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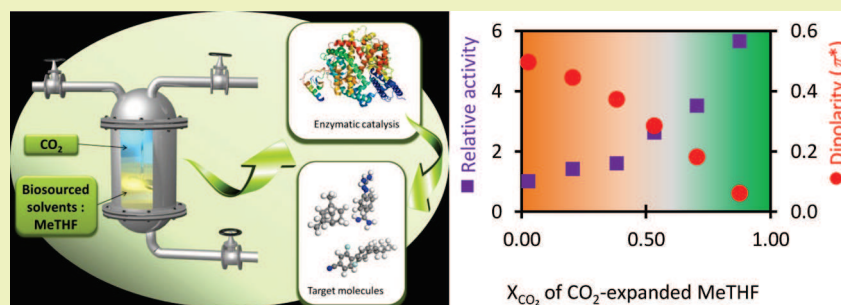
# Modulating Biocatalytic Activity toward Sterically Bulky Substrates in CO<sub>2</sub>-Expanded Biobased Liquids by Tuning the Physicochemical Properties

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**ABSTRACT:** The study of CO<sub>2</sub>-expanded liquids using a green component such as a biobased solvent has been recently raised as a new concept for an alternative solvent and yet been largely unexplored in the literature for neither fundamental nor application studies. On the other hand, structural bulkiness of substrates remains one of the main limitations to promote enzymes as an efficient versatile catalytic tool for organic synthesis, especially biocatalysis in nonconventional solvents. Herein, we report a detailed investigation of CO<sub>2</sub>-expanded biobased liquids as reaction media for improved biocatalysis of sterically hindered compounds. We found that CO<sub>2</sub> acts as a crucial trigger for various lipases to catalyze transesterification of challenging bulky alcohols in CO<sub>2</sub>-expanded 2-methyltetrahydrofuran (MeTHF). Furthermore, this study determines the physicochemical and transport properties of CO<sub>2</sub>-expanded MeTHF for the first time, which were then utilized for modulating biocatalytic activity. It was found that lipase activity increased with the accordingly decrease of the dipolarity of CO<sub>2</sub>-expanded MeTHF, which is tunable by altering the concentration of CO<sub>2</sub> in the solvent system.

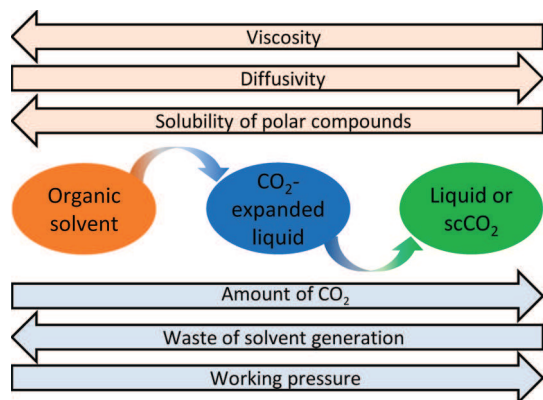
**KEYWORDS:** Pressurized carbon dioxide, *Candida antarctica* lipase B, 2-Methyltetrahydrofuran, Transesterification, Kinetic resolution, Substrate specificity, Solvatochromism, Green solvent engineering

## INTRODUCTION

Solvents, which play an important role in chemical and pharmaceutical industries, have often been claimed as a major source of waste generation and associated environmental and economic burdens.<sup>1–3</sup> With the eco-environmental urge of developing sustainable processes, academic and industrial intentions have been focused both on minimizing the overall solvent usage and on replacing traditional organic solvents by more environmentally friendly alternatives.<sup>4–6</sup>

A novel class of alternative solvents is gas-expanded liquids,<sup>7–9</sup> more particularly CO<sub>2</sub>-expanded liquids (CXLs).<sup>10</sup> They are considered as tunable solvents and very promising as they inherit the advantages of pressurized CO<sub>2</sub> and of traditional solvents in an optimal fashion: (1) the dissolved

CO<sub>2</sub> can tune the physicochemical and transport properties of the solvent such as viscosity, density, and diffusivity across a large polarity range, and (2) the component organic solvent improves the solubility of polar compounds (Figure 1). The use of CXLs, on the other hand, can overcome the sustainable drawbacks of the required high working pressure of supercritical CO<sub>2</sub> and subsequently reduces the installation cost of equipment and energy consumption of operation.<sup>11</sup> Other important environmental advantages include the reduced waste of organic solvents because CXLs are generated by substantially



**Figure 1.** Green and sustainable use of carbon dioxide to tune the physical properties of solvents.

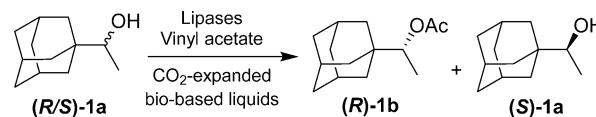
replacing the organic solvents with the environmentally benign dense CO<sub>2</sub>.

On the other hand, the biocatalytic processes, which has been well established as a topic of chemical research,<sup>12–14</sup> shares many attractive features in the context of green chemistry and sustainability.<sup>15</sup> Traditionally, biocatalysts have been used in aqueous solvents, which sometimes results in inevitable side reactions and low solubility toward hydrophobic compounds. The scope of biocatalysis, especially in organic synthesis,<sup>14</sup> has been extended through the use of enzymes in organic solvents<sup>16</sup> and in nonconventional solvents<sup>17,18</sup> such as supercritical<sup>19</sup> and liquid<sup>20–22</sup> CO<sub>2</sub>, ionic liquids,<sup>23</sup> and deep eutectic solvents.<sup>24,25</sup> To date, however, the study of CXLs as reaction media for a biocatalysis process has remained less explored.

Although the use of CXLs adheres to some principles of green chemistry and sustainability, such as reduced waste of organic solvents and energy cost, usually a CXL system employs a petroleum-sourced volatile organic compound, which is depletable and often harmful to the environment. Therefore, classical CXLs cannot be adequately satisfactory as green solvents. An alternative way out is to replace the conventional harmful organic solvents with environmentally friendly biobased liquids.<sup>26–28</sup> Previously we reported CXLs using biobased liquids such as 2-methyltetrahydrofuran (MeTHF) as reaction media for *Candida antarctica* lipase B (Novozym 435, CALB) to effectively catalyze kinetic resolution of various *sec*-alcohols.<sup>29</sup> MeTHF, which can be derived from lignocellulosic biomass, has recently gained much interest as a promising and an emerging biobased solvent for various synthesis applications<sup>30–32</sup> including biocatalysis.<sup>33,34</sup> However, as there has been no studies on the solvent properties of CO<sub>2</sub>-expanded MeTHF reported, the biocatalytic behavior in this novel medium has not yet been addressed.<sup>29</sup>

In the present study, by using 1-adamantylethanol **1a** as a challenging bulky model substrate, it was found out that CO<sub>2</sub> acts as a crucial trigger for various lipases to catalyze transesterification in a wide range of CO<sub>2</sub>-expanded biobased liquids (Scheme 1). In order to explain our observation, the physicochemical and transport properties of CO<sub>2</sub>-expanded MeTHF were studied both experimentally and by molecular modeling. Thus, the vapor–liquid equilibrium and polarity of the expanded phase were determined experimentally; in particular, the polarity of the expanded phase was determined by solvatochromic measurements. The density and viscosity of the CO<sub>2</sub>-expanded phase were obtained by molecular dynamics.

**Scheme 1.** Kinetic Resolution of *rac*-1-Adamantylethanol **1a** by Lipases in CO<sub>2</sub>-Expanded Biobased Liquids



The improved lipase activity can be attributed to important changes in solvent properties, of which transport properties are enhanced in CO<sub>2</sub>-expanded MeTHF by the decrease of viscosity and density, while the dipolarity/polarizability parameter naturally decreases to a certain degree that is ideal for the lipase-catalyzed reaction. Significantly, this study shows for the first time that the activity of a biocatalysis process in CO<sub>2</sub>-expanded MeTHF at different temperatures was closely correlated to the physicochemical properties, which are modulated by tuning the CO<sub>2</sub> molar fraction ( $X_{\text{CO}_2}$ ).

## RESULTS AND DISCUSSION

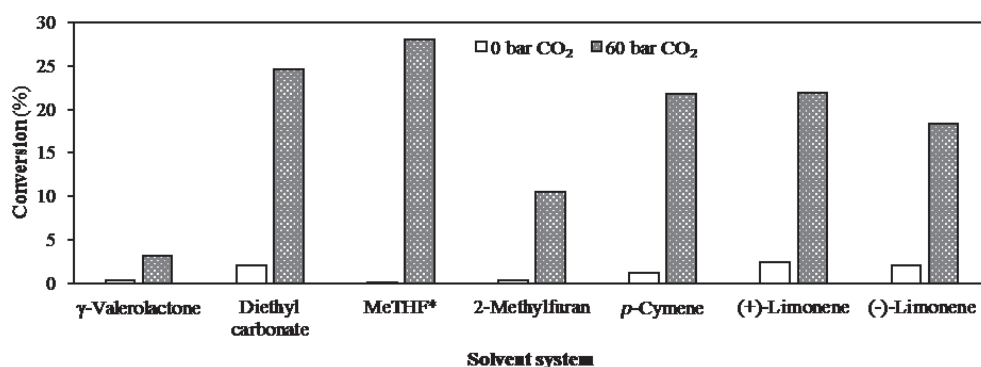
**Lipase-Catalyzed Transesterification of 1a.** First, transesterification of *rac*-1-adamantylethanol **1a** using vinyl acetate as an acyl donor was investigated in MeTHF and CO<sub>2</sub>-expanded MeTHF using various lipases. Because MeTHF has a low hydrophobicity ( $\log P = 1.0$ , Supplementary Table S1), which could be unfavorable for lipase-catalyzed reaction in organic solvents,<sup>35</sup> we also performed the same reactions in hexane ( $\log P = 3.5$ , Supplementary Table S1) and CO<sub>2</sub>-expanded hexane for comparison (Table 1). Vinyl acetate was used in a small volume (50  $\mu\text{L}$ ) compared to a bulk amount of MeTHF or hexane to minimize the solvent effect of vinyl acetate, which can also contribute as a cosolvent if used in a large amount. As can be seen from Table 1, most of the used enzymes exhibited no or very low catalytic activity for the transesterification of **1a**, although they have potent catalytic activity toward general *sec*-alcohols such as 1-phenylethanol. The low reactivity of those lipases toward the bulky substrate 1-adamantylethanol **1a** may resulted from (1) the nature of the lipase, of which the substrate-binding cavity is not large enough to accommodate the bulky substrate **1a**, or (2) the low stability of the used lipase under nonaqueous solvents. Among the examined enzymes, *Candida antarctica* lipase B (CALB) and lipase TL showed high conversions in CO<sub>2</sub>-expanded liquids. Notably, when used in CO<sub>2</sub>-expanded systems, CALB and PS-C performed much higher than in neat MeTHF or hexane. To elucidate the mechanism of this phenomenon, we selected CALB for further investigations because it showed high conversion and improved activity in CO<sub>2</sub>-expanded liquids.

**Effect of Biobased Liquids with and without CO<sub>2</sub> Expansion on Conversion of CALB-Catalyzed Transesterification of 1a.** The transesterification of *rac*-1-adamantylethanol **1a** catalyzed by CALB was performed under various biobased liquids (Figure 2) and compared with the reactions in their CO<sub>2</sub>-expanded counterparts. Seven biobased liquids with different hydrophobicity ( $\log P$ ) (Supplementary Table S1) were used in this study. Three lignocellulosic biomass derivatives ( $\gamma$ -valerolactone, MeTHF, and 2-methylfuran),<sup>36,37</sup> diethyl carbonate,<sup>38,39</sup> and two citrus-derived alkanes (*p*-cymene and (+)-limonene)<sup>40</sup> have been reported as promising solvents for reactions,<sup>32</sup> including some biocatalysis processes.<sup>41</sup> (–)-Limonene is not a natural-derived source but was employed for a comparison with its natural counterpart (+)-limonene. The green credentials of these

**Table 1. Lipase-Catalyzed Transesterification of *rac*-1-Adamantylethanol **1a**<sup>a</sup>**

lipase (organism, carrier)	neat MeTHF		CO <sub>2</sub> -expanded MeTHF		neat hexane		CO <sub>2</sub> -expanded hexane	
	conversion (%)	<i>E</i> value	conversion (%)	<i>E</i> value	conversion (%)	<i>E</i> value	conversion (%)	<i>E</i> value
CALB ( <i>Candida antarctica</i> , acrylic resin)	<1 <sup>b</sup>	n.d.	29 <sup>b</sup>	>200	3.1 <sup>b</sup>	n.d.	29	>200
TL ( <i>Pseudomonas stutzeri</i> , beige powder)	47	>200	45	>200	45	>200	36	>200
PS-D ( <i>Burkholderia cepacia</i> , diatomite)	<1	n.d.	3.5	>200	6.9	>200	<1	n.d.
PS-C ( <i>Burkholderia cepacia</i> , ceramic)	1.3	n.d.	7.4	>200	5.1	>200	12	>200
LIP 301 ( <i>Pseudomonas sp.</i> , hyflo supercel)	6.3	>200	4.5	>200	22	>200	<1	N.d.
LPL 311 ( <i>Pseudomonas sp.</i> , lyophilized powder)	24	>200	2.1	>200	14	>200	<1	N.d.

<sup>a</sup>Conditions: 0.10 mmol *rac*-1-adamantylethanol **1a**, 0.53 mmol vinyl acetate, 10 mg lipase, 10 mL solvent (CO<sub>2</sub>-expanded liquid 10% v/v concentration, 60 bar), 293 K, 24 h. Lipase AK (*Pseudomonas fluorescens*, lyophilized powder), AH (*Burkholderia cepacia*, beige powder), Lipozyme (*Mucor miehei*, ion-exchange resin) resulted in no or very low conversion (<2%) in all solvent systems. <sup>b</sup>Data taken from ref 29.



**Figure 2.** Effect of biobased liquids with and without CO<sub>2</sub> expansion on conversion of CALB-catalyzed transesterification of **1a**. Reaction conditions: 0.10 mmol of *rac*-1-adamantylethanol **1a**, 0.53 mmol of vinyl acetate, 10 mg of Novozym 435, 10 mL of CO<sub>2</sub>-expanded liquid (biobased liquid concentration 10% v/v, 60 bar), 293 K, 24 h. \*Data taken from ref 29 and Table 1.

biobased liquids selected have been discussed and ranked in a recent solvent selection guide.<sup>6</sup> None of the biobased liquids used in this work are considered hazardous or highly hazardous. They all were found to solubilize CO<sub>2</sub> in such great quantity that volumetric expansion of CO<sub>2</sub>-expanded phase is up to 10-fold (Supplementary Figure S1), which leads us to categorize these fluids as class II liquids according to Jessop and Subramaniam.<sup>9</sup>

The conversion of CALB-catalyzed transesterification of *rac*-1-adamantylethanol **1a** was very low in all neat biobased solvents (Figure 2). After 24 h, the highest conversion of less than 3% was found in neat (+)-limonene. It should be noticed that many of the biobased solvents used have been reported as effective platforms for CALB to catalyze a typical esterification of hexyl laurate,<sup>41</sup> which suggested that the bulkiness of the substrate **1a** determined the low conversion of CALB to catalyze the reaction in this study. In contrast, the lipase exhibited impressively high conversion under all corresponding CO<sub>2</sub>-expanded liquid systems (Figure 2).

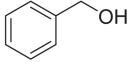
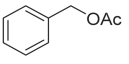
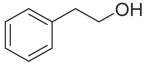
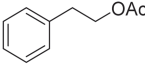
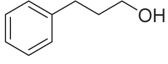
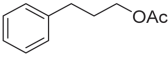
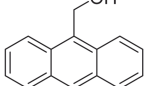
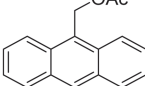
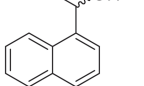
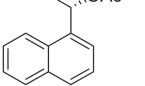
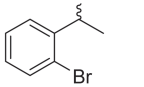
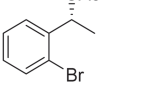
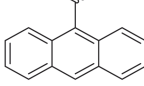
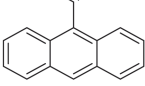


The highest conversions were observed in CO<sub>2</sub>-expanded MeTHF (29%) and CO<sub>2</sub>-expanded diethyl carbonate (25%). There were no significant differences between (+) and (-)-limonene on the CALB-catalyzed reaction. With CO<sub>2</sub>-expanded  $\gamma$ -valerolactone, a conversion of 3.1%, but higher than neat  $\gamma$ -valerolactone, was observed. The much higher conversions observed in all CO<sub>2</sub>-expanded systems implied the crucial role of the presence of CO<sub>2</sub> pressure to accelerate the lipase to mediate the reaction. Lipases have been reported to generally afford higher activity in more hydrophobic solvents (solvents with higher log *P*)<sup>16</sup> compared to other conventional solvents for (trans)esterification.<sup>21,29,41,42</sup> It should be noticed that addition of CO<sub>2</sub> to a hydrophilic solvent such as MeOH

can increase the nonpolarity and hydrophobicity of this solvent.<sup>29</sup> However, since all conversions in hexane (log *P* = 3) and biobased solvents (with different log *P* values, Supplementary Table S1) were low (<4%), the hydrophobicity (log *P*) alone cannot explain the high reaction conversions obtained in CO<sub>2</sub>-expanded liquids. Without the presence of CO<sub>2</sub>, the enzyme failed to mediate the reaction of bulky **1a** even in neat hydrophobic solvents such as hexane (Table 1) or *p*-cymene (Figure 2). Thereby, the high conversion observed for CALB to transform bulky **1a** in CO<sub>2</sub>-expanded systems could contribute to important changes in the enzyme structure which are induced by pressurized CO<sub>2</sub>.

**Effect of Reaction Pressure on Conversion of CALB-Catalyzed Transesterification of **1a**.** As stated above, CO<sub>2</sub> pressure may induce increased conversions in CO<sub>2</sub>-expanded liquids, which can be differentiated into the effect of pressure and of CO<sub>2</sub> molecules. To study the effect of pressure, reactions under high pressure were performed at 60 bar of vinyl acetate, hexane, or MeTHF by using the same apparatus but pumping solvent liquids instead of CO<sub>2</sub> gas.<sup>43</sup> However, the reaction did not proceed at high pressure (60 bar) without using CO<sub>2</sub>. Only traces of conversions were seen with MeTHF and vinyl acetate (<1%). Although a small increase of performance was observed in the case of hexane at high pressure (5.3% conversion at 60 bar compared with 3.1% conversion at ambient pressure), it was not significant when compared with CO<sub>2</sub>-expanded system (29%, Table 1). This behavior suggests that high pressure is not the main factor to increase the high conversion for the lipase to catalyze transesterification of bulky substrate **1a**.

**Effect of Incubation of CALB with CO<sub>2</sub>-Expanded MeTHF on Conversion of the CALB-Catalyzed Transesterification of **1a**.** To investigate whether pressurized CO<sub>2</sub>

**Table 2. Effect of Substrate Bulkiness on CALB-Catalyzed Transesterification in MeTHF and CO<sub>2</sub>-Expanded MeTHF**

Substrate	Solvent	Time <sup>a</sup> (h)	Product	Conversion (%)	<i>E</i> value
2a 	MeTHF	0.5 <sup>b</sup>	2b 	23	-
	CO <sub>2</sub> -expanded MeTHF			19	-
3a 	MeTHF	0.5 <sup>b</sup>	3b 	18	-
	CO <sub>2</sub> -expanded MeTHF			33	-
4a 	MeTHF	0.5 <sup>b</sup>	4b 	36	-
	CO <sub>2</sub> -expanded MeTHF			33	-
-----					
5a 	MeTHF	48	5b 	1.5	-
	CO <sub>2</sub> -expanded MeTHF			44	-
6a 	MeTHF	1	6b 	2.6	N.d.
	CO <sub>2</sub> -expanded MeTHF			23	>200
7a 	MeTHF	5	7b 	<1	N.d.
	CO <sub>2</sub> -expanded MeTHF			19	>200
8a 	MeTHF	24	8b 	<1	N.d.
	CO <sub>2</sub> -expanded MeTHF			<1	N.d.
9a 	MeTHF	28	9b 	1.6	N.d.
	CO <sub>2</sub> -expanded MeTHF			26	>200

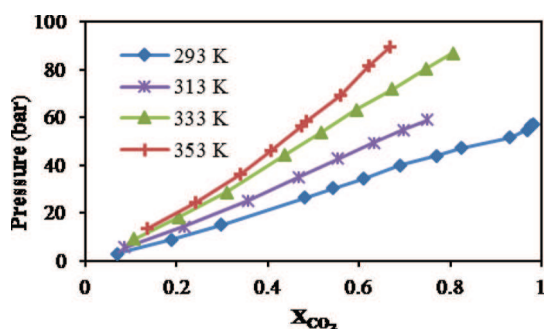
<sup>a</sup>The reactions were stopped before reaching 50% conversion to show differences between the reactions in the two media and differences among substrates. <sup>b</sup>Novozym 435, 5 mg. <sup>c</sup>Reaction conditions: alcohol (2–4a 0.40 mmol, 5–8a 0.10 mmol, 9a 0.02 mmol), vinyl acetate (0.53 mmol), Novozym 435 (10 mg), in 10 mL of MeTHF or in 10 mL of CO<sub>2</sub>-expanded MeTHF (MeTHF concentration 10% v/v, 60 bar) at 293 K. n.d.: not determined due to low conversion observed.

contributes to an irreversible or reversible conformation change of the enzyme structure to expand substrate specificity, CALB was pretreated by the incubation in CO<sub>2</sub>-expanded MeTHF (10% v/v MeTHF, 60 bar) for 7 h before employing for transesterification of *rac*-1-adamantylethanol 1a.<sup>44</sup> The pretreated CALB resulted in a very low conversion in neat MeTHF (<1%), while it retained high conversion in CO<sub>2</sub>-expanded MeTHF with excellent enantioselectivity (conversion 31%, *E* > 200). Therefore, the effect induced by CO<sub>2</sub> is reversible, and the presence of CO<sub>2</sub> in the medium is mandatory for the reaction to take place.

**Effect of Substrate Bulkiness on Conversion of CALB-Catalyzed Transesterification in Neat MeTHF and in CO<sub>2</sub>-Expanded MeTHF.** The comparison of CALB-catalyzed transesterification of nonbulky and bulky alcohols was studied in CO<sub>2</sub>-expanded MeTHF and in neat MeTHF (Table 2). For those simple *pro*-alcohols (2–4a), which do not have a bulky substituent at the  $\alpha$  position, the reaction resulted in very high conversions in both neat MeTHF and CO<sub>2</sub>-expanded MeTHF. However, if a bulky substituent is present such as anthracene (5a), the reaction can only proceed in CO<sub>2</sub>-expanded MeTHF. The phenomenon was further confirmed with the same observation with other sterically hindered bulky *sec*-alcohols 6–7a. The bulkiness of 1-naphthyl as in 6a was also the main cause for the very low reactivity of CALB observed in a hydrolysis reaction.<sup>45</sup>

Unfortunately, CALB failed to mediate *rac*-9-(hydroxyethyl)anthracene 8a in the CO<sub>2</sub>-expanded system, suggesting its substrate scopes limitation. The reaction of CALB toward an interesting molecule 9a (1-(7-phenyl-1,7-dicarba-*closo*-dodecaboran-1-yl)ethanol),<sup>46</sup> bearing an icosahedral boron cluster (*m*-carborane, C<sub>2</sub>B<sub>10</sub>H<sub>12</sub>) with a bulky spherical surface, was investigated in the two media. The reaction again resulted in very high conversion in CO<sub>2</sub>-expanded MeTHF but poor conversion observed in neat MeTHF. To explain the high conversion observed for CALB to transform bulky molecules, plausible assumptions related to three factors are considered: (1) conformational changes of the lipase by formation of carbamates from CO<sub>2</sub> and the free amine groups (such as lysine) on the surface of the lipase,<sup>47</sup> (2) higher flexibility<sup>48</sup> and less compactness<sup>48</sup> when CALB is soaked in CO<sub>2</sub> media, causing the lipase to be more tolerant to accept more bulky substrates, and (3) enhanced transport and physicochemical properties of the expanded MeTHF compared to neat solvent.

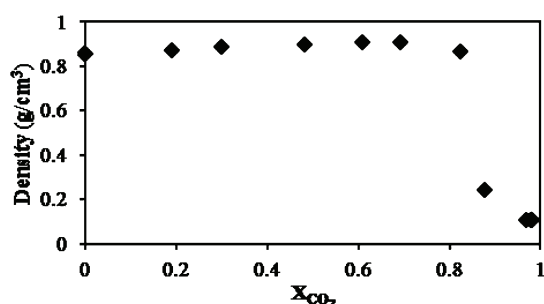
**Phase Equilibrium.** When studying gas-expanded liquids, phase equilibrium knowledge is necessary to understand the physicochemical behavior of the expanded phase. Physical equilibrium data between phases were needed in order to correctly model the expanded phases. However, the vapor-liquid equilibria (VLE) data for CO<sub>2</sub>-expanded MeTHF were not yet available. Figure 3 shows VLE results obtained experimentally using the visual method. It is clear that MeTHF can dissolve large amounts of CO<sub>2</sub> and expands



**Figure 3.** Vapor–liquid equilibria curves for MeTHF/CO<sub>2</sub> mixture at different temperatures. CO<sub>2</sub> molar fraction is defined as the ratio of CO<sub>2</sub> moles over the total moles of MeTHF and CO<sub>2</sub>.

volumetrically as a class II liquid according to the classification system proposed by Jessop and Subramaniam.<sup>9</sup> As a consequence, CO<sub>2</sub>-expanded MeTHF undergoes significant changes in almost every physical property, of which polarity presents important changes,<sup>9</sup> and also prominently relates to enzymatic behavior in organic solvents.<sup>35</sup>

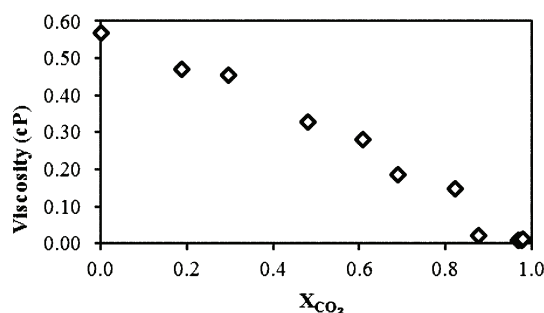
**Molecular Modeling of CO<sub>2</sub>-Expanded MeTHF.** *Density.* Figure 4 shows the results obtained from MD calculations



**Figure 4.** Density as a function of CO<sub>2</sub> molar fraction of CO<sub>2</sub>-expanded MeTHF at 293 K.

for the density of the CO<sub>2</sub>-expanded MeTHF phase as a function of CO<sub>2</sub> molar fraction at 293 K. The dependence of density on pressure is similar to that of CO<sub>2</sub>-expanded systems with other class II liquids such as ethyl acetate or 1,4-dioxane that show a distinctive maximum in the liquid density.<sup>49</sup> Density shows a great decrease after  $X_{\text{CO}_2} > 0.8$ .

*Viscosity.* Calculated viscosities at 293 K are shown in Figure 5 as a function of CO<sub>2</sub> molar fraction. The standard deviation for all systems falls under  $5 \times E-10$  due to integration over time smoothing. CO<sub>2</sub> in the vapor, liquid, and supercritical phase has



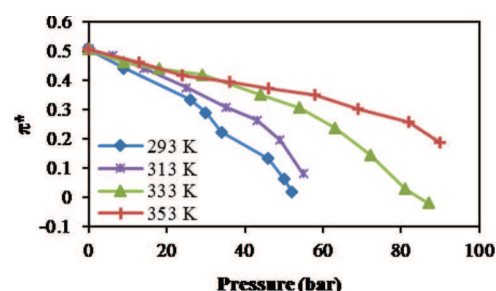
**Figure 5.** Viscosity as a function of CO<sub>2</sub> molar fraction of CO<sub>2</sub>-expanded MeTHF at 293 K.

much lower viscosity than most organic liquids. As more CO<sub>2</sub> dissolves in the liquid state, the viscosity decreases accordingly.<sup>50</sup> As expected, the viscosity of CO<sub>2</sub>-expanded MeTHF decreased gradually with the increase of CO<sub>2</sub> concentration. However, a great decrease can be observed at  $X_{\text{CO}_2}$  higher than 0.8.

The density and viscosity of the CO<sub>2</sub>-expanded phase show that at  $X_{\text{CO}_2} > 0.8$  a great decrease in these properties is obtained. These results suggest that the transport properties of the medium are clearly enhanced by CO<sub>2</sub> addition, which will have an indubitable impact on the reactions performed in this medium.

**Polarity.** Different measures of polarity can be used to compare solvents. For gas-expanded liquids, the Kamlet–Taft parameters (KT)<sup>51–54</sup> are of great interest as they can be used to differentiate acidity ( $\alpha$ , ability to donate a proton in a solvent–solute solvent–solute hydrogen bonding), from dipolarity/polarizability ( $\pi^*$ , ability to stabilize a charge or hydrogen bond), and from basicity ( $\beta$ , ability to accept a proton in a dipole). The dipolarity/polarizability parameter is among the most common polarity measures, which changes significantly as the CO<sub>2</sub> pressure increases, whereas the other solvent properties such as the hydrogen-bond-accepting ability  $\beta$  and especially the hydrogen-bond-donating ability  $\alpha$  are less strongly affected by the expansion.<sup>9,55</sup>

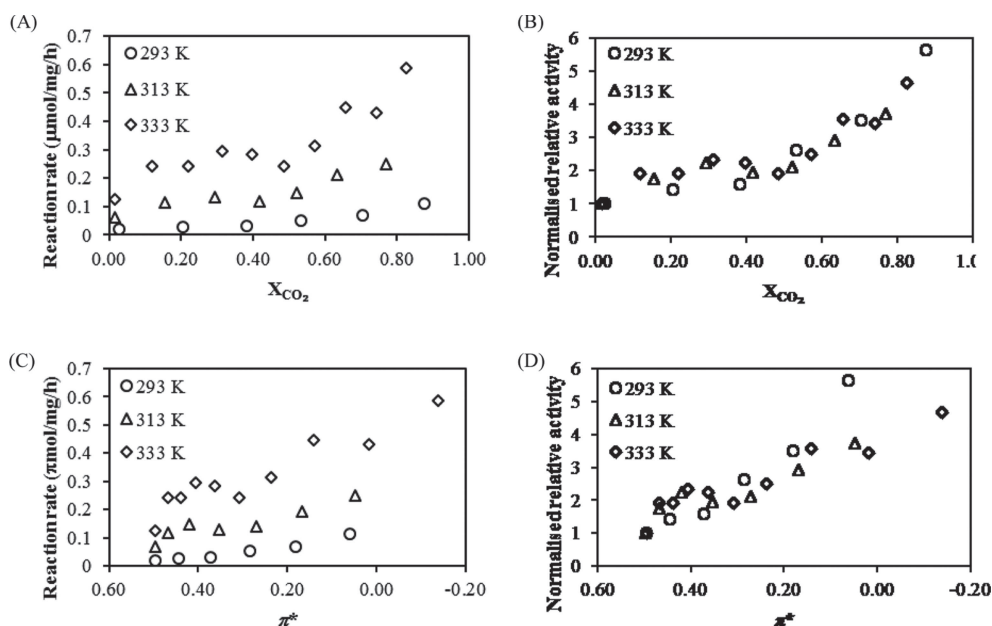
Figure 6 shows the behavior of the corrected  $\pi^*$  parameter against pressure for the CO<sub>2</sub>-expanded MeTHF system at



**Figure 6.** Dependence of  $\pi^*$  on pressure of CO<sub>2</sub>-expanded MeTHF at different temperatures.

different pressures and at temperatures from 293 to 353 K. As expected, the  $\pi^*$  value decreases with increasing pressure of carbon dioxide. The decrease of  $\pi^*$  values is more pronounced at low temperatures as a result of the higher concentration of CO<sub>2</sub> dissolved in MeTHF at lower temperatures.

**Activity of CALB as a Function of  $X_{\text{CO}_2}$  and  $\pi^*$ .** As mentioned above, the conversion of CALB-catalyzed transesterification of bulky **1a** after 24 h of reaction was largely dependent on the presence of CO<sub>2</sub> in CO<sub>2</sub>-expanded biobased liquids. We subsequently studied the dependence of CALB activity on CO<sub>2</sub> mole fraction of CO<sub>2</sub>-expanded MeTHF at different temperatures (Figure 7A). The  $X_{\text{CO}_2}$  in CO<sub>2</sub>-expanded MeTHF was controlled by the pressure of CO<sub>2</sub> based on the VLE of the CO<sub>2</sub>/MeTHF system (Figure 3). Figure 7A shows that the enzymatic activity increased with the increase of CO<sub>2</sub> concentration in the expanded MeTHF. To correct the difference in activity caused by temperature, relative activities based on the activity at  $X_{\text{CO}_2} = 0$  are shown in Figure 7B. Interestingly, the increase of the relative activity against CO<sub>2</sub> mole fraction was similar regardless of the temperatures. This showed that the CALB activity in CO<sub>2</sub>-expanded MeTHF can



**Figure 7.** Tuning CALB activity in CO<sub>2</sub>-expanded MeTHF as a function of CO<sub>2</sub> mole fraction  $X_{CO_2}$  (A and B) and dipolarity/polarizability  $\pi^*$  (C and D). Reaction conditions: 0.10 mmol of *rac*-1-adamantylethanol **1a**, 0.53 mmol of vinyl acetate, 10 mg of Novozym 435, 1.0 mL of MeTHF, pressures from 1 to 90 bar. Reaction rate ( $\mu\text{mol}$  substrate/mg enzyme/h) was calculated at conversions less than 10% (A and C) and normalized to 1 for the reaction at  $X_{CO_2} = 0$  for each temperature (B and D).

be modulated by accordingly controlling the mole fraction of CO<sub>2</sub>.

On the other hand, the hydrophobicity of solvents is often reported as a factor affecting lipase activity in organic solvents: that lipases usually exhibit higher activity in higher hydrophobic solvents.<sup>16,35,41</sup> A recent report attempted to investigate the relationship between reaction rate and different solvent properties including polarity; however, it failed to elucidate any statistically significant correlations with solvent polarity ( $\pi^*$ )<sup>42</sup> because it employed different solvents. Herein, we replotted CALB activity as a function of  $\pi^*$  parameter in CO<sub>2</sub>-expanded MeTHF by varying the amount of CO<sub>2</sub> instead of employing a series of solvents with different  $\pi^*$  values. Therefore, the structural effect of the solvents, such as size and functional groups,<sup>56,57</sup> can be reduced. Figure 7C shows that the enzyme activity increased with the decrease of the  $\pi^*$  values of the solvent systems at different temperatures. To correct the difference in activity caused by temperature, relative activities based on the lipase activity at  $\pi^*$  values of neat MeTHF at each temperature were normalized to 1 and are shown in Figure 7D. It shows that the increased activity of CALB was coordinately dependent on the  $\pi^*$  parameter. This is the first evidence that the enzyme activities were closely correlated to physicochemical properties of the solvents, which is controlled by tuning the CO<sub>2</sub> concentration of the solvent systems.

## CONCLUSION

We demonstrated that CO<sub>2</sub>-expanded MeTHF and other biobased liquids are feasible solvents for lipase-catalyzed transesterification of bulky substrates. Enhancement of the reaction conversion observed was attributed to important changes in the transport and physicochemical properties of the solvent system. According to our results, the transport properties are enhanced in CO<sub>2</sub>-expanded MeTHF by the decrease of viscosity and density. Dipolarity/polarizability  $\pi^*$

naturally decreases with  $X_{CO_2}$  to a certain degree, which is ideal for the enzyme-catalyzed reaction, which results in higher reaction conversions and enzymatic activities. The decrease in  $\pi^*$  with pressure is more pronounced at low temperatures, which is due to CO<sub>2</sub> solubility in MeTHF. Finally, CALB activity in CO<sub>2</sub>-expanded MeTHF at different temperatures was found to be closely correlated to the dipolarity/polarizability of the solvent system, which can be modulated by tuning the CO<sub>2</sub> mole fraction. This study will contribute to a more efficient usage of biobased solvents for enhanced biocatalysis by utilization of CO<sub>2</sub>.

## EXPERIMENTAL AND MOLECULAR MODELING

### Typical Procedure for Reaction in CO<sub>2</sub>-Expanded Liquids.

Lipase, 1.0 mL of biobased liquid, alcohol, vinyl acetate, and a magnetic bar were added and sealed in a high-pressure-resistant stainless-steel vessel (10 mL volume) at 293 K. CO<sub>2</sub> gas was sent into the vessel by the CO<sub>2</sub> pump until the desired pressure was achieved. The reaction mixture was magnetically stirred. At the end of the reaction, the vessel was gradually depressurized on ice. An aliquot (100  $\mu\text{L}$ ) was filtered through EXTrelut and diluted with diethyl ether before being analyzed by GC or HPLC.

Conversion ( $c$ ) was calculated as  $c = ee_{\text{substrate}} / (ee_{\text{product}} + ee_{\text{substrate}})$  or by the GC molar response factor of acetate/alcohol. The enantiomeric ratio ( $E$ ) value was calculated as  $E = \ln[1 - c(1 + ee_{\text{product}})] / \ln[1 - c(1 - ee_{\text{product}})]$  or  $E = \ln[(1 - c)(1 - ee_{\text{substrate}})] / \ln[(1 - c)(1 + ee_{\text{substrate}})]$ .<sup>58</sup>

The GC/HPLC profiles for enantiomeric excess measurement and <sup>1</sup>H NMR data of the synthesized *rac*-alcohols and *rac*-acetates as authentic samples for GC/HPLC analysis were included in the Supporting Information. The absolute configurations of lipase-synthesized compounds were determined by comparing the signs of optical rotations with those of the literature.

**Phases Equilibrium.** Experimental data concerning phase equilibria along the bubble point curve were obtained by the procedure described hereafter. A known volume, usually 10 mL, of MeTHF was loaded inside the cell. Before starting the experiment, the air contained inside the cell was evacuated by reducing the volume to its minimum until no more air bubbles were observed. The remaining



amount of air inside the cell was very small and considered negligible. The cell was then tightly closed, and a known amount of CO<sub>2</sub> was pumped into the cell by using a 260D high-pressure Teledyne Isco syringe pump. Pressure inside the cell was carefully controlled by the syringe pump and the piston of the cell. The mixture thus obtained had a known composition. When the system was equilibrated at the chosen temperature, it was initially biphasic. At that point, the piston was progressively moved in order to decrease the volume and to bring the biphasic system to a monophasic system at a higher pressure. Once the pressure was stabilized it was slowly decreased by opening the piston until a second phase appeared. The system was then successively compressed and decompressed in order to define the narrowest range of pressures for the phase transition. The stabilization had to be reached after each modification of pressure. Temperatures studied were 293, 313, 333, and 353 K, and the pressures studied were from 0 to 100 bar.

**Polarity by Solvatochromic Measurements.** Experimental determinations of Kamlet–Taft parameters (KT)<sup>51–54</sup> such as  $\pi^*$  are based in solvatochromism, which is the effect induced by the solvent on the spectral shift of certain colored indicators. This shift reflects the probe's environment (solvent molecules) interaction with the probe.<sup>59</sup>

Nile Red (NR) has been widely used<sup>60</sup> as a solvatochromic probe due to its large shift in excitation maxima, which goes from around 484 nm in nonpolar solvents to around 591 nm in polar solvents. When the  $\pi^*$  dipolarity/polarizability parameter increases, the absorbance spectra show a bathochromic shift.<sup>61</sup> Inversely, when this parameter decreases, a hypsochromic shift is observed. The relation between this shift and the  $\pi^*$  parameter has been reported in the literature<sup>62,63</sup> for hydrogen-bonding donor solvents (HBD) and non-hydrogen-bonding solvents (NHD,  $\alpha$  parameter = 0); for the case of MeTHF (NHD), it is given by eq 1:<sup>63</sup>

$$\lambda_{\max} = 19\,993 - 1725 \times \pi^* \quad (1)$$

Normalized absorption spectra of Nile Red in CO<sub>2</sub>-expanded MeTHF at different pressures (Supplementary Figure S3) and the effect of the temperature in the absorption wavelength of Nile Red in neat MeTHF (Supplementary Figure S4) were taken into account to correct the obtained  $\pi^*$  values in Figure 6.

Solvatochromic determinations were performed as follows: the view cell was filled with the MeTHF and with NR, flushed with CO<sub>2</sub>, and then tightly closed. The temperature was kept constant by the thermostated bath, and the content of the cell was agitated by a magnetic stirrer. Previously, a reference spectrum was obtained for the pure MeTHF. CO<sub>2</sub> was then injected with the ISCO pump until the desired pressure was reached inside the cell. Once the equilibrium was reached, UV–vis spectra of the NR in the expanded phase were acquired.

**Molecular Dynamics (MD) Modeling.** The density and viscosity of the saturated liquid phase were calculated using molecular dynamics (MD). For these calculations a box containing CO<sub>2</sub> and MeTHF molecules at the desired composition was simulated at fixed pressure and temperature. The required liquid-phase equilibrium composition data was obtained from our own determinations presented in this work.

Molecular dynamics potentials were selected after testing those available in the Materials Processes and Simulations (MAPS) Suite from Scienomics<sup>64</sup> and in the Large-Scale Atomic/Molecular Massively Parallel Simulator (LAMMPS) code from Sandia National Laboratories.<sup>65</sup> After several tests the “Amber\_Cornell\_Extended” force field was chosen, which includes the Harris and Yund model for the CO<sub>2</sub> molecule. The geometries of MeTHF molecules were optimized at the DFT/6-31+G\*\* level using the B3LYP exchange-correlation potential. The partial charges were then fitted using the electrostatic potential method. The simulated boxes were set with periodic boundaries in all directions containing a total of 1000 molecules in the isobaric–isothermal ensemble. A typical simulation box is shown in Figure 8. This box size was revealed as being the best compromise between a cell being large enough for minimizing the volume effects and small enough for an efficient computational

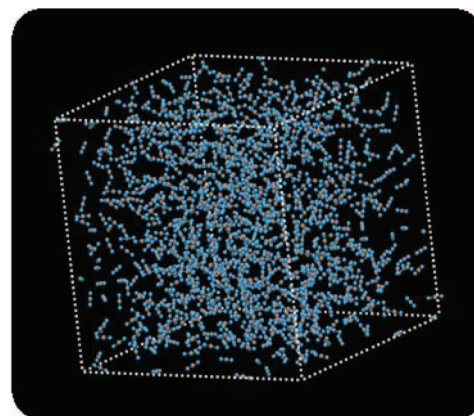


Figure 8. Molecular modeling typical box.

treatment. The simulated boxes were set with periodic boundaries in all directions containing a total of 1000 molecules in the isobaric–isothermal ensemble. The simulations were performed as follows: NPT equilibration and density production runs of 1 200 000 steps. Afterward, NVT equilibration runs for 400 000 steps were followed by NVE viscosity production runs for 1 200 000 steps. All runs were performed with a time step of 1 fs. The L–J and Coulombic cutoff was set to 8 Å with a switching distance of  $r = 12$  Å. The simulations were started with randomly assigned velocities, the total momentum of the system was set to zero, and Newton's equations of motion were solved with the standard velocity–Verlet algorithm. Calculations were performed with the EOS supercalculator of CALMIP. Even if experimental data concerning density and viscosity of CO<sub>2</sub>-expanded MeTHF cannot be found in the literature, the MD method used in this study has been successfully used to predict these properties in other CO<sub>2</sub>-expanded solvents with errors inferior to 5%.<sup>26</sup>

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsuschemeng.7b03018.

General experimental conditions including experiment apparatus and analysis methods; log  $P$  of used neat biobased liquids, hexane, and vinyl acetate; volume expansion of biobased liquids, hexane, and vinyl acetate with CO<sub>2</sub>; normalized absorption spectra of Nile Red in CO<sub>2</sub>-expanded MeTHF at different pressures; effect of the temperature in the absorption wavelength of Nile Red in neat MeTHF; <sup>1</sup>H NMR data and GC/HPLC profiles of synthesized compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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- (44) Reaction conditions: 0.10 mmol of *rac*-1-adamantylethanol **1a**, 0.53 mmol of vinyl acetate, 10 mg of pretreated Novozym 435 in CO<sub>2</sub>-expanded MeTHF (10% v/v MeTHF, 60 bar CO<sub>2</sub>, 7h), 10 mL of MeTHF or 10 mL of CO<sub>2</sub>-expanded MeTHF (10% v/v MeTHF, 60 bar), 293 K, 24 h.
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