Adv Biochem Eng Biotechnol (2022) 181: 53–72
https://doi.org/10.1007/10_2021_173

Published online: 14 September 2021

Parameters Influencing Lipase-Catalyzed Glycolipid Synthesis by (Trans-) Esterification Reaction

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Contents

Abstract Glycolipids are biodegradable, non-toxic surfactants with a wide range of applications. Enzymatic esterification or transesterification facilitated in reaction media of low water activity is a reaction strategy for the production of tailor-made glycolipids as a high structural diversity can be achieved. Organic solvents, ionic liquids, and deep eutectic solvents have been applied as reaction media. However, several challenges need to be addressed for efficient (trans-)esterification reactions, especially for the lipophilization of polar substrates. Therefore, crucial parameters in (trans-)esterification reactions in conventional and non-conventional media are discussed and compared in this review with a special focus on glycolipid synthesis.

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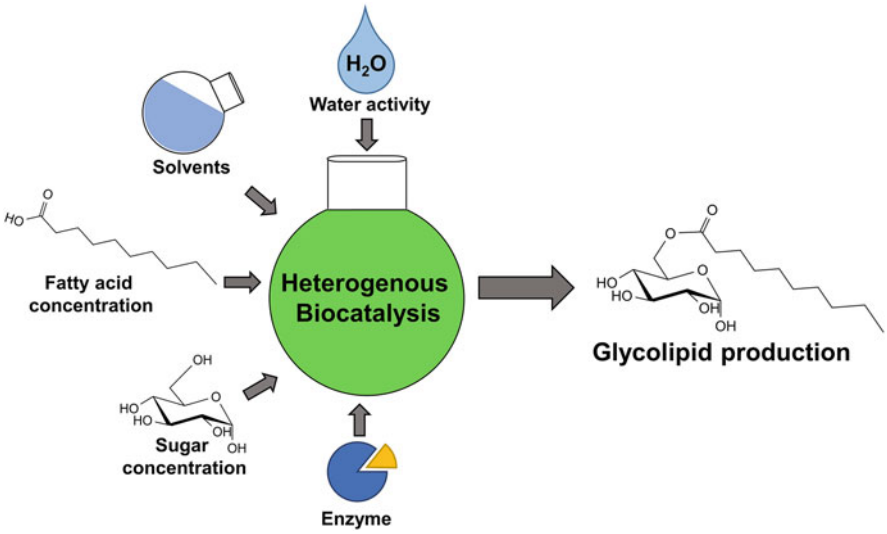
Graphical Abstract



Keywords Biosurfactants, Deep eutectic solvents, Enzymatic synthesis, Glycolipids, Lipases

1 Introduction

Glycolipids are non-ionic surfactants that are not of fossil origin and can be produced entirely based on renewables. They are more ecofriendly than petrochemically-derived surfactants as they pose no risk of accumulation in the environment because they are readily biodegradable [1–6]. Moreover, glycolipids are considered as non-toxic exhibiting no mutagenic potential, low toxicity toward invertebrate and zebra fish, as well as low cytotoxicity against human epidermal keratinocytes [3, 4, 6, 7].

Glycolipids were shown to have excellent surface properties: high surface activities in combination with an efficient lowering of surface tension [8–10]. They efficiently stabilize emulsions and foams [9, 11–13]. Therefore, they present a sustainable alternative to petrochemical surfactants.

Generally, surfactants have a wide field of applications in everyday life, as well as in industry. They are used in detergents, cosmetics and foods, as well as in fire-fighting and petrochemistry [14, 15]. Sucrose esters are glycolipids already approved for application in food industry [16]. Due to their drug permeability enhancing effects glycolipids are also of relevance for the pharmaceutical industry [17]. Moreover, antibacterial, anti-adhesive, antiviral, and tumor inhibiting activities are reported for glycolipids [10, 13, 18–20].

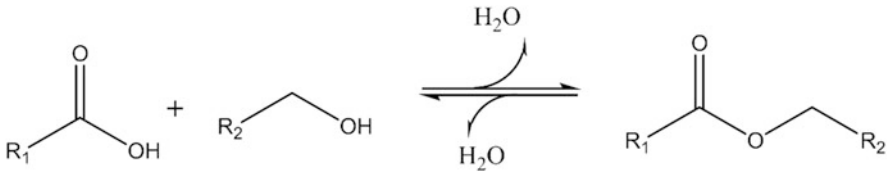


Fig. 1 Reaction scheme of reversed hydrolysis

Chemical synthesis, microbial fermentation, and enzymatic synthesis are possible strategies for glycolipid production. Chemical glycolipid synthesis is industrially established on a large scale by Fischer glycosylation, which ensures low cost production with high yields [21–23]. However, chemical synthesis also has a number of disadvantages: harsh reaction conditions are necessary using high temperatures and acidic catalysts [21–23]. Product mixtures are generated and products are formed which make a costly purification necessary [21–23].

Rhamnolipids, sophorolipids, and mannosylerythritol lipids are microbial lipids with commercial applications in cosmetic and detergent industry [14]. However, structural variety of glycolipids in microbial fermentation is limited to the metabolism of the host. Low glycolipid titers in fermentation broth render purification laborious and costly [24, 25].

Enzymatic synthesis is a method enabling the production of a nearly unlimited diversity of glycolipids [9, 11, 26–29]. Thus, the tailor-made production of glycolipids gets possible. Enzymatic synthesis is based on reverse hydrolysis, which can be catalyzed enzymatically under conditions of reduced water activity (Fig. 1). Hence, organic solvents, ionic liquids, and deep eutectic solvents (DES) are applicable reaction media [30–35]. The use of DES enables glycolipid production entirely based on renewables. A process solely based on lignocellulosic biomass was presented in 2018 by Siebenhaller et al. [36]. By application of a microwave reactor, even a one-pot synthesis of glycolipids from yeast biomass without previous extraction and transesterification of fatty acids was achieved [37].

This review discusses the latest findings on different parameters influencing enzymatic transesterification. Section 2 deals with deep eutectic solvents as they emerged only recently as green alternative to common solvents. Their properties and their health and environmental risk assessment will be addressed. Section 3 presents crucial parameters for enzymatic transesterification. Here, the role of different enzymes (Sect. 3.1), the impact of the sugar loading (Sect. 3.2), the influence of the fatty acid concentration (Sect. 3.3), and the role of water in the reaction systems (Sect. 3.4.) are discussed, as well as the impact of solvent nucleophilicity and solvent hydrophobicity (Sect. 3.5.).

2 Deep Eutectic Solvents

Deep eutectic solvents were first described in 2003 by Abbott et al. [38]. They are a mixture of two solid components, a hydrogen bond donor and a hydrogen bond acceptor, which result in a liquid at room temperature after heating or freeze-drying. DES are considered as supramolecular structures with hydrogen bond interactions [39–41]. A wide range of hydrogen bond donors and acceptors are applicable for DES formation which enable tailoring of the physicochemical properties of DES [42, 43]. There are hydrophilic, water-miscible DES and hydrophobic, water-immiscible DES, binary and ternary DES, as well as acidic, neutral, and alkaline DES covering a wide range of polarities [39, 44–49]. Due to this diversity, DES can be applied as "designer-solvents." DES have a high dissolution power, e.g. choline chloride: urea- and choline chloride: glycerol-DES, as well as ternary DES consisting of choline chloride or guanidine hydrochloride combined with ethylene glycol, propylene glycol or glycerol and p-toluenesulfonic acid are reported to dissolve up to 80% of xylan and lignin from biomass [50, 51]. DES are reported to have stabilizing effects on enzymes while their individual components lead to enzyme denaturation. Urea leads to denaturation and inactivation of *Candida antarctica* lipase B (CalB) by disrupting hydrogen bonds of the enzyme [52]. In choline chloride: urea- DES, diffusion of urea is limited due to the strong hydrogen bond network within the DES and the enzyme remains stable and active [52]. The DES forms hydrogen bonds with the surface of the enzyme resulting in a more rigid structure of the enzyme and an increased thermal stability [52]. In dissolutions of hydrophilic DES the supramolecular structure of DES is remained even with addition of up to 50% water, as water gets incorporated into the hydrogen bond network, only at higher dissolution the structure of DES gets disrupted [39, 41, 53].

In contrast to organic liquids DES are non-volatile and non-flammable [42, 43]. DES have some further advantages over ionic liquids: DES are easier to prepare than ILs and due to the low cost raw materials, DES cost only about 20% of ILs [54]. Furthermore, DES have a higher biodegradability and lower toxicity compared to ILs (see Sects. 2.1 and 2.2).

The applicability of DES-buffer mixtures for fed-batch and continuous processes was shown for the enzymatic esterification of glycerol and benzoic acid in 2019 [55]. Recently, also scalability of a DES system for glycolipid synthesis was proven [56].

2.1 Toxicity of DES

DES are less cytotoxic than ILs [57]. Choline chloride: amino acid DES show about 10 times lower inhibitory effects on enzymes than the imidazolium-based IL [Bmim][BF4] on acetyl choline esterase and the minimal inhibitory concentration toward catalase was even 600–800 times higher than those toward acetyl choline esterase

[58]. DES cytotoxicity is cell line dependent and depends on the hydrogen bond donor used [57]. DES with urea as hydrogen bond donor are less toxic than those with glycerol, ethylene glycol or triethylene glycol [57]. Interestingly, these DES show lower cytotoxicity than aqueous solutions of their single components which indicates a reduced reactivity after DES formation due the strong hydrogen bond network. Glucose based DES are less harmful than fructose based DES [59]. The sugars are metabolized differently in the cells which leads to a higher formation of reactive oxygen species in fructose metabolism compared to glucose metabolism [59]. The cytotoxic effects of DES are related to an increased cell membrane permeability and an increase in reactive oxygen species level [57, 59].

Toxicity of hydrophobic DES has still to be assessed more thoroughly. It is merely known that menthol: lauric acid DES exhibit cytotoxicity toward HACaT cells similar to pure menthol [60].

Choline chloride: amino acid DES also showed 10–200 times lower toxicity toward bacteria than imidazolium or pyridinium derived ILs [58]. DES based on choline chloride or choline acetate as hydrogen bond acceptors and acetamide, glycerol, ethylene glycol or urea as hydrogen bond donors exhibit low toxicity to bacteria at concentrations below 75 mM while they show antibacterial activity at high concentrations [61]. Inhibitory effects toward gram-negative bacteria were higher than toward gram-positive bacteria, suggesting a different mode of action than conventional bacteriocides, e.g. increasing cell permeability [58, 62].

Inhibitory effects of DES based on cholinium and alkanoates on growth of filamentous fungi decreased with increasing alkyl chain. The minimal inhibitory concentrations of all cholinium alkanoates were higher than those of SDS and benzalkonium chloride [63].

Choline chloride based DES show phytotoxic effects depending on the hydrogen bond donor, while the use of ethylene glycol and acetamide shows phytotoxic effects on garlic, urea- and glycerol-DES exhibited no significant phytotoxic effect on garlic [61].

Hydras are freshwater invertebrate used for ecotoxicological studies. Choline based DES exhibit lower toxicity on hydra than their single components and therefore also a lower ecotoxicological burden [61, 64].

2.2 Biodegradability of DES

Biodegradability of the solvents plays a major role in the evaluation of the environmental burden of manufacturing processes. Therefore, this is an important criterion in the selection of reaction media.

DES based on choline chloride with urea or acetamide are characterized as readily biodegradable while those with glycerol and ethylene glycol only showed biodegradability comparable to IL [61]. DES based on ChCl:amino acids were also readily biodegradable [58]. Likewise, the more hydrophobic DES consisting of cholinium hydrogen carbonate and fatty acids showed biodegradability [63]. In DES, a

correlation between low toxicity and high biodegradability was observed [58]. This simplifies solvent selection compared to ILs, since ILs of low toxicity usually show low biodegradability and therefore a high environmental burden [58]. However, there are only a few studies existing on the biodegradability of hydrophobic DES while these data are still missing for most hydrophobic, water-immiscible DES.

3 Enzymatic Synthesis

Success of biotransformations is strongly related to the choice of appropriate reaction conditions. Several parameters are already identified as crucial for enzymatic synthesis of glycolipids in organic solvents as well as in uncommon reaction media. Besides the selection of a suitable enzyme, the water content, substrate concentrations, and solvent properties such as nucleophilicity and hydrophobicity are decisive for efficient enzymatic synthesis (Table 1). These parameters will be discussed in detail in the following chapter.

Enzymatic glycolipid synthesis was demonstrated with three different enzyme classes: lipases, glycosidases, and proteases. Glycolipid production using proteases or glycosidases was less investigated than lipase-catalyzed synthesis.

Protease catalyzed synthesis of sugar fatty acid esters was successfully conducted in organic solvents using subtilisin and *Bacillus pseudofirmus* Al-89 protease [65–67]. 90% conversion was reached in a DMF/water-mixture using subtilisin [65] and 98% conversion to sucrose laurate in 9 h using Protex 6L protease in a tert-amyl alcohol/DMSO/water solvent mixture [67]. In a comparative study, Bernal et al. [68] reached 57% lactulose yield within 24 h using subtilisin and 61% using *Thermomyces lanuginosus* lipase in acetone [68]. So far, no studies on glycolipid

Table 1 Parameters positively influencing the efficiency of transesterification reactions

Parameter	Organic solvents	Ionic liquids	Deep eutectic solvents
Sugar loading	Supersaturated solution	Supersaturated solution	Supersaturated solution
Molar ratio of sugar and fatty acid	Equimolar	n.e.d.	n.e.d.
Water activity	$a_w < 0.2$	$a_w \sim 0.2$	$0.15 < a_w < 0.25$
Water content	Water removal system		Addition of water up to 10%
Solvent nucleophilicity	Low nucleophilicity	Low nucleophilicity	Low nucleophilicity
Solvent hydrophobicity		Medium polarity	
Others		Low halide content	

Table 1 shows which parameters were shown to work out most efficient for enzymatic glycolipid synthesis in the different solvent systems, using Novozym 435 as biocatalyst. *n.e.d.* not enough data for a clear evidence

synthesis using proteases in DES are available. Albeit, it was shown that subtilisin exhibits transesterification activity in choline chloride: urea DES [69].

Glycosidase catalyzed synthesis of glycolipids was conducted in organic solvents and biphasic systems [70, 71]. Miranda-Molina et al. [72] reported the first glycosidase catalyzed glycolipid synthesis in DES [72]. Organic acid containing DES inactivated α-amylase within 4 h while hydrolytic activity was still measureable after 4 h in choline chloride: urea, propanediol: choline chloride: water, choline chloride: glucose: water, and choline chloride: sucrose: water DES. However, at least 20% of the cosolvent water was necessary to maintain alcoholysis activity of α-amylase, in choline:chloride: glucose: water even 60% water was mandatory. At high DES concentrations reaction rates of hydrolysis and alcoholysis reaction were decreased with hydrolysis being affected more strongly. Selectivity of methyl-glucoside synthesis was higher in DES containing reaction media than in pure buffer [72]. Therefore, DES has potential for further investigations as solvent for glucosidase catalyzed glycolipid synthesis.

First lipase-catalyzed lipophilization of polar substrates in DES was reported 2013 by Durand et al. [73]. Water activity, solvent hydrophobicity, and solvent nucleophilicity are parameters that have already been identified as crucial for enzymatic glycolipid synthesis using lipases (Table 1).

3.1 Different Lipases for Transesterification

Several lipases have been screened for activity in DES. Novozym 435 revealed to be the most effective lipase for biodiesel production in DES, followed by Lipozyme TLIM while lipases from *Penicillium expansum*, *Aspergillus niger*, *Aspergillus oryzae,* and *Rhizopus chinensis* showed no or only little activity [64]. The study of Zhao et al. [74] demonstrated that the transesterification activity of Novozym 435 in DES is also higher than that of Amano lipase, porcine pancreas lipase, *Pseudomonas cepacia* lipase, and *Candida cylindracea* lipase in DES [74]. Novozym 435 also proved to be a more active enzyme in the synthesis of trehalose diesters compared to Lipozyme TLIM, porcine pancreas lipase, and *Carica papaya* lipase [12]. Moreover, Novozym 435 was the most effective lipase in sorbitol laurate synthesis in a 2-in-1-DES system consisting of sorbitol and choline chloride [56].

In a two-phase system of an IL and t-butanol Novozym 435 was the most active enzyme for glucose laurate synthesis with a conversion of 59%, while *T. lanuginosa* lipase reached 33% and *R. miehei* 8% [32]. *Pseudomonas cepacia* lipase, *Aspergillus sp.* acylase, *Candida antarctica* lipase A, *Candida rugosa* lipase were also tested in that system, but showed conversions of less than 5% [32].

In organic solvents Novozym 435 was also revealed as efficient biocatalyst. Novozym 435 showed superior performance in glycolipid synthesis in several studies compared to Lipozyme IM, *Candida antarctica* lipase A, and lipases from *Rhizomucor miehei*, *Thermomyces lanuginosa*, *Pseudomonas cepacia,* and *Fusarium solani* [35, 75, 76].

Novozym 435 was more active and stable than CalB covalently immobilized on activated silica supports, activated alumina supports, epoxy-activated sepharose, and tresylated sepharose. Native CalB loses activity exponentially in a first order deactivation pattern, while Novozym 435 shows a much slower deactivation pattern [77]. Due to its robustness and high activity, Novozym 435 is a promising biocatalyst for enzymatic glycolipid synthesis in DES (Table 2).

3.2 *Influence of Water Activity on Lipase-Catalyzed Transesterification*

Hydration of enzymes is important for their stability and activity [78–81]. However, for transesterification reaction almost anhydrous conditions are necessary in order to reverse the enzymes' activity from hydrolysis to esterification [82, 83]. Therefore, water activity is a crucial parameter in enzymatic glycolipid synthesis. Water removal systems were improving reaction yields of glucose fatty acid esters and trehalose diesters in different organic solvents with conversions up to 95% [12, 84, 85].

Novozym 435 is an enzyme widely applied in transesterification reaction due to its beneficial properties. Due to the immobilization of *Candida antarctica* lipase B on a hydrophobic polymeric resin, the carriers do not strip off water from the enzyme and a sufficient hydration level is possible also at low water content of the media [77]. In 2-methyl-2-butanol, highest glucose palmitate yields were reached at a water activity of 0.07, however at such low water content enzyme selectivity was reduced and the diester was produced as side product [31]. Lee et al. [33] reported an optimal water activity of 0.2 for transesterification reactions in ILs with Novozym 435, 0.4 with *Candida rugosa* lipase, and 0.5 with Lipozyme IM. At higher water activities the reaction rates decreased [33]. However, due to the strong hydrogen bond network, a defined water content is necessary for biocatalysis in DES in order to make substrates accessible. Low conversions of phenolic acids were observed without addition of water, while at 8–10% of water (water activity between 0.15 and 0.25) almost complete transesterification occurred [73]. Arabinose laurate yield in DES was significantly increased by an addition of 4% water compared to the reaction in DES without addition of water [86] and also sorbitol laurate conversion in DES was highest with addition of 5% water [37, 56].

3.3 *Influence of Sugar Loading on Enzymatic Glycolipid Synthesis*

Sugar solubility is rather poor in organic solvents applied for glycolipid synthesis, such as acetonitrile, acetone, t-butanol, hexane, or 2-methyl-2-butanol [85]. Ionic

Table 2 Conversions of different lipases in organic solvents, ionic liquids, and deep eutectic solvents

Solvent	Lipase	Reaction conditions	Conversion	Reference
Organic solvents	Novozym 435	Acetone, 45°C, 72 h, glucose palmitate, transesterification	93	[75]
		t-Butanol, 45°C, 72 h, glucose palmitate, transesterification	88%	[75]
		2-Methyl-2-butanol, 40°C, 72 h, fructose palmitate, esterification	53%	[35]
	Rhizomucor miehei	Acetone, 45°C, 72 h, glucose palmitate, transesterification	2%	[75]
		t-Butanol, 45°C, 72 h, glucose palmitate, transesterification	3%	[75]
		2-Methyl-2-butanol, 40°C, 72 h, fructose palmitate, esterification	30%	[35]
	Thermomyces lanuginose	Acetone, 45°C, 72 h, glucose palmitate, transesterification	28%	[75]
		t-Butanol, 45°C, 72 h, glucose palmitate, transesterification	32%	[75]
	Pseudomonas cepacia	Acetone, 45°C, 72 h, glucose palmitate, transesterification	–	[75]
		t-Butanol, 45°C, 72 h, glucose palmitate, transesterification	3%	[75]
Ionic liquids	Novozym 435	60°C, 72 h, glucose fatty acid esters, transesterification [BMIM][BF4]: t-butanol or [BMIM][PF6]: t-butanol (3:2)	59	[32]
	Rhizomucor miehei		8	
	Thermomyces lanuginose		33	
	Pseudomonas cepacia		<5	
	Candida rugosa		<5	
	Candida antarctica lipase A		<5	
Deep eutectic solvents	Novozym 435	50°C, 48 h, transesterification of *Millettia pinnata* seed oil, choline acetate: glycerol	55	[64]
	Lipozyme TLIM		45	
	Penicillium expansum		8	
	Novozym 435 Lipozyme TL IM CalA Immo 150 Lipase TL CLEA Lipozyme 435	50°C, 48 h, sorbitol laurate, transesterification, sorbitol: choline chloride	20 <10 <10 <10 20	[56]

liquids and DES are solvents with a wide range of different physical properties, so that in some, such as [Bmim][TfO] and hydrophilic DES, the sugar solubility is very good while in others it is as limited as in organic solvents [33, 36]. A limited sugar solubility and thus reactant availability can strongly influence the synthesis efficiency and is therefore a crucial parameter.

Flores et al. [85] showed that the dissolution of the excess sugar is not as fast as initial reaction rate in transesterification in 2-methyl-2-butanol [85]. Glucose dissolution rate was enhanced by crystalline ß-glucose and amorphous glucose resulting in higher dissolution rates and higher initial reaction rates. However, only for amorphous glucose a slightly higher yield was observed. A four times higher initial reaction rate and an 18% higher yield were achieved by the application of supersaturated glucose solution [85]. Acylation rates of disaccharides in organic solvents also depend on the dissolved sugar. Higher conversions were reported for disaccharides with a higher solubility. For the production of butanoate esters in tert-butanol yields were improved by using amorphous disaccharides compared to less soluble crystalline disaccharides [87].

Lee et al. [33] could correlate enzyme activity with the dissolved sugar concentration for glycolipid synthesis in ionic liquids [33]. Higher reaction rates and yields were achieved using supersaturated glucose solution than using saturated glucose solution in ionic liquids [33]. These results are in accordance with Shin et al. [88] who reported higher reaction rates, yields and productivities using supersaturated sugar solutions for glucose, fructose, and sucrose laurate synthesis in ionic liquids [88].

A beneficial effect of increased sugar amounts on initial reaction rates and yields was also shown in DES. Higher initial sugar addition resulted in a ninefold increase in glucose monodecanoate yield in a hydrophobic (−)-menthol: decanoic acid DES [28].

3.4 Influence of Fatty Acid Concentration on Transesterification Reactions

Inhibiting effects of high fatty acid concentrations were observed in transesterification reactions in organic solvents. Equimolar ratios of fatty acid and sugar led to highest yields in glucose myristate synthesis in organic solvents while fatty acid excess resulted in reduced conversions [89, 90]. An inhibitory effect of high fatty acid concentrations was also observed in other transesterification reactions catalyzed by *Candida antarctica* lipase B, *Candida rugosa* lipase, and *Rhizopus oryzae* lipase [91–96]. The inhibiting effect of fatty acids is due to the formation of non-productive complexes between fatty acids and the enzyme that are reported for reactions following ping-pong mechanism [91, 93, 96].

Lin et al. [97] reported an optimal fatty acid to sugar ratio of 1:5 for a biphasic system of ionic liquid and 2-methyl-2-butanol while productivity decreased with

higher fatty acid concentrations [97]. Ha et al. [98] investigated sugar to fatty acid ratio from 1:1 to 1:10 in ionic liquids with highest enzyme activity for an equimolar ratio of sugar and fatty acid [98]. However, Mai et al. [99] reported highest glucose laurate yields with an excess of fatty acid (sugar: fatty acid, 1:7.6) and also Galonde et al. [100] reported beneficial effects of a strong excess of fatty acid on mannosyl myristate synthesis in pure ionic liquids [100]. In ionic liquid with DMSO as cosolvent (DMSO:IL, 1:20) a sugar to fatty acid ratio of 3:1 resulted in highest conversions while at equimolar ratios or a greater excess of fatty acid yields decreased [101]. The difference in these studies might be explained by the fact that Ha et al. used free fatty acids and supersaturated sugar solutions in an esterification while Mai et al. and Galonde et al. used vinylated fatty acids and sugar concentrations below saturation in a transesterification reaction. Therefore, the mechanism of the reaction as well as the overall substrate loading differed between the studies limits their comparability. During esterification reaction water is released as a side product which shifts the reaction toward hydrolysis. While in transesterification ethenol is released which tautomerizes to acetaldehyde and evaporates. Thus, the reaction gets shifted toward transesterification and is, therefore, thermodynamically favored.

In DES, an inhibitory effect of excess fatty acid was observed similar to the studies in organic solvents [27].

While fatty acids show in general good solubility in the organic solvents applied in transesterification, fatty acid solubility is limited in many ionic liquids and deep eutectic solvents [27, 102]. Therefore, fatty acids are not necessarily dissolved in ionic liquids and DES, but fatty acid-solvent emulsions may be formed. This inherent difference between the solvent systems might also be an explanation for the varying observations in suitable fatty acid ratios for transesterification reaction.

3.5 Influence of Solvent Hydrophobicity and Nucleophilicity on Lipase-Catalyzed Transesterification

Furthermore, solvent hydrophobicity and nucleophilicity are parameters that are identified as crucial for transesterification reactions. For transesterification of 2-phenyl-1-propanol with vinyl acetate, transesterification rates were higher in more hydrophobic organic solvents: methyl-t-butyl-ether>hexane>toluene>tetrahydrofuran>acetonitrile>dimethylsulfoxide [80]. In organic solvents, higher sugar ester yields were achieved in less nucleophilic solvents. For transesterification using Novozym 435, Šabeder et al. [35] reported higher conversions in butanone and acetone than in t-butanol [35] and Bouzaouit and Bidjou-haiour [30] reported higher reaction rates in tetrahydrofuran and butanone than t-butanol [30]. t-butanol is more polar than butanone and tetrahydrofuran according to the solvatochromic parameter E_T^N [103]. The same pattern was observed using *Candida antarctica* lipase B, *Mucor miehei* lipase and *Pseudomonas*

cepacia lipase for lactose and sucrose ester synthesis, yields were higher in 2M2B than in acetone and lowest in methyl ethyl ketone [104]. Less hydrophilic solvents have lower ability to strip off water from the enzyme [79–81].

It has also been shown for ionic liquids that the enzyme activity depends on the properties of the solvent. For transesterification of benzyl alcohol with vinyl acetate, enzyme stability and enzyme activity was dependent on hydrophobicity of the ionic liquid used [105]. More nucleophilic ILs like [Bmin][TfO] enabled lower enzyme activity and stability than less nucleophilic, more hydrophobic IL [105]. In a transesterification study by Kaar et al. [106], enzyme activity in the ionic liquid [Bmim][PF6] was higher than in organic solvents [106]. However, no transesterification occurred by varying the anions resulting in more hydrophilic ILs. Re-suspension of the enzyme in water revealed that inhibition was reversible with acetate and methylsulfonate anions while nitrate anions exhibited irreversible inactivation of enzymes [106]. Immobilization could not enhance enzyme stability in hydrophilic ionic liquids [106]. Investigations of enzyme structure using IR analysis revealed a loss of the secondary structure of the enzyme in ionic liquids with ethyl sulfate, nitrate, or lactate anions [107]. In these solvents transesterification activity of Novozym 435 was strongly reduced, indicating that nucleophilicity, strong hydrogen bond accepting and donating properties of ionic liquids lead to reduced lipase activity [107]. Similar effects were also reported for transesterification of 2-phenyl-1-propanol with vinyl acetate: transesterification rates were higher in more hydrophobic ILs with higher reaction rates in [Emim][Tf2N] than in [C2OC1mim][Tf2N] and [C2OHmim][Tf2N] [80].

Ganske and Bornscheuer [32] reported no activity of *Candida antarctica* lipase B for synthesis of glycolipids in pure [Bmim][BF4]. However, a conversion of 59% to glucose laurate was achieved by adding t-butanol to the ionic liquid resulting in a two-phase system [32]. In the less nucleophilic ionic liquids [Bmim][TfO] and [Hmim][TfO], Zhao et al. [108] reported up to 26% conversion in pure ionic liquids [108]. In ionic liquids with the more nucleophilic anion methyl sulfate lower conversion was achieved even though sugars were highly soluble in that system [108]. Also for those ionic liquids, higher conversion rates were achieved after mixing with an organic solvent [108]. Lin et al. [97] reported also that ionic liquids with methyl sulfate anion showed low conversions, while conversions in ionic liquids were better with increasing hydrophobicity of the cations. In a comparative study with four different ionic liquids and their mixtures, highest productivities combined with a high lipase stability were reported for mixtures of hydrophilic and hydrophobic ionic liquids [33].

Effects of deep eutectic solvents are less thoroughly investigated than in organic solvents or ionic liquids. However, some similarities between DES, organic solvents, and ionic liquids could already be observed. Hollenbach et al. [27, 28] showed that an increased solvent hydrophobicity increases glycolipid yields and also initial reaction rates were higher in the hydrophobic (−)-menthol: decanoic acid DES than in hydrophilic ones [27, 28]. Full conversion to menthyl laurate was reported for transesterification reaction using *Candida rugosa* lipase in a hydrophobic menthol: lauric acid DES [109, 110].

Moreover, the anion of the hydrogen bond donor affected transesterification reactions in DES. Zhao et al. [108] investigated glucose laurate synthesis in two-phase systems of 2-methyl-2-butanol and DES. Almost no conversion was observed (Lipozyme TLIM and Novozym 435) in choline chloride: urea and choline chloride: glycerol-DES, neither with Novozym 435 nor with Lipozyme TLIM, while higher conversion rates were obtained in choline acetate based DES, which were nevertheless lower than 15% [108]. Also for biodiesel production, choline acetate based DES were better suited than choline chloride based ones [64]. Glycerol and ethylene glycol as hydrogen bond donor resulted in higher activity than urea or acetamide for the production of biodiesel [64]. It was suggested that the hydrogen bonding network of the polyols would have an activating effect on the enzyme by interacting with a serine residue [64]. Elgharbawy [111] demonstrated increased hydrolytic lipase activity in choline chloride based DES with sugars as hydrogen bond donor for porcine pancreas lipase, Novozym 435, Immobead 150, and *Rhizopus niveus* lipase, while *Candida rugosa* lipase and Amano lipase PS stayed unaffected [111]. Contrarily, malonic acid and glycerol as hydrogen bond donors showed some inhibitory effects [111]. Oh et al. [47] investigated lipase activity and lipase stability in various DES [47]. Lipase was more active in DES with an amide hydrogen bond donor than with a polyol hydrogen bond donor, but for lipase stability the relation was reversed [47]. Still, they could not identify a correlation between solvatochromic properties of the DES and lipase activity [47].

4 Conclusion

The selection of the reaction conditions is a crucial step in biotransformation. For lipophilization of polar substrates, some parameters could already be identified as decisive for synthesis success independent of the solvent type.

High sugar concentrations and the use of supersaturated sugar solutions were revealed as beneficial for transesterification yields in all solvent types. In organic solvents an equimolar ratio of sugar and fatty acids resulted in highest conversion rates as an excess of fatty acids might lead to inhibitory effects. For ionic liquids and deep eutectic solvents, there are still more studies necessary to provide clear evidence as the field of applicable ionic liquids and deep eutectic solvents is a widely diverse field and solubility of fatty acids in these solvents varies considerably.

Low water activity is necessary to prevent hydrolysis of the ester products in organic solvents, as well as in ionic liquids and deep eutectic solvents. However, a certain water addition is mandatory in deep eutectic solvents to allow for an efficient reaction.

Solvent nucleophilicity and solvent hydrophobicity were also crucial no matter what type of solvent was used. Selecting a solvent with low nucleophilicity promises the highest yields as no water will be stripped off from the enzyme and solvents of low nucleophilicity do not disturb enzyme structure. Nevertheless, comparative

studies with solvents of different nucleophilicity and hydrophobicity are still needed, especially for DES, as the currently available studies do not cover the broad spectrum of possible DES systems.

Acknowledgments *Conflicts of Interest:* The authors declare no conflict of interest.

Author Contributions: Conceptualization, R.H.; writing – original draft preparation, R.H.; writing – review and editing, R.H., K.O and C.S.; supervision, C.S.; funding acquisition, C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work by R.H. was supported by the European Regional Development Fund and the Ministry of Science, Research and the Arts of the State of Baden-Württemberg within the research center ZAFH InSeL (Grant#32-7545.24-20/6/3). We gratefully thank the Open Access Publishing Fund of Karlsruhe Institute of Technology.

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