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Lipase Immobilization by Adsorption Techniques on The Hydrophobically Modified Matrix: A Review

Edy Subroto[#1], Siti Nurhasanah[1], S. Joni Munarso[2]

[1] *Department of Food Industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Jl.Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang 40600, Indonesia*

[2]*Indonesia Center for Agricultural Postharvest Research and Development, Jl. Tentara Pelajar No. 12 Bogor 16114, Indonesia*

edy.subroto@unpad.ac.id

Abstract — *Lipase immobilization has been carried out by various methods, each of which has various advantages. One of the rapidly developing lipase immobilization techniques is the adsorption method. This review describes the immobilization of lipase with adsorption techniques, especially about the use of various types of matrices and matrix surface modification techniques that can improve the lipase microenvironment. The adsorption method has several advantages over other ways, including easy preparation, the low cost can be regenerated, and the matrix's surface can be modified to improve lipase activity and stability. Hydrophobic modification of the surface matrix can encourage the lid lipase to open easily, thereby increasing its activity. Adsorption immobilized lipases have also been shown to be effective and stable over several applications for various reactions. However, the challenge in the hydrophobic modification is that the loading of lipase protein is generally relatively lower than that of the hydrophilic matrix. Therefore, further, development is needed by utilizing various types of matrices, hydrophobic groups, lipases, and modification techniques to increase their loading capacity, activity, and stability.*

Keywords — *Immobilization, lipase, Adsorption, hydrophobic modification.*

I. INTRODUCTION

Enzymes, including lipases, are well-known as one of the leading biological catalysts (biocatalyst) of a wide variety of processes that are highly effective and efficient catalysts characterized by high selectivity and activity to accelerate biochemical reactions [1]. As a natural catalyst, lipases contribute to commercially essential processes, especially in various food and pharmaceutical industries. Lipases are a powerful tool for biotransformation on a broad substrate range. Lipases catalyze the alteration of substrates into products by reducing reaction activation energy, carried out under mild conditions (physiological pH and temperature), excellent selectivity, and substrate activity [2]–[4]. Catalyst is used in almost every sector of the chemical industry: in basic chemistry, in polymerization chemistry, in refining, in reactions of fluid catalytic cracking, in a variety of industrial processes (food, cosmetic, etc.) [1], [5]–[7].

The advantages of lipases can reduce the number of hazardous solvents needed; the total reaction steps make the process cheaper and more environmentally friendly. Other benefits of using lipases are mild reaction conditions, low energy consumption, biodegradability, and yields of a pure product [8], [9]. Lipase is one of the biocatalysts with great potential proven to contribute to the non-exploited million-dollar lipid bio-industry. It has been used in ex situ lipids and Insitu multifaceted industrial applications [10], [11].

The lipase from *Thermomyces lanuginosus* expressed in *Aspergillus oryzae* is the first commercialized recombinant lipase familiar as Lipolase [10]. Lipase or also known as triacylglycerol acyl hydrolase, is an enzyme that can work reversibly, catalyze the hydrolysis of triacylglycerol to glycerol and free fatty acids and, or partial hydrolysis to diacylglycerols (DAGs) and monoacylglycerols (MAGs) [12]. Lipases are also used to catalyze hydrolysis, forming free fatty acids and glycerol from the reaction of fats with water. This enzyme is an esterase group, a sub-class of hydrolytic enzymes, also known as carboxylic acid esterases. These fats' formation can be carried out through various types of reactions, including esterification, transesterification, and acidolysis [13]. This enzyme is produced by plants, animals, and microorganisms. Among the various sources, microbial lipase was given more attention from industries because of its ability to remain active at extreme temperatures, pH, organic solvents, exhibit a high enantio-selectivity, possess broad substrate specificity, and do not require cofactors [8], [14], ability to target certain fatty acids (typoselectivity), the capability to distinguish between sn-1 and sn-3 TAG molecular positions (stereospecificity), and the capability to differentiate two external positions from a TAG (regioselectivity) [10], [15].

Functional oil and fat production can be catalyzed by lipase. Fats/oils can be improved physicochemical and nutritional properties using a lipase catalyst [16], [17]. Unsaturated fatty acids in triglycerides are mostly in the sn-2 position. The lipase specificity of sn-1,3 can be used to catalyze the transesterification reaction while maintaining sn-2 fatty acids [18]. The engineering of

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lipases for industrial applications with specific properties or better performance continues to be developed by isolating new lipases from new sources so that the industry's need for lipases is fulfilled [19]. To be efficient, the supporting matrix is ensured to be inert, regenerative to prevent enzyme washing, minimal Adsorption, economical, and can maintain catalytic activity [4].

Lipases can be used in the form of free lipases directly with ease and high catalytic activity. In addition to their advantages, free lipases also have disadvantages such as can't be separated from the reaction system (lipase is an enzyme that dissolves in an aqueous solution), cannot be recovered and reused, easily degraded, and inactivation, so they lack catalytic performance under extreme conditions such as excessive mechanical pressure, narrow temperature tolerance, poor tolerance to acid and alkali conditions (sensitive to the environment), incompatible organic solvents, surfactants or denaturants, etc. [8], [20], [21]. Lipase immobilization has been shown to upgrade enzyme activity, stability, and selectivity [2], [22]. Immobilized lipases are highly efficient for commercial use; this technique facilitates its separation and recovery for reuse. Immobilized lipases offer various advantages over free lipases or dissolved lipases, higher stability, including economic convenience, prolonged duration of catalysis, increased rate of catalysis, the likelihood to be easily separated from the reaction mixture, guiding to the isolation of a pure product, and importance for continuous flow biocatalysis using immobilized enzymes [1].

II. LIPASE IMMOBILIZATION

Enzyme immobilization is interpreted as enzyme entrapment, limiting the enzyme's mobility in various matrices so that it can be used repeatedly, more economical, higher activity, stable, and prevents product contamination [23]. Thus, the enzyme-modified to its water-insoluble form by the appropriate technique fulfills the definition of an immobilized enzyme. In enzyme immobilization, functional groups such as amino groups, carboxyl, sulfhydryl from cysteine, histidine imidazoles, phenolics, and hydroxyl groups of threonine serine need attention [24], [25]. In the reaction process for lipase immobilization, functional groups located in the center of the active site should not be involved because they can affect enzyme activity. Furthermore, the tertiary structure of enzyme proteins is preserved by relatively weak binding forces, such as hydrophobic, ionic, and hydrogen bonds, so that the immobilization process must prevent enzyme deactivation [26].

The purpose of the enzyme immobilization into a solid supporting matrix is to maintain biologically active three-dimensional structures and prevent structural deformation from increasing enzyme stability and catalytic performance [27]. The immobilization technique's choice is of great importance for the fruitfulness application and optimal immobilization to maintain the enzyme's stability, catalytic, and activity. Diverse techniques enzyme immobilization can be categorized as covalent bonding, Adsorption, crosslinking of the enzyme by short molecular linkers, and entrapment or encapsulation of the enzyme in permeable materials. Selection of appropriate method must be careful, including turn on the type and condition of the kind of enzyme and conditions of the catalytic process [3], [5], [27]–[29].

Immobilization carrier of biocatalysts for optimizing the catalytic performance is a very important aspect and a major problem for success. Besides that, the interaction formed between the support materials and the enzyme used also needs to be considered. Various materials could be used as a support for the immobilization of enzymes. These materials could be divided into composite, organic, and inorganic materials. The main requirement is to maintain high catalytic activity so that the material must be adequate to maintain the enzyme structure against harsh reaction conditions [1]. Various enzyme immobilization carriers, including glass beads, macropore resins, mesoporous silica, sol-gel materials, magnetic particles, carbon nanotubes [28]. Characteristic immobilization matrix that needs attention: surface area and porosity, surface functional groups, mechanical and chemical stability, size and shape, microbial resistance, hydrophobic/hydrophilic properties [30].

Immobilization carrier should be good mechanical strength and good stability to realize enzyme reusability, to increase the amount of immobilization, have porous structure or large specific surface area, easy surface modification or coating, abundant sources, low cost, environmentally friendly, most importantly it does not have a negative effect on enzyme structure and does not interfere the enzyme to create stable enzyme–matrix interactions [31], [32]. Enzyme stability is also affected by immobilization supports with high enzyme loads that can cause enzyme crowding [3], [5]

Several requirements must be met in enzyme immobilization; namely, the matrix or polymer must be inert and generally made of inorganic materials. In addition, the ideal matrix must meet characteristics such as good physical strength (not easily destroyed), good stability to the environment such as temperature and pH, regeneration ability, ability to increase enzyme activity, not inhibit product formation, and not be contaminated by microbes [26]. Enzyme immobilization has various advantages, namely more stable enzymes, easier separation of biocatalysts and products, easy application of enzymatic processes in continuous reactors, regeneration can be conducted, enzymes can be used repeatedly, reduced operating costs, and the absence of biocatalyst contamination in products [33].

TABLE 1. ADVANTAGES AND DISADVANTAGES OF VARIOUS PREPARATION METHODS FOR LIPASE IMMOBILIZATION

Immobilization methods	Advantages	Disadvantages
Physical Adsorption	Easy preparation and operation, low cost, and can be	Weak bonding, low activity, and specificity

	regenerated	
Ionic bonding	Easy preparation, low cost, high activity, and can be regenerated	The low specificity and weak bonding
Covalent binding	The bond is strong, the activity and specificity are high	Preparation is difficult, high cost, and cannot be regenerated
Crosslinking	The bond is strong, the activity and specificity are high	Preparation is difficult, high cost, and cannot be regenerated
Entrapment	High activity, strong bonds, and low cost	Preparation and operation is difficult and cannot be regenerated

Immobilization changes the nature of the catalyst from homogeneous to heterogeneous. The method of enzyme immobilization can be categorized into three ways, namely (i) carrier-binding method, where the enzymes are bound to a water-insoluble carrier, (ii) cross-linking method, where enzymes are bound to one another by several reagents, (iii) entrapping method, which combines several enzymes in a semipermeable gel lattice or enclosing enzymes in a semipermeable polymer [23], [34].

The carrier binding method is the first immobilization method invented. This method is based on the direct binding of the enzyme to a water-insoluble matrix. It can be differentiated into three types based on the binding method: physical Adsorption, ionic bonds, and covalent bonds [23], [35]. Matrices that can be used for immobilization with a bonding system include water-insoluble polysaccharides (dextran, cellulose, and agarose derivatives), organic materials (silica and porous glass), synthetic polymers (polyacrylamide gels and ion exchange resins), and proteins (albumin and gelatin). Some aspects that need to be considered are surface area, particle size, molar ratio, hydrophobic or hydrophilic properties, and chemical composition [34], [36]. Some of the advantages and disadvantages of the preparation method and the characteristics of lipase immobilization using the carrier binding method are presented in Table 1.

Based on Table 1, lipase immobilization using the physical adsorption method is interesting to continue to be developed because it has several advantages, namely, easy preparation, low cost, and easy regeneration. However, some of the weaknesses, such as low activity and low specificity, need to be improved, among which can be conducted by various modifications to the matrix's surface to increase lipase activity and stability [8], [37].

III. LIPASE IMMOBILIZATION USING ADSORPTION TECHNIQUE THROUGH MATRIX SURFACE MODIFICATION

The physical adsorption method has advantages over other methods, where preparation is easier, regeneration can be done more efficiently, and the costs required for operation are lower than other methods [37]. However, the low enzyme activity and the weak bond are the obstacles to the immobilization of lipase using physical adsorption techniques. The lipase can be immobilized after several carriers have been attached to the matrix or modified surface of the matrix. Modifications can be conducted by hydrophobic surface modifications that can improve the microenvironment, activity, and stability of lipases [38]. Various surface modification techniques and types of matrices for lipase immobilization can be noticed in Table 2.

Based on Table 1, lipase immobilization using Matrix surface modification has increased lipase activity and stability. Matrix surface modification can be conducted by adding hydrophilic or hydrophobic groups. However, hydrophobic modification is preferred because it increases lipase activity and stability, improves the lipase microenvironment, and particularly suitable for interesterification reactions rather than hydrolysis. The adsorption method only involves physical interactions such as electrostatic interactions, van der Waals, and hydrogen bonds on a porous matrix with a hydrophobic surface and a charged surface [35], [39]. Hydrophobic interaction also refers to the adsorption method. Lipase will be adsorbed on the surface, then change its conformation when the matrix has a hydrophobic character, thereby increasing its activity. This type of Adsorption is somewhat difficult to control because enzyme activity depends on how the enzyme binds. In a situation where the active site of the immobilized lipase is open to accept the substrate, the activity can increase, and vice versa [40].

TABLE 2. VARIOUS SURFACE MODIFICATIONS AND TYPES OF THE MATRIX ON THE IMMOBILIZATION BY PHYSICAL ADSORPTION METHOD

Type of matrix modification	Matrix	Lipase	Activity/application	References
Hydrophobic	Amberlite IRA-96	*Candida rugosa* lipase	Synthesis of Fructose Oleic Ester was 75.96% for 24 h reaction time.	[41]
	Octyl- and cyanogen bromide agarose beads	*Pseudomonas cepacia* and *Thermomyces lanuginosus*	Octyl agarose resulted in open form stabilized lipase than the covalent preparation.	[40]

		lipase		
	Amberlite IRA-96	*Candida Antarctica* lipase B	The immobilized lipase was used for glycerolysis of palm stearin-palm olein blend. The yield of MAG and DAG were 7.86% and 27.34%, respectively, and conversion rates were 0.45 and 0.45%/h, respectively, for 24 h reaction time.	[9], [42]
	Silk woven fabric	*Candida sp. 99-125* lipase	Immobilized lipase can be used up to 27 times with a high yield of esterification of around 97%	[43]
	Methyl-modified silica aerogels	*Candida rugosa* lipase type VII	The lipase activity was 19.87 µmol/ min.mg protein; adsorption capacity was 67.42 mg/g; and activity retention about 56.44%	[37]
	Poly(ethylene terephthalate) grafted acrylamide (PET-g-AAm) fiber	*Candida rugosa* lipase	Immobilized lipase has the remaining activity of about 0.06U and can be used up to 10 times for the hydrolysis of vegetable oils	[44]
	poly-methacrylate particles (PMA)	*Thermomyces lanuginosus, Rhizomucor miehei, Candida sp., Mucor javanicus*, and porcine pancreatic lipase	*Thermomyces lanuginosus*–PMA yielded protein loading of about 37.5 mg/g, and the highest hydrolytic activity was about 217.8 IU/g. Immobilized lipase was suitable for n-octyl oleate synthesis; the conversion up to 95.1 %	[45]
Hydrophilic-Hydrophobic	Amberlite IRA-96	*Candida rugosa* lipase	Lipase adsorption was 9.27 mg protein/g matrix; Synthesis of Fructose Oleic Ester was 85.29% for 18 h reaction time.	[41]
	Magnetic Silica Aerogel	*Candida rugosa* lipase	Specific activity was 15.34 U/mg protein for hydrophobic matrix; and 11.21 U/mg protein for hydrophilic matrix	[46]
Hydrophilic or Unmodified matrix	Chitosan beads	*Candida rugosa* lipase	Immobilized lipase was 21.93 µg/g-chitosan. The conversion of transesterification of cooking oil with methanol reached 73%.	[47]
	Santa Barbara Amorphous-15 (SBA-15)	*Candida antarctica* lipase B (CALB)	Immobilized lipase was used for glycerolysis of corn oil, resulted in about 53.6 wt% of diacylglycerols, and can be used up to 5 times.	[34]
	Celite-545, Sephadex G-25, and chitosan	*Candida rugosa* lipase	Celite-545 in acetone has the highest catalytic efficiency and can be used up to 6 times, which only about 38.21% loss of activity	[48]
	Biochar	*Candida rugosa* lipase	Lipase immobilization increased stability by more than 80% compared to free lipase. The conversion rate of immobilized lipase reached about 95% for oleic acid esterification.	[49]

The adsorption method with a hydrophobic surface generally has low enzyme loading properties. This is related to the reversible nature of the bond between protein and matrix, which is greatly affected by pH during the immobilization process [35], [36]. One of the compounds that can modify the matrix is 2-Phenylpropionaldehyde (PPA), where this compound has an aldehyde group end that can react with an amine group to form an imine group;

and an aromatic ring of PPA, which gives a hydrophobic property [41].

Many modifications with the addition of hydrophobic compounds have been carried out with satisfactory results. Chen et al. [43] modified woven fabrics with polydimethylsiloxane (PDMS) to make the fabric surface hydrophobic. The lipase activity for esterification and hydrolysis in PDMS fabric increased two times compared to without modification. Immobilized lipases have stability over a wider range of temperature and pH than free lipases and can be used repeatedly for esterification reactions at constant yield. Immobilized lipase can be used up to 27 times with a high yield of esterification of around 97%. Öztürk [50] made immobilized *Candida rugosa* lipase on the hydrophobic Octyl-sepharose CL-4B surface by Adsorption. Immobilized lipase has good activity and stability. The best conditions for immobilization of the amount of lipase adsorbed were achieved using pH 5-7, temperature 37 ° C, and 0.5 M NaCl, for 1 minute of Adsorption.

The immobilization of *Candida rugosa* lipases on the hydrophobic Amberlite IRA-96 was performed by Hilmanto et al. [41] by augmenting of 2-Phenylpropionaldehyde (PPA) 12.5%. Lipases could be completely immobilized in a hydrophobic matrix. The faster equilibrium was achieved when the hydrophobic properties of the matrix were obtained [51]. Hydrophobic modification of Amberlite for the immobilization of *C. rugosa* lipase also results in hydrophobic interactions between the enzyme-matrix. It has high activity for the synthesis of fructose oleic esters [41]. Subroto et al. [42] also modified the hydrophobic Amberlite IRA-96 by adding PPA for immobilization of *Candida Antarctica lipase B*. The optimum conditions for lipase immobilization were achieved with the addition of PPA in methanol and a lipase concentration of 12 mg/mL. The immobilized lipase was then used for glycerolysis of the mixture of palm olein and palm stearin blend at 50 °C for 24 hours, yielding monoacylglycerol diacylglycerol of 7.86% and 27.34%, respectively.

Lipase immobilization on hydrophobic supports was also carried out by Manoel et al. [40], who used octyl and cyanogen bromide agarose beads to immobilize *Pseudomonas cepacia* and *Thermomyces lanuginosus* lipase. The results showed that lipase immobilization in octyl agarose produced a relatively higher catalytic activity than cyanogen bromide agarose. Immobilized lipase activity in *Thermomyces lanuginosus* and *Pseudomonas cepacia* lipase was 106.6 and 56.6 µmoles of substrate hydrolyzed per minute, respectively. In another study, Gao et al. [37] immobilized *Candida rugosa* lipase on a hydrophobic modified silica-aerogel matrix with the addition of a methyl group. The results showed that the droplet and pore diameter became smaller, mass transfer increased, and immobilized lipase was obtained with an adsorption capacity of 67.42 mg/g with relatively stable activity retention. Another advantage is that immobilized lipases can occupy the interface between oil and water used for reactions in the emulsion system. These conditions are very suitable for the properties of lipase, which require interface conditions for its activity [52].

Lipase immobilization in the hydrophobic matrix was also carried out by Bassi et al. [45], who used a matrix in the form of poly-methacrylate particles. Immobilization was applied to various lipases, including *Thermomyces lanuginosus, Rhizomucor miehei, Candida sp, Mucor javanicus*, and porcine pancreatic lipase. The lipase obtained has good interfacial activity. Lipase has high hydrolysis activity but is also effective for esterification, so it is suitable for the synthesis of octyl oleate through the esterification of oleic acid n-octanol in a solvent-free system. Immobilized lipase also has the resistance to be used multiple times up to 12 reactions.

Lipases immobilized in hydrophobically modified matrices generally have good activity for esterification and interesterification. However, hydrophobic modification can also be used for hydrolysis reactions. Yigitoglu and Temoçin [44] immobilized *C. rugosa* lipase on a hydrophobic modified glutaraldehyde-activated polyester fiber matrix, then used for hydrolysis of olive oil, soybean oil, sunflower oil, canola oil, and corn oil. The results showed that hydrolysis of olive oil and canola oil using immobilized lipase resulted in a higher yield than using free lipase. The use of isooctane as a solvent also increases the rate of the hydrolysis reaction.

Modification of matrix is restricted to compounds that provide hydrophobic characters, but the modification using the hydrophilic groups has been widely used, but the hydrophilic matrix modification can lead lipases to lief water and promote hydrolysis than esterification reactions [14], [41]. [46] compared *C. rugosa* lipase immobilized in the hydrophobic and hydrophilic modified Magnetic Silica Airgel matrix. Lipases in the hydrophobic matrix had higher specific activity than the hydrophilic matrix, namely 15.34 U/mg-protein and 11.21 U/mg-protein. Modification by combining hydrophilic and hydrophobic properties of the matrix is an alternative to improve immobilized lipase activity. The hydrophilic side can improve lipase loading on the matrix because the surface portion of the lipase protein is mostly dominated by hydrophilic protein so that the lipase is easily adsorbed on the matrix. Meanwhile, the hydrophobic side functions to improve the microenvironment of the lipase's active site, which is closed with a lid, which is hydrophobic so that it opens easily and catalyzes the hydrophobic substrate. Hilmanto et al. [41] modified the Amberlite IRA-96 matrix in a hydrophilic-hydrophobic combination to immobilize *Candida rugosa* lipase. The lipase obtained is very suitable for synthesizing oleic fructose by the esterification of oleic acid with fructose; the yield of oleic fructose reaches 85.29% at 60 ° C for 18 hours.

Supports or matrix modification for lipase immobilization continues to develop by utilizing various types of matrices, hydrophobic groups, lipases, and modification techniques to increase their stability and activity. The hydrophobic matrix modification proved to be effective in increasing the activity and stability of lipase. However, the challenge in the hydrophobic modification is that the loading of lipase protein is generally relatively

lower than that of the hydrophilic matrix. Therefore, various studies to increase the loading of adsorbed lipases on the hydrophobic matrix.

IV. CONCLUSIONS

Lipase immobilization through Adsorption on the surface of the matrix has various advantages, especially easy preparation and operation, low cost, and can be regenerated. The surface of the matrix can be improved by modifying the matrix primarily to make the matrix hydrophobic, which has been shown to increase lipase activity and stability by improving the microenvironment on the catalytic site of the lipase. Hydrophobic modification is proven to be effective for reaction catalysis, hydrolysis, and esterification, including hydrolysis and glycerolysis of vegetable oil to produce monoacylglycerols diacylglycerols, esterification for ester sugar synthesis, and interesterification for structured lipid synthesis.

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