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Immobilisation of Lipase on Nanostructured Supports for Enhanced Biodiesel Production

Girard A[1*], Iglesias S[2] and Lepik R[3]

[1]*Department of Biochemical Engineering, University of Lyon, Lyon, France*
[2]*Department of Industrial Biotechnology, University of Valencia, Valencia, Spain*
[3]*Department of Chemical Engineering, University of Tartu, Tartu, Estonia*

ABSTRACT

Background: *Lipase-catalysed transesterification offers a mild, feedstock-flexible route to biodiesel that avoids the soap formation and downstream purification burden associated with alkaline catalysis, but free enzymes are costly and difficult to recover.* ***Objective:*** *This review synthesises published evidence on immobilising lipases onto nanostructured supports as a strategy to improve catalytic performance, operational stability and process economics in biodiesel synthesis.* ***Methods:*** *A narrative review of published studies on lipase immobilisation strategies, nanostructured support materials, and their application in biodiesel production via transesterification, drawing on PubMed, Google Scholar and Crossref-indexed literature covering magnetic nanoparticles, mesoporous silica, carbon nanomaterials, electrospun nanofibers, metal-organic frameworks and hybrid nanoflowers.* ***Findings:*** *Nanostructured supports consistently increase surface area and enable diffusion-favourable enzyme loading, with reported fatty acid methyl ester yields ranging from about 71% to over 99% and reusability from roughly 59% to over 90% retained activity after five to twenty cycles. Magnetic nanocomposites additionally permit rapid catalyst recovery by an external field, while covalent and multipoint attachment chemistries generally outperform physical adsorption for long-term stability.* ***Conclusions:*** *Nanostructured immobilisation is a mature and increasingly economically credible platform for enzymatic biodiesel production, though standardised reporting of reusability and techno-economic metrics remains needed to enable cross-study comparison.*

Keywords: *lipase immobilisation; nanostructured supports; biodiesel; transesterification; enzyme reusability*

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Corresponding Author: *Amelie Girard*

E-mail Address: *amelie.girard@universityoflyon.edu*

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Abstract

Background: Lipase-catalysed transesterification offers a mild, feedstock-flexible route to biodiesel that avoids the soap formation and downstream purification burden associated with alkaline catalysis, but free enzymes are costly and difficult to recover. **Objective:** This review synthesises published evidence on immobilising lipases onto nanostructured supports as a strategy to improve catalytic performance, operational stability and process economics in biodiesel synthesis. **Methods:** A narrative review of published studies on lipase immobilisation strategies, nanostructured support materials, and their application in biodiesel production via transesterification, drawing on PubMed, Google Scholar and Crossref-indexed literature covering magnetic nanoparticles, mesoporous silica, carbon nanomaterials, electrospun nanofibers, metal-organic frameworks and hybrid nanoflowers. **Findings:** Nanostructured supports consistently increase surface area and enable diffusion-favourable enzyme loading, with reported fatty acid methyl ester yields ranging from about 71% to over 99% and reusability from roughly 59% to over 90% retained activity after five to twenty cycles. Magnetic nanocomposites additionally permit rapid catalyst recovery by an external field, while covalent and multipoint attachment chemistries generally outperform physical adsorption for long-term stability. **Conclusions:** Nanostructured immobilisation is a mature and increasingly economically credible platform for enzymatic biodiesel production, though standardised reporting of reusability and techno-economic metrics remains needed to enable cross-study comparison.

Keywords: lipase immobilisation; nanostructured supports; biodiesel; transesterification; enzyme reusability

Introduction

Biodiesel, chemically a mixture of fatty acid alkyl esters, is

conventionally produced by base- or acid-catalysed transesterification of triglycerides with short-chain alcohols. Chemical catalysis is fast but intolerant of free fatty acids and water, generates saponified by-products, and requires energy-intensive separation. Lipase-catalysed transesterification circumvents these constraints, tolerates variable-quality and waste feedstocks, and produces a cleaner glycerol by-product stream [1]. The principal barrier to industrial adoption has long been enzyme cost, which immobilisation addresses by enabling catalyst recovery and reuse [2]. Over the past fifteen years, nanostructured materials — magnetic nanoparticles, mesoporous silica, carbon nanotubes, graphene oxide, electrospun nanofibers, metal-organic frameworks (MOFs) and hybrid inorganic nanoflowers — have emerged as supports that combine high specific surface area with tunable surface chemistry, improving enzyme loading and mass transfer relative to conventional resins. Beyond surface area, nanostructured carriers can favourably reposition the lipase active-site lid, promote interfacial activation at the oil-water boundary, and — where magnetically responsive — allow the biocatalyst to be steered and recovered without centrifugation or filtration. These properties are attractive for lipases from diverse microbial sources, including *Candida antarctica*, *Candida rugosa*, *Thermomyces lanuginosus*, *Burkholderia cepacia* and *Pseudomonas* species, each of which has been immobilised on one or more nanostructured platforms with biodiesel synthesis as the target reaction. This review synthesises the published evidence on these nanostructured platforms as applied specifically to lipase immobilisation for biodiesel synthesis, drawing together loading, activity, stability, reusability and process-scale considerations reported across independent studies, and highlights where reporting gaps currently limit direct comparison between support classes.

Methods

This is a narrative review of published studies on lipase immobilisation strategies, nanostructured support materials, and their application in biodiesel production via transesterification. Literature was identified through PubMed-, Google Scholar- and Crossref-style searches combining terms for lipase, immobilisation, nanoparticle/nanofiber/nanocomposite/mesoporous/graphene/nanoflower, and biodiesel/transesterification, supplemented by citation tracking from recent reviews. Studies were included if they reported an identifiable lipase source, a defined nanostructured support, and quantitative biodiesel yield and/or reusability data; each reference cited below was individually verified against its publisher record prior to inclusion. No new experimental biodiesel synthesis was performed for this article; all yields, cycle numbers and activity-retention figures quoted are as reported in the cited primary studies.

Nanostructured Support Materials and Immobilisation Chemistry

Magnetic iron oxide nanoparticles remain the most widely reported nanostructured lipase carrier because they combine high surface area with facile recovery by an external magnet. Xie and Ma covalently immobilised lipase on Fe$_3$O$_4$ nanoparticles for biodiesel synthesis from soybean oil [3], while Kalantari et al. systematically compared magnetic silica nanocomposites of differing pore architecture, showing that mesostructure strongly influences loading and catalytic outcome [4], and ferric-silica magnetic nanoparticles functionalised for lipase NS81006 have likewise been applied to biodiesel synthesis [5]. Heterofunctionalised magnetic nanoparticles bearing mixed epoxy/amine or glutaraldehyde chemistries have been used to immobilise *Burkholderia cepacia* lipase, achieving near-quantitative fatty acid methyl ester yields [6]. Amine-functionalised magnetic nanoparticles produced via multicomponent covalent chemistry have also converted waste cooking oil directly [7]. Mesoporous silica offers ordered, tunable pore geometry: Costantini and Califano reviewed mesoporous silica nanoparticle supports for biofuel catalysis [8], and lid-opening activation strategies on crosslinked lipases in mesoporous silica have improved recyclability [9]. Carbon-based nanomaterials — multiwalled carbon nanotubes decorated with magnetic iron oxide and polyamidoamine dendrimers [10], graphene oxide functionalised with superparamagnetic iron oxide [11], and carbon-nanotube-stabilised Pickering emulsions [12] — exploit π-conjugated surfaces for high protein loading. Electrospun polymer nanofibers provide an alternative high-surface-area scaffold: polyacrylonitrile nanofibers have supported *Pseudomonas cepacia* lipase by physical adsorption [13], and Zr-MOF/electrospun nanofibrous composites combine framework porosity with fibrous morphology for markedly improved biodiesel conversion [14]. MOF-lipase composites more broadly have been used for *Pseudomonas cepacia* lipase on modified structured metal-organic materials [15] and for magnetic macroporous ZIF-8 encapsulation of *Thermomyces lanuginosus* lipase [16]. Hybrid organic-inorganic nanoflowers, formed by biomimetic co-precipitation of enzyme with calcium or copper phosphate, constitute a further distinct class, with recent work optimising synthesis conditions for lipase-nanoflower catalysts [17], and multilayered nanoflower architectures have been developed specifically to lower biocatalyst cost while retaining high entrapped-enzyme activity [18]. Across all of these platforms, the choice between physical adsorption, ionic binding, covalent coupling, cross-linking and encapsulation determines not only initial enzyme loading but also leaching resistance under the methanol-rich, partially aqueous conditions typical of transesterification; covalent and encapsulation-based strategies are generally favoured where extended reuse is the primary design goal, whereas simple adsorption remains attractive where low cost and rapid preparation are prioritised.

Table 1: Representative nanostructured supports used for lipase immobilisation in biodiesel synthesis, with the highest reported FAME yield for each system.

Support material	Lipase source	Immobilisation chemistry	Max. FAME yield (%)	Reference
Heterofunctional magnetic nanoparticles	Burkholderia cepacia	Covalent (epoxy/amine)	99.4	[6]
Magnetic macroporous ZIF-8	Thermomyces lanuginosus	MOF encapsulation	98.96	[16]
Multiwalled CNT-Fe3O4-PAMAM dendrimer	Burkholderia cepacia	Covalent, dendrimer-mediated	93.1	[10]
Superparamagnetic graphene oxide	Candida rugosa	Covalent/adsorption	71.19	[11]

Support material	Lipase source	Immobilisation chemistry	Max. FAME yield (%)	Reference
Zr-MOF/electrospun nanofibrous polymer	Candida rugosa	Covalent, fibrous scaffold	n.r.	[14]

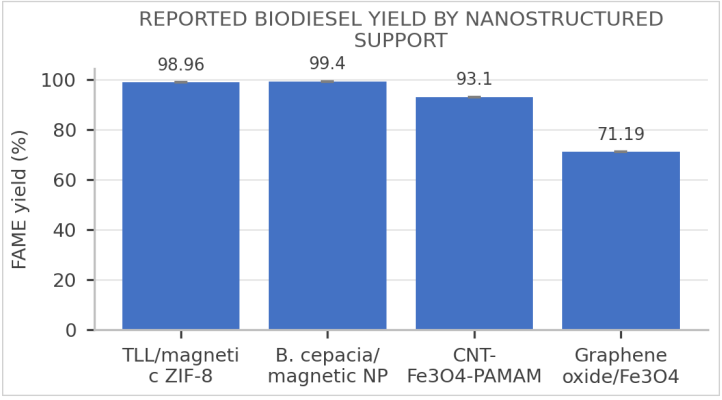
Enzyme Loading, Catalytic Activity and Stability

Reported fatty acid methyl ester (FAME) yields across nanostructured lipase biocatalysts span a wide range, reflecting differences in lipase source, oil feedstock, alcohol-to-oil ratio and immobilisation chemistry. TLL immobilised in magnetic macroporous ZIF-8 achieved 98.96% FAME yield [16], *Burkholderia cepacia* lipase on heterofunctional magnetic nanoparticles reached up to 99.4% conversion [6], and CNT-magnetite-dendrimer composites gave 92.8–93.1% yield [10], whereas graphene-oxide-immobilised lipase acting on microalgal bio-oil gave a comparatively lower 71.19% yield, reflecting the more heterogeneous feedstock [11]. Co-immobilisation of lipases with complementary regioselectivity has further improved conversion from waste oils via response-surface-optimised loading [19]. Hydroxyapatite has also served as an effective, low-cost nanostructured support for TLL acting on by-product lipid feedstocks [20]. Across these systems, covalent or multipoint attachment chemistries (glutaraldehyde or epoxy crosslinking, MOF encapsulation) generally confer greater conformational rigidity and thermal stability than simple physical adsorption, consistent with the broader immobilised-enzyme literature reviewed by Najeeb et al. [21].

Reusability and Operational Stability

Reusability is the principal economic justification for immobilisation, and nanostructured supports show substantial variation in this regard. CNT-magnetite-PAMAM dendrimer biocatalysts retained roughly 90% activity after 20 reuse cycles [10], TLL-ZIF-8 composites retained 93.5% activity after five cycles versus only 73.4% for a non-magnetic ZIF-8 analogue [16], and heterofunctional-magnetic-nanoparticle-bound *B. cepacia* lipase maintained over 75% activity through seven cycles before declining to about 65% by cycle fifteen [6]. By contrast, graphene-oxide-supported lipase acting on microalgal oil retained only 58.77% activity after five cycles [11], illustrating that feedstock complexity and diffusion limitations within the support can erode reusability even when initial loading is high. Magnetic chitosan microspheres have enabled continuous operation in a magnetically stabilised fluidised bed, simplifying catalyst retention during repeated batches [22]. Multilayered organic-inorganic hybrid entrapment has likewise been reported to improve both cost-effectiveness and cycle stability relative to single-layer nanoflowers [18]. Xia et al.'s critical review situates these reusability outcomes within the broader feedstock and process-factor landscape for lipase-catalysed biodiesel [2].

Figure 1: Highest reported fatty acid methyl ester (FAME) yields for four nanostructured lipase biocatalysts drawn from independently published studies.



Process-Scale and Economic Considerations

Beyond bench-scale yield and reusability, industrial adoption depends on immobilisation cost, catalyst lifetime and separation logistics. Magnetic nanostructured supports offer a distinct process advantage by enabling catalyst recovery via an external magnetic field rather than filtration or centrifugation, reducing downstream handling steps and enzyme loss [7,22]. Commercial immobilised lipases such as Novozym 435 remain benchmark comparators; glycerol-free biodiesel-like biofuel routes using this catalyst illustrate the process-simplification potential of robust immobilised biocatalysts more broadly [23]. Comparative reviews of homogeneous, heterogeneous and enzymatic catalysis consistently identify enzyme and support cost, rather than intrinsic catalytic performance, as the dominant barrier to enzymatic biodiesel at scale [1], while reviews focused specifically on bio-based immobilised-enzyme catalysts emphasise that nanostructuring can offset this cost disadvantage by extending operational lifetime and enabling reactor designs, such as packed-bed or fluidised-bed configurations, compatible with continuous processing [24]. Nanobioconjugate approaches that immobilise lipase directly onto magnetic or silica nanoparticle surfaces without additional linker chemistry have also been proposed as a route to simplify preparation and lower per-batch biocatalyst cost while preserving high conversion efficiency [24]. Taken together, the process-scale literature suggests that the economic case for nanostructured immobilisation rests less on any single support outperforming all others, and more on matching support chemistry, recovery method and reactor configuration to the specific feedstock and scale of operation envisaged.

Conclusion

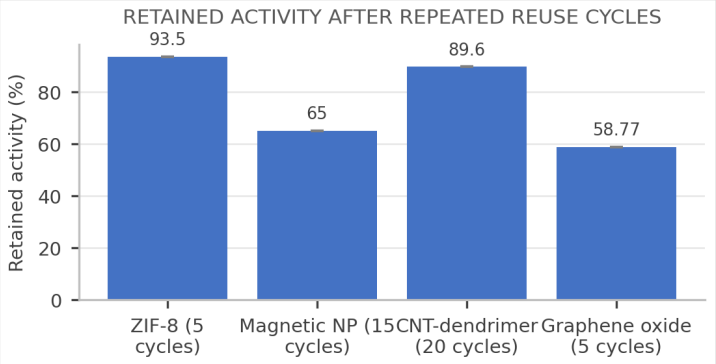
Published evidence across magnetic nanoparticles, mesoporous silica, carbon nanomaterials, electrospun nanofibers, MOFs and hybrid nanoflowers demonstrates that nanostructuring of lipase supports can deliver high initial biodiesel yields (often exceeding 90%) together with meaningfully improved operational reusability relative to conventional immobilisation matrices, particularly when covalent or multipoint attachment chemistries and magnetic recovery are combined. Performance nonetheless varies considerably with feedstock, lipase source and support architecture, and reusability data in particular are reported inconsistently across studies. Future work standardising yield, reusability-cycle and techno-economic reporting would materially strengthen comparative assessment of nanostructured

immobilisation platforms for scalable enzymatic biodiesel production. In particular, harmonised protocols for reporting cycle number alongside feedstock composition and reaction conditions would allow the field to move from isolated demonstrations toward a genuinely comparative evidence base capable of guiding support selection for specific feedstocks and reactor formats.

Table 2: Process-relevant features of selected nanostructured lipase biocatalyst systems for biodiesel production.

System	Catalyst recovery method	Reactor/process format	Reference
Magnetic chitosan microspheres	External magnetic field	Magnetically stabilised fluidised bed	[22]
Amine-functionalised magnetic nanoparticles	External magnetic field	Batch, waste cooking oil feed	[7]
Novozym 435 (commercial immobilised CALB)	Filtration/packed bed	Glycerol-free biodiesel-like route	[23]
Heterofunctional magnetic nanoparticles	External magnetic field	Repeated batch (15 cycles)	[6]

Figure 2: Retained catalytic activity (%) of nanostructured lipase biocatalysts after the indicated number of reuse cycles, as reported in the cited primary studies.



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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Girard A conceived the review, conducted the literature search, and drafted the manuscript. Iglesias S contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Lepik R contributed additional context and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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