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Review

A General Overview of Support Materials for Enzyme Immobilization: Characteristics, Properties, Practical Utility

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Abstract: In recent years, enzyme immobilization has been presented as a powerful tool for the improvement of enzyme properties such as stability and reusability. However, the type of support material used plays a crucial role in the immobilization process due to the strong effect of these materials on the properties of the produced catalytic system. A large variety of inorganic and organic as well as hybrid and composite materials may be used as stable and efficient supports for biocatalysts. This review provides a general overview of the characteristics and properties of the materials applied for enzyme immobilization. For the purposes of this literature study, support materials are divided into two main groups, called *Classic* and *New materials*. The review will be useful in selection of appropriate support materials with tailored properties for the production of highly effective biocatalytic systems for use in various processes.

Keywords: enzymes; enzyme immobilization; inorganic and organic supports; hybrid materials; biocatalysts and bioprocesses

1. Introduction

Enzymes are well-known as highly effective and efficient catalysts of a wide variety of processes characterized by high selectivity and activity. Additionally, enzymes may reduce the number of reaction steps and quantities of hazardous solvents needed and thus make a process more inexpensive and environmentally friendly [1]. For these reasons enzymes have become extremely important catalysts which exhibit great potential in many practical applications in industries ranging from food to pharmaceuticals [2]. The use of enzymes in multiple catalytic processes has resulted in studies leading to significant improvement of the enzyme properties. One of the most important and widely used techniques is enzyme immobilization in which catalysts are attached to a solid support that is insoluble in the reaction mixture [3]. The greatest advantage of immobilization is that it significantly improves the stability of the biomolecules under various reaction conditions and enhances the reusability of biomolecules over successive catalytic cycles [4]. Moreover, after binding the enzyme molecules, the catalysts change from a homogeneous to a heterogeneous form, which facilitates simple separation of the biocatalytic system from the reaction mixture and results in products of higher purity [5,6]. Various immobilization techniques have been developed, including adsorption, covalent binding, entrapment, encapsulation and cross-linking [7]. These differ in the type and character of the interactions formed and in the form and type of the support materials used. Selection of the most appropriate immobilization method and support material depends strongly on the type and conditions

of the catalytic process as well as the type of the enzyme [8]. However, it should be emphasized that the selection of the support materials is the most crucial challenge due to the major impact the support material may have on the properties of the biocatalytic system.

A very broad variety of materials of various origins can be used as supports for enzyme immobilization. These materials may, in general, be divided into organic, inorganic and hybrid or composite. The support should protect the enzyme structure against harsh reaction conditions and thus help the immobilized enzyme to retain high catalytic activity [9]. Moreover, use of a suitable material, for example hydrophobic carriers in lipase immobilization, may additionally increase the activity of the biocatalyst [10,11]. However, there are some limitations in this area, because the matrix must not have a negative effect on the structure of the enzyme and should not disturb the enzyme more than is required to create stable enzyme–matrix interactions. Additionally, there should be affinity between the functional groups of the two materials to allow the formation of these enzyme–matrix interactions and effective binding of the enzyme to the support. This is particularly important in the case of covalent immobilization [12]. The carrier should expose the active sites of the catalyst for easy attachment of substrate molecules and to reduce diffusional limitations of the substrates and products [13]. The main required features of support materials for effective enzyme immobilization are summarized in Figure 1.

Figure 1. Main features of support materials used for enzyme immobilization.

Nevertheless, it should be remembered that the appropriate selection of a matrix is directly related to the type of enzyme and to the process in which the biocatalytic system will be used. For the purposes of this literature review, the materials used as supports for enzyme immobilization have been classified as *Classic materials* (Section 2), which are the most commonly used and *New materials*, which offer especially desirable properties (Section 3). The latter type is also currently used

in immobilization and not only allow effective enzyme binding but also increase the applicability of the resulting biocatalytic systems.

2. *Classic Support Materials* for Enzyme Immobilization

Since the beginning of the work to develop the immobilization techniques, there has been a need to define a group of materials to which enzymes may be attached. In general, materials have been sought which offer high stability, availability, relatively low price and high affinity to the bound enzymes. A wide variety of materials of both inorganic and organic origin have been evaluated as effective supports for biocatalysts and are classified for the purposes of this review as *Classic materials* (Figure 2). Although *Classic materials* have been less frequently applied in recent years, they remain an important group of materials used for the immobilization of enzymes.

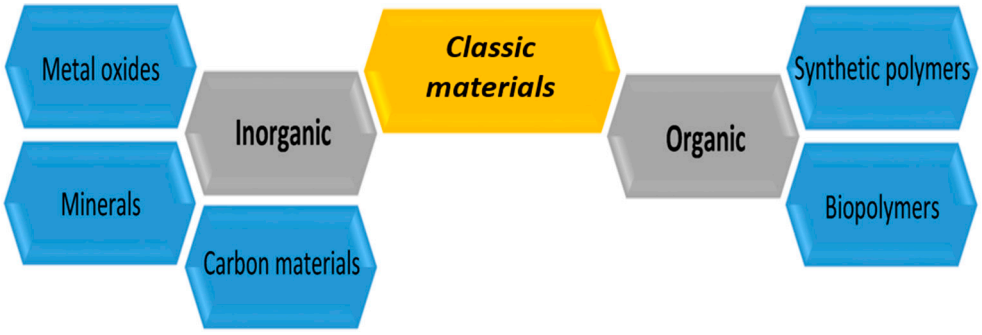

Figure 2. Selected examples of *Classic materials* of inorganic and organic origin used for enzyme immobilization.

2.1. Inorganic Materials

2.1.1. Silica and Inorganic Oxides

Silica is one of the most frequently used inorganic support materials for enzyme immobilization. Its high thermal and chemical resistance and good mechanical properties make it a suitable material for many practical applications. Silica offers good sorption properties due to its high surface area and porous structure. These properties allow effective enzyme attachment and reduce diffusional limitations [14,15]. Moreover, the presence of many hydroxyl groups on the surface of silica facilitates enzyme attachment and favours its functionalization with surface modifying agents such as glutaraldehyde or 3-aminopropyltriethoxysilane (APTES) [16]. Another advantage of this material is that it can be used in many different forms. Enzymes belonging to many catalytic classes, for example oxidoreductases, transferases, hydrolases and isomerases, have been immobilized with the use of sol-gel silica, fumed silica, colloidal silica nanoparticles and silica gel as supports [17–21]. The biocatalytic systems obtained demonstrate high catalytic activity retention and good thermal and pH resistance. For example, lipases immobilized on a silica gel matrix and on mesoporous silica retained respectively 91% and 96% of the activity of the free enzyme [22,23].

In previous studies, among other inorganic oxides, titanium, aluminium and zirconium oxides have also been used for the immobilization of many enzymes, for example lipase, cysteine, urease and α-amylase [24–27]. These supports are known for their high stability, mechanical resistance and good sorption capacity. Moreover, these materials are inert under various reaction conditions, which facilitates their application as supports for various classes of enzymes. Due to the presence of many hydroxyl groups on their surface, these materials are highly hydrophilic; this enhances enzyme immobilization and surface modification modified that favours the formation of relatively stable enzyme–matrix interactions.

2.1.2. Mineral Materials

Minerals are also used as support materials to produce recoverable biocatalytic systems with enhanced enzyme stability under reaction conditions. They are abundant in nature, are easily available, offer high biocompatibility and can be used as obtained without further advanced treatment and purification, which makes them relatively cheap [28]. Moreover, the presence of many functional groups (such as –OH, COOH, C=O, –SH, $–NH_2$) on the surface of the minerals allows the formation even of covalent bonds between the enzyme and the support and facilitates modification of the minerals. When additional functional groups are introduced, the adhesion area and hydrophobicity of the support increases while steric hindrances may be reduced [29]. The minerals used as supports for enzyme immobilization are mainly clay materials such as bentonite, halloysite, kaolinite, montmorillonite and sepiolite [30–32] though the group also includes the mineral hydroxyapatite known as calcium apatite [33,34]. In theory, enzymes belonging to many catalytic classes can be attached without limitation to the surface of mineral materials but in practice the most often immobilized are lipases, α-amylases, tyrosinases and glucose oxidases. Enzymes immobilized on minerals are used mainly in environmental engineering for waste and wastewater treatment as well as in biosensors to improve linear range and detection limit [35]. For example, according to Chrisnasari et al., glucose oxidase immobilized on bentonite modified by tetramethylammonium hydroxide retains over 50% of its initial activity after five repeated catalytic cycles [36].

2.1.3. Carbon-Based Materials

Carbon-based materials such as activated carbons and unmodified and modified charcoals have been used as effective and valuable support materials in enzyme immobilization, especially during the last two decades. The well-developed porous structure of these materials, with pores of various sizes and volumes and the high surface area (up to 1000 m^2/g) mean that these materials contain numerous contact sites on their surface for enzyme immobilization [37]. High adsorption capacity, the abundance of many functional groups and minimal release of fine particulate matter make carbon-based materials suitable carriers for the adsorption immobilization of various enzymes [38]. For example, unmodified charcoal support was used for the immobilization of amyloglucosidase. The immobilized enzyme when used for starch hydrolysis without any additional treatment retained over 90% of the free enzyme catalytic activity [39]. According to Silva et al. use of activated carbon for adsorption attachment of pancreatin allows a total immobilization yield that results in the creation of biocatalytic systems with good catalytic properties [40].

2.2. Organic Materials

It is well known that there is no universal support material suitable for all enzymes for all of their applications. Inorganic carriers have certain limitations, such as limited biocompatibility, lower affinity to biomolecules and reduced possibilities to create various geometrical shapes. Moreover, a cross-linking agent such as glutaraldehyde is usually required to create covalent bond between the enzyme and an inorganic support. Due to these reasons, some materials of organic origin are also used for the immobilization of various enzymes under different immobilization protocols. In general, organic support materials can be divided into two groups: (i) synthetic materials (mainly polymers) and (ii) renewable materials obtained from natural sources (biopolymers). Both groups have been widely used since the beginning of enzyme immobilization for the attachment of different types of biocatalysts.

2.2.1. Synthetic Polymers

The greatest advantage of synthetic polymers as support materials is that the monomers that build the polymeric chain can be selected according to the requirements of the enzyme and process in which the product of immobilization will be used [41,42]. The type and quantity of the monomers

determine the chemical structure and properties of the polymer. The composition of the monomers strongly affects the solubility, porosity, stability and mechanical properties of the polymer. A chemical feature that is directly related to the monomer structure is the presence of reactive chemical moieties in the polymeric chain. A very wide range of verified chemical functional groups may be observed in the structure of polymers. They include, for example, carbonyl, carboxyl, hydroxyl, epoxy, amine and diol groups, as well as strongly hydrophobic alkyl groups and trialkyl ammine moieties [43,44]. These groups facilitate effective enzyme binding and also functionalization of the polymer surface. The type of functional groups determines whether the enzyme is anchored to the matrix via for example adsorption or by the formation of covalent bonds, since it is mainly these two types of immobilization that take place when synthetic polymeric supports are used. Additionally, the type and quantity of functional groups determine the hydrophobic/hydrophilic character of the matrix and therefore its ability to form polar or hydrophobic interactions with the enzyme [45]. Moreover, by using polymeric supports, control of the length of the matrix–enzyme spacers has been achieved. Longer spacers allow the enzyme to retain higher conformational flexibility, while shorter spacers can protect the biomolecules against thermal inactivation and reduce leaching of the enzyme [46].

Various polymer materials can be used as effective supports and improve properties of the immobilized enzyme such as thermal stability and reusability. The polymer layers play a very important role in protecting the active sites of the enzyme from negative effects of the ingredients of the reaction mixture and the process conditions. However, it should be noted that synthesis of a polymer with the desired properties and functional groups is usually a time-consuming and costly process. Different polymers containing various functional groups have been used for enzyme immobilization. For example, α-amylase was covalently immobilized on polyaniline via –NH groups, while tyrosinase was immobilized via –NH and C=O groups on polyamide 66 (Nylon 66) without any linkers [47,48]. In another study, commercial lipase was immobilized by covalent binding on strongly hydrophobic polystyrene microspheres activated by epoxy groups [49]. In a hydrophobic environment, lipase exhibits extremely high catalytic properties which are related to the phenomenon called interfacial activation. Furthermore, polyurethane foam has been used for covalent immobilization of inulinase [50]. Bai et al. used polyvinyl alcohol modified by glutaraldehyde as a support for the immobilization of laccase via –OH groups. After immobilization, as a result of the strong interactions, the product was characterized by good storage stability and reusability which make it suitable for use in biosensors to detect bisphenol A [51]. Glucose oxidase, an antimicrobial enzyme, was immobilized on amino- and carboxyl-plasma-activated polypropylene film. The introduction of these groups enhanced the affinity of the polymer to the enzyme [52]. Commercially available ion exchange resins—for example Amberlite and Sepabeads—have also been used, respectively, for the immobilization of enzymes such as α-amylase and alcohol dehydrogenase [53,54].

2.2.2. Biopolymers

An alternative to the use of synthetic polymers as matrices for enzymes is the use of biopolymers—polymers of natural origin. Biopolymers include carbohydrates but also proteins such as albumin and gelatin [55]. Materials such as collagen, cellulose, keratins and carrageenan as well as chitin, chitosan and alginate are examples of biopolymers used for immobilization [56–59]. Biopolymers possess a unique set of properties, from biodegradability to harmless products, biocompatibility and non-toxicity, to an outstanding affinity to proteins, which make them suitable supports for enzymes [60]. Their natural origin and biocompatibility minimizes their negative impact on the structure and properties of enzymes and thus the immobilized proteins retain high catalytic activities. Furthermore, the availability of reactive functional groups in their structure—mainly hydroxyl but also amine and carbonyl moieties—enables direct reaction between the enzyme and matrix and facilitates modification of their surface [61]. Above all, however, these materials are renewable and easy to obtain; in many cases they are by-products of various industries, which makes them inexpensive and reduces the costs associated with the immobilization process [62]. Biopolymers

are used for immobilization by adsorption and covalent binding; however, their ability to create various geometrical configurations and propensity for gel formation mean that they are also used for immobilization by encapsulation and entrapment.

Chitosan on the basis of a literature survey can be considered the most frequently used biopolymer for enzyme immobilization. Chitosan can be applied in various forms and shapes. For example, Shi et al. used chitosan microspheres cross-linked by glutaraldehyde for the immobilization of nuclease, which is an important enzyme in genetic engineering [63]. In another study, glucose isomerase was adsorbed in macroporous chitosan beads prepared by chelation with various metal ions [64]. As reported by Kim et al. cellulose nanocrystals obtained from cotton linter cellulose can be used for immobilization by non-specific adsorption interactions of *Candida rugosa* lipase with high loading efficiency [65], whilst lipase was immobilized by entrapment by Tumturk et al. using κ-carrageenan hydrogels [66]. Vegetable and marine sponges characterized by an open fibrous network that reduces diffusional limitations have also been used as matrices for the immobilization of lipases, mainly via hydrogen bonds [67,68]. It may be concluded that biopolymers can be used to immobilize enzymes belonging to various catalytic classes with the retention of good catalytic properties. Moreover, the produced biocatalytic systems offer improved thermal stability and in general are noted for their good reusability.

Special attention should also be paid to alginates. Their remarkable abilities for gelation, mainly using sodium or calcium ions and for the creation of capsules in which single or multiple enzymes can be immobilized, mean that these materials are used principally for encapsulation and entrapment [69]. However, due to the relatively low mechanical stability of alginate gels and diffusional limitations in the transport of the substrates and products, their utilization in immobilization is restricted to a few applications only [70]. For example, Betigeri and Neau immobilized lipase in calcium alginate beads by entrapment, while Kocaturk and Yagar encapsulated polyphenol oxidase in copper alginate beads and obtained high immobilization yields [71,72]. The products exhibited good catalytic activity retention but their reusability was poor due to leaching of the enzyme from the matrix.

Agarose is a popular choice among biopolymers for use in enzyme immobilization. This linear heteropolysaccharide biopolymer consists of β-D-galactose and 3,6-anhydro-α-L-galactose units, linked by β-1-4 glycosidic and α-1-3 glycosidic bonds [73]. Like alginates, agarose also exhibits great ability for gelation which can occur at temperatures of agarose solution below 35 °C, without addition of any ions and results in formation of highly ordered stable and rigid structures [74]. It is worth mentioning that the 3-D architecture of this hydrophilic material remains almost unaltered in the presence of various organic solvents and does not shrink or swell under such conditions [75]. The ability of agarose gel to form various forms, such as beads, capsules or fibres, has led to this organic support material being of the great interest for industrial applications. For example, Prakash and Jaiswal used agarose beads for simple physical entrapment of thermostable α-amylase. The practical application of the resulting biocatalytic system was tested for removal of starch stains from clothes and the reusability of agarose immobilized enzyme was found to be up to five cycles [76]. In another study, a cross-linked agarose bead support highly activated with aldehyde groups was applied for multipoint covalent attachment of the commercial enzyme Depol™ 333MDP (β-1,4-endoxylanase). A high immobilization efficiency of over 85% was observed and the immobilized enzyme showed significant improvement of thermal stability compared to free protein [77].

2.3. Summary of Classic Materials

Classic materials used for enzyme immobilization of both inorganic and organic origin have been described in the above sections. Inorganic support materials, such as inorganic oxides, minerals or carbon-based materials, are characterized mainly by good thermal and chemical stability as well as by excellent mechanical resistance. These materials are also known for their good sorption properties which are a result of their well-developed porous structure and usually high surface area that ensures numerous contact sites for effective enzyme immobilization. In contrast, synthetic polymers and

biopolymers, also grouped under *Classic materials*, offer numerous functional groups that facilitate even covalent binding of enzymes without cross-linking agents. Additionally, biopolymers are usually characterized by high protein affinity as well as biocompatibility that limits negative effects of the support on the structure of enzymes. Moreover, irrespective of their origin, *Classic materials* for enzymes immobilization are usually abundant in nature (mineral, biopolymers) or are easy to synthesize (inorganic oxides, synthetic polymers) which makes them relatively cheap. These facts have meant that these support materials still play an important role as carriers for use for immobilization of enzymes.

The *Classic materials* and types of enzymes that may be immobilized using of these supports are summarized in Table 1 together with information about immobilization type, cross-linking agents and binding group.

Table 1. Summary and selected examples of *Classic materials* of both inorganic and organic origin applied for enzymes immobilization.

Support Material	Binding Groups	Cross-Linking Agent	Immobilization Type	Immobilized Enzyme	Reference
Inorganic Materials					
Sol-gel silica	–OH	–	adsorption	lipase from *Aspergillus niger*	[20]
Silica gel	–OH, C=O	glutaraldehyde	covalent binding	commercial lipase	[22]
γAl_2O_3	–OH	–	adsorption	cysteine proteinases from *Solanum granuloso-leprosum*	[25]
ZrO_2	–OH	–	adsorption	α-amylase from *Bacillus subtilis*	[26]
Montmorillonite	–OH	3-aminopropyl-triethoxysilane	covalent binding	glucoamylase from *Aspergillus niger*	[31]
Hydroxyapatite	–OH	–	adsorption	glucose oxidase from *Aspergillus niger*	[34]
Bentonite	–OH, $–NH_2$	tetramethyl ammonium hydroxide	covalent binding	glucose oxidase from *Aspergillus niger*	[36]
Commercial activated carbon	–OH, C=O	–	adsorption	cellulose from *Aspergillus niger*	[37]
Activated charcoal	–OH, C=O, COOH	–	adsorption	papain	[38]
Activated charcoal	–OH, C=O, COOH	–	adsorption	amyloglucosidase	[39]
Organic materials					
polyaniline	–N–H, C=O	glutaraldehyde	covalent binding	α-amylase	[47]
polystyrene	C=O, epoxy groups	poly(glycidyl methacrylate)	covalent binding	lipase	[49]
poly(vinyl alcohol)	–OH, C=O	glutaraldehyde	covalent binding	laccase from *Trametes versicolor*	[51]
polypropylene	–OH	plasma activated	covalent binding	Glucose oxidase	[52]
Cellulose nanocrystals	–OH	–	adsorption	lipase from *Candida rugosa*	[65]
Luffa cylindrica sponges	–OH, C=O, COOH	–	adsorption	lipase from *Aspergillus niger*	[67]
chitosan	–OH, $–NH_2$	–	entrapment	lipase from *Candida rugosa*	[71]
agarose	–OH	–	entrapment	α-amylase	[76]

3. *New Support Materials* for Enzyme Immobilization

Possibilities for practical applications of immobilized enzymes are continuing to grow. For this reason, discovery and use of *New materials* with desired properties, tailored to particular enzymes, has recently become extremely important. These materials, of both organic and inorganic origin, are characterized by exceptional thermal and chemical stability and very good mechanical properties. Moreover, these support materials are produced in various morphological shapes with controllable particle sizes, usually at nanoscale, which make them suitable for use with enzymes. Furthermore, these materials possess remarkable quantities of various functional groups, corresponding to the chemical groups of the proteins, which enhance enzyme binding and surface modification [78]. However, particularly during the last decade, scientific attention has been directed towards hybrid and composite materials, which combine properties of both composite precursor types and thus maximize their advantages [79]. Hence, with the use of *New materials* (see Figure 3) as enzyme supports, control of the technological process is improved, the immobilized enzymes exhibit enhanced catalytic efficiency

and the purity and quality of the reaction products increase compared to the processes catalysed by enzymes immobilized using the *Classic materials*.

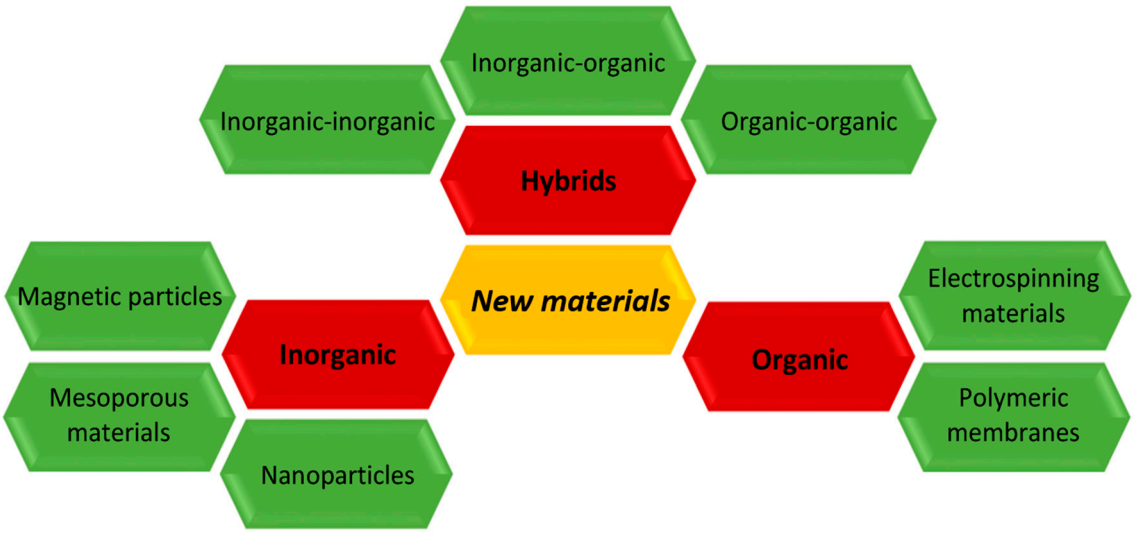

Figure 3. Selected examples of *New materials* of inorganic, organic and hybrid origin, applied for enzyme immobilization.

3.1. Inorganic Materials

3.1.1. Magnetic Particles

The separation of biocatalysts from the reaction mixture after the catalytic process is one of the crucial problems that must be solved when immobilized enzymes are used. One possible solution is attachment of the enzyme molecules to magnetic iron oxide nanoparticles (MNPs) and simple separation of the biocatalytic system with the use of an external magnetic field [80]. MNPs are also known for their large surface area and the abundance of hydroxyl groups on their surface which enables their easy modification and strong (covalent) binding of the enzyme. These are very important features. High mechanical stability and low porosity, however, which minimize steric hindrances, are also relevant for the creation of a stable enzyme–matrix biocatalytic system [81]. According to Netto et al. many enzymes grouped within the oxidoreductases, hydrolases or transferases can be immobilized on the surface of magnetic nanoparticles to create generally stable systems offering high reusability and easy separation from the reaction mixture [82]. There are several examples that demonstrate these advantages. Mehrasbi et al. immobilized lipase on magnetic nanoparticles functionalized with 3-glycidoxypropyltrimethoxysilane. The immobilized enzyme when used to catalyse the production of biodiesel from waste cooking oil, maintained 100% of its initial activity even after six reaction cycles [83]. In another study, Aber et al. immobilized glucose oxidase by adsorption on unmodified MNPs and used the resulting system for decolourization of Acid Yellow 12. After 15 catalytic cycles the immobilized glucose oxidase retained more than 90% of its initial properties [84]. Atacan et al. used magnetic nanoparticles, pre-treated with gallic acid, for the covalent immobilization of trypsin. Their results showed that the MNPs-trypsin biocatalyst can degrade bovine serum albumin with high efficiency [85].

3.1.2. Mesoporous Materials

A feature that distinguishes mesoporous supports from other materials used for enzyme immobilization is the possibility of tailoring the properties of the support to the biomolecules by adjusting the synthesis conditions and obtaining matrices with a desired pore structure [86,87].

These kinds of carriers contain mesopores with diameters of usually 2 to 50 nm, with a narrow and regular pore arrangement, surface areas as high as 1500 m^2/g and pore volumes of around 1.5 cm^3/g, which make them suitable supports for various biomolecules [88]. Enzymes can be immobilized on mesoporous materials by covalent binding or by encapsulation. In both techniques of immobilization, however, the enzyme is placed in the pores of the support, which means that the structure of the protein is protected and good catalytic properties are generally retained. When the enzyme is located in the pores of the carrier, diffusional limitations must also be taken into account because the transport of substrates and products is restricted [89]. Nevertheless, in view of their water insolubility, thermal and chemical stability, hydrophilicity and the presence of sufficient chemical groups for the binding of catalysts, mesoporous materials fulfil most of the requirements for effective enzyme matrices [90].

Materials such as zeolites, carbons and sol-gel matrices, as well as precipitated and ordered mesoporous oxides are included in the mesoporous group [91–93]. For example, various mesoporous silica materials are frequently used. Lipases from *Candida antarctica* and from *Candida rugosa* have been immobilized via non-specific interactions without any intermediate agents on SBA 15 and MCM 41 mesoporous silica, respectively and used as catalysts in organic synthesis [94,95]. The reusability of the immobilized enzymes was found to be significantly improved, because they could be reused for at least five reaction cycles without significant loss of their activity. SBA 15 and MCM 41 silicas were also used for adsorption immobilization of alkaline protease [96]. The immobilized enzyme attained its maximum loading (589.43 mg/g) when SBA 15 silica was used. The products of immobilization also had good pH and temperature stability. In another study, Mangrulkar et al. immobilized tyrosinase on the mesoporous silica MCM-41 and used the resulting biocatalytic system for the detection of phenol. The lowest concentration of phenol detectable by the immobilized tyrosinase was found to be 1 mg/L [97]. As mentioned above, mesoporous materials can also be used for enzyme encapsulation. Wang and Caruso used mesoporous silica spheres for the immobilization of catalase, protease and peroxidase and showed that after immobilization the lifetime of all tested enzymes was improved compared to their free forms [98].

3.1.3. Nanoparticles

Nanoparticles of both inorganic and organic origin with diameters of up to 30 nm have been extensively studied in recent years as potential supports for enzyme immobilization. However, for the purpose of this review, attention is focused on nanoparticles of inorganic origin as these materials are attracting growing interest due to the fact that they generally significantly improve the immobilization yield and the efficiency of the biocatalytic system obtained [99,100]. Nanoparticles provide a large surface area for enzyme binding that leads to higher loading of the enzyme on the matrix surface and increased immobilization yield. The greatest advantage, however, of nanoparticles over other inorganic materials is their ability to minimize diffusional limitations. Enzyme molecules are attached to the surface of the nonporous particles and their active sites are exposed for wide contact with substrates [101,102]. This means that biocatalytic systems based on nanoparticles usually provide high catalytic activity retention [103].

Various inorganic nanomaterials such as nanogold [104] and graphene [105] can be used as matrices for enzyme immobilization. Most frequently, however, inorganic oxide nanoparticles are used. For example, Hou et al. used titania nanoparticles for the immobilization of carbonic anhydrase by glutaraldehyde and immobilized over 160 mg of the enzyme per gram of the matrix. The product was used for biomimetic conversion of CO_2 [106]. In another study, lipase from *Rhizomucor miehei* was covalently immobilized on silica nanoparticles modified by octyltriethoxysilane and glycidoxypropyltrimethoxysilane. The immobilized lipase proved to be a very thermostable biocatalyst [107]. Notably, both biocatalytic systems achieved over 90% catalytic activity retention.

3.1.4. Ceramic Materials

Ceramic materials are known for their extremely high resistance to temperature, pressure and chemicals (organic solvents, bases, acids). These features make them very promising materials for use as supports for industrial applications of immobilized enzymes [108]. Ceramic supports also offer good mechanical stability. Hence, when the enzymes become catalytically inactive, they can be relatively easily regenerated and used for the immobilization of a new biocatalyst [109]. Hydroxyl groups are mainly present on the surface of these materials. This favours mainly adsorption immobilization of enzymes, based on non-specific interactions. For covalent attachment of biomolecules, additional surface modification is necessary. Ceramic materials such as alumina, zirconia, titania, silica, iron oxide and calcium phosphate have been used as biomolecule carriers. It should be added that these materials can also be used in the form of ceramic foam or composite (TiO_2/Al_2O_3) ceramic membranes [110,111].

Ebrahimi et al. physically immobilized β-galactosidase using a ceramic material in the form of a membrane as a support. The system was used to catalyse a transgalactosylation reaction of lactose to produce galactosyl-oligosaccharides. The immobilized enzyme was used for continuous production of oligosaccharides and its efficiency reached 40% under optimal process parameters [112]. Wang et al. immobilized horseradish peroxidase on a ceramic material (cordierite) which had been modified by *N*-β-amino-ethyl-γ-aminopropyl-trimethoxysilane for covalent attachment of the enzyme. The biocatalytic system was used to remove oil from wastewater. The highest recorded removal efficiency was close to 92% and the system exhibited good reusability [113].

3.1.5. Carbon Nanotubes

Carbon nanotubes are a new type of support material that has been more and more widely used in recent years. Both single-walled and multi-walled carbon nanotubes are characterized by an ordered, nonporous structure, large surface area and biocompatibility. Moreover, they exhibit outstanding thermal, chemical and mechanical resistance [114,115]. Carbon nanotubes are also relatively amenable to functionalization to further increase their affinity to enzymes and favour the formation of strong enzyme–matrix interactions [116]. Unlike other materials, carbon nanotubes enhance the transfer of electrons between the substrate and the immobilized enzyme. They are thus most frequently used for the immobilization of oxidoreductases and are applied in biosensors for the detection of various compounds such as phenol and its mono- and multi-substituent derivatives, bisphenols or pharmaceuticals, i.e., diclofenac or tetracycline. However, other groups of enzymes (transferases and hydrolases) have also been immobilized with the use of carbon nanotubes [117,118].

In one reported study, glucose oxidase was immobilized on carbon nanotubes and further cross-linked with chitosan. The results confirmed that the transfer rate of electrons was strongly enhanced [119]. In another study α-glucosidase was covalently immobilized on multi-walled carbon nanotubes functionalized by amine groups. The system so obtained was used in a biosensor for measuring the antidiabetic potential of medicinal plants [120]. The immobilized enzymes used in both biosensors exhibited greatly improved sensitivity and time of response and thus better detecting properties. The biosensors also offered relatively good storage stability as indicated by their activity which remained almost unaltered over 30 days.

3.1.6. Graphene and Graphene Oxide

Graphene and graphene oxide (GO) among carbon-based materials have also attracted great attention as support materials for enzymes. This interest reflects their unique features such as biodegradability, two-dimensional structure, high surface area and pore volume as well as good thermal and chemical stability [121]. Additionally, the presence of many various functional groups, such as carboxylic (COOH), hydroxyl (–OH) or epoxide groups, facilitates creation of strong enzyme-matrix interactions without linking agents or modification of the graphene surface [122]. Due to these features, enzymes like lipases [123] or peroxidases [124] can be immobilized on GO

surfaces mainly by adsorption, covalent binding or entrapment [125]. It is also worth mentioning that graphene-based materials may even enhance enzyme biocatalytic activity. Moreover, graphene-based supports are characterized by antioxidant properties and may enhance removal of free radicals (i.e., hydroxyl or dithiocyanate) from reaction mixtures [126]. This results in improved protection of enzyme molecules from inactivation.

For example, horseradish peroxidase (HRP) was covalently immobilized on reduced graphene oxide nanoparticles functionalized with glutaraldehyde. Kinetic parameters (turnover number (k_{cat}) and catalytic efficiency (k_{cat}/K_M)) of the immobilized enzyme increased after its attachment, thus demonstrating enhancement of the catalytic properties of the HRP. Moreover, reusability was also significantly improved, as indicated by immobilized enzyme which maintained over 70% of its initial activity after 10 catalytic cycles [127]. In another study, D-psicose 3-epimerase (DPEase) was immobilized on non-modified graphene oxide and applied for production of the rare sugar D-psicose, an epimer of D-fructose. After immobilization, the efficiency of biocatalytic conversion of D-fructose to D-psicose was improved. Thermal stability of immobilized DPEase was also significantly enhanced, as the graphene-bounded enzyme exhibiting a half-life of 720 min that was 180 times higher than the half-life of free D-psicose 3-epimerase (4 min) [128].

3.2. Organic Materials

As already mentioned, the properties of the immobilized enzyme are strongly dependent on the structure and characteristics of the matrix. In general, the organic materials classified here as *New materials* are mainly the same materials and containing very similar chemical groups as those described in Section 2.2. However, here these organic materials appear in completely different forms and as a result have very different properties. These materials may for example be used in the form of single particles, fibres of nanometre size, or membranes. These supports increase the performance, efficiency and stability of the immobilized biomolecules and render the latter more reusable. Moreover, control of the process is improved and processes can be carried out in a continuous manner, which makes them cost-effective [129]. Hence, biocatalytic systems based on these materials can become attractive for applications in industrial-scale processes. Selected cases of the use of *New materials* of organic origin for enzyme immobilization are discussed below.

3.2.1. Electrospun Materials

The great potential of electrospun materials as supports for enzyme immobilization results from their many functional and structural advantages. These materials are known for their length (electrospun nanofibers), uniformity of diameter and diversity of composition. They also have high porosity and surface areas, which lead to high enzyme loading [130]. The nanometre sizes of these materials provide additional benefits mainly related to low hindrance of mass transfer and reduced diffusional limitations. As a result, the efficiency of the immobilized enzyme can be increased. Electrospun support materials are also known for other useful properties, for example their biocompatibility, nontoxicity, biodegradability, high mechanical strength and hydrophilicity, which make them suitable matrices for various types of biocatalysts [131,132]. It should also be noted that due to the presence of various functional moieties on their surface, electrospun nanomaterials can be easily modified to favour enzyme attachment and increase the activity of the enzyme [133]. A very wide range of synthetic polymers such as poly(vinyl alcohol), polystyrene, polyacrylamide and polyurethane, as well as biopolymers such as chitin, chitosan, alginate and cellulose, may be used to produce electrospun carriers [134–136]. Their great advantage over other matrices is the fact that because of the variety of materials used to produce electrospun supports, they can be obtained with properties tailored to the enzyme and the process. Enzymes can be immobilized on these materials by adsorption and covalent binding (surface attachment) as well as by encapsulation carried out at the same time as the support is formed, which additionally reduces the costs of the process [137].

For example, Canbolat et al. used poly(ε-caprolactone) to immobilize catalase by encapsulation using layering methods. Moreover, to enhance the catalytic activity of the enzyme, its molecules were combined with cyclodextrin before immobilization. The results indicated that the addition of cyclic oligosaccharides had a positive effect on the catalytic properties of the biocatalyst. It should also be noted that immobilization by encapsulation results in higher stability of the catalase because the enzyme is protected from negative effects of the reaction conditions [138]. Weiser et al. used polyvinyl alcohol nanofibers for the immobilization by entrapment of five different types of lipase for the kinetic resolution of racemic secondary alcohols by acylation in inorganic media. The study proved that the activity of all tested lipases was enhanced by stabilization of the active conformation of the enzyme. Furthermore, after immobilization of the biomolecules, the turnover frequency of the reaction increased due to a reduction in mass transfer limitations, which was directly related to the structural properties of the polymeric nanofibers [139]. In another study, poly(vinyl alcohol) or polylactic acid nanofibers in the form of a membrane were used for adsorption immobilization of *Candida antarctica* lipase B. The biocatalytic system was used for the kinetic resolution of 1-phenylethanol and 1-phenyl acetate. Polyvinyl alcohol membrane with immobilized lipase may be used in both organic and water media; however, the lipase immobilized on polylactic acid fibres preserved higher activity and exhibited higher enantiomer selectivity. Nevertheless, due to the protection afforded by the nanofibers, both immobilized systems demonstrated excellent stability even over 10 reaction cycles [140].

3.2.2. Polymeric Membranes

As has been described above, polymeric materials can be used in enzyme immobilization in the form of beads, powders, fibres or foams. However, in recent years there has been growing interest in the use of commercially available polymeric membranes. This is mainly because they have easily tuneable properties which make them suitable for many groups of enzymes [141,142]. These membranes have a large surface area that allows efficient enzyme attachment. The membranes also offer good porosity as well as well-defined pore sizes and structure, which facilitate the immobilization of biomolecules not only on the surface of the support but also in its pores [143]. The reaction mixture passing through the membrane therefore has relatively easy access to the active sites of the enzyme, which reduces diffusional limitations. Moreover, membranes with tailored properties can easily be prepared in different shapes and various geometrical configurations [144]. The membranes are also generally insoluble and are known for their mechanical stability, hence they fulfil the requirements for performing as suitable carriers for enzyme immobilization.

Many types of synthetic polymers can be used for the preparation of membranes, for example poly(vinyl alcohol), polyurethane and poly(vinylidene fluoride) [145,146]. However, membranes made from biopolymers, for example from chitosan or cellulose, can also be used as enzyme support materials [147]. Depending on the type of material used, various functional groups are present in the membrane that not only favour effective enzyme immobilization but also enable modification of the membrane for covalent binding of biomolecules. It should also be noted that in addition to membranes obtained on a laboratory scale, commercially available ultra- and nanofiltration membranes can also be used for enzyme immobilization and examples such as GR51PP, NF270 and NTR7450 may be mentioned [148–150]. However, the greatest advantage of polymeric membranes as biomolecule support matrices, in comparison with other materials, is the fact that no additional separation and purification of the reaction mixture is required; the catalytic process can take place and products can be isolated from the reaction mixture in the same step [151]. It is also worth noting that polymeric membranes, as supports for enzymes, play a crucial role particularly in the case of enzymatic membrane reactors (EMR).

3.3. Hybrid and Composite Materials

In the light of the above-mentioned unique features and properties of inorganic and organic matrices, many studies have been carried out with the aim of combining them to maximize their

benefits. The possibility of selecting and combining precursors to meet the requirements of a given enzyme and the process in which it is to be used facilitates more precise control of the enzyme immobilization process. Additionally, the biocatalytic systems thus produced can be used in a wider range of practical applications [152]. Combined and reinforced materials usually exhibit properties not observed for their individual components. Hybrids and composites usually make it possible to stabilize the interactions between an enzyme and a support and make biocatalysts more mechanically resistant and stable under reaction conditions. It should be added that hybrid supports in general provide a suitable environment for biomolecules that favours the retention of high catalytic properties by the immobilized enzyme, makes the biocatalytic system reusable and protects it against conformational changes during storage [153]. An additional benefit of the use of composite supports is the fact that these materials are suitable for enzymes belonging to all catalytic classes. Hence, in the quest for support materials for enzyme immobilization, particular attention should be paid to hybrid or composite materials obtained by the conjugation of: (i) organic-organic; (ii) inorganic-inorganic, or (iii) organic-inorganic precursors.

3.3.1. Organic-Organic Hybrids

As described above, many types of polymers of synthetic and natural origin can be used as supports for enzymes. However, to increase their usability, they can be combined to obtain products with enhanced properties which are better suited to the enzyme and the technological process. Organic-organic hybrids may be synthesized by connecting together the following: (i) two synthetic materials, for example polyaniline and polyacrylonitrile, polyethyleneimine, with epoxy-activated acrylate copolymer or poly(acrylic acid) and polyvinyl alcohol [154–156]; (ii) a synthetic polymer with a biopolymer, such as poly(acrylic acid) and cellulose, polyvinyl alcohol and chitosan, or polyaniline and chitosan [157–159]; (iii) two biopolymers, such as chitosan and alginate, chitin and lignin or cellulose and dextran [160–162]. For instance, combining a synthetic polymer having good pH and thermal resistance and mechanical stability with a biopolymer known for its biocompatibility and high affinity to biocatalysts and using the resulting hybrid as an enzyme support, could result in a stable, reusable biocatalytic system with the retention of good catalytic properties [163]. Additionally, through appropriate selection of precursors, the hydrophobic/hydrophilic character of the matrix can be controlled to increase the strength of the interactions formed between the enzyme and support and to enhance the catalytic activity of the biomolecule [164]. The greatest advantage of these materials is the possibility of forming them into various shapes and sizes. The materials can be used as enzyme supports in the form of particles, fibres, tubes, beads, membranes or sheets [165–167]. The wide variety of available organic-organic hybrids makes these materials suited for immobilization of enzymes belonging to all catalytic classes by adsorption, covalent binding and also by entrapment or encapsulation [168,169]. For example, two monomers, polylactic acid and polyethylene glycol, were used to produce micro- or nanofibers by an electrospinning technique. The material thus produced had high porosity, a large surface area, the ability to incorporate functional additives, which are all excellent properties as a matrix for enzyme attachment. In the study, alkaline phosphatase was immobilized via biotin-streptavidin interactions on surface of the matrix. The bound enzyme exhibited good stability and reusability over a long storage time so this system may be a promising platform for the development of biosensors [170]. Polyvinyl alcohol–hypromellose is an example of an organic hybrid produced by merging a synthetic polymer with a semisynthetic biopolymer which is an inert derivative of methylcellulose. This material was used for the immobilization of lipase from *Burkholderia cepacia* without any cross-linkers. The resulting biocatalytic system was used for the synthesis of phenethyl butyrate in nonpolar medium and proved to be a successful catalyst, achieving 99% conversion of the phenethyl alcohol and vinyl butyrate used as substrates. The protective effect of the polymeric hybrid support resulted in the retention of high catalytic activity by the immobilized lipase and the activation energy of the reaction was found to be lower when the immobilized enzyme was used [171]. Matto and Husain employed a calcium alginate–starch hybrid gel as a carrier for

adsorption immobilization and entrapment of peroxidase. The presence of many hydroxyl groups in the starch structure enhanced the surface attachment of the enzyme, while the capacity of alginate for gelation favoured the entrapment of peroxidase. The entrapped enzyme was found to be significantly more stable against pH, temperature, solvents and inhibitors like urea or heavy metals, compared to the surface-immobilized enzyme. Moreover, this form of immobilized peroxidase retained over 70% of its original activity even after seven repeated reaction cycles [172]. In a study by Abdulla and Ravindra, equal proportions of alginate and κ-carrageenan were used to produce a novel biopolymeric hybrid for lipase immobilization by entrapment. The resulting biocatalyst was employed in biodiesel production from *Jatropha* oil and ethanol. Under optimal process conditions, total transesterification of triglycerides to fatty acid ethyl esters was achieved. This biocatalytic system also demonstrated good reusability as indicated by retention of over 75% of its initial activity after six cycles. The results suggest that lipase immobilized on an alginate–κ-carrageenan hybrid could be an efficient and environmentally friendly biocatalyst for biodiesel production [173]. Another application of a composite built from two biopolymers was reported by Nupur et al. who combined chitosan and calcium alginate and used the resulting hybrid in the form of beads for entrapment immobilization of penicillin G amidase (PGA). The immobilized PGA demonstrated high thermal and storage stability and good reusability. The enzyme entrapped in alginate–chitosan hybrid beads was found to have several advantages and could be used in the organic synthesis of 6-aminopenicillanic acid with high efficiency [174].

3.3.2. Organic-Inorganic Hybrids

A very wide range of materials of both organic and inorganic origin can be combined to create hybrid or composite supports for the immobilization of enzymes. The most frequently used inorganic precursors include silica, inorganic oxides such as zinc and titanium oxides, as well as minerals, carbon materials and magnetic nanoparticles [175–178]. They can be combined with polymers of synthetic origin, for example polyacrylonitrile, polyethyleneimine and polyvinyl alcohol [179,180], as well as with biopolymers such as chitosan, lignin and alginate [181,182]. These materials are mainly used for the adsorption or covalent immobilization of hydrolases, oxidoreductases and transferases but the encapsulation of these enzymes in carriers of this type has also been reported [183]. Organic-inorganic hybrids display great potential as support materials for enzymes; such hybrids provide good stability and mechanical resistance and very high affinity to biological molecules. The high stability and often also chemical inertness are related to features of the inorganic precursor. The good ability to bind enzymes is due to the organic components since synthetic polymers and biopolymers have many functional moieties in their structures that are able to interact with the chemical groups of biocatalysts [184]. As a result of their stability and functionality, hybrids in this group can be used for many practical applications, which is their greatest advantage. Many different combinations of organic and inorganic substances have been used to produce functional organic-inorganic composites and hybrids for enzyme immobilization. For example, Zhao et al. combined the stability and mechanical resistance of silica with the biocompatibility and gelation properties of chitosan and achieved immobilization of glucose isomerase on silica–chitosan hybrid microspheres via simple in situ encapsulation. The immobilized enzyme was further used as a catalyst for the conversion of glucose to fructose. The relative activity of the enzyme was found to be above 90% over a wide pH range of 6–8, a temperature range of 40–80 °C, a storage time of 3 months and after 15 repeated catalytic cycles [185]. In another study, a silica–dialdehyde starch (SiO_2-DAS) mixed hybrid, offering high enzyme binding capacity due to the presence of polysaccharide in the structure, was used for the immobilization of cellulose. The product maintained higher activity over broader pH and temperature ranges than the free enzyme. Moreover, the immobilized cellulase exhibited higher affinity to the substrates as well as better reusability and storage stability. This product might therefore be used as an effective biocatalyst for cellulose bioconversion [186]. Miranda et al. reported the synthesis of an eco-friendly poly-L-leucine-rehydrated hydrotalcite nanohybrid material and its effective use for tyrosinase immobilization without bifunctional linkers. They used the resulting biocatalytic system in

the asymmetric epoxidation reaction of chalcone. The nanohybrid-based biocatalyst exhibited excellent activity and enantioselectivity. The product also demonstrated good reusability, with unaltered activity after five consecutive runs. Thus, this biocatalytic system could find potential applications in protein engineering, biomedicine and catalysis [187]. Chang et al. combined natural clay composed of montmorillonite and layer silicates with chitosan, which exhibits good gelation abilities. The inorganic-organic hybrid was further cross-linked with glutaraldehyde and finally applied in the form of wet and dry beads for the immobilization of β-glucosidase. The covalently bound enzyme exhibited high stability over wide pH and temperature ranges. Additionally, the properties of wet and dry beads were compared and it was found that use of the dried materials led to higher catalytic efficiency [188]. In another study, the conductive properties of carbon nanospheres were combined with the gelation ability of sodium alginate for entrapment immobilization of glucose oxidase and its use for biosensing of glucose. Under optimal measurement conditions, the biosensor achieved very good performance for glucose over a wide linear concentration range, with a detection limit of 0.5 µM. The biosensor also exhibited satisfactory reproducibility and good long-term stability [189].

3.3.3. Inorganic-Inorganic Hybrids

Besides the types discussed above, inorganic-inorganic hybrid and composite materials also possess many features which make them interesting potential supports for enzyme immobilization. In general, inorganic hybrids exhibit good pH and thermal stability, mechanical resistance and chemical inertness. Moreover, their precursors are easily available and in many cases their synthesis is simple and hence relatively cheap [190]. A variety of functional groups such as carbonyl (C=O), carboxyl (COOH), amine ($-NH_2$) and epoxy are present on the surface of inorganic-inorganic materials, although the most frequently observed are hydroxyl (–OH) groups. These groups determine the hydrophilic character of inorganic composite supports and increase their affinity to biomolecules [191]. Additionally, the presence of many functional groups enhances immobilization efficiency and allows easy functionalization of the surface [192]. Besides simple modification, the presence of various chemical moieties allows the production of combined materials with desired technological features and high affinity for enzymes, which makes these materials promising for practical applications. For example, sol-gel derived silica was combined with multi-walled carbon nanotubes (MWCNTs) and used for the non-specific immobilization of lipase from *Candida rugosa*. The biocatalytic system thus obtained was applied in esterification reactions in organic media, with high efficiencies. The immobilized lipase also exhibited good reusability as indicated by almost unaltered initial activity after five catalytic cycles due to the protective effect of the MWCNTs [193]. In another study, Zhu et al. used carboxyl-functionalized silica-coated magnetic nanoparticles (SCMNPs) as a carrier for covalent immobilization of porcine pancreatic lipase. The addition of the magnetic nanoparticles to the composite enabled easy separation of the biocatalytic system from the reaction mixture using a magnetic field. The immobilized lipase exhibited enhanced activity compared to the free enzyme and good thermal resistance, with high catalytic efficiency at 70 °C [194].

The presence of many functional moieties enables the attachment of enzymes belonging to many catalytic classes, including hydrolases and oxidoreductases [195,196] and the potential use of all known immobilization techniques. For example, glucose oxidase was entrapped in nanozeolites combined with magnetic nanoparticles and multi-walled carbon nanotubes [197]. In another case, β-glucosidase, α-chymotrypsin and lipase B from *Candida antarctica* were successfully covalently immobilized on the surface of magnetic nanoparticles activated by *N*,*N*-disuccinimidyl carbonate [198]. In general, silica is one of the most commonly used precursors for inorganic hybrids. However, other inorganic components such as inorganic oxides, minerals, clays, noble metal nanoparticles and carbon-based materials may also act as precursors for inorganic-inorganic enzyme supports [199–202]. An interesting example of an inorganic-inorganic composite used for enzyme immobilization is a combination of calcium carbonate and gold nanoparticles ($CaCO_3$-AuNPs). This support was used by Li et al. for adsorption immobilization of horseradish peroxidase and further to produce a mediator-free hydrogen

peroxide biosensor. Due to the good electrical conductivity of the $CaCO_3$-AuNPs inorganic hybrid, as well as the favourable orientation of the enzyme molecules on the surface of the matrix, the biosensor exhibited strong activity toward H_2O_2 reduction and achieved a good linear response over a wide range of hydrogen peroxide concentrations and a relatively low limit of detection (1.0×10^{-7} M) [203]. In another study, the ability of carbon nanotubes to direct electron transfer and the high surface area of titanium dioxide were exploited to create TiO_2–carbon nanotube microparticles for adsorption immobilization of glucose oxidase by non-specific interactions. The immobilized enzyme was further used to build a biosensor for glucose detection. Electrochemical analysis showed the biosensor to have high efficiency, sensitivity and reproducibility and the capacity to detect glucose up to concentrations of 3 mM [204]. In another such system, multi-walled carbon nanotubes provided a highly porous conductive network that enhanced electrochemical transduction, while $CaCO_3$ acted as a host carrier for immobilization of tyrosinase by entrapment. This inorganic hybrid with immobilized enzyme was used as a highly effective and sensitive dopamine or catechol biosensor. The biocatalytic system was shown to be resistant to the effects of inhibitors and interferents and its parameters remained unaltered in the presence of uric and ascorbic acid [205].

3.4. Summary of New Materials

Table 2 summarizes the most important types of *New materials* and enzymes that may be immobilized by using these carriers. Moreover, information about binding groups, immobilization type or cross-linking agents are also presented.

Table 2. Summary and selected examples of *New materials* of inorganic, organic and hybrid origin applied for enzymes immobilization.

Support Material	Binding Groups	Cross-Linking Agent	Immobilization Type	Immobilized Enzyme	Reference
Inorganic Materials					
magnetic nanoparticles	epoxy groups	3-glycidoxypropyl-trimethoxylsilane	covalent binding	lipase from *Candida antarctica*	[83]
	–OH	–	adsorption	glucose oxidase from *Aspergillus niger*	[84]
silica SBA-15	–OH	–	adsorption	alkaline protease	[96]
mesoporous silica	–OH	–	encapsulation	catalase	[98]
silica mesoporous nanoparticles	epoxy groups	3-glycidoxypropyl-trimethoxylsilane	covalent binding	lipase from *Rhizomucor miehei*	[107]
TiO_2 nanoparticles	–OH	–	adsorption	carbonic anhydrase	[106]
cordierite	$–NH_2$	*N*-*β*-aminoethyl-*γ*-aminopropyl-trimethoxysilane	covalent binding	horseradish peroxidase	[113]
multi-walled carbon nanotubes	$–NH_2$	3-aminopropyl-triethoxysilane	covalent binding	α-glucosidase	[120]
reduced graphene oxide	C=O	glutaraldehyde	covalent binding	horseradish peroxidase	[127]
Organic materials					
electrospinning fibres of polycaprolactone	C=O	-	adsorption	catalase	[138]
electrospinning nanofibers of polyvinyl alcohol	–OH	-	encapsulation	lipase from *Burkholderia cepacia*	[140]
polyethersulphone membrane	-	-	adsorption	Phosphotriesterase lactonase from *Sulfolobus solfataricus*	[145]
NTR7450 membrane	-	-	adsorption	casein glycomacropeptide	[150]
Hybrid/composite materials					
polyaniline-polyacrylonitrile composite	–N–H	-	encapsulation	glucose oxidase	[154]
cellulose-poly(acrylic acid) fibres	–OH, COOH	-	covalent binding	horseradish peroxidase	[157]
chitosan-alginate beads	$–NH_2$, –OH	-	entrapment	amyloglucosidase	[160]
graphene oxide-Fe_3O_4	–OH, C=O	cyanuric chloride	covalent binding	glucoamylase	[177]
silica-lignin	–OH, C=O	-	adsorption	glucose oxidase form *Aspergillus niger*	[178]
polyacrylonitrile-multi-walled carbon nanotubes	–N–H, C=O, –OH	*N*-Hydroxy-succinimide	covalent binding	catalase	[179]
silica-graphene oxide particles	–OH, C=O	*N*-Hydroxy-succinimide	covalent binding	cholesterol oxidase	[199]
ZnO-SiO_2 nanowires	–OH	–	cross-linking	horseradish peroxidase	[202]
$CaCO_3$-gold nanoparticles	–OH, C=O	–	adsorption	horseradish peroxidase	[203]

New materials with tailored properties are increasingly frequently used as supports for enzymes both due to limitations in application of *Classic materials* and also to improve the properties of the immobilized enzymes. Materials belonging to the *New materials* group can facilitate easy separation of biocatalytic systems from reaction mixtures (magnetic nanoparticles) or enable avoidance of enzyme particles overloading on the surface of the carrier (nanoparticles and mesoporous materials). Moreover, materials such as graphene or graphene oxide enhance transfer of electrons between immobilized enzyme and substrate and result in increased catalytic activity of the biomolecules. The greatest advantage of the materials of organic origin classified as *New materials* is that they can be formed in various geometrical shapes such as fibres or membranes and reduce diffusional limitations and improve the efficiency of the biocatalytic processes. Within *New materials*, there is continuing and growing interest in hybrid materials. Hybrid supports may be synthesized through the combination of precursors of different origin and their properties can be tailored to the requirements of the biocatalysts as well as to the technological process in which the product will be used after immobilization. Hybrid supports are characterized by good thermal and chemical stability and mechanical resistance and usually ensure stable, covalent binding of the enzyme.

4. Summary

Inorganic materials such as inorganic oxides, minerals and carbon-based materials and materials of organic origin, including synthetic and natural polymers, may be classified as *Classic support materials* used for the immobilization of enzymes. These carriers are characterized by good stability under harsh reaction conditions, high availability or relatively simple synthesis and consequently low price. In general, these groups of support materials can be used for the immobilization of all classes of enzymes through the use of all immobilization techniques although adsorption immobilization is the most common. In view of the properties of these materials, the formation of highly specific interactions between the enzyme and the support is usually limited. Therefore, immobilization is based on non-specific hydrogen interactions. In some cases, however, where for example there is a high affinity of functional groups, the formation of covalent bonds cannot be excluded. The wide use of *Classic materials* as supports for enzymes is also linked to the more cost-effective immobilization process since these carriers do not require complicated preparation procedures and immobilization is usually fast and simple. *Classic materials* will continue to play a significant role as supports for the immobilization of enzymes from many catalytic classes for many practical applications ranging from the synthesis of highly pure chemical compounds to food processes and wastewater treatment.

The wide group of support materials which for the purposes of this review are called *New materials* for enzyme immobilization can offer properties designed for particular enzymes or for the requirements of a given technological process. These materials have come into use due to certain defects of the *Classic materials* and to the relatively low efficiencies of catalytic conversions carried out with biocatalysts bound to *Classic materials*. Inorganic materials in the *New materials* group include magnetic nanoparticles, which enhance fast and simple separation of the immobilized enzyme from the reaction mixture by means of an external magnetic field and mesoporous materials with a hierarchic pore structure, which ensures the uniform distribution of biomolecules in the matrix pores and thus reduces overloading of the enzymes and maintains catalytic activity at a high level. There are also materials of organic origin, such as electrospun membranes; depending on the material used for their production, these materials can significantly increase the transfer of electrons, which is a key step in many biocatalytic transformations. However, special interest particularly in recent years has been paid to applications of hybrid and composite *New materials* for enzyme immobilization. As has been described, these materials are synthesized from combinations of precursors of inorganic, organic and mixed inorganic and organic origin. They are designed to offer high stability, good affinity for enzymes and the presence of many chemical functional groups compatible with the chemical moieties present in the protein structure. Because of these features, biomolecules are attached mainly via covalent bonds and this ensures the good reusability and operational stability of the resulting biocatalytic

systems. *New materials* will be employed more and more extensively in the future for immobilization of enzymes. Their applications will not be limited to the creation of biocatalytic systems for use in synthesis; hybrids and composite materials will also be used to production of biocatalytic cells and biosensors for the detection of various compounds in medicine and in environmental monitoring, as well as for remediation of hazardous compounds.

It should be emphasized that in presented literature review the materials have been considered regardless of the type/mode of enzyme immobilization methodology employed, thus the treatise is universal with respect to this regard. However, for creation of stable and efficient biocatalytic systems, the attachment technique must be optimized individually for the specific enzyme, the specific material and the biocatalytic process to be employed.

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