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Kinetic Resolution of *rac*-1-Phenylethanol with Immobilized Lipases: A Critical Comparison of Microwave and Conventional Heating Protocols[||]

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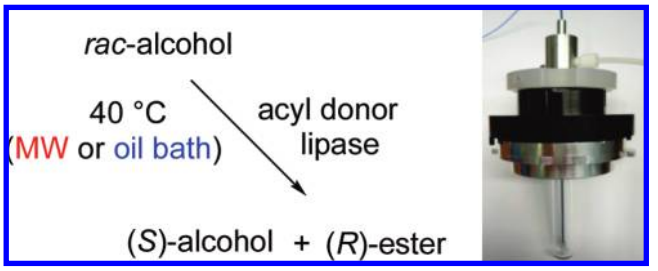

The lipase-catalyzed kinetic resolution of *rac*-1-phenylethanol with vinyl acetate as acyl donor and cyclohexane as solvent has been investigated applying both microwave dielectric heating and conventional thermal heating in order to probe the existence of nonthermal microwave effects. All transformations were conducted at 40 °C in a dedicated reactor setup that allowed accurate internal reaction temperature measurements with use of fiber-optic probes. Quartz reaction vessels that allow higher levels of microwave power to be administered to the reaction mixture were used for all experiments. For all five studied immobilized lipases, the observed reactivities and enantioselectivities in microwave and oil bath experiments were identical and thus not related to the presence of the microwave field. The effect of magnetic stirring proved critical as too rapid stirring in some instances destroyed the enzyme support structure and led to altered reactivities and selectivities.

Introduction

Biocatalysis with enzymes is an attractive alternative to other catalytic methods (for example metal- or organocatalysis) for effecting chemical transformations.[1] In general, high levels of chemo-, regio-, and enantioselectivities can be achieved due to highly selective recognition of the substrate by the enzyme.[1] Among the many enzyme classes used as biocatalysts for synthetic organic transformations, lipases (triacyl glycerol hydrolases EC 3.1.1.3) are among the most useful enzymes applied in both aqueous and nonaqueous media.[2] Lipases possess wide substrate specificity, have an excellent ability to recognize chirality, and do not require labile cofactors. The kinetic resolution of secondary alcohols or their carboxylates either via esterification or hydrolysis has been extensively studied with a variety of different lipases, where the enzyme discriminates between the two enantiomers present in the racemic mixture.[2]

[||] Dedicated to the memory of Professor Octavio A. C. Antunes.

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In the field of synthetic organic chemistry, microwave dielectric heating for the past two decades has provided a powerful method to enhance chemical processes and to provide, in many cases, improved yields and cleaner reaction profiles in significantly shorter reaction times.[3] More recently, microwave heating has also been successfully applied in the biosciences field, including areas such as peptide synthesis, protein digestion (proteomics), or DNA amplification by polymerase chain reaction (PCR).[4] As far as synthetic enzymatic transformations are concerned, a significant number of reports over the past few years have described microwave-assisted, mostly lipase-catalyzed esterifications, transesterifications, or hydrolysis reactions.[5−12] In most instances, the published results suggest that microwave irradiation can have an influence on enzyme stability and activity,[5−7] in addition to altering/enhancing reaction rates[5−10] and/or enantioselectivities.[5,11,12] In the majority of these publications so-called nonthermal microwave effects[13,14] have been invoked to rationalize the observed effects since control experiments with conventional heating in a similar temperature range often led to different reaction rates or selectivities.[5−12] In fact, a 2008 publication has presented evidence that microwave irradiation can induce changes in the tertiary structure/conformation of a hyperthermophilic enzyme not related to a macroscopic temperature change, which resulted in high biocatalytic hydrolysis rates at bulk solution temperatures far below the thermal optimum of the enzyme.[10,15] On the other hand, a recently conducted careful analysis of lipase-catalyzed transesterification reactions led to the conclusion that, in contrast to previous literature reports, no differences between microwave heating and conventional heating existed.[16]

Prompted by the ongoing and controversial debate on the involvement of nonthermal microwave effects in biocatalytic transformations,[5] we herewith describe a critical comparison of microwave and conventionally heated kinetic resolutions of a racemic secondary alcohol using immobilized lipases in nonaqueous media. The kinetic resolution of a secondary alcohol appears to be an ideal probe for investigating nonthermal microwave effects in enzymatic transformations, since apart from the standard parameter "conversion" that is typically monitored in comparing conventional heating and microwave heating experiments, the "enantioselectivity" parameter can also be studied here.[17] The enantioselectivity for a lipase-catalyzed kinetic resolution would be expected to be rather sensitive to temperature and therefore a good probe to distinguish between thermal and nonthermal microwave effects. Previous investigations of lipase-catalyzed kinetic resolutions of secondary alcohols with microwave irradiation have in several cases demonstrated significant enhancements in both rate and/or enantioselectivity compared to the results obtained applying conventional heating.[11] To distinguish between thermal and nonthermal microwave effects in these reactions we herewith present a critical evaluation of lipase-catalyzed kinetic resolutions of a secondary alcohol using accurate and fast responding internal fiber-optic temperature probes.[14,17,18]

Results and Discussion

General Considerations. As a simple and representative model system for our planned comparison studies between microwave and conventionally heated kinetic resolutions using secondary alcohols and lipases we have chosen *rac*-1-phenylethanol (**1**) as the alcohol component and vinyl acetate (**2**) as the acyl donor (Scheme 1). This system has

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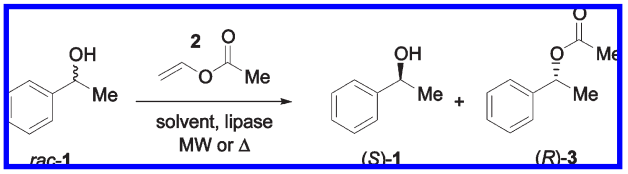
SCHEME 1



been studied numerous times in the literature and the parameters effecting the kinetic resolution of this alcohol by lipases are well understood.[19] As far as the enzyme source is concerned we have selected a variety of immobilized lipases (rather than working with the free enzymes) in our screening campaigns since immobilization of the enzyme will generally improve thermal stability and thus make the enzymes more suitable for transformations at higher temperatures. Since it is known that the properties of the immobilized enzyme strongly depend on the microenvironment of the support surface, a selection of lipases immobilized on different supports was chosen.

Initially, the following lipases were selected: Novozym 435 (*candida antartica* lipase B, CAL-B, immobilized on a macroporous polyacrylate resin); Amano Lipase PS-C I (*Pseudomonas cepacia* lipase immobilized on a ceramic support); and Amano Lipase AK (*Pseudomonas fluorescens* lipase, PFL lipase). All of the above immobilized lipase preparations can be considered as thermostable, having their optimum range of activity between 55 and 80 °C,[20] and therefore appear well suited for the planned microwave irradiation studies.

Reaction Optimization. We began our study with the optimization of reaction conditions for the kinetic resolution of *rac*-1-phenylethanol (**1**) catalyzed by the immobilized lipases Novozym 435, Amano Lipase PS-C I, and Amano Lipase AK using conventional heating. At this point, the transesterification reactions (Scheme 1) were performed in parallel directly in standard sealed HPLC/GC autosampler vials equipped with small stir bars. The vials were fitted inside the 20 wells (5 × 4 matrix) of a silicon carbide reaction block, placed on a standard hot plate (Figure S1, Supporting Information).[21] Because of the high thermal conductivity of silicon carbide,[22] rapid and homogeneous heating of the reaction mixtures contained inside the HPLC/GC vials to the selected target temperatures was generally achieved within less than 2 min (Figure S2, Supporting Information). The outcome of the kinetic resolutions was established by directly monitoring conversion by GC-FID and enantiomeric excess of the substrate (*S*)-**1** and the product (*R*)-**3** by chiral HPLC. The enantiopreference for all lipases was always as displayed in Scheme 1 and the correct assignment of absolute configuration for alcohol (*S*)-**1** was confirmed by comparison with an authentic sample.[19,23]

Several parameters were initially evaluated for the optimization of the reaction conditions, including solvent, reaction temperature, enzyme source, and acyl donor concentration. Clearly, the proper choice of solvent is important in lipase-catalyzed kinetic resolutions, as the solvent can influence reaction rates and the enantioselectivity for a given reaction. As expected,[24] unpolar solvents such as hexane or cyclohexane provided the highest activity in kinetic resolutions of alcohol *rac*-**1** (Scheme 1) in test runs performed at 70 °C for 2 h, using 2 equiv of acyl donor **2**, 10% (w/w, based on substrate) enzyme loading, and a substrate concentration of 0.11 M (Figure S3, Supporting Information). In a subsequent experiment, the effect of the acyl donor concentration on the conversion was evaluated. Utilizing Amano Lipase PS-C I in cyclohexane as a model system and the conditions detailed above (70 °C, 2 h, 10% w/w enzyme loading) the optimum concentration of the acyl donor was found to be 2 equiv. Neither lower (1 equiv) nor higher concentrations (3 and 5 equiv) of vinyl acetate led to an improvement of the transesterification rate (Figure S4, Supporting Information).

Of most interest for our planned comparison studies between microwave and conventional heating was the influence of reaction temperature on enzyme activity (reaction rate) and enantioselectivity. On the basis of the initial optimization studies described above, a series of experiments was performed with 10% (w/w) Novozym 435 and Amano Lipase PS-C I in cyclohexane with 2 equiv of the acyl donor in a temperature range of 25−70 °C. For Amano Lipase AK results with 10% enzyme loading were unsatisfactory and therefore 100% (w/w) enzyme loading was used. Table 1 summarizes the conversions and selectivities obtained after 2 h of reaction time. The superiority of Novozym 435 over the other enzymes in terms of reaction rate and selectivity was clearly evident. Even at room temperature, high conversions and excellent selectivities (*E*) were obtained. Increasing the temperature to 70 °C did not significantly change conversion or selectivities, confirming the good thermal stability of this enzyme. In contrast, Amano Lipase PS-C I exhibited a somewhat lower conversion in the range from 25 to 50 °C, but showed perfect conversion at 60 and 70 °C. For Amano Lipase AK only a moderate increase in the reactivity level was obtained, going from 25 to 70 °C (Table 1).

In addition to the experiments described above, the recyclability of the immobilized enzymes was investigated. For both Novozym 435 and Amano Lipase PS-C I reusability of the enzyme was excellent. After 10 cycles at 70 °C under the conditions described in Table 1, both the recorded activity and selectivity levels remained excellent, with only a minor decrease seen after the eighth cycle (Figures S5 and S6, Supporting Information).

Microwave versus Oil-Bath Heating. To accurately compare the results obtained by direct microwave heating with the outcome of a conventionally heated reaction we have used a reactor system that allows us to perform both types of transformations *in the identical reaction vessel* and to

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TABLE 1. Temperature Dependence of the Kinetic Resolution of *rac*-1-Phenylethanol by Different Lipases in Cyclohexane (Scheme 1)[a]

entry	enzyme	temp (°C)	conv (%)[b]	ee_S (%) (*S*-**1**)[c]	ee_P (%) (*R*-**3**)[c]	E[d]
1	Novozym 435	25	51	98	99	>200
2	Amano PS-C I	25	31	51	98	164
3	Amano AK	25	7	97	97	>200
4	Novozym 435	40	54	97	98	>200
5	Amano PS-C I	40	33	53	99	>200
6	Amano AK	40	23	95	97	>200
7	Novozym 435	50	48	99	98	>200
8	Amano PS-C I	50	31	55	97	114
9	Amano AK	50	23	98	97	>200
10	Novozym 435	60	51	98	98	>200
11	Amano PS-C I	60	50	97	99	>200
12	Amano AK	60	18	97	97	>200
13	Novozym 435	70	52	97	99	>200
14	Amano PS-C I	70	52	97	97	>200
15	Amano AK	70	30	97	98	>200

[a] *rac*-1-Phenylethanol (**1**) (20 mg, 0.16 mmol), vinyl acetate (**2**) (27.5 mg, 0.32 mmol, 2 equiv), and 2 mg (10% w/w) of the corresponding immobilized enzyme (100% for Amano AK) were reacted in cyclohexane (1.5 mL) for 2 h. For further details, see the Experimental Section. [b] Based on GC analysis (HP-5MS column). [c] Determined by chiral HPLC analysis (chiral column OD-H) employing heptane:2-propanol (95:5) as the mobile phase at 0.5 mL/min (254 nm). [d] Selectivity was calculated as $E = f(ee_S,ee_P)$ according to ref 25.

monitor the internal reaction temperature in both experiments directly with a fiber-optic probe device.[17] Similar to the setup first described by Maes and co-workers[26] we have used a CEM Discover single mode microwave reactor equipped with a fiber-optic probe for directly monitoring the internal reaction temperature in a 10 mL sealed reaction vessel made either out of Pyrex or fully microwave transparent quartz glass.[17] This setup can be immersed either into the cavity of the microwave reactor or into a preheated and temperature equilibrated oil bath placed on a magnetic stirrer (Figure S7, Supporting Information). In both cases, the software of the microwave instrument is recording the internal temperature and similar heating profiles can be obtained (see below). This system has the advantage that the same reaction vessel and the same method of temperature measurement is used. In this way all parameters apart from the mode of heating are identical and therefore a fair comparison between microwave heating and thermal heating can be made.

Since the chosen optimum solvent for the lipase-catalyzed kinetic resolution of alcohol *rac*-**1** was microwave-transparent cyclohexane ($\tan\delta < 0.01$),[27] the use of quartz reaction vessels for all microwave experiments was mandatory. On the basis of our previous experience with low absorbing solvents under microwave conditions, we recognized that the standard Pyrex reaction vessels are not truly microwave transparent and will absorb a significant amount of microwave energy, thereby acting as "passive heating elements".[22] For the planned microwave-assisted kinetic resolutions in cyclohexane this was readily demonstrated by comparing the heating and power profiles of a typical reaction mixture (1.5 mL of cyclohexane, 20 mg of *rac*-**1**, 27.5 mg of **2**, and 2 mg of Novozym 435) in a standard microwave Pyrex vessel ($\tan\delta = 10 \times 10^{-4}$)[28] and in a custom-made quartz vessel ($\tan\delta = 0.6 \times 10^{-4}$)[28] of exactly the same geometry. In the case of the Pyrex vessel experiment, the temperature of the reaction mixture measured by the fiber-optic probe sensor was rapidly raised to 70 °C within 2 min. In contrast, the same experiment in the quartz vessel required 45 min, and even using the full 300 W maximum nominal magnetron output power of the microwave reactor, the internal reaction temperature could not be raised above 70 °C (Figure S8, Supporting Information). Apparently, the comparatively small amounts of polar reagents **1** and **2** contained in the reaction medium do not influence the overall microwave absorptivity of the reaction mixture enough to allow efficient heating by microwave dielectric heating effects.[22,29] Heating of the reaction mixture in the Pyrex vial thus undoubtedly occurs mostly by conductive heating via the hot glass surface from the self-absorbing reaction vessel, not unlike in a conventional oil bath experiment. In contrast, the use of a quartz reaction vessel ensures that most of the microwave irradiation will be able to directly interact with the chemistry inside the reaction vessel, thereby allowing the potential observation of nonthermal microwave effects.

On the basis of the observations made above, the comparison experiments between oil bath and microwave heating for the kinetic resolution of *rac*-1-phenylethanol (**1**) with vinyl acetate (**2**) as acyl donor (Scheme 1) were therefore performed in a quartz reaction vial with cyclohexane as solvent at a fixed temperature of 40 °C measured internally by a fiber-optic probe. The comparatively low reaction temperature selected for these studies ensured that thermal effects could be separated from nonthermal microwave effects, since although the temperature was relatively low, a high magnetron output power still had to be applied in all microwave experiments in order to reach 40 °C (see above). Our initial model system involved Novozym 435 as immobilized lipase keeping the other reaction parameters essentially as described in Table 1. To avoid weighing/pipetting errors, stock solutions of *rac*-1-phenylethanol (**1**) and

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(27) The ability of a specific solvent to convert microwave energy into heat at a given frequency and temperature is determined by the so-called loss tangent ($\tan\delta$) expressed as the following quotient, $\tan\delta = \varepsilon''/\varepsilon'$. A reaction medium with a high $\tan\delta$ at the standard operating frequency of a microwave synthesis reactor (2.45 GHz) is required for good absorption and, consequently, for efficient heating. Solvents used for microwave synthesis can be classified as high ($\tan\delta > 0.5$), medium ($\tan\delta$ 0.1−0.5), and low microwave absorbing ($\tan\delta < 0.1$). See the following references for further details: (a) Gabriel, C.; Gabriel, S.; Grant, E. H.; Halstead, B. S.; Mingos, D. M. P. *Chem. Soc. Rev.* **1998**, *27*, 213. (b) Mingos, D. M. P.; Baghurst, D. R. *Chem. Soc. Rev.* **1991**, *20*, 1.

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TABLE 2. Comparison of Microwave and Conventional Heating in the Kinetic Resolution of *rac*-1-Phenylethanol by Different Lipases in Cyclohexane (Scheme 1, 40 °C, 2 h)[a]

entry	enzyme/loading (%)	heating method[b]	conv (%)[c]	ee_s (%) (*S*-**1**)[d]	ee_P (%) (*R*-**3**)[d]	*E*[e]
1	Novozym 435/10	MW (200 W)	50	97	98	>200
2	Novozym 435/10	oil bath	50	97	99	>200
3	Amano PS C I/10	MW (200 W)	35	51	97	109
4	Amano PS C I/10	oil bath	33	53	98	168
5	Amano AK/100	MW (200 W)	41	56	97	115
6	Amano AK/100	oil bath	38	51	96	81
7	Lipozyme TL IM/100	MW (200 W)	23	33	84	15
8	Lipozyme TL IM/100	oil bath	25	30	83	14
9	Lipozyme RL IM/100	MW (200 W)	11	25	79	10
10	Lipozyme RL IM/100	oil bath	14	25	81	12

[a] *rac*-1-Phenylethanol (**1**) (20 mg, 0.16 mmol), vinyl acetate (**2**) (27.5 g, 0.32 mmol, 2 equiv), and 10−100% (w/w) of the corresponding immobilized enzyme were reacted in cyclohexane (1.5 mL) for 2 h. For further details, see the Experimental Section. All experiments have been performed three times and the given values reflect median values. [b] Both microwave and oil bath experiments were performed in the same 10 mL quartz reaction vessel with magnetic stirring at 100 rpm (oil bath) or by using the setting "low" (MW), respectively. [c] On the basis of GC analysis (HP-5MS column). [d] Determined by chiral HPLC analysis (Chiralcel OD-H) employing heptane:2-propanol (95:5) as the mobile phase at 0.5 mL/min (254 nm). [e] Selectivity was calculated as $E = f(ee_S, ee_P)$ according to ref 25.

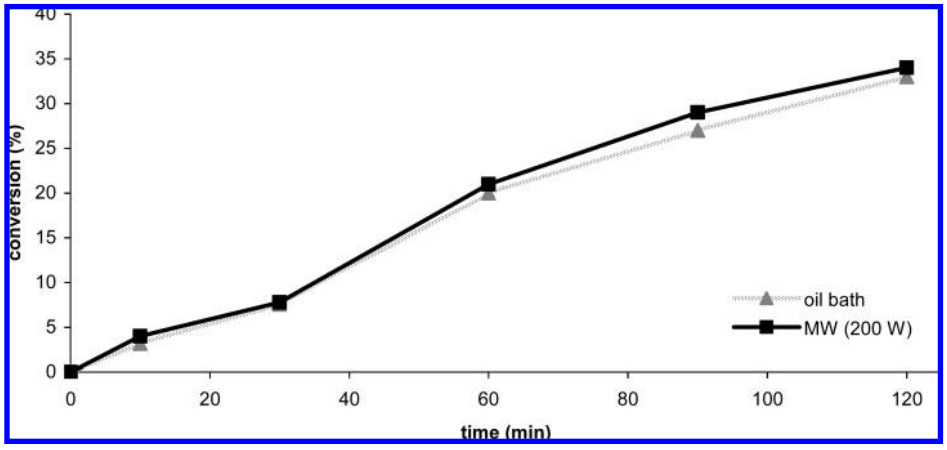

FIGURE 1. Comparison of microwave and conventional heating for the kinetic resolution of *rac*-1-phenylethanol by Amano PS-C I lipase in cyclohexane at 40 °C (Scheme 1, Table 2).

vinyl acetate (**2**) in cyclohexane were employed in both the microwave and oil bath runs. In the microwave experiments a maximum nominal magnetron output power of 200 W was selected so that the heating profiles obtained in the oil bath mimicked the heating rates achieved by dielectric effects in the microwave reactor (Figure S9, Supporting Information). Given the comparatively long reaction times of 2 h the initial heating rate in these transformations was, however, less important and similar results were achieved with 50 or 100 W of initial magnetron power (data not shown).[30]

In the experiments with Novozym 435 at 40 °C an apparently significant nonthermal microwave effect was initially observed, comparing the activities and selectivities achieved in the oil bath with the activities/selectivities obtained under the influence of microwave irradiation at the same temperature after 2 h. These experiments were repeated a number of times and seemed to indicate that both the activity (conversion) and the selectivity of the enzyme in all cases was significantly lower in the microwave experiment as compared to the oil bath runs. After considerable experimentation and variations of conditions we realized that the stirring speed in the microwave reactor (set on "high") did not match the stirring speed used in the oil bath experiments (100 rpm).[31] Adjusting the stirring speed in the microwave instrument to "low", the obtained activity and selectivity much closer resembled the data seen with conventional heating (Table 2, entries 1 and 2). This effect is clearly related to the destruction of the immobilized enzyme support (polyacrylate resin) by the frictional forces exerted by the magnetic stir bar and the quartz vessel if too rapid stirring is performed, and can be evidenced by investigating the immobilized enzymes after treatment (Figure S10, Supporting Information).[32]

To additionally support the notion that the stirring speed has a major role on this enzymatic reaction we have performed a control experiment at room temperature (no microwave power applied) in the microwave reactor involving Novozym 435 and *rac*-1-phenylethanol (**1**) under otherwise identical reaction conditions (Table 2, entry 1). In agreement with the observations discussed above, there was a significant effect of the stirring speed on the activities/selectivities obtained in these transformations, with

(30) Glasnov, T. N.; Findening, S.; Kappe, C. O. *Chem.—Eur. J.* **2009**, *15*, 1001.

(31) Three different stirring levels can be set on the CEM Discover microwave reactor (low, medium, high). The correlation with actual stirring speeds in rpm are not disclosed by the instrument vendor.

(32) Kvittingen, L.; Sjursnes, B.; Halling, P.; Anthonsen, T. *Tetrahedron* **1992**, *48*, 5259.

higher stirring speeds leading to reduced activities/selectivities (see Table S1 in the Supporting Information).

Because of the fact that Novozym 435 displays near perfect selectivities for the investigated kinetic resolution displayed in Scheme 1 over a wide temperature range (Table 1), this enzymatic system is apparently not well suited for a study on nonthermal microwave effects. We have therefore looked at Amano Lipase PS-C I where our initial temperature screen has revealed a comparatively lower selectivity at 40 °C bulk temperature in addition to a temperature dependence on reactivity (Table 1). Under the conditions used for the comparison of microwave and oil bath heating in the 10 mL quartz tube, an excellent agreement between the conversion and selectivity values obtained via the two heating modes was obtained. All experiments were repeated three times each with nearly identical results being obtained in all cases (Table 2, entries 3 and 4). In addition, the degree of conversion was monitored after different time intervals and also here no significant difference between microwave and oil bath heating to 40 °C was seen (Figure 1). Interestingly, the stirring speed with Amano Lipase PS-C I is less critical, as the ceramic support is apparently more resistant to degradation. Even with use of the 400 rpm stirring speed, the results were still the same as with low stirring (data not shown)

Finally, we wanted to evaluate if microwave irradiation could perhaps improve the performance of lipases that display very poor selectivity in kinetic resolutions. Apart from Amano Lipase AK (see above) we have additionally performed experiments with Lipozyme TL IM (*Thermomyces lanuginosus* lipase immobilized on porous silica) and Lipozyme RM IM (*Mucor miehei* lipase immobilized on anionic resin). In all three cases, the observed conversions and selectivities at 40 °C with conventional heating were extremely low. However, no changes in both activity and selectivity of the immobilized lipases under the influence of microwave irradiation was observed.

Conclusions

In summary, we have conducted a detailed investigation on the existence of nonthermal microwave effects in the kinetic resolution of a secondary alcohol with five different immobilized lipases. For all the studied lipases and experimental conditions we have shown that no difference between heating in an oil bath or heating by microwave irradiation exist. The observed reactivities and selectivities in all cases were −within experimental error−identical, clearly demonstrating the absence of any nonthermal microwave effects in these enzymatic transformations. This is despite the fact that the reaction mixtures containing the enzymes were exposed to significant amounts of microwave power for an extended time period (100−200 W for 2 h). Of critical importance for our work was the use of microwave transparent quartz reaction vessels. Only by using quartz vessels did it prove possible to deliver a substantial amount of microwave irradiation to the reaction mixture. Utilizing standard Pyrex glass, most of the applied microwave energy was consumed by heating the vessel material and not the reaction mixture.

As an additional critical factor in these experimental setups involving immobilized enzymes we have identified the stirring speed. Apparently, some enzyme preparations (for example Novozym 435) do not withstand intensive stirring with a magnetic stir bar as already previously described. Choosing a too high stirring speed will lead to a destruction of the immobilized enzyme support by the frictional forces exerted by the magnetic stir bar and the vessel. These changes in the enzyme support structure may lead to apparent altered reactivity/selectivity levels and can therefore mimic microwave effects if different stirring speeds in the oil bath and microwave experiments were selected. We would like to emphasize that performing meaningful comparison experiments between conventional and microwave heated transformations must therefore not only carefully consider accurate internal temperature measurements and identical vessel geometries, but also the stirring speed.

Experimental Section

Microwave Irradiation Experiments. Microwave irradiation experiments were performed with a single-mode Discover System from CEM Corporation,[17] using either custom-made high purity quartz or standard Pyrex vessels (capacity 10 mL). The temperature profiles for microwave and oil bath experiments were recorded with use of a fiber-optic probe protected by a sapphire immersion well inserted directly into the reaction mixture (Figure S7 in the Supporting Information).

Parallel Screening of Enzyme Reactivity and Selectivity for the Kinetic Resolution of *rac*-1-Phenylethanol by Different Lipases with Conventional Heating (Table 1). The 1.5 mL stock solution of *rac*-1-phenylethanol (**1**) (20 mg, 0.16 mmol) and vinyl acetate (**2**) (27.5 g, 0.32 mmol, 2 equiv) in cyclohexane was charged with 10−100% w/w of the immobilized lipases in standard sealed HPLC/GC autosampler vials equipped with small stir bars. The vials were fitted inside the 20 wells (5 × 4 matrix) of a silicon carbide reaction block[21] and placed on a standard hot plate (Figure S1, Supporting Information). The mixture was subsequently heated applying the appropriate temperature (Table 1). Samples (0.1 mL) were taken from the reaction vessel to monitor the conversion of the reaction (GC-FID). After completion of the reaction, the enzyme was removed by filtration and washed five times with cyclohexane and placed to dry under ambient temperature. The crude reaction mixture was subjected to HPLC analysis with use of a chiral column (Table 1).

Microwave and Conventional Heating in the Kinetic Resolution of *rac*-1-Phenylethanol by Different Lipases in Cyclohexane (Table 2). To a 1.5 mL stock solution of *rac*-1-phenylethanol (**1**) (20 mg, 0.16 mmol) and vinyl acetate (**2**) (27.5 g, 0.32 mmol, 2 equiv) in cyclohexane were added the appropriate immobilized lipases (10−100% w/w). The mixture was subsequently heated with stirring in a 10 mL microwave process vial (Figure S7, Supporting Information) for 2 h applying the appropriate mode of heating and stirring (Table 2). Samples (0.1 mL) were taken from the reaction vessel to monitor the conversion of the reaction (GC-FID). After the reaction was completed, the enzyme was removed by filtration, washed five times with cyclohexane, and dried under ambient temperature. The crude reaction mixture was subjected to HPLC analysis with use of a chiral column (Table 2).

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Supporting Information Available: Temperature/power profiles and pictures of equipment used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.