

Research Article

## Glycerolysis of Esters with *Candida antarctica* Lipase B in Glycerol

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**Abstract** Glycerol was successfully used as a green solvent and as the acyl acceptor in the transesterification of amyl and benzyl acetate using immobilized *Candida antarctica* lipase B. It was found that increasing either the reaction temperature or the enzyme to substrate ratio increased the conversion of both esters. Using glycerol as the solvent also enabled the separation of product by simple extraction with diethyl ether and catalyst recycling.

**Keywords** glycerol; transesterification; lipase; green solvent; benzyl acetate

### 1 Introduction

The split of an ester to the corresponding carboxylic acid and alcohol is a basic organic transformation [7]. In general, it can be accomplished by two main routes: hydrolysis in the presence of water, which yields a carboxylic acid and an alcohol, or alcoholysis (transesterification) in the presence of an alcohol, which extracts the carboxyl group from the ester and releases the corresponding alcohol. Both routes are equilibrium reactions that usually require excess amounts of acyl acceptor to yield high conversions and the presence of a chemo- or bio-catalyst that is either homogenous or heterogeneous [2, 15]. The most commonly used catalysts are soluble and solid acids or bases. In recent years, however, free and immobilized lipases have also been exploited as more environmentally friendly catalysts for industrial applications.

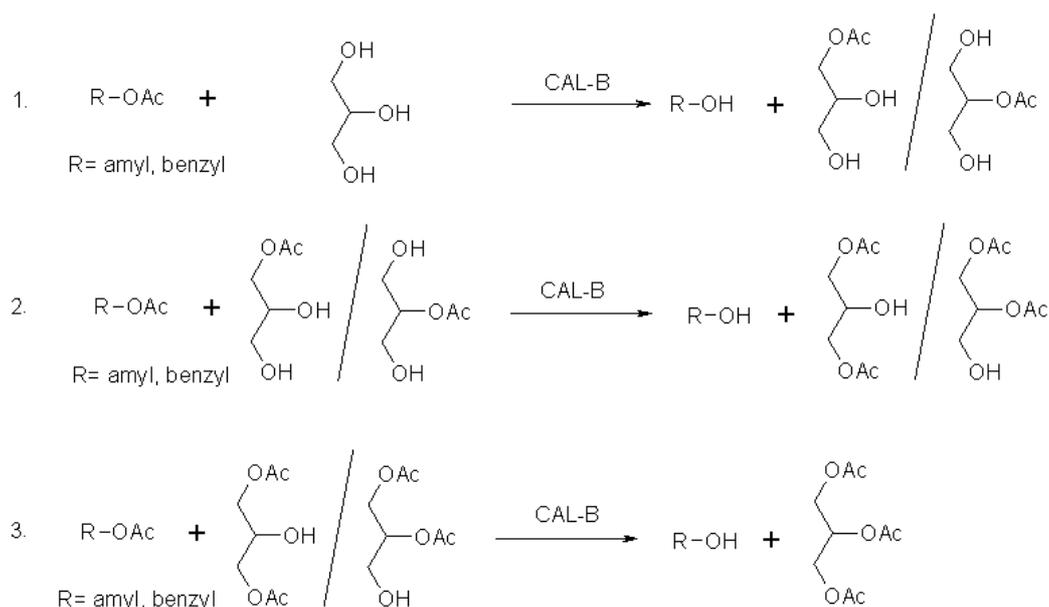
Acetyls are frequently used as a protecting group in multi-step organic synthesis to protect free hydroxyl groups by forming the corresponding ester [5, 6, 7]. The presence of an acetyl allows chemoselective transformations, and it can be easily released afterward by hydrolysis or alcoholysis. However, as many organic molecules have low miscibility in water and as water itself can attack and react with various functional groups and catalysts, using transesterification to extract the acetyl group and release the hydroxyl group selectively is preferred. For the same reason of chemoselectivity, employing a free or immobilized lipase as the catalyst is usually preferred over acids or bases.

Different alcohols can be used for the alcoholysis of an ester, and short chain alcohols such as methanol and ethanol are usually used. We recently showed that glycerol can be successfully used as both the solvent and the acyl acceptor in the kinetic resolution of ester racemates via transesterification using immobilized *Candida antarctica* lipase B (CAL-B) as the catalyst [1]. Likewise, glycerol triacetate (triacetin) was used as both solvent and acyl donor in the resolution of alcohol racemate but also in the production of isoamyl acetate, a characteristic banana flavor ester used in the food industry, using CAL-B or acidic ion exchange as the catalyst [9, 13]. The use of triacetin produced high alcohol conversion, and the resulting esters were easily separated by simple extraction with petroleum ether.

As a by-product from the conversion of oils and fats in the production of oleochemical and biodiesel, glycerol, whose supply has increased tremendously in the last decade, has consequently decreased substantially in price. Glycerol was employed as the raw material in various chemical syntheses [4, 16]. In addition, due to its promising physical, chemical, and biological properties that make it non-volatile, non-hazardous, recyclable, and biodegradable, we recently reported the use of glycerol as a reaction medium in wide variety of catalytic and non-catalytic organic syntheses [10, 11, 12]. Though glycerol has a relatively high viscosity at room temperature, its viscosity decreases as reaction temperature increases, and it does not prevent the reaction from proceeding.

As previously mentioned, glycerol can be simultaneously used as green solvent and as reactant in organic reactions, where it also allows easy product separation and catalyst recycling [1]. Hence, as glycerol has three hydroxyl groups, it was suggested that it can be efficiently used as solvent and acyl acceptor in the transesterification of esters, which produces the corresponding alcohol that can then be easily isolated from the reaction mixture by simple extraction.

In this paper, we report on our study about the alcoholysis of two representative esters, amyl and benzyl acetate,



**Figure 1:** Transesterification of esters in glycerol.

using immobilized CAL-B in various alcohols and glycerol, the latter of which was used both as a green solvent and as an acyl acceptor (Figure 1). The effects of alcohol type and amount of glycerol, reaction temperature, and the enzyme to substrate ratio on the ester alcoholysis yield were studied, as was the enzyme separation process and recycling. Simple extraction with diethyl ether was used to separate the reaction product from the catalyst.

## 2 Experimental

In a typical procedure, 0.8 mmol of ester was added together with 0.01 g immobilized CAL-B to a vial with 5 mL of alcohol (all purchased from Aldrich). The mixture was placed in a preheated oil bath and heated to the required temperature (40–110 °C) after which it was magnetically stirred for 1–5 h. At the end of the reaction, the reaction mixture was cooled and extracted with 3 × 10 mL diethyl ether. The organic phase was concentrated under reduced pressure, and the resulting crude product was analyzed by GC analysis using an HP-5 column (30 m × 0.25 mm, 0.25 μm thick). The product yield was calculated by dividing the molar amount of the product, benzyl or amyl alcohol, at the end of the reaction by the molar amount of the ester, benzyl or amyl acetate, at the beginning of the reaction.

Several catalyst recycling methods were tested. The first reaction cycle in all the experiments was run as follows: 1 g of benzyl acetate and 0.1 g of immobilized CAL-B were added to a vial with 10 g of glycerol. The vial underwent shaking at 300 rpm and was then heated to 90 °C in an Excella E24 incubator (New Brunswick Scientific) for 5 h. For the first catalyst recycling method, the immobilized

lipase was filtrated at the end of the reaction and the product was extracted by 5 × 10 mL diethyl ether and analyzed by GC. The filtrated immobilized enzyme was then added to a fresh mixture of benzyl acetate in glycerol, and the reaction was repeated. For the second method, both the catalyst and the glycerol were reused by filtrating the catalyst at the end of the reaction, extracting the product by 5 × 10 mL diethyl ether, and then adding the reused catalyst together with fresh benzyl acetate to the reused glycerol. Finally, for the last recycling method, the product was first extracted by 5 × 10 mL diethyl ether from the glycerol-catalyst mixture, to which was added fresh benzyl acetate.

## 3 Results and discussion

As previously mentioned, esters can be enzymatically alcoholized through transesterification using an alcohol, which can be simultaneously used as the solvent and as the acyl acceptor using CAL-B as the catalyst. Hence, the investigation began by testing the yields of the transesterification of amyl acetate with various alcohols as both solvents and acyl acceptors (Table 1).

As illustrated in Table 1, employing simple short chain alcohols in excess yielded negligible reactions (entries 1–4), while using 1-octanol (entry 5) as the solvent and the acyl acceptor resulted in high product yield. On the other hand, employing alcohols with more than one hydroxyl group (polyols), such as ethylene glycol and glycerol, improved the reaction yield (entry 6–8). In addition, while increasing reaction time from 1 to 3 h increased the product yield in simple alcohols with one hydroxyl group, it only slightly changed amyl alcohol yield in the three tested polyols. It

Entry	Alcohol	Yield (%), 1 h	Yield (%), 3 h
1	Methanol	0	0
2	Ethanol	0	0
3	1-Propanol	1.1	5.6
4	2-Propanol	1.8	6.3
5	1-Octanol	39.3	55.6
6	1,2-Ethandiol	61.5	63.4
7	1,2-Propandiol	26.1	30.4
8	Glycerol	73.4	75.5

<sup>a</sup>Reaction conditions: 0.1 g amyl acetate, 0.01 g immobilized CAL-B, 5 mL alcohol, 70 °C.

**Table 1:** Transesterification of amyl acetate in various alcohols<sup>a</sup>.

Entry	Molar ratio glycerol/amyl acetate	Yield (%)
1	5	25.9
2	3	23.7
3	2	23.1
4	1	21.4
5	0.67	20.8
6	0.33	17.7

<sup>a</sup>Reaction conditions: 0.1 g amyl acetate, 0.01 g immobilized CAL-B, 70 °C, 1 h.

**Table 2:** Effect of glycerol to amyl acetate ratio on the transesterification yield<sup>a</sup>.

might be that the reaction in polyols reached its equilibrium conversion faster due to the high excess of alcohol groups.

Besides the high product yields associated with its use, glycerol produced as a by-product of the transesterification of oil from renewable sources has recently gained increased attention as an alternative sustainable solvent for catalytic and non-catalytic organic transformations [10,11,12]. In addition to glycerol's ability to dissolve many organic and inorganic compounds, its use also allows easy product separation and simple catalyst recycling. Because glycerol was used in high excess (~ 90 fold on a molar basis, Table 1, entry 8), we also studied how that affected amyl alcohol yield (Table 2), and the tests were run for 1 h to exclude equilibrium conversion. From the results in Table 2, it can be seen that decreasing the molar ratio of glycerol to amyl acetate from 5 : 1 to 1 : 1 or even to 1/3 : 3 (equimolar ratio based on hydroxyl groups) only slightly changed the conversion after 1 h of reaction, revealing that the amount of glycerol can be decreased to a minimum as each molecule of glycerol can extract more than one acyl group.

Besides its easy separation and recycling, immobilized CAL-B is also preferable to the parent lipase because it is more stable at high temperatures in most organic solvents. However, the increased temperatures that promote higher reaction rates cause more rapid enzyme deactivation.

Entry	Temperature (°C)	S/E (g/g)	Amyl alcohol yield (%)	Benzyl alcohol yield (%)
1	30	10	12.5	27.2
2	50	10	29.7	35.3
3	70	10	73.4	38.0
4	90	10	57.2	39.5
5	110	10	55.6	48.1
6	70	6.7	83.9	51.5
7	70	5	86.9	52.3

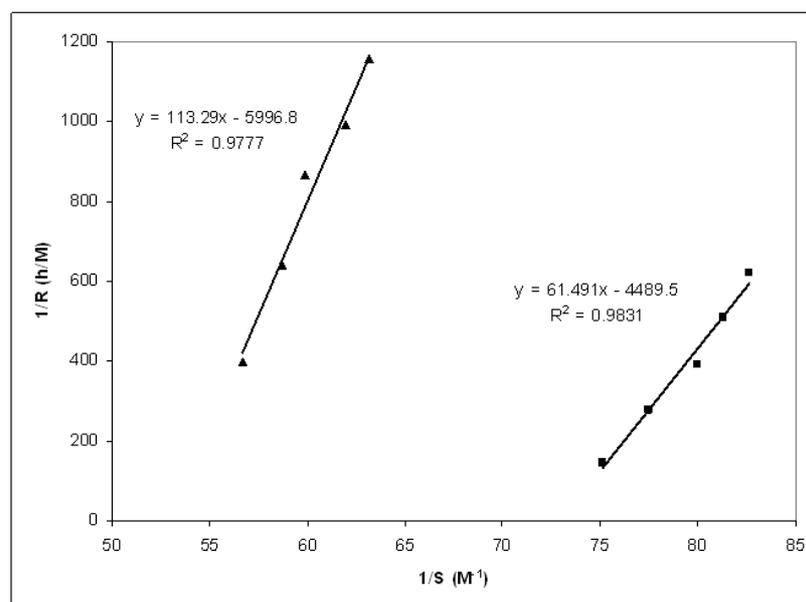
<sup>a</sup>Reaction conditions: 0.1 g ester, immobilized CAL-B, 1 h.

**Table 3:** Effect of reaction conditions on the transesterification yield<sup>a</sup>.

Therefore, the effect of reaction temperature on the reaction rate using heterogeneous lipase in glycerol was studied with two representative esters, amyl and benzyl acetate, in the range of 40–120 °C (Table 3, entries 1–5). It can be seen from the results in Table 3 that up to a temperature of 50 °C amyl acetate as the representative alkyl ester was less active than the representative benzyl ester, benzyl acetate, while above 50 °C the aliphatic ester was more active. As expected, the product yield increased with the increase in reaction temperature, but when amyl acetate was employed as the substrate, maximum activity was detected at 70 °C.

It is well known that the substrate to enzyme (S/E) ratio also affects the catalytic activity of enzymatic reactions. Therefore, the effect of catalyst loading on the yield of amyl and benzyl alcohol in glycerol was also studied (Table 3, entries 3, 6 and 7). As expected, decreasing the S/E ratios by increasing the loading of the lipase increased the overall yield. At high enzyme ratios, however, when there was enough enzyme for all the available substrate, the addition of enzyme did not increase the reaction rate, and the conversion rate was changed negligibly.

The progress of product yield with reaction time in glycerol was also studied with the two representative esters and a reaction mechanism was proposed based on equilibrium expressions (Figure 2). As transesterification is an equilibrium reaction, the kinetic model was calculated at low temperatures to avoid equilibrium conversion (30 °C for amyl acetate and 70 °C for benzyl acetate). Moreover, since transesterification is a two-substrate reaction, it can be classified as a ping-pong, or sequential, reaction mechanism, yet when one of the substrates is in excess, as in this study, the reaction mechanism is similar to a one-substrate mechanism, e.g. Michaelis-Menten kinetics [3]. As can be seen from the results in Figure 2, it obeys the equation  $v/v_{\max} = [S]/(K + [S])$ , where  $v$  is current reaction rate,  $v_{\max}$  is maximum reaction rate, and  $[S]$  is substrate concentration. A similar mechanism was reported for other lipase catalyzed transesterification reactions [8, 14].



**Figure 2:** Lineweaver-Burk plot of amyl and benzyl acetate in glycerol. Reaction conditions: 0.8 mmol ester, 0.01 g immobilized CAL-B, 5 g glycerol. (▲) amyl acetate, 30 °C; (■) benzyl acetate, 70 °C.

At the end of the reaction, amyl acetate was separated from the reaction mixture either by distillation or by extraction with diethyl ether. Although the use of glycerol as the acyl acceptor in alcoholysis (glycerolysis) may yield glycerol monoacetate, glycerol diacetate, and/or triacetin as by-products, the high boiling points and high solubilities of these potential by-products in glycerol allowed the product to be easily separated from the reaction mixture.

Finally, the efficiency of product separation and catalyst recycling are also environmentally and economically important. As previously mentioned, using glycerol as the solvent facilitated product separation by simple extraction with diethyl ether, while simple filtration can be used to separate the immobilized enzyme from the reaction medium. However, the partial erosion of the immobilized lipase by the magnetic stirrer during the reaction precluded easily recovering the lipase in its entirety. In addition, the erosion was harsher with increased reaction temperature. Alternatively, shaking the reaction medium that contained the catalyst enabled full recovery of the catalyst after its filtration. Catalyst recycling tests, therefore, were done in a laboratory shaker incubator.

Although the highest benzyl alcohol yield was detected at 110 °C, at this temperature the catalyst underwent partial degradation during shaking of the reaction mixture. Hence to test the viability of enzyme recycling for the heterogeneous lipase, the reaction mixture was run for 5 h at 90 °C, after which the catalyst was filtrated and the product and the residual substrate were fully extracted by diethyl ether (Table 4, entry 1). After the first reaction cycle, the

Entry	Procedure	Conversion (%)
1	First cycle	71
2	Second cycle of the catalyst, fresh glycerol	69
3	Third cycle of the catalyst, fresh glycerol	68
4	Second cycle of the catalyst and glycerol	70
5	Second cycle of the catalyst and glycerol <sup>b</sup>	71
6	Third cycle of the catalyst and glycerol <sup>b</sup>	70
7	Forth cycle of the catalyst and glycerol <sup>b</sup>	71

<sup>a</sup>Reaction conditions: 1 g benzyl acetate, 0.1 g immobilized CAL-B, 10 g glycerol, 90 °C, 5 h. Catalyst separation by filtration and product extraction by diethyl ether.

<sup>b</sup>Recycling of the catalyst and the glycerol after extraction of the product but without first isolating the catalyst.

**Table 4:** Recycling of immobilized CAL-B and glycerol<sup>a</sup>.

filtrated catalyst was added to a fresh mixture of benzyl acetate in glycerol and the reaction was run again under similar conditions. Two cycles of catalyst reuse resulted in comparable reaction conversions (Table 1, entries 2, 3). Since glycerol was both solvent and acyl acceptor, recycling of the glycerol together with the heterogeneous catalyst was also tested by filtration of the catalyst at the end of the first reaction cycle, after extraction of the product and residual substrate and subsequent addition of the reused catalyst and fresh benzyl acetate to the used glycerol (Table 1, entry 4). The observed benzyl alcohol yield was similar to those recorded in the previous recycling experiments, showing that glycerol recycling is also possible. Finally, to avoid catalyst filtration, after the first reaction cycle the

product was extracted from the reaction mixture, which still included the catalyst, and fresh benzyl acetate was added to the reused reaction mixture. This method also yielded similar benzyl alcohol yields (Table 1, entries 5–7) relative to the reaction with the reused catalyst that was separated before product extraction (Table 1, entry 4).

#### 4 Conclusions

Glycerol can be successfully used as a green solvent and an acyl acceptor in the transesterification of esters using immobilized CAL-B as the catalyst. It was found that increasing the reaction temperature or decreasing the S/E ratio increased the conversion. Using glycerol as the solvent also allowed for easy separation of the product and catalyst recycling.

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