ELSEVIER

Novel materials through Nature's catalysts

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The use of enzymes in materials science allows the development of unique and innovative functional materials. As biomolecules, enzymes can provide exceptional features such as substrate specificity, rate acceleration, regio-, chemo-, and stereoselectivity within catalyzed reactions. Many enzymes are suitable for biomaterials modification because of their natural origin or because they catalyze a desired reaction between non-natural substrates. In their native form, or after tailoring by genetic engineering, enzymes can complement the traditional approaches of materials science by following Nature principles. Multiple technologies involve enzymes in the fabrication and processing of materials, such as direct enzymatic treatment of materials and the assembly of novel bio-hybrid materials. In this review we highlight the most recent applications of enzymes by illustrating selected examples from the last 3 years, and indicate future trends in the field of materials science.

Introduction

Enzymes were established over the last decades as biocatalysts in various important industrial processes and can be found in many products of daily life [1–3]. Besides the conventional areas of enzyme application, such as pharma [4–6], food [7], or the washing agent industry [8], the application of enzymes in materials chemistry is still modest. By combining properties like protein stability, substrate and/or reaction selectivity with enormous reaction rate acceleration [9], enzymes can be considered as optimally evolved functional structures. They can give access to chemical processes that cannot be realized by classical synthetic approaches under the same conditions [2,10–12]. Against common prejudices, enzymes can accept multiple substrates, are relatively stable, able to function in an non-physiological environment, and are, due to recombinant production strategies, relatively inexpensive [2,3]. The most difficult task for applications of enzymes in industry is the selection or discovery of enzymes for a certain reaction as well as to integrate them stably into processes/devices while maintaining and preserving their properties. However, it is nowadays possible to develop enzymes with improved features in the laboratory [13–17]. Although the *de novo* design of new catalytic reactions is still in its infancy, some protein catalysts that cannot be found in Nature have been already designed [18–21]. In any case, nature provides a large pool of yet unexploited enzymes that continuously enlarge the toolbox of biocatalysts for the application in materials science [22–28].

An understanding of materials or enzymes based on the etymology falls short. The term 'materials' derives from the Latin word *materialis* which describes all things made out of matter, and the term *enzyme* itself is derived from Greek words *en* and *zýmē* pointing to their occurrence 'in yeast'. Note, a narrow definition of the term 'material' is not possible and probably not appropriate. This can also be concluded from general descriptive approaches where materials are 'identified as substances in the condensed states (liquid, solid, colloidal) designed or manipulated for technological ends' [29]. Others quote that 'materials can be said to emerge by human action: a material is a *substance with* a present or an expected future *application* for mankind. So, not all substances are materials' [30]. Similarly, 'materials are defined as solids used by man to produce items which constitute the support for his living environment' [31].

To a substantial extent it is also not easy to give one definition for enzymes, especially one including a general relationship

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between amino acid sequence and enzyme function. However, in our understanding there are obvious traits of enzymes that cannot be found in any material. Materials and enzymes can be classified according to their physico–chemical and biochemical properties, respectively (Table 1).

Even though enzymes and materials might be extremely different in many aspects, their common 'language' is chemistry. As soon as their reactive chemical moieties are compatible, the combination of materials and enzymes is feasible. Biomaterials such as polyhydroxyalkanoates (PHAs), cellulose, and lignin, are directly connected to enzymes, since enzymes are involved in their biosynthesis and modification [32,33]. As shown in Fig. 1 enzymes are intrinsically related to many fields of materials applications [34]. The way enzymes are applied for materials modification range from isolated enzymes over immobilized enzymes, cell free extracts up to microbial cells (in form of living cells, resting cells or biofilms).

The application of enzymes in materials science combines two rather different scientific approaches. Materials science is predominantly an engineering discipline, aiming at the intentional assembly of building blocks to achieve desired material properties. In contrast, enzyme technology is a discovery-based discipline. This is reflected by the fact that not all enzymes can be easily synthesized on demand so far. While the limits of the individual approaches have been critically discussed elsewhere [35,36], the unique properties of both, materials and enzymes, can be combined in a synergistic way leading to advanced materials.

A schematic overview showing possible interactions of enzymes with materials is depicted in Fig. 2. In general, enzymes can be used to modify materials *via* direct catalysis at the surface of the material (I), to activate substrates which can further react with the material (II), or enzymes can be immobilized or integrated directly into materials (III). Additionally, polymeric materials can be enzymatically degraded into oligomers, monomers and other compounds (IV) or constructed from individual building blocks (V). Moreover, enzymes can be directly used to synthesize building blocks for the assembly of materials (VI). Combinations of all methods are possible. Common synthetic themes in all fields are direct and/or indirect conversion of the enzyme substrates.

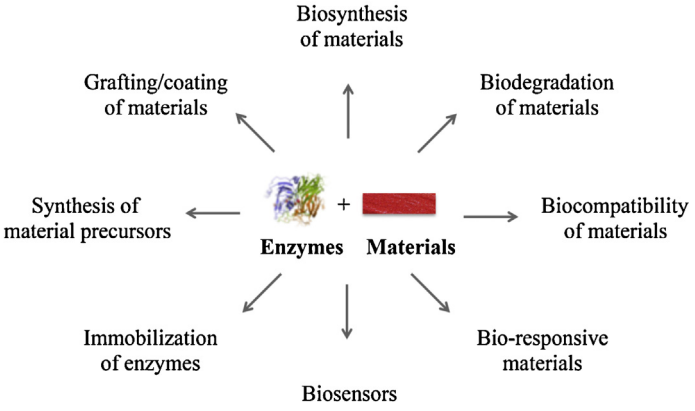

FIGURE 1

Fields of enzyme application in materials science.

Several challenges have to be considered when combining materials and enzymes. When enzymes are used for surface modification of polymeric substrates, issues such as the substrate accessibility, the selection of appropriate reaction systems, the analytics, and the downstream processing of the generated product play a crucial role. Future challenges for the realization of innovations in the field of enzymes and materials might be similar to the ones discussed in the article from Kunz and Müllen [37] where they address the question 'whether natural product and material chemistries are separated forever'. Following their arguments, 'every sub-discipline requires extensive focus' which may lead to further separation of these disciplines. Accordingly, the exploration of enzymes for innovative materials is possible within highly interdisciplinary consortia among biologists, materials scientists, chemists, and physicists. The fabrication of materials by using enzymes can in our opinion be regarded as an emerging field where complementary technologies lead to exceptional

TABLE 1

Comparative view on enzymes and materials.

Enzymes	Materials
Composition/state	
Biopolymers, composed of a polypeptide backbone made of predominantly 20 natural amino acid building blocks, Functional structures dissolved in aqueous solution	Mainly condensed phases comprising diverse substances (liquid, solid and colloidal), Unlimited number of precursors
Main descriptors	
Biocatalysts	Compounds, often designed or manipulated for various technological ends
Origin	
Naturally evolved or generated by enzyme engineering Produced from renewable precursors under biological conditions	Naturally or chemically synthesized Often produced from non-renewable precursors, under non-natural conditions
General properties	
Intrinsically dynamic	Exhibit various physical or chemical properties upon external triggers
Classification system	
According to type of catalyzed reaction; structure or biochemical characteristics	According to physico-chemical traits and chemical structure, for example, polymers, metals, ceramics, wood
Applications	
Widely applied in catalytic processes (washing agents, synthesis, biosensors, *etc.*)	Used for technical or high performance applications (construction, energy storage, textiles, *etc.*)

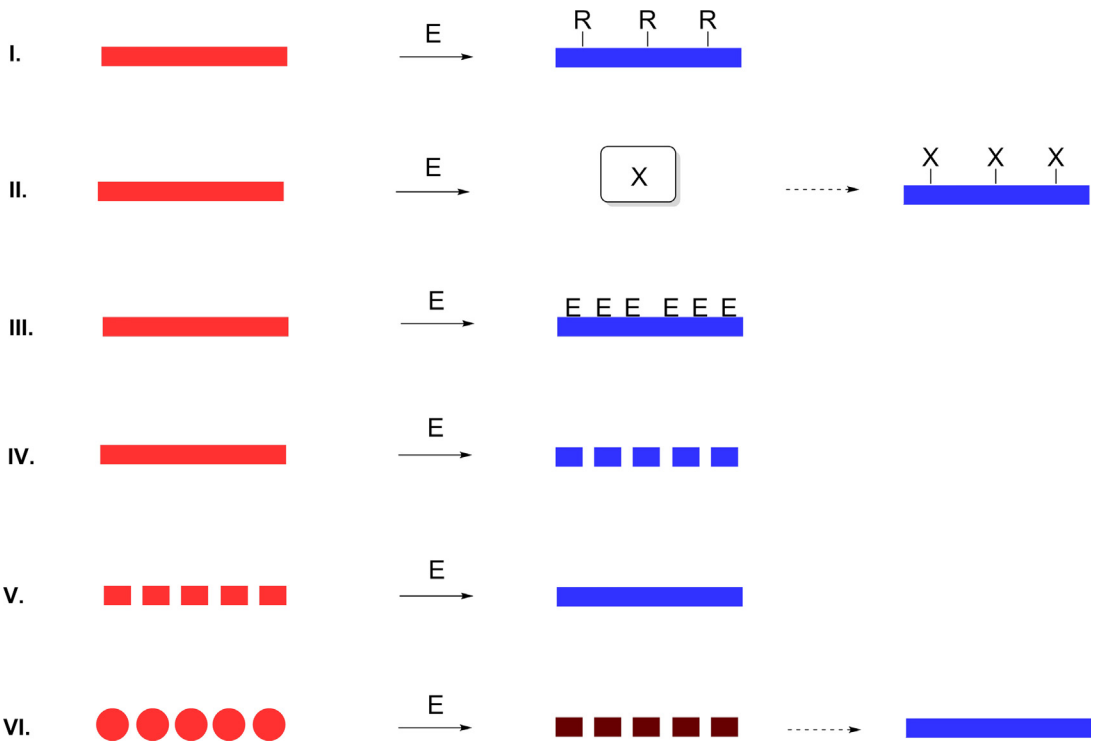

FIGURE 2

Different strategies for materials functionalization with enzymes (E). (I) Direct enzyme catalyzed modifications, where R indicates a novel functional group introduced by enzymatic treatment, (II) indirect enzyme mediated modification of polymers, where X can be an enzyme-activated substrate or an enzyme-bound transition state of a substrate, (III) direct immobilization or integration of enzymes on/into materials, (IV) enzyme-mediated polymer degradation, (V) enzyme-mediated polymer formation, and (VI) enzymatic synthesis of building blocks for materials fabrication.

solutions. Apart from the fabrication and processing of materials or bio-hybrid materials, enzymes contribute to the development of sustainable processes [38].

The present article points out selected enzymatic reactions relevant for the synthesis or modification of materials, and processes that lead to hybrid materials of unique functionality. With this review we want to demonstrate that materials science and biocatalysis provide complementary strategies towards innovative solutions which cannot be realized by one discipline alone.

I: Direct enzyme catalyzed modifications of materials

This section describes processes where enzymes directly catalyze reactions on polymeric substrates. One field where enzymes are extensively used for polymer modification is in textile industry. They are included in almost every step during textile processing (Table 2). The applied enzymes belong primarily to the families of hydrolases (EC 3.) and oxidoreductases (EC 1.).

An interesting case of polymer modifying enzymes is their use for the conservation and restoration of fabrics [42–44]. A common method to strengthen, for example, silk-based historic textiles is the introduction of synthetic polymers with molecular structures different from the silk protein. Alternatively, transglutaminases can be applied for the restoration of silk. The covalent cross-linking of glutamyl and lysyl residues increases the stability and resistance of the silk protein towards degradation. Besides strengthening the textile, the morphology of the fibers is improved without influencing other properties such as textile color [44].

II: Indirect enzyme mediated modifications of materials

In contrast to direct modifications with enzymes, the indirect enzyme mediated modifications of materials are processes where either certain activated substrate–enzyme intermediates are formed or where enzymes first activate substrates that subsequently react non-enzymatically with the polymer.

Exemplary biocatalysts for oxidation reactions in materials science, where an activated intermediate state occurs, are laccases (Fig. 3). These promiscuous multi-copper oxidases catalyze the oxidation of various aromatic and some non-aromatic compounds with concomitant reduction of oxygen to water (Fig. 3a). The generated radicals may undergo diverse rearrangement or coupling reactions. Due to the free radical formation, laccases can initiate a broad spectrum of reactions, including polymerization and depolymerization [27,45–47]. Moreover, laccases are very useful enzymes for diversity-oriented synthetic approaches where the concept 'one enzyme–many products' is desired [12]. These features, along with high stability and activity, make laccases widely used in the paper, textile, cosmetics and pharmaceutical industry [26,48].

In materials science, laccases can be used to generate novel hybrid polymers by combining organic and siloxane materials such as a lignin-siloxane copolymers for use in adhesives and coatings. Lignin, an organic polymer, was activated by enzymatic oxidation and mixed with siloxanes that resulted in the incorporation of 60% of the activated lignin into this polymer [49]. One example where laccases are applied to surface modification in MedTech devices is the functionalization of urinary catheters to prevent biofilm formation and thus infections in patients [50]. To achieve this, laccase, catechin – an antimicrobial flavonoid, and

TABLE 2

Enzymes used in textile industry.

Processing step	Enzyme (EC number)	Function
Desizing	Amylases (3.2.1.1.) Lipases (3.1.1.3.)	Removal of size material like starch and grease added for fabric protection during weaving
Scouring	Amylases (3.2.1.1.) Cellulases (3.2.1.4.) Proteases (3.4.2.) Pectinases (3.2.1.15.) Lipases (3.1.1.3.)	Removal of natural impurities that impede equal dying like starch, wax, grease, pectin
Finishing	Cellulases (3.2.1.4.) Lipases (3.1.1.3.) Cutinases (3.1.1.74.)	Modification of fiber surfaces to improve properties like softness, gleaming, defuzzing, a certain look (biostoning) and general handling
Composting	Cellulases (3.2.1.4.) Proteases (3.4.2.)	Textile and fiber degradation

sodium hydroxide-pre-treated silicone catheter were co-incubated. In the result of this reaction, the catheter was coated with poly(catechin) leading to reduced bacterial adhesion and subsequent biofilm formation [50] (Fig. 3b).

Another novel technology is the application of enzymes for non-natural reactions. For example in atom transfer radical polymerization (ATRP) reactions, various oxidoreductases such as laccases from *Trametes versicolor*, horseradish peroxidase (HRP), and catalase from bovine liver have been used as protein-based catalysts [51,52] (Fig. 3c). Using this technology, electrospun lignin nanofibers with PNIPAM-brush-like structures (poly(N-isopropylacrylamide)) were synthesized that can be used in filter devices, medical diagnostics, and sensor technologies [53].

Besides laccases, tyrosinases can be used to indirectly modify polymers (Fig. 4). By converting tyrosyl side chains to reactive *o*-quinones, tyrosinases catalyze the covalent crosslinking of proteins harboring tyrosine residues within regions of low complexity. The addition of small phenolic substrates can be beneficial for the production of crosslinked aggregates of proteins that are not natural substrates for tyrosinase [54]. Genetic engineering of the substrate protein can provide additional exposed tyrosine residues in the substrate protein for direct crosslinking [55] and material functionalization. In this way, fluorescent proteins such as phycocyanin, an interesting target for biomedical and diagnostic approaches, was successfully immobilized to amino-modified polystyrene beads [56,57].

In contrast to the tyrosinase and laccase-based approaches that lead to relatively unspecific surface modification, enzymes like

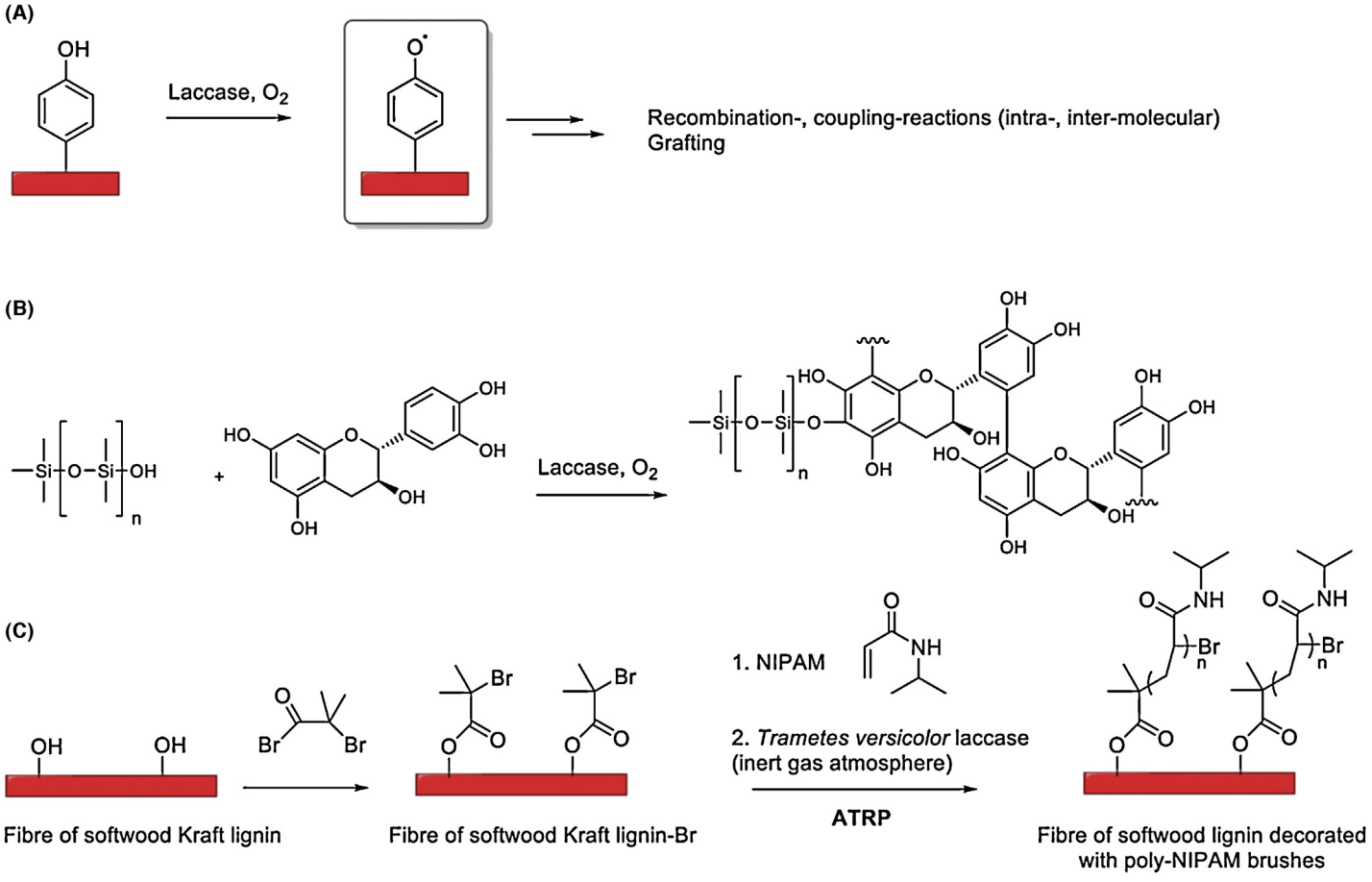

FIGURE 3

Examples of laccase-dependent reactions. (A) Chemistry of laccase-catalyzed reaction. The generated radical can undergo several subsequent rearrangement or coupling reactions. (B) Polymerization of catechin on the silicone catheter [50]. (C) Enzyme-mediated ATRP for the brush-like modification of lignin with PNIPAM [53].

sortase A (SrtA) enable access to site-specific modifications. The most widely studied sortase is SrtA from *Staphylococcus aureus* that was used for oriented polymer modification [25]. SrtA is mainly applied to immobilize proteins on polymeric surfaces to improve materials properties. During catalysis, SrtA forms an activated acyl-enzyme intermediate with target proteins carrying an exposed LPXTG amino acid sorting motif close to their C-terminus, where X represents any natural amino acid. Subsequently, the enzyme catalyzes the transfer of the target protein to the free N-terminus of an oligoglycine-functionalized surface, leading to the formation of a new peptide bond (Fig. 5). Protein hybrid materials generated by the use of SrtA include various proteins, such as fluorescent proteins [22,58–60], enzymes [61], antibody fragments [62], and functional proteins [63,64] immobilized on different types of surfaces. With a recently developed flow cytometry-based assay the immobilization of green-fluorescent protein on polystyrene particles could be followed in real time, enabling the direct comparison of the reaction characteristics of different sortase variants [65].

III: Enzyme immobilization on materials

By directly immobilizing enzymes on a material surface, the properties and functionality of the material are expanded. Enzyme immobilization can be achieved by simple protein adsorption, by affinity or by covalent coupling. This can enable unique applications in, for example, MedTech, biosensing and catalysis. The functionalization of surfaces with particular enzymes becomes increasingly important for the fabrication of antimicrobial surfaces. To prevent biofilm formation, extracellular DNA (eDNA) from bacteria has been identified as target as it is involved in the initial step of bacteria attachment. To hydrolyze the eDNA, DNaseI was covalently immobilized on polymethylmethacrylate (PMMA) *via* polydopamine films, which were established by coating the PMMA surface with L-dopamine under alkaline conditions. The modified surfaces showed hydrolytic activity towards free DNA and provided unprecedented reduction in biofilm growth of *Pseudomonas* sp. and *Staphylococcus* sp. In contrast the attachment of *human osteosarcoma U2OS* cells was not affected [66]. In a different way lysozyme was immobilized on a 3D silicon nanowafer structure for a synergistic capture and lysis of pathogenic bacteria. Thus, in antimicrobial tests the number of bacteria on the surface could be drastically reduced [67]. Another example of enzyme immobilization is the production of a polydopamine-based biocomposite with integrated carbon-nanotubes and laccases to confer unique surface properties. The obtained bionanocomposite is implemented as an electrochemical biosensor and as biocathode in a biofuel cell [68].

Complex enzyme cascade reactions, interesting for biocatalysis, can also be realized on materials surfaces. Recently, three enzymes of the menaquinone biosynthesis pathway have been co-immobilized on CdSe-ZnS quantum dots *via* hexa-histidine tag affinity-driven self-assembly to synthesize 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate [69].

A relatively new approach to immobilize enzymes on surfaces makes use of natural protein–protein interactions. The SpyTag-SpyCatcher system is based on the fibronectin-binding protein FbaB from *Streptococcus pyogenes* [70]. Attachment or incorporation of the SpyCatcher on or into matrices can then be used to covalently immobilize any protein carrying the SpyTag [71]. In a comparable manner, the FimGt/DsF system, which is based on donor strand complementation, can be used [72]. In contrast to the SpyTag-SpyCatcher system, the FimGt/DsF system does not rely on covalent bond formation between the protein and the peptide, but on hydrophobic and electrostatic interaction. Nevertheless, with a K_D in the range of 10^{-20} M, it forms the most stable protein–ligand complex known so far. Also split-intein-triggered trans-splicing can be used to immobilize proteins on surfaces. Until now, this technique was mainly applied for protein-crosslinking to form hydrogels [73]. For this purpose, the two fragments of the naturally split DnaE intein from *Nostoc punctiforme* are connected to the proteins of interest. By attaching or incorporating one half of the split intein into a matrix, this setup can also be used to directly immobilize proteins on surfaces [74].

IV: Enzyme mediated degradation of materials

Hydrolases like proteases and endonucleases, which cleave (poly)-peptides and oligonucleotides, respectively, can be used to degrade or disassemble materials containing peptide or phosphodiester bonds. Moreover, different microbial hydrolases are described for hydrolysis of a variety of aliphatic, aromatic, and aliphatic-aromatic copolyesters [75]. For example, PET hydrolases are capable of hydrolyzing polyethylene terephthalate (PET). Different enzymes such as lipases and cutinases belong to the class of PET hydrolyzing enzymes. Besides degrading PET, treatment with these enzymes provides access to the modification of PET for further functionalization [76]. For instance, PET-nanoparticle suspensions made from recycled PET fibers, films, and granulates were monitored *via* turbidimetric assays during enzymatic treatment with cutinase from *Thermobifida fusca* KW3. According to these studies, the main factor determining the degradability of PET fibers is its flexibility [24]. Potentially, these enzymes can be exploited for anti-pilling effects of polyester fibers or for surface functionalization.

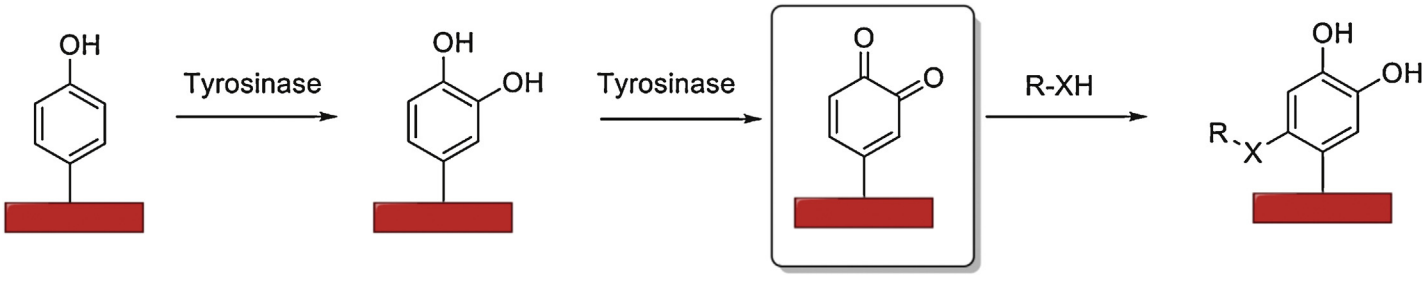

FIGURE 4

Tyrosinase-catalyzed reactions. The reactive *o*-quinone is highlighted.

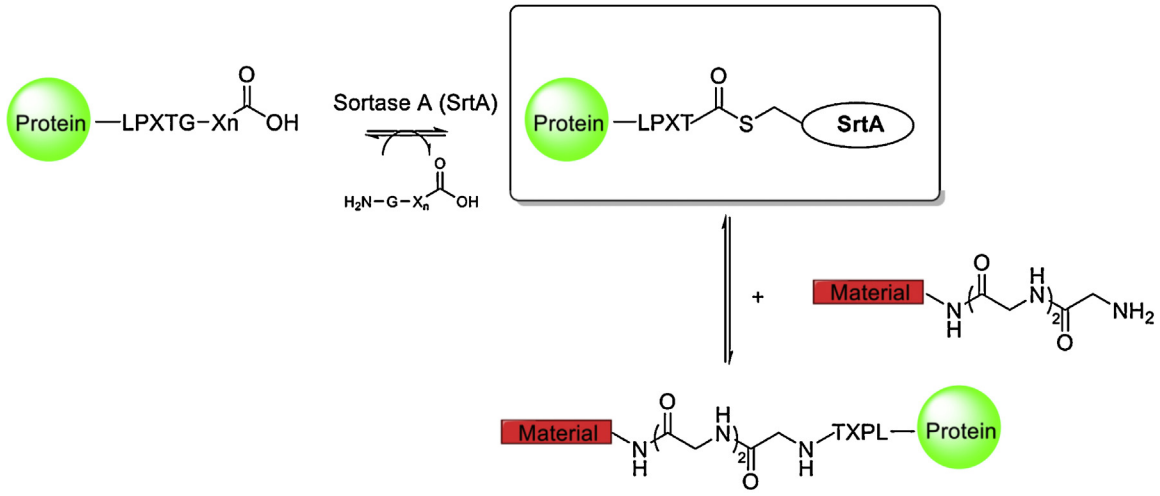

FIGURE 5

Sortase A-catalyzed site directed immobilization of proteins on material surfaces. The reactive acyl-enzyme intermediate is highlighted. X stands for every canonical proteinogenic amino acid [65].

An innovative application for polymer modifying enzymes is the degradation of polyvinylalcohol (PVA), a water-soluble synthetic polymer frequently used as sizing agent in the textile industry. After textile desizing PVA is washed from the fiber and released in the environment. For the treatment of PVA-contaminated wastewater several microorganisms have been identified that can degrade PVA. However, the corresponding enzymes are not fully characterized yet [39–41].

Polymer degrading enzymes are often used to design enzyme responsive materials (ERM). Such materials change their composition and thus functionality due to the activity of an enzyme. ERM are especially interesting for medical applications, since a natural trigger, like a change in native enzyme or metabolite concentrations, can be harnessed. For example, to achieve drug-targeting in case of pulmonary drugs, a poly(ethylene glycol)-based hydrogel, with peptides incorporated into the polymer chain, was designed [77]. Metalloproteases, which are upregulated during pulmonary diseases, allow drug release by cleaving the incorporated peptide bonds of the ERM (Fig. 6a). In another study mesoporous silica nanoparticles were functionalized on the outer surface with ε-poly-L-lysine to prevent drug leakage. The potential of this system for controlled drug delivery was tested by encapsulating the cytotoxic drug camptothecin. The capped silica nanoparticles were taken up by HeLa cells and targeted to the lysosome. Due to amide bond cleavage by lysosomal proteases the entrapped drug was released, causing targeted cell death [78]. Similarly, mesoporous silica material was coated with Konjac oligosaccharides as a gatekeeper to prevent drug release (Fig. 6b). Upon contact with colonic bacteria that secrete mannanase, the oligosaccharides were degraded and the drug was released [79,80].

V: Enzyme mediated formation of materials

Besides degrading materials, enzymes can also trigger the formation of materials [27,81,82]. For example, transglutaminase was used to crosslink soy protein isolate to generate hydrogels [83]. One example in the MedTech area is a wound dressing that was designed to form a hydrogel upon wound contact. In this system, hydrogelation is initiated by pouring a PVA derivative and the enzymes HRP and glucose oxidase (GOx) into the wound. To be accessible for crosslinking by the enzyme HRP, the employed PVA had been functionalized with phenolic hydroxyl groups. The required co-substrate of HRP, H_2O_2, is provided by GOx, which generates hydrogen peroxide upon oxidation of wound internal glucose [84] (Fig. 7).

Also hydrolytic enzymes such as subtilisin [85] or thermolysin [86] can be applied to trigger self-assembly of peptides, leading to the formation of hydrogels. It was demonstrated that the peptidase thermolysin can catalyze the formation of self-assembling peptides from non-self-assembling shorter precursors. In a first step a peptide sequence is partially cleaved. If the peptide concentration is high enough, the enzyme catalyzes the synthesis of longer fragments, which can then self-assemble into β-sheet-rich fibers [87].

Other hydrolytic enzymes, oxidoreductases and transferases have been successfully applied in polymer synthesis including polyesters, polyethers and polyamides [28,88–90]. Recently, a 2 step polymerization to obtain bio-based poly(butylene succinate) by applying Novozyme 435 was described, starting from succinic anhydride, succinic acid and butane-1,4-diol. First different precursors were synthesized *via* atmospheric polycondensation and these precursors were further polymerized enzymatically [91].

A novel chemoenzymatic cascade reaction for the synthesis of methylacrylates was described for copper-mediated ATRP of 2,2,2-trifluoroethyl methacrylate (TFEMA) as a model compound and using ethyl-2-bromoisobutyrate (EB*i*B) as initiator. Concomitantly transesterified TFEMA derivatives resulting from Novozyme 435 catalysis in the presence of primary, secondary or tertiary alcohols were copolymerized. In the latter case copolymerization and enzymatic transesterification proceeded coherently [92].

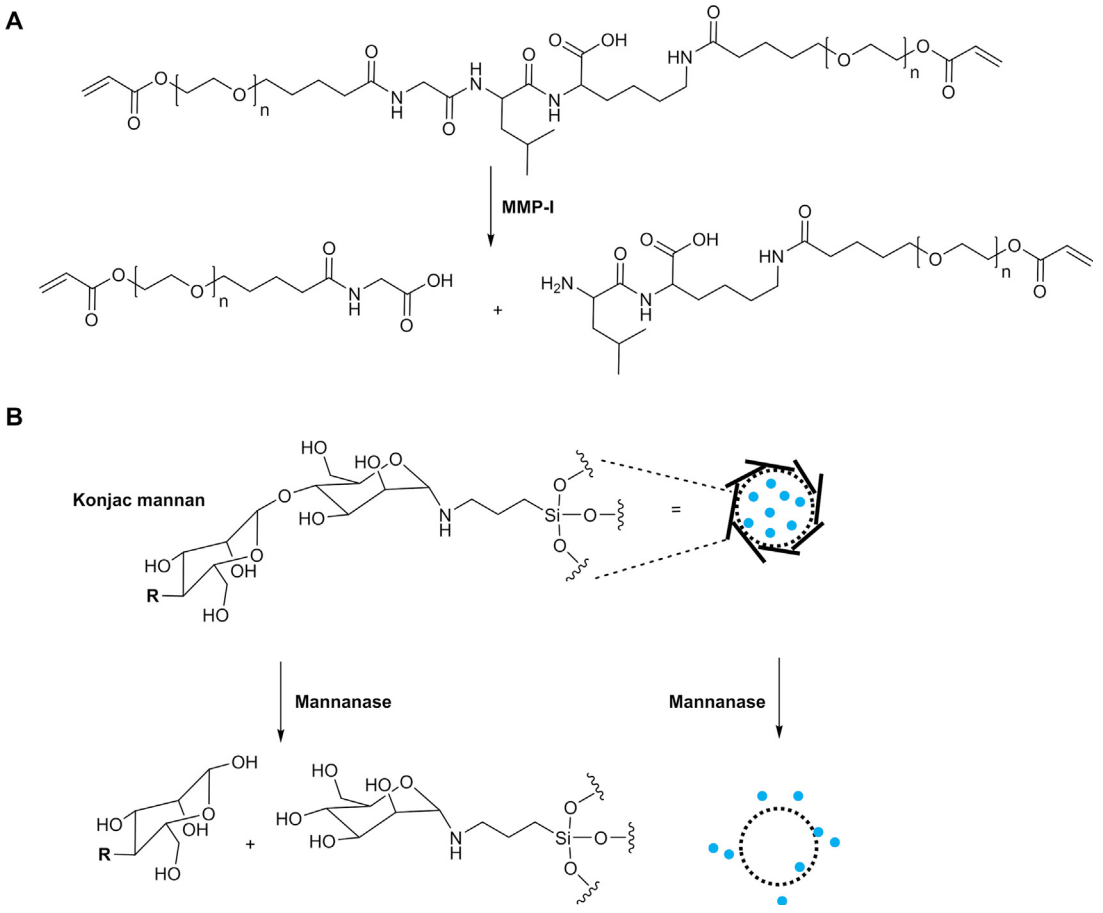

FIGURE 6

Enzyme responsive materials for prospective medical devices. (A) Microparticles composed of a poly(ethylene glycol)-peptide-copolymer for pulmonary drug delivery: upon cleavage of the peptide bonds by the metalloprotease MMP-I that is overexpressed in pulmonary diseases, an encapsulated drug can be released. (B) Oligosaccharide-functionalized silica particles for targeted drug release: the modified silica-capsule retains the drug (blue dot) until the bacteria-secreted enzyme mannanase cleaves the glycosylic bond, which triggers drug release. The figure is adapted from [79].

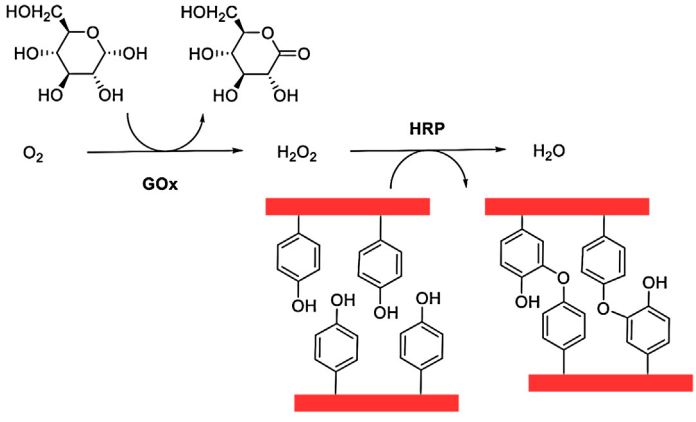

FIGURE 7

Hydrogel formation upon wound contact. Hydrogelation is initiated by the enzyme horseradish peroxidase (HRP). The required co-substrate hydrogen peroxide (H_2O_2) is provided by the enzyme glucose oxidase (GOx), which uses wound internal glucose as a substrate.

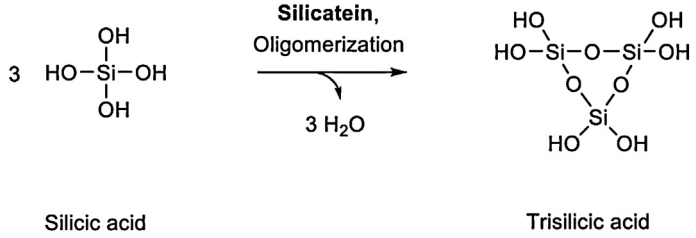

FIGURE 8

Enzymatic polymerization of silicic acid catalyzed by silicateins.

Unlike the above mentioned enzymes that react exclusively with substrates of organic nature, silicateins represent an exceptional group of enzymes that catalyze the bio-polycondensation of monomeric inorganic silicic acid precursors into polymeric biosilica structures (Fig. 8). Biosilica formation by silicateins leads to the deposition of pure amorphous silica structures found, for example, in the skeletal spicules of marine sponges. Besides their catalytic activity, filamentous aggregates of silicateins adopt a structure-guiding function, providing a template for biosilica deposition.

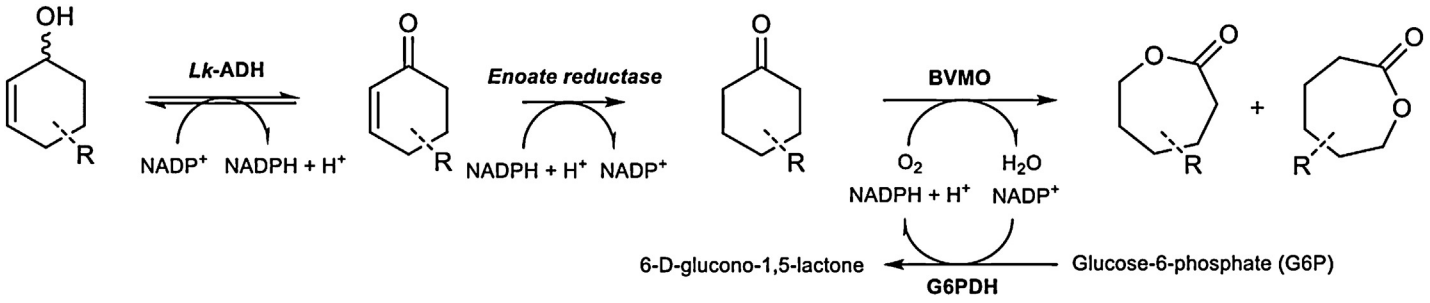

FIGURE 9

Enzyme cascade for the synthesis of lactones that can be used for polyester production [94].

The finding that silica structures can be enzymatically assembled around organic matrices suggests useful applications for bio-polycondensation reactions, for example, for the production of glass fibers within waveguides or the fabrication of silica layers with electrically insulating properties to be used in the field of microelectronics [93].

VI: Enzymatic synthesis of building blocks for material fabrication

Besides acting directly on materials, enzymes can produce building blocks for polymer fabrication. The synthesis of polymer precursors according to the principles of green chemistry is of great importance in order to avoid the use of petro-chemical resources. P450 cytochromes have been shown to be potentially useful for the enzymatic synthesis of hydroxy-acids that can further be employed as starting materials for the synthesis of polyesters. Baeyer-Villiger monooxygenases (BVMOs) were included into a three-enzyme cascade by using cell free extracts of *E. coli* containing the heterologously expressed enzymes. The starting material involved compounds from renewable sources containing a cyclohexenol moiety that was converted to the corresponding ketone *via* subsequent steps of alcohol dehydrogenase from *Lactobacillus kefir* and the enoate reductase from *Saccharomyces carlsbergensis*. The produced ketone is then converted by a BVMO from *Acinetobacter* sp. to yield lactone with good regio-, and enantioselectivity (Fig. 9). All corresponding lactones were formed with good to excellent conversion rates and in a reasonable time. This demonstrates a novel synthetic approach to the synthesis of functional and biorenewable polymer building blocks that again can be polymerized by using enzymes [81,82,94,95].

In a comparable manner the building block styrene can be manufactured *via* an enzymatic cascade reaction. Styrene is used for the production of polystyrene, a very common packaging material. For this purpose, the enzymes ammonia lyase from *Arabidopsis thaliana* and the enzyme phenylacrylate decarboxylase from *Saccharomyces cerevisiae* were transformed into a polyalanine-overproducing *E. coli* strain. While the first enzyme catalyzed the conversion of L-phenylalanine to *trans*-cinnamate, the latter enzyme catalyzed the subsequent formation of styrene [96].

Outlook

Enzymes provide exceptional solutions for the fabrication of innovative functional materials. Different strategies for materials functionalization span from direct enzymatic reactions on materials surfaces to the integration of enzymes into materials. Biocatalysts intrinsically bear unique substrate specificity, potential chemo-, regio-, and stereoselectivity and act under ambient conditions. Moreover, enzymes match the principles of green chemistry. Thus, the combined use of enzymes and materials paves the way for applications targeting issues such as biocompatibility, biocatalysis, and biosensing.

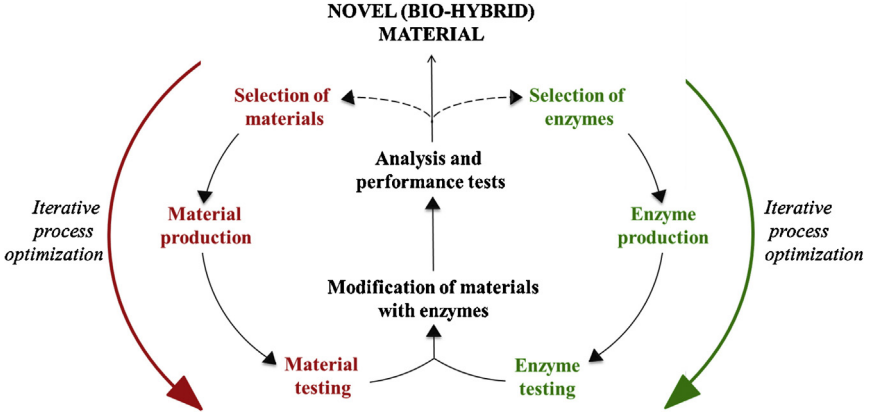

FIGURE 10

Double spinning wheel illustrating routes for the development of new (bio-hybrid) materials.

The field of enzyme-modified materials is strongly multidisciplinary. Hence, process development of enzyme-modified materials involves the improvement of both, material and enzyme to eventually merge them into one novel product (Fig. 10).

Challenges for the production of bio-hybrid materials include the selection of enzymes, the precise analytics including protein quantification for determination of enzyme activity before and after immobilization, and the fabrication of defined architectures on surfaces. The combined approach of materials and enzymes can readily be extended to include functional proteins in general. The increasing number of tools and know-how for the selection of proteins and enzymes, based on structural data and biochemical properties of known enzymes, will certainly support future innovations. The plethora of proteins provided by Nature offers certainly many possibilities that can be transferred to materials science. Bio-hybrid materials carry unique functionalities, especially when biocompatibility issues are addressed. These innovative solutions will have an impact on the society as they offer sustainable and green processes that are highly demanded today.

References

[1] U.T. Bornscheuer, et al. Nature 485 (2012) 185.
[2] K. Faber, Biotransformations in Organic Chemistry, Springer, Berlin, Heidelberg/New York, 2011.
[3] A. Schmid, et al. Nature 409 (2001) 258.
[4] J. Bongaerts, et al. Angew. Chem. Int. Ed. 50 (2011) 7781.
[5] M. Karpf, R. Trussardi, Angew. Chem. Int. Ed. 48 (2009) 5760.
[6] M. Müller, Angew. Chem. Int. Ed. 44 (2005) 362.
[7] R. DiCosimo, et al. Chem. Soc. Rev. 42 (2013) 6437.
[8] K.-H. Maurer, in: M.C. Flickinger (Ed.), Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, John Wiley & Sons, Inc., 2010.
[9] M. Eigen, Cc/Phys. Chem. Earth (1990) 16.
[10] M.-R. Kula, Introduction, in: Enzyme Catalysis in Organic Synthesis, Wiley-VCH Verlag GmbH, 2008. p. 1.
[11] J.E. Leresche, H.P. Meyer, Org. Process Res. Dev. 10 (2006) 572.
[12] H.-P. Meyer, et al. Catal. Sci. Technol. 3 (2013) 29.
[13] M. Goldsmith, D.S. Tawfik, Curr. Opin. Struct. Biol. 22 (2012) 406.
[14] E.M. Brustad, F.H. Arnold, Curr. Opin. Chem. Biol. 15 (2011) 201.
[15] S.D. Copley, Trends Biochem. Sci. 40 (2015) 72.
[16] C. Jäckel, et al. Annu. Rev. Biophys. 37 (2008) 153.
[17] K.J. Woycechowsky, et al. Advances in Enzymology, John Wiley & Sons, Inc, 2010
[18] N. Preiswerk, et al. Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 8013.
[19] J.B. Siegel, et al. Science 329 (2010) 309.
[20] G. Kiss, et al. Angew. Chem. Int. Ed. 52 (2013) 5700.
[21] D. Hilvert, Annu. Rev. Biochem 82 (2013) 447.
[22] X. Guo, et al. Bioconjug. Chem. 23 (2012) 650.
[23] N. Kashiwagi, et al. Biotechnol. Lett. 36 (2014) 2311.
[24] R. Wei, et al. J. Mol. Catal. B Enzym. 103 (2014) 72.
[25] T. Proft, Biotechnol. Lett. 32 (2010) 1.
[26] A. Mikolasch, F. Schauer, Appl. Microbiol. Biotechnol. 82 (2009) 605.
[27] F. Hollmann, I.W.C.E. Arends, Polymers 4 (2012) 759.
[28] A.R.A. Palmans, A. Heise (Eds.), Enzymatic Polymerisation, Springer, Heidelberg/Dordrecht/London/New York, 2010.
[29] Ganesh, Nature Materials, Guide to Authors, Nature Publishing Group, 2011 http://www.nature.com/nmat/pdf/nmat_gta.pdf.
[30] E.J. Mittemeijer, Fundamentals of Materials Science, in: The Microstructure–Property Relationship Using Metals as Model Systems, Springer, Heidelberg/Dordrecht/London/New York, 2011.
[31] J.P. Mercier, G. Zambelli, W. Kurz, in: J.P. Mercier, G. Zambelli, Kurz (Eds.), Introduction to Materials Science, Elsevier, Oxford, 2002.
[32] R. Vanholme, et al. Plant Physiol. 153 (2010) 895.
[33] R.A.J. Verlinden, et al. J. Appl. Microbiol. 102 (2007) 1437.
[34] A. Rajagopalan, W. Kroutil, Mater. Today 14 (2011) 144.
[35] M. Jansen, J.C. Schön, Angew. Chem. Int. Ed. 45 (2006) 3406.
[36] W. Kogge, M. Richter, Stud. Hist. Philos. Biol. Biomed. Sci. 44 (2013) 181.
[37] H. Kunz, K. Müllen, J. Am. Chem. Soc. 135 (2013) 8764.
[38] P.T. Anastas, J.C. Warner, Green Chemistry: Theory and Practice, Oxford University Press, New York, 1998.
[39] F. Kawai, X. Hu, Appl. Microbiol. Biotechnol. 84 (2009) 227.
[40] A. Yamatsu, et al. Appl. Microbiol. Biotechnol. 72 (2006) 804.
[41] T. Heck, Isolation and Identification of Microorganisms for the Biodegradation of Polyvinylalcohol, Empa, unpublished data, 2013.
[42] H.E. Ahmed, Conserv. Mag. 4 (2007) 177.
[43] A. Blüher, et al. Paper Conserv. 21 (1997) 37.
[44] Z. Zhu, et al. Heritage Sci. 1 (2013) 1.
[45] F. Hollmann, et al. Macromolecules 41 (2008) 8520.
[46] R. Reiss, et al. BMC Biotechnol. 11 (2011) 9.
[47] P. Sharma, et al. World J. Microbiol. Biotechnol. 23 (2007) 823.
[48] S.R. Couto, J.L.T. Herrera, Biotechnol. Adv. 24 (2006) 500.
[49] E.N. Prasetyo, et al. Biotechnol. J. 7 (2012) 284.
[50] I. Goncalves, et al. Biocatal. Biotransform. 32 (2014) 2.
[51] Y.-H. Ng, et al. Chem. Commun. 47 (2011) 6464.
[52] Y.-H. Ng, et al. Polym. Chem. 2 (2011) 589.
[53] G. Gao, et al. Green Chem. 16 (2014) 3890.
[54] M. Fairhead, L. Thöny-Meyer, J. Biotechnol. 150 (2010) 546.
[55] G. Faccio, et al. Sci. Rep. 4 (2014).
[56] M.J.C. Long, L. Hedstrom, Chembiochem 13 (2012) 1818.
[57] G. Faccio, et al. RSC Adv. 5 (2015) 22319.
[58] L. Chan, et al. PLoS ONE 2 (2007) e1164.
[59] R. Parthasarathy, et al. Bioconjug. Chem. 18 (2007) 469.
[60] S. Wu, T. Proft, Biotechnol. Lett. 32 (2010) 1713.
[61] T. Ito, et al. Biochemistry 49 (2010) 2604.
[62] M.K.M. Leung, et al. Angew. Chem. Int. Ed. 51 (2012) 7132.
[63] F. Clow, et al. Biotechnol. Lett. 30 (2008) 1603.
[64] R. Jiang, et al. Bioconjug. Chem. 23 (2012) 643.
[65] T. Heck, et al. Bioconjug. Chem. 25 (2014) 1492.
[66] J.J.T.M. Swartjes, et al. Adv. Funct. Mater. 23 (2013) 2843.
[67] Y.Q. Li, et al. Angew. Chem. Int. Ed. 53 (2014) 5837.
[68] Y.M. Tan, et al. J. Phys. Chem. B 114 (2010) 5016.
[69] W. Kang, et al. Bioconjug. Chem. 25 (2014) 1387.
[70] B. Zakeri, et al. Proc. Natl. Acad. Sci. U. S. A. 109 (2012) E690.
[71] H. Abe, et al. Bioconjug. Chem. 24 (2013) 242.
[72] C. Giese, et al. Angew. Chem. Int. Ed. 51 (2012) 4474.
[73] M. Ramirez, et al. J. Am. Chem. Soc. 135 (2013) 5290.
[74] K. Min, et al. Biochip J. 7 (2013) 288.
[75] A.A. Shah, et al. Appl. Microbiol. Biotechnol. 98 (2014) 3437.
[76] E. Herrero Acero, et al. Macromolecules 44 (2011) 4632.
[77] E. Secret, et al. ACS Appl. Mater. Interfaces 6 (2014) 10313.
[78] L. Mondragon, et al. Chemistry 20 (2014) 5271.
[79] W. Guo, et al. Langmuir 30 (2014) 243.
[80] K. Katsuraya, et al. Carbohydr. Polym. 53 (2003) 183.
[81] H. Kikuchi, et al. Polym. J. 34 (2002) 835.
[82] S. Kobayashi, Encyclopedia of Polymer Science and Technology, John Wiley & Sons, Inc., 2002.
[83] F. Song, L.M. Zhang, J. Phys. Chem. B 112 (2008) 13749.
[84] S. Sakai, et al. J. Mater. Chem. B 1 (2013) 5067.
[85] A.K. Das, et al. Small 4 (2008) 279.
[86] J.B. Guilbaud, et al. Langmuir 26 (2010) 11297.
[87] J.B. Guilbaud, et al. Biomacromolecules 14 (2013) 1403.
[88] J.-I. Kadokawa, S. Kobayashi, Curr. Opin. Chem. Biol. 14 (2010) 145.
[89] A. Khan, et al. ChemSusChem 7 (2014) 379.
[90] S. Kobayashi, A. Makino, Chem. Rev. 109 (2009) 5288.
[91] L. Ren, et al. Macromol. Chem. Phys. 216 (2015) 636.
[92] C. Fu, et al. Chem. Commun. 48 (2012) 9062.
[93] W.E. Müller, et al. Chemistry 19 (2013) 5790.
[94] N. Oberleitner, et al. J. Biotechnol. 192 (Part B) (2014) 393.
[95] S. Schmidt, et al. Angew. Chem. Int. Ed. 54 (2015) 2784.
[96] R. McKenna, D.R. Nielsen, Metab. Eng. 13 (2011) 544.