Immobilized cellulase: interactions between cellulase and nanostructured supports

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Abstract. In this review, we will analyze the main aspects of immobilization of cellulase - an enzyme for processing cellulosic biomass waste - on nanostructured supports. Such substrates provide a large surface area, increased enzymatic load and a favorable environment for increasing the efficiency of cellulase and its stability, which leads to the creation of nanobiocatalysts for the production of biofuels and chemicals with added value. Here we will discuss nanostructured supports, methods of cellulase immobilization, the interaction between the enzyme and the support, as well as factors affecting the activity of the enzyme to achieve maximum conversion of cellulose biowaste into fermentable sugars.

1 Introduction

Decline of fossil fuel reserves and environmental pollution have led to the need to use alternative sustainable and renewable energy sources, such as biofuels. In this context, lignocellulose biomass is considered as a strategic fuel source. Cellulases are a class of hydrolytic enzymes that convert cellulose into fermentable sugars [1]. A wide range of materials of various origins, such as inorganic, organic, hybrid and/or composite ones, can be used as a support matrix for the immobilization of enzymes. The materials used should not only maintain the structural conformation of the enzymes, but also create a stable interaction with the enzyme [2]. The choice of a suitable support matrix is related to the type of enzyme and the process in which it is proposed to use these immobilized systems [3, 4]. Nevertheless, it should be emphasized that the choice of support materials is the most important task due to the serious influence of the support material on the properties of the biocatalytic system.

The development of a highly efficient biocatalyst is an urgent requirement for the production of biofuels, in particular biodiesel/bioethanol. To circumvent the minimal efficiency of traditionally used biocatalysts, nanotechnology is paving the way by using nanoparticles (NPS) as supports of biocatalysts. The nanobiocatalysts thus obtained are used as a tool for recycling a wide range of biomass-related molecules into biofuels. Disadvantages of conventional biocatalysts, such as catalyst deactivation, mass transfer, poisoning, and a long reaction time can be outpaced by new nanobiocatalysts. The nanobiocatalyst increases the catalytic activity; and this higher activity is due to the

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increased surface-to-volume ratio, and therefore it can also act as a deoxygenation catalyst. In recent years, using modern tools for the synthesis and characterization of nanoparticles, high-quality optimized and conditioned nanocatalyst systems such as metal oxide nanoparticles, magnetic nanoparticles and carbon nanotubes have been obtained to increase biofuel production productivity. Lipases and cellulases immobilized in nanomaterials are predictably innovative catalysts with remarkable properties [5, 6].

Typical methods of cellulase immobilization on nanostructured supports are similar to those used for other enzymes on various supports. These include adsorption on the surface of the support, encapsulation in the support, covalent attachment and crosslinking. In recent years, covalent attachment has become common, since it provides higher stability of immobilization and does not affect the structure of the enzyme, if the tether used allows protecting the secondary structure of the enzyme. On the other hand, encapsulation and crosslinking can also be useful for the operation of a biocatalyst if the conformation of the enzyme is preserved. In this review, the greatest attention will be paid to the methods of covalent binding and adsorption for the immobilization of cellulase on nanostructured supports.

2 Typical methods of cellulase immobilization

2.1 Covalent binding

The activity of a covalently attached enzyme depends on the size of the support material, its shape and composition, as well as on the nature of the functional groups and the specific conditions of the combination reaction. [7]. Covalent bonding usually occurs between chemically active groups on the support surface and nucleophilic groups on enzymes. Reactive functional groups can be added to the support matrix without modification, or the support matrix is functionalized to create activated groups. Most enzymes are covalently attached using lysine amino groups, which are present on the surface of the protein in large quantities and have a high reactivity [8]. From a chemical point of view, covalent binding provides a strong connection between the support matrix and the enzyme, prevents its leaching into the reaction medium and ensures reuse [9]. After the immobilization reaction, the support must be sufficiently inert to prevent the formation of undesirable interactions between the support material and the enzyme molecule [10, 11]. For optimal covalent binding, the immobilization conditions should favor the reactivity of the enzyme-support complex: long reaction time, alkaline pH and moderately high temperatures [12]. It is promising to attach enzymes to the surface of a support having a large number of reactive groups, which can be realized by using glyoxyl supports. In addition, the deactivation of the enzyme by distortion is also prevented if the adsorption of the enzyme has a constructive effect on its stabilization. Changing the orientation of the enzyme on the support by varying the conditions of immobilization is an additional advantage of the versatility of glutaraldehyde [10].

Covalent attachment is often preferred for cellulase immobilization because it provides increased stability, which is often combined with improved enzyme activity - important advantages of this approach. Covalent immobilization, however, requires the functionalization of the support, unless the support initially possesses functional groups [13-15]. In addition, a suitable linker is needed to preserve the conformation of the enzyme [16]. The most commonly used bifunctional linker is glutaraldehyde, which interacts with amino groups under environmental conditions and does not require any catalyst [16, 17]. Despite the fact that the length of glutaraldehyde is only 0.75 nm, it seems to provide sufficient distance to prevent nonspecific adsorption of the enzyme.

Cellulase covalently immobilized on amino-functionalized Fe_3O_4@SiO_2 core-shell LPS provided high stability at various pH values and temperatures during enzymatic saccharification of poplar wood [18]. This biocatalyst provides an enzymatic saccharification rate of 38.4% in 72 hours, which is promising for the decomposition of lignocellulose biomass. The same principle of cellulase immobilization using amino groups was used on a completely different support: a hybrid conductive nanohydrogel obtained using polyaniline (PANI) nanorods formed on a cationic poly(e-caprolactone) hydrogel containing a macromolecule of cationic phosphinoxide [19].The hybrid nanobiocatalyst showed good results in the hydrolysis of cellulose substrates, without showing a loss of activity compared to the free enzyme.

Proper functionalization of the support may be crucial for effective covalent attachment of the enzyme. This pathway was investigated by Gao et al. who modified graphene oxide (GO) sheets using p-β-sulfuric acid ester ethyl sulfone aniline, which creates a hydrophobic linker for further rapid immobilization of cellulase (~10 min) after diazotization [20]. It is noteworthy, however, that the reliability of rapid cellulase attachment is opposed by a complex functionalization procedure, which makes this achievement doubtful.

In the original work, a sortase-mediated enzyme immobilization method (called sorting labeling) was developed and tested on microgels for five different enzymes, including cellulase [21]. This method provides site-specific immobilization of the enzyme due to covalent attachment to stimulus-sensitive microgel particles based on poly (N-vinylcaprolactam)/glycidyl methacrylate.

2.2 Adsorption

The review [1] noted that mesostructured silica nanoparticles offer unique opportunities in the field of biocatalysis due to their outstanding properties. The adjustable pore size in the mesopore range allows the immobilization of bulk enzyme molecules. A large surface area increases the catalytic efficiency by increasing the loading of enzymes and fine dispersion of biocatalyst molecules. Easily customizable pore morphology allows you to create the right environment for the placement of the enzyme. The limiting effect of mesopores can improve the stability of the enzyme and its resistance to extreme pH values and temperatures. When considering the immobilization of enzymes in general and, in particular, cellulases, there are factors that need to be taken into account in order to maximize absorption. Firstly, the surface charge of the enzyme and the support should be opposite, since electrostatic interactions have a great influence on the adsorption and desorption of the enzyme on the support. The second is the correspondence between the mesopore size and the molecular diameter of the enzyme, that is, the pore size should be large enough to accommodate the enzyme, but not too large to promote desorption. Finally, the functionalization of the silica surface by hydrophobic groups can also play an important role in the adsorption and desorption of enzymes, since cellulases have high hydrophobicity.

The authors synthesized mesoporous silica materials with pore sizes of 17.6 nm and 3.8 nm (hereinafter referred to as MS-17.6 nm and MS-3.8 nm, respectively) were synthesized in the manner of a seeded-growth method [22]. The amounts of adsorbed cellulase showed a clear correlation with the pore size of the sorbents; i.e., the amount of adsorption of MS-17.6 nm (410 mg g-1) with a pore size similar to the long axes of the cellulase molecules was higher than that of MS-3.8 nm (315 mg g-1) with a pore size similar to the short axes of the molecules cellulases. But at the same time, MS-17.6 nm revealed a lower specific activity (35.8%) compared to MS-3.8 nm, which showed 63.3% of the activity of free cellulase. According to the image model of the forms of the adsorption mechanism (Figure

1), when cellulase was adsorbed into the sorbent MS-17.6 nm pore, there was a suitable space for the molecules not to volatilize, but to line up in a dense and orderly arrangement.

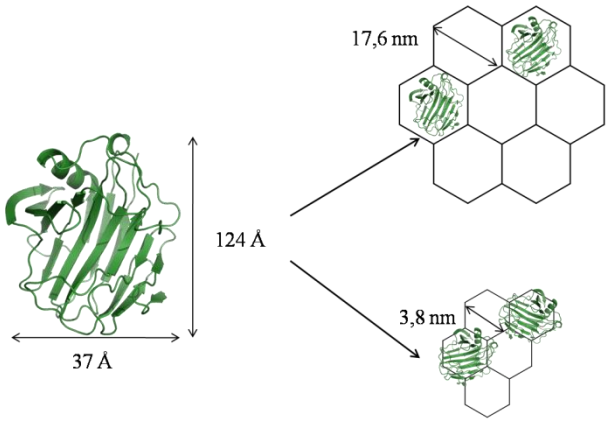

Fig. 1. Structural model of immobilized cellulase on MS-17.6 nm and MS-3.8 nm

However, the dense and ordered arrangement prevented the conformational flexibility of cellulase, since cellulase molecules need to change conformation during the interaction between cellulase and substrate. For the MS-3.8 nm sorbent, the molecules stuck at the mouth into the pores and the active centers of the cellulase molecules were preserved and the activity and stability of the immobilized form were higher. Thus, despite any possible preconceived opinion that smaller pores can damage the structure of secondary cellulase, the authors' thoughtful choice of pore sizes in two mesoporous materials allowed a deeper understanding of the limitations or their absence when choosing nanoporous substrates.

In another work, the authors immobilized cellulase by adsorption on wrinkled silica nanoparticles (WSNs), obtaining an active and stable biocatalyst.[23] The physicochemical characteristics of the biocatalyst demonstrated an improvement in catalytic properties caused by an increase in the distance between wrinkles for adsorbed cellulase. It was found that the adsorption energy is 25 kJ/mol, indicating that the driving force of cellulase adsorption in WSN is probably the hydrogen bond between polar cellulase residues and silanol groups on the silica surface. The authors suggest that monolayer adsorption occurs as the most likely mechanism involved in cellulase adsorption on WSNs. The authors believe that it is the hydrogen bond that ensures the adsorption of a homogeneous layer from a mixture of cellulase enzymes, which are cellulolytic enzymes rich in hydrogen bond groups. If we assume an electrostatic or hydrophobic interaction, then given that cellulolytic enzymes have different isoelectric points and hydrophobic characteristics, then crowding and multilayer uneven distribution of the enzyme on the surface would be observed. The results of catalytic analyses and operational stability confirmed the key role of pore size, morphology and distribution in the successful outcome of the cellulase immobilization process.

In [24], the authors immobilized β-glucosidase (BG) by adsorption on wrinkled silica nanoparticles (WSNs) with central radial pores and on tannic acid-templated mesoporous silica nanoparticles (TA-MSNPs) with disordered channel-like pores.The results demonstrated that the catalytic characteristics of the immobilized enzyme depend on the pore size of the sorbent, but the key factor is the pore morphology. The catalyst with the best characteristics is BG/WSNs, in which the support has a centrally radial pore structure and a hierarchical trimodal micromesoporous pore size. This peculiar morphology allows the enzyme to settle in a place where interaction with the walls is maximized, increasing its

conformational rigidity. In addition, the enzyme is predominantly localized inside the pores, so that the pores do not close completely and there is substrate access.

In [25, 26], the authors used polyporous biochar in combination with a magnetic particle γ-Fe_2O_3 for cellulase adsorption.The behavior of cellulase adsorption showed that the endothermic process proceeds more easily at high temperatures (studied at 20, 35 and 50 °C, respectively), which leads to a high degree of adsorption. Considering that the pore size was from 2.7 to 3.6 nm, and Cellulase is an elongated object, and the horizontal and vertical are 12.4 and 3.7 nm, which is larger than the average pore size, then adsorption occurs on the surface.

An interesting work is presented by the authors [27]. The main idea is to test iron oxide nanoparticles with different degrees of oxidation, which affects the binding behavior of enzymes with nanoparticles and especially a significant difference in enzymatic activity. The authors believe that the binding is mainly based on electrostatic interactions and coordination bonds of carboxylic acids with the surface of iron oxide nanoparticles. The authors synthesized nanoparticles of magnetite, semi-oxidized magnetite and maghemite (Figure 2). It turned out that nanoparticles based on magnetite as a support material with the highest binding capacity demonstrate the lowest activity (1.52 U/g). Nanoparticles based on semi-oxidized magnetite and maghemite demonstrate significantly higher activity with 5.82 U/g and 5.17 U/g, respectively. Using the XPS method, it was shown that the load on magnetite-based nanoparticles was also lower compared to more oxidized particles. The authors hypothesized that the explanation of the different behavior is the aggregation of the immobilized enzyme, which is strongly influenced by differences in support materials.

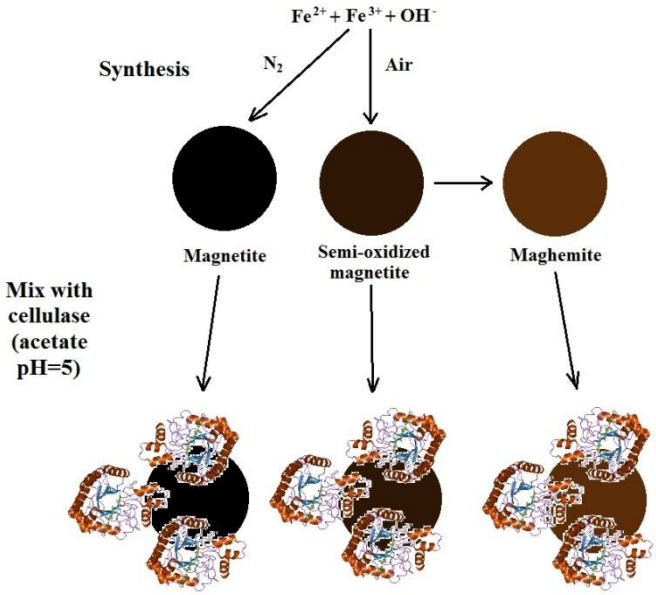

Fig. 2. Scheme of iron oxide nanoparticle synthesis and immobilization of cellulase

This hypothesis is confirmed by the analysis of the hydrodynamic diameter of the immobilized enzyme using an optical centrifuge. According to this method, the diameter of the immobilized enzyme increases with a decrease in the degree of oxidation, starting with magnetite (1450 nm), semi-oxidized magnetite (790 nm), maghemite (620 nm). This difference is a clear indicator of the dependence of the enzyme on the surface. The active center of cellulase usually consists of peptide domains rich in glutamic and aspartic acids, which are prone to coordination binding to magnetite surfaces. In all likelihood, the

interaction occurs so strongly that large aggregates of the "folded" enzyme are formed on the surface.

A simple approach to encapsulation of enzymes in nanogels is described in [28]. Nanogels were constructed by direct chemical crosslinking of water-soluble reactive copolymers of poly(N-vinylpyrrolidone-co-N-methacryloxysuccinimide) with proteins such as enhanced green fluorescent protein (EGFP) and cellulase in the "water in oil" emulsion (Figure 3).

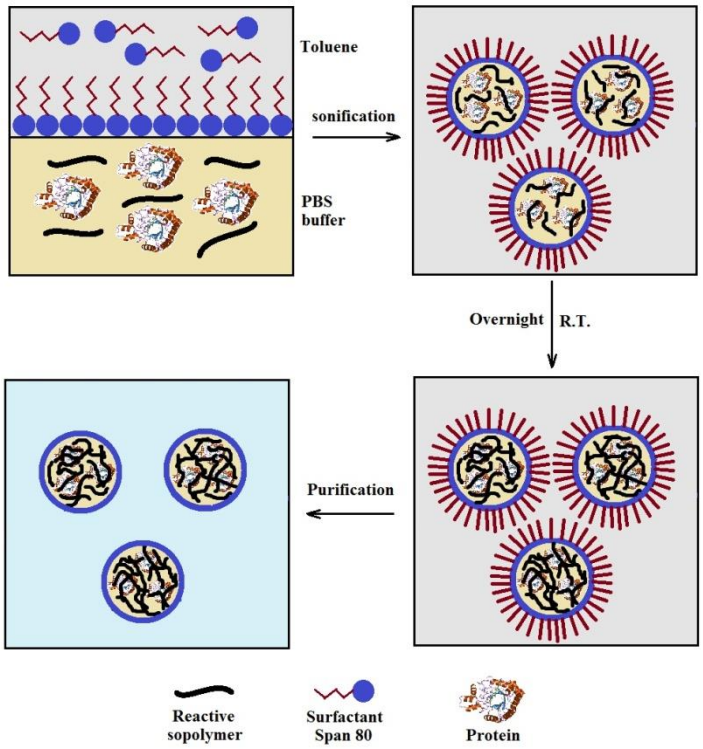

Fig. 3. Synthetic procedure to obtain biohybrid nanogels via cross-linking in "water in oil" emulsion

A study based on the resonance transfer of fluorescence energy (Fluorescence resonance energy transfer FRET) showed that biohybrid nanogels with a lower degree of crosslinking provide a higher rate of substrate transport, facilitating the substrate's access to the active center of the enzyme. Circular dichroism CD spectra showed that the proteins were chemically bound in the nanogel grid with a change in the secondary structure. The effect on the secondary structure of the enzyme was enhanced by increasing the density of cross-links. Proteins captured in nanogels with a lower degree of crosslinking were in a more expanded state, which led to higher enzymatic activity. Biohybrid nanogels have demonstrated significantly improved stability in maintaining enzymatic activity compared to free cellulase. Functional biohybrid nanogels with regulated enzymatic activity and improved stability are promising candidates for application in the fields of biocatalysis, biomass conversion or energy utilization.

The authors [29] studied the immobilization of cellulase on poly (methyl methacrylate) (PMMA) nanoparticles by mini-emulsion polymerization. The immobilization of the enzyme and the synthesis of polymer nanoparticles (support) occur simultaneously. The

type of surfactant (nonionic and ionic) and the pH of latex showed a great influence on the activity of cellulase. High activity values were obtained only with the simultaneous use of a nonionic surfactant (Lutensol AT50) and a buffer agent ($NaHCO_3$). The presence of cellulase did not affect the rate of polymerization of MMA and the final conversion of the monomer. The maximum immobilization efficiency (60%) was obtained using 6 wt.% cellulase and obtaining stable nanoparticles of PMMA (133 nm). The authors showed that the concentration of cellulase affects its distribution on the surface of PMMA nanoparticles. It was shown that there is a saturation point at which the surface of the nanoparticle is completely covered by the enzyme and the packing coefficient is maximal. With an increase in the concentration of the enzyme above this point, the effectiveness of immobilization decreases.

3 Nanostructured supports for cellulase immobilization

The main nanostructured supports used for cellulase immobilization include nanoporous materials (MOF, biochars, porous silica, etc.), nanohydrogels, polymer NP, magnetic NP, etc. Most of these substrates have been used for many years to immobilize enzymes, but over the past five years we can observe innovations in the manufacture or modification of these nanomaterials for better adaptation to the load of cellulase and its functioning.

Porous materials with different pore sizes, including hierarchical porosity, were studied for the immobilization of cellulase. New wrinkled mesoporous silica nanoparticles with radial and hierarchical structures of open pores were developed and used for enzyme immobilization [30]. By varying the (smaller, WSN, and larger, WSN-p) distance between the folds, which, in turn, depended on the conditions for obtaining NP silica, the authors were able to successfully adsorb BG and cellulase, most likely due to hydrogen bonds, without damaging the secondary structures of the enzyme [30]. Despite the absence of chemical bonds between the enzymes and the substrate, the nanobiocatalysts demonstrated high stability, probably due to the combination of a large surface area and specific pores/folds trapping enzymes.

The combination of a mesoporous Fenton catalyst (Fe-CM-48) and cellulase immobilized on a single support makes it possible to effectively depolymerize chitosan [31]. This achievement demonstrates an innovation in the use of very different catalysts on the same substrate and in the same complex process. There are only a few examples of nanogels in the modern literature, although they seem to have a clear advantage: the swollen state of the hydrogel can provide better access to immobilized cellulase, thereby increasing enzymatic activity. For cellulase adsorption, a poly (acrylic acid)-based nanogel obtained by reverse-phase microemulsion polymerization was used [32]. It has demonstrated high temperature resistance, maintaining 75% activity at 80°C, as well as higher pH tolerance. A hybrid nanogel substrate in which PANI nanorods were formed in situ inside a nanogel obtained by electrospinning was discussed in the section on covalent bonding [19]. In this work, a higher activity of the immobilized enzyme was observed in the same temperature range compared to the free enzyme, but the immobilized cellulase demonstrated higher thermal stability and storage stability. Nanohydrogels were formed by grafting carboxymethyl cellulase to acrylic polymers in the presence of GO sheets, whose role was to provide double crosslinking through hydrogen bonds [33]. After encapsulation of cellulase, the nanobiocatalyst was used for enhanced hydrolysis of lignocellulose biomass and showed a significant increase in the conversion of beet pulp treated with alkali. We believe that an additional advantage of nanogels can be realized when they react to pH or temperature, which allows the removal of hydrolysis products that may linger in the nanogel.

Polymer particles can be useful for surface covalent attachment of enzymes if polymers have functional groups. Polymer NPS were obtained from a cross-linked styrene and maleic anhydride copolymer using precipitation polymerization without a stabilizer, followed by covalent addition of cellulase via anhydride groups [34]. This hierarchical structure made it possible to expose cellulase to the reaction medium and preserve the conformation of cellulase due to the soft substrate. Chitosan-cellulase nanohybride was obtained by self-assembly of chitosan in the presence of cellulase, followed by immobilization on alginate granules [35].

Magnetically responsive nanostructured supports are usually based on magnetic nanoparticles. The use of magnetic nanoparticles for the development of nanobiocatalysts has increased dramatically in recent years due to the simplicity of magnetic separation, which allows the repeated use of nanobiocatalysts and makes the processes more reliable, economically and environmentally friendly. Magnetic nanoparticles (most commonly iron oxide nanoparticles) are usually functionalized to provide enzyme attachment. To achieve this, such nanoparticles are either coated with silicon dioxide with subsequent addition of functional (amino groups), or with a polymer containing reactive groups, for example, chitosan or other functional polymers. The addition of metal ions (for example, copper) to aminofunctionalized magnetic LPS makes it possible to improve the immobilization of cellulase due to metal affinity [36]. For better protection of iron oxide nanoparticles Purakbar et al. a gold shell around magnetic nanoparticles was used, followed by a silica shell and functionalization with PEG and L-aspartic acid for covalent addition of cellulase [37]. Another way to synthesize a magnetic nanobiocatalyst is realized by introducing magnetic nanoparticles into porous or polymer materials [38]. Even pure magnetite nanoparticles were used for cellulase adsorption [39] or after functionalization with glutaraldehyde [40].

When Fe_3O_4 nanoparticles coated with SiO_2 were additionally functionalized with a copolymer shell consisting of poly(N-isopropylacrylamide-co-glycidyl methacrylate) P(NIPAM-GMA), additional possibilities for configuring the nanobiocatalyst opened up [41]. PGM provides a covalent attachment of cellulase, while PNIPAM is a thermosensitive polymer that allows you to control swelling and delamination with temperature changes. GO sheets modified with PEG quadrilateral macromolecules containing amino-terminal groups were applied to Fe_3O_4 magnetic nanoparticles and used for cellulase immobilization [42]. A similar functionalization was investigated by the same group using exclusively magnetic NPS as support [43].

In another example, in order to minimize the number of Fe_3O_4 nanoparticles used, the authors [44] adsorbed them on MWCNTs and used them as a support for cellulase adsorption. Considering that MWCNTs are not a cheaper material than magnetite nanoparticles, the whole idea of this design seems questionable. To create a favorable substrate for the immobilization of cellulase, Papadopoulou et al. formed magnetic iron oxide nanoparticles in hierarchical porous carbons containing macropores (>50 nm), as well as interconnected meso- and micropores [45]. The authors investigated both covalent and non-covalent (adsorption) addition of cellulase and determined that covalent immobilization provides higher activity and stability during reuse. Magnetic (Fe_3O_4) nanoparticles coated with quaternized lignosulfonate and having pH-dependent properties were synthesized to immobilize and extract cellulase from bio-processing waste [46]. Cellulase was immobilized or desorbed when the pH changed due to electrostatic interactions. Schnell et al. A similar behavior was demonstrated based on electrostatic and coordination interactions on the surface of bare iron oxide nanoparticles with carboxylic acid groups of cellulase [27]. The authors also found that the ratio of Fe^{2+}:Fe^{3+} affects the loading and activity of the enzyme.

An interesting magnetic support was proposed by Raza et al. [47]. For its fabrication, the first hollow polymer particles were obtained by precipitation from biophenylpropene. This is followed by the addition of amino-functionalized Fe_3O_4 NPS with further modification by glutaraldehyde to form multilayer magnetic hollow particles for covalent attachment by cellulase.

Metal-organic frameworks (MOFs) with a magnetic core and a shell for cellulase immobilization were obtained by growing UIO-66-NH_2 on the surface of modified poly(4-sodium styrene sulfonate) Fe_3O_4 nanoparticles [48]. This substrate provided a high loading capacity of the enzyme and demonstrated high pH and thermal stability, as well as better tolerance of formic acid and vanillin, which are common inhibitors of intermediates of lignocellulose hydrolysis.

The original approach was proposed by Tan et al. to obtain a nanocomposite with oriented cellulase on chitosan/Fe_3O_4 nanoparticles [49]. To achieve this goal, the authors mixed cellulase with cellulose (consumable matrix), tightly attaching the enzyme to the latter. The mixture was then immersed in chitosan, followed by the formation of magnetic nanoparticles on the periphery of chitosan and cellulose hydrolysis. As a result, a complete magnetically recoverable structure with cellulase in the stretched and most active conformation was obtained.

4 Conclusions

Nanobiocatalysts based on cellulase immobilized on nanostructured supports were mainly used for catalytic hydrolysis of biomass. An analysis of the latest trends presented in this review shows there have been impressive innovations in immobilization methods and support structures. Covalent attachment is often preferred for cellulase immobilization because it provides increased stability. Covalent immobilization, however, requires the functionalization of the support and a suitable linker is needed to preserve the conformation of the enzyme. The adsorbed cellulase was significantly stabilized and activated due to the modification of the carrier by macromolecules that change the charge, or macromolecules that change the balance of hydrophobicity and hydrophilicity.

Finally, ways to optimize the contact between immobilized cellulase and cellulosic biomass with the help of stimulus-responsive materials seem favorable for further development of nanobiocatalysts for processing cellulosic biomass.

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References

1. V. Califano, A. Costantini, Catalysts, **10**, 706 (2020).
2. J. Zdarta, A. Meyer, T. Jesionowski, M. Pinelo, Catalysts, **8(2)**, 92 (2018).
3. A.B. Muley, K.H. Mulchandani, R.S. Singhal, Methods Enzymol. **630**, (2020).
4. U. Guzik, K. Hupert-Kocurek, D. Wojcieszynska, Molecules, **19, (**2014).
5. Nisha Singh , B.S. Dhanya, Madan L. Verma, Materials Science for Energy Technologies, **3** (2020).
6. A.M. Sulman, V.G. Matveeva, L.M. Bronstein, Nanomaterials, **12,** 3796, (2022).
7. P. Zucca, E. Sanjust, Molecules, **19**, (2014).
8. D. Brady, J. Jordaan, Biotechnol Lett. **31**, (2009).
9. E.T. Hwang, M.B. Gueng, Life Sci. **13**, (2013).

10. C. Mateo, JM. Palomo, G. Fernandez-Lorente // Enzyme Microb Technol. **40**, (2007).
11. A. Salis, M. Pisano, M. Monduzzi, J Mol Catal B: Enzym. **58**, (2009).
12. J. Pedroche, M. del Mar, C. Yust Mateo, Enzyme Microb Technol. **40**, (2007).
13. R. Ahmad, S.K. Khare, Bioresour. Technol. **252**, (2018).
14. A. Amaro-Reyes, A. Diaz-Hernandez, J. Gracida, B.E. Garcia-Almendarez, M. Escamilla-Garcia, T. Arredondo-Ochoa, C. Regalado, Catalysts, **9**, 966, (2019).
15. T. Zheng, L. Yang, M. Ding, C. Huang, J.Yao, Bioresour. Technol. **344,** (2022).
16. H. Mo, J. Qiu, C. Yang, L. Zang, E. Sakai, J. Chen, Enzyme Microb. Technol. **138,** (2020).
17. P. Kaur, M.S. Taggar, A. Kalia, Biomass Convers. Biorefin. **11,** (2021).
18. Y.-Y. Huang, P. Zhan, F. Wang, L.-S. Shao, L. Zhang, Y. Qing, J.-N. Chen, Chem. Pap. **76**, (2022).
19. A. Zarei, F. Alihosseini, D. Parida, R. Nazir, S. Gaan, ACS Appl. Mater. Interfaces, **13,** (2021).
20. J. Gao, C.-L. Lu, Y. Wang, S.-S. Wang, J.-J. Shen, J.-X. Zhang, Y.-W. Zhang, Catalysts, **8**(5), 180 (2018).
21. Z. Zou, E. Gau, I. El-Awaad, F. Jakob, A. Pich, U. Schwaneberg, Bioconjugate Chem. **30**, (2019).
22. Baiyi Chen, Jianhui Qiu, H. Mo, Yanling Yu, Kazushi Ito, E. Sakai, H. Feng, **41**(17), (2017).
23. A. Costantini , V. Venezia , G. Pota , A. Bifulco , V. Califano , F. Sannino, Nanomaterials, **10**, (2020).
24. V. Califano, A. Costantini, B. Silvestri, V. Venezia, S. Cimino, F. Sannino, Pure Appl. Chem. **91**(10), 2019.
25. H. Mo, Jianhui Qiu, Polymers, **12**, (2020).
26. H. Mo, Jianhui Qiu, . Chao Yang, L. Zang . E. Sakai, Cellulose, **27**, (2020).
27. F. Schnell, M. Kube, S. Berensmeier, S.P. Schwaminger. ChemNanoMat. **5**, (2019).
28. H. Peng, K. Rübsam, F. Jakob, U. Schwaneberg, A. Pich. Biomacromolecules, **17**, (2016).
29. P. Simon, J.S. Lima, A. Valério, D. de Oliveira, P.H. Araújo, C. Sayer, A.A.U. de Souza, S.M.A. G.U. de Souza, Brazilian Journal of Chemical Engineering, **35**(2) (2018).
30. G. Pota, A. Sapienza Salerno, A. Costantini, B. Silvestri, J. Passaro, V. Califano, Langmuir, **38**, (2022).
31. H. Geng, Z. Mou, Z. Liu, F. Li, C. Yang, Catalysts, **10**, 604 (2020).
32. I.N. Ahmed, R. Chang, W.-B. Tsai, Colloids Surf., B, **152**, (2017).
33. S. Ariaeenejad, E. Motamedi, G. Hosseini Salekdeh, Carbohydr. Polym. **230**, (2020).
34. Y. Wang, D. Chen, G. Wang, C. Zhao, Y. Ma, W.Yang, Chem. Eng. J. (Amsterdam, Neth.), **336**, (2018).
35. K. Saha, P. Verma, J. Sikder, S. Chakraborty, S. Curcio, Renewable Energy, **133**, (2019).
36. M. Abbaszadeh, P. Hejazi, Food Chem. **290**, (2019).
37. E. Poorakbar, A.A. Saboury, B. Laame Rad, K. Khoshnevisan, Protein J., **39**, (2020).

38. N. Jannah Sulaiman, A.F. Mansor, R.A. Rahman, R.M. Illias, S.M. Shaarani, Chem. Eng. Technol., **42**, (2019).
39. F. Schnell, M. Kube, S. Berensmeier, S.P. Schwaminger, ChemNanoMat. **5**, (2019).
40. A.P. Ingle, J. Rathod, R. Pandit, S.S. da Silva, M. Rai, Cellulose (Dordrecht, Neth.), **24**, (2017).
41. J. Han, J. Rong, Y. Wang, Q. Liu, X. Tang, C. Li, L.Ni, Bioprocess Biosyst. Eng. **41**, (2018).
42. J. Han, P. Luo, Y. Wang, L. Wang, C. Li, W. Zhang, J. Dong, L. Ni, Int. J. Biol. Macromol. **119**, (2018).
43. J. Han, L. Wang, Y. Wang, J. Dong, X. Tang, L. Ni, L. Wang, Biochem. Eng. J. **130**, (2018).
44. H.A.N. Yasmin, S. Naresh, B. Kunasundari, S.H. Shuit, Chem. Pap.**76**, (2022).
45. A. Papadopoulou, D. Zarafeta, A.P. Galanopoulou, H. Stamatis, Protein J. **38**, (2019).
46. R.-j. Dong, D.-f. Zheng, D.-j. Yang, X.-q. Qiu, Bioresour. Technol. **294**, (2019).
47. S. Raza, X. Yong, J. Deng, Microporous Mesoporous Mater. **285**, (2019).
48. B. Qi, J. Luo, Y. Wan, Bioresour. Technol. **268**, (2018).
49. L. Tan, Z. Tan, H. Feng, J. Qiu, New J. Chem. **42**, (2018).