CHEMICAL ENGINEERING TRANSACTIONS
VOL. 49, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Tajalli Keshavarz

ISBN 978-88-95608-40-2; **ISSN** 2283-9216
DOI: 10.3303/CET1649012

Immobilization of Peroxidases on Textile Carrier Materials and their Use in Bleaching Processes

Klaus Opwis*, Katharina Kiehl, Jochen S. Gutmann

Deutsches Textilforschungszentrum Nord-West gGmbH, Adlerstr. 1, D-47798 Krefeld, Germany
opwis@dtnw.de

The economical use of often high-priced enzymes in chemical synthesis can be improved by the immobilization of the catalyst on a suitable carrier. Particularly some synthetic or natural textile fiber materials such as polyester, polyamide or viscose are well-suited carrier materials, which are comparatively inexpensive. The flexibility of the textile media allows the use in reactors of any geometry and a fast and residue-free removal after the end of each reaction. Enzymatically catalyzed reactions combine a number of advantages compared to conventional chemical processes. For instance enzymes can be used at moderate temperatures, generally in the pH ranges close to neutral and stay unchanged after the reaction. Therefore, often very small quantities are enough for a sufficiently high implementation rate. Other advantages are their high substrate selectivity, their biodegradability and their mostly safe and easy handling. Especially in food industries bleaching processes with chemical additives such as benzoyl peroxide could be replaced by innovative methods of 'white biotechnology'. Here, we present an efficient method for the permanent immobilization of peroxidases on modified textile carrier materials and their use in the gentle enzymatic degradation of food colors (e.g. norbixin) in whey from cheese dairy. The textile-fixed peroxidase shows a distinct bio-catalytc activity over at least 15 reactions cycles. In addition, the fiber-fixed enzyme is able to bleach industrial whey completely.

1. Introduction

Enzymes are biological catalysts with high selectivities. Because of their natural origin they have been used in the food industry for hundreds of years. Today, they have established an important role in many industries (washing agents, textile manufacturing, pharmaceuticals, pulp and paper). Peroxidases (POD) are enzymes that catalyze the chemical reduction of peroxides, mainly hydrogen peroxide. Plants and animals are using peroxidases for a quick and efficient decomposition of toxic peroxides. Therefore, in nature peroxidases are part of the antioxidative protection system. At the same time, an electron donor is oxidized. In doing so, peroxidases can be used for the post-treatment of industrial waste waters (e.g. phenol removal) or textile dyeing bathes (decolorization of dyestuffs). In food industry they are used commercially for the bleaching of whey after the production of highly colored cheese such as cheddar or gouda. For instance, the annual US production of cheddar amounts to 1.5 million tons resulting in 0.5 million tons dried whey (USDA, 2011). In order to use whey commercially, e.g. as whey protein concentrate, a chemical bleaching with hydrogen peroxide or benzoyl peroxide is mandatory. Unfortunately, this harsh bleaching step is typically accompanied by the composition of undesired side products ("off-flavors") (Croissant et al., 2009, Listiyani et al., 2012). Therefore, the bio-catalytic bleaching with hydrogen peroxide in the presence of POD at mild pH and temperature conditions is an appropriate alternative to the use of benzoyl peroxide. Yellow or orange cheese varieties are typically colored with an isomeric mixture of norbixin (annatto, E 160 b). The chemical structure of norbixin is shown in Figure 1. Peroxidases are able to catalyze the oxidation of the chromophoric groups of such natural dyestuffs in the presence of hydrogen peroxide (Scheibner et al., 2008, Zorn et al., 2003, 2006). Commercial POD products promise to work fast and efficiently under regular whey conditions (around pH 6) at low temperatures between 5 and 50 °C (DSM Product Information). In addition, the reaction occurs without side reactions resp. off-flavors. Because of the substitution of benzoyl peroxide by hydrogen peroxide no benzoate residues remain in the bleached whey powder. However, the use of peroxidases in food applications

Please cite this article as: Opwis K., Kiehl K., Gutmann J.S., 2016, Immobilization of peroxidases on textile carrier materials and their use in bleaching processes, Chemical Engineering Transactions, 49, 67-72 DOI: 10.3303/CET1649012

can be further improved by the permanent immobilization of the enzyme on a suitable carrier material to use it repeated or even continuously (Hill et al., 2005, Wiseman, 1985). Here, we present results on the immobilization of two commercial peroxidases on textile carrier materials and their use in the gentle enzymatic degradation of food colors (e.g. norbixin) in whey from cheese dairy. The investigations are based on our former studies on the use of fibrous supports for enzymes. Our different wet chemical and photochemical strategies for the immobilization of biocatalysts on textile carrier materials are already published (Opwis et al., 2004, 2005, 2007, 2013, 2014). Beside the immobilization procedure, we provide results on the activity of the fiber-fixed proteins in repeated use, and explain various practically relevant applications.

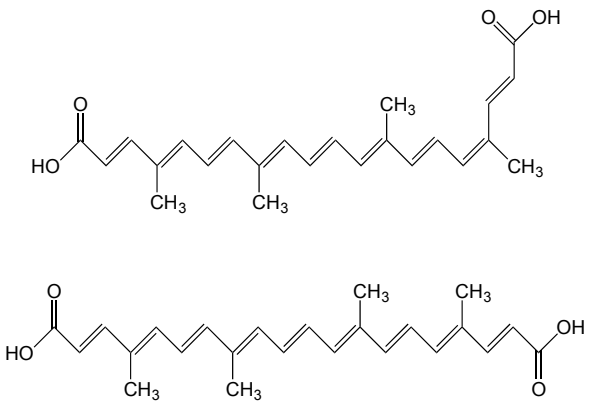


Figure 1: Chemical structures of cis-norbixin (above) and trans-norbixin (below).

2. Experimental

2.1 Peroxidase immobilization

Commerical polyvinylamine (10 - 15 wt. % PVAm, Lupamin® 9095, BASF, Germany) was diluted 1:1 with distilled water. Afterwards the pH value was adjusted with NaOH to 10. A PET needle-felt nonwoven filter material (Heimbach Filtration, Düren, Germany) was wetted fully with PVAm solution and squeezed well-defined. The material was pre-dried for 30 min at 80 °C and afterwards thermally fixed for 15 min at 130 °C. To remove unbound PVAm, the material was washed intensely with hot water. The textiles were stirred for 2 h at 25 °C in 5 % glutardialdehyde (GDA) solution. The textiles were washed consecutively 3 min in distilled water, 3 min in 1.5 M NaCl and 10 min in water (ultrasonic bath). Finally, 6 mL Baylase® (Lanxess, Germany,) respectively 6 mL MaxiBright® (DSM Food Specialities, The Netherlands) were pipetted on the modified textiles. The textiles were dried overnight at room temperature. For comparison, the PVAm-modified textiles were pipetted with the same peroxidase amount without previous GDA treatment.

2.2 Analyses, enzyme activity and reuse of immobilized peroxidase

The immobilization of peroxidase on textile carrier materials was analyzed qualitatively using FT-IR-spectroscopy (FTS-45, Biorad) with an ATR-unit (Silver Gate) and UV-Vis-spectroscopy (Cary 5E). The peroxidase loads on the textiles were determined quantitatively by ICP-OES (Varian 720-ES) after the chemical decomposition in suitable concentrated acids measuring the iron concentration of these solutions. The activity of immobilized peroxidase was determined by the time depending oxidation of ABTS. The immobilized peroxidase was reused up to 15 times in fresh ABTS solutions. Between each repetition the samples were stored for one day in distilled water.The decolorization of norbixin colored whey with textile-immobilized peroxidase was carried out at 25 °C with a norbixin concentration of 6.4 mg/L and a H_2O_2 starting concentration of 40 mg/L.

3. Results

3.1 Enzyme Immobilization

First, in order to increase the number of functional surface groups for the subsequent immobilization step, the textile polyester material (PET) was finished thermically with polyvinylamine (PVAm). The intermediate product is shown schematically in Figure 2. The established surface amino groups were qualitatively detected

by a NH_2-selective color reaction with 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Figure 3). The PVAm-modified material exhibits a strong orange coloring as significant proof for the successful implementation of numerous amino groups (Figure 3, center), whereas the blank PET substrate shows no coloring (Figure 3, left).

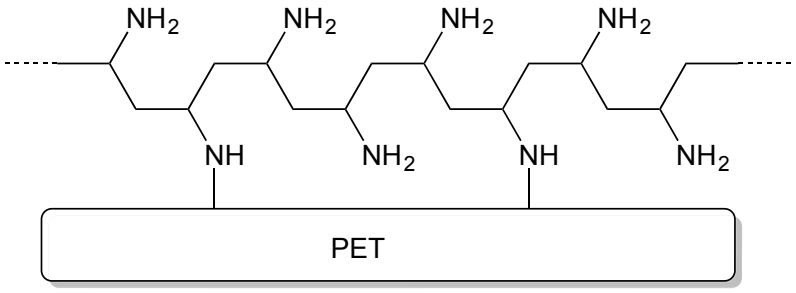
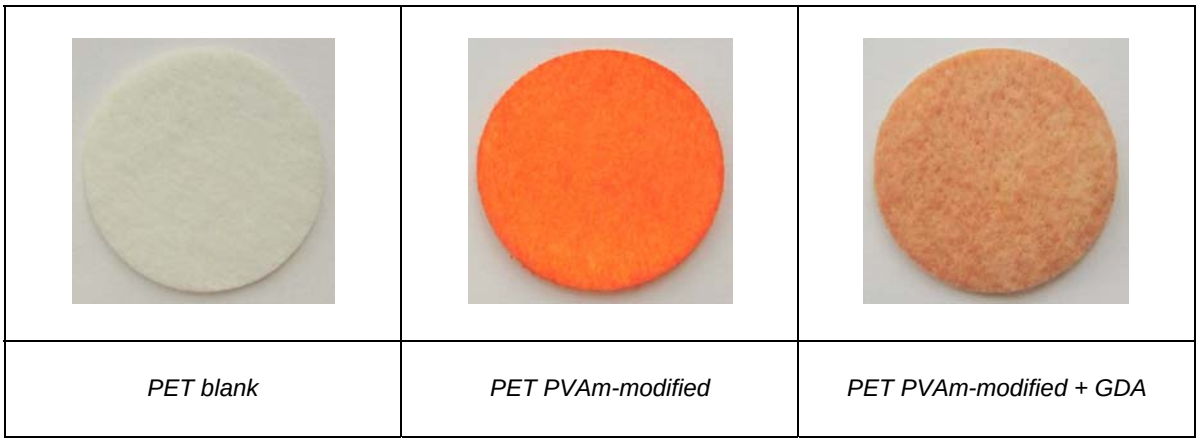


Figure 2: Pre-functionalized polyester textile finished with polyvinylamine.



PET blank	*PET PVAm-modified*	*PET PVAm-modified + GDA*

Figure 3: TNBS assay for the qualitative detection of amino groups.

Secondly, the polyvinylamine layer was partially cross-linked by glutaraldehyde (GDA) to enhance the adhesion of PVAm to the textile support. In addition, GDA acts as anchor molecule for the subsequent peroxidase fixation. The successful saturation of free amino groups by GDA can be easily detected by the TNBS assay. The GDA treated textile shows a significantly lower orange color (Figure 3, right). The overall immobilization procedure of POD via GDA is shown schematically in Figure 4. One aldehyde function of GDA reacts with the amino groups of PVAm, the other with amino groups of the enzyme. In addition, cross-linking between PVAm/PVAm resp. enzyme/enzyme is possible too.

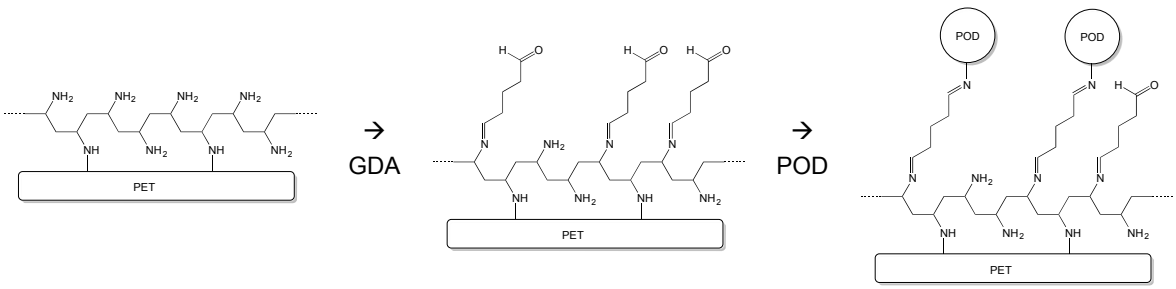


Figure 4: Covalent immobilization of peroxidase on PVAm-modified polyester via glutaraldehyde (schematic).

Surface-sensitive spectroscopic methods such as UV-Vis remission measurements and ATR-IR-spectroscopy are useful for the qualitative proof of the successful immobilization procedure. Figure 5 shows exemplarily UV-Vis remission spectra of textile-fixed peroxidase Baylase® compared to the blank material and the control experiments with the PVAm-modification respectively GDA treatment. The blank material and the samples treated only with PVAm respectively PVAm plus GDA exhibit no UV-Vis absorbance near 410 nm, where the protein peroxidase has an absorption maximum. After the final POD immobilization step, a significant signal appears around 410 nm.

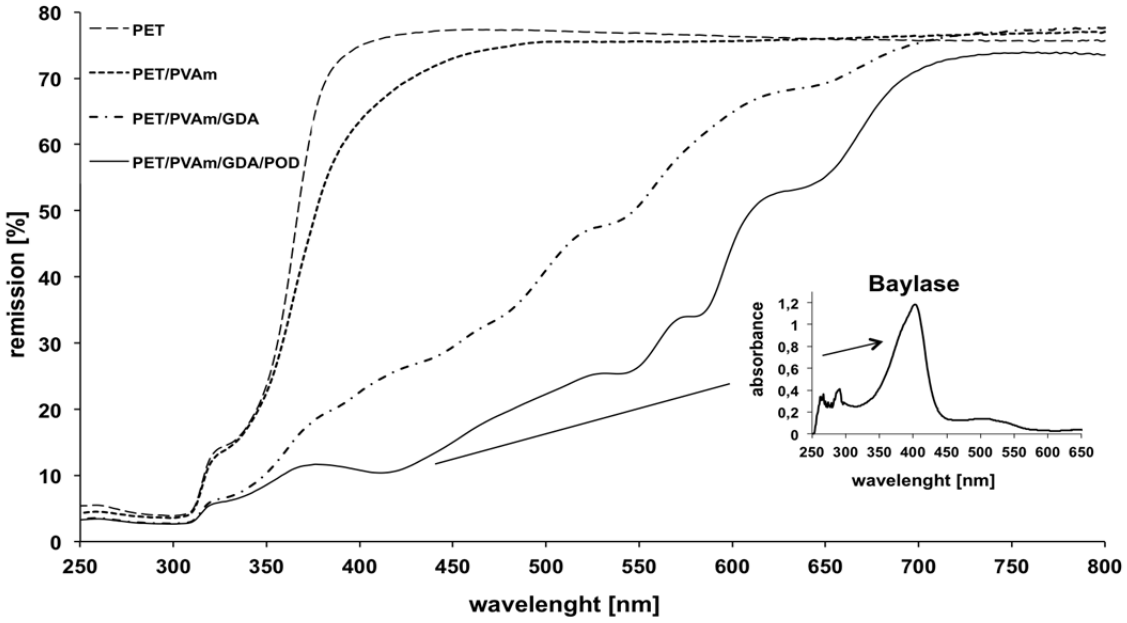


Figure 5: UV-Vis remission spectra of PET textiles at different stages of the POD immobilization procedure (here Baylase®).

Further quantitative evidence for the POD immobilization on fibrous PET is given by ICP-OES analyses. Because of its iron content, the peroxidase load can be analyzed quantitatively by ICP-OES. Depending on the used peroxidase up to 20 mg enzyme/g carrier can be fixed durably on the textile material. Afterwards the investigations were focused on the bio-catalytic activity of the in such a way immobilized POD.

3.2 Bio-catalytic activity and reuse

Peroxdidases catalyze the reduction of peroxides while various organic electron donors will be oxidized. The bio-catalytic activity of POD is measured typically by the ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)). The reaction of ABTS with hydrogen peroxide yields a green, soluble and stable end product that can be easily quantified by UV-Vis spectroscopy at its absorption maximum of 420 nm. Figure 6 summarises the bio-catalytc activity of textile-fixed POD in repeated use. Even after 15 reactions cycles the textile show a distinct and stable catalytic behavior. After demonstrating the successful immobilization and the remaining of its activity we succeed in bleaching real colored whey from cheese dairy. Figure 7 shows photographs of various whey samples. In the absence of POD the whey stays yellow even if hydrogen peroxide was added (fourth cuvette). In the case of adding both, textile-fixed peroxidase and hydrogen peroxide, the norbixin is fully oxidized to uncolored compounds (third cuvette). The color impression meets the yellowness of natural, uncolored whey (first cuvette).

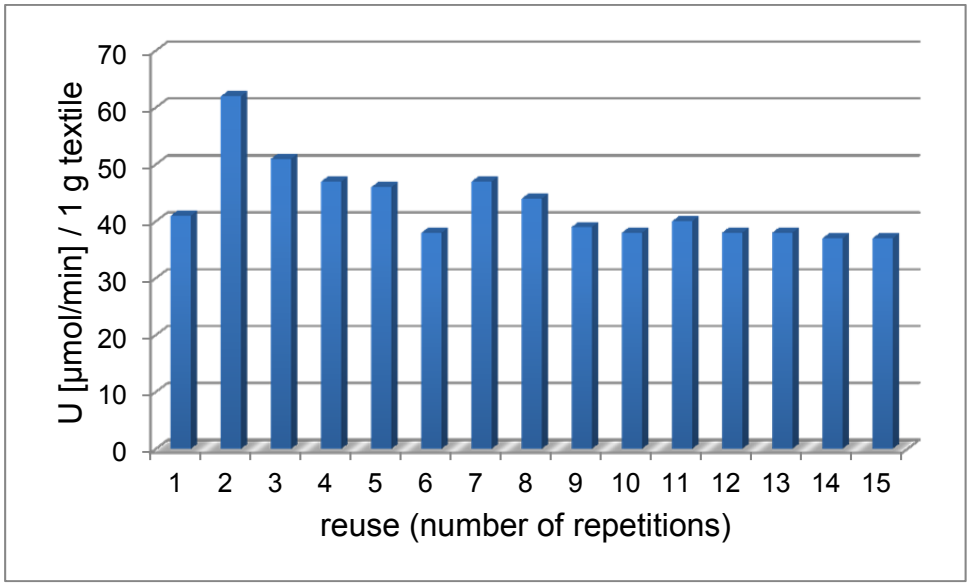


Figure 6: Bio-catalytical activity of textile-fixed peroxidase in repeated use (ABTS assay, here MaxiBright®).

Figure 7: Photographs of whey samples (cuvettes from left to right: uncolored whey, norbixin-colored whey, norbixin-colored whey treated with hydrogen peroxide in the presence of textile-fixed POD, norbixin-colored whey treated with hydrogen peroxide without immobilized POD.

4. Conclusions

In our former studies we have identified low-cost textile fabrics made of polyester, polyamide and cotton as alternative and high performing carrier materials for bio-catalytic active enzymes. With a low preparative and economic expense textiles with high protein load, high activity and sufficient permanence against enzyme desorption can be realized using various anchor molecules and additional cross-linking agents. Morever, we are able to increase the density of the required functional groups for the covalent enzyme fixation dramatically by functionalizing the textiles with amino groups containing compounds. The drapable and connected construction of textiles allows reactor constructions of arbitrary geometry and a quick, residue-free removal of the catalyst after its use. In addition, the fibrous structure enables an optimal substrate flow-through and, therefore, turn-over. In summary, our innovative concept for the permanent fixation of enzymes and other catalytsts on textile carrier materials opens a new class of technical textiles with widespread applications and

prospects in pharmaceutics, chemistry and biochemistry. With its use in the bleaching of whey from cheese dairy, now, we have widen the general concept to a practical food application. In this context, our recent work is focused on the up-scale of the procedure. First experiments with whey volumina of 10 - 50 L are carried out actually.

Acknowledgments

This work is funded by the German Federal Ministry of Education and Research (BMBF) under the project “POD-Immobilisierung”, grant identifier 03X0135C.

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