{Glycerol + Ethylene carbonate} \rightarrow \text{Glycerol carbonate + Ethyleneglycol} \quad (4)
\]
where \( r_1 \) (mol\( \cdot \)L\(^{-1} \)\cdot min\(^{-1} \)) is the reaction rate for the Gly transesterification driven by the lipase; \( k_1 \) (L\( \cdot \)mol\(^{-1} \)\cdot min\(^{-1} \)) is a second-order kinetic constant, \( n_{\text{Gly}} \) is the molar concentration of Gly and \( n_{\text{EC}} \) the molar concentration of EC.

\[
\text{Ethylene carbonate} \leftrightarrow [\text{Ethylene carbonate}]_x
\]

\[
r_2 = k_2 \cdot n_{\text{EC}}^2
\]

\[
r_3 = k_3 \cdot n_{\text{PEC}}
\]

Here, \( r_2 \) (mol L\(^{-1} \)\cdot s\(^{-1} \)) is the reaction rate for the ring-opening polymerization of EC; and \( r_3 \) is the inverse reaction (ring-closing depolymerization to EC) (mol L\(^{-1} \)\cdot s\(^{-1} \)). Their kinetic constants are \( k_2 \) (L\( \cdot \)mol\(^{-1} \)\cdot min\(^{-1} \)) and \( k_3 \) (min\(^{-1} \)); \( n_{\text{EC}} \) is the molar concentration of EC and \( n_{\text{PEC}} \) is the molar concentration of the polymer, whose production can account for the increasing turbidity with time molar excess of EC and reaction temperature.

The suggested model with three reactions happening in parallel was fitted to data from runs performed with different excesses of EC and at temperatures ranging from 50 to 70 °C.

Results shown in Figure 10 and Table 1 indicate that the excess of EC only helps slightly the formation of GC and EG. As expected, values for the kinetic constants do not depend on the excess of EC, taking into account the respective absolute errors for such constants. The kinetic model fits perfectly to retrieved experimental data, as indicated by the very high values for Fisher’s F parameter, and very low values of SQR and RMSE, together with the evident goodness of fit shown by lines (calculated with the model) and experimental points in the figure. Finally, the maximum error intervals at 95% confidence for the kinetic constants are very small, rendering very narrow confidence intervals for such constants.

Table 1. Kinetic and goodness-of-fit parameters for the proposed model and the different runs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 )</td>
<td>0.0170 ± 0.0006</td>
<td>0.0174 ± 0.0006</td>
<td>0.0193 ± 0.0011</td>
<td>0.0284 ± 0.0022</td>
<td>0.058 ± 0.0051</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>0.0046 ± 0.0007</td>
<td>0.0058 ± 0.0007</td>
<td>0.0032 ± 0.0013</td>
<td>0.0089 ± 0.0013</td>
<td>0.019 ± 0.008</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>0.0068 ± 0.0033</td>
<td>0.0132 ± 0.0016</td>
<td>0.0082 ± 0.0008</td>
<td>0.0036 ± 0.0024</td>
<td>0.0012 ± 0.0006</td>
</tr>
<tr>
<td>SQR</td>
<td>0.000336</td>
<td>0.0000518</td>
<td>0.000552</td>
<td>0.0018</td>
<td>0.00838</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.0105</td>
<td>0.012</td>
<td>0.0012</td>
<td>0.021</td>
<td>0.017</td>
</tr>
<tr>
<td>F-value</td>
<td>23140</td>
<td>51179</td>
<td>113139</td>
<td>6883</td>
<td>4883</td>
</tr>
</tbody>
</table>

Common conditions: enzyme load: 3% w/w; solvent: tert-butanol (30% v/v); closed stirred batch reactor; agitation speed: 400 rpm; \( T = 50 \) °C for runs 1, 2 and 3; EC:Gly 3:1 molar ratio for runs 1, 4, and 5.

From the kinetic model, it can be inferred that the effect of increasing temperature would seem to affect both parallel reactions (transesterification and the couple polymerization-depolymerization of ethylene carbonate). Results of the fit are shown in Figure 11 and Table 1, and they indicate that temperatures as high as 70 °C affect all reaction rates dramatically, while 50 and 60 °C lead to very similar results (slightly higher reaction rates for 60 °C). In general, these observations correspond to the relatively high activation energies for transesterification (55.21 kJ\( \cdot \)mol\(^{-1} \)), polymerization by ring-opening (63.88 kJ\( \cdot \)mol\(^{-1} \)) and depolymerization by ring-closing (77.96 kJ\( \cdot \)mol\(^{-1} \)). It is interesting to observe that the latter reaction tends to disappear at high temperatures, so the lipase effectively does not act as a good catalyst at high temperatures for this reaction in particular. It is unusual to observe negative activation energies, a phenomenon that can be related to a notably negative enthalpy change with temperature [43], to protein folding dynamics [44] or to deactivation or denaturation of the enzyme, which can happen at least partially [45]. In this latter case, the effect could affect only some of the reactions catalyzed by the enzyme. It should be considered that all kinetic constants here calculated include the activity of the enzyme towards the reaction under consideration.
Figure 10. Fitting of the kinetic model proposed to runs 1, 2, and 3 (molar ratios EC:Gly 1:3, 1:6, and 1:9, respectively). Subfigure (A) shows results for glycerol, (B) for ethylene carbonate, (C) for glycerol carbonate, and (D) for ethylene glycol. Other conditions: enzyme load: 3% w/w; solvent: tert-butanol (30% v/v); closed stirred batch reactor; agitation speed: 400 rpm; T = 50 °C.

Figure 11. Fitting of the kinetic model proposed to runs 1, 4, and 5 (50 °C, 60 °C, and 70 °C, respectively). Subfigure (A) shows results for glycerol, (B) for ethylene carbonate, (C) for glycerol carbonate, and (D) for ethylene glycol. Other conditions: enzyme load: 3% w/w; solvent: tert-butanol (30% v/v); closed stirred batch reactor; agitation speed: 400 rpm; molar ratio EC:Gly 1:3.
4. Conclusions

Glycerol carbonate can be synthesized from glycerol and organic carbonates like dimethyl carbonate and ethylene carbonate employing enzymatic processes. In fact, the latter reaction is presented for the first time here using an enzymatic transesterification reaction. The two reactions are first conducted with different free and immobilized enzymes and their performance was compared. For the transesterification with dimethyl carbonate, CALB was the free enzyme of choice, whereas Eversa Transform 2.0 performed better for the reaction with ethylene carbonate, a reaction, that in the end, showed a much better performance.

For this reason, subsequent studies with this reaction and enzyme optimized the operation conditions using tert-butanol as solvent in a closed system to minimize possible evaporation, a concentration of enzyme Eversa Transform 2.0 of 3% \( \text{w/}\text{w} \), a molar excess of EC:Gly from 3:1 to 9:1 and a temperature from 50 to 70 °C. The evolution of the concentration of the components involved was determined by ion exclusion HPLC, while a kinetic model was proposed to fit data retrieved in runs performed in a closed reactor at several molar ratios (runs 1 to 3) and at several temperatures (runs 1, 4, and 5). The model proposed and validated included a transesterification reaction taking place in parallel to a polymerization-depolymerization reaction affecting EC. The first one was very influenced by temperature, as its reaction rate rocketed between 60 and 70 °C.

Author Contributions: M.L. and J.E. conceived the research; M.L. designed the experiments; A.G.-L.; D.V. and D.E.B. performed the experiments and analyzed the samples; J.E. developed the original analytical procedures, while A.G.-L. and D.V. developed a modified analytical HPLC procedure for this reacting system and analyzed the retrieved data, including the preliminary statistical analysis; J.E., P.Y. and M.L. performed the final statistical analysis and wrote the paper. All the authors read and approved the final manuscript.

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