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Microwave Irradiated Immobilized Lipase Catalyzed Synthesis of Alkyl Benzoate Esters by Transesterification: Mechanism and Kinetic Modeling

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ABSTRACT: Alkyl benzoate esters have gained importance due to their application in a variety of industries such as flavor, cosmetics, and pharmaceuticals. Effect of microwave irradiation in the enzymatic transesterification of methyl benzoate with different alcohols, viz., *n*-butanol, *n*-pentanol, *n*-hexanol, *n*-octanol, benzyl alcohol, isoamyl alcohol, and 2-ethyl-1-hexanol, was investigated. Synthesis of *n*-hexyl benzoate was chosen as the model reaction. Different enzymes such as Novozym 435, Lipozyme TL IM, Lipozyme RM IM, and Lipase AYS Amano were screened under microwave irradiation. Novozym 435 was the most active catalyst. To establish the kinetics and mechanism for Novozym 435 catalyzed transesterification of methyl benzoate with *n*-hexanol, the effects of various parameters affecting the conversion and rate of reaction were studied. Under microwave synergism, an increase in initial rates up to 6.5-fold was observed. Twenty millimoles of methyl benzoate and 10 mmol of *n*-hexanol in *n*-heptane with immobilized *Candida antarctica* lipase B, i.e., Novozym 435, as biocatalyst showed an optimal conversion of 97% at 60 °C in 6 h. Based on initial rate and progress curve data, the reaction was found to follow the ternary complex ordered bi−bi mechanism with inhibition by *n*-hexanol.

1. INTRODUCTION

In recent years, industrial biotechnology, also called white biotechnology, for the production of chemicals has gained great importance due to its wide applications in industries ranging from the pharmaceutical to bulk chemicals.[1] The exploitation of enzymes as catalysts in chemical synthesis has been much in evidence. Lipases and esterases are the most widely used biocatalysts among them. Lipases have been reported to catalyze many reactions in organic solvents which include esterification, interesterification, transesterification, hydrolysis, amidation, thioesterification, trans-thioesterification, and epoxidation.[2−8] These reactions were reported to kinetically proceed via the ping-pong bi−bi mechanism, ternary complex ordered bi−bi mechanism, or ternary complex random bi−bi mechanism. It involved, in some cases, inhibition by either substrate or product or both.[8−13] For instance, 4,8-dimethylnon-7-en-1-ol substrate inhibition was observed in Novozym 435 catalyzed cinnamate ester synthesis with *n*-heptane as solvent[14] while in the esterification of lauric acid with geraniol in isooctane, lauric acid inhibition was proposed.[12]

Alkyl benzoate esters are mainly used in perfumes and the flavor industry, in cosmetics, in pharmaceuticals, as dye carriers, in the paint industry, in adhesives, as plasticizers in surface coatings, and as the solvents of cellulose acetate, nitrocellulose, and insect repellents.[15−17] They are synthesized by chemical[17] and enzymatic routes.[3,15,18,19] The most commonly used Fischer esterification process for synthesis of alkyl benzoate employs sulfuric acid and *p*-toluene sulfuric acid (PTSA). These catalysts generate tremendous acidic waste which is hazardous to nature. Therefore, an environmentally friendly and inexpensive process that could avoid homogeneous liquid acids is the most desirable for organic transformations.[20−22] Several general routes of ester preparation have been listed among which heterogeneous solid acids as catalysts could be used under high temperature.[23] In contrast to solid acids, biocatalysts allow synthesis of esters to be performed at moderate temperatures.[5,14,24] However, biocatalytic methods reported in previous literature for alkyl benzoate synthesis possess low reaction rates and require long reaction times to obtain good conversions. In a biocatalytic transesterification processes, maintenance of water activity is easy and important. In most of the cases, increased water activity in reaction media showed decreased conversion and reaction rates.[25,26] However, the lipases exhibited an independent water activity profile for maximum transesterification and esterification reactions.[27−30]

Microwave radiation is established as an efficient heating source for a variety of chemical reactions, where high yields and reaction selectivity can be achieved at reduced times of reaction.[31−34] Hence it was thought to be beneficial to employ microwave radiation to increase the reaction rates and conversions. Microwave irradiation results in an instantaneous localized superheating which is achieved due to dipole rotation or ionic conduction.[14,35−38] Kinetic modeling for enzymatic transesterification of methyl benzoate with different alcohols under microwave irradiation has not been reported so far. The kinetics and mechanism for the lipase catalyzed transesterification of methyl benzoate with different alcohols under microwave irradiation was investigated to propose a suitable model. The effects of various parameters such as various solvents, speed of agitation, biocatalyst concentration, temperature, and substrate concentration on the conversion and rate of reaction were studied systematically.

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2. EXPERIMENTAL SECTION

2.1. Enzyme and Chemicals. Lipozyme RM IM, Lipozyme TL IM, and Novozym 435 were obtained as free gift samples from Novo Nordisk, Denmark. Lipase AYS Amano was a gift sample from Amano Enzyme Inc. Japan. Lipozyme RM IM is *Rhizomucor miehei* lipase immobilized on an anionic exchange resin (activity of 30 U g^{-1}, based on tristearin assay), whereas Lipozyme TL IM is *Thermomyces lanuginosus* immobilized on silica. Novozym 435 is *Candida antarctica* lipase B (CALB) immobilized on macroporous polyacrylic resin beads (bead size 0.3−0.6 mm, bulk density 0.430 g cm^{-3}, water content 3%, activity 7000 PLU g^{-1}). Lipase AYS Amano is *Candida rugosa* lipase in the form of a lyophilized powder (activity 30 000 U g^{-1}).

Chemicals used in the study were AR grade, purchased from a reputed firm and used as such with no further purification: methyl benzoate, *n*-butanol, *n*-pentanol, *n*-hexanol, *n*-octanol, benzyl alcohol, isoamyl alcohol, 2-ethyl-1-hexanol, *n*-heptane, *n*-hexane, toluene, tetrahydrofuran, and 1,4-dioxane (SD. Fine Chemicals Pvt. Ltd., Mumbai, India).

2.2. Analytical Method. Specifications for the gas chromatographic (GC) instrument, GC−MS, and the capillary column used for the analysis are the same as reported earlier.[14] The analytical method adopted in this study is mentioned here, while other conditions maintained for analysis remained the same. The initial temperature of the GC oven was 70 °C for 2 min, and then it was increased to 90 °C at a ramp rate of 10 °C min^{-1} and held constant for 1 min. Further, it was increased to 230 °C at a ramp rate of 10 °C min^{-1} and held constant for 1 min. Percentage conversion was calculated based on the area under the curve of the limiting reactant as follows: conversion (%) = $[[(A_0/I_0) - (A/I)](100)]/(A_0/I_0)$, where A_0 and A are the areas under the curve of the limiting reactant at times $t = 0$ and $t = t$ min; I_0 and I are the areas under the curve of the internal standard at times $t = 0$ and $t = t$ min. The product was confirmed by GC−MS.

2.3. Experimental Setup and Procedure. *2.3.1. Conventional Heating.* The experimental setup used for conventional heating studies was the same as reported earlier.[14] A typical reaction procedure for lipase catalyzed synthesis of benzoate esters contained 20 mmol of methyl benzoate and 10 mmol of *n*-hexanol, diluted to 15 mL with *n*-heptane as a solvent. The reaction mixture was agitated at 60 °C for 15 min at a speed of 300 rpm. Reaction was initiated by adding a known fixed quantity of lipase. Samples were collected at regular intervals, filtered to eliminate particulate matter, if any, and analyzed by GC. The blank reactions were conducted without enzyme as well as only under conventional heating.

2.3.2. Microwave Reactor. The microwave reactor (Discover, CEM-SP 1245 model) setup used in the study is the same as reported earlier.[14] The reactor was a 120×10^{-6} m^3 capacity fully baffled 4.5×10^{-2} m (i.d.) cylindrical glass vessel with provision for mechanical stirring (Figure 1). A standard four-bladed-turbine impeller of 1.5×10^{-2} m diameter was used for agitation. However, the actual reactor volume exposed to the microwave irradiation was 45×10^{-6} m^3. The CEM Discover microwave reactor could be used to carry out reactions up to a microwave power of 300 W. The experiments were carried out at constant temperature. A constant microwave irradiation was provided (30−40 W). A typical reaction procedure for lipase catalyzed synthesis of benzoate esters under microwave irradiation studies was the same as mentioned for conventional heating, unless otherwise stated. The blank reactions were conducted without enzyme as well as only under microwave heating.

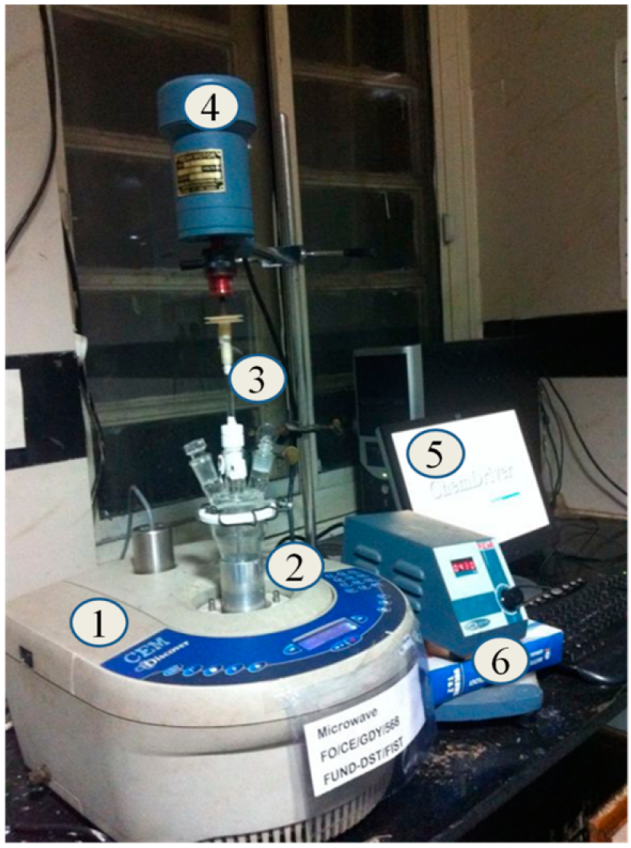

Figure 1. Experimental setup for lipase catalyzed methyl benzoate transesterification under microwave heating. 1, CEM microwave reactor; 2, glass slurry reactor; 3, glass stirrer; 4, Remi's lab stirrer; 5, computer control unit for microwave; 6, Remi's speed regulator.

2.4. Enzyme Kinetics. Different kinetic parameters were studied to elucidate the kinetics of lipase catalyzed transesterification of methyl benzoate with *n*-hexanol. Concentrations of substrates were systematically varied over a wide range to study the effect on the rate of reaction using 300 mg of Novozym 435. Various concentrations of *n*-hexanol (B) from 3 to 20 mmol were studied at different fixed quantities of methyl benzoate (A) (10−30 mmol). In another set of experiments, different concentrations of methyl benzoate (A) from 10 to 30 mmol were studied at a fixed quantity of *n*-hexanol (3−20 mmol). The quantified data obtained were used to calculate initial rates of reaction.

3. RESULTS AND DISCUSSION

3.1. Conventional Heating versus Microwave Irradiation. Enzymatic transesterification of methyl benzoate and *n*-hexanol (Scheme 1) under conventional heating versus microwave irradiation conditions was compared. Under conventional and microwave heating, the initial rate was found to be increased from 7.256×10^{-4} to 4.703×10^{-3} mol L^{-1} min^{-1} respectively at 60 °C (Figure 2). A 6.5-fold increase in initial rate was observed under microwave irradiation when compared with that under conventional heating, which resulted in reduced reaction time to achieve the same conversion. The polar compounds have more tendencies to absorb microwave radiation than less polar compounds, resulting in super-

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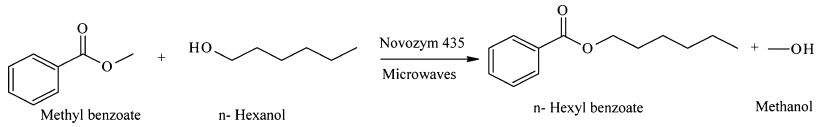
Scheme 1. Microwave Irradiated Novozym 435 Catalyzed Synthesis of *n*-Hexyl Benzoate



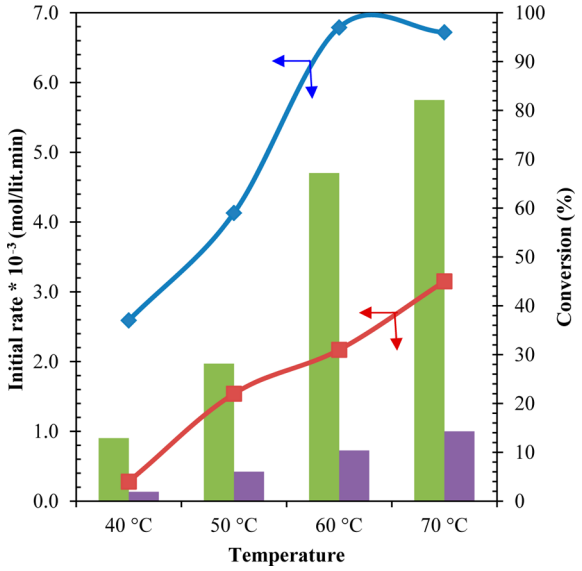


Figure 2. Effect of conventional heating versus microwave heating. Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, *n*-heptane up to 15 mL; temperature, 60 °C; speed of agitation, 300 rpm; Novozym 435, 0.02 g cm^{-3}. ◆, microwave conversion; ■, conventional conversion; green bar, microwave initial rate (mol L^{-1} min^{-1}); purple bar, conventional initial rate (mol L^{-1} min^{-1}).

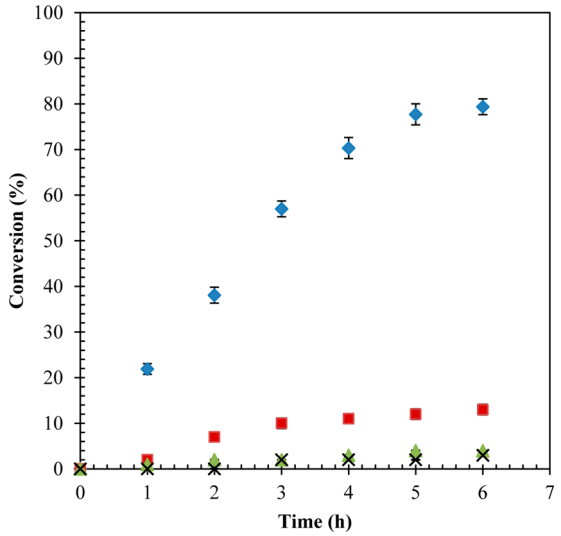


Figure 3. Effects of various biocatalysts. Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, toluene up to 15 mL; temperature, 60 °C; speed of agitation, 300 rpm; catalyst, 0.02 g cm^{-3}. Biocatalyst: ◆, Novozym 435; ■, Lipozyme RM IM; ▲, Lipozyme TL IM; ×, Lipase AYS Amano.

molecular heating. An increase in temperature causes molecules to move about more rapidly, which leads to a greater number of more energetic collisions. This occurs much faster with microwave energy, due to the high instantaneous heating of the substance(s) above the normal bulk temperature, and is the primary factor for the observed rate enhancements.[35] This suggests that the microwave capturing nature of the reactants (methyl benzoate and *n*-hexanol) was contributing to the elevated reaction rate. This is further confirmed experimentally through calculations of the collision frequency factor (A) under microwave versus conventional heating and is discussed in detail in section 3.8. Recently, a similar effect for enzymatic transformation under microwave irradiation for various reactions was reported.[14,39] Enzyme under microwave irradiation may possibly behave to some extent in a different way and turn out to be more effective. This is due to conformational modification in the enzyme which is capable of assisting the substrate to come close to the active site of the enzyme more easily under microwave irradiation. The control experiments were conducted without enzyme as well as only under microwave irradiation in the absence of enzyme. In either case, no conversion was observed. This clearly indicates the synergistic effect between microwave irradiation and enzyme catalysis.

3.2. Effects of Various Biocatalysts. Enzymatic transesterification of methyl benzoate with *n*-hexanol was selected as the model reaction to study the effects of different immobilized lipases on conversion and the rate of reaction. It can be seen from Figure 3 that conversion varied noticeably with the type of lipase. Lipozyme RM IM and Novozym 435 showed conversions of 13 and 79%, respectively, whereas very much less conversion was obtained with Lipozyme TL IM and Lipase AYS Amano. The objective for studying these different enzymes was to discover if any significant activation could be attained owing to microwave irradiation, regardless of their well-known applications. Versatile Novozym 435 enzyme has been found to be very effective under microwave irradiation. The microenvironment around the active site pocket of Novozym 435 favors proper interaction of substrate in nonaqueous media. The flapping lid of *R. miehei* (Lipozyme RM IM) and *T. lanuginosus* (Lipozyme TL IM) projects into the binding pocket, thereby creating steric hindrance in binding of substrate at the active site.[40] Novozym 435, being the most efficient biocatalyst among the studied enzymes, was used in further experiments.

3.3. Effects of Various Solvents. Different solvents such as 1,4-dioxane, tetrahydrofuran, toluene, *n*-hexane, and *n*-heptane were used to study the effect on conversion and the rate of reaction (Figure 4). The nature of the solvent has a great impact on the activity of enzyme which requires essential water activity for maintaining the native, catalytically active enzyme conformation in the organic solvent. For nonaqueous enzymatic transformations, hydrophobic solvents are more favored than hydrophilic solvents, because hydrophilic solvents cause stripping of the necessary water layer around the enzyme, resulting in reduced enzyme activity.[24,41] Maximum conversion of 97% was obtained using *n*-heptane as a solvent and hence was used for further studies.

3.4. Effect of Speed of Agitation. The optimal speed of agitation and enzyme loading of optimum particle size can be used to minimize the external mass transfer and internal

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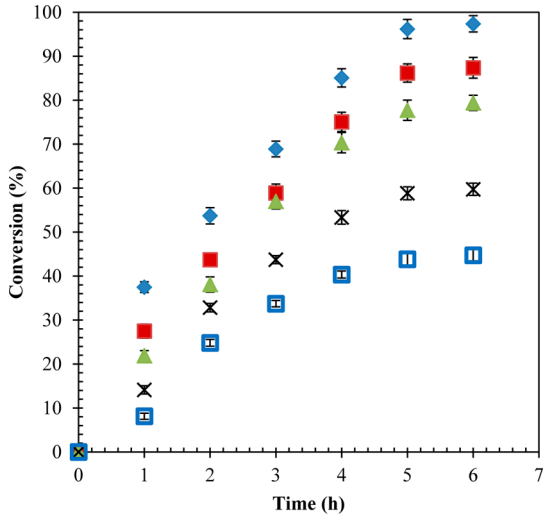

Figure 4. Effects of different solvents. Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, up to 15 mL; temperature, 60 °C; speed of agitation, 300 rpm; Novozym 435, 0.02 g cm^{-3}. Solvent: ◆, *n*-heptane; ■, *n*-hexane; ▲, toluene; □, tetrahydrofuran; ×, 1,4-dioxane.

diffusion limitations. Hence, the effect of the speed of agitation in the range 100−400 rpm was studied using Novozym 435 as biocatalyst and *n*-heptane as solvent (Figure 5). The conversion

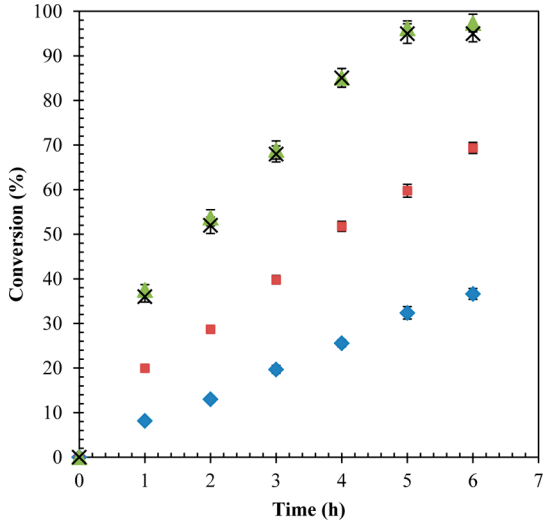

Figure 5. Effect of speed of agitation. Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, *n*-heptane up to 15 mL; temperature, 60 °C; Novozym 435, 0.02 g cm^{-3}. Speed of agitation: ◆, 100 rpm; ■, 200 rpm; ▲, 300 rpm; ×, 400 rpm.

profile and initial rate data were obtained. An increase in conversion from 37 to 97% was found when the speed of agitation was increased from 100 to 300 rpm. On the other hand, there was no significant increase in the rate and conversion at 300 and 400 rpm. At higher speeds of agitation, the biocatalyst particles were thrown out of the reaction medium on the reactor wall, thus resulting in reduced effective biocatalyst loading. Thus, further studies were carried out at an optimum speed of 300 rpm.

3.5. Effects of Different Alcohols. Transesterification of methyl benzoate was studied with different alcohols such as *n*-butanol, *n*-pentanol, *n*-hexanol, *n*-octanol, benzyl alcohol, 2-ethyl-1-hexanol, and isoamyl alcohol under otherwise similar conditions. It was observed that the conversion obtained with primary alcohols, viz., *n*-butanol, *n*-pentanol, *n*-hexanol, *n*-octanol, and isoamyl alcohol, was 72, 80, 97, and 87%, respectively (Figure 6). Transesterification with the branched

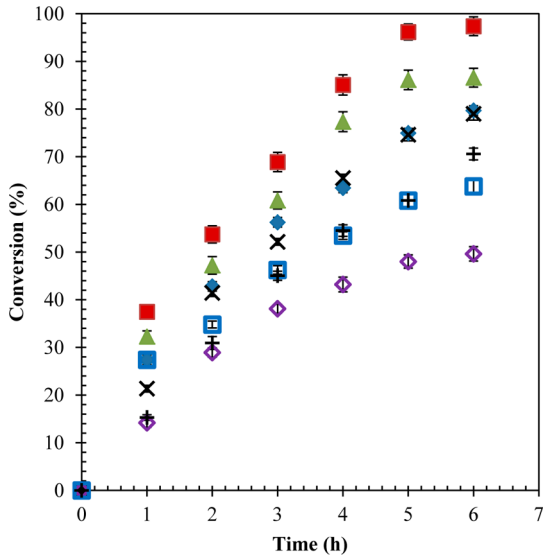

Figure 6. Effects of different alcohols. Reaction conditions: methyl benzoate, 20 mmol; alcohol, 10 mmol; solvent, *n*-heptane up to 15 mL; temperature, 60 °C; speed of agitation, 300 rpm; Novozym 435, 0.02 g cm^{-3}. Alcohol: +, *n*-butanol; ◆, *n*-pentanol; ■, *n*-hexanol; ▲, *n*-octanol; □, isoamyl alcohol; ×, benzyl alcohol; ◇, 2-ethylhexyl alcohol.

chain alcohol 2-ethyl-1-hexanol showed a lower conversion of 50%, whereas aromatic alcohol, i.e., benzyl alcohol, showed a good conversion of 79%. The difference in conversion with chain length and the nature of the alcohol can be related to the effect of a number of factors such as the molecular size of the alcohol, solubility in reaction solvent, and the affinity of lipase for a specific individual alcohol.[42]

3.6. Effect of Biocatalyst Amount. The effect of the amount of Novozym 435 varying from 0.007 to 0.027 g cm^{-3} for the transesterification of methyl benzoate was investigated under microwave irradiation while molar ratio of substrates was maintained constant. It can be seen from Figure 7 that the conversion increased linearly with an increase in biocatalyst amount up to the loading of 0.02 g cm^{-3}. Above 0.02 g cm^{-3} biocatalyst loading, no significant increase in the conversion was found, which clearly shows that the biocatalyst loading was much higher than needed and the rate was limited by the external mass transfer. Hence, a biocatalyst loading of 0.02 g cm^{-3} under specified conditions was considered be the most efficient and optimal.

3.7. Effect of *n*-Hexanol Concentration. The transesterification of methyl benzoate with *n*-hexanol was studied at different moles of *n*-hexanol, keeping the moles of methyl benzoate (20 mmol) constant at constant liquid volume. Maximum conversion and rate of reaction were achieved at 10 mmol of *n*-hexanol (Figure 8). By increasing the moles of *n*-hexanol from 5 to 10 mmol, conversion and the reaction rate were increased. The conversion was decreased with further increase in *n*-hexanol concentration. This could be related to the inhibitory effect of *n*-hexanol at high concentration on Novozym 435 enzyme. The hydrophobic tail of *n*-hexanol and

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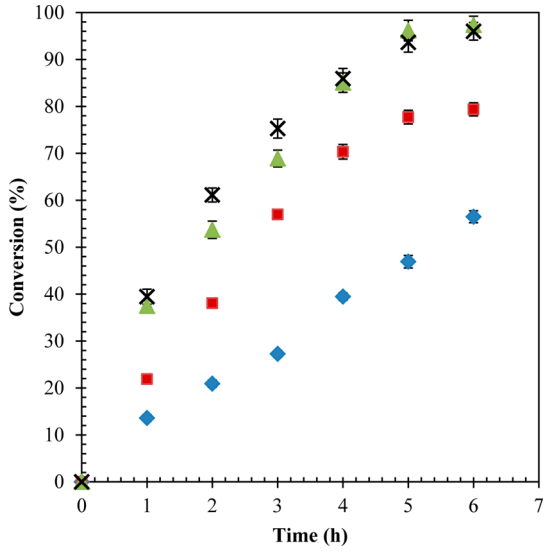

Figure 7. Effect of catalyst loading. Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, *n*-heptane up to 15 mL; temperature, 60 °C; speed of agitation, 300 rpm. Novozym 435: ◆, 0.007 g cm^{-3}; ■, 0.013 g cm^{-3}; ▲, 0.02 g cm^{-3}; ×, 0.027 g cm^{-3}.

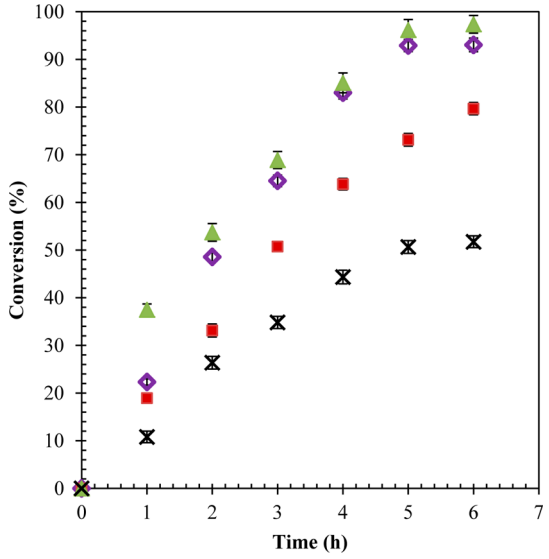

Figure 8. Effect of *n*-hexanol concentration. Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol 5−30 mmol; solvent, *n*-heptane up to 15 mL; temperature, 60 °C; speed of agitation, 300 rpm; Novozym 435, 0.02 g cm^{-3}. Concentration: ◇, 5 mmol; ■, 10 mmol; ▲, 20 mmol; ×, 30 mmol.

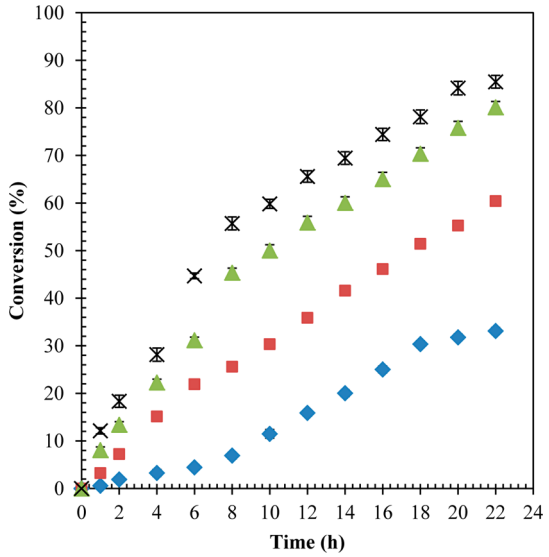

Figure 9. Effect of temperature (conventional heating). Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, *n*-heptane up to 15 mL; speed of agitation, 300 rpm; Novozym 435, 0.02 g cm^{-3}. Temperature: ◆, 40 °C; ■, 50 °C; ▲, 60 °C; ×, 70 °C.

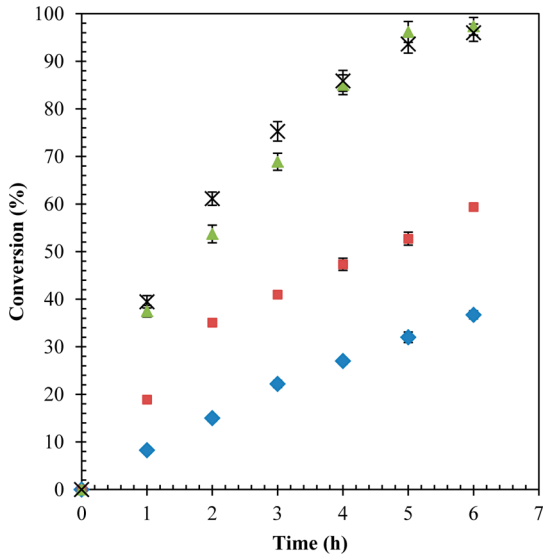

Figure 10. Effect of temperature (microwave heating). Reaction conditions: methyl benzoate, 20 mmol; *n*-hexanol, 10 mmol; solvent, *n*-heptane up to 15 mL; speed of agitation, 300 rpm; Novozym 435, 0.02 g cm^{-3}. Microwave heating: ◆, 40 °C; ■, 50 °C; ▲, 60 °C; ×, 70 °C.

the hydrophobic part of lipase enzyme may interact to form a hydrophobic−hydrophobic interaction. This will increase the residence time of *n*-hexanol. Because of a close contact with the neighboring hydrophobic residues, the enzyme−*n*-hexanol complex would have to be partially dehydrated, which may destabilize the native conformation of the enzyme; this leads to decreased conversion and initial rate.

3.8. Effect of Temperature. The effect of different temperatures on conversion and the initial rate under conventional heating and microwave irradiation was investigated. As discussed in section 3.1, under microwave irradiation the overall conversion and the rate of reaction for transesterification of methyl benzoate were higher (Figure 10) than under conventional heating (Figure 9). With an increase in temperature in the range 40−70 °C under microwave irradiation, the initial rate and conversion were found to be increased from 3.01×10^{-3} to 19.16×10^{-3} mol $L^{-1}min^{-1}$ g^{-1} enzyme and from 37 to 97%, respectively. This is attributed to the momentum provided by microwave energy to overcome the energy barrier and thus the reaction is completed more quickly than under conventional heating. Further, under microwave irradiation, high instantaneous heating of the substance(s) above the normal bulk temperature results in a greater number of more energetic collisions, which is the primary factor for the observed rate enhancements. The activation energy values were obtained by the Arrhenius plot (Figure 11) as 13.79 and 14.14 kcal mol^{-1} under microwave and conventional heating,

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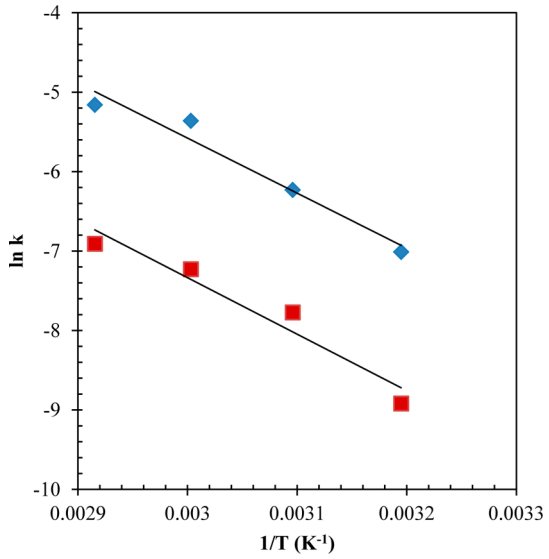


Figure 11. Arrhenius plot: ◆, microwave; ■, conventional.

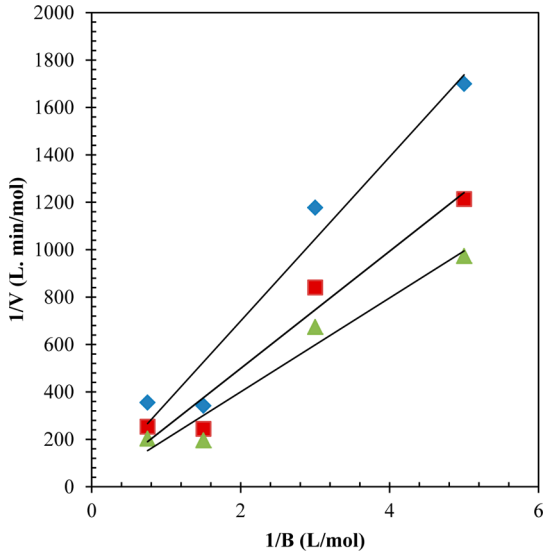


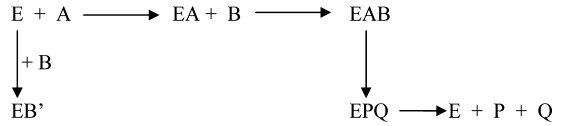
Figure 12. Lineweaver–Burk plot of 1/initial rate (L min mol^{-1}) versus 1/[*n*-hexanol] (L mol^{-1}) at different constant methyl benzoate concentrations: ◆, 10 mmol; ■, 20 mmol; ▲, 30 mmol.

respectively. These are not very different, thereby suggesting that the pre-exponential factor in the Arrhenius equation is more enhanced due to microwaves resulting in more fruitful collisions. This has ultimately resulted in higher reaction rates and conversions at reduced time.

3.9. Effect of Reusability. The reusability of Novozym 435 under optimized process parameter conditions was studied to examine the stability and recyclability of the enzyme. The enzyme after first use was filtered, washed with *n*-hexane after each use, dried at room temperature, and reused for further studies. Similar steps were followed for each further reusability study. A marginal decrease in conversion from 97 to 82% after three reuses was observed for transesterification of methyl benzoate with *n*-hexanol, which may possibly be because of the loss of enzyme during handling. Thus, Novozym 435 was quite stable for reuse.

3.10. Kinetic Model Based on Initial Rate Measurements. Based on initial rates (V) obtained under specified conditions, the kinetic model for transesterification of methyl benzoate with *n*-hexanol was proposed. Initial rates for *n*-hexyl benzoate ester synthesis were determined systematically from the linear section of the conversion plot. With an increase in the *n*-hexanol (B) concentration at different fixed methyl benzoate (A) concentrations, the initial rate was increased and attained the maximum at the critical concentration. The reaction rate decreased with further increase in the concentration of B, and thus the *n*-hexanol inhibition was significant. Thus, it is believed that *n*-hexanol at high concentrations forms a dead-end inhibitory complex with the lipase. However, there was inhibition observed by methyl benzoate (A) at any concentration tested. The Lineweaver–Burk plot of 1/initial rate (L min mol^{-1}) versus 1/[*n*-hexanol] (L mol^{-1}) at different constant methyl benzoate concentrations was made (Figure 12). The possibility of a ping-pong bi–bi mechanism is ruled out as there are no parallel lines seen in Figure 12. In fact, a ternary complex mechanism with inhibition by *n*-hexanol was suggested since the lines intersected at a point.

The mechanism for a ternary complex with inhibition by *n*-hexanol is depicted in Cleland's notation, as shown below.



According to the ternary complex mechanism, the acyl donor, i.e., methyl benzoate (A), first binds with the enzyme and forms an acyl–enzyme complex (EA). The second reactant, *n*-hexanol (B), then combines with EA to form ternary complex EAB. This ternary complex then isomerizes to another ternary complex EPQ, which then releases the products *n*-hexyl benzoate (P) and methanol (Q). However, at high concentrations of *n*-hexanol, the dead-end binary complex between *n*-hexanol and enzyme is formed. The reaction mechanism may be depicted as follows:

$$\mathrm{E} + \mathrm{A} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{EA} \tag{1}$$

$$\mathrm{EA} + \mathrm{B} \underset{k_4}{\overset{k_3}{\rightleftarrows}} \mathrm{EAB} \tag{2}$$

$$\mathrm{EAB} \Leftrightarrow \mathrm{EPQ} \tag{3}$$

$$\mathrm{EPQ} \underset{k_6}{\overset{k_5}{\rightleftarrows}} \mathrm{P} + \mathrm{EQ} \tag{4}$$

$$\mathrm{EQ} \underset{k_8}{\overset{k_7}{\rightleftarrows}} \mathrm{E} + \mathrm{Q} \tag{5}$$

inhibition step:

$$\mathrm{E} + \mathrm{B}' \underset{k_{10}}{\overset{k_9}{\rightleftarrows}} \mathrm{EB}' \tag{6}$$

The rate equation for the above ternary complex mechanism with inhibition by alcohol[24] is as follows:

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$$V = (V_{max}[A][B]) \left\{ K_{iA}K_{mB}\left(1 + \frac{[B]}{K_i^B}\right) + K_{mB}[A] + K_{mA}[B] \left(1 + \frac{[B]}{K_i^B}\right) + [A][B] \right\}^{-1} \quad (7)$$

where [A] is the methyl benzoate concentration (mol L^{-1}), [B] is the *n*-hexanol concentration (mol L^{-1}), K_{mA} is the Michaelis constant for methyl benzoate (mol L^{-1}), K_{mB} is the Michaelis constant for *n*-hexanol (mol L^{-1}), K_{iA} is the dissociation constant for the enzyme−methyl benzoate complex, and K_i^B is the inhibition constant due to *n*-hexanol. V and V_{max} are the initial rate and maximum rate (mol L^{-1} min^{-1}), respectively.

Kinetic constants were calculated by using Polymath 6.0 software as follows: V_{max} (mol L^{-1}) = 0.012, K_{mA} (mol L^{-1}) = 1.014, K_{mB} (mol L^{-1}) = 0.026, K_{iA} = 0.015, and K_i^B = 0.151. A parity plot showed an excellent correlation coefficient between experimental and simulated rates. This validates the proposed ternary complex mechanism with inhibition by *n*-hexanol.

4. CONCLUSIONS

Industrially important alkyl benzoate esters were synthesized using Novozym 435 as biocatalyst under the synergistic effect of microwave irradiation. Enzymatic transesterification of methyl benzoate was also studied with different alcohols such as *n*-butanol, *n*-pentanol, *n*-hexanol, *n*-octanol, benzyl alcohol, isoamyl alcohol, and 2-ethyl-1-hexanol. Optimized kinetic parameters obtained were 300 rpm for the speed of agitation, *n*-hexane for the solvent, 0.02 g cm^{-3} for Novozym 435 loading, and 60 °C for the temperature. A kinetic model was postulated based on initial rate data and conversion profiles. The ternary complex ordered bi−bi mechanism with *n*-hexanol substrate inhibition was assumed for enzymatic transesterification of methyl benzoate with *n*-hexanol. The proposed mechanism appropriately fitted the data well for the microwave assisted Novozym 435 biotransformation reaction.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Woodley, J. M.; Breuer, M.; Mink, D. A future perspective on the role of industrial biotechnology for chemicals production. *Chem. Eng. Res. Des.* **2013**, *91*, 2029−2036.

(2) Wolski, E.; Menusi, E.; Mazutti, M.; Toniazzo, G.; Rigo, E.; Cansian, R. L.; Mossi, A.; Oliveira, J. V.; Luccio, M. D.; de Oliveira, D.; Treichel, H. Response surface methodology for optimization of lipase production by an immobilized newly isolated *Penicillium* sp. *Ind. Eng. Chem. Res.* **2008**, *47*, 9651−9657.

(3) Vosmann, K.; Wiege, B.; Weitkamp, P.; Weber, N. Preparation of lipophilic alkyl (hydroxy) benzoates by solvent-free lipase-catalyzed esterification and transesterification. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 29−36.

(4) Sovova, H.; Zarevucka, M.; Bernasek, P.; Stamenic, M. Kinetics and specificity of Lipozyme-catalysed oil hydrolysis in supercritical CO_2. *Chem. Eng. Res. Des.* **2008**, *86*, 673−681.

(5) Yadav, G. D.; Shinde, S. D. Kinetic modeling and optimization of immobilized *Candida antarctica* lipase B catalysed synthesis of butyl-4-methyl-3-oxopentanoate using response surface methodology. *Int. J. Chem. React. Eng.* **2012**, *10*, A70.

(6) Meng, Z.; Geng, W.-X.; Li, J.-W.; Yang, Z.-Q.; Jiang, J.; Wang, X.-G.; Liu, Y.-F. Enzymatically catalyzed synthesis of anti-blooming agent 1,3-dibehenoyl-2-oleoyl glycerol in a solvent-free system: optimization by response surface methodology. *J. Agric. Food Chem.* **2013**, *61*, 10798−10806.

(7) Jakovetić, S. M.; Luković, N. D.; Bošković-Vragolović, N. M.; Bezbradica, D. I.; Picazo-Espinosa, R.; Kneževič-Jugović, Z. D. Comparative study of batch and fluidized bed bioreactors for lipase-catalyzed ethyl cinnamate synthesis. *Ind. Eng. Chem. Res.* **2013**, *52*, 16689−16697.

(8) Kraai, G.; Winkelman, J. G. M.; de Vries, J. G.; Heeres, H. J. Kinetic studies on the *Rhizomucor miehei* lipase catalyzed esterification reaction of oleic acid with 1-butanol in a biphasic system. *Biochem. Eng. J.* **2008**, *41*, 87−94.

(9) Perez, V. H.; Miranda, E. A.; Valenca, G. P. Kinetics of gas-phase hydrolysis of ethyl acetate catalyzed by immobilized lipase. *Appl. Biochem. Biotechnol.* **2007**, *136*, 23−37.

(10) Romero, M. D.; Calvo, L.; Alba, C.; Daneshfar, A.; Ghaziaskar, H. S. Enzymatic synthesis of isoamyl acetate with immobilized *Candida antarctica* lipase in *n*-hexane. *Enzyme Microb. Technol.* **2005**, *37*, 42−48.

(11) Segel, I. H. *Enzyme Kinetics*; Wiley-Interscience: New York, 1975.

(12) Vazquez Lima, F.; Pyle, D. L.; Asenjo, J. Reaction kinetics of the esterification of lauric acid in iso-octane using an immobilized biocatalyst. *Appl. Biochem. Biotechnol.* **1996**, *61*, 411−422.

(13) Yadav, G. D.; Lathi, P. S. Synergism between microwave and enzyme catalysis in intensification of reactions and selectivities: Transesterification of methyl acetoacetate with alcohols. *J. Mol. Catal. A: Chem.* **2004**, *223*, 51−56.

(14) Yadav, G. D.; Shinde, S. D. Synergism of Microwave Irradiation and Immobilized Lipase Catalysis in Synthesis of 4,8-dimethylnon-7-en-1yl (2*E*)-3-phenylpro-2-enolate. *Int. Rev. Chem. Eng.* **2012**, *4*, 589−596.

(15) Krause, P.; Hilterhaus, L.; Fieg, G.; Liese, A.; Bornscheuer, U. Chemically and enzymatically catalyzed synthesis of C6−C10 alkyl benzoates. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 194−201.

(16) Thum, O. Enzymatic production of care specialties based on fatty acid esters. *Tenside, Surfactants, Deterg.* **2004**, *41*, 287−290.

(17) Asai, S.; Nakamura, H.; Tanabe, M.; Sakamoto, K. Synthesis of triphenyl phosphate and benzyl benzoate with phase-transfer catalyst in heterogeneous liquid-liquid reaction system. *Ind. Eng. Chem. Res.* **1994**, *33*, 1687−1691.

(18) Gryglewicz, S.; Jadownicka, E.; Czerniak, A. Lipase catalysed synthesis of aliphatic, terpene and aromatic esters by alcoholysis in solvent-free medium. *Biotechnol. Lett.* **2000**, *22*, 1379−1382.

(19) Shintre, M. S.; Ghadge, R. S.; Sawant, S. B. Kinetics of esterification of lauric acid with fatty alcohols by lipase: effect of fatty alcohol. *J. Chem. Technol. Biotechnol.* **2002**, *77*, 1114−1121.

(20) Krishna, S. H.; Sattur, A. P.; Karanth, N. G. Lipase-catalyzed synthesis of isoamyl isobutyrate—optimization using a central composite rotatable design. *Process Biochem. (Oxford, U. K.)* **2001**, *37*, 9−16.

(21) Yadav, G. D.; Bokade, V. V. Novelties of heteropoly acid supported on clay: etherification of phenethyl alcohol with alkanols. *Appl. Catal. A: Gen.* **1996**, *147*, 299−323.
(22) Yadav, G. D.; Kirthivasan, N. Single-pot synthesis of methyl tert-butyl ether from tert-butyl alcohol and methanol—dodecatungstophosphoric acid supported on clay as an efficient catalyst. *J. Chem. Soc., Chem. Commun.* **1995**, 203−204.
(23) Yadav, G. D.; Mehta, P. H. Heterogeneous catalysis in esterification reactions—preparation of phenethyl acetate and cyclohexyl acetate by using a variety of solid acidic catalysts. *Ind. Eng. Chem. Res.* **1994**, *33*, 2198−2208.
(24) Yadav, G. D.; Devendran, S. Lipase catalyzed synthesis of cinnamyl acetate via transesterification in non-aqueous medium. *Process Biochem. (Oxford, U. K.)* **2012**, *47*, 496−502.
(25) Zhang, D.-H.; Li, Y.-Q.; Li, C.; Lv, Y.-Q.; Li, Y. Kinetics of enzymatic synthesis of L-ascorbyl acetate by lipozyme TLIM and novozym 435. *Biotechnol. Bioprocess Eng.* **2012**, *17*, 60−66.
(26) Goderis, H. L.; Ampe, G.; Feyten, M. P.; Fouwé, B. L.; Guffens, W. M.; Van Cauwenbergh, S. M.; Tobback, P. P. Lipase-catalyzed ester exchange reactions in organic media with controlled humidity. *Biotechnol. Bioeng.* **1987**, *30*, 258−266.
(27) Herbst, D.; Peper, S.; Niemeyer, B. Enzyme catalysis in organic solvents: influence of water content, solvent composition and temperature on *Candida rugosa* lipase catalyzed transesterification. *J. Biotechnol.* **2012**, *162*, 398−403.
(28) Chamouleau, F.; Coulon, D.; Girardin, M.; Ghoul, M. Influence of water activity and water content on sugar esters lipase-catalyzed synthesis in organic media. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 949−954.
(29) Chowdary, G. V.; Prapulla, S. G. The influence of water activity on the lipase catalyzed synthesis of butyl butyrate by transesterification. *Process Biochem. (Oxford, U. K.)* **2002**, *38*, 393−397.
(30) Léonard-Nevers, V.; Marton, Z.; Lamare, S.; Hult, K.; Graber, M. Understanding water effect on *Candida antarctica* lipase B activity and enantioselectivity towards secondary alcohols. *J. Mol. Catal. B: Enzym.* **2009**, *59*, 90−95.
(31) Loupy, A. *Microwaves in Organic Synthesis*; Wiley-VCH: Weinheim, Germany, 2002.
(32) Liao, C.-C.; Chung, T.-W. Optimization of process conditions using response surface methodology for the microwave-assisted transesterification of Jatropha oil with KOH impregnated CaO as catalyst. *Chem. Eng. Res. Des.* **2013**, *91*, 2457−2464.
(33) Hoseinzadeh Hesas, R.; Arami-Niya, A.; Daud, W. M. A. W.; Sahu, J. N. Preparation of granular activated carbon from oil palm shell by microwave-induced chemical activation: Optimisation using surface response methodology. *Chem. Eng. Res. Des.* **2013**, *91*, 2447−2456.
(34) Jeon, S. G.; Kwak, N. S.; Rho, N. S.; Ko, C. H.; Na, J.-G.; Yi, K. B.; Park, S. B. Catalytic pyrolysis of Athabasca bitumen in H_2 atmosphere using microwave irradiation. *Chem. Eng. Res. Des.* **2012**, *90*, 1292−1296.
(35) Hayes, B. L. *Microwave Synthesis: Chemistry at the Speed of Light*; CEM Publishing: Matthews, NC, USA, 2002.
(36) Kappe, O. C.; Pieber, B.; Dallinger, D. Microwave effects in organic synthesis: myth or reality? *Angew. Chem., Int. Ed.* **2013**, *52*, 1088−1094.
(37) (a) Yadav, G. D.; Borkar, I. V. Kinetic modeling of microwave assisted chemo-enzymatic epoxidation of styrene to styrene oxide. *AIChE J.* **2006**, *52*, 1235−1247. (b) Yadav, G. D.; Devi, K. M. Enzymatic synthesis of perlauric acid using Novozym 435. *Biochem. Eng. J.* **2002**, *10*, 93−101.
(38) Liao, C.-C.; Chung, T.-W. Analysis of parameters and interaction between parameters of the microwave-assisted continuous transesterification process of *Jatropha* oil using response surface methodology. *Chem. Eng. Res. Des.* **2011**, *89*, 2575−2581.
(39) Yu, D.; Tian, L.; Ma, D.; Wu, H.; Wang, Z.; Wang, L.; Fang, X. Microwave-assisted fatty acid methyl ester production from soybean oil by Novozym 435. *Green. Chem.* **2010**, *12*, 844−850.
(40) Yadav, G. D.; Borkar, I. V. Kinetic modeling of immobilized lipase catalysis in synthesis of *n*-butyl levulinate. *Ind. Eng. Chem. Res.* **2008**, *47*, 3358−3363.
(41) Wehtje, E.; Adlercreutz, P. Lipases have similar water activity profiles in different reactions. *Biotechnol. Lett.* **1997**, *19*, 537−540.
(42) Varma, M.; Madras, G. Effect of chain length of alcohol on the lipase-catalyzed esterification of propionic acid in supercritical carbon dioxide. *Appl. Biochem. Biotechnol.* **2010**, *160*, 2342−2354.