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# Enzymatic processing of renewable glycerol into value-added glycerol carbonate

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Increased production of biodiesel has led to excess glycerol production worldwide, which has resulted in a significant drop in glycerol prices. Glycerol carbonate is a multifunctional compound used as chemical intermediates, solvents, additives and monomers. In this study, the enzymatic synthesis of glycerol carbonate from glycerol and a dialkyl carbonate was investigated. Glycerol carbonate was formed when reacting glycerol with dimethyl carbonate, diethyl carbonate or dibutyl carbonate in the presence of *Candida antarctica* lipase B (Novozym 435), using *tert*-butanol as a solvent. Nearly 100% glycerol conversion was reached after 12 h, with glycerol carbonate being the primary product. The effects of reaction parameters including solvent choice and biocatalyst loading were also examined. The highest activity was found at restricted water conditions and when using *tert*-butanol as a solvent.

## Introduction

The expansion of biodiesel production around the world has resulted in an excess of crude glycerol. This surplus glycerol offers an interesting opportunity to produce biomass-derived raw materials and value-added chemical intermediates that have previously been manufactured from petroleum fractions. Recently, the conversion of glycerol into various commodity chemicals has been reported, including acid-catalyzed dehydration of glycerol to acrolein under supercritical conditions[1] and at lower pressures,[2] low pressure hydrogenolysis of glycerol to 1,2-propanediol,[3] polymerization of glycerol to polyglycerols and polyglycerol esters,[4] covalent incorporation into thermosetting melamine formaldehyde resins,[5] and gasification to form syngas.[6] Microbial fermentation using glycerol as a feedstock has enabled production of 1,3-propanediol,[7,8] glyceric acid,[9] citric acid and erythritol,[10,11] polyhydroxybutyrate,[12,13] as well as small molecule fuels such as hydrogen,[14] ethanol,[15] butanol,[16] and methane.[17]

Glycerol carbonate (4-(hydroxymethyl-1, 3-dioxolan-2-one)) is a colorless, stable liquid with low toxicity. It is useful as a green, nonvolatile solvent for paints, plastics and resins, such as cellulose acetate, nylon, and nitrocellulose.[18] Additional applications include coatings, gas separation membranes, as a novel electrolyte in lithium ion batteries,[19] and as a raw material for polymer and other organic synthesis reactions.[20,21] Glycerol carbonate is also emerging as a viable biosolvent for enzymatic synthesis reactions.[22]

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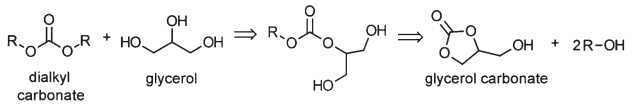
Glycerol carbonate is traditionally produced with phosgene (toxic) and glycerol.[23] Newer routes have focused on a) carboxylation of glycerol with carbon dioxide using zeolites, ion exchange resins,[24,25] or Sn-based catalysts;[26] and b) reaction of glycerol and a cyclic carbonate (such as ethylene carbonate) in the presence of basic[27] or organometallic catalysts.[28] Direct carboxylation suffers from low glycerol carbonate yields (25–35%), while use of the heterogeneous catalysts requires neutralization of the catalyst and difficult purifications of salts or coproducts from the glycerol carbonate. Ochoa-Gomez *et al.* have investigated a transesterification route using CaO and triethylamine as catalysts.[29,30]

Lipases (EC 3.1.1.3, triacylglycerol hydrolases) offer a greener pathway to glycerol carbonate synthesis. They catalyze the hydrolysis of lipids, and are capable of synthesizing aliphatic, aromatic, and other esters in nonaqueous systems. They exhibit the regioselectivity and enantioselectivity of other enzymes, but they are able to catalyze reactions with a wide variety of non-natural acyl acceptors and donors, such as alcohols,[31] amines,[32] prochiral and *meso* diols,[33] sugars,[34] and polymers.[35,36]

Glycerol has both primary and secondary hydroxyl groups, which offers two possible reaction routes. However, the effect of hydroxylation position on the esterification reaction has been studied.[37] 1- and 2-propanol were reacted with dibutyl and dibenzyl carbonate. Reactions with 1-propanol formed mono- and di-substituted products at a 2 : 1 ratio, while only mono-substituted products were formed when 2-propanol was used as the alcohol. Drawing correlations between these results and the position of the hydroxyl groups on glycerol indicate that using glycerol as the alcohol will result in the formation of glycerol carbonate. Scheme 1 shows the proposed two-step reaction between a dialkyl carbonate and glycerol.




**Scheme 1** Proposed two step reaction between a dialkyl carbonate and glycerol, resulting in the formation of glycerol carbonate.



Dialkyl carbonates have been shown to be acceptable substrates in lipase-catalyzed hydrolysis and transesterification reactions.[38] A lipase-catalyzed reaction between diphenyl carbonate and various alcohols was shown to produce mono- and di-substituted carbonate.[39] This alkoxycarbonylation reaction was catalyzed by lipases from *Aspergillus*, *Candida*, *Mucor*, *Pseudomonas*, and *Rhizopus*. As these studies show, conversion of the alcohol was greatly influenced by variations in the chain length or ring size of the carbonate substrate.

Recently, Kim *et al.*[40] reported the use of *Candida antarctica* lipase B (CalB) and *Candida rugosa* lipase to produce glycerol carbonate from glycerol and dimethyl carbonate in THF. Other reports testing other lipases, solvents, and supports have subsequently been reported.[41–43]

Table 1 shows a comparison of these results, which indicate the inherent shortcomings of these systems. High conversion was achieved using CalB, but required either long reaction times (30–48 h), high ratios of DMC to glycerol (10 : 1), or extremely high enzyme loading relative to glycerol (55% w/w). Reaction systems using lipase from *Aspergillus niger* were successful in producing glycerol carbonate more rapidly (4–6 h), but still required significant excess DMC, and overall conversion was less than 75%. It was our intention to develop a CalB reaction system that used relatively shorter reaction times, low DMC excess, relatively low CalB loading, while achieving high conversion and selectivity.

In this study, the lipase-catalyzed production of glycerol carbonate from glycerol and several dialkyl carbonates is reported. Lipases from various bacterial and fungal sources were screened for catalytic performance. Factors affecting the selectivity and reaction rate, including substrate choice (dialkyl and diaryl carbonates), solvent, substrate molar ratios, lipase loading and reusability, were investigated.

## Results and discussion

### Lipase screening

Lipases from *Candida antarctica*, *Candida rugosa*, *Pseudomonas fluorescens*, *Aspergillus niger*, *Mucor miehei*, *Rhizomucor miehei*, and *Burkholderia cepacia* were screened for activity in the reaction between glycerol and dimethyl carbonate. *Candida antarctica* lipase B (CalB) was the only screened lipase that showed any detectable activity. Glycerol conversion reached 72% after 20 h. Glycerol carbonate was the primary product of this reaction, with approximately 1% (area) coproducts. Based on these results, CalB was used in all subsequent reactions.

As expected, CalB showed significantly more catalytic activity in this system than the other lipases examined. This confirms a higher solvent tolerance as well as increased substrate range in synthetic reactions. Although it was recently reported that *Candida rugosa* lipase showed some activity (~15% glycerol conversion) for this reaction in THF,[40] none was seen when performed in *tert*-butanol. It is possible that the disparity results from the solvent change. However, *Candida rugosa* lipase is not a single protein, but rather a mixture of isoenzymes. The relative abundance of each enzyme in the mixture varies with strain and growth conditions.[44] Combined with changes in the enzyme physical state (resin-immobilized *versus* lyophilized or cross-linked aggregates), changes in the relative abundance of each isoform could also account for the lack of activity.

### Dialkyl carbonate

The choice of dialkyl carbonate affected both the reaction rate and selectivity for glycerol carbonate formation. The two diaryl carbonates, dibenzyl and diphenyl carbonate, displayed little to no glycerol carbonate formation. Dibenzyl and diphenyl carbonate also presented something of a challenge in reaction set up, as they are both solids that dissolve slowly in the *tert*-butanol and glycerol.

Fig. 1 shows the glycerol conversion seen with each dialkyl carbonate. Dimethyl, diethyl, and dibutyl carbonate all reached nearly 100% glycerol conversion after 24 h. However, significant differences in the reaction rates were seen in the first six hours of reaction time. Conversion rates increased with alkyl chain length, with dimethyl carbonate, diethyl carbonate, and dibutyl carbonate yielding 65%, 69%, and 79% conversion after six hours, respectively. This suggests that the increasing chain lengths are more readily recognized by the lipase active site as a viable substrate. It is likely that the

**Table 1** Comparison of enzymatic routes to glycerol carbonate synthesis

| % Glycerol Conversion | % Product Selectivity | Conditions |
|---|---|---|
| 94 | 94 | 60 °C, 30 h, 1 : 1 DMC to glycerol, THF, 55% CalB loading (w/w glycerol): Kim[40] |
| 90 | >90 | 70 °C, 48 h, 10 : 1 DMC to glycerol, glycerol coated on silica gel; 5–20% CalB loading (w/w glycerol): Lee[41] |
| 74 | 80.3 | 60 °C, 4 h, 10 : 1 DMC to glycerol, 12% *Asp. niger* lipase (w/w glycerol): Tudorache[42] |
| 48.6 | 85 | 60 °C, 6 h, 10 : 1 DMC to glycerol, 2–8% *Asp. niger* lipase (w/w glycerol) immobilized on magnetic particles: Tudorache[43] |

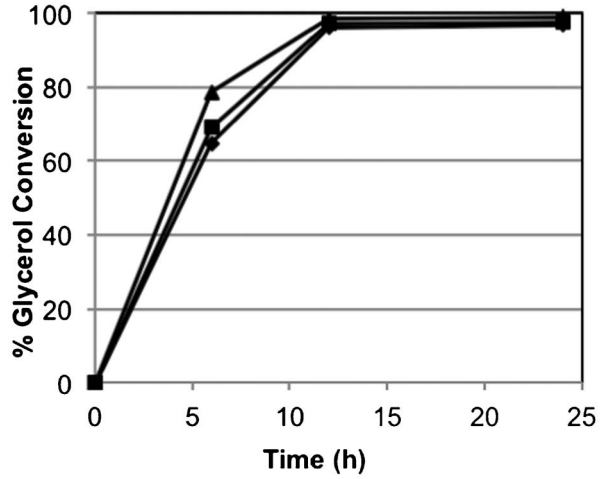



**Fig. 1** Effect of dialkyl/diaryl carbonate choice on glycerol conversion: dimethyl carbonate (◆), diethyl carbonate (■), and dibutyl carbonate (▲). Reaction conditions: 20 mmoles glycerol 40 mmoles DAC, 5 mL *tert*-butanol, and 0.1 g CalB. Run at 50 °C with stir rate of 350 RPM.

longer chain aids in the correct orientation of the substrate within the active site. However, the lack of conversion when using diphenyl and dibenzyl carbonate indicates that the active site is more suited to straight-chain substrates.

Dialkyl carbonate choice also affected the product selectivity (Fig. 2). Glycerol carbonate elutes in an asymmetrical peak, resulting in a retention time shift for larger quantities of glycerol carbonate. The primary product when using dimethyl carbonate is glycerol carbonate. Diethyl carbonate reacts to form some glycerol carbonate, but the peak overlaps with the primary product, believed to be glycidol. Dibutyl carbonate also resulted in the formation of glycerol carbonate, but there were four unknown products that far exceeded glycerol carbonate in peak area.

Time course studies indicate glycerol carbonate forms first, with additional products showing up at later time points. This indicates that the additional peaks are not a stable intermediate (say a glycerol–dimethyl carbonate complex), but rather are products of secondary reactions, most likely between glycerol carbonate and the dialkyl carbonate.

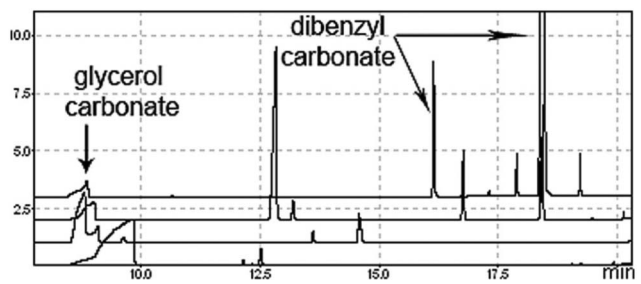



**Fig. 2** Chromatograms displaying the products when using (from the bottom) dimethyl carbonate, diethyl carbonate, dibutyl carbonate, and dibenzyl carbonate.

### Substrate molar ratios

Fig. 3 shows the effect of DMC to glycerol molar ratios on glycerol conversion. The highest conversion is seen at a DMC : glycerol ratio of 10 : 1. This ratio also resulted in a much higher activity, with 84% glycerol conversion after only 4 h.

However, the switch to reagent grade dimethyl carbonate lowered overall conversion across all ratios. The reagent grade contains 1.5% impurities, with water and methanol making up the bulk. Enzymatic synthesis reactions in organic solvents are sensitive to water, as it shifts the reaction equilibrium in favor of hydrolysis and limits overall conversion. Interestingly, the 10 : 1 DMC to glycerol ratio was also found to produce the highest conversion level in solventless systems.[42] An enzyme study in THF[40] found an optimum ratio for the best conversion and selectivity to be 1 : 2.

Dialkyl carbonate choice and substrate molar ratios were the only parameters that affected the product selectivity of glycerol carbonate. Fig. 4 shows representative chromatograms for DMC to glycerol ratios of 10 : 1 and 1 : 1. The first cluster of peaks is glycerol carbonate. The larger peaks are the R- and S-conformations of glycerol, while the small peak in the middle is due to thermal degradation of the carbonate ring structure during analysis. The average area percentage of glycerol carbonate in the products decreased as DMC concentration increased, with the 10 : 1 DMC to glycerol ratio resulting in ~50% GC product formation (Fig. 5). The higher DMC concentrations resulted in larger amounts of higher molecular weight co-products being formed. Although reactions containing higher concentrations of glycerol compared to DMC were tested, they resulted in total conversions uniformly lower than the 1 : 1 ratio. However, reactions with excess glycerol showed limited formation (less than 5%) of alternate products. As glycerol concentration increased, formation of the higher MW products decreased to less than 0.5%.

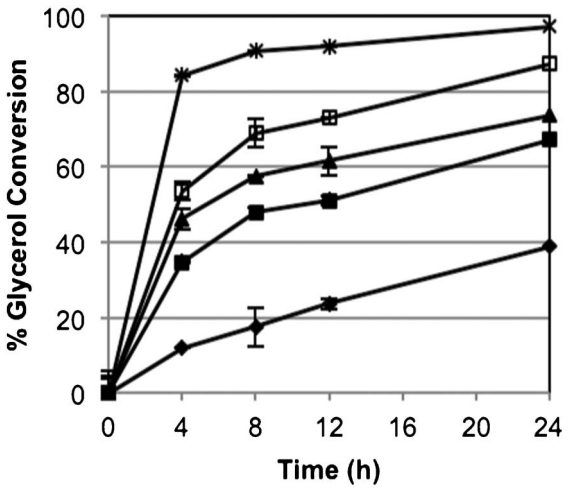



**Fig. 3** Effect of DMC to glycerol molar ratio. DMC : glycerol ratios: 1 : 1(◆), 2 : 1(■), 3 : 1 (▲), 5 : 1(□), and 10 : 1(*). Reaction conditions: 5 mL total reaction volume 0.02 g CalB, 4 mmoles glycerol, varied DMC (4, 8, 12, 20, 40 mmoles), 60 °C, shaken at 250 RPM.

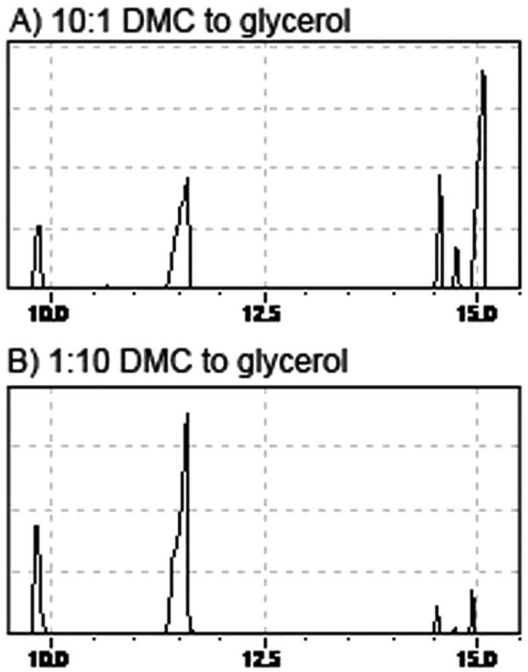



**Fig. 4** Different product profiles for varying DMC : glycerol ratios. A) DMC to glycerol ratio of 10 : 1; B) DMC to glycerol ratio of 1 : 1 after 24 h. The first cluster of peaks is glycerol carbonate, the second cluster is glycerol dicarbonate.

The lack of other products when glycerol is in great excess suggests that the later peaks are from secondary reactions involving DMC. The lack of these other peaks when glycerol is in excess indicates that the remaining products most likely result from the remaining hydroxyl group on the glycerol carbonate molecule reacting with the dimethyl carbonate to form glycerol dicarbonate (4-(methoxycarbonyloxymethyl)-1,3-dioxolan-2-one). Glycerol dicarbonate can then react with glycerol carbonate to form diglycerol tricarbonate (Scheme 2).

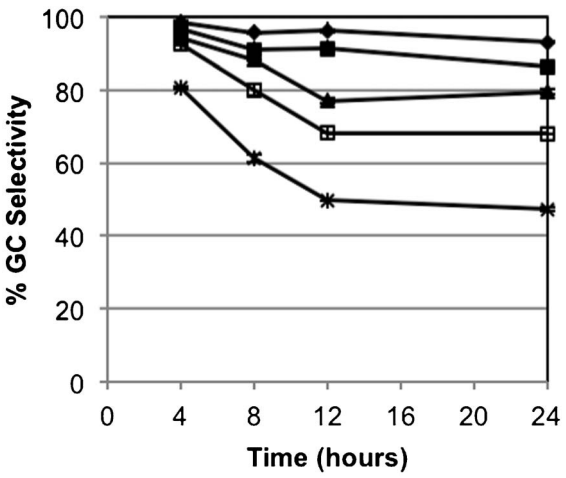
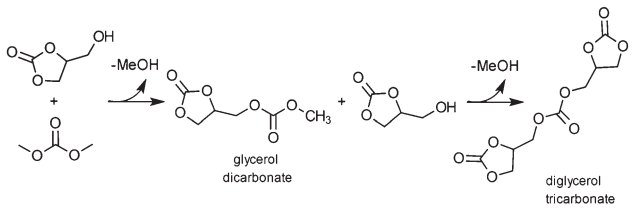



**Fig. 5** Effect of DMC to glycerol molar ratio on glycerol carbonate selectivity. DMC : glycerol ratios: 1 : 1(◆), 2 : 1(■), 3 : 1 (▲), 5 : 1(□), and 10 : 1(*). Reaction conditions: 5 mL total reaction volume 0.02 g CalB, 4 mmoles glycerol, varied DMC (4, 8, 12, 20, 40 mmoles), 60 °C, shaken at 250 RPM.




**Scheme 2** Synthesis of glycerol dicarbonate and diglycerol tricarbonate from DMC and glycerol carbonate.

While reaction chemistry indicates that both of these products are capable of forming, it is postulated that only glycerol dicarbonate is formed in this system. The two largest peaks that elute between 14 and 15 min are likely the R- and S-conformations of glycerol dicarbonate, with the small middle peak being a thermal degradation product of the carbonate ring structure, as was seen with glycerol carbonate analysis.

### Solvent

Solvent studies included several hydrophobic (hexane and toluene) and hydrophilic (*tert*-butanol, isopropanol, ethanol, 1-propanol and *tert*-amyl alcohol) organic solvents, as well as a solvent free system. Solvents were chosen because all have provided successful environments for enzymatic synthesis reactions.[45–47] *Tert*-butanol is chemically inert in this reaction, encourages a mono-phasic system at reaction temperature, and does not interfere with analysis of the products. Hexane and toluene are also chemically inert, but they result in two liquid phases. The straight chain alcohols, while promoting a single liquid phase, resulted in no measurable conversion, suggesting either competitive inhibition or lipase deactivation. While *tert*-amyl alcohol did result in some conversion, the reaction system was tri-phasic and quantitative analysis was not viable. The solvent-free system was bi-phasic.

Table 2 summarizes the results. The reaction running in *tert*-butanol reached nearly 100% glycerol conversion. After 24 h, hexane and toluene both showed approximately 55% conversion. The solvent free system showed ~48% conversion.

Solvent had a large effect on overall conversion. Use of *tert*-butanol resulted in nearly complete conversion, while use of non-polar solvents toluene and hexane resulted in only 55% conversion. It is believed that the significant difference is due in part to the biphasic system produced in the presence of non-polar solvents. Two distinct liquid phases were present, even at high stir rates. This limits the reaction to the interface

**Table 2** Glycerol conversion after 24 h when using different solvents[a]

| Solvent | % Conversion |
|---|---|
| *Tert*-butanol | 97 |
| Hexane | 55 |
| Toluene | 55 |
| No Solvent | 49 |

[a] Reaction conditions: 4 mmoles glycerol, 8 mmoles DMC, 1 mL solvent, 0.02 g CalB, 50 °C with a stir rate of 350 RPM.

between the two phases, while single-phase systems utilize the entire reaction volume. It is also believed that the polar solvent allowed the methanol produced by the reaction to mix with the reaction volume instead of isolating it near the catalyst beads, thus limiting its adverse effect on activity.

The remaining solvents tested were polar, but showed no conversion whatsoever. This was expected, as primary and secondary alcohols can act as competitive inhibitors. Because no additional products were seen with these solvents, it appears that the alcohols were not participating in the reaction itself, but merely reducing the number of active sites available for the desired reaction. *Tert*-butanol avoids this problem, as CalB shows no activity toward tertiary alcohols.

The solvent free system also resulted in a lower conversion. It is likely that the drop in conversion is in part due to mass transfer limitations, as the viscosity of glycerol discourages effective mixing in the bulk solution and slows diffusion into the pores of the support bead. This same phenomenon can be seen in biodiesel production, as both the reactant methanol and the product glycerol have low solubility in oil, and thus can exist in small areas of high concentration. The high concentration of glycerol can coat the immobilized catalyst and reduce activity,[48] while methanol can inactivate the lipase. It was found that the addition of *tert*-butanol allowed a single phase to form and improved the operational stability and catalytic activity of the lipase.[49,50]

Molecular dynamics simulations of CalB in organic solvents suggest that *tert*-butanol provides increased enzyme flexibility, which allows the enzyme to more easily bind substrates and alter conformation to facilitate reaction. The distance between catalytic residues also point towards increased formation of hydrogen bonds, which enable chemical transformations in the active site.[51]

The benefits of *tert*-butanol are threefold. By promoting a single phase, the reaction is not limited to an interface and methanol inactivation can be avoided. Finally, *tert*-butanol positively affects the conformational flexibility of the enzyme, resulting in higher catalytic activity.

### Lipase loading

Fig. 6 shows glycerol conversion at different lipase loading values. After increasing the catalyst loading to 0.2 g (~10% of initial glycerol weight), glycerol conversion reached 80% after only six hours. However, all catalyst loading led to nearly complete conversion within twelve hours.

These results indicate that even a small amount of lipase (~1% of glycerol weight) is capable of producing nearly 100% conversion after 12 h. Moreover, it indicates that the liberated methanol is not sufficient to inhibit the reaction even in lipase-limited environments. The significant increase in reaction rate when using a higher catalyst loading, however, indicates that the tradeoff between enzyme cost and process time must be carefully tuned. Lipase reusability is a key component to realizing an economical and timely process.

### Lipase reusability

A series of five concurrent reactions were run for 12 h using the same lipase. The lipase retained 85% retention of initial activity by the fifth run (Fig. 7). However, observation of the

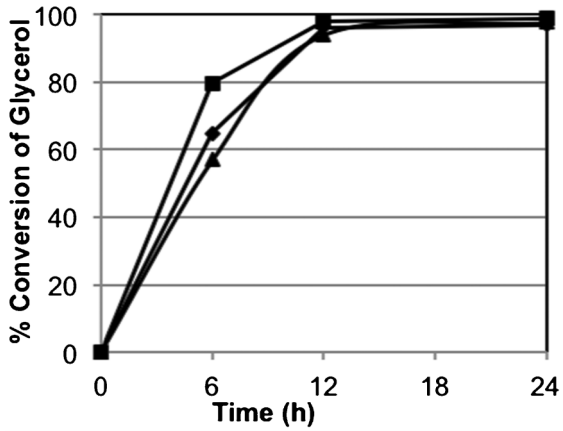



**Fig. 6** Effect of lipase loading on % glycerol conversion. Reaction conditions: 20 mmoles glycerol, 40 mmoles DMC, 5 mL *tert*-butanol, 50 °C, 350 RPM stir rate. Lipase loading included 1% (▲), 5% (◆), and 10% (■) w/w glycerol weight (0.02 and 0.2 g, respectively).

reaction vials indicated that loss of activity might result from a breakdown of the lipase beads during the runs due to mechanical stress from the micro stir rods. Subsequent reactions were agitated in an incubating shaker at 250 RPM. Although there were variations between runs, glycerol conversion remained near or over the initial 12-hour conversion rate. This confirmed that the acrylic lipase beads are subject to mechanical breakdown, and that the *tert*-butanol wash effectively prevents irreversible inhibition by methanol.

### Performance summary and analysis

Dialkyl carbonate choice affected the reaction rate, with initial conversion increasing with chain length. However, all substrates reached near complete glycerol conversion after 12 h, rendering the increased reaction rate less significant. The presence of additional products with the larger chain dialkyl carbonates indicates that secondary reactions are occurring,

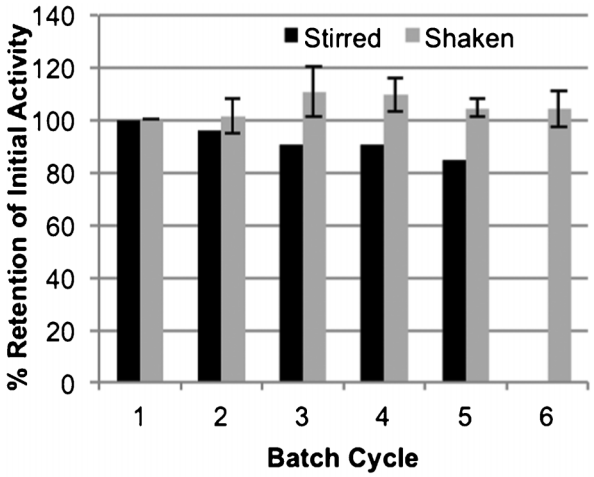



**Fig. 7** % Retention of initial activity. Reaction conditions: 4 mmoles glycerol, 8 mmoles DMC, 1 mL *tert*-butanol, and 0.02 g CalB. 12 h at 50 °C and stirred at 350 RPM (■) or shaken at 250 RPM (■).

possibly involving the alcohol released during the formation of glycerol carbonate as a substrate. Dimethyl carbonate resulted in first glycerol carbonate, followed by additional products believed to be products of a second reaction between glycerol carbonate and dimethyl carbonate, such as glycerol dicarbonate.

Our results correspond to a study of the effect of alkyl group size on alkoxycarbonylation reactions, specifically between a dialkyl carbonate and 1-propanol, using Candida antarctica lipase B and *tert*-butanol as a solvent.[37] Using dimethyl, diethyl and dibutyl carbonate as substrates, conversion after 48 h was 13%, 48%, and 66% respectively, indicating an activity increase with increasing alkyl chain length. This is unsurprising as lipase active sites are designed to accommodate hydrophobic fatty acid chains. However, the conversion dropped to 56% when dibenzyl carbonate was used. This was attributed to steric hindrance from the ring structure;[37] however the effect may also be due to charge delocalization within the active site.[52]

Mass spectra suggest that our system is forming glycerol dicarbonate; however, no diglycerol tricarbonate formation was observed after 24 h reaction time. Formation of glycerol dicarbonate and diglycerol tricarbonate was observed previously in a system using $K_2CO_3$ as a catalyst to produce glycerol carbonate, in the presence of excess dimethyl carbonate. Formation of diglycerol tricarbonate occurred at longer reaction times, while formation of glycerol dicarbonate required a higher temperature (90 °C) and progressive methanol removal.[20]

In our system, excess dimethyl carbonate results in higher formation of glycerol dicarbonate, but an equimolar mix of dimethyl carbonate and glycerol will also form glycerol dicarbonate in less than two hours reaction time at 50 °C. This suggests that the orientation and binding of the glycerol carbonate molecule in the active site negates the reactivity-restricting effect of the carbonate group that is seen when using the $K_2CO_3$ catalyst. While this contributes to the formation of co-products, the flexibility observed with this enzyme indicates that it might be useful in the synthesis of higher-MW polyglycerols.

CalB is unique among lipases in that it does not require interfacial activation. Solvents resulting in two liquid phases showed lower conversions in this study, presumably limited by mass transfer. The choice of *tert*-butanol as a solvent had a profound effect. The tertiary alcohol promoted a single liquid phase, which allowed for free movement of reaction components, without competing for active site space. The polar solvent allowed dilution of the liberated methanol throughout the reaction volume, checking its inhibitive effect. Additionally, the narrow temperature range at which it is a liquid (25–83 °C) will allow for easier product separation and solvent recovery. As expected,[45] it was also important to limit the water content in the system. If anhydrous or non-dried materials were not used, overall conversion dropped to less than 50%. The use of molecular sieves would be useful when processing crude glycerol.

The results achieved with this system are superior to those reported in the literature for similar enzymatic routes for glycerol carbonate synthesis. The system reported in this paper results in nearly 100% conversion in a shorter time period and at a lower temperature than other reports using CalB. Catalyst loading was also reduced by a factor between 2 and 11. Fig. 8 compares the ultimate glycerol carbonate yield for our system to those presented in Table 1, and also indicates the ultimate yield normalized by the Time (in hours), the enzyme Loading (in wt. percent relative to glycerol), and the DMC/glycerol mole Ratio used (or TLR yield). The TLR yield essentially indicates the reaction efficiency and rate on a specific enzyme basis. This metric clearly distinguishes the performance of our reaction system from enzymatic reactions reported previously.

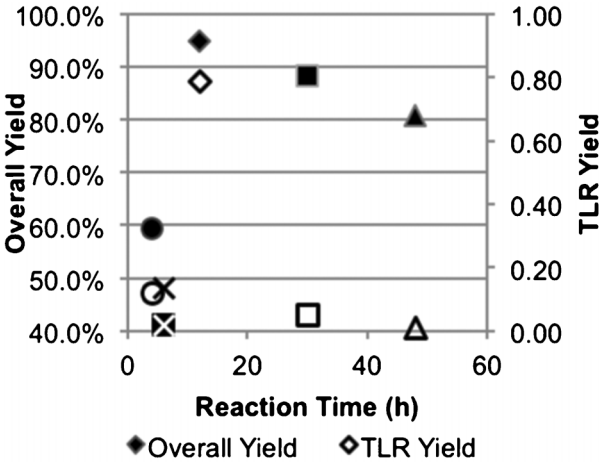



**Fig. 8** Glycerol carbonate product yield. Overall (filled) and TLR (outlined) yields for this study (◆) compared to other enzymatic GC syntheses: Tudorache (●)[42] and (X),[43] Kim (■),[40] and Lee (▲).[41] See Table 1 for reaction details.

As noted in the introduction, non-enzymatic routes to glycerol carbonate synthesis are being investigated. The most promising of these is transesterification of glycerol and dimethyl carbonate using triethylamine as a homogeneous catalyst.[30] When using a DMC to glycerol ratio of 4 : 1 and a catalyst to glycerol molar ratio of 0.3, conversion was 62% at 55 °C, with a glycerol carbonate yield of 60% after 8 h. When reflux was added to remove methanol, conversion of 99% was reached after only 1.25 h. However, addition of reflux resulted in formation of glycerol dicarbonate. The authors suggested that glycerol dicarbonate formation could be avoided by halting the reaction at lower conversion. A liquid–liquid extraction technique was developed to facilitate purification of glycerol carbonate from unreacted glycerol. This separation methodology could easily be applied to glycerol carbonate reactions systems using different catalysts and solvents.

Meaningful comparison of catalyst loading between this triethylamine protocol and the CalB protocol is difficult, as active sites per gram of immobilized CalB have not been quantified. However, it is clear that CalB requires a longer reaction time than triethylamine, particularly without reflux. This is not unexpected, as enzymatic reactions often require longer reaction times compared to their traditional catalyst counterparts. However, preliminary results in our laboratory show that transferring the CalB reaction from a closed batch process to a packed bed reactor offers high conversion and glycerol carbonate yields at reaction times similar to those

seen with the triethylamine catalyst under reflux. (Data not shown.)

## Materials and methods

### Materials

Lipases from *Candida antarctica* (Novozym 435), *Candida rugosa*, *Pseudomonas fluorescens*, *Aspergillus niger*, *Mucor miehei*, *Rhizomucor miehei*, and *Burkholderia cepacia* were purchased from Sigma-Aldrich. *Candida antarctica* lipase B was immobilized on a macroporous acrylic resin. This resin had a water content of 1–2% and a specific activity of 11,200 PLU $g^{-1}$, where 1 PLU $g^{-1}$ is defined as 1 μmole of propyl laurate formed per minute per gram enzyme in a reaction between lauric acid and 1-propanol at standard conditions. The remaining lipases were lyophilized and had reported activities of ~10 000 PLU $g^{-1}$.

Anhydrous glycerol and *tert*-butanol, glycerol carbonate, and reagent grade dimethyl carbonate were purchased from Sigma-Aldrich. Anhydrous dimethyl carbonate was purchased from Fisher Scientific. Diethyl carbonate, dibutyl carbonate, and dibenzyl carbonate were purchased from City Chemical (West Haven, CT). Reagents that were not purchased in anhydrous form were mixed with 4 Å molecular sieves overnight at room temperature before use to remove water.

Anhydrous dimethyl carbonate was used for the lipase screening, dialkyl carbonate, solvent and lipase loading studies. Reagent grade dimethyl carbonate was used for the molar ratio and reusability studies.

### Methods

**Lipase screening.** Lipase screening reactions consisted of glycerol and dimethyl carbonate (3 : 1 molar ratio), 5 mL *tert*-butanol, and 0.1 g lipase. Unless otherwise noted, all lipase loading is done at ~5% glycerol weight. Although the reaction mixture exhibited two liquid phases at room temperature, the addition of *tert*-butanol resulted in a single phase at reaction temperature. The reactions were run at 45 °C with a stir rate of 350 RPM for 24 h. Reactions were run at 60 °C for *Candida antarctica* and 45 °C for the other lipases. The temperature choices were based on the optimum temperature ranges for the respective lipases found in literature.

**Dialkyl carbonate choice.** In order to determine the effect that dialkyl carbonate choice had on glycerol conversion and product selectivity, three different dialkyl carbonates were tested. Glycerol and a dialkyl carbonate (dimethyl carbonate, diethyl carbonate, or dibutyl carbonate), 20 mmoles and 40 mmoles respectively, were combined with 5 mL of *tert*-butanol and 0.1 g of immobilized lipase in a 22.2 mL screw-top glass vial. Reactions were run at 50 °C with a stir rate of 350 RPM for 24 h with samples taken periodically. Two diaryl carbonates, dibenzyl and diphenyl carbonate, were also tested. Dimethyl carbonate was used in all subsequent reactions.

**Substrate molar ratios.** A range of substrate concentrations and relative molar ratios were screened. Five mL reactions were set up with varying ratios of DMC to glycerol. Each reaction contained 4 mmoles of glycerol mixed with 4, 8, 12, 20 or 40 mmoles of DMC. Each reaction was loaded with 0.02 g of CalB and allowed to shake at 250 RPM and 60 °C. Samples were withdrawn periodically.

**Solvent effect.** Solvent effect was examined by replacing *tert*-butanol with a variety of non-aqueous solvents. Reactions were set up in 5.5 mL vials, and reactions were scaled down to 1/5 the size of the standard reaction (4 mmoles glycerol, 8 mmoles dimethyl carbonate, 1 mL solvent, 0.02 g lipase) to improve analysis accuracy. Hexane, toluene, isopropanol, 1-propanol, ethanol, pentanol, and *tert*-amyl alcohol were all investigated. Reactions were run at 50 °C with a stir rate of 350 RPM for 24 h, with samples taken at the end. Due to the high polarity of glycerol, non-polar solvents resulted in a multi-phase system.

**Lipase loading.** The effect of lipase loading was investigated using the same reaction mix as above, containing 20 mmoles of glycerol, 40 mmoles of dimethyl carbonate, and 5 mL of *tert*-butanol. Lipase loading varied from 1% to 10% glycerol weight (0.02 and 0. 2 g, respectively). Reactions were run at 50 °C with a stir rate of 350 RPM for 24 h with samples taken periodically.

**Lipase stability and reusability.** In order to determine if the lipase was reusable, a series of five consecutive reactions were run using the same lipase sample. Reactions were allowed to run for 12 h at 50 °C and stirred at 350 RPM. A second set of reactions was run under the same conditions, except they were shaken at 250 RPM in an incubating shaker. After 12 h, the remaining reaction volume was removed *via* centrifugation and the lipase was washed once with *tert*-butanol, and then incubated for 1 h in 2 ml of *tert*-butanol. The *tert*-butanol/lipase mixture was then centrifuged and the *tert*-butanol layer was poured off. The lipase was then transferred to a new vial for the subsequent reaction.

**Sample analysis.** Samples were withdrawn at regular intervals, mixed with an equal volume of methanol to immediately quench the reaction, and then centrifuged at 13 000 RPM for 15 min to remove any enzyme. Aliquots were then diluted with methanol as necessary for analysis.

Analysis of reaction products was performed using a Shimadzu QP2010 GC-MS equipped with a Shimadzu SHRXI-5MS column (30 m × 0.25 mm ID × 0.25 μm df). Injections were done at 300 °C with a split ratio of 50 and constant column flow of 1.3 mL $min^{-1}$. The column temperature started at 50 °C for 1 min, ramped up to 100 °C at 15 °C per minute and held for 4 min, then increased to 150 °C at 10 °C per minute and held for 2 min, before being increased to 300 °C at a rate of 25 °C per minute. The interface temperature was 300 °C, while the ion source was kept at 200 °C. Samples were typically diluted 1 : 100 in methanol for analysis, but lower dilutions were used to obtain clean mass spectra for unknown compounds.

Analysis of the reaction products from the substrate molar ratio and the lipase reusability assays was performed on the same instrument, but using a Phenomenex ZB-5MSi column (30 m × 0.25 mm ID × 0.25 μm df). The split ratio was lowered to 20 in order to more closely monitor product formation.

## Conclusions

Lipase-catalyzed glycerol carbonate synthesis from glycerol and a dialkyl carbonate was studied. CalB was selected as the most effective catalyst, and the influence of reaction conditions was investigated. A reaction system was identified that offers dramatically higher productivity than previously reported.

Our system offers high glycerol conversion and glycerol carbonate selectivity without the use of environmentally toxic solvents[53] or additional treatment of the reactants, and requires a minimal amount of excess reactant (DMC). Through control of the reaction parameters, particularly the relative substrate concentrations, the reaction can be tuned to deliver high conversion as well as high selectivity towards glycerol carbonate. Additionally, substitution of an alternate dialkyl carbonate offers the possibility of an expanded product profile. As new glycerol chemistries are being explored as a basis for renewable biorefineries, these side products deserve further study.

As biofuel production expands, new strategies for enhancing the economics of the process must be implemented. Glycerol carbonate production from waste glycerol offers not only an economic advantage, but will allow biodiesel facilities to recycle their waste stream into a sustainable chemical source. Furthermore, as CalB is a suitable catalyst for both biodiesel and glycerol carbonate production, practical implementation of these processes can be streamlined. In addition, we observed increased conversion rates and interesting side products when using diethyl and dibutyl carbonate in place of dimethyl carbonate. These additional products show the substrate versatility of CalB and indicate the potential of glycerol as a feedstock for biorefineries.

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