Engineering bio-chemo catalytic reactions

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Laboratory of Industrial Chemistry and Reaction Engineering
Process Chemistry Centre
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Åbo Akademi University
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Sevgili anne ve babama

"There are three ways of doing things around here: the right way, the wrong way, and the way that I do it."
Ace Rothstein

PREFACE

The present work was carried out at the Laboratory of Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Department of Chemical Engineering, Åbo Akademi University during the academic year 2009-2010. The research is a part of the activities performed at Åbo Akademi Process Chemistry Centre within the Finnish Centre of Excellence Programmes (2000-2011) by the Academy of Finland.

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Finally, I wish to thank my family for their unlimited and unreserved support and encouragement. *Sevgili annecim ve babacim, benden esirgemediginiz maddi ve manevi her turlu desteginiz icin sonsuz tesekkurler.* My special thanks go to Yagut Allahverdiyeva-Rinne and Marja Hakala-Yatkin as well as Emrah Yatkin and Petri Rinne whom I consider as a part of my family. I am grateful for their immense care and unconditional love.

At last but not least, I would like to thank my beloved Sayra for his love, passion, inspiration, understanding and blissful existence in my life. We made it together!

Åbo, September 2010

Serap Şahin

ABSTRACT

Engineering chemo-bio catalytic reactions

Serap Şahin

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Keywords: one-pot synthesis, chemo-bio, kinetic resolution, immobilized lipase.

The improvements in process efficiency can be achieved through reduction of the number of synthesis steps. In recent years, utilization of cascade methodology in which the intermediate product is converted in the forthcoming consecutive reactions without any separation in the same reaction pot has been practiced for use mainly in the pharmaceutical and chemical industries.

Heterogeneously conducted one-pot synthesis of *(R)*-1-phenylethyl acetate in a consecutive reaction starting from the catalytic hydrogenation of acetophenone and following the acylation reaction of the formed *(R)*-enantiomer of racemic 1-phenylethanol in one-pot was performed over the palladium catalysts on different supports in combination with an immobilized lipase. The catalytic performances of palladium catalysts supported on C, MgO, ZrO_2, Al_2O_3, H-MCM-41, Al_2O_3-UOP, Si-MCM-41, SiO_2 and the affect of the reaction steps on each other and/or on the overall reaction system were investigated. The catalysts were characterized by means of XRD, SEM, TEM, CO chemisorption and N_2-physisorption, finding the optimum formula in developing a cascade catalytic process in such a way that the hydrogenation proceeds under conditions where the immobilized lipase maintains its activity. The challenge was to achieve very high selectivity in the hydrogenation step, since the support acidity had a crucial effect on the selectivity towards the desired product. Therefore, palladium catalysts on VGCF supports which contain basic N-surface groups deposited either with incipient-wetness impregnation or with sol immobilization were tested. VGCFs possess unique properties such as adjustable surface properties, high surface area and high mechanical stability. The effect of the preparation method on the synthesis of *(R)*-1-phenylethyl acetate in one-pot with an immobilized lipase as well as the effect of catalyst treatment procedure was investigated.

The experiments related to one-pot synthesis were carried out in two different reactor systems; a glass reactor and an autoclave in batch mode. The typical experiments starting from acetophenone hydrogenation in glass reactor were performed at 70 ^{o}C under atmospheric pressure of hydrogen over supported palladium based catalyst with an immobilized lipase in ethyl acetate which was used as an acyl donor as well. However, toluene was used as solvent only in the experiments carried out over palladium catalyst on N-VGCFs. Therefore, these two experimental sets were not comparable. The transformation of racemic 1-phenylethanol was performed in an autoclave at 50 °C under

5.6 bar total pressure of hydrogen or argon in ethyl acetate. Acylation of racemic 1-phenylethanol was conducted in the presence of an immobilized lipase.

The preparation of chiral drugs as single enantiomers, which are valuable building blocks for mainly pharmaceutical and fine-chemical industries, is one of the most industrially important reactions since the biological activities displayed by the enantiomeric pairs are different. Kinetic resolution of chiral alcohols with enzymes is the most widely used method for separating the two enantiomers of a racemic mixture. The kinetic resolution of racemic 1-phenylethanol with ethyl acetate was performed in a down-flow fixed-bed reactor operated with continuous mode. In a standard experiment, the kinetic resolution of racemic 1-pheylethanol with ethyl acetate was performed at the molar ratio of 1:3 in toluene at 70 ^{o}C. The catalytic activity of the immobilized lipase was investigated for this reaction by performing the following experiments; (i) applying different flow rates, and (ii) applying different substrate concentrations, (iii) investigating the effect of organic compounds related to one-pot synthesis, such as ethyl acetate, ethyl benzene, acetic acid, acetophenone etc., (iv) the inhibitory effect of either the desired or the stoichiometric products (*(R)*-1-phenylethyl acetate and ethanol, respectively), (v) elucidating the inhibitory effect of water on the activity and stability of the immobilized lipase, (vi) investigating reactor hydrodynamics by applying step changes. The comparison between the continuous flow reactor and the batch reactor modes for the kinetic resolution of racemic 1-phenylethanol was discussed.

Finally, the kinetics in the one-pot synthesis of *(R)*-1-phenylethyl acetate was systematically investigated by using different amounts of hydrogenation catalyst and an immobilized lipase. The kinetics revealed that there is an interrelation between the catalytic performance of the supported palladium catalyst and an immobilized lipase indicating that these catalysts were not acting independently but in concert. A detailed mechanism for the overall reaction was presented and a kinetic model was proposed. The estimated kinetic constants were identified by parameter sensitivity analysis plots using Markov Chain Monte Carlo (MCMC) method based on Bayesian approach. The results revealed an interaction between both catalysts where the activity of the heterogeneous catalyst was hindered due to the presence of increased amounts of enzyme.

REFERAT

Undersökning av kombinerade katalytiska och enzymatiska reaktioner

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Keywords: one-pot-syntes, kemisk-biologiska katalysatorer, kinetisk upplösning, immobiliserad lipas.

Effektiviteten av en kemisk process kan uppnås genom att minska på antalet syntessteg. Under de senaste åren har utnyttjandet av kaskadteknologin, i vilken mellanprodukter omsätts i konsekutiva reaktioner utan separering i samma reaktionskärl blivit aktuell i farmaceutisk och kemisk industri

Heterogent katalyserad one-pot-syntes av *(R)*-1-fenyletylacetat i en konsekutiv reaktion, som startar med katalytisk hydrering av acetofenon och följs av en reaktion av den bildade *(R)*-enantiomeren av racemisk 1-fenyletanol genomfördes på ett kombinerat system bestående av palladiumkatalysatorer med olika bärarmaterial och en enzymatisk katalysator, lipas.

Den katalytiska prestandan av palladiumkatalysatorer på följande bärarmaterial undersöktes: C, MgO, ZrO_2, Al_2O_3, H-MCM-41, Al_2O_3-UOP, Si-MCM-41, SiO_2. Växelverkan mellan reaktionsstegen och reaktionsstegens effekt på hela systemet undersöktes experimentellt. Katalysatorerna karakteriserades med flera kemiska och fysikaliska metoder: röntgendiffraktion, svepelektronsmikroskopi, transmissions-elelektronmikroskopi, kemisorption av kolmonoxid och fysisorption av kväve för att hitta ett optimalt koncept för utveckling av en kaskadprocess, där den heterogent katalyserade hydreringen framskrider under sådana betingelser som bevarar den enzymatiska katalysatorns aktivitet.

Utmaningen var att uppnå en hög selektivitet i hydreringssteget, eftersom bärarmaterialets surhet hade en avgörande inverkan på den önskade produktens selektivitet. Därför undersöktes palladiumkatalysatorer på VGCF-bärarmaterial, som hade kväveinnehållande ytgrupper. Porimpregnering och sol-gelmetod användes för katalysatorprepareringen. VGCF har unika egenskaper, så som justerbara ytegenskaper, hög ytarea och hög mekanisk stabilitet. Inverkan av preparationsmetoden och katalysatorförbehandlingen på syntes av *(R)*-1-fenylacetat studerades ingående.

Experimenten genomfördes i två olika reaktorsystem: en glasreaktor samt en satsvis autoklav. Typiska experiment för acetofenonhydrering i glasreaktorn genomfördes vid 70 ^{o}C under atmosfäriskt tryck av väte på palladiumkatalysatorer och immobiliserad lipas i

ethylacetat, som även fungerade som acyldonor. Toluen användes som lösningsmedel endast i experiment, som genomfördes på palladium-

N-VGCF-katalysatorer. Därför kunde dessa två experimentella system inte jämföras. Transformation av racemisk 1-fenyletanol genomfördes i autoklavreaktorn vid 50 °C under 5.6 bar totaltryck av väte eller argon i etylacetat. Acylering av racemisk 1-fenyletanol genomfördes i närvaro av immobiliserad lipas.

Preparering av kirala komponenter som rena enantiomerer, som är värdefulla byggelement för farmaceutisk och finkemikalieindustri, hör till de viktigaste industriella reaktionerna, eftersom den biologiska aktiviteten av molekylpar av de olika enantiomererna är olika. Kinetisk upplösning av kirala alkoholer med enzymer är den mest utnyttjade metoden för separering av två enantiomerer ur en racemisk blandning.

Kinetisk upplösning av racemisk 1-fenyletanol med etylacetat genomfördes i en kontinuerlig packad bäddreaktor med nedåtströmmande reaktionsvätska. I standard experiment studerades kinetisk upplösning av racemisk 1-fenyletanol med etylacetat med molförhållandet 1:3 i toluen vid 70 ^{o}C. Den katalytiska aktiviteten av immobiliserad lipas undersöktes i avseende på följande experimentella parametrar: olika strömningshastigheter, olika reaktantkoncentrationer, organiska komponenters effekt, inhiberande effekt på önskade eller stökiometriska produkter (*(R)*-1-fenyletylacetat och etanol), vattnets inhiberande effekt på aktiviteten och stabiliteten av lipas och reaktorns hydrodynamik. Den kontinuerliga bäddreaktorns och satsreaktorns beteende jämfördes.

I slutskedet av arbetet undersöktes kinetiken av syntesen av *R*-1-fenyletylacetat systematiskt genom att använda olika mängder av hydreringskatalysatorer och immobiliserad lipas. De kinetiska studierna avslöjade att det existerar ett samband mellan prestandan av heterogena palladiumkatalysatorer och immobiliserad lipas, vilket tyder på att dessa katalytiska komponenter inte agerar separat utan växelverkar.

En detaljerad mekanism för reaktionerna presenterades och en kinetisk modell föreslogs. De estimerade kinetiska konstanterna identifierades med en känslighetsanalys genom att använda Monte Carlo-metod med Markov-kedjor (MCMC) i regressionsanalys. Resultaten avslöjade en växelverkan mellan båda katalysatorerna: den heterogena katalysatorns aktivitet minskade i närvaro av ökade mängder av den enzymatiska katalysatorn.

LIST OF PUBLICATIONS

The thesis consists of the following publications, which are referred to, in the text by their Roman numerals.

I P. Mäki-Arvela, S. Sahin, N. Kumar, J.-P. Mikkola, K. Eränen, T. Salmi, D. Yu. Murzin, One-pot utilization of heterogeneous and enzymatic catalysis: Synthesis of *R*-1-phenylethyl acetate from acetophenone. Catalysis Today 140 (2009) 70-73.

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2 S. Sahin, P. Mäki-Arvela, K. Eränen, T. Salmi, D. Yu. Murzin, Mechanistic studies in lipase-catalyzed kinetic resolution in a down-flow continuous reactor. 9^{th} Congress on Catalysis Applied to Fine Chemicals, Zaragoza, Spain, September 13^{th} - 16^{th} 2010. Oral presentation.

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5 S. Sahin, P. Mäki-Arvela, J.-P. Tessonnier, A. Villa, L. Shao, D. Sheng Su, R. Schlög, D. Yu. Murzin, Effect of carbon nano tube basicity in Pd/N-CNT catalysts on the synthesis of *R*-1-phenylethyl acetate. 10th International Symposium of Scientific Bases for the Preparation of Heterogeneous Catalysts, Louvain-la-Neuve, Belgium, July 11th - 15th 2010. Poster presentation.

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TABLE OF CONTENTS

1
INTRODUCTION

1.1 Why one-pot synthesis?

The application of cascade catalysis has been recently studied intensively both in academia and in industry due to the advantages of cascade reactions over multi-step processes in producing fine chemicals. The idea is to perform several reactions in one reactor pot, thus producing less waste, saving equipment and maintenance costs compared to conventional methods. The synthesis of complex molecules is traditionally performed by separate steps, each of which requires its own conditions, solvent and catalyst. After each reaction is completed, the solvent and the waste products are removed and discarded and the intermediate product is separated and purified. The need for environmentally tolerable procedures by reducing the waste and the responsible treatment of resources are utmost important to achieve process intensification in chemical production. The improvements in process efficiency can be achieved through reduction of the number of synthesis steps [1]. One-pot syntheses offer efficient transformations of starting materials to the final products without any separation of intermediates (Figure 1.1). Moreover, this approach reduces the handling and storage of process intermediates as the intermediate products of multi-step organic synthesis are often highly active or toxic, thus one-pot methodology may prevent the exposure of the operators and environment to such chemicals.

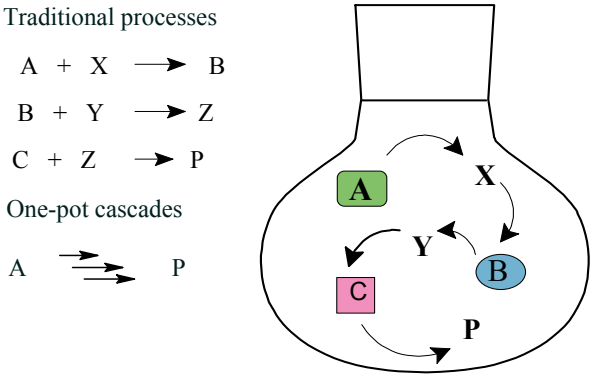


Figure 1.1. One-pot multi-step synthesis.

1.2 The concept of one-pot methodology

A variety of examples of cascade conversions involving different combinations of enzymes exist in the literature. The classification of one-pot synthesis is not a simple task, given the wide variety of different reaction types, their order of occurrence, and the different reaction conditions that are possible. Numerous classification types can be found in the literature. However, the most common approaches have been categorized as bio-bio, bio-chemo and chemo-chemo cascades [2].

Most of the examples of bio-bio cascades have been reported from the 1970s in the field of carbohydrates using combinations of conversions for enzymatic redox reactions towards amino and hydroxy acids. Combined two-enzymatic action of glucose dehydrogenase and aldehyde dehydrogenase for two-enzyme enantioselective amination of small amino acids in carbonyl reduction in one-pot conversions can be given as another example [3]. Fat hydrolysis coupled with fatty acid oxidation by lipase and lipoxygenase enzymes in ambient temperature with up to 96% yield after 5.5 hours can be presented as one of the most attractive applications of bio-bio cascades [4]. Moreover, modern detergent formulations containing up to six different enzymes including lipase is an industrial example of bio-bio one-pot conversions [5].

The conversion of glucose into mannitol is the very first example involving the combined action of an enzyme and a metal catalyst studied abundantly [6]. Conversion of glucose into mannitol in the concept of one-pot reaction is three times less expensive than conversion at glucitol, the sole product from conventional glucose hydrogenation. The enzyme called isomerase converts glucose into glucose-fructose mixture and takes care that this mixture remains in equilibrium. At the same time the heterogeneous copper catalyst is used to hydrogenate preferentially fructose from this equilibrium into mannitol. It has been observed that high temperature (>70 ^{o}C) and hydrogen pressure (70 atm) had no negative effects on the enzyme's activity or stability. The application of combined action of a transition-metal catalyst for the racemization and a lipase for the esterification in a consecutive step in organic solvents for the conversion of racemic secondary alcohols into their esters in high yields and high optical purities can be given as another example of bio-chemo cascade approach [7]. This type of synthesis for enantiomerically pure compounds from relatively inexpensive racemates has attracted attention from the fine chemical industry. Racemization-esterification reactions over ruthenium and lipase via bio-chemo one-pot synthesis concept have been demonstrated in the literature [8-9]. Only one of the enantiomers of a racemic secondary alcohol is esterified by lipase enzyme, while ruthenium catalyst was used as a racemization catalyst.

Synthesis of the drug sertraline can be given as an example of a chemo-chemo cascade application [7]. In this type of synthesis less solvents and recovery operations resulting in less waste were used whereas 20% higher yield was obtained compared to a traditional process. The chemo-chemo cascade application reduced the solvent requirement thus saved the costs and eliminated the environmental burden. One-pot chemo-chemo three steps preparation of taxol without isolation of intermediates was one of the most impressive applications of chemo-chemo cascades in large scale [7].

Chemical synthesis has been less favored than catalytic ones, since in the former cases such problems as poor selectivity, low yields and high costs of manufacturing can occur.

1.3 Substrate

Hydrogenation of carbonyl compounds is eminent and hydrogenation of acetophenone is a typical example of this category. Acetophenone (Figure 1.2a), a pro-chiral ketone, is widely used in heterogeneous catalytic reduction [10].

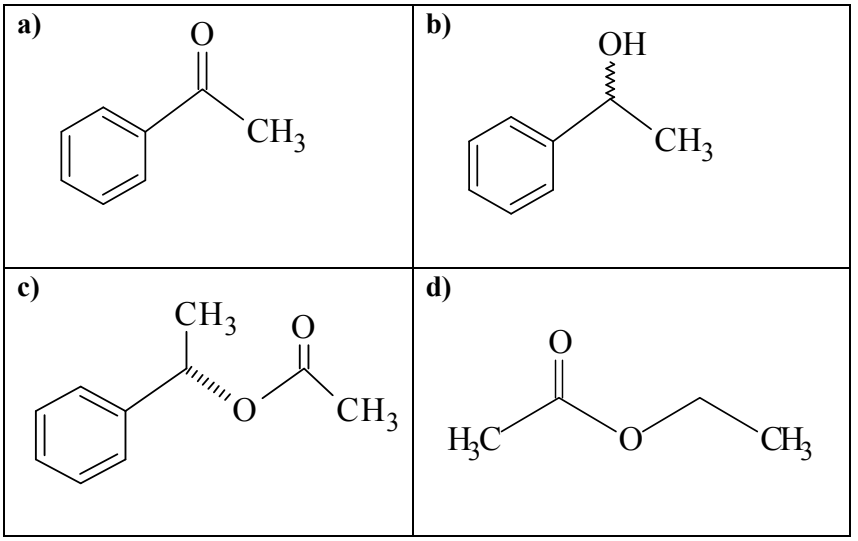


Figure 1.2. a) Acetophenone, b) racemic 1-phenylethanol, c) *(R)*-1-phenylethyl acetate, d) ethyl acetate.

1.3.1 Characteristic features of acetophenone hydrogenation

Acetophenone is one of the simplest molecules that includes two kinds of functional groups; carbonyl (C=O bond) and phenyl (a benzene ring as a substituent on a carbon chain, C=C bond) groups (Figure 1.2a). Besides, the control of hydrogenation selectivity of a molecule containing both carbonyl and phenyl groups is commercially important. The hydrogenation of acetophenone can produce racemic 1-phenylethanol (Figure 1.2b) through carbonyl hydrogenation process and ethyl benzene as a side product via dehydration of racemic 1-phenylethanol to styrene and its further hydrogenation to ethyl benzene.

1.3.2 The selection of metal catalyst

Palladium (Pd), platinum (Pt), nickel (Ni), and ruthenium (Ru) catalysts have been used as heterogeneous catalysts in hydrogenation. In the field of fine chemicals, Pd based catalysts are widely applied in the hydrogenation of acetophenone due to their high selectivity to hydrogenate only the carbonyl groups. Pd is the most active catalyst for hydrogenation of aromatic ketones to alcohols and it is used under mild conditions in many fine chemical operations. The properties of palladium based heterogeneous catalysts can be easily modified in order to increase selectivity. The performances of the catalysts are significantly affected by their pretreatment conditions. Supports also play an important role in the catalytic activity and product selectivity of precious metal catalysts.

1.4 Why lipases?

Lipases (triacylglycerol esters, EC 3.1.1.3) are ubiquitous enzymes that catalyze the hydrolysis of triglycerols to glycerol and fatty acids. Lipases are serine hydrolases in which the active site is generally characterized by the triad of amino acids composed of serine, histidine and aspartate [11]. Lipases are the most frequently used enzymatic catalysts in organic synthesis because they are stable at elevated temperatures over an extended pH range, commercially available with low costs, and do not require co-factors. These versatile

enzymes exhibit chiral properties and they can be used with a wide substrate range. Acyl–enzyme complexes are the crucial intermediates in all lipase-catalyzed reactions. Many lipases are active in organic solvents where they catalyze a number of reactions including esterification, transesterification (Figure 1.3), regioselective acylation etc. Lipase-catalyzed reactions of organic chemicals including kinetic resolution of chiral alcohols to organic esters were studied after establishing the stability of enzymes in organic media.

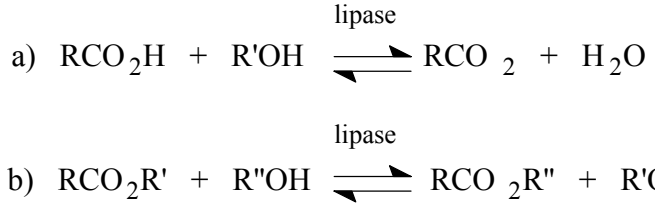


Figure 1.3. Lipase catalyzed a) esterification and b) transesterification.

The activation of lipases occurs only in the presence of a hydrophobic interphase such as lipid-water interface. The active site of lipases contains an amphipathic polypeptide unit so-called lid which covers the active site when the enzyme is in its inactive form [12]. Due to the interaction with a hydrophobic interface, the lid undergoes a movement in such a way that exposes the active site to the medium increasing the lipase activity by providing free access for the substrate (interfacial activation).

In many cases, the low catalytic efficiency and stability of native enzymes are considered as the main barriers for the development of industrial applications. The immobilization of lipases using hydrophobic materials promotes the thermal stability, the activity and the reusability. Moreover, the application of immobilized enzymes minimizes the cost of product isolation and provides operational flexibility.

In addition to their applications in organic chemistry in the synthesis of fine chemicals and pharmaceuticals, lipases are widely used in the processing of fats and oils, detergents and degreasing formulations, food processing, paper manufacture and production of cosmetics.

1.4.1 Challenges and advantages of lipase action in organic solvents

The use of enzyme-catalyzed reactions in organic solvents has several advantages such as (i) solubilization of hydrophobic substrates, (ii) easy recovery of the products and heterogeneous catalysts, (iii) enhanced thermostability of biocatalysts and (iv) shifting of thermodynamic equilibria in favor of synthesis over hydrolysis. Enzymes in non-aqueous solvents can catalyze a variety of reactions such as esterification, transesterification, aminolysis etc. catalyzed by lipases, while on the contrary these processes are completely suppressed by hydrolysis in water. Lipases are particularly gained acceptance to perform stereoselective biotransformations, such as kinetic resolutions, in organic solvents due to the fact that lipases are highly enantioselective, accept a multitude of substrates, usually retain high activity and stability which can be influenced by both the type and the concentration of a solvent. It has been shown that changing solvent influences the essential water associated with the enzyme. Solvents have different effects on the enzyme activity because of their ability to strip off the essential water from the catalyst is different [13].

Essential water which is the water around the enzyme to preserve the 3D structure of the protein in a catalytically active form acts as a molecular lubricant resulting in greater flexibility. Many enzymes have hydrophobic active sites folded inside of the molecule. The hydrophobic active site intend to disperse resulting in unfolding of the molecule with increasing the hydrophilicity of the solvent meaning that the enzyme activity decreases with increasing the polarity of the organic solvent. In order to predict the enzyme activity, both the hydrophobicity of the solvent and the reactants must be considered in order to retain enzymatic activity. Solvent hydrophobicity can be sufficiently characterized by dielectric constant and the logP value, indicating the logarithm of the partition coefficient in an octanol-water system [14]. The solvents with a log$P$ < 2 are not favorable for enzymatic systems, because they strongly distort the water-enzyme interaction, which is essential for the enzyme activity. Solvents with a log$P$ between 2 and 4 are weak water distorters and can affect enzymatic activity in an unpredictable manner. Finally, solvents with a log$P$ > 4 are not able to distort the water-enzyme interactions, thus the biocatalyst remains in an active state.

1.4.2 Lipase catalyzed kinetic resolution

Kinetic resolution with enzymes is the most widely used method for separating the two enantiomers of a racemic mixture. The majority of kinetic resolution of racemates has been performed with lipases. Kinetic resolution (Figure 1.4) begins with a racemic mixture and is based on the fact that the two enantiomers of a racemic mixture react at different rates. One of the enantiomers of the racemic mixture is transformed into the desired product while the other is recovered unchanged. After reaction, the mixture is enriched in the slower-reacting enantiomer.

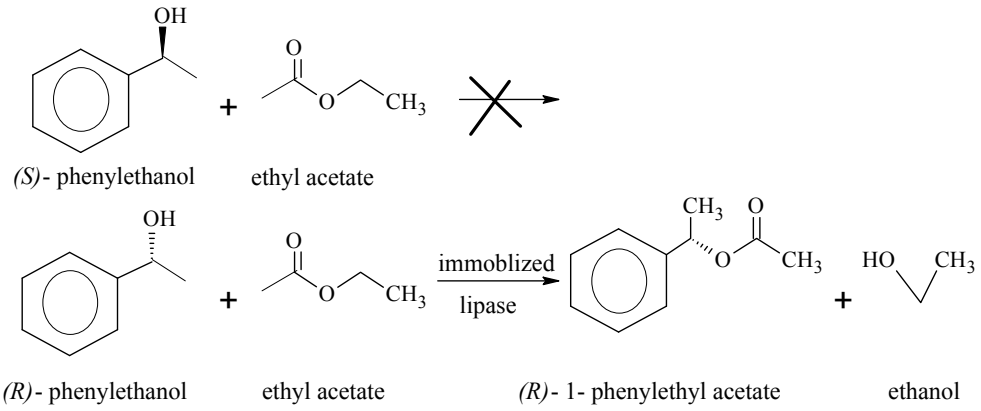


Figure 1.4. Reaction scheme of the enantioselective enzymatic kinetic resolution of racemic 1-phenylethanol with ethyl acetate over immobilized lipase.

The main drawback of this method, however, is that the chemical yield is limited only to 50%. Some additional processes, such as separation, racemization and repeated resolution can be performed to increase the yield. Furthermore, *in situ* racemization of the unwanted enantiomer over a racemisation catalyst together with the enzyme is another way to increase the yield and thus to overcome this limitation.

1.5 Aim and the scope of the research

The main objective of this work was to study a catalytic process for hydrogenation of acetophenone over a supported palladium catalyst followed by acylation reaction over an immobilized lipase at the same time in one reaction pot **[Publications I-VII]**. Mechanistic aspects of this reaction **[Publication I]** by using several different types of supported palladium catalysts **[Publications II-IV]** and different reactors **[Publication V]** were investigated. The main challenge, however, was to optimize the reaction conditions where the enzyme will maintain its activity whereas the hydrogenation reaction proceeds **[Publications I-III]**. Combination of the hydrogenation and acylation steps by using a heterogeneous palladium catalyst and an immobilized lipase for acylation in one-pot was the main driving force of this study. Achieving very high selectivity in the hydrogenation step is crucial since the support acidity highly effects the product distribution **[Publications IV-VI]**. Not only had the support of the metal catalyst but also its pretreatment had a massive impact on the activity and selectivity of the catalyst **[Publication VII]**.

The utilization of the continuous flow reactor for the kinetic resolution of racemic 1-phenylethanol with ethyl acetate in toluene over an immobilized lipase was performed to investigate the behavior of the immobilized lipase in a down-flow continuous reactor **[Publication VIII]**. The catalytic activity of the immobilized lipase, transient kinetics, mechanistic investigation of the effects of different compounds and their concentrations were studied for this reaction. The obtained data was linked to the one-pot synthesis carried out in a batch mode.

In spite of the fact that several kinetic studies have been carried out, only limited amount of appropriate kinetic analysis has been performed. Most of the kinetic modeling studies are based on initial reaction rates. There is a dearth of literature on kinetics and modeling of lipase-mediated chemo-bio cascade reactions. For these reasons, the purpose of this work was to propose a reaction mechanism which could describe kinetics of chemo-bio synthesis of *(R)*-1-phenylethyl acetate over Pd-Al_2O_3 and an immobilized lipase in ethyl acetate **[Publication IX]**.

2
EXPERIMENTAL

2.1 **Catalyst preparation**

2.1.1 Preparation of Pd-Al_2O_3-(UOP)-C, Pd-H-MCM-41C and Pd-SiO_2 catalysts

5% (w/w) Pd-Al_2O_3-(UOP)-C and 5% (w/w) Pd-SiO_2 (Merck) catalysts were prepared by vacuum evaporation impregnation (VEI) method in a rotary evaporator using an aqueous solution of palladium nitrate (Degussa) as a precursor. The catalysts were dried at 110 ^oC and calcined in a muffle oven.

2.1.2 Preparation of Pd-$CaCO_3$ catalyst

Pd $(NO_3)_2$ was used as a precursor. 3 g $CaCO_3$ was dissolved in 300 mL water at room temperature by stirring for 2 min. After precipitation the catalyst was dried at 60 ^oC overnight. The reduction of the catalyst was done under H_2 flow at 100^oC for 30 min with the temperature ramp 2 ^oC per min – 250 ^oC (2 hours). After cooling down to room temperature it has been passivated 48 hours under 1% O_2 in N_2.

2.1.3 Preparation of Pd-ZrO_2 catalyst

Pd $(NO_3)_2$ has been used as a precursor. 3 g ZrO_2 was dissolved in 300 mL water at room temperature by stirring for 5 min to obtain milky slurry. pH of the slurry was adjusted to 10 by NaOH addition. Clear water was detected after the precipitation. The same reduction and passivation methods have been followed as explained above.

2.1.4 Preparation of Pd-MgO catalyst

5% (w/w) Pd-MgO was prepared according to the method explained as in [15]. Precursor, $NaPdCl_4$, was prepared by a drop-wise addition of 0.1M NaOH to the solution of $PdCl_2$ with 30 min stirring. The final ratio for preformed solution, NaCl to $PdCl_2$, was 2.5 to 1, respectively. 2 g of MgO dissolved in 200 mL water, added to the preformed solution and stirred for 2 hours at room temperature. After the precipitation water has been removed, 200 mL of 0.1M NaOH was added, stirred for 2 hours and washed with 600 mL deionized water. The catalyst was dried for 24 hours at 60 ^oC. The same reduction and passivation methods have been followed as explained in 2.1.2.

2.1.5 Preparation of Pd-Al_2O_3 catalyst

0.5 g Pd-acetyl acetonate ($Pd(acac)_2$) was used as a precursor. A versal VGL type of aluminum with the fraction below 63 μm was mixed with the precursor in 200 mL technical acetone. The suspension was left overnight at room temperature for impregnation. Acetone was evaporated at 80 ^oC for 2 hours. The catalyst was reduced at 100 ^oC for 2 hours with temperature ramp 2 ^oC per minute and was passivated for 24 hours analogously to the catalyst 5% (w/w) Pd-$CaCO_3$.

2.1.6 Preparation of Pd-Si-MCM-41 catalyst

Synthesis of the Si-MCM-41 mesoporous molecular sieve was carried out in a 300 ml autoclave. The synthesis was performed by preparing solutions A, B and C. Solution A was prepared by mixing fumed silica (Aldrich) with distilled water under continuous stirring. Solution B was prepared by adding tetramethylammonium silicate (Sachem) to sodium silicate (Merck) and stirring for 15 minutes. Solution C was prepared by dissolving tetradecyl trimethyl ammonium bromide (Aldrich) to distilled water. Solution B was added to solution A slowly and stirred for 20 min, subsequently solution C was introduced under vigorous stirring. After measuring pH of the prepared gel it was introduced in a teflon cup, which was then inserted in an autoclave. The synthesis was performed at 100 ^{o}C in an oven. After completion of the synthesis, the reactor was quenched and mesoporous material was filtered and washed thoroughly with distilled water. Synthesized Si-MCM-41 was dried at 110 ^{o}C and calcined at 550 ^{o}C. 5% (w/w) Pd loaded Pd-Si-MCM-41 catalyst was prepared by VEI method in a rotator evaporator (Buchi) using aqueous solution of palladium nitrate as precursor for Pd. The catalysts were dried at 110 ^{o}C and calcined in a muffle oven.

2.1.7 Preparation of Pd-N-VGCF catalysts

Vapor-grown carbon fibers (Pyrograf Products, USA, product PR24-PS) were used as starting support. Vapor-grown carbon fibers (VGCFs) were first oxidized with concentrated nitric acid for 2 h at 100 ^{o}C. After washing and drying, the oxidized N-VGCFs were further treated with gaseous ammonia at 200 ^{o}C, 400 ^{o}C or 600 ^{o}C for 4 h, respectively. 2% (w/w) Pd/N-VGCF catalysts were subsequently prepared either by sol immobilization or incipient-wetness impregnation. The samples prepared sol immobilization obtained by direct reduction of Pd chloride with $NaBH_4$ in a PVA solution where the samples prepared by incipient-wetness impregnation using Pd nitrate was diluted in HNO_3. After drying at room temperature for overnight, the samples were calcined in air at 350 ^{o}C for 2 h.

2.2 Catalyst characterization

2.2.1 Transmission electron microscopy **[Publication VII]**

The microstructure of the supported palladium material was observed by transmission electron microscopy (TEM). The Pd-catalysts were reduced at 100 ^{o}C for 30 min under H_2 flow before the TEM analysis until otherwise stated. The microstructure of the reduced catalysts was investigated by high-resolution transmission electron microscopy (HRTEM), using a Philips CM200 FEG TEM operated at 200 kV. The samples were dispersed in chloroform and deposited on a holey carbon film supported on a Cu grid.

2.2.2 Scanning electron microscopy **[Publications IV, VII]**

The morphology and homogeneity of the samples were investigated with a Hitachi S-4800 scanning electron microscope (FE-SEM) equipped with SE and YAG-BSE detectors for imaging. The samples were loosely dispersed on conductive carbon tape. Images were first acquired with both SE and YAG-BSE detectors using an acceleration voltage of 15 kV in order to check the homogeneity of the samples, in particular the absence of any large metal aggregate. EDX spectra were also acquired at 15 kV primary electron energy. Quantification was done using the standard-less ZAF correction method in the Genesis

software from EDAX. High magnification images were acquired using an acceleration voltage of 1.5 kV for better resolution of surface features.

The SEM images of fresh and spent immobilized lipase catalysts were taken with the scanning electron microscope system Leo Gemini 1530 with a Thermo Scientific UltraDry Silicon Drift Detector (SDD) equipped with SE (secondary electron) and BSE (backscattered electron) detectors including an In-Lens detector.

2.2.3 X-ray diffraction **[Publications III, IV]**
The Pd crystallite size was determined by X-ray powder diffraction measurements on a Bragg-Brentano θ/2θ reflection geometry based Philips PW1820 diffractometer using nickel filtered Cu Ka (l=1.542 A˚) radiation operated at 40 kV/50 mA.

2.2.4 Carbon monoxide chemisorption **[Publications I, II, VII]**
The exposed surface area of the active metal phase and metal dispersion were studied with a pulse CO chemisorption method by applying a Micromimetics Autochem 2910 instrument, by reducing the catalysts with flowing hydrogen in situ at 100 ^{o}C for 2 h prior to the adsorption of CO for 30 min, unless otherwise stated. A gas mixture containing 10 vol% CO in helium was used for these measurements. The stoichiometric relationship between CO and Pd was assumed to be unity [16].

2.2.5 Temperature programmed reduction of hydrogen and temperature programmed desorption of carbon monoxide **[Publications IV, VI, VII]**
Temperature programmed desorption (TPD) is based on heating a sample in vacuum and simultaneously detecting the residual gas by means of a mass analyzer. Temperature is usually slowly raised (Micromeritics, Autochem 2910 apparatus). Certain species will escape as a result of temperature rise. The desorbed species will be detected as arise in pressure for a certain mass. Since desorption is performed in a vacuum, once a molecule has desorbed it is rapidly eliminated by the pumps. Moreover, because pressure is always very low and re-desorption could be neglected. The rate constant for desorption increases with increasing temperature and when it is high enough the coverage drops to zero as if all molecules left the surface thus the desorption goes through a maximum. The temperature of the peak maximum provides information on the binding energy of the bound species.

2.2.6 Nitrogen physisorption **[Publications II, III, VI, VII]**
The specific surface area of different powder catalysts was measured with N_2 physisorption at -196 ^{o}C (Carlo Erba Instruments, Sorptometer 1900). The samples were outgassed at 150 ^{o}C for 3 h prior to the measurements and the Brunauer, Emmett and Teller adsorption isotherm (B.E.T.) was used for calculations of the surface area.

2.2.7 Acidity measurements **[Publications II, IV, VI, VII]**
The acidity of Al_2O_3-(UOP) and H-MCM-41 supports was studied by pyridine adsorption using infrared spectroscopy (ATI Mattson FTIR). Molar extinction coefficient for pyridine was determined by [17]. The acidity of the MgO and SiO_2 supports was titrated using 0.01M NaOH and 0.01M KCl with the Titralab TIM 880 apparatus. The acid-base titrations of N-VGCF supports were performed to characterize the surface chemistry. Typically, **100**

mg of sample was dispersed in 50 mL of 10^{-3}M KCl solution and stirred for overnight. Prior to measurements, the mixture was degassed under Ar for at least 1 h until the pH value was constant. The titration was performed under Ar, using 10^{-2}M HCl solution. The initial pH ($pH_{initial}$) values of the solution were recorded.

2.3 Product analysis by gas chromatography

The products were analyzed by a gas chromatograph (Agilent Technologies) equipped with a chiral column (CP Chirasil Dex (250 μm×250 μm×25 m)) and a flame ionization detector. The samples were analyzed by using the following temperature programme 100 °C (1 min)-0.30 °C/min-130 °C-15 °C/min-200 °C (10 min). The temperature of the injector and the split ratio were 280 °C and 100:1, respectively. The GC-method was calibrated with the following chemicals: *(R)*-1-phenylethanol (Sigma), racemic 1-phenylethanol (Fluka, ≥98%) and ethyl benzene (Fluka, >99%).

2.4 Experimental set-up

2.4.1 Glass reactor

The glass reactor system has three subsidiary apparatus shown in Figure 2.1. The reactor was purged with Ar (AGA, 99.999%) to obtain air-free environment after transferring the chemo-catalyst to the reactor. The typical experiments were performed at 70 °C under atmospheric pressure of hydrogen (AGA, 99.999%) with a volumetric flow rate of 295 mL/min. The aim of the condenser was to minimize the evaporation of acyl donor and volatile compounds.

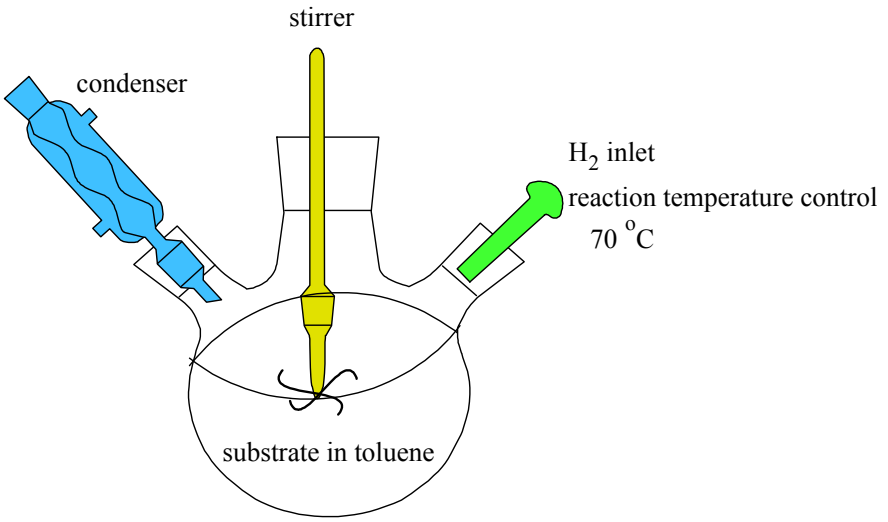


Figure 2.1. The glass reactor set-up.

2.4.2 Autoclave

The pressurized reactor system consisted of five sections as shown in Figure 2.2. Air-free environment was obtained by flushing the reactor with Ar. The typical experiments were carried out at 50 ^{o}C under 6 bars pressure of H_2 (AGA, 99.999%) with 650 rpm stirring speed.

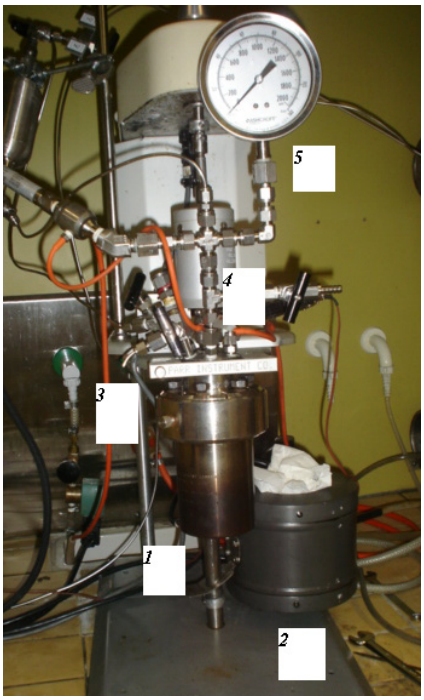


Figure 2.2. The pressurized reactor set-up: 1; reactor, 2; heater, 3; sampling unit, 4; stirrer, 5; H_2 inlet.

2.4.3 Fixed-bed reactor

The kinetic resolution of racemic 1-phenylethanol (Figure 1.4) with ethyl acetate was performed in a down-flow continuous reactor (Figure 2.3). The internal diameter and the length of the reactor are 1 cm and 12 cm, respectively. The reactor was filled with 3.5 cm quartz sand (> 355 μm) from the bottom on top of a 1 cm quartz wool layer. The catalyst bed, 2.25 cm, containing a mixture of 0.125 g immobilized lipase (Novozym 435) and 1.5 g quartz sand had a height of 2.9 cm. The uppermost quartz layer which dimension is 3.5 cm in length distributes the liquid flow above the catalyst bed.

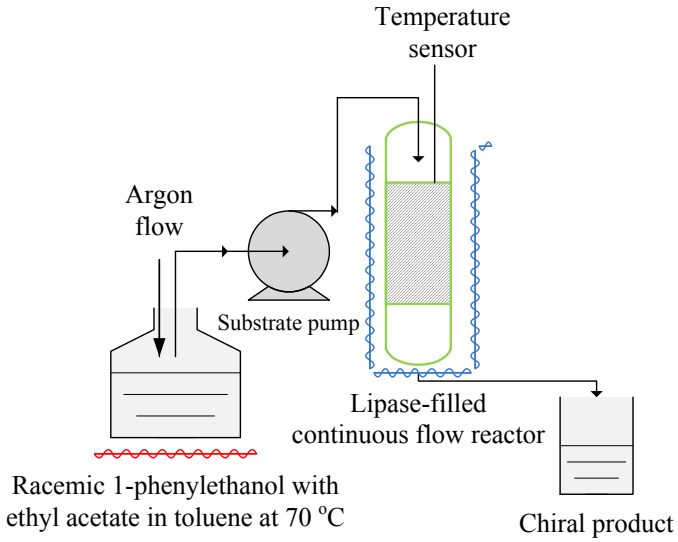


Figure 2.3. A simplified illustration of the experimental apparatus (~~: temperature control unit).

2.5 **Hydrogenation in batch mode**

Combination of the hydrogenation and acylation steps by using a heterogeneous chemo-catalyst, palladium catalysts (Pd) on several different supports, and an immobilized lipase Novozym 435, *Candida antarctica* lipase B produced by submerged fermentation of a genetically modified Aspergillus microorganism and immobilized on macroporous polyacrylate resin beads, bead size 0.3-0.9 mm, S= 95.50 m^2/g, average pore diameter 17.9 ηm, bulk density 430 kg m^{-3}, activity of 7,000 PLU g^{-1}, Sigma, USA [4, 18], for acylation in one-pot represents the current approach investigated in this study. Acetophenone (Acros, 99%) with 0.02 mol/L concentration was used as substrate if otherwise not stated. The hydrogenation experiments were performed under H_2 (AGA, 99.999%) flow.

2.5.1 Hydrogenation over chemo-catalysts in glass reactor

The typical experiments were performed at 70 °C under atmospheric pressure of H_2 with a volumetric flow rate of 295 mL/min in ethyl acetate (Sigma-Aldrich, >99.5%) which was used as an acyl donor as well. The stirring speed was 370 rpm. The liquid phase volume and the initial reactant concentration were 250 mL and 0.02 mol/L, respectively. The catalytic hydrogenation of acetophenone was carried out over Pd supported on different supports and the formed *(R)*-1-phenylethanol was acylated in the same pot to *(R)*-1-phenylethyl acetate with an immobilized lipase (Novozym 435). The masses of both hydrogenation catalyst and the immobilized lipase were 125 mg, if other amounts were not specified. The hydrogenation catalyst was pre-reduced with following hydrogen at 100 °C for 30 min, if otherwise was not stated, prior to injection of the lipase, substrate and the solvent.

2.5.1.1 Hydrogenation over Pd-N-VGCF catalysts in glass reactor
The experiments were performed at 70 ^{o}C in toluene (J.T. Baker, 99.5%) under atmospheric pressure of H_2 with a volumetric flow rate of 295 mL/min. The stirring speed was 500 rpm. The liquid phase volume and the initial reactant concentration were 125 mL and 0.02 mol/L, respectively. Ethyl acetate with the concentration of 0.06 mol/L was used as an acyl donor. The catalytic hydrogenation of acetophenone was carried out over 2% (w/w) Pd-N-HVGCF (312.5 mg) and the formed *(R)*-1-phenylethanol was acylated in the same pot to *(R)*-1-phenylethyl acetate with an immobilized lipase (Novozym 435, 62.5 mg). The experiments were performed with the following catalysts if otherwise was not specified; i) catalysts without pre-reduction, ii) catalysts reduced at 100 ^{o}C for 30 min, iii) catalysts reduced at 200 ^{o}C for 120 min under H_2 flow prior to injection of the lipase, substrate and the solvent.

2.5.2 Transformation of racemic 1-phenylethanol over metal catalysts in autoclave
The transformation of racemic 1-phenylethanol (Fluka, >98%, 305 mg, 2.5 mmol) was performed in an autoclave at 50 °C under 5.6 bar total pressure of H_2 or Ar (AGA, 99.999%). Acylation of racemic 1-phenylethanol was conducted in the presence of an immobilized lipase (Novozym 435). In a typical experiment, 125 mg Pd supported catalyst and 125 mg immobilized lipase were loaded into the reactor together with the liquid containing 0.02 mol/L the substrate. Ethyl acetate (125 mL) was used as a solvent and as an acyl donor. Thereafter, the reaction mixture was heated to the desired temperature and pressurized either with H_2 or with Ar. The reaction kinetics was measured under vigorous stirring (650 rpm).

2.6 Kinetic resolution in down flow continuous mode
The kinetic resolution of racemic 1-phenylethanol (Figure 1.4) with ethyl acetate was performed in a down-flow fixed-bed reactor operating in a continuous mode (Figure 2.3). In a standard experiment, the kinetic resolution of racemic 1-pheylethanol with ethyl acetate was perfomed at the molar ratio of 1:3 in 400 mL toluene at 70 ^{o}C. The volumetric flow rate was 3 mL min^{-1} under atmospheric pressure unless otherwise stated. The liquid phase including substrates was saturated with Ar prior pumping into the reactor. The reactor bed was washed with toluene at room temperature for 15 min before and after each and every experiment. The immobilized lipase was kept at +4 ^{o}C overnight between the experiments.

2.7 Transient kinetics
The following aspects were investigated; (i) the effect of different residence times by applying different flow rates, and (ii) the effect of different substrate concentrations, (iii) the effect of organic compounds related to one-pot synthesis, such as ethyl acetate, ethyl benzene, acetic acid, acetophenone etc., on the activity and stability of the immobilized lipase, (iv) the inhibitory effect of either the desired or the stoichiometric products (*R*-1-phenylethyl acetate and ethanol, respectively) on the activity and stability of the immobilized lipase, (v) the inhibitory effect of water on the activity and stability of the immobilized lipase, (vi) transient kinetics by applying step changes. The comparison between the continuous flow reactor and the batch reactor modes for the kinetic resolution of racemic 1-phenylethanol was discussed.

2.7.1 Residence time distribution by applying step changes and reactor hydrodynamics

Residence time distribution was investigated by applying step changes using either ethanol (Etax Aa, 99.5%) or hexane (J.T. Baker, 99%) or ethanol and hexane in toluene. The hydrodynamics of the reactor system was studied by following the responses to step changes in feed concentrations. The experiments were performed at room temperature (25 ^{o}C) due to suppress the possible evapotaion of corresponding compounds. The following experiments were done; 0.06M of i) ethanol or, ii) hexane was pumped into the reactor, iii) 0.06M ethanol and 0.06 M hexane mixture was pumped to the reactor. Reactions started with pumping toluene to the reactor for 10 min. The samples were collected in every 30 sec time-on-stream interval both from the inlet and outlet streams.

2.7.2 Transient kinetics of chemicals

The effect of acetophenone (Acros, 99%), ethyl benzene (Aldrich), acetic acid (J.T. Baker, 99-100%), H_2O on the activity and stability of the immobilized lipase was investigated. Moreover, the inhibitory effect of either the desired or the stoichiometric products (*(R)*-1-phenylethyl acetate (Acros, 96+%) and ethanol (Etax Aa, 99.5%), respectively) on the activity and stability of the immobilized lipase was studied as well. The concentrations of racemic 1-phenylethanol and ethyl acetate were kept constant, 0.02M and 0.06M, respectively. After each start of the reaction, the known concentration of the compounds investigated (0.02 M) was added to the known concentration of the liquid phase at definete time intervals.

2.7.4 Catalyst deactivation

The activity of the immobilized lipase was determined by comparing the activities obtained over fresh and spent catalysts. The experiments were performed by using 0.02 mol/L racemic 1-phenylethanol and 0.06 mol/L ethyl acetate in 400 mL toluene with 3 mL min^{-1} flow rate. The very same experiments were carried out 30 days after the first experiment. SEM images of the fresh and spent catalysts were taken in order to visualize any morphological changes to explain activity decrease.

3
EXPERIMENTAL RESULTS AND DISCUSSION

3.1 Catalyst characterization [I-IV, VI-VII]

The specific surface areas of the catalysts calculated by BET are given in Table 3.1. The largest BET specific surface areas were obtained for Pd-C (Aldrich) and Pd-C (Degussa) 1214 m^2/g_{cat} and 949 m^2/g_{cat}, respectively. The third highest BET specific surface area was obtained for Pd-H-MCM-41C (Table 3.1), whereas all the other catalysts exhibited the BET surface areas in a range of 75-379 m^2/g_{cat}.

The metal particle sizes were determined by XRD for Pd-Al_2O_3 and for Pd-H-MCM-41 (Table 3.1, Figure 3.1a). For Pd-H-MCM-41 catalyst reduced at 100 °C for 30 min the following Pd peaks were found: Pd(111), at 2(39.962 °C, peak Pd(200) at 47.208 °C, peak Pd(220) at 68.083 °C and Pd(311) at 81.2 °C at 81.2. An average Pd crystallite size for this catalyst was 3.9 nm. Analogously for Si-MCM-41 the average Pd crystallite size was determined from XRD (Figure 3.1b) being 7.3 nm. The same phases were observed for Pd-Si-MCM-41 as for Pd-H-MCM-41, but the angles were slightly shifted to larger levels for the former catalyst. For reduced Pd-Al_2O_3 catalyst only three forms of Pd were observed namely Pd(111), 40.1°2θ, Pd(200), 46.1°2θ and Pd(311), 82.2°2θ giving an average Pd crystallite size of 7.1 nm. Pd particles were smaller in the mesoporous Pd-H-MCM-41 exhibiting both a higher concentration of Brønsted acid sites as well as 2.9 fold larger BET surface area than that of Pd-Al_2O_3. This result indicated that the larger BET surface area and the higher concentration of Brønsted acid sites facilitated the preparation of more dispersed Pd particles over H-MCM-41 than over Pd-Al_2O_3 since the nominal metal loading was the same. Furthermore the Pd crystallites were the largest in Pd-Si-MCM-41 from the three studied catalyst, while the Pd crystallite size from Pd-SiO_2 was not determined, since its activity in the test reaction was low.

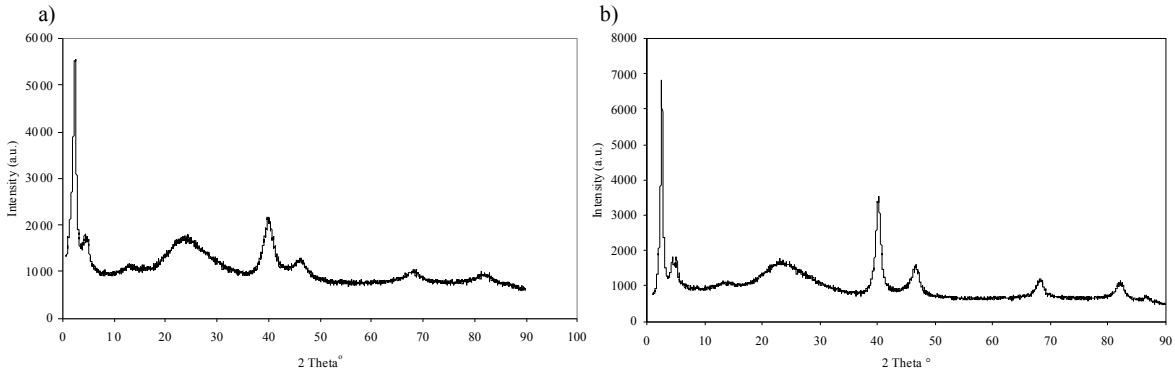


Figure 3.1. The XRD pattern of the reduced a) Pd-H-MCM-41 and b) Pd-Si-MCM-41. Reduction procedure: 100 °C for 30 min.

Table 3.1. Catalyst characterization results.

Catalyst	BET specific surface area (m^2/g_{cat})	Dispersion (%)	Concentration of Brønsted acid sites ($\mu mol/g_{support}$)	Concentration of Lewis acid sites ($\mu mol/g_{support}$)	Avarage Pd crystallite size with XRD (nm)	Publication
Pd-C (Aldrich)	1214	42	-	-	-	**II-V**
Pd-C (Degussa)	949	54	-	-	3.1	**VI**
Pd-H-MCM-41	902	-	89	168	3.9	**IV**
Pd-Si-MCM-41	379	-	0	0	7.3	**IV**
Pd-SiO_2	351	-	0	0	n.m.	**IV**
Pd-Al_2O_3-UOP	306	-	7	156	7.1	**IV**
Pd-Al_2O_3 (Aldrich)	115	-	7	156	-	**IV**
Pd-MgO	106	7	-	-	-	**II**
Pd-ZrO_2	75	11	-	-	-	**I**

n.m.: not measured.

The amount of hydrogen desorbed was determined for Pd-SiO_2 and for Pd-Al_2O_3. The latter catalyst exhibited 3.4 fold higher amount of desorbed hydrogen than the former one (Table 3.1). Furthermore, hydrogen desorption had two maxima in case of Pd-Al_2O_3, i.e. at 307 °C and at 367 °C indicating that there are two energetically different adsorption sites available on the catalyst surface (Figure 3.2), whereas only one temperature maximum for hydrogen desorption was seen for Pd-SiO_2.

Table 3.2. H_2 TPD results for Pd-Al_2O_3 and for Pd-SiO_2. The TPD was 25 °C-10 °C/min-700 °C (60 min)

Catalyst	Total amount of hydrogen desorbed (mmol/$g_{cat.}$)	T_{max} (°C)
Pd-Al_2O_3	0.0067	307, 367
Pd-SiO_2	0.002	364

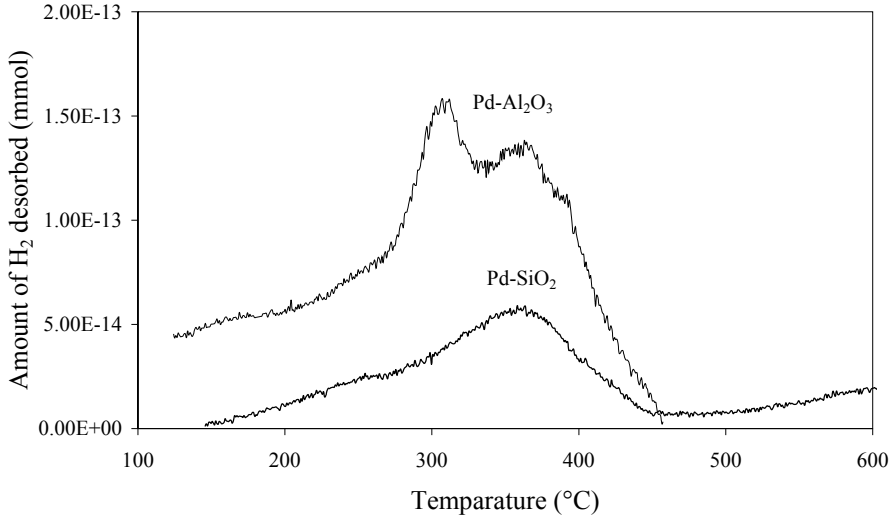


Figure 3.2. Hydrogen TPD from Pd-SiO_2 and Pd-Al_2O_3 catalysts.

The acidity of the two supports, Al_2O_3 and H-MCM-41 was determined with pyridine adsorption (Table 3.1). The former one exhibited only a small concentration of Brønsted acid sites; whereas their concentration in H-MCM-41 was 12.7 fold that of the former one. In both support materials the concentration of Lewis acid sites was about the same (**Publication IV**).

The low metal specific surface areas of Pd-N-VGCFs are due to the wall thickness of the N-VGCFs [14]. The catalyst support must preferably possess a high surface area in order to allow a good dispersion of the active phase [14]. The total surface area determined from the BET equation (Tables 3.3 and 3.4). The total surface area of the samples ranges from 29 $m^2\ g^{-1}$ to 35 $m^2\ g^{-1}$ for the catalysts prepared by sol immobilization (Table 3.3) and from 43 $m^2\ g^{-1}$ to 49 $m^2\ g^{-1}$ for the catalysts prepared by incipient-wetness

impregnation (Table 3.4) (**Publications VI, VII**). This observation is in agreement with a previous study showing the correlation between specific surface area, carbon nanotube diameter and number of walls [16].

The metal dispersions were higher for the catalysts prepared by incipient-wetness impregnation than the ones prepared by sol immobilization (Table 3.3, **Publication VII**). The metal dispersions for the catalysts prepared by sol immobilization decreased with increasing gaseous ammonia treatment temperature when the catalysts were reduced at 100 ^{o}C for 30 min under H_2 flow. Slightly higher metal dispersions were, however, achieved when these catalysts were reduced at 200 ^{o}C for 120 min under H_2 flow than when reduced at 100 ^{o}C for 30 min under H_2 flow. This result can be explained by the fact that Pd was not totally reduced at 100 ^{o}C according to TPR results (see below) and thus more metallic Pd was available when the reduction was performed at 200 ^{o}C. Furthermore, at 200 ^{o}C, no sintering took place. The highest metal dispersions were achieved with the catalysts prepared by incipient-wetness impregnation and treated with gaseous ammonia at 200 ^{o}C **(Publication VII).**

Table 3.3. Catalyst characterization results of the catalysts prepared by sol immobilization.

Catalyst	NH_3 treatment temperature (^{o}C)	BET specific surface area (m^2/g_{cat})	Dispersion (%)
Pd-N-VGCF	200[a]	32	13
	200[b]	32	18
Pd-N-VGCF	400[a]	35	11
	400[b]	35	19
Pd-N-VGCF	600[a]	29	9
	600[b]	29	16

[a, b] Pd catalysts were pre-reduced at 100 ^{o}C for 30 min and 200 ^{o}C for 120 min under H_2 flow, respectively.

Table 3.4. Catalyst characterization results of the catalysts prepared by incipient-wetness impregnation.

Catalyst	NH_3 treatment temperature (^{o}C)	BET specific surface area (m^2/g_{cat})	Dispersion (%)
Pd-N-VGCF	200[a]	43	54
Pd-N-VGCF	400[a]	49	32
Pd-N-VGCF	600[a]	43	41

[a] Pd catalysts were pre-reduced at 100 ^{o}C for 30 min under H_2 flow.

TPR was performed for the fresh catalysts. Temperature reduction results for the fresh catalysts prepared by sol immobilization exhibited that high reduction temperatures

needed for the catalyst treated at 200 °C with gaseous ammonia since the highest hydrogen uptake was at 310 °C (Figure 3.3). The hydrogen uptake temperatures decreased with increasing gaseous ammonia treatment temperatures of the catalysts prepared by sol immobilization. In the case of catalysts prepared by sol immobilization, a small negative peak with maximum at around 78 °C can be attributed for Pd existing in another oxidic state, such as Pd-polyhydroxyl [17]. The negative hydrogen uptake in catalysts prepared by sol immobilization is related to formation of palladium hydride and their decomposition [18]. According to the literature [18] the amount of hydride and its desorption temperature increase with decreasing metal dispersion. The maximum temperatures for hydrogen uptake for the catalysts prepared by sol immobilization and treated with gaseous ammonia either at 200 °C, 400 °C or at 600 °C prior to Pd addition were 310 °C, 242 °C and 225 °C, respectively (Figure 3.3). This result indicated that when the catalyst was treated at higher temperature with gaseous ammonia, it was easier to reduce the metal. The highest temperatures for the hydrogen uptake for the catalysts prepared by incipient-wetness immobilization and treated with NH_3 at 200 °C, 400 °C or 600 °C prior to Pd addition were 120 °C, 188 °C and 310 °C, respectively (Figure 3.4). This result, on the other hand, showed an opposite trend compared to the trend achieved for the catalysts reduced by sol immobilization method.

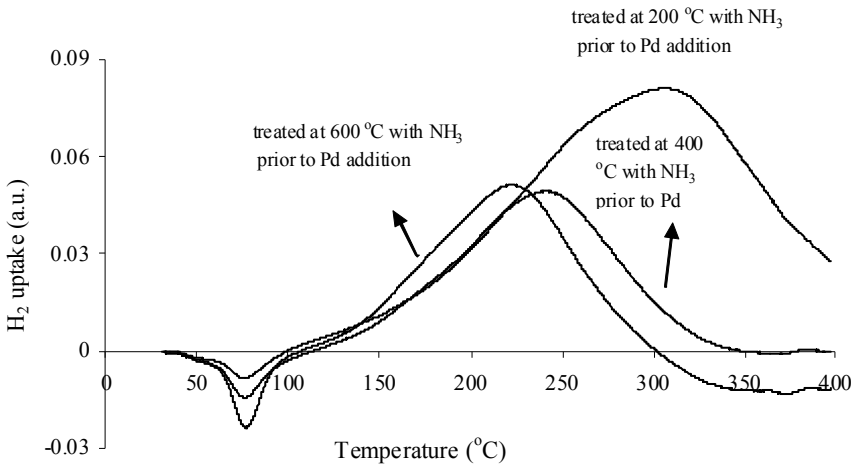


Figure 3.3. Temperature programmed reduction for the fresh catalysts prepared by sol immobilization.

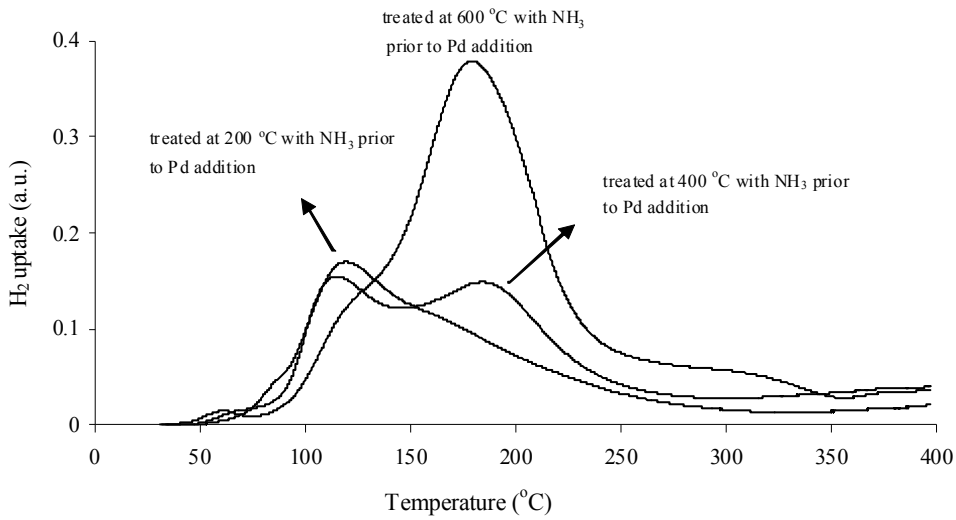
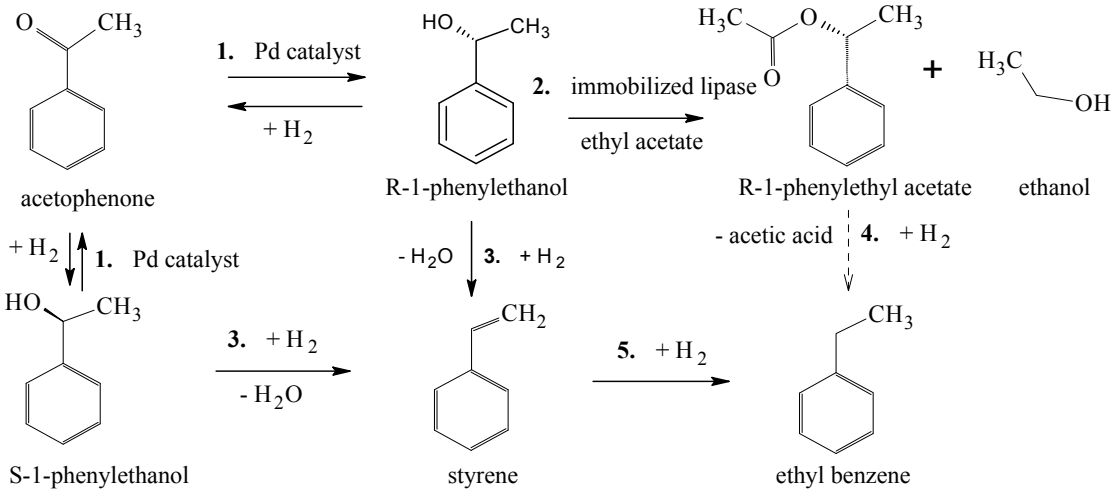


Figure 3.4. Temperature programmed reduction for the fresh catalysts prepared by incipient-wetness impregnation.

3.2 Catalyst screening [I-VII]

In the catalyst screening study, the performances of the catalysts were evaluated due to the following definitions: initial hydrogenation and acylation rates which were defined as mmol per time unit and amount of catalyst; the fractional yield, i.e., the formed moles of the desired product divided by the initial amount of the reactant (acetophenone) and the selectivity as a ratio between the moles of desired product divided by the total molar amount of products. The liquid phase mass balance was calculated as addition of the molar amounts of acetophenone, racemic 1-phenylethanol, *(R)*-1-phenylethyl acetate, ethyl benzene, water and taking into account stoichiometric amounts of ethanol and it was close to 100%. The formation of acetic acid was also taken into account.

The combination of hydrogenation and acylation steps using a heterogeneous Pd supported catalyst for selective hydrogenation of acetophenone and an immobilized enzyme for the acylation step was investigated. The proposed reaction scheme is demonstrated in Scheme 3.1.



Scheme 3.1. Reaction scheme for cascade one-pot synthesis of *(R)*-1-phenylethyl acetate starting from acetophenone hydrogenation. Reaction steps: 1. hydrogenation of acetophenone to racemic 1-phenylethanol, 2. acylation of *(R)*-1-phenylethanol to *(R)*-1-phenylethyl acetate over immobilized lipase and formation of ethanol, 3. dehydration of racemic 1-phenylethanol in the presence of an acidic catalyst, 4. debenzylation of *(R)*-1-phenylethyl acetate and formation of acetic acid, 5. hydrogenation of styrene to ethyl benzene.

Hydrogenation of acetophenone over Pd leads to racemic 1-phenylethanol and is followed by the consecutive acylation of *(R)*-1-phenylethanol to *(R)*-1-phenylethyl acetate over lipase. The main side reaction is dehydration of racemic 1-phenylethanol to styrene, which occurs on the supports, possessing acidic properties. Due to the availability of hydrogen the subsequent hydrogenation of styrene over Pd catalyst to ethyl benzene is feasible and in fact proceeds very fast, since the amounts of styrene in the reaction mixtures were below 1%.

3.2.1 Catalytic screening of Pd on C, MgO, ZrO_2, Al_2O_3 **[I-II]**

The highest acetophenone conversions were achieved over Pd-ZrO_2 catalyst followed by Pd-C, Pd-Al_2O_3 (Aldrich) and Pd-MgO. The latter catalyst exhibited the lowest conversion among all four types of catalysts (Table 3.5), which can be explained by the relatively low surface area and low Pd dispersion (Table 3.1). Moreover this catalyst was neutralized with NaOH (*2.1.4*). The conversion levels after prolonged reaction times over the three most active catalysts were close to 100%. Over Pd-MgO the catalytic activity was low and the conversion of acetophenone remained at 27% after 1300 min reaction time (Table 3.5).

The main reaction product was ethyl benzene with the yield of 62% after 1600 min of reaction time in the preliminary experiments over Pd-C and immobilized lipase catalysts. Ethyl benzene is mainly formed from the fast hydrogenation of styrene which

is the dehydration product of racemic 1-phenylethanol, while the yield of the desired product, *(R)*-1-phenylethyl acetate, was below 1wt% at the end of the reaction carried out over Pd-C. Ethanol was formed in the stoichiometric amounts related to the desired product (Scheme 3.1).

Table 3.5. Catalytic synthesis of *(R)*-1-phenylethyl acetate over Pd on C, MgO, ZrO_2, Al_2O_3.

Catalyst	Conversion after 1360 min (%)	Selectivity to *(R)*-1-phenylethyl acetate (%)[a]
Pd-C (Aldrich)	99	2[a]
Pd-ZrO_2	98	4[a]
Pd-Al_2O_3 (Aldrich)	97	4[a]
Pd-MgO	27	30[b]

[a] maximum selectivity at 95% conversion, [b] at conversion of 25%.

3.2.2 Catalytic screening of Pd on H-MCM-41, Al_2O_3-UOP, Si-MCM-41 and SiO_2 **[III-IV]**

The total initial transformation rate of acetophenone decreased as follows: Pd-H-MCM-41 > Pd-Al_2O_3 > Pd-Si-MCM-41 > Pd-SiO_2. The third lowest activity was observed for Pd-Si-MCM-41 exhibiting the largest Pd crystallite size of the three studied catalysts in XRD (Table 3.1). This result indicates that smaller Pd particles enhanced hydrogenation of acetophenone. Furthermore, the two most active catalysts possessing Brønsted acidity (Table 3.1) catalyzed both hydrogenation and dehydration of the formed racemic 1-phenylethanol.

The catalyst exhibiting the lowest activity, i.e. Pd-SiO_2 was characterized by hydrogen desorption technique and compared with the performance and hydrogen desorption capacity of Pd-Al_2O_3 (Tables 3.4 and 3.2, Figure 3.2). The latter catalyst was relatively active in the hydrogenation, since conversion of acetophenone over Pd-SiO_2 catalyst was 7% while this value was 47% over Pd-Al_2O_3 catalyst (Table 3.4). When correlating this value to the amounts of hydrogen desorbed, it was clearly visible that the amount of hydrogen desorbed from the former catalyst was only 30% of the value for Pd-Al_2O_3 indicating that there was a correlation between the acetophenone conversion and the amount of hydrogen desorbed form the catalyst. The temperatures at which the maximum amounts of desorbed hydrogen were measured for these two catalysts were about the same thus indicating that only the different amounts of hydrogen available at the catalyst surfaces affected the rates, since the adsorption strengths for hydrogen for the two catalysts should be similar (Table 3.2, Figure 3.2).

Table 3.4. Catalytic synthesis of *(R)*-1-phenylethyl acetate over Pd on H-MCM-41, Al_2O_3-UOP, Si-MCM-41 and SiO_2.

Catalyst	Conversion after 1600 min (%)	Selectivity to *(R)*-1-phenylethyl acetate (%)
Pd-H-MCM-41	92	7[a], 20 (93)[b]
Pd-Al_2O_3-UOP	47	16[a], 47 (46)[b]
Pd-Si-MCM-41	15	0[a], 49[c]
Pd-SiO_2	7	47[d]

[a] 40% conversion, [b] Selectivity after 1700 min, conversion in parenthesis in %, [c] 15% conversion, [d] 7% conversion.

The conversions over four different catalysts after prolonged reaction times decreased as follows: Pd-H-MCM-41 > Pd-Al_2O_3 > Pd-Si-MCM-41 > Pd-SiO_2 (Table 3.4). The deactivation of Pd catalysts was clearly visible after prolonged reaction times. Several possible reasons for Pd catalysts deactivation were investigated. The results were discussed extensively in **Publication IV**.

3.2.3 Catalytic screening of Pd on C and N-VGCF **[I, II, V-VII]**

Activated carbons (C) as catalyst supports present several advantages being relatively inexpensive and inert materials [19]. They are one of the most used materials as catalytic support in industrial reactions mainly because of their inertness, high surface area, easy recovery of metal phase in the spent catalyst, low deactivation and mechanical strength [20]. However, they also exhibit a major drawback as their surface properties can vary from batch to batch. Furthermore, typically Pd-C catalysts exhibit acidic surface groups, such as carbonyl, carboxylic, phenolic hydroxyl, lactone and quinone groups [21]. It was reported that Brønsted acid sites enhance the hydrogenolysis of secondary alcohols, such as 1-phenylethanol (**Publications II**, **V**, **VI**). The results showed that the selection of the support material is crucial in the one-pot cascade catalytic synthesis of *R*-1-phenylethyl acetate. Over an acidic Pd-C and lipase catalytic system the main product was ethyl benzene with the yield of 30% after 300 min reaction time (**Publications I**, **II**). It should be noted that the amount of the ethyl benzene was dependent on the catalyst acidity, since with non-acidic supports only below 4% of ethyl benzene was formed.

Since the results with Pd-C catalysts were not very good due to side reactions, Pd catalysts supported on vapor-grown carbon nanofiber with nitrogen-containing surface groups (N-VGCF) were tested in one-pot synthesis of *(R)*-1-phenylethyl acetate starting from acetophenone hydrogenation (**Publications VI**, **VII**). Incipient-wetness impregnation was applied for catalyst synthesis or sol immobilization catalysts were applied. Not only the effect of the catalyst preparation method on the synthesis of *(R)*-1-phenylethyl acetate in one-pot with an immobilized lipase but also the effect of catalyst treatment procedure was investigated. VGCFs possess unique properties such as adjustable surface properties, high surface area and high mechanical stability [22]. Typically, the dispersion of the metal can be improved by pre-treating the carbon nanotubes in order to introduce functional groups (e.g. oxygen-containing surface

groups) to the surface to enhancing interactions between the support and the catalyst precursor [23, 24].
The catalytic activity of Pd catalysts either on C or on N-VGCF was studied in the one-pot synthesis of *(R)*-1-phenylethyl acetate. The aim of this work was to investigate the influence of the acid-base properties of the support on the catalytic activity by treating oxidized VGCFs with gaseous ammonia (NH_3) at different temperatures in order to introduce various amounts of basic N-containing groups on the surface [25], the effect of preparation methods and the effect of the catalyst pretreatment temperature on the catalytic activity.

The highest acetophenone conversion was obtained over 5% (w/w) Pd-C. However, the yield of *(R)*-1-phenylethyl acetate was only 13%, since ethyl benzene was formed as a major product due to the acidic support (**Publication VI**). The maximum acetophenone conversion over 2% (w/w) Pd-N-VGCF was 75% corresponding to 34% selectivity over 312.5 mg of 2% (w/w) Pd-N-VGCF catalyst in combination with 62.5 mg of immobilized lipase. Furthermore, the yield of (*R*-1)-phenylethyl acetate as well as the conversion of acetophenone increased with an increased basicity of the support material. At the same conversion level, the results showed that the most selective catalyst was Pd-N-VGCF in which the support was treated at 200 ^{o}C with NH_3 prior to Pd addition exhibiting the lowest acidity of the three studied N-VGCF-catalysts as discussed in **Publication VI** (Table 3.5).

Table 3.5. Kinetic results using Pd either on C or N-VGCF catalysts. Pd-N-VGCF catalysts prepared by incipient-wetness impregnation and reduced at 200 ^{o}C for 120 min under H_2 flow.

Catalyst	Conversion after 480 min (%)	Selectivity to *(R)*-1-phenylethyl acetate (%)
Pd/N-VGCF[a]	26	41[d]
Pd/N-VGCF[b]	66	36[e]
Pd/N-VGCF[c]	75	34[e]
Pd/C	96	23[e]

[a, b, c] N-VGCF treated with NH_3 at 200 ^{o}C, 400 ^{o}C, 600 ^{o}C, respectively, [d] selectivity to *(R)*-1-phenylethyl acetate at 26% conversion after 480 min, [e] selectivity to *(R)*-1-phenylethyl acetate at 66% conversion.

The highest acetophenone conversion was 98% after 480 min corresponding to 36% *(R)*-1-phenylethyl acetate selectivity over the catalyst prepared by sol immobilization treated with gaseous ammonia at 400 ^{o}C prior to palladium addition which was used without the pre-reduction. Although, the catalyst prepared by sol immobilization treated with gaseous ammonia (NH_3) at 600 ^{o}C prior to palladium addition and used without reduction displayed the same acetophenone conversion, 98% after conversion 480 min reaction times, the selectivity to *(R)*-1-phenylethyl acetate was 30% at this acetophenone conversion over 312.5 mg of Pd-catalyst in combination with 62.5 mg of immobilized lipase (**Publication VII**). The lowest selectivity at 90% acetophenone conversion was obtained with the catalyst prepared by incipient-wetness impregnation treated with gaseous ammonia at 200 ^{o}C and further treated at 100 ^{o}C for 30 min under H_2 flow

where the highest selectivity, 38%, at 90% conversion was with the catalyst prepared by incipient-wetness impregnation treated with gaseous ammonia at 600 ^{o}C and further treated at 100 ^{o}C for 30 min under H_2 flow. At the same conversion levels, 90%, the most selective catalyst was 2% (w/w) Pd-N-VGCF prepared by incipient-wetness impregnation and treated at 400 ^{o}C with gaseous ammonia prior to Pd addition and reduced at 100 ^{o}C for 30 min under H_2 flow prior to the experiment. The catalysts prepared by incipient-wetness impregnation and treated with gaseous ammonia at 400 ^{o}C especially catalyzed the formation of ethyl benzene resulting from diffusion limitations and secondary reactions [26] which often lead to low selectivity of the desired product.

Table 3.6. Catalytic synthesis of *(R)*-1-phenylethyl acetate over Pd-N-VGCF prepared by sol immobilization.

Catalyst	NH_3 treatment temperature (^{o}C)	Conversion after 300 min (%)	Conversion after 480 min (%)	Selectivity to *(R)*-1-phenylethyl acetate (%)
Pd-N-VGCF[a]	200[a]	67	82 (97)	19[d] (25)[e]
	200[b]	33	47 (78)	38[d] (19)[f]
	200[c]	7	10 (30)	17[d] (38)[g]
Pd-N-VGCF[b]	400[a]	95	98 (99)	15[d] (33)[e]
	400[b]	76	89 (98)	26[d] (33)[e]
	400[c]	32	45 (75)	32[d] (21)[h]
Pd-N-VGCF[c]	600[a]	96	98 (98)	6[d] (24)[e]
	600[b]	77	88 (98)	22[d] (31)[e]
	600[c]	54	71 (94)	27[d] (18)[e]

[a, b, c] Pd catalysts without pre-reduction, pre-reduced at 100 ^{o}C for 30 min and at 200 ^{o}C for 120 min, respectively; [d] selectivities to *(R)*-1-phenylethyl acetate at 30 % reactant conversion; [e] selectivities to *(R)*-1-phenylethyl acetate at 90 % reactant conversion; [f, g, h] selectivities to *(R)*-1-phenylethyl acetate at 78%, 10% and 75% reactant conversions, respectively. The conversions in parenthesis were obtained after 1320 min reaction times.

Table 3.7. Catalytic synthesis of *(R)*-1-phenylethyl acetate over over Pd-N-VGCF prepared by incipient-wetness impregnation.

Catalyst	NH_3 treatment temperature (^{o}C)	Conversion after 300 min (%)	Conversion after 480 min (%)	Selectivity to *(R)*-1-phenylethyl acetate (%)
Pd-N-VGCF[a]	200[a]	52	83 (98)	27[c] (17)[d]
	200[b]	57	75 (96)	18[c] (8)[d]
Pd-N-VGCF[b]	400[a]	93	98 (98)	3[c] (17)[d]
	400[b]	75	86 (98)	17[c] (15)[d]
Pd-N-VGCF[c]	600[a]	93	98 (99)	20[c] (25)[d]
	600[b]	85	94 (98)	21[c] (38)[d]

[a, b] Pd catalysts without pre-reduction and pre-reduced at 100 ^{o}C for 30 min, respectively; [c] selectivities to *(R)*-1-phenylethyl acetate at 30 % reactant conversion; [d] selectivities to *(R)*-1-phenylethyl acetate at 90 % reactant conversion. The conversions in parenthesis were obtained after 1290 min reaction times.

The increasing ammonia treatment temperature for the catalysts prepared by either sol immobilization or incipient-wetness impregnation increased acetophenone conversions after prolonged reaction times. Furthermore, the fresh catalysts exhibited higher acetophenone conversions than the reduced ones. The catalysts prepared by sol immobilization and reduced at 200 ^{o}C for 120 min exhibited lower conversions than the catalysts reduced at 100 ^{o}C for 30 min under H_2 flow prior to the experiments.

The hydrogenation rates and conversions were not affected so much by the catalyst reduction temperatures when the average particle sizes were small (Table 3.1) as in the case of the catalysts prepared by incipient-wetness impregnation (Tables 3.6 and 3.7). TPR-results showed that it is easier to reduce the catalysts prepared by incipient-wetness impregnation than the catalysts prepared by sol immobilization. The results were discussed in detail in **Publication VII**.

3.3 Catalyst testing [III]

The aim of the current work was to study systematically the kinetics in one-pot hydrogenation-acylation reaction using 5% (w/w) Pd-Al_2O_3 in combination with immobilized lipase but varying the amounts of both the chemo-catalyst and the enzyme. The following series of experiments were performed: i) experiments using Pd-Al_2O_3 as a hydrogenation catalyst with and without lipase, ii) experiments with varying amount of Pd-Al_2O_3 while keeping the mass of lipase constant, iii) experiments with varying amount of lipase while keeping the mass of Pd-Al_2O_3 constant and iv) keeping the mass ratio of Pd-Al_2O_3 to lipase constant but varying the amount of the catalysts.

The conversion of acetophenone was slightly higher in the absence of lipase than in the presence of it being 13% and 10% after 1400 min, respectively in the first series. These results indicate that there is an effect of lipase on the hydrogenation step as well. An additional experiment was performed over Pd-Al_2O_3 catalyst without lipase in ethanol which is a stoichiometric product of acylation step (see Scheme 3.1). The conversion of acetophenone was 92% after 1660 min, indicating that the chemo-catalyst deactivation in ethanol as the solvent is not significant.

In the second series with varying amounts of Pd-Al_2O_3 and keeping the amount of lipase constant in the hydrogenation, the conversion levels of acetophenone after 1400 min reaction time were 10 mol%, 46 mol% and 97 mol% with 62.5 mg, 125 mg and 250 mg Pd/Al_2O_3, respectively. The catalyst deactivation became dominant when high reactant-to-chemo-catalyst mass ratios were used.

Varying amounts of lipase in combination with a constant amount of Pd-Al_2O_3 were used in the third series of the experiments. The conversion of acetophenone was the highest with the lowest amount of lipase, whereas with 125 mg and 250 mg of lipase, the conversion of acetophenone was between 46-48%, respectively. The hydrogenation rates were very slow with higher amounts of lipase.

In the fourth series the mass ratio of Pd-Al_2O_3 to lipase was kept constant equal to 2, but the amounts of catalysts were increasing twofold. The acetophenone conversion was

close to 100% conversions using 250 mg Pd-Al_2O_3. However, the catalyst deactivation is more prominent on the Pd surface, when at the same time the mass ratio between acetophenone to Pd was increased.

Table 3.7. Catalytic synthesis of *(R)*-1-phenylethyl acetate over Pd-Al_2O_3 prepared by incipient-wetness impregnation.

Amount of Pd/Al_2O_3 (mg)	Amount of lipase (mg)	Conversion after 1400 min (%)	Selectivity to *(R)*-1-phenylethyl acetate at 80% acetophenone conversion (%)
62.5	0	13	0
62.5	125	10	a (a)
125	62.5	82	5 (31)
125	125	46	14 (b)
125	250	48	40 (c)
250	125	97	3 (5)

[a] 10% conversion reached within 1500 min giving the selectivity to *(R)*-1-phenylethyl acetate 49%, [b] 46% conversion reached within 1500 min, [c]49% conversion reached within 1500 min.

3.4 Kinetic resolution [VIII]

The utilization of the continuous flow reactor for the kinetic resolution of racemic 1-phenylethanol with ethyl acetate in toluene over an immobilized lipase was studied. The aim of this work was to elucidate the behavior of the immobilized lipase for the kinetic resolution of racemic 1-phenylethanol with ethyl acetate in a down-flow continuous reactor. The obtained data were linked to the one-pot synthesis performed in a batch mode. The reaction rates for the continuous and the batch reactors were found as $1.2\ 10^{-6}$ mol/s.g and $2\ 10^{-6}$ mol/s.g, respectively.

3.4.1 Transient kinetics by applying step changes and reactor hydrodynamics

The hydrodynamics of the reactor system was studied by following the responses to step changes in feed concentrations. The deviation from plug flow was then evaluated by performing a rough time domain fit to measured concentration responses, such as that shown in Figure 3.5.

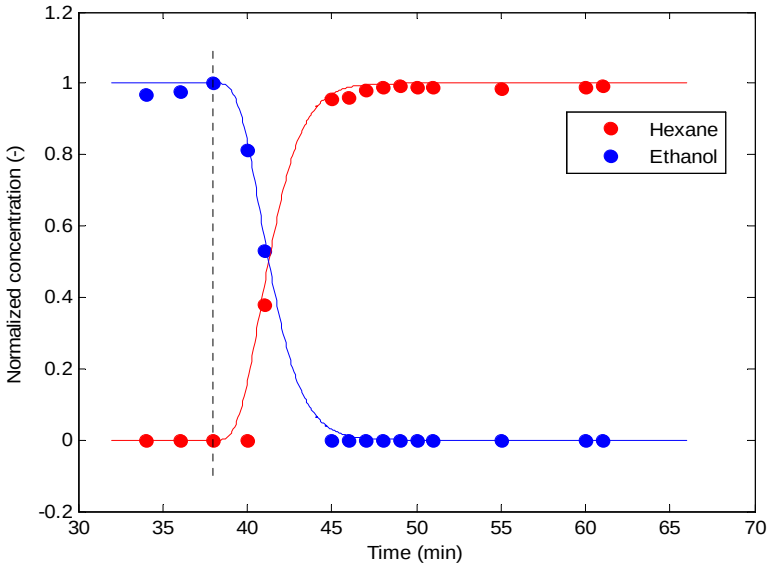


Figure 3.5. Sample step response of the studied system; ●, measured concentration; line, modeled concentration response; $\dot{V} = \quad ml/min, n = 6, \bar{t} \approx 3\,min$.

The residence time distribution for the tanks-in-series is given by

$$F(t) = \frac{c}{c_0} = 1 - e^{-n \cdot t/\bar{t}} \left[\sum_{i=1}^{n} \frac{(n \cdot t/\bar{t})^{i-1}}{(i-1)!} \right] \tag{3.1}$$

and the corresponding washout function, *W(t)*, is obtained from the usual relationship

$$W(t) = 1 - F(t) \tag{3.2}$$

Six mixing cells used a mean residence time of roughly 3 minutes, which is more than three times longer than the residence time calculated based on reactor volume and volumetric flow, in order to get a satisfactory description (Figure 3.5) of the measured hydrodynamics. The results showed that the pump and inlet lines constitute much of the total volume in the studied system. The deviations from ideal flow for the investigated system are small enough that the results discussed in depth in **Publication VIII** can be attributed to intrinsic kinetic and possibly adsorption effects.

3.4.2 Transient behavior with different chemicals

The aim of this study was to obtain understanding about the behavior of the immobilized enzyme and reactor hydrodynamics (**Publications I-VII**). The results were linked to the one-pot reactions conducting hydrogenation and kinetic resolution in the same reaction pot. Transient kinetics was studied by applying the step changes of organic compounds

such as acetophenone, ethyl benzene and acetic acid. Since acetophenone was used as a substrate in one-pot reactions (**Publications I-VII**), the effect of known concentration of acetophenone was investigated. Since ethyl benzene was observed as a major side product in the one-pot reactions due to the hydrogenolysis of racemic 1-phenylethanol over acidic catalysts as well as acidic acid formed due to water formation during the hydrogenolysis (see section 3.2), the effects of both ethyl benzene and acetic acid in the enzyme activity and stability was studied. The results showed that acetophenone slightly inhibited the lipase activity while no effect of ethyl benzene on it was observed. However, acetic acid significantly retarded the acylation activity of lipase, as expected, since enzymes are very sensitive to pH changes as pH significantly influences the secondary, tertiary and quaternary structure of proteins thus affecting enzyme deactivation kinetics [27] (**Publication VIII**).

3.4.2.1 The effect of residence time and different substrate concentrations

The effect of residence time ($\dot{t}$=V/$\dot{V}$, where V is the catalyst bed volume and $\dot{V}$ is the volumetric flow rate of the liquid, respectively) was investigated by applying different volumetric flow rates. The results showed that doubling the residence time increased twofold *(R)*-1-phenylethanol conversion, as expected, whereas the concentrations of substrates were kept constant. The highest *(R)*-1-phenylethanol conversion was 30% after 300 min time-on-stream corresponding to 100% selectivity of the desired acetate (*(R)*-1-phenylethyl acetate) (**Publication VIII**). No deactivation of the immobilized lipase was observed.

The effect of different substrates concentrations were also investigated. Initially, 0.02M racemic 1-phenylethanol with 0.06M ethyl acetate in toluene was transfererd to the reactor with a constant volumetric flow rate. Thereafter, varying substrate concentrations was applied. The results showed that decreasing the concentration of ethyl acetate decreased the desired product concentration by half whereas doubling substrate concentration enhanced the desired product concentration, *(R)*-1-phenylethyl acetate, by 50%. Furthremore, the highest desired product concentration was obtained with excess of ethyl acetate. However, the ratio between the substrates is an important parameter since higher desired product yields were obtained when smaller substrate ratios were applied (**Publication VIII**).

3.4.2.2 The effect of the desired or the stoichiometric products

One of the most throughly addresses issue in analysing activity and stability of enzymes is the product inhibition. Therefore, the effects of the desired product, *(R)*-1-phenylethyl acetate, and stoichiometric product, ethanol, excess on the lipase activity were studied. The enzyme activity decreased by 30% due to the high desired product concentration indicating that the product should be removed from the reaction media continuously to achieve high desired product yields. Furthermore, the most extensive inhibition was observed with the excess of ethanol decreasing acylation significantly (**Publication VIII**). The inhibition of lipase due to high concentration of ethanol can be explained by the influence of hydrophilic solvents on the enzyme activity in organic solvents as discussed in **Publication VIII** in depth.

3.4.3 The effect of water

Essential water is needed to keep the enzyme active even though the reaction media contains mainly organic solvent and/or substrates. On the other hand, water in the reaction mixture can cause unfavorable equilibrium in hydrolysis and lead to hydrolytic side reactions in transferase reactions. Thus, this study focused on the effect of water added to the reaction media. The results showed that an excess of water significantly diminished enzyme activity (**Publication VIII**).

3.4.4 Catalyst deactivation

The activity of the immobilized lipase decreased as a function of time by about 40% after 30 days. The decrease in the catalytic activity of the immobilized enzyme was also observed in the experiments conducted in different time intervals. The SEM pictures from fresh and spent catalysts clearly showed that the activity decrease was due to the loss of the 3D structure of the enzyme (**Publication VIII**). The SEM pictures taken from the fresh catalyst (Figure 3.6a) demonstrate smooth surface of the catalyst. The same pictures for the spent catalysts (Fig 3.7a) give a clear image of the destruction of the catalyst integrity, which might be a result of the physical changes of the immobilized enzyme during the reactions in organic reaction media.

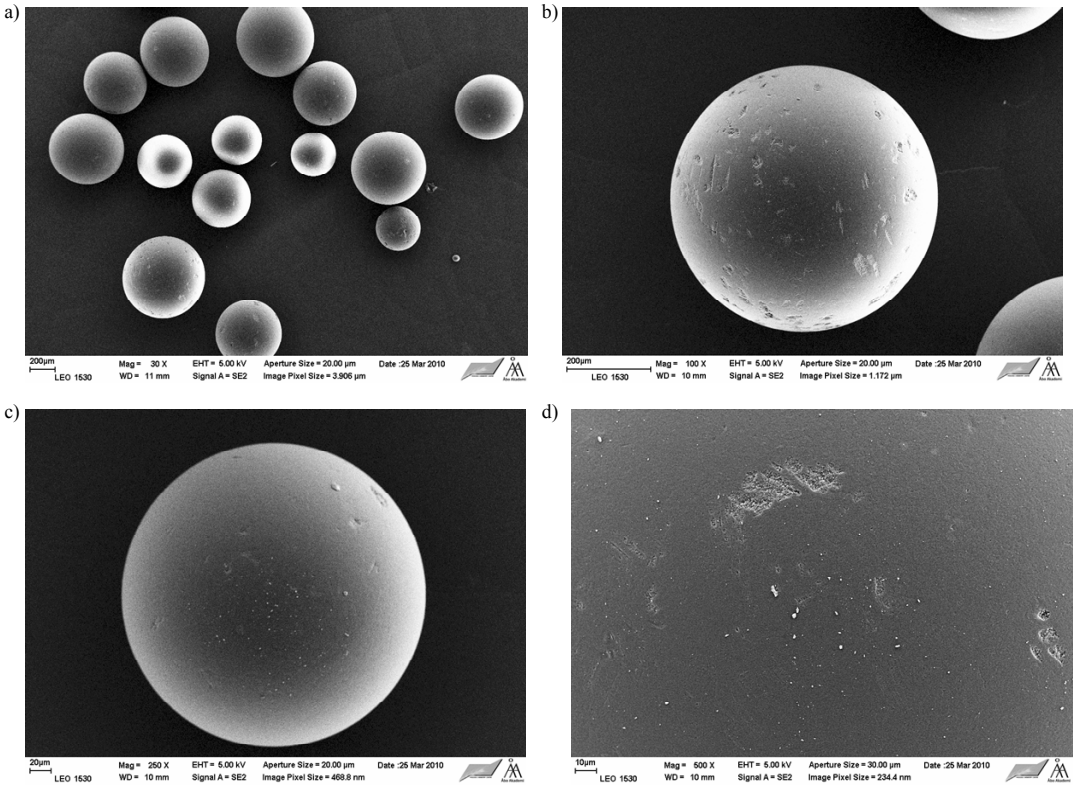


Figure 3.6. SEM images of the fresh immobilized lipase. a) 30x, b) 100x, c) 250x, d) 500x.

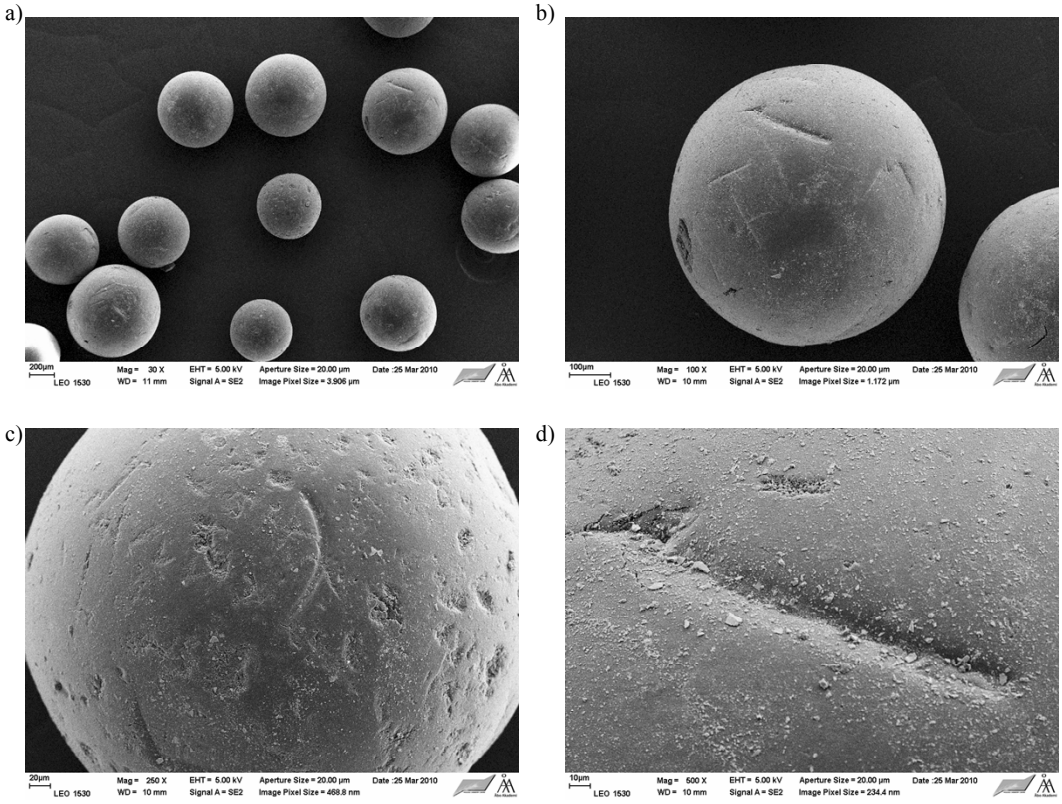


Figure 3.7. SEM images of the spent immobilized lipase. a) 30x, b) 100x, c) 250x, d) 500x.

4
KINETIC MODELING RESULTS

It is essential to find an accurate model, which is capable of simulating the behavior of the process and estimating the value of the reaction rate constants in order to predict reactor performance. The kinetic model of a certain reaction system should consider all the important parameters since the model plays an important role not only in the predicting the system performance under different operating conditions but also process optimization, reactor design, scale-up and control of the system etc [28]. The predicted behavior obtained by solving the formulated model equations is compared with experimental data. Any significant differences between the experimental data and the predicted data imply that there may be some other important effects not considered. The kinetic constants are estimated by fitting the model equation to the experimental data [29].

4.1 Kinetic modeling

Kinetic modeling of lipase-catalyzed organic phase reactions has been scarcely investigated. Most extended kinetic models are based on the application of Michaelis-Menten assumptions. This type of model is usually valid for the simplest enzymatic reactions [30]. More complex models, however, are needed to explain sequential actions of enzymes. Ping-pong model [31, 32] is the most widely used model for lipase catalyzed reactions. This mechanism, with inhibition of both substrate and acyl donor, was proposed for the transesterification of several alcohols over lipase with vinyl acetate at 30 ^{o}C [31]. Studies conducted with ethyl acetate as an acyl donor and isoamyl alcohol as an acyl acceptor in the synthesis of isoamyl acetate over an immobilized lipase in hexane were described with the ping-pong bi-bi mechanism with competitive inhibition by substrates and a stoichiometric product, ethanol [32]. In fact, it is often necessary to consider enzyme inhibition effects in the kinetic modeling of enzymatic reactions. Dead-end complexes which do not participate in the reaction can be formed between the enzyme and other substrates present in the reaction media as a result of enzyme inhibition. Competitive inhibition was considered as the most common inhibition mechanism by many researchers [32, 33].

4.2 Qualitative kinetics

Ethyl acetate was chosen to be a solvent and an acyl donor in this study. It is a hydrophobic solvent which is found to be highly soluble in 95% ethanol with 0.67 log*P* value [34]. The stability of enzyme can be further increased by several orders of magnitude if it is transferred from a water-rich environment to an organic medium. This phenomenon was explained by the fact that when the lipase was transferred to an organic solvent, the dehydration caused the lipase to be blocked in its native catalytically active conformation [33]. It is known that an optimum amount of water is essential for the immobilized enzyme for maintaining the enzymatic activity. Since hydrophilic solvents cause stripping of the

essential water layer around the enzyme, which is necessary for enzyme activity, hydrophobic solvents are more preferred.

The acylation reaction did not occur in the absence of the enzyme at 70 ^{o}C. The hydrogenation of acetophenone produced racemic 1-phenylethanol and the reaction was irreversible. The reversible reaction between *(R)*-1-phenylethanol and ethyl acetate resulted in the desired ester (*(R)*-1-phenylethyl acetate) and ethanol. Lipases are known to lose their activity as time elapses [35]. Such processes are frequently characterized by first-order kinetics [36]. The most frequent inhibition is competitive inhibition due to the formation of binary complexes between the free enzyme and the alcohol or the ester [33]. The reason for this is that inhibitor is a substrate analogue, and fits precisely where the substrate should be attached in the enzyme's active site. Low-molecular weight alcohols, such as ethanol can disrupt the three-dimensional architecture of the lipase [35]. Immobilized lipases are more stable than their free counterparts facilitating their applications under harsher conditions such as higher temperature, variable pH and presence of organic solvents because the enzyme molecule becomes more rigid upon multi-point attachment to a solid carrier.

4.3 Detailed kinetic modeling for Pd-Al_2O_3 catalyst

In spite of the fact that several kinetic studies have been carried out, only limited amount of appropriate kinetic analysis has been performed. Most of the kinetic modeling studies are based on initial reaction rates. There is a dearth of literature on kinetics and modeling of lipase-catalyzed chemo-bio cascade reactions. For these reasons, the purpose of this work was to propose a reaction mechanism which could describe kinetics of chemo-bio synthesis of *(R)*-1-phenylethyl acetate over Pd-Al_2O_3 and immobilized lipase in ethyl acetate. Overall reactions are summarized in Table 4.1 and extensively discussed in **Publication IX**.

Table 4.1. Proposed mechanism and reaction sequence.

		$N^{(1)}$	$N^{(2)}$	$N^{(3)}$	$N^{(4)}$	$N^{(5)}$	N(6)
I.	$A + Z \equiv ZA$	1	1	0	0	0	0
II.	$ZB \equiv Z + B$	1	0	0	0	-1	0
III.	$ZC \equiv Z + C$	0	1	0	0	0	-1
IV.	$P + Z \equiv PZ$	0	0	0	1	0	0
V.	$ZF \equiv Z + F$	0	0	0	1	1	1
1.	$ZA + H_2 \xrightarrow{k_1} ZB$	1	0	0	0	0	0
2.	$ZA + H2 \xrightarrow{k_2} ZA$	0	1	0	0	0	0
3.	$Q + E \underset{k_{-3}}{\overset{k_3}{\Leftrightarrow}} EQ + I$	0	0	1	0	0	0
4.	$EQ + B \underset{k_{-4}}{\overset{k_4}{\Leftrightarrow}} E + P$	0	0	1	0	0	0
5.	$PZ \xrightarrow{k_5} ZF + AcOH$	0	0	0	1	0	0
6.	$ZB \xrightarrow{k_6} ZS + H_2O$	0	0	0	0	1	0
7.	$ZC \xrightarrow{k_7} ZS + H_2O$	0	0	0	0	0	1
7′.	$ZS + H_2 \xrightarrow{fast} ZF + H_2O$	0	0	0	0	1	1

$N^{(1)}$: $A + H_2 = B$; $N^{(2)}$: $A + H_2 = C$; $N^{(3)}$: $Q + B = I + P$; $N^{(4)}$: $P = F + AcOH$; $N^{(5)}$: $B + H_2 = F + H_2O$; $N^{(6)}$: $C + H_2 = F + H_2O$

In table 4.1 Z is the adsorption site on which acetophenone (A) is adsorbed and hydrogenated to *(R)*-1-phenylethanol (B) and to *(S)*-1-phenylethanol (C) over Pd/Al_2O_3 followed by acylation of B to *(R)*-1-phenylethyl acetate (P) over an immobilized lipase. It is assumed that the acyl donor, ethyl acetate (Q), was bound first with the free enzyme (E) forming a non-covalent enzyme-acyl complex (EQ), which released ethanol (I). It has been previously established that the lipase first forms an acyl-enzyme complex with the acyl donor [37]. The substrate in acylation step, *(R)*-1-phenylethanol (B), combines with EQ which subsequently releases *(R)*-1-phenylethyl acetate (P) and E. Styrene was obtained dehydration of B and C over $Pd\text{-}Al_2O_3$. The side product ethyl benzene (F) is formed as a result of fast hydrogenation of S and de-acylation of P in the presence of H_2 releasing acetic acid (AcOH) to the media. On the right hand side of equations, the stoichiometric numbers for the six routes ($N^{(1)}$ – $N^{(6)}$) are given. These numbers are selected in such a way that the overall chemical equations do not contain intermediates. The total number of reaction routes was determined by the expression proposed by Horiutu and further developed by Temkin [38]. In this case it is 6 (number of routes) = 13 (number of steps) – 9 (number of intermediates) + 2 (number of equations). The symbol ≡ denotes adsorption-desorption steps I – V, which are in equilibria. Kinetically significant steps 1 -7 are either irreversible (→) or reversible (↔). Step 7′ is considered to be fast and thus kinetically not significant.

The rate equations was derived according to the well established procedures [39]

$$r_1 = \frac{k_1 K_A [A]}{D} C_{cat} \tag{4.1}$$

$$r_2 = \frac{k_2 K_A [A]}{D} C_{cat} \tag{4.2}$$

$$r_3 = k_3 [B] C_{enzyme} \tag{4.3}$$

$$r_5 = \frac{k_5 K_P [P]}{D} C_{cat} \tag{4.4}$$

$$r_6 = \frac{k_6 K_B [B]}{D} C_{cat} \tag{4.5}$$

$$r_7 = \frac{k_7 K_C [C]}{D} C_{cat} \tag{4.6}$$

$$D = 1 + K_A [A] + K_B [B] + K_C [C] + K_P [P] + K_F [F] \tag{4.7}$$

where [A], [B], [C], [P], [F] are respectively the concentration of acetophenone, *(R)*-1-phenylethanol, *(S)*-1-phenylethanol, *(R)*-1-phenylethyl acetate, ethyl benzene; K_A, K_B,

K_C, K_P, K_F are the adsorption constants for the above mentioned compounds, k_i reaction rate constants of a particular step, r_i the rates of reactions. C_{cat} and C_{enzyme} are the concentrations of the metal catalyst and the immobilized enzyme, respectively.

The acylation reaction was assumed to be irreversible and therefore the rate is equal to the rate of step 3 and, moreover, EQ complex concentration is low. Furthermore, it should be noted that k_1 and k_2 contain implicitly also a hydrogen pressure dependence. The kinetic model was further simplified for data fitting by assuming that k_1 and k_2, k_6 and k_7, K_B and K_C are equal to each other, respectively. The value of K_B was estimated and used together with the estimated value of k_6K_B to evaluate k_6. Moreover, adsorption of ethyl benzene was neglected in the kinetic model.

A simplified model is written as follows

$$r_1 = \frac{k_1 K_A [A]}{D'} C_{cat} \tag{4.8}$$

$$r_2 = \frac{k_2 K_A [A]}{D'} C_{cat} \tag{4.9}$$

$$r_3 = k_3 [B] C_{enzyme} \tag{4.10}$$

$$r_5 = \frac{k_5 K_P [P]}{D'} C_{cat} \tag{4.11}$$

$$r_6 = \frac{k_6 K_B [B]}{D'} C_{cat} \tag{4.12}$$

$$r_7 = \frac{k_6 K_B [C]}{D'} C_{cat} \tag{4.13}$$

$$D' = 1 + K_A [A] + K_B [B] + K_B [C] + K_P [P] \tag{4.14}$$

The generated rates for each compound were derived by taking into account the stoichiometry:

$$\frac{dC_A}{dt} = (-r_1 - r_2)\ q_{deact} \tag{4.15}$$

$$\frac{dC_B}{dt} = (r_1 - r_6)\ q_{deact} - r_3 \tag{4.16}$$

$$\frac{dC_C}{dt} = (r_2 - r_7)\ q_{deact} \tag{4.17}$$

$$\frac{dC_P}{dt} = r_3 - r_5\, q_{deact} \tag{4.18}$$

$$\frac{dC_D}{dt} = (r_5 + r_6 + r_7)\ q_{deact} \tag{4.19}$$

where q_{deact} is the activity function. Since deactivation was observed in the hydrogenation step after prolonged reaction times, it was taken into account by the following activity function

$$q_{deact} = \exp\left((-k_{-d} + k_{+d}) \frac{m_{enzyme}}{m_{cat}} t \right) \tag{4.20}$$

where k_{+d} and k_{-d} are the forward and the reverse deactivation reaction rate constants, m_{enzyme} the mass of immobilized enzyme, m_{cat} the mass of the metal catalyst and t is time.

The results showed that the kinetics was dependent on the mass ratio of Pd-Al_2O_3 to immobilized lipase. The larger the ratio the more deactivation of Pd-Al_2O_3 occurred (**Publication IX**). The model predictions and kinetic parameters were acquired by using parameter estimation software ModEst 6.0 [40].

4.4 Reactor modeling

A batch reactor model was utilized. The mass balances for liquid-phase components (*i*) where ρ_{cat} and ρ_{enzyme} are the bulk densities of the hydrogenation catalyst and the immobilized enzyme, respectively, in a batch reactor were written as:

$$\frac{dC_A}{dt} = (-r_1 - r_2)\ \rho_{cat}\, q_{deact} \tag{4.21}$$

$$\frac{dC_B}{dt} = (r_1 - r_6)\ \rho_{cat}\, q_{deact} - r_3\ \rho_{enzyme} \tag{4.22}$$

$$\frac{dC_C}{dt} = (r_2 - r_7)\ \rho_{cat}\, q_{deact} \tag{4.23}$$

$$\frac{dC_P}{dt} = r_3\, \rho_{enzyme} - r_5\, \rho_{cat}\, q_{deact} \tag{4.24}$$

$$\frac{dC_D}{dt} = (r_5 + r_6 + r_7)\ \rho_{cat}\, q_{deact} \tag{4.25}$$

The software Modest 6.1 was used to minimize the objective function, and the rate constants were estimated with the Simplex method and then switched to a Levenberg–Marquardt method [40]. The ordinary differential equations (ODEs) describing the reactor model were solved by the backward difference method. The equations for the reaction kinetic model are ODEs which can be integrated starting from the initial conditions. In comparing how well competing models fit the experimental data, a convenient way defined in Modest 6.1 is the calculation of a coefficient of determination, the R^2-value:

$$R^2 = 1 - \frac{\sum_{i=1}\sum_{j=1}\sum_{t=1}\left[\left(c_{i,j,t} - \hat{c}_{i,j,t}\right)^2\right]}{\sum_{i=1}\sum_{j=1}\sum_{t=1}\left[\left(c_{i,j,t} - \hat{c}_{i,j,t}\right)^2\right]} \tag{4.26}$$

where i denotes each component in the reaction mixture, j denotes the different data sets and t refers to the data points, $c_{i,j,t}$ is the experimental value; $\hat{c}_{i,j,t}$ is the corresponding model prediction; $\bar{c}_{i,j,t}$ is the average of all the data points. The estimated sum of squares is divided by the sum of squares of the simplest possible model, the average of the values. This implies that R^2 values approaching 100% are desired. Typically values exceeding 95% represent a good fit.

4.5 Sensitivity

The estimated kinetic constants were identified by parameter sensitivity analysis plots using the Markov Chain Monte Carlo (MCMC) method. The MCMC analysis for the

predictions of model parameters showed that the model fits the measured data well (Figure 4.1). In this method, which is based on the Bayesian approach, all the uncertainties in the data as well as the modeling results are treated as statistical distributions [41]. It is important to consider the possible cross-correlation of the parameters. The prediction distributions revealed to which extent the parameter uncertainty is relevant with respect to the model predictions.

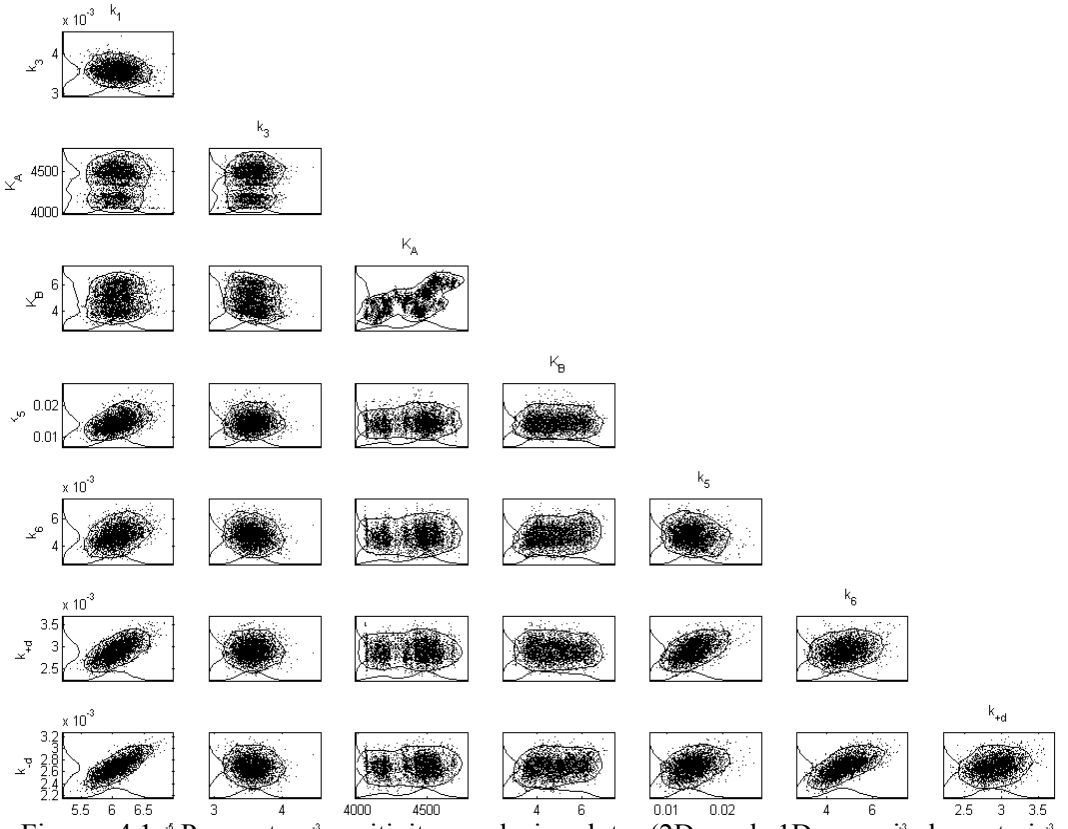


Figure 4.1 Parameter sensitivity analysis plots (2D and 1D marginal posterior distribution plots for all parameters).

4.6 Summary

Kinetic modeling of one-pot acetophenone hydrogenation over 5% (w/w) Pd-Al_2O_3 and further acylation with an immobilized lipase was performed. The fit of the model to the experimental data is extensively discussed and the estimated rate constants are presented in **Publication IX**. The results revealed an interaction between both catalysts where the activity of the heterogeneous catalyst was hindered due to the presence of increased amounts of enzyme. The values are valid for the experiments carried out under the specified reaction conditions. The estimated kinetic constants were identified by parameter sensivity analysis plots using MCMC method. The MCMC analysis for the prediction of model parameters showed that the model fits the measured data well (**Publication IX**).

5
CONCLUSIONS

A detailed study of the reaction system including catalyst selection, catalyst testing, characterization, transient kinetics as well as the kinetic investigation was carried out. Chemo-bio catalytic one-pot synthesis for *(R)*-1-phenylethyl acetate starting from selective hydrogenation of acetophenone to racemic 1-phenylethanol over a heterogeneous palladium catalyst on different supports and acylation of the formed *(R)*-1-phenylethanol to the corresponding ester, *(R)*-1-phenylethyl acetate, with an immobilized lipase was thoroughly investigated in the present work. The experiments were performed in a batch mode either in a glass reactor under atmospheric pressure at 70 ^{o}C or in an autoclave under 5.6 bars of H_2 at 50 ^{o}C. Ethyl acetate was used as a solvent as well as an acyl donor. The most promising catalyst pair for the one-pot synthesis of *(R)*-1-phenylethyl acetate was 5% (w/w) Pd-Al_2O_3-UOP with an immobilized lipase giving maximally 45% selectivity to *(R)*-1-phenylethyl acetate at 47% conversion under optimal conditions. The results revealed that the water formation resulting in *(R)*-1-phenylethyl acetate hydrolysis was due to the acidic character of the catalysts such as Pd-H-MCM-41, Pd-Al_2O_3 (Aldrich) Pd-C, promoting the dehydration of the intermediate products racemic 1-phenylethanol. Thus, palladium catalysts supported on a vapor-grown carbon nanofiber having adjustable surface properties with nitrogen-containing surface groups (N-VGCF) were also tested in a glass reactor under atmospheric pressure of H_2 at 70 ^{o}C in toluene. The effect of the preparation method on the synthesis of *(R)*-1-phenylethyl acetate in one-pot with an immobilized lipase and the effect of catalyst treatment procedure of the palladium deposited on vapor-grown carbon nanofibers (VGCFs) either with incipient-wetness impregnation or with sol immobilization catalysts were investigated. The maximum acetophenone conversion over 2% (w/w) Pd-N-VGCF was 75% corresponding to 34% selectivity of the desired product over this catalyst in combination with 62.5 mg of immobilized lipase.

The systematic kinetic experimentation using 5% (w/w) Pd-Al_2O_3-UOP in combination with an immobilized lipase but varying the amounts of both the chemo-catalyst and the enzyme in one-pot hydrogenation-acylation reaction was performed. Additionally, the kinetics was investigated in the absence and in the presence of the immobilized lipase. The results showed that these two different types of catalysts, 5% (w/w) Pd-Al_2O_3-UOP and immobilized lipase, do not act independently, but in concert.

The utilization of the continuous flow reactor for the kinetic resolution of racemic 1-phenylethanol with ethyl acetate in toluene over an immobilized lipase was also studied in the present work. The aim of this study was to elucidate the behavior of the immobilized lipase for the kinetic resolution of racemic 1-phenylethanol with ethyl acetate in a down-flow continuous operation mode by performing several experiments

including transient ones and elucidating residence time distributions by step changes. The obtained data were linked to the one-pot synthesis performed in a batch mode. Thus, the effects of ethyl benzene, ethanol, acetic acid, water, etc. on acylation were studied. The results provided an understanding about the deactivation mechanism of the immobilized lipase which was also observed in one-pot reactions operated in a batch mode. The acylation reaction was retarded mostly by ethanol, acetic acid and water. Furthermore, product inhibition by *(R)*-1-phenylethyl acetate was observed. Additionally, the hydrodynamics of the continuous reactor revealed that this reactor was operated close to the plug-flow mode.

Finally, quantitative kinetic model including catalyst deactivation during batch operation was proposed. The results revealed an interaction between both catalysts where the activity of the heterogeneous catalyst was hindered due to the presence of increased amounts of enzyme.

6
NOTATION

TEM	transmission electron microscopy
HRTEM	high-resolution transmission electron microscopy
SEM	scanning electron microscopy
TPD	temperature programmed desorption
BET	Brunauer, Emmett and Teller adsorption isotherm
FTIR	infrared spectroscopy
XRD	X-ray diffraction
N-VGCF	nitrogen-containing vapor-grown carbon nanofiber
$\dot{V}$	volumetric flow rate of the liquid (mL/min)
t	residence time ((S)-^{1})
c(t)	concentration at outlet (mol/L)
c_0	concentration of incoming flow (mol/L)
n	number of mixing cells (or number of tanks in series)
i	index
t	time
t	mean residence time
F	residence time distribution
W	washout function
Pe	Peclet number (dimensionless)
u	superficial velocity (m/s)
L	length (m)
D	axial dispersion (m^2/s)
≡	adsorption-desorption steps
(↔)	reversible reaction
(→)	irreversible reaction
[A]	concentration of acetophenone
[B]	concentration of *(R)*-1-phenylethanol
[C]	concentration of *(S)*-1-phenylethanol
[P]	concentration of *(R)*-1-phenylethyl acetate
[F]	concentration of ethyl benzene
K_A	adsorption constants for acetophenone
K_B	adsorption constants for *(R)*-1-phenylethanol
K_C	adsorption constants for *(S)*-1-phenylethanol
K_P	adsorption constants for *(R)*-1-phenylethyl acetate
K_F	adsorption constants for ethyl benzene

k_i	reaction rate constants of a particular step
r_i	reaction rate
C_{cat}	concentrations of the metal catalyst
C_{enzyme}	concentrations of the immobilized enzyme
q_{deact}	the activity function
k_{+d}	forward deactivation reaction rate constant
k_{-d}	reverse deactivation reaction rate constant
m_{enzyme}	mass of immobilized enzyme
m_{cat}	mass of the metal catalyst
ρ_{cat}	bulk density of the hydrogenation catalyst
ρ_{enzyme}	bulk density of the immobilized enzyme
ODE	ordinary differential equations
i	each component in the reaction mixture
j	different data sets
t	data points
$\hat{c}_{i,j,t}$	experimental value
$\underline{c}_{i,j,t}$	model prediction
$\bar{c}_{i,j,t}$	average of all the data points
MCMC	Markov Chain Monte Carlo

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