catalysts

MDPI

Article

Lipase Immobilized on MCFs as Biocatalysts for Kinetic and Dynamic Kinetic Resolution of *sec*-Alcohols

Dominika Stradomska [1], Monika Heba [2], Aleksandra Czernek [2], Nikodem Kuźnik [2], Danuta Gillner [2,3], Katarzyna Maresz [4], Wojciech Pudło [1], Andrzej Jarzębski [1,4] and Katarzyna Szymańska [1,*]

[1] Department of Chemical Engineering and Process Design, Silesian University of Technology, 44-100 Gliwice, Poland; dominika.stradomska@polsl.pl (D.S.); wojciech.pudlo@polsl.pl (W.P.); andrzej.jarzebski@polsl.pl (A.J.)
[2] Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, 44-100 Gliwice, Poland; monika.heba@polsl.pl (M.H.); aleksandraczernek@wp.pl (A.C.); nikodem.kuznik@polsl.pl (N.K.); danuta.gillner@polsl.pl (D.G.)
[3] Biotechnology Center, Silesian University of Technology, 44-100 Gliwice, Poland
[4] Institute of Chemical Engineering, Polish Academy of Sciences, 44-100 Gliwice, Poland; k.kisz@iich.gliwice.pl
* Correspondence: katarzyna.szymanska@polsl.pl

Abstract: Dynamic kinetic resolution (DKR) is one of the most attractive methods for enantioselective synthesis. In the reported studies, lipase B from *Candida antarctica* (CALB) immobilized on siliceous mesoporous cellular foams (MCF) functionalized with different hydrophobic groups, and two ruthenium complexes with substituted cyclopentadienyl ligands were investigated as catalysts for the chemoenzymatic DKR of (*rac*)-1-phenylethanol, using Novozym 435 as a benchmark biocatalyst. Studies on the (*rac*)-1-phenylethanol transesterification reaction showed that CALB supported on MCFs grafted with methyl groups is a promising biocatalyst and isopropenyl acetate is a preferable acylation agent. Both Ru-complexes activated by K_3PO_4 or *t*-BuOK, proved to be effective catalysts of the racemization reaction. The final DKR experiments using all catalysts combinations singled out, gave 96% conversion, and (*R*)-1-phenylethyl acetate enantiomeric excess of 98% in 8 h using K_3PO_4 activator.

Keywords: chiral secondary alcohols; resolution of enantiomers; dynamic kinetic resolution; DKR; synthesis of enantiomers; bicatalytic tandem

Citation: Stradomska, D.; Heba, M.; Czernek, A.; Kuźnik, N.; Gillner, D.; Maresz, K.; Pudło, W.; Jarzębski, A.; Szymańska, K. Lipase Immobilized on MCFs as Biocatalysts for Kinetic and Dynamic Kinetic Resolution of *sec*-Alcohols. **2021**, *11*, 518. https://doi.org/10.3390/catal11040518

Academic Editors: Karolina Labus and Luciana R. B. Gonçalves

Received: 31 March 2021
Accepted: 19 April 2021
Published: 20 April 2021

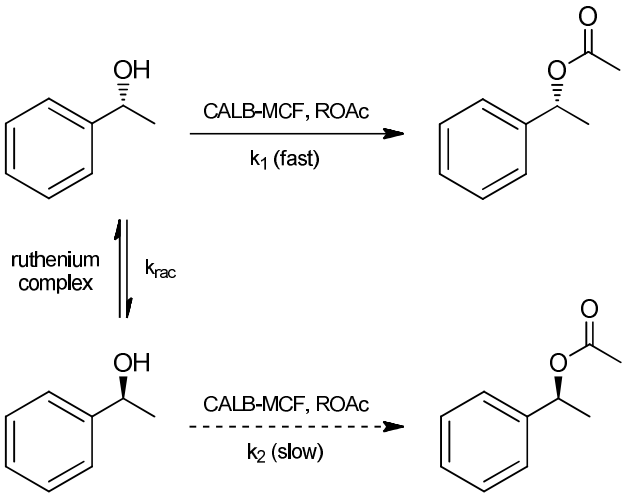
1. Introduction

The augmented awareness of the importance of chirality and its strong association with biological activity, critical especially for pharmaceutical, food and agricultural industries, created an immense need for development of enantiomerically pure compounds at a reduced cost [1]. One of the important group of compounds are chiral *sec*-alcohols, and, in particular, chiral 1-phenylethanol, which is used as an ophthalmic preservative, a solvatochromic dye, an inhibitor of cholesterol absorption, as a mild floral fragrance and as precursor material for the synthesis of enantiomerically pure active pharmaceutical ingredients [2–4]. Consequently, various methods for its preparation have been developed: chromatographic separation using porous coordination polymers, asymmetric bioconversion of acetophenone using whole cells, direct hydrogenation of acetophenone using Ru based Noyori catalyst, chemo- and biocatalytic stereoselective reduction from pro-chiral ketones, or enantioselective resolution of racemates [5–12]. Among several production routes, kinetic resolution (KR), and more recently, its dynamic kinetic mode (DKR) appears to be the most effective, and, thus, widely studied method. In this context, chemoenzymatic dynamic kinetic resolution, which combines the enzyme-catalyzed kinetic resolution (KR) of racemic *sec*-alcohols with the in situ chemocatalytic racemization (Scheme

1), affords the desired product as a single enantiomer in up to 100% yield [5,13,14]. Prominent examples of such DKRs have been demonstrated by Bäckvall et al. [13,15,16] or Kim end Park et al. [17–20]. In an efficient DKR, the main challenge is to select a suitable, i.e., active and stable bio- and chemo-catalyst for the one pot process. In many cases DKR catalysts require specific, often different reaction conditions. Transition-metal complexes are typically employed for the racemization reaction, whereas lipases are used for kinetic resolution [13,15,17]. Lipases, formally triacylglycerol hydrolases (EC 3.1.1.3), belong to the most versatile biocatalysts applied in organic synthesis, either in hydrolysis reactions or ester synthesis (acylation reactions), in which they usually exhibit high enantioselectivity also in organic solvents. Their specific structure features an active center covered by a polypeptide chain (lid), which opens up under favorable conditions. Therefore, due to the hydrophobic surrounding of their active center, lipases can be immobilized in an open conformation. Its so-called interfacial activation on hydrophobic supports has been reported to be a simple and efficient method of purifying and immobilizing lipases [21–23]. The most popular hydrophobic carriers are organic polymers such as poly(methyl methacrylate) (PMMA) which constitutes the core of the Novozym 435 (the most popular commercial lipase-based biocatalyst). However, the major disadvantage of polymer carriers is their low stability in organic solvents, which was shown among others by Zhao, Jose and Kowalczykiewicz [24–26]. For this very reason the search for stable, hydrophobic carriers for lipase is still an important issue [27].

Mesoporous silicates (MS) prepared by templating method, and mesoporous cellular foams (MCF) in particular, demonstrate considerable potential for enzyme immobilization [28–30]. They show very high chemical, thermal, mechanical, and biological resistance, and they are environmentally acceptable. While silica is hydrophilic by nature, its surface can be covered with various anchor groups, e.g., hydrophobic ones, thus changing and even modulating its character.

Here we present the results of studies on the dynamic kinetic resolution of 1-phenylethanol racemic mixture (Scheme 1), with the use of MCFs, functionalized with different hydrophobic groups (methyl, octyl and hexadecyl) as lipase carriers. Such heterogeneous biocatalysts were tested in the kinetic resolution of 1-phenylethanol via transesterification reaction with different types of acyl donors. After selection of the best acylating agent, the lipase immobilized on MCFs was tested in the DKR process using the ruthenium complexes as a racemization catalyst.



Scheme 1. Dynamic kinetic resolution of *sec*-alcohols.

2. Results and Discussion

2.1. Carriers Characterization

An enzyme immobilization is a successful method to improve its properties: resistance to harsh reaction conditions, catalytic stability or reusability (facilitating separation). Enzymes can be immobilized by covalent or non-covalent (adsorption) interaction with support [28]. Lipases (especially lipase B from *Candida antarctica*), due to their specific structure, prefer hydrophobic carriers and non-covalent immobilization method [26,31]. In the presented study, MCFs were applied as carriers for lipase immobilization. Their open three-dimensional structure, made of spherical pores (cells) connected by windows, and large pore sizes provide more favorable conditions for the expression very high enzymatic activity [29,30]. Pristine MCFs feature very high specific surface area (Table 1), densely covered by hydroxyl groups (Figure 1). To modulate its chemical nature towards more hydrophobic one, different hydrophobic groups (methyl, Me; octyl, Oc; hexadecyl, Hd) were incorporated, using either a suitable disilazane (Me) or silanes (Oc and Hd). Note that these two distinct groups of organosilane compounds have substantially different structures, and thus various abilities to condense on and modify the silica surface. These differences were also observed in the properties of thus obtained materials. The two different functional groups load was applied: 1.5 and 3.0 mmol per 1 g of the silica. As expected, the specific surface area (S_{BET}) decreased after modification (Table 1). In the case of MCF-Me samples, the change in S_{BET} was strongly influenced by the concentration of the applied disilazane, in contrast to MCF-Oc and MCF-Hd samples, where only slight decrease in the surface area was observed, despite doubling the concentration of the silane used. In order to estimate the amount of the attached functional groups, thermogravimetric measurements were performed (Table 1, Figures S1–S4). They showed that the hydrophobic groups introduced on the carrier did not exceed 3.5% w/w in the case of disilazane (MCF-3.0Me), and 5–6% *w*/*w* in the case of silanes (MCF-3.0Oc and MCF-3.0Hd). Smaller Me groups of the disilazane precursor, more spread out over the silica surface, tend to explain the former, whereas the larger size of the hexadecyl entities than of the octyl groups is the most likely explanation of the latter. A similar amount of functional groups (3.2–3.4% *w*/*w*) on MCF-1.5Me and MCF-3.0Me, suggest almost complete surface coverage even for a smaller load of methyl groups. This trend is not observed in the other silane modified samples (MCF-Oc and MCF-Hd). Thermogravimetric evaluation of water content in samples (weight lost in the temperature range of 25 to 100 °C) may be useful in determining the surface hydrophobicity. Silica functionalized with Oc- and Hd-groups appeared to adsorb similar amount of water, whereas those functionalized with Me-groups adsorbed three times less (Table 1). This result confirms the hypothesis (*vide supra*) that surface coverage with Me-groups in the MCF-3.0Me sample is much more dense than in samples of the MCF-Oc and MCF-Hd families.

The presence of hydrophobic groups was additionally confirmed by FTIR measurements. Figure 1 shows the FTIR spectra of MCF material in its pristine form and after modification. The unmodified silica featured a broad band between 3200 and 3600 cm^{-1} that could be ascribed to the Si-OH stretching vibrations, additionally, a sharp and intense band at 3740 cm^{-1}, could be unambiguously assigned to the OH symmetric stretching vibration of isolated silanol groups [32]. Adsorption bands around 1000–1250 cm^{-1} and 800 cm^{-1} are attributed to asymmetric and symmetric stretching vibrations of the Si-O-Si framework [33]. After hydrophobization, the intensity of the band at 3740 cm^{-1} was notably reduced, due to incorporation of organic groups. Strong absorption asymmetric stretching bands at 2930–2950 cm^{-1}, and symmetric stretching bands at 2860–2900 cm^{-1} of methylene groups were observed for all samples after hydrophobization [29].

Table 1. Characteristics of biocatalysts.

Carriers	S_{BET} [m²/g]	Amount of Water * [% *w*/*w*]	Amount of Functional Groups * [% *w*/*w*]	Protein Load ** [$mg_{protein}/g_{silica}$]	Immobilization Yield [%]
MCF	560	-	-	-	-
MCF-1.5Me	421	0.90	3.2	18.90 ± 0.08	94.7
MCF-3.0Me	352	0.86	3.4	18.67 ± 0.07	95.0
MCF-1.5Oc	438	2.5	1.3	17.99 ± 0.07	94.7
MCF-3.0Oc	420	2.80	4.9	18.52 ± 0.01	93.4
MCF-1.5Hd	431	2.52	2.0	18.24 ± 0.03	92.6
MCF-3.0Hd	408	2.40	5.8	18.63 ± 0.03	93.7

* Measured by TG; ** Protein content: CALB—12.00 ± 0.28 [$mg_{protein}/g_{enzyme\ preparation}$]; Novozym 435—54.31 ± 1.06 [$mg_{protein}/g_{biocat.}$].

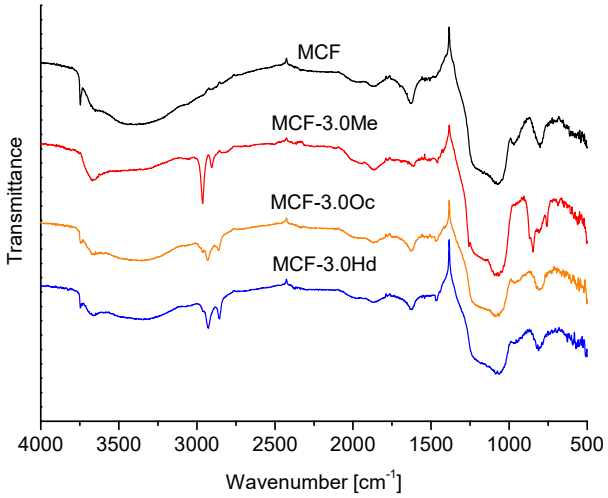


Figure 1. FTIR spectra of the pristine MCF and after grafting with: methyl (Me), octyl (Oc), and hexadecyl (Hd) groups.

2.2. Biocatalysts Characterization

Surprisingly enough, despite the varying degree of silica functionalization all the supports bound a similar amount of lipase, and immobilization yield was ca. 92–95% (Table 1). The latter values prove the extremely high sorption capacity of the functionalized silica. As could be expected, both the amount and type of functional groups significantly influenced the activity of the immobilized lipase (Table 2), which was studied in two types of reactions: hydrolysis of *p*-nitrophenyl acetate (aqueous solution) and in the transesterification of (*rac*)-1-phenylethanol (organic solvent). In both cases, free CALB and Novozym 435 were used as benchmarks. Silica functionalized with methyl groups (MCF-Me) showed the highest activity both in hydrolysis and transesterification reactions. Furthermore, in transesterification reaction, the specific activity of lipase immobilized on MCF-3.0Me was ca. 20 to 37 times higher than that of free CALB and only ca. 20% lower than of Novozym 435 (Table 2). These results clearly indicate, that interfacial activation of the lipase molecule has a critical effect on the expressed catalytic activity and MCF-Me material is an attractive support for lipase. It is noteworthy that activity of the biocatalysts supported on MCF with octyl and hexadecyl groups (with over 2.5% *w*/*w* water content) was lower than that of MCF-Me based catalysts, by ca. 25 and 30–50%, respectively, both in the hydrolysis and transesterification reaction. This indicates a direct correlation between

the activity of the MCF-bound lipase and the hydrophobicity of the silica surface (Table 1). Note that the hydrolytic specific activity of MCF-3.0Me supported lipase was three times higher than in Novozym 435, whereas in organic solvent performance of the latter was slightly better. Again, it can be explained by the solvent–carrier interaction. Polymeric carrier of Novozym 435 features higher affinity to organic solvents, whereas silica to water. However, despite the good affinity of Novozyme 435 for organic solvents, its stability is rather poor [24–27].

Table 2. Biocatalysts performance in hydrolysis of *p*-nitrophenyl acetate (aqueous environment) and in transesterification (TR) of (*rac*)-1-phenylethanol (organic solvent).

Biocatalysts	Hydrolytic Activity [(μmol/min)/$g_{biocat.}$]	Specific Activity in Hydrolysis Reaction [(μmol/min)/$mg_{prot.}$]	TR Activity [(μmol/min)/$g_{biocat.}$]	Specific Activity in TR Reaction [(μmol/min)/$mg_{prot.}$]
CALB	126.38	10.53	4.36	0.36
Novozym 435	81.82	1.51	916.77	16.88
CALB-MCF-1.5Me	45.64	2.42	144.98	7.67
CALB-MCF-3.0Me	82.17	4.36	248.18	13.29
CALB-MCF-1.5Oc	38.95	2.17	99.92	5.55
CALB-MCF-3.0Oc	60.05	3.24	146.94	7.93
CALB-MCF-1.5Hd	36.68	2.01	75.00	4.11
CALB-MCF-3.0Hd	60.44	3.24	173.56	9.32

2.3. Kinetic Resolution of (rac)-1-Phenylethanol

Enzymatic acylation of alcohols can be performed with a broad variety of acyl donors. In appropriate conditions, i.e., in the presence of a suitable acylating agent, one enantiomer of the racemic mixture is selectively transformed to the corresponding ester [34]. However, since esterification is an equilibrium reaction, the desired product cannot be obtained in quantitative yield [35]. The most common solution is to apply an enol ester as the acyl donor [35], which shifts the equilibrium toward the formation of an acylated product. The enol, formed as a by-product, instantly tautomerizes to the keto-form, thus, reaction is irreversible. Here, the experiments were conducted for two types of esters: alkyl (ethyl and isopropyl; low price esters) and enol (vinyl and isopropenyl) acetates, and the reaction was catalyzed by commercial Novozym 435 and by CALB immobilized on silica modified with methyl groups (MCF-3.0Me). As can be seen from Figure 2, for both CALB-MCF-3.0Me and Novozym 435, enol acetates were the most effective acylating agents. For the MCF-based catalyst a significant difference in the efficiency between vinyl and isopropenyl acetate was observed (20 and 38% conversion after 5 h, respectively) (Figure 2A). This difference could be attributed to the effect of the by-product formed. Nucleophilic addition to vinyl acetate releases vinyl alcohol, which is tautomerized to acetaldehyde, while isopropenyl acetate gives acetone in the same way. As Kim et al. reported [36], the sol-gel silica is an excellent adsorbent for the volatile organic compounds (VOC) such as acetaldehyde, but it can hardly be regenerated by simple methods. We believe that this might offer some explanation why the kinetic resolution of 1-phenylethanol with vinyl acetate using CALB-MCF-3.0Me is less efficient than using Novozym 435. It is likely that acetic aldehyde formed during the reaction adsorbs on silica and it affects the enzyme. Additionally, since ethanal may interfere with the metal catalyst [37], which is envisaged as racemization catalyst for the targeted DKR of (*rac*)-1-phenylethanol, isopropenyl acetate was selected as the acyl donor in further experiments.

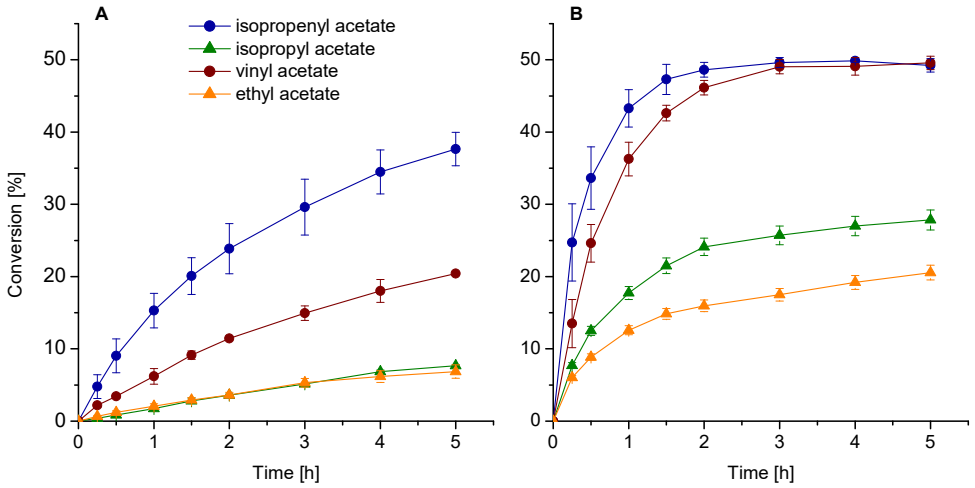
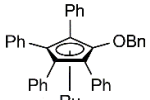
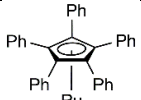


Figure 2. Effect of the acyl donor on the performance of CALB-MCF-3.0Me catalyst (**A**) and Novozym 435 (**B**) in the transesterification of (*rac*)-1-phenylethanol.

2.4. Racemization of (S)-1-Phenylethanol

Prior to investigation of the CALB-MCF-3.0Me performance in the dynamic kinetic resolution of secondary alcohols, studies on the racemization of the slow-reacting 1-phenylethanol enantiomer were carried out. While a number of metal complexes, such as vanadium, iridium, rhodium, and ruthenium proved to catalyze the racemization reaction, only few have successfully cooperated with the enzyme [38]. In the present study we used two most recently developed ruthenium complexes with a substituted cyclopentadienyl ligands (Table 3) [39]. Due to this bulky aromatic ligand, they are stable and neutral to electrophilic or nucleophilic factors and recommended as very efficient isomerization catalysts [40]. Moreover, as these complexes operate at room temperature, and small amounts are needed to carry out the reaction [18], we considered them most suitable for the operation in tandem with the enzyme catalyst in the targeted DKR process. Racemization reactions were performed in the inert atmosphere, at ambient temperature with 4 mol% of the catalyst. To activate the ruthenium complexes, one organic (*t*-BuOK) and two inorganic bases (K_2CO_3 or K_3PO_4) were used. Complete racemization of (*S*)-1-phenylethanol with the ruthenium complex **2** occurred in a much shorter time for all of the tested bases, and *t*-BuOK was the most efficient base for both catalysts, while K_3PO_4 came second (Table 3). The reaction was completed in less than 5 min, in the presence of Ru complex **2** and *t*-BuOK activator (entry 6) and 2 h with inorganic bases, thus proving enormous potentials of this ruthenium complex. Since in the presence of K_2CO_3 worse results were obtained for Ru complex **1**, K_3PO_4 and *t*-BuOK were selected for further research.

Table 3. Racemization of (*S*)-1-phenylethanol by two ruthenium complexes with different activators.

Entry	Catalyst	Base	Base amount [eq]	Time [h]
1	 **1**	K_2CO_3	1.000	48
2		K_3PO_4	1.000	4
3		*t*-BuOK	0.052	1
4	 **2**	K_2CO_3	1.000	2
5		K_3PO_4	1.000	2
6		*t*-BuOK	0.052	<5 min

2.5. DKR of (rac)-1-Phenylethanol

Finally, the activity of CALB-MCF-3.0Me was tested in the DKR of (*rac*)-1-phenylethanol with isopropenyl acetate as acyl donor. To shift the equilibrium toward product formation, the acylating agent was used in a large excess (typically 3 equivalents). Reactions were carried out using both ruthenium complexes and two activating bases - K_3PO_4 and *t*-BuOK. K_3PO_4 played a role of base and desiccant, and thus 2 equivalents were applied. In the reaction performed with *t*-BuOK, Na_2CO_3 (1 equivalent) was utilized as a drying agent. The combination of CALB-MCF-3.0Me and ruthenium complex **2** with K_3PO_4 activator, appeared to be the most efficient among all tested systems. It gave 96% conversion of (*rac*)-1-phenylethanol with almost 100% enantiomeric excess of (*R*)-1-phenylethyl acetate after 8 h (Figure 3). Surprisingly, the system with complex **1** was almost as efficient as that with complex **2**, despite the fact that in the racemization experiments it was much less active (Table 3). After 8 h it gave the conversion of 91%, with 98% enantiomeric excess of the acylated product (*R* enantiomer), and that is also respectable (Figure 3). However, using a bi-catalytic system of complex **2** and Novozym 435, the conversion of (*rac*)-1-phenylethanol of ca. 98% and (*R*)-1-phenylethyl acetate enantiomeric excess of ca. 100% was achieved in ca. 4 h and this is even more attractive (Figure 4). It can be mostly ascribed to over two-fold protein load in Novozym 435 as in CALB-MCF-3.0Me (Table 1). In experiments carried out with *t*-BuOK no racemization was observed, thus the conversions obtained for both complexes did not exceed 50%. Exactly the same was observed for the DKR process carried out using Novozym 435 as biocatalyst (Figure 4). The additional use of Na_2CO_3 had no positive effect, so as the additional distillation of isopropenyl acetate before the reaction. Probably due to traces of water, necessary to maintain the flexible protein structure, and thus its catalytic properties, *t*-BuOK partially decomposed and hydrolyzed to *t*-BuOH and KOH.

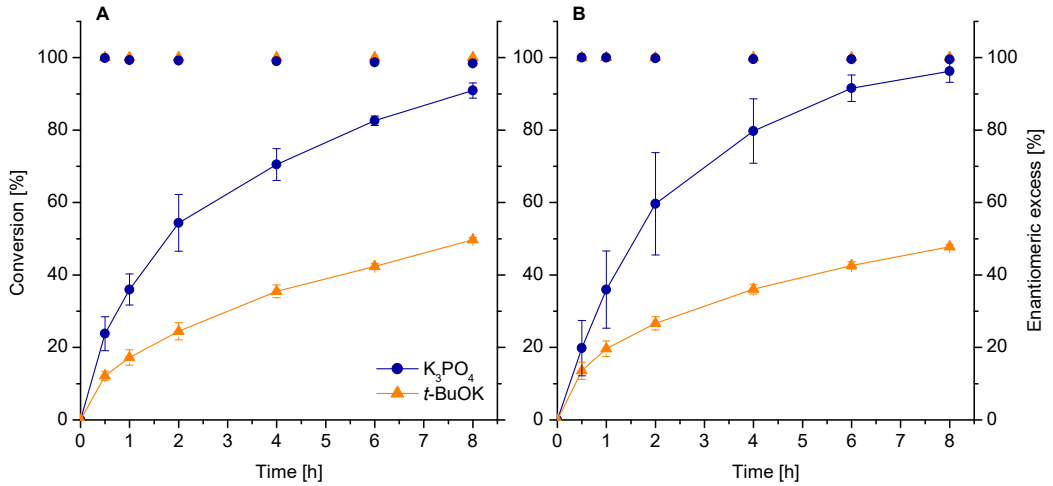


Figure 3. Conversion and enantiomeric excess of the (*R*)-1-phenylethyl acetate *vs* time in the dynamic kinetic resolution of (*rac*)-1-phenylethanol catalyzed by CALB-MCF-3.0Me and ruthenium complexes: **1** (**A**) and **2** (**B**), using two activators (K_3PO_4 and *t*-BuOK).

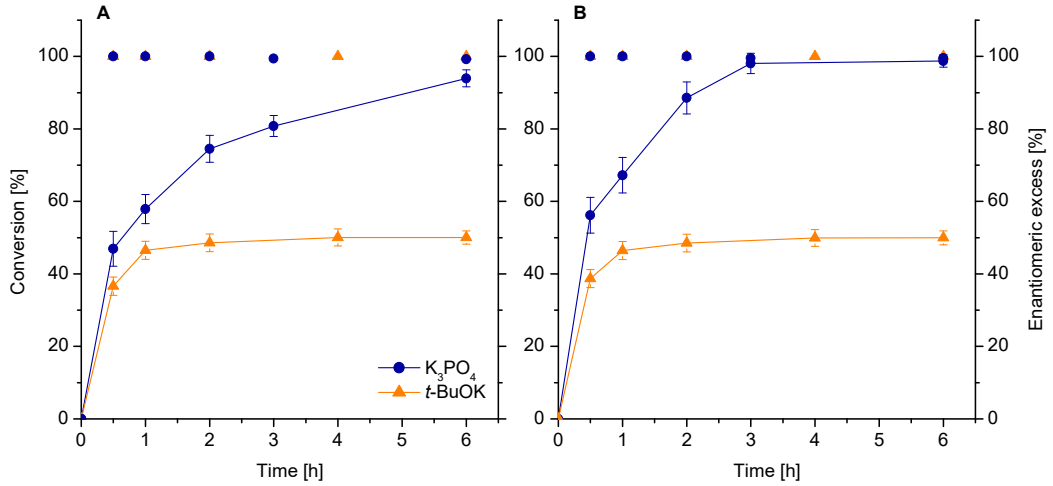


Figure 4. Conversion and enantiomeric excess of the (*R*)-1-phenylethyl acetate *vs* time in the dynamic kinetic resolution of (*rac*)-1-phenylethanol catalyzed by Novozym 435 and ruthenium complexes: **1** (**A**) and **2** (**B**), using two activators (K_3PO_4 and *t*-BuOK).

3. Materials and Methods

3.1. Materials

Isopropenyl acetate +99%, isopropyl acetate +99%, *p*-nitrophenyl acetate 97% (*p*-NPA), (*S*)-1-phenylethanol 99%, potassium *tert*-buthoxide +98%, and anhydrous potassium phosphate 97% were purchased from Acros Organics (Geel, Belgium). *n*-Decane +99% and vinyl acetate 99% (stabilized with hydroquinone) were from Alfa Aesar (Kandel, Germany). Hexamethyldisilazane +99% (Me) was from Fluka Analytical (Munich, Germany), *n*-octyltriethoxysilane (Oc) was from abcr GmbH (Karlsruhe, Germany). Pluronic P123, mesitylene 98%, tetraethyl orthosilicate 98% (TEOS), hexadecyltrimetoxysilane +85% (Hd), lipase B from *Candida antarctica*, Novozym 435, (*rac*)-1-phenylethanol 98%, and ethyl acetate +99.7% were obtained from Sigma–Aldrich (St Louis, MO, USA). Ammonium fluoride was from Avantor Performance Materials (Gliwice, Poland).

3.2. General Synthesis of Ruthenium Complex **1**

Ruthenium complex **1** was synthesize in pursuance of literature [18] however, the synthesis time was reduced to 22 h. ^{1}H NMR (CD_3COCD_3) δ: 7.59–7.64 (m, 4H); 7.00–7.28 (m, 21H), 4.81 (s, 2H, CH_2) ppm. ^{13}C NMR (CD_3COCD_3) δ: 197.5 (CO); 135.9; 132.9; 132.2; 129.9; 129.9; 128.6; 128.5; 128.3; 12.3; 127.8; 102.6; 88.8; 75.3 (CH_2) ppm.

3.3. General Synthesis of Ruthenium Complex **2**

Ruthenium complex **2** was synthesize in pursuance of literature [15] ^{1}H NMR ($CDCl_3$) δ: 7.17–7.20 (m, 5H); 7.08–7.12 (dd, J = 7.10 Hz, 10H); 7.02–7.06 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 196.9; 132.2; 128.4; 127.8; 127.5; 106.5 ppm.

3.4. Synthesis of the MCF Material

The preparation procedure of siliceous MCF was the same as described in the literature [29,30]. In brief Pluronic P123 (4 g) was dissolved in 30 mL of H_2O and 120 mL of 2 M HCl solution at room temperature. Then, NH_4F (0.046 g) and 1,3,5-trimethylbenzene (11.6 mL) were added under vigorous stirring at 40 °C. After 1 h of stirring TEOS was added (9.4 mL), whereupon the mixture was stirred for 1 h, and then stored at 40 °C for 20 h, and then at 100 °C for 24 h. After cooling the precipitate was filtered under vacuum, dried at room temperature and calcinated at 500 °C for 8 h.

3.5. Modification of the MCF Surface

Before grafting of functional groups, MCFs were contacted with water vapor for 5 h, and subsequently dried at 200 °C for 2 h. Hydrophobic groups were grafted onto MCFs surface, to obtain the load of functional moiety of about 1.5 or 3.0 mmol/g of silica, by direct reacting of organosilanes and organodisilazane (Me, Oc, Hd) with hydroxy groups present on the silica surface under reflux (24 h, 85 °C). In particular, 30 mL of the solution containing organosilanes and organodisilazane were stirred under reflux with 1 g of MCF for 24 h, after which the solvent was evaporated.

3.6. Characterisation of the Supports

Nitrogen adsorption/desorption isotherms from the silica materials were obtained using a Micrometrics ASAP 2020 instrument at −196 °C to calculate the specific surface area (S_{BET}), pore volume (V_{pN2}), and their average size (dm). The size of the pores was obtained using the Barret–Joyner–Halenda (BJH) method. The amounts of functional groups and adsorbed water into the functionalized silica matrices were determined by a thermogravimetric method. The measurements were performed using a Mettler Toledo STAR851 thermobalance. Samples of approximately 10–13 mg were heated at a rate of 10 °C/min in a standard 150 μL Pt crucibles under an air flow of 50 mL/min. A Thermo Scientific FTIR Nicolet 6700 spectrometer was used for the spectroscopic studies of functionalized samples. The spectra were recorded in the range of 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}.

3.7. Lipase Immobilization on the Modified MCF Carrier

The functionalized MCFs (0.5 g) were rinsed by centrifugation (9000 rpm, 20 min) with ethanol, distilled water and 0.1 M phosphate buffer (pH 7.0), three times for each solution. Then 16.5 mL of native *Candida antarctica* lipase B solution in 0.1 M phosphate buffer (pH 7.0) was added and incubated with silicas for 2.5 h at room temperature and then overnight at 6 °C. After that they were washed off with 20 mL of 0.1 M phosphate buffer (pH 7.0) to remove protein excess. Finally the biocatalysts obtained were dried at 4 °C under vacuum. Eluates from the immobilization steps were collected and amount of protein was determined by the Lowry Assay. As a standard bovine serum albumin (BSA) was used.

3.8. Protein Desorption from the Surface of Novozym 435

Desorption procedure was performed as described in the literature [41]. To Novozym 435 (0.5 g) 2% (*v*/*v*) Triton X-100 solution in 0.1 M phosphate pH 7 (25 mL) was added and then incubated for 24 h at room temperature.

3.9. Activity Assay

3.9.1. General Procedure for Hydrolytic Activity of the Immobilized Lipase

The hydrolytic activity of biocatalysts was determined using the procedure described by Gustafsson et al. [42]. The substrate solution (5.5 mM *p*-nitrophenyl acetate in acetonitrile, 15 mL) was dissolved in 50 mM phosphate buffer pH 7 (85 mL) and incubated for 10 min at 37 °C. Then the CALB-MCFs or Novozym 435 (5 mg) were added and reaction was carried out for 15 min at rotation speed of 500 rpm. Periodically, during the reaction, 1 mL of the samples were collected (and the catalyst was filtered off) to monitor progress of the reaction. The amount of released *p*-nitrophenol was measured spectrophotometrically at 400 nm and calculated using the calibration curve.

3.9.2. General Procedure for Transesterification of (*rac*)-1-Phenylethanol (Kinetic Resolution of (*rac*)-1-Phenylethanol)

(*rac*)-1-Phenylethanol (0.625 M in toluene, 3 mL with 0.30 mmol of *n*-decane as an internal standard) was added to the flask with CALB-MCF or Novozym 435 (10 mg/mmol). After few minutes acylating agent (1.5 equivalent in toluene, 3 mL) was inserted. The resulting mixture was stirred under ambient conditions for 5 h. Progress of the reaction was monitored by collecting 100 μL samples of the reaction mixture (the catalyst was filtered off) and analyzing by chiral GC.

3.10. General Procedure for Racemization of (S)-1-Phenylethanol

(*S*)-1-Phenylethanol (0.625 M in toluene, 0.4 mL with 0.04 mmol of *n*-decane as an internal standard) was added to a vial containing solution of Ru catalyst (4.0 mol%) and base (5.2 mol% for *t*-BuOK or 1.0 equivalent for K_3PO_4 and K_2CO_3) in toluene (0.4 mL). The reaction mixture was stirred at 25 °C in argon atmosphere. Periodically, during the reaction, 100 μL of the samples were collected (and the catalyst was filtered off) to monitor progress of the reaction and analyzed by chiral GC.

3.11. General Procedure for the Dynamic Kinetic Resolution of (rac)-1-Phenylethanol

(*rac*)-1-Phenylethanol (0.625 M in toluene, 0.4 mL with 0.04 mmol of *n*-decane as an internal standard) was added to a vial with Ru catalyst (4.0 mol%) and K_3PO_4 (2.0 equivalents), previously filled with argon. The resulting mixture was stirred under ambient conditions for 30 min and then CALB-MCF (10 mg/mmol) and isopropenyl acetate (3.0 equivalents in toluene, 0.4 mL) was added. Progress of the reaction was monitored by collecting 100 μL samples of the reaction mixture (the catalyst was filtered off) and analyzing by chiral GC (SI, Figures S5–S7).

4. Conclusions

The lipase B from *Candida antarctica* (CALB) immobilized on siliceous mesoporous cellular foams, finely functionalized with methyl groups using the disilazane precursor, together with two most recently developed ruthenium complexes with substituted cyclopentadienyl ligands, appear to form catalytic tandems very effective in the chemoenzymatic dynamic kinetic resolution of (*rac*)-1-phenylethanol. The best system (with complex **2**) gave 96% conversion and nearly 100% enantiomeric excess of (*R*)-1-phenylethyl acetate in 8 h long one pot process, and the second one (with complex **1**) was nearly as good. However, using Novozym 435 as catalyst of acylation reaction, owing much larger enzyme load, the time of nearly full resolution could be significantly reduced. The proposed technology paves the way to the efficient synthesis of others enantiomers of *sec*-

alcohols in larger scales, as well as to further development of even more effective systems for flow syntheses.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/11/4/518/s1, Figures S1–S3 Thermogravimetric analysis, Figure S4 Process stability of immobilized lipase, Figures S5–S7 GC chromatograms.

Author Contributions: D.S. Data Curation, Formal analysis, Investigation, Methodology, Writing - Original Draft, Visualization; M.H. Data Curation, Formal analysis; A.C. Data Curation; N.K. Writing - Original Draft, Conceptualization, Writing –Supervision; D.G. Writing—Original Draft, Conceptualization, Writing—Supervision; K.M. Formal analysis; W.P. Formal analysis; A.J. Funding acquisition, Writing—Review & Editing, Conceptualization; K.S. Writing—Original Draft, Writing - Review & Editing, Conceptualization, Writing—Supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Science Centre of Poland, grant number 2016/23/B/ST8/00627.

Data Availability Statement: Data is contained within the article.

Conflicts of interest: There is no conflict to declare.

References

1. Ahmed, M.; Kelly, T.; Ghanem, A. Applications of enzymatic and non-enzymatic methods to access enantiomerically pure compounds using kinetic resolution and racemisation. *Tetrahedron* **2012**, *68*, 6781–6802, doi:10.1016/j.tet.2012.05.049.
2. Galvão, W.S.; Pinheiro, B.B.; Golçalves, L.R.B.; De Mattos, M.C.; Fonseca, T.S.; Regis, T.; Zampieri, D.; Dos Santos, J.C.S.; Costa, L.S.; Correa, M.A.; et al. Novel nanohybrid biocatalyst: Application in the kinetic resolution of secondary alcohols. *J. Mater. Sci.* **2018**, *53*, 14121–14137, doi:10.1007/s10853-018-2641-5.
3. Vieira, G.A.B.; Araujo, D.M.D.F.; Oliveira, M.D.C.F.D.; Melo, V.M.M.; Lemos, T.L.G.; De Mattos, M.C.; De Gonzalo, G.; Gotor-Fernandez, V.; Gotor, V. Candida tropicalis CE017: A new Brazilian enzymatic source for the bioreduction of aromatic prochiral ketones. *J. Braz. Chem. Soc.* **2010**, *21*, 1509–1516, doi:10.1590/s0103-50532010000800015.
4. Cen, Y.; Li, D.; Xu, J.; Wu, Q.-S.; Wu, Q.; Lin, X.-F. Highly Focused Library-Based Engineering of Candida antarctica Lipase B with (S)-Selectivity Towards sec-Alcohols. *Adv. Synth. Catal.* **2019**, *361*, 126–134, doi:10.1002/adsc.201800711.
5. De Almeida, L.A.; Marcondes, T.H.; Milagre, C.D.F.; Milagre, H.M.S. Lipase-oxovanadium heterogeneous catalysis system: A robust protocol for the dynamic kinetic resolution of sec-Alcohols. *ChemCatChem* **2020**, *12*, 2849–2858, doi:10.1002/cctc.202000292.
6. Satska, Y.A.; Komarova, N.P.; Gavrilenko, K.S.; Polunin, R.A.; Manoylenko, O.V.; Kolotilov, S.V. Chromatographic Separation of Racemates of Alcohols Using Porous Coordination Compounds of Zinc and Vanadium (IV) with Lactate and β-Cyclodextrin. *Theor. Exp. Chem.* **2017**, *53*, 204–209, doi:10.1007/s11237-017-9517-7.
7. Chen, B.-S.; De Souza, F.Z.R. Enzymatic synthesis of enantiopure alcohols: Current state and perspectives. *RSC Adv.* **2019**, *9*, 2102–2115, doi:10.1039/c8ra09004a.
8. Shende, V.S.; Singh, P.; Bhanage, B.M. Recent trends in organocatalyzed asymmetric reduction of prochiral ketones. *Catal. Sci. Technol.* **2018**, *8*, 955–969, doi:10.1039/c7cy02409f.
9. Mane, S. Racemic drug resolution: A comprehensive guide. *Anal. Methods* **2016**, *8*, 7567–7586, doi:10.1039/c6ay02015a.
10. Dub, P.A.; Gordon, J.C. The role of the metal-bound N–H functionality in Noyori-type molecular catalysts. *Nat. Rev. Chem.* **2018**, *2*, 396–408, doi:10.1038/s41570-018-0049-z.
11. Atak, G.B.; Bayraktar, E.; Mehmetoglu, Ülkü Optimization of the asymmetric synthesis of (S)-1-phenylethanol using Ispir bean as whole-cell biocatalyst. *Green Process. Synth.* **2019**, *8*, 525–532, doi:10.1515/gps-2019-0021.
12. Sengupta, A.; Sunder, A.V.; Sohoni, S.V.; Wangikar, P.P. The effect of CO2 in enhancing photosynthetic cofactor recycling for alcohol dehydrogenase mediated chiral synthesis in cyanobacteria. *J. Biotechnol.* **2019**, *289*, 1–6, doi:10.1016/j.jbiotec.2018.11.002.
13. Verho, O.; Bäckvall, J.-E. Chemoenzymatic Dynamic Kinetic Resolution: A Powerful Tool for the Preparation of Enantiomerically Pure Alcohols and Amines. *J. Am. Chem. Soc.* **2015**, *137*, 3996–4009, doi:10.1021/jacs.5b01031.
14. Xu, Y.; Wang, M.; Feng, B.; Li, Z.; Li, Y.; Li, H.; Li, H. Dynamic kinetic resolution of aromatic sec-alcohols by using a heterogeneous palladium racemization catalyst and lipase. *Catal. Sci. Technol.* **2017**, *7*, 5838–5842, doi:10.1039/C7CY01954H.
15. Martín-Matute, B.; Edin, M.; Bogár, K.; Kaynak, F.B.; Bäckvall, J.-E. Combined Ruthenium (II) and Lipase Catalysis for Efficient Dynamic Kinetic Resolution of Secondary Alcohols. Insight into the Racemization Mechanism. *J. Am. Chem. Soc.* **2005**, *127*, 8817–8825, doi:10.1021/ja051576x.
16. Martín-Matute, B.; Edin, M.; Bogár, K.; Bäckvall, J.-E. Highly Compatible Metal and Enzyme Catalysts for Efficient Dynamic Kinetic Resolution of Alcohols at Ambient Temperature. *Angew. Chem. Int. Ed.* **2004**, *43*, 6535–6539, doi:10.1002/anie.200461416.

17. Kim, H.; Choi, Y.K.; Lee, J.; Lee, E.; Park, J.; Kim, M.-J. Ionic-Surfactant-Coated Burkholderia cepacia Lipase as a Highly Active and Enantioselective Catalyst for the Dynamic Kinetic Resolution of Secondary Alcohols. *Angew. Chem. Int. Ed.* **2011**, *50*, 10944–10948, doi:10.1002/anie.201104141.
18. Kim, N.; Ko, S.-B.; Kwon, M.S.; Kim, M.-J.; Park, J. Air-Stable Racemization Catalyst for Dynamic Kinetic Resolution of Secondary Alcohols at Room Temperature. *Org. Lett.* **2005**, *7*, 4523–4526, doi:10.1021/ol051889x.
19. Ko, S.-B.; Baburaj, B.; Kim, A.M.-J.; Park, J. Air-Stable Racemization Catalysts for the Dynamic Kinetic Resolution of Secondary Alcohols. *J. Org. Chem.* **2007**, *72*, 6860–6864, doi:10.1021/jo071065o.
20. Kim, C.; Lee, J.; Cho, J.; Oh, Y.; Choi, Y.K.; Choi, E.; Park, J.; Kim, M.-J. Kinetic and Dynamic Kinetic Resolution of Secondary Alcohols with Ionic-Surfactant-Coated Burkholderia cepacia Lipase: Substrate Scope and Enantioselectivity. *J. Org. Chem.* **2013**, *78*, 2571–2578, doi:10.1021/jo3027627.
21. Fernandez-Lorente, G.; Cabrera, Z.; Godoy, C.; Fernandez-Lafuente, R.; Palomo, J.M.; Guisan, J.M. Interfacially activated lipases against hydrophobic supports: Effect of the support nature on the biocatalytic properties. *Process. Biochem.* **2008**, *43*, 1061–1067, doi:10.1016/j.procbio.2008.05.009.
22. Blanco, R.M.; Terreros, P.; Fernández-Pérez, M.; Otero, C.; Díaz-GonzálezG. Functionalization of mesoporous silica for lipase immobilization. *J. Mol. Catal. B Enzym.* **2004**, *30*, 83–93, doi:10.1016/j.molcatb.2004.03.012.
23. Fernández-Lorente, G.; Palomo, J.M.; Cabrera, Z.; Guisán, J.M.; Fernández-Lafuente, R. Specificity enhancement towards hydrophobic substrates by immobilization of lipases by interfacial activation on hydrophobic supports. *Enzym. Microb. Technol.* **2007**, *41*, 565–569, doi:10.1016/j.enzmictec.2007.05.004.
24. José, C.; Bonetto, R.D.; Gambaro, L.A.; Torres, M.D.P.G.; Foresti, M.L.; Ferreira, M.L.; Briand, L.E. Investigation of the causes of deactivation–degradation of the commercial biocatalyst Novozym® 435 in ethanol and ethanol–aqueous media. *J. Mol. Catal. B Enzym.* **2011**, *71*, 95–107, doi:10.1016/j.molcatb.2011.04.004.
25. Zhao, H.; Song, Z. Migration of reactive trace compounds from Novozym® 435 into organic solvents and ionic liquids. *Biochem. Eng. J.* **2010**, *49*, 113–118, doi:10.1016/j.bej.2009.12.004.
26. Kowalczykiewicz, D.; Szymańska, K.; Gillner, D.; Jarzębski, A.B. Rotating bed reactor packed with heterofunctional structured silica-supported lipase. Developing an effective system for the organic solvent and aqueous phase reactions. *Microporous Mesoporous Mater.* **2021**, *312*, 110789, doi:10.1016/j.micromeso.2020.110789.
27. Brito, M.J.P.; Bauer, L.C.; Santos, M.P.F.; Santos, L.S.; Bonomo, R.C.F.; Fontan, R.D.C.I.; Veloso, C.M. Lipase immobilization on activated and functionalized carbon for the aroma ester synthesis. *Microporous Mesoporous Mater.* **2020**, *309*, 110576, doi:10.1016/j.micromeso.2020.110576.
28. Hudson, S.; Cooney, J.; Magner, E. Proteins in Mesoporous Silicates. *Angew. Chem. Int. Ed.* **2008**, *47*, 8582–8594, doi:10.1002/anie.200705238.
29. Szymańska, K.; Bryjak, J.; Mrowiec-Białoń, J.; Jarzebski, A.B. Application and properties of siliceous mesostructured cellular foams as enzymes carriers to obtain efficient biocatalysts. *Microporous Mesoporous Mater.* **2007**, *99*, 167–175, doi:10.1016/j.micromeso.2006.08.035.
30. Jarzębski, A.B.; Szymańska, K.; Bryjak, J.; Mrowiec-Białoń, J. Covalent immobilization of trypsin on to siliceous mesostructured cellular foams to obtain effective biocatalysts. *Catal. Today* **2007**, *124*, 2–10, doi:10.1016/j.cattod.2007.03.023.
31. Drozdz, A.; Chrobok, A.; Baj, S.; Szymańska, K.; Mrowiec-Białoń, J.; Jarzębski, A. The chemo-enzymatic Baeyer–Villiger oxidation of cyclic ketones with an efficient silica-supported lipase as a biocatalyst. *Appl. Catal. A Gen.* **2013**, *467*, 163–170, doi:10.1016/j.apcata.2013.07.009.
32. Karbowiak, T.; Saada, M.-A.; Rigolet, S.; Ballandras, A.; Weber, G.; Bezverkhyy, I.; Soulard, M.; Patarin, J.; Bellat, J.-P. New insights in the formation of silanol defects in silicalite-1 by water intrusion under high pressure. *Phys. Chem. Chem. Phys.* **2010**, *12*, 11454–11466, doi:10.1039/c000931h.
33. Ciemięga, A.; Maresz, K.; Mrowiec-Białoń, J. Meervein-Ponndorf-Vereley reduction of carbonyl compounds in monolithic siliceous microreactors doped with Lewis acid centres. *Appl. Catal. A Gen.* **2018**, *560*, 111–118, doi:10.1016/j.apcata.2018.04.037.
34. Ghanem, A.; Aboul-Enein, H.Y. Application of lipases in kinetic resolution of racemates. *Chirality* **2004**, *17*, 1–15, doi:10.1002/chir.20089.
35. Hanefeld, U. Reagents for (ir)reversible enzymatic acylations. *Org. Biomol. Chem.* **2003**, *1*, 2405–2415, doi:10.1039/b302109b.
36. Kim, M.I.; Seo, B.; Lim, C.-S. Adsorption of acetaldehyde at room temperature in a continuous system using silica synthesized by the sol-gel method. *Korean J. Chem. Eng.* **2017**, *34*, 2773–2779, doi:10.1007/s11814-017-0170-6.
37. Hoyos, P.; Pace, V.; Alcántara, A.R. Dynamic Kinetic ResolutionviaHydrolase-Metal Combo Catalysis in Stereoselective Synthesis of Bioactive Compounds. *Adv. Synth. Catal.* **2012**, *354*, 2585–2611, doi:10.1002/adsc.201200365.
38. Csjernyik, G.; Bogár, K.; Bäckvall, J.-E. New efficient ruthenium catalysts for racemization of alcohols at room temperature. *Tetrahedron Lett.* **2004**, *45*, 6799–6802, doi:10.1016/j.tetlet.2004.07.013.
39. Heba, M.; Stradomska, D.; Szymańska, K.; Jarzębski, A.; Ambroziak, K.; Masternak, M.; Kolanowska, A.; Pudło, W.; Kuźnik, N. Engineering and Performance of Ruthenium Complexes Immobilized on Mesoporous Siliceous Materials as Racemization Catalysts. *Catalysts* **2021**, *11*, 316, doi:10.3390/catal11030316.
40. Crabtree, R.H. *The Organometallic Chemistry of the Transition Metals*, 2nd ed.; John Wiley & Sons: New York, NY, USA, 1994; p. 487.

41. Cabrera, Z.; Fernandez-Lorente, G.; Fernandez-Lafuente, R.; Palomo, J.M.; Guisan, J.M. Novozym 435 displays very different selectivity compared to lipase from Candida antarctica B adsorbed on other hydrophobic supports. *J. Mol. Catal. B Enzym.* **2009**, *57*, 171–176, doi:10.1016/j.molcatb.2008.08.012.
42. Gustafsson, H.; Thörn, C.; Holmberg, K. A comparison of lipase and trypsin encapsulated in mesoporous materials with varying pore sizes and pH conditions. *Colloids Surf. B Biointerfaces* **2011**, *87*, 464–471, doi:10.1016/j.colsurfb.2011.06.012.