CHEMICAL ENGINEERING TRANSACTIONS
VOL. 38, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Taj Keshavarz

ISBN 978-88-95608-29-7; **ISSN** 2283-9216
DOI: 10.3303/CET1438038

Various Strategies for the Immobilization of Biocatalysts on Textile Carrier Materials

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Textile fabrics made of polyester (PET), polyamide (PA) or cotton represent alternative carrier materials for the immobilization of enzymes. In contrast to conventional carriers, fiber materials are considerably inexpensive. The flexible construction of fabrics enables reactor constructions of any geometry and a quick removal of the catalyst without any residues after the reaction. Moreover, their open structure guarantees an optimal substrate turn-over and the active surface is easily adjustable by the fiber diameter. We have demonstrated successfully, that fabrics with a high enzyme load, a high relative activity and good permanence against enzyme desorption can be produced with low preparative and economic expense. Here, we report various methods for the permanent fixation of enzymes on fiber forming polymers such as photochemical grafting, the use of bifunctional anchor molecules, monomeric and polymeric cross-linking agents or specific enzyme modification for direct immobilization. In addition, we compare the strategies in terms of load, catalytic activity and reusability.

1. Introduction

The potential of the so-called “White Biotechnology” as an ecological advantageous and moreover economical beneficial technology is beyond all question. Caused of the ever-growing costs for energy and polluted waste waters, enzymatic technologies will stay in the focus of science and technique and their relevance will be increased significantly in future. Enzymes, biological catalysts with high selectivities, have been used in the food industry for hundreds of years and have established an important role in many other industries (washing agents, textile manufacturing, pharmaceuticals, pulp and paper). Currently, enzymes are becoming increasingly important in sustainable technology and green chemistry.

However, the application of enzymes is often hampered by major limitations such as high costs. Therefore, in modern bio-catalysis especially the use of immobilized enzymes is getting more and more attractive. If an enzyme is fixed on an adequate carrier material, the limitations can be overcome as the immobilized enzyme enables easy separation, the possibility of reuse and simple operation. Moreover, the reaction products are free from proteinous residues (Hill et al., 2005). Indeed, some immobilized enzymes such as glucose isomerase and penicillin G acylase have reached large-scale industrial application, and the immobilization of other enzymes has been of great interest in research and development.

Besides encapsulation techniques, adsorptive or ionic fixation the enzymes mostly are bonded covalently to a functionalized carrier material (Wiseman, 1985). Here, we present various successful approaches for the immobilization of enzymes on textile carrier materials. Besides the negligible low prize compared to conventional carriers, textiles exhibit several other material-inherent advantages such as flexibility, mechanical strength and high surface area. The woven structure allows a high substrate through-put and therefore a high turn over. The investigations were carried out using the enzyme catalase (E.C. 1.11.1.6) as an example. Catalase is an iron-containing protein, which in nature catalyzes the decomposition of hydrogen peroxide to water and molecular oxygen as a detoxifying mechanism. In addition to its crucial biological relevance, this enzyme has many industrial applications, including the elimination of hydrogen peroxide after milk sterilization in the dairy industry or the prevention of inactivation of other oxidases in the presence of

Please cite this article as: Opwis K., Straube T., Kiehl K., Gutmann J.S., 2014, Various strategies for the immobilization of biocatalysts on textile carrier materials, Chemical Engineering Transactions, 38, 223-228 DOI: 10.3303/CET1438038

high peroxide concentrations. Moreover, catalase is also from interest in textile processes. Catalase can be used after bleaching to decompose residual hydrogen peroxide, which disturbs the following dyeing process (Paar et al., 2002).

2. Experimental

2.1 Enzyme used

A catalase from bovine liver (Fluka) was used (M = ~ 240,000 g/mol, activity 2500 U/mg, pH-value optimum 7, temperature optimum 25 °C).

2.2 Immobilization Method 1: Anchor molecules and additional cross-linking (on cotton and polyamide 6)

4.0 g Cotton fabric (Testex, plain weave, 102 g/m^2) were covered with 15 ml NaOH solution (5 %). After 15 min 135 ml water and 0.025 g sodium dodecylsulfate (SDS) were added. The pH-value reached 12.5 to 13. After adding 1.5 g cyanuric chloride (CC) the solution was stirred for 3 h at 25 °C. Cyanuric chloride was completely dissolved and the pH-value decreased to 11.5 to 12. The cotton was washed several times with distilled water and dried in air. In the case of polyamide 6 (PA 6) 8.0 g PA 6 fabric (VBL, plain weave, 65 g/m^2) were stirred for 2 h at 25 °C in 100 ml of a glutardialdehyde (GDA) solution (5 %). The textile was washed several times with distilled water and dried in air. The activated cotton resp. PA 6 material was stirred for 24 h at 25 °C in 100 ml buffer solution (citrate/NaOH, pH 6), which contains 400 mg dissolved catalase. For additional cross-linking 1.0 ml GDA solution (25 %) was added after 2 h. Afterwards the samples were stirred for 0.5 h in 100 ml aqueous 0.5 Vol.-% Marlipal O13/80 (Sasol) solution. After this treatment all samples were washed five times with 100 ml distilled water and dried overnight.

2.3 Immobilization Method 2: Immobilization by use of polycarbodiimide crosslinker (on polyester)

500 mg Catalase were dissolved in 28.8 ml phosphate buffer pH 7 and 1.2 ml commercial polycarbodiimide Permuthan® XR-5577 (Stahl Europe) was added. Afterwards 10 g PET fabric (10 pieces each 1.0 g) were wetted with the protein-solution and heated 30 min at 90 °C. After drying overnight the material was washed and dried as described before.

2.4 Immobilization Method 3: Photo-induced Crosslinking (on polyester and polyamide 6.6)

The experiments were carried out using commercial polyester (PET) and polyamide 6.6 (PA 6.6) fabrics as carrier materials. Starting with 94.5 ml distilled water, 5.0 ml of diallylphthalate (DAP, > 98 %, Acros Organics) or cyclohexane-1,4-dimethanoldivinylether (CHMV, > 98 %, Merck) were emulsified by adding 0.5 ml Marlipal O13/80 under stirring. 50.0 mg catalase was dissolved in 3.0 ml of the DAP- or CHMV-emulsion. 1.0 g of the PET or PA 6.6 fabric was wetted with the protein-emulsion. For comparison, experiments were also carried out without reactive media. A KrCl*-excimer-lamp (BlueLight BLC 222/300, Heraeus Noblelight) emitting nearly monochromatic light at 222 nm served as UV-source. The fabrics (wetted either with or without reactive compound) were irradiated in a steel reactor with a transparent polyethylene window. The irradiation takes place in an argon atmosphere avoiding a photo-oxidation by air oxygen. The distance between the light source and the sample was 8 cm. The irradiation time was 5.0 min per each fabric side. Afterwards the samples were washed and dried as described before.

2.5 Immobilization Method 4: Wet chemical enzyme modification and photochemical immobilization (on polyester)

20 Vol.-% Allylglycidylether (AGE, > 98 %, Acros Organics) were dissolved with 1 vol.-‰ Marlipal in distilled water under strong stirring. 150 mg catalase was added to 0.5 ml of the AGE solution, which is filled up to a final volume of 5.0 ml with water. The mixture was stirred 45 min. 1.0 g PET fabric was wetted with 3.0 ml of the catalase containing solution (corresponds to 90 mg catalase). A KrCl*-excimer-lamp (BlueLight BLC 222/300, Heraeus Noblelight) emitting nearly monochromatic light at 222 nm served as UV-source. The fabrics (wetted with the modified catalase) were irradiated with a monochromatic excimer UV lamp (222 nm). Afterwards the samples were washed and dried as described before.

2.6 Analyses, enzyme activity and reuse of immobilized catalase

The immobilization of catalase 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). Scanning electron microscopy images (SEM) of fabrics were made using a Topcon microscope ATB-55. The catalase load on the fabrics was determined quantitatively by atomic absorption spectroscopy (SpectrAA 800, Varian) after the chemical decomposition in suitable concentrated acids measuring the iron concentration of these solutions (Opwis et al., 2004a). The activity of immobilized catalase was determined by the time depending degradation of hydrogen peroxide. The enzymatic reactions with immobilized catalase were carried out at 25 °C with 1.0 g treated fabric in 50 ml hydrogen peroxide solution (c = 6.0 g/l, m = 300.0 mg). After 1 min

the H_2O_2-concentration was analyzed quantitatively by High Performance Anion Exchange Chromatography (Dionex, HPAEC-PAD, column Carbo Pac PA 1, eluent 0.15 m NaOH). The immobilized catalase was reused up to 30 times in fresh hydrogen peroxide solutions (50 ml, c = 6.0 g/l). Between each repetition the samples were stored for one day in distilled water.

3. Results

3.1 Enzyme Immobilization

Fabrics made of cotton, polyamide 6, polyamide 6.6 and polyester were used for the immobilization of catalase. The methods are optimized for the individual carrier-specific properties. Figure 1 summarizes the methods schematically. The wet chemical method 1 takes advantage of the functional groups of cotton and polyamide (Opwis et al., 2004b). In the case of cotton, the enzyme was fixed by a cyanuric chloride anchor, where the chlorine atoms may react with the hydroxyl groups of the cellulosic carrier on the one side and with the amino groups of the protein on the other side. In the case of polyamide, glutardialdehyde reacts with both, the amino groups of the textile substrate and the amino groups of the enzyme. The total enzyme load can be easily increased by an additional cross-linking step with excess glutardialdehyde. In the case of polyester polycarbodiimide can be used as anchor and cross-linker at the same time (method 2) (Opwis et al., 2013).

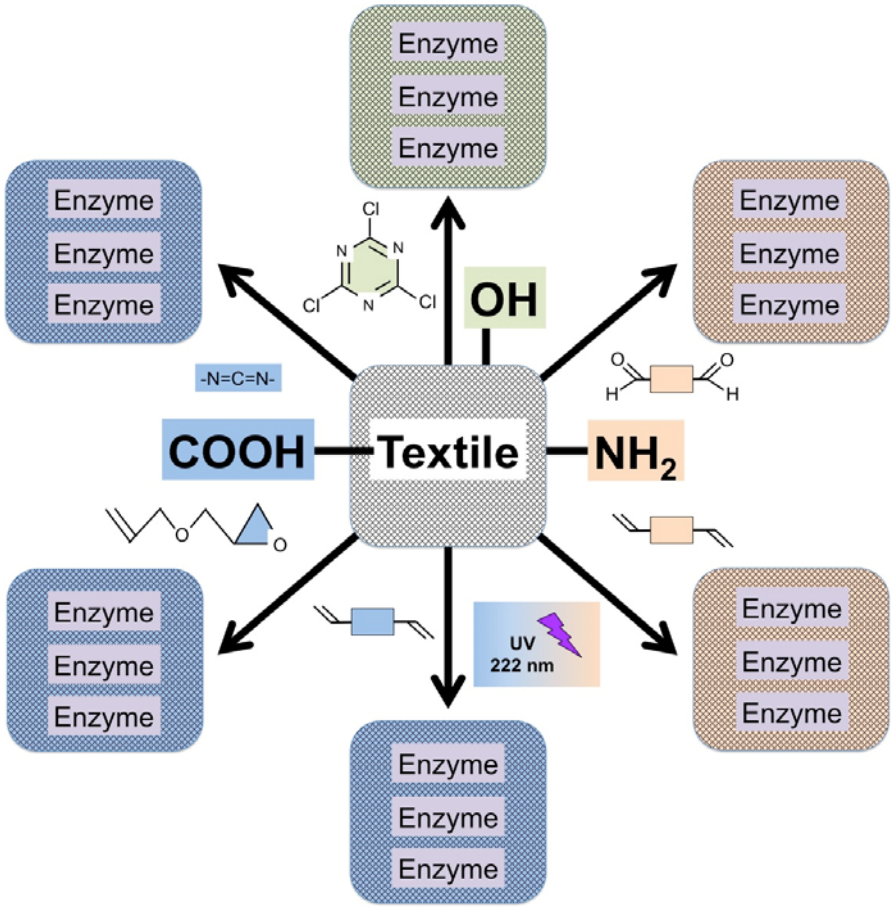

Figure 1: Various strategies for the immobilization of enzymes on textile carrier materials.

Beside the wet chemical approaches we also succeeded using monochromatic excimer UV lamps, which are able to cleave bonds in UV absorbing materials such as polyester and polyamide (method 3) (Opwis et al., 2005). In the presence of photo-active cross-linking agents and enzymes the yielding radicals can react with neighbored radical species forming new covalent bonds resulting in a cross-linked structure surrounding the fibrous material. Method 4 combines wet chemical and photo-chemical elements (Opwis et al., 2007). In

a first step the enzyme is modified with allylglycidylether. Afterwards, the allyl-functionalized protein can be grafted photo-chemically on polyester fibers.

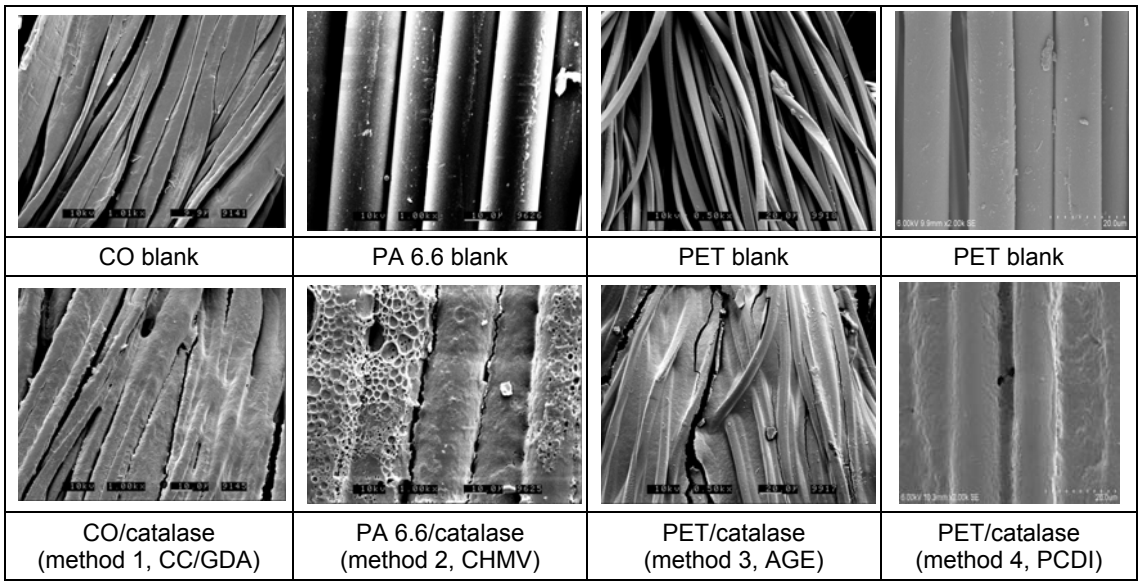

Figure 2: SEM of various textile carrier fabrics treated with catalase using different approaches (Opwis et al., 2004b, 2005, 2007, 2013).

Figure 2 shows various SEM pictures of the textile materials before and after the immobilization of catalase using different methods. In all cases the three-dimensional covering of the fibers with the cross-linked protein is visible impressively. In addition, surface-sensitive spectroscopic methods such as UV-Vis remission measurements and ATR-IR-spectroscopy are useful for the qualitative proof of the successful immobilization procedures. Figure 3 shows exemplarily spectra of textile-fixed catalase compared to the blank material and the control experiments with the used cross-linker respectively catalase.

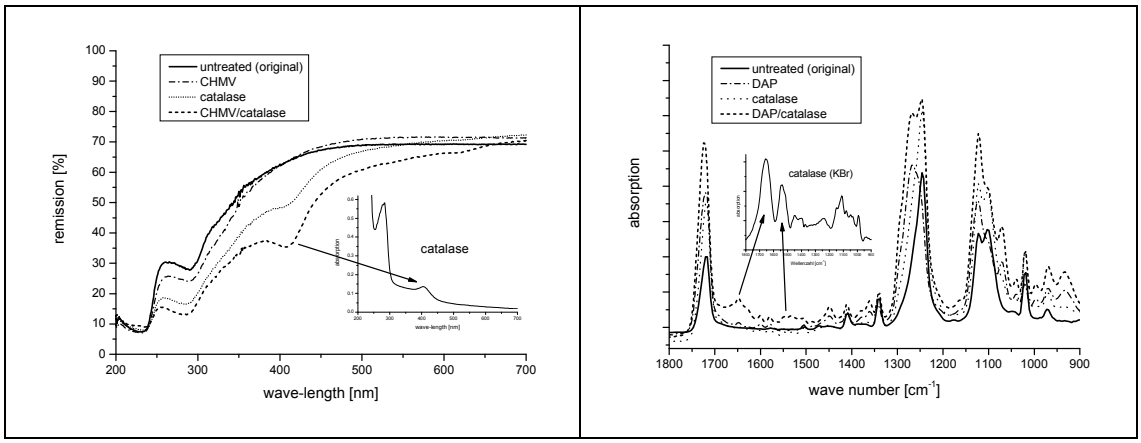

Figure 3: UV-Vis-spectra of PA 6.6 fabrics (left) and FT-IR(ATR)-spectra of PET fabrics (right) before and after UV-irradiation in the presence of the used cross-linker, catalase and both together (Opwis et al., 2005).

The blank material and the sample treated only with the cross-linking agent CHMV shows no UV-Vis absorbance near 410 nm, where the protein catalase has an absorption maximum. Following the irradiation in the presence of catalase, a significant signal is visible in this area showing that the photochemical immobilization of catalase on PA 6.6 works also without cross-linking agent. A higher yield of immobilized catalase can be achieved using the enzyme in combination with the cross-linking agent; the absorbance of the polymer carrier at 410 nm is strongly enhanced. Further qualitative evidence for the enzyme

immobilization on textile carrier materials is given by FT-IR-spectroscopy using the ATR-technique. The irradiation in the presence of the cross-linking agent DAP causes a significant variation of the spectra between 1200 cm^{-1} and 1300 cm^{-1}. The experiment in the presence of catalase without DAP yields no detectable change of the spectra. Without a cross linking agent, no catalase immobilization on PET takes place. Using a combination of catalase and the cross-linking agent DAP, the spectrum shows two characteristic signals of the enzyme between 1500 cm^{-1} and 1700 cm^{-1} (see KBr spectra of catalase powder).

Table 1: Load of immobilized catalase on cotton, PA 6, PA 6.6 and PET using various methods (Opwis et al., 2004b, 2005, 2007, 2013).

carrier material	Immobilization method	anchor molecule resp. cross-linking agent	catalase load [mg/g carrier]
CO	1	CC/GDA	48.7
PA 6	1	GDA	50.4
PA 6.6	2	DAP	20.8
PA 6.6	2	CHMV	22.0
PET	2	DAP	32.2
PET	2	CHMV	23.9
PET	3	AGE	70.0
PET	4	PCDI	27.4

Moreover, because catalase is an iron-containing enzyme, the protein load can be analyzed quantitatively by atomic absorption spectroscopy (AAS) (Opwis et al., 2004a). The results are summarized in Table 1. Depending on the used method 20 to 70 mg/g of the enzyme can be fixed durably on the textile materials. Further investigations focused on the bio-catalytic activity of the in such a way immobilized catalase.

3.2 Bio-catalytic activity and reuse

The enzyme catalase catalyzes the disproportionation of hydrogen peroxide into oxygen and water. By measuring the enzymatic decomposition of hydrogen peroxide as a function of time, it is possible to calculate the relative activity of immobilized enzymes in comparison to free catalase. The results are summarized in Table 2. All immobilization products show a distinct bio-catalytic activity even after 20 reuses. Depending on the used procedure the integral activity over 20 respectively 30 reuses differs from nearly 80 to more than 350 %. The highest relative activity of nearly 18 % (compared to the free system) was found when the photochemical approach with the cross-linker DAP on PA 6.6 was used. On the other hand a high catalase load does not necessarily lead to a high integral activity. By using the combined method 4 the highest protein load was detected (70 mg/g) but the relative activity was rather poor.

Table 2: Integral activity of immobilized catalase after 20 resp. 30 reuses in comparison to free catalase (Opwis et al., 2004b, 2005, 2007, 2013).

carrier material	immobilization method	anchor molecule resp. cross-linking agent	relative activity [%]	reuses	integral activity [%]
CO	1	CC/GDA	15.5	20	≥ 310
PA 6	1	GDA	15.2	20	≥ 304
PA 6.6	2	DAP	18.3	20	≥ 366
PA 6.6	2	CHMV	11.3	20	≥ 226
PET	2	DAP	9.6	20	≥ 192
PET	2	CHMV	11.3	20	≥ 226
PET	3	AGE	5.1	30	≥ 153
PET	4	PCDI	4,0	20	≥ 80
free catalase	-	-	100	1	100

4. Conclusions

Low-cost textile fabrics made of cotton, polyamide or polyester were identified as alternative carrier materials for the immobilization of enzymes. With a low preparative and economic expense fabrics with a high protein load, a high relative activity and good permanence against enzyme desorption can be produced using anchor molecules and cross-linking agents by various wet chemical and photo-chemical approaches. The flexible

construction of fabrics enables reactor constructions of arbitrary geometry and a quick removal of the catalyst without any residues after the reaction. Moreover, their open structure guarantees an optimal substrate turn-over and the active surface is easily adjustable by the fiber diameter. In summary, our innovative concepts for the permanent fixation of enzymes on textile carrier materials are able to compete with conventional immobilization procedures respectively common substrates. Recently, our photochemical approach was transferred successfully to organic catalysts (Lee et al., 2013). These improved textiles can be used for various chemical syntheses of industrial relevance without a significant loss of its catalytic activity for more than 250 reaction cycles. In conclusion, our *Permanently Textile-Fixed Catalysts* may provide a totally new class of technical textiles with widespread applications and prospects in pharmaceutics, chemistry and biochemistry in the near future.

Acknowledgement

Based on a decision of the German Bundestag the IGF project 16884 N of the Forschungskuratorium Textil e.V. (Berlin) was supported via Arbeitsgemeinschaft industrieller Forschungsvereinigungen e.V. (AiF) within the program Industrielle Gemeinschaftsforschung (IGF) from resources of the Bundesministerium für Wirtschaft und Technologie (BMWi). In addition, we wish to thank the Ministry for Science and Research of the country of Northrhine-Westphalia (Germany) for financial support. This support was granted within the project DTNW/Support for attainment of further funds.

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