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# Nanosupport bound lipases their stability and applications

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## ABSTRACT
This review article demonstrates the immobilization of lipases by using various kinds of nanoparticles and nanocomposites. Lipases have successfully been immobilized on/in different nanomaterials by maintaining very high enzyme activities and yields of immobilization. Nanomaterials bound lipases showed enhanced activity and enantioselectivity, very high stability against varying types of parameters such as pH, temperatures and detergents. Moreover, lipases immobilized on nanocarriers have demonstrated very high stability in the presence of numerous types of water miscible and immiscible organic solvents, thus making these enzymes suitable for catalyzing unique reactions such as acylation, esterification, interesterification and transesterification in nonaqueous media apart from their naturally occurring reactions. Nanomaterials bound lipases exhibited very high operational stability, reusability and applicability in continuous reactors and biosensors. Furthermore, the immobilized lipases have demonstrated synthesis/production of novel compounds such as biopolymers, biodiesel, enantiopure pharmaceuticals, agrochemicals, flavor & fragrance compounds, fine chemicals, esters and amino acid derivatives. Nanocarriers-lipases composites have successfully been employed as biosensor for rapid and efficient analysis of triglycerides and other related compounds in serum and other biological samples. Lipases attached to the electrodes via nanocarriers have validated remarkably their potential as perfect biosensors. Nanocomposite bound lipases have also used as a tool to remove environmental pollutants.

**Keywords:** *Lipase; Immobilization; Nanoparticles; Nanocomposites, Reusability; Stabilization; Regioselectivity, Enantioselectivity*.
**Abbreviations**: *3-APTES, (3-aminopropyl) triethoxysilane; ANL, Aspergillus niger lipase; AOL, Aspergillus oryzae lipase; BCL, Burkholderia cepacia lipase, BSL, Bacillus stearothermopillus Li lipase; BTL, Bacillus thermocatenulatus lipase; CALB, Candida Antarctica lipase B; CLEA, crosslinked enzyme aggregate; CS, chitosan; CRL, Candida rugosa lipase; CDI, carbodiimide; GCE, glassy carbon electrode; GA, glutaraldehyde; GC, gas chromatographic; GO, graphene oxide; MNPs, magnetic nanoparticles; MP, mesoporous; MJL, Mucor javanicus lipase; MWCNTs, multiwalled carbon nanotubes; NFs, nanofibers; NCs, nanocomposites; MNA, magnetic nanobiocatalyst aggregates; MRL, Mucor racemosus lipase; NMs, nanomaterials; NPG, nanoporous gold; NPs, nanoparticles; PDA, polydopamine; PEG, polyethyleneglycol; PEI, polyethyleneimine; PMMA, polymethylmethacrylate; PPL, porcine pancreatic lipase; PLA, polylactic acid; PS, polystyrene; PVA, polyvinyl alcohol; PCL, Pseudomonas cepacia lipase; PFL, Pseudomonas fluorescens lipase; RML, Rhizomucor miehei lipase; ROL, Rhizopus oryzae lipase; RSM, response surface methodology; TLL, Thermomyces lanuginosus lipase; TGs, triglycerides; WCO, waste cooking oil.*

## 1. INTRODUCTION
Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are the enzymes that act on carboxylic ester bonds. The physiologic role of lipases is to hydrolyze triglycerides (TGs) into diglycerides, monoglycerides, fatty acids and glycerol. These are subclasses of the enzymes esterases those act at a specific position on the glycerol moiety of lipid substrates (A1, A2 or A3). They perform significant roles in the digestion, transport and processing of dietary lipids in most of the living organisms. Human pancreatic lipase is one of the main enzymes that hydrolyzes dietary fats in digestive system (small intestine), converts TGs, substrates found in ingested oils to monoglycerides and two fatty acids. Lipases are engaged in various types of biological functions ranging from routine metabolism of dietary TGs to cell signaling and inflammation [1,2]. These enzymes are widely found in bacteria, yeasts, fungi, plants and animals. Lipases have attracted the utmost scientific attention as they catalyze some novel reactions such as acylation, esterification, interesterification and transesterification in nonaqueous media besides their natural reactions [3]. Fig. 1 illustrates novel reactions catalyzed by lipases. This versatile nature of lipases makes them of great choice for potential applications in various industries such as food, fuel, biosensors, bioremediation, pharmaceutical, detergent, leather, cosmetics, textile, paper, nutraceuticals and oil degumming [4-6]. Fig. 2 demonstrates various industrial applications of lipases. Moreover, the lipases have successfully been exploited for the synthesis and production of novel biotechnological products in nonconventional media like biopolymers, biodiesel, enantiopure pharmaceuticals, agrochemicals, flavor & fragrance compounds, fine chemicals, cosmetic compounds, esters and amino acid derivatives etc [7,8]. Fig. 3 depicts different biotechnological products synthesized by lipases.

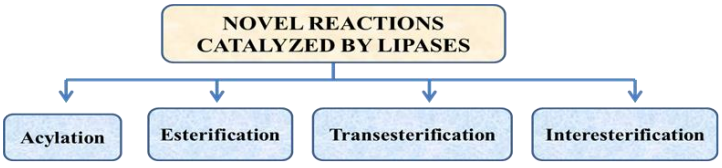


**Fig. 1.** Illustrates novel reactions catalyzed by lipases.

The applications of free lipases have their own demerits such as high cost, poor stability, difficulty to reuse them again again in batch processes and problem of application in continuous reactors. In order to overcome these limitations, immobilization of enzymes is one of the preferred choices [9-12]. The immobilization is making enzymes reusable, more stable, applicable in continuous reactors and also minimizing the cost of the processes. A large number of organic and inorganic supports have been employed for the immobilization of enzymes but the classical methods of enzyme immobilization have their own demerits [13-15]. Recently nanomaterials (NMs) have attracted the attention of enzymologists as an enzyme immobilizing support due to unique properties of such materials [16-18]. Nanobiotechnology is an emerging field of biotechnology. In recent years the researchers have developed unique nanoscaffolds to prepare highly stable nanobiocatalysts in order to exploit them for the synthesis of novel compounds. Enzymes, mainly hydrolases, have been extensively immobilized on NMs for long-term stabilization resulting in enhancement of pH, heat, operational and storage stability [19-21]. The novel nanoscaffold variants employed in the recent past for lipase immobilization are nanocrystals, nanofibres (NFs), nanoparticles (NPs), nanorods (NRs), nanotubes (NTs), nanopores, nanosheets and nanocomposites (NCs). These nanosupports have remarkably large surface area that permits the binding of huge amounts of enzymes and consequently high volumetric enzyme activity. NMs provides high robustness and resistance to breakage mediated by mechanical shear in the running reactors making them suitable for several repeated uses. The optimization of various nanosupports process parameters, such as source of enzyme and the selection of a suitable method for the immobilization of enzyme may be helpful in the development of an effective enzyme based reactor [22-25]. Such kinds of materials may also be applied for the development of other kinds of stable nanobiocatalytic systems, which may be useful in resolving environmental problem by increasing production of green energy. NMs bound lipases have already demonstrated their potential in the conversion of various oils to biodiesel which is a new field of research towards green energy production [26].

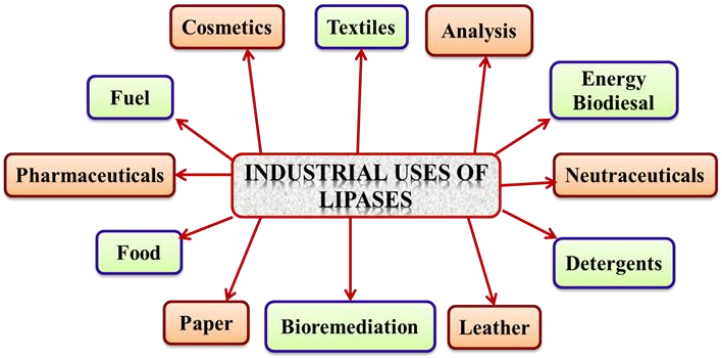


**Fig. 2.** Demonstrates various industrial applications of lipases.

In this article, I have reviewed literature based on immobilization of lipases from different sources on various kinds of magnetic and nonmagnetic nanosupports. The effect on the activity and stability of lipases bound to nanosupports has been critically discussed in detail. The kinetics and stability properties of lipases immobilized on/in nanosupports have been described. The applications of nanosupports bound lipases in varying industrial, clinical and biosensor fields have also been illustrated.

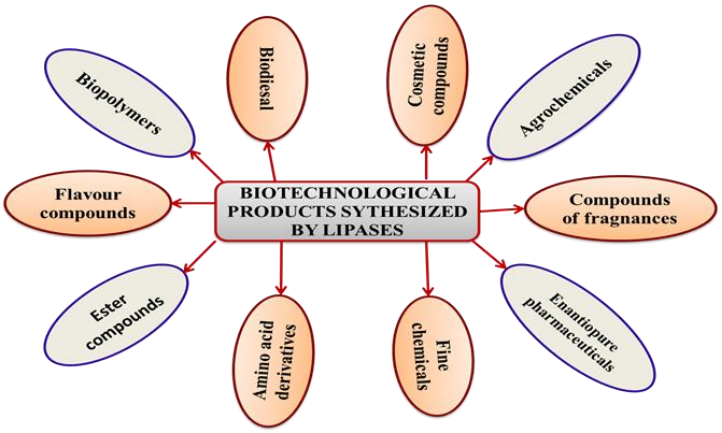


**Fig. 3.** Depicts different biotechnological products synthesized by lipases.

## 2. NON-MAGNETIC NANOSUPPORTS BOUND LIPASES

**2.1**. **Physically adsorbed lipases on NMs**. Table 1 depicts various kinds of lipases physically adsorbed on non-magnetic nanocarriers, their improved properties and applications. Nanostructured polystyrene (PS) and polymethylmethacrylate (PMMA) have considered as supports for the immobilization of lipolytic enzymes; *Candida rugosa* lipase (CRL) and *Pseudomonas cepacia* lipase (PCL). Lipases adsorbed on polymeric NPs demonstrated improved activity and selectivity with respect to the enzymes adsorbed on the similar non-nanostructured supports. CRL and PCL immobilized on nanostructured PMMA and PS retained 60% and 74% of their initial activity, respectively. Moreover, it has been noticed that enantioselectivity, pH and thermal stability of the enzymes were enhanced after immobilization. These findings have focused that new protein conformers with enhanced enantioselectivity were stabilized after adsorption on NPs [27]. In a further study, Miletic et al. [28] evaluated the effect of pH on the binding of *Candida Antarctica* lipase B (CALB) on PS NPs. The pH of the buffer solution did not exhibit any influence on enzyme loading, while the activity of immobilized enzyme was highly dependent on the pH of adsorption. Immobilized CALB expressed higher activity at pH 6.8 than the free enzyme powder and Novozyme 435. Chen et al. [29] for the first time used carboxylic surfactant modified ZrNPs for the adsorption of CRL and PCL. The immobilized lipases showed enhanced activity during resolution of (R,S)-ibuprofen and (R,S)-1-phenylethanol via esterification and

acylation in isooctane organic solvent. Lipases immobilized on erucic acid-modified ZrNPs exhibited significantly high activity and enantioselectivity compared to crude lipase powders. These NC bound lipases were found quite stable and reused for 8-times without any loss in its activity. Nano-$SiO_2$ adsorbed lipase showed activity yield of 3867 U $g^{-1}$ carrier. The immobilized lipase showed temperature optimum at 45°C that was 5°C higher than free enzyme. The optimal-pH for immobilized enzyme dropped to 5.5 compared to free enzyme, pH 7.0. The immobilized lipase illustrated a marked improvement in pH and heat stability. The immobilized neutrophil lipase catalyzed esterification of different oils to produce biodiesel such as soybean oil, rapeseed oil and waste oil. The esterification rate of rapeseed oil was highest [30]. Lipase was immobilized on chitosan (CS) NPs of two different diameters; 7 and 10 nm. The lipase adsorption capacity of NPs of 7 nm in size was 156 mg $g^{-1}$ and activity retention compared to free enzyme was as high as 66.7%. The immobilized lipase retained 91% activity after 5-repeated uses [31].

An inexpensive nanostructured material, fumed silica, was used for the immobilization of CALB. It involved a two step process, firstly the enzyme molecules were physically adsorbed on the surface of non-porous fumed silica NPs with the participation of silanol groups and secondly water was removed by lyophilization. This technique was successfully applied to immobilize CALB and the acivity of fumed silica-bound lipase was examined in hexane. The simple esterification of geraniol and the enantioselective transesterification of (R,S)-1-phenylethanol was the model reactions for CALB nanobiocomposite. The observed catalytic activity was remarkably higher compared to commercially available preparation [32]. Porcine pancreatic lipase (PPL) was reversibly attached on a monolithic polymer support containing thiol functionalities prepared within confines of a fused silica capillary and functionalized with AuNPs. Use of NPs enabled rejuvenation of the activity of the deactivated reactor simply by removing inactive enzyme from NPs using 2-mercaptoethanol and subsequent immobilization of fresh lipase. This flow through enzymatic reactor was then used to catalyze hydrolysis of glyceryl tributyrate (tributyrin). Highest activity was found within a temperature range of 37-40°C. $V_{max}$ for the immobilized enzyme was 1,000 times faster than the free lipase. The fast reaction rate enabled to achieve 86.7% conversion of tributyrin only in 2.5 min and an almost complete conversion in 10 min. The reactor maintained over 90% of its activity even after continuous pumping through it a solution of substrate equaling 1,760 reactor volumes. The potential application of the enzymatic reactor was determined with the transesterification of TGs from kitchen oil to FAME, which demonstrated the ability of the reactor to produce biodiesel [33]. Zhao et al. [34] studied the immobilization of CRL on the silica NPs and evaluated its enantioselectivity in organic solvent at high pressures under different water activities. The high hydrostatic pressures (50-200 MPa) enhanced the activity of immobilized CRL 4-6 times during transesterification of (R)-1-phenylpropan-2-ol with vinyl acetate. Moreover, the immobilized CRL showed a remarkable alteration in its selectivity, shifting the enantiomeric excess from the (R)-towards (S)-1-phenylpropan-2-yl acetate product at atmospheric pressure. The application of high pressures led to either enantiomeric excess towards (R)-1-phenylpropan-2-yl or no enantiomeric selectivity, depending on the water activities in the organic solvent and the level of pressures. This unique property of immobilized CRL under high pressures opens new avenues to modulate enzyme functions via combination of high pressures and enzyme immobilization. *Burkholderia cepacia* lipase (BCL) @tannic acid (TA)-mesoporous silica NPs (MP-silica-NPs) exhibited good thermal stability; strong tolerance to organic solvents such as methanol, ethanol, isooctane, n-hexane, and tetrahydrofuran; and high reusability when BCL@TA-MP-silica-NPs was used in esterification and transesterification reactions. Over 85% yield was retained after 15 repeated uses in the transesterification reaction for biodiesel production [35]. Kalantari et al. [36] used octadecylalkyl modified C18-MP-silica-NPs with a high C18 content (~19 wt. %) and tunable pore sizes (1.6-13 nm) for high yield immobilization of lipase. The immobilized enzyme showed a loading capacity of 711 mg $g^{-1}$ and a specific activity 5.23 times higher than free enzyme. Moreover, the bound enzyme maintained about 93% activity after 5-repeated uses.

Polyethylene glycol (PEG)-decorated PS NPs modified by Congo red and used for the adsorption of CRL at 24±1°C and pH 7.0. The immobilized lipase illustrated significantly very high $K_m$, $V_{max}$ and $k_{cat}$ values. In addition, immobilized enzyme exhibited good heat stability and maintained 90% or 70% of the initial activity at 40°C or 25°C after 7-repeated uses, respectively [37]. BCL and CALB were immobilized effectively by adsorption onto the fibrous material as well as by entrapment within the electrospun NFs of polyvinyl alcohol (PVA) or polylactic acid (PLA). The catalytic activity of membrane biocatalysts system was examined for the kinetic resolution of racemic 1-phenylethanol (rac-1) and 1-phenylethyl acetate (rac-2). Fine dispersion of lipases in polymer matrix and large surface area of NFs provided very high rise in the activity of the membrane biocatalyst compared to their free counterparts. PLA immobilized lipase was found far superior over PVA immobilized enzyme in terms of its activity and stability. Lipase activity and enantiomer selectivity was much higher for PLA-entrapped enzyme compared to PVA-entrapped lipase. The electrospun membrane forms of CALB showed high mechanical stability in the repeated acylations and hydrolyses than commercial forms of CALB immobilized on polyacrylamide beads, Novozyme 435 and IMMCALB-T2-150 [38]. A main problem in lipase immobilization for catalysis is to open the lipase lid and maintain it in an open conformation in order to expose its active site. Mathesh et al. [39] designed graphene-based nano-supports for effective lipase immobilization via molecular engineering, which is a great challenge to control biophysicochemical interactions at nano-biointerface. It was seen that increasing hydrophobic surface increased lipase activity due to opening of helical lid present on its surface. The molecular mechanism of lid-opening revealed in molecular dynamics simulations highlighted the role of hydrophobic interactions at the interface. It has been demonstrated that the open and active form of lipase was obtained and mediated with an optimized activity via

chemical reduction of graphene oxide (GO). This work has provided a novel NM which can be used as a platform for enhancing enzyme activity after immobilization. BCL was immobilized on the TA-MPNPs via physical adsorption.

Table 1: List of lipases physically adsorbed on non-magnetic NPs, their improved properties and applications.

| Name of enzyme | Name of support | Property/ Enhanced | Reference |
|---|---|---|---|
| CRL/PCL | PS/PMMA NPs | Enhanced enantio-selectivity, pH & heat stability | 27 |
| CRL | MWCNTs | Enhanced stability & transesterification activity in water immiscible organic solvents | 40 |
| CRL/PCL | Eurocic acid modified Zr NPs | Very high activity, enantioselectivity & resolution of (R,S)-ibuprofen to (R,S)-1-phenylethanol via esterification/& acylation | 29 |
| Lipase | CS NPs of sizes 7 & 10 nm | Very high adsorption capacity/reusability | 31 |
| CALB | PS NPs | Very high activity at pH 6.8 | 28 |
| NL | Nano- $SiO_2$ | Improved pH & heat stability; biodiesel from soybean, rapeseed & waste oils; rapeseed oil was highest esterified | 30 |
| CALB | COOH, $NH_2$-& ester functionalized MWCNTs | High catalytic activity, storage, operational & organic solvents stability | 41 |
| CALB | Nonporous fumed silica NPs | Very high esterification of geraniol & enantioselective transesterification of (R,S)-phenylethanol | 31 |
| PPL | AuNPs functionalized fused silica capillary | Very high activity & stability, biodiesel production | 32 |
| PPL | PEG-Ag/IONPs | Enhanced activity, heat & storage stability | 34 |
| CRL | PS/PEG NPs & PS/PEG/CR NPs | Retained remarkably high activity, heat stability & reusability | 37 |
| BCL | Modified MWCNTs | Decreased reaction time | 42 |
| CRL/ CALB | MWCNTs-DVD,MWCNTs-BA,MWCNTs-OA | Biodiesel production | 47,48 |
| CRL | MWCNTS-COOH | Higher production of methyl oleate in nonionic surfactant/isooctane | 43,44 |
| CALB | PMMA NPs | Good reusability | 51 |
| CRL | *o*-MWCNTs | Two-fold more synthesis of geranyl propionate, an ester employed in flavoring & fragrances | 45,46 |
| CRL | Silica NPs | Enhanced transesterification of (R)-1-phenylpropan-2-ol with vinyl acetate by 4-6 times | 34 |
| Lipase | C-18 MSNs | High yield & loading of enzyme, catalytic efficiency, storage stability & reusability | 36 |
| BCL/ CALB | Electrospan NFs of PVA/PLA | PLA entrapped CALB had high mechanical stability in repeated acylation & hydrolysis | 38 |
| Lipase | Gr based nanosupports | Very high activity | 39 |
| BCL | TA-MSN NPs | High heat stability & operational reusability; strong tolerance to organic solvents | 35 |

Shah and coworkers [40] have investigated the adsorption of CRL on the multiwalled carbon nanotubes (MWCNTs). MWCNTs bound enzyme retained very high activity, 97%. The immobilized CRL showed 2.2 and 14-fold increase in transesterification activity in anhydrous hexane and water immiscible ionic liquid [Bmim] [PF6], respectively compared to lyophilized enzyme. The interaction of CRL with the hydrophobic surface of the NTs resulted in the conformational changes that led to 'open lid' structure of enzyme. The immobilized enzyme produced 64% of butylbutyrate in nearly anhydrous hexane over 24 h whereas the free enzyme catalyzed only 14% formation of this product. Similarly, the immobilized enzyme allowed 71% conversion in ionic liquid while the free enzyme catalyzed about 16% conversion. The immobilized lipase also showed high enantioselectivity as determined by kinetic resolution of (±) 1-phenylethanol in [Bmim] [PF6]. However, the free CRL gave only 5% conversion after 36 h, the immobilized enzyme resulted in 37% conversion with > 99% enantiomeric excess. CALB was immobilized via physical adsorption on MWCNTs functionalized with carboxyl-, amine- and ester- terminal groups on their surface. The enzyme loading was attained as 25% by weight of CNTs. MWCNT-lipase bioconjugate exhibited high catalytic activity and increased storage and operational stability. The adsorbed CALB maintained more than 55% of its activity after 6 months at 4°C, whereas it showed about 25% of the initial activity after 30 days of incubation in hexane at 60°C. The catalytic behaviour of the immobilized enzyme depends on the terminal group of the CNTs, the concentration of the enzyme and the immobilization method employed [41]. Modified MWCNTs was considered for BCL immobilization and was further optimized via single factorial experiments and response surface methodology (RSM). The enzyme activity was 50 200 U $g^{-1}$, 54 fold under standard conditions that of the free lipase in resolution of 1-phenylethanol, taking place in a very short time from 30 h of the free lipase to 10 min for immobilized enzyme. MWCNTs-BCL demonstrated great advantages and possesses promising potential in industrial

applications [42]. In a recent investigation, acid functionalized MWCNTs were used as support for the adsorption of CRL and this nanobiocatalyst was exploited for the production of methyl oleate. CRL-MWCNTs nanobiocatalyst was used to produce higher quantity of methyl oleate, 79.85% in 11 h, an about 1.5-fold improvement over the free enzyme. The highest percentage of esterification (83.62%) was observed following the use of nonionic surfactant when compared to anionic and cationic ones. MWCNTs nanobiocatalyst maintained 50% of its activity after 5-repeated uses [43]. Moreover, these researchers have used same MWCNTs bound CRL for the production of methyl oleate in isooctane medium. The CRL-MWCNTs resulted in 30-110% improvement in the production of methyl oleate over the free CRL. The CRL-MWCNTs attained its highest yield (84.17 %) at 50°C, molar ratio of acid/alcohol of 1:3, 3 mg $mL^{-1}$ of enzyme loading, and iso-octane (log P 4.5) as solvent [44]. Oxidized MWCNTs adsorbed CRL was employed for the synthesis of geranyl propionate in *n*-heptane. There was a 2-fold improvement in the conversion of geranyl propionate by immobilized CRL compared to free enzyme. The highest yield of geranyl propionate was obtained in a molar ratio acid: alcohol of 1:5 in 6 h at 55°C and with the presence of 1.0 g desicant. CRL-MWCNTs retained 50% activity after 6 repeated uses [45, 46]. Rastian et al. [47] used functionalized MWCNTs with carboxyl and hydroxyl groups for CRL adsorption in different weight ratio of enzyme to support. Immobilized lipase was used for conversion of oleic acid and butanol to butyl oleate ester. Further same group evaluated immobilization of CALB on the MWCNTs-COOH and amidated via butylamine (MWCNTs-BA) or octadecylamine (MWCNTs-OA). The CALB loading on the MWCNTs-BA and MWCNTs-COOH was 20 mg $g^{-1}$ protein, while the value for MWCNTs-OA was 11 mg $g^{-1}$ protein. The immobilized CALB was used for the methanolysis of rapeseed oil. The yield of biodiesel, fatty acid methyl ester (FAME) production by CALB bound to MWCNTs-BA was 92%, while 86% yield was reported in case of lipase immobilized on MWCNTs-OA. The immobilized CALB preparations showed very high catalytic activity, heat stability and reusability in batch processes [48].

**2.2. Covalently immobilized lipases on nanomaterials.** Table 2 demonstrates various covalently bound lipases onto nonmagnetic nanosupports, their improved properties and applications. Farshad et al. [49] studied the immobilization of *Thermomyces lanuginosa* lipase (TLL) on polyacrolein/MCM-41 NCs via covalent bonds formation with aldehyde groups. The immobilized lipase exhibited better operational stability such as pH tolerance, heat and storage stability. Moreover, NCs bound lipase was easily recollected and retained about 74% of its initial activity after 15 repeated uses. Kisukuri et al. [50] investigated covalent immobilization of BCL and PPL on thiol-functionalized AgAu nanoshells (NS). The catalytic performance of AgAuNS-lipase was determined by measuring kinetic resolution of (R,S)-1-(phenyl). BCL attached to the AgAuNS with largest spacer mercaptoundecanoic acid showed fastest conversion rate compared to lipase bound to a smaller spacer such as cysteamine or mercaptoacetic acid and free enzyme. The enzyme maintained 90% activity after 3 repeated uses. The findings of the work demonstrated that the size of the spacer played an important role in optimizing lipase activity in metallic NS as solid supports. Dumri and Hung-Anh [51] immobilized a commercial available PPL on polydopamine (PDA)/AgNPs (10-20 nm) complex surface via covalent bonds formation. Thus a tailor-made immobilized enzyme was obtained which was the complex of PPL/PDA/AgNPs. Gas chromatographic (GC) analysis showed a remarkable biodiesel production yield of 95% by immobilized lipase at 40°C for 6 h, whereas 86% yield was achieved by free lyophilized lipase. Immobilized PPL was reused 7-times by retaining the conversion rate of soybean oil to 27%. CALB was immobilized in PMMA NPs. The immobilized enzyme maintained about 40% activity after 20-repeated uses [52]. Aldehyde-functionalized silica and silica NPs (SBA-15) were considered for covalent immobilization of *Rhizomucor miehei* lipase (RML) via a multicomponent reaction under extremely mild conditions at 25 and pH 7. The findings showed very fast immobilization of 10 and 60 mg of RML on 1 g of aldehyde-functionalized silica and SBA-15 after 10 and 30 min, respectively. The heat stability and co-solvent stability of the immobilized enzyme preparations in the presence of three polar organic solvents (1-propanol, 2-propanol and dioxane) were remarkably enhanced compared to soluble enzyme. Both immobilized enzyme preparations have been applied to catalyze transesterification of colza oil with methanol to produce FAMEs. RML immobilized on SBA-15 showed improved conversion yield in presence of 40% (v/v) of *t*-butanol [53].

Lipase was immobilized onto hybrid protein: sugar nanofibrils via photochemical reaction. The nanofibrils were obtained via aggregation of hen white egg lysozyme induced by the highly sulfated glycosaminoglycan heparin. The new hybrid NM could be easily functionalized using photochemical reaction in order to attach lipases via dityrosine covalent bonds. The photo-immobilized lipase demonstrates better thermostability and enhanced resistance to non-conventional environment than the free enzyme. Structural and morphological characterization of nanofibrils showed that they were compatible with amyloid-like aggregates. Moreover, the supramolecular arrangement of heparin and lysozyme within the building unit of the nanofibrils has also been suggested. This procedure was found highly useful in designing a new generation of insoluble biocatalyst by a single photo-click method that is clearly more ecofriendly and faster than conventional chemical cross-linked procedures [54]. Patel et al. [55] grafted ZnO NPs with polyethyleneimine (PEI) and modified with glutaraldehyde (GA) in order to use it as a linkage for binding lipase to the support. This immobilized lipase was applied for the synthesis of geranyl acetate in *n*-hexane. Among all the three prepared NCs; ZnO + PEI, ZnO + PEI + succinic acid anhydride (SAA), ZnO + PEI + GA, CRL immobilized on ZnO-PEI-GA was found far superior in the synthesis of higher amount of ester. *Bacillus thermocatenulatus* lipase (BTL) was covalently immobilized on carboxylated-nanographene oxide via Ugi four-component assembly process (Ugi 4-CAP), in which amine, aldehyde, isocyanide, and acid components come together in a one-pot reaction to generate hydrophobic-, hydrophilic-, or

amphiphilic multifunctionalized graphene composites. The immobilized BTL exhibited very high catalytic efficiency and stability. The enzymatic production of biodiesel by methanolysis of canola oil was studied using self-made biocatalysts [56]. MP SBA-15 NPs support functionalized with 3-glycidyloxypropyl trimethoxysilane was used for the covalent immobilization of three lipases; CALB, TLL and RML. Immobilized lipases were found significantly more heat stable and methanol tolerant compared to their native forms. The water had little effect in increasing FAME yield when SBA-RML was used as catalyst whereas SBA-TLL catalyzed almost 98% FAME production in presence of 20 wt% water by substrate weight. Moreover, *t*-butanol had a great effect on yield, with almost complete conversion for SBA-RML and SBA-TLL. The immobilized TLL was quite stable and was reused 20-times without significant loss in its activity (6%). RML and CALB retained 95% of their initial activities after 7 and 15 repeated uses, respectively [57]. *Rhizopus oryzae* lipase (ROL) immobilized onto GO nanosupport prepared by Staudenmaier and Brodie methods. The enzyme was immobilized by various methods; physical adsorption, covalent attachment, and additional crosslinking. Covalently immobilized lipase demonstrated markedly more resistance to heat inactivation compared to its soluble counterpart. Physically adsorbed lipase obtained 100% of the initial activity in a series of organic solvents. These findings showed enhanced heat stability and solvent tolerance of GO immobilized enzyme. It will put a profound impact on practical industrial scale uses of enzymes for the conversion of lipids into fuels [58].

Functionalized MWCNTs was used for covalent immobilization of TLL and this immobilized lipase exhibited broader pH and temperature optima compared to soluble enzyme. Lipase immobilized on MWCNTs exhibited significantly high reusability even after 10 reuses and improved thermal stability [59]. Raghavendra et al. [60] demonstrated two approaches for covalent attachment of CALB on MWCNTs. In one method of enzyme immobilization carbodiimide (CDI) was used while in the second approach the cross linker 3-aminopropyltriethoxysilane (3-APTES) followed by succinic acid anhydride (SAA) were employed prior to CDI activation. MWCNTs bound lipase was exploited for the formation of flavor ester, pentyl valerate, in cyclohexane. The immobilized CALB prepared by CDI found highly stable and retained nearly 79% activity while the nanobiocatalyst obtained using 3-APTES and SAA retained approximately 30% activity. Immobilized lipase preparations were repeatedly used 50-times without much loss in their activities. Three liquid phases (viz. aqueous, nonaqueous, and reverse micelles (RMs)) were scrutinized as medium for attachment of CRL onto MWCNTs. The NTs were functionalized to attain carboxyl and amino groups on their surfaces before enzyme conjugation. High enzyme loadings associated with the functionalized CNTs were observed when RMs were used as the attachment medium. In addition, high activity in terms of ester synthesis in organic solvents was also obtained. The nanobiocatalyst prepared using RMs were found to be highly resistant and exhibited remarkably high operational stability. Carboxylated NTs bound lipase retained about 95±3% of the esterification activity at end of 20$^{th}$ reuse while the aminated NTs attachéd enzyme maintained 90±5% of its activity at the end of only 10$^{th}$ cycle. It demonstrated high potential of RMs as the attachment medium for surface active enzymes such as CRL onto CNTs [61].

**Table 2.** List of lipases covalently attached onto various non-magnetic NPs and their improved properties.

| Name of enzyme | Name of support | Property/properties Enhanced | Reference |
|---|---|---|---|
| BCL/PPL | SH-functionalized AgAu NPs | Very high reusability, size of spacer played an important role in binding | 50 |
| TLL | Polyacrolein/MCM-41 NC | Better heat, pH and storage stability, significantly very high reusability | 49 |
| CALB | MWCNTs-CDI/ MWCNTs-COOH, MWCNTs-$NH_2$ in RMs | Enhanced activity, heat & storage stability, formation of flavor ester, pentyl valerate in cyclohexane with greater efficiency | 60,61 |
| PPL | PDA/Ag NPs (10-20 nm) | Remarkably very high biodiesel production, good reusability | 51 |
| ROL | GO nanosupport | Enhanced heat stability & tolerance to organic solvents | 58 |
| Lipase | Protein-sugar nanofibrils | Better heat stability & resistance to non-conventional environment | 54 |
| RTL | Nano-GO-COOH | Very high catalytic efficiency & stability, & biodiesel production from canola oil+methanol | 56 |
| Lipase | ZnO NPs - PEI & GA | Used in the production of geranyl acetate in *n*-hexane | 55 |
| CALB,TLL& RML | MS SBA-15 NPs-epoxy activated | TLL was quite stable & highly reusable; RML & CALB had good reusability. | 57 |
| RML | MWCNTs-COOH & Gr-COOH | High stability & tolerance to organic solvents | 62 |
| CRL | o-MWCNTS | High binding by covalent method compared to adsorption method | 63 |
| PFL | $NH_2$-, alkyl-, OH- & COOH-MWCNTs | MWCNTs-$NH_2$ was most active & MWCNTs-COOH best enantioselective, high storage stability & reusability | 64 |

Mohammadi and coworkers [62] obtained high loaded preparation of RML on MWCNT-COOH and carboxylated graphene nanosheets (Gr-COOH) by using an isocyanide-based four-component reaction. In this approach the -$NH_2$ groups of the enzyme react with -COOH group of the supports and the reaction was done in water at 25$^{o}$C. The maximum loading capacity of 530 mg and 680 mg was obtained for Gr-COOH and MWCNT-COOH, respectively. The immobilized preparations showed

significantly enhanced heat and co-solvent stability compared to soluble enzyme. Prlainović et al. [63] adopted two approaches for immobilization of CRL on oxidized MWCNTs (o-MWCNTs). One method involved activating agents to promote covalent binding and the other illustrated simply adsorption of CRL on o-MWCNTs. o-MWCNTs adsorbed CRL retained 37 μg $mg^{-1}$, while the amount of enzyme was more than double of it in case of covalently immobilized lipase, 80 μg $mg^{-1}$ CNTs. In a further study *Pseudomonas fluorescens* lipase (PFL) was covalently attached to the functionalized-MWCNTs containing aminoalkyl-, OH- and COOH-groups, respectively. Amino- and epoxy-functionalized MP-silica (f-MP-SBA-15) were used as the reference support. Transesterification of vinyl *n*-butyrate by racemic Solketal with GC traced kinetics was selected as the model reaction. The studies revealed that different chemical functionalization of morphologically identical nanotube supports led to various enzyme loadings, catalytic activities and enantioselectivities. MWCNTs-$NH_2$-based nanobiocatalyst was found most active composite among all the examined systems (yield 20%, t = 0.5 h, 1321 U g−1), i.e.12-times more active than the free enzyme. Lipase immobilized on MWCNTs-COOH was found to be the best enantioselective system (ex aequo with SBA-$NH_2$) (eeR=74%, t=0.5 h at yield of 3–5%). MWCNT-$NH_2$-based nanobiocatalyst retained only 40% its original activity after 8 cycles of transesterification, whereas the activity of SBA-$NH_2$-bound enzyme remained unchanged. Moreover, the stability of all MWCNTs-bound lipase preparations were completely preserved over prolonged storage or even enhanced in case of enzyme immobilized on MWCNT-OH [64].

*Aspergillus niger* lipase (ANL) was immobilized with $SiO_2$NPs in sol-gel powders prepared via base-catalyzed polymerization of tetramethoxysilane and methyltrimethoxysilane. The immobilized lipase retained 92% of loaded protein and 94% of the initial activity. The bound enzyme exhibited significantly high heat and pH stability than its free form [65]. CRL was immobilized on fumed nano-silica (FNS), amino-FNS (AFNS) and cyanuric chloride-AFNS (CCAFNS). The amount of immobilized enzyme was increased with increasing enzyme concentration, achieving loadings of 121.3, 104.8 and 61.2 mg $g^{-1}$ on the FNS, CCAFNS and AFNS, respectively. Lipase immobilized on CCAFNS carrier in 0.1 M buffer expressed highest lipolytic activity, 1320 U $g^{-1}$ support, while more stable preparations were obtained in 1.0 M buffer. The immobilization was maintained by different kinds of interactions on FNS and AFNS and it was mainly due to adsorption, whereas the initial adsorption of lipase on CCAFNS favoured reorientation and amino groups of the enzyme formed a covalent bond with the chlorine atom of the modified carrier. Improved thermal and operational stability of lipase immobilized on CCAFNS led to the conclusion that electrostatic interactions have played a major role in the immobilization [66]. ANL was covalently immobilized onto PANI/Ag/GO via GA crosslinking. ANL@PANI/Ag/GO NCs showed activity recovery of 88.5% and immobilization yield of 94%. The NCs bound enzyme exhibited high optimum temperature and pH, greater catalytic efficiency and enzyme-substrate affinity. ANL@PANI/Ag/GO retained more than 86% of the activity after 11 repeated uses and demonstrated very high heat and solvent tolerance. The enhanced stability of the immobilized lipase at high temperatures and in various organic solvents revealed that this immobilized enzyme preparation has a lot scope in industrial application [67]. In a most recent study, Asmat and co-workers [68] investigated the immobilization of ROL on polypyrrole-methylanthranilate (Ppy-MA) $TiO_2$ NC via both physical adsorption and GA activated covalent binding. The covalently immobilized lipase showed remarkbly higher catalytic activity yield than the physically adsorbed enzyme. The obtained nanobiocatalysts showed improved solvent tolerance (150% and 125% activity recovery in acetone and isopropanol) and high heat, pH, and storage stability compared to its free form. Nano cellulose (NCe) fused Ppy/GrO NC was employed for efficacious immobilization of CRL via physical adsorption. The catalytic activity, stability against pH, heat, organic solvents and storage for CRL@NCe-PPy/GO were remarkably enhanced compared to soluble enzyme. CRL-NCe-PPy/GO NC retained 85% of its activity after 10 repeated uses. The nanobiocatayst was employed for the synthesis of flavour compound, ethyl acetoacetate. The immobilized lipase successfully synthesized flavour compound in solvent free media and *n*-hexane having 27.5% and 75.5% ester yields, respectively [Author unpublished work].

**2.3. Biosensor applications of NMs bound lipases.** The enzymes immobilized via nanocomposites have successfully been employed in various biosensors on their repeated uses. Recently, enzymes immobilized on electrodes via NC and their biosensors applications for the analysis of different compounds in clinical, environmental and food samples have been reviewed [69]. For TGs biosensor design, protein immobilization is necessary so as to create interface between enzyme and electrode. Narang and Pundir [70] demonstrated the construction of a novel amperometric TG biosensor based on covalent co-immobilization of lipase, glycerol kinase and glycerol-3-phosphate oxidase onto CS and ZnONPs composite film deposited on the surface of Pt electrode. The sensor showed optimum response within 6 s at pH 7.5 and 35°C. The sensor measured current due to electrons generated at 0.4V against Ag/AgCl from $H_2O_2$, which was produced from triolein by co-immobilized enzymes. A linear relationship between a wide triolein concentration range (50-650 mg $dl^{-1}$) and current (mA) under optimum conditions was obtained. The biosensor has demonstrated high sensitivity, low detection limit, 20 mg $dl^{-1}$ and good storage stability, $t_{1/2}$ of 7 months at 4°C. The biosensor was not influenced by serum substances at their physiological concentrations. The biosensor was successfully employed for the analysis of TG in sera in apparently healthy subjects and persons suffering from hypertriglyceridemia. In a further study, the same group has constructed an amperometric TG biosensor by coimmobilizing all three enzymes onto Au-Ppy-NCs decorated poly indole-5-carboxylic acid electrodeposited on the surface of an Au-electrode. Biosensor exhibited optimum response within 4 s at pH 6.5 and 35°C, when polarized at +0.1 V against Ag/AgCl. There was a linear relationship between sensor response and triolein concentration in the range 50-700 mg $dl^{-1}$. The biosensor

showed a detection limit of 20 mg $dl^{-1}$ and it was monitored with 91-95% recovery of added triolein in sera and 4.14 and 5.85% within and between batch coefficients of variation, respectively. There was a good correlation between sera TG values by standard method (enzymatic colorimetric) and the present method. The biosensor was also successfully used without any inhibition by serum components at physiological concentration. It was stored at 4°C for more than 7 months and retained above 50% activity after its 100 reuses [71]. Wu et al. [72] modified a glassy carbon electrode (GCE) with lipase-nanoporous gold (NPG) biocomposite, which was designated as lipase/NPG/GCE). Due to highly conductive, porous and biocompatible 3-D structure, NPG is suitable for enzyme immobilization. In cyclic voltammetry experiment, the lipase/NPG/GCE bioelectrode demonstrated surface-confined reaction in a phosphate buffer. Linear responses were obtained for tributyrin ranging from 50 to 250 mg $dl^{-1}$ and olive oil ranging from 10-200 mg $dl^{-1}$. The apparent $K_m$ for tributyrin was 10.67 mg $dl^{-1}$ and the detection limit was 2.68 mg $dl^{-1}$. The bioelectrode exhibited strong anti-interference ability against urea, glucose, cholesterol and uric acid as well as a long shelf-life. The values obtained by bioelectrode for the detection of TGs in human serum were in quite similar with those obtained by using an automatic analyzer. These properties along with a long shelf-life make this electrode an excellent choice for the construction of TGs biosensor.

## 2.2. MAGNETIC NANOSUPPORT BOUND LIPASES

### 2.2.1. Binding of lipases on simple and activated MNPs.

Table 3 summarizes various magnetic nanosupports bound lipases, their improved properties and applications. Several types of simple and activated MNPs have been used for high yield immobilization of lipases and provided them high stability [73]. CRL immobilized on γ-$Fe_2O_3$ MNPs showed very high activity and stability. The immobilization strategies were either involved enzyme amine groups to the NP surface acetyl or amine groups. In the first case, the enzyme was attached via C=N bond, while in the latter it was linked by GA [74]. Lipase was covalently attached to the $Fe_3O_4$ MNPs (12.7 nm) via CDI. The 100% binding efficiency of lipase was obtained when the weight ratio of lipase bound to $Fe_3O_4$ MNPs was below 0.033. The bound lipase exhibited 1.41-fold enhanced activity, 31-fold improved stability and better resistance to change in pH compared to native enzyme. The immobilized enzyme was stable and active for over 30 days. [75]. Maghemite NPs immobilized CRL exhibited high stereoselectivity in kinetic resolution of racemic carboxylates and improved long term stability compared to free enzyme. Immobilized lipase has been repeatedly employed for a series of chiral resolution reactions [76]. Lauric acid-stabilized MNPs was employed for the covalent immobilization of CRL via CDI activation. Resolution of (±)-menthol was performed by the immobilized lipase-catalyzed enantioselective esterification with propionic anhydride and as a result, (-)-menthyl propionate with a yield higher than 96% and over 88% enantiomeric excess of products was obtained. The immobilized lipase showed better conversion and enantioselectivity, when catalyzed reaction at 30°C for 2.5 h with 0.2 mol $l^{-1}$ of (±)-menthol. Hexane appeared as a best solvent, and the activity as well as enantioselectivity of the immobilized lipase decreased gradually with increasing water activity. Immobilized lipase exhibited good durability during resolution of (±)-menthol [77]. Lipase was covalently attached to the amine-functionalized MNPs using GA as a coupling reagent with the activity recovery of 70% and the enzyme binding efficiency of 84%. Moreover, the immobilized lipase efficiently catalyzed transesterification of soybean oil with methanol to produce FAME, biodiesel. The conversion of soybean oil to biodiesel fuels reached over 90% by the three-step addition of methanol when 60% immobilized lipase was used. In addition, it has revealed that the immobilized lipase retained its full activity even after 4-repeated uses [78]. Further, these workers covalently immobilized TLL onto magnetic $Fe_3O_4$ NPs by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) as an activating agent and the bound TLL catalyzed transesterification of vegetable oils with methanol to produce FAME. The immobilized lipase showed high resistance to heat and pH inactivation compared to its free form. Soybean oil was converted into FAME over 90% in a three-step transesterification process when 40% immobilized TLL was applied. Moreover, immobilized lipase was used 3 times without much loss in its activity [79]. Lipase was covalently immobilized via GA on a facile nano-sized magnetic carrier. The amount of enzyme loading and its activity recovery were 43.6 mg $g^{-1}$ support and 58.2%, respectively. The immobilized lipase demonstrated good heat stability and reusability [80]. The lipase-coated MNPs were applied in a reactive extraction process that allowed separation of products formed during transesterification. Kinetics data for triolein and ethanol consumption during biodiesel (ethyl oleate) synthesis together with a thermodynamic phase equilibrium model were used for simulation of batch and continuous processes. The analysis demonstrated the possibility of applying this biocatalytic system in the reactive zone using external magnetic fields. The immobilized lipase was efficiently used in the reactors for continuous production of biodiesel [81]. BCL was immobilized onto superparamagnetic NPs via adsorption and chemisorption procedures and was used in the kinetic resolution of (RS)-1-(phenyl)ethanols via transesterification reactions. (R)-esters and the remaining (S)-alcohols were obtained with excellent enantiomeric excess (>99%), which relates to an efficient process of enzymatic kinetic resolution (conversion 50%, E> 200). The transesterification reactions catalyzed by BCL immobilized by GA method showed best results in terms of reusability, maintaining the enzyme activity (conversion 50%, E> 200) after 8-repeated uses [82]. CRL was immobilized on magnetite NPs, size of 15±5 nm with an average surface area of 112.15 $m^2$ $g^{-1}$. The activation energy was 1.9-fold lower for the immobilized enzyme for hydrolytic reactions compared to native lipase. The immobilized lipase showed lower $K_m$ and higher $V_{max}$ values, thus it exhibited high catalytic efficiency of the immobilized lipase. The immobilized lipase demonstrated higher esterification efficiency

during synthesis of ethyl isovalerate than the soluble enzyme. Immobilized lipase retained 91.5% esterification efficiency for ethyl isovalerate synthesis even after 10 reuses [83]. In order to avoid chemical modification of enzyme and hence structural and functional deterioration, lipase has been immobilized simply by adsorption onto MNPs via electrostatic interaction [84]. CRL was covalently immobilized onto GA coated MNPs (13 nm). The immobilized enzyme showed good esterification of oleic acid with butyl alcohol [85]. PFLp1 was immobilized onto amino silane modified superparamagnetic MNPs. The mixture of biodiesel was produced from waste cooking oil (WCO) in the presence of methanol by using magnetite immobilized PFLp1. The obtained biodiesel was the mixture of methyl esters; oxiraneundecanoic acid, 3-pentyl-methyl ester, hexadecanoic acid methyl ester, 10-octadecenoic acid methyl ester [86].

Lipase immobilized on magnetic support has been used in the synthesis of fine chemicals, i.e., precursors for drugs [87-89]. Lipase was efficiently immobilized onto super paramagnetic MNPs surface-modified with gluconic acid. The immobilized lipase was found highly stable against the denaturation mediated by change in pH and temperature and on prolonged storage. This bound enzyme maintained significantly high activity even after several repeated uses and was easily recollected magnetically [90]. MNPs loaded MWCNTs NCs was used for the immobilization of lipase. Magnetic MWCNT bound lipase demonstrated significantly improved resolution of (R,S)-1-phenyl ethanol in heptane. The catalytic activity of bound lipase was not changed even after 30 min sonication, while the catalytic activity of free lipase was remarkably decreased during similar treatment [91].

**Table 3.** List of various MNPs support bound lipases, their mode of immobilization and improved properties.

| Name of enzyme | Name of support | Mode of immobilization | Property/properties Enhanced | Reference |
|---|---|---|---|---|
| CRL | γ-$Fe_2O_3$ MNPs, GA | Covalent binding | Enhanced heat & storage stability | 74 |
| Lipase | $Fe_3O_4$ (12.7 nm), CDI | Covalent binding | Enhanced activity, several folds improved stability, high resistance to pH changes, high storage stability | 75 |
| CRL | Maghemite NP | Adsorption | High storage stability, high stereoselectivity in kinetic resolution of racemic carboxylates | 76 |
| CRL | Lauric acid- MNPs, CDI | Covalent binding | Very high enantioselectivity, High stability during resolution of (±)-menthol | 77 |
| Lipase, Tll | $NH_2$-MNPs, GA; $Fe_3O_4$ NPs, EDAC | Covalent Binding | High transesterification of vegetable oils with methanol to biodiesel, high resistance to heat & pH, high reusability | 78, 79 |
| Lipase | Nanosized magnetic support | Covalent binding | Good heat stability & reusability | 80 |
| Lipase | MNPs | Adsorption | Efficient transesterification of triolein with $C_2H_5OH$ into biodiesel in batch process & column reactors | 81 |
| BCL | Superparamagnetic NPs, | Adsorption & chemisorptions Covalent binding | Good reusability, High resolution of (RS)-1-(phenyl)ethanols via transesterification, Covalenly immobilized BCL showed better performance | 82 |
| CRL | Magnetite NPs (15±5 nm) | Adsorption | Higher esterification efficiency during synthesis of ethyl isovalerate, lower $K_m$ & high $V_{vax,}$ high reusability during ethyl isovalerate synthesis | 83 |
| CRL | GA-MNPs (13 nm) | Covalent binding | High esterification of oleic acid with butyl alcohol | 85 |
| PFLP1 | $NH_2$ -superparamagnetic MNPs | Adsorption | Biodiesel production from WCO & methanol | 86 |
| Lipase | Super paramagnetic MNPs-gluconic acid | Adsorption | Highly stable against the change in pH & temperature & on storage, maintained significantly high reusability | 90 |
| Lipase | MNPs-MWCNTs NC | Adsorption | High resolution of (R, S)-1-phenyl ethanol in heptanes, No change in ativity after 30 min sonication | 91 |
| PCL | MNPs | Adsorption | High biodiesel production from WCO & methanol. | 92 |
| TLL, CALB | Core-shell structured MNPs 80 nm | Covalent binding | Biodiesel production from grease with methanol, very high reusability | 94 |
| CRL | $NH_2$/COOH activated $Fe_3O_4$ clusters | Covalent binding | Very high thermal stability & reusability | 95,96 |
| Lipase | $Fe_3O_4$NPs, EDAC & Sulfo-NHS | Covalent binding | Remarkably high heat stability and reusability. | 97 |
| CALB | Carbon coated cobalt NPs | Covalent binding | Excellent stability & reusability | 100 |
| Lipase | Core-shell super-paramagnetic NPs | Adsorption | Good reusability, pharmaceutically important chiral isomers from meso-cyclopent-2-en-1,4-diacetate | 101 |
| MJL | $NH_2$-MNPs | Covalent binding | Specific activity increased to 10-fold retained 90% activity after 10 reuses. 1,3-DAGs of lauric, myristic, palmitic, stearic, oleic & linoleic acid were efficiently synthesized | 102 |
| Lipase | 3-APTES- MNPs, GA | Covalent binding | Higher synthesis of ethyl acetate & transesterification of vegetable oil into biodiesel, remarkably very high shelf life | 103 |
| Lipase | $NH_2$- $Fe_3O_4$@C NPs | Adsorption | High enzyme loading, heat stability and reusability | 104 |
| TLL | DA-functionalized $Fe_3O_4$ NPs-oleic acid | Covalent binding | High immobilization efficiency, yield & enzyme loading, High reusability & improved heat stability | 106 |
| ANL | $Fe_3O_4$ NPs | Adsorption | Higher synthesis of benzothiazepine & spirobenzothiazine | 109 |

**Nanosupport bound lipases their stability and applications**

| Name of enzyme | Name of support | Mode of immobili-zation | Property/properties Enhanced | Refere nce |
|---|---|---|---|---|
| | | | chroman derivatives via three-component reaction of coumarine-3-carboxylic acid derivatives, 2-aminothiophenol & alkyl isocyanides | |

Yu et al. [92] produced biodiesel from WCO by PCL immobilized onto MNPs. The optimal dosage of lipase-bound MNP was 40% (w/w) of oil and there was little difference between stepwise addition of methanol at the intervals of 12 h and 24 h. RSM optimized various reaction parameters such as temperature 44.2°C, substrate molar ratio (methanol/oil) of 5.2, and water content of 12.5% (w/w) of oil. The predicted and experimental molar conversions of FAME were 80% and 79%, respectively. TLL was covalently attached to the MNPs and hydroxylated nonporous glass beads via GA. The yield and efficiency for immobilized lipase on MNPs were $72 \pm 2.4$ and $63 \pm 3.5\%$, whereas yield and efficiency for TLL immobilized on glass beads were $60 \pm 2.1$ and $55 \pm 4.1\%$, respectively. Immobilized TLL was compared with six other available lipases for regioselective acetylation of prednisolone. The highest and lowest yields of the product were reported for Novozym 435 and glass beads immobilized TLL, respectively. Immobilized lipase retained its full bioacetylation activity of prednisolone after 5 repeated uses [93]. TLL and CALB were covalently immobilized on core-shell structured MNPs of 80 nm size, respectively, followed by freeze-dry to give magnetic nanobiocatalyst aggregates (MNA) with high yield (80-89%) and enzyme loading (61 mg TLL or 22 mg CALB $g^{-1}$ MNA). MNA-TLL among the lipases studied gave best yield of biodiesel, 99% yield in 12 h. MNA-TLL was easily separated by applying magnetic field and was reused by maintaining 88% activity after 11 repeated uses. MNA CALB was also successfully converted above 97% FFA in grease (17 wt. % FFA) to FAME in 12 h. Herein results showed that the green and efficient production of biodiesel from waste grease containing high amount of FFA has been successfully achieved using MNA bound lipases [94]. A highly water-dispersible size-tunable magnetite $Fe_3O_4$ nanocrystal clusters end-functionalized with amine or carboxyl groups were employed for the immobilization of CRL. The immobilized lipase exhibited remarkably very high heat stability and reusability compared to native enzyme. This novel support bound enzyme had shown its potential in organocatalysis [95, 96].

Lipases were covalently attached to the $Fe_3O_4$ NPs via EDAC and Sulfo-NHS activated coupling to synthesize magnetically responsive lipases. The immobilized lipase showed high heat stability and reusability [97]. CALB was immobilized directly and as a crosslinked aggregate of enzyme (CLEA) on the magnetic particles. The hydrolysis of TGs in aqueous media and the transesterification reactions to synthesize biodiesel and biosurfactants in organic solvents were performed by CLEA immobilized on magnetic support [98]. Talbert et al. [99] demonstrated the use of a hierarchical assembly immobilization strategy to enhance physicochemical properties and stability of CALB. CALB was complexed with MNPs followed by interfacial assembly at the surface of an oil-in-water emulsion. Immobilized lipase exhibited enhanced heat and pH stability, providing 72% activity retention after 24 h at 50°C, pH 7.0 and 62% activity retention after 24 h at pH 3.0, 30°C; conditions resulting in complete deactivation of free enzyme. Lipase B was covalently immobilized on the carbon coated cobalt magnetic NPs. Conjugated enzyme showed good activity and stability and was efficiently recycled within very short times from milliliter to liter scales [100]. Lipase immobilized on a novel high surface area core-shell superparamagnetic NPs and used as an efficient reusable catalysts for the selective production of pharmaceutically important chiral isomers from meso-cyclopent-2-en-1,4-diacetate [101]. Zhuang et al. [102] described the covalent immobilization of *Mucor javanicus* lipase (MJL) on amine-group-activated MNPs and used this preparation for the synthesis of 1,3-DAGs. The specific activity of lipase was increased to 10-folds after immobilization and the immobilized enzyme retained 90% activity at 55°C after 10 repeated uses. 1,3-DAGs of lauric, myristic, palmitic, stearic, oleic and linoleic acid were efficiently obtained by employing immobilized enzyme, all with yields greater than 90% and the reaction time was also significantly minimized. Lipase from Pseudozyma sp. NII 08165 was covalently coupled to 3-APTES functionalized MNPs via GA and was employed for biotransformation reactions including synthesis of ethyl acetate and transesterification of vegetable oil into biodiesel. MNP immobilized lipase showed long shelf life without any loss in its activity [103]. Lipase was immobilized on the amine-functionalized $Fe_3O_4$@C NPs and the amount of enzyme loading was about 115.6 mg $g^{-1}$ support with the retention of 93% of the initial activity. The heat stability of the immobilized lipase was remarkably very high and it retained over 68% activity after 10 reuses [104]. Haritha et al. [105] investigated the immobilization of PPL onto MNPs by co-precipitation method via ECH-CS cross-linking, under mild and eco-friendly conditions. The immobilized lipase was exploited for the synthesis of organic disulphides. The immobilized lipase exhibited broad range of substrate specificity in synthesizing organic disulphides, which involved both intra and inter-molecular disulphide bond formation under anaerobic conditions. The synthesized disulphide compounds also exhibited a promising antimicrobial activity.

A nanocarrier, dopamine (DA) functionalized $Fe_3O_4$ NPs coated with oleic acid (OA), was simply used for the immobilization of TLL via covalent binding. A high immobilization efficiency of 88% and yield of 97% with enzyme loading of 13.09 mg $g^{-1}$ support was obtained under optimal experimental conditions in a buffer of pH 7.0, enzyme concentration at 0.27 mg $mL^{-1}$ and reaction time of 10 h. The heat stability of immobilized TLL was improved by 2.9-fold at 60°C than the soluble enzyme. The immobilized lipase retained more than 75% of the initial activity after 10-reuses. The $V_{max}$ and $K_m$ of the native and immobilized lipase were determined as 2.51 mmol $min^{-1}$, 2.13 mmol $min^{-1}$ and 8.86 mM, 10.50 mM, respectively. The finding of the work showed that DA functionalized $Fe_3O_4$ NPs coated with OA appeared an effective support for lipase immobilization [106]. $Fe_3O_4$ MNPs functionalized by 3-APTES

and (3-mercaptopropyl) trimethoxysilane (MPTMS) have been employed as supports for covalent immobilization of ANL NS81006 using GA. A biodiesel yield of 89% and 81% was obtained by lipase immobilized on APTES-$Fe_3O_4$ and MPTMS-$Fe_3O_4$ MNPs, respectively under optimized conditions of oil to methanol molar ratio 1:3 with three steps addition of methanol, at 45°C for 12 h. These immobilized ANL preparations were easy to recollect magnetically after the reaction [107]. CRL7 and TLL were immobilized on the MNPs functionalized for thiol and urea using MPTMS and 1-(3-trimethoxysilyl propyl) urea (TMSPU) and employed these preparations for transesterification reactions. The loading capacities of CRL7 and TLL on MNPs-TMSPU were 410 and 440 mg $g^{-1}$ MNPs, 5.9 and 5.5 times higher than those on the non-functionalized MNPs, respectively. The initial activities of both CRL7 and TLL on MNPs-TMSPU were higher than those on MNPs-MPTMS. MNPs-TMSPU-TLL retained 2.5 times more activity after 30 min incubation at 80°C compared to free enzyme. The remaining activities of the biocomposites assayed after each use in transesterification reaction demonstrated that TLL-MNPs-TMSPU biocomposite has a high yield, 92%. The immobilized TLL on MNPs-TMSPU and MPTMS retained 80% and 70% of their initial activities after 8 repeated uses, respectively [108]. ANL was attached to $Fe_3O_4$ NPs. The obtained nanostructure ANL was exploited for the synthesis of benzothiazepine and spirobenzothiazine chroman derivatives via three-component reaction of coumarine-3-carboxylic acid derivatives, 2-aminothiophenol and alkyl isocyanides at room temperature [109]. In a further study, ANL was immobilized onto magnetic barium ferrite NPs and used for biodiesel production. The results illustrated that immobilized lipase retained 83% biodiesel production activity after 5 reuses. The maximum biodiesel production from WCO by immobilized lipase was obtained at 45°C, 4 h and 400 rpm [110].

**2.2.2. Lipase bound to CS-MNPs NCs.** Table 4 lists different CS-MNPs NCs immobilized lipases, their improved properties and applications. Wu et al. [111] have studied immobilization of lipase onto magnetic $Fe_3O_4$-CS NPs. The adsorption capacity of lipase was 129 mg $g^{-1}$ and the maximal enzyme activity was 20.02 µmol $min^{-1}$ $mg^{-1}$ proteins. The activity retention was 55.6% at a certain loading amount. The $Fe_3O_4$-CS NPs was taken for the covalent immobilization of CRL using EDAC and NHS as coupling agents. The RSM and ridge max analysis optimized immobilization conditions; the reaction time 2.14 h, pH 6.37, and enzyme/support ratio 0.73 (w/w); the highest activity obtained was 20 U $g^{-1}$ $Fe_3O_4$-CS. The immobilized lipase retained over 83% of its activity after 20 repeated uses and showed better operational stability, including wider thermal and pH ranges, and remained stable for over 13 days of storage at 25°C [112]. CRL was immobilized on superparamagnetic alginate CSNS via adsorption and covalent attachment. Magnetic alginate/CSNS was activated by oxidic (PEG) in order to create aldehyde groups for covalent binding. Covalently bound lipase exhibited superior stability compared to physically adsorbed enzyme [113]. In a further study the layered assemblies of CRL onto the magnetic alginate/CSNSs via both electrostatic adsorption and covalent binding were obtained. The coating of lipase was made by alternative layers of alginate and CS. Covalently immobilized CRL was remarkably more stable than the physically adsorbed enzyme. Additional layering of immobilized lipase with covering layers showed an enhancement in enzyme stability [114].

**Table 4.** List of various CS-MNPs support bound lipases, their mode of immobilization and improved properties.

| Name of enzyme | Name of support | Mode of immobilization | Property/properties Enhanced | Reference |
|---|---|---|---|---|
| Lipase | Magnetic $Fe_3O_4$-CS NPs | Adsorption | Good reusability & stability | 111 |
| CRL | $Fe_3O_4$-CS NPs, EDAC & NHS | Covalent binding | Very binding, pH, heat, operational and storage stability & high reusability | 112 |
| CRL | superparamagnetic alginate CSNSs | Adsorption, Covalent binding | High enzyme loading, reusability & stability | 113,114 |
| ROL | Functionalized magnetic $Fe_3O_4$-CS beads | Covalent binding | High immobilization yield, & reusability & stability during esterification of phenolic acid in isooctane | 116 |
| CRL | $Fe_3O_4$ MNPs-CS NPs & GA | Covalent binding | Good storage and operational stability & high reusability, synthesis of hexyl and butyl oleate in *n*-hexane | 118 |
| CALB | $Fe_3O_4$ MNPs-CS NPs & GA | Covalent binding | Rapid synthesis of ethyl oleate, high reusability | 119 |
| BCL | CS-MNPs | Adsorption | Improved relative activity & stability | 120 |
| CRL | MNPs-GO-CS | Adsorption | Excellent immobilization capacity | 122 |
| TLL | $Fe_3O_4$ @-CS NPs | Entrapment, Covalent binding | High pH & heat stability & reusability, higher synthesis of ascorbyl palmitate in *t*-butanol | 121 |
| ANL | CS-MNPs & GA & glycidol | Covalent binding | Enhanced pH & heat stability, high storage stability & reusability, low $K_m$ | 123 |
| CRL | Collagen, CS & CS-collagen-MNPs, GA & squaric acid | Covalent binding | High activity & reusability | 124 |
| CRL | CS encapsulated MNPs, GA & genipin | Covalent binding | High pH, heat, storage & operational stability & reusability | 125 |

CS–poly [*N*–benzyl–2-(methacryloxy)–*N*,*N*–dimethyl-ethanaminium bromide] coated MNPs was obtained by co-precipitation via epichlorohydrin-CS cross-linking and this magnetic NC was used to immobilize lipase via EDAC/sulfo-NHS. The immobilized lipase was successfully used to produce (*S*)-methyl ester of ibuprofen with high enantioselectivity

(*E*=50.6). The chiral compounds that obtained by the application of MNPs were analyzed using chiral stationary phases. Moreover, magnetic supports immobilized lipase maintained very high

enantioselectivity after repeated uses [115]. ROL was covalently immobilized onto functionalized magnetic $Fe_3O_4$-CS beads and 86.60% of lipase was bound to the support. CS-beads bound lipase was successfully used for the esterification of phenolic acid which resulted in esterification of phenolic acid in isooctane solvent reaction system for 8 recycles (totally 384 h), 72.6% of its initial activity was retained, demonstrating its high stability in pharmaceutical and industrial applications [116]. El-Hadi et al. [117] immobilized *Mucor racemosus* NRRL 3631 lipase on magnetic $Fe_3O_4$-CS NPs and the obtained immobilization efficiency was 95.6%. The immobilized lipase demonstrated better pH, heat and operational stability. The pH optimum of the immobilized enzyme shifted towards acidic side and was stable in the pH range of 3-5 as compared to free form pH 5.6. Lipase immobilized by $Fe_3O_4$-CS NPs remained fully active up to 40°C. The immobilized enzyme maintained 72.9% activity after 1 h incubation at 60°C whereas its free form retained only 40% activity under similar exposure. The values of $V_{max}$ and $K_m$ for immobilized lipase were 250 U $mg^{-1}$ and 20 mmol $l^{-1}$, respectively. The immobilized lipase exhibited better resistance to pH and heat inactivation compared to free enzyme and it retained over 63.5% activity even after 8 repeated uses. CRL was immobilized on CS coated and GA functionalized $Fe_3O_4$MNPs with loading capacity of 87 mg $g^{-1}$ under optimal conditions. The immobilized enzyme illustrated good operational and storage stability and it remained stable over 30 days of storage at 4°C. $Fe_3O_4$MNPs bound CRL retained about 61% of its activity after 20 repeated uses. Finally enzymatic synthesis of butyl and hexyl oleate at 40°C with shaking, 200 rpm was realized in *n*-hexane and confirmed by GC analysis [118]. CALB was successfully immobilized on magnetite $Fe_3O_4$ MNPs functionalized with CS and GA. The obtained magnetic catalyst performance was evaluated in solvent-free synthesis of ethyl oleate at room temperature. The performance of this biocatalyst was compared to commercial Novozym 435. It was found that using 33 mg of the biocatalyst it was possible to reach almost the same activity that was obtained using 12 mg of Novozym 435. Furthermore, this new biocatalyst presents the advantages of not being degraded by short alcohols, being easily recovered from the reaction media using magnetic decantation and low fabrication cost. The immobilized enzyme maintained a significant activity up to 8-reuses [119].

Ghadi and coworkers [120] studied immobilization of BCL on CS magnetic core-shell NPs (CSMNPs). The immobilized lipase retained its activity, 32 U $mg^{-1}$ CSMNPs at optimum pH and temperature. CSMNPs were used in transesterification reaction in order to evaluate the activity of immobilized enzyme compared to free enzyme. Immobilization of BCL on CSMNPs improved stability and total relative activity of the enzyme. $Fe_3O_4$@CS NPs support was used for covalent immobilization of TLL by chemical conjugation after electrostatic entrapment. The immobilization efficiency and protein loading under optimal conditions were 75% and 16.8 mg $g^{-1}$ support, respectively. Immobilized TLL showed high pH and heat stability and maintained 70% activity after 10-reuses. Ascorbyl palmitate synthesis by immobilized lipase was performed in *t*-butanol at 50°C, and the conversion of ascorbic acid was higher than 50% [121]. Magnetic GO-CS NCs immobilized CRL showed excellent immobilization capacities and high stability [122]. Osuna et al. [123] studied covalent immobilization of ANL on CS-coated MNPs (CS-MNP) via GA and glycidol. The derivatives showed activities of 309.5 ± 2.0 and 266.2 ± 2.8 U $g^{-1}$ CS-MNPs treated by GA and glycidol, respectively. Immobilization enhanced the lipase stability at different pH and temperatures. The obtained $K_m$ of enzyme immobilized via GA was 1.7 times lower than the native lipase. The immobilized lipase showed remarkably high stability over 15 days storage and maintained over 80% of its hydrolytic activity after 15 reuses. Thus this immobilized enzyme preparation demonstrated a great potential in various biotechnological applications. The immobilization of CRL was done on the surface of collagen, CS and CS-collagen coated MNPs and these preparations were crosslinked with GA and squaric acid. All of lipase-biopolymers coated NPs were characterized with good activity recovery. A marginal hyperactivation of lipase immobilized on NPs with squaric acid was noticed. All NPs-immobilized lipase preparations exhibited very activity on repeated uses [124]. Liu et al. [125] described immobilization of CRL on CS encapsulated MNPs (CSMNPs) using two different crosslinkers, genipin and GA. The genipin-CSMNPs-CRL showed its optima at pH 8.0 and 40°C and retained more than 95% of its activity after 7 days storage at 25°C. Genipin-CSMNPs-CRL maintained over 80% activity after 5-repeated uses while the GA-CSMNPs-CRL showed only 26% of its initial activity. Both CRL immobilized preparations preserved higher substrate affinity than the free form. FT-IR analysis showed that the variance of β-sheet element in the secondary structure of CRL might contribute to the stability and activity enhancement of genipin-CSMNPs-CRL. Genipin-CSMNPs-CRL showed higher pH, heat, storage and operational stabilities than GA-CSMNPs-CRL. From the findings of this work, it has been concluded that genipin is a safer cross-linker than GA for lipase immobilization on CSMNPs.

**2.2.3. Lipase bound to silica-MNPs NCs.** Table 5 depicts silica-MNPs NCs immobilized lipases, their improved properties and applications. Numerous kinds of silica-MNPs NCs have been prepared for their various applications. These NCs have also been found quite successful in high yield immobilization and in enahancing the stability of enzymes. Kim et al. [126] immobilized *Bacillus stearothermopilus* L1 lipase (BSL) via His tagged onto magnetic particles coated with silica and charged with $Cu^{2+}$ ions via a multidentate ligand, IDA. The specific activity of immobilized enzyme was observed in the following orders: $Cu^{2+}$-charged silica-MNP>silica-MNP>$Cu^{2+}$-charged silica gel>silica gel. BSL immobilized onto $Cu^{2+}$-charged silica-MNPs preserved nearly 70% activity after 5 -repeated uses while the silica gel immobilized enzyme lost its full activity just after 3-repeated uses. The immobilization of CRL on MNPs supported ionic liquids containing varying cation chain lengths ($C_1$, $C_4$ and $C_8$) and anions ($Cl^-$, $BF_4^-$ and $PF_6^-$) was performed. MNPs supported ionic liquids were obtained by covalent bonding of ionic liquids-silane on silica-MNPs. The immobilized enzyme exhibited 118.3% activity and retained 60% activity at 80°C. In addition, the bound lipase maintained about 60% activity after 8-repeated uses [127]. Burkholderia sp. C20 strain lipase was immobilized on $Fe_3O_4$ core with silica shell. The NPs treated by dimethyl octadecyl [3-(trimethoxysilyl) propyl] $NH_4Cl$ were used as a support for lipase immobilization.

**Table 5.** Silica-MNPs support bound lipases, their mode of immobilization and improved properties.

| Name of enzyme | Name of support | Mode of immobilization | Property/properties Enhanced | Reference |
|---|---|---|---|---|
| BSL | $Cu^{2+}$-charged silica-MNPs | Bioaffinity binding | Enhanced reusability | 126 |
| PCL | $NH_2$-nonporous & MS silica-magnetite cluster NCs, GA | Covalent binding | High transesterification of soybean oil in $CH_3OH$, into biodiesel. high stability & reusabilitys | 131 |
| Recombinant TTL | 3-APTES-modified $Fe_3O_4$@$SiO_2$ MNPs | Covalent binding | High resistance to heat, pH, metal ions, inhibitors & detergents; good reusability, low $K_m$ | 130 |
| CRL | Silica-based β-CD - MNPs | Entrapment | Very high conversion & enantioselectivity | 134 |
| Lipase | $Fe_3O_4$@$SiO_2$ NPs | Covalent binding | Higher relative activity, better stability, broader pH range & high reusability | 133 |
| PPL | COOH- $SiO_2$-MNPs | Covalent binding | Enhanced heat stability | 135 |
| TLL | Succinated PEI grafted on silica coated $Fe_3O_4$ MNPs, GA, HMD | Adsorption/ covalent binding | Good reusability & high stability in organic solvents | 136 |
| CRL | Magnetic silica aerogel | Adsorption | High pH & heat stability, Very high adsorption capacity & activity | 137 |
| Lipase | $Fe_3O_4$@$SiO_2$MNPs in organic solvents | Adsorption | Easy recovery, high stability & enhanced activity in organic solvents Very high reusability, immobilization yield of 97% & relative activity of 124% | 138 |
| CRL | Magnetite NPs coated with the MCM-41 silica | Adsorption | Better catalytic activity towards interesterification of lard and soybean oil | 139 |
| Lipase | Magnetically separable (PANI-MS@$Fe_3O_4$) NCs | Adsorption | The Lower $K_m$ & higher $V_{max}$ & higher affinity towards substrate, high reusability | 140 |
| CRL | $Fe_3O_4$@$SiO_2$ NPs dip-coated-NCM via a low temperature, 3-APTES. GA | Covalent binding | Improved relative activity & loading capacity, decreased $K_m$ and $V_{max,}$ Very high heat, storage, & operational stability | 144 |
| CALB | Silica-MNPs-glycidyloxypropyl trimethoxysilane | Covalent binding | Maintained 100% at 55°C significant improvement in heat stability & methanol tolerance | 142 |
| Lipase | PAA-coated $Fe_3O_4$ cluster@$SiO_2$ NC | Covalent binding | Excellent performance in a broad range of temperature & pH; high heat & storage stability, & reusability | 141 |
| CRL | $Fe_3O_4$@$SiO_2$/PAMAM magnetic nanocarriers | Adsorption | Improved tolerance towards wider ranges of pH and temperature. | 143 |
| Lipase NS81006 | APTES & MPTMS-$Fe_3O_4$@$SiO_2$ core magnetic NCs, GA | Covalent binding | Maximum activity recovery (84% by APTES, 83% by MPTMS) & biodiesel yield (>90%) | 145 |

The protein binding efficiency on alkyl-functionalized $Fe_3O_4$-$SiO_2$ was determined as 97%, while the efficiency was only 76% on non-modified $Fe_3O_4$-$SiO_2$. The optimum adsorption capacity of lipase on alkyl-functionalized $Fe_3O_4$-$SiO_2$ was measured 29.45 mg $g^{-1}$ based on Langmuir isotherm. The hydrolytic kinetics of the immobilized lipase followed Michaelis-Menten model with $V_{max}$ and $K_m$ values of 6251 U $g^{-1}$ and 3.65 mM, respectively. Moreover, the immobilized lipase (11 wt%) efficiently catalyzed transesterification of olive oil with methanol to produce FAME, which showed a substrate conversion of about 90% within 30 h in a batch process. The immobilized lipase was reused for 10-times without any loss in its transesterification activity [128]. Karimi et al. [129] immobilized lipase onto MP-$SiO_2$-superparamagnetic $Fe_3O_4$ core-shell NPs. The effects of $CH_3OH$ ratio to WCO, lipase concentration, water content and reaction time on the synthesis of biodiesel were analyzed by utilizing RSM. A quadratic response surface equation for calculating FAME content as the objective function was described by experimental data obtained in accordance with the central composite design. Hybrid RSM-genetic algorithm predicted the maximum FAME content, 91% at the optimum level of medium variables: methanol ratio to WCO 4.34, lipase content 43.6%, water content 10.22% and reaction time 6 h. Moreover, the immobilized lipase retained its full activity even after 4-repeated uses. A recombinant thermostable lipase from *Thermus thermophilus* WL expressed in *E. coli* was immobilized onto 3-APTES-modified $Fe_3O_4$@$SiO_2$ supermagnetic NPs. As the immobilized lipase expressing very low $K_m$ than its free form, it demonstrated improved affinity of the enzyme for its substrate. The immobilized enzyme exhibited remarkably high stability against the inactivation mediated by heat, pH, metal ions, inhibitors and detergents compared to native lipase and retained over 79.5% of its original activity after 10 reuses [130]. PCL was covalently attached to the amine-functionalized nonporous and MP-silica-coated magnetite cluster NCs via GA. The immobilized lipase was employed as a recoverable biocatalyst in a transesterification reaction to convert soybean oil in methanol into biodiesel. Enzyme immobilization has provided higher stabilities and conversion values as compared to free enzyme. Moreover, the silica structure put a remarkable effect on stability and catalytic activity of immobilized enzymes. The immobilized lipase retained about 55% of their initial conversion capability after 5 reuses [131]. Superparamagnetic $Fe_3O_4$/SiOx NPs or $Fe_3O_4$-APTES NPs were used for the immobilization of neutrophil lipase (NL) directly via adsorption or by covalent coupling with GA. The heat stability and reusability of the enzyme immobilized by covalent binding was remarkably superior compared to physically adsorbed lipase [132].

Liu et al. [133] immobilized lipase on $Fe_3O_4$@$SiO_2$ NPs. The immobilized enzyme illustrated significantly high stability at

elevated temperatures and the relative activity of immobilized enzyme was 5.8 fold higher than the soluble lipase at 70°C after 10 h incubation. $Fe_3O_4$@$SiO_2$ NPs bound lipase retained higher activity in the range of pH 7.0-9.5. The immobilized enzyme has advantages like higher relative activity, better stability, broader pH range and easy recovery. It proved that $Fe_3O_4$@$SiO_2$ NPs immobilized enzyme has a great future at commercial level. A macrocyclic compound with magnetic property was prepared by immobilizing silica-based β-cyclodextrin (β-CD) on MNPs and β-CD-grafted MNPs were encapsulated along with CRL in sol-gel matrices using alkoxysilane precursors. The encapsulated lipase showed very high conversion and enantioselectivity that has an ee value of S-naproxen acid of nearly 98% [134].

A carboxyl functionalized silica-coated MNPs was employed for the immobilization of PPL via EDAC/NHS coupling reaction. The obtained $K_m$ and $V_{max}$ for immobilized enzyme were 0.02 mM and 6.40 U·$mg^{-1}$ enzyme, respectively. Immobilized PPL showed enhanced enzyme activity, reusability, heat and storage stability compared to its native form. Moreover, the immobilized enzyme maintained 60% of its original activity at 70°C. PPL-MNPs NCs was tested for enzyme inhibition assays by taking orlistat and two natural products obtained from oolong tea [135]. Magnetic nanospheres containing PEI and succinated PEI grafted on silica coated $Fe_3O_4$ were used for the adsorption or covalent attachment of TLL via GA or hexamethylene diisocyanate (HMD). MNPs@PEI-GA-lipase retained 80% of its activity after 12 repeated uses and employed for the synthesis of ethyl valerate. The immobilized lipase when incubated in *n*-hexane and solvent free media for 24 h maintained 72.9% and 28.9%, esterification activity, respectively [136]. A nanomagnetic silica aerogel support was used for CRL immobilization via adsorption. The influence of the sonication amplitude and time in lipase immobilization yield and activity were studied using RSM. Comparison between the performance dispersed and non-dispersed supports revealed the positive effect of dispersion process on immobilization yield and enzyme activity. Maximum adsorption capacity of CRL was 81.9 mg $g^{-1}$ based on Langmuir isotherm [137]. Shi et al. [138] carried out adsorption of lipase onto $Fe_3O_4$@$SiO_2$ MNPs in various organic solvents. Toluene appeared as most appropriate solvent for the immobilization of lipase among the solvents considered for this study. A maximum immobilization yield, 97% and relative activity, 124% were obtained in toluene at 30°C. The optimal temperature, enzyme loading and water activity were 30°C, 1.25 mg $mg^{-1}$ support and 0.48 aw, respectively. The immobilized lipase retained 67% activity after 10-repeated uses.

The magnetite NPs coated MCM-41 silica core-shell structured material was successfully used for the immobilization of CRL. The catalytic efficiency of the bound lipase was applied in the interesterification of lard and soybean oil. The immobilized lipase exhibited better catalytic activity towards interesterification reaction. The slip melting point of the final product was lower than that of the original blend, and the interesterification led to the clear variation in the microstructure of the product [139]. Magnetic PANI-MP-$SiO_2$-@$Fe_3O_4$ was employed for the immobilization of lipase via electrostatic adsorption. The BET surface area was calculated as 779.27 $m^2$ $g^{-1}$, 425 $m^2$ $g^{-1}$, and 230.45 $m^2$ $g^{-1}$ for magnetic MP-$SiO_2$-@$Fe_3O_4$, PANI-MP-$SiO_2$-@$Fe_3O_4$ NC, and lipase immobilized PANI-MP-$SiO_2$-@$Fe_3O_4$ NC, respectively. The immobilized lipase exhibited slightly higher optimal pH and temperature with a wider pH-activity and temperature stability profiles than the soluble enzyme. The lower $K_m$ (0.25 mM) and higher $V_{max}$ (0.0341 mM $min^{-1}$) for immobilized lipase revealed the higher affinity of immobilized lipase towards its substrate. The bound lipase retained nearly 70% of its initial activity after 7-repeated uses [140]. Esmaeilnejad-Ahranjani et al. [141] described immobilization of lipase onto core-shell structured PAA-coated $Fe_3O_4$ cluster@$SiO_2$ NCs. Low- or high-molecular-weight (1800 and 100 000) PAA molecules were covalently attached onto surface of amine-functionalized magnetic silica NCs. Once lipase was covalently immobilized onto NPs with an average diameter of 210 ± 50 nm, resulting from high binding sites concentrations on the low- and high-molecular-weight PAA-coated NPs, high lipase immobilization efficiencies of 86.2% and 89.9%, respectively and loading capacities 786 and 816 mg $g^{-1}$, respectively were obtained. The lipases immobilized onto low- and high-molecular-weight PAA-coated particles exhibited maximum activities at 55°C and 50°C, respectively, which were ∼28% and ∼15% higher than the free lipase at 40°C, respectively. The immobilized lipase demonstrated excellent activity in a broader range of pH and temperature and high heat and storage stability, as well as superior reusability. Glycidyloxypropyl trimethoxysilane functionalized $SiO_2$ coated magnetite NPs was used for the immobilization of CALB. The immobilized CALB maintained 100% of its activity at 55°C while free enzyme lost its full activity under similar conditions. Immobilized CALB showed a significant improvement in thermal stability and $CH_3OH$ tolerance compared to soluble enzyme [142].

Zhao et al. [143] used three types of amine reagents to graft dendritic macromolecules on $Fe_3O_4$ NPs and obtained a variety of $Fe_3O_4$@$SiO_2$/PAMAM magnetic nanocarriers with different generations for the immobilization of CRL. The density of surface functional groups, the structure, and the length (generation) of the flexible chain play an important role in immobilizing CRL. HMD grafted dendritic magnetic carriers with the fourth generation (Gc-4) showed remarkably superior performance in terms of immobilizing CRL. Then, PEI instead of HMD was grafted to the Gc-4 to obtain a higher activity with respect to immobilized CRL, 955.53 U $g^{-1}$ and free lipase. The immobilized CRL demonstrated improved tolerance towards wider ranges of pH and temperature. CRL was covalently immobilized on NC membrane (NCM), the $Fe_3O_4$@$SiO_2$ NPs were dip-coated onto the UFM surface via a low temperature hydrothermal process, and then reacted with 3-APTES. GA was used as a coupling agent to covalently immobilize lipase on the NCM surface. It was found that the activated NCM remarkably improved the relative activity and loading capacity as compared to unmodified NCM. The $K_m$ and $V_{max}$ were decreased due to immobilization which exhibited increasing substrate affinity and decreasing catalytic activity of immobilized enzyme. The immobilized enzyme demonstrated very high thermal, storage, and operational stability [144]. Thangaraj et al. [145] immobilized lipase on APTES and MPTMS-modified $Fe_3O_4$@$SiO_2$ core magnetic NCs and explored their potential application in biodiesel production. $Fe_3O_4$ MNPs prepared by co-precipitation method were coated with various ratios of $SiO_2$ as per Stöber method and further functionalized by different organosilane compounds APTES and MPTMS. Lipase NS81006

was covalently immobilized using GA as crosslinking agent on the functionalized $Fe_3O_4$@$SiO_2$ MNPs. The immobilization efficiency and activity recovery were reduced by increasing the ratio of silica coating on $Fe_3O_4$. Maximum activity recovery (84% by APTES, 83% by MPTMS) and biodiesel yield (>90%) were obtained by lipase immobilized on $Fe_3O_4$@$SiO_2$ support when the $Fe_3O_4$ and $SiO_2$ (TEOS) ratio was low (1:0.25).

**2.2.4. Lipase bound to polysaccharide MNPs NCs.** Carboxymethyl cellulose (CMC) and diethylaminoethyl cellulose-C (DEAE-C); micropores supports were developed by penetrating with MNPs and these carriers were used for the surface immobilization of cross-linked lipase aggregates (CLLAs) prepared by GA. The activity of CLLAs coated on DEAE was nearly twice of the enzyme aggregates coated on CMC. This is explained by the fact that CLLAs with amine groups were more efficient than those with carboxyl groups. After a 96 h enantioselective ibuprofen esterification reaction, 6% ibuprofen propyl ester was produced from the racemic mixture of ibuprofen using DEAE-lipase and 2.8% using CMC-lipase [146]. Gum Arabic coated MNPs support was used for covalent immobilization of lipase via GA. The surface of CRL was initially coated by various surfactants in order to stabilize enzyme in its open form and then immobilized on the support. The immobilized lipase was used as a biocatalyst for the production of ethyl isovalerate, a flavor ester. Various surfactants were employed among these non-ionic surfactant performed better, showing about 80% esterification yield in 48 h as compared to cationic/anionic surfactants. Enhanced activity due to interfacial activation was observed for immobilized non-ionic surfactant-lipase complex. The immobilized surfactant coated lipase retained its full activity even after 7 reuses. CAL was covalently immobilized on the surface of cellulose acetate-coated $Fe_2O_3$ NPs. The immobilized lipase was studied for production of MG and diglyceride (DG) by glycerolysis of olive oil in a solvent medium. The optimum condition for MG and DG formation was found at 50°C and 0.025 g of lipase with the molar ratio of glycerol to oil 1.5:1 in 5 h. The $K_m$ and $V_{max}$ values of immobilized lipase were found to be 25 mM and 0.58 mM $min^{-1}$, whereas for free lipase these values were 52.63 mM and 1.75 mM $min^{-1}$, respectively. The activation and deactivation energy were decrease for cellulose acetate-coated $Fe_2O_3$ NPs immobilized lipase [147].

Khoobi et al. [148] evaluated the immobilization of lipase on PEI and β-CD grafted NCs with magnetic core. Lipase - MNPs@hydroxyapatite/PEI/β-CD showed remarkably high heat, pH and storage stability, and reusability. The best immobilized lipase preparation was chosen for the synthesis of ethyl valerate which catalyzed production of ethyl valerate to 79% and 62% in *n*-hexane and dimethyl sulfoxide within 24 h, respectively. Magnetic cellulose nanocrystals were used for the successful immobilization of PCL. The obtained enzyme loading was 82.2 mg $g^{-1}$ nanosupport and activity recovery was 95.9%. Immobilized PCL showed a marked enhancement in its stability and solvent tolerance, due to increase in enzyme structural rigidity. The pH and temperature optima for NC bound PCL were higher than the free enzyme. The immobilized lipase manifested relatively higher substrate affinity and catalytic efficiency. Moreover, bound lipase was quite successful in catalyzing asymmetric hydrolysis of ketoprofenethyl ester with high yield of 43.4% and product e.e. of 83.5%. Immobilized PCL maintained nearly 66% of its activity after 6-repeated uses [149].

**2.2.5. Lipase bound to other polymer-MNP NCs.** Functionalized superparamagnetic particles were prepared by graft polymerization of glycidyl methacrylate and methacryloxyethyl trimethyl ammonium chloride onto the surface of modified-$Fe_3O_4$ NPs. The results showed that the polymer chains had been effectively grafted onto the surface of $Fe_3O_4$ NPs. Lipase was immobilized on the magnetic particles under mild conditions by electrostatic adsorption and covalent binding with the activity recovery up to 70.4%. The immobilized lipase showed better heat stability than the free enzyme [150]. Ren et al. [151] reported immobilization of lipase onto PDA coated MNPs with 73.9% yield and 429 mg $g^{-1}$ support loading capacity. Immobilized enzyme was found significantly more stable against pH and heat exposure than the free enzyme and retained over 70% activity even after 21-repeated uses. PEI-$Fe_3O_4$ NP was considered for CRL adsorption via electrostatic interactions. The procedure resulted in marginal simultaneous purification and immobilization of enzyme. Immobilized CRL exhibited 110×higher transesterification activities in low-water media. It was also efficient in kinetic resolution of (±)-1-phenylethanol with eep of 99% and E =412 within 24 h. The immobilized lipase was reused at least 4-times by retaining almost its full activity. This environmentally friendly method for the enzymatic transesterification has a great future in the field of biodiesel production [152]. Further, lipase immobilized on the PEI-MNPs was used to synthesize vitamin A palmitate from vitamin A acetate and palmitic acid. The reuse of immobilized lipase has been extended to 8- times by removing water during esterification with a conversion rate above 80% in 12 h [153]. Sol-gel encapsulation of CRL was done with and without calix[n]arene carboxylic acid derivative grafted onto MNPs or in the presence of calix[n]arene carboxylic acid derivative with $Fe_3O_4$ MNPs as an additive. These results showed that the encapsulated lipase without MNPs NC performed lower conversion and enantioselectivity compared to Calix[n]COOH-based encapsulated lipase. Calix[4]COOH-based encapsulated lipase maintained excellent enantioselectivity (enantiomeric ratio (E) >400) as compared to encapsulated-free lipase enantioselectivity (E=137) and it has also an enantiomeric excess value of ~98% for S-naproxen [154].

Some workers have focused on sonication mediated immobilization of PPL onto PEG-Ag-$Fe_3O_4$ hybrid NPs. This immobilized PPL preparation exhibited increased heat and storage stability and enhanced lipase activity. The industrial uses of the antibacterial and magnetically recyclable system were vouched by its excellent compatibility with other commercial detergents for oil destaining [155]. TLL immobilized via physical adsorption onto three different metal chelated MNPs (Fe@PEI-M). Fe@PEI-Co and Fe@PEI-Cu showed better activity at extreme temperature and pH compared to free enzyme. Lipase immobilized on Fe@PEI-Co retained above 60% and 80% of its activity after 10-repeated uses and 14 days of storage, respectively. This adsorbed lipase was used for the synthesis of ethyl valerate and the yields of esterification were 70% and 60% in *n*-hexane and DMSO after 24 h, respectively [156]. PCL was covalently attached to magnetite NPs coated with a thin PDA film and employed for the conversion of soybean oil into biodiesel in the presence of methanol. The

proposed strategy explored the direct immobilization of the enzyme *via* Michael addition and aldolic condensation reactions at catechol rings without use of any crosslinker. The MNPs bound PCL retained very high catalytic activity. In the biodiesel production, the transesterification reaction was carried out directly in soybean oil by the stepwise addition of methanol, in order to circumvent its inactivation effect on the enzyme. A better yield was obtained in relation to native enzyme, achieving 90% yield at 37$^{o}$C. However, the catalysis became gradually less effective after 3$^{rd}$ cycle, due to its prolonged exposure to methanol [157]. Core-shell $Fe_3O_4$/PS-PAA NPs modified with -COOH groups were used to immobilized CRL and PPL. The amount of CRL loading on $Fe_3O_4$/PS-AA NPs obtained as 87.7 mg protein $g^{-1}$ particles. The immobilized CRL showed better stability and retained more than 56.1% of original activity after its 10 repeated uses. Moreover, the immobilized PPL exhibited a marked tolerance to organic media and maintained high esterification activity in *n*-hexane. This work illustrated a facile and general method to synthesize magnetic functional NC for applications in biocatalytic fields [158].

**2.2.6. Lipase bound to hydrophobic MNPs NCs.** Wang and co-workers [159] studied direct immobilization of lipase on the magnetite NPs coated by alkyl silanes of varying chain lengths via hydrophobic interaction. The activity of immobilized lipase was enhanced with increasing chain length of alkyl silane. Moreover, the catalytic activity of lipase immobilized on trimethoxyl octadecyl silane ($C_{18}$) bound $Fe_3O_4$ was just double of the values reported from other surface immobilized systems. The activity of lipase immobilized on $C_{18}$ modified MNPs retained 65% of the initial activity after 7-repeated uses; it exhibited a marked enhancement in stability. MNPs immobilized lipase was easily separated and recycled by retaining high activity. The activity of immobilized lipase increased by increasing alkyl chain length of the alkyl trimethoxy silanes used in surface modification of magnetite NPs. PFL was immobilized onto $Fe_3O_4$ MNPs via hydrophobic bonding. The enzyme loading and immobilization yield were as 21.4±0.5 mg $g^{-1}$ support and 49.2±1.8%, respectively. Bound enzyme was successfully used for resolution of 2-octanol with vinyl acetate and the preferred isomer for the enzyme was (R)-2-octanol. The highest enantioselectivity (E=71.5±2.2) was achieved with a greater lipase activity (0.197±0.01 μmol $mg^{-1}min^{-1}$). The immobilized enzyme maintained nearly 89% of the activity and the enantioselectivity remained unchanged even after 5 reuses [160]. Liu et al. [161] developed a novel oriented method for covalent immobilization of *Yarrowia lipolytica* lipase $LIP_2$ on functionalized $Fe_3O_4$ MNPs in RMs system. The observed activity recovery was 382% as compared to 29% in aqueous phase and further increased to 1425% under optimum conditions. The immobilized lipase was used to enrich polyunsaturated fatty acids in fish oil; a 90% increase of docosahexanenoic acid content was obtained after 12 h. Over 80% of relative degree of hydrolysis was retained after 20 repeated uses.

## 3. CONCLUSIONS

Recently lipases have drawn attention of the biotechnologists because these enzymes can also catalyze acylation, esterification, interesterification and transesterification reactions in nonaqueous media besides their natural hydrolytic reactions. This uniqueness of lipases makes them potential candidates for their possible applications in various industrial sectors, i.e., food, fuel, pharmaceutical, detergent, leather, cosmetics, textile, paper, nutraceuticals and oil degumming. Moreover, their biotechnological uses have successfully been applied for the synthesis and production of biopolymers, biodiesel, enantiopure pharmaceuticals, agrochemicals, compounds of flavor & fragrance, fine chemicals, esters and amino acid derivatives, use as biosensor and bioremediation. With the advent of nanobiotechnology, the NMs have been exploited as carrier for enzyme immobilization. NMs have shown their superiority in the high yield immobilization of lipases due to their unique properties. Such type of material has also provided high robustsness and mechanical strength to the enzymes. Magnetic and non-magnetic both kinds of NMs have successfully been employed for high yield immobilization of lipases. Lipases immobilized on NMs have been found highly stable against various kinds of denaturants such as pH, heat, organic solvents, and detergents, and found less inhibitory to their specific inhibitors. Immobilized lipases have shown very high activity, enantioselectivity, operational stability and reusability. NMs bound lipases were quite successful in catalyzing acylation, esterification, interesterification and transesterification reactions in nonaqueous media. Lipases from various sources have successfully been immobilized on NMs (Fig. 4). Several organic compounds and oils have effectively been transformed into biodiesel by using nanocarriers bound lipases (Table 6 and Fig. 5). Several lipases attached to the electrode via nanocarriers have proved their high potential as efficient biosensors. Biosensors prepared by using NMs have repeatedly been used several times without much loss in their initial activity on prolonged storage. Nanocarriers bound lipases were also used as a tool to treat environmental pollutants.

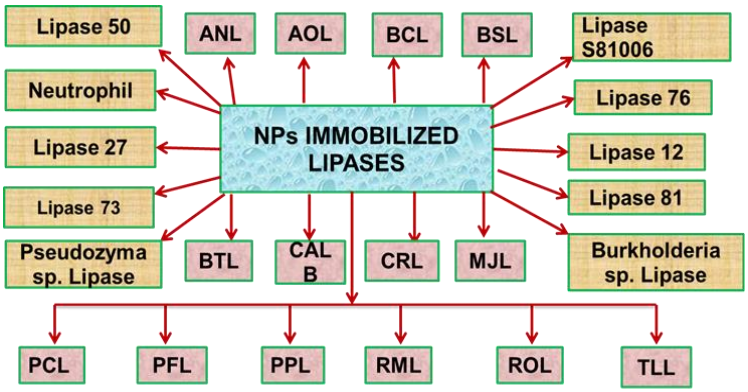


**Fig. 4.** Summarizes lipases from various sources immobilized on NMs.

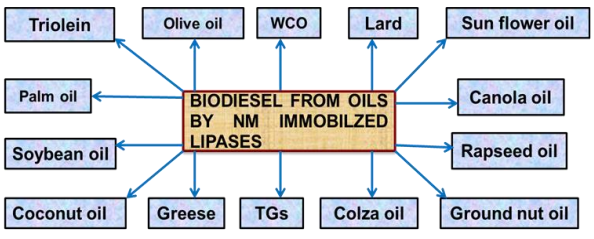


**Fig. 5.** Represents biodiesel production from oils by lipases immobilized on NMs.

**Table 6.** Summarizes the synthesis of biodiesel production by using nanocarrier bound lipases.

| Enzyme | Nanocarrier | Substrates | Product | Reference |
|---|---|---|---|---|
| NL | Nano-$SiO_2$ | Soybean, rapeseed & waste oils | Biodiesel | 30 |
| PPL | Fused silica capillary-AuNPs | TGs from kitchen | Biodiesel | 33 |
| BCL | TA-MP-silica NPsN | Oils | Biodiesel | 35 |
| CRL | MWCNTs-OA, MWCNTs-BA | Rapseed oil & methanol | Biodiesel | 48 |
| PPL | PDA/AgNPs | Soybean oil & Methanol | Biodiesel | 51 |
| RML | Aldehyde silica, silica NPs (SBA-15) | Colza oil & methanol | Biodiesel | 53 |
| BTL | Nanographene oxide | Colza oil & methanol | Biodiesel | 56 |
| CALB, TLL, RML | MP-SBA-15,NPs-epoxy activated | Canola oil & methanol | Biodiesel | 57 |
| Lipase | $NH_2$-MNPs | Soybean oil & methanol | Biodiesel | 78 |
| TLL | Amino-MNPs, EDAC | Vegetable oil & methanol | Biodiesel | 79 |
| Lipase | MNPs | Triolein & methanol | Biodiesel | 81 |
| PFL LP1 | $NH_2$-silane-MNPs | WCO & methanol | Biodiesel | 86 |
| PCL | MNPs | WCO & methanol | Biodiesel | 92 |
| TLL, CALB | MNPs | Grease & methanol | Biodiesel | 95 |
| CALB | CLEA-MNPs | TGs | Biodiesel & biosurfactant | 98 |
| Pseudozyma sp. lipase | 3-APTES-MNPs via GA | Vegetable oil & methanol | Biodiesel | 103 |
| ANL S81006 | APTES-MNPs, MPTMS-MNPs | Oils & methanol | Biodiesel | 107 |
| ANL | Magnetic barium ferrite NPs | WCO & Methanol | Biodiesel | 110 |
| Burkholderia sp. lipase | Alkyl-functionalized $Fe_3O_4$ $SiO_2$ | Olive oil & methanol | Biodiesel | 128 |
| Lipase | MP-$SiO_2$-magnetic $Fe_3O_4$ core shell NPs | WCO & methanol | Biodiesel | 129 |
| PCL | Nonporous & MP $SiO_2$-magnetic clusters NCs, GA | Soybean oil & methanol | Biodiesel | 131 |
| CRL | MNPs-MCM-4NC | Lard & soybean oil | Biodiesel | 139 |
| PCL | Magnetite NPs-PDA | Soybean oil & methanol | Biodiesel | 157 |

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## 6. ACKNOWLEDGEMENT

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## 7. CONFLICT OF INTEREST

There is no conflict of interest in publishing this manuscript.