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Process Integration of Fermentation and Catalysis for the Production of Succinic Acid Derivatives

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1 Introduction

Prior to the end of the 18th century, the economy was largely based on agriculture and forestry. In the 19th century, the arrival of the industrial revolution and the emergence of the organic chemical industry completely modified the structure of the economy. Fossil energies (petroleum, coal, natural gas) were then used as main energy resources, as well as major raw materials for the new chemical industry. The current chemical industry still relies on these non-renewable resources and consumes more than 1 billion barrels of oil per year (Paster et al., 2003). Considering this consumption and the limited nature of fossil fuels, it comes not as a surprise that the economy faces several problems: a continuously rising price for oil, political and economic tension related to the unequal distribution of the remaining oil stocks and increasingly severe environmental impact due to the use of these resources and the by-products generated. These problems have motivated the industry to find a replacement for fossil fuels as feedstocks.

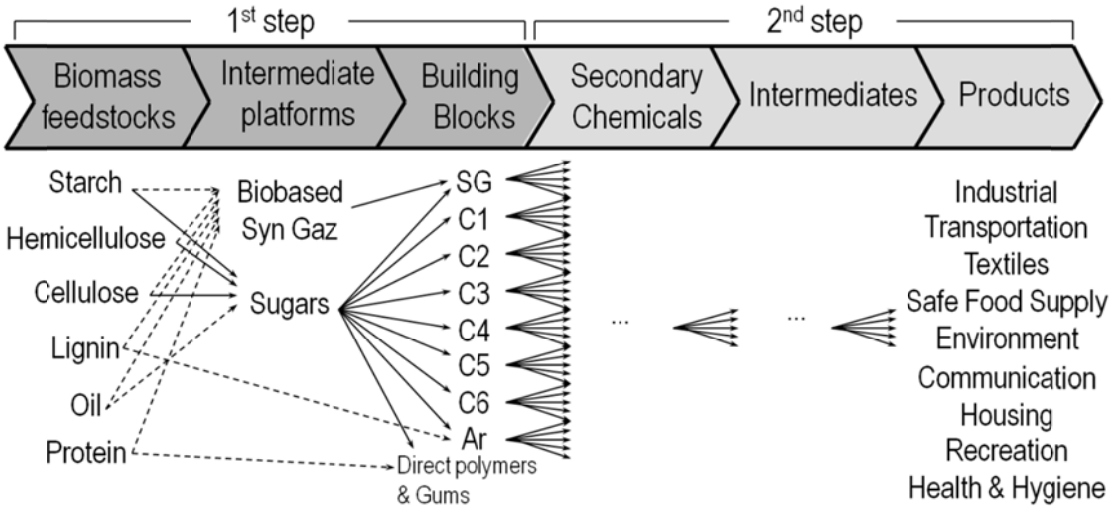


Figure 1-1: Bio-based chemical industry combining white biotechnology and green chemistry (Kamm and Kamm, 2007; Werpy and Petersen, 2004).

Considerable effort is being invested in biotechnology and "green chemistry" to develop a chemical industry based on renewable resources as at least a partial substitute for the dwindling fossil fuels. The idea of using biomass as starting material led to the new concept of "biorefineries", which dual structure is presented in Figure 1-1. This two step approach is currently being developed. In a first step, fermentation of biomass is used for the production of renewable platform chemicals,

which could replace the C_1 to C_6 petroleum-derived building-block chemicals. In a second step, these bio-derived platform chemicals must be transformed into valuable chemicals. Sometimes though, these building-block chemicals are difficult to purify, so that the further chemical or biotechnological transformations of those should be performed directly in the non-purified aqueous solutions produced in the first step (e.g. in the fermentation broth). New chemical catalysts and new reaction pathways must therefore be developed for the aqueous catalysis of the bio-derived bulk chemicals. This is a new challenge that the biotechnological and chemical industries must face together for the development of suitable processes for the future bio-chemical industry.

In this thesis, the emphasis is placed on the C_4 platform. Among the potential replacements for the oil-derived C_4 maleic anhydride, succinic acid is a key bioderived chemical that has attracted a lot of attention in the last decade and that could be used for the production of a large range of derivatives.

2 Motivation and objectives

2.1 Motivation

Among the potential platform chemicals of the future biorefineries, succinic acid has been reported many times as a promising candidate (Patel et al., 2006; Werpy and Petersen, 2004). This dicarboxylic acid is indeed an intermediate of the tricarboxylic acid (TCA) cycle that could replace the maleic anhydride produced nowadays from oil as a C_4 building-block chemical. The optimization of the biotechnological production of succinic acid has been investigated with many strains in the last decade with final concentrations up to 146 g l^{-1} (Okino et al., 2008; Raab et al., 2010; Song and Lee, 2006; Zeikus et al., 1999). One of the bottlenecks of this process is, however, the expensive purification of succinic acid (Kurzrock and Weuster-Botz, 2010; Kurzrock and Weuster-Botz, 2011; Song and Lee, 2006), with purification costs that represent in general up to 50 - 80 % of the total process costs. As succinic acid is an intermediate, it would thus be profitable to perform the catalytic reactions for the production of the derivatives directly in the fermentation broth. This would avoid the expensive purification of succinic acid from the broth. The resulting derivatives should have different physico-chemical properties that will ease their final purification. This constitutes the primary motivation of the present research effort.

However, it is challenging to perform catalysis in water, because of the stability issue for the catalyst and the need of new reaction pathways. Furthermore, if water is produced during the reaction, the use of water as reaction solvent will push the equilibrium backwards and limit hence the final conversion. Besides, difficulties can arise from the presence of the by-products or the medium components of the fermentation that could deactivate or inhibit the catalysts.

2.2 Objectives

Among the interesting derivatives of succinic acid, its reduced products and its esters are of particular interest. The aim of this project was therefore to develop strategies for the production of these two types of compounds in an aqueous medium.

For both the hydrogenation and the esterification of succinic acid, catalysts and process options had to be first researched in the literature, then tested experimentally in small-scale batch experiments and eventually selected given the following criteria:

- conversion / yield,
- selectivity,
- rate / activity,
- stability of the catalyst in water under the reaction conditions applied,
- mild reaction conditions (e.g. low temperature and pressure, neutral pH...),
- recovery and reusability of the catalyst if possible,
- cost.

In other words, the goal of this study was to raise and to the best current knowledge answer the following questions:

- What are the potential catalysts for the hydrogenation and the esterification of succinic acid in aqueous solutions and in fermentation broths?
- What are the successful and unsuccessful process options for these two reactions?

2.3 Methodology

Little information was available on some of the reactions of interest. In this case, similar reactions in organic solvent and/or with similar substrates were screened as an initial research step. The knowledge gained previously was then used to transfer the reaction into water using succinic acid as substrate.

In a second step, provided that the reaction could be performed successfully in water, the reaction was further studied in distilled water with the following goals:

- selection of potential catalysts based on the conversion, the rate and their costs,
- determination of optimal reaction conditions using a single- or multiple-parameter optimization so that the rate, the conversion (and the selectivity) could be improved.

In a third step, succinic acid contained in real fermentation broths was tested as substrate. The best catalyst and reaction conditions were selected for the process integration, having the following desired criteria in mind:

- little impact of the fermentation by-products and medium components on the reaction rate and final conversion,
- low cost,
- reusability of the catalysts for the reaction in the broth if possible,
- easy purification of the product leading to high purity.

Lastly, in a fourth step, the potential of such a process for industrial applications was investigated and documented.

2.4 Outline of the thesis

The outcome of this study will be presented in the following chapters. The literature review that provided the theoretical background and the state of knowledge for catalysis, hydrogenation and esterification will be reported in Chapter 3. The materials and methods that were used for the laboratory experiments, as well as all relevant calculations or computational details, will be described in Chapter 4. A thorough presentation of the experimental work and of the related findings will follow and will be subdivided into two separate chapters dealing with the hydrogenation (Chapter 5) and the esterification (Chapter 6), respectively. Finally, and based on those findings, conclusions for the whole research effort and implications for potential industrial applications and the design of future research will be covered in Chapter 7.

3 Theoretical background

3.1 Succinic acid: a platform chemical

3.1.1 Succinic acid

Succinic acid, also called butanedioic acid or, in the IUPAC system, ethane-1,2-dicarboxylic acid, is a dicarboxylic acid with four carbon atoms, that was first extracted from amber (*succinum* in Latin) by Georgius Agricola in 1546 (Song and Lee, 2006). It is a common metabolite in plants, animals and microorganisms (Zeikus et al., 1999). It can be found in three forms: diprotonated, monoprotonated and non-protonated (see Figure 3-1), the ratios of which are determined by the its pK_a (pK_{a1} = 4.21 ; pK_{a2} = 5.64). Its physico-chemical properties are summarized in Annex (see Section 9.2.3).

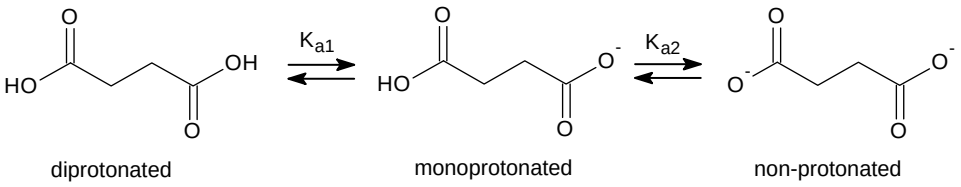


Figure 3-1: Acid-base equilibriums between the different forms of succinic acid (diprotonated, monoprotonated and non-protonated).

The chemical industry currently produces succinic acid mostly from oil, but fermentations from biomass are promising production alternatives. Succinic acid is a platform chemical for the synthesis of many chemical compounds.

3.1.2 Current synthesis of succinic acid from oil

The industrially used succinic acid is nowadays mainly produced from the petroleum-derived maleic anhydride. The latter can be hydrogenated into succinic anhydride that is then hydrated into succinic acid. Maleic anhydride can also be first hydrated to maleic acid, which is then hydrogenated into succinic acid, as presented in Figure 3-2.

Maleic anhydride was produced on a commercial scale from 1930s to 1980s through phase-oxidation of benzene. But due to the price increase of benzene, its toxicity and some security issues, maleic anhydride is nowadays mainly produced from *n*-butane or mixture of *n*-butenes and *n*-butane in fixed-bed, fluidized-bed or transport-bed processes (Felthouse et al., 2001; Lohbeck et al., 2000).

The hydrogenation of maleic anhydride or maleic acid into succinic anhydride / acid is catalysed by metal supported catalysts mainly in organic solvent.

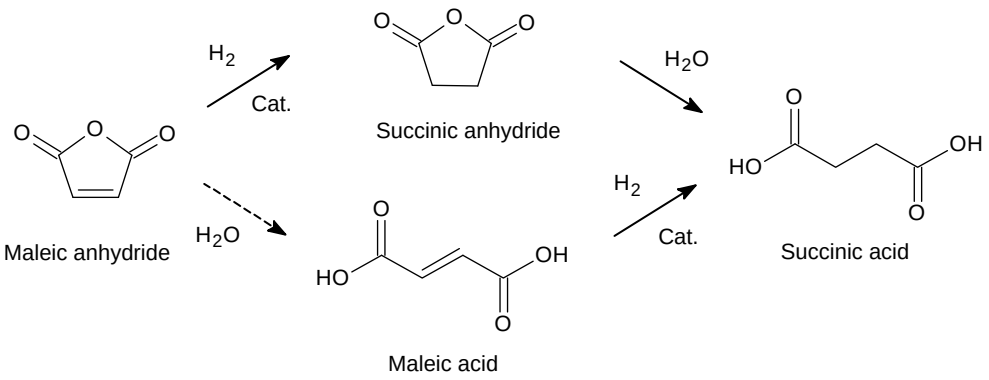


Figure 3-2: Synthesis of succinic acid from maleic anhydride through succinic anhydride or maleic acid.

3.1.3 Biotechnological production of succinic acid

Besides the chemical production from maleic anhydride, succinic acid can also be produced from biomass with different microorganisms. This pathway is to date only relatively limited in industry and the succinic acid produced from the fermentation of carbohydrates is mostly used in the food and beverage industry.

Succinic acid is an intermediate of the aerobic tricarboxylic acid (TCA) cycle but is also produced by many anaerobic microbes as major end-product of their energy metabolism (Song and Lee, 2006). The anaerobic fermentation routes to succinic acid allow the fixation of CO_2 and this process is therefore a green technology. It could be combined with the biotechnological production of ethanol that produces CO_2 to reduce the carbon loss of such process (Zeikus et al., 1999). From the electron balance between glucose (24 e^-) and succinic acid (14 e^-), ~ 1.71 mol succinate can be theoretically produced per mol glucose. In the presence of CO_2 and an additional reducing agent such as hydrogen, this theoretical yield can be increased up to 2 mol succinate per mol glucose (McKinlay et al., 2007). The experimental yields will be lower, because of carbon transfer to biomass and alternative by-products. The goal of the fermentative production of succinic acid is to reach the product concentrations and the space-time yields obtained for glutamic acid, i.e. ~ 150 g l^{-1} at 5 g l^{-1} h^{-1}, at a maximum yield (McKinlay et al., 2007).

Two strategies have been used to develop succinic producing strains: on the one hand, natural producing strains were screened and optimized; on the other hand, platform

organisms (e.g. *Corynebacterium glutamicum*, *Escherichia coli* or *Saccharomyces cerevisiae*) were forced, by metabolic engineering, into producing succinic acid.

Regarding the natural succinic acid producing strains, many microorganisms have been screened, among other fungi, few gram-positive bacteria and several gram-negative ones. The most interesting strains to date are *Anaerobiospirillum succiniciproducens* isolated from human and animal feces and *Actinobacillus succinogenes* and *Mannheimia succiniciproducens* isolated from rumen. These three organisms use the PEP carboxylation pathway to form succinic acid (Song and Lee, 2006). While these organisms produce naturally succinic acid, they often lead to a mixture of different acids, increasing the purification cost. However, more information on the metabolism and more genetic tools should be available to allow metabolism changes and hence prevent the formation of by-products. Finally, these strains could be pathogenic, limiting their industrial applications.

On the contrary, platform microorganisms, such as *E. coli* or *C. glutamicum*, do not produce succinic acid naturally or only in little amount, but they can be more easily genetically engineered and are not pathogenic. Modifications are often made to inactivate the enzymes which compete with succinic acid pathways, amplify those involved in it and introduce heterologous enzymes catalyzing the reaction towards an increased succinic acid formation (Song and Lee, 2006). With these strategies, high titers of succinate (i.e. 99 and 146 g l^{-1}, respectively) could be obtained by genetically modified *E. coli* or *C. glutamicum* (see Table 3-1).

The development of new strains that produce high titers of succinate but low concentrations of by-products in a defined medium will lower both the operating and purification costs of the process. The purification of succinate from the fermentation broth is indeed extremely challenging and represents the major part of the process costs. The different purification options have been reviewed in detail by Kurzrock and Weuster-Botz (2010). Among others, precipitation techniques with calcium hydroxide or ammonia have been reported, as well as electrodialysis, sorption, ion exchange, extraction with predispersed solvent and reactive extraction. The latter process seems to be a promising approach for the selective extraction of succinic acid from the fermentation broth.

However, since succinic acid has only a relative small market as end-product, it would be preferable to directly perform the catalytic production of its derivatives in the fermentation broth. The end-products should have different physico-chemical properties

that will facilitate the end-purification. This will considerably lower the production costs of succinic acid derivatives, making it more attractive for replacing the current oil-derived C_4 platform chemical.

Table 3-1: Strains for the production of succinate: maximum concentration of succinate achieved, yield, advantages and disadvantages (McKinlay et al., 2007; Song and Lee, 2006; Zeikus et al., 1999)

Organism	Max. conc. of Succ., g l^{-1}	Yield, mol mol^{-1}	Lit. Ref.	Advantages	Disadvantages
Anaerobio-spirillum succinici-producens (wild-type)	50	1.37	Glassner and Datta, 1992	• Many C sources • Natural production of high conc. of succinate	• Production of mixed acid • No growth at glucose conc. ≥ 70 g l^{-1} • Unknown metabolism • Need of genetic tools • Strict aerobe • Potential human virulence
Actinobacillus succinogenes (natural mutant)	106	1.27	Guettler et al., 1996	• Many C sources • Stability at different pHs • Tolerance to glucose conc. up to 160 g l^{-1} • High end conc. of succinate • Resistance to high conc. of succinate	• Production of propionic acid, pyruvic acid • Limited genetic tools • Unknown pathogenicity
Mannheimia succinici-producens (recombinant)	52	1.16	Lee et al., 2006	• Many C sources • Stability at different pHs	• Need of more genetic tools • Unknown pathogenicity • Many auxotrophies
Coryne-bacterium glutamicum (recombinant)	146	1.40	Okino et al., 2008	• Different C sources • Genetic tools available • High conc. of succinate and productivity • Fast growth • No need of complex media during the succinate production phase	• Need of bicarbonate • Acetic acid as main by-product
Escherichia coli (recombinant)	99	1.78	Vemuri et al., 2002	• Genetic tools available • High conc. of succinate	• Lower productivities than the natural succinate producers • Complex media • Co-production of other acids

3.1.4 Succinic acid as platform chemical

Nowadays, succinic acid is used as surfactant, foaming agent, and ion chelator. It has also a market in the food industry as acidulant or pH modifier, flavoring agent and antimicrobial agent, with a global market of 16,000 to 30,000 ton/a (Werpy and Petersen, 2004; Zeikus et al., 1999). Finally, it is a starting material for the production of health-related agents in the pharmaceutical industry. However, if its production price can decrease from \$2,000 - 3,000 to \$550 per ton, it could be used as replacement of the maleic anhydride C_4 platform and as polymer intermediate (source: UK's National Centre for Biorenewable Energy, Fuels and Materials). Its market is thus expected to grow greatly (market potential: 270,000 ton/a (Willke and Vorlop, 2004)). Succinic acid could indeed be the starting material for the production of a wide range of derivatives, as presented in Figure 3-3, notably of reduced derivatives and esters.

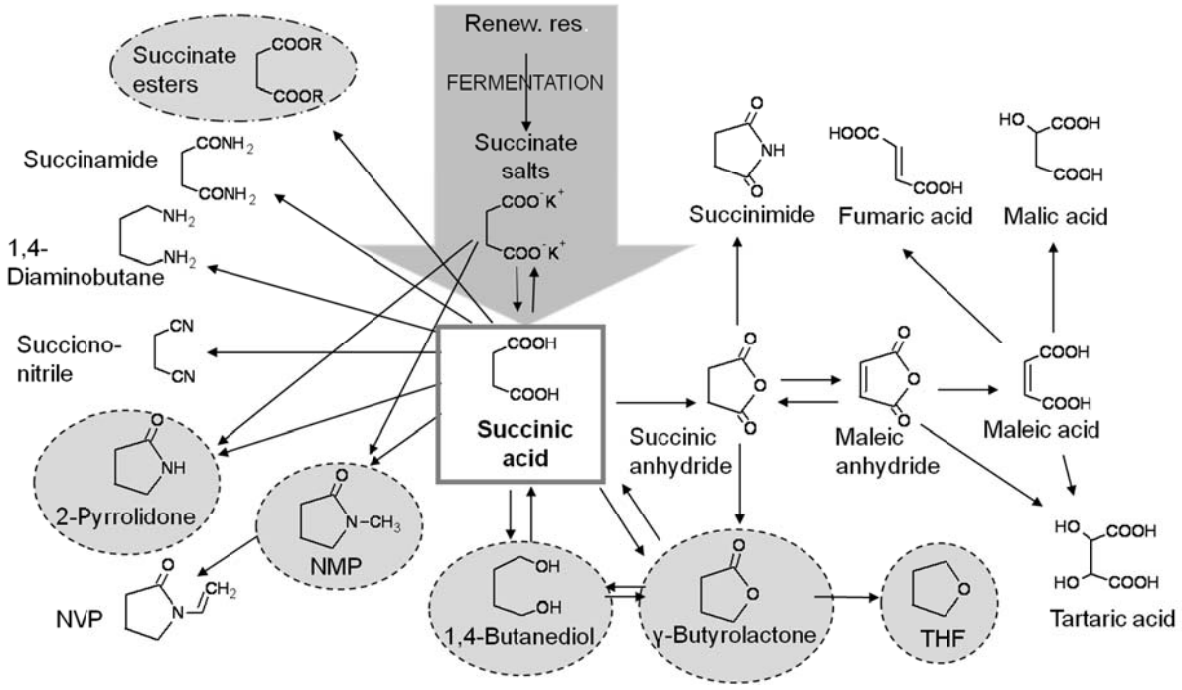
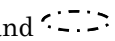


Figure 3-3: Succinic acid as platform chemical (Kamm and Kamm, 2007): reduced derivatives and  succinate esters are particularly of great interest and their production will be studied in this project. (NVP = N-vinyl pyrrolidone; NMP= N-methyl-2-pyrrolidone; THF = tetrahydrofuran).

3.1.5 Reduced derivatives of succinic acid

The reduced products of succinic acid have one of the largest markets among its derivatives. Its main reduced products are γ-butyrolactone (GBL), 1,4-butanediol (BDO),

tetrahydrofuran (THF), 2-pyrrolidone (2-pyrr) and *N*-methyl-2-pyrrolidone (NMP). Their applications, market and price are summarized in Table 3-2.

Table 3-2: Applications, world market and price of succinic acid reduced derivatives (source: Delhomme et al., 2009).

Derivative	Applications	World market, t/a	Price, $/kg
γ-Butyrolactone (GBL)	Used as the starting material for the synthesis of NMP and other pyrrolidones, in particular *N*-vinylpyrrolidone and its polymer which is widely used in medicine. It can also be utilized as a solvent.	250,000	-
1,4-Butanediol (BDO)	Intermediate mainly for the synthesis of THF and polybutylene terephtalate.	1,300,000	0.30-0.41
Tetrahydrofuran (THF)	Used as a monomer for the production of PTMEG, as a solvent in PVC cement, in pharmaceuticals and coatings, or as a reaction solvent.	439,000	0.70-0.77
2-Pyrrolidone (2-pyrr)	Intermediate in the preparation of nylon-4 type polymers or in the synthesis of pharmaceuticals, medicines and agrochemicals.	-	-
N-methyl-2-pyrrolidone (NMP)	Solvent, used e.g. for polyurethanes, polyacrylonitriles and heterocyclic polymers of high melting points. It is also used as an extracting solvent for acetylene and butadiene. NMP can also be used as a replacement for chlorinated solvents as its low volatility results in lower VOC emissions.	-	-

Currently, these reduced derivatives of succinic acid are produced mainly from maleic anhydride by hydrogenation with metal supported catalysts in organic solvents. Only few alternatives starting from maleic acid and using water as reaction medium have been developed (Delhomme et al., 2009). Should maleic anhydride be replaced by succinic acid as C_4 building block chemical, a new reaction pathway would have to be developed from this compound. In particular, it is important to search for water-tolerant catalysts that could be compatible with the aqueous solutions of succinic acid resulting from its biotechnologically production.

3.1.6 Succinate esters

Among succinic acid derivatives, its esters are of a high interest for the chemical, pharmaceutical, food and cosmetic industries, as they have a broad spectrum of applications (see Table 3-3).

Table 3-3: Succinate esters and their applications, alcohols required for their synthesis and water solubility of the alcohols.

Esters	Applications	Alcohol and its water solubility	Lit. ref.
Dimethyl succinate	Green solvent, often used in mixtures with other dimethyl carboxylic esters. It shows low toxicity, is biodegradable and non-VOC. It is used as coating solvent, paint remover, chemical intermediate, resin/adhesive cleanup, etc.	Methanol (C_1), miscible	Cukalovic and Stevens, 2008; the Dow Chemical Company[a] (ESTASOL™)
Monoethyl succinate	Used in treatment of diabetes.	Ethanol (C_2), miscible	Cukalovic and Stevens, 2008; Saravanan and Pari, 2006
Diethyl succinate	Green solvent for replacement of methylene chloride, e.g. employed in fuel oxygenate mixtures. Its incorporation in diesel fuels results in a reduction in particulate emissions. It is also used as flavour in the food industry.	Ethanol (C_2), miscible	Cukalovic and Stevens, 2008; Wisconsin Biorefining Development Initiative[b]
Dipropyl succinate	Used as solvent, GC stationary liquid, fragrance and in manufacturing plastic.	1-Propanol (C_3), miscible	Shaanxi Baoji Baoyu Chemical Co., LTD[c]
Mono or dibutyl succinate	Additive in fuels for reduction of particulates.	Butanol (C_4), 80 g l^{-1}	Berglund, 2009
Diisobutyl succinate	Solvent used in mixtures with other diisobutyl carboxylic esters. This solvent mixture is a non-VOC, low odour, cost effective coalescing agent for paints.	Isobutanol (C_4), 87 g l^{-1}	The Dow Chemical Company[a] (COASOL™)
Diethoxyethyl succinate	Solvent and moistering agent in the cosmetic industry.	2-Ethoxyethanol (C_4), misc.	Ohmori et al., 2008
Diamyl succinate	Solvent and plastic additive.	Pentanol (C_5), 22 g l^{-1}	Shaanxi Baoji Baoyu Chemical Co., LTD[c]
Diisoamyl succinate	Solvent, organic intermediate, plastic additive.	Isoamyl alcohol (C_5), 28 g l^{-1}	Shaanxi Baoji Baoyu Chemical Co., LTD[c]
Dibenzyl succinate	Antispasmodic.	Benzyl alcohol (C_7), 40 g l^{-1}	Downs, 1934
Di-*p*-cresyl succinate	Flavour compounds in the food industry.	*p*-Cresol (C_7), 20 g l^{-1}	Reddy et al., 2005b
Dioctyl succinate	Cold-resistant plasticizers, solvents, intermediates for organic synthesis and GC eluents.	1-Octanol (C_8), 0. 3 g l^{-1}	Zheng et al., 2010
Diethylhexyl succinate	Emollient and glossing agent, known as Crodamol OSU® (Croda International PLC.) used in the cosmetic industry, film forming, plasticiser, solvent.	2-Ethylhexanol (C_8), 1.1 g l^{-1}	Croda International PLC[d]; Huang et al., 1993
Didecyl succinate	Lubricant.	Decanol (C_{10}), insoluble	Clarke, 1981
Oley monoester succinate (OES)	Aqueous viscoelastic fluids.	Oleyl alcohol (C_{18}), insoluble	Hughes et al., 2008

[a] : The Dow Chemical Company: http://www.dow.com/ (2011.05.07)
[b] : Wisconsin Biorefining Development Initiative: http://www.wisbiorefine.org/prod/sacid.pdf (2011.05.07)
[c] : Shaanxi Baoji Baoyu Chemical Co., LTD: http://www.baoyuchem.com/ (2011.05.07)
[d] : Croda International PLC: http://www.croda.com/home.aspx?s=1 (2011.05.07)

Table 3-3 (Con't): Succinate esters and applications; alcohols required for their synthesis and water solubility of the alcohols.

Esters	Applications	Alcohol and its water solubility	Lit. ref.
Tocopherol succinate	Analogue of vitamin E, promising molecule for cancer treatment.	α-Toropherol insoluble	Murray et al., 2006
Chloramphenicol succinate	Precursor of chloramphenicol, used for parenteral administration of chloramphenicol, effective antibiotic in the treatment of typhoid fever.	Chloramphenicol, 2.5 g l^{-1}	Ti et al., 1990
Hydrocortisone succinate	Synthetic corticosteroid administered when the body is deficient in the natural hormone. Used in the treatment of inflammation, allergy, etc.	Hydrocortisol, 0.28 g l^{-1}	Rigge and Jones, 2005
Oestriol succinate	Drug precursor used in hormonal treatment.	Oestriol, slightly soluble	Rauramo et al., 1978
Polyol succinate	Pharmaceutical utility in the treatment and prophylaxis of diseases characterized by a dysfunction in the metabolism and energy status, e.g. diabetes, endotoxemia, etc.		Bjorkling and Malaisse, 2000

To date, these esters are mainly produced from the succinic acid derived from maleic anhydride. If succinic acid becomes a platform chemical, its esters could be directly produced in the fermentation broth. For the final purification, their lower polarity will make them easier to remove from the broth than the very polar succinate.

3.2 Catalysis

Since the syntheses of the aforementioned derivatives (i.e. reduced chemicals and esters) are slow, catalysts must be added to perform the reactions at higher rates. Nowadays, catalysis is widely used in the industry in order to reach economically relevant rates. A catalyst is, by definition, a substance that accelerates the reaction without changing the final equilibrium. This increase of the reaction rate is achieved by creating a new reaction path lowering the energy barriers. A homogeneous catalyst is defined as a catalyst dissolved in the reaction phase, while a heterogeneous catalyst constitutes another phase than the reactants and products. Catalysts are often described in terms of activity, selectivity and stability.

The activity can be determined by the reaction rate and the rate constant as presented in equation (3-1).

$$r = \frac{1}{\nu_i}\frac{dC_i}{dt} = k \cdot f\left(C_i, C_j, C_k, \dots\right) \tag{3-1}$$

with	r	*reaction rate*	*mol l^{-1} h^{-1}*
	k	*rate constant*	*dependent on f*
	$C_{i,j.k}$	*concentrations of the different substrates and products*	*mol l^{-1}*
	ν_i	*stoichiometric coefficient of the substance i*	-
	t	*time*	*h*
	$f(\ldots)$	*function of the concentrations of substrates and products*	

Sometimes, the activity is also described in terms of Turnover Frequency (TOF). It is the specific activity of a catalytic center in defined reaction conditions and for a special reaction and is defined by equation (3-2). It is often calculated from the highest slope of the conversion *vs.* time curve or from the average of the slope on a specific time range.

$$TOF = \frac{1}{n_{cat.cent.}} \frac{1}{\nu_B} \frac{dn_B}{dt} \tag{3-2}$$

with	TOF	*turnover frequency*	*h^{-1}*
	n_B	*moles of a product B in the reaction system*	*mol*
	ν_B	*stoichiometric coefficient of the substance B*	-
	$n_{cat.\ cent.}$	*moles of catalytic center in the reaction system*	*mol l^{-1}*

The Turnover Number TON can also be used to quantify the catalyst activity. It is defined as the number of moles of products per moles of catalytic center during the whole catalyst lifetime (see equation (3-3)). However, the latter is not always available.

$$TON = TOF \cdot catalyst\ lifetime \tag{3-3}$$

with	TON	*turnover number*	-

Besides, the reaction is characterized in terms of conversion, yield and selectivity, as defined in equations (3-4) to (3-7). The selectivity is only defined when multiple products are produced at the same time from different reactions.

$$X = \frac{n_{A,0} - n_A}{n_{A,0}} . 100 \tag{3-4}$$

$$Y_P = \frac{\nu_A}{\nu_P} \frac{n_P}{n_{A,0}} . 100 \tag{3-5}$$

$$S_P = \frac{\nu_A}{\nu_P} \frac{n_P}{\left(n_{A,0} - n_A\right)} . 100 \tag{3-6}$$

$$Y_P = \frac{X \cdot S_P}{100} \tag{3-7}$$

with	X	*conversion*	*%*
	Y_P	*yield of product P*	*%*
	S_P	*selectivity of product P*	*%*
	n_A, ν_A	*moles of substrate with its stoichiometric coefficient*	*mol, -*
	n_P, ν_P	*moles of product P with its stoichiometric coefficient*	*mol, -*

3.2.1 Mass transfer

The observed kinetics is not always the intrinsic kinetics of the reaction. Different transport mechanisms (such as the transports of the substrate and the product into or out of the reaction sites of a heterogeneous catalyst, or in a multi-phasic system the transports of these chemicals between the different phases) take place simultaneous and can be limiting, influencing therefore the observed kinetics. Two types of mass transfer (for either the substrate or the product) can be distinguished:

1. external mass transfer (i.e. between the bulk of the fluid phase and the external surface of the heterogeneous catalyst or between two fluid phases),
2. internal mass transfer (i.e. between the catalysts surface and the reaction sites in an heterogeneous catalyst).

As presented in Figure 3-4, the observed reaction is finally a result of the different steps.

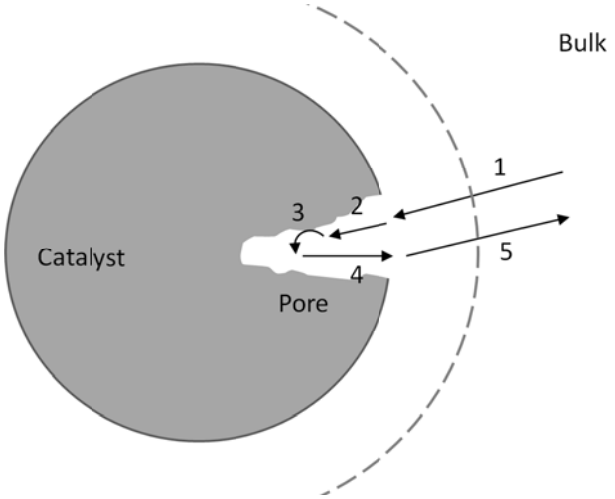


Figure 3-4: Different steps of a heterogeneous catalytic reaction: 1: external mass transfer of the substrate from the bulk to the catalyst surface, 2: internal mass transfer of the substrate in the pores, from the surface of the catalyst to the reaction site, 3: chemical / biological reaction, 4: internal mass transfer of the product in the pores, from the reaction site to the surface of the catalyst, 5: external mass transfer of the product from the catalyst to the bulk.

If three phases are present in the system (two fluid phases and a solid phase), external mass transfers also occur between the two fluid phases. These additional transfers will not be discussed in detail here.

Because of mass transfer limitations, concentration profiles appear in the reactor, as shown in Figure 3-5. It is important to understand that these profiles may change the observed kinetics. In order to characterize a reaction, it is therefore crutial to select the right reaction conditions, so that the observed rate is the intrinsic reaction rate and not the rate of the limiting step, e.g. the internal or external mass transfer. Otherwise, the determination of the kinetics would be falsified by the mass transfer.

Only the mass transfer limitations, and not the heat transfer limitations, will be assessed here, since the studied reactions are not highly exo- or endothermic.

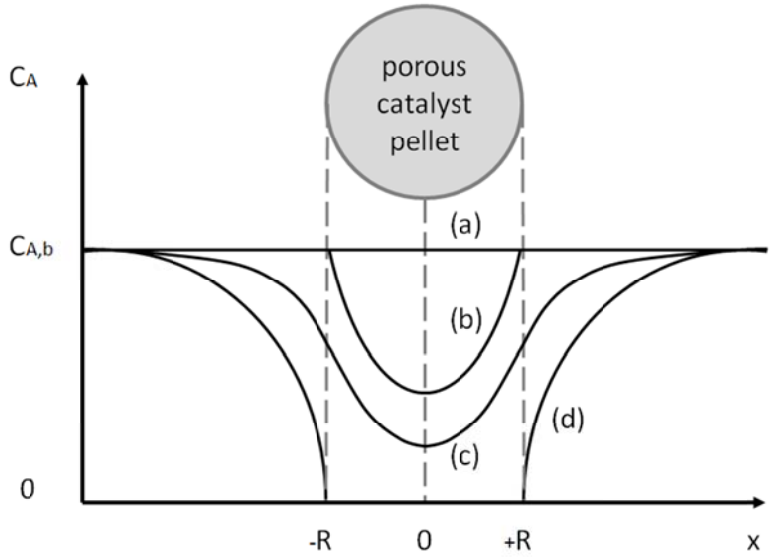


Figure 3-5: Concentration profiles in the fluid and in the porous catalyst pellet when: (a) the reaction is the limiting step, (b) the pore diffusion is limiting, (c) the internal and external mass transfers are limiting, (d) the external mass transfer is limiting (Schmidt, 2005).

3.2.1.1 Pure external mass transfer

The mass transfer from the bulk of the fluid phase to the external surface of the catalyst is called "external mass transfer" and can be described by the "film model" (see Figure 3-6).

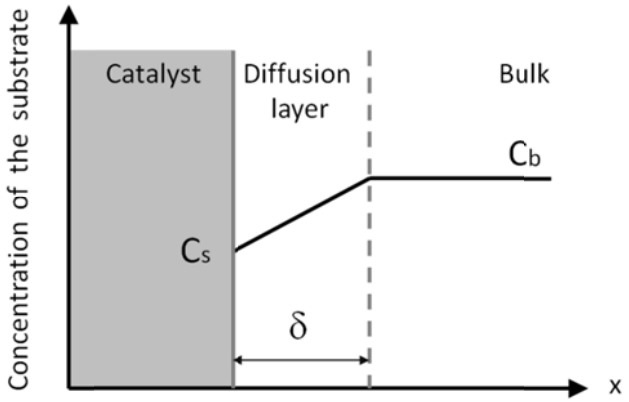


Figure 3-6: Film model applied to the interface between the catalyst and the liquid phase, with C_b the concentration of the substrate in the bulk of the liquid phase and C_s its concentration at the surface of the catalyst.

This model assumes the existence of a stagnant boundary layer of thickness δ surrounding the catalyst interface, and that the complete resistance to mass transfer is located in this layer. The molar flux, J in mol m^{-2} s^{-1}, of the substrate to the catalyst surface through the interface is given by the integration of the Fick's first law on the film layer as presented in equation (3-8).

$$J = -\frac{D}{\delta}(C_b - C_s) = -k_l(C_b - C_s) \tag{3-8}$$

with	J	*molar flux*	*mol m^{-2} s^{-1}*
	D	*diffusion coefficient of the substrate in the liquid phase*	*m^2 s^{-1}*
	δ	*diffusion layer thickness*	*m*
	k_l	*mass transfer coefficient in the liquid phase*	*m s^{-1}*
	C_b	*concentration of the substrate in the bulk of the liquid*	*mol m^{-3}*
	C_s	*concentration of the substrate at the catalyst surface*	*mol m^{-3}*

At steady state, the rate of the external mass transfer is equal to the rate of the surface reaction, leading to equation (3-9).

$$r_{v,p}^{obs} = k_l \cdot a \cdot (C_b - C_s) = k_{v,p} \cdot C_s^n \tag{3-9}$$

with	$r_{v,p}^{obs}$	*observed rate per unit particle volume*	*mol m^{-3} s^{-1}*
	a	*specific surface of the catalyst (surface by volume)*	*m^{-1}*
	$k_{v,p}$	*reaction coefficient per unit of volume catalyst*	*$m^{3(n-1)}$ $mol^{-(n-1)}$ s^{-1}*
	n	*reaction order*	-

If the reaction is irreversible and of first order, equation (3-9) can be simplified to equation (3-10), by eliminating the unknown concentration C_s at the surface.

$$r_{v,p}^{obs} = \frac{1}{\frac{1}{k_{v,p}} + \frac{1}{k_l a}} C_b = \frac{1}{\tau_T + \tau_R} C_b \tag{3-10}$$

with τ_T *external mass transfer resistance* *s*
τ_R *chemical reaction resistance* *s*

A dimensionless number, the Damköhler number I, defined by equation (3-11), can be introduced to describe the external mass transfer limitation.

$$Da = \frac{\tau_T}{\tau_R} = \frac{k_{v,p}}{k_l a} \tag{3-11}$$

with *Da* *Damköhler number I* -

Large values of *Da* correspond to strong mass transfer limitations where the observed rate is the rate of the mass transfer. For small values of *Da*, the external mass transfer can be neglected and the observed rate is the rate of the chemical reaction. However $k_l a$ is not easy to determine and it must be calculated from different equations based on the Sherwood number, that is defined by equation (3-12). The Sherwood number is also calculated from different dimensionless numbers such as the Reynold number and the Schmidt number.

$$Sh = \frac{convective\ mass\ transfer\ coefficient}{diffusive\ mass\ transfer\ coefficient} = \frac{k_l \cdot l}{D} \tag{3-12}$$

with *Sh* *Sherwood number* -
l *characteristic length in the system* *m*

Finally, an external effectiveness factor, η_e, can be introduced and is defined by equation (3-13).

$$\eta_e = \frac{observed\ reaction\ rate}{reaction\ rate\ at\ bulk\ fluid\ conditions} = \frac{r_{v,p}^{obs}}{k_{v,p} \cdot C_b^n} \tag{3-13}$$

with η_e *external effectiveness factor* -

3.2.1.2 Pure internal mass transfer

If the reaction does not occur at the surface of the catalyst but in its pores, the diffusion of the substrate in the catalyst's pores might be limiting and a concentration profile in the pores might occur. The molar flux in the pores, *J* in mol m^{-2} s^{-1}, is defined by equation (3-14) in the case of equimolar counter-diffusion.

$$J = -D_e \frac{dC}{dz} \tag{3-14}$$

with	J	*molar flux*	*mol m^{-2} s^{-1}*
	D_e	*effective diffusion coefficient*	*m^2 s^{-1}*
	z	*particle coordinate*	*m*

However, D_e is relatively difficult to calculate, because it depends on the porosity of the catalyst particule (ε_p), the tortuosity of the pores (τ), the molecular (D_m) and the Knudsen diffusivities (D_k).

In order to quantify the limitation due to the internal mass transfer, the internal effectiveness factor, η_i, can be defined by equation (3-15).

$$\eta_i = \frac{observed\ reaction\ rate}{reaction\ rate\ at\ external\ surface\ conditions} = \frac{r_{v,p}^{obs}}{k_{v,p} \cdot C_s^n} \tag{3-15}$$

with	η_i	*internal effectiveness factor*	-

At steady state conditions, the rate of disappearance of the reactant $r_{v,p}^{obs}$ is equal to the flux through the external surface (van Santen et al., 1999). Through a complex set of equations that depends on the catalyst geometry, it is possible to show that the internal effectiveness factor, η_i, is a function of the dimensionless Thiele modulus, ϕ (see equation (3-17) for spherical catalyst particles), defined by equation (3-16) for a first order irreversible reaction.

$$\phi = R\sqrt{\frac{k_{v,p}}{D_e}} \tag{3-16}$$

$$\eta_i = \frac{3}{\phi}\left[\frac{1}{tanh(\phi)} - \frac{1}{\phi}\right] \tag{3-17}$$

with	ϕ	*Thiele modulus*	-
	R	*radius of the catalyst spherical particle*	*m*

The square of Thiele modulus can be compared to the Damköhler number I since it is the ratio of the internal diffusion resistance to the chemical reaction resistance. Small values of ϕ and therefore $\eta_i \sim 1$ correspond to a situation where the internal diffusion is not limiting. Internal concentration gradients can hence be neglected. Conversely, large values of ϕ ($\eta_i \sim 0$) represent high internal diffusion limitations. The asymptote for large ϕ corresponds to $\eta_i \sim 3/\phi$.

3.2.1.3 Simultaneous external and internal mass transfers

When external and internal mass transfers occur simultaneously, the rate of the external transfer is equal, at steady state, to the rate of surface reaction with internal diffusion, so that the equation (3-18) can be derived. After elimination of the unkown C_s concentration, equation (3-19) can be obtained.

$$r_{v,p}^{obs} = r_{ext} = k_l \cdot a \cdot (C_b - C_s) = r_{rx+int} = \eta_i \cdot k_{v,p} \cdot C_s^n \tag{3-18}$$

$$r_{v,p}^{obs} = \frac{\eta_i \cdot k_{v,p} \cdot C_b}{1 + \frac{\eta_i \cdot k_{v,p}}{k_l \cdot a}} \tag{3-19}$$

with	r_{ext}	*rate of the external mass transfer*	*mol m^{-3} s^{-1}*
	r_{rx+int}	*rate of the reaction with internal mass transfer*	*mol m^{-3} s^{-1}*

3.2.2 Effect of the temperature

In the **kinetic regime**, the effect of the temperature is given by Arrhenius' law (see equation (3-20)), where E_{kin} is the intrinsic activation energy for the rate constant $k_{v,p}$.

$$k_{v,p} = A \cdot \exp\left(-\frac{E_{kin}}{RT}\right) \tag{3-20}$$

with	A	*pre-exponential factor*	*m$^{3(n-1)}$ mol$^{-(n-1)}$ s^{-1}*
	E_{kin}	*activation energy of the chemical reaction*	*J mol^{-1}*
	R	*gas constant (= 8.314472)*	*J mol^{-1} K^{-1}*
	T	*temperature*	*K*

However, if mass transfer limitations arise in the system, the apparent energy of activation will not be the intrinsic one. Depending on the limitation, the temperature can have a different impact on the reaction rate, since the rate is given, for a nth order reaction, by:

$r_{v,p}^{obs} \sim k_{v,p} \cdot C_b^n$	for kinetic regime (limitation by the chemical reaction)
$r_{v,p}^{obs} \sim \eta_i \cdot k_{v,p} \cdot C_b^n$	for internal mass transfer limitation
$r_{v,p}^{obs} \sim k_l \cdot a \cdot C_b$	for external mass transfer limitation

If **internal mass transfer** limitations occur, provided that the Thiele modulus is high (ϕ > 10) and that the external transport resistance is negligible (Roberts, 2009), the

equation (3-21) can be obtained by substituting η_i by $1/\phi$ and replacing ϕ by its expression (see equation (3-16)).

$$ln(\eta_i \cdot k_{v,p}) \sim \frac{1}{2} ln(k_{v,p}) + \frac{1}{2} ln(D_e) - ln(R) \tag{3-21}$$

The value of D_e is generally not very sensible to temperature. Since the activation energy of the diffusivity (~ 5 - 20 kJ mol^{-1}) is much lower than those of the intrinsic reaction (~ 50 - 300 kJ mol^{-1}), the apparent activation energy can be approximated by: $E_{app} \sim E_{kin}/2$.

If the **external mass transfer** is limiting, the rate is almost independent of the temperature, which leads to an apparent activation energy equal to zero.

The **dependence of the rate coefficient with the temperature** can be summarized by Figure 3-7. At low temperature, the kinetic reaction is so slow that it is the limiting step. When increasing the temperature, the reaction rate increases faster than the internal mass transfer, so that the latter becomes limiting. As the temperature further increases, the internal mass transfer rate is further enhanced, whereas the external mass transfer is not affected by the temperate. At high temperature, the external mass transfer becomes thus the limiting step.

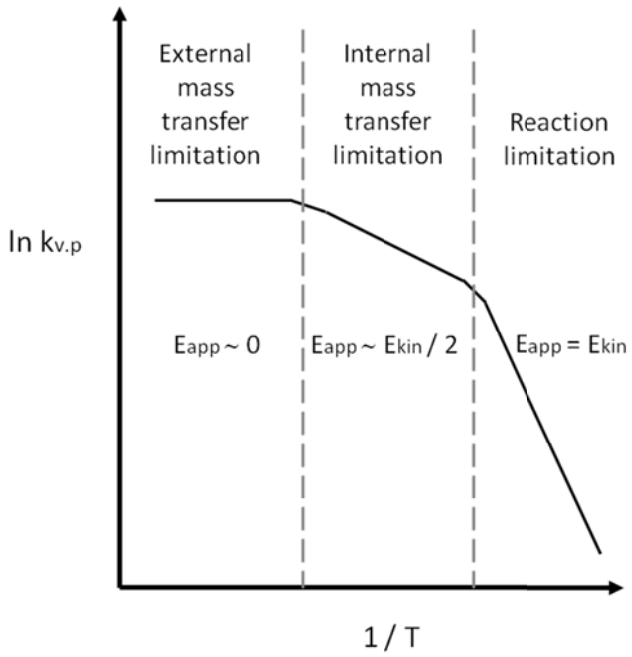


Figure 3-7: Impact of the temperature on the reaction coefficient (Arrhenius' plot).

3.2.3 Effect on the order of reaction

While studying the kinetics of the reaction, it is important to be in reaction conditions where external and internal mass transfers are avoided. Otherwise, the studied kinetics will not be the kinetics of the reaction. In the case of an irreversible n^{th} order reaction, the observed order of reaction n_{obs} under severe diffusional limitations (ϕ >10, $\eta_i \sim 1/\phi$) is not the intrinsic reaction order n but (n+1)/2 (Roberts, 2009). In this case, the experimental data will show the intrinsic concentration dependence only when the reaction is of first order. Otherwise the order will be falsified.

3.2.4 Effect of the catalyst particle size

A possibility to detect an internal mass transfer limitation is to test the effect of the catalyst particle size on the kinetics. When η_i is low, the actual reaction rate is indeed inversely proportional to l_c, the characteristic dimension of the particle. If the internal mass transfer is limiting, the reaction rate will be affected by the dimension of the particle with a constant mass of catalyst.

In conclusion, it is important to understand the different mechanisms that take place in the reaction system, to determine the intrinsinc kinetics of the reaction and derive the optimal reaction conditions for industrial applications.

3.2.5 Two types of catalysis

Two types of catalysts were tested during this study: enzymes and chemical catalysts. Some aspects of the enzymatic catalysis will be briefly discussed in Subsection 3.2.5.1. Concerning the chemical catalysts, the particular case of coordination chemistry will be presented in the Subsection 3.2.5.2. Finally, a possible immobilization strategy will be discussed in Subsection 3.2.5.3.

3.2.5.1 Particularity of enzymatic catalysis

Biological processes are catalyzed and controlled by a wide variety of enzymes. Those are very efficient and selective catalysts. Due to the 3D-conformation of their active sites, they accept only a limited range of substrates and do not react with other functions present on the substrates. Those functions often have to be protected when chemical catalysts are used to prevent undesired reactions.

For a simple one-substrate / one-product mechanism, the enzymatic reaction can be simplified, as represented in equation (3-22). The substrate (S) binds to the enzyme (E) forming the enzyme-substrate complex (ES) that can then be dissociated back into the two initial entities or can react further giving the product (P) and the free enzyme in its initial state.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \tag{3-22}$$

One standard model for describing the kinetics of enzymes was derived by Leonor Michaelis and Maud Menten and is known as the Michaelis-Menten kinetics. It is based on the following assumptions:

- the activated enzyme-substrate complex (ES) is formed by one enzyme entity and one substrate molecule.
- the change in concentration of the ES complex is much slower than those of the substrate (S) and of the product (P), so that d[ES]/dt ~ 0 (pseudo-steady-state assumption).
- the overall reaction is far from the thermodynamic equilibrium and a reverse reaction of the last step is negligible. This can be assumed when initial reaction rates are determined.
- the dissociation of the ES into the product and the free enzyme (k_2) is the rate limiting step.

From these assumptions, the reaction rate (v) can be derived and is given by the Michaelis-Menten kinetic (see equation (3-23)).

$$v = v_{max} \cdot \frac{[S]}{K_m + [S]} \tag{3-23}$$

with	v	*reaction rate*	*mol s^{-1}*
	v_{max}	*maximal reaction rate*	*mol s^{-1}*
	$[S]$	*substrate concentration*	*mol l^{-1}*
	K_m	*half saturation constant*	*mol l^{-1}*

3.2.5.2 Particularity of coordination chemistry catalysis

Coordination chemistry is nowadays very important in catalysis. It deals with compounds usually referred to as metallic complexes, in which a ligand (L) i.e. molecule or ion carrying suitable donor groups, is capable of binding (or coordinating covalently) to a central atom, commonly a metal (M). An atom or molecule can act as a ligand if it shows at least one lone pair of electrons present on what is called the "donor atom" or "donor group". If a ligand presents a single donor atom with a lone pair for binding to a metal ion and thus occupies only one coordination site (i.e. $M^{n+}\leftarrow:L$), it is called a *monodentate* ligand. Many ligands offer several donor groups capable of binding to the same metal and are therefore *polydentate* or *chelate* ligands (Lawrance, 2010). The most commonly used group of metals is the transition metals (e.g. ruthenium, rhodium). Because many transition elements have the capacity to exist in a range of stable oxidation states, even one element can offer different chemistry as a result of the differing *d* electrons present in the diverse oxidation states (Lawrance, 2010).

One of the advantages of these metallic complex catalysts is that the ligands can be engineered to allow the desired selectivity towards the substrate. Furthermore, changes in the ligand set can greatly modify the chemistry of the metal (Crabtree, 2009) and are therefore of great importance.

Among the commonly used ligands, tertiary phosphines PR_3 are a group of ligands in which electronic and steric properties can be altered in a systematic and predictable way over a very wide range by varing R (Crabtree, 2009).

This type of chemistry can allow the development of highly active and selective catalysts. However, the reaction pathways and the effect of the ligands are sometimes not completely understood, making the development of adapted ligands difficult.

3.2.5.3 Particularity of catalyst immobilization on polymer

Different strategies have been developed for the immobilization of metal complexes and many supports have been tested. Among other, the immobilization on polymer is of great interest, because it can allow reactions in a pseudo homogeneous phase when the swelling solvent is well selected.

In 1963, Merrifield introduced the polymer-supported chemistry by publishing his work on "solid phase" peptide synthesis (Merrifield, 1963). The immobilized complexes were synthesized following the scheme presented in Figure 3-8 (a). The most commonly used

polymer is polystyrene (PS) because it is a quite inexpensive material that is chemically inert, robust under mechanical stress and easily functionalized (McNamara et al., 2002). However, as PS is hydrophobic and non-polar, the resin beads only swell in non-protic solvents such as dioxane, dichloromethane, DMF, THF or toluene, but not in polar solvents (e.g. water, alcohols) or in apolar aprotic solvents (e.g. alkanes) (Santini et al., 1998), leading to low pore accessibility.

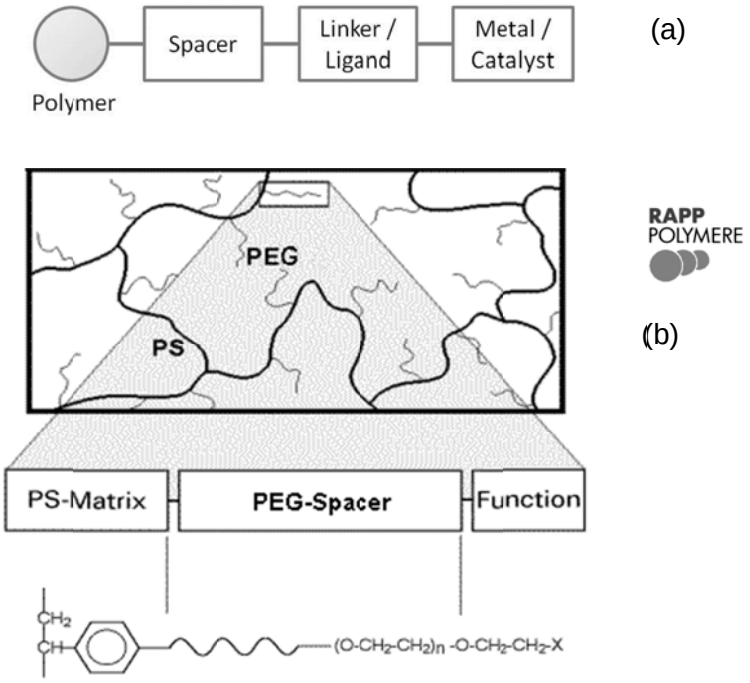


Figure 3-8: Structure of catalyst immobilized on a polymer (Haag and Roller, 2004); (b) chemical architecture of TentaGel® resins (source: Rapp Polymere, Tübingen, Germany), where the function X (e.g. NH_2, OH, Cl) is used to anchore the ligand.

More recently amphiphilic and water-soluble polymers were developed for the solid-phase peptide synthesis. These amphiphilic polymers were synthesized by grafting long poly(ethylene glycol) (PEG) side chains on the 1 % crosslinked polystyrene-divinyl benzene (PS-DVB) matrix (see Figure 3-8 (b)). TentaGel® resins – PS-PEG copolymers – are commercially available with different terminal functional groups (e.g. NH_2, OH or Cl) and are swelling very well in water (i.e. up to 4 ml g^{-1}, source: Rapp Polymere, Tübingen, Germany). These gel type polymers consist of a network of chains in molecular contact with each other. Since the resin has a small surface area in the dry state, it must be swollen in an appropriate solvent, allowing then the access by small molecules to the polymer network (Sherrington, 1998). The solvated PEG chains have then a high degree of mobility and behave like a homogeneous phase (Bayer, 1991).

Besides, the swollen beads are stable under pressures up to 20 MPa, allowing reaction under pressure or plug flow packing (Bayer, 1991). Furthermore, these polymers present a good biocompatibility with enzyme and cells (Park et al., 1997). One drawback of these PS-PEG grafted resin (e.g. TentaGel® S loading: 0.2-0.35 mmol g^{-1}, source: Rapp Polymere, Tübingen, Germany) is, however, the lower loading with respect to PS resins (0.5-2 mmol g^{-1}). This could limit the concentration of immobilized ligands in the polymer. Finally, the immobilization of catalysts on this type of polymer allows aqueous reactions under pressure, while providing the advantages of heterogeneous catalysis (e.g. easy recovery by filtration).

3.3 Hydrogenation

On the particular case of the catalysis of the succinic acid, two main reactions were studied in this project: the hydrogenation that will be discussed in this Subchapter, and the esterification that will be described in the next Subchapter (3.4).

As mentioned in Section 3.1.5, the reduced derivatives of succinic acid – GBL, BDO and THF – are currently produced from maleic anhydride in organic solvent. However, since succinic acid might replace maleic anhydride as bulk chemical in the future, new reaction pathways starting from this compound must be derived. Information was hence researched on potential catalysts and reaction conditions for such new pathways.

Different options for the aqueous hydrogenation of succinic acid or maleic acid, using metal supported heterogeneous catalysts under high temperatures and pressures have first been reviewed and will be shortly summarized in Section 3.3.1. Then, new homogeneous catalysts working under milder conditions have been researched. As no aqueous hydrogenation of succinic acid with metal complexes under mild conditions had been yet reported in the literature, information on the same reaction in organic solvent (starting from succinic anhydride) was gathered and will be reviewed in Section 3.3.2. Afterwards, the aqueous hydrogenation of similar substrates (especially levulinic acid) was considered and will be presented in Section 3.3.3. Finally, a literature survey on the solvent hydrogenation of carboxylic acid esters has been done to pave the way for the synthesis of GBL, BDO and THF starting from succinate esters. This new route will be introduced in Section 3.3.4.

3.3.1 Hydrogenation of succinic or maleic acid in water with metal supported catalysts

The production of succinic acid reduced derivatives is nowadays realized in organic solvent. Nonetheless, several patents have reported the use of water-tolerant metal-supported catalysts for the aqueous hydrogenation of succinic or maleic acid (see Table 3-4). Two reviews (Cukalovic and Stevens, 2008; Delhomme et al., 2009) summarize the different heterogeneous catalysts published or patented for such reactions.

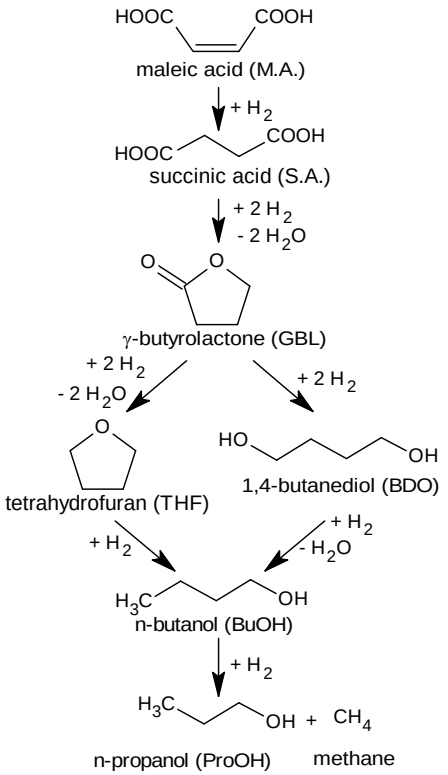


Figure 3-9: Complex network of hydrogenation reactions for the production of reduced products from maleic or succinic acid with metal supported catalysts (Deshpande et al., 2002).

Different metals or metal combinations have been immobilized on diverse supports to achieve high selectivities. The most active catalysts contain group VIII metals, combinations of them or combinations with other metals such as rhenium, tin, etc. In a complex network of reactions, such as the hydrogenation routes from succinic acid shown in Figure 3-9, different metals can promote and/or inhibit diverse reactions and have synergetic interactions, so that the selectivity towards the desired product can be increased. The choice of the metals is hence crucial for the nature of the end-product(s), the yield and the selectivity.

Table 3-4: Metal containing heterogeneous catalysts for the aqueous hydrogenation of maleic or succinic acid into 1,4-butanediol (BDO), tetrahydrofurane (THF) or γ-butyrolactone (GBL).

Company / Institute	Catalyst	T, °C	P, MPa	S*, %	Y* , %	Lit. Ref.
E.I. Du Pont de Nemours	3 % Pd-3 % Re on C	190-200	17	90 (THF)	-	Griffiths and Michel, 1987; Mabry et al., 1985 and 1986
		180	17	79 (BDO)	-	
	1 % Pd-4 % Re on TiO_2	200	3.5	90 (GBL)	90	Rao, 1988
		200	6.9	90 (BDO)	89	
	1 % Ru-4 % Re on C	250	8.3	83 (THF)	82	Schwartz, 1995 and 1996
	7 % Ru-5 % Sn on ZrO_2	225	14	96 (BDO)	94	Tooley and Black, 1999
	1) 1 % Pd on C 2) 1 % Ru-6 % Re on C	1) 120 2) 175	20.7	- (BDO)	82	Bockrath et al., 1999
	1.5 % Ru-3 % Re-0.6 %Sn on C	250	13.8	- (THF)	67	
	1 % Ru-6 % Re on C	250	13.9	48-56 (GBL)	-	Chaudhari et al., 2003
		270	13.9	72 (THF)	-	
	1 % Ru-6 % Re on C	270	15	78 (THF)	-	Thakar et al., 2003
	1 % Pt-6 % Re-0.8 % Sn on C	250	13.9	94 (THF)	-	Campos and Sisler, 2003
	4 % Ru-1.4 % Sn-1.3 % Mo on TiO_2	250	13.9	89 (THF)	-	Campos, 2004
Standard Oil Company	3 % Pd-3 % Re on C	175	9	80 (THF)	-	Budge et al., 1995
	3.3 % Pd-3.2 % Ag-6.6 % Re on C	175	9	74 (BDO)	-	
	3 % Pd-3 % Ag-6 % Re on oxidized C	140	17.2	93 (BDO)	-	Pedersen, 1997
	Pd-Ag-Re-Al on oxidized C	152	17.2	91 (BDO)	-	Budge et al., 1999
	Pd-Ag-Re on oxidized C	1) 130 2) 162	27.6	70 (BDO)	-	Budge et al., 2002
ISP Investments	4 % Pd-4 % Ag-4 % Re on C	-	-	-	-	Hepfer et al., 2006
BP Inc.	3 % Pd-6 % Ag-3 % Re	230-260	5-8	98 (GBL)	-	Kitson and Williams, 1991 and 1992
INEOS USA LLC	1) 0.5 % Pd on Rutile TiO_2 2) 5 % Re on Rutile TiO_2	1) 110[a] / 100[b] 2) 165[a] / 177[b]	17.2-27.6	94 (BDO)[a] 89 (BDO)[b]	91[a] 90[b]	Bhattacharyya and Maynard, 2006a, 2006b and 2007
Battelle Memorial Inst.	5 % Pd-5 % Zr on C	225	17	92 (GBL)	90	Werpy et al., 2002 and 2003

[a]: first set of reaction conditions; [b]: second set of reaction conditions; *: S = selectivity, Y = yield

Schwartz (1995; 1996) highlighted also the need for catalysts to have a very high degree of metal dispersion that would remain constant throughout the many repetitive runs. The formation of unwanted microstructures over time can indeed lead to catalyst aging and activity losses. To that end, different deposition strategies were developed (Schwartz, 1995; Schwartz, 1996; Werpy et al., 2003). The metals are generally deposited on various inert supports (Tooley and Black, 1999). Carbon supports, in spite of their inert behavior with respect to the hydrogenation reaction itself, high surface area and low cost, lead to the formation of carbon fines during the reaction that can plug void spaces. Therefore, new catalyst supports were developed, such as oxidized carbon supports (Budge et al., 1995; Pedersen, 1997), TiO_2 and ZrO_2 supports (Tooley and Black, 1999) and supports in rutile form of TiO_2 (Bhattacharyya and Maynard, 2006a).

Apart from the metals, the reaction conditions can also favour the production of one reduced derivative. The studies on the effect of reaction parameters on the THF selectivity revealed that its selectivity increases with the catalyst loading, the pressure and the temperature (Chaudhari et al., 2003; Mabry et al., 1985; 1986) and is favoured at lower liquid velocities or long liquid residence times (Thakar et al., 2003). Furthermore, continuous vapor removal of the product from the hydrogenation promotes the production of THF at the expense of BDO. Therefore, Campos and Sisler (2003) pointed out that slurry reactors or constantly stirred reactors are optimal for the production of THF. In contrast, BDO formation is favoured at lower temperature and with low temperature liquid removal. Campos and Sisler (2003) recommended therefore the use of a fixed bed catalyst reactor for the production for BDO. Finally, the hydrogenation of maleic acid or succinic acid to GBL is generally difficult to accomplish because GBL can be further hydrogenated. The metals must be hence carefully selected to decrease the rate of the unwanted reactions (Kitson and Williams, 1991).

Concluding remarks

In summary, selecting the right metals and supports for the metal supported catalysts, as well as optimizing the reaction conditions can have a crucial impact on the selectivity towards the desired products. However, considering the data presented in Table 3-4, the optimal reaction conditions reported are generally relatively severe (temperatures up to 270 °C and pressures up to 27.6 MPa). This has a major negative impact on the operating and equipment costs. Therefore, developing novel types of selective catalysts, which are active under milder conditions, is of great interest.

Finally, it should be noted that one major problem with the hydrogenation process of maleic acid is the highly corrosive impact of this product on equipment at temperatures exceeding 140 °C (Bhattacharyya and Maynard, 2006a; Hepfer et al., 2006; Tooley and Black, 1999). Since succinic acid is much less corrosive at elevated temperature, the use of the bio-derived platform chemical (i.e. succinic acid) instead of the oil derived one (i.e. maleic acid or anhydride) has the advantage of avoiding the hazardous handling of the latter.

3.3.2 Hydrogenation of succinic anhydride in solvent with metal complexes

Metallic complexes could be of great interest for developing new catalytic systems for the hydrogenation of succinic acid in water under mild reaction conditions. These complexes have been often reported to work under mild conditions. Since their structure can be modified, high selectivities might be achieved. However, extremely little information can be found on the use of such catalysts for the reduction of carboxylic acids in water.

Nevertheless, the use of metallic complexes for the hydrogenation of carboxylic acid anhydrides in organic solvents has been examined in greater detail and different complexes have thus been synthesized to date. For such reactions, mainly ruthenium and rhodium complexes with phosphine ligands have been reported (see Table 3-5). The hydrogenation product is generally the corresponding lactone (i.e. γ-butyrolactone (GBL) for succinic anhydride). Apart from this, some studies of unsymmetrical anhydrides enabled a better understanding of the reaction mechanism as well as assessment of the enantioselectivity of the reaction. In contrast to heterogeneous catalysts, the metallic complexes do not seem to promote over-hydrogenation of GBL and therefore their use could be interesting for the commercial production of this chemical. Furthermore, these metallic complexes are generally active at much lower pressures and temperatures (often 100-200 °C and 1-3 MPa).

3.3.2.1 Hydrogenation using ruthenium complexes

The first article mentioning this reaction type was published in 1975 by Lyons. He studied several complexes with triphenylphosphine (PPh_3) ligands and different metal centers. Among $[IrCl(CO)(PPh_3)_2]$, $[RhCl(CO)(PPh)_3]$, $[Co_2(CO)_8]$ and $[RuCl_2(PPh_3)]$, the ruthenium complex was the only one active for the production of lactone under the conditions investigated in this article (1 MPa, 100 °C, toluene as solvent). Lyons also mentioned, as would be observed by other researchers later, that the water produced

during the reaction had the unwanted effect of hydrolyzing succinic anhydride to the less active succinic acid. Five years later, Bianchi et al. (1980) tested other ruthenium complexes in dioxane on different carboxylic acids – both aliphatic and aromatic ones – and on dicarboxylic acids and their corresponding anhydrides. The hydrogenations of succinic acid and anhydride were investigated in the presence of the complex $[H_4Ru_4(CO)_8(PBu_3)_4]$. The reaction rate (at 150 °C, 13 MPa) was disappointingly low but a yield and a selectivity of 100 % were finally achieved within 48 h.

Table 3-5: Homogeneous metallic complexes for the hydrogenation of succinic anhydride (or similar substrates) into GBL (or similar products) in organic solvent.

Reaction	Solvent / Reactants	Catalyst	T, °C	P, MPa	X*, %	Y*, %	TOF*	Lit ref.
S.Anh. → GBL	H_2 + toluene	$[RuCl_2(PPh_3)_3]$	100	1	100	50	2	Lyons, 1975
S.Ac. (or Anh.) → GBL	H_2 + dioxane	$H_4Ru_4(CO)_8(PBu_3)_4$	180	13	100	100	2	Bianchi et al., 1980; Frediani et al., 2007
S.Anh. (or Ac.) → GBL	H_2 + TGM	$[Ru(acac)_3]$, P(octyl)$_3$, *p*-TsOH	200	1-3	97	95	257	Hara et al., 2000; Hara and Takahashi, 2000; 2002
Asym S.Ac. → Asym GBL	H_2 + toluene	H_2-$[RuCl_2(PPh_3)_3]$ (or $LiAlH_4$ or Na-EtOH)	100	2.1	-	70-75	-	Morand and Kayser, 1976
Asym S.Ac. → Asym GBL	H_2 + Net_3	$[RhCl_2(PPh_3)_3]$ or $Ru_2Cl_4(diop)_3$ or $RuCl_2(ttp)$	120	~1	-	52-62	1	Ikariya et al., 1984
Asym substrate → Asym lactone	H_2 + toluene	$[RuCl_2(PPh_3)_3]$ or $[RuH_2(PPh_3)_4]$ or $[RhCl(PPh_3)_3]$	180	1	-	56-99	-	Osakada et al., 1982

* : TOF = Turnover Frequency in $mol_{product}\ mol_{metal}^{-1}\ h^{-1}$; X = conversion; Y = yield.

More recently, Hara et al. (2000) from the Mitsubishi Chemical Corporation presented a more systematic development of an organometallic catalytic system for the hydrogenation of succinic anhydride to GBL. Hara et al. described how the ruthenium catalysts reported by Lyons (1975) and Bianchi et al. (1980), despite their ability to produce GBL and no other hydrogenation products, presented some technological drawbacks such as low activities or unfavourable halogen ligands that might corrode the reactor. In the case of the catalyst examined by Lyons (1975), Hara et al. also mentioned that the PPh_3 ligand complexes were not stable at high temperatures (above 180 °C).

Accordingly, there were attempts to develop a catalyst system consisting of a ruthenium salt, alkyl phosphines and an acid promoter in an organic solvent, with the aim of reinforcing the interaction between the substrate and the catalyst (Hara et al., 2000). Among the three types of organometallic complexes – anionic, neutral and cationic –, a cationic complex was expected to make the carbonyl group of the substrate more accessible to the Ru metal and hence to increase the activity. There were attempts to synthesize $[RuHX(PPh_3)_3]$ complexes, with X being more acidic than Cl. These complexes were formed through the reaction between the corresponding Brønsted acid of the anion and a ruthenium complex of the type $[H_2RuP_4]$, P being the phosphine ligand. Although the complex could not be isolated in the pure form, the authors assumed that the catalyst had the structure presented in Figure 3-10.

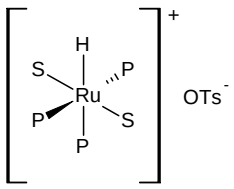


Figure 3-10: Structure of the ruthenium complex from $Ru(acac)_3$ and the phosphine ligand (P) in solvent (S) (Hara et al., 2000).

Through screening of various catalysts, it was found that weakly coordinating anions, like OTs and PF_6, yielded higher activities. Additionally, Brønsted acids not only enhanced the catalytic activity but also the selectivity toward GBL. A structural change in the Ru complexes was induced, leading to cationic complexes, with an increased stability. Among the acids studied, *p*-TsOH was found to be the best candidate because of its solubility, resistance to reduction and low price.

The effect of the ligands on the catalytic properties was then examined and linear trialkyl phosphines, like PBu_3 or $P(octyl)_3$, gave the best recorded activity. The optimal ratio $P(octyl)_3$/Ru was found to be between 5 and 10. However, trialkyl phosphines not only stabilized the ruthenium metal, but also acted as a strong base. Hence, without the presence of the Brønsted acid, formation of spiro dilactone was monitored, leading to a GBL selectivity of only 50 %. With the addition of *p*-TsOH, free $P(octyl)_3$ disappeared and was transformed into a phosphonium salt of $P(octyl)_3$ and *p*-TsOH. Among the solvents studied, tetraethylene glycol dinethyl ether (tetraglyme), dodecyl benzene, and sulfolane allowed high GBL yields. As succinic anhydride is not very soluble in the last two solvents, tetraglyme was regarded as the optimal solvent.

The technology utilizing this novel homogeneous catalytic system to produce GBL was commercialized in 1997 by the Mitsubishi Chemical Corporation. A construction of a plant with a capacity up to 15,000 t/a was finalized in 2002.

3.3.2.2 Reaction mechanism of the hydrogenation of succinic anhydride

A lot of effort has been put into better understanding the reaction mechanism of the hydrogenation of dicarboxylic acid anhydrides to lactones. To that end, the hydrogenation of unsymmetrical anhydrides into asymmetrical lactones has been studied. Two reaction pathways are possible (see Figure 3-11). Morand and Kayser (1976) tried to achieve the regioselective reduction of the less hindered carbonyl group of the anhydride to yield the corresponding asymmetrical lactones **A**. They found that the catalyst proposed by Lyons (1975) was able to catalyze such a reaction in toluene at 100 °C and 2.1 MPa, contrary to $LiAlH_4$ or Na-EtOH, which preferentially catalyzed the reduction of the more hindered carbonyl group (**B**). Ikariya et al. (1984) also investigated the catalytic reaction of unsymmetrical anhydrides with the four Ru catalysts $RuCl_2(PPh_3)_3$, $Ru_3Cl_4(DIOP)_3$, $RuCl_2(TTP)$ and $Ru_2Cl_4(DPPB)_3$. They also obtained the hydrogenation product of the less hindered carbonyl group (**A**) as the major product. As shown in Figure 3-11, they assumed that the reaction occurred by an initial attack of ruthenium to the carbonyl group and successive C-O bond cleavage of one of the C–O bonds.

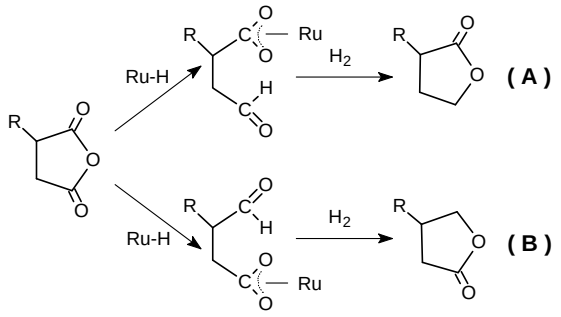


Figure 3-11: Reaction mechanisms of the hydrogenation of asymmetrical anhydrides using ruthenium complexes, proposed by Ikariya et al. (1984).

Ikariya et al. (1978) and then Osakada et al. (1982) tried to explain more precisely the reaction mechanism of the transformation of succinic anhydride into GBL using $[RuH_2(PPh_3)_4]$. Osakada et al. (1982) isolated the intermediate complexes formed by the initial catalyst complex with the anhydride by C–O bond cleavage. Then, upon contact with hydrogen at elevated temperatures (180 °C, 1.2 MPa), or with hydrogen chloride, or

carbon monoxide at atmospheric pressure, these intermediate complexes released the lactones through reduction of formyl or acyl groups in the carboxylate ligands followed by intramolecular condensations.

Concluding remarks

For the hydrogenation of succinic anhydride in organic solvents, ruthenium complexes with mostly phosphine ligands were studied in the literature and gave a GBL selectivity of 100 %. The reaction conditions mentioned were much less severe than those reported for heterogeneous metallic catalysts (see Section 3.3.1). Finally, more work should be invested in transferring this type of reaction to water so that succinic acid could be hydrogenated in fermentation broth under mild reaction conditions.

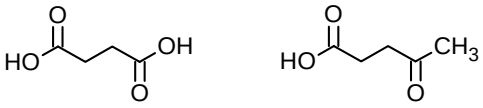
3.3.3 Hydrogenation of levulinic acid in water

As presented in the previous Section, different catalytic systems have been developed for the hydrogenation of succinic anhydride in organic solvents. However, the same reaction in water from succinic acid with metal complexes did not attract much attention. Nevertheless, a similar chemical – levulinic acid (LA) – has been hydrogenated using metal complexes both in organic solvent and in water. This reaction will hence be further investigated, since it might present similarities with the hydrogenation of succinic acid in water.

3.3.3.1 Interest in levulinic acid

Levulinic acid is a C_5 chemical with a carbon structure similar to the one of succinic acid. As shown in Figure 3-12, it has a carboxylic function on one end of the linear carbon chain and bears a ketone function on the other end, contrary to succinic acid, which has a second carboxylic function. In 2004, the US Department of Energy (DoE) cited levulinic acid in his list of promising biomass-derived compounds that could serve as building-block chemicals for the development of new biorefineries (Werpy and Petersen, 2004). Six years later, Bozell and Petersen (2010) confirmed the potential of levulinic acid as C_5 bulk chemical. Levulinic acid, also called 4-oxopentanoic acid, is produced by the chemical hydrolysis of hydroxymethylfurfural (HMF) from biomass. Several processes have been developed from wood, cellulose, starch or glucose (Rackemann and Doherty, 2011). Besides, its production at a commercial scale has already been proven to be feasible (Fitzpatrick, 2002).

Levulinic acid is a very versatile chemical that can be used for the production of many different derivatives, among them γ-valerolactone (GVL) (Yan et al., 2009). GVL can be used for polyester synthesis or as food additive (Yan et al., 2009). Recently it has been reported to be a promising green solvent and fuel additive. It shows a lot of advantages: renewable, easy and safe to store, low melting point, high boiling and flash points, low vapour pressure, low toxicity, stable in aqueous environment and in air (Horváth et al., 2008). Finally, aqueous solutions of γ-valerolactone (GVL) can be converted to liquid alkenes for transportation fuels (Bond et al., 2010).



Succinic acid (C_4) Levulinic acid (C_5)

Figure 3-12: Structures of succinic acid – a dicarboxylic acid – and levulinic acid – a carboxylic acid containing a ketone function. Succinic acid is a C_4 compound whereas levulinic acid is a C_5 chemical.

The hydrogenation of levulinic acid (LA) or its esters into γ-valerolactone (GVL) – a similar lactone to γ-butyrolactone (GBL) produced from succinic acid – has been reported mainly in organic solvents (Bullock et al., 2002; Manzer, 2002; Manzer, 2003; Manzer, 2004; Starodubtseva et al., 2005; Starodubtseva et al., 2007; Yan et al., 2009). However, since it would be more attractive to directly treat the aqueous solution of levulinic acid produced from biomass, hydrogenations in water were also performed with both heterogeneous and homogeneous catalysts.

3.3.3.2 Aqueous hydrogenation of levulinic acid

Mehdi et al. (2008) reported the hydrogenation of levulinic acid with Ru(III) acetylacetonate ($Ru(acac)_3$) and tri-*n*-butyl phosphine (PBu_3), with ammonium hexafluorophosphate (NH_4PF_6) as additive at 10 MPa, 135 °C in water. These conditions are similar to those used by Hara et al. (2000) for the hydrogenation of succinic anhydride in tetraethylene glycol dimethyl ether (tetraglyme). Mehdi et al. performed also the hydrogenation of levulinic acid with $Ru(acac)_3$ as metal precursor and 3,3′,3″-phosphinidynetris(benzenesulfonic acid) trisodium salt (TPPTS) as ligand in distilled water, at 6.9 MPa and 140 °C. They obtained a 95 % yield after 12 h. In addition, they performed the transfer hydrogenation of levulinic acid in water at 70 °C with [(η^6-

C_6Me_6)Ru(bpy)(H_2O)][SO_4] and with sodium formate as hydride donor. After 18 hours of reaction, it resulted in 25 % γ-valerolactone (GVL) and 25 % 1,4-pentandiol.

For the hydrogenation of a continuous flow of aqueous levulinic acid (50 wt %), Serrano-Ruiz et al. (2010) reported the use of the metal supported catalyst 5 % Ru/C at 150 °C and 3.5 MPa achieving 100 % conversion and 96 % selectivity towards GVL.

Two other articles recently reported the transfer hydrogenation of levulinic acid in aqueous solution using formic acid as hydride donor. Deng et al. (2009) used ruthenium (III) trichloride ($RuCl_3$) with triphenylphosphine ligand with pyridine or triethylamine as base to reduced levulinic acid (50 wt % in water) at 150 °C, with yield up to 95 %. Kopetzki and Antonietti (2010) suggested another process in hydrothermal conditions at temperatures up to 220 °C with phosphate salts showing high basicity at high temperature and achieving finally 19 % yield in 20 min.

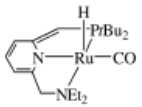
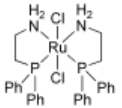
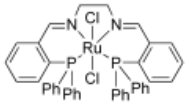
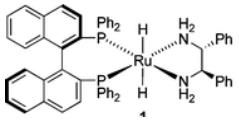
Concluding remarks

The development of the aqueous hydrogenation of the ketone function of levulinic acid is still at its beginning, but metal complexes seem to be promising alternatives for performing such reaction. Hopefully, similar complexes might also be active for the aqueous hydrogenation of succinic acid.

3.3.4 Hydrogenation of esters in solvents using metal complexes

As mentioned earlier, the hydrogenation of succinic acid in water is a new research field that must be developed and studied in detail before finding industrial relevant processes. However, the reduced derivatives of succinic acid might also be produced from its esters. The succinic acid from the fermentation broth could indeed be first esterified (see Subchapter 3.4) and the more easily recovered esters (see Section 3.1.6) could then be hydrogenated in organic solvent. If the use of water as solvent for the hydrogenation of succinic acid turns out to be problematic, this reaction pathway would allow the production of the reduced products. Hydrogenation of esters with metallic complexes has not been as intensively studied as the hydrogenation of ketones. However, several articles could be found in the literature and are summarized in Table 3-6.

Table 3-6: Hydrogenation of carboxylic acid esters in organic solvents.

Substrate	Products	Catalytst	Additive	Solvents	Time	Conversion (X) / Yield (Y), %	TON/TOF, h^{-1}	T, °C	P, MPa	Lit. Ref.
Dimethyl oxalate	Methyl glycolate (E) Ethylene glycol (A)	$Ru(acac)_3$ + Triphos	Zn	Methanol Methanol (dry)	16 h 16 h	X100 Y_E95 X100 Y_E84	160 / 10 857 / 53.5	100	7	Teunissen and Elsevier, 1997
Dimethyl phthalate	Phthalide(L) 1,2-Bis(hydroxyethyl)-benzene (A)	$Ru(acac)_3$ + Triphos	NEt_3 HBF_4 HBF_4	Methanol Methanol Propan-2-ol	16 h 16 h 16 h	X87 Y_L82 Y_A0 X91 Y_L79 Y_A0 X100 Y_L18 Y_A78	56 / 3.5 53 / 3.4 103 / 5.6	100	8.5	Teunissen and Elsevier, 1998
Benzyl benzoate	Benzyl alcohol	$Ru(acac)_3$ + Triphos	NEt_3 NEt_3	Propan-2-ol FIPA[a]	16 h 16 h	X87 Y82 X97 Y95	105 / - 2071/ -	120	8.5	
Dimethyl maleate	Butane-1,4-diol	$Ru(acac)_3$ + Triphos	NEt_3	FIPA[a]	16 h	X100 Y100	2019 / -	120	8.5	
Methyl palmitate	Hexadecan-1-ol	$Ru(acac)_3$ + Triphos	NEt_3	FIPA[a]	16 h	X94 Y94	596 / -	120	8.5	
Dimethyl oxalate	Methyl glycolate (E) Ethylene glycol (A)	$Ru(acac)_3$ + $TriSulf^{Bu}$ $Ru(acac)_3$ + Triphos $Ru(acac)_3$ + P(*n*-Oct)$_3$	Zn Zn Zn	Methanol Methanol Methanol	69 h 5.7 h 304 h	Y_E87 Y_A100 Y_E100	87 / 3.2 200 / 50.3 100 / 0.3	100	8	Boardman et al., 2006
Methyl phenylacetate	2-Phenylethanol	$Ru(acac)_3$ + P(*n*-Oct)$_3$	Zn	Xylene	5 h	Y98		180	1	Nomura et al., 2001; 2002
Benzoate esters Hexyl hexanoate Ethyl butyrate Ethyl acetate *tert*-Butyl acetate Dimethyl terphthalate	Benzyl alcohol 1-Xexanol 1-Butanol Ethanol Ethanol 1,4-Dimethanolbenzene		-	Dioxane	4 h 5 h 4 h 12 h 24 h 5 h	up to X100 Y97 X82 Y82 X100 Y97 X86 Y86 X11 Y11 X100 X97		115	0.54	Zhang et al., 2006
Methyl benzoate Other aromatic or aliphatic esters	Benzyl alcohol Corresponding products		NaOMe 5%	THF	1 h 2.5-4 h	Y99 Y82-99		100	5	Saudan et al., 2007
Aromatic or aliphatic methyl esters	Corresponding products		NaOMe 5%	THF	2.5-4 h	Y83-95		100	5	
Benzoate esters Ethyl hexanoate Methyl cinnamate	Benzyl alcohol Hexanol 3-Phenyl-1-propanol		KO^tBu	THF	3 h 3 h 3 h	up toY100 Y61 Y100		50 30 30	0.4	Takebayashi and Bergens, 2009

[a] *FIPA: 1,1,1,3,3,3,-hexafluropropan-2-ol*

3.3.4.1 Use of phosphine ruthenium complexes

In one of the first articles on the use of metallic complexes for the hydrogenation of esters, Grey et al. (1981) reported that the hydrogenation of non-activated esters was quite difficult, whereas esters with electron-withdrawing substituents were more easily hydrogenated. The best catalyst reported for the activated esters was the potassium hydrido(phosphine)ruthenate anionic complex $K_2^+[(Ph_3P)_3(Ph_2P)Ru_2H_4]^{2-}.2C_6H_{14}O_3$. However, this catalyst that gave high activity towards ketones led to only decarbonylation of methyl acetate. Following this work, different ruthenium catalytic systems were tested for the reduction of esters in organic solvents, especially phosphine ligand with ruthenium metal precursor.

A tridental phosphine – 1,1,1-Tris(diphenylphosphinomethyl)ethane (Triphos) – was used by several groups for the hydrogenation of a wide range of esters with $Ru(acac)_3$ as metal precursor. Teunissen and Elsevier (1997; 1998) reported that a ruthenium complex with a *fac* coordination ligand (such as Triphos) was essential for a high catalytic activity of the complex towards the hydrogenation of dimethyloxate into ethylene glycol. Moreover, van Engelen et al. (2003) underlined the importance of using ruthenium complexes with increased electron density on the metal centre in order to enhance the nucleophilicity of the intermediate hydride towards the less polar carbonyl function of the esters (in comparison with the ketones). Teunissen and Elsevier (1997; 1998) also added zinc to the catalytic system in order to initiate a fast reduction of the acetylacetonate ruthenium complex (Teunissen and Elsevier, 1997). Besides, the formed Zn^{II} can act as a Lewis acid and can activate the ester carbonyl function by coordinating to it and hence ease the attack by the ruthenium complex (van Engelen et al., 2003). However, for the hydrogenation of dimethyl phthalate, the addition of zinc had a negative effect on the production of phthalide, whereas the addition of triethylamine (NEt_3) or fluoroboric acid (HBF_4) (Teunissen and Elsevier, 1998) improved the yields of phthalide.

The choice of the solvent is also crucial. Teunissen and Elsevier noted an improvement of the activity while replacing methanol by propan-2-ol for the hydrogenation of benzyl benzoate. They also tested fluorinated alcohols (e.g. 1,1,1,3,3,3,-hexafluropropan-2-ol (FIPA)) as solvents, leading to a high enhancement of the turnover number. Instead of a transesterification-hydrogenation mechanism, they finally attributed the high catalytic activity in FIPA solvent to an ionic hydrogenation, so that the remarkable activity in the sulfonate alcohols compared to propan-2-ol was probably related to the pK_a of the

alcohols and not to the transesterification. Finally, they could efficiently hydrogenate dimethyl maleate into 1,4-butanediol (BDO) in FIPA solvent. Van Engelen et al. (2003) also underlined the importance of using dry solvents since the presence of water might lead to the hydrolysis of the esters and the decarbonylation of the acid.

Recently, Rosi et al. (2010) used the same catalytic system ($Ru(acac)_3$ and Triphos ligand with zinc) for the hydrogenation of succinic acid in methanol at 120 °C and 8 MPa, leading to the formation of dimethyl succinate as intermediate. However, the reaction times were quite long (from 24 to 72 h). GBL and BDO were produced simultaneously so that the selectivity of the process was limited. The suggested mechanism is presented in Figure 3-13.

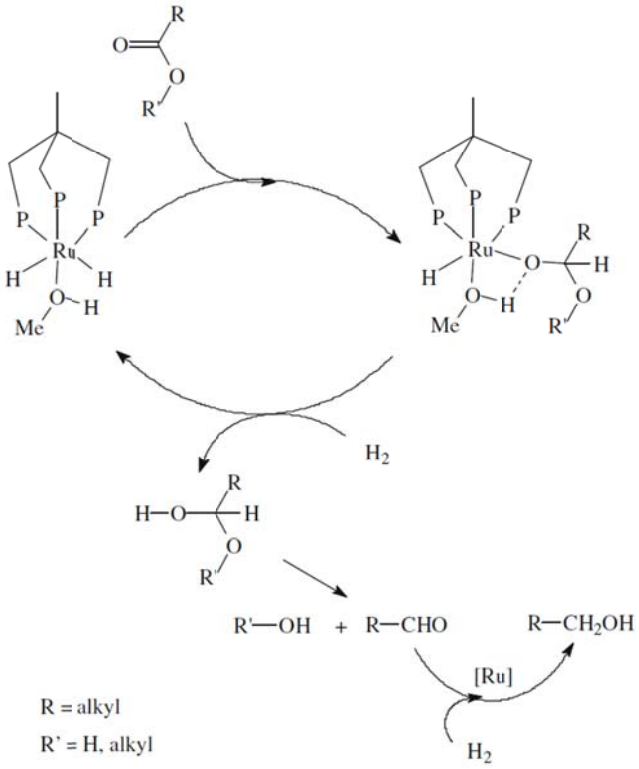


Figure 3-13: Hydrogenation mechanism of carboxylic functions with $Ru(acac)_3$ and Triphos ligand as catalytic system in methanol (Rosi et al., 2010).

Linear phosphine ligands were also used by Nomura et al. (2001; 2002), who developed, based on Hara's work (Hara et al., 2000; Hara and Takahashi, 2000; Hara and Takahashi, 2002), a catalytic system formed *in situ* from the metal precursor $Ru(acac)_3$ and the ligand trioctylphosphine P(*n*-Oct)$_3$ for the hydrogenation of methyl phenylacetate at 180 °C and 1 MPa in tetraglyme solvent. The same catalytic system was indeed reported by Hara et al. for the hydrogenation of succinic anhydride into GBL

at 200 °C and 5 MPa. Similar activities were reported in both tetraglyme and xylene. Trioctylphosphine was more efficient than the Triphos ligand for the hydrogenation of methyl phenylacetate in tetraglyme. Zinc, copper or titanium isopropoxide had a beneficial impact on the activity, whereas methanesulfonic acid and *para*-toluenesulfonic acid, reported as efficient reaction promoter for the synthesis of GBL, slightly increased the activity.

3.3.4.2 Use of other complexes

Similarly to Triphos ligands, sulphur ligands – 1,1,1-tris(*n*-butylthiomethyl)ethane ($TriSulf^{Bu}$) – were synthesized by Boardman et al. (2006) and tested for the hydrogenation of dimethyl oxylate in methanol. The sulphur ligand ruthenium catalysts were formed with or without zinc, but the latter reduced the induction period and increased the rate. The catalytic system with $TriSulf^{Bu}$ gave a selectivity towards methyl glycolate whereas Triphos or P(*n*-Oct)$_3$ produced mainly ethylene glycol. Finally the complex with the linear trioctylphosphine showed limited turnover frequency.

Aside from phosphine (or phosphine-like ligand) ruthenium complexes, research groups developed also different ruthenium complexes with both phosphine and amine ligands for the hydrogenation of esters in organic solvent. Those were similar to some highly active complexes designed for the ketone reduction. Zhang et al. (2006) developed a PNN ligand ruthenium complex for the hydrogenation of non-activated aromatic and aliphatic esters in dioxane under low pressure (0.54 MPa) and limited temperature (115 °C). The comparison with the analogous PNP system revealed a major ligand effect, attributed to the hemilability of the PNN ligand. The dissociation of the amide side can indeed provide a site for the ester coordination to the metal center. They concluded hence that the mechanism differs from the ones reported for ruthenium-catalyzed ketone reduction, in which the binding of the ketone to the metal is not required, as hydrogenation takes place by a concerted hydride/proton transfer.

Saudan et al. (2007) proposed PN ligand ruthenium complexes for the hydrogenation of aromatic and aliphatic esters using THF as solvent, since methanol gave no activity. Best yields were indeed reported in ethereal solvents. A base was also required to transform the ruthenium complex into an active catalyst. Sodium methoxide (NaOMe) (1 - 10 %) gave the best results, whereas triethylamine (NEt_3) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were inefficient.

Finally, Takebayashi and Bergens (2009) reported the use of a ruthenium complex with (S)-(-)-2,2'-bis(di-*p*-tolylphosphino)-1,1'-binaphthyl ((S)-binap) ligand and diamine ligands. The hydrogenation of esters or lactones was performed in THF under low pressure (0.4 MPa) and low temperature (30-50 °C). The hydrogenation of ethyl hexanoate was, however, limited by product inhibition. In opposition to what was reported by Zhang et al. (2006) with their PNN ruthenium complex, they suggested that the (S)-binap diamine ruthenium complex undergoes facile addition of lactones and esters in a similar mechanism as the addition of ketones. The additions either proceed in a bifunctional manner or form the hemiacetal hydrogen bonded to a ruthenium-amide group, which then changes into a hemiacetaloxide. Alternatively, the bifunctional addition forms the hemiacetal oxide direct through a partial Ru-oxygen bond in the transition state. A competition might take place during the hydrogenation between the alcohol products that react with the amide and the ester that reacts with the dihydride. This competition could be the origin of the product inhibition. However, several substrates such as methyl benzoate and methyl cinnamate were hydrogenated with yields up to 100 % at 30 °C or 50 °C.

Concluding remarks

Ruthenium complexes with Triphos ligands seem to be good candidates for the catalytic hydrogenation of succinate esters in organic solvent. However, little is understood on the role of the additive (e.g. zinc) and of the solvent. Furthermore, the highly expensive fluorinated alcohols, which gave high activity for non-activated esters, are not well suited for industrial applications. Therefore other alternatives should be developed for the fast catalysis of non-activated esters. Kinetic studies and more systematic screenings of ligands, metals, additives and reaction conditions must still be realized for a better understanding of the reaction mechanism.

3.4 Esterification

Aside from the reduced derivatives of the bioderived succinic acid, its esters are also of great interest for the chemical, pharmaceutical, food and cosmetic industries as presented in Table 3-3. Since the esters are easier to purify than the corresponding carboxylic acid, performing the esterification of the succinic acid in the fermentation broth without prior purification would be favourable. This process must be hence developed for industrial applications. The principle of the esterification (see Sections

3.4.1 and 3.4.2) and the state of the art of the esterification with chemical and biological catalysts (see Sections 3.4.3 and 3.4.4) will be presented here.

3.4.1 Principle and mechanism

An esterification reaction is the reversible dehydrative reaction of a carboxylic acid (RCOOH) with an alcohol (R'OH) leading to the formation of an ester (RCOOR') and a water molecule, as presented in equation (3-24). The reverse reaction of the esterification is the hydrolysis of the esters.

$$RCOOH + R'OH \rightleftharpoons RCOOR' + H_2O \quad (3\text{-}24)$$

An esterification is generally an extremely slow process. Therefore catalysts are often required to perform the reaction with relatively high reaction rates. Two types of catalysts are often reported for such a reaction: chemical catalysts, mainly Brønsted and Lewis acid, or enzymes (lipases or esterases). The reaction mechanisms with these two types of catalysts differ strongly.

The esterification catalyzed by chemical catalysts follows the Fischer-Speier mechanism. This reaction was first described in 1805 by Emil Fischer and Arthur Speier (1895). The Brønsted acid, by definition, acts as a proton donor, whereas the Lewis acid is an electron donor, enhancing both the electrophilicity of the carbonyl carbon for an easier nucleophilic attack of the alcohol. Similar to the mechanism with the Lewis acid, the reaction with the Brønsted acid, shown in Figure 3-14 (Carey, 2008), consists of the following steps:

1. An alkyloxonium ion is formed by proton transfer from the acid catalyst to the alcohol.
2. The carboxylic acid is protonated on its carbonyl oxygen by the alkyloxonium ion.
3. A molecule of the alcohol acts as a nucleophile and attacks the carbonyl carbon.
4. The oxonium ion formed in step 3 loses a proton to give the tetrahedral intermediate in its neutral form.
5. The tetrahedral intermediate is protonated on one of its hydroxyl oxygens.
6. This intermediate loses a molecule of water to give the protonated form of the ester.
7. Deprotonation of the species formed in step 6 gives the neutral form of the ester product.

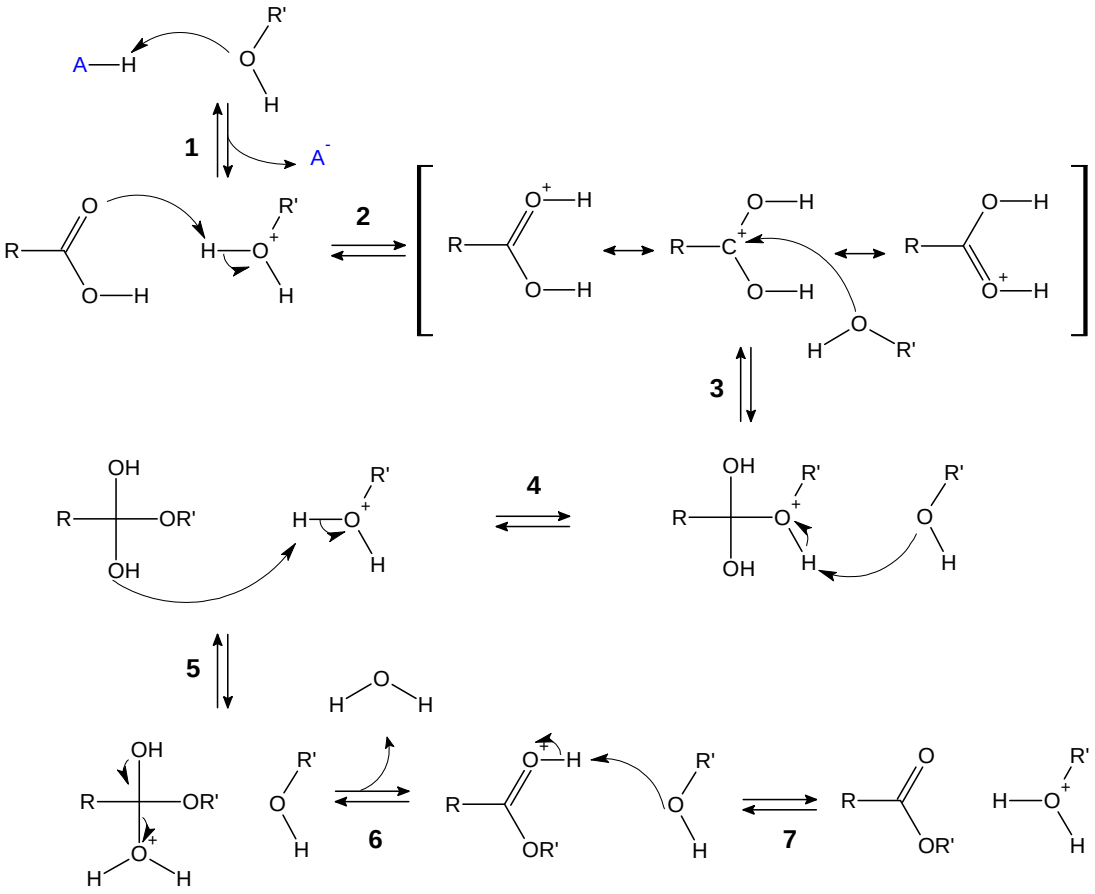


Figure 3-14: Fischer-Speier mechanism for the esterification of a carboxylic acid with an alcohol under acidic conditions (Carey, 2008).

The reaction mechanism with enzymes is different. In lipases, a triad of amino acids (serine (Ser), histidine (His) and aspartic acid (Asp)) is mainly responsible for the catalytic activity of the enzyme. This triad promotes the charge delocalization and therefore enhances the nucleophilicity of the serine. The reaction mechanism for the lipase B from *Candida antarctica* has been reported by Kwon et al. (2007) and Li et al. (2010a) and is presented in Figure 3-15. In the first step of this reaction, the serine is acylated by the carbonyl function of the carboxylic acid forming the first tetrahedral intermediate. The oxyanion is stabilized by three hydrogen bonds in a so-called "oxyanion hole". After the release of a water molecule (if the initial substrate is a carboxylic acid (R^2 = H)), the alcohol (R^3OH) acts as a nucleophile and attacks the carbonyl carbon attached to the serine and a second tetrahedral intermediate is formed. Here also, the oxyanion of the tetrahedral intermediate is stabilized by the oxyanion hole. Finally, the ester is released from the active site. The mechanism follows a Ping-Pong-Bi-Bi mechanism and can be simplified as presented in the diagram of Figure 3-16.

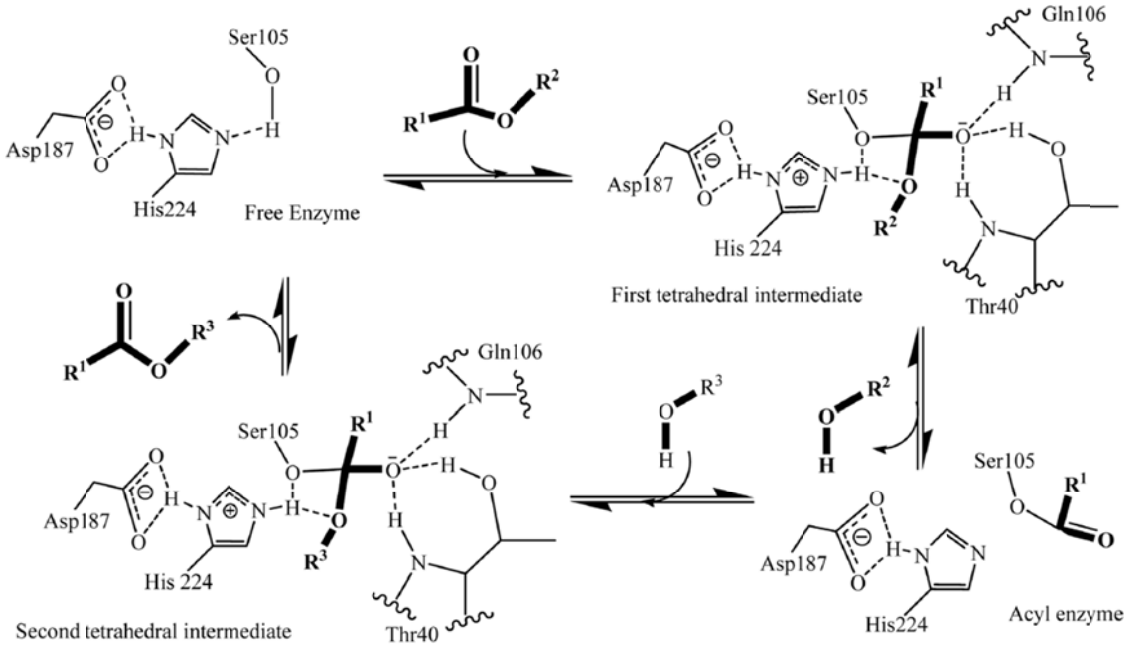


Figure 3-15: Esterification mechanism in the lipase B from *Candida antarctica* (Kwon et al., 2007).

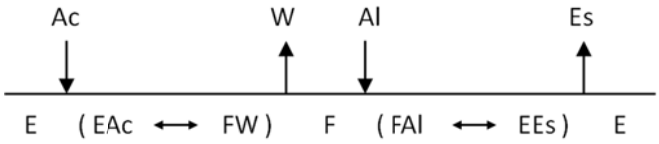


Figure 3-16: Ping-Pong Bi-Bi Mechanism for the esterification of a carboxylic acid (Ac) with and alcohol (Al) producing water (W) and the ester (Es). E represents the enzyme and F the intermediate state of the enzyme.

3.4.2 Biphasic esterification

An esterification is a dehydrative condensation, i.e. a condensation of a carboxylic acid with an alcohol molecule with the liberation of water. The equilibrium is hence shifted backward while working in aqueous media. The esterification in water is therefore challenging because it is limited by the equilibrium. However, the esterification of succinic acid in water is of great interest since it allows the direct production of derivatives with high market potential. This reaction can be either performed in a monophasic system if the alcohol is water-soluble or in a biphasic one if the alcohol is not miscible or if a co-solvent is added. If a two-phase strategy is chosen, it is important to understand the different reactions or equilibriums that take place simultaneously in the different phases (see Figure 3-17):

- the pure extraction of succinic acid from the aqueous phase into the organic phase,
- the acid / base equilibrium between the different species of succinic acid in the aqueous phase (diprotonated, monoprotonated and non-protonated forms),
- the esterification of succinic acid into monosuccinate ester and then into its diester.

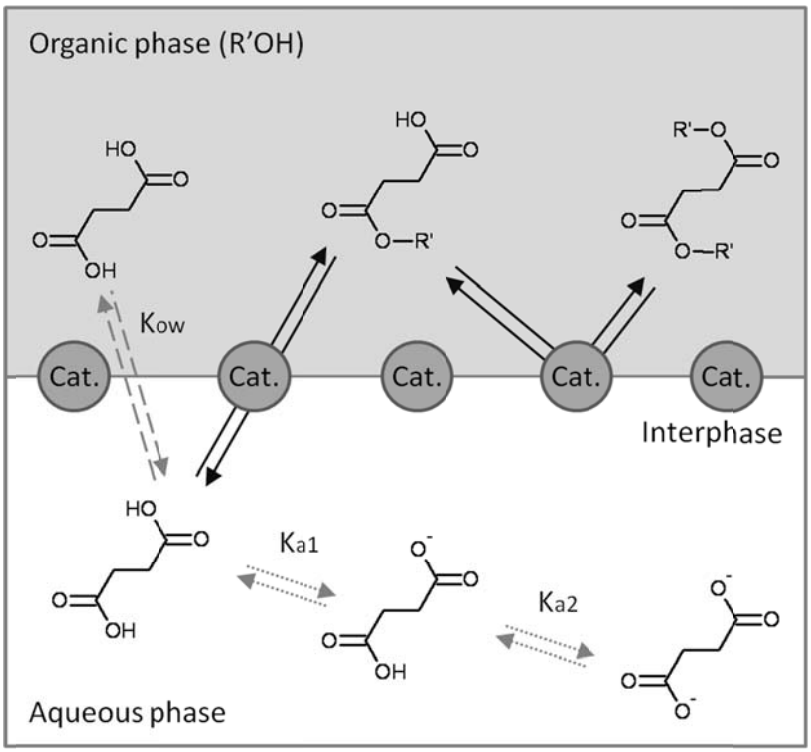


Figure 3-17: Biphasic esterification of succinic acid with a non-water miscible alcohol (R'OH): ⟶ represents the esterification or hydrolysis reactions, ⇢ the pure extraction of succinic acid into the organic phase and ⋯▸ the equilibrium reactions between the difference species (diprotonated, monoprotonated and non-protonated forms) of succinic acid.

First, even if no esterification is taking place, the succinic acid contained in the aqueous phase can simply be extracted into the alcohol phase (e.g. here 1-octanol). The concentration of the succinic acid extracted into the organic phase and the concentration of succinic acid in the aqueous phase at equilibrium are linked to the logarithm of the constant of extraction in water / 1-octanol system (log P) and can be calculated from equations (3-25) and (3-26). Log P for succinic acid has been reported to be of -0.59 (Hans et al., 1995). The extraction is much faster than the esterification reaction and can be considered to happen instantaneously.

$$K_{ow} = \frac{[AH_2]_{org}^{eq}}{[AH_2]_{aq}^{eq}} \quad (3\text{-}25)$$

$$logP = log(K_{ow}) \quad (3\text{-}26)$$

with	*P or K_{ow}*	*constant of extraction for water / 1-octanol system*	-
	$[AH_2]_{org}^{eq}$	*concentration of diprotonated succinic acid at equilibrium in the organic phase*	*mol l^{-1}*
	$[AH_2]_{aq}^{eq}$	*concentration of diprotonated succinic acid at equilibrium in the aqueous phase*	*mol l^{-1}*

Second, in the aqueous phase, depending on the pH, different forms (diprotonated, monoprotonated and non-protonated) of succinic acid are present. The concentrations of the different species are regulated by the equilibrium constants (K_{a1} and K_{a2}) presented in equations (3-27) and (3-28). These constants are characterized by their decimal logarithm (pK_{a1} = 4.21 and pK_{a2} = 5.64 for succinic acid).

$$K_{a1} = \frac{[AH^-]_{aq}[H^+]_{aq}}{[AH_2]_{aq}} \quad (3\text{-}27)$$

$$K_{a2} = \frac{[A^{2-}]_{aq}[H^+]_{aq}}{[AH^-]_{aq}} \quad (3\text{-}28)$$

with	*K_{a1}*	*equilibrium constant for the first acidity of succinic acid*	*mol l^{-1}*
	K_{a2}	*equilibrium constant for the second acidity of succinic acid*	*mol l^{-1}*
	$[AH_2]_{aq}$	*concentration of diprotonated succinic acid in aq. phase*	*mol l^{-1}*
	$[AH^-]_{aq}$	*concentration of monoprotonated succinic acid in aq. phase*	*mol l^{-1}*
	$[A^{2-}]_{aq}$	*concentration of non-protonated succinic acid in aq. phase*	*mol l^{-1}*
	$[H^+]_{aq}$	*proton concentration in the aqueous phase*	*mol l^{-1}*

With these two equations (3-27) and (3-28), the molar ratio of the different species can be calculated given the pH of the aqueous phase as shown in Figure 3-18.

Finally, the esterification of succinic acid into its diesters takes place in two steps with first the production of the monoester of succinic acid and then its conversion into the diester. Both species are supposed to be almost instantaneously extracted into the organic phase as they bear a long aliphatic chain on the ester functions.

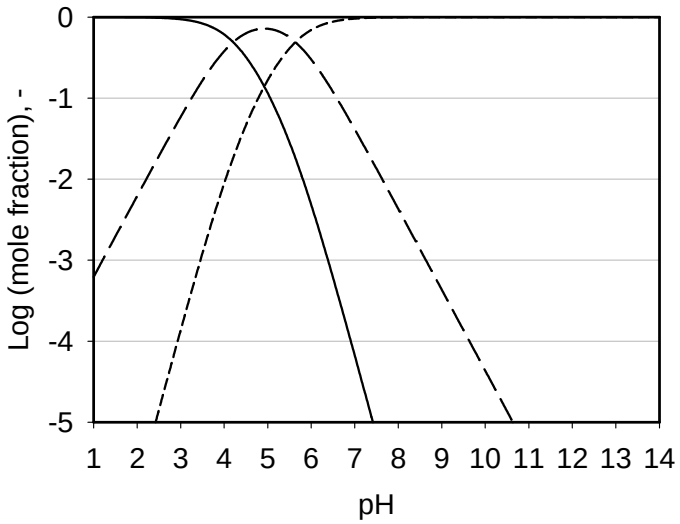


Figure 3-18: Distribution of the different species of succinic acid over the pH range: logarithm of the mole fraction of the different species of succinic acid *vs.* pH (—— diprotonated form AH_2, – – monoprotonated form AH^- and - - - non-protonated form A^{2-}).

For the esterification in mono- or biphasic systems, different approaches have been reported in the literature and will be summarized in Section 3.4.3 for the chemical catalysts and in Section 3.4.4 for the enzymes.

3.4.3 State of the art for the chemical catalysis

Classic catalysts for the esterification of carboxylic acid are chemical ones, mainly Brønsted or Lewis acids. Since this reaction is dehydrative, the esterification has mainly been reported in solvent-free conditions or in organic solvents (see Table 3-7). Nevertheless, a few articles reported esterification reactions in presence of water, as shown in Table 3-8.

3.4.3.1 Catalysts for reactions in organic solvents

The chemical catalysts can be separated into two classes, the homogeneous catalysts and the heterogeneous ones. In the field of homogeneous catalysis, Lewis or Brønsted acids such as sulfuric acid (H_2SO_4) (Krbechek, 1994), toluenesulfonic acid (*p*-TsOH), methanesulfonic acid, 4-(dimethylamino)-pyridin (DMAP), tertiary amines (Bauduin et al., 2009; Datta et Tsai, 1998) and I_2 (Ramalinga et al., 2002) have widely been used so far for esterifications.

Table 3-7: Chemical catalysts for the esterification of carboxylic acids with alcohols in solvent-free conditions or in organic solvents.

Carboxylic acid	Alcohol	Solvent	Catalyst	Yield (Y) / conversion (X), %	Lit. Ref.
Isooctanoic acid	Methanol	-	H_2SO_4	Y92 (10 h)	Krbechek, 1994
Lactic and butyric acid	Ethanol	-	DMAP or *p*-TsOH	Y19-41 (72 h)	Datta and Tsai, 1998
			Amberlyst 5 or XN-1010	Y99	
Methacrylic, propionic, hexahydrophthalic, phthalic acid	Methanol, isobutanol, isoocyl alcohol	-	$SO_4^=/ZrO_2$, $SO_4^=/TiO_2$, NH_4ZSM-5, Nafion NR-50	Y99	Wu et al., 1998
Hexanoic acid	1-Octanol	Cumene	Nafion SAC-13	Y92 (5 h)	Nijhuis et al., 2002
			Zeolite BEA	Y99	
Acetic acid	Methanol	THF	Nafion SAC-13	X75 (11 h)	Liu et al., 2006b
			H_2SO_4	X80	
Succinic acid (SA) and diverse carbox. acids	Methanol or ethanol	Toluene	Fe^{3+}-MTM* clay	SA: Y70 (4.5 h)	Kantam et al., 2002
Phenyl-acetic acid	*p*-Cresol	Toluene	Al^{3+}-MTM* clay	Y77 (6 h)	Reddy et al., 2004
Succinic acid	*n*-Butanol	o-Xylene, toluene, benzene, dioxane	M^{n+}-MTM* clay (Al^{3+}, Fe^{3+}, Cr^{3+}, …)	Y94 (8 h)	Reddy et al., 2005
Succinic anhydride	*p*-Cresol	Toluene	M^{n+}-MTM* clay (Al^{3+}, H^+, Cr^{3+}, …)	Y78 (12 h)	Reddy et al., 2005b
			p-TsOH		
Succinic acid	Ethanol	-	Amberlyst 15	Y~70 (13 h)	Kolah et al., 2008
Maleic anhydride	Methanol	-	Al-MCM-41 (mesoporous molecular sieves)	▫ Y78 (9 h)	Bhagiyalakshmi et al., 2004
			Hß-zeolite	▫ X100 (9 h)	

*: MTM = Montmorillonite

As for heterogeneous catalysts, a lot of different catalysts have been developed and screened for this type of reaction mainly in organic solvents. Ion exchange resins, especially Amberlysts™, are widely used for solvent esterification (Datta and Tsai, 1998; Kolah et al., 2008). These solid catalysts from the DOW Chemical Company have the advantages of replacing mineral and organic acids and bases in various syntheses. The Amberlyst resins used for esterification often contain $–SO_3H$ functions. Different strategies have also been studied to immobilize $–SO_3H$ or $–SO_4$ functions on diverse supports: Nafion® is, for example, a sulfonated tetrafluoroethylene based fluoropolymer-copolymer discovered in the late 1960s by Walther Grot of E. I. Du Pont de Nemours and Company (see Figure 3-19). This polymer has also been immobilized on silica supports and tested for esterification reactions by Nijhuis et al. (2002) and Liu et al. (2006b) and is referred as Nafion SAC-13. As for Wu et al. (1998), they tried to immobilize $-SO_4$ groups on diverse metal oxides.

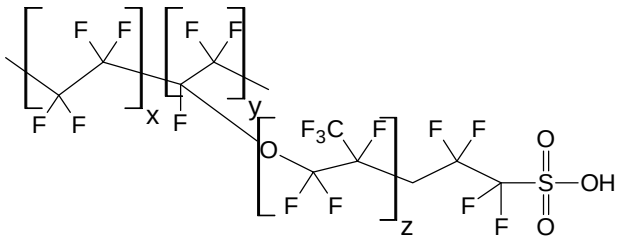


Figure 3-19: Nafion, a sulfonated tetrafluoroethylene based fluoropolymer-copolymer discovered in the late 1960s by DuPont.

Besides, a silicate-based clay, Montmorillonite, has been investigated for the esterification of different carboxylic acids in organic solvent (Kantam et al., 2002; Reddy et al., 2004; 2005a; 2005b). It is a very soft phyllosilicate from the smectite family, with two tetrahedral sheets surrounding a central octahedral sheet (i.e. 2:1 clay) as shown on Figure 3-20. This type of clay is of high interest for environmentally benign and reusable catalyst applications (Kawabata et al., 2005). Montmorillonite clay has the ability to swell in different solvents and especially water. The hydrated cations on the interlamellar surfaces might be replaced with different cations by utilising simple ion exchange methods. Even neutral molecules can be intercalated between the silicate layers (Pinnavaia, 1983). Incorporating mono or complex oligomeric cations leads to a porous solid that possesses some properties of zeolites, such as strong acidity and regular porosity (Singh et al., 2007). For esterification reactions, mainly H^+, Al^{3+} and Fe^{3+} pillared Montmorillonites have proven to be the best catalysts in organic solvent.

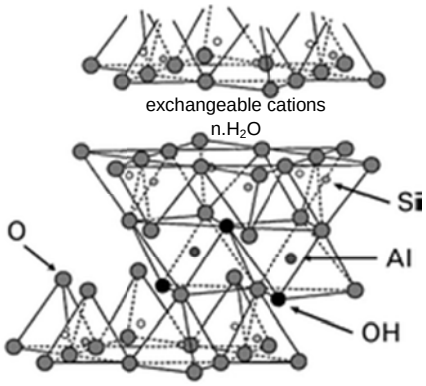


Figure 3-20: Montmorillonite structure (Kowal-Fouchard et al., 2004) $(Na,Ca)_{0.3}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot n(H_2O)$

Finally, different heterogeneous molecular sieves have also been tested: zeolite BEA (Nijhuis et al., 2002), Al-MCM-41 and Hß-zeolite (Bhagiyalakshmi et al., 2004). Zeolites, microporous aluminosilicate minerals, can also accommodate different cations in their porous structures. They are powerful solid-state acids in their hydrogen form and are therefore used as catalyst in different syntheses.

3.4.3.2 Alternatives for reactions in presence of water

It is important to underline that, even in organic solvents, the water formation during the esterification reaction often reduces dramatically the catalyst activity so that water scavenger must be introduced in the reaction system. In homogeneous conditions, the activity loss caused by water has been attributed to the reverse hydrolysis and to competitive protonation steps involving water and the alcohol (Liu et al., 2006a). If the reaction has to be performed in water, it is very challenging to achieve the esterification with a reasonably high activity. Different strategies have hence been developed to reduce the contact of the reaction sites or of the esters formed with water.

The use of Brønsted acid surfactants have been developed by Manabe et al. (2002) for the esterification of organic substrates in water. Among them, dodecylbenzenesulfonic acid (DBSA) is a relatively cheap and widely used commercial surfactant that forms reverse microemulsion (see Figure 3-21). The emulsion droplets are dispersed in water and are hydrophobic enough to protect water-labile substrates or intermediates from hydrolytic decomposition. The surfactant molecules are also concentrating protons onto the surface of the droplets so that the reaction rate will be enhanced. In addition, water is removed directly from the droplets due to hydrophobic interactions inside the droplet core, thus lowering the water inhibition as shown in Figure 3-21 (b).

Table 3-8: Chemical catalysts for the esterification of carboxylic acids with alcohols in presence of water.

Carboxylic acid	Alcohol	Catalyst	Principle	Yield (Y) / conversion (X), %	Lit. Ref.
Diverse	Diverse	I_2		Up to Y98 (4-20 h)	Ramalinga et al., 2002
Lauric acid	3-Phenyl-1-propanol	DBSA	Surfactant	Y84 (170 h)	Manabe et al., 2002
Diverse non-polar acids	Diverse non-polar alcohols	Polystyrene supported sulfonic acid (DBSA like)		Y74 (24 h)	Manabe and Kobayashi, 2002
Butyric acid	Diverses	Polystyrene supported sulfonimide (PS-SI)		Up to Y92 (48 h)	Zhang et al., 2007
Succinic acid	Ethanol	Starbon		Y90 (7 h)	Budarin et al., 2007c
Succinic, fumaric, itaconic, levulinic acid	Ethanol	Starbon		Y99 (8 h)	Budarin et al., 2007a; 2007b
Succinic acid, Lactic acid	Ethanol	Amberlyst XN-1010, Nafion NR-50	Pervaporation		Benedict et al., 2006
Acetic acid	Ethanol	Heteropolyacids	Reactive distillation	Up to Y80	Fujita et al., 2004
Acetic acid	*n*-Butanol, *iso*-amyl alcohol	Indion 130 *(macroporous ion-exchange resin)*	Reactive distillation column	Up to X58	Saha et al., 2000
Succinic acid	Butanol	*p*-TsOH, methanesulfonic acid, DMAP, tertiary amines Amberlyst 15, Amberlyst 19, DPT 1	Reactive distillation	Up to Y97	Bauduin et al., 2009

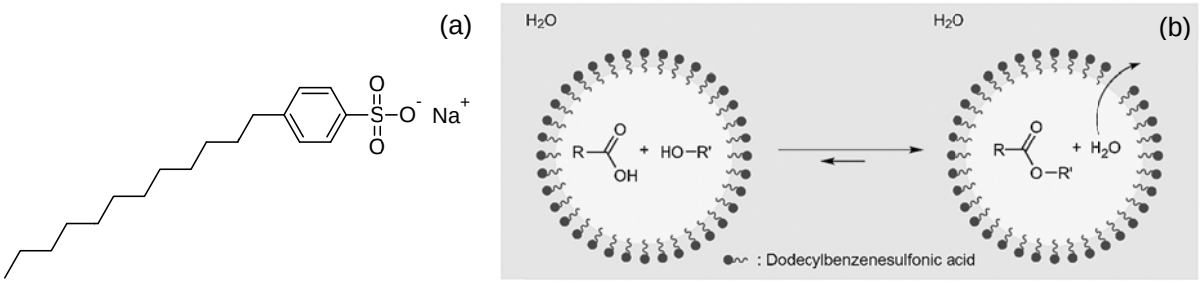


Figure 3-21: (a) Dodecylbenzenesulfonic acid (DBSA); (b) aqueous esterification with DBSA emulsion (Manabe et al., 2002).

Molecules such as DBSA can also be attached on different supports. Manabe and Kobayashi (2002) indeed immobilized an amphiphilic Brønsted acid onto polystyrene beads. Nonetheless, this approach with immobilized or non-immobilized Brønsted acid surfactant has only been so far reported for aqueous reactions of non-water soluble carboxylic acids and alcohols. The field of immobilized Brønsted acids is growing rapidly. Most reported catalysts consist of sulfonic acid functionalized polymers. Because of a high acid strength, sulfonimide groups or sulfonmethide groups were also immobilized on polymers and are currently under investigation for the development of new polymer-supported Brønsted acids. These sulfonimide supported on polystyrene beads have already been reported by Zhang et al. (2007) to catalyse the aqueous esterification of water-soluble carboxylic acids, such as butyric acid.

A second approach for the aqueous esterification of carboxylic acids consists in designing the acid support with appropriate hydrophobic reaction sites, so that water is constantly removed from the sites where the reaction takes place. The local surface properties of the solid sites must hence be properly designed for water removal (Budarin et al., 2007c). This strategy has been applied by Budarin et al. (2007b) while designing their Starbon® support. This catalyst is a mesoporous carbonaceous material produced from high surface area forms of starch and other expanded polysaccharides after pyrolysis at different temperatures (Budarin et al., 2007a). This catalyst has also the advantage of being produced from renewable resources (starch or other polysaccharides). Using this type of catalyst, high yields in diethyl succinate esters have been obtain after 8 hours for reactions in water.

Finally, similar catalysts as those mentioned for non-aqueous esterifications – both homogeneous and heterogeneous – can be used for reactions in water, while using either pervaporation (Benedict et al., 2006) or distillation (Bauduin et al., 2009; Fujita et al., 2004; Saha et al., 2000) to push the equilibrium towards the esterification using product

removal. In case of dilute reaction solutions, these strategies can be expensive and are hence unsuitable for industrial applications.

Concluding remarks

Many chemical catalysts can catalyse esterifications in organic solvents. However, the presence of water has been reported to be detrimental for such a reaction. For aqueous esterifications, three approachs seem to be very promising: 1) Brønsted acid surfactants (free or immobilized); 2) catalysts with reaction sites of appropriate hydrophobicity; 3) reaction systems, in which the contact between the esters and the water is limited.

3.4.4 State of the art for the enzymatic catalysis

Another promising approach for the production of succinate ester is the use of enzymes as catalysts for the esterification of succinic acid. The use of these water-stable biocatalysts for such reactions often results in improved selectivity – especially when substrates are bearing different functional groups (Torres and Otero, 2001) – under milder reaction conditions (Rees and Robinson, 1995). These high selectivities are required in the flavour and fragrance, pharmaceutical and specialty chemicals industries, where esters have a broad market potential (Meissner and Carta, 2002).

Two types of enzymes are widely used for the esterification of both hydrophobic or hydrophilic substrates: carboxylesterases (E.C. 3.1.1.1) and mainly lipases (E.C. 3.1.1.3). Lipases are present in many oilseed plants. Some lipases catalyze only the hydrolysis reaction of fats and oils, whereas others show catalytic activity towards both hydrolysis and esterification reactions (Yesiloglu and Kilic, 2004). Their activity is greatly increased at the lipid-water interface, a phenomenon known as interfacial activation (Verger, 1997). Given their wide availability, their relative low cost, their general robustness under operating conditions and their lack of cofactor requirement, lipases and esterases are good candidates for industrial processes.

However, when esterases and lipases are used for esterification reactions, they also catalyze the backward reaction, i.e. the hydrolysis of the esters. The reaction is hence limited by its thermodynamical equilibrium. High water concentrations can dramatically reduce the final conversion, by pushing backwards the equilibrium to hydrolysis. That is why solvent-tolerant lipases have been developed and used for esterification in organic solvents, where the water content can be controlled. However, developing esterifications in presence of large amounts of water remains challenging. The different enzymes used

for the esterification of relatively short chain carboxylic acids ($C_{\leq 10}$) will now be reviewed for applications both in organic solvents (see Subsection 3.4.4.1) and in reaction systems containing water (see Subsection 3.4.4.2).

3.4.4.1 Enzymatic esterification in solvents

Many esterification reactions with lipases have been developed for organic solvent or solvent-free applications in order to limit the water content in the system. Different esterifications performed on relative short carboxylic acids ($C_{\leq 10}$) in organic solvent are summarized in Table 3-9. The esterification of more hydrophobic acids was not reviewed here, since they will differ too much from the polar succinic acid and the lipases reported for such acids might not catalyse the esterification of succinic acid.

Mainly lipases have been screened so far for solvent esterifications, because some lipases are solvent-tolerant enzymes working among others on lipophilic substrates. A common lipase – the lipase B from *Candida antarctica* in its free or immobilized form (i.e. "Novozym 435" from Novo Nordisk) – has been widely screened for esterification of different substrates in organic solvents or ionic liquids. The lipase B from *Candida antarctica* can be classified as an esterase, since it shows little or no interfacial activation and only slowly hydrolyzes long chain triglycerides (Bornscheuer and Kazlauskas, 2006). This enzyme does not seem to be very specific as it catalyzes the esterification of acetic acid (Bélafi-Bakó et al., 2003), lactic acid (Wei et al., 2003; Yu et al., 2008), acrylic acid, methacrylic acid (Park et al., 2004), succinic acid (Abdul Rahman et al., 2010; Springer et al., 2009) and oleic acid (Radzi et al., 2005) in organic solvents. This immobilized enzyme has hence a broad spectrum of applications but often with limited activities.

A wide range of other lipases has been tested. Among them, the lipase from *Candida rugosa* (free or immobilized) has been screened for the esterification of succinic acid with ethanol without success (Springer et al., 2009), but could esterify 2-chloropropanoic acid with butan-1-ol (Gubicza et al., 2003). The lipase from *Burkhoderia cepacia* gave also slow activity towards succinic acid and ethanol (Springer et al., 2009). Besides, a lipase from *Mucor miehei* has been reported to catalyse the esterification of propionic acid with 2-ethyl-1,3-hexanediol (Meissner and Carta, 2002) and demonstrated activity towards adipic or sebacid acids and polyethylene glycol (PEG) (Das and Bhattacharyya, 2006). Additionally, lipases from *Rhizopus oryzae* (Huang and Yang, 2005) and *Yarrowia lipolytica* (Yu et al., 2008) both catalysed the esterification of lactic acid with ethanol and propyl-glycoside respectively, whereas the lipase from *Thermomyces lanuginosus* slowly

catalysed the esterification of lactic acid with propyl-glycoside (Wei et al., 2003). Finally, the lipoprotein lipase (LPL) from *Pseudomonas sp.*, free or immobilized, catalyzed the esterification of *n*-butyl acid with *n*-butanol in solvents (Ikeda et al., 2002).

While performing the esterification in solvent, the latter must be selected carefully, in order to limit its cell toxicity. The log P (where P is the partition coefficient of a given chemical in a water/1-octanol biphasic system) is commonly used as parameter to describe the solvent polarity and its possible effect on biological system (enzyme or whole cells). In general solvents with log P > 4.0 (nonpolar) are more favourable solvents for biological reactions, and especially enzymatic esterifications (Radzi et al., 2005; Yesiloglu and Kilic, 2004).

Finally, large amounts of water have been reported to lower the final conversions of the enzymatic esterifications in organic solvent (Springer et al., 2009), whereas a minimum of water is generally still necessary to ensure the optimal conformation and activity of the enzyme (Giacometti et al., 2001; Yesiloglu and Kilic, 2004). Several options have been presented to limit the water concentration in the solvent system: pervaporation (Gubicza et al., 2003), hollow fiber reactor (Huang and Yang, 2005) and molecular sieves (Giacometti et al., 2001; Park et al., 2004; Torres and Otero, 2001; Wei et al., 2003) – either in the reaction medium or in an external packed column (Yoo et al., 2007). Finally, if molecular sieves are used as desiccant, 5-Å sieves must be preferentially used, because 3-Å ones have been reported to inactive the enzyme due probably to stripping of essential water (Giacometti et al., 2001).

Concluding remarks

For the development of new esterification reactions of succinic acid in water, the lipase B from *Candida antarctica*, in its free or immobilized form (i.e. Novozym 435), and the lipoprotein lipase (LPL) from *Pseudomonas sp.,* reported for the solvent esterification of a small monocarboxylic acid (C_4) with a small alcohol (C_4) (Ikeda et al., 2002), could be potential catalysts. However, it is not sure that the solvent enzymatic reactions presented above will be transferable to water containing systems, because of the limited activities reported in presence of a large amount of water. Alternatives for esterifications in water must therefore be researched in the literature, by looking for enzymes which catalyze reactions in presence of larger amounts of water.

Table 3-9: Enzymatic esterifications in solvent-free or in organic solvents.

Organism	Yield, %	Acid	Alcohol	Solvent	T, °C	Additive / Remarks	Lit. Ref.
Candida antarctica (lipase B) (immob.[a])		Acetic acid (C_2)	Ethanol	Heptane or solvent-free	40		Bélafi-Bakó et al., 2003
Mucor miehei (immob. [a])		Propionic acid (C_3)	2-Ethyl-1,3-hexanediol	Hexane		Simulated Moving Bed Reactor	Meissner and Carta, 2002
Rhizopus oryzae (immob. [a])		Lactic acid (C_3)	1-Octanol	+ Alamine 336		Hollow fiber reactor	Huang and Yang, 2005
Candida antartica (immob. [a]) *Yarrowia lipolytica*		Lactic acid (C_3)	Ethanol	2-Octanol + alamine 336			Yu et al., 2008
Candida antarctica (lipase B) (immob.[a]) *Rhizomucor miehei* *Thermomyces lanuginosus*	58 15 15	Lactic acid (C_3)	Propyl-glycoside	Acetonitrile, acetone, 2M-2B, *n*-hexane	50-80	Silica gel, Molecular sieve	Wei et al., 2003
Candida rugosa	52	2-Chloropropanoic acid (C_3)	Butan-1-ol	Hexane, toluene, tetrahydrofurane, ILs[b]	40-60	Pervaporation	Gubicza et al., 2003
*Pseudomonas sp. (*LPL[c]) *Pseudomonas sp. (*LPL[c]) (immob. [a] on celite and cellulose acetate with TiO_2)		*n*-Butyric acid (C_4)	*n*-Butanol	Hexane, heptane (saturated with phosphate buffer solution pH 7.2)	40-50		Ikeda et al., 2002
Burkholderia cepacia *Candida rugosa* Lipase Lipozyme IM *Candida antarctica* (lipase B) (immob.[a])	6 - - 80	Succinic acid (C_4)	Ethanol	Tetradecan, octan, diphyl KT	80-90	Stripping	Springer et al., 2009
Candida antarctica (lipase B) (immob.[a])	85	Succinic acid (C_4)	Oleyl alcohol	Hexane	41		Abdul Rahman et al., 2010
Mucor Miehei	64	Adipic acid (C_6) Sebacic acid (C_{10})	PEG		70-75		Das and Bhattacharyya, 2006

[a]: immob. = immobilized; [b]: IL = ionic liquid; [c]: LPL = lipoprotein lipase

Table 3-10: Enzymatic esterifications in presence of water.

Organism	Yield, %	Acid	Alcohol	Aqu. phase	Orga. phase	T, °C	Additive / Remarks	Lit. Ref.
Mucor miehei "Sp 225" (Novo)	-	Propionic acid (C_3)	Oleyl alcohol C_{18}	Complex fermentation medium, pH = 4	Alcohol	30		Aires-Barros et al., 1989
Mucor miehei "Lipozyme" (Novo)	-		C_4, C_5, C_6, C_8, C_{10}, C_{12} *n*-linear alcohol					
Chromobacterium viscosum LPL	Optimal							
Porcine pancreas	-							
Rhizopus sp.	-							
Penicillium sp.	-							
Aspergillus niger	-							
Chromobacterium viscosum	Better for acids $C_{\geq 6}$	C_4 to C_{12} linear acid	Ethanol or octan-2-ol	Diglycerine buffer, pH = 8	Chloroform:n-heptane (1:1)	40	CTAB	Rees and Robinson, 1995
Chromobacterium viscosum	85	Hexanoic acid (C_6)	1-Pentanol	Phosphate buffer, pH = 7	Iso-octane	25	SDS	Backlund et al., 1995
Candida antartica (lipase A)	80 (24 h)	Linear (C_7-C_{12}) carboxylic acids	ω-phenyl-labeled primary alcohol (C1-C5)	Water, pH = 3.8	Hexadecane	40	Lutensol AT50	Aschenbrenner et al., 2009
Penicillum cammemberti	-							
Burkholderia cepacia	80 (5 h)							
Hog pancreas	-							
Rhizopus arrhizus	20 (24 h)							
Aspergillus oryzae (Esterase)	80 (24 h)							
α-Chymotrypsin (CT) from bovine pancreas (Sigma)	90	N-acetyl-L-leucine (C_8), -phenylalanine (C_{11}), -tryptophan (C_{13})	Ethanol	Phosphate buffer, pH = 6.8	Ethanol (+ chloroform)	30		Kise et al., 1987
Candida Rugosa	34	Decanoic acid (C_{10})	D-sorbitol	Phosphate buffer, pH = 7 + sorbitol	Decanoic acid	35	Two-phase membrane reaction	Janssen et al., 1990
Chromobacterium viscosum								
Porcine pancreas	-							
Aspergillus niger								
Pseudomonas fluoresens	-							
Rhizopus delemar								

Table 3-10 (Con't): Enzymatic esterifications in presence of water.

Organism	Yield, %	Acid	Alcohol	Aqu. phase	Orga. phase	T, °C	Additive / Remarks	Lit. Ref.
Aspergillus niger (Feruloyl esterase)	60	Ferulic acid (C_{10})	*n*-Pentanol	Phosphate buffer, pH = 6	Hexane + n-pentanol	40	Cetyltrimethylammoniumbromide (CTAB)	Giuliani et al., 2001
Rhizomucor miehei *Mucor miehei* *Candida antarctica* (Lipase B)	90	Lauric acid (C_{12})	1-Propanol	Tris-HCl buffer, pH = 7.5	Hexane	30		Zoumpanioti et al., 2006
Rhizomucor miehei *Humicola insolens* Lipozyme immobilized on polyurethane foams	85 92	Sunflower oil (C_{16}-C_{18})	Methanol	Water buffer, pH = 5	Sunflower oil	40		Oliveira and Rosa, 2006
Mucor miehei *Rhizopus delemar* *Pseudomonas cepacia* *Pseudomonas fluoresecens* *Rhizopus arrhizus* *Candida deformans* *Candida parapsilosis*	 - - - Optimal	Fatty acids (C_{16}-C_{18})	Methanol (or 1-butanol)	Phosphate buffer, pH = 6.5	Fatty acids	45		Lecointe et al., 1996
Rhizomucor miehei		Oleic acid (C_{18})	Ethanol	Phosphate buffer, pH = 5.6	Oleic acid	30		Oliveira et al., 1998; 2001
Rhizomucor miehei	82	Oleic acid (C_{18})	1-Butanol	Phosphate buffer, pH = 5.6	Heptane + Oleic acid	50	Continuous process	Kraai et al., 2008
Candida deformans		Oleic acid (C_{18}) Linoleic acid (C_{18})	Methanol	Phosphate buffer, pH = 6.5 - 7	Acid	40	Polyvinyl alcohol	Boutur et al., 1995

3.4.4.2 Enzymatic esterification in 2-phase systems including water

The esterification of succinic acid from fermentation broth involves dilute solutions of succinic acid. However, water is itself a product of the esterification and could hence limit the final conversion. As in chemical esterifications, water could be stripped out of the system, but for dilute solutions coming from fermentations, this technique would be too expensive and is not applicable at an industrial scale. However, if the ester formed is enough hydrophobic, it could be extracted in a second phase to drive the equilibrium to the esterification side. Two-phase systems could therefore be of great interest.

Aires-Barros et al. (1989) developed a reactive extraction for propionic acid and other carboxylic or dicarboxylic acids (including succinic acid) from fermentation broth using a long-chain alcohol as second phase. They showed that the extraction of the acid could be increased if the acid was simultaneously esterified enzymatically. To perform the catalysis, seven lipases were tested and Aires-Barros et al. (1989) claimed that the lipase from *Chromobacterium viscosum* gave the best results. However, the kinetics of the esterification with this enzyme was extremely slow and the equilibrium could only be reached after 50 h. Nevertheless, the extraction of the carboxylic acids with long-chain alcohols could be increased by at least 5-fold by coupling the process with a lipase-catalyzed esterification.

The lipase from *Chromobacterium viscosum* was also tested successfully by other groups for the esterification of hexanoic acid with 1-pentanol using iso-octane as solvent (Backlund et al., 1995), or C_4 to C_{12} linear acids with ethanol or 2-octanol using chloroform:*n*-heptane (1:1) as solvent (Rees et Robinson, 1995).

For the esterification of linear C_7 to C_{12} carboxylic acids with ω-phenyl-labelled primary alcohols in hexadecane, Aschenbrenner et al. (2009) tested different lipases and an esterase. The most efficient one was the lipase PS from *Burkholderia ceparia* (Amano). Chirazyme L-5 (lipase A from *Candida antarctica*) (Roche) and the esterase 009 from recombinant *Aspergillus oryzae* (Jülich Chiral Solution GmbH) gave also satisfactory conversions after 24 h. Lipase PS showed even a substrate specificity towards nonanoic acid.

Similarly to the study of Aires-Barros et al. (1989) on the coupled esterification-extraction of propionic acid, Oliveira et al. (2066) developed a strategy for the removal of ethanol during its production by fermentation with lipase from *Rhizomucor miehei* and *Humicola insolens*, since fermentation of high glucose concentrations are often limited by

product inhibition (Oliveira et al., 2001; Oliveira and Rosa, 2006; Oliveira et al., 1998). The hydrophobic oleic acid was used as second phase.

Besides, while esterifying a carboxylic acid, Aires-Barros et al. (1989) showed that the pH of the aqueous solution has to be chosen so that the acid is mainly in its uncharged form. However, as lower pHs are not always compatible with enzymatic catalysis, a compromise must be found between the enzymatic activity and stability and the concentration of the uncharged form of the acids.

As for the organic solvent of the biphasic system, it should carefully be selected. It must satisfy the following requirements: (i) high partition coefficient of the esters towards the solvent phase; (ii) high selectivity for the product over water; and (iii) little or no toxicity towards biocatalysts (Aires-Barros et al., 1989). To limit the use of extra solvent, the acid or the alcohol can be used as second phase if enough hydrophobic. Otherwise a co-solvent can be introduced as second phase. For the esterification-extraction of propionic acid, water-immiscible alcohols with chain lengths from C_4 to C_{18} were screened. The partition coefficient of the acid decreases with the increase of the chain length of the alcohol, and therefore the least toxic solvents are the worst extractants. Fortunately, the improvement in the extraction with the coupled process was greater for the less-toxic long chain alcohols (Aires-Barros et al., 1989). For water-miscible short chain alcohols (such as ethanol or propanol), a co-solvent as dodecane could be used successfully as second non-water-miscible phase.

Lipases have interfacial activation and their activity is thus influenced by the quality of the emulsion (Boutur et al., 1995). As the reaction takes place at the interface, a large interfacial area is desirable for achieving fast conversions. For such reactions, mini- or microemulsions are therefore highly beneficial, as they are isotropic, thermodynamically stable, present a large interfacial area and allow the solubilisation of widely differing polarities in a single phase (Rees and Robinson, 1995; Zoumpanioti et al., 2006). Microemulsions have hence been reported to lead to higher reaction rates than macroemulsion or homophase reactions (Aschenbrenner et al., 2009). The major parts of the mini- or microemulsions reported in the literature for lipase catalyzed reactions are water in oil (w/o) emulsions. However, for the development of an esterification-extraction process for succinic acid from fermentation broth, an oil in water (o/w) micro- or mini-emulsion would be more suitable.

Aschenbrenner et al. (2009) reported such an oil in water (o/w) mini-emulsion for the esterification of C_7 to C_{12} linear carboxylic acids with ω-phenyl-labelled primary alcohols

in hexadecane. Droplets are stabilized against coagulation by either an ionic or non-ionic surfactant. However, the enzyme / surfactant must be carefully selected as certain enzymes are unstable while in contact with some surfactants. A high-shear force process such as ultrasound is applied for the formation of uniform-size droplets. The enzyme is located at the interface of the droplets and the water is evacuated out of the hydrophobic droplets during the reaction.

Finally, the enzyme for the biphasic reaction can be immobilized on different supports. Oliveira et al. (1998) reported an increase of the specific activity of the lipase "Palatase M1000L" from *Rhizomucor miehei* after immobilization on an hydrophobic support – Accurel EP700. They made the assumption that the hydrophobic support aggregates at the aqueous-organic interface, facilitating the action of the lipase. Furthermore, the immobilization of the enzyme allows the recycling of the expensive biocatalysts, making the process more attractive for industrial applications.

Concluding remarks

From this literature search on two-phase esterification of carboxylic acids, several potential candidates for the esterification of dilute solutions of succinic acid could be found: the lipoprotein lipase (LPL) from *Chromobacterium viscosum* reported for the esterification of propionic acid from a fermentation broth with diverses alcohols (Aires-Barros et al., 1989). The lipase from *Chromobacterium viscosum,* mentioned for the esterification of C_4 to C_{12} linear carboxylic acids with ethanol or 2-octanol and chloroform:n-heptane as solvent (Rees and Robinson, 1995) and of hexanoic acid with 1-pentanol with iso-octane as co-solvent (Backlund et al., 1995), might also be successful for the aqueous esterification of succinic acid. Finally, the lipase from *Burkholderia cepacia* could also be of interest, since it has been reported to catalyse the esterification of linear C_7 to C_{12} carboxylic acids (Aschenbrenner et al., 2009).

4 Materials and methods

In this Chapter, the various materials and methods used for the experiments during this project will be described. The different chemicals as well as the equipment used for this study were purchased from diverse producers and are listed in Subchapter 9.2 (see Annex). The abbreviations of the different chemicals are indicated in Subchapter 9.1 (see Annex). The different steps of the experimental work will be detailed in this Chapter: the synthesis of catalysts (in Subchapter 4.1), the hydrogenation (in Subchapter 4.2), the esterification (in Subchapter 4.3) and the process integration (in Subchapter 4.4). Then will be described the analytical techniques (in Subchapter 4.5), as well as the calculation and optimization methods (resp. in Subchapters 4.6 and 4.7).

4.1 Synthesis of catalysts

Certain catalysts used for the hydrogenation and esterification were synthesized, whereas others were bought. The protocol for the Montmorillonite catalysts (see Section 4.1.1) and the ruthenium phosphine complex immobilized on polymer (see Section 4.1.2) are presented in the following Sections.

4.1.1 Montmorillonite (MTM) clay catalysts

4.1.1.1 Synthesis of the metal supported MTM clays used for the hydrogenation

The synthesis was performed according to the procedure published by Kawabata et al. (2003) for the preparation of Scandium immobilized clay catalyst. Under inert atmosphere (argon gas), 1 g Montmorillonite clay (K-10, Al pillared or KSF) was added to 67 ml of an aqueous solution of 5 mM of $RuCl_3$ or $IrCl_3$. The solution with the suspended clay was stirred for 24 h under inert atmosphere. The solution was then centrifuged and the supernatant discarded. The clay was then washed with 100 ml of distilled water and the solution was centrifuged again. This washing step was repeated four times. The obtained clays were black or grey for the Ruthenium pillared clays and grey for the Iridium pillared one. The clays were finally dried for 3 h at 100 °C in an oven.

4.1.1.2 Synthesis of the ion exchanged MTM clays used for the esterification

A similar protocol to the method presented in Subsection 4.1.1.1 and to the procedure described by Reddy et al. (2005b) was used for the synthesis of the ion exchanged MTM clays with Ag^+, Co^{2+}, Al^{3+} and H^+. Aqueous solutions of $AgNO_3$, $CoCl_2.6H_2O$, $AlCl_3.6H_2O$

and HCl at 0.5 M were prepared. Then, 100 ml of the aqueous solution were added to 2.5 g of MTM K-10 and the mixture was stirred for 24 h at 150 rpm. The washing and drying procedures were the same as those mentioned in Subsection 4.1.1.1.

4.1.2 Immobilized phosphine catalysts on amphiphilic polymers

The immobilization of phosphine catalysts on amphiphilic polymers required to first produce the polymeric ligands and then form the complex.

4.1.2.1 Synthesis of the polymer immobilized ligands

The procedure used for the first step was derived from Uozumi and Nakai (2002) and is presented in Figure 4-1.

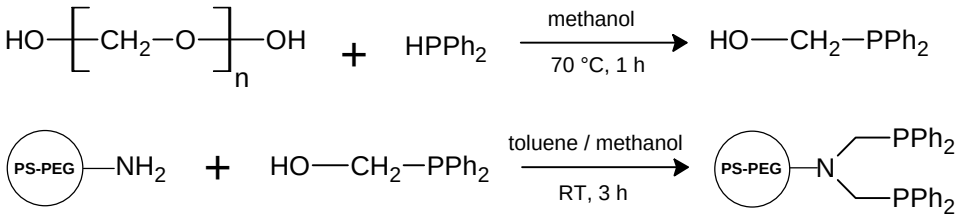


Figure 4-1: Methodology developed by Uozumi and Nakai (2002) to immobilize phenylphosphine ligands on PS-PEG amphiphilic polymer beads.

For the synthesis of diphenylphosphinomethanol, the paraformaldehyde (0.213 g, 7.09 mmol) was slowly stirred with the diphenylphosphine (0.675 ml, 3.88 mmol) at 70 °C for 1 h in methanol (15 ml) under argon atmosphere. The mixture was then cooled to room temperature. TentaGel S NH_2 (S 30 902, Rapp polymer, Tübingen, Germany) (1.00 g, 0.26 mmol g^{-1}, 90 µm) and toluene (15 ml) were added into the mixture and the suspension was stirred for 3 h at room temperature. The reaction mixture was filtered and the resin was washed three times with 15 ml of methanol. The resin was dried under reduced pressure. Finally, the polymeric ligands were analyzed by MAS ^{31}P-NMR (i.e. Magic Angle Spinning Phosphorus-31 Nuclear Magnetic Resonance spectroscopy) and elemental analysis.

4.1.2.2 Synthesis of the polymer immobilized complex

The procedure of Kayaki et al. (2003) was applied to the polymer supported phosphine ligands produced previously to form the complex presented in Figure 4-2.

The following steps were applied: Tris(triphenylphosphine)ruthenium(II) dichloride [$RuCl_2(PPh_3)_3$] (0.19 g, 0.2 mmol) was added to a benzene suspension (10 ml) of resin-supported diphosphine ligands (0.4 g) at room temperature under inert argon atmosphere. The mixture was then stirred slowly for 18 h. After filtration, the resin was washed 3 times with 10 ml of benzene and dried under vacuum to give brown beads of product. Finally, the polymer immobilized complex was analyzed by MAS ^{31}P-NMR and elemental analysis.

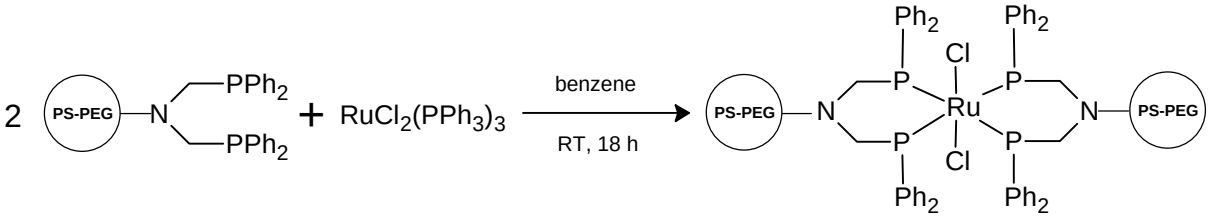


Figure 4-2: Methodology developed by Kayaki et al. (2003) to synthesize a ruthenium complex ($RuCl_2$(PS-PEG-adppp)$_2$) with the phenyphosphine ligands immobilized on PS-PEG polymer beads.

4.2 Hydrogenation

Using the different synthesized and purchased catalysts, various hydrogenation reactions were performed in an autoclave presented in Figure 4-3 and following the procedures described in this Subchapter. The autoclave BR-100 (Berghof, Eningen, Germany) allows reactions to be performed at conditions up to 20 MPa and 230 °C.

4.2.1 Aqueous hydrogenation of levulinic acid with metal complexes

The procedure for the hydrogenation of levulinic acid was derived from the protocol described by Mehdi et al. (2008). In 40 ml of degassed water, the substrate (26.4 mmol), the metal precursor, Ru(III) acetylacetonate ($Ru(acac)_3$) (0.044 mmol), and the ligand (0.44 mmol) were introduced in a flask and stirred until total dissolution. The solution was then transferred under argon into the autoclave polytetrafluoroethylene (PTFE) insert that was subsequently introduced in the Berghof BR-100 autoclave (Eningen, Germany). The autoclave was sealed and flushed with nitrogen for several minutes. The reaction was warmed up to 140 °C at an agitation rate of 1400 rpm. When the temperature was reached, 5.5 MPa of hydrogen were introduced into the autoclave. The samples were taken regularly through the liquid sampling valve. The samples were then filtered on filter paper and deep-frozen for later analysis by HPLC (i.e. High Performance Liquid Chromatography) (see Section 4.5.1). At the end of the reaction, the autoclave was cooled down to 50 °C in a water bath and the pressure was released. The

reactor could then be opened and the last sample was taken. The samples were stored at – 20 °C until their analysis by High Performance Liquid Chromatography (HPLC).

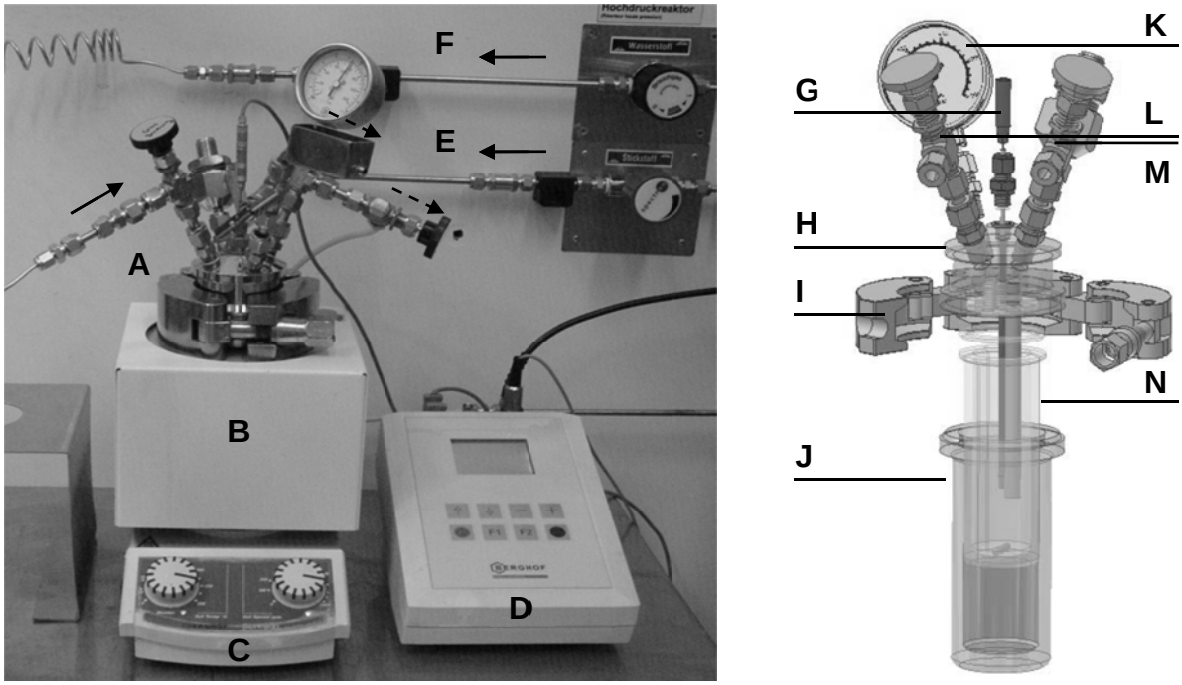


Figure 4-3: The stainless steel autoclave BR-100 (Berghof, Eningen, Germany) (A) placed in a heating jacket (B) connected to a heating and magnetic stirring plate BLH-800 (C). The pressure is indicated on the manometer (K) and the temperature probe (G) is connected to a temperature controller BTC-3000 (Berghof, Eningen, Germany) (D). The solution is added in the 100 ml PTFE reactor insert (N) that is placed in the reactor vessel (J). The reactor is closed with the reactor lid (H) and sealed with the conical flange lock (I). Hydrogen (pipe F) or nitrogen (pipe E) is added into the reactor through the gas inlet valve (L) and samples are taken through the sampling tube closed by a valve (M). The inlet gas is represented by → and the out gas by - → (source: Berghof, Eningen, Germany).

4.2.2 Hydrogenation of succinic anhydride in solvents with metal complexes

The hydrogenation of succinic anhydride was realized in similar conditions to the one reported by Hara et al. (2000). The solvent used was tetraethylene glycol dimethyl ether (tetragyme). The metal precursor (20 mmol), the ligand (0.05 mmol) and the acid (*para*-toluenesulfonic acid) (0.4 mmol) or the base (sodium isopropoxide) (1 mmol) were introduced under argon into 16 ml of tetraglyme in a 100 ml-flask. If it was already formed, the complex (0.05 mmol) was simply added in the tetraglyme. Decane was introduced as internal standard at a concentration of 80 mM. The solution was stirred until the different components were dissolved and then transferred under argon into the

autoclave PTFE insert. The insert was introduced in the autoclave that was then sealed. The autoclave was flushed with nitrogen for several minutes. The reaction mixture was then heated up to 150 °C at a stirring rate of 1000 rpm. When the temperature was reached, the hydrogen pressure was set to 1 MPa. Samples were taken after 0, 1, 2.5, 4 and 5.5 h. A final sample was taken the next morning after about 20 h. The samples were stored at – 20 °C. Before analysis, they were diluted 5 times with ethyl acetate and then injected on a GC-MS (i.e. Gas Chromatography - Mass Spectroscopy) (see Section 4.5.2).

4.2.3 Aqueous hydrogenation of succinic acid with different catalysts

The protocol of the aqueous hydrogenations of succinic acid is similar to the one presented in Section 4.2.1 for the hydrogenation of levulinic acid. The methodology slightly differs from one catalyst to another. For the metallic complexes tested at low or high temperature and pressure (see Section 5.2.2), the metal precursor and the ligands were first dissolved in water in a flask under argon. Succinic acid was introduced directly in the autoclave insert and the catalyst solution was transferred under argon into the reactor insert.

As for the metal supported Montmorillonite catalysts (see Section 5.2.3), the catalysts, the water and the substrate were directly added into the autoclave insert under argon.

During the reaction, samples were taken, filtered and stored at – 20 °C for analysis. For the reaction at high temperature (see Subsection 5.2.2.2), samples could not be taken during the reaction due to the loss in the gas phase. Only an end-point sample was taken after cooling the autoclave down to 50 °C.

The samples of the reactions with the metal complexes (see Section 5.2.2) were analysed by HPLC. For the reaction with MTM (see Section 5.2.3), the samples were extracted with ethyl acetate for analysis on a GC-MS, since the nature of the side-products could not be determined on a HPLC device. The extraction was realized with 300 µl of the aqueous sample and 900 µl of a solution of 5 µl ml^{-1} of diethylene glycol butyl ether (DEGDBE) in ethyl acetate. It was extracted for 20 min in a mixer mill (MM 200, Retsch, Haan, Germany) at 1800 rpm at room temperature and the phases were separated by centrifugation for 15 min at 3500 rpm. The organic phase was then analyzed (see Section 4.5.2) by GC-FID (i.e. Gas Chromatography – Flame Ionisation Detector).

4.2.4 Hydrogenation of succinate esters in solvents

The reaction conditions were derived from the literature search presented in Section 3.3.4. The solvents that were used for the reaction were degassed and water-free. The metal precursor ($Ru(acac)_3$), the ligand (Triphos) and the substrate (dimethyl succinate, DMS) were introduced in a flask under inert atmosphere with the solvent. After dissolution of the chemicals, the solution was transferred under argon to the reactor insert which already contained zinc. The following procedure is similar to the one reported in Section 4.2.1.

Before analysis, the samples taken during the reaction were defrosted. Then 10 µl of a standard solution of 1 g ml^{-1} of mesitylene in methanol was added to 190 µl of the sample for analysis by GC-MS (see Section 4.5.2).

4.3 Esterification

For the esterification, the reactions were realized in different set-ups depending on the type of catalysts used or the amount of reactions simultaneously performed. The procedures for the chemical and the enzymatic esterifications will be described respectively in Sections 4.3.1 and 4.3.2. The scale up of the reaction to 200-ml and the purification of the esters will be then depicted in Section 4.4.2. Finally, the hydrolysis of the esters will be introduced in Section 4.4.3.

4.3.1 Chemical catalysis

4.3.1.1 Experimental set-up

The chemical esterifications were performed in a 12 Carousel Reaction Station (Radleys, Essex, UK). As presented in Figure 4-4, it consists of 12 magnetically stirred tubes standing on a metallic support connected to a stirring hot plate controlled by a temperature probe. Samples can be taken through the sampling port located in the cap of the tubes.

In this parallel reactor system, both the esterification of succinic acid with ethanol in monophasic systems and the biphasic esterification of succinic acid with chemical catalysts were performed (see Subsections 4.3.1.2 and 4.3.1.3).

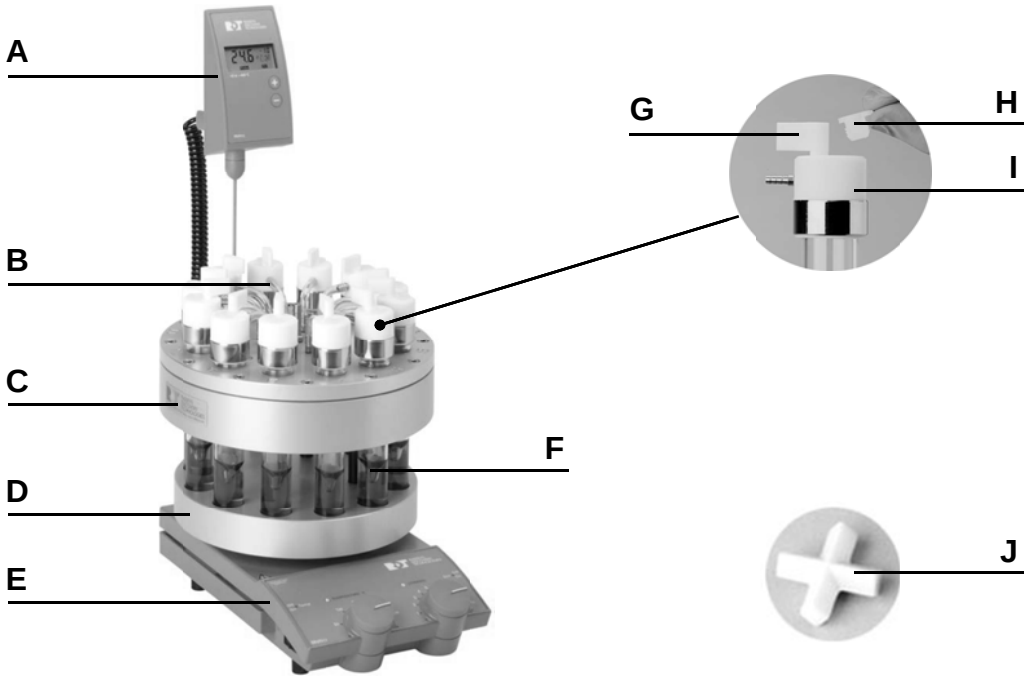


Figure 4-4: Experimental set-up for the chemical esterification of succinic acid: 12 Carousel Reaction Station from Radleys (Essex, UK): the 12 reaction threaded glass tubes (F) are heated by a heating jacket (D) connected to a heating and stirring plate (E) controlled by a temperature controller (A). The reactors are cooled at the top by a cooling jacket (C) for reactions at reflux. The tubes are sealed with a PTFE reaction cap (I) with a sampling port closed by a suba seal (H). The tubes can be supplied through a pipe (B) and a valve (G) in gas. The solutions in the tubes are stirred with a cross magnetic stirrer (J).

4.3.1.2 One-phase esterification with ethanol

For the one-phase esterification with ethanol, solutions of succinic acid at 0.4 M were prepared in diverse mixtures of ethanol and distilled water. The solutions contained 100, 75, 50 or 25 % ethanol (i.e. 0, 25, 50 or 75 % water). The reactions were performed in a parallel reactor system from Radleys (as shown in Figure 4-4). For each catalyst, 0.5 g was introduced in the glass tube. For the liquid catalyst (DBSA and iodine), 10 and 5 mol % respectively were added. The tubes were then filled with the succinic acid solutions. A t_0 sample was taken in each reaction tube as reference and the tubes were sealed with the caps. The tubes were then heated up to 80 °C at a stirring rate of 1000 rpm. A sample was taken in each tube after 24 h of reaction and was deep frozen at – 20 °C before analysis. The defrosted samples were then analyzed by HPLC (see Section 4.5.1).

4.3.1.3 Two-phase esterification

For the biphasic esterification of succinic acid with for example 1-octanol, solutions of succinic acid at different concentrations and pHs were prepared in distilled water for further experiments. pH was set using HCl 10 % and NaOH 10 %. The standard reaction conditions (SC) for the chemical esterification with DBSA and Nafion NR-50 are indicated in Table 4-1. When testing the impact of different reaction conditions, only one variable was changed at a time, while the other reaction conditions were the standard ones. In the following protocol, standard conditions are reported.

Table 4-1: Standard reaction conditions (SC) for the biphasic esterification of succinic acid with 1-octanol using chemical catalysts.

	DBSA	Nafion NR-50
	SC	SC
Succinate	0.8 M	0.8 M
Aqueous phase	phosphate buffer*	phosphate buffer*
pH	2	2
Organic phase	1-octanol	1-octanol
Catalyst mass	0.131 g	0.5 g
Temperature	80 °C	80 °C
Aqueous phase	5 ml	5 ml
Organic phase	5 ml	5 ml
Agitation speed	1000 rpm	1000 rpm

* : phosphate concentration at 43.35 M

The reactions were performed in the Radleys Carousel 12 Reaction Station (see Figure 4-4). The catalyst (131 mg of DBSA or 0.5 g Nafion NR-50) was introduced in the glass tubes that were then filled with 5 ml of the aqueous phase and sealed with the caps. The tubes were then heated up to 80 °C at a stirring rate of 1000 rpm. A reference sample was taken from the aqueous phase before contact with the organic phase. In order to start the reaction, 5 ml of the organic phase (1-octanol) was introduced through the sampling port located on the top of the PTFE caps. 0.3 to 0.4 ml-samples were taken with a 1-ml syringe through the sampling port after 0, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h and 24 h. In some cases, two more samples were taken after 48 h and 72 h. Samples were introduced in 1.5 ml-Eppendorf tubes. The Eppendorf tubes were immediately deep-frozen until treatment for the analytics. Before analysis, the samples

were defrosted and homogenised at 30 °C for 5 min. The Eppendorf tubes were then centrifuged for 1 min at 13000 rpm with a centrifuge Mikro 20 (Hettich, Tuttlingen, Germany). 50 or 100 µl of the aqueous phase respectively was pipetted out of the Eppendorf tube and introduced in a glas vial containing 950 µl or 400 µl of distilled water respectively. The dilution was chosen to fit the linear calibration curve of succinic acid established for the HPLC column. The diluted aqueous phase was then injected on the HPLC column (see Section 4.5.1).

4.3.2 Enzymatic catalysis

Enzymatic esterifications were first performed for a "one-at-a-time" screening and then for a three-variable optimization. The reaction set-ups used for these two types of reactions were slightly different and are presented respectively in Subsection 4.3.2.1 and 4.3.2.2. The protocol for the biphasic enzymatic esterification of succinic acid will be described in Subsection 4.3.2.3.

4.3.2.1 Experimental set-up for the "one-at-a-time" optimization

The enzymatic esterification for the "one-at-a-time" optimization was realized in a small reactor unit prototype provided by 2mag AG (Munich, Germany), containing 8 magnetically inductive driven stirred tank bioreactors on a 10-ml scale (Weuster-Botz et al., 2005), as shown in Figure 4-5. The S-stirrers were designed for the homogenization of particle suspensions at high solid content (Riedlberger and Weuster-Botz, 2010).

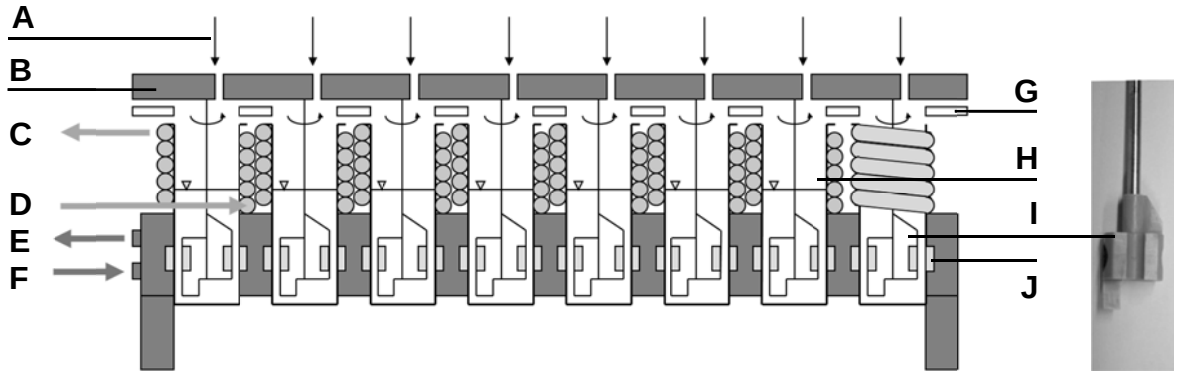


Figure 4-5: Small reactor unit prototype (2mag, Munich, Germany) containing 8 magnetically inductive driven (J) stirred tank bioreactors (H) with a heating jacket (warm water inlet (F) and outlet (E)) and a cooling jacket at their top (cold water inlet (D) and outlet (C)). The tubes are agitated with designed S-stirrers (I) (Riedlberger and Weuster-Botz, 2010). The reactors are closed by a cover (B) with a silicone joint (G), through which samples can be taken (A).

4.3.2.2 Experimental set-up for the three-variable optimization

For the three-variable optimization, the reactions were realized in triplicate and a bigger reactor unit was necessary to perform the multiple reactions in parallel. Therefore a bioreactor unit consisting of 48 parallel miniaturized stirred tank reactors (Weuster-Botz et al., 2005) was used (see Figure 4-6). The reactors were similar to those presented in Figure 4-5.

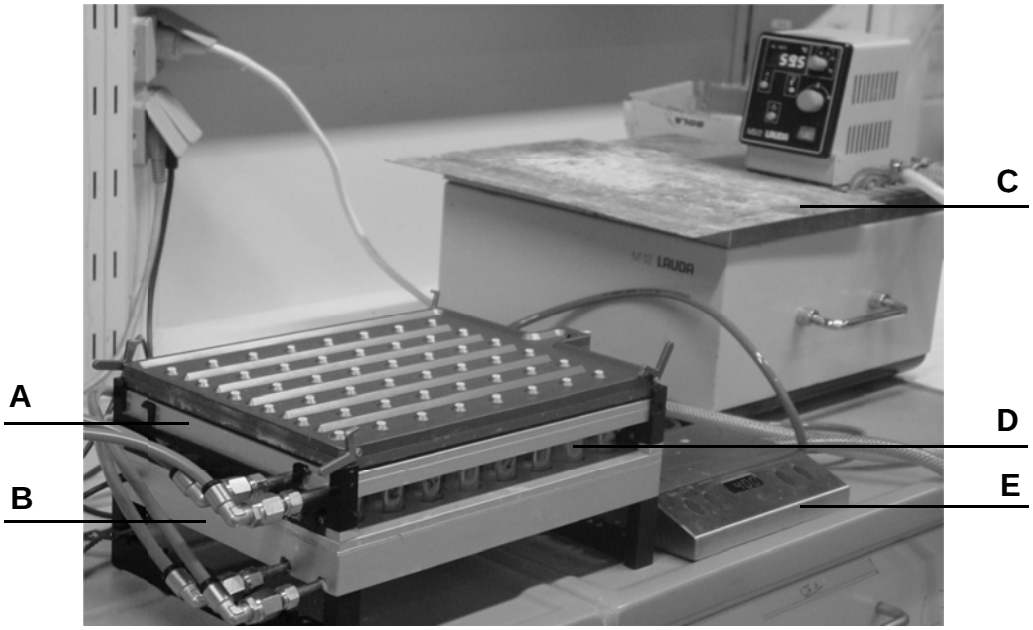


Figure 4-6: Bioreactor unit with the 48 parallel miniaturized stirred tank bioreactors (D) (2-mag, Munich, Germany) with a heating jacket (B) at the bottom of the reactor tubes connected with a water bath (C) and a cooling jacket (A) at their top. The agitation is controlled magnetically by a controller (Mix control, 2mag, Munich, Germany) (E).

4.3.2.3 Two-phase enzymatic esterification

Whether the enzymatic esterification of succinic acid was performed in a small bioreactor unit (see Figure 4-5) or in a larger unit containing 48 parallel reactors (see Figure 4-6), the protocol of the enzymatic esterification remained the same and will be hereafter described for the standard conditions (see Table 4-2).

Solutions of succinic acid were prepared at the wanted concentration in distilled water. The pH was set with HCl 10 % or NaOH 10 %. The enzyme was directly weighted in the reaction tubes and 5 ml of the aqueous solution of succinic acid was added. For the enzyme screening, the enzymes purchased in liquid solutions were introduced in the tubes after the addition of the aqueous phase. The reactors were then placed in the

reactor unit and heated up to 50 °C at an agitation rate of 800 rpm. A reference sample of the aqueous phase was taken before it got in contact with the organic phase. When the reaction temperature was reached, 5 ml of 1-octanol was introduced by the sampling port in order to start the reaction. 0.3 ml-samples were then taken during the reaction at 0, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 24 h, and eventually also at 8 h, 48 h and 72 h. The samples were directly centrifuged for 1 min at 13000 rpm with a centrifuge Mikro 20 (Hettich, Tuttlingen, Germany). Finally, 100 µl of the aqueous phase was diluted with 400 µl of distilled water. The diluted samples were either directly analyzed by HPLC (see Section 4.5.1) or deep-frozen at – 20 °C until analysis.

Table 4-2: Standard reaction conditions (SC) for the biphasic enzymatic esterification of succinic acid with 1-octanol using Novozym 435.

	SC
Succinate	0.15 M
Aqueous phase	phosphate buffer*
pH	4
Organic phase	1-octanol
Catalyst mass	0.04 g
Temperature	50 °C
Aqueous phase	5 ml
Organic phase	5 ml
Agitation speed	800 rpm

* : phosphate concentration at 43.35 M

4.3.3 Additional experiments

During the study of the chemical and enzymatic esterification, additional experiments were also performed in order to assess some important factors for the selection of an appropriate catalyst for the esterification. The protocols of these experiments are presented in this Section.

4.3.3.1 pH change

The pH change was measured for the chemical and enzymatic esterification at different initial pHs. To follow the evolution of the pH of the aqueous phase over time, the reaction was done as presented in Sections 4.3.1 and 4.3.2; however, the sample treatment slightly differed from the aforementioned procedure. In this case, 0.5 ml was

sampled from the reaction tube instead of 0.3 or 0.4 ml and centrifuged. About 0.3 ml of the aqueous phase was placed in another 1.5ml-Eppendorf tube. The pH of this phase was then determined with a small pH electrode (HI 1330, Schott, Mainz, Germany) (pH-meter PHD 2, PCE, Meschede, Germany). 50 or 100 µl of the aqueous phase were then diluted as mentioned in Sections 4.3.1 and 4.3.2 for HPLC analyses (see Section 4.5.1).

4.3.3.2 Reuse of the Nafion NR-50

The reuse of the heterogeneous catalyst Nafion NR-50 was also tested. The same reaction protocol as the procedure presented in Section 4.3.1 was followed. The Nafion NR-50 beads were filtered at the end of the reactions and then washed and dried at 80 °C in an oven (Binder, Tuttlingen, Germany) with different methodologies. During the washing steps, the beads were placed in small vessels and agitated at 400 rpm in the washing solvent.

4.3.3.3 Phase separation with DBSA

The phase separation after the esterification of succinic acid with 1-octanol using DBSA was mimicked with a simple biphasic system of distilled water and 1-octanol with DBSA. In a 15 ml-falcon tube, 131 mg of DBSA as well as 5 ml of distilled water and 5 ml of 1-octanol were introduced. Different additives were also introduced in the falcon tube. The biphasic solution was agitated and let at rest for several hours. Pictures of the biphasic system were taken after 20 min, 1 h, 4 h and 17 h.

4.4 Process integration

4.4.1 Fermentation broth

The fermentation broth containing succinic acid was also used for testing realistic solutions for a coupled process of fermentation and esterification.

The biotechnological production of succinate was realized with an *Escherichia coli* K-12 MG1655 ΔldhA ΔadhE Δack-pta strain with an overexpression of the pyruvate carboxylase (Sánchez et al., 2005). The fermentation was realized following a dual phase procedure consisting first of an aerobic growth and then of an anaerobic production under carbon dioxide (Jiang et al., 2010; Vemuri et al., 2002; Wang et al., 2011). The final titer in succinic acid was of 0.257 M. The main side product was pyruvate (5 to 10 g l^{-1}), whereas only small amount (< 1 g l^{-1}) of ethanol, lactate, acetate and formate could be measured.

The obtained fermentation broth was centrifuged to remove the cells and cell debris. The pH was then adjusted to 2 (for the chemical reaction) and 3.33 (for the enzymatic esterification) by adding HCl. The yellow broth was then used either for small scale reaction (10-ml) following the protocol described in Sections 4.3.1 and 4.3.2 or at a 200-ml scale for the ester purification (see Section 4.4.2).

4.4.2 200-ml scale esterification with DBSA

4.4.2.1 Reaction set-up and protocol

The set-up used for the biphasic esterification with DBSA at a 200 ml-scale is depicted in Figure 4-7. First, 100 ml of the aqueous phase (either a pure solution of succinic acid in distilled water or the fermentation broth) at pH = 2 was introduced in a 500 ml two neck round-bottom flask connected to a condenser. The flask was placed in an oil bath that was heated up to 90 °C and a first sample of the aqueous phase was taken as reference. In order to start the reaction, 100 ml of 1-octanol were added by the second neck of the flask. After 8 h, the reaction was stopped by cooling down the solution to room temperature. The reaction mixture was then transferred to a decanter if fermentation broth was used or to a centrifuge in the case of distilled water. The aqueous phase was then analyzed by HPLC (see Section 4.5.1) in order to determine the conversion and the organic phase was then further processed for the purification of the dioctylsuccinate.

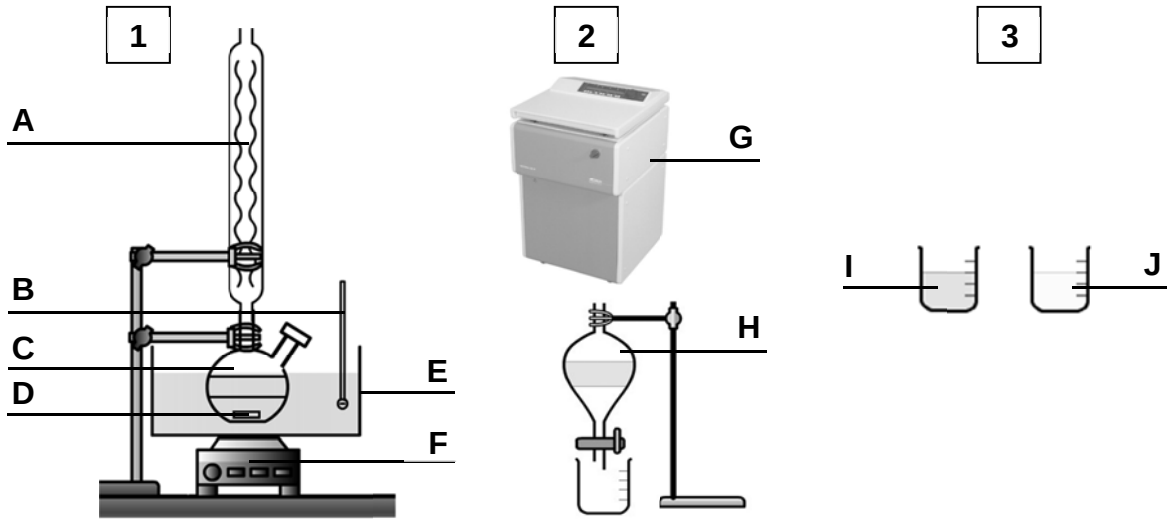


Figure 4-7: Esterification of succinic acid with 1-octanol using DBSA at the 200 ml-scale: **1**: Reaction set up: condenser (A), temperature probe (B), two neck round bottom flask (C), magnetic stirrer (D), oil bath (E) and stirring hot plate (F). **2**: Phase separation: either in a centrifuge Rotixa 50 RS (Hettich, Tuttlingen, Germany) (G) or in a decantor (H). **3**: Separated phases: aqueous phase (J) and the organic phase (I).

4.4.2.2 Purification of the esters

The organic phase obtained after esterification of succinic acid in fermentation broth or in distilled water with 1-octanol and using DBSA was further transferred to a rotary evaporator LABOROTA 4003 (Heidolph, Schwabach, Germany) shown in Figure 4-8. The organic phase was heated up to 130 °C in an oil bath and put under reduced pressure (500 Pa). The distillate was the lighter 1-octanol (bp = 194-195 °C), whereas the dioctyl succinate esters (bp ~ 375 °C) and other heavier compounds of the fermentation broth (if the broth was used as aqueous phase) remained in the flask. The heavy fraction was analyzed by ^{1}H-NMR (i.e. proton NMR spectroscopy) to determine the nature of the product and its purity. Finally, the heavy fraction was filtered on silica gel using dichloromethane as eluent. The dichloromethane was then evaporated in the rotary evaporator and the esters were analyzed again by ^{1}H-NMR.

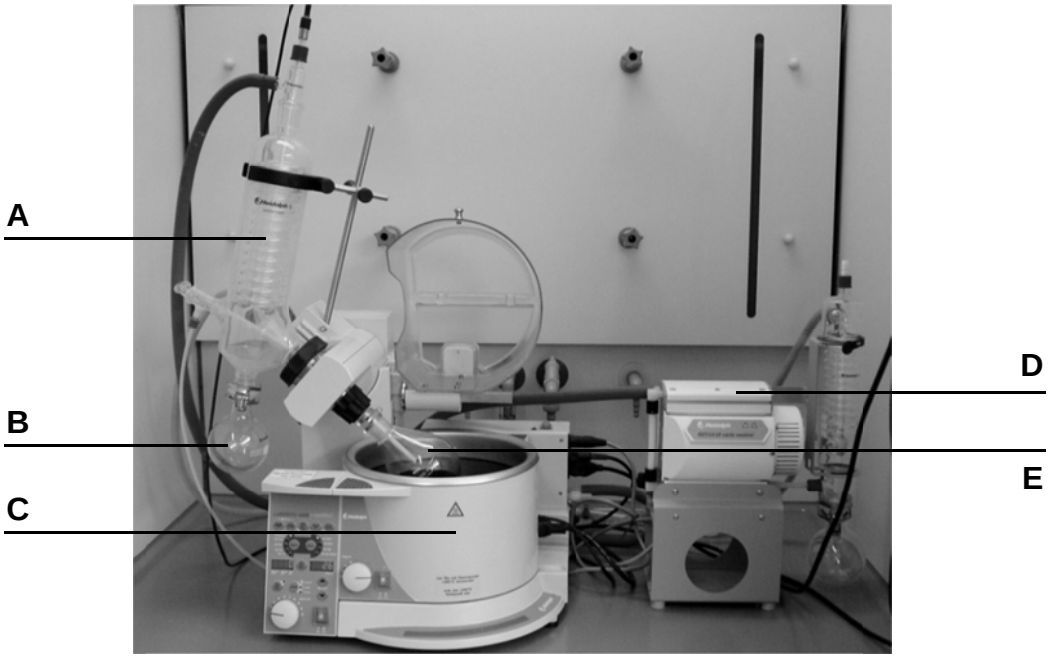


Figure 4-8: Rotary evaporator LABOROTA 4003 (Heidolph, Schwabach, Germany) for the purification of the dioctyl succinate esters: condenser (A), flask for the condensate (B), oil bath (C), vacuum pump (D) and flask containing initially the liquid to distillate and at the end the heavier fraction (E).

4.4.3 Hydrolysis of the esters

A large amount of esters was produced from a pure solution of succinic acid following the protocol mentioned in Section 4.4.2. These esters (purity ~ 91 %) were used for hydrolysis experiments with both chemical and enzymatic catalysts.

4.4.3.1 Chemical hydrolysis

Chemical catalysts were tested in the same set-up as the one reported for the esterification and presented in Figure 4-4. In the standard conditions, 5 ml of distilled water was introduced in the reaction tubes of the Carousel 12 Reaction Station (Radleys, Essex, UK), with the catalyst (0.25 g of the heterogeneous catalysts or 65.5 mg DBSA). The reaction solutions were heated up to 90 °C and agitated at 1000 rpm. When the reaction temperature was reached, 0.5 ml (~ 0.586 g) of ester was introduced in each tube. Samples of 150 µl were regularly taken. After centrifugation at 13000 rpm for 1 min, 40 µl of the aqueous phase was diluted with 160 µl of distilled water and these diluted samples were analyzed by HPLC (see Section 4.5.1) for the determination of the succinic acid yield.

4.4.3.2 Enzymatic hydrolysis (both 10 ml and 1 ml scale)

For the enzymatic hydrolysis, the reaction was either performed in the reaction set-up used for the esterification shown in Figure 4-6 or at a 1ml-scale in a thermo shaker RiO (Quantifoil Instruments, Jena, Germany). For the larger scale experiments, a similar procedure as the protocol described for the chemical hydrolysis in Subsection 4.4.3.1 was used. However, the reaction conditions tested at first were slightly changed: 40 µg or 40 µl of enzyme, 5 ml of 2.5 M phosphate buffer at pH = 7.5 with or without gum arabic (5 g l^{-1}), 37 °C, 0.5 ml of esters and at 1000 rpm.

The experiments at the 1 ml-scale were realized similarly as in the larger scale equipment but with 1 ml of buffer and with 0.1 ml of esters and 8 mg enzyme at 1400 rpm. Different temperatures, pHs and phosphate concentrations were screened. Only an end point measurement was realized after 5 h of reaction. The experiments were performed in triplicate.

4.5 Analytics

4.5.1 High Performance Liquid Chromatography (HPLC)

The aqueous samples were mainly analyzed by High Performance Liquid Chromatography (HPLC) (Agilent Technologies, Santa Clara, CA, USA). The measurement conditions were:

Column	Aminex HPX-87H (Bio-rad Laboratories, Inc., Hercules, USA)
Eluent phase	Aqueous solution of 5 mM sulphuric acid
Eluent flow rate	0.7 ml min^{-1}
Oven temperature	55 °C
RI Detector	1200 Serie G1362A (Agilent Technologies, Santa Clara, USA)
RI Detector temperature	55 °C
UV Detector	S 3300 (Knauer, Berlin, Germany)

Typical retention times of phosphate, succinic acid, GBL, BDO, THF, levulinic acid and GVL are listed in Table 4-3.

Table 4-3: Typical retention times of the different chemicals on the HPLC column using detections with RI and UV detectors.

Compound	RI retention time, min	UV retention time, min
Phosphate	7.44	-
Succinic acid	11.5	10.1
BDO	19.0	-
GBL	20.5	20.0
THF	29.4	-
Levulinic acid	13.4	13.2
GVL	24.8	24.5

The concentration of succinic acid can be easily determined by a calibration with succinic acid solutions of known titers. In most cases, the phosphate contained in the aqueous phase was used as internal standard. The concentration of succinic acid could be calculated based on the equation (4-1).

$$[SA]_{aq}(t) = \frac{Area_{IS}(t) \cdot [SA]_{tot,0}}{Area_{IS,tot,0}} \quad (4\text{-}1)$$

with	$[SA]_{tot,0}$ & $[SA]_{aq}(t)$	*aqueous concentration of succinic acid: initial and at time t*	*mol* l^{-1}
	$Area_{IS,tot,0}$ & $Area_{IS}(t)$	*area of the Internal Standard peak: initial and at time t*	-

4.5.2 Gas Chromatography (GC)

The analyses of organic samples and of aqueous samples extracted in organic solvents were realized by Gas Chromatography (GC). Gas Chromatography was used for the analysis of the samples from the hydrogenation of succinic anhydride in solvent (see Section 5.2.1), of the aqueous hydrogenation of succinic acid with metal supported Montmorillonite clays (see Section 5.2.3) (after the samples were extracted in solvents) and of the hydrogenation of dimethyl succinate in solvent (see Subchapter 5.3). Depending on the chemicals analyzed, different columns and methods were used for the analysis of the samples. These different analytical methods are summarized in Table 4-4.

Table 4-4: GC methods for organic samples of hydrogenation reactions.

	Method 1	Method 2	Method 3
Hydrogenation reaction	Succinic anhydride in solvent	Succinic acid with metal supported MTM clays in water	Dimethyl succinate in solvent
GC	CP-3800 GC (Varian)	HP 6890 (Hewlett Packard)	CP-3800 GC (Varian)
Column	VF-200 ms (Agilent)	DB-225 ms (Agilent)	Optima 5 Amine (Macherey-Nagel)
Detector	MS (1200 L Quadrupole MS/MS, Variant)	MS (HP 5973 Mass Selective Detector)	FID
Internal standard	Decane	Diethylene glycol dibutyl ether (DEGDBE)	Mesitylene
Injector temperature, °C	330	320	320
Temperature ramp	60 °C, hold 4 min to 100 °C at 20 °C $min^{-1,}$ hold 4 min to 115 °C at 10 °C min^{-1}, hold 8 min to 300 at at 40 °C min^{-1}, hold 2 min	100 °C, hold 2 min to 120 °C at 20 ° C min^{-1} to 128 °C at 10 °C min^{-1}, hold 3.2 min to 240 °C at 11 °C min^{-1}, hold 3 min	80 °C, hold 5 min to 140 °C at 4 °C min^{-1} to 300 °C at 20 °C min^{-1}, hold 2 min
Typical retention times, min	Decane: 4.7 / GBL: 9.0 / Succ. Anh.: 11.7 / Succ. Ac.: 13.6	GBL: 6.5 / DEGDBE: 9.2 / Succ. Ac.: 12.0	GBL: 11.9 / BDO: 12.8 / mesitylene: 14.7 / DMS: 16.9

For each method, substance and internal standard, an Internal Response Factor (IRF) is calculated by equation (4-2) during calibration measurements. The unknown concentration of a sample is then calculated using the IRF.

$$IRF(Cpd/IS) = \frac{Area_{IS} \cdot [Cpd]}{[IS] \cdot Area_{Cpd}} \quad (4\text{-}2)$$

with	*IRF(Cpd/IS)*	*Internal Response Factor of the compound (Cpd) with the Internal Standard (IS)*	-
	[*Cpd*]	*concentration of the compound*	*mol l^{-1}*
	[*IS*]	*concentration of internal standard*	*mol l^{-1}*
	Area$_{Cpd}$	*area of the compound peak on the GC spectrum*	-
	Area$_{IS}$	*area of the Internal Standard peak on the GC spectrum*	-

4.5.3 Other analysis methods

The polymer ligand and complexes synthesized as described in Section 4.1.2 were analyzed by Elemental Analysis. These analyses were made by the Microanalytical Laboratory of the Technische Universität München, Germany.

The polymer ligand and complexes were also analyzed by ^{31}P-MAS NMR (i.e. Phosphorus-31 Magic Angle Spinning NMR) on a Bruker Avance 300 MHz (8 kHz, 4 mm Rotor, $(NH_4)H_2PO_4$ as external reference (1.11 ppm *vs.* H_3PO_4) with a High Power Decoupling (HPDEC) acquisition program (recycle delay of 10 s and pulse length of 1.50 µs).

Finally, the esters produced from succinic acid and 1-octanol were analyzed by ^{1}H-NMR. The spectra were recorded on a Bruker Avance UltraShield 400 MHz spectrometer at 300 K.

4.6 Calculations

4.6.1 pH calculation

When needed, the pH of the aqueous phase during an esterification can be theoretically calculated from the succinic acid remaining in the aqueous phase. To that end, the equations (3-27) and (3-28) must be used. If the reaction is performed in a phosphate buffer, similar equations must be used for the phosphate given its pK_as ($pK_{a,p1}$ = 2.16, $pK_{a,p2}$ = 7.21, $pK_{a,p3}$ = 12.32) (see equations (4-3) to (4-5)).

$$K_{a,p1} = \frac{[H_2PO_4^-]_{aq}[H^+]_{aq}}{[H_3PO_4]_{aq}} \tag{4-3}$$

$$K_{a,p2} = \frac{[HPO_4^{2-}]_{aq}[H^+]_{aq}}{[H_2PO_4^-]_{aq}} \tag{4-4}$$

$$K_{a,p3} = \frac{[PO_4^{3-}]_{aq}[H^+]_{aq}}{[HPO_4^{2-}]_{aq}} \tag{4-5}$$

A balance on the mole of hydrogen is necessary to calculate the pH at time t from the initial one. The balance is given by equation (4-6).

$$n_H = \left([H^+]_{aq} + [AH^-]_{aq} + 2\cdot[AH_2]_{aq} + 3\cdot[H_3PO_4]_{aq} + 2\cdot[H_2PO_4^-]_{aq} + [HPO_4^{2-}]_{aq}\right)\cdot V_{aq} + 2\cdot n_{orga} \tag{4-6}$$

with

n_H	*total mole of protons*	*mol*
$[AH_2]_{aq}$	*aqueous conc. of the diprotonated form of succinic acid*	*mol l^{-1}*
$[AH^-]_{aq}$	*aqueous conc. of the monoprotonated form of succinic acid*	*mol l^{-1}*
n_{orga}	*total mole of esters or diprotonated succinic acid contained in the organic phase*	*mol*

The total mole of esters is contained in the balance because the esterification equation does not produce or consume any proton. They are hence considered as the reactant (i.e. the diprotonated form of succinic acid). n_{orga} can be calculated from a balance on the disappearance of succinic acid from the aqueous phase, as shown in equation (4-7).

$$n_{orga} = \left([SA]_{tot,0} - [SA]_{aq}(t)\right)\cdot V_{aq} \tag{4-7}$$

Finally, equations (3-27), (3-28) and (4-3) to (4-7) can be combined to equation (4-8).

$$n_H = \left[[H^+]_{aq} + \frac{[H^+]_{aq}^2 + 2\cdot[H^+]_{aq}\cdot K_{a1}}{[H^+]_{aq}^2 + [H^+]_{aq}\cdot K_{a1} + K_{a1}\cdot K_{a2}}\cdot[SA]_{aq}(t)\right]\cdot V_{aq} + \frac{3\cdot[H^+]_{aq}^3 + 2\cdot[H^+]_{aq}^2\cdot K_{ap,1} + [H^+]_{aq}\cdot K_{ap,1}\cdot K_{ap,2}}{[H^+]_{aq}^3 + [H^+]_{aq}^2\cdot K_{ap,1} + [H^+]_{aq}\cdot K_{ap,1}\cdot K_{ap,2} + K_{ap,1}\cdot K_{ap,2}\cdot K_{ap,3}}\cdot[Phos]_{aq,0}\cdot V_{aq} + 2\cdot\left([SA]_{tot,0} - [SA]_{aq}(t)\right)\cdot V_{aq} \tag{4-8}$$

with

$[Phos]_{aq,0}$	*initial concentration of phosphate in the aq. phase*	*mol l^{-1}*

Equation (4-8) is a polynomial of 8th order in $[H^+]_{aq}$ and can be solved by a Microsoft Excel Solver in order to determine the proton concentration and hence the pH of the solution from the concentration of succinic acid contained in the aqueous phase at time t.

4.6.2 Conversion

The conversion of the esterification is defined by the disappearance of the succinic acid from the aqueous phase. The conversion at time t is given by equation (4-9).

$$X(t) = \frac{[SA]_{tot,0} - [SA]_{aq}(t)}{[SA]_{tot,0}} \tag{4-9}$$

with	*X(t)*	*conversion of succinic acid at time t*	-
	$[SA]_{tot,0}$	*initial concentration of succinic acid of the aqueous phase before any contact with the organic phase*	*mol l-1*
	$[SA]_{aq}$	*concentration of succinic acid in the aqueous phase at time t*	*mol l-1*

The conversion defined above includes therefore the pure extraction of the succinic acid into the organic phase.

4.6.3 Initial consumption rate and rate constant for the esterification

The esterification of succinic acid with an alcohol is a reversible reaction. If the reaction is realized in a biphasic system, the evolution of the succinic acid aqueous concentration $[SA]_{aq}$ is governed by a complex system of extraction, esterification and hydrolysis reactions. The disappearance of the aqueous succinic acid can be fitted by an exponential decay given by equation (4-10) using the program SigmaPlot® (Systat Softwater Inc., Chicago, IL). The initial molar consumption ($r_{mol,0}$) in mol l^{-1} h^{-1} can then be simply calculated from equation (4-12).

$$[SA]_{aq} = m + a \cdot e^{-b \cdot t} \tag{4-10}$$

$$r_{mol} = \frac{d[SA]_{aq}}{dt} = -a \cdot b \cdot e^{-b \cdot t} \tag{4-11}$$

$$r_{mol,0} = \left(\frac{d[SA]_{aq}}{dt}\right)_{t=0} = -a \cdot b \tag{4-12}$$

with	$r_{mol,0}$	*initial molar consumption rate*	*mol l^{-1} h^{-1}*
	$[SA]_{aq}$	*concentration of succinic acid in the aqueous phase at time t*	*mol l^{-1}*
	m	*parameter of the exponential fitting*	*mol l^{-1}*
	a	*parameter of the exponential fitting*	*mol l^{-1}*
	b	*parameter of the exponential fitting*	*h^{-1}*

Rate contants that are not depending on the initial succinic acid concentration can also be calculated. Here the calculations are based on the initial rate as described in equations (4-13) and (4-14). First, the rate constant can be calculated from the molar reaction rate $r_{mol,0}$ divided by the initial aqueous concentration ($[SA]_{aq,0}$) of succinic acid (see equation (4-13)). This rate constant is designated as "aqueous (initial) rate constant" or $k_{aq,0}$ (in h^{-1}). It is useful for comparing processes with different initial aqueous concentrations after contact with the organic phase (e.g. when different phase ratios are used with the same initial total concentrations of succinic acid, or with diverse alcohols in which succinic acid shows different partition coefficients).

However, the initial aqueous concentration after contact of the two phases is sometimes difficult to assess, because a lot of parameters influence the pure extraction of succinic acid into the organic solvent. It is hence easier to calculate the rate constant with regard to the initial total concentration of succinic acid ($[SA]_{tot,0}$) as shown in equation (4-14). $[SA]_{tot,0}$ is the succinic acid concentration in the fermentation broth or in the pure solution of succinic acid before they are put in contact with the organic phase. This concentration is therefore an important parameter of the process and is easily determined. The corresponding rate constant is designated as "total (initial) rate constant" or $k_{tot,0}$ (in h^{-1}). It will be often used as the parameter to describe the process.

$$k_{aq,0} = \frac{1}{[SA]_{aq,0}} \left(\frac{d[SA]_{aq}}{dt} \right)_{t=0} = -\frac{a \cdot b}{[SA]_{aq,0}} \tag{4-13}$$

$$k_{tot,0} = \frac{1}{[SA]_{tot,0}} \left(\frac{d[SA]_{aq}}{dt} \right)_{t=0} = -\frac{a \cdot b}{[SA]_{tot,0}} \tag{4-14}$$

with	$k_{aq,0}$	*aqueous (initial) rate constant*	*h^{-1}*
	$k_{tot,0}$	*total (initial) rate constant*	*h^{-1}*
	$[SA]_{aq,0}$ & $[SA]_{aq}$	*concentration of succinic acid in the aqueous phase, initially and at time t after contact of the two phases*	*mol l^{-1}*
	$[SA]_{tot,0}$	*initial total concentration of succinic acid in the aqueous phase before contact of the two phases*	*mol l^{-1}*

4.7 Optimization

To optimize the reaction conditions for the biphasic esterification, both a "one-at-a-time" strategy and a multiple-variable optimization were realized during this study.

The "one-at-a-time" optimization consists in varying one variable at a time with the others set as in the standard conditions. Finally, the optima of each experiment are taken as optimal set of reaction conditions. This method has a very simple experimental plan and can give a lot of information on transfer limitations, reaction order, etc… However, this method does not take the interaction of the different variables into account.

To that end, a multiple-variable optimization was performed using the simple Response Surface Methodology (RSM) (Montgomery, 2009). The RSM technique consists in optimally planning and performing a series of experiments by varing the input variables and fitting the responses with second order polynomial functions with interaction terms, such as presented in equation (4-15).

$$y=\beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum\sum \beta_{ij} x_i x_j \qquad (4\text{-}15)$$

with y *output variable*

x_i *input variables*

$\beta_0, \beta_i, \beta_{ii}, \beta_{ij}$ *zero, first, second order and interaction parameters*

This methodology was used for optimizing both the total initial rate constant of succinic acid and the conversion after 6 h with the temperature (T), the pH and the succinic acid initial concentration (C) as input variables. The reactions were performed in triplicate in a parallel reactor unit as described in Subsections 4.3.2.2 and 4.3.2.3. For both optimizations, the experiments were planned following the faced Central Composite Design (CCD), also known as face centered Box-Wilson design. The temperature was varied between 30 °C and 70 °C, the concentration of succinic acid from 0.15 M to 0.8 M and the pH from 2 to 4. As some temperature fluctuations happened during the reaction, the average temperature was used as input variable. The center of the faced Central Composite Design was hence slightly shifted to lower temperature (at 47.5 °C instead of 50 °C).

The computational calculations were realized with Matlab (Mathworks 2010a) with the least square method. To that end, "coded" variables were defined: these are variables

centered on the range center and scaled so that they vary between -1 and +1 over the ranges tested. They are labelled as C^*, pH^* and T^* and can be calculated from the normal input variables C, pH and T by equation (4-16).

$$Z = \frac{Z_{max} + Z_{min}}{2} + Z^* \cdot \frac{Z_{max} - Z_{min}}{2} \quad (4\text{-}16)$$

with Z *input variable*: *concentration (C), pH or temperature (T)*

Z^* *coded input variable*

Z_{min}, Z_{max} *minimum and maximum value of the input variable range tested*

Finally, the models were simplified by removing the non-significant coefficients in the second order polynomials, in order to lower the *P* value. The *P* value is used in hypothesis testing and is the probability that an observed effect is simply due to chance. Values close to 0 indicate that the observed difference is unlikely to be due to chance, whereas a *P* value close to 1 suggests there is no difference between groups other than that due to random variation (Whitley and Ball, 2002).

5 Hydrogenation

As noted in Section 3.1.4, the potential of succinic acid relies among other on its reduced derivatives and on its esters. In the experimental part of this project (see Chapters 5 and 6), catalysts and process options were successively tested for the hydrogenation and the esterification of the succinic acid. The present Chapter deals with the hydrogenation which aims to produce chemicals such as 1,4-butanediol (BDO), γ-butyrolactone (GBL), tetrahydrofuran (THF) or pyrrolidone, which have a broad range of applications. This reaction has been widely reported in the literature, in organic solvents and with different metal supported catalysts. However, as the biotechnological production of succinic acid generates aqueous solutions of this compound, it would be more desirable to develop its hydrogenation in aqueous media.

Different options have been suggested for the aqueous hydrogenation of succinic or maleic acid using mostly metal supported catalysts working at high temperatures and pressures (up to 270 °C and 27.6 MPa) (Delhomme et al., 2009). The published catalysts combine many different metals in order to enhance the process selectivity, which is difficult to control for such an intricate network of hydrogenation reactions. Water-tolerant catalysts working under milder reaction conditions with high selectivities would be extremely attractive for industrial applications. Since metallic complexes are catalysts that have been reported as working in general under mild conditions with high selectivities, this approach was studied here. However, very little information has been published to date on the use of such complexes for the aqueous hydrogenation of carboxylic acids. This field is hence challenging.

In Subchapter 5.1, tests were first conducted on the aqueous hydrogenation with metallic complexes of a similar substrate, levulinic acid, for which some literature could be found. Similar metallic complexes as well as heterogeneous clay catalysts were then tested for the hydrogenation of succinic acid or its anhydride both in water and in solvent. Results will be presented in Subchapter 5.2. Lastly, the hydrogenation of succinate esters with metallic complexes in solvent will be addressed as alternative route in Subchapter 5.3, prior to concluding remarks.

5.1 Aqueous hydrogenation of levulinic acid with metallic complexes, as a preliminary study

Before the possibility of hydrogenating succinic acid in water using metallic complexes could be tested, levulinic acid, a similar substrate for which already published protocols exist for its aqueous hydrogenation (see Section 3.3.3), was used as the starting point of this work.

The hydrogenation of levulinic acid (LA) to γ-valerolactone (GVL), which is shown in Figure 5-1, has only been reported by few groups and the state of the art has been presented in Subsection 3.3.3.2. In this study, similarly to the work of Mehdi et al. (2008), several ruthenium complexes with water-soluble phosphine ligands were screened for the hydrogenation of levulinic acid.

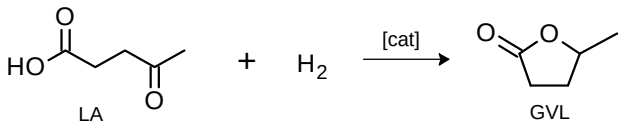
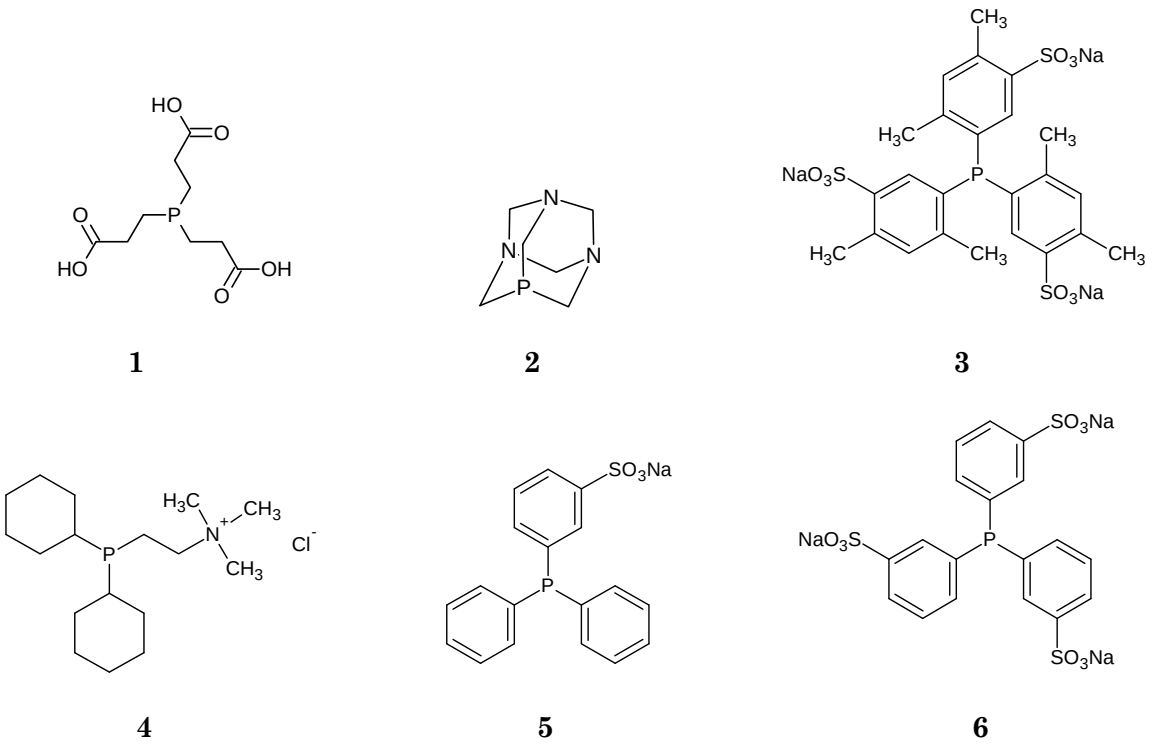


Figure 5-1: Hydrogenation of levulinic acid (LA) into γ-valerolactone (GVL).

5.1.1 Aqueous hydrogenation of levulinic acid using ruthenium complexes with water-soluble phosphine ligands

Several water-soluble ligands with Ru(III) acetylacetonate ($Ru(acac)_3$) as metal precursor were tested for the aqueous hydrogenation of levulinic acid at 140 °C and 5 MPa H_2. The metal complexes were formed *in-situ*. The different phosphine ligands tested for this reaction were: Tris(2-carboxyethyl)phosphine (**1**), 1,3,5-Triaza-7-phosphaadamantane (PTA) (**2**), Tris(2,4-dimethyl-5-sulfophenyl) phosphine trisodium salt (**3**), [2-(Dicyclohexylphosphino)ethyl] trimethylammonium chloride (TXTPS) (**4**), 3-(Diphenylphosphino) benzenesulfonic acid sodium salt (TPPMS) (**5**), 3,3′,3″-Phosphinidynetris(benzenesulfonic acid) trisodium salt (TPPTS) (**6**). These ligands are presented in Figure 5-2. As a comparison, the alumina supported 5 % Ru catalyst, similar to the catalyst suggested by Serrano-Ruiz et al. (2010), was also tested. The results of the hydrogenation are presented in Table 5-1.



1 **2** **3**

4 **5** **6**

Figure 5-2: Ligands used for the aqueous hydrogenation of levulinic acid into γ-valerolactone: **1**: Tris(2-carboxyethyl) phosphine; **2**: 1,3,5-Triaza-7-phosphaadamantane (PTA); **3**: Tris(2,4-dimethyl-5-sulfophenyl) phosphine trisodium salt (TXTPS); **4**: [2-(Dicyclohexylphosphino)ethyl] trimethylammonium chloride; **5**: 3-(Diphenylphosphino)benzenesulfonic acid sodium salt (TPPMS); **6**: 3,3′,3″-Phosphinidynetris(benzenesulfonic acid) trisodium salt (TPPTS).

Table 5-1: Gas hydrogenation of levulinic acid with 1.1 mM ruthenium and 11 mM of ligand at 140 °C and 5 MPa H_2 in 40 ml water: Turnover Frequency (TOF), conversion (X) and selectivity (S) after 5 or 21 h.

N°	Catalyst	TOF, mol_{GVL} mol_{Ru}^{-1} h^{-1}	After 5 h		After 21 h	
			X, %	S, %	X, %	S, %
1	$Ru(acac)_3$ + **1**	10	1	-	62	39
2	$Ru(acac)_3$ + **2**	13	3	100	34	14
3	$Ru(acac)_3$ + **3**	117	23	95	26	100
4	$Ru(acac)_3$ + **4**	135	59	100	84	91
5	$Ru(acac)_3$ + **5**	194	94	94	100	92
6	$Ru(acac)_3$ + **6**	202	99	97	100	92
7	$Ru(acac)_3$	569	100	98	N.D.*	N.D.*
8	Ru 5 % on Al_2O_3	575	100	98	100	86

*: N.D.: not determined.

Levulinic acid was reduced by all the screened catalytic systems. The turnover frequencies (TOF) were calculated using equation (3-2).

Ligands **1** and **2** gave poor activities for the aqueous hydrogenation of levulinic acid into γ-valerolactone (TOFs < 20 mol_{GVL} mol_{Ru}^{-1} h^{-1}). Whereas yellow ruthenium complexes were initially formed for all ligands tested, a fast discolouring of the aqueous solution was observed for the ligands 1 and 2 after only 2 h of reaction. The low TOFs can hence be explained by a fast deactivation of the complex, due probably to a change in its structure.

On the contrary, phosphines with non-linear side chains such as **3**, **4**, **5** and **6** gave satisfactory TOFs from 116 to 202 mol_{GVL} mol_{Ru}^{-1} h^{-1}. Concerning the ligand TXTPS (**3**), good TOFs could be achieved in the first 2 h of reaction. Notwithstanding, the conversion did not increase much afterwards (from 19 % after 2 h to 26 % after 21 h). Here also, a fast discolouring of the solution was observed after 4 h of reaction and the formation of black particles was noticed, which was an evidence of the complex decomposition.

Nevertheless, TOF reported with ligand **3** before deactivation was just slightly lower than the TOF of the ligand **6**. The two similar ligands have been reported to have comparable Lewis basicity i.e. electron donating effects, since the CO stretching frequencies of *trans*-[L_2Rh(CO)Cl] complexes (L = ligands) measured by Moore et al. (2008) were of 1993 cm^{-1} for the two ligands. The two additional methyl groups (*para* and *ortho* positions) on the TXTPS (**3**) have nevertheless steric effects on the complex. This could be observed by the change in cone angles reported by Moore et al. (2008) (cone angles of 165° for TPPTS and 210° for TXTPS). These steric effects of the alkyl groups could be responsible for the smaller TOF. Furthermore, Gulyás et al. (2004) attributed the observed decomposition of their rhodium complex with TXTPS ligands into a black Rh precipitate to the steric effects of the ligands on the relatively small Rh center. The decomposition observed here for the ruthenium complex might be hence caused by the steric effects of the additional methyl groups.

The best phosphine ligands tested here were TPPMS (**5**) and TPPTS (**6**), with TOFs of 194 and 202 mol_{GVL} mol_{Ru}^{-1} h^{-1} respectively. These two ligands are well-known water-soluble phosphines, TPPTS being already used for industrial applications (Joó, 2001). With these two ligands, high conversions (94 and 99 % resp.) could be achieved after only 5 h with high selectivities (94 and 97 % resp.). The two ligands only differ from one another by the number of sulfonic groups: TPPTS (**6**) bears indeed a sulfonate function in *meta* position on all the three phenyl groups, whereas only one phenyl group of

TPPMS (**5**) has a *meta* sulfonate function. Sulfonate groups are electron withdrawing groups that have an influence on the Lewis basicity of the ligand. As reported by Monflier and Mortreux (1994) for $Co_2(CO)_6L_2$ complexes (L = ligand), the C=O stretching frequency (ν_{CO}) is higher in the complex with TPPTS (**6**) than in the one containing TPPMS (**5**) (1955 *vs.* 1949 cm^{-1}). This shows the higher Lewis basicity of TPPMS, i.e. a higher electron donating effect. On the contrary, a higher amount of sulfonic functions will decrease the electron density on the metal. This might be necessary for the reaction mechanism of the reduction of the ketone function of levulinic acid. Steric effects of the two ligands are also different, because of the additional sulfonic groups, leading to cone angles of 151° for TPPMS and 165° for TPPTS (Monflier and Mortreux, 1994). The steric effect might also be responsible for the change in TOFs.

Surprisingly, the metal precursor ($Ru(acac)_3$) alone gave a high TOF (569 mol_{GVL} mol_{Ru}^{-1} h^{-1}) similar to the one obtained with the heterogeneous metal supported catalyst Ru 5 % on alumina (Al_2O_3) (575 mol_{GVL} mol_{Ru}^{-1} h^{-1}). However, it should be noted that a fast discolouring (from the red $Ru(acac)_3$ to a colourless solution) took place after only 1 h of reaction and black particles were formed. The decomposed catalyst, however, catalysed efficiently the hydrogenation of levulinic acid into GVL. The similar TOFs of the reactions with $Ru(acac)_3$ and with Ru 5 % on alumina seem to be in agreement with the reaction being catalyzed by the ruthenium clusters (i.e. the observed black particles) formed by the decomposition of $Ru(acac)_3$. Finally, the heterogeneous catalyst Ru 5 % on alumina remained the most active catalyst tested here. Nevertheless, it should be noted, that for the reaction with this catalyst, the selectivity of GVL slowly decreased after 3 hours of reaction (from 99 % after 3 h to 86 % after 21 h) possibly due to a further hydrogenation of GVL into more reduced products.

Concluding remarks

Levulinic acid could be reduced into GVL in water at 140 °C and 5 MPa (H_2) using ruthenium complexes with a wide range of water-soluble phosphines. Both the steric effects and the Lewis basicity of the ligands must be taken into account for selecting the best appropriate catalytic system. On the one hand, the sterically highly hindered TXTPS ligand (large cone angle) led to a smaller TOF and to the deactivation of the catalyst. On the other hand, electron withdrawing groups (such as sulfonic groups) on the ligand (e.g. in TPPTS), which decreased the electron density on the metal center, seemed to increase the TOF. In the end, the best homogeneous catalytic system with regard to the activity, the stability and the selectivity consisted of $Ru(acac)_3$ with TPPTS

ligands (**6**). Nonetheless, the metal supported catalyst 5 % Ru on alumina gave a higher TOF than the homogeneous metal complexes tested.

Ultimately, one of the main drawbacks of homogeneous catalysis is the waste of catalyst at the end of the reaction, since the catalyst can often not be easily removed from the reaction system, so that heterogeneous catalysts are often more suitable for industrial applications. As ruthenium complexes with water-soluble modified triphenylphosphine ligands (**5** and **6**) were good catalysts for the aqueous hydrogenation of levulinic acid, a strategy to synthesize heterogeneous catalysts by immobilizing similar ligands was tested and will be presented in the next Section.

5.1.2 Immobilization of the ligands

Among the different strategies and supports reported for the immobilization of ligands, the immobilization on polymer was investigated. This method allows indeed a good contact of the catalyst with an aqueous phase and hence with the substrate if the selected polymer swells well in water (see Subsection 3.2.5.3). This approach has already been studied for several types of reactions, including hydrogenation in presence of water (Kayaki et al., 2003).

In this study, the catalyst developed by Kayaki et al. (2003) was synthesized and then tested for the aqueous hydrogenation of levulinic acid into GVL. This analog of $RuCl_2(PPh_3)_4$ was initially developed for the hydrogenation of supercritical carbon dioxide.

5.1.2.1 Synthesis of the immobilized complex

For the synthesis of the immobilized complex, the ligand was first attached to the polymer to form the polymeric ligand. The PS-PEG resin-supported N-anchored 2-aza-1,3-bis(diphenylphosphino)propane (adppp) ligands were synthesized from PS-PEG resins with NH_2 terminal groups (Rapp Polymere, Tübingen, Germany) following the protocol published by Uozumi and Nakai (2002) (see Subchapter 4.1.2). Then the ruthenium complex was formed with two bidental polymeric ligands to produce the final immobilized complex. The complex ($RuCl_2$(PS-PEG-adppp)$_2$) was synthesized accordingly to Kayaki et al. (2003) and its structure is presented in Figure 5-3.

The analysis of the dry polymeric ligand was done with solid phase phosphor NMR (^{31}P HPDEC MAS NMR, 4 mm Rotor, 8 kHz, 2300 scans, ext. ref. $(NH_4)H_2PO_4$ 1.11 ppm *vs.* H_3PO_4). Uozumi and Nakai (2002) mentioned a product peak at δ = – 28.7 ppm (s). The

spectrum of the synthesized ligand showed a peak at δ = – 28.2 ppm (see Annex 9.3.1) with two small side peaks at δ = – 14.2 and + 27.0 ppm, which natures could not be determined.

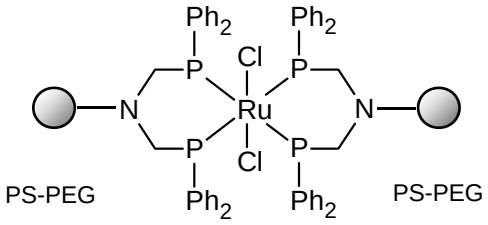


Figure 5-3: Ruthenium complex with two polymer supported phosphine ligands ($RuCl_2$(PS-PEG-adppp)$_2$) (with adppp = 2-aza-1,3-bi(diphenylphosphino)propane).

From the elemental analysis presented in Table 5-2, it was calculated that the immobilization of the ligand was achieved successfully with 91 % conversion, whereas the conversion of the complex formation did not exceed 81 % (if only one type of complexation is considered).

A P to Ru molar ratio of 3.24 was obtained for the immobilized complex, whereas the expected ratio would be of 4 (cf. 4 P atoms should be bonded to the Ru center). Furthermore the phosphorus content in the polymeric complex was higher as expected. Either not all the polymer ligands are bound to a ruthenium centre, or not all Ru centers are linked to two polymeric ligands. Other complex conformations, such as the one presented in Figure 5-4, were probably also formed.

Table 5-2: Elemental analysis of the immobilized ligand (adppp) and complex ($RuCl_2$(PS-PEG-adppp)$_2$).

Element	Ligand		Complex	
	Measured content, %	Theoretical content, %	Measured content, %	Theoretical content, %
C	65.96		64.53	
H	8.19		8.17	
N	0.23	0.33	0.23	0.32
P	1.33	1.46	1.59	1.43
Ru	-	-	1.60	1.17

These side-products were also observed on the ^{31}P MAS NMR spectrum of the immobilized complex (^{31}P HPDEC MAS NMR, 4 mm Rotor, 8k Hz, 6401 scans), which showed a main peak at δ = – 4.1 ppm and side peaks at δ = – 11.5 ppm and in the positive δs (mainly δ = + 25.6 ppm). Kayaki et al. (2003) mentioned a peak at δ = – 3.9 ppm for the ruthenium complex with two polymeric ligands. Therefore, it could be confirmed that the bipolymeric ligand complex was formed but together with other complexes.

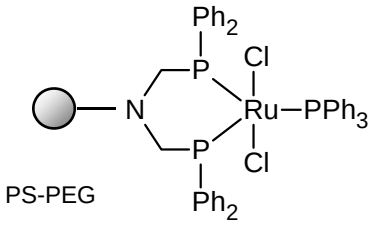


Figure 5-4: Possible other conformation of the immobilized ruthenium complex with ($RuCl_2$(PS-PEG-adppp)(PPh_3)).

In the initial procedure, Kayaki et al. (2003) used ArgoGel, a similar PS-PEG copolymer, as starting polymer instead of TentaGel. This polymer has a higher loading capacity (i.e. ~ 0.37 mmol g^{-1} for Argogel and 0.2 to 0.3 mmol g^{-1} for TentaGel). Due to the higher loading capacity of Argogel, the molar ratio between the ligand and the Ru complex is higher in Kayaki et al.'s (2003) protocol than in the methodology used here (since the same polymer ligand weight as in Kayaki et al.'s protocol was used). The quantity of polymer ligands and Ru complex must thus be optimized to achieve the synthesis of the pure bi-polymer-ligand complex.

Concluding remarks

The immobilized ruthenium phosphine complexes could be synthesized successfully. However, some impurities were observed, probably due to the formation of complexes with a single polymer ligand. The formation of the unwanted complexes might be reduced by using a larger amount of ligand with respect to the [$RuCl_2(PPh_3)_3$] concentration. With an increased ratio (PS-PEG-adppp : Ru complex), the formation of the mono-polymer-ligand complex might be less probable.

5.1.2.2 Application of the immobilized complex for the hydrogenation of levulinic acid

The immobilized complex $RuCl_2$(PS-PEG-adppp)$_2$ described in Subsection 5.1.2.1 was then used for the aqueous hydrogenation of levulinic acid at 140 °C and 5.5 MPa. As a comparison, an *in-situ* formed complex of $RuCl_3$ with TPPTS ligands (**6**) was tested with a similar P to Ru ratio (3.24) as the one determined by elemental analysis in the immobilized complex. Finally, the non-water soluble complex $RuCl_2(PPh_3)_3$ was also tested for comparison purposes. The turnover frequencies, the conversions and the selectivities of the hydrogenation with these three catalytic systems are presented in Table 5-3.

Table 5-3: Gas hydrogenation of levulinic acid with 0.0158 mM ruthenium with if necessary 0.0513 mM of ligand at 140 °C and 5.5 MPa H_2 in 40 ml water: Turnover Frequency (TOF), conversion (X) and selectivity (S) after 5 or 24 h.

N°	Catalyst	TOF, mol_{GVL} mol_{Ru}^{-1} h^{-1}	X after 5 h, %	S after 5 h, %	X after 24 h, %	S after 24 h, %
9	$RuCl_2$(PS-PEG-adppp)$_2$	78	49	81	97	87
10	$RuCl_3$ + **6**	210	74	91	90	86
11	$RuCl_2(PPh_3)_3$	92	22	86	23	97

The homogeneous catalyst system consisting of a water soluble complex of $RuCl_3$ with TPPTS (ligand **6**) gave similar TOF (210 mol_{GVL} mol_{Ru}^{-1} h^{-1}) as the one observed with the same ligand and $Ru(acac)_3$ as metal precursor (202 mol_{GVL} mol_{Ru}^{-1} h^{-1}, entry 6 in Table 5-1). The anionic ligand does not seem to have a high impact on the initial TOF.

The immobilized complex showed a lower TOF (78 mol_{GVL} mol_{Ru}^{-1} h^{-1}), but after 24 h, a higher conversion was achieved with the polymer catalyst than with the water-soluble complex (97 % *vs.* 90 %). The lower TOF might result from the absence of electron-withdrawing groups on the phenyl groups of the ligands (in comparison to the TPPTS ligands), leading to a higher electron density on the Ru center. That is why it might be preferable to immobilize phosphine ligand with electron-withdrawing groups instead of the simple triphenylphosphine ligands.

As expected, the non-water-soluble complex $RuCl_2(PPh_3)_3$ tested for comparison purpose gave a low TOF (92 mol_{GVL} mol_{Ru}^{-1} h^{-1}). Furthermore, after 3 h of reaction, the conversion

did not increase anymore, reaching only 23 % after 24 h. The non-water soluble catalyst did not dissolve in the reaction system and the outer surface was probably deactivated by the water.

Concluding remarks

Although the homogeneous water-soluble catalyst system was the one allowing the highest TOF and conversion after 5 h, the immobilized catalyst with polymeric ligand eventually led to the highest final conversion by maintaining a similar TOF throughout the reaction without showing any deactivation like the free water-insoluble complex. Furthermore, the polymer catalyst can easily be removed from the reaction system and might be reused in further reactions.

In conclusion, the hydrogenation of levulinic acid can be performed with homogeneous or immobilized metallic complexes in water. However, the ketone function of levulinic acid is reduced during this reaction and not its carboxylic function. Therefore, the possibility of using such complexes for succinic acid must still be proven.

5.2 Hydrogenation of succinic acid or anhydride

After the aqueous hydrogenation of levulinic acid was successfully performed with metallic complexes as described in Subchapter 5.1, the reduction of succinic acid or of its anhydride was then attempted. Since little information could be found in the literature on this reaction using metallic complexes in water, the hydrogenation of succinic anhydride in organic solvent was first tested with such catalysts and the results will be presented in Section 5.2.1. Then, the reaction was transferred to water, using succinic acid as substrate and reaction conditions similar to the one derived for levulinic acid, as well as with another metallic complexe at higher temperature. The outcome of these tests will be discussed in Section 5.2.2. Finally another strategy with metal supported clay catalysts was tested and will be described in Section 5.2.3.

5.2.1 Validation of the hydrogenation of succinic anhydride in organic solvents with metallic complexes

As indicated before, the hydrogenation of succinic anhydride into γ-butyrolactone (GBL) in an organic phase was first tested and is shown in Figure 5-5. This reaction is currently performed mostly with metal supported catalysts for the production of reduced derivatives of succinic acid. Nevertheless, it has also been reported with metallic

complexes in solvent (Hara and Inagaki, 1991; Hara et al., 2000; Hara and Takahashi, 2000; Hara and Takahashi, 2002) (see Section 5.2.1). Hara et al., for instance, hydrogenated succinic anhydride in tetraethylene glycol dimethyl ether (tetraglyme) at 200 °C and 3 MPa in acidic condition (with *p*-Toluenesulfonic acid (*p*-TsOH)) using a catalytic system consisting of $Ru(acac)_3$ and tri-*n*-octylphosphine ($P(octyl)_3$).

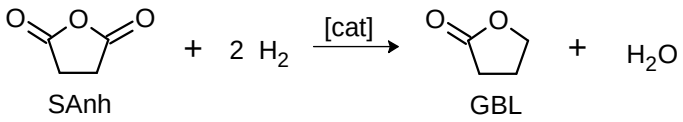


Figure 5-5: Hydrogenation of succinic anhydride (SAnh) into γ-butyrolactone (GBL).

The approach adopted here was similar to the methodology used for the hydrogenation of levulinic acid: homogenous catalysts were first screened and then an immobilized complex was tested.

5.2.1.1 Screening of homogeneous catalytic systems

For the screening, different ruthenium complexes, presented in Figure 5-6, were tested for the hydrogenation of succinic anhydride in tetraglyme. Three systems with phosphine ligands with a ruthenium center were screened since Hara et al. (2000) reported them to be successful catalysts for such a reaction. A ruthenium complex with P-N ligands was also tested, as it has been reported to be a good catalyst for the hydrogenation of ketone in solvent. In addition, two carbene complexes were tested because, unlike phosphine ligands, these catalysts are stable in air, which makes their handling very easy and more suitable for industrial applications. They can also be modified for reactions in water (Hong and Grubbs, 2006).

The reaction conditions were derived from Hara et al. (2000). Tetraglyme was used as solvent and the reaction was performed at 1 MPa H_2 and 150 °C under acidic (addition of *p*-TsOH) or basic (addition of sodium isopropoxide (C_3H_7NaO)) conditions. Lower pressure and temperature were used here (1 MPa instead of 3 MPa and 150 °C instead of 200 °C) for technical reasons. The molar ratios between the reactants were:

Substrate : Metal : (Ligand) : Acid or Base

400 : 1 : (10) : 8 or 20

The three ruthenium phosphine complexes were only tested in acidic conditions, since Hara et al. (2000) reported that, if no acid was used, the trialkyl phosphine acted as strong base and promoted the formation of spirodilactone instead of GBL. The other catalytic systems were tested both in basic and in acidic conditions.

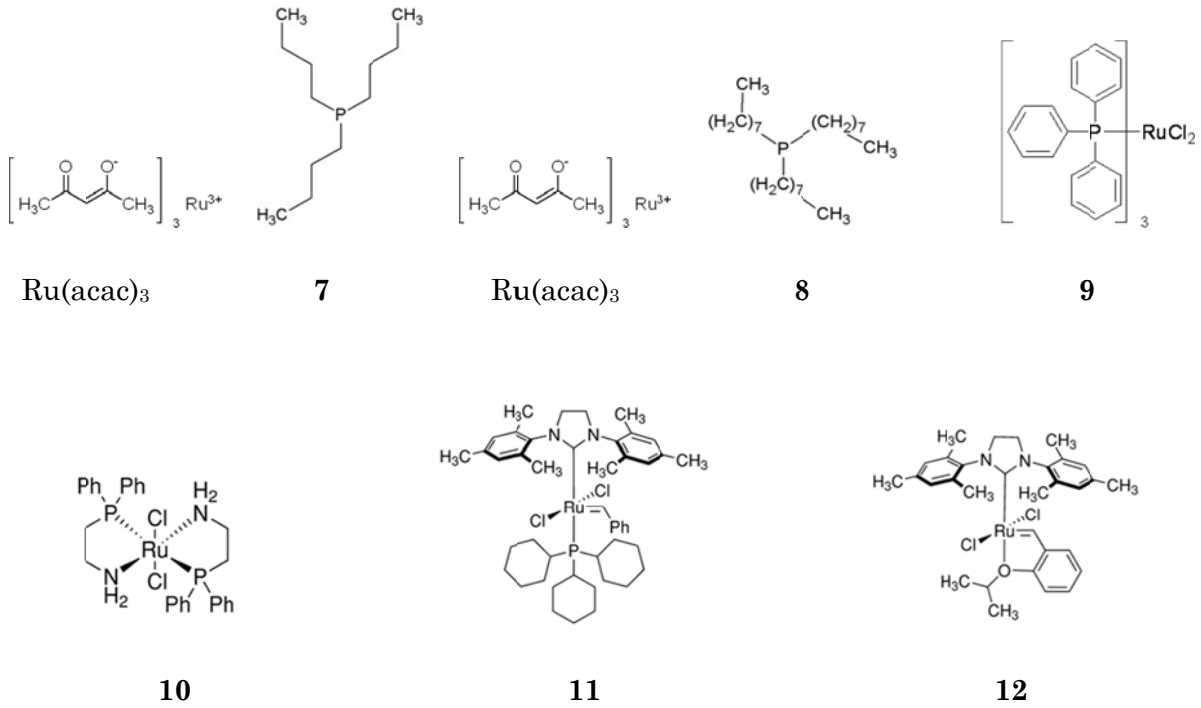


Figure 5-6: Catalytic systems used for the aqueous hydrogenation of succinic anhydride into γ-butyrolactone: Ru(III) acetylacetonate ($Ru(acac)_3$) with **7**: tri-*n*-butylphosphine ($P(Bu)_3$) or with **8**: tri-*n*-octylphosphine ($P(octyl)_3$); **9**: Dichloro-tris[triphenylphosphine]ruthenium[II]; **10**: Dichlorobis(2-(diphenylphosphino) ethylamine)ruthenium(II); **11**: 2nd Generation Grubbs Catalyst; **12**: 2nd Generation Hoveyda-Grubbs Catalyst.

Due to the gas chromatography column used for analysis, only the yield of GBL could be measured with precision, whereas succinic anhydride and acid could not be measured with high enough resolution. The yields and the turnover frequencies are reported in Table 5-4.

From the catalytic systems tested, the linear phosphines (**7** and **8**) reported by Hara et al. (2000) seemed to be the best ligands for the hydrogenation of succinic anhydride in tetraglyme at 1 MPa and 150 °C, under acidic conditions, with TOFs of 95 and 76 $mol_{GBL}\ mol_{Ru}^{-1}\ h^{-1}$ respectively. The TOFs reported by Hara et al. (2000) are higher (i.e. 132 $mol_{GBL}\ mol_{Ru}^{-1}\ h^{-1}$ for the complex with ligand **7** and 120 for the one with ligand 8)

than those measured here, but they performed the reactions at higher temperature (200 °C) and pressure (3 MPa).

The complex with the aromatic phosphines (**9**) gave much lower TOF (17 mol_{GBL} mol_{Ru}^{-1} h^{-1}). Hara et al. (2000) found also a lower TOF for this complex and reported a fast deactivation of the catalyst due to the replacement of the Cl ion by a carboxylate ion derived from the succinic anhydride. LiCl had to be added continuously to maintain good activities. Besides, Hara et al. (2000) mentioned that triphenylphosphine complexes might not be stable enough under high temperature and will completely decompose at temperatures above 180 °C.

Table 5-4: Homogeneous hydrogenation of succinic anhydride in tetraglyme at 150 °C, 1 MPa H_2, with 1.25 M succinic anhydride, 3.12 mM Ruthenium, 31.2 mM ligand if necessary, 25 mM *p*-toluenesulfonic acid (*p*-TsOH) or 62.5 mM sodium isopropoxide (C_3H_7NaO), 80 mM *n*-decane as internal standard.

N°	Catalyst	Additive	TOF, mol_{GBL} mol_{Ru}^{-1} h^{-1}	Y after 5.5 h, %	Y after ~ 21 h, %
12	$Ru(acac)_3$ + **7**	*p*-TsOH	95	37	42
13	$Ru(acac)_3$ + **8**	*p*-TsOH	76	36	48
14	**9**	*p*-TsOH	17	15	22
15	**10**	*p*-TsOH	2	1	8
16	**10**	C_3H_7NaO	17	11	15
17	**11**	*p*-TsOH	26	23	26
18	**11**	C_3H_7NaO	25	17	32
19	**12**	*p*-TsOH	7	6	8
20	**12**	C_3H_7NaO	80	35	39

The carbene complex named 2nd generation Hoveyda-Grubbs catalyst (**12**) appeared to be also a good candidate with TOF of 80 mol_{GBL} mol_{Ru}^{-1} h^{-1} in basic conditions. To the best of our knowledge, it is the first time that such a carbene catalyst is reported for the solvent hydrogenation of anhydrides. The other carbene complex, i.e. the 2nd generation Grubbs catalyst (**11**), gave, however, a low TOF (i.e. 25 mol_{GBL} mol_{Ru}^{-1} h^{-1}) in basic conditions. Similarly to the ruthenium triphenyl phosphine complex (**9**), the triphenyl phosphine containing complex **11** might be deactivated at the temperature of the reaction. In acidic conditions, the use of the two carbenes lead to low TOFs (i.e. 26 mol_{GBL} mol_{Ru}^{-1} h^{-1} for **11** and 7 mol_{GBL} mol_{Ru}^{-1} h^{-1} for **12**).

Lastly, it should be noted that, for the best three catalysts, a fast drop of the TOF could be observed after 2.5 h of reaction, resulting in low yields after 21 h (from 39 to 48 %), the best yield being obtained for $Ru(acac)_3$ with ligands **8**. As reported by Hara et al. (2000), this could be due to the formation of water during the reaction (see Figure 5-5). Succinic anhydride would thus be hydrated into succinic acid (see Figure 5-7), which showed TOFs at least 3 times lower than the one obtained with succinic anhydride as substrate (Hara et al., 2000). This hypothesis might be tested by repeating the reaction with water added already at the beginning of the reaction and by recording the impact on the TOF. This phenomenon could be problematic if the reaction had to be transferred to the aqueous phase.

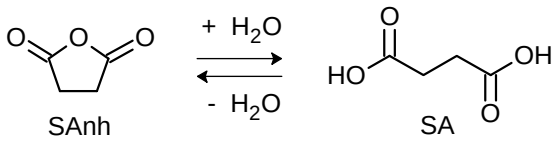


Figure 5-7: Hydration of succinic anhydride (SAnh) into succinic acid (SA) and reverse dehydration.

Concluding remarks

Ruthenium complexes with linear phosphine ligands under acidic conditions and the 2nd generation Hoveyda-Grubbs catalyst under basic conditions were the best catalysts for the hydrogenation of succinic anhydride into GBL in tetraglyme. Accordingly, linear phosphines ruthenium complexes and carbenes seem to be good candidates for the development of new water-soluble ligands for the aqueous hydrogenation of succinic acid. However, the lower activities of the complexes towards succinic acid reported by Hara et al. (2000) might be a major drawback for aqueous hydrogenations.

5.2.1.2 Impact of the reaction conditions on the homogeneous catalysis

As the reported yields were rather moderate for the hydrogenation of succinic anhydride in tetraglyme, the reaction with the linear tri-*n*-octyl phosphine ligand (**8**) was repeated at a higher temperature, since Hara et al. (2000) performed their reactions at 200 °C. The impact of the temperature change (from 150 to 200 °C) on the turnover frequency and the yields after 5.5 h and ~ 21 h, reported in Table 5-5 and the impact on the evolution of the yield over time in Figure 5-8.

Table 5-5: Impact of the temperature on the homogeneous hydrogenation of succinic anhydride in tetraglyme, 1 MPa H_2, with 1.25 M succinic anhydride, 3.12 mM Metal, 31.2 mM ligand, 25 mM *p*-TsOH, 80 mM n-decane as internal standard: Turnover Frequency (TOF); yield after 5.5 h and at the end of the reaction (21-22 h).

N°	Catalyst	Temperature, °C	TOF, mol_{GBL} mol_{Ru}^{-1} h^{-1}	Y after 5.5 h, %	Final Y, %
13	$Ru(acac)_3$ + **8**	150	76	36	48
21	$Ru(acac)_3$ + **8**	200	65	75	99

When increasing the temperature from 150 to 200 °C, the maximal TOF did not vary much (76 mol_{GBL} mol_{Ru}^{-1} h^{-1} at 150 °C and 65 mol_{GBL} mol_{Ru}^{-1} h^{-1} at 200 °C). However, the final yields increased extremely from 48 % to 99 %. As seen in Figure 5-8, the activity dropped greatly after 2.5 h for the reaction performed at 150 °C contrary to the one at 200 °C (i.e. TOFs of 10 ± 4 and 33 ± 2 mol_{GBL} mol_{Ru}^{-1} h^{-1} from 2.5 to 5 h at 150 and 200 °C resp). This could not only be explained by the impact of the temperature on the reaction rate due to the Arrhenius' law. Other reactions are hence taking place. This might be due to the dehydration of the formed succinic acid, only happening at higher temperature. This reaction is indeed performed industrially at 200 °C (Fumagalli, 2006). However, the water formed by the hydrogenation might prevent the dehydration from taking place even at higher temperature.

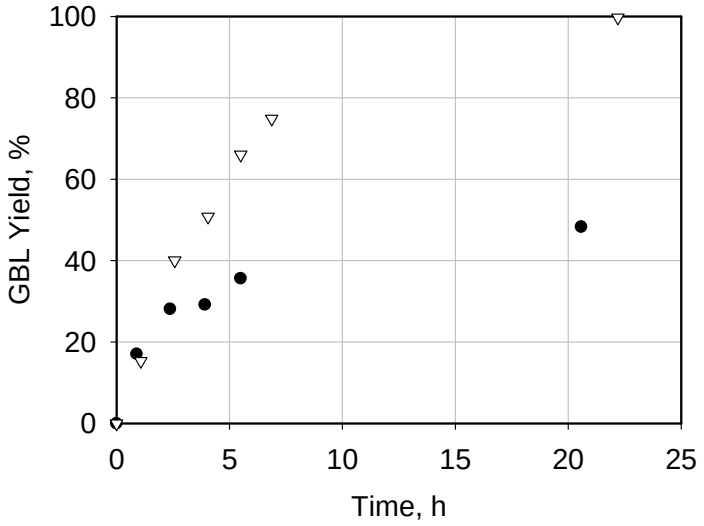


Figure 5-8: Impact of the temperature (150 °C ● and 200 °C ▽) on the homogeneous hydrogenation of succinic anhydride in tetraglyme, 1 MPa H_2, with 1.25 M succinic anhydride, 3.12 mM metal, 31.2 mM ligand, 25 mM *p*-TsOH, 80 mM *n*-decane as internal standard.

Finally, it should be noted that, since the maximal TOF did not change much while increasing the temperature, the higher TOF reported by Hara et al. (2000) (i.e. 120 mol_{GBL} mol_{Ru}^{-1} h^{-1}) might only be achieved at high pressure (3 MPa). The concentration of hydrogen dissolved in the organic phase might be limiting at 1 MPa so that higher pressures are necessary to enhance the reaction rate.

Concluding remarks

The reaction should be performed at high temperature (200 °C) in order to achieve almost completion after 22 h, whereas at 150 °C the activity dropped drastically after 2.5 h. The increase in temperature might be responsible for the conversion of the succinic acid produced with water into the more active succinic anhydride. Finally, a higher TOF might also be achieved at higher pressure (such as 3 MPa).

5.2.1.3 Comparison of a homogeneous system and an immobilized one

Homogenous catalysts are usually difficult to remove from the reaction system. It was thus decided, as for the levulinic acid hydrogenation, to also test the interest in using an immobilized catalyst for the succinic anhydride hydrogenation in organic solvent. The immobilized complex $RuCl_2$(PS-PEG-adppp)$_2$, the synthesis of which was reported in Subsection 5.1.2.1, was therefore compared with the similar free complex $RuCl_2(PPh_3)_3$ (**9**), which was already tested for such a reaction in Subsection 5.2.1.1 (see Table 5-4). Even if the immobilization of the complex on the PS-PEG polymer was developed for the reaction in water, this immobilized complex can also be used for reactions in solvents, since the PS-PEG copolymer has been reported to swell in a lot of different solvents (water, methanol, ethanol, dichloromethane, toluene, dimethylformamide, acetonitrile, tetrahydrofurane and dimethylsulfoxide) (source: Rapp Polymere, Tübingen, Germany).

The reaction conditions were close to the ones reported in Subsection 5.2.1.1, but the ratios between the different components was changed, since a lower amount of the polymer catalyst was available and high concentrations of substrate were necessary for analysis:

Substrate	:	Metal	:	Acid
1600	:	1	:	16

The results of the hydrogenation of succinic anhydride with both the immobilized and free complexes are reported in Table 5-6 and Figure 5-9.

Table 5-6: Comparison of immobilized and free triphenyl phosphine ruthenium complexes for the hydrogenation of succinic anhydride (1.25 M) into GBL in tetraglyme (solvent), at 150 °C, 1 MPa, in acidic conditions (*p*-TsOH) (12.5 mM), with $RuCl_2$(PS-PEG-adppp)$_2$ or with $RuCl_2(PPh_3)_3$ (0.78 mM Ru).

N°	Catalyst	Additive	TOF, mol_{GBL} mol_{Ru}^{-1} h^{-1}	Y after 5.5 h, %	Final Y, %
22	[$RuCl_2$(PS-PEG-adppp)$_2$]	*p*-TsOH	16	4	16
23	**9**	*p*-TsOH	17	3	5

As seen in Table 5-6, the immobilized (entry 22) and the free (entry 23) catalysts gave similar TOFs. Even if the ratio between the substrate and the metal was changed with respect to the reaction presented in Table 5-4, the reported TOFs were simitar to the one reported in entry 14 (17 mol_{GBL} mol_{Ru}^{-1} h^{-1}, cf. Table 5-4). However, the obtained yields were lower, since the molar ratio of substrate to ruthenium was much higher here. While the TOFs in the first 3 h were relatively similar for the immobilized and the free catalysts (see Figure 5-9), the non-immobilized complex seemed to deactivate after 4 h of reaction (TOF of 2 ± 1 mol_{GBL} mol_{Ru}^{-1} h^{-1} from 4 to 27 h), while the immobilized one showed similar TOFs over the 20 h of reaction (13 ± 2 mol_{GBL} mol_{Ru}^{-1} h^{-1} from 1 to 20 h).

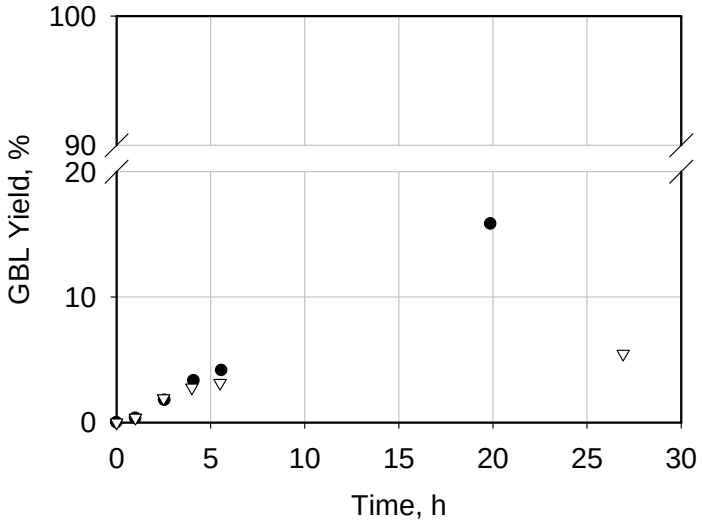


Figure 5-9 : Comparison of the immobilized and free triphenyl phosphine ruthenium complexes for the GBL yield of the hydrogenation of succinic anhydride (1.25 M) in tetraglyme (solvent), at 150 °C, 1 MPa, in acidic conditions (*p*-TsOH) (12.5 mM), with $RuCl_2$(PS-PEG-adppp)$_2$ ● or with $RuCl_2(PPh_3)_3$ ▽ (0.78 mM Ru).

Concluding remarks

The immobilization of the catalyst on the support stabilizes the catalyst and prevents its deactivation. Since high TOFs can be maintained for a longer time, the immobilized catalyst might be reused several times after filtration, hence lowering the catalyst costs.

5.2.2 Aqueous hydrogenation of succinic acid with homogenous metallic complexes

5.2.2.1 Transfer of the previous approaches to the aqueous phase

Since the feasibility of the hydrogenation with metallic complexes has been proven in organic solvent for succinic anhydride and in water for levulinic acid, a similar catalytic system was subsequently searched for the aqueous hydrogenation of succinic acid. As phosphines had been reported to be good candidates for the hydrogenation of levulinic acid in water and of succinic anhydride in organic solvent, the standard water-soluble phosphine ligand TPPMS was used with both $RuCl_3$ and $Ru(acac)_3$ as metal precursor, at different temperatures, pressures, pH and sometimes with NaI as additive. The experiments are summarized in Table 5-7.

Reaction conditions similar to the one reported for the aqueous hydrogenation of an aldehyde (Mahfud et al., 2007) (entries 24 to 31) were first tested. It was first supposed that the pH could have an impact on the hydrogenation, since the non-protonated form of carboxylic function of succinic acid could act as ligand on the metal center. However, even if the pH of the aqueous phase was varied from 2 to 7 (entries 24 to 26), no reaction occurred. The reaction was next performed in water instead of a phosphate buffer (entries 26 and 27) to limit the possible interaction of the phosphate ions with the complex, but still no reduction was observed. Different pressures (from 1 to 5 MPa) (entries 27 to 29) and temperatures (40 to 80 °C) (entries 27, 39, 31) were then tested, unfortunately without success.

According to the catalytic systems that Mehdi et al. (2008) tested for the reaction of levulinic acid in water, the metal precursor was replaced by $Ru(acac)_3$ (entry 32) but no reduction occurred either. Finally, the reaction conditions reported by Mehdi et al. (2008) that were successfully tested in Subchapter 5.1 for levulinic acid were applied here for succinic acid (entry 33), without enabling the reaction to take place.

Table 5-7: Hydrogenation of succinic acid in water with *in-situ* formation of the complex.

N°	Ru	L*	T, °C	P, MPa	pH	Aqueous phase	V*, ml	SA, mmol	Ru, mmol	L*, mmol	NaI, mmol	TOF mol_{GBL} mol_{Ru}^{-1} h^{-1}
24	$RuCl_3$	**5**	80	5	7	PB* 0.2 M	16	3.25	0.1461	0.2922	1.28	0
25	$RuCl_3$	**5**	80	5	5	PB* 0.2 M	16	3.25	0.1461	0.2922	1.28	0
26	$RuCl_3$	**5**	80	5	2	PB* 0.2 M	16	3.25	0.1461	0.2922	1.28	0
27	$RuCl_3$	**5**	80	5	~2.5	Water	32	3.25	0.1461	0.2922	1.28	0
28	$RuCl_3$	**5**	80	3	~2.5	Water	32	3.25	0.1461	0.2922	1.28	0
29	$RuCl_3$	**5**	80	1	~2	Water	32	3.25	0.1461	0.2922	1.28	0
30	$RuCl_3$	**5**	60	5	~2.5	Water	32	3.25	0.1461	0.2922	1.28	0
31	$RuCl_3$	**5**	40	5	~2.5	Water	32	3.25	0.1461	0.2922	1.28	0
32	$Ru(acac)_3$	**5**	80	5	~3	Water	32	3.25	0.1461	0.2922	1.28	0
33	$Ru(acac)_3$	**6**	140	5	~2.5	Water	40	8.8	0.044	0.44	-	0

* *L = ligand; V = total volume; PB = phosphate buffer*

Concluding remarks

Contrary to the hydrogenation of levulinic acid, which was catalyzed by a large spectrum of organometallic complexes, and especially ruthenium phosphine complexes, the hydrogenation of succinic acid could not be performed with such catalysts in similar reaction conditions. This change in reactivity can be first attributed to the presence of the more reactive ketone function in levulinic acid. The less polar carboxylic acid function might require an increase of the electron density on the metal center to enhance the nucleophilie of the intermediate hydride towards the carbonyl function. This was indeed suggested for ester function by van Engelen et al. (2003). Similarly, the linear phosphine ligands tested for the hydrogenation of succinic anhydride were more basic (i.e. were more electron donors to the metal) than the ligand tested here.

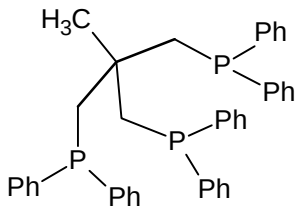
A second parameter might also be taken into account. Contrary to the hydrogenation of levulinic acid, the reduction of succinic acid is a dehydrative reaction and performing this reaction in water is therefore unfavourable, because of the thermodynamic equilibrium. To confirm this hypothesis, the hydrogenations of both succinic and

levulinic acid would have to be performed in a polar organic solvent where both substrates are soluble or in deuterated water. If a deuteration of succinic acid is observed in deuterated solvent, the complex is active toward the substrate but the reaction might not happen because of the large amount of water.

Finally, the reaction might only happen at high temperature, since much higher temperatures (~ 200 °C) were reported for the hydrogenation of succinic anhydride in solvent (see Subsection 5.2.1.2). It was thus decided to change the reaction conditions.

5.2.2.2 New approach at high temperature with Triphos ligands

Since the previous attempts for the hydrogenation of succinic acid in water remained unsuccessful, new reaction conditions were searched in the literature. A catalytic system consisting of $Ru(acac)_3$ and 1,1,1-Tris(diphenylphosphinomethyl)ethane (Triphos) ligand (ligand **13**) (see Figure 5-10) – a non-water-soluble tridentate ligand – has been reported by Wood et al. (2007 and 2009) for the hydrogenation in water of carboxylic acids and especially maleic acid and succinic acid at high temperature. In this patent, water was used as solvent for the hydrogenation of maleic acid, whereas a mixture of water and N-methyl-2-pyrrolidone (NMP) was used for the hydrogenation of succinic acid at 250 °C and 7 MPa.



13

Figure 5-10: 1,1,1-Tris(diphenylphosphinomethyl)ethane ligand or Triphos (**13**).

Tests were performed for the hydrogenation of succinic acid in both water and NMP, at 230 °C and 5.5 MPa (since no higher temperature and pressure were allowed in the autoclave BR-100, Berghof, Eningen, Germany). No kinetic curves could be recorded for this reaction, because the sampling at 230 °C was not possible without a loss of different compounds in the gas form. The final conversions and selectivities are presented in Table 5-8.

Contrary to what was observed at lower temperature (see Subsection 5.2.2.1), succinic acid could be reduced efficiently using $Ru(acac)_3$ with Triphos ligand as catalytic system at 230 °C and 5.5 MPa, both in water and in water / NMP mixtures, with conversions up to 99 % after 18 h. In Subsection 5.2.2.1, it was supposed that performing the reaction in

water should be thermodynamically unfavourable, since water is a product of the hydrogenation. Notwithstanding, Wood et al. (2007; 2009) supposed that the presence of water had a positive impact on the hydrogenation, because the CO from the decarbonylation of the products or intermediates could react with water to form carbon dioxide and hydrogen (water gas shift reaction), avoiding the poisoning of the catalyst by CO. This reaction is only promoted at high temperature so that the reaction has to be done at higher temperature. This hypothesis should be still investigated with a detailed analysis of the catalyst structure in presence or in absence of water.

Table 5-8: Hydrogenation of succinic acid at 230 °C and 5.5 MPa H_2 in 40 ml water or 28.6 ml water and 11.4 ml NMP with $Ru(acac)_3$ (2.17 mM) and Triphos ligands: conversion X, selectivity S.

N°	Solvent	SA*, M	Triphos : $Ru(acac)_3$ molar ratio	Reaction time, h	X, %	S (GBL), %	S (BDO), %	S (THF), %	∑ S, %
34	Water + NMP	0.8	13	18	99	37	27	14	78
35	Water	0.8	13	18	99	32	25	30	87
36	Water + NMP	0.8	1.2	18	99	32	33	23	88
37	Water	0.8	1.2	18	98	34	35	24	93
38	Water	0.8	1.2	6	90	48	32	2	82

* *SA = succinic acid*

The obtained selectivities were moderate both in water and in water / NMP mixtures. The two ligand concentrations tested did not have a high influence on the selectivities. Nevertheless, it should be noted that the selectivities changed over time. The selectivity of GBL was indeed higher after a shorter reaction time (48 % at 6 h (entry 37) *vs.* 34 % at 18 h (entry 38)), whereas the selectivity of THF increased greatly after 18 h of reaction (2 % at 6 h *vs.* 24 % at 18 h). The selectivity of BDO did not change much (35 % after 6 h *vs.* 32 % after 18 h). In the complex network of hydrogenation reaction (see Figure 5-11), GBL and BDO are hence formed at first and GBL is then reduced further into THF.

However, the selectivity of GBL obtained after 6 h was still not high enough for industrial applications.

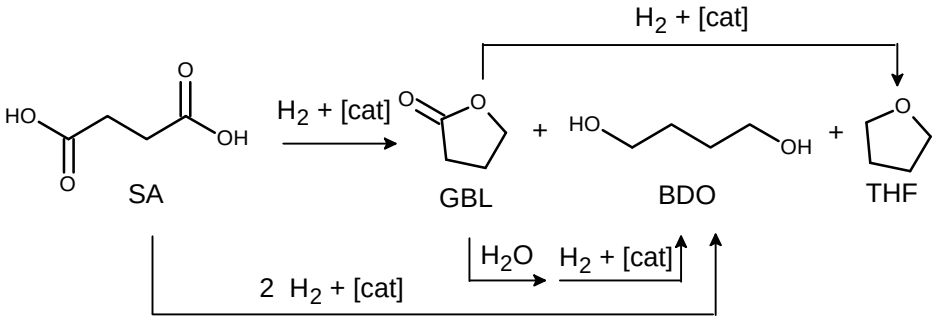


Figure 5-11: Hydrogenation of succinic acid (SA) into γ–butyrolactone (GBL), 1,4-butanediol (BDO) and tetrahydrofuran (THF).

The solvent of the reaction had a small impact on the THF selectivity, since the production of the latter was favoured in water (14 % in water / NMP (entry 34) *vs.* 30 % in water (entry 35)). This could be caused by either a ligand effect of NMP or a solvent or solubility effect. The two effects could not be differenciated here.

At the end of the 18 h of reaction, a yellow sticky precipitate was observed for entries 34 to 37. While analysing the solid by ^{31}P-NMR, only the ligand peak of Triphos could be observed, whereas the aqueous solution did not show any complex or ligand peak. It is thus difficult to determine if the reaction was catalyzed homogeneously or heterogeneously. If changes occur in the catalyst structure, it might lead to the formation of different catalytically active species, lowering significantly the selectivities. The formation of this solid happens probably after several hours of reaction, as less yellow particles could be observed for the reaction stopped after 6 h.

Concluding remarks

The non-water soluble catalytic system consisting of $Ru(acac)_3$ and Triphos ligands allowed the production of the reduced products of succinic acid in water. However, the process is still unselective even for a short reaction time (6 h). The reduction of succinic acid happens in a complex pathway of reactions and the selectivity is therefore difficult to control. It could be shown here that GBL and BDO are first produced but GBL is then further hydrogenated into THF. This complex network of reactions must still be further investigated by performing a kinetic study. This could not be performed here, as the sampling system applied did not allow the sampling at high temperature. A detailed

study of the nature of the catalyst (e.g. homogeneous *vs.* heterogeneous) should be performed to understand how the reaction takes place in the system. More effort must hence be invested in characterizing the reaction mechanism and finding selective systems for this reaction.

5.2.2.3 Conclusions

It has been shown that succinic anhydride could be easily reduced in organic solvent, using either $Ru(acac)_3$ and linear phosphine in acidic conditions or the 2nd generation Hoveyda-Grubbs carbene catalyst under basic conditions. Nonetheless, the transfer of this reaction in water using succinic acid as substrate was extremely challenging. Whereas the aqueous hydrogenation of levulinic acid could be performed successfully with different water-soluble phosphines, the aqueous hydrogenation of succinic acid at temperatures up to 140 °C remained unsuccessful. Only if the temperature was raised to 230 °C and $Ru(acac)_3$ and Triphos were used as catalytic system, succinic acid could be hydrogenized efficiently in water with up to 99 % conversion.

However, the selectivities toward GBL, BDO and THF were only moderate, because these three chemicals are produced in a complex network of reactions. Since an enhanced activity of ester hydrogenation with $Ru(acac)_3$ and Triphos has been reported with zinc (van Engelen et al., 2003), this metal might be added in order to reduce the ruthenium precursor and it might increase the reaction rate. Nevertheless, it is not certain that it will increase the selectivity. In another article describing the use of a similar catalytic system for the hydrogenation of itaconic acid (a dicarboxylic acid that has a similar structure as succinic acid), in dioxane at 195 °C and 10 MPa (Geilen et al., 2010), it could be shown that the selectivities toward the lactone, the diols and the cyclic ether could be modified by adding different acids or bases, or by changing the ligand and solvent. For example, the selectivity of the cyclic ether could be highly increased by adding both *para*-toluenesulfonic acid (*p*-TsOH) and ammonium hexafluorophosphate (NH_4PF_6), whereas using 1,4-bis(diphenylphosphino) butane (dppb) ligand and THF as solvent favoured the production of the diol. Different additives and ligands should be screened for the aqueous hydrogenation of succinic acid. But more importantly, the reaction mechanisms of the complex network of hydrogenations must be better understood and characterized by kinetic analyses.

Since the aqueous hydrogenation of succinic acid with metallic complexes remained unselective, heterogeneous catalysts were also tested for the sake of comparison.

5.2.3 Aqueous hydrogenation of succinic acid with metal supported clay catalysts

As the metal supported catalysts have been applied successfully for the aqueous hydrogenation of succinic acid (Delhomme et al., 2009), this approach was also tested in this study and other supports were searched for the immobilization of metal clusters. Among the possible supports, a silicate-based clay – Montmorillonite – was selected here because of its good swelling in water and its biocompatibility. It presents strong Lewis acid sites and possesses a combination of cation exchange, intercalation and swelling properties which make it unique (Pinnavaia, 1983). Metal supported Montmorillonite clays have been reported as catalysts for different hydrogenations in organic solvents (Albertazzi et al., 2005; Manikandan et al., 2008; Marín-Astorga et al., 2005) and for other types of reactions in water (e.g. Sc^{3+} pillared clay for Michael reaction of 1,3-dicarbonyls (Kawabata et al., 2003), Sc^{3+} or Cu^{2+} pillared clay carbon-carbon forming reaction (Kawabata et al., 2005)).

Ruthenium or iridium immobilized Montmorillonite clays were synthesized and these catalysts were used for the hydrogenation of succinic acid in water. Whereas the iridium MTM-K10 clay catalyst gave no conversion of succinic acid, the ruthenium one led to the formation of GBL and other side-products. The impacts of the support and of the temperature were hence further investigated with the ruthenium immobilized Montmorillonite.

5.2.3.1 Impact of the support on the GBL yield

Following the protocol derived from Kawabata et al. (2003) and described in Subsection 4.1.1.1, three different ruthenium immobilized clays were synthesized using the following Montmorillonite supports: K-10, KSF and Aluminium pillared MTM. The hydrogenation of succinic acid in water at 180 °C and 5.5 MPa was realized with these three catalysts. The results are presented in Table 5-9 and Figure 5-12.

The ruthenium immobilized KSF Montmorillonite gave a slow conversion of the succinic acid into GBL (i.e. TOF of 0.9 $mmol_{GBL}\ g_{cat}^{-1}\ h^{-1}$), whereas the ruthenium K-10 and Al pillared clays gave similar turnover frequencies (10.1 and 9.1 $mmol_{GBL}\ g_{cat}^{-1}\ h^{-1}$ respectively). While synthesizing the ruthenium clay catalyst, it could be observed that the initially black solutions of $RuCl_3$, in which the beige K-10 and the Al pillared MTM clays were introduced, discoloured to grey after 24 h and black clays were obtained. As for the KSF clay, the metal solution remained black and the obtained clay was grey,

probably due to a low metal loading onto the KSF clay surface. This might be then responsible for the 10 times lower TOF of the KSF immobilized catalyst with respect to the other two supports. In order to confirm this hypothesis, the formed clays should still be analysed by elemental analysis to determine their ruthenium content.

Table 5-9: Impact of the Montmorillonite support (K-10, Aluminium pillared or KSF) of the Ruthenium pillared catalyst (2.5 g l^{-1}) on the hydrogenation of succinic acid (0.66 M) in water (40 ml), at 180 °C and 5.5 MPa: Turnover Frequency (TOF), yield (Y) and selectivity (S).

N°	Support	TOF, $mmol_{GBL}$ g_{cat}^{-1} h^{-1}	Y after 5 h, %	S after 5 h, %	Y after ~ 22 h, %	S after ~ 22 h, %
39	MTM K-10	10.1	15	51	6	7
40	MTM Al pillared	9.7	16	52	6	7
41	MTM KSF	0.9	2	30	4	11

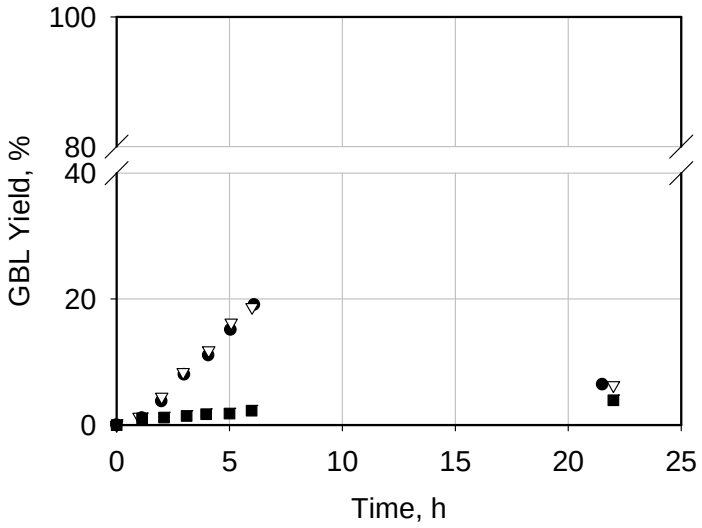


Figure 5-12: Impact of the Montmorillonite support (K-10 ●, Al pillared ▽ or KSF ■) of the Ruthenium pillared catalyst (2.5 g l^{-1}) on the GBL yield of the hydrogenation of succinic acid (0.66 M) in water (40 ml), at 180 °C and 5.5 MPa.

Nevertheless, even for the best two catalysts, the yield of GBL remained low (under 20 %). As shown in Figure 5-12, the yields of GBL increased linearly during the first 6 h of reaction with the K-10 and Al pillared clays but decreased sharply overnight. This might be due to further hydrogenations of GBL, leading to the low selectivities but high conversions (88 and 92 % for K-10 and Al pillared MTM resp.) obtained at the end of the reaction (entries 39 and 40). The production of propionic and butanoic acid could be

observed simultaneously to the production of GBL with gas chromatography analysis coupled with mass spectroscopy. Decarbonylation reactions of the substrate might be responsible for the by-product formation.

Concluding remarks

Only K-10 and Al pillared allowed a high loading of ruthenium and could therefore hydrogenate in water. Nevertheless, relative low selectivities were recorded, because of probable decarbonylation reactions. The impact of the temperature on the selectivity was then tested with ruthenium Al pillared clay, with the aim of obtaining better selectivities.

5.2.3.2 Impact of the temperature on the GBL yield

Different temperatures ranging from 140 °C to 180 °C were tested for the aqueous hydrogenation of succinic acid with the ruthenium Al pillared MTM clay, in order to determine the impact of temperature on the turnover frequency and the selectivity. The resultats are summarized in Table 5-10 and Figure 5-13.

As expected, the reactions performed at higher temperatures gave higher TOFs (up to 9.7 $mmol_{GBL}$ g_{cat}^{-1} h^{-1} at 180 °C), following the effect represented by the Arrhenius' law. The highest yields after 5 h was hence recorded at 180 °C (16 %), whereas the selectivities were relatively constant (~ 51 - 52 %).

Table 5-10: Impact of the temperature on the hydrogenation of succinic acid (0.66 M) in water (40 ml), at 180 °C and 5.5 MPa with ruthenium pillared MTM (Aluminium pillared support) (2.5 g l^{-1}): Turnover Frequency (TOF), GBL yield (Y) and selectivity (S).

N°	Temperature, °C	TOF, $mmol_{GBL}$ g_{cat}^{-1} h^{-1}	Y after 5 h, %	S after 5 h, %	Y after ~ 22 h, %	S after ~ 22 h, %
42	140	2.2	4	N.D.*	22	47
43	160	5.5	10	51	19	27
40	180	9.7	16	52	6	7

*: N.D. = not determined

However, the yields and selectivities changed drastically overnight, so that the lowest yield (6 %) and selectivity (7 %) were finally obtained after ~ 22 h at the highest temperature (180 °C, entry 40). Only at the lower temperature, the TOF was maintained, leading to the highest yield (22 %) and selectivity (47 %) after ~ 22 h. The

drop in yields and selectivities observed at 160 °C and 180 °C are most probably due to the further hydrogenation of GBL, which is certainly promoted at higher temperature.

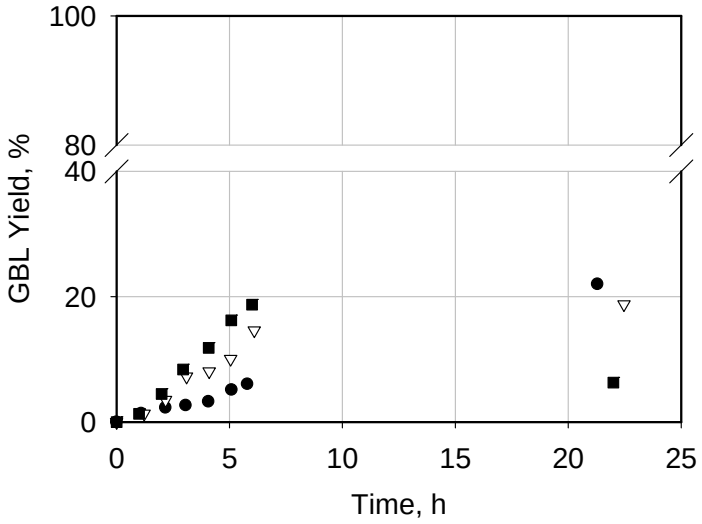


Figure 5-13: Impact of the temperature on the GBL yield of the hydrogenation of succinic acid (0.66 M) in water (40 ml), at 180 °C and 5.5 MPa H_2 with ruthenium pillared MTM (Aluminium pillared support) (2.5 g l^{-1}): 140 °C ●, 160 °C ▽ or 180 °C ■

Concluding remarks

The temperature cannot be used to control the selectivity of the reaction, since it has obviously a similar impact on the formation of GBL than on the side reactions. The increase of the temperature hence increased the TOF but lowered the selectivities at the end of the reaction.

5.2.3.3 Conclusion

The ruthenium immobilized clay catalysts have thus proved to hydrogenate succinic acid into GBL in aqueous solutions. However, the reaction was again unselective (max. 52 %), as decarbonylation reactions might happen simultaneously. Similarly to what has been mentioned in the literature for the metal supported catalysts (see Section 3.3.1), diverse metals must probably be immobilized simultaneously in order to control the selectivity. This kind of multiple-metal immobilization into MTM clays has been published among others by Albertazzi et al. (2005). This strategy might be applied here to achieve higher selectivities. Furthermore, it has been reported that metallic complexes or ligands could be absorbed, exchanged or anchored into MTM clays (Aldea and Alper, 1998; Breu et al., 2001; Choudary et al., 1985; Margalef-Català et al., 1999; Yan et al., 1992). The

ruthenium complex with Triphos described in Subsection 5.2.2.2 for the aqueous hydrogenation of succinic acid at high temperature might for instance be immobilized onto MTM in order to perform the reaction heterogeneously.

5.3 Hydrogenation of succinate esters in solvents

Since succinic acid could not be reduced selectively in water with the catalysts tested so far in this study, another approach was finally investigated. The production of succinic acid reduced derivatives was considered using succinate esters as starting material and performing the hydrogenation in solvent.

5.3.1 Hydrogenation of dimethyl succinate in solvents

Esters are often used as substrate for hydrogenations instead of the corresponding carboxylic acid because of their higher reactivity and higher solubility in organic solvents. Succinic acid could be first esterified in the fermentation broth and the recovered esters could be then used as starting material for the production of BDO, GBL and THF, as shown in Figure 5-14. Since succinate esters are also interesting derivatives of succinic acid, their production would be anyway of great interest.

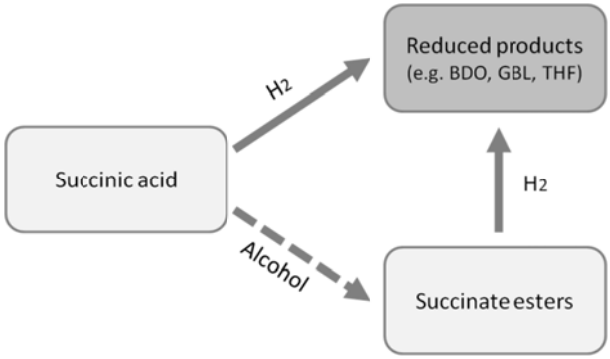


Figure 5-14: Routes to the reduced derivatives of succinic acid: → corresponds to hydrogenation reactions and ⇢ to esterification ones.

Only few articles reported the hydrogenation of carboxylic acid esters with metallic complexes as indicated in Section 3.3.4. Furthermore, the esters tested in the literature had often electron withdrawing groups, aryl groups or other aromatic ones, whereas succinate alkyl esters are non-activated esters. For this study, the catalytic system reported by Teunissen and Elsevier (1997; 1998), van Engelen et al. (2003) and Rosi et al. (2010), consisting of $Ru(acac)_3$ and 1,1,1-Tris(diphenylphosphinomethyl)ethane

(Triphos) ligands (ligand **13**), was eventually tested. It is the same catalytic system as the one mentioned by Wood et al. (2007 and 2009) and tested in Subchapter 5.2.2.2 for the aqueous hydrogenation of succinic acid at high temperature. Here, dimethyl succinate esters were used as substrate and were hydrogenized in organic solvent at 120 °C and 5.5 MPa. The reaction path was described by Rosi et al. (2010) and is presented in Figure 5-15.

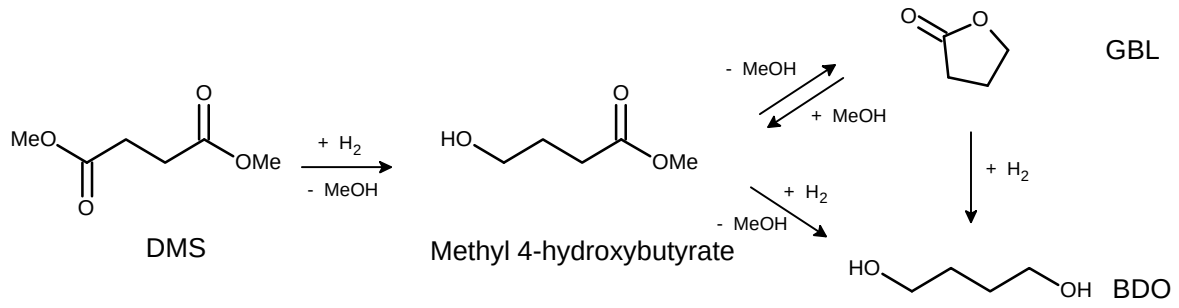


Figure 5-15: Hydrogenation of dimethylsuccinate (DMS) in methanol with the formation of methyl 4-hydroxybutyrate, γ-butyrolactone (GBL) and 1,4-butanediol (BDO) (Rosi et al., 2010).

In this study, the impact of the solvents, the ligand concentration and the additive concentration on the turnover frequency of the production of GBL and BDO was investigated.

5.3.1.1 Impact of the solvents

Four different solvents were tested for the hydrogenation of DMS with $Ru(acac)_3$ and Triphos ligands: the polar protic solvent methanol, the polar aprotic solvents dimethylformamide (DMF) and tetrahydrofuran (THF) and the non-polar solvent toluene. The tested solvents were degazed before use. Even though fluorinated alcohols have been reported to enhance dramatically the turnover frequencies (Teunissen and Elsevier, 1998), the hydrogenation of succinate esters was not tested in these solvents, because they are extremely expensive and therefore not suitable for industrial applications. The turnover frequencies for GBL and BDO recorded in the different solvents are reported in Figure 5-16.

As clearly seen in Figure 5-16, significant TOFs could only be found in methanol. Nonetheless, GBL and BDO were produced simultaneously, hence limiting the selectivity. All the other solvents gave either no production of GBL and BDO (for toluene and THF) or extremely little amounts of the two products (in DMF).

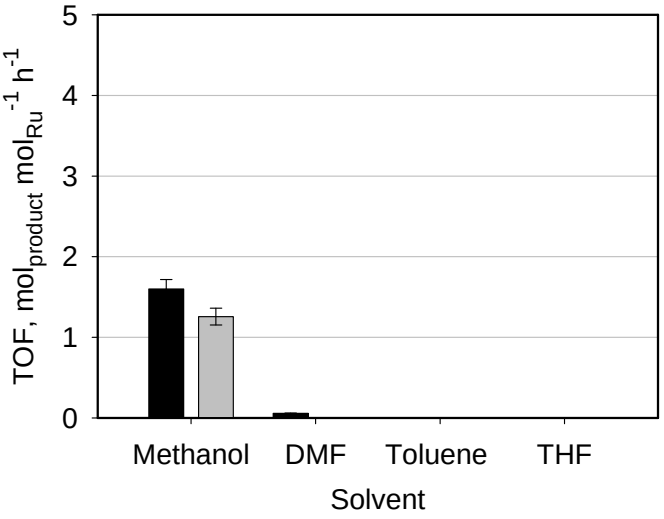


Figure 5-16: Impact of the solvent on the Turnover Frequencies of GBL ■ and BDO □ for the hydrogenation of DMS with $Ru(acac)_3$ (1.1 mM) and Triphos (1.55 mM) in 40 ml of solvent with 2.2 mM zinc as additive, 120 °C, 5.5 MPa.

Even though toluene has been reported to be a good solvent for the hydrogenation of activated carboxylic esters with ruthenium complexes (Grey et al., 1981), no formation of reduced products could be observed for the catalytic system consisting of $Ru(acac)_3$ and Triphos. For the same catalytic system, Rosi et al. (2010) reported the importance of the alcohol in the reaction mechanism. They indeed supposed that the $Ru(Triphos)(acac)_2$ complex reacted with hydrogen to give an hydride, which was subsequently interacting with the alcohol in order to form a solvento species (see Figure 5-17). Finally, the solvento species reacted with the substrate by the addition of the Ru-H moiety to the CO group. They hence suggested that the absence of alcohol as solvent might make this complex formation more difficult and lower its activity. This could explain why no or little activity could be observed with the other solvents tested. Nevertheless, Rosi et al. (2010) reported the hydrogenation of fumaric acid into GBL using THF as solvent. However, they observed that no BDO was produced in THF, whereas both GBL and BDO were produced in methanol. In this study, though, neither GBL nor BDO was produced in THF.

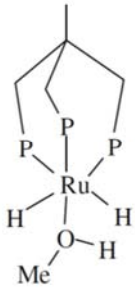


Figure 5-17: Complex prepared from $Ru(acac)_3$ and Triphos ligand in methanol with hydrogen and zinc. Complex reported by Rosi et al. (2010) for the hydrogenation of the esters.

Concluding remarks

Methanol seems to be the only solvent tested here that is suitable for the hydrogenation of succinate esters, whereas the fluorinated alcohols were considered as too expensive for industrial applications. Furthermore, since the production of the DMS from succinic acid would have to be performed with the substrate methanol, using the latter as solvent for the hydrogenation presents the advantage of using the same solvent for both the esterification and the subsequent hydrogenation of the DMS.

5.3.1.2 Impact of the ligand concentration

The ligand concentration might also be of great importance, since it might change the conformation of the complex or change the acidity / basicity of the reaction system. Ligand concentrations from 1.1 to 5.5 mM were hence tested and the impact on the GBL and BDO turnover frequencies is shown in Figure 5-18.

The increase of the ligand concentration lowered both the GBL and BDO's TOFs as clearly shown in Figure 5-18. The lowest concentration corresponds to a molar ratio of Ruthenium to ligand of 1:1. The complex reported by Rosi et al. (2010) and shown in Figure 5-17, presents indeed one mole Triphos ligand per mole ruthenium.

An excess of phosphine might have a detrimental effect on the hydrogenation. The excess of phosphine has been reported by Hara et al. (2000) to be detrimental to the production of GBL from succinic anhydride in solvent since side products were produced instead of GBL.

However, it should be noted, that the ratio of the TOFs of GBL to BDO increased from 1.6 to 5.2 when increasing the ligand concentrations from 1.1 to 5.5. The ligand excess might hence slightly increase the selectivity toward GBL.

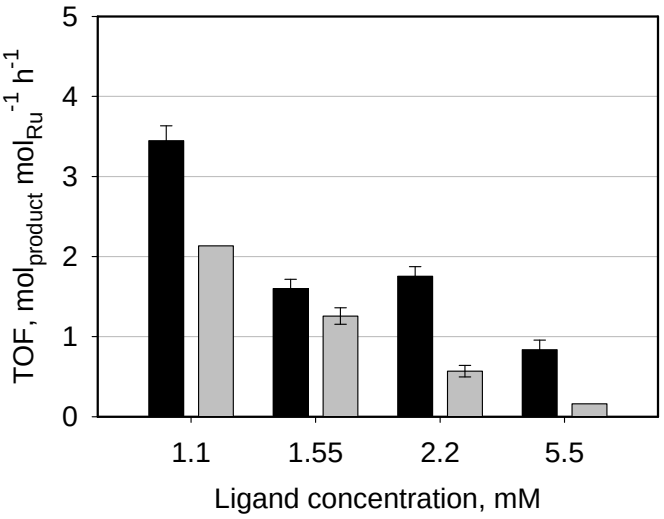


Figure 5-18: Impact of the ligand concentration on the Turnover Frequencies of GBL ■ and BDO □ for the hydrogenation of DMS with $Ru(acac)_3$ (1.1 mM) and different concentrations of Triphos ligand (1.1, 1.55, 2.2 or 5.5 mM) in 40 ml of methanol with 2.2 mM zinc as additive, 120 °C, 5.5 MPa.

Concluding remarks

A molar ratio of ruthenium to ligand of 1:1 is optimal for the hydrogenation of DMS in methanol. An excess of phosphine led indeed to the reduction of the turnover frequencies of GBL and BDO, but seemed to increase the selectivity toward GBL. However, the two products were still produced simultaneously.

5.3.1.3 Impact of zinc concentration

Finally, the impact of the zinc concentration was tested. The addition of zinc was indeed accounted for the reduction of ruthenium (van Engelen et al., 2003), thus enhancing the catalytic activity. The influence of the zinc concentration on the Turnover Frequencies of GBL and BDO was tested and is presented in Figure 5-19.

The increase of the zinc concentration by 25 times enhanced the TOF of GBL by a factor of 2.7 and TOF of BDO by 2.6. Zinc has indeed been reported to promote the fast reduction of the $Ru(acac)_3$ complex. The formed Zn^{II} can then act as Lewis acid and activate the ester carbonyl function by coordinating to it (van Engelen et al., 2003).

However, the ratio of the TOFs of GBL to BDO were not improved by increasing the zinc concentration (ratio of 1.2 to 1.3), so that both products were still produced simultaneously. Lastly, no inhibition by zinc could be observed.

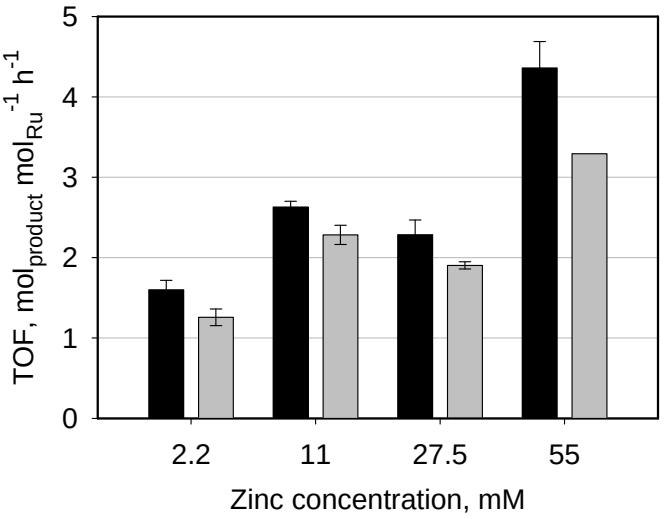


Figure 5-19: Impact of the zinc concentration on the Turnover Frequencies of GBL ■ and BDO □ for the hydrogenation of DMS with $Ru(acac)_3$ (1.1 mM) and Triphos (1.55 mM) in 40 ml of methanol with 2.2, 11, 27.5 or 55 mM zinc as additive, 120 °C, 5.5 MPa.

Concluding remarks

An increased concentration of zinc enhanced the production of both GBL and BDO similarly, preventing an increase of the process selectivity. Since zinc is a cheap additive, the highest concentration of zinc (55 mM) should be used, but more effort must still be done to control the selectivity of the process.

5.3.2 Conclusions

The hydrogenation of dimethyl succinate could be performed successfully by the catalytic system consisting of $Ru(acac)_3$ and Triphos ligands. However, as reported by Rosi et al. (2010), GBL and BDO were simultaneously produced. This considerably lowers the process selectivity. A ratio of metal to ligand of 1 and the increase of the zinc concentration allowed the turnover frequencies to be enhanced. The ratio of the TOFs could only be slightly increased at high ligand concentration, at the expense of the rates.

More efforts must be invested in developing the hydrogenation of succinate esters in organic solvents, before reaching the desired selectivities for industrial applications. The impact of other additives such as acids, bases, copper or titanium isopropoxide, might be also tested. Changing the solvent to propan-2-ol or the fluorinated alcohol FIPA might also increase the TOF. However, if the reaction had to be performed in the fluorinated alcohol, its recycling would be of major importance since it is extremely expensive.

5.4 Concluding remarks on hydrogenation

During the first experimental part of this project, dedicated to the aqueous hydrogenation of succinic acid, three series of preliminary tests provided fruitful results. First, the hydrogenation of a slightly different carboxylic acid, levulinic acid, was performed successfully with a variety of water-soluble phosphines and $Ru(acac)_3$ as metal precursor at 140 °C and 5 MPa. The ligand was even immobilized on PS-PEG copolymer for the aqueous hydrogenation of levulinic acid. Second, succinic anhydride – the dehydrated form of succinic acid – was easily reduced with $Ru(acac)_3$ and linear phosphines under acidic conditions. For the first time, it could then be shown that the 2nd generation Hoveyda-Grubbs ruthenium carbene catalyst efficiently reduced succinic anhydride under basic conditions in tetraglyme solvent at 180 °C and 1 MPa, with similar TOFs as the one obtained with the catalytic system developed by Hara et al. (2000). This approach must definitively be further investigated, as the air-stable carbene complex can be easily immobilized and could be of great interest for industrial applications, contrary to the less stable phosphine complexes. Lastly, it could be shown that the PS-PEG immobilized complex of triphenyl phosphine ligand was active for the hydrogenation of succinic anhydride in tetraglyme at 180 °C and 1 MPa. Furthermore the immobilized catalyst maintained its activity for 22 h, whereas the free catalyst underwent deactivation, underlining the necessity to immobilize the catalyst for an easy recovery and an increased stability.

For the hydrogenation of succinic acid in water, however, the reduction was quite difficult. The hydrogenation of a dicarboxylic acid remains a difficult field since the selectivity of such reactions is extremely difficult to control due to over-hydrogenations and decarbonylation reactions. Furthermore, the presence of water can deactivate the catalytic system or lead to other side reactions.

The homogeneous catalytic systems consisting of ruthenium and a water-soluble phosphine were not successful for the aqueous hydrogenation of succinic acid, at temperatures up to 140 °C and pressures up 5 MPa in aqueous solution from pH 2 to 7. A strategy at a higher temperature (230 °C) was derived from Wood et al. (2007; 2009). The catalytic system consisting of $Ru(acac)_3$ and Triphos ligand successfully reduced succinic acid up to 99 % conversion but it was relatively unselective, producing GBL and BDO at first and reducing then GBL into THF. The heterogeneous alternative consisting of ruthenium catalysts immobilized on clay could also reduce succinic acid in water, but GBL and BDO were also produced simultaneously.

Since the aqueous hydrogenation of succinic acid could not be performed selectively, the hydrogenation of succinic acid esters was also investigated in organic solvents using $Ru(acac)_3$ and Triphos as catalytic system. Even if the TOFs could be increased while changing the solvent, the ligand and zinc concentrations, GBL and BDO were still simultaneously produced, reducing the process selectivity. The alternatives tested here are therefore not yet suitable for industrial applications. More selective strategies should be developed and different additives, solvents and ligands should be screened for such reactions. Complexes with different metals might be simultaneously used for the hydrogenation of succinic acid in water, since metal supported catalysts have been reported to give low selectivities with only one metal but good selectivities with multiple-metal immobilization. This strategy has been described by Behr and Brehme (2002) for the hydrogenation of carboxylic acids and lactones for the production of alcohols and diols in dioxane at temperatures up to 200 °C and pressures up to 15 MPa. Finally, temperature stable catalysts must be developed, since the aqueous hydrogenation of succinic acid could only be performed at high temperature (above 180 °C) and pressure (above 5 MPa) in order to reach high activities.

In conclusion, only when such new catalytic systems are made available, may the production of reduced derivatives of succinic acid by hydrogenation in a fermentation broth become an efficient and selective industrial process.

6 Esterification

Apart from the reduced derivatives of succinic acid, its esters are also interesting chemicals that have a broad range of applications. That is why the esterification of succinic acid from fermentation broth was studied in the second part of this project. As indicated in Subchapter 3.4, the esterification of succinic acid in aqueous solutions has not been widely developed so far. Catalysts have only been published for the esterification with small alcohols such as ethanol or butanol (Bauduin et al., 2009; Benedict et al., 2006; Budarin et al., 2007a; Budarin et al., 2007b; Budarin et al., 2007c). A wider range of catalysts and new process approaches had hence to be tested, to derive suitable process options for the esterification of succinic acid from fermentation broth.

The work presented in this Chapter can be divided into 3 steps. In the first step described in Subchapter 6.1, different catalysts were screened for the esterification of solutions of succinic acid in distilled water. The best catalysts were then further investigated in the second step, first, by applying a "one-at-a-time" approach (see Subchapter 6.2) and, then, by using a 3-variable optimization (see Subchapter 6.3). From these optimization strategies, optimal reaction conditions were selected. In the third step, the esterification of succinic acid was tested in real fermentation broths with the best catalyst in the best reaction conditions, and finally, different options for the use of the formed esters will be discussed. These process integration aspects will be presented in Subchapter 6.4.

6.1 Screening of catalysts

A wide range of both chemical and enzymatic catalysts were studied for the esterification of succinic acid in aqueous media, in order to determine the most suitable ones. The screening of the chemical catalysts is presented in the Section 6.1.1 and the different enzymes are introduced in the Section 6.1.2.

6.1.1 Screening of chemical catalysts

As presented in Section 3.4.3, different chemical catalysts have been mentioned in the literature for esterifications in pure solvents, in aqueous media or in biphasic systems. From those reported in Table 3-7 and Table 3-8, fifteen catalysts were first selected here for the esterification of succinic acid in monophasic conditions and the five best performers were then screened in biphasic conditions. Most of the fifteen catalysts

screened were heterogeneous ones, since they are easily recovered and thus often more suitable for industrial applications. Iodine (I_2) and 4-dodecylbenzenesulfonic acid (DBSA) were the only two homogeneous catalysts tested in the present study.

6.1.1.1 Monophasic esterification of succinic acid with chemical catalysts

The esterification of succinic acid using chemical catalysts was first examined with ethanol. Fifteen catalysts were screened for this reaction in a mixture of alcohol and water with different water contents (0, 25, 50 and 75 % v/v). The first half of the screened catalysts was selected among those, which have been reported for esterifications in pure organic solvents (see Table 3-7) and consists in: Amberlyst (Amb) resins 15, 16, 36 and 131, Montmorillonite clays (MTM) with Ag^+, Co^{2+},Ru^{3+}, Al^{3+}, H^+ as immobilized cations and Nafion® SAC-13. The second half was chosen from the catalysts that have been published for esterifications in presence of water (see Table 3-8) and comprises iodine (I_2), 4-dodecylbenzenesulfonic acid (DBSA), Nafion® NR-50, and two polymers with immobilized sulfonic acid functions (polystyrene sulfonic acid (PS-Sulf. Ac.) and ScavengePore® Benzenesulfonic acid H^+ form (Scav. Pore)). The reaction conditions used were derived from the literature: 80 °C, 0.4 M succinic acid, 1000 rpm. The pH of the reaction solution was not set. The results are presented in Figure 6-1.

Catalysts were first tested in pure ethanol (0 % v/v water). First, Montmorillonite clays (MTM) gave limited diethyl succinate (DES) yields after 24 h (yields from 4.8 to 53.0 %). Yet the ions exchanged into the clays seemed to have an impact on the activity: H^+ and Al^{3+} pillared MTM gave the best yields (53.0 and 47.3 % resp.) in pure ethanol, whereas Ag^+ and Co^{2+} showed little activity (yields ≤ 7 %). Second, Amberlyst resins allowed relatively high DES yields in pure ethanol (up to 81.0 % for Amberlyst 15). Last, the catalysts reported in the literature for aqueous esterifications (I_2, DBSA, Nafion NR-50, PS-Sulf. Ac. and Scav. Pore) and Nafion SAC-13 gave the best final yields of DES after 24 h in pure ethanol (yields ≥ 85 %). Among them, the surfactant DBSA and Nafion SAC-13 allowed the esterification to go almost to completion in pure ethanol after 24 h (DES yields of 99.8 % and 100 % respectively), making them the most suitable catalysts for the water-free esterification of succinic acid.

The introduction of water in the reaction solutions drastically decreased the final yields of DES for all catalysts. Even with only 25 % v/v of water, yields were often reduced by more than 50 %. If the reaction solutions contained 75 % v/v of water, yields did not exceed 15.1 %. For example, Montmorillonite catalysts were dramatically affected by water (yields ≤ 6.3 % with 25 % v/v of water). As for the Amberlysts resins, only

Amberlyst 131 and Amberlyst 16 maintained yields above 30 % for reaction media containing 25 % v/v of water (yields of 39.7 and 31.9 % resp.), while for the other Amberlyst catalysts (15 and 36), the losses in yield exceeded 50 % (i.e. yields ≤ 24.5 %). From the catalysts that gave high yields in pure ethanol, some were relatively strongly affected by the introduction of water: e.g. Nafion SAC-13 and I_2 showed a large loss in DES yield when introducing water in the system (DES yield of resp. 24.5 or 24.8 % for reaction solutions with 25 % v/v of water). Finally, the best catalysts for aqueous reactions were DBSA, Nafion NR-50 and PS-sulfonic acid with DES yields of 54.6, 52.7 and 55.4 % respectively, in aqueous solutions with 25 % v/v of water.

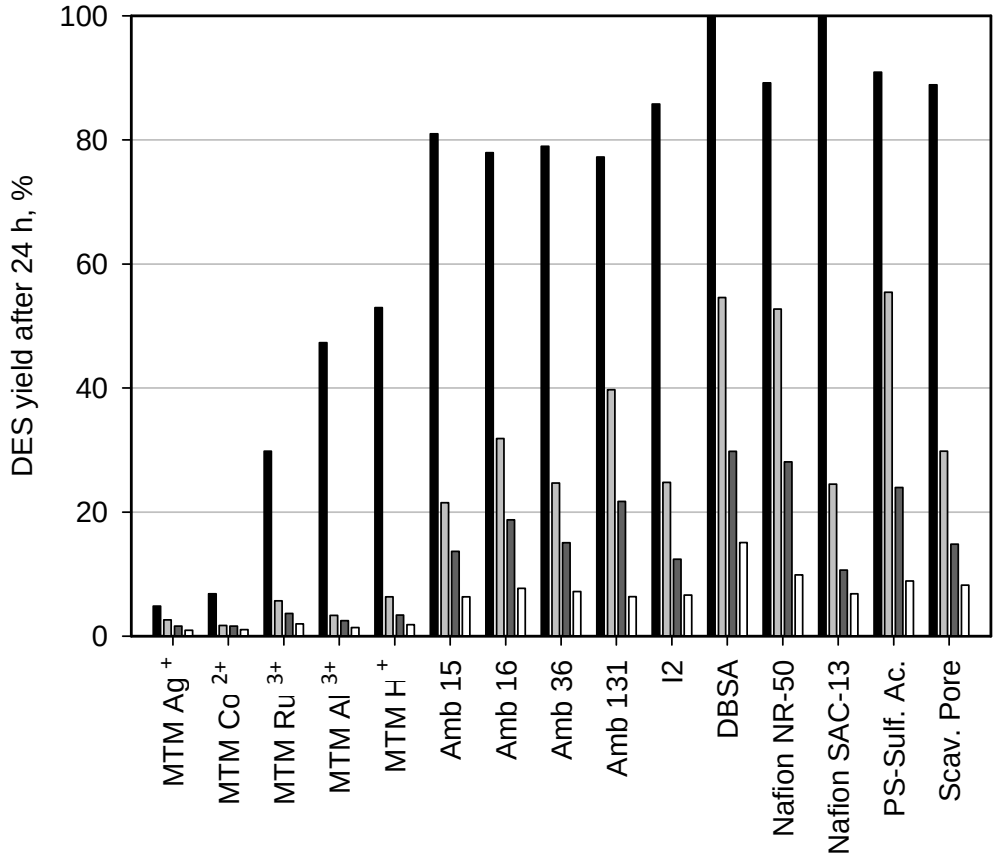


Figure 6-1: Screening of chemical catalysts for the esterification of succinic acid with ethanol in monophasic system: diethyl succinate (DES) yields after 24 h in solvents with different water/ethanol volumetric ratios (0 % v/v ■ , 25 % v/v ▭ , 50 % v/v ▭ , 75 % v/v ▭ of water). Reaction conditions: 80 °C, 0.4 M succinic acid, 10 mol % DBSA, 5 mol % I_2 or 0.5 g solid catalyst, 10 ml of water/ethanol mixture, 1000 rpm.

The aforementioned dramatic decrease in yields could be explained by the potential hydrolysis of the esters in the presence of water. The esterification reaction is indeed a reversible dehydrative reaction, the back reaction of which is the hydrolysis of the ester. In the presence of water, the formed ester could hence be directly hydrolysed back to

succinic acid or to the monoethyl succinate. Furthermore, competitive protonation steps between the alcohol and the water molecules might also be responsible for the inhibitory effect of water (Liu et al., 2006a).

For the heterogeneous catalysts, the high losses might also be due to the ability of certain catalysts to absorb high quantities of water in their pores, thus increasing local water concentrations and thereby shifting the equilibrium towards the hydrolysis of the esters. For example, regarding MTM, the high capacity of this clay to swell in water has been reported by Ravina and Low (1972). Amberlyst 15 has also been reported to strongly absorb water (Darge and Thyrion, 2007). Very recently, after this study was completed, a new more water-tolerant resin – Amberlyst BD-20 – has been studied by Park et al. (2010) and might be more suitable for such applications. Lastly, Nafion SAC-13, which gave yields up to 100 % in pure alcohol but only low yields in aqueous ethanol, is supported on a porous silica matrix that is hydrophilic due to its –OH groups. To conclude, catalysts should be engineered in order to get pores with an optimized hydrophilicity / hydrophobicity balance, in order to limit high water concentrations in the pores and thus avoid the hydrolysis of the esters (Budarin et al., 2007c).

Finally, it was surprising that, even though I_2, DBSA, Nafion NR-50, PS-Sulf. Ac. and Scav. Pore were reported in the literature as catalyzing esterifications in aqueous solutions or as being tolerant to water (see Table 3-8), not all of them gave good yields in the presence of water. Regarding iodine, although Ramalinga et al. (2002) claimed that the esterification of lactic acid with butanol using iodine was tolerant to 12 % v/v water, the catalysts might react with water, becoming a Brønsted acid as shown in equation (6-1) and leading to another reactivity.

$$I_{2\,liq} + H_2O \rightleftharpoons OI^-_{aq} + 2H^+_{aq} + I^-_{aq} \qquad (6\text{-}1)$$

As for the other aforementioned catalysts, strategies presented in the literature for reactions in the presence of water limit the contact of the esters and the catalysts with the aqueous phase, c.f. biphasic systems for DBSA and polystyrene supported sulfonic acid (Manabe et al., 2002), pervaporation for Nafion NR-50 (Benedict et al., 2006) and reactive distillation with many other catalysts (Bauduin et al., 2009; Fujita et al., 2004; Fujita et al., 2004; Saha et al., 2000). When these strategies are not used to limit the hydrolysis of the esters, those catalysts seem to be unsuitable for the esterification of aqueous solution of succinic acid.

Concluding remarks

The esterification of succinic acid in pure ethanol could be performed easily with a broad range of catalysts. DBSA and Nafion SAC-13 were the best catalysts with DES yields of resp. 99.8 and 100 % in pure ethanol. When water was introduced into the system, yields dropped drastically because of the reverse hydrolysis of the esters and of competitive protonation steps between the water and the alcohol. The best catalysts found for the aqueous esterification of succinic acid with ethanol were: DBSA, Nafion NR-50, PS-Sulfonic acid and Amberlyst 131 (yields up to 56 % for solutions with 25 % v/v of water). Nevertheless, the monophasic strategy seems limited for the aqueous esterification of succinic acid with the chemical catalysts tested here.

Conversely, a two-phase reaction could be well-suited for the esterification of succinic acid as the less polar esters would be directly extracted in the organic phase, hence limiting their hydrolysis. In addition, the extraction of the esters in the organic phase would simplify their purification. Besides, there is a broad range of interesting esters that could be produced from non-water-miscible alcohols (see Table 3-3). These alcohols could hence be used as second phase. As for the small alcohols (methanol or ethanol), a co-solvent might be introduced as second phase. As several options have already been published for the esterification of succinic acid with small alcohols (Bauduin et al., 2009; Budarin et al., 2007a; Budarin et al., 2007b, Budarin et al., 2007c), this work will concentrate on the use of longer chain alcohols for the two-phase esterification of aqueous solutions of succinic acid.

6.1.1.2 Biphasic esterification of succinic acid with chemical catalysts

As mentioned before, a two-phase strategy for the esterification of longer chain alcohols would allow the production of a wide range of interesting esters of succinic acid. Therefore, biphasic systems were also studied here in order to assess their suitability for preventing the hydrolysis of the formed esters and leading to high yields in presence of water. To that end, the best five chemical catalysts of the screening with ethanol (DBSA, Nafion NR-50, PS-Sulf. Ac., Amberlyst 131 and Nafion SAC-13) (see Subsection 6.1.1.1) were tested for the esterification of aqueous solutions of succinic acid with different non-water miscible alcohols (1-butanol, 1-hexanol, 1-octanol, 1-nonanol, 1-decanol and 1-undecanol). For the biphasic reactions, the alcohol phase and the aqueous solution of succinic acid were introduced in equal volumes into the reaction systems. This corresponds to a high excess in alcohol. The other reaction parameters were: 80 °C, 0.8 M succinic acid in distilled water (pH ~ 2.1) and stirring at 1000 rpm. The conversions of

these reactions were calculated as the percentage of the succinic acid that disappeared from the aqueous phase. The conversions after 24 h are presented in Figure 6-2.

As shown in Figure 6-2, the type of alcohol had a high impact on the conversion of succinic acid. For the shortest chain alcohol (1-butanol), high conversions after 24 h could be reached: 93, 90, 90, 85 and 69 % for DBSA, Nafion NR-50, PS-sulf.ac., Amberlyst 131 and Nafion SAC respectively. However as the chain length of the alcohol increased, the conversions strongly dropped for the heterogeneous catalysts (≤ 11 % for 1-undecanol), while the only homogeneous catalyst – the surfactant DBSA – retained good conversions (93, 92, 90, 89 and 87 % with 1-hexanol, 1-octanol, 1-nonanol, 1-decanol and 1-undecanol respectively). The only heterogeneous catalyst that could maintain satisfactory conversion (75 %) with 1-octanol was Nafion NR-50.

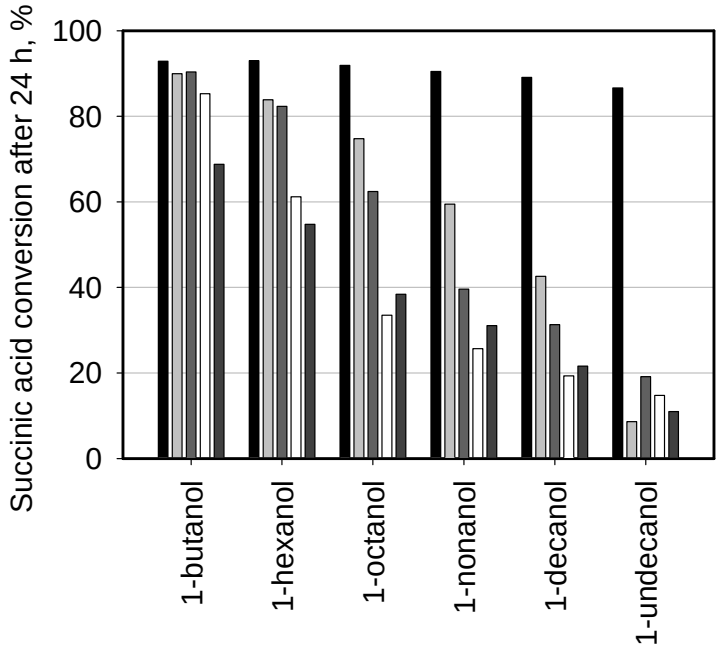


Figure 6-2: Chemical catalyst screening for the biphasic esterification of succinic with different alcohols: conversion of succinic acid from the aqueous phase after 24 hours. Reaction conditions: 80 °C, 0.8 M succinic acid in distilled water at pH = 2.1 (pH not set), 10 mol % DBSA (131 mg) or 0.5 g heterogeneous catalysts, 5 ml aqueous phase, 5 ml alcohol. Catalysts tested: DBSA , Nafion NR-50 , PS-sulf. ac. , Amberlyst 131 and Nafion SAC-13 .

The observed differences between the heterogeneous and the homogeneous catalysts could be due to the higher hydrophobicity of the longer chain alcohols and the hydrophilicity of the pores of the heterogeneous catalysts. For example, amberlyst resins and polystyrene polymers with sulfonic acid groups have been shown to swell or absorb water (Darge and Thyrion, 2007; Gagarin et al., 2008) and Nafion SAC-13 is supported

on a hydrophilic silicate matrix. When the pores of the catalysts are hydrophilic, the water will be strongly absorbed into the pores in presence of hydrophobic alcohols, lowering the interface between the two phases and hence the contact between the two substrates, and enhancing the ester hydrolysis that takes place mainly in the aqueous phase.

Concluding remarks

For all catalysts, the shorter the alkyl chain of the alcohol, the higher the conversion. Unfortunately, the two shorter chain alcohols, i.e. 1-butanol and 1-hexanol, will be unsuitable for biphasic applications because of their high water solubilities: 80 g l^{-1} (20 °C) and 6 g l^{-1} (25 °C) respectively. It would result in high quantities of alcohol to be lost in the aqueous phase, which would be detrimental in the case of an industrial process where the excess of alcohol should be recycled. On the contrary, 1-octanol with a much lower water solubility (0.30 mg l^{-1} at 20 °C) could be used as second phase for the esterification of succinic acid with both DBSA and Nafion NR-50, which were the best catalysts tested (total rate constant of succinic acid initial consumption ($k_{tot,0}$) of 0.329 ± 0.009 h^{-1} and 0.036 ± 0.020 h^{-1} resp.). The biphasic strategy with these two catalysts will hence be further investigated.

6.1.2 Screening of enzymatic catalysts

As mentioned in Section 3.4.4, enzymes can also catalyze the esterification of carboxylic acids. In order to assess the feasibility of an enzymatic esterification of aqueous solutions of succinic acid, several lipases were tested at different temperatures, since the thermal stability can be a key factor for enzymatic reactions. The biphasic systems with 1-octanol were used, since they have shown to limit ester hydrolysis and because they could facilitate the ester purification. This screening experiment helped determining potential enzymes that could be used for the esterification of succinic acid contained in a fermentation broth.

6.1.2.1 Screening of enzymes for the biphasic esterification of succinic acid with 1-octanol

Seven lipases were tested for the biphasic esterification of succinic acid with 1-octanol at 30 °C, 50 °C and 70 °C. From the literature review reported in Section 3.4.4, the following enzymes were studied: lipase from *Burkholderia cepacia* (BCL), immobilized lipase B from *Candida antarctica* (Novozym® 435), lipase from *Candida rugosa* (CRL), lipase from *Thermomyces lanuginosus* (TLL), lipoprotein lipase from *Chromobacterium*

viscosum (CVLPL), lipoprotein lipase from *Pseudomonas sp.* (PSLPL) and Amano lipase from *Pseudomonas fluorescens* (PFL). The result of this screening experiment is presented in Figure 6-3 and Figure 6-4.

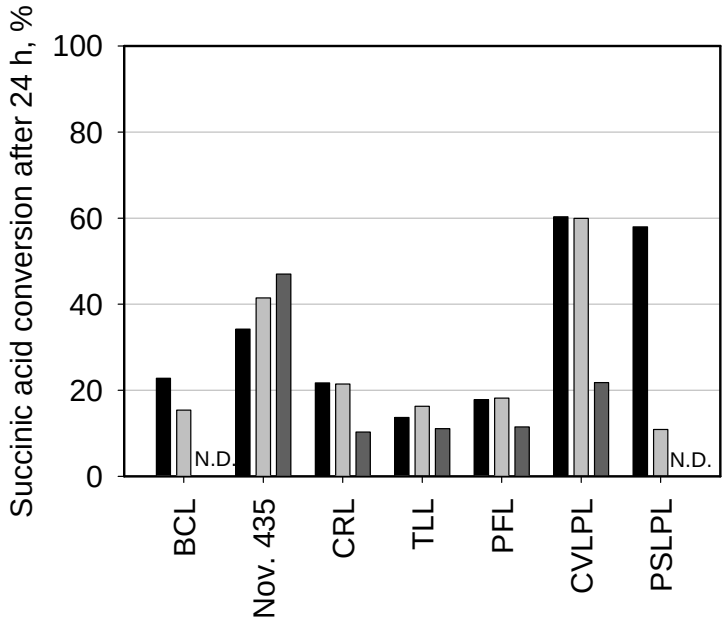


Figure 6-3: Enzyme screening for the biphasis esterification of succinic acid at different temperatures: conversion of succinic acid from the aqueous phase after 24 hours at three different temperatures. Reaction conditions: 0.15 M succinic acid in phosphate buffer (43 mM) at pH = 4, 40 mg or 40 µl, 5 ml aqueous phase, 5 ml 1-octanol. Temperature: 30 °C ■ , 50 °C □ and 70 °C ▬ . (N.D. = not determined).

From the lipases screened, the two lipoprotein lipases were the enzymes that gave the highest conversions (i.e. 60 and 58 % with the CVLPL and the PSLPL respectively) after 24 h. For the CVLPL, these final conversions were even achieved after 4 hours at 30 °C and 3 hours at 50 °C. An increase of the temperature from 30 °C to 50 °C was favourable for the CVLPL, whereas it deactivated the PSLPL. However, a further increase of the temperature up to 70 °C dramatically reduced the final conversion (22 %) with the CVLPL. The highest total initial rate constants ($k_{tot,0}$) were thus achieved at 50 °C with CVLPL (0.843 ± 0.036 h^{-1}) and at 30 °C with PSLPL (0.340 ± 0.069 h^{-1}). The decrease in yield at high temperature is probably due to a thermal deactivation of the proteins. Similarly, the free lipoprotein lipase from *P. fluorescens* has been reported to show little thermal stability at temperature above 50 °C (Hayashi and Ikada, 1990; Itoyama et al.,

1994). A thermal deactivation of the PSLPL at temperature > 50 °C and pH = 7.0 has also been reported by Toyobo Enzyme, Japan[1].

It should also be noted that, although the lipoprotein lipases showed high rate constants, they reached their maximal conversion after only several hours, but they did not bring the reaction to completion. Final conversions significantly lower than those obtained in biphasic systems with chemical catalysts were hence observed (up to 60 % for enzymes *vs.* up to 92 % for chemical catalysts with 1-octanol, see Figure 6-2). This could not be due to the thermodynamic equilibrium as the chemical catalysts gave higher equilibrium conversions. It might, however, be due to a deactivation of the enzymes or to the influence of another reaction parameter (such as the pH). This last hypothesis will be further investigated in Section 6.2.4.

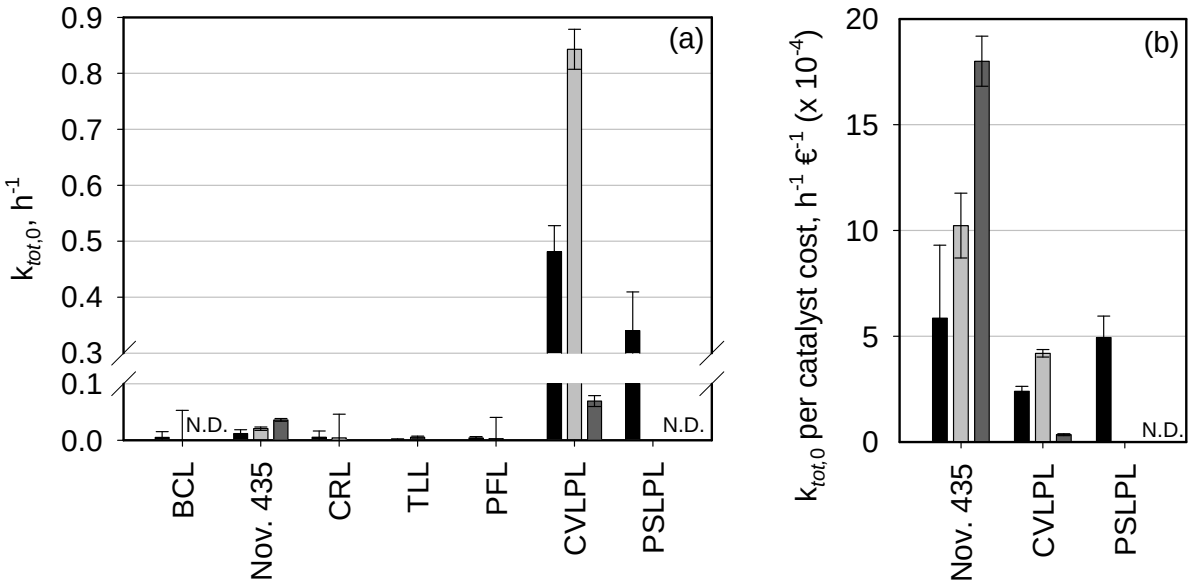


Figure 6-4: Enzyme screening for the biphasis esterification of succinic acid at different temperatures: (a) total rate constant of the succinic acid initial consumption $k_{tot,0}$ (h^{-1}) at three different temperatures, (b) total rate constant per catalyst cost (h^{-1} €$^{-1}$) for Novozym 435, CVLPL and PSLPL. Reaction conditions: 0.15 M succinic acid in phosphate buffer (43 mM) at pH = 4, 40 mg or µg, 5 ml aqueous phase, 5 ml 1-octanol. Temperature: 30 °C ■ , 50 °C □ and 70 °C ■ . (N.D. = not determined).

Lastly, the third best enzyme was the Novozym 435, with which conversions of 34 %, 41 %, 47 % could be achieved resp. at 30 °C, 50 °C and 70 °C. This enzyme seems

[1] Toyobo Enzyme, Toyobo Co., LTD.
http://www.toyobo.co.jp/e/seihin/xr/enzyme/pdf_files/201_204LPL_311.pdf, consulted on 04.04.2011

thermostable and it enabled higher total rate constants when increasing the temperature (up to 0.036 ± 0.002 h^{-1} at 70 °C).

Concluding remarks

The three enzymes that gave conversions ≥ 40 % after 24 h for the esterification of succinic acid in two-phase system with 1-octanol were the lipoprotein lipases and Novozym 435. It should be noted that Novozym 435 and the PSLPL were the two promising enzymes determined from the literature search for enzymatic strategies in solvent reported in Section 3.4.4. The lipoprotein lipases gave significantly higher total rate constants (see Figure 6-4). Unfortunately, besides their thermal instability, the lipoprotein lipases are extremely expensive (2010 € g^{-1} for CVLPL and 688 € g^{-1} for PSLPL), whereas Novozym 435 has a relatively low price (20 € g^{-1}) (source: Sigma-Aldrich, 2010). That is why the increase in activity does not compensate the higher costs due to the use of the lipoprotein lipases as it is shown in Figure 6-4 (b). Those enzymes could therefore only be used in the industry if they were immobilized and reused many times. Several approaches for the immobilization of LPL have been reported (Emi et al., 1994; Hayashi and Ikada, 1990; Itoyama et al., 1994), but no reusability tests were performed so far. In the end, Novozym 435 seems at the moment to be a cheaper alternative with a relatively good activity (total rate constant per € of $(1.8 \pm 0.1) \cdot 10^{-3}$ h^{-1} $€^{-1}$ at 70 °C) and high thermal stability pH = 4 (see Anderson et al., 1998). Furthermore, it has the advantage to be already immobilized, hence simplifying purification steps.

6.1.2.2 Enzymatic biphasic esterification of succinic acid with different alcohols

As it was done for the chemical catalysts, the best enzyme (Novozym 435) was then tested with a large spectrum of non-water-miscible alcohols for the esterification of aqueous solutions of succinic acid. The same alcohols were used: 1-butanol, 1-hexanol, 1-octanol, 1-nonanol, 1-decanol and 1-undecanol. The results are presented in Figure 6-5.

Similarly to DBSA, Novozym 435 could esterify succinic acid from aqueous solutions with all the alcohols tested. The highest conversions after 24 h were recorded for the esterification with the three shorter chain alcohols (45, 44 and 44 % with 1-butanol, 1-octanol and 1-hexanol resp.), whereas only slightly lower conversions were measured for the other alcohols (37, 38, 38 % with 1-nonanol, 1-decanol and 1-undecanol resp.). Contrary to the heterogeneous chemical catalyst, the conversions with longer chain alcohols did not drop with the immobilized enzyme. The macroporous hydrophobic acrylic resin, on which the enzyme is immobilized, does not really absorb water unlike the tested heterogeneous chemical catalysts.

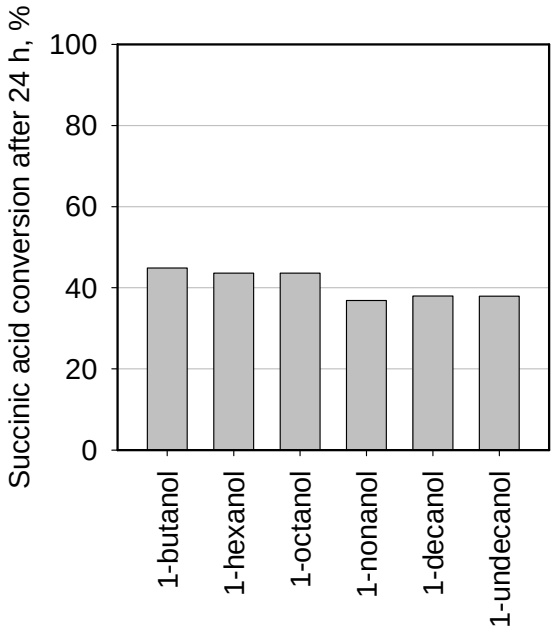


Figure 6-5: Two-phase esterification of aqueous solutions succinic acid with different alcohols using Novozym 435: succinic acid conversions after 24 h ▭. Reaction conditions: standard conditions (SC) reported in Table 4-2, except for the alcohol.

Finally, Novozym 435 seems to be a promising catalyst for the biphasic esterification of succinic acid, since good conversions could be obtained with a wide range of alcohols.

6.1.3 Conclusions on the selection of the best catalysts and reaction system for further process study

For the esterification of succinic acid with chemical catalysts, a monophasic reaction was first tested for the esterification with short chain alcohols such as ethanol. Whereas DES yields up to 100 % were measured in pure ethanol, the yields dropped drastically when water was introduced in the system for all catalysts tested. These limited yields were attributed to the hydrolysis of the formed esters due to the water present in the system and in the catalyst pores. A biphasic strategy was hence tested with longer chain alcohols in order to extract the formed esters, prevent their hydrolysis and facilitate their purification.

Different chemical and enzymatic catalysts were screened for such a biphasic reaction. Among the chemical catalysts, DBSA (a homogeneous surfactant) and Nafion NR-50 (a heterogeneous fluorinated polymer) showed good conversions for reactions with 1-octanol. While the homogeneous surfactant DBSA could esterify succinic acid with a wide range of alcohols, the heterogeneous catalysts gave lower conversions with increasing chain length of the alcohol. As for the enzymes, lipoprotein lipases were the most active catalysts, but their high prices currently prevent any industrial application.

Finally, Novozym 435 (an immobilized lipase on acrylic resin) gave good activities and conversions for the esterification of succinic acid with 1-octanol in a biphasic process. This enzyme also gave satisfactory conversions with a broad range of alcohols.

These three catalysts (DBSA, Nafion NR-50 and Novozym 435) were hence further examined for the esterification of aqueous solutions of succinic acid in biphasic system with 1-octanol. In Subchapter 6.2, a screening of different reaction parameters will be presented and their impact on the three catalysts will be reported in order to search for an optimal set of reaction conditions for the three catalysts.

6.2 Impact of the reaction conditions: comparison of DBSA, Nafion NR-50 and Novozym 435 and optimization

In Subchapter 6.1, three best catalysts (DBSA, Nafion NR-50 and Novozym 435) were selected for the esterification of aqueous solutions of succinic acid in a biphasic system using 1-octanol as second phase. In order to optimize the reaction conditions, the esterification with these three catalysts was first characterized in more details with a separate screening of different reaction conditions. Although this "one-at-a-time" optimization, which does not take the interaction of the variables into account, may lead to a suboptimal set of parameters, it is an easy and fast methodology, with a simple experimental design. Furthermore, a lot of information can be derived from such a study. To that end, the impact of the following variables was tested: agitation rate, catalyst concentration, temperature, pH, succinic acid concentration and volumetric ratio of the two phases. For each study, only one variable was varied while the others were kept as in the standard conditions (see Table 4-1 and Table 4-2). Besides, some technical aspects were also investigated, such as the phase separation for the surfactant DBSA and the recycling of the heterogeneous catalyst Nafion NR-50. Finally, the sets of reaction conditions derived for the three catalysts by this "one-at-a-time" approach were tested for the esterification of succinic acid in biphasic systems.

6.2.1 Impact of the agitation rate

The agitation rate has an influence on the convection in the reaction system. It can have hence an impact on the external mass transfer. If the reaction kinetics has to be studied, it is important that mass transfer limitations are avoided. Otherwise, the observed rate will not be the intrinsic reaction rate but the rate of the limiting step, e.g. the external or internal mass transfer. In order to assess if the external mass transfer is limiting, the

agitation rate was increased stepwise while following up the resulting change in the observed rate of the reaction, i.e. here the total rate constant of the succinic acid initial consumption, $k_{tot,0}$. This experiment is summarized in Figure 6-6.

The agitation rates did not have a significant impact on the conversion and the total initial rate constant. The conversions remained indeed relatively constant over the range of agitation rate tested (from 89 to 92 % for DBSA, from 64 to 69 % for Nafion NR-50 and 45 to 53 % for Novozym 435). Similarly, the rate constants did not vary much while increasing the agitation rate (rate constants of 0.234 ± 0.005 to 0.342 ± 0.009 h^{-1} for DBSA, 0.022 ± 0.017 to 0.033 ± 0.004 h^{-1} for Nafion NR-50 and 0.023 ± 0.004 to 0.038 ± 0.006 h^{-1} for Novozym 435).

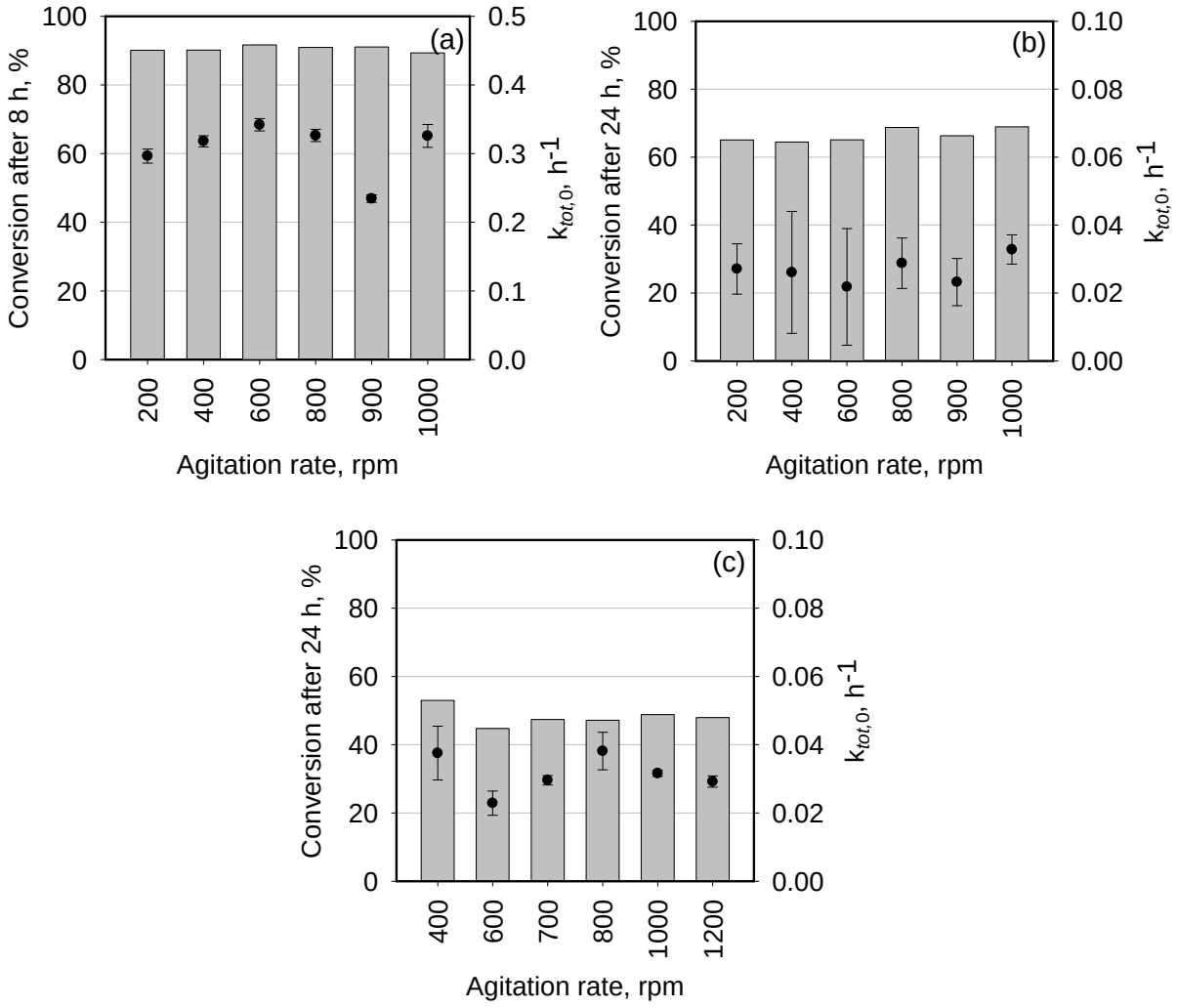


Figure 6-6: Impact of the agitation rate on the conversion after 8 h or 24 h ▭ and on the total rate constant of the succinic acid initial consumption $k_{tot,0}$ ● with DBSA (a), Nafion NR-50 (b) and Novozym 435 (c) for the biphasic esterification of succinic acid with 1-octanol. Reaction conditions: standard conditions (SC) (see Table 4-1 and Table 4-2) except for the agitation rates (from 200 to 1000 rpm for the chemical catalysts and 400 to 1200 rpm for the enzymes).

Concluding remarks

The external mass transfer of the esterification in the two phase systems with the three catalysts does not seem hence to be limiting in the reaction conditions tested here. The observed reaction rate is therefore either the rate of the internal mass transfer or the real reaction rate. It is important to avoid mass transfer limitations to use the full potential of the catalyst and observe the real kinetics of the reaction.

6.2.2 Impact of the catalyst concentration

The impact of the catalyst concentration can also give information on potential mass transfer limitation. This is why this variable has been studied here for the three best catalysts reported previously. Different catalyst masses were introduced into the system and the conversion of succinic acid was recorded over the reaction time. Catalyst masses ranging from 0.008 g to 0.393 g of DBSA, from 0.048 g to 1.510 g of Nafion NR-50 and from 0.005 g to 0.080 g of Novozym 435 have been tested. The results, in terms of total rate constant of the succinic acid initial consumption *vs.* catalyst mass, are presented in Figure 6-7.

For the three catalysts, the rate constant at low catalyst concentrations was proportional to the mass of the catalyst. However, as the mass increased, the activity tended to level off, especially for DBSA and Nafion NR-50. The observed non-linearity at high catalyst masses can be due to mass transfer limitations in the pores in the case of the two heterogeneous catalysts or to external mass transfer for the three catalysts. By increasing the catalyst mass, new reaction sites are indeed introduced in the reaction system, enhancing the reaction rate. However, if the reaction rate becomes higher than the mass transfer rate, the latter will become the limiting step. For a heterogeneous catalyst, (such as Nafion NR-50 or Novozym 435), the hypothesis of internal mass transfer limitations might be tested using heterogeneous catalysts with different pore sizes or different particle sizes for a constant mass of catalyst. Nevertheless, those were not available. As for homogeneous catalysts like DBSA, the levelling off of the rate constant can only be explained by external mass transfer limitations. However, since DBSA is also a surfactant, the increase of the catalyst concentration up to 131 mg could also increase the interfacial area. But with a further increase of the mass, the bubbles of the emulsions might keep a constant diameter. The additional DBSA would then not be at the interface but in the bulk of the organic phase, where mass transfers could be limiting.

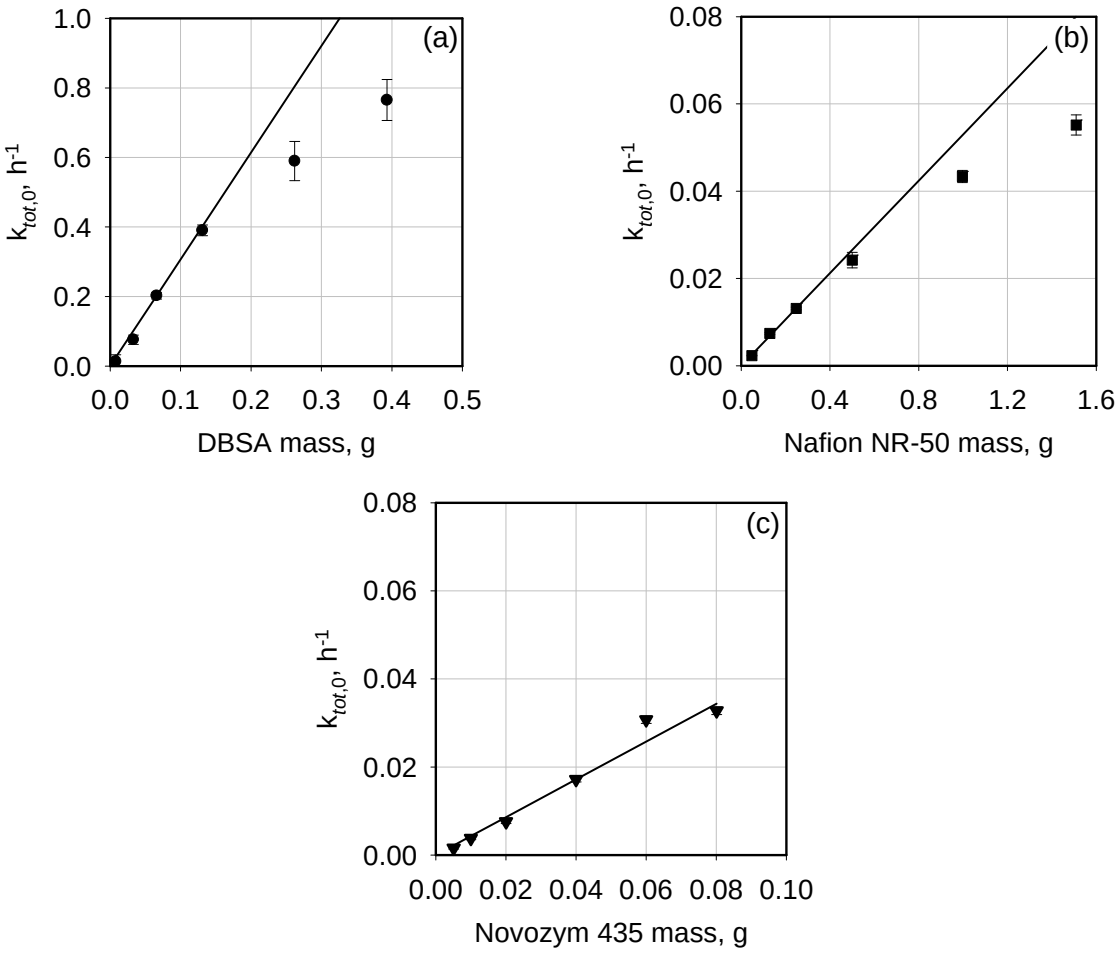


Figure 6-7: Impact of the catalyst mass on the total rate constant of the succinic acid initial consumption $k_{tot,0}$, for its esterification with 1-octanol in biphasic system using DBSA ● (a), Nafion NR-50 ■ (b) and Novozym 435 ▼ (c). The solid line ⎯ depicts a theoretical proportionality between the catalyst mass and the initial total consumption rate. Reaction conditions: standard reaction conditions (SC) (see Table 4-1 and Table 4-2) except for the catalyst mass (from 0.008 to 0.393 g of DBSA, from 0.05 to 1.5 g of Nafion NR-50).

Concluding remarks

The rate constant was proportional to the catalyst mass for the three catalysts at low mass. However, the increase of the rate constant tented to level off for DBSA and Nafion NR-50, due to mass transfer limitations. In the industry, the optimal catalyst concentration is often taken slightly above the linear range, in order to have high activities but not to add a high quantity of catalyst that would not be used at its full capacity. Following this rule, the optimal concentrations were selected as 131 mg for DBSA and 750 mg for Nafion NR-50. As for Novozym 435, the rate seemed to stay proportional to the mass added. However, at 60 mg and to a greater extent at 80 mg, the solutions are difficult to mix. That is why concentrations of maximum 60 mg should be used for the enzymatic esterification.

6.2.3 Impact of the temperature

The temperature has a large impact on catalytic reactions and enzymes and chemical catalysts are not always stable at large temperature. Furthermore, as the reaction rate increases with the temperature, mass transfer can become limiting. That is why it is important to determine the adapted range of temperature for a catalysis process. For the chemical catalysts, the impact of the temperature was tested from 50 °C to 90 °C and for the enzymatic catalysis, milder temperatures were screened (30 °C to 70 °C) as enzyme are often less thermostable. The results are presented in the form of Arrhenius plots (ln (activity) *vs.* 1/T, see equation (3-20)) in Figure 6-8.

For the three catalysts, an increase of the temperature led to an enhancement of the activity. The highest rate constants for the three catalysts were the following: 0.361 ± 0.002 h^{-1} at 90 °C for DBSA, 0.036 ± 0.003 h^{-1} at 90 °C for Nafion NR-50 and 0.037 ± 0.001 h^{-1} at 70 °C for Novozym 435. However, for DBSA and especially for Novozym 435, the activity increased less rapidly at high temperatures (low 1/T) (for temperatures > 70 °C for DBSA and > 50 °C for the enzyme). As for Nafion NR-50, the Arrhenius' law seemed to be valid for the whole range of temperatures tested.

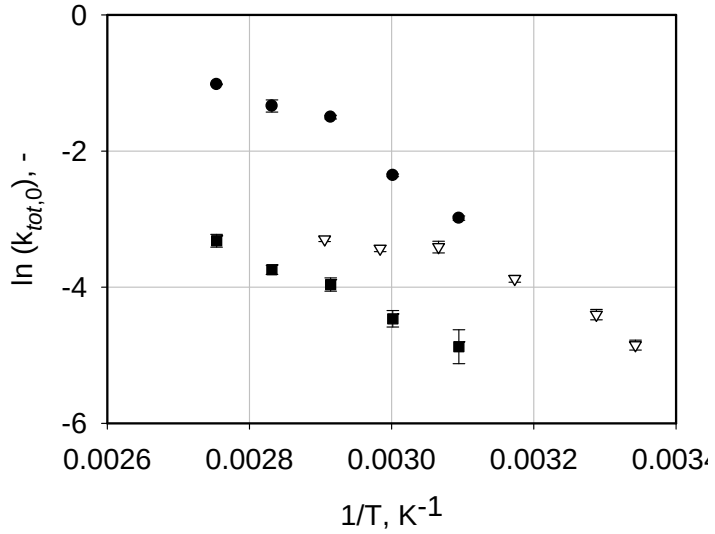


Figure 6-8: Arrhenius' plot for esterification of succinic acid with 1-octanol in biphasic system with DBSA ●, Nafion NR-50 ■ and Novozym 435 ▽: natural logarithm of the total rate constant of the succinic acid initial consumption ($k_{tot,0}$) *vs.* 1/T. Reaction conditions: standard conditions (SC) (see Table 4-1 and Table 4-2) except for the temperatures from 50 °C to 90 °C of the chemical catalysts and from 30 °C to 70 °C for the enzyme.

The slower increase of the rate constant at high temperature for DBSA and Novozym 435 could be due either to mass transfer limitations or to potential partial deactivation of

the catalyst (for the enzyme). In the case of DBSA, very high rate constants were indeed observed at 90 °C (0.361 ± 0.002 h^{-1}), so that the mass transfer between the two phases might slowly become limiting. As for the enzyme, an increase of the temperature could be responsible for small changes in the 3D-structure of the enzyme, levelling off the activities. Besides, transfer in the pores of the immobilized enzyme could also become limiting. However, this latest hypothesis could not be verified, as no Novozym 435 with different pore sizes could be tested.

Activation energies for the three catalysts were determined from the linear part of the Arrhenius' plot: 68 ± 7 kJ mol^{-1} for DBSA, 37 ± 2 kJ mol^{-1} for Nafion NR-50 and 43 ± 3 kJ mol^{-1} for Novozym 435. The determined values were different for the three catalysts but in the same order of magnitude. For DBSA and Nafion NR-50, the reaction mechanism should be similar, since both catalysts bear analogous sulfonic groups. Therefore similar activation energies would have been expected. Nonetheless, in the heterogeneous catalyst (Nafion NR-50), the reaction could be limited by pore diffusion, leading to an apparent activation energy about half the real one (see Section 3.2.2). This is almost the case here. It is not surprising that the internal mass transfer might be limiting in Nafion NR-50, since most of its active sites have been reported to be inaccessible (Ledneczki et al., 2005). Similar activation energies (61 kJ mol^{-1}) to the estimated value obtained with DBSA have been reported for the esterification of acetic acid with methanol using sulfuric acid when water was introduced in the system (Liu et al., 2006a). For the enzymatic catalyst Novozym 435, the activation energy is intermediate. However, the mechanism of reaction is different from the Fischer-Speier mechanism for the chemical catalysts (see Section 3.4.1). It is therefore difficult to compare the estimated activation energies.

Concluding remarks

In the lower range of the temperatures, the rate constants were following the Arrhenius's law, although the activation energy determined for Nafion NR-50 might be altered by the internal mass transfer. Higher temperatures (90 °C) were found optimal for DBSA and Nafion NR-50, as no complete deactivation could be observed. Regarding the enzyme, a slowly increasing "plateau" was obtained at high temperatures. However, as no complete deactivation was obtained, the highest temperature (70 °C) was also taken as the optimal one, similarly to what have been done for the chemical catalysts.

6.2.4 Impact of the pH

The pH could also be an important factor for the process development of the esterification of succinic acid since the substrate of the reaction is a dicarboxylic acid. Furthermore, the chemical catalysts are Brønsted acids and enzymes are often only active within a certain pH range. In order to assess the impact of the pH, aqueous solutions of succinic acid with different initial pHs were tested. The change in pH was also recorded throughout the reaction time. Figure 6-9 shows the impact of the pH on the conversions after 24 h and on the total rate constant of the succinic acid initial consumption, while Figure 6-10 presents the pH change during the reaction at different initial pHs.

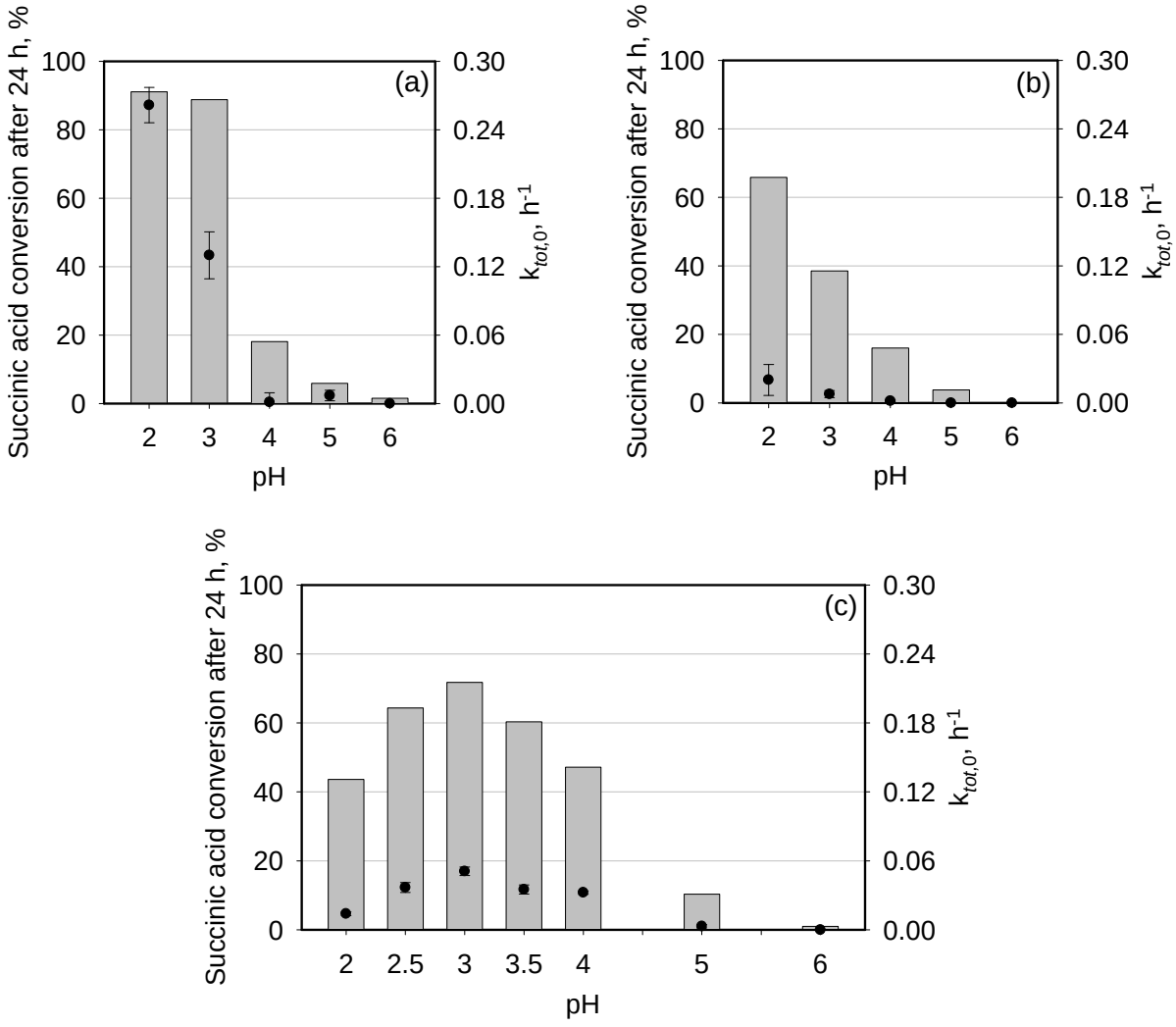


Figure 6-9: Impact of the pH on the esterification of succinic acid with 1-octanol in biphasic system with DBSA (a), Nafion NR-50 (b) and Novozym 435 (c): conversion of succinic acid from the aqueous phase after 24 h ▭ and total rate constant of the succinic acid initial consumption $k_{tot,0}$ ●. Reaction conditions: standard conditions (SC) (see Table 4-1 and Table 4-2) except for the pH (from 2 to 6).

As shown in Figure 6-9, the pH had a significant impact on the conversion of succinic acid from the aqueous phase with both the chemical catalysts (DBSA (a) and Nafion NR-50 (b)) and the enzyme Novozym 435 (c).

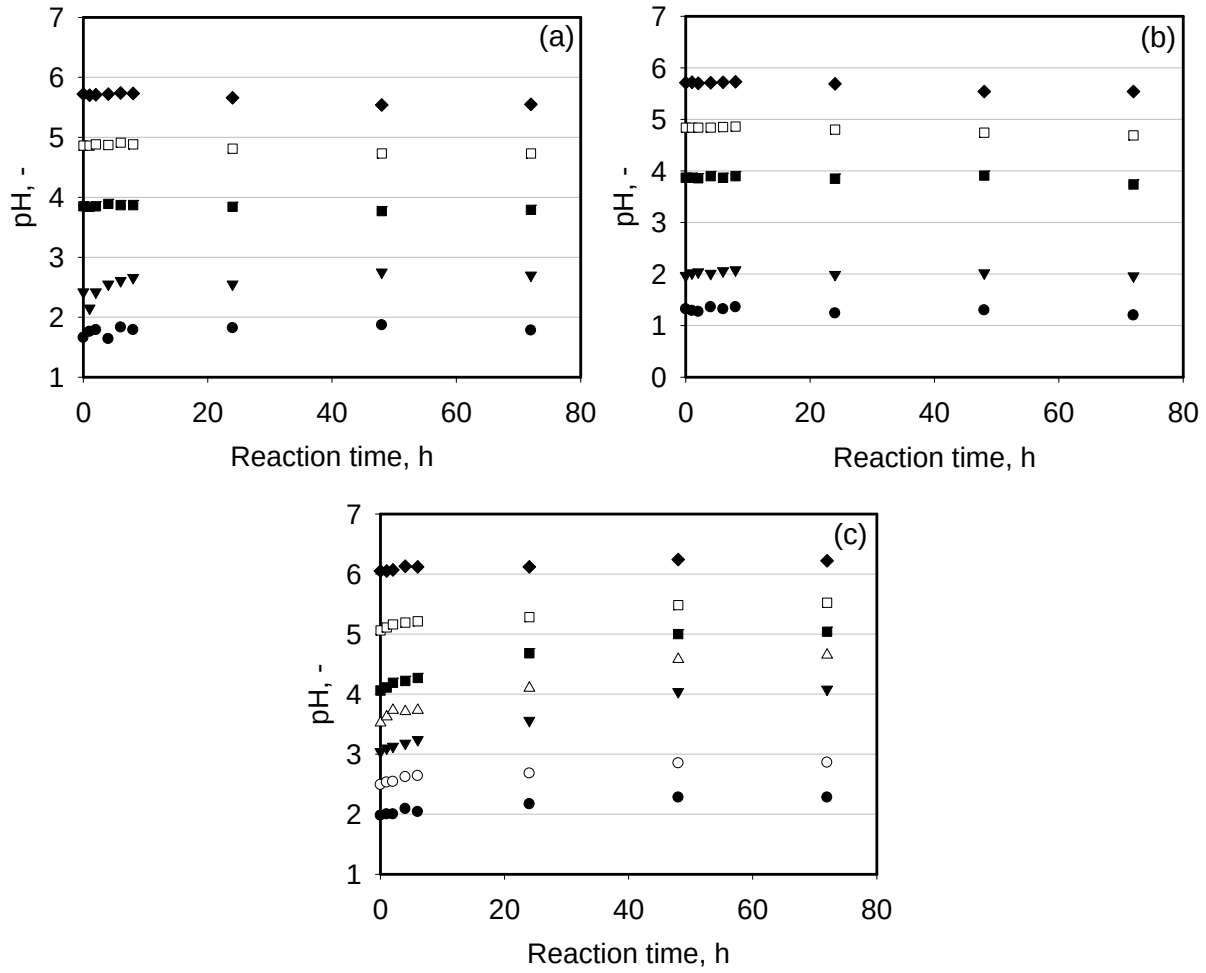


Figure 6-10: pH change during the biphasic esterification of succinic acid with 1-octanol DBSA (a), Nafion NR-50 (b) and Novozym 435 (c): aqueous solutions at initial pHs of 2 ●, 2.5 ○, 3 ▼, 3.5 △, 4 ■, 5 □ and 6 ◆. Reaction conditions: standard conditions (SC) (see Table 4-1 and Table 4-2) except for the pH (from 2 to 6).

Regarding the chemical catalysts, the final conversions after 24 h were relatively limited at pH ≥ 4 (i.e. ≤ 18 % using DBSA and Nafion NR-50), whereas good conversions could be achieved after 24 h in buffers at pH = 2 and 3 (for DBSA, 91 and 89 % resp. and for Nafion NR-50, 66 and 37 %). However, a clear decrease of the total initial rate constant could be observed for both DBSA and Nafion NR-50, if the initial pH was changed from 2 to 3: for DBSA, the rate constant dropped from 0.262 ± 0.015 h^{-1} to 0.130 ± 0.021 h^{-1} and for Nafion NR-50, the rate constant diminished from 0.020 ± 0.014 h^{-1} to 0.008 ± 0.003 h^{-1}. This decrease in activity happened at a pH close to the pK_a of DBSA

($pK_{a,DBSA}$ = 2.55; Massoumi et al., 2009) and of Nafion NR-50[2]. The phenomenon might therefore be caused by the change in the ratio of the protonated to non-protonated form of DBSA or of Nafion NR-50. According to the Fischer-Speier esterification mechanism (see Figure 3-14), the catalyst indeed acts as a proton donor to the alcohol, so that only the protonated form of the catalyst present at lower pH can catalyze the reaction. In order to test this hypothesis, the rate constants per mmol protonated DBSA were calculated at both pH = 2 and 3, taking into account that the pH of the buffers varied after the addition of the catalyst (see Figure 6-10). These two rate constants per mmol protonated DBSA were finally relatively similar (0.75 ± 0.40 and 0.62 ± 0.10 h^{-1} $mmol_{prot.\ DBSA}^{-1}$ for pH = 2 and 3 resp.), although the total rate constants were very different (0.262 ± 0.015 h^{-1} and 0.130 ± 0.021 h^{-1} resp.). The loss in activity is therefore most probably due to the decrease of the amount of the protonated active form of the catalyst at initial pHs ≥ 3.

Concerning the pH change, shown in Figure 6-10 (a) and (b), pH is relatively stable throughout the reactions with the chemical catalysts, although it can be seen that the recorded pHs are slightly lower than the pHs of the aqueous solutions set initially. It could be explained by the addition of an acid species (i.e. the catalyst) into the system, hence lowering the pH of the reactions.

Regarding the enzymatic catalysts, the impact of the pH was more complex. On the one hand, increasing the pH up to values ≥ 5 dramatically diminished the final conversions (only 10 and ~ 0 % at pH = 5 and 6 resp. after 24 h). On the other hand, lowering the pH down to values ≤ 2.5 had also a negative impact on the final conversions (conversion of only 44 % at pH = 2). The conversion reached a maximum at pH = 3 with a conversion of 72 % after 24 h. The total initial rate constant showed a similar maximum at pH = 3 with a maximal rate constant of 0.051 ± 0.004 h^{-1}. Similar activity or conversion *vs.* pH curves were observed by Buthe et al. (2005) and Domínguez de María et al. (2009) for the esterification of different carboxylic acids with 1-butanol using a *Thermomyces lanuginosus* lipase (TLL) and a lipase from *Candida rugosa* (CRL) in biphasic water-heptane systems. The moderate conversions at lower pHs can be explained by a partial denaturing of the enzyme at low pHs. Notwithstanding, the Novozym 435 is still relatively stable at lower pHs, contrary to TLL that gave extremely

[2] The pK_a of Nafion NR-50 is probably similar to the pK_a of DBSA as it possesses comparable sulphonic functions.

narrow optima with a steep decrease in activities at pH ≤ 3. The loss in activity at higher pHs was ascribed by Buthe et al. (2005) and Domínguez de María et al. (2009) to the fact that the protonated form of the carboxylic acid was the only possible substrate of the lipase. In the present study, a similar decrease of the activity above the first pK_a of succinic acid (pK_{a1} = 4.21) was indeed observed. For initial concentrations of succinic acid of 0.15 M and, at initial pHs of 3, 4, 5 and 6, the initial concentrations of the diprotonated succinic acid are only of 0.13, 0.088, 0.016 and 0.0007 M resp. At pH ≥ 5, almost no substrate is therefore available for the enzymes. "Corrected" conversions of the sole diprotonated succinic acid can be calculated from the initial and final amount of the diprotonated form of succinic acid. For initial pHs of 3, 3.5 and 4, these "corrected" conversions were of 76, 75 and 82 % whereas the total conversions after 24 h were of 72, 60 and 47 %. This confirms that succinic acid can only be used as substrate by the enzyme in its diprotonated form. Buthe et al. suggested that electrostatic repulsions could repel the substrate out of the active site. That could explain why the negatively charged monoprotonated succinic acid cannot be used as substrate, even if it bears a protonated carboxylic acid on one side.

As for the pH change during the reaction with the enzyme, shown in Figure 6-10 (c), no pH shift could be seen at the beginning but the pH slowly increased over the reaction time (see Table 6-1). The biggest change was recorded for the pH at which the rate was the highest (i.e. initial pH of 3). This pH increase can be explained by the consumption of the succinic acid, an acidic species. This phenomenon does not happen for DBSA and Nafion NR-50, as these acid catalysts might have buffered the solutions. The increase of the pH can be calculated theoretically from the equations presented in Subchapter 4.6.1 and the measured conversions. The theoretical calculations, reported in Table 6-1, slightly underestimated the measured pHs, but an increase in pH similar to the observed one could be very well estimated theoretically.

Table 6-1: Measured and predicted pH variations after 24 h (see Subchapter 4.6.1).

Initial pHs	2	2.5	3	3.5	4	5	6
Measured pHs after 24 h	2.2	2.7	3.6	4.1	4.7	5.3	6.1
Predicted pHs after 24 h	2.0	2.6	3.4	4.0	4.6	5.1	6.0

It has already been reported in Subsection 6.1.2.1 that even the fastest enzymes could not reach conversions higher than 60 %, whereas the chemical catalysts led to equilibrium conversions of 92 %. The increase of the pH recorded here for Novozym 435

(see Figure 6-10 (c)) could be responsible for this limitation in final conversions. Since the diprotonate succinic acid is the only possible substrate for the enzymes, the higher pH at the end of the reaction consequently limits the amount of available diprotonated species. For instance, an equilibrium conversion of 57 % was indeed recorded for Novozym 435, after 72 h for an initial pH of 4. As the pH had increased from 4 up to ~ 5 after 72 h, there was only ~ 0.007 M left of diprotonated succinic acid, hence limiting the equilibrium conversion. Since conversely the chemical catalysts were tested at a lower pH (pH = 2.1) (see Subsection 6.1.1.2), all the succinic acid was available in its diprotonated form, allowing much higher equilibrium conversions. Accordingly, for enzymatic reactions, a titration system should be used to maintain the pH throughout the reaction time.

Concluding remarks

In summary, optimal activities and conversions for the two chemical catalysts could be reached at pH = 2, since the two catalysts were in their protonated form at such a pH and could act as proton donor according to the Fischer-Speier esterification mechanism. Regarding Novozym 435, an optimal pH for the enzymatic esterification could be found around pH = 3. On the one hand, low pHs were required, as the diprotonated form of succinic acid was the only substrate esterified by the enzyme. On the other hand, the stability of the enzyme was relatively limited at very low pHs and a compromise had to be found between the available diprotonated succinic acid and the enzyme stability.

6.2.5 Impact of the succinic acid concentration

The impact of the initial concentration of succinic acid might be of great importance. At a high concentration, inhibitory effects might indeed take place. Furthermore, the study of the rate can give useful indications on the order of reaction. To that end, pure solutions of succinic acid with initial concentrations ($[SA]_{tot,0}$) ranging from 0.005 to 0.8 M were tested, with a constant mass of the three catalysts. The conversions after 8 h or 24 h and the initial molar consumption rates ($r_{mol,0}$) are reported in Figure 6-11.

It must be first noted that the conversions stayed constant over the range of concentrations tested: from 89 to 91 % for DBSA, from 60 to 68 % for Nafion NR-50 and from 41 to 46 % for Novozym 435. The initial molar consumption rate ($r_{mol,0}$) seems to be proportional to the initial concentration of succinic acid ($[SA]_{tot,0}$). A small levelling off can, however, be observed for DBSA and Novozym 435 at concentrations as high as 0.8 M, and might be due to a too low concentration of catalytic active sites available.

From the molar rates, total rate constants ($k_{tot,0}$) can be calculated using equation (4-14). These rate constants are almost stable over the concentration ranges. Total rate constants ranging from 0.248 ± 0.016 h^{-1} to 0.289 ± 0.012 h^{-1} were obtained for DBSA. The rate constants varied between 0.029 ± 0.002 h^{-1} and 0.034 ± 0.002 h^{-1} for Nafion NR-50. Finally, the total rate constants of the enzymatic reaction ranged from 0.025 ± 0.002 h^{-1} to 0.030 ± 0.006 h^{-1}.

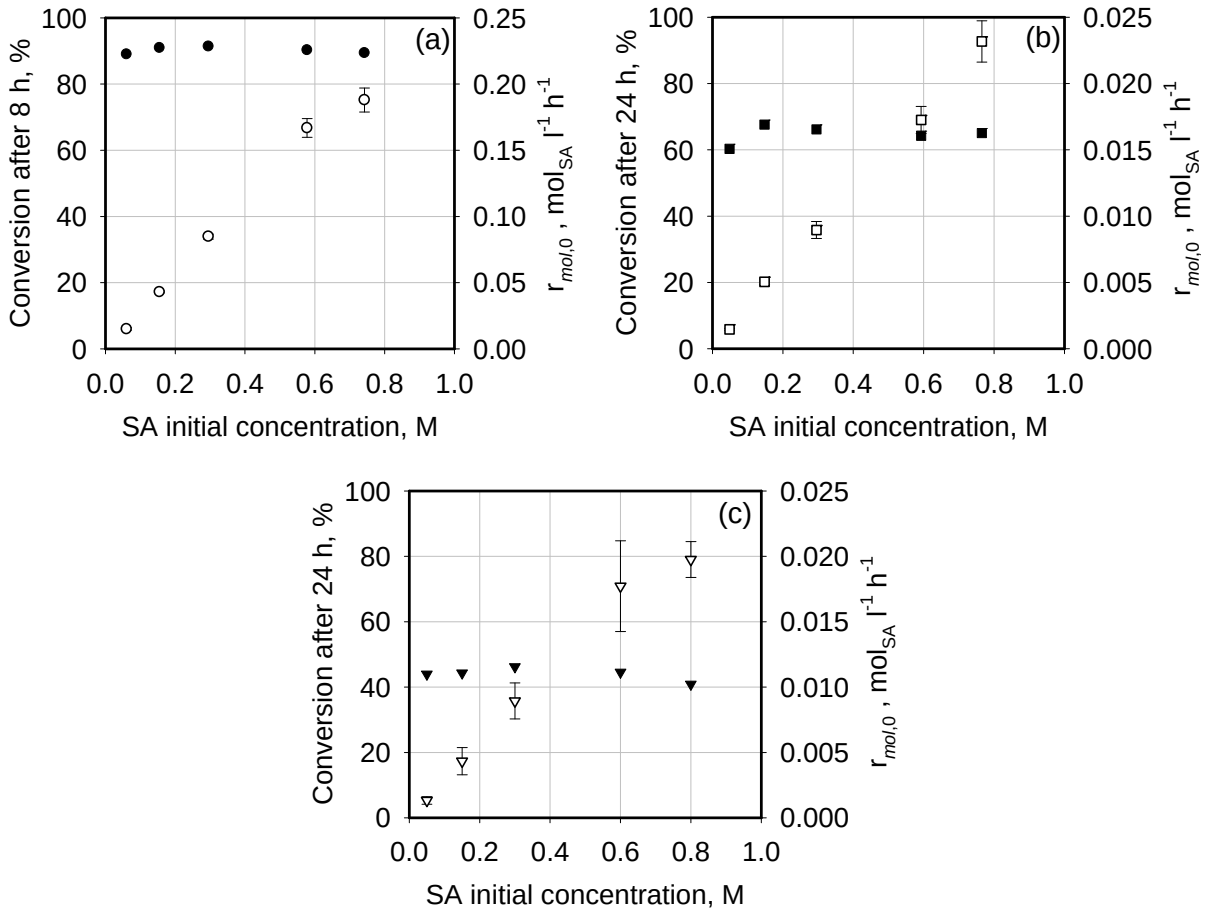


Figure 6-11: Impact of the initial concentration of succinic acid on the conversion (closed symbols: ●, ■,▼) and the initial molar consumption rate of succinic acid ($r_{mol,0}$) (open symbols: ○, □,▽) for its esterification with 1-octanol in biphasic system using DBSA ● (a), Nafion NR-50 ■ (b) and Novozym 435 ▼ (c). Reaction conditions: standard conditions (SC) (see Table 4-1 and Table 4-2) except for the concentration of succinic acid from 0.05 to 0.8 M.

The proportionality of the molar rate with respect to the initial concentration of succinic acid ($[SA]_{tot,0}$) could be due to a first order reaction rate. However, this apparent first order of reaction might not be the "real" order of the reaction, if the reaction is limited by mass transfer. As presented in Section 3.2.3, though, the observed reaction rates is of (n+1)/2, when internal mass transfer is limiting. But if the reaction is of first order, the observed reaction rate will hence also be of first order and no distinction can be done

between the observed and real reaction rate. Therefore, the intrinsic reaction seems to well be of first order in succinic acid, with all three catalysts tested.

Concluding remarks

The molar rate is proportional to the initial concentration of succinic acid. The half-life of the reaction (i.e. time required to reduced the concentration of succinic acid to half of its initial value) is thus independent of its initial concentration. And no significant inhibition effect could be observed at high concentrations, although a very small levelling of the molar rate might be observed for DBSA and Novozym 435 at 0.8 M of succinic acid. Since the aim of the present study is to develop an esterification process for the succinic acid contained in real fermentation broth and fermentations have been reported to yield concentrations up to 146 g l^{-1} (i.e. 1.24 M) (Okino et al., 2008), it is important that the esterification could be performed at such high initial concentrations of succinic acid. This can be done with the three catalysts tested here, without a loss in rate.

6.2.6 Impact of the volumetric ratio of the two phases

The volume ratio of the two phases could also be of great importance for industrial applications. On the one hand, it would be most convenient to have a large ratio of water to alcohol in order to use a smaller excess of the latter. One the other hand, a large ratio of water could limit the conversion by pushing the equilibrium backwards to the hydrolysis of the esters. Besides, a large amount of alcohol could be inhibitory or enhance only the pure extraction of succinic acid but not the reaction itself. However, a large excess of alcohol might be needed to push the reaction toward the esterification.

In order to assess these complex impacts, different values of the volumetric ratio w/o (i.e. of the volume of water phase divided by the volume of organic phase) were hence tested. The ratio of the initial moles of succinic acid by the mass of catalyst was kept constant for the reactions with the same catalyst in order to have comparable results. Even if the initial total concentrations of succinic acid $[SA]_{tot,0}$ were kept constant for all reactions, the initial aqueous concentration of succinic acid $[SA]_{aq,0}$ after pure extraction were relatively different. In order to normalize the rates to the initial aqueous concentration of succinic acid, the aqueous rate constant ($k_{aq,0}$) (see equation (4-13)) was used as comparison factor. The conversions after 24 h and the aqueous rate constant ($k_{aq,0}$) at different phase volumetric ratios are presented in Figure 6-12.

The phase volumetric ratio happened to have a great impact on the **conversion** using the two chemical catalysts. With a high amount of water (ratios > 1), the conversions

indeed dropped significantly, down to 67 % for a volumetric ratio of 3 using DBSA and down to 46 % for a volumetric ratio of 5 using Nafion NR-50. The impact of the phase ratio on the conversion of the enzymatic reaction was much more limited. The conversions only diminished from 46 to 38 % at phase ratio increasing from 0.33 to 5. The impact of the phase ratio can be explained by the two different phenomena: at a low phase ratio (i.e. high content of alcohol), the pure extraction of succinic acid is higher, artificially increasing the conversion, whereas at a high phase ratio, the high concentration of water might push back the equilibrium towards the hydrolysis of the ester, thereby lowering the final concentration of the esters at the thermodynamic equilibrium. These two effects could cause the decrease of the conversion when increasing the phase ratio (w/o).

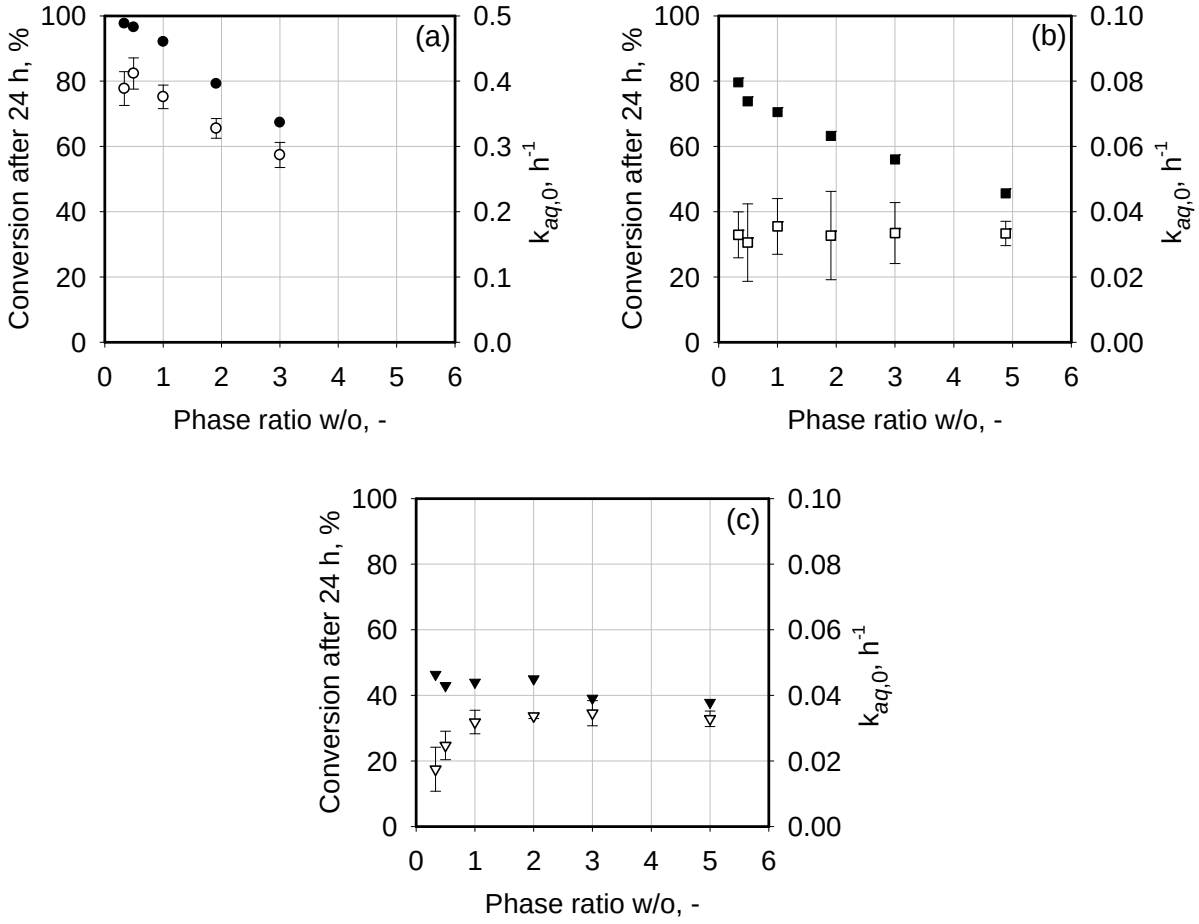


Figure 6-12: Impact of the phase ratio on conversion (closed symbols: ●, ■, ▼) and the aqueous rate constant of the succinic acid initial consumption ($k_{aq,0}$) (open symbols: ○, □, ▽) for its esterification with 1-octanol in biphasic system using DBSA ● (a), Nafion NR-50 ■ (b) and Novozym 435 ▼ (c). Reaction conditions: standard conditions (SC) (see Table 4-1 and Table 4-2) except for the phase ratio (from 0.33 to 5) with a total volume of 10 ml. The ratio mole of succinic acid per mass catalyst was kept constant as in the standard conditions.

As for the **aqueous rate constant**, the impact of the phase ratio was very different depending on the catalyst used. For DBSA, the aqueous consumption rate decreased as the phase ratio increased. This can be caused by several factors. With the increase of the water content, the emulsion with the surfactant DBSA changes from a water-in-oil emulsion at phase ratio < 1 to an oil-in-water emulsion at higher phase ratio. The entrapment of water micelles at ratios < 1 might lower the hydrolysis of the formed esters. Conversely, when the emulsion is reversed to an oil-in-water one, the hydrolysis might be more important due to a larger contact surface of the esters with the water. Furthermore at a very high ratio or a very low ratio, the surface area between the two phases might decrease as one of the two phases is present in large excess. As the catalyst is present at the interface, this might then decrease the observed rate.

Regarding Nafion NR-50, the phase ratio did not seem to have any impact on the aqueous consumption rate. The molar consumption rate of succinic acid is hence only depending on the concentration of succinic acid in the aqueous phase. No inhibition could be observed at high water or alcohol concentration. The loss in conversion might be due to the decreased pure extraction.

Finally, for Novozym 435, the rate increased until the volumetric ratio reached 1 and then the rate was relatively constant. The reaction might be inhibited at high concentrations of alcohols, whereas no inhibition by the water could be observed at high phase ratio. The inhibition of the alcohol would have to be tested separately with different concentrations of 1-octanol in a co-solvent as second phase.

Concluding remarks

The impact of the phase ratio on the rate constant and conversion is very complex, as it has an influence on many different phenomena (e.g. pure extraction of succinic acid, hydrolysis of the esters, interfacial area and inhibition of the alcohol). Even if high phase ratios might be desirable for an industrial process, lower conversions were reported at high water contents, which would prevent from using phase ratios much larger than 1. Consequently, a phase ratio of 1 was found to give the best compromise between industrial requirement (no high excess of 1-octanol) and high rates and conversions. Only for the enzyme, the reaction might be performed at phase ratio higher than 1, as the conversion and rate are not too affected at ratio > 1.

6.2.7 Other process parameters (phase separation and reusability)

6.2.7.1 Phase separation for the homogeneous catalyst

DBSA seems to be a promising alternative for the esterification of succinic acid. Nevertheless, it is also a surfactant and stabilizes therefore the emulsion of the aqueous phase and the 1-octanol. The phase separation at the end of the reaction is hence made difficult. On the laboratory scale, it is realized by centrifugation. However, the centrifugation is quite cost demanding at a production scale. That is why an alternative process step allowing a simple phase separation would be preferable. Many chemicals have an impact on the phase tension between the two phases and can sometimes influence the phase separation. In order to test this possibility, different additives at several concentrations were added to a two-phase system consisting of 5 ml distilled water and 5 ml 1-octanol with 131 mg DBSA. The phases were well mixed and pictures were taken after 20 min, 1 h, 4 h and 17 h to monitor the rate of the phase separation. A blank with no additive was prepared to mimic the long phase separation observed after the esterification with DBSA.

As seen in Figure 6-13 (n° 8), the phase separation of the emulsion of 5 ml distilled water and 5 ml 1-octanol with 131 mg DBSA (blank) did not happen after 1 h, and not even after 17 h at rest. Therefore, it is necessary to find an additive that could allow a fast and simple phase separation. The additives tested in the present study and the masses or volumes added are reported in Table 6-2. Pictures representing the phase separation after 1 h are presented in Figure 6-13.

Table 6-2: Additive for the phase separation of DBSA containing samples.

N°	Additive	Mass / volume added
1	NaCl	500 mg
2	NaCl	250 mg
3	NaCl	50 mg
4	NaCl	5 mg
5	NaOH 10 %	0.5 ml
6	NaOH 10 %	2 ml
7	Hexadecyl trimethyl ammonium bromide	10 mg
8	-	-

The best additive seems to be sodium chloride (NaCl) that allowed a fast phase separation (after only 1 h) at concentrations ≥ 50 mg (see Figure 6-13 (1), (2), (3)). However, concentrations ≥ 250 mg seemed to be necessary to have a clear phase separation. The phase separation at high concentrations of salt can be explained by a reduction of the interfacial tension, destabilizing therefore the emulsion (Sams and Zaouk, 2000).

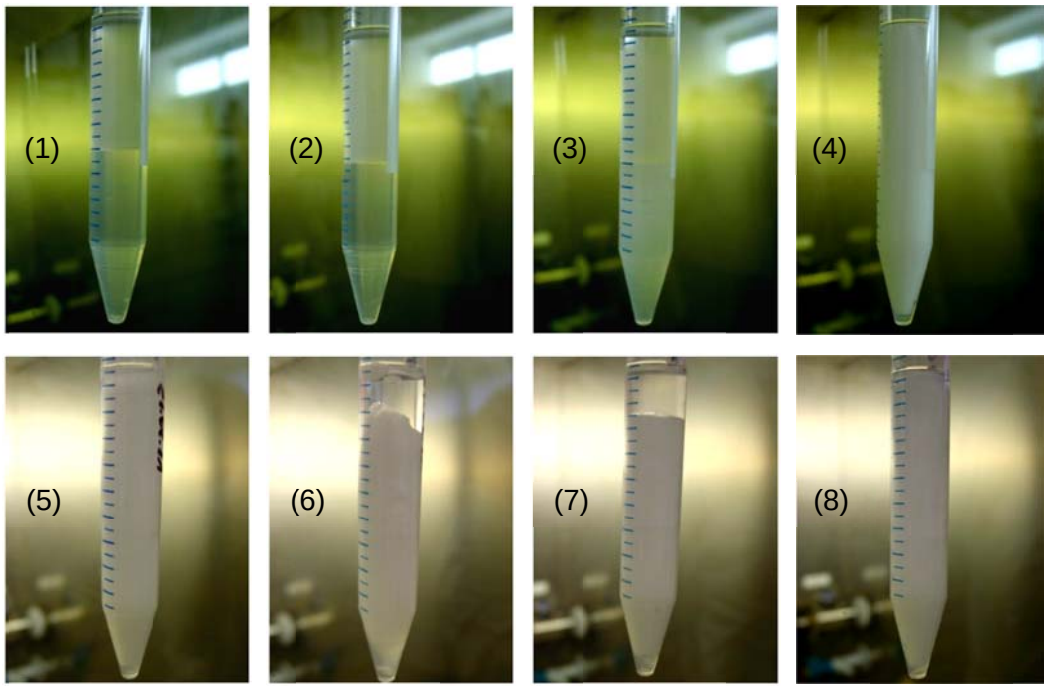


Figure 6-13: Phase separation of a two-phase system consisting of 5 ml distilled water and 5 ml 1-octanol with 131 mg DBSA after 1 h. Additives introduced in the emulsion: NaCl 500 mg (1), 250 mg (2), 50 mg (3), 5 mg (4), NaOH 10 % 0.5 ml (5), 2 ml (6), Hexadecyl trimethyl ammonium bromide 10 mg (7) and no additive (8).

Concluding remarks

Sodium chloride (NaCl), a very cheap additive, allowed a simple phase separation of the "water / 1-octanol / DBSA" system after only 1 h. This strategy could be used at an industrial scale to prevent performing an expensive centrifugation. With this simple separation method, the surfactant DBSA remains a potential catalyst for the biphasic esterification of succinic acid for industrial applications.

6.2.7.2 Reusability for the heterogeneous catalysts

In order to increase the industrial potential of heterogeneous catalysts, they should be reused several times to lower the catalyst costs. The recycling of the catalyst is therefore of great importance and will be examined in this Subsection. The heterogeneous enzyme

– Novozym 435 – could unfortunately not be recycled in the present study, as the S-stirrers used for the reaction crushed part of the beads during the 24 hours of reaction. Therefore, only the recycling of Nafion NR-50 was studied here. Little is mentioned in the literature about the washing / regeneration methods for the recycling of Nafion NR-50. Doyle and Plummer (1993) washed successively the Nafion NR-50 resin with acetone, water and 25 % nitric acid. The resin was finally washed several times with water until a neutral pH was obtained. In order to test if a regeneration step with strong acid is necessary, different washing methods were tested in this study. They are presented in Table 6-3. Water, acetone and/or HCl 10 % were used for the washing steps. The resin was dried at 80 °C either for 2 h or overnight. After each reaction, the resin was recovered by filtration, washed and dried following the chosen method and finally reused for a next esterification cycle. The changes in the appearance of the beads before and after the 1st first washing / drying procedure and after the 4th washing / drying procedure are presented in Figure 6-14.

Table 6-3: Method of washing and drying of the Nafion NR-50 beads for their recycling.

Method	1st washing step (30 min)	2nd washing step (30 min)	3rd washing step (1h)	Drying step
A	10 ml water	10 ml water	10 ml water	2 h at 80 °C
B	10 ml acetone	10 ml acetone	10 ml acetone	2 h at 80 °C
C	10 ml water	10 ml acetone	10 ml water	2 h at 80 °C
D	10 ml water	10 ml HCl 10%	10 ml water*	2 h at 80 °C
E	10 ml water	10 ml water	10 ml water	overnight at 80 °C
F	10 ml acetone	10 ml acetone	10 ml acetone	overnight at 80 °C

* = replace the water every 10 min until pH ≈ 6-7

As seen in Figure 6-14, the appearance of the Nafion NR-50 beads changed significantly after the washing / drying steps, even after the first cycle. The changes of color happened after the drying of the beads. The color of the beads washed in the end with acetone turned to dark-brown (see (B) and (F) for (ii) and (iii)). Furthermore, they are a bit smaller than the other ones. The beads washed with water and dried overnight in the oven at 80 °C showed also a small color change from transparent to orange-brown (see (E) for (iii)). Acetone is probably evaporated much faster from the resin than the water.

This fast drying might cause changes in the structure of the polymer, leading to a darker coloration. This phenomenon has been also observed by Doyle and Plummer (1993).

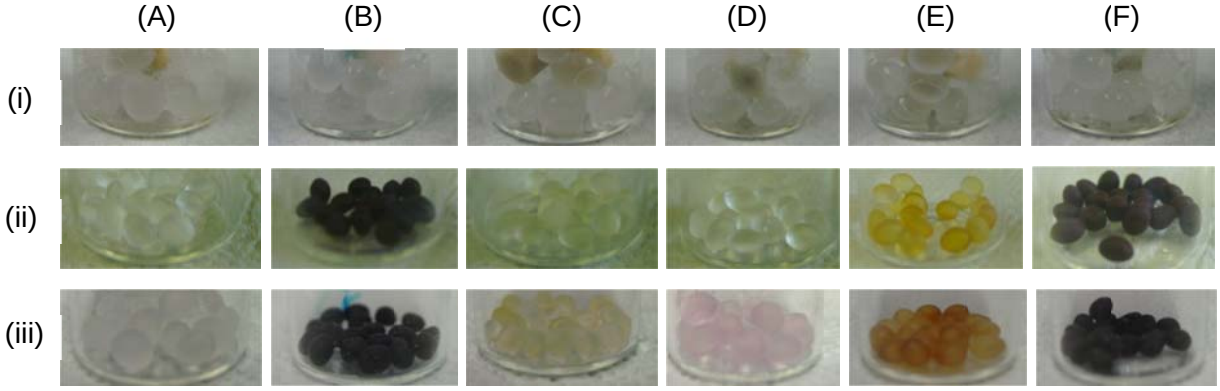


Figure 6-14: Recycling of the Nafion NR-50 beads for the esterification of succinic acid in distilled water: appearance of the beads before (i) and after (ii) the 1st washing / drying procedure and finally after the 4th washing / drying procedure (iii), using six different washing / drying methods: (A), (B), (C), (D), (E) and (F) (see Table 6-3).

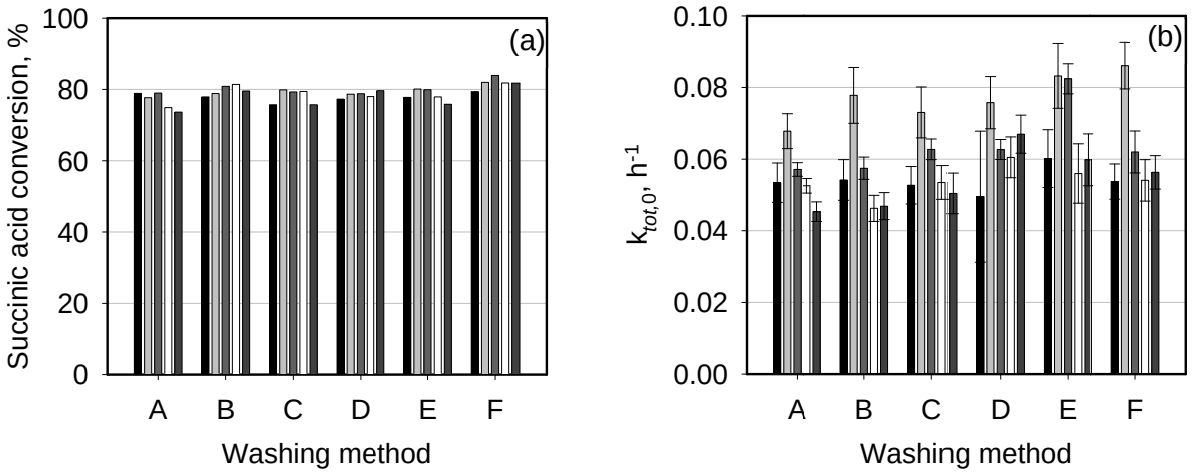


Figure 6-15: Impact of the washing method on the recycling of Nafion NR-50 for the esterification of succinic acid in distilled water: (a) succinic acid conversion, (b) total rate constant of the succinic acid initial consumption ($k_{tot,0}$). Cycle: 1st ■, 2nd ▒, 3rd ▓, 4th □ and 5th ▓. Washing methods: A & E: three times with water; B & F: three times with acetone; C: water, acetone, water; D: water, aqueous HCl 10 %, water; A to D drying for 2 h at 80 °C, E and F drying over night at 80 °C. Reaction conditions: optimal conditions (OC) for Nafion NR-50 (see Table 6-4).

Even if this surprising change in colours happened throughout the recycling, high conversions (74 to 84 %) and high rate constants (0.045 ± 0.002 to 0.086 ± 0.006 h^{-1}) could be kept for 5 cycles as presented in Figure 6-15. The resin is hence still active despite its dark color. The sulfonic acid groups of the resin are probably not affected by the structural change of the resin.

Concluding remarks

Even if a change in appearance could be observed while washing and recycling the Nafion NR-50 beads, they could maintain high conversions and activities for the biphasic esterification of succinic acid with 1-octanol during 5 cycles, regardless of the washing and drying procedure. These beads might be recycled for many cycles in order to lower the catalyst costs.

6.2.8 Optimized conditions

From the "one-at-a-time" optimization, the impact of different parameters on the conversions and the rates could be tested (see in Sections 6.2.1 to 6.2.3). Sets of potential optimal conditions (see Table 6-4) were therefore derived from these single-variable tests. These sets of reaction conditions were tested for the esterification of succinic acid with 1-octanol in biphasic systems using DBSA, Nafion NR-50 and Novozym 435. The comparison of the conversions and the rate constants in the standard conditions and the optimized conditions is reported in Table 6-5 (entries 1 to 3).

Table 6-4: Standard (SC) and potential optimal (OC or OC 1) reaction conditions for the biphasic esterification of succinic acid with 1-octanol using chemical catalysts.

	DBSA		Nafion NR-50		Novozym 435	
	SC	OC	SC	OC	SC	OC 1
Succinate	0.8 M	0.8 M	0.8 M	0.8 M	0.15 M	0.8 M
Aqueous phase	phosphate buffer*	phosphate buffer*	phosphate buffer*	phosphate buffer*	phosphate buffer*	phosphate buffer*
pH	2	2	2	2	4	3
Organic phase	1-octanol	1-octanol	1-octanol	1-octanol	1-octanol	1-octanol
Catalyst mass	0.131 g	0.131 g	0.5 g	0.75 g	0.04 g	0.06 g
Temperature	80 °C	90 °C	80 °C	90 °C	50 °C	70 °C
Aqueous phase	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Organic phase	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Agitation speed	1000 rpm	1000 rpm	1000 rpm	1000 rpm	800 rpm	800 rpm

* : phosphate concentration at 43.35 M

For DBSA and Nafion NR-50, the potential optimal conditions derived from the study of all variables separately allowed the increase of the total rate constant by a factor 1.5 for DBSA and 2.3 for Nafion NR-50. DBSA hence enabled the reaction to reach the equilibrium at 91 % conversion after only 8 h of reaction. The optimization for Nafion NR-50 allowed an increase of the conversion after 24 h from 66 to 84 %.

Concerning Novozym 435, the set of potential optimized parameters gave surprisingly a complete deactivation of the enzyme, with only 22 % conversion, mostly corresponding to the pure extraction of succinic acid into the 1-octanol phase at pH = 3.

Table 6-5: Comparison of the standard conditions (SC) and the potential optimal conditions (OC or OC 1) for the esterification of pure aqueous solution of succinic acid with 1-octanol in biphasic systems.

N°	Catalyst	X (SC)	X (OC 1)	Total initial rate constant (SC), h^{-1}	Total initial rate constant (OC 1), h^{-1}
1	DBSA	89 % (8 h)	91 % (8 h)	0.26 ± 0.02	0.39 ± 0.02
2	Nafion NR-50	66 % (24 h)	84 % (24 h)	0.029 ± 0.003	0.067 ± 0.004
3	Novozym 435	47 % (24 h)	22 % (24 h)	0.032 ± 0.002	0

Concluding remarks

This simple optimization was realized by taking the optima of the tests on each variable separately. However, taking the set of those reaction conditions found by separate screenings as the optimal set of conditions (OC) supposes that the variables are independent. This approach worked well with DBSA and Nafion NR-50, but the variables cannot always be considered as independent, as here for the enzyme. For example, temperature and pH have both impacts on the 3D-structure of the enzyme. At high temperature, the hydrogen bonds are weakened. Lower pH can cause changes in the electrostatic interactions between the charged amino acids. Finally, low pH and high temperature can cause deamination through peptide bond hydrolysis. As both pH (from 4 to 3) and temperature (from 50 °C to 70 °C) were simultaneously varied when testing the OC, a modification of the 3D structure of the enzyme might have happened.

Contrary to the chemical catalysts, this simple approach using an optimization of each reaction condition in turn can therefore not be used for the enzyme. A multiple-variable

optimization taking into account their interactions had thus to be performed for the enzymatic esterification and it will be reported in Subchapter 6.3.

6.2.9 Conclusions

During the reaction condition screening, it has been shown that while the succinic acid concentration and the agitation had no significant effect on the total rate constants of the succinic acid initial consumption and the conversions, the volumetric phase ratio, the catalyst concentration and especially the pH and the temperature had an important impact. The optimal conditions derived from separate variable screening allowed an optimization of conversions and rates for the two chemical catalysts. However, the set of conditions derived from the "one-at-a-time" screening for the enzyme led to its complete deactivation. A multiple-variable optimization was therefore necessary for the optimization of the reaction conditions for the enzymatic reaction.

6.3 Three-variable optimization of the enzymatic esterification in biphasic systems

A multi-variable screening was required for the optimization of the reaction conditions of the enzymatic esterification. For doing so, the following three input variables were considered: temperature, pH and substrate concentration. These three variables were indeed those, which varied most from the standard conditions (SC) to the potential optimal conditions (OC 1) tested in Section 6.2.8. The experimental design consists in optimally planning a series of experiments and fitting the "responses" (i.e. total initial rate constant of succinic acid and the conversion after 6 h) with second order polynomial functions (see Subsection 4.7). The temperature was varied between 30 °C and 70 °C, the concentration of succinic acid from 0.15 M to 0.8 M and the pH from 2 to 4.

6.3.1 Optimization of the total initial rate constant

6.3.1.1 Model fitting and validation

Using the Response Surface Methodology, the total rate constant of the succinic acid initial consumption was first modelled with the succinic acid concentration (C), the initial pH (pH) and the reaction temperature (T) as input variables. The raw data of the RSM optimization are presented in Table 9-16 (see Annex). The equation (4-15) in Subchapter 4.7 was adapted for this set of input and output variables and the fitted surface was finally represented by equation (6-2). The first order term in pH, the

interaction coefficients of C and pH, and C and T were not kept in the final equation (6-2) as they were not significant. The statistical parameters of the fitting are given in Table 6-6. The response surface obtained for the initial reaction rate is presented in Figure 6-16.

$$k_{tot,0} = 0.0335 - 0.0022 \cdot C^* + 0.0072 \cdot pH^* + 0.0075 \cdot pH^* \cdot T^* + 0.0083 \cdot C^{*2} - 0.0125 \cdot pH^{*2} - 0.0131 \cdot T^{*2} \quad (6\text{-}2)$$

with	$k_{tot,0}$	*initial total rate constant*	h^{-1}
	C^*	*initial concentration of succinic acid*	*M*
	pH^*	*initial pH*	-
	T^*	*reaction temperature*	°C

N.B.: *C^*, pH^* and T^* are "coded" variables (see Subsection 4.7).*

Validating the model is an important step of the modelling. This validation was realized with a new set of data (i.e. other than the central composite design (CDD) ones) and is presented in Figure 6-17. The validation raw data are given in Table 9-17 (see Annex). The validation was satisfying. The model slightly overestimated the rates but the only point () really out of scale was the former optimal point from the "one-at-a-time" optimization (i.e. the reaction conditions OC 1: 70 °C, pH = 3 and 0.8 M) where a complete deactivation was observed. The model does not predict such a complete deactivation. The other points are well predicted by the model. The experimental R^2 was of 0.7876.

Table 6-6: Statistical parameters of the surface fitting for the total initial rate constant.

Statistical parameter	Value
R^2	0.9558
Adjusted R	0.9227
P value	$5.31 \cdot 10^{-5}$

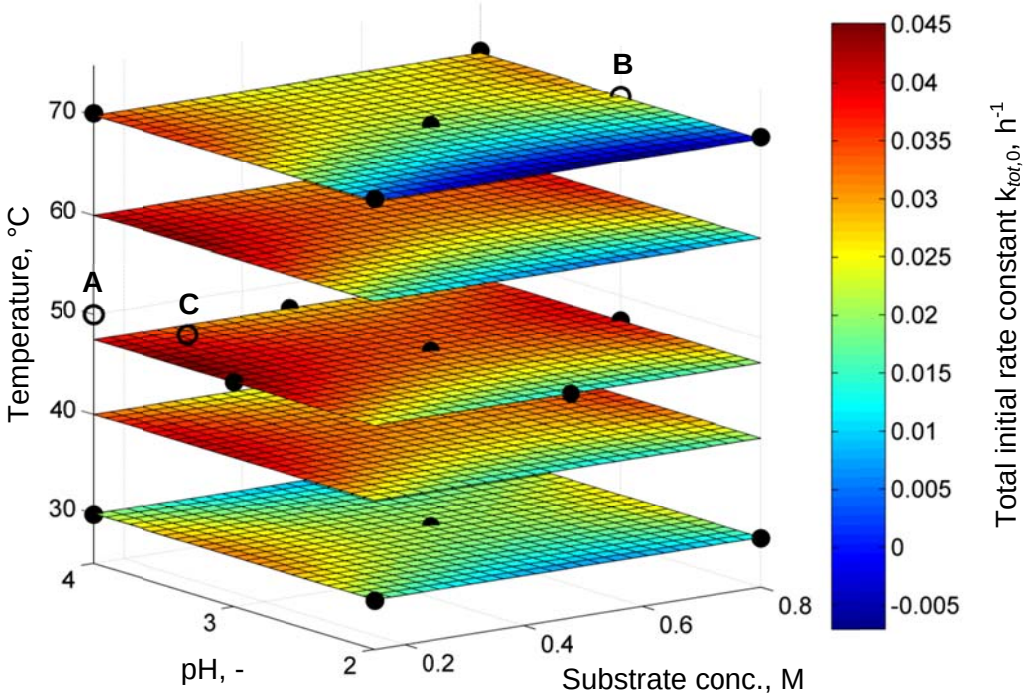


Figure 6-16: Response surface of the total initial rate constant with respect to the three variables: temperature, pH and succinic acid initial concentration. The reactions were performed in parallel reactors with 40 mg Novozym 435, 5 ml aqueous solutions of succinic acid and 5 ml 1-octanol, 800 rpm. The black points ● represent the experimental data points. The open circle ○ "A" (50 °C, pH = 4, 0.15 M) represents the standard conditions. The open circle ○ "B" (70 °C, pH = 3, 0.8 M) represents the optimized conditions from the "one-at-the-time" optimization. The open circle ○ "C" represents the optimal conditions found with the Response Surface Methodology (RSM) for the initial rate.

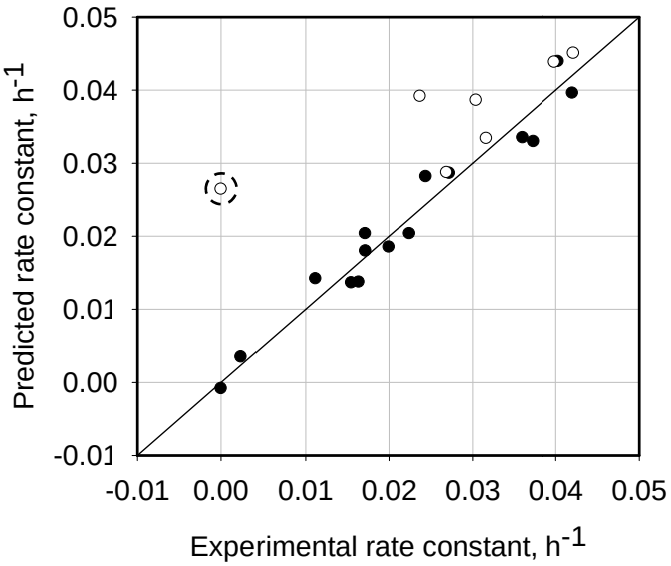


Figure 6-17: Validation of the model for the total initial rate constant. Predicted rate by the model *vs.* experimental rate measured in the parallel reactor unit with 40 mg of Novozym 435, 5 ml aqueous phase and 5 ml 1-octanol and with an agitation rate of 800 rpm (● = data set used for the fitting, ○ = validation data set).

6.3.1.2 Determination of the optimal conditions

The influence of the three variables (substrate concentration, pH and temperature) can be derived from the partial derivatives of $k_{tot,0}$ presented in equations (6-3) to (6-5).

$$\frac{\partial k_{tot,0}}{\partial C^*} = -0.0022 + 0.0166 \cdot C^* \tag{6-3}$$

$$\frac{\partial k_{tot,0}}{\partial pH^*} = 0.0072 + 0.0075 \cdot T^* - 0.0250 \cdot pH^* \tag{6-4}$$

$$\frac{\partial k_{tot,0}}{\partial T^*} = 0.0075 \cdot pH^* - 0.0262 \cdot T^* \tag{6-5}$$

The **inital concentration of succinic acid** is not coupled to the other two variables, as shown in equation (6-3). The extremum of $k_{tot,0}$ in C^* is therefore independent of pH^* and T^*. This minimum (see negative 1st-order coefficient and positive 2nd-order) lies around C^* = 0.133 (i.e. C = 0.518 M). This can be observed in Figure 6-16, where a relatively flat minimum, independent of T and pH, can be seen. Over the range of concentrations considered (0.15 to 0.8 M), the maximal rate constants can therefore be observed at the lower end of the range (i.e. C ~ 0.15 M), since the minimum is slightly shifted to the higher concentrations.

The two larger coefficients of equations (6-2) are the 2nd-order ones in **temperature and pH**, indicating that these two factors have the highest impact on the rate constant. Furthermore, a clear coupling could be seen between the two variables, as shown in equations (6-2) or (6-4) and (6-5). The coupling between the pH and the temperature can be observed in Figure 6-16 where, for example, for C = 0.15 M, the pH maximizing the rate constant increases linearly in temperature. The extremum is, however, independent of the concentration. This maximum (see positive 1st-order coefficient in pH and negative 2nd-order coefficients in pH and temperature) was obtained by setting the two partial derivatives in both pH and temperature to zero. This maximum lies at pH^* = 0.315 and T^* = 0.090 (i.e. pH = 3.33 and T = 51.8 °C).

The optimal conditions derived from the model were thus: T = 51.8 °C, pH = 3.33 and 0.15 M of succinic acid and the predicted rate was 0.0451 h^{-1}. The rate determined experimentally at 52 °C, pH = 3.33 and 0.15 M was of 0.0421 ± 0.0014 h^{-1}. The deviation from the predicted rate to the experimental value is extremely small and corresponds to ~ 7 % error.

Concluding remarks

From the RSM optimization, the influence and the coupling of the concentration, pH and temperature could be studied in more detail. The concentration was not coupled to the other two variables and showed a flat minimum in rate constant over the range tested. The temperature and the pH had a major and coupled impact on the rate constant. They led to a maximum in rate constant lying around the centre of the pH and temperature ranges tested. A new set of optimal reaction conditions was thus derived from this optimization and gave the best total initial rate constant so far. From the standard conditions (50 °C, pH = 4, 0.15 M, total initial rate constant: 0.0304 ± 0.0017 h^{-1}), the initial rate constant was hence multiplied by 1.4. The three-variable optimization was therefore successful.

Finally, it could be observed in Figure 6-17 that, with the optimal set of reaction conditions derived from the "one-at-a-time" approach (open circle B), the three variables were varied simultaneously from the initial conditions (open circle A), likely causing the changes in the 3D-structure of the enzyme which led to its total deactivation. The choice of high temperature was certainly detrimental but, if only two variables, e.g. the temperature and the pH or the concentration and the pH, had only been varied, a total deactivation would not have taken place.

6.3.2 Optimization of the conversion after 6 h

6.3.2.1 Model fitting and validation

A second empirical model was identified for the conversion after 6 h. However, it must be first noted that the conversion of the reactive extraction is defined by the succinic acid that disappears from the aqueous phase. This can be caused by two different phenomena: first the pure extraction of succinic acid from the aqueous phase into the organic phase without reacting and second the conversion of the succinic acid into its esters. On the one hand, the pure extraction of succinic acid is strongly dependent on the pH since only the diprotonated form of succinic acid can be extracted in the 1-octanol phase. On the other hand, the transformation of succinic acid into its esters is related to the consumption rate constant, the optimization of which has been reported in Subsection 6.3.1.1. Therefore, it is expected that, because of the higher pure extraction at lower pHs, the optimal reaction parameter range obtained for the conversion model will be slightly shifted towards lower pHs from the one obtained for the model of the rate constant.

The identified polynomial function is given by equation (6-6). The interaction parameter of C and pH was not kept in the final equation, as it was not significant. The statistic parameters of the fitting are given in Table 6-7. The response surface obtained for the conversion after 6 h is presented in Figure 6-18.

$$X_{6h} = 35.189 - 4.5900 \cdot C^* - 1.3700 \cdot pH^* - 1.1100 \cdot T^* - 2.8625 \cdot C^* \cdot T^* + 5.4875 \cdot pH^* \cdot T^* + 4.7389 \cdot C^{*2} - 10.3611 \cdot pH^{*2} - 10.3611 \cdot T^{*2} \quad (6\text{-}6)$$

with	X_{6h}	*conversion after 6 h*	*%*
	C^*	*initial concentration of succinic acid*	*M*
	pH^*	*initial pH*	-
	T^*	*reaction temperature*	*°C*

N.B.: *C^*, pH^* and T^* are "coded" variables (see Subsection 4.7).*

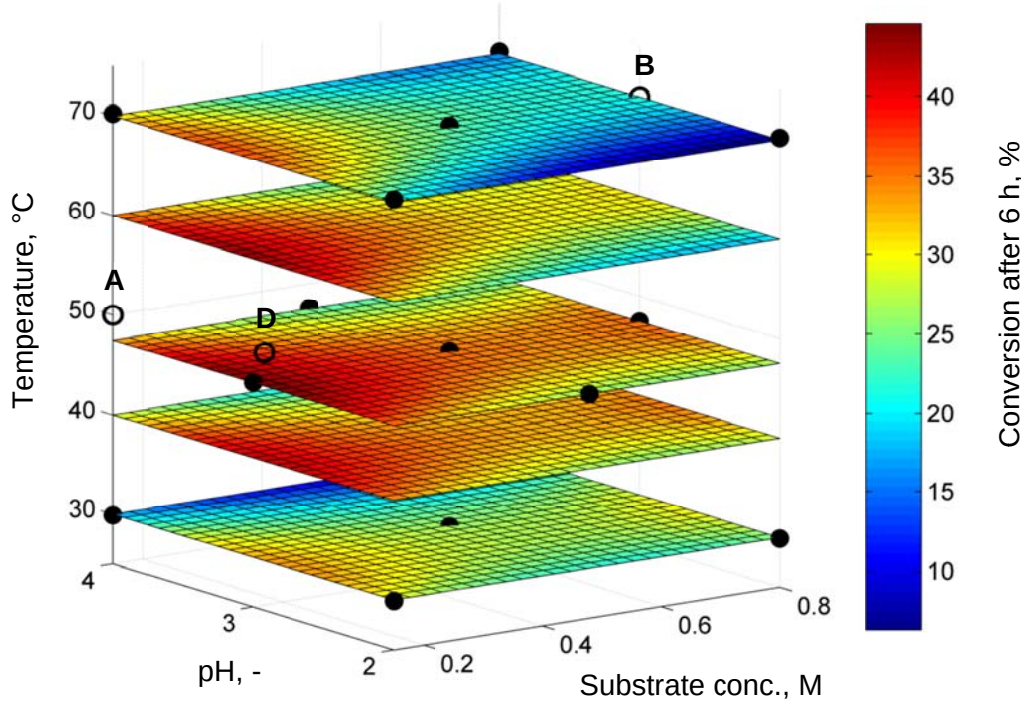


Figure 6-18: Response surface of the conversion after 6 h with respect to the three variables: temperature, pH and succinic acid initial concentration. The reactions were performed in parallel reactors with 40 mg Novozym 435, 5 ml aqueous solutions of succinic acid and 5 ml 1-octanol, 800 rpm. The black points ● represent the experimental data points. The open circle ○ "A" (50 °C, pH = 4, 0.15 M) represents the standard conditions. The open circle ○ "B" (70 °C, pH = 3, 0.8 M) represents the optimized conditions from the "one-at-the-time" optimization. The open circle ○ "D" represents the optimal conditions found with the Response Surface Methodology (RSM) for the conversion.

Table 6-7: Statistical parameters of the surface fitting of the conversion after 6 h.

Statistical parameter	Value
R^2	0.9608
Adjusted R	0.9087
P value	0.001097

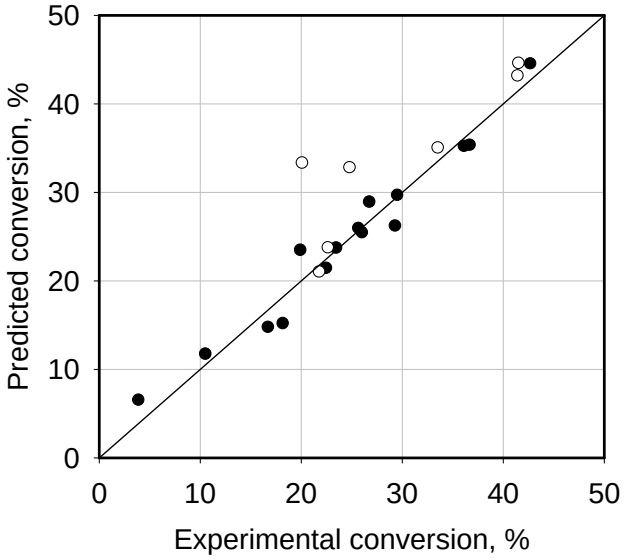


Figure 6-19: Validation of the model for the conversion after 6 h. Predicted conversion by the model *vs.* experimental conversion measured in the parallel reactor unit with 40 mg of Novozym 435, 5 ml aqueous phase and 5 ml 1-octanol and with an agitation rate of 800 rpm (● = data set used for the fitting, ○ = validation data set).

The model validation was also realized with a new set of data and is presented in Figure 6-19. The validation was successful. The new conversions are well predicted by the model. The experimental R^2 was of 0.9320.

6.3.2.2 Model validation and determination of the optimal conditions

Similarly to what was done for the rate constant, the partial derivatives were calculated and are presented in equations (6-7) to (6-9).

$$\frac{\partial X_{6h}}{\partial C^*} = -4.5900 - 2.8625 \cdot T^* + 9.4778 \cdot C^* \quad (6\text{-}7)$$

$$\frac{\partial X_{6h}}{\partial pH^*} = -1.3700 + 5.4875 \cdot T^* - 20.7222 \cdot pH^* \quad (6\text{-}8)$$

$$\frac{\partial X_{6h}}{\partial T^*} = -1.1100 - 2.8625 \cdot C^* + 5.4875 \cdot pH^* - 20.7222 \cdot T^* \quad (6\text{-}9)$$

Contrary to the model identified for the rate constant, all variables are coupled. For the **initial concentration of succinic acid**, the conversion passes through a minimum (negative 1st-order coefficient and positive 2nd-order one) that depends also on the temperature (see equation (6-7)). As with the model of the rate constant, the minima are slightly shifted to the higher concentration, as observed in Figure 6-18, and accordingly the maximal conversions on the concentration range tested will be found at the lowest end of the range (i.e. C ~ 0.15 M). Notwithstanding, it should be noted, that, contrary to what was observed for the rate constant in Figure 6-16, the conversion stays relatively low, when increasing the concentrations up to 0.8 M. High concentrations of succinic acid seem hence to be more detrimental for achieving high conversions than getting high rate constant.

As with the rate constant model, the larger coefficients are the 2nd-order ones in **temperature and pH**, indicating that these two variables have the highest impact. From equations (6-8) and (6-9), it can be observed that the maximum in pH is also depending on the temperature, whereas the maximum in temperature depends on the pH and to a lesser extent on the concentration. As low concentrations are desirable, it can be calculated from the same two equations that, for C = 0.15 M (i.e. C^* = -1), the maximum of the conversion is obtained at pH^* = -0.046 and T^* = 0.072 (i.e. pH = 2.95 and T = 51.4 °C). For a succinic acid concentration of 0.15 M, the coupled effect of the temperature and the pressure on the conversion, which can be seen in Figure 6-18, is very similar to the one observed previously for the rate constant in Figure 6-16.

Finally, the optimal conditions derived from the model were: T = 51 °C, pH = 2.95 and 0.15 M of succinic acid. Under the optimal set of conditions, the predicted conversion was of 44.6 %, whereas the experimental value was of 42.4 ± 2.1 %.

Concluding remarks

The empirical polynomial model identified for the conversion showed a more complex interaction of the three variables than the rate constant one. Since both the esterification and the extraction influence the conversion and show different

dependence in concentration, pH and temperature, a more complex coupling was obtained for the model of the conversion. Similarly to what was found for the model of the rate constant, a minimum of the conversion was observed over the tested concentration range, so that the best conversions were obtained at C ~ 0.15 M. On the contrary, a maximum was obtained over the temperature and pressure ranges. As expected, the optimal conditions found for the conversions are quite similar to those obtained for the initial rate constant. However, the optimal pH is slightly lower here, which is not surprising, since at lower pHs, the pure extraction of the diprotonated acid is more efficient. Finally, the optimization of the conversion allowed its increase from 25 % (in the standard conditions) to 42 % (in the optimal ones).

6.3.3 Optimized conditions

The Response Surface Methodology (RSM) was used to optimize both the total initial rate constant of succinic acid and the conversion after 6 h. Therefore, two slightly different sets of optimal conditions were obtained, which gave either the optimal total initial rate constant or the optimal conversion.

The reason for performing these two optimizations is that they correspond to two different options regarding the process of interest. As mentioned before, the conversion is defined as the percentage of the extracted succinic acid out of the aqueous phase at a certain time either converted in its esters or purely extracted (see definition in Section 4.6.2). Therefore by optimizing the conversion, the pure extraction of succinic acid into the organic phase is indirectly optimized as well. If the goal of the process is to purify succinic acid and therefore extract it from the fermentation broth in its normal or esterified form, an optimization of the conversion should be performed.

If the process of interest is the production of the diesters of succinic acid, the total initial rate constant should be taken as the parameter to be optimized. As the pure extraction of succinic acid is almost instantaneous, the observed initial consumption rate is indeed characteristic of the esterification of succinic acid.

Finally, the sets of optimal conditions obtained from the two models led to extremely similar conversions and rate constants (41.4 ± 1.6 % *vs.* 42.4 ± 2.1 % after 6 h and 0.0421 ± 0.0014 h^{-1} *vs.* 0.0401 ± 0.0028 h^{-1} for the models of the rate constant and of the conversion resp.). The optimal set of conditions obtained for the total initial rate constant

was eventually chosen as the optimal one, since the pH was a bit higher and fermentations are normally realized at higher pHs.

These optimal conditions (labelled as OC 2 and presented in Table 6-8) derived from the three-variable RSM optimization were then compared with the standard conditions in 24 h experiments and results are presented in Table 6-9. Through an increase of the total initial total rate constant by 1.3 times, the conversion after 24 h could be highly enhanced, from 47 % in the standard conditions to 72 % in the optimal conditions (OC 2).

Table 6-8: Standard (SC) and optimized (OC 1 and 2) reaction conditions for the biphasic enzymatic esterification of succinic acid with 1-octanol. OC 1: optimized conditions from the "one-at-the-time" optimization, OC 2: optimized conditions from the three-variable RSM optimization (for the total initial rate constant).

	SC	OC 1	OC 2
Succinate	0.15 M	0.8 M	0.15 M
Aqueous phase	phosphate buffer*	phosphate buffer*	phosphate buffer*
pH	4	3	3.33
Organic phase	1-octanol	1-octanol	1-octanol
Catalyst mass	0.04 g	0.06 g	0.04 g
Temperature	50 °C	70 °C	51 °C
Aqueous phase	5 ml	5 ml	5 ml
Organic phase	5 ml	5 ml	5 ml
Agitation speed	800 rpm	800 rpm	800 rpm

* : phosphate concentration 43.35 M

Table 6-9: Comparison of the standard conditions (SC) and the optimal conditions (OC 2) for the enzymatic esterification of pure aqueous solution of succinic acid with 1-octanol in biphasic systems. Optimization realized with the Response Surface Methodology.

N°	Catalyst	X (SC)	X (OC 2)	Total initial rate constant (SC), h^{-1}	Total initial rate constant (OC 2), h^{-1}
4	Novozym 435	47 % (24 h)	72 % (24 h)	0.032 ± 0.002	0.042 ± 0.001

Concluding remarks

Finally, it should be noted, that, while the simple "one-at-a-time" optimization for the enzymatic esterification reported in Subchapter 6.2 was unsuccessful, the three-variable optimization realized with the RSM gave the highest initial rate constant reported so far. This underlines the importance of applying an appropriate optimization methodology. Enzymes are indeed highly sensitive to a lot of reaction conditions, since the protein folding responsible for their activity is depending on, among others, the pH, the temperature and the ionic strength (Kelly, 1996).

Lastly, the RSM approach yields a deeper insight into the evolution of the two output variables of interest, i.e. conversion and rate constant, as a function of the input variables. From Figure 6-16 and Figure 6-18, it appears, for instance, that, at temperatures around 50 °C, and for pH around 3, the substrate concentration could be increased up to 0.8 M, without too much decreasing the rate constant and the conversion values (i.e. ~ 0.04 h^{-1} and ~ 35 % after 6 h). This potential use of high concentrations of succinic acid without a high decrease in rate constant and conversion is very important, since the goal of this study is to use real fermentation broth, in which the succinic acid titer might reach 146 g l^{-1} (i.e. 1.24 M).

6.4 Process integration

In Subchapters 6.2 and 6.3, the optimization of the reaction conditions for the esterification of pure solutions of succinic acid with DBSA, Nafion NR-50 and Novozym 435 was presented. The process should now be tested on real fermentation broth, as the goal of this project is the esterification of biotechnologically produced succinic acid. In Section 6.4.1, different medium components and by-products from the fermentation will be tested separately in solutions of succinic acid in distilled water, with only one additional chemical at a time. The emulsion problem encountered with DBSA will be addressed in Section 6.4.2. The comparison of the three catalysts for the esterification of succinic acid in real fermentation broth will be next reported in Section 6.4.3. Then, Section 6.4.4 will describe how, using the best catalyst, the esterification process could be scaled up to 200-ml and the ester could be recovered after the reaction and purified. Finally, different uses of the obtained esters will be presented and discussed in Section 6.4.5.

6.4.1 Impact of the fermentation by-products for the three catalysts

First, the impact of different medium components and by-products should be assessed separately. To do so, several solutions of pure succinic acid were prepared in distilled water with one additional compound, such as a medium component or a by-product. These additional chemicals were selected from the compounds often found in *E. coli* fermentation broths for the production of succinic acid (Lu et al., 2009). The solutions were tested in the optimal conditions derived previously for the three catalysts and compared both with the pure solution of succinic acid and with a real fermentation broth from *E. coli* containing 0.257 M of succinic acid (see Section 4.4.1). The pH of the fermentation broth was set at pH = 2 for the chemical catalysts and at pH = 3.33 for the enzyme by addition of HCl. The results are summarized in Table 6-10.

For the chemical catalysts, only the high concentration of phosphate salts (entry 5) seemed to have a negative impact on the final conversions (83 % *vs.* 91 % for DBSA and 67 % *vs.* 84 % for Nafion NR-50 resp. with and without phosphate salts (entries 5 and 15)). The other medium components and by-products did not change the final conversions much (89 % to 91 % for DBSA and 80 % to 84 % for Nafion NR-50). As for the real fermentation broth (entry 16), the conversion achieved with DBSA (78 %) was sightly lower than the one obtained in pure phosphate buffer (entry 5), whereas for Nafion NR-50, the conversion reached with the real fermentation broth (70 %) was a bit higher.

In the case of Nafion NR-50, the high concentration of phosphate salts might be responsible for the exchange of small cations (K^+ and Na^+) with the H^+ of the resins, partially deactivating the catalyst. The high salt concentrations contained in the fermentation broth probably caused a similar deactivation.

As for DBSA, the lower conversions obtained in the case of high phosphate concentration and in the fermentation broth could be explained by the destabilisation of the emulsion in presence of salts. This fact could be assessed visually, as the reaction mixture becomes white when a 1-octanol / water emulsion is formed, whereas it remains clear when no stable emulsion is created. At high concentration of phosphate salts and for the fermentation broth, the solutions were here almost transparent. The presence of salts might indeed have reduced the interfacial tension and destabilized the emulsion (Sams and Zaouk, 2000). Furthermore, it could be shown that by adding the simple salt NaCl, the emulsion of 5 ml of distilled water with 5 ml of 1-octanol and 131 mg DBSA was disrupted at concentrations of the salt ≥ 10 g l^{-1} (see Section 6.2.7). The concentrations

added for entry 5 are in this range. Other salts have also been tested (entries 6 to 8) but in much lower concentrations. The high salt concentration contained in the fermentation broth probably disturbed the emulsion similarly. If the emulsion is not formed, the surface area where the surfactant catalyst is in contact with the two phases is strongly reduced and so are therefore the activity and the final conversions.

Table 6-10: Impact of the medium components and by-products from the fermentation broth on the esterification of succinic acid with 1-octanol using DBSA, Nafion NR-50 and Novozym 435 at the optimal reaction conditions.

N°	Components	Conc, g l^{-1}	DBSA Conv (8 h)	Nafion NR-50 Conv (24 h)	Novozym 435 Conv (24 h)
5	$Na_2HPO_4.12H_2O$; KH_2PO_4; $(NH_4)_2HPO_4$	4; 8; 8	83	67	70
6	NH_4Cl	0.20	91	81	68
7	$(NH_4)_2SO_4$	0.75	90	80	66
8	$MgSO_4.7H_2O$	1	91	83	67
9	Glucose	5	91	83	70
10	Ethanol	1	91	81	74
11	Acetate	1	89	83	76
12	Formiate	1	91	82	77
13	Pyruvate	5	90	82	74
14	Lactate	1	91	84	75
15	-	-	91	84	68
16	Fermentation broth	-	78	70	70

As for the enzymatic catalysts, the conversions varied less (from 66 % to 75 %) even with high phosphate concentrations. Small increases of the conversions could be observed when other carboxylic acid salts or ethanol were present. However, these small variations are difficult to explain and no real tendency could be derived from these data. Finally, the conversions, obtained with the fermentation broth (entry 16) and the pure solution of succinic acid (entry 15), were very similar (70 % and 68 % resp.). The enzyme is therefore not inhibited by any component or by-product from the fermentation broth.

Concluding remarks

Succinic acid from fermentation broth could be esterified efficiently with the three catalysts with conversions of 78 % for DBSA and 70 % for both Nafion NR-50 and Novozym 435. The high concentrations of salts are slightly reducing the conversions for DBSA and Nafion NR-50, due to emulsion destabilisation and ion exchange, respectively. However, no real inhibition of fermentation by-products or medium components could be observed and satisfactory conversions were maintained. These three catalysts are hence potential catalysts for industrial applications of the esterification of succinic acid in fermentation broth.

6.4.2 Emulsion in the fermentation broth with DBSA

As it was shown in Section 6.4.1, the high salt concentration present in the fermentation broth limits the emulsion formation and hence decreases the final conversion obtained after 24 h. This could be a problem for processes at the industrial scale. Higher concentrations of the surfactant DBSA were thus introduced in the fermentation broth, in order to restore the emulsion, increase the final conversion and shorten the reaction time. The screening of different masses of DBSA for the esterification of succinic acid in the fermentation broth is presented in Figure 6-20.

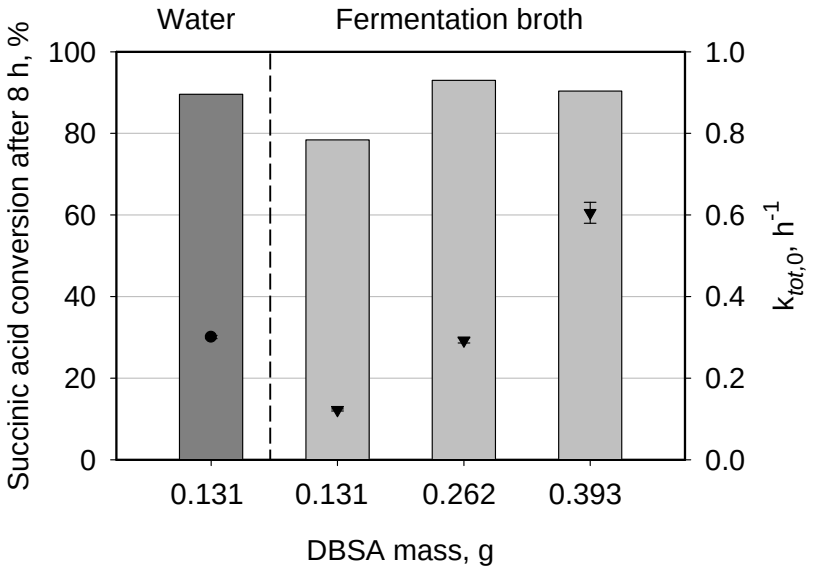


Figure 6-20: Impact of the mass of DBSA for the esterification in fermentation broth in comparison with the esterification in distilled water: conversions after 8 h (in distilled water and in fermentation broth) and total rate constant of the succinic acid initial consumption $k_{tot,0}$ (● in distilled water and ▼ in fermentation broth). Reaction conditions: optimal conditions for the esterification with DBSA (see Table 6-4) except for the mass of DBSA introduced.

As already mentioned in Table 6-10, performing the reaction with DBSA in fermentation broth made the conversion drop from 91 % in distilled water to 78 % after 8 h of reaction. Similarly, the total initial rate constant greatly diminished from 0.301 ± 0.004 to 0.122 ± 0.003 h^{-1} for the same mass of DBSA used (0.131 g) (see Figure 6-20). However, when the mass of catalyst was increased in the fermentation broth, the rates and conversions were enhanced, and with a mass of DBSA of 0.262 g, very similar conversion (93 %) and rate constant (0.293 ± 0.006 h^{-1}) as those initially obtained in distilled water with 0.131 g DBSA could be obtained.

Concluding remarks

It was possible to reach similar rate constant and conversions in the fermentation broth as in the distilled water by only doubling the catalyst concentration. The increase of the DBSA mass might both enhance the emulsion formation and create more acid sites for the catalysis, allowing therefore higher rate constants. Finally, 0.262 g of DBSA allowed the reaction to be completed in 8 h and this mass is therefore relevant for industrial applications. It will be used for further reactions in fermentation broth.

6.4.3 Comparison of the three alternatives for real fermentation broth

Since it has been shown previously that the three catalysts (DBSA, Nafion NR-50 and Novozym 435) could esterify the succinic acid contained in real fermentation broths, the best catalyst for such a process should finally be selected, with two major criteria in mind. On the one hand, rate constant and conversion must be as high as possible. On the other hand, the price of catalysts per gram esters produced must be taken into account for any industrial process. Finally, in order to reduce catalyst costs, it is desirable to recycle the catalyst. These three aspects will thus be discussed in turn in this Section.

6.4.3.1 Rate constant and conversion

As mentioned before, kinetics of the esterification of succinic acid from the fermentation broth with 1-octanol using the three catalysts must be first compared in order to select the best option. The evolutions of the conversion over the reaction time for the three catalysts are presented in Figure 6-21. As mentioned in Section 6.4.2, a higher concentration of DBSA should be used to maintain optimal conversions after 8 h in the fermentation broth, because of emulsion formation problems. This slightly higher concentration of DBSA (262 mg instead of 131 mg DBSA for 5 ml of fermentation broth) was hence used for this comparison.

The total initial rate constants were of 0.295 ± 0.008 h^{-1} for DBSA, 0.036 ± 0.002 h^{-1} for Nafion NR-50 and 0.050 ± 0.001 h^{-1} for Novozym 435 and conversions of 94 % could be obtained after 8 h for DBSA, whereas only 70 % conversions were obtained after 24 h for Nafion NR-50 and Novozym 435.

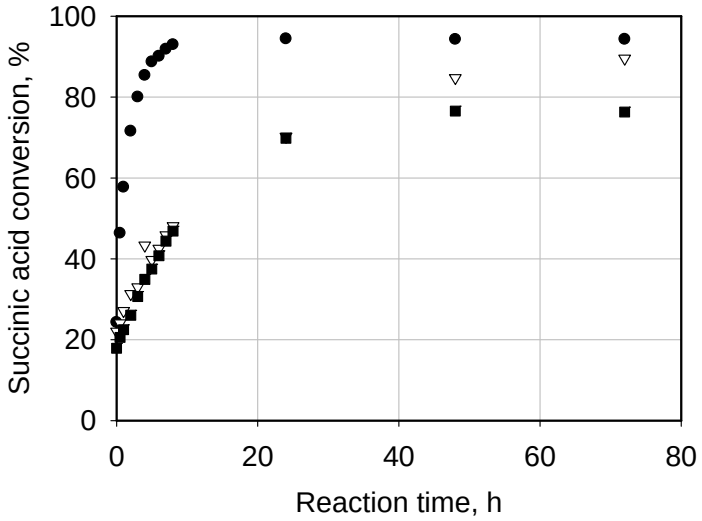


Figure 6-21: Comparison of the evolution of the conversions of succinic acid in fermentation broth over time for its esterification with 1-octanol using DBSA ●, Nafion NR-50 ■ and Novozym 435 ▽. Reaction conditions: real fermentation broth with 0.257 M succinic acid at pH = 2 for DBSA and Nafion NR-50 or pH = 3.33 for Novozym 435, 0.262 g DBSA, 0.75 g Nafion NR-50, 0.04 g Novozym 435, 90 °C for the chemical catalysts or 52 °C for the enzyme, 5 ml aqueous phase, 5 ml 1-octanol.

The reaction hence reached almost completion in 8 h with DBSA, whereas Nafion NR-50 reached similar conversions (90 %) only after 72 h. As for the enzyme, such high conversions could not be achieved, since the conversion stagnated at 76 % after 48 h. This could be due to a loss in activity of the immobilized enzyme or a rise of the pH during the reaction following the consumption of the succinic acid, which was only observed for the enzyme in Subchapter 6.2.4. Only a small amount of diprotonated succinic acid would hence be present at the end of the reaction, hence limiting the conversion to 76 %. Accordingly, a pH control should be used during the enzymatic reaction to keep the pH at 3.33 (below the pK_{a1} of succinic acid) and to achieve higher equilibrium conversions.

Concluding remarks

Nafion NR-50 and Novozym 435 are heterogeneous catalysts, which is often a desirable feature for industrial applications, since they can be easily removed from the reaction solution. However, their activity (i.e. rate constant) is at least 6 times lower than the

activity of DBSA. The latter is unfortunately lost at the end of the reaction, but it allowed the reaction to be completed in 8 h, while similar yields were only reached after 72 h with Nafion NR-50. DBSA seems thus to be the best catalyst from the three tested catalysts, in terms of conversion and rate.

6.4.3.2 Cost

In order to select the best option for the esterification of succinic acid from fermentation in a two-phase system with 1-octanol, the cost must still be taken into account. Catalyst costs per gram of diester produced in 8 h (DBSA) or 24 h (Nafion NR-50 or Novozym 435) are presented in Figure 6-22. The catalyst costs per gram ester produced were of 0.07 € g^{-1} for DBSA, 20.98 € g^{-1} for Nafion NR-50 and 55.62 € g^{-1} for Novozym 435.

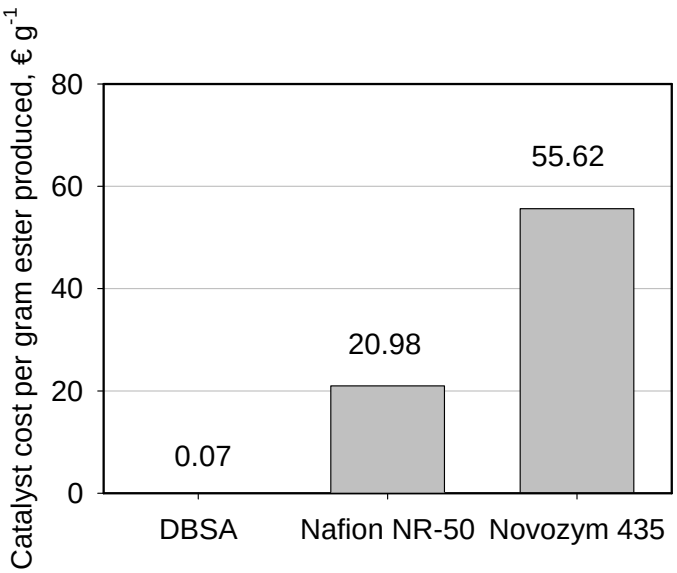


Figure 6-22: Catalyst cost ▭ for the production of dioctyl esters by esterification of succinic acid from fermentation broth with DBSA (in 8 h), Nafion NR-50 (in 24 h) and Novozym 435 (in 24 h).

Concluding remarks

DBSA is about 800 times less expensive than Novozym 435 and 300 times than Nafion NR-50. The use of the last two catalysts is therefore only competitive if they can be recycled a few hundred times. The reuse of the heterogeneous catalysts must hence be assessed, in order to determine if these two catalysts could be used for industrial applications.

6.4.3.3 Reusability

Both heterogeneous catalysts must be reused in order to reduce catalyst costs and be suitable candidates for industrial applications. Unfortunately, the recycling of the enzyme could not be tested here as the stirrers used for the reaction crushed repeatedly part of the beads, after 24 hours of reaction. As for Nafion NR-50, its reuse with pure solutions of succinic acid has already been presented in Section 6.2.7. It could then be shown that Nafion NR-50 could be reused five times for pure solutions of succinic acid with simple washing steps with water or/and acetone with final conversions after 24 h of 73 % to 84 %. The same washing procedures (see Table 6-3) were tested here for the reaction in fermentation broth. The conversions and the total initial rate constants for the 5 cycles are presented in Figure 6-24 (a) and (b).

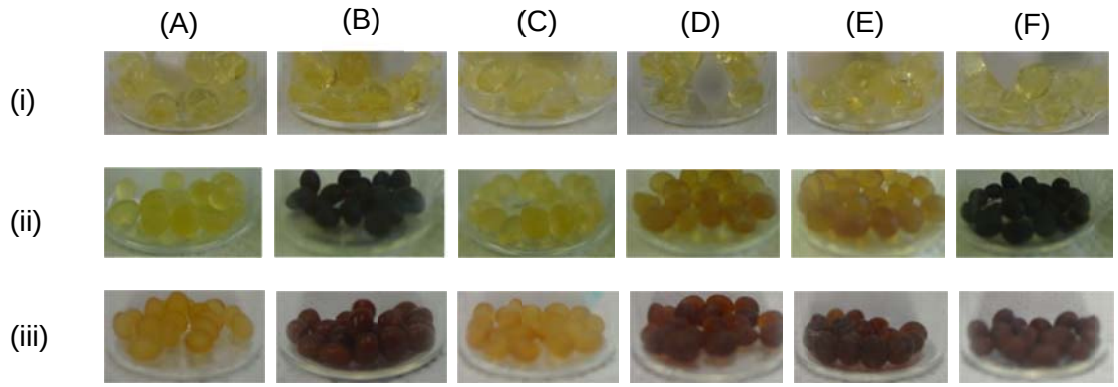


Figure 6-23: Recycling of the Nafion NR-50 beads for the esterification of succinic acid in fermentation broth: appearance of the beads before (i) and after (ii) the 1st washing / drying procedure and finally after the 4th washing / drying procedure (iii), using six different washing / drying methods: (A), (B), (C), (D), (E) and (F) (see Table 6-3).

As shown in Figure 6-23 (i), the beads recovered after the first cycle of the esterification of succinic acid in fermentation broth were yellow, in opposition to the colorless beads recovered after the reaction in pure solutions of succinic acid (see Figure 6-14). The broth from the fermentation of *E. coli* for the production of succinic acid is indeed yellow and the colored chemicals seemed to be absorbed into the resin. Even after the washing procedure (ii), the beads remained yellow. Similarly to the recycling with pure solutions of succinic acid (see Subsection 6.2.7.2), a fast darkening of the beads was observed for the beads washed with acetone in the last step. After 4 cycles, the beads were all darker.

Contrary to the recycling of Nafion NR-50 with solutions of succinic acid in distilled water, the washing method seemed to have an important impact on the recycling of the

catalysts when using fermentation broth. The conversions and the initial rate constants of the first cycle varied from 67 to 75 % and from 0.027 ± 0.003 to 0.041 ± 0.004 h^{-1}. Only the method "D" comprising a washing step with an aqueous solution of 10 % HCl seemed to maintain relatively good conversions (62 to 84 %) and initial rate constants (0.027 ± 0.002 to 0.033 ± 0.001 h^{-1}) for the 2nd to the 5th cycle of reuse. For the other methods of washing, the conversions and the rates dropped below 45 % and 0.018 h^{-1} even after the first cycle.

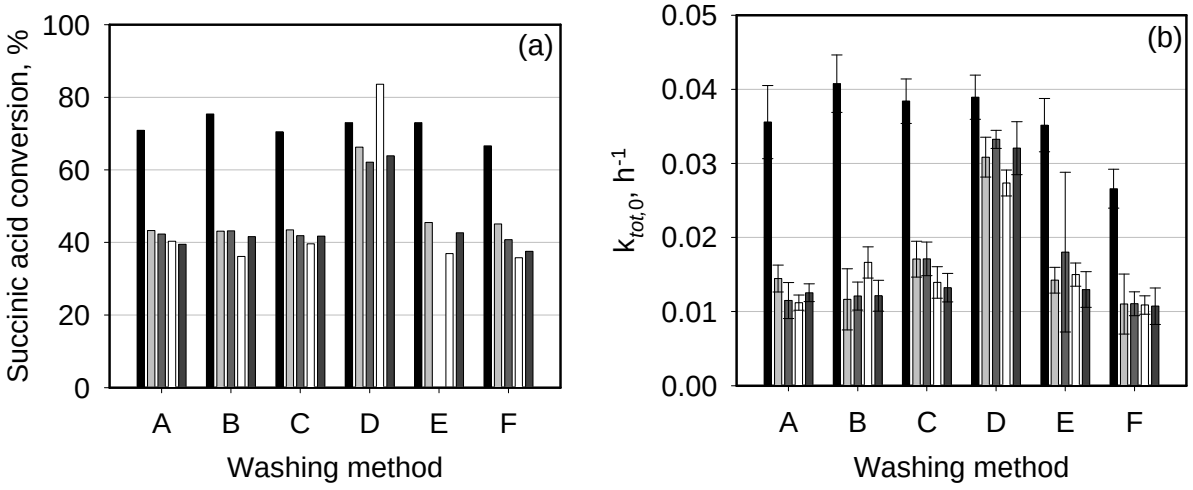


Figure 6-24: Impact of the washing method on the recycling of Nafion NR-50 for the esterification of succinic acid in fermentation broth: (a) succinic acid conversion, (b) total rate constant of the succinic acid initial consumption. Cycle: 1st ■, 2nd ■, 3rd ■, 4th ■ and 5th ■. Washing methods: A & E: three times with water; B & F: three times with acetone; C: water, acetone, water; D: water, aqueous HCl 10 %, water; A to D drying for 2 h at 80 °C, E and F drying over night at 80 °C. Reaction conditions: 90 °C, 0.75 g Nafion NR-50 (recycled), 5 ml of fermentation broth containing 0.257 M succinic acid at pH = 2, 5 ml of 1-octanol, 1000 rpm.

It is surprising that this impact of the washing step was not observed with the pure solutions of succinic acid. This suggests that a component of the fermentation broth is deactivating the resin. As a regeneration step with HCl is necessary to maintain good activities, this could indicate that some H^+ ions of the Nafion NR-50 might be lost during the reaction in the fermentation broth. As discussed previously, the fermentation broth contains indeed high concentrations of small cations that could replace the protons in the resin. If no regeneration of the H^+ ions is realized with aqueous solutions of HCl, the proton donor efficiency of the resin is hence much lower after the first cycle. Since the esterification is catalyzed by the proton donor catalyst, this drastically lowers the rate of the reaction.

Concluding remarks

The Nafion NR-50 polymer should be regenerated with aqueous solutions of HCl in order to maintain good conversions and rate constants, because of probable ion exchange with small cations contained in the fermentation broth. In the present study, the Nafion NR-50 beads were recycled over five cycles. However, Nafion NR-50 should be recycled at least hundred times to be competitive with the cheap DBSA. Since a regeneration procedure with HCl is necessary to maintain high conversions, this washing step will introduce additional costs and might not be suitable for industrial applications.

6.4.3.4 Conclusion

With regards to conversion, rate and cost, DBSA seems to be the most promising alternative for the esterification of succinic acid from fermentation broth in biphasic system, allowing the reaction to be completed in 8 h. However, this catalyst is homogeneous and cannot be recovered easily at the end of the process. Its use could thus contaminate the product. That is why the purification of the ester must still be examined at a higher scale. This will be presented in the next Section.

6.4.4 Process integration at a 200 ml-scale using DBSA

In order to analyze the feasibility of the esterification of succinic acid from fermentation broth with DBSA, the reaction had to be realized at a higher scale and the esters formed had to be recovered and purified. The biphasic esterification was hence realized at a 200 ml-scale using a fermentation broth, as well as a pure solution of succinic acid as a comparison. The reaction with the fermentation broth was realized with two different concentrations of the DBSA (13.1 and 26.2 g l_{tot}^{-1}), as it had been shown that the high salts concentration of the broth cause the disruption of the emulsion. The reactions were stopped after 8 h and the conversions were determined. The phases were then separated by centrifugation for the pure solution of succinic acid or by simple settling for the broth. 1-octanol was then evaporated out of the organic phase in a Rotary Evaporator and recovered with a purity of 95 % to 98 %. The recovered esters were analyzed by ^{1}H-NMR. The esters were then filtered on silica gel and analyzed again by ^{1}H-NMR. The conversions of the esterification, the purity of the esters before and after filtration are reported in Table 6-11.

The conversions (91 % for the pure solution and 85 % and 93 % for the broth) were similar as those obtained at the 10-ml scale (91, 78 and 93 %, see Table 6-10, entries 15 and 16 and Figure 6-20). The purity of the esters produced from the pure solution of

succinic acid was high (90 %), whereas it remained limited for the esters from the fermentation broth (63 % and 50 % for 1.31 and 2.62 g l_{tot}^{-1} DBSA). The lower purity of the esters from the fermentation broth could be caused by the extraction of different less polar by-products or medium components from the fermentation broth into the 1-octanol phase. Other carboxylic acids from the fermentation broth might also have been esterified by DBSA and extracted in the 1-octanol phase. Concerning the extracted succinic acid into the 1-octanol phase, however, it should not contaminate the esters much (~ 1 %, based on calculations).

Table 6-11: Conversion and purity of the esters for the biphasic esterification of succinic acid in fermentation broth and in distilled water under the optimized conditions.

Aqueous phase	Conc DBSA g l_{tot}^{-1}	Conv (8 h)	Diester					
			Filtration	Color	Purity (NMR)	Purity (Calc.*)	% DBSA (NMR)	% SA (Calc.*)
Distilled water	1.31	91 %	without	Pale yellow	90 %	88 %	6 %	1 %
			with	Pale yellow	95 %		5 %	
Broth	1.31	85 %	without	Brown	63 %	65 %	4 %	1 %
			with	Yellow	83 %		4 %	
Broth	2.62	93 %	without	Brown	50 %	57 %	9 %	< 1 %
			with	Yellow	74 %		11 %	

* = Calculated from the conversion and the mass of esters recovered after evaporation of the 1-octanol.

The increase of the amount of DBSA enhanced the conversion but lowered the purity of the final esters. DBSA was found in the esters at a low percentage (≤ 6 %) for the reaction in distilled water and in the fermentation broth with the lower starting concentration of DBSA. However, when the concentration of DBSA was doubled in the broth, the percentage of DBSA in the recovered esters increased up to 9 %. The surfactant has indeed a long unpolar aliphatic chain and is probably mainly recovered in the 1-octanol phase. As its boiling point is higher than 225 °C, it is not removed from the esters simultaneously with the 1-octanol (boiling point of 195 °C).

Because of the relatively limited purity of the esters from the broth, the recovered product was filtered on silica gel and analyzed again by ^{1}H-NMR. After filtration, the

purity was significantly improved for the fermentation broth (83 % and 74 % for initial DBSA concentrations of 1.31 and 2.62 g l_{tot}^{-1} resp). The filtration also increased the purity of the esters from the pure solution of succinic acid (95 %). The colour of the esters from the fermentation also improved from brown to yellow after filtration.

In order to prevent the extraction of the contaminants from the broth, a well-selected co-solvent, in which those compounds have a lower solubility, might be added to the 1-octanol for the biphasic reaction. If the co-solvent has a lower boiling point than the ester, it could be easily removed from the organic phase simultaneously with the 1-octanol.

Conversely, the filtration did not lower the DBSA content, as 4 and 11 % DBSA were found in the filtered esters from the broth (for 1.31 and 2.62 g_{DBSA} l_{tot}^{-1} resp.). The filtration on silica gel can therefore not be used to remove the DBSA from the esters. DBSA might be removed more efficiently by an increase of the temperature during the vacuum distillation, since the esters have an extremely high boiling point (~ 375 °C), but the surfactant has been reported to decompose at high temperature producing toxic fumes of oxides of sulfur[3], which would preclude the use of this method.

Concluding remarks

DBSA allowed the esterification of succinic acid contained in fermentation broths in two-phase systems with conversions up to 93 % at a 200 ml-scale. After the reaction, the esters could be separated from the 1-octanol phase. Since the 1-octanol could be recovered at a purity of 95 % to 98 %, the excess used for the reaction could be reused for further esterifications, lowering the process costs.

By-products or medium components were extracted from the fermentation, lowering the purity. A co-solvent selected carefully may decrease the solubility of these chemicals in the organic phase and increase the final ester purity.

As for the DBSA concentration, it should be noted that, at high concentration (2.62 g l_{tot}^{-1}), the reaction was faster and a higher conversion was achieved after 8 h (93 % instead of 85 % with 1.31 g_{DBSA} l_{tot}^{-1} resp.), but the contamination of the esters by the surfactant was increased (i.e. DBSA content up to 11 w/w %), reducing the final purity to 74 %. A compromise should thus be made between the rate of the esterification (i.e. the time to completion) and the purity of the recovered esters. DBSA removal methods must

[3] Source : CAMEO Chemicals, Database of Hazardous Materials, US government.

thus be further investigated to facilitate the ester purification. However, in this study, final purity up to 83 % could still be achieved with 1.31 g_{DBSA} l_{tot}^{-1}.

One study has reported the synthesis of a DBSA-like polymer for esterification in biphasic system (Manabe and Kobayashi, 2002). Since this heterogeneous catalyst will not contaminate the esters, this polymer could be useful for industrial applications, provided that it shows similar reactivity as DBSA. Using this catalyst might be a promising approach in the near future. However, the recycling of such a polymer catalyst should be also studied, since, in the present study, the polymeric Nafion NR-50 showed a fast deactivitation, if the beads were not washed with HCl.

6.4.5 Potential use of the esters produced from bioderived succinic acid

6.4.5.1 Synthesis of a broader range of succinate esters

As shown previously, DBSA can efficiently esterify the succinic acid present in a fermentation broth into its dioctyl esters. These esters can then be used as solvent, intermediate for organic synthesis and antifreezing agent (Zheng et al., 2010). Even if the process has been developed with 1-octanol, it would be advantageous if the same catalyst could esterify the succinic acid from fermentation broths into a wide range of different esters just by changing the alcohol of choice. A lot of esters of succinic acid are indeed of great interest as mentioned in Table 3-3. It has previously been shown that DBSA can esterify the succinic acid in distilled water with 1-butanol, 1-hexanol, 1-nonanol, 1-decanol and 1-undecanol at high conversions (≥ 87 %) (see Figure 6-2). In order to test the ability of DBSA to efficiently esterify succinic acid with a broad range of alcohols in fermentation broth, the esterification was performed in the fermentation broth with six alcohols, namely isobutylalcohol, isoamylalcohol, benzyl alcohol, *p*-cresol, 1-decanol, and 1-dodecanol. The results are presented in Figure 6-25.

Succinic acid from the recombinant *E. coli* fermentation broth could be easily esterified using DBSA with conversions after 24 h of 94 %, 95 %, 85 %, 92 % and 90 % for isobutylalcohol, isoamylalcohol, benzyl alcohol, 1-decanol, and 1-dodecanol, respectively. Only the conversion using *p*-cresol remained low (39 %), corresponding almost to the purely extraction of the succinic acid in the *p*-cresol phase. Still DBSA can definitely be used for a broad range of alcohols enabling the production of a lot of interesting diesters.

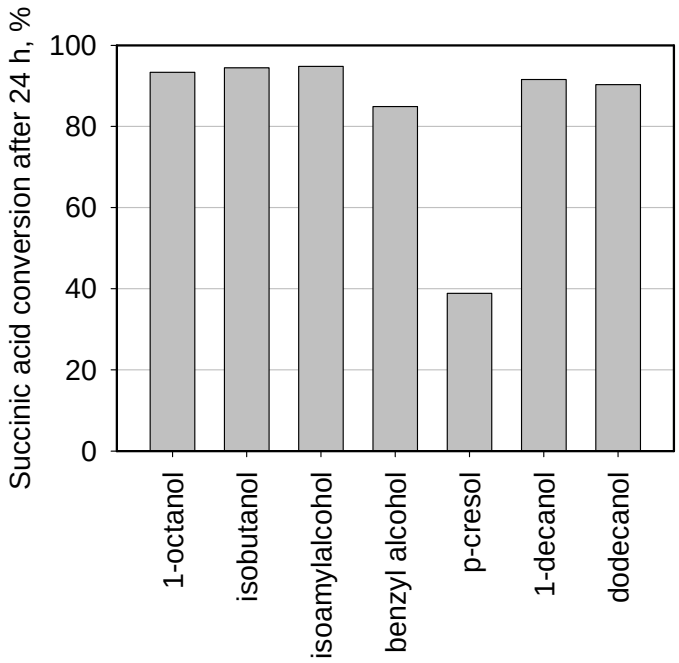


Figure 6-25: Esterification of succinic acid in fermentation broth with different alcohols using DBSA as catalyst: conversion after 24 h ▭. Reaction conditions: optimal conditions for DBSA (Table 6-4) except for the alcohol used.

6.4.5.2 Ester hydrolysis for a two-step purification of succinic acid

As mentioned in Chapter 3, the recovery and purification of succinic acid from fermentation broth is still challenging and represents a high percentage of the production costs. The esterification of succinic acid in two-phase systems could be used as a reactive extraction of the carboxylic acid from the broth. The hydrophobic esters are indeed much more easily extracted in the organic phase than the polar succinic acid itself. The formed esters can be then hydrolyzed back into succinic acid after separation. This esterification-hydrolysis process can be seen as a purification method for the succinic acid in the fermentation broth. The hydrolysis of the esters was hence shortly studied, in order to test the feasibility of this approach.

a) <u>Chemical and enzymatic catalysts screening for the hydrolysis</u>

Similar catalysts as for the esterification (i.e. both enzymes and chemical catalysts) were tested for the hydrolysis of dioctyl succinate esters produced from the esterification of succinic acid with DBSA (purity of 91 %).

Different chemical catalysts were tested at 90 °C, in 5 ml distilled water, with 0.5 ml esters and 0.25 g catalyst or 65.5 mg DBSA: Amberlysts 15, 16, 36 and 131, Nafion SAC, DBSA, PS-Sulf. Ac. and Scavenger Pore Benzensulfonic acid. Concerning the enzymatic hydrolysis, the following enzymes were tested: immobilized lipase B from *Candida*

antarctica (Novozym 435), Amano lipase from *Burkholderia cepacia* (BCL), Amano lipase from *Pseudomonas fluorescens* (PFL), lipase from *Candida rugosa* (CRL) and lipase from *Thermomyces lanigunosus* (TLL). The enzymatic reactions were performed at 37 °C, 1000 rpm with 40 mg or 40 µl enzyme, with 5 ml of Tris-HCl buffer (2.5 M) with Triton X-100 (5 g l^{-1}) at pH = 7.5 and with or without gum arabic (1 g l^{-1}), a standard additive for hydrolysis with lipases. The results of the catalyst screening are presented in Figure 6-26 (a) for the chemical catalyst and (b) for the enzymatic ones.

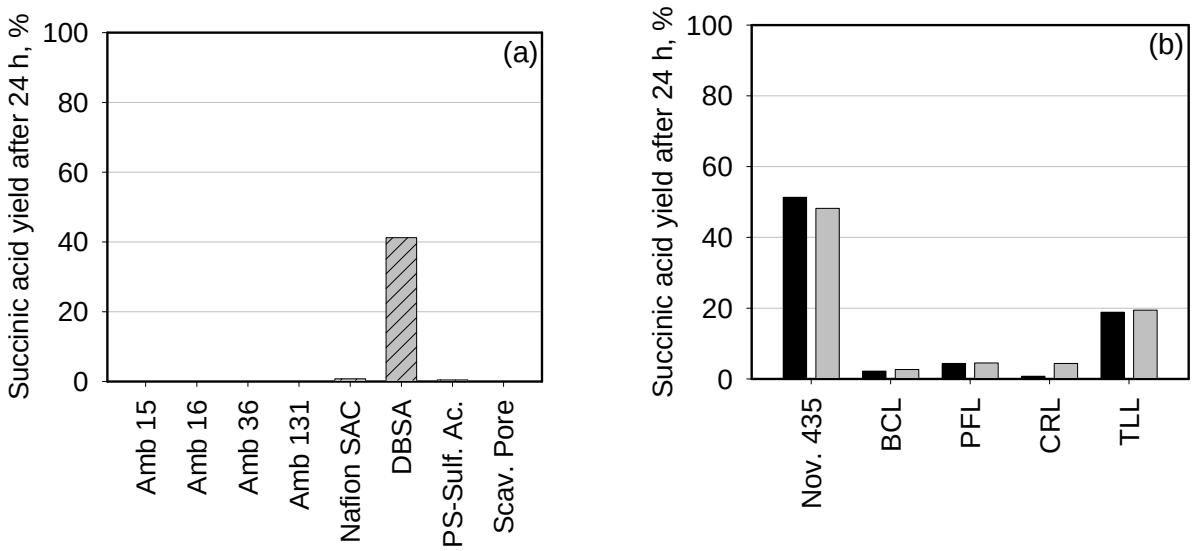


Figure 6-26: Catalyst screening for the hydrolysis of dioctyl succinate esters with chemical ▨ (a) and enzymatic (b) catalysts with ■ or without □ gum arabic. Reaction conditions: for the chemical catalyst: 0.25 g catalyst or 65.5 mg DBSA, 90 °C, 0.5 ml dioctyl ester, 5 ml distilled water. For the enzymes: 37 °C, reaction performed in the parallel reactor unit, with 40 mg or µl enzyme, 5 ml of Tris-HCl buffer (2.5 M), Triton X-100 (5 g l^{-1}) at pH = 7.5 with or without gum arabic (1 g l^{-1}), 0.5 ml dioctyl ester, 1000 rpm.

As it can be seen in Figure 6-26 (a), among the chemical catalysts, only DBSA gave a significant yield of succinic acid after 24 h (41 %), whereas the other catalysts allowed only yields below 1 %. DBSA was hence the only chemical catalyst being able to catalyze the hydrolysis of dioctyl succinate esters into succinic acid.

Regarding the enzymatic catalysts, the BCL, the PFL and the CRL gave only low yields of succinic acid (< 5 %), whereas the TLL allowed a yield of 19 % (with and without gum arabic). Finally, only the immobilized lipase B from *Candida antarctica* (Novozym 435) could achieve a yield of 48 % and 51 %, without and with gum arabic respectively. Besides, gum arabic did not have a high impact on the final yield. As this product would

increase the cost and complicate the purification of succinic acid, it was not used in further experiments.

Finally, it should be noted that the same catalysts (i.e. DBSA and Novozym 435) catalyzing the esterification of succinic acid catalyse its hydrolysis in other reaction conditions. Further experiments were realized with these two catalysts.

b) <u>Parameter screening for the enzymatic hydrolysis</u>

The screening of different parameters was only realized with the enzyme since, for the chemical catalyst, a preliminary study had shown that the pH of the aqueous phase had no impact on the yield of succinic acid. Furthermore, it was supposed that higher temperature were optimal for the chemical catalysis. As the reaction parameters have a much more complex impact on enzymatic reactions, their influence was studied at a 1-ml scale in a thermo shaker. These small scale reactions allowed a fast screening of reaction conditionss. The experiments were realized in triplicate and only an end-point was taken after 5 h. To that end, different temperatures, pHs and buffer concentrations were tested (see Figure 6-27 (a), (b) and (c)). Contrary to the "one-at-a-time" screening used for the esterification, the reaction conditions were not kept as the standard conditions throughout the screening phase, but the optimal condition found in one experiment was used for further experiments to accelerate the screening. The experiments were realized with 1 ml buffer and 0.1 ml esters.

First of all, it can be seen that lower yields (e.g. 5 ± 2 % at pH = 7.5 and 30 °C) were achieved in the thermo shaker in comparison to the experiment in the parallel stirred tank reactor unit, where 37 % yield was achieved after 5 h (data not shown) at pH = 7.5 and 37 °C. These lower yields can be explained by a worse mixing in the thermo shaker, where only axial shaking is achieved.

For the hydrolysis, a clear maximum of the yield (19 ± 1 %) was observed at 60 °C (see Figure 6-27 (a)). Similarly to what was eventually found for the esterification, higher temperatures are probably unfavourable, due to changes in the protein folding.

The screening of pH was thus realized at 60 °C and the pH was varied from 5 to 9 (cf. Figure 6-27 (b)). pHs lower or equal to 7 gave the best yields (36 ± 3 %, 43 ± 3 %, 37 ± 3 %, 42 ± 2 % for pH = 5, 6, 6.5 and 7 resp.). At pHs higher than 7.5, the yields dropped considerably, down to 3 ± 1 % for pH = 9. Since the succinic acid stays in the non-protonated form throughout the pH range tested, this cannot have had any impact on

the rate. However, a high pH can also cause changes in the 3D-structure of the enzyme leading maybe to an almost complete deactivation.

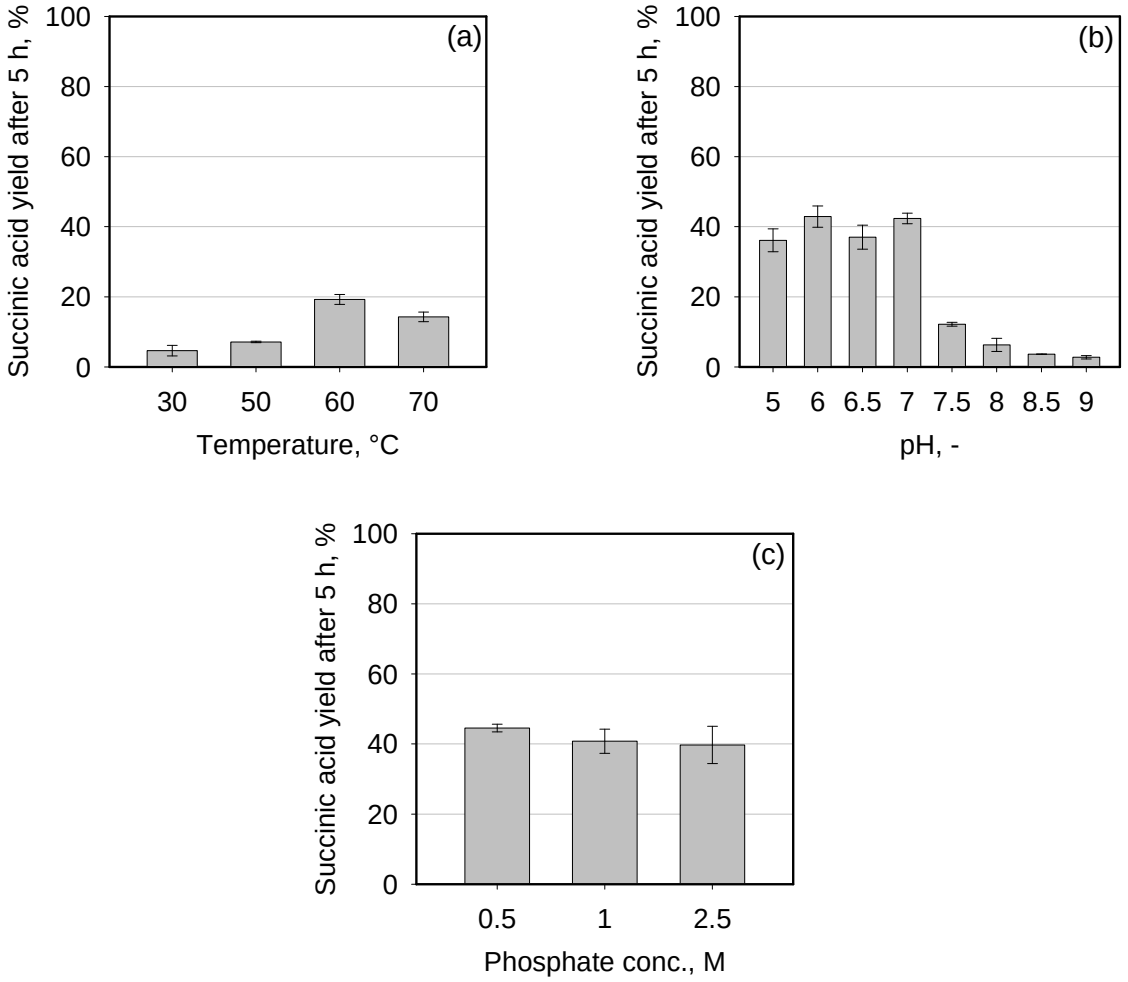


Figure 6-27: Parameter screening for the enzymatic hydrolysis of dioctyl succinate esters: (a) Impact of the temperature, (b) impact of the pH and (c) impact of the phosphate concentration of the buffer on the yield of succinic acid after 5 h ▭. Reaction conditions: 8 mg Novozym 435, 1 ml of buffer, 0.1 ml esters, 1400 rpm; (a) pH = 7.5 in Tris buffer; (b) 60 °C, for pH 5 to 6.5: phosphate buffer at 2.5 M and for pHs ≥ 7, Tris-HCl buffer at 2.5 M with Triton X-100 (5 g l^{-1}); (c) pH = 6.5, 60 °C.

Lastly, the impact of the concentration of the phosphate buffer was tested at pH 6.5 and 60 °C. The concentration of phosphate did not have a high influence on the conversion (conversions from 45 ± 1 % to 40 ± 5 % for concentrations from 0.5 to 2.5 M). The lowest concentration was selected for further experiments, as it will diminish the cost and increase the purity of the succinic acid in the aqueous phase.

A pH of 6.5 with a phosphate concentration of 0.5 M and a temperature of 60 °C were finally selected as optimal conditions for further reactions at a larger scale.

c) <u>Impact of the ester concentration on the chemical and enzymatic hydrolysis</u>

It would be interesting to be able to hydrolyze the ester up to concentrations as high as those produced by fermentation. To that end, different masses of esters were initially introduced in the reaction system with the two catalysts, keeping a constant volume of water. The results are presented in Figure 6-28.

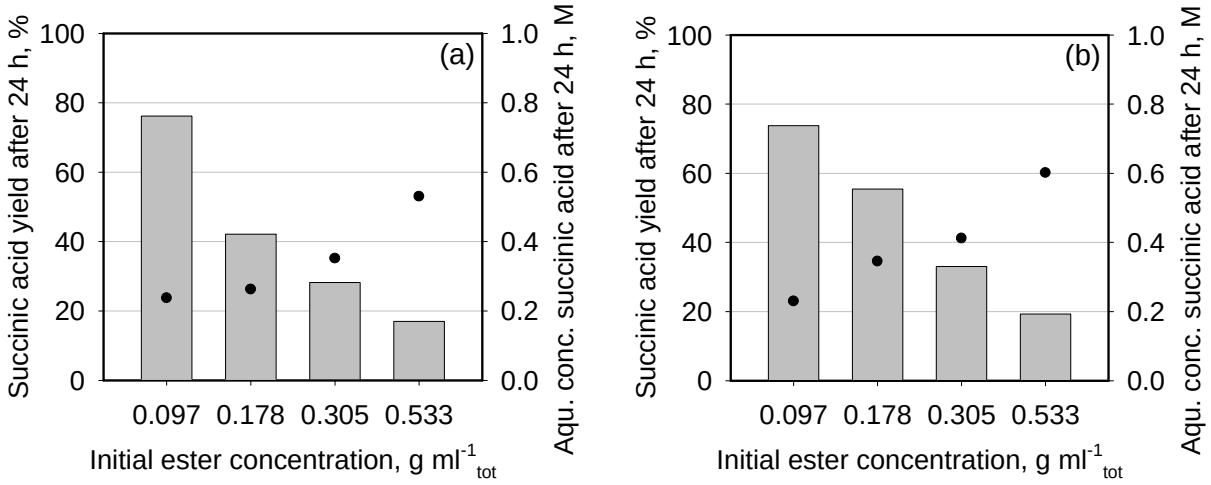


Figure 6-28: Impact of the initial ester concentration on the hydrolysis of dioctyl succinate esters with DBSA (a) and Novozym 435 (b): succinic acid yield after 24 h ▭ and aqueous concentration of succinic acid after 24 h ●. Reaction conditions: 66 mg DBSA or 20 mg of Novozym 435, 2.5 ml of distilled water or of 0.5 M phosphate buffer at pH = 6.5, 60 °C or 90 °C.

As the initial ester concentration increased in the reaction system, the final yield after 24 h decreased (from 76 to 17 % for DBSA and from 75 to 19 % for Novozym 435 while increasing the concentrations from 0.097 to 0.533 g ml^{-1}_{tot}). The aqueous concentrations of succinic acid achieved after 24 h during the experiments, however, increased up to 0.53 M for DBSA and 0.60 M for Novozym 435 but not proportionally to the initial mass of the esters.

These limited yields could be caused by many factors. First, succinic acid could have an inhibitory effect on the esterification. Second, the production of succinic acid might lower the pH, possibly decreasing the activity of the enzyme (the impact of pHs < 5 were not tested in Figure 6-27). However, this decrease was also recorded with DBSA that showed no difference in activity at pH 2 to pH 9 (data not shown).

The inhibitory effect of succinic acid was thus tested with Novozym 435 using aqueous solutions containing initially 0.1, 0.3 and 0.6 M of succinic acid. At an initial concentration of succinic acid of 0.6 M, only a small impact on the final yield could be

seen, with a yield after 24 h of 72 %, whereas 81, 83 and 90 % could be achieved with initial concentrations of 0, 0.1 and 0.3 M of succinic acid. Nevertheless, a large decrease in conversions such as the one observed in Figure 6-28 could not be satisfactorily explained by a potential inhibition by succinic acid.

Finally, higher masses of catalysts (up to 131 mg of DBSA and 80 mg of Novozym 435) were added for the highest initial mass of ester (2.67 g), in order to determine if a higher conversion could be achieved. However, no higher conversion could be recorded. Further experiments should therefore be performed to investigate if larger final concentrations of succinic acid can be obtained while still reaching large yields.

Despite this pending issue, it has been shown that the dioctyl esters of succinic acid could be successfully hydrolyzed back into pure succinic acid. As a consequence, since DBSA can also catalyze the hydrolysis of the esters, the traces of the catalyst found in the synthesized esters would not cause any problem if the esters are to be hydrolyzed back into pure succinic acid, which would alleviate the problem mentioned in Section 6.4.4.

6.4.5.3 Hydrogenation

Finally, the esters might also be used for the production of reduced products from succinic acid, such as γ-butyrolactone, 1,4-butanediol or tetrahydrofuran, since the hydrogenation of esters using alcohol as solvents have been reported (Teunissen HT, 1998) (Boardman et al., 2006). This reaction path was previously tested in Subchapter 5.3. More efforts must be developed in the domain of the metallic complexes to develop selective catalyst.

6.4.6 Conclusions

The great potential of the esterification of succinic acid in biphasic systems with DBSA was highlighted in this last Subchapter. This process option could first produce many esters of industrial relevance. It could also be used as a mean of purification for the succinic acid from fermentation broth through a coupled esterification-hydrolysis process, since DBSA and Novozym 435 were found to be two suitable catalysts for the hydrolysis. Lastly, the esters produced could be then hydrogenated into the reduced derivatives of succinic acid for the production of a large range of chemicals.

6.5 Concluding remarks on esterification

Through the study presented in this Chapter, it could be shown that biphasic reaction systems constitute an efficient alternative for the esterification of succinic acid in fermentation broth with non-water soluble alcohols. From the wide range of chemical and biological catalysts tested, three catalysts (DBSA, Nafion NR-50 and Novozym 435) were selected as the best options in terms of activity, conversion and cost. Different reaction conditions were screened for the three catalysts for a simple "one-at-a-time" optimization. The pH was a variable of major importance for both the chemical and the biological catalyses. Whereas the chemical catalysts were only active in their protonated form, i.e. at low pHs, the enzyme showed a maximum in activity around pH = 3 in the standard reaction conditions. This optimum in pH was the result of a deactivation of the enzyme at low pHs and of a substrate selectivity of the enzyme only toward the diprotonated form of succinic acid. The temperature study was also of great interest and revealed a potential internal mass transfer limitation in the Nafion NR-50 pores. At high temperatures, a levelling off of the reaction rate constant could be observed for DBSA and especially for Novozym 435. Lastly, the succinic acid concentration showed little impact on the rate constant. That is why the high concentrations of succinic acid contained in fermentation broth should be esterified without any succinic acid inhibition.

From these single-variable optimizations, a set of optimal reaction conditions was derived and tested. It led to an increase of the rate constants by a factor 1.5 for DBSA and 2.3 for Nafion NR-50 and to conversions of 91 % (in 8 h) and 84 % (in 24 h) respectively. However, a complete deactivation of the enzyme was observed. Since the interactions of the variables were not taken into account in this simple optimization, a three-variable RSM was used for the optimization of the rate constant and the conversion of the enzymatic esterification with the temperature, the pH and the succinic acid concentration as input variables. These three-variable models underlined the interaction of pH and temperature. The optima in rate constant and conversion for the enzyme were eventually found to lie around pH ~ 3 and 51 °C for a low succinic acid concentration. With this three-variable optimization, the rate constant for Novozym 435 could be increased by a factor 1.3 and conversions up to 72 % after 24 h were achieved. This result emphasizes the necessity of taking the variable interactions into account for the development of enzymatic reactions, due to the sensibility of the 3D-structure of the active site to many reaction conditions, whereas chemical catalysts present often less complex interaction mechanisms.

The three catalysts were then tested on real fermentation broth and conversions of 94 % (after 8 h) for DBSA and 70 % (after 24 h) for the other two catalysts were measured. Due to cost and reaction rate considerations, DBSA was the selected as optimal catalyst for the biphasic esterification of succinic acid in fermentation broth with 1-octanol. Indeed, it appeared that the heterogeneous Nafion NR-50 and Novozym 435 catalysts should be recycled at least a few hundred times to achieve competitive costs with the homogeneous DBSA catalysts. Furthermore, the recycling of Nafion NR-50 could only be achieved with a regeneration step with HCl that would introduce additional costs.

DBSA was finally tested at a larger scale for the biphasic esterification and the dioctyl esters were subsequently purified. A compromise had to be reached between a high rate of esterification and the purity of the esters. Due to the high concentration of salts in the fermentation broth, the emulsion formation was less efficient in the broth than in pure water, so that the concentration of DBSA had to be increased to reach the reaction completion in 8 h. However, since the DBSA cannot easily be removed from the esters at the end of the reaction, this higher concentration of catalysts caused a higher contamination in the recovered esters. The use of a DBSA-like polymer (Manabe and Kobayashi, 2002) could be an alternative to the homogeneous DBSA, so that the catalyst could be easily removed at the end of the reaction. However, these catalysts should retain their activity throughout many reaction cycles to be potential catalysts for industrial applications. This field should hence be further investigated.

Finally, the potential of the biphasic esterification process using DBSA was investigated. It could be shown that the process could be transposed easily to a wide range of other non-water soluble alcohols, allowing the production of many succinate esters. It was also demonstrated that the esterification could be used as a reactive extraction process of succinic acid from fermentation broth, when coupled with the hydrolysis of the esters in a second step. Both DBSA and Novozym 435 have shown activity towards the hydrolysis reaction. Lastly, as presented in Subchapter 5.3, the succinate esters could be used as starting material for the production of the reduced products of succinic acid. These multiple applications of the biphasic esterification of succinic acid underline its relevance for industrial utilization.

7 Conclusions and outlook

The aim of this work was to develop strategies for the production of succinic acid derivatives in aqueous media and especially in fermentation broth, in order to demonstrate the suitability of C_4 dicarboxylic acid to replace the oil-derived maleic anhydride as a platform chemical. Different process options were screened for the hydrogenation and the esterification of succinic acid.

In the first part of this thesis, as a preliminary study for the aqueous hydrogenation of succinic acid, other hydrogenation reactions were tested for comparison purposes. Some very interesting results could be drawn from these tests.

First, concerning the aqueous hydrogenation of levulinic acid at 140 °C and 5 MPa, it could be shown that this reaction can be performed with $Ru(acac)_3$ and different water-soluble phosphine. Phosphines with electron withdrawing groups, such as TPPTS, seemed to increase the turnover frequency. A ruthenium complex with immobilized triphenyl phosphine ligand on a PS-PEG amphiphilic polymer also catalyzed the reaction without deactivation, contrary to the corresponding non-water-soluble free catalyst.

Second, for the hydrogenation of succinic anhydride, $Ru(acac)_3$ with linear phosphine in acidic conditions was confirmed as a good catalytic system for reactions in tetraglyme. The 2nd generation Hoveyda-Grubbs catalyst also gave a similar TOF in basic conditions. This is the first time that such a carbene catalyst was used for this reaction. The use of this air-stable catalyst is hence a very promising approach for the solvent hydrogenation of succinic anhydride. Besides this, the immobilization of a triphenyl phosphine ruthenium catalyst on PS-PEG has shown to increase the catalyst stability, allowing relatively good TOFs during at least 22 h. In addition to the simplified catalyst recovery, this further underlines the advantages of catalyst immobilization.

Furthermore, different process strategies were either tested or reviewed from the literature for the **aqueous hydrogenation of succinic acid** and they are summarized in Table 7-1. The metal supported catalysts reported in the literature (Delhomme et al., 2009) must contain a combination of different metals in order to obtain the desired selectivity towards 1,4-butanediol (BDO), tetrahydrofuran (THF) and γ-butyrolactone (GBL). In addition, they are only active at high temperatures and pressures (up to 270 °C and to 27.6 MPa), which greatly enhance the process costs.

Table 7-1: Different strategies for the aqueous hydrogenation of succinic acid: advantages, drawbacks and outlook for the different methods.

Strategy	Advantages	Drawbacks	Outlook
Metal supported catalysts from the literature (reviewed)	• Good selectivity • Heterogeneous	• Needs many metals to control the selectivity • High pressures and temperatures • No knowledge on the reaction mechanism	
Metal supported clays	• Biocompatible • Easily synthesized • Heterogeneous	• Low selectivity of GBL • Probable decarbonylation reactions	• Simultaneous immobilization of different metals • Immobilization of metallic complexes • Kinetics study
Metallic complexes	• Limited pressure • Tunable catalyst • Low amount of ligand needed • Probably less reactions of decarbonylation	• Low selectivity (GBL, BDO, THF produced) • High temperature needed	• Impact of different additives and ligands on the selectivity • Kinetics study • Multiple metal complexes • Immobilization
Metallic complexes for the hydrogenation of succinate esters	• Tunable catalyst	• Low selectivity (GBL and BDO produced simultaneously)	• Impact of additives, ligands and solvents on the selectivity • Kinetics study • Multiple metal complexes • Immobilization

As a milder alternative, metal supported clays were hence tested in this study. The main advantage of this type of catalyst is its biocompatibility and its easy synthesis. MTM K-10 and Al pillared MTM were the only supports suitable for the aqueous hydrogenation of succinic acid into GBL. However, the obtained selectivities of GBL were low. A ruthenium clay catalyst would be very suitable for GBL production, provided that the decarbonylation reactions, which seem to take place simultaneously, can be prevented. More effort should be invested to understand the reaction pathways and their kinetics and to optimize the reaction conditions and the catalyst design, with the aim of increasing further the GBL selectivity. Different metals could for instance be simultaneously immobilized into the MTM clay and this support could also be used for the immobilization of metal complexes.

The hydrogenation of either succinic acid in water or its esters in solvent was also catalyzed by metallic complexes. The same catalytic system (i.e. $Ru(acac)_3$ and Triphos ligands) was tested in both cases. For the aqueous reaction, high temperatures were necessary to obtain the reduced derivatives. However, GBL, BDO and THF were produced in a complex network of reactions at high temperatures, drastically lowering the obtained selectivities. As for the hydrogenation of the esters, methanol was the only suitable solvent and the addition of zinc increased the turnover frequency. But here too, GBL and BDO were produced simultaneously and no satisfactory selectivities were obtained.

Finally, the metal supported catalysts, even though they combine many different metals and they only work under severe reaction conditions, remain for the moment the only suitable alternative for the hydrogenation of succinic acid in water solution. However, a better understanding of the hydrogenation mechanisms with metallic complexes, both in solvent and in water, should still be developed through kinetics studies and screenings of catalytic systems and reaction conditions, with the aim of developing catalysts of industrial relevance. For example, multi-complex alternatives could be considered, as different metals might enhance the selectivity.

In the second part of this project, different process strategies were screened for the **esterification of succinic acid** from fermentation broths. Both chemical and enzymatic catalyses were considered for this reaction, in mono- or biphasic systems. Whereas the monophasic strategy was unsuitable in presence of water with the catalysts tested, biphasic reactions have proven to be very suitable for aqueous chemical esterifications. They indeed prevent the hydrolysis of the formed esters and enable an easier recovery of the esters from the fermentation broth.

For this type of reaction system, different catalysts were thus screened for the aqueous esterification of succinic acid and are summarized in Table 7-2. Among the enzymatic catalysts screened, lipoprotein lipases showed high activity toward succinic acid. However, they were deactivated at temperatures above 50 °C and are extremely expensive. Even though this approach seems to be very promising, the production costs of the enzyme must thus be considerably lowered and immobilization strategies should be developed, in order to consider them for industrial applications.

From the catalyst screening, Novozym 435 (the immobilized lipase B from *Candida antarctica*), Nafion NR-50 and dodecylbenzyl sulfonic acid (DBSA) were found to be interesting for future industrial applications, as far as costs, activity and conversion are

concerned. It was also shown that, whereas single-variable optimizations were suitable for the chemical catalysts, a multiple-variable strategy was necessary for enzymes as many variables jointly influence the 3D-structure of their active sites. One variable of major importance was the pH: the chemical catalysts had to be in their protonated form, allowing reaction only at low pHs, whereas the enzyme showed a maxima in activity at pH ~ 3 due to combined effects of low stability at low pHs and substrate selectivity only toward the diprotonated succinic acid. The temperature had to be selected in accordance with the pH for enzymatic esterification due to a coupled effect of the two variables. From these optimizations, the activities and conversions could be further increased for the three catalysts. Rate constants were increased up to 0.39 h^{-1}, 0.069 h^{-1} and 0.042 h^{-1} for DBSA, Nafion NR-50 and Novozym 435. Conversions of 91 % (in 8 h), 84 % and 72 % (in 24 h) were finally achieved in distilled water with the three catalysts respectively.

Table 7-2: Catalysts for the biphasic esterification of succinic acid in distilled water or from fermentation broth: advantages, drawbacks and outlook for the different process options.

Catalyst	Advantages	Drawbacks	Outlook
DBSA	• High rate (reaction in 8 h) • Catalyst and surfactant • Cheap (0.11 € g^{-1}) • Easy phase separation with salt addition • Large range of alcohols possible	• High conc. of DBSA needed in fermentation broth because of the less good emulsion • High conc. of DBSA can contaminate the product	• DBSA-like polymer • Recovery of DBSA
Nafion NR-50	• Recyclable • Easy recovery by filtration	• Moderate rate • Regeneration with HCl for broth • Expensive (7.7 € g^{-1}) • Not suitable for very long chain alcohols	• Select a support with an appropriate hydrophobicity and in which pore diffusion limitation will be prevented
Novozym 435	• Biocompatible • Green catalyst • Different alcohols possible	• Moderate rate • Expensive (15.2 € g^{-1})	• Recycling of the catalyst
Lipoprotein lipases	• Biocompatible • Green catalyst • High rate (reaction in 3 h)	• Less thermostable • Very expensive (2010.00 € g^{-1})	• Immobilization • Promising catalyst if its production price decreases

The possibility of a process integration of fermentation and esterification was then successfully tested and the best conversion in the broth (94 %) was obtained with DBSA after 8 h, whereas the other catalysts reached 70 % conversions only after 24 h. As for costs, DBSA was found to be at least 800 times cheaper than Novozym and 300 times less expensive than Nafion NR-50. Catalyst recycling may therefore not compensate for this. Even though, for example, the latter was recycled, a HCl regeneration was required due to ion exchange with the ions of the fermentation broth. This regeneration step would lead to extra costs.

The acid surfactant DBSA best catalyzed the esterification of succinic acid in distilled water with different non-water-soluble alcohols. However, the emulsion was disrupted by the large amount of salts in the fermentation broth. A compromise had to be made between the reaction rate and the esters purity, since the larger amount of DBSA introduced for reactions in the fermentation broth contaminated the esters recovered from the organic phase. Thereby the reaction could still be performed in about 15 h with a final ester purity of 83 %. However, procedures for DBSA removal should be developed to lower the potential contamination. DBSA-like polymers that have been developed by Manabe and Kobayashi (2002) would for example be of great interest to facilitate the catalyst recovery.

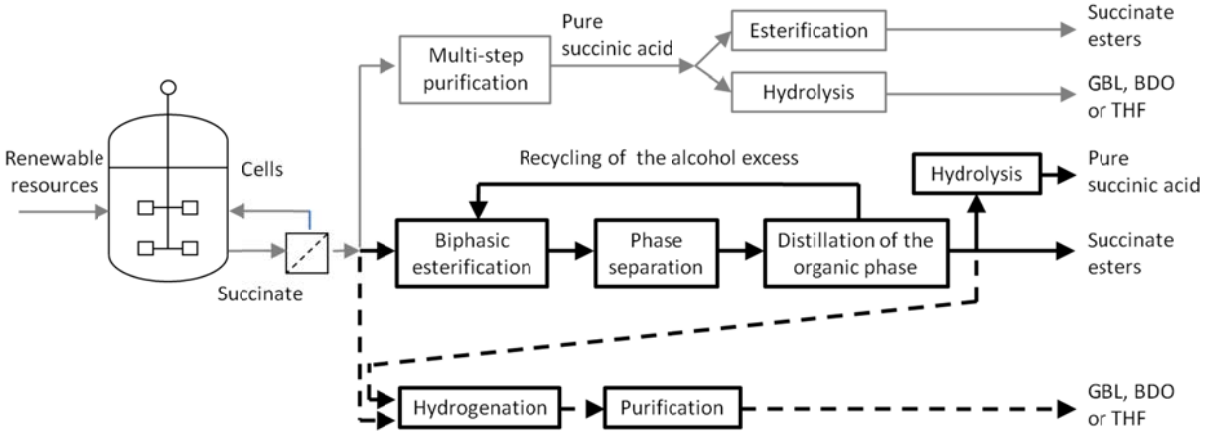


Figure 7-1: Production of pure bio-derived succinic acid and its derivatives (existing processes, process developed in this study, process studied, needs to be further developed).

Finally, the esterification process with DBSA, which can be applied on a 200 ml-scale, has a lot of applications. Not only can it produce diverse esters with a broad range of non-water-soluble alcohols, but it can also be used as a purification strategy for succinic

acid from the fermentation broth, in a coupled esterification-hydrolysis approach. As DBSA can also catalyze the hydrolysis of the esters, the small amount of DBSA found in the esters will not be problematic. Lastly, a coupled esterification-hydrogenation process can be considered for the production of the reduced derivatives.

To summarize, new paths for the production of pure succinic acid and of its derivatives were studied and developed for some of them during this thesis, as represented by Figure 7-1. These new catalytic processes from succinic acid in fermentation broths will lower its production costs by avoiding its expensive purification. Only with this cost reduction, succinic acid may become a suitable platform for new biorefineries. Finally, the new reaction pathways developed from it in this work will increase its potential as a bioderived bulk chemical.

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9 Annex

9.1 Abbreviations and symbols

9.1.1 Abbreviations

9.1.1.1 Chemical and biological substances

adppp	N-anchored 2-aza-1,3-bis(diphenylphosphino) propane
Al-MCM-41	microporous aluminosilicate mineral
Amb	Amberlyst resin
Asp	aspartic acid
BCL	Amano lipase from *Burkholderia cepacia*
BDO	1,4-butanediol
BEA	type of zeolite
(S)-binap	(S)-(-)-2,2'-bis(di-*p*-tolylphosphino)-1,1'-binaphthyl
bpy	2,2'-bipyridine
CRL	lipase from *Candida rugosa*
CTAB	cetyltrimethylammoniumbromide
CVLPL	lipoprotein lipase from *Chromobacterium viscosum*
DBSA	4-dodecylbenzenesulfonic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEGDBE	diethylene glycol butyl ether
DES	diethyl succinate
DIOP	diisooctyl phtalate
DMAP	4-(dimethylamino)-pyridin
DMF	dimethylformamide
DMS	dimethyl succinate
DOS	dioctyl succinate
dppb or DPPB	1,4-bis(diphenylphosphino) butane
E	enzyme
ES	enzyme-substrate complex
FIPA	1,1,1,3,3,3,-hexafluropropan-2-ol
GBL	γ-butyrolactone
GVL	γ-valerolactone
HBF_4	fluoroboric acid
His	histidine
HMF	hydroxymethylfurfural
Hß-zeolite	type of zeolite
L	ligand
LA	levulinic acid
LPL	lipoprotein lipase
M	metal

MTM	Montmorillonite clay
MTM K-10, Al pillared & KSF	MTM supports for catalyst immobilization
Nafion NR-50	perfluorinated resinsulfonic acid
Nafion SAC-13	Nafion polymer immobilized on a silica support
NEt_3	triethylamine
NMP	*n*-methyl-2-pyrrolidone
Novozym® 435	immobilized lipase B from *Candida antarctica*
OES	oleyl monoester succinate
OTs	tosylate group
P	product (or phosphine ligand)
p-TsOH	*p*-toluenesulfonic acid (or *p*-tosylic acid)
P(*n*-Oct)$_3$ or P(octyl)$_3$	tri-*n*-octylphosphine
PB	phosphate buffer
PBu_3	tri-*n*-butyl phosphine
PEG	poly(ethylene glycol)
PEP	phosphoenolpyruvic acid
PFL	Amano lipase from *Pseudomonas fluorescens*
PPh_3	triphenylphosphine
PR_3	tertiary phosphine
PS	polystyrene
PS-DVB	polystyrene-divinyl benzene
PS-PEG	polystyrene-poly(ethylene glycol)
PS-Sulf. Ac.	polystyrene sulfonic acid
PSLPL	lipoprotein lipase from *Pseudomonas sp.*
PTA	1,3,5-triaza-7-phosphaadamantane
PTFE	polytetrafluoroethylene
PTMEG	polytetramethylene ether glycol
PVC	polyvynil chloride (or polychloroethene)
2-pyrr	2-pyrrolidone
R'OH	alcohol
RCOOH	carboxylic acid
RCOOR'	ester
Ru(acac)$_3$	Ru(III) acetylacetonate
S	substrate or solvent
SA	succinic acid
SAnh	succinic anhydride
Scav. Pore	ScavengePore® benzenesulfonic acid
SDS	sodium dodecyl sulfate
Ser	serine
TCA	tricarboxylic acid
TentaGel® resin	type of PS-PEG copolymer
Tetraglyme or TGM	tetraethylene glycol dimethyl ether
THF	tetrahydrofuran

TLL	lipase from *Thermomyces lanigunosus*
TPPMS	3-(diphenylphosphino) benzenesulfonic acid sodium salt
TPPTS	3,3′,3″-phosphinidynetris(benzenesulfonic acid) trisodium salt
Triphos	1,1,1-tris(diphenylphosphinomethyl)ethane
Tris	tris(hydroxymethyl)aminomethane
Tris-HCl	tris(hydroxymethyl)aminomethane hydrochloride
$TriSulf^{Bu}$	1,1,1-tris(*n*-butylthiomethyl)ethane
Triton X-100	t-octylphenoxypolyethoxyethanol
TTP	a linear chiral triphosphine
TXTPS	tris(2,4-dimethyl-5-sulfophenyl) phosphine trisodium salt
VOC	volatile organic compound

9.1.1.2 Other abbreviation

% v/v	volumetric percentage
% w/w	mass percentage
^{1}H-NMR	proton NMR spectroscopy
^{31}P-NMR	phosphorus-31 NMR
CCD	central composite design
FID	flame ionization detector
GC	gas chromatography
GC-MS	gas chromatography - mass spectroscopy
HPLC	high performance liquid chromatography
IRF	internal response factor
log P	logarithm of the constant of extraction
MAS ^{31}P-NMR	phosphorus-31 magic angle spinning NMR
pH	negative logarithm of the H^+ concentration in a solution
pK_a	negative logarithm of the acidity equilibrium constant
R^2	statistical coefficient of determination
rpm	round per minute
RSM	response surface methodology

9.1.2 Symbols of the variables

Symbol	Meaning	Unit
$[A^{2-}]_{aq}$	*concentration of non-protonate succinic acid in aq. phase*	*mol l^{-1}*
$[AH^{-}]_{aq}$	*concentration of monoprotonated succinic acid in aq. phase*	*mol l^{-1}*
$[AH_2]_{aq}$	*concentration of diprotonated succinic acid in aq. phase*	*mol l^{-1}*
$[AH_2]^{eq}_{aq}$	*concentration of diprotonated succinic acid at equilibrium in the aqueous phase*	*mol l^{-1}*
$[AH_2]^{eq}_{org}$	*concentration of diprotonated succinic acid at equilibrium in the organic phase*	*mol l^{-1}*
$[Cpd]$	*concentration of the compound*	*mol l^{-1}*
$[H^{+}]_{aq}$	*proton concentration in the aqueous phase*	*mol l^{-1}*
$[IS]$	*concentration of internal standard*	*mol l^{-1}*
$[Phos]_{aq,0}$	*initial concentration of phosphate in the aq. phase*	*mol l^{-1}*
$[S]$	*substrate concentration*	*mol l^{-1}*
$[SA]_{aq}$ *or* $[SA]_{aq}(t)$	*concentration of succinic acid in the aqueous phase at time t*	*mol l^{-1}*
$[SA]_{aq,0}$	*initial concentration of succinic acid in the aqueous phase (after contact of the two phase)*	*mol l^{-1}*
$[SA]_{tot,0}$	*initial concentration of succinic acid in the aqueous phase (before contact of the two phase)*	*mol l^{-1}*
a	*specific surface of the catalyst (surface by volume)*	*m^{-1}*
A	*pre-exponential factor*	*m$^{3(n-1)}$ mol$^{-(n-1)}$ s^{-1}*
$Area_{Cpd}$	*area of the compound peak on the GC spectrum*	-
$Area_{IS}$	*area of the Internal Standard peak on the GC spectrum*	-
$Area_{IS,tot,0}$	*initial area of the Internal Standard peak*	-
$Area_{IS}(t)$	*area of the Internal Standard peak at time t*	-
β_0, β_i, β_{ii}, β_{ij}	*zero, first, second order and interaction parameters*	*dependent on the variables*
$C_{i,j.k}$	*concentrations of the different substrates and products*	*mol l^{-1}*
C^*	*initial concentration of succinic acid ("coded" variable)*	*M*
C_b	*concentration of the substrate in the bulk of the liquid*	*mol m^{-3}*
C_s	*concentration of the substrate at the catalyst surface*	*mol m^{-3}*
D	*diffusion coefficient of the substrate in the liquid phase*	*m^2 s^{-1}*
δ	*diffusion layer thickness*	*m*
D_a	*Damköhler number I*	-
D_e	*effective diffusion coefficient*	*m^2 s^{-1}*
E_{app}	*apparent activation energy*	*J mol^{-1}*
E_{kin}	*activation energy of the chemical reaction*	*J mol^{-1}*
ϕ	*Thiele modulus*	-
f(…)	*function of the concentrations*	
η_e	*external effectiveness factor*	-
η_i	*internal effectiveness factor*	-
IRF(Cpd/IS)	*Internal Response Factor of the compound Cpd) (IS) with the Internal Standard*	-

Symbol	Meaning	Unit
J	*molar flux*	$mol\ m^{-2}\ s^{-1}$
k	*rate constant*	*dependent on f*
K_{a1}	*equilibrium constant for the first acidity of succinic acid*	$mol\ l^{-1}$
K_{a2}	*equilibrium constant for the second acidity of succinic acid*	$mol\ l^{-1}$
$k_{aq,0}$	*aqueous (initial) rate constant*	h^{-1}
k_l	*mass transfer coefficient in the liquid phase*	$m\ s^{-1}$
K_m	*half saturation constant*	$mol\ l^{-1}$
$k_{tot,0}$	*total (initial) rate constant*	h^{-1}
$k_{v,p}$	*reaction coefficient per unit of volume catalyst*	$m^{3(n-1)}\ mol^{-(n-1)}\ s^{-1}$
l	*characteristic length in the system*	m
m, a & b	*parameters of the exponential fitting*	$mol\ l^{-1}$, $mol\ l^{-1}$, h^{-1}
n	*reaction order*	-
$n_{cat.\ cent.}$	*moles of catalytic center in the reaction system*	$mol\ l^{-1}$
n_H	*total mole of protons*	mol
n_i	*stoichiometric coefficient of the substance i*	-
n_i	*moles of a substance i in the reaction system*	mol
n_{orga}	*total mole of esters (or diprotonated succinic acid) contained in the organic phase*	mol
P or K_{ow}	*constant of extraction for water / 1-octanol system*	-
pH^*	*initial pH ("coded" variable)*	-
r	*reaction rate*	$mol\ l^{-1}\ h^{-1}$
R	*radius of the catalyst spherical particle*	m
R	*gas constant*	$J\ mol^{-1}\ K^{-1}$
r_{ext}	*rate of the external mass transfer*	$mol\ m^{-3}\ s^{-1}$
$r_{mol,0}$	*initial molar consumption rate*	$mol\ l^{-1}\ h^{-1}$
r_{rx+int}	*rate of the reaction with internal mass transfer*	$mol\ m^{-3}\ s^{-1}$
$r_{v,p}^{\ obs}$	*observed rate per unit particle volume*	$mol\ m^{-3}\ s^{-1}$
Sh	*Sherwood number*	-
S_P	*selectivity of product P*	%
t	*time*	h
T	*temperature*	K or $°C$
T^*	*reaction temperature ("coded" variable)*	$°C$
TOF	*turnover frequency*	h^{-1}
TON	*turnover number*	-
τ_R	*chemical reaction resistance*	s
τ_T	*external mass transfer resistance*	s
v	*enzymatic reaction rate*	$mol\ s^{-1}$
v_{max}	*maximal enzymatic reaction rate*	$mol\ s^{-1}$
X	*conversion*	%
$X(t)$	*conversion at time t*	-
$X_{6\ h}$	*conversion after 6 h*	%

Symbol	Meaning	Unit
x_i	*input variables*	*dependent on the variables*
y	*output variable*	*dependent on the variables*
Y_P	*yield of product P*	*%*
z	*particle coordinate*	*m*
Z	*input variable: C (concentration), pH or T (temperature)*	*dependent on the variables*
Z^*	*coded input variable*	-
Z_{min}, Z_{max}	*minimum and maximum value of the input variable range*	*dependent on the variables*

9.2 Material

9.2.1 Equipment

Table 9-1: Standard laboratory equipment

Equipment	Name / Code	Manufacturer, location
Oven	E 28	Binder, Tuttlingen
Centrifuge	Rotixa 50 RS	Hettich, Tuttlingen
Table-centrifuge	Mikro 20	Hettich, Tuttlingen
pH-electrode	BlueLine 14 pH	Schott, Mainz
pH-Meter	CG 843	Schott, Mainz
pH-minielectrode	HI 1330	Schott, Mainz
Mini pH-meter	PCE-PHD 2	PCE, Meschede
Balance	Extend	Sartorius, Goettingen
Balance	Explorer	Ohaus, Nänikon, Switzerland
Thermomixer	Comfort	Eppendorf, Hamburg
Thermo shaker	RiO	Quantifoil Instruments, Jena
Schwingarmmühle	MM200	Retsch, Haan
Vortex	Vortex Genie 2	Scientific Industies, Bohemia, NY, USA
Freezer	Öko_Arctis	AEG
Rotary evaporator	LABOROTA 4003	Heidolph, Schwabach
Vacuum pump	ROTAVAC vario control	Heidolph, Schwabach

Table 9-2: Autoclave

Equipment	Name / Code	Manufacturer, location
Autoclave	BR-100	Berghof, Enningen
Magnetic stirring hot plate	BLH-800	Berghof, Enningen
Heating plate jacket	BAH-100	Berghof, Enningen
Temperature controller	BTC-3000	Berghof, Enningen
PTFE Insert 100 ml		Berghof, Enningen

Table 9-3: Radleys carousel

Equipment	Name / Code	Manufacturer, location
Carousel	Carousel 12 Reaction Station	Radleys, Essex, UK
Thermometer controller	EKT Hei-Con	Heidolph, Schwabach
Stirring heating plate	RCT basic	KIKA Labortechnik, Staugen

Table 9-4: Small block reactor

Equipment	Name / Code	Manufacturer, location
Reactor	Prototype	2mag, Munich
Power control	Mix control	2mag, Munich

Table 9-5: Big block reactor

Equipment	Name / Code	Manufacturer, location
Reactor	Prototype	2mag, Munich
Power control	Mix control	2mag, Munich

Table 9-6: High Performance Liquid Chromatography (HPLC)

Equipment	Name / Code	Manufacturer, location
HPLC	Agilent 1100 Series	Agilent Technologies, Santa Clara, CA, USA
Quat. Pump	DE14918242	Agilent Technologies, Santa Clara, CA, USA
Autosampler	DE14918655	Agilent Technologies, Santa Clara, CA, USA
A/D-Converter	CN 00003423	Agilent Technologies, Santa Clara, CA, USA
Degasser:	JP05033450	Agilent Technologies, Santa Clara, CA, USA
Software	A.05.01 + Rev E.01.02	Agilent Technologies, Santa Clara, CA, USA
RI-Detector	1200 Series G1362A	Agilent Technologies, Santa Clara, CA, USA
UV-Detector	S 3300	Knauer, Berlin
Column	HPX-87H	Biorad,München
Guard	HPLC Cation H Refill, 30*4.6MM	Biorad,München

9.2.2 Chemicals

9.2.2.1 Chemicals for the hydrogenation

Table 9-7: Substrates for the hydrogenation

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Dimethyl succinate	$C_6H_{10}O_4$	106-65-0	≥ 95 %	Merck	8.20150.0250
Levulinic acid	$C_5H_8O_3$	116.12	98 %	Aldrich	L2009
Succinic acid	$C_4H_6O_4$	110-15-6	≥ 99 %	Merck	8.22260.1000
Succinic anhydride	$C_4H_4O_3$	108-30-5	99 %	Alfa Aesar	A12245

Table 9-8: Solvents for the hydrogenation

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
DMF	C_3H_7NO	68-12-2	≥ 99 %	Sigma	D4551
Methanol	CH_4O	67-56-1	≥ 99.98 %	Roth	HN41.1
Tetraglyme	$C_{10}H_{22}O_5$	143-24-8	≥ 98 %	Merck	8.20959.1000
THF	C_4H_8O	109-99-9	≥ 99 %	Sigma-Aldrich	360589

Table 9-9: Catalysts for the hydrogenation

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
[2-(Dicyclohexyl phosphino)ethyl] trimethyl ammonium chloride	$C_{17}H_{35}ClNP$	181864-78-8		Aldrich	574287
Diphenylphosphine	$(C_6H_5)_2PH$	829-85-6	≥ 95 %	Fluka	43154
Grubbs Catalyst, 2nd Generation	$C_{46}H_{65}Cl_2N_2PRu$	246047-72-3		Aldrich	569747
Hoveyda-Grubbs Catalyst 2nd Generation	$C_{31}H_{38}Cl_2N_2ORu$	301224-40-8		Aldrich	569755
Montmorillonite K-10		1318-93-0		Aldrich	281522
Montmorillonite KSF		1318-93-0		Aldrich	281530
Montmorillonite Aluminium pillared clay		139264-88-3		Aldrich	69907
Ruthenium, 5 % on alumina powder		7440-18-8		Alfa Aesar	11749
Ruthenium acetylacetonate	$C_{15}H_{21}O_6Ru$	14284-93-6		Alfa Aesar	10568
Tentagel S-NH_2				Rapp Polymere	S 30 902
1,3,5-Triaza-7-phosphaadamantane	$C_6H_{12}N_3P$	53597-69-6	97 %	Aldrich	695467
Tris(2-carboxyethyl) phosphine hydrochloride	$C_9H_{15}O_6P \cdot HCl$	51805-45-9		Aldrich	C4706
Tris(2,4-dimethyl-5-sulfophenyl) phosphine trisodium salt	$((C_6H_2)(CH_3)_2(SO_3Na))_3P$	443150-11-6	95 %	Aldrich	667382
1,1,1-Tris(diphenylphosphino methyl)ethane (Triphos)	$CH_3C[CH_2P(C_6H_5)_2]_3$	22031-12-5	≥ 97 %	Fluka	93322
Tris(triphenylphos-phine)ruthenium(II) dichloride	$[(C_6H_5)_3P]_3RuCl_2$	15529-49-4	97 %	Aldrich	223662

Table 9-10: Other chemicals for the hydrogenation

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Benzene	C_6H_6	71-43-2	99.5 %	Roth	7173.1
Diethylene glycol dibutyl ether	$C_{12}H_{26}O_3$	112-73-2	≥ 99 %	Aldrich	205621
Iridium trichloride	$IrCl_3$ H_2O	14996-61-3	99.9 %	Aldrich	203491
Paraformaldehyde	$(CH_2O)_n$	30525-89-4	97 %	Alfa Aesar	A11313
Ruthenium trichloride hydrate	$RuCl_3$ xH_2O	14898-67-0	99.98 %	Aldrich	463779

9.2.2.2 Chemicals for the esterification

Table 9-11: Substrate for the esterification

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Amylalcohol	$C_5H_{12}O$	123-51-3	≥ 99 %	Merck	8.18969.1000
Benzyl alcohol	C_7H_8O	100-51-6	≥ 99 %	Merck	8.22259.1000
1-Butanol	$C_4H_{10}O$	71-36-3	≥ 99.5 %	Merck	1.01990.1000
p-Cresol	C_7H_8O	106-44-5	≥ 98 %	Merck	8.05223.1001
1-Decanol	$C_{10}H_{22}O$	112-30-1	99 %	Sigma	150584
1-Dodecanol	$C_{12}H_{26}O$	112-53-8	98 %	Aldrich	126799
Ethanol	C_2H_6O	64-17-5	≥ 99.9 %	Roth	P076.2
1-Hexanol	$C_6H_{14}O$	111-27-3	≥ 98 %	Merck	8.04393.0100
Iso-butanol	$C_4H_{10}O$	201-148-0	≥ 99 %	Merck	1.00984.2500
1-Nonanol	$C_9H_{20}O$	143-08-8	≥ 98 %	Merck	8.06866.0250
1-Octanol	$C_8H_{18}O$	111-87-5	≥ 99 %	Merck	8.20931.2500
Succinic acid	$C_4H_6O_4$	110-15-6	≥ 99 %	Merck	8.22260.1000

Table 9-12: Chemical catalysts for the esterification

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Aluminium chloride hexahydrate	$AlCl_3 . 6 H_2O$	7784-13-6	97 %	Merck	1.01084.1000
Amberlyst 15 hydrogen form, wet		39389-20-3		Aldrich	216399
Amberlyst 16 hydrogen form, wet		125004-35-5		Fluka	86317
Amberlyst 36		39389-20-3		Aldrich	436712
Amberlyst 131		56451-56-0		Sigma	A2461
Cobalt chloride	$Cl_2Co . 6 H_2O$	7791-13-1	99 %	Merck	1.02539.0100
4 –Dodecylbenzyl Sulfonic acid (DBSA)	$C_{18}H_{30}O_3S$	204-489-3	~90 %	Fluka	44198
Iodine	I_2	7553-56-2	≥ 99 %	Sigma	03002
Montmorillonite K-10		1318-93-0		Aldrich	281522
Nafion NR-50		31175-20-9		Aldrich	309389
Nafion SAC-13		31175-20-9		Aldrich	474541
Nafion NR - 50		31175-20-9		Aldrich	309389
Polystyrene sulfonic acid				Rapp Polymere	H 400 0430
ScavengerPore Benzenesulfonic acid				Rapp Polymere	SC11014
Silver nitrate	$AgNO_3$	231-853-9	≥ 99.8 %	Merck	1.1510.0050

Table 9-13: Biological catalysts for the esterification

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Amano Lipase PS, from *Burkholderia cepacia*		9001-62-1	≥ 30,000 U g^{-1}	Aldrich	534641
Lipase from *Candida rugosa*		9001-62-1	≥ 700 U mg^{-1}	Sigma	L1754
Lipase from *Thermomyces lanuginosus*		9001-62-1	≥ 100,000 U g^{-1}	Sigma	L0777
Lipoprotein Lipase from *Chromobacterium viscosum*		9004-02-8	2500 U mg^{-1}	Fluka	62333
Lipoprotein Lipase from *Pseudomonas sp.*		9004-02-8	≥ 1200 U mg^{-1}	Fluka	62335
Novozym 435		9001-62-1		Sigma	L4777

Table 9-14: Other chemicals for the esterification

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Ammonium chloride	NH_4Cl	12125-02-9	≥ 99 %	Roth	5470.1
Ammonium phosphate dibasic	$(NH_4)_2HPO_4$	7783-28-0	≥ 98 %	Roth	P736.1
Calcium lactate	$C_6H_{10}CaO_6 \cdot 5\ H_2O$	28305-25-1	≥ 98 %	Roth	4071.1
Diammonium hydrogen phosphate	$(NH_4)_2HPO_4$	7783-28-0	≥ 97 %	Roth	0268.3
Di-sodium hydrogen phosphate dodecahydrate	$Na_2HPO_4 \cdot 12\ H_2O$	10039-32-4	≥ 99 %	Roth	T106.2
α-D-(+)Glucose monohydrate	$C_6H_{12}O_6 \cdot H_2O$	14431-43-7	≥ 99.5 %	Roth	6780.2
Hexadecyl trimethyl ammonium bromide	$C_{19}H_{42}BrN$	57-09-0	≥ 99%	Sigma-Aldrich	52365
Hydrochloric acid	HCl	7647-01-0	37 %	Roth	4625.2
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7\ H_2O$	10034-99-8	≥ 99%	Roth	P027.3
o-Phosphoric acid	H_3PO_4	7664-38-2	≥ 85 %	Roth	6366.1
Potassium di hydrogen phosphate	KH_2PO_4	7778-77-0	≥ 99 %	Roth	3904.3
Pyruvic acid	$C_3H_4O_3$	127-17-3	≥ 98 %	Merck	8.20170.0100
Sodium acetate	$C_2H_3NaO_2$	127-09-3	≥ 99 %	Roth	6773.2
Sodium chloride	NaCl	7647-14-5	≥ 99.5 %	Roth	3957.2
Sodium formiate	$NaCHO_2$	205-488-0	≥ 98 %	Fluka	71540
Sodium hydroxide	NaOH	1310-73-2	> 99 %	Roth	6771.2
Sulfuric acid	H_2O_4S	7664-93-9	95-97 %	Merck	100731.1000

9.2.2.3 Chemicals for hydrolysis

Table 9-15: Chemicals for the hydrolysis

Chemical	Formula	CAS-number	Purity	Manufacturer	Product number
Gum arabic				Sigma-Aldrich	51200
Tris HCl	$C_4H_{11}NO_3 \cdot HCl$	1185-53-1	≥ 99 %	Roth	9090.3
Triton X-100	$C_{33}H_{60}O_{10}$			Roth	3051.3

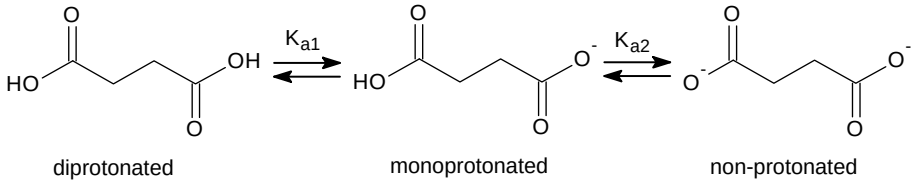
9.2.3 Physico-chemical properties of important chemicals

9.2.3.1 Principal substrates and products of the hydrogenation

Succinic acid

CAS number: 110-15-6

$C_4 H_6 O_4$

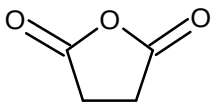


Property	Symbol	Value	Unit	Source
Molecular weight	MW	118.09	g mol^{-1}	
Specific gravity		1.57	- (25 °C)	Mallinckrodt-Baker, Inc.
Melting point	T_{melt}	185 - 190	°C	Acros organics
Boiling point	T_{eb}	235	°C	Acros organics
Flash point		206	°C	Acros organics
Autoignition		630	°C	Chemicalland21
Vapor density		3.04	-	Chemicalland21
pK_{a1}		4.21	-	
pK_{a2}		5.64	-	
Solubility in water		80	g l^{-1} (20 °C)	Acros organics
Partition coefficient	Log P	-0.59	-	Hans et al., 1995

Succinic anhydride

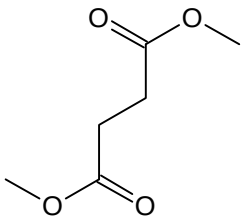
CAS number: 108-30-5

$C_4 H_4 O_3$



Property	Symbol	Value	Unit	Source
Molecular weight	MW	100.07	g mol^{-1}	
Specific gravity		1.234	- (25 °C)	ScienceLab
Melting point	T_{melt}	> 118	°C	Acros organics
Boiling point	T_{eb}	261	°C	Acros organics
Vapor density		3.7	-	Chemicalland21
Solubility in water		20	g l^{-1} (decompose)	Chemicalland21

Dimethyl succinate



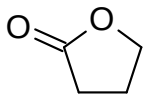
CAS number: 106-65-0

$C_6 H_{10} O_4$

Property	Symbol	Value	Unit	Source
Molecular weight	MW	146.14	g.mol-1	Acros organics
Density		1.11		Acros organics
Refractive index		1.4185-1.4205		Acros organics
Melting point		18-19	°C	Acros organics
Boiling point		200	°C	Acros organics
Flash point		85	°C	Acros organics
Solubility in water		75	g l^{-1} (20 °C)	Merck
Partition coefficient	Log P	0.20 ± 0.24		ACD/Labs

Gamma-butyrolactone

CAS number: 96-48-0

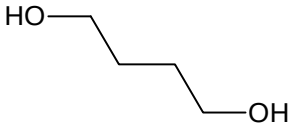


$C_4 H_6 O_2$

Property	Symbol	Value	Unit	Source
Molecular weight	MW	86.09	g.mol-1	Acros organics
Density		1.12		Acros organics
Refractive index		1.4355-1.4375		Acros organics
Melting point		-45	°C	Acros organics
Boiling point		204-205	°C	Acros organics
Flash point		98	°C	Acros organics
Solubility in water		soluble		Merck

1,4-Butanediol

CAS number: 110-63-4



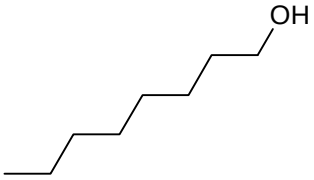
$C_4 H_{10} O_2$

Property	Symbol	Value	Unit	Source
Molecular weight	MW	90.12	g.mol-1	Acros organics
Density		1.01		Acros organics
Refractive index		1.4442-1.4462		Acros organics
Melting point		20	°C	Acros organics
Boiling point		229.2	°C	Acros organics
Flash point		135	°C	Acros organics
Solubility in water		soluble		Merck

9.2.3.2 Principal substrates and products of the esterification

1-Octanol

CAS number: 111-87-5



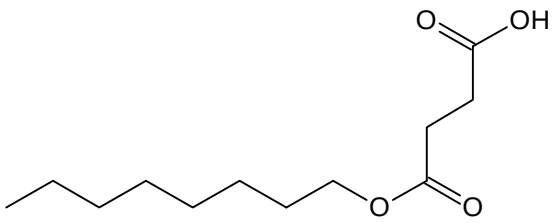
$C_8 H_{18} O$

Property	Symbol	Value	Unit	Source
Molecular weight	MW	130.23	g mol^{-1}	Acros organics
Density	ρ	0.824	-	Acros organics
Melting point	T_{melt}	- 16	°C	Acros organics
Boiling point	T_{eb}	195	°C	Acros organics
Flash point		81	°C	Acros organics
Autoignition		270	°C	ScienceLab
Vapor density		4.5		Mallinckrodt-Baker, Inc.
Vapor pressure		0.07	mm Hg (25°C)	Mallinckrodt-Baker, Inc.
Refractive index		1.428 - 1.431		Acros organics
Solubility in water		0.30	mg l^{-1}	

Monooctyl succinate

CAS number:

C_{12} H_{22} O_4

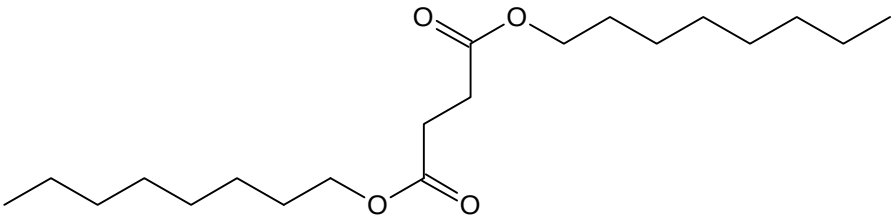


Property	Symbol	Value	Unit	Source
Molecular weight	MW	230.30	g mol^{-1}	ACD/Labs
Density		1.024 ± 0.06	-	ACD/Labs
Flash point		124	°C	ACD/Labs
Boiling point		347	°C	ACD/Labs
Partition coefficient	Log P	3.51 ± 0.37	-	ACD/Labs

Dioctyl succinate

CAS number: 111-87-5

C_{20} H_{38} O_4



Property	Symbol	Value	Unit	Source
Molecular weight	MW	342.51	g mol^{-1}	ACD/Labs
Density		0.936 ± 0.06	-	ACD/Labs
Flash point		168	°C	ACD/Labs
Boiling point		375	°C	ACD/Labs
Partition coefficient	Log P	7.64 ± 0.25	-	ACD/Labs

9.3 Data

9.3.1 ^{31}P-NMR spectra of the immobilized ligand and Ru complex

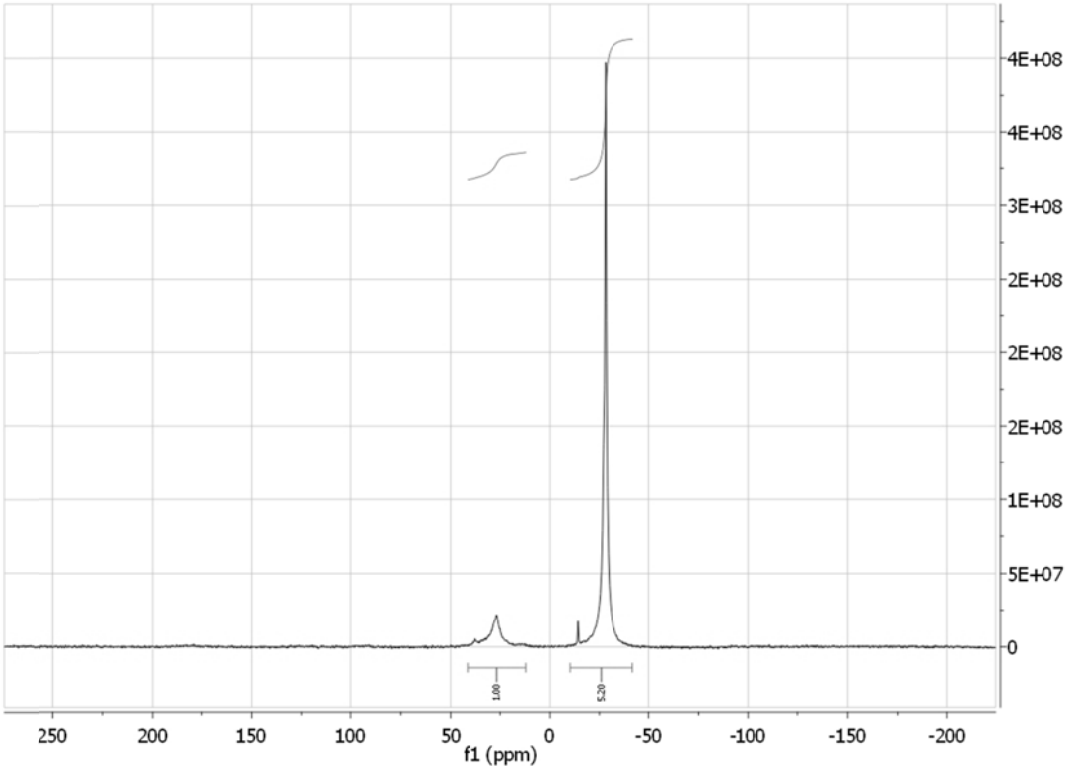


Figure 9-1: MAS ^{31}P-NMR spectrum of the immobilized ligand

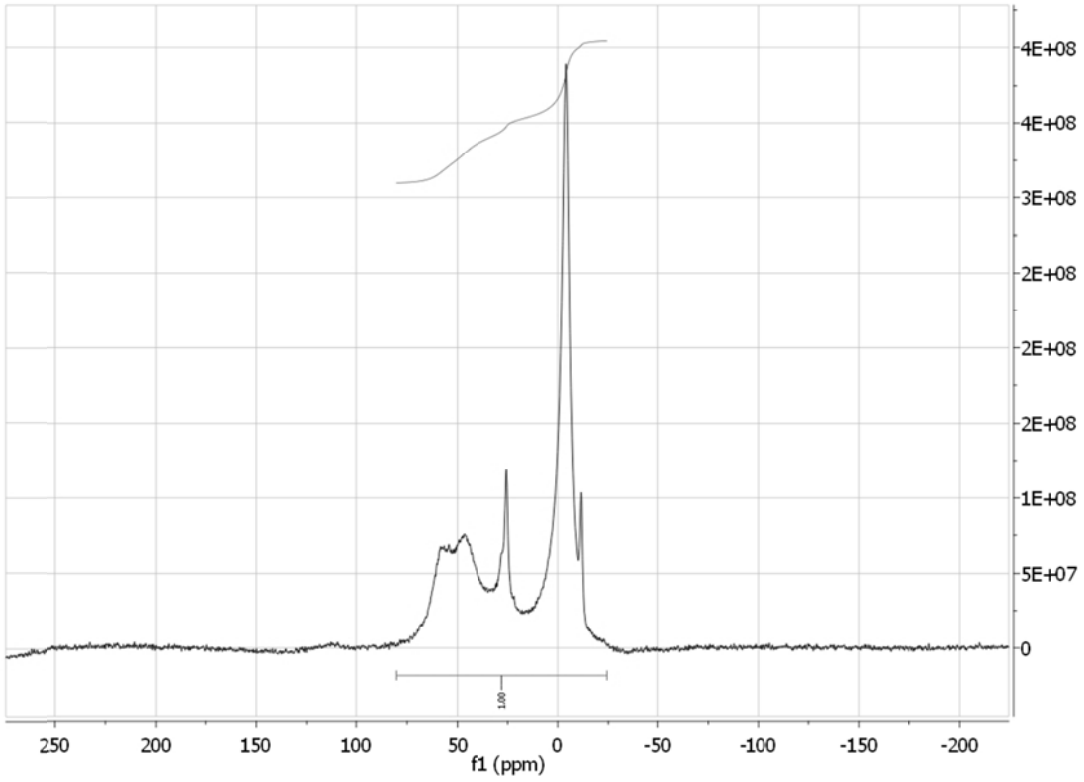


Figure 9-2: MAS ^{31}P-NMR spectrum of the immobilized complex

9.3.2 ^{1}H-NMR spectra of the esters

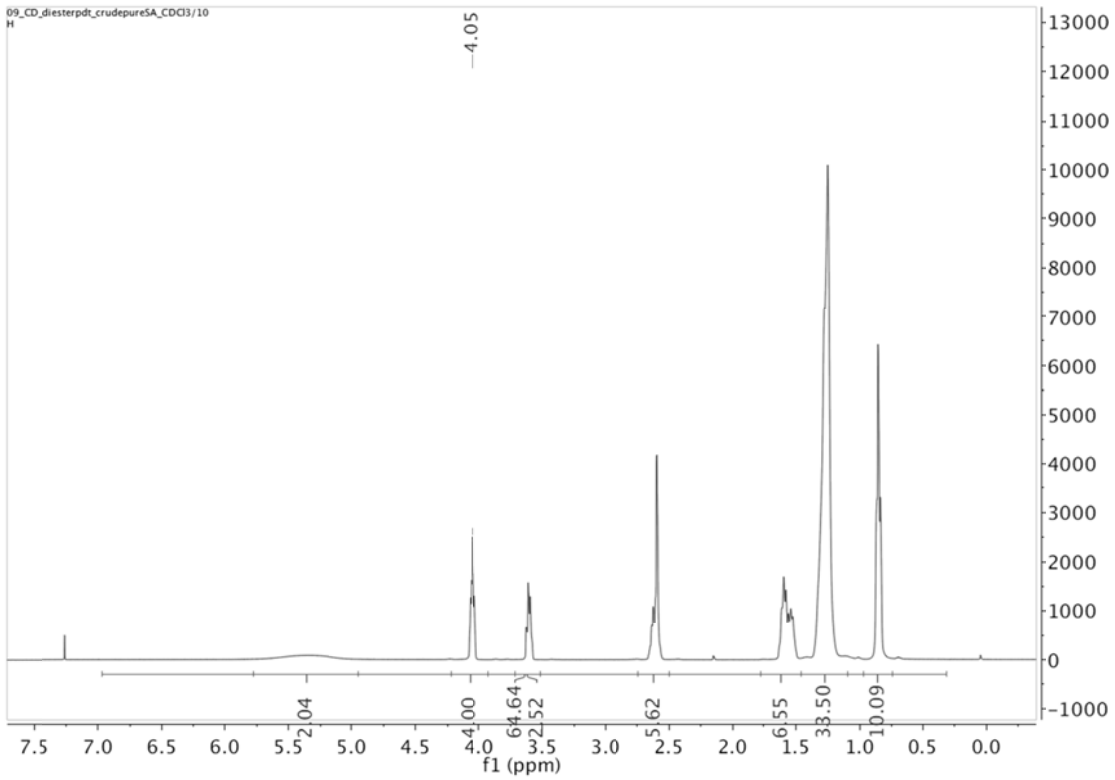


Figure 9-3: ^{1}H-NMR spectrum of the esters from succinic acid in distilled water before filtration.

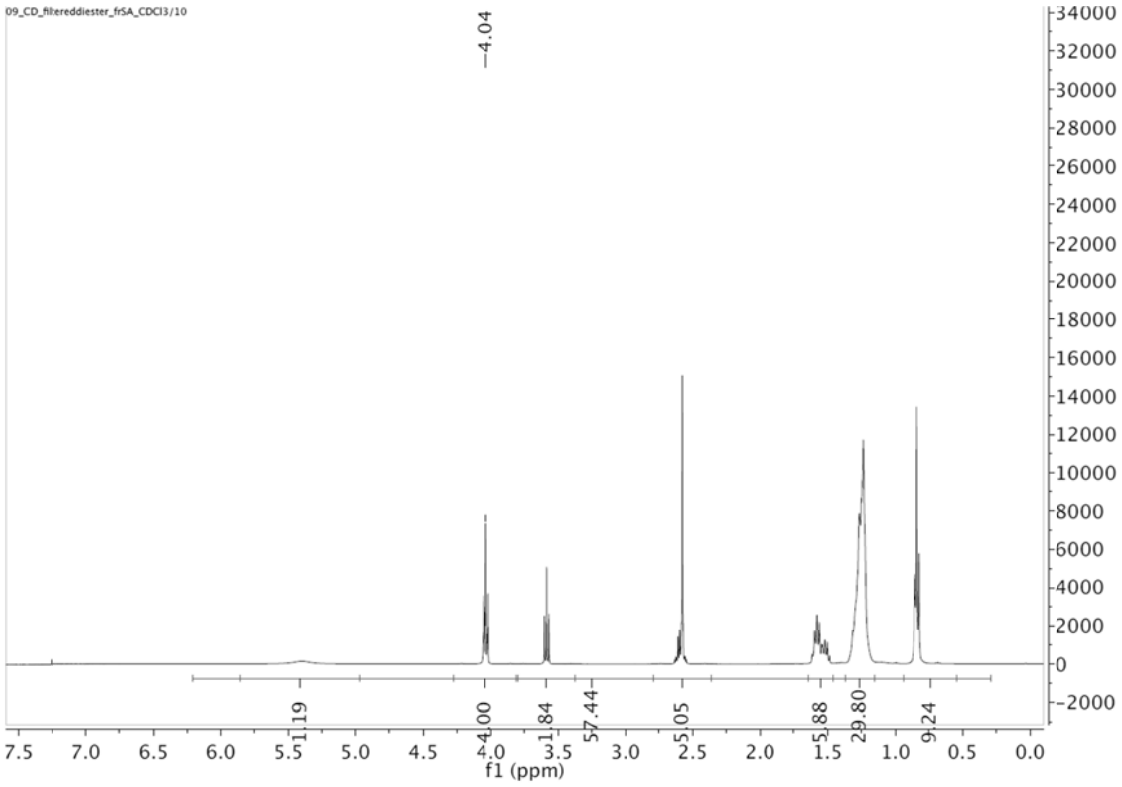


Figure 9-4: ^{1}H-NMR spectrum of the esters from succinic acid in distilled water after filtration.

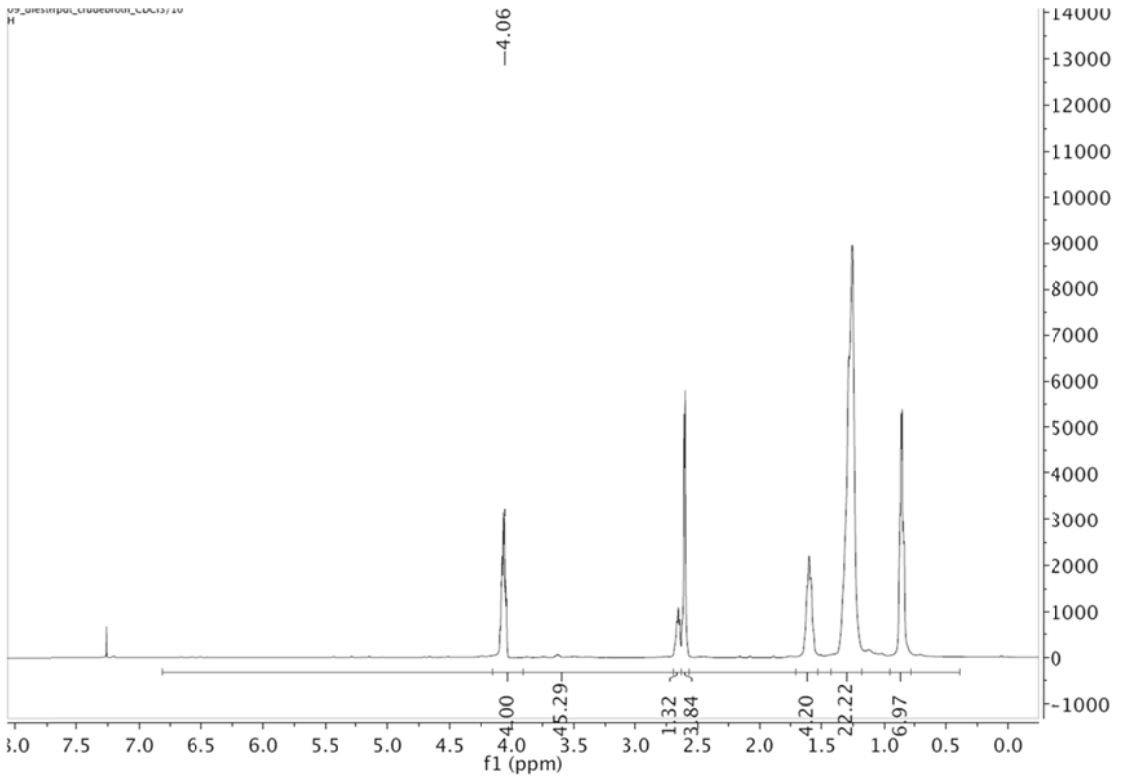


Figure 9-5: ^{1}H-NMR spectrum of the esters from succinic acid in the fermentation broth with DBSA at 1.31 g l $^{tot-1}$ before filtration.

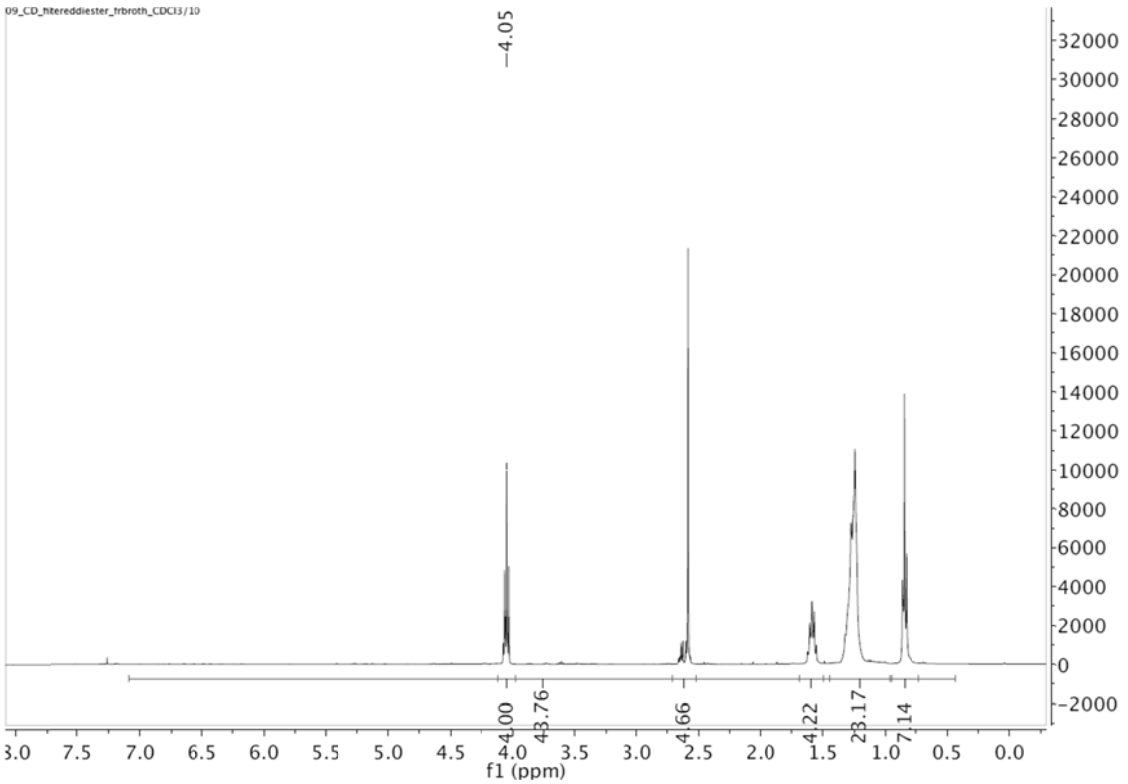


Figure 9-6: ^{1}H-NMR spectrum of the esters from succinic acid in the fermentation broth with DBSA at 1.31 g l $^{tot-1}$ after filtration.

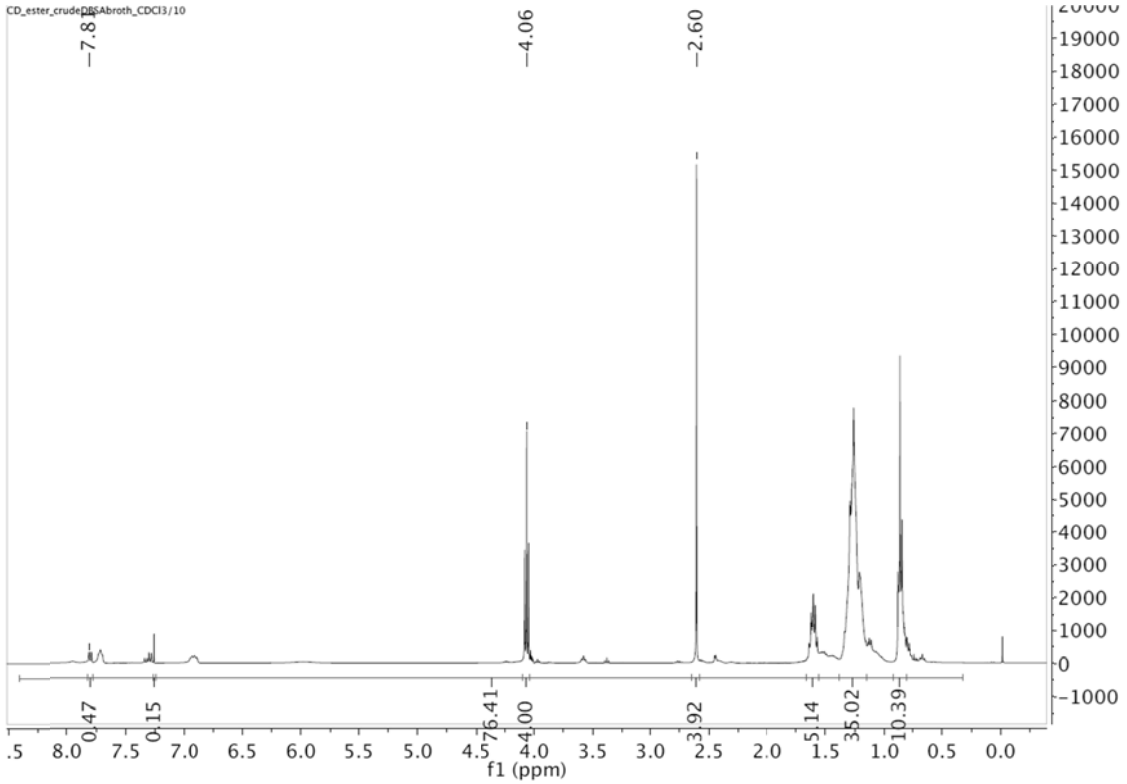


Figure 9-7: ^{1}H-NMR spectrum of the esters from succinic acid in the fermentation broth with DBSA at 2.62 g l $^{tot-1}$ before filtration.

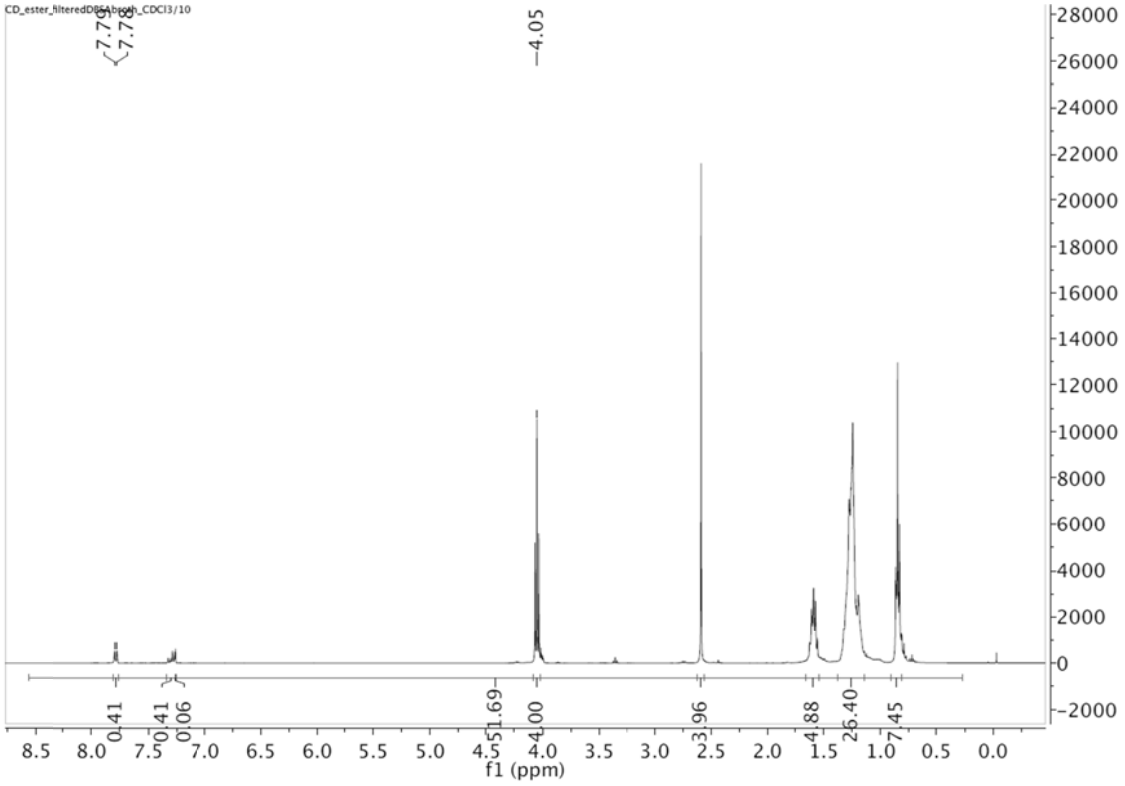


Figure 9-8: ^{1}H-NMR spectrum of the esters from succinic acid in the fermentation broth with DBSA at 2.62 g l $^{tot-1}$ after filtration.

9.3.3 Experimental design for the RSM optimization of the total initial rate constant and the conversions (raw data)

Table 9-16: Raw data of the RSM optimization of the rate contant and the conversion after 6 h.

N°	Init. succinic acid conc., M	pH, -	T, °C	Rate constant, h^{-1}	St. Dev.	Conversion after 6 h, %	St. Dev.
1	0.15	2.00	29.9	0.0199	0.0009	26.8	1.1
2	0.15	2.00	70.2	0.0024	0.0021	22.5	1.2
3	0.15	4.00	29.9	0.0171	0.0019	18.2	0.9
4	0.15	4.00	70.2	0.0373	0.0008	29.5	1.5
5	0.80	2.00	29.9	0.0112	0.0016	26.0	0.1
6	0.80	2.00	70.2	0.0000	0.0000	3.9	1.1
7	0.80	4.00	29.9	0.0155	0.0011	10.5	0.5
8	0.80	4.00	70.2	0.0272	0.0049	16.7	2.8
9	0.15	3.00	47.5	0.0403	0.0049	42.7	1.5
10	0.80	3.00	47.5	0.0420	0.0065	36.7	2.7
11	0.48	2.00	47.5	0.0163	0.0037	29.3	1.5
12	0.48	4.00	47.5	0.0244	0.0005	19.9	0.8
13	0.48	3.00	29.9	0.0224	0.0002	25.7	0.2
14	0.48	3.00	70.2	0.0171	0.0042	23.5	1.3
15	0.48	3.00	47.5	0.0360	0.0040	36.1	1.4

Table 9-17: Validation of the identified polynomials for the rate constant and the conversion after 6 h as functions of initial succinic acid concentration, pH and temperature.

N°	Init. succinic acid conc., M	pH, -	T, °C	Rate constant, h^{-1}	St. Dev.	Conversion after 6 h, %	St. Dev.
16	0.15	4.00	47.5	0.0304	-	24.8	-
17	0.80	3.00	47.5	0	-	21.8	-
18	0.15	3.33	52.4	0.0421	0.0014	41.4	1.5
19	0.15	3.00	52.4	0.0398	0.0013	41.5	1.3
20	0.48	4.00	52.4	0.0268	0.0016	22.7	1.2
21	0.48	3.00	52.4	0.0316	0.0034	33.5	2.0
22	0.15	4.00	52.4	0.0237	0.0022	20.1	1.4