processes

MDPI

Review

Recent Progress in Non-Aqueous Biocatalysis of Immobilized Enzymes

Jiayun Ma, Luyao Wang, Yan Chu, Yitong Wang, Kequan Chen and Hui Li *

State Key Laboratory of Materials—Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211816, China
* Correspondence: lihui11@njtech.edu.cn

Abstract: Non-aqueous biocatalysis has attracted broad interest recently due to its differences from traditional aqueous catalysis and increased substrate solubility, which reduces feedback inhibition, improving enantiomer selectivity and completing synthesis reactions that cannot be performed in an aqueous solution. This approach shows remarkable application value in producing natural products, chemical products, pharmaceutical intermediates, and foods. This study aims to provide a concise overview of the current state of non-aqueous biocatalysis and its sustainability, summarizing the mechanism of non-aqueous biocatalysis and recent progress using immobilization technology. It includes different non-aqueous systems, such as organic phase systems, two-phase systems, ionic liquid systems, deep eutectic solvent systems, and non-solvent systems. Finally, this manuscript illustrates the challenges of non-aqueous catalysis and the prospects of the future areas of non-aqueous catalysis research.

Keywords: non-aqueous; biocatalysis; immobilization

Citation: Ma, J.; Wang, L.; Chu, Y.; Wang, Y.; Chen, K.; Li, H. Recent Progress in Non-Aqueous Biocatalysis of Immobilized Enzymes. *Processes* **2024**, *12*, 1571. https://doi.org/10.3390/pr12081571

Academic Editor: Adina Musuc

Received: 19 June 2024
Revised: 20 July 2024
Accepted: 23 July 2024
Published: 26 July 2024

1. Introduction

Biocatalysis, a green technology in synthetic organic chemistry, offers a novel method for synthesizing products in an environmentally friendly way. Because enzymes have specific active centers, they can provide chemoselectivity, regioselectivity, or stereoselectivity for products more easily than chemical catalysis. Biocatalysis can accomplish transformations that cannot be performed by chemical methods [1–4]. These advantages have made biotransformation increasingly popular in synthesizing natural products, chemicals, pharmaceuticals, and foods [5–9]. Because water is a natural solvent, most current research on targeted biosynthesis is usually founded on aqueous solutions [10]. However, not all reactions can be performed in an aqueous environment. For example, when some drugs or chemicals are utilized as hydrophobic substrates, the aqueous phase system will cause the enzyme to separate from the substrate, thus hindering reaction progress.

Therefore, non-aqueous phase biocatalysis, as an emerging area in industrial production, has been proposed to overcome such problems. Aqueous phase catalysis can result in costly purification processes because water has a higher boiling point and lower vapor pressure. In addition, biocatalytic reactions in the aqueous phase produce undesirable side reactions, including hydrolysis, racemization, polymerization, and decomposition, which are detrimental to process production and economic efficiency.

In the mid-1980s, Klibanov discovered that enzymes retain their catalytic function even in highly dehydrated organic solvents. Notably, some enzymes, including lipase and esterase, may have higher catalytic activity in organic solvents relative to aqueous environments. Over the next two decades, non-aqueous enzyme-catalyzed reactions rapidly emerged and found diverse applications. These studies have uncovered numerous advantages of enzyme-catalyzed reactions in non-aqueous phase systems. They enhance the solubility of organic substrates, allowing for more efficient reactions. Additionally, they alter the equilibrium direction of the

reaction, driving it toward the desired products and increasing the stereoselectivity of the reaction, leading to the formation of specific products with precise configurations. Furthermore, non-aqueous enzyme-catalyzed reactions inhibit side reactions involving water and readily eliminate substrate and product inhibition.

Except for some lipases and esterases, other enzymes are highly sensitive to organic solvents and less stable in organic media than in aqueous environments. Therefore, to improve the catalytic efficiency and recovery of enzymes, some modifications are often made to the enzymes. The enzyme can be genetically engineered, as Cai et al. obtained recombinant methanol-stabilized esterase by gene engineering. In his study, the *Bacillus subtilis* DSM13552 gene estGSU753 was cloned, sequenced, and overexpressed in *Escherichia coli* BL21. The new gene had an open reading frame of 753 bp and encoded a 250 amino acid esterase. The esterase retained 50% of its original activity even after 35 h of incubation in 90% methanol. In addition, modified enzymes can synthesize short-chain flavor esters and a more than 99% conversion rate is obtained within 6 h [11].

Enzymes can be modified with materials to improve their stability. Enzyme immobilization has been an extraordinary approach for large-scale applications because of the ease of catalyst recycling, continuous operation, enzyme separation, diverse choice of reactors, easy product purification, and low downstream processing costs [12,13]. Researchers have examined various enzyme immobilization strategies, including affinity adsorption, covalent binding, cross-linking, anti-micelle, and capture/encapsulation in emulsions, as well as organic polymers like polyallylamine, activated charcoal, and chitosan and inorganic polymers such as nano graphene oxide, nano-silica, iron oxide, and nano-gold [14]. Golombek et al. entrapped the solvent-sensitive enzyme mandelate racemase in (cross-linked) polymersomes to protect it from the organic phase. Mandelate racemase in (cross-linked) polymersomes remained active in highly dispersed biphasic systems for over 24 h. The free enzyme, in contrast, was completely inactivated within 1 h, illustrating the potential of polymersomes as nano-reactors in biphasic reaction setups. This is because the covalent cross-linking of individual chains of the block copolymer poly (2-methyloxazoline) 15-poly (dimethylsiloxane) 68-poly (2-methyloxazoline) 15 via terminal methacrylates leads to enhanced membrane stability, higher mass transfer, and faster conversion [15].

To provide researchers in biology, chemistry, pharmacy, and medicine with a quicker and more up-to-date understanding of non-aqueous phase biocatalysis in recent years, we identify that biocatalysis shows remarkable value in the aqueous phase system, organic phase system, ionic liquid system (IL), two-phase system, deep eutectic solvent system (DES), and non-solvent system (Figure 1). Specifically, we summarize the properties of five non-aqueous catalytic reaction media, focusing on their mechanisms. Then, we introduce the immobilized enzyme catalysis in different types of solvents. Finally, we outline the current challenges the non-aqueous phase catalysis faces and propose future directions.

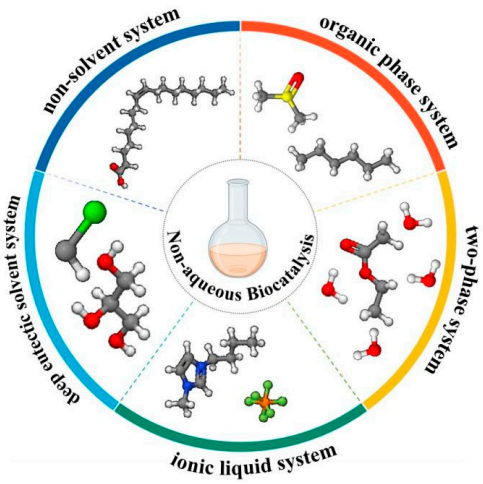

Figure 1. The main classification of non-aqueous biocatalysis.

2. Organic Phase System

Biotransformation that takes place in a completely pure organic phase offers significant advantages. For instance, it will increase the solubility of non-polar substrates, reverse the thermodynamic equilibrium of the hydrolysis reaction, inhibit the water-dependent side reactions, alter the substrate specificity, and eliminate microbial contamination. It has greatly facilitated the use of organic solvents in industrial production. Enzymatic reactions are carried out in the organic phase, which not only offers the benefit of being able to solubilize hydrophobic substrates but also avoids emulsions generated by a two-phase aqueous/organic solvent system. As two-phase emulsion makes product separation and post-processing more challenging, when pure organic media are used, downstream processing and unit operation steps can be simplified via simple filtration or decantation [16,17]. It will make biocatalysis of organic phases ideally suited for producing bulk chemicals [18].

However, the application of enzymes in organic media is limited because the majority of enzymes are less active and less stable in organic solvents and not all enzymes can operate in organic solvents. Some micro-organisms from *Bacillus* sp., *Rhodococcus* sp., and *Staphylococcus* sp. are more tolerant to organic solvents [19,20] and their extracellular lipases have better stability in organic solvents, often used in industrial production [21]. Senaite Leykun et al. successfully isolated a thermophilic lipase SHI-160, an organic solvent-tolerant lipase from *Bacillus subtilis*, from a hot spring in the Rift Valley, East Africa. SHI-160 can catalyze the reaction at an optimal temperature of 65 °C and retain over 90% of its activity after incubation at 70 °C for 1 h. The enzyme could catalyze both polar and non-polar reactions at high temperatures. The enzyme is stable in both polar and non-polar organic solvents. This suggests the potential of lipase SHI-160 to catalyze reactions in non-aqueous media for synthesizing valuable compounds [22].

While organic solvents disrupt the integrity and stability of cell membranes [23–25], some organic-solvent-tolerant bacteria can still thrive in toxic environments. Because whole-cell catalysts benefit from protecting the cellular matrix, enzymes in crude extracts, especially when purified, may be more fragile than whole-cell catalysts. Therefore, researchers determined that the tolerance of purified enzymes to organic solvent toxicity can be enhanced by protein engineering and physicochemical methods such as immobilizing enzymes through modification or embedding [26–28], which can attain higher catalytic efficiency. Iuliano et al. reported the use of waste fish oil as a raw material for wax ester production by esterification [29]. They proposed an approach to catalyze emollient synthesis using immobilized lipase in the presence of oleol alcohol. Through ion exchange, interfacial activation, and covalent anchoring, they immobilized lipase from *Candida acuraculus* on a magnetic amino-functionalized super-cross-linking resin. They not only successfully achieved a 90% immobilization rate but the yield reached 94% at 45 °C after 12 h. Table 1 summarizes the literature on the catalysis of immobilized enzymes in the organic phase and their properties.

Table 1. Recent progress in organic phase-based immobilized enzyme catalysis.

Organic Phase System	Carrier	Time (h)	Catalyst	Substrate	Product	Conversion (%)	Reusability	Ref.
Cyclohexane	Super-absorber	24	OxdB	N-octanaloxime	N-octanenitrile	>99	-	[30]
N-hexane	Macro-porous resin	10	*Candida rugosa* lipase	Lauric acid Phytosterol	Phytosterol ester	96.6	6	[31]
N-hexane	Magnetic amino-functionalized hyper-cross-linked resin	12	*Candida rugosa* lipase	Waste fish oil	Wax ester	94	10	[29]

Table 1. *Cont.*

Organic Phase System	Carrier	Time (h)	Catalyst	Substrate	Product	Conversion (%)	Reusability	Ref.
Chloroform	Acrylic resin	-	Novozym 435 lipase	Lactic acid Ethanol	Ethyl lactate	88	5	[32]
MTBE	Acrylic resin	24	Novozym 435 lipase	N-trans-4-coumaroyltyramine	Coumaroyltyramine	65	-	[33]
N-hexane	Iron magnetic nano-particles	8	*Candida antarctica* lipase B	Butyric acid Methanol	Methyl butyrate	96.8	12	[34]
Tert-butanol	Macro-porous resin	3	Yarrowia lipolytica lipase	P-Nitrophenyl laurate	Nitrophenol Lauric acid	-	5	[35]
N-heptane	Santa barbara amorphous-15	3	Thermophilic lipase QLM	Palmitic acid 2-ethyl hexanol	2-ethylhexyl palmitate	99	10	[36]
Petroleum ether	Macro-porous resin HPD826	6	*Candida antarctica* lipase B	Vitamin A acetate Palmitic acid	Vitamin A palmitate	84	15	[37]
2-methyl-2-butanol	Acrylic resin	24	*Candida antarctica* lipase B	Bixin Sorbitol	Sorbitol ester of norbixin	50	-	[38]
Tetrahydrofuran	Acrylic resin	72	*Candida antarctica* lipase B	3-(1-acetoxyethyl) phenyl acetate	(S) and (R) enantiomers of 3-(1-hydroxyethyl) phenol	50	-	[39]

3. Two-Phase System

Two-phase biocatalysis typically involves the use of two immiscible liquid phases, often aqueous and non-aqueous. The non-aqueous phase contains organic solvents [40–42] and ionic liquids [43,44], widely used for the transformation of non-hydrophilic substrates. Two-phase is used narrowly to refer specifically to the aqueous-oil phase to distinguish it from ionic liquid systems and low-eutectic systems. The advantages and disadvantages of two-phase catalysis and its progress will be outlined.

Two-phase catalysis has various advantages, including the presence of water protecting the enzyme conformation and maintaining enzyme stability. Water is directly or indirectly involved in all non-covalent interactions, maintaining the natural conformation of the enzyme for catalysis [45,46]. If the enzyme possesses a completely anhydrous environment for a long period of time, the spatial conformation of the enzyme will be distorted and lead to inactivation. Therefore, the two-phase system is more rational because it can overcome the problem of isolation and purification. Under the limiting conditions, once the requirement for water is satisfied, the remaining water will not affect the enzyme activity, even if it is replaced by an organic solvent.

As organic compounds only exhibit limited hydrophilicity, the load of the substrate in biocatalytic transformations is usually restricted to the low millimolar range, which is far lower than the titer required in industrial applications [47]. Various strategies and process designs have been developed to enhance substrate concentration and limit solvent usage. Enzymatic reactions typically occur in the aqueous phase at substrate concentrations near the solubility limit, with the non-aqueous phase (usually the organic phase) operating as a substrate reservoir, continuously delivering the substrate to the aqueous phase. Simultaneously, the non-aqueous phase is a product sink, removing reaction products from the aqueous phase. In addition to achieving an extremely high substrate concentration, two-phase biocatalysis offers several essential advantages, including preventing inhibition

or changes in reaction equilibrium through continuous product removal and separating products through phase separation [48].

Although oil–water systems are widely used in enzyme-catalyzed synthesis, it is difficult for the enzyme to make contact with the substrate due to the large mass transfer resistance in the two-phase system. It is often necessary to use co-solvents, surfactants, or vigorous agitation. However, using co-solvents and surfactants can make it difficult to separate and purify products. Additionally, intense stirring requires a significant amount of energy and creates shear forces that can permanently alter the tertiary structure of the enzyme, leading to a reduction in its activity or deactivation.

Pickering emulsions (PEs) can be widely cited in the field of biocatalysis as an excellent system for catalytic hydrophobic substrates. PE was first proposed by Ramsden in the 20th century [49]. In the system, the use of surfactants was abandoned and some solid particles were used as emulsifiers to stabilize the whole system. After the emulsifier enters the oil–water phase, the irreversible adsorption of these solid particles at the oil/water interface forms a dense film at the interface, blocking the collision between the two-phase molecules; in addition, the surface adsorption of granulation will also increase the repulsion between the emulsion droplets, which are also the stabilizing mechanisms of the currently accepted PE. In Xu's research [50], *Candida antarctica* lipase B modified with a metal-organic framework operated as biphasic biocatalysts, which can prepare oil-in-water (*o*/*w*) PE with an oil/water volume ratio of 3 by homogenizing p-nitrophenyl palmitic acid n-heptane solution into a ZIF-8@CALB aqueous dispersion. The enzyme-catalyzed reaction had a conversion rate of up to 48.9% within 0.5 h, while the p-NPP n-heptane solution system containing free CALB only achieved a stable product conversion rate of 6.8%. In all eight cycles, the hydrolytic equilibrium conversion rate of p-NPP was maintained at 40%, reflecting the high catalytic efficiency and enzyme reuse ability of the PE [50]. This study offered a new opportunity for practical application of the design of enzyme-MOFs-based Pickering interface biocatalysts.

In addition, Pickering emulsions can be used to solve the problems caused by stirring mentioned above. For the first time, Yang et al. used a non-stirring Pickering emulsion as a reaction platform to overcome the problem of inefficient enzymatic reactions in organic-aqueous systems [51]. In Yang's experiment, a silica nano-sphere was used as the emulsifier and *Candida antarctica* lipase B as the catalyst. The experimental results show that the reaction efficiency of the Pickering emulsion reaches the maximum value without stirring. This result shows that in PEs, due to the large interface generated by the emulsifier, the enzyme and the substrate can easily contact and react at the interface and the catalysis of the aqueous solution can protect the activity of the enzyme well, so the factor affecting the reaction efficiency is not the stirring rate but the size of the droplet. Although the Pickering emulsion system achieves good substrate utilization and high enzyme recovery, the problem of two-phase separation complicating the formation of a stable emulsion should also be considered [52]. Advances in two-phase-based biocatalysis are listed in Table 2.

Table 2. Recent progress in two-phase-based immobilized enzyme catalysis.

Two-Phase System	Ratio	Carrier	Catalyst	Substrate	Product	Conversion (%)	Ref.
N-heptane/Water	3	ZIF-8	*Candida antarctica* Lipase B	Cinnamic acid	Benzyl cinnamate	48.9	[50]
Methanol/Water	4:1 (*v*/*v*)	Nano-fibrous membrane	*Candida antarctica* lipase B	2-Bromoethyl ketone Salicylaldehyde	Benzofuran-2-yl (phenyl) methanone	88	[53]
Acetonitrile/DMSO	3:2	XAD1180 resin	Lipase UM1	Dihydromyricetin	Vitamin E succinate	99	[54]

Table 2. *Cont.*

Two-Phase System	Ratio	Carrier	Catalyst	Substrate	Product	Conversion (%)	Ref.
Dichloromethane/Water	-	Metal-surfactant nano-capsules	Phospholipase D	Phosphatidylcholine	Phosphatidylserine	91.9	[55]
Ethyl acetate/Water	1:1	Mesoporous silica cube	Phospholipase D	Phosphatidylcholine	Phosphatidylserine	91.2	[56]
Castor oil/Water	1.60:1	Fe_3O_4@chitosan	*Candida rugosa* lipase	Castor oil	Ricinoleic acid	46.8	[57]
Paraffin oil/Water	1:1 (*v*/*v*)	Lignin/Chitosan nano-particles	*Candida rugosa* lipase	P-nitrophenol palmitate	Nitrophenol Palmitic acid	100	[58]
Hexane/Water	3:2	Coffee ground	*Candida rugosa* lipase	4-Nitrophenol palmitate	4-nitrophenol	74	[59]
Ethyl acetate/Sodium acetate	1:1	Sodium Alginate	*β*-glucosidase	Genipin	Geniposide	47.81	[60]

4. Ionic Liquid System

Ionic liquid (IL) systems have been among the most widely researched fields in the past decade [61,62]. The traditional definition of ionic liquids is a class of fluid at low temperatures due to the formation of bulky chloroaluminate or chlorozincate ions at eutectic compositions of the mixture; however, ionic liquids are now commonly referred to as solvents composed of ions only. The traditional definition was first employed to describe ionic liquids based on chloroaluminate salts. The first generation of ionic liquids were those derived from organic cations with $AlCl_3$ and $ZnCl_2$ [61]. Such ionic liquids are fluid at low temperatures due to large volumes of chloroaluminate or chlorozincate ions in the eutectic components of the mixture. This reduces the charge density of the ions, which reduces the lattice energy of the system, producing a lower freezing point. The second generation of ionic liquids consists of discrete ions instead of the complex eutectic mixtures of ions that are found in first-generation ionic liquids.

Wilkes and Zaworotko, using alkyl imidazolium salts, found that air- and moisture-stable liquids could be synthesized by replacing $AlCl_3$ in low eutectic ionic liquids with discrete anions (tetrafluoroborate and acetate). HF is a useful tool for synthesizing low eutectic ionic liquids [63]. Several studies have observed that exposure to moisture could impact some chemical and physical properties. Water content increases as HF is produced [64]. The stability of ionic liquids can be improved by the addition of more hydrophobic anions, such as trifluoro thanesulfonate (CF_3SO^{3-}), bis (trifluoromethane sulphonyl) imide [$(CF_3SO_2)_2N^-$], and tris (trifluoromethane sulphonyl) methide [$(CF_3SO_2)C^-$] [40,64,65]. These systems have the additional benefit of large electrochemical windows, allowing less noble metals, inaccessible from chloroaluminate liquids, to be electrodeposited [64].

ILs have the potential to be versatile solvents and can be easily adapted to specific applications. Firstly, ILs are widely utilized in the synthesis of biodiesel. Lozano et al. reported a straightforward strategy using a mixture of the hydrophobic [C_{16}mim] [NTf_2] with the hydrophilic [Bmim] [Cl] as a solvent to extract algae triglycerides and biotransform them into biodiesel. After reacting for 2 h at 60 °C, a 100% biodiesel yield was attained. Furthermore, the unique sponge-like [C_{16}mim] [NTf_2] permits clean separation of the biodiesel product easily (via cooling or centrifugation) as well as the recovery and reuse of the biocatalyst and IL system. Combining the unique properties of ionic liquids with biocatalysts provides a sustainable solution for the synthesis of biofuel and enhances the possibility of developing green industrial processes [66]. Fan et al. also studied the hydroxyl-functionalized ionic liquid [C_1C_3OHPyr]NTf_2 and used it as a medium for lipase to produce biodiesel by transesterification. Furthermore, recycling ILs and lipase is relatively easy via water and acetone washing, respectively [67].

Additionally, ILs can operate as bifunctional solvents. This indicates that IL operates as both a solvent and a catalyst. Chen et al. developed a novel, efficient, and simple method for the synthesis of benzothiazoles under solvent-free conditions. They utilized [Bmim] PF_6 as a catalyst and 2-aminobenzenethiols and aldehydes as raw materials. In this reaction, there is no requirement for additional organic solvents and it has a wide substrate scope and excellent yield [68]. In Wei's study, the synthesis of sulfonic acid (-SO_3H) and sulfhydryl group (-SH) bifunctional was designed in ionic liquids. The BFIL catalyst could attain nearly 100% conversion of 9-fluorenone and high selectivity (95.2%) for 9,9-bis (4-hydroxyphenyl) fluorene. As a solvent, IL does not have an advantage over other non-aqueous catalysis and developing its new applications is an urgent problem. However, ILs did improve the reaction efficiency and solve the problems of enzyme production as well as recycling. A summary of ILs is presented in Table 3.

Table 3. Recent progress in IL-based immobilized enzyme catalysis.

Ionic Liquid System	Carrier	Catalyst	Substrate	Product	Conversion (%)	Ref.
[Bmim] PF_6	Acrylic resin	Novozym 435 lipase	ε-caprolactone	Poly (ε-caprolactone)	97	[69]
[EMIM] Ac /[BMIM] [BF]	Acrylic resin	Novozym 435 lipase	Chitosan	Long-chain chitosan ester	-	[70]
[Bmim] [Tf_2N]	Acrylic resin	Novozym 435 lipase	Ethyl ferulate Phosphatidylcholine	Feruloylated lysophospholipids	50.79	[71]
[EMIM] [BF_4]	Santa Barbara Amorphous-15	Mucor miehei lipase	Licylaldehyde Indole cyclohexane-1,3-dione	Indolyl 4H-Chromenes	98	[72]
[Bmim] [TfO] /[Bmim] [Tf_2N]	Acrylic resin	Novozym 435 lipase	Glucose Fatty acid	Glucose fatty acid ester	55	[73]
[Bmim] [PF_6]	Acrylic resin	Novozym 435 lipase	Palmitic acid Glucose	Glucose palmitate	-	[74]
[C_{16}mim] [NTf_2] /[Bmim] [Cl]	Acrylic resin	Novozym 435 lipase	Algal oil	Biodiesel	100	[66]
[C_{16}tma] [NTf_2]	Acrylic resin	Novozym 435 lipase	Aliphatic acids Alcohol	Flavor ester	100	[75]
[Emim] [Tf_2N]	Acrylic resin	Novozym 435 lipase	Caffeic acid Phenylethanol	Caffeic acid Phenethyl ester	63.75	[76]
[Bmim] [PF_6]	Acrylic resin	Novozym 435 lipase	Sterol	(1R,3R)-N-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl) dodecanamid	23	[77]
[C_1C_3OHPyr] NTf_2	Acrylic resin	Novozym 435 lipase	Soybean oil	Biodiesel	82.4	[67]

5. Deep Eutectic Solvent System

To further develop green manufacturing and protect the environment, a deep eutectic solvent system (DES), a new generation of green solvents, has shown great potential for application across many fields [78–82]. DES are two- or three-component sub-eutectic mixtures of hydrogen bond acceptors (HBAs) (quaternary salts) and hydrogen bond donors (HBDs) (amines, carboxylic acids, alcohols, and carbohydrates) with a specific stoichiometry, and a freezing point significantly lower than the pure substance [83–85]. To understand the properties of DESs, Abbott et al. categorized DESs into four types: Type I (quaternary salt and metal halide), Type II (quaternary salt and hydrated metal halide), Type III (quaternary salt and hydrogen bond donor), and Type IV (metal halide and hydrogen bond donor) [84].

Compared to traditional solvents, DES is greener, biodegradable, and has higher substrate solubility. Additionally, the majority of DES preparation is easier and cheaper [86,87]. DES has gained wide attention and application as an alternative solvent to ionic liquid systems [88–90]. Many researchers have focused on using DES as a replacement for conventional solvents in enzyme-dependent biocatalytic synthesis. In 2003, Abbot et al. obtained DES via the interaction of choline chloride (melting point 302 °C) with urea (melting point 133 °C). This combination of solids yielded a low eutectic mixture that was liquid at ambient temperatures, exhibiting unusual solvent properties [84]. Hydrogen bonding and van der Waals forces can

interfere with the ability of the initial compound to crystallize. HBA can shield the charge when near certain HBDs and obtain DES [91].

To minimize the environmental impact of this process, as green co-solvents, the use of natural deep eutectic solvents (NADES) in enzymatic conversions has attracted attention. Mero et al. developed a sustainable biorefinery approach that combines the use of NADESs for recovering polyphenolic compounds and bio-based ionic liquids to treat and convert the remaining lignocellulosic residue into an ionogel. They evaluated the efficiency and selectivity of different types of NADESs by changing both the hydrogen bond acceptor HBA and the hydrogen bond donor HBD. Compared to traditional solvents, the majority of ChCl-based DESs improve the total phenol extraction up to twofold and possess excellent catalysis behavior. As a solvent, DES activates the proteins and improves the efficiency of enzymatic reactions [92]. In Zhao's study, newly synthesized eutectic ILs derived from choline acetate or choline chloride were documented and coupled with biocompatible hydrogen-bond donors, such as glycerol. According to experiments, these eutectic solvents have favorable properties, including low viscosity, high biodegradability, and excellent compatibility with Novozym 435 lipase. Additionally, they reached high conversion (97%) of the triglyceride obtained within 3 h under optimal conditions, suggesting that these novel eutectic solvents are worthy of further exploration as putative mediums in the enzymatic production of biodiesel [93]. Table 4 presents further examples of the application of DES to enhance production.

DES plays a key role as a reaction medium and has novel applications in many fields. Firstly, DES is widely used in high-purity extraction and separation. Qi et al. designed and synthesized different types of DESs to separate DMC-MeOH binary azeotropes. They reported that the intermolecular hydrogen bond between ChCl-urea and DMC was the strongest, performing better in isolation and purification [94]. Many studies have applied DES as an additive to extract and isolate polysaccharides or other bioactive compounds from natural products [95]. Moreover, DES has been used to modify some materials (polymers or silica) [96]. These materials have been utilized for extraction and separation. The physical properties of materials govern their potential applications. For instance, DES with very low surface tension can be employed as a binder or wetting agent. DES with high electrical conductivity can be utilized in the electrochemical industry [97].

Table 4. Recent progress of DES-based immobilized enzyme catalysis.

Deep Eutectic Solvent System	Molar Ratio	Carrier	Catalyst	Substrate	Product	Conversion (%)	Ref.
ChCl/Glycerol	1:2	XAD1180 resin	MAS1 lipase	Glycerol n-3 PUFA	Triacylglycerols	55.80	[98]
ChCl/Glc	1:2	Chitosan micro-spheres	β-D-glucosidase	Tyrosol	Salidroside	>50	[99]
ChCl/Glycerol–DMSO	1:2	PD-MNP	*Aspergillus niger* lipase	Dihydromyricetin	DMY-16-acetate	91.6	[100]
ChCl/Glyceeol	1:2	Cross-linking aggregates	*Pseudomonas* stutzeri lipase	Benzoic acid	Glyceryl α-monobenzoate	>20	[101]
ChCl/Glyceeol	1:3	Cross-linking aggregates	Lipase	Benzoic acid	Glyceryl α-monobenzoate	50	[102]
ChCl/Urea	1:2	PA@MNCC	Papain	N-(benzyloxycarbonyl)-alanyl methyl ester (Z-Ala-OMe)	N-(benzyloxycarbonyl)-alanyl-histidine	68.40	[103]
ChCl/Glycol	7:3 (v/v)	Magnetic nano-crystalline cellulose	Penicillin acylase	7-ACCA	Cefaclor	84	[104]
ChCl/Glycerol	1:2	Acrylic resin	Novozym 435 lipase	Waste oil Ethanol	Fatty acid ethyl ester	93.33	[105]

Table 4. *Cont.*

Deep Eutectic Solvent System	Molar Ratio	Carrier	Catalyst	Substrate	Product	Conversion (%)	Ref.
(-)-Menthol/Decanoic acid	-	Acrylic resin	*Candida antarctica* lipase B	Glucose	Glucose monodecanoate	-	[106]
Chcl/Glycerol	1:2	Acrylic resin	Novozym 435 lipase	Waste oil Butyl-3-Methylimidazolium hexafluorophosphate	Biodiesel	44	[107]
Chcl/Glycerol	1:2	Acrylic resin	Novozym 435 lipase	Soybean oil	Biodiesel	88	[108]
ChOAc/Glycerol	1:1.5	Acrylic resin	Novozym 435 lipase	Miglyol 812	Biodiesel	97	[93]

6. Non-Solvent System

Enzymatic reactions in organic media are separated into two systems: those in organic solvent systems and those in non-solvent systems. In the non-solvent system, the reaction mixture consists only of liquid organic substrates (such as liquid oil) without any organic solvent. This system offers high volumetric performance and economic advantages over the organic solvent system, especially for large-scale production. Therefore, the use of a non-solvent system for enzymatic reactions offers higher volumetric performance and economic advantages compared to organic solvent systems. This is also ideal for synthesizing food-grade products, where stringent safety regulations must be observed [10].

Due to the specificity of the organic substrate, biological enzyme catalysis in non-solvent systems is often propelled by lipase. Factors like tolerance to the substrate, solubility, and stability of the enzyme must be accounted for. To extend the advantages of an economical and green biological system, these lipases are modified by materials such as resins and gels. Venturi et al. used lipase B from *Candida antarctica*, immobilized on acrylic resin. Using immobilized enzymes, the reaction was performed under optimized conditions of 5 Å zeolite, 1:6 substrate molar ratio, 70 °C, reaching a 95% conversion rate after 30 min. Moreover, the immobilized lipase exhibited a good reusability and recovery rate, maintaining the same activity over five reaction cycles. These findings indicate that microwave-assisted lipase-catalyzed transesterification reactions in solvent-free systems are an excellent and sustainable catalytic approach for producing geranyl alcohol esters [109].

Jawale et al. analyzed the reaction kinetics and mechanism for the synthesis of propyl benzoate in a non-solvent system. They used an embedding method to immobilize lipase on a hydroxypropylmethylcellulose and polyvinyl alcohol polymer blend. Among the different carriers, *Candida-cylindrica*-immobilized lipase showed outstanding activity, with a loading efficiency of 94.56%. According to the study, the enzyme activity of the immobilized enzyme (99% yield) was higher than the free enzyme (40% yield). The catalysts were recoverable for up to four catalytic cycles and a 40% retention of activity was observed in the fourth cycle [110].

In Cirillo's study, cetyl palmitate was synthesized via esterification of cetyl alcohol with palmitic acid in a non-solvent system. The Lipozyme RM IM was immobilized and the reaction conversion only decreased by 6.8% after 15 uses. They proposed a novel kinetic model based on the random-sequential bi-bi mechanism and experimentally demonstrated that the substrate conversion rate could reach 100% at the optimal reaction conditions of 480 rpm, 70 °C, 1.0% enzyme, and 1:1 M ratio [111]. These findings show that the catalyst can be reused and remains stable after being immobilized, increasing its attractiveness. As no solvents are used in the reaction, it is a more environmentally friendly method of synthesis and eliminates the drawbacks associated with flammability and toxicity. Therefore, the entire process is environmentally friendly and economical in the non-solvent system, as outlined in Table 5.

Table 5. Recent progress in non-solvent-based immobilized enzyme catalysis.

Carrier	Catalyst	Substrate	Product	Conversion (%)	Ref.
Lewatit VP OC 1600	Novozymes eversa transform 2.0	2-ethylhexyl alcohol Palmitic acid	2-ethylhexyl palmitate	97	[112]
Polyacrylate beads	Fermase CALBex 10,000	Polyethylene glycol 600 Stearic acid	Polyethylene glycol stearate	86.98	[113]
Octyl agarose	*Candida rugosa* lipase	Glycerol Ethylene carbonate	Glycerol carbonate	>99	[114]
Lewatit VP OC 1600	Novozym 435 lipase	Borneol Linoleic acid	Bornyl linoleate	92.62	[115]
Acrylic resin	Novozym 435 lipase	Geranyl ester	Polyhydroquinolines	95	[109]
Hydroxypropyl methylcellulose	*Candida cylindracea* lipase	N-propyl alcohol Vinyl benzoate	Propyl benzoate	99	[110]
Lewatit VP OC 1600	Novozym 435 lipase	Lauric acid Pyridoxine	Pyridoxine monolaurate	94.45	[116]
Acrylic resin	Novozym 435 lipase	Free fatty acids Xylitol	Xylitol fatty acid esters	95	[117]
Polyacrylate beads	Fermase CALB™ 10,000	Levulinic acid Amyl alcohol	Amyl levulinate	73.20	[118]
Lewatit VP OC 1600	Thermomyces lanuginosus Eversa lipase	Adipic acid Isononyl alcohol	Diisononyl adipate	100	[119]
Acrylic resin	Novozym 435 lipase	2,5-bis-(Hydroxymethyl) Furan	2,5-bis-(Hydroxymethyl) Furan fatty acid	97	[120]
Acrylic resin	*Candida rugosa* lipase	Oleic acid	Pine sterol ester	95.10	[121]
Acrylic resin	Novozym 435 lipase	Sucrose Fructose	Sugar ester	96.60	[122]
Macro-porous ionexchange resin	Rhizomucor miehei lipozyme RM IM	Cetyl alcohol Palmitic acid	Cetyl palmitate	100	[111]
Macro-porous resin	Novozym 435 lipase	Caprylic acid N-butanol	Butyl caprylate	92	[123]
Acrylic resin	Novozym 435 lipase	Eraniol Ethyl acetate	Geranyl acetate	83	[124]
Micro-porous resins	Lipase NS 88,011	Oleic acid Monoethylene glycol	Ethylene glycol oleate	99%	[125]
Acrylic resin	Novozym 435 lipase	Methanol phenylacetic acid	Methyl phenylacetate	-	[126]
Acrylic resin	Novozym 435 lipase	Vinyl methacrylate	5-O-methacryloylcytidine	36	[127]
Rice straw filaments	Pseudomonas fluorescens lipase	Citronellol Vinyl acetate	Citronelly acetate	99.8	[128]

7. Summary and Outlook

In this review, we summarize the recent advances in non-aqueous catalysis, including the application of enzymes in non-aqueous catalysis and immobilized enzyme modification methods. Additionally, the challenges of different non-aqueous catalytic systems, including organic phase, oil-water two-phase, ionic liquid, deep eutectic solvent, and solvent-free system, are also summarized.

With the promotion of the concept of green development, non-aqueous catalysis should also develop in the direction of environmental friendliness. It is true that aqueous catalysis is green, safe, and sustainable, but at present, there are some bottlenecks restricting the development of aqueous catalysis. For example, hydrogen bonding and hydrophobic interactions carried by water itself can have an impact on yield and chirality. Secondly, when the properties of the water-soluble substrate are close to the product, it is difficult to bypass the organic solvent to separate and extract the product. Thirdly, the reaction in aqueous solution generally cannot be carried out out of pH. In addition, which is most important, aqueous catalysis cannot solve the problem that the substrate is a hydrophobic organic. Enhancing the hydrophobicity of the catalyst surface can improve the adsorption capacity of the catalyst and organic matter but, to a certain extent, it will also cause the catalyst to aggregate, affecting its dispersion and catalytic activity in the aqueous phase.

Therefore, non-aqueous catalysis inevitably dominates the synthesis of some reactions that use organic matter as substrates.

At present, catalysis for non-aqueous systems is also focused on how to be more economical and environmentally friendly. Due to the features of non-aqueous biocatalysis, it has been widely used in the production of natural products, chemical products, health care products, and food. In this paper, we summarize some cases of using enzymes as catalysts, which have high resistance to organic solvents and low demand for aqueous solution. And some enzymes can be reused more than a dozen times. By modifying enzyme and solvent systems, the requirements of various substrates and catalysts for media diversity are met. In addition, the solvents used should also be screened, and it is important to consider whether the benefits of using non-aqueous catalysis outweigh the environmental impact and the potential safety of the solvent. This requires the continuous upgrading of non-aqueous catalytic systems. It can be seen that the ionic liquid system is gradually being replaced by the deep eutectic solvent system, which is a good phenomenon. In conclusion, non-aqueous enzymatic reactions will progress green biocatalysis and open up new methods for the preparation and production of drugs, foods, and materials.

Author Contributions: Conceptualization, H.L.; project administration, H.L. and K.C.; supervision, L.W., K.C. and Y.W.; validation, Y.C. and Y.W.; writing—original draft, J.M.; writing—review and editing, J.M. and H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program of China (2021YFC2104100) and the National Natural Science Foundation of China (22378200).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Winkler, C.K.; Schrittwieser, J.H.; Kroutil, W. Power of Biocatalysis for Organic Synthesis. *ACS Cent. Sci.* **2021**, *7*, 55–71. [CrossRef] [PubMed]
2. Bell, E.L.; Finnigan, W.; France, S.P.; Green, A.P.; Hayes, M.A.; Hepworth, L.J.; Lovelock, S.L.; Niikura, H.; Osuna, S.; Romero, E.; et al. Biocatalysis. *Nat. Rev. Methods Primer* **2021**, *1*, 46. [CrossRef]
3. Arnold, F.H. Directed Evolution: Bringing New Chemistry to Life. *Angew. Chem. Int. Ed.* **2018**, *57*, 4143–4148. [CrossRef] [PubMed]
4. Sandoval, B.A.; Hyster, T.K. Emerging strategies for expanding the toolbox of enzymes in biocatalysis. *Curr. Opin. Chem. Biol.* **2020**, *55*, 45–51. [CrossRef] [PubMed]
5. Chakrabarty, S.; Romero, E.O.; Pyser, J.B.; Yazarians, J.A.; Narayan, A.R.H. Chemoenzymatic Total Synthesis of Natural Products. *Acc. Chem. Res.* **2021**, *54*, 1374–1384. [CrossRef] [PubMed]
6. Devine, P.N.; Howard, R.M.; Kumar, R.; Thompson, M.P.; Truppo, M.D.; Turner, N.J. Extending the application of biocatalysis to meet the challenges of drug development. *Nat. Rev. Chem.* **2018**, *2*, 409–421. [CrossRef]
7. Stout, C.N.; Renata, H. Reinvigorating the Chiral Pool: Chemoenzymatic Approaches to Complex Peptides and Terpenoids. *Acc. Chem. Res.* **2021**, *54*, 1143–1156. [CrossRef] [PubMed]
8. Fryszkowska, A.; Devine, P.N. Biocatalysis in drug discovery and development. *Curr. Opin. Chem. Biol.* **2020**, *55*, 151–160. [CrossRef] [PubMed]
9. Hughes, G.; Lewis, J.C. Introduction: Biocatalysis in Industry. *Chem. Rev.* **2018**, *118*, 1–3. [CrossRef]
10. Kumar, A.; Dhar, K.; Kanwar, S.S.; Arora, P.K. Lipase catalysis in organic solvents: Advantages and applications. *Biol. Proced. Online* **2016**, *18*, 2. [CrossRef]
11. Cai, X.; Lin, L.; Shen, Y.; Wei, W.; Wei, D.Z. Functional expression of a novel methanol-stable esterase from Geobacillus subterraneus DSM13552 for biocatalytic synthesis of cinnamyl acetate in a solvent-free system. *BMC Biotechnol.* **2020**, *20*, 36. [CrossRef]
12. Lee, J.S.; Hong, S.; Lee, J.; Choi, T.S.; Rhie, K.; Khim, J.S. Evaluation of residual toxicity of hypochlorite-treated water using bioluminescent microbes and microalgae: Implications for ballast water management. *Ecotoxicol. Environ. Saf.* **2019**, *167*, 130–137. [CrossRef] [PubMed]
13. Parandi, E.; Safaripour, M.; Mosleh, N.; Saidi, M.; Nodeh, H.R.; Oryani, B.; Rezania, S. Lipase enzyme immobilized over magnetic titanium graphene oxide as catalyst for biodiesel synthesis from waste cooking oil. *Biomass Bioenergy* **2023**, *173*, 106794. [CrossRef]
14. Mohidem, N.A.; Mohamad, M.; Rashid, M.U.; Norizan, M.N.; Hamzah, F.; Mat, H.B. Recent Advances in Enzyme Immobilisation Strategies: An Overview of Techniques and Composite Carriers. *J. Compos. Sci.* **2023**, *7*, 488. [CrossRef]
15. Golombek, F.; Castiglione, K. Polymersomes as Nanoreactors Enabling the Application of Solvent-Sensitive Enzymes in Different Biphasic Reaction Setups. *Biotechnol. J.* **2020**, *15*, 1900561. [CrossRef]

16. Carrea, G.; Riva, S. (Eds.) *Organic Synthesis with Enzymes in Non-Aqueous Media*; Wiley Online Books; John Wiley & Sons: Hoboken, NJ, USA, 2024.
17. Datta, S.; Christena, L.R.; Rajaram, Y.R.S. Enzyme immobilization: An overview on techniques and support materials. *3 Biotech* **2013**, *3*, 1–9. [CrossRef] [PubMed]
18. Homaei, A.A.; Sariri, R.; Vianello, F.; Stevanato, R. Enzyme immobilization: An update. *J. Chem. Biol.* **2024**, *6*, 185–205. [CrossRef]
19. Nielsen, P.M.; Brask, J.; Fjerbaek, L. Enzymatic biodiesel production: Technical and economical considerations. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 692–700. [CrossRef]
20. Anbu, P.; Hur, B.K. Isolation of an organic solvent-tolerant bacterium *Bacillus licheniformis* PAL05 that is able to secrete solvent-stable lipase. *Biotechnol. Appl. Biochem.* **2014**, *61*, 528–534. [CrossRef]
21. Romero, C.M.; Pera, L.M.; Loto, F.; Vallejos, C.; Castro, G.; Baigori, M.D. Purification of an organic solvent-tolerant lipase from *Aspergillus niger* MYA 135 and its application in ester synthesis. *Biocatal. Agric. Biotechnol.* **2012**, *1*, 25–31. [CrossRef]
22. Leykun, S.; Johansson, E.; Vetukuri, R.R.; Ceresino, E.B.; Gessesse, A. A thermostable organic solvent-tolerant lipase from *Brevibacillus* sp.: Production and integrated downstream processing using an alcohol-salt-based aqueous two-phase system. *Front. Microbiol.* **2023**, *14*, 1270270. [CrossRef] [PubMed]
23. Heipieper, H.J.; Neumann, G.; Cornelissen, S.; Meinhardt, F. Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 961–973. [CrossRef] [PubMed]
24. Sharma, S.; Kanwar, S.S. Organic Solvent Tolerant Lipases and Applications. *Sci. World J.* **2014**, *2014*, 625258. [CrossRef] [PubMed]
25. Sardessai, Y.; Bhosle, S. Tolerance of bacteria to organic solvents. *Res. Microbiol.* **2002**, *153*, 263–268. [CrossRef] [PubMed]
26. Sharma, S.; Kanwar, S.S.; Dogra, P.; Chauhan, G.S. Gallic Acid-Based Alkyl esters Synthesis in a Water-Free System by Celite-Bound Lipase of *Bacillus licheniformis* SCD11501. *Biotechnol. Prog.* **2015**, *31*, 715–723. [CrossRef] [PubMed]
27. Adlercreutz, P. Immobilisation and application of lipases in organic media. *Chem. Soc. Rev.* **2013**, *42*, 6406–6436. [CrossRef] [PubMed]
28. Badillo-Zeferino, G.L.; Ruiz-Lopez, I.I.; Oliart-Ros, R.; Sanchez-Otero, M.G. Improved expression and immobilization of *Geobacillus thermoleovorans* CCR11 thermostable recombinant lipase. *Biotechnol. Appl. Biochem.* **2017**, *64*, 62–69. [CrossRef] [PubMed]
29. Iuliano, M.; Ponticorvo, E.; Cirillo, C.; Castaldo, R.; De Pasquale, S.; Gentile, G.; Sarno, M. Wax esters from waste fish oil catalysed by immobilized *Candida rugosa* lipase. *Process Biochem.* **2023**, *130*, 386–400. [CrossRef]
30. Hinzmann, A.; Adebar, N.; Betke, T.; Leppin, M.; Gröger, H. Front Cover: Biotransformations in Pure Organic Medium: Organic Solvent-Labile Enzymes in the Batch and Flow Synthesis of Nitriles. *Eur. J. Org. Chem.* **2019**, *2019*, 6888. [CrossRef]
31. Jiang, Z.; Yu, M.; Ren, L.; Zhou, H.; Wei, P. Synthesis of phytosterol esters catalyzed by immobilized lipase in organic media. *Chin. J. Catal.* **2013**, *34*, 2255–2262. [CrossRef]
32. Koutinas, M.; Yiangou, C.; Osório, N.M.; Ioannou, K.; Canet, A.; Valero, F.; Ferreira-Dias, S. Application of commercial and non-commercial immobilized lipases for biocatalytic production of ethyl lactate in organic solvents. *Bioresour. Technol.* **2018**, *247*, 496–503. [CrossRef]
33. Zeng, F.; Zhang, H.; Xu, M.; Huang, K.; Zhang, T.; Duan, J. Immobilized lipase catalytic synthesis of phenolamides and their potential against α-glucosidase. *J. Biotechnol.* **2021**, *334*, 51–57. [CrossRef] [PubMed]
34. de Souza, M.C.M.; dos Santos, K.P.; Freire, R.M.; Barreto, A.C.H.; Fechine, P.B.A.; Goncalves, L.R.B. Production of flavor esters catalyzed by lipase B from *Candida antarctica* immobilized on magnetic nanoparticles. *Braz. J. Chem. Eng.* **2017**, *34*, 681–690. [CrossRef]
35. Sun, J.; Chen, Y.; Sheng, J.; Sun, M. Immobilization of *Yarrowia lipolytica* Lipase on Macroporous Resin Using Different Methods: Characterization of the Biocatalysts in Hydrolysis Reaction. *BioMed Res. Int.* **2015**, *2015*, 139179. [CrossRef]
36. Wang, L.; Zhang, Y.; Zhang, Y.; Zheng, L.; Huang, H.; Wang, Z. Synthesis of 2-Ethylhexyl Palmitate Catalyzed by Enzyme Under Microwave. *Appl. Biochem. Biotechnol.* **2018**, *185*, 347–356. [CrossRef] [PubMed]
37. Yao, C.; Lin, W.; Yue, K.; Ling, X.; Jing, K.; Lu, Y.; Tang, S.; Fan, E. Biocatalytic synthesis of vitamin A palmitate using immobilized lipase produced by recombinant Pichia pastoris. *Eng. Life Sci.* **2017**, *17*, 768–774. [CrossRef]
38. Jahangiri, A.; Møller, A.H.; Danielsen, M.; Madsen, B.; Joernsgaard, B.; Vaerbak, S.; Adlercreutz, P.; Dalsgaard, T.K. Hydrophilization of bixin by lipase-catalyzed transesterification with sorbitol. *Food Chem.* **2018**, *268*, 203–209. [CrossRef]
39. Braia, N.; Merabet-Khelassi, M.; Aribi-Zouioueche, L. Efficient access to both enantiomers of 3-(1-hydroxyethyl)phenol by regioselective and enantioselective CAL-B–catalyzed hydrolysis of diacetate in organic media by sodium carbonate. *Chirality* **2018**, *30*, 1312–1320. [CrossRef] [PubMed]
40. Carrea, G. Biocatalysis in water-organic solvent two-phase systems. *Trends Biotechnol.* **1984**, *2*, 102–106. [CrossRef]
41. Bühler, B.; Bollhalder, I.; Hauer, B.; Witholt, B.; Schmid, A. Use of the two-liquid phase concept to exploit kinetically controlled multistep biocatalysis. *Biotechnol. Bioeng.* **2003**, *81*, 683–694. [CrossRef]
42. Antonini, E.; Carrea, G.; Cremonesi, P. Enzyme catalysed reactions in water—Organic solvent two-phase systems. *Enzyme Microb. Technol.* **1981**, *3*, 291–296. [CrossRef]
43. Oppermann, S.; Stein, F.; Kragl, U. Ionic liquids for two-phase systems and their application for purification, extraction and biocatalysis. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 493–499. [CrossRef]
44. Maciejewski, H. Ionic Liquids in Catalysis. *Catalysts* **2021**, *11*, 367. [CrossRef]
45. Thomas, E.; Creighton, W.H. (Eds.) *Proteins: Structures Molecular Properties*, 2nd ed.; Freeman: New York, NY, USA, 1992; ISBN 0-7167-7030-X.

46. Tooney, N.M. Biophysical chemistry—Part I: The conformation of biological, macromolecules, Cantor and Schimmel, W.H. Freeman and Company, San Francisco, 1980, 341 pp. *J. Polym. Sci. Polym. Lett. Ed.* **1980**, *18*, 643–644. [CrossRef]
47. Xiang, L.; Kaspar, F.; Schallmey, A.; Constantinou, I. Two-Phase Biocatalysis in Microfluidic Droplets. *Biosensors* **2021**, *11*, 407. [CrossRef] [PubMed]
48. Grundtvig, I.P.R.; Heintz, S.; Krühne, U.; Gernaey, K.V.; Adlercreutz, P.; Hayler, J.D.; Wells, A.S.; Woodley, J.M. Screening of organic solvents for bioprocesses using aqueous-organic two-phase systems. *Biotechnol. Adv.* **2018**, *36*, 1801–1814. [CrossRef]
49. Ramsden, W.; Gotch, F. Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation)—Preliminary account. *Proc. R. Soc. Lond.* **1997**, *72*, 156–164.
50. Xu, C.; Sun, Y.; Sun, Y.; Cai, R.; Zhang, S. High Internal Phase Pickering Emulsion Stabilized by Lipase-Coated ZIF-8 Nanoparticles towards Recyclable Biphasic Biocatalyst. *Catalysts* **2023**, *13*, 383. [CrossRef]
51. Wei, L.; Zhang, M.; Zhang, X.; Xin, H.; Yang, H. Pickering Emulsion as an Efficient Platform for Enzymatic Reactions without Stirring. *ACS Sustain. Chem. Eng.* **2016**, *4*, 6838–6843. [CrossRef]
52. Mathys, R.G.; Schmid, A.; Witholt, B. Integrated two-liquid phase bioconversion and product-recovery processes for the oxidation of alkanes: Process design and economic evaluation. *Biotechnol. Bioeng.* **1999**, *64*, 459–477. [CrossRef]
53. Aghababaie, M.; Beheshti, M.; Razmjou, A.; Bordbar, A.K. Covalent immobilization of *Candida rugosa* lipase on a novel functionalized Fe_3O_4@SiO_2 dip-coated nanocomposite membrane. *Food Bioprod. Process.* **2016**, *100*, 351–360. [CrossRef]
54. Li, W.; Lin, S.; Lan, D.; Wang, Y. Efficient enzymatic synthesis of vitamin E succinate using an organic solvent-stable immobilized lipase. *J. Am. Oil. Chem. Soc* **2024**. *Early View*. [CrossRef]
55. Li, H.; Pang, Y.; Wang, X.; Cao, X.; He, X.; Chen, K.; Li, G.; Ouyang, P.; Tan, W. Phospholipase D encapsulated into metal-surfactant nanocapsules for enhancing biocatalysis in a two-phase system. *RSC Adv.* **2019**, *9*, 6548–6555. [CrossRef] [PubMed]
56. Zhang, Y.; Zhu, L.; Wu, G.; Wang, X.; Jin, Q.; Qi, X.; Zhang, H. A novel immobilized enzyme enhances the conversion of phosphatidylserine in two-phase system. *Biochem. Eng. J.* **2021**, *172*, 108035. [CrossRef]
57. Zhao, K.; Chen, B.; Li, C.; Li, X.F.; Li, K.B.; Shen, Y.H. Immobilization of *Candida rugosa* Lipase on Glutaraldehyde-Activated Fe_3O_4@Chitosan as a Magnetically Separable Catalyst for Hydrolysis of Castor Oil. *Eur. J. Lipid Sci. Technol.* **2018**, *120*, 1700373. [CrossRef]
58. Tang, C.; Chai, Y.; Wang, C.; Wang, Z.; Min, J.; Wang, Y.; Qi, W.; Su, R.; He, Z. Pickering Emulsions Stabilized by Lignin/Chitosan Nanoparticles for Biphasic Enzyme Catalysis. *Langmuir* **2022**, *38*, 12849–12858. [CrossRef] [PubMed]
59. Girelli, A.M.; Chiappini, V.; Amadoro, P. Immobilization of lipase on spent coffee grounds by physical and covalent methods: A comparison study. *Biochem. Eng. J.* **2023**, *192*, 108827. [CrossRef]
60. Yang, Y.S.; Zhang, T.; Yu, S.C.; Ding, Y.; Zhang, L.Y.; Qiu, C.; Jin, D. Transformation of Geniposide into Genipin by Immobilized β-Glucosidase in a Two-Phase Aqueous-Organic System. *Molecules* **2011**, *16*, 4295–4304. [CrossRef] [PubMed]
61. Welton, T. Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis. *Chem. Rev.* **1999**, *99*, 2071–2084. [CrossRef]
62. Armand, M.; Endres, F.; MacFarlane, D.R.; Ohno, H.; Scrosati, B. Ionic-liquid materials for the electrochemical challenges of the future. *Nat. Mater.* **2009**, *8*, 621–629. [CrossRef]
63. Wilkes, J.S.; Zaworotko, M.J. Air and water stable 1-ethyl-3-methylimidazolium based ionic liquids. *J. Chem. Soc. Chem. Commun.* **1992**, *13*, 965–967. [CrossRef]
64. Endres, F.; Zein El Abedin, S. Air and water stable ionic liquids in physical chemistry. *Phys. Chem. Chem. Phys.* **2006**, *8*, 2101–2116. [CrossRef] [PubMed]
65. MacFarlane, D.R.; Meakin, P.; Sun, J.; Amini, N.; Forsyth, M. Pyrrolidinium Imides: A New Family of Molten Salts and Conductive Plastic Crystal Phases. *J. Phys. Chem. B* **1999**, *103*, 4164–4170. [CrossRef]
66. Lozano, P.; Bernal, J.M.; Gomez, C.; Alvarez, E.; Markiv, B.; Garcia-Verdugo, E.; Luis, S.V. Green biocatalytic synthesis of biodiesel from microalgae in one-pot systems based on sponge-like ionic liquids. *Catal. Today* **2020**, *346*, 87–92. [CrossRef]
67. Fan, Y.; Wang, X.; Zhang, L.; Li, J.; Yang, L.; Gao, P.; Zhou, Z. Lipase-catalyzed synthesis of biodiesel in a hydroxyl-functionalized ionic liquid. *Chem. Eng. Res. Des.* **2018**, *132*, 199–207. [CrossRef]
68. Chen, X.; Zhong, Q.; Ma, X.; Li, S.; Sun, W.; Liu, C. Synthesis of benzothiazoles catalyzed by [Bmim]PF6 ionic liquid in solvent-free condition. *J. Catal.* **2024**, *429*, 115274. [CrossRef]
69. Yuan, J.; Dai, Y.; Yu, Y.; Wang, P.; Wang, Q.; Fan, X. Biocatalytic synthesis of poly(ε-caprolactone) using modified lipase in ionic liquid media. *Eng. Life Sci.* **2016**, *16*, 371–378. [CrossRef]
70. Zhao, G.; Lang, X.; Wang, F.; Li, J.; Li, X. A one-pot method for lipase-catalyzed synthesis of chitosan palmitate in mixed Ionic liquids and its characterization. *Biochem. Eng. J.* **2017**, *126*, 24–29. [CrossRef]
71. Yang, H.; Han, X.; Saleh, A.S.M.; Shao, C.; Duan, Y.; Xiao, Z.G. Lipase-catalyzed Synthesis of Feruloylated Lysophospholipid in Toluene-Ionic Liquids and Its Antioxidant Activity. *J. Oleo Sci.* **2021**, *70*, 531–540. [CrossRef] [PubMed]
72. Zhang, W.; Zhao, Z.; Wang, Z.; Guo, C.; Wang, C.; Zhao, R.; Wang, L. Lipase-Catalyzed Synthesis of Indolyl 4H-Chromenes via a Multicomponent Reaction in Ionic Liquid. *Catalysts* **2017**, *7*, 185. [CrossRef]
73. Shin, D.W.; Mai, N.L.; Bae, S.W.; Koo, Y.M. Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids. *Enzyme Microb. Technol.* **2019**, *126*, 18–23. [CrossRef] [PubMed]
74. Findrik, Z.; Megyeri, G.; Gubicza, L.; Bélafi-Bakó, K.; Nemestóthy, N.; Sudar, M. Lipase catalyzed synthesis of glucose palmitate in ionic liquid. *J. Clean. Prod.* **2016**, *112*, 1106–1111. [CrossRef]

75. Alvarez, E.; Rodriguez, J.; Villa, R.; Gomez, C.; Nieto, S.; Donaire, A.; Lozano, P. Clean Enzymatic Production of Flavor Esters in Spongelike Ionic Liquids. *ACS Sustain. Chem. Eng.* **2019**, *7*, 13307–13314. [CrossRef]
76. Wang, J.; Li, J.; Zhang, L.; Gu, S.; Wu, F. Lipase-catalyzed Synthesis of Caffeic Acid Phenethyl Ester in Ionic Liquids: Effect of Specific Ions and Reaction Parameters. *Chin. J. Chem. Eng.* **2013**, *21*, 1376–1385. [CrossRef]
77. Kanojia, S.V.; Chatterjee, S.; Chattopadhyay, S.; Goswami, D. A chemoenzymatic synthesis of ceramide trafficking inhibitor HPA-12. *Beilstein J. Org. Chem.* **2019**, *15*, 490–496. [CrossRef] [PubMed]
78. Patiño, J.; Gutiérrez, M.C.; Carriazo, D.; Ania, C.O.; Parra, J.B.; Ferrer, M.L.; Del Monte, F. Deep eutectic assisted synthesis of carbon adsorbents highly suitable for low-pressure separation of CO_2-CH_4 gas mixtures. *Energy Environ. Sci.* **2012**, *5*, 8699–8707. [CrossRef]
79. Hansen, B.B.; Spittle, S.; Chen, B.; Poe, D.; Zhang, Y.; Klein, J.M.; Horton, A.; Adhikari, L.; Zelovich, T.; Doherty, B.W.; et al. Deep Eutectic Solvents: A Review of Fundamentals and Applications. *Chem. Rev.* **2021**, *121*, 1232–1285. [CrossRef] [PubMed]
80. Zhang, Q.; Vigier, K.D.O.; Royer, S.; Jerome, F. Deep eutectic solvents: Syntheses, properties and applications. *Chem. Soc. Rev.* **2012**, *41*, 7108–7146. [CrossRef] [PubMed]
81. Zhao, N.; Liu, S.; Xing, J.; Zheng, Z.; Pi, Z.; Song, F.; Liu, Z. Enhanced one-step sample pretreatment method for extraction of ginsenosides from rat plasma using tailor-made deep eutectic mixture solvents. *Anal. Methods* **2019**, *11*, 1035–1042. [CrossRef]
82. Yue, Y.; Huang, Q.; Fu, Y.; Chang, J. A quick selection of natural deep eutectic solvents for the extraction of chlorogenic acid from herba artemisiae scopariae. *RSC Adv.* **2020**, *10*, 23403–23409. [CrossRef]
83. Abbott, A.P.; Barron, J.C.; Ryder, K.S.; Wilson, D. Eutectic-based ionic liquids with metal-containing anions and cations. *Chem-Eur. J.* **2007**, *13*, 6495–6501. [CrossRef] [PubMed]
84. Abbott, A.P.; Capper, G.; Davies, D.L.; Rasheed, R.K.; Tambyrajah, V. Novel solvent properties of choline chloride/urea mixtures. *Chem. Commun.* **2003**, 70–71. [CrossRef] [PubMed]
85. Wang, T.; Guo, Q.; Yang, H.; Gao, W.; Li, P. pH-controlled reversible deep-eutectic solvent based enzyme system for simultaneous extraction and in-situ separation of isoflavones from Pueraria lobata. *Sep. Purif. Technol.* **2022**, *292*, 120992. [CrossRef]
86. Li, X.; Row, K.H. Development of deep eutectic solvents applied in extraction and separation. *J. Sep. Sci.* **2016**, *39*, 3505–3520. [CrossRef] [PubMed]
87. Li, X.; Row, K.H. Exploration of Mesoporous Stationary Phases Prepared Using Deep Eutectic Solvents Combining Choline Chloride with 1,2-Butanediol or Glycerol for Use in Size-Exclusion Chromatography. *Chromatographia* **2015**, *78*, 1321–1325. [CrossRef]
88. Yang, W.; Gu, Q.; Zhou, J.; Liu, X.; Yu, X. High-Value Bioconversion of Ginseng Extracts in Betaine-Based Deep Eutectic Solvents for the Preparation of Deglycosylated Ginsenosides. *Foods* **2023**, *12*, 496. [CrossRef] [PubMed]
89. Zhang, S.; Ma, C.; Li, Q.; Li, Q.; He, Y.C. Efficient chemoenzymatic valorization of biobased D-fructose into 2,5-bis(hydroxymethyl)furan with deep eutectic solvent Lactic acid:Betaine and Pseudomonas putida S12 whole cells. *Bioresour. Technol.* **2022**, *344*, 126299. [CrossRef] [PubMed]
90. Annunziata, F.; Guaglio, A.; Conti, P.; Tamborini, L.; Gandolfi, R. Continuous-flow stereoselective reduction of prochiral ketones in a whole cell bioreactor with natural deep eutectic solvents. *Green. Chem.* **2022**, *24*, 950–956. [CrossRef]
91. Harris, R.C. Physical Properties of Alcohol Based Deep Eutectic Solvents. Ph.D. Thesis, University of Leicester, Leicester, UK, 2009.
92. Mero, A.; Mezzetta, A.; De Leo, M.; Braca, A.; Guazzelli, L. Sustainable valorization of cherry (*Prunus avium* L.) pomace waste via the combined use of (NA)DESs and bio-ILs. *Green Chem.* **2024**, *26*, 6109–6123. [CrossRef]
93. Zhao, H.; Baker, G.A.; Holmes, S. New eutectic ionic liquids for lipase activation and enzymatic preparation of biodiesel. *Org. Biomol. Chem.* **2011**, *9*, 1908–1916. [CrossRef]
94. Qi, J.; Qu, Y.; Zhou, M.; Su, Z.; Zhang, X.; Wei, R.; Xue, K.; Zhu, Z.; Meng, F.; Wang, Y. Phase behavior and molecular insights on the separation of dimethyl carbonate and methanol azeotrope by extractive distillation using deep eutectic solvents. *Sep. Purif. Technol.* **2023**, *305*, 122489. [CrossRef]
95. Tang, B.; Zhang, H.; Row, K.H. Application of deep eutectic solvents in the extraction and separation of target compounds from various samples. *J. Sep. Sci.* **2015**, *38*, 1053–1064. [CrossRef] [PubMed]
96. Tang, B.; Row, K.H. Exploration of deep eutectic solvent-based mesoporous silica spheres as high-performance size exclusion chromatography packing materials. *J. Appl. Polym. Sci.* **2015**, *132*, 42203. [CrossRef]
97. Francisco, M.; van den Bruinhorst, A.; Kroon, M.C. Low-Transition-Temperature Mixtures (LTTMs): A New Generation of Designer Solvents. *Angew. Chem.-Int. Ed.* **2013**, *52*, 3074–3085. [CrossRef] [PubMed]
98. Wang, X.; Zhao, X.; Qin, X.; Zhao, Z.; Yang, B.; Wang, Y. Immobilized MAS1 Lipase-catalyzed Synthesis of n − 3 PUFA-rich Triacylglycerols in Deep Eutectic Solvents. *J. Oleo Sci.* **2021**, *70*, 227–236. [CrossRef] [PubMed]
99. Chen, J.; Cheng, Q.; Ma, Q.; Wu, Y.; Zhang, L. Salidroside synthesis via glycosylation by β-D-glucosidase immobilized on chitosan microspheres in deep eutectic solvents. *Biocatal. Biotransform.* **2024**, *42*, 227–240. [CrossRef]
100. Cao, S.L.; Deng, X.; Xu, P.; Huang, Z.X.; Zhou, J.; Li, X.H.; Zong, M.H.; Lou, W.Y. Highly Efficient Enzymatic Acylation of Dihydromyricetin by the Immobilized Lipase with Deep Eutectic Solvents as Cosolvent. *J. Agric. Food Chem.* **2017**, *65*, 2084–2088. [CrossRef] [PubMed]
101. Guajardo, N.; Ahumada, K.; Domínguez de María, P. Immobilization of *Pseudomonas stutzeri* lipase through Cross-linking Aggregates (CLEA) for reactions in Deep Eutectic Solvents. *J. Biotechnol.* **2021**, *337*, 18–23. [CrossRef] [PubMed]

102. Guajardo, N.; Ahumada, K.; Domínguez de María, P. Immobilized lipase-CLEA aggregates encapsulated in lentikats® as robust biocatalysts for continuous processes in deep eutectic solvents. *J. Biotechnol.* **2020**, *310*, 97–102. [CrossRef]
103. Xiong, J.; Cao, S.L.; Zong, M.H.; Lou, W.Y.; Wu, X.L. Biosynthesis of Alanyl-Histidine Dipeptide Catalyzed by Papain Immobilized on Magnetic Nanocrystalline Cellulose in Deep Eutectic Solvents. *Appl. Biochem. Biotechnol.* **2020**, *192*, 573–584. [CrossRef]
104. Wu, X.; Xiong, J.; Huang, Z.; Cao, S.; Zong, M.; Lou, W. Improving biocatalysis of cefaclor with penicillin acylase immobilized on magnetic nanocrystalline cellulose in deep eutectic solvent based co-solvent. *Bioresour. Technol.* **2019**, *288*, 121548. [CrossRef]
105. Suo, H.; Hao, X.; Zhang, G.; Zhang, Q.; Du, S. A kinetic study of the ultrasonically assisted ethyl esterification of fatty acids using an immobilized lipase catalyst and deep eutectic solvent. *Int. J. Chem. Kinet.* **2022**, *54*, 400–412. [CrossRef]
106. Hollenbach, R.; Ochsenreither, K.; Syldatk, C. Enzymatic Synthesis of Glucose Monodecanoate in a Hydrophobic Deep Eutectic Solvent. *Int. J. Mol. Sci.* **2020**, *21*, 4342. [CrossRef]
107. Merza, F.; Fawzy, A.; AlNashef, I.; Al-Zuhair, S.; Taher, H. Effectiveness of using deep eutectic solvents as an alternative to conventional solvents in enzymatic biodiesel production from waste oils. *Energy Rep.* **2018**, *4*, 77–83. [CrossRef]
108. Zhao, H.; Zhang, C.; Crittle, T.D. Choline-based deep eutectic solvents for enzymatic preparation of biodiesel from soybean oil. *J. Mol. Catal. B-Enzym.* **2013**, *85–86*, 243–247. [CrossRef]
109. Venturi, V.; Presini, F.; Trapella, C.; Bortolini, O.; Giovannini, P.P.; Lerin, L.A. Microwave-assisted enzymatic synthesis of geraniol esters in solvent-free systems: Optimization of the reaction parameters, purification and characterization of the products, and biocatalyst reuse. *Mol. Divers.* **2023**. [CrossRef]
110. Jawale, P.V.; Bhanage, B.M. Synthesis of propyl benzoate by solvent-free immobilized lipase-catalyzed transesterification: Optimization and kinetic modeling. *Bioprocess. Biosyst. Eng.* **2021**, *44*, 369–378. [CrossRef] [PubMed]
111. Cirillo, N.A.; Quirrenbach, C.G.; Corazza, M.L.; Voll, F.A.P. Enzymatic kinetics of cetyl palmitate synthesis in a solvent-free system. *Biochem. Eng. J.* **2018**, *137*, 116–124. [CrossRef]
112. Choi, S.; Kim, B.H.; No, D.S.; Yoon, S.W.; Lee, M.W.; Im, D.J.; Kim, I.H. Lipase-catalyzed synthesis of 2-ethylhexyl palmitate in a solvent free system using step changes in temperature. *Biochem. Eng. J.* **2022**, *177*, 108261. [CrossRef]
113. Nhivekar, G.S.; Rathod, V.K. Optimization of lipase-catalyzed synthesis of polyethylene glycol stearate in a solvent-free system. *Green. Process Synth.* **2019**, *8*, 30–37. [CrossRef]
114. Gonzalez-Miranda, D.; Carballares, D.; Pedregal, T.; Fernandez-Lafuente, R.; Ladero, M.; Bolivar, J.M. Enzyme-Catalyzed Synthesis of Glycerol Carbonate in Solventless Liquid One Phase Conditions: Role of Reaction Medium Engineering on Catalytic Performance. *Ind. Eng. Chem. Res.* **2023**, *62*, 15798–15808. [CrossRef]
115. Wang, X.; Zhao, Y.; Jiang, C.; Chang, M.; Huang, J.; Xie, D. Enzymatic synthesis of bornyl linoleate in a solvent-free system. *Food Biosci.* **2021**, *41*, 100947. [CrossRef]
116. Yu, H.; Kim, S.; Chang, P.S. Lipase-catalyzed production of pyridoxine monolaurate in solvent-free bioreactor system. *Food Chem.* **2023**, *399*, 133949. [CrossRef] [PubMed]
117. Nieto, S.; Villa, R.; Donaire, A.; Lozano, P. Ultrasound-assisted enzymatic synthesis of xylitol fatty acid esters in solvent-free conditions. *Ultrason. Sonochem.* **2021**, *75*, 105606. [CrossRef] [PubMed]
118. Jaiswal, K.S.; Rathod, V.K. Green synthesis of amyl levulinate using lipase in the solvent free system: Optimization, mechanism and thermodynamics studies. *Catal. Today* **2021**, *375*, 120–131. [CrossRef]
119. Lee, A.; Kim, H.; Choi, N.; Yoon, S.W.; Kim, Y.; Kim, H.R.; Kim, I.H. Preparation of diisononyl adipate in a solvent-free system via an immobilized lipase-catalyzed esterification. *Enzyme Microb. Technol.* **2019**, *131*, 109340. [CrossRef] [PubMed]
120. Lăcătuş, M.A.; Dudu, A.I.; Bencze, L.C.; Katona, G.; Irimie, F.D.; Paizs, C.; Toşa, M.I. Solvent-Free Biocatalytic Synthesis of 2,5-bis-(Hydroxymethyl)Furan Fatty Acid Diesters from Renewable Resources. *ACS Sustain. Chem. Eng.* **2020**, *8*, 1611–1617. [CrossRef]
121. Zhang, Y.; Ma, G.; Wang, S.; Nian, B.; Hu, Y. Study on the synthesis of pine sterol esters in solvent-free systems catalyzed by *Candida rugosa* lipase immobilized on hydrophobic macroporous resin. *J. Sci. Food Agric.* **2023**, *103*, 7849–7861. [CrossRef]
122. Ye, R.; Hayes, D.G.; Burton, R.; Liu, A.; Harte, F.M.; Wang, Y. Solvent-Free Lipase-Catalyzed Synthesis of Technical-Grade Sugar Esters and Evaluation of Their Physicochemical and Bioactive Properties. *Catalysts* **2016**, *6*, 78. [CrossRef]
123. Sose, M.T.; Bansode, S.R.; Rathod, V.K. Solvent free lipase catalyzed synthesis of butyl caprylate. *J. Chem. Sci.* **2017**, *129*, 1755–1760. [CrossRef]
124. Bhavsar, K.V.; Yadav, G.D. Synthesis of geranyl acetate by transesterification of geraniol with ethyl acetate over *Candida antarctica* lipase as catalyst in solvent-free system. *Flavour Fragr. J.* **2019**, *34*, 288–293. [CrossRef]
125. Cordeiro, E.d.S.; Henriques, R.O.; Deucher, E.M.; de Oliveira, D.; Lerin, L.A.; Furigo, A., Jr. Optimization, kinetic, and scaling-up of solvent-free lipase-catalyzed synthesis of ethylene glycol oleate emollient ester. *Biotechnol. Appl. Biochem.* **2021**, *68*, 1469–1478. [CrossRef] [PubMed]
126. Bayout, I.; Bouzemi, N.; Guo, N.; Mao, X.; Serra, S.; Riva, S.; Secundo, F. Natural flavor ester synthesis catalyzed by lipases. *Flavour Fragr. J.* **2020**, *35*, 209–218. [CrossRef]

127. Chea, S.; Nguyen, K.T.; Rosencrantz, R.R. Microwave-Assisted Synthesis of 5′-O-methacryloylcytidine Using the Immobilized Lipase Novozym 435. *Molecules* **2022**, *27*, 4112. [CrossRef]
128. Yuan, M.; Cong, F.; Zhai, Y.; Li, P.; Yang, W.; Zhang, S.; Su, Y.; Li, T.; Wang, Y.; Luo, W.; et al. Rice straw enhancing catalysis of Pseudomonas fluorescens lipase for synthesis of citronellyl acetate. *Bioprocess. Biosyst. Eng.* **2022**, *45*, 453–464. [CrossRef]