

Photochemical Immobilization of Enzymes on Textile Carrier Materials

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Low-cost textile fabrics made of polyester (PET) or polyamides (PA) are alternative carrier materials for the immobilization of enzymes. With a low preparative and economic expense fabrics with a high enzyme load, a high relative activity and good permanence against enzyme desorption can be produced by photo-induced cross-linking and grafting processes using monochromatic excimer-UV-lamps. The flexible and open construction of fabrics allows a high substrate conversion. Moreover, conventional immobilization products are mostly produced as granulates or pellets, which must be filtered after the enzymatic reactions. Fabrics can be removed very quickly from the reactor without any filtration step and the solution contains no proteinous residues after the enzymatic reaction. Here, the enzyme catalase was immobilized on PET and PA fabrics by different photochemical processes. Depending on the support and the used reactive agents 20 - 70 mg enzyme per gram carrier could be fixed durably, which can be quantitatively analyzed by atomic absorption spectroscopy due to the iron content of catalase. The efficiency of the immobilization products were investigated by measuring the enzymatic decomposition of hydrogen peroxide in comparison to the free enzyme. The relative activity of the catalase after the immobilization was 5 - 20 % of the free, not fixed catalase. The immobilized enzyme showed even after 20 reuses a distinct activity and the integral activity over all reuses was nearly 3.5 times higher than the activity of the free catalase. Therefore, these promising new photo-induced immobilization techniques open widespread applications in bio-catalysis in the future.

1. Introduction

Photochemical treatments allow a permanent surface modification of several synthetic fibers such as polyesters, polyamides or polyolefines by irradiating them with UV light in the presence of different reactive media. These processes are based on a homolytic bond cleavage initiated by the absorption of energetic photons and leading to the generation of radicals on the irradiated polymer surfaces. This allows manifold subsequent reactions such as photo oxidations, graftings and cross-linking, which are described in numerous publications [1-4].

In particular, monochromatic UV lamps, e.g. the so-called excimer UV lamps, allow a selective irradiation taking advantage of substrate and agent specific absorption bands. Since many years the Deutsches Textilforschungszentrum Nord-West e.V. (DTNW)

investigates photochemical surface modifications of fiber materials using such excimer UV lamps. In the case of poly(ethylene terephthalate) (PET) the use of a monochromatic KrCl^{*}-excimer lamp with a wavelength of 222 nm is appropriate, because PET strongly adsorbs radiation between 220 nm and 250 nm. In the presence of oxygen (air) such irradiations initiate a photo-oxidation of the substrate resulting in high surface energies and, therefore, a significant hydrophilization of the material [5]. Thus, composites are available without using adhesion-promoting agents [6]. Using reactive media other than atmospheric oxygen the surface properties are directly assessable in wide ranges. Unsaturated compounds show an improved affinity for the addition to radicals. The photochemical grafting of such unsaturated compounds with second functionalities transfers the functionality of the attached molecule to the irradiated polymer. Thus, the UV irradiation of polyester in the presence of allylic compounds with terminal hydroxyl groups leads to a permanent hydrophilization [7]. In contrast, hydrophobic molecule parts, e.g. perfluorinated carbon groups, results in hydrophobic and even oleophobic wetting properties of the photochemically modified material [8]. Using diallylic compounds barrier layers on PET can be achieved, which, e.g., protect the material against alkaline hydrolysis [9]. Moreover, in photochemically cross-linkable matrices molecules with totally new properties can be integrated. For example, the excimer UV lamps can be applied to immobilize enzymes durable on textile carrier materials. These fixed bio-catalysts are practical for the permanent use in technical flow-through reactors. The work presented here summarizes different published approaches concerning the permanent photochemical immobilization of catalase on textile carrier materials [10-12] and gives an outlook for further investigations in this field.

2. Experimental

2.1 Materials and Methods

The experiments were carried out using commercial Polyester (PET) and Polyamide 6.6 (PA 6.6) fabrics as carrier materials. A catalase from bovine liver with a molecular weight of 240.000 g/mol and an activity of 2300 U/mg at 25 °C and pH 7.0 was used. Diallylphthalate (DAP) and cyclohexane-1,4-dimethanoldivinylether (CHMV) were acting as crosslinking-agents and allylglycidylether (AGE) was taken to modify the catalase. A KrCl^{*}-excimer-lamp (BlueLight BLC 222/300, Heraeus Noblelight) emitting nearly monochromatic light at 222 nm served as UV-source. The irradiation takes place in an argon atmosphere avoiding a photooxidation by air oxygen. After the irradiations, the samples were stirred for 0.5 h in 100 ml Marlipal[®] (0.5 Vol.-%) containing water and washed another five times with 100 ml distilled water respectively. Further information on the preparation of protein-emulsions and polymer wetting are given in references [10-12].

2.2 Analyses

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 after the chemical decomposition in suitable concentrated acids measuring the iron concentration of these solutions [13]. 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 ($c = 6.0 \text{ g/l}$, $m = 300.0 \text{ mg}$). After 1 min the H_2O_2 -concentration was analyzed quantitatively by HPAEC-PAD. The decay of hydrogen peroxide showed the activity of the immobilized catalase. The immobilized catalase was reused 20 times in fresh hydrogen peroxide solutions (50 ml, $c = 6.0 \text{ g/l}$). Between each repetition the samples were stored for one day in distilled water.

3. Results

As described in the introduction excimer UV lights are able to initiate cross-linking or grafting reactions on polymer surfaces. Taking benefit from the special properties of textile fabrics (high surface area, high substrate throughput and turn-over, flexibility, low costs etc.) these materials are appropriate carrier materials for catalysts - namely enzymes. Therefore, studies were carried out to immobilize enzymes by photo-initiated reactions directly onto textile materials. A first approach used water-based emulsions of enzymes and photo-reactive cross-linking agents such as DAP and CHMV.

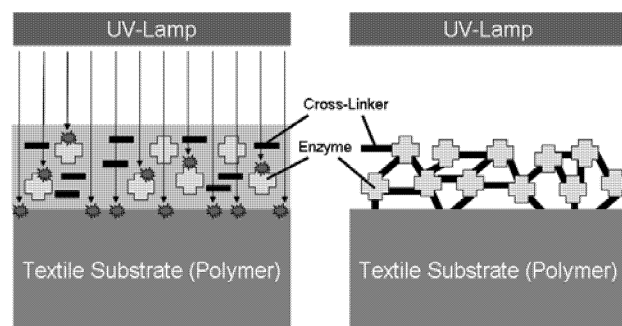


Figure 1: Photochemical immobilization of enzymes on textile carrier materials by cross-linking.

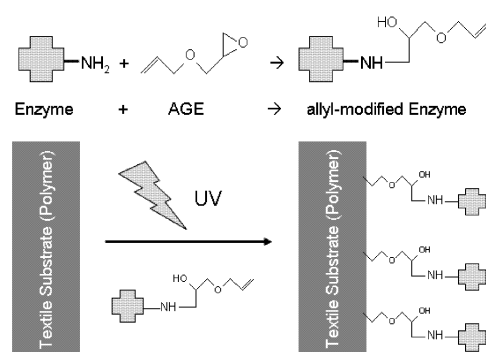


Figure 2: Photochemical immobilization of enzymes on textile carrier materials by grafting of functionalized enzymes.

The textiles were wetted with these protein-containing mixtures and irradiated by UV light in inert atmospheres. The process is described schematically in Figure 1. The irradiation starts the generation of radicals on the polymer and protein surfaces by absorption of the energy-rich UV-light (homolytic bond-cleavage, Figure 1 left). The addition of at least bifunctional cross-linkers yields a film formation on the polymers surface (Figure 1 right). Another approach focuses the pre-functionalization of the enzyme before the photo-induced fixation on the textile carrier material. In this study the enzyme catalase was treated with the bifunctional molecule AGE. The reaction, which is described in Figure 2, occurs spontaneously at room temperature and yields an allyl-functionalized enzyme, which is able to be grafted on the textile carrier material by UV exposure. Both strategies for a photochemical immobilization of enzymes on textile substrates lead to a three-dimensional structure of the fixed catalase surrounding the fibre material. Figure 3 shows SEM pictures of PET and PA fabrics with and without the immobilized catalase using different reaction agents and techniques.

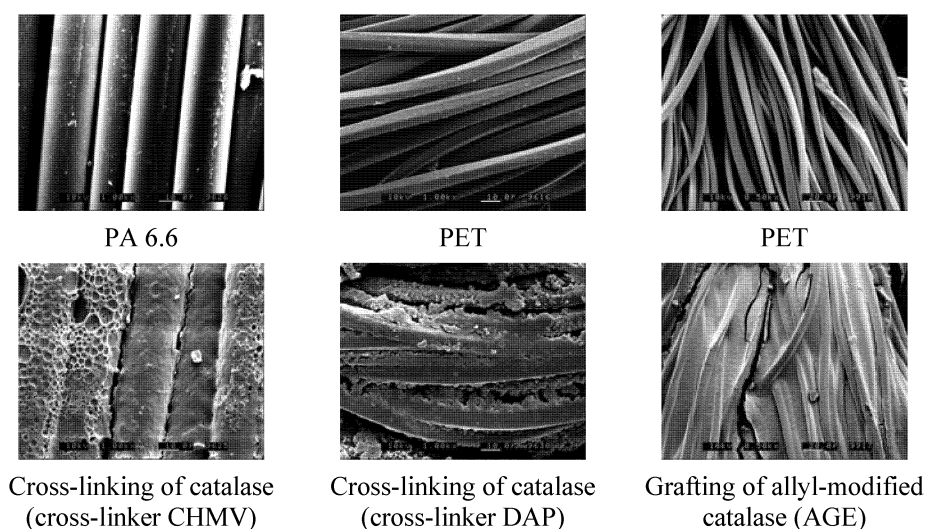


Figure 3: SEM of different fabrics after photochemical immobilization of catalase using different reaction agents and techniques.

The enzyme catalase catalyzes the degradation of hydrogen peroxide into oxygen and water. Covalent immobilization of enzymes is always accompanied by a decrease of activity because of the loss of mobility. Moreover, covalent bonds impair the ability of the enzyme to form the enzyme-substrate-complex (relative activity = activity/activity of free catalase). A successful enzyme immobilization must include a minimum of activity loss and a maximum of possible reuses. The aim of each enzyme fixation in economic terms is an increased activity over all reuses (integral activity = relative activity x number of reuses, assuming rel. activity ~ constant). By measuring the enzymatic decomposition of hydrogen peroxide as a function of time, it is possible to calculate the relative activity of the immobilized enzyme in comparison to free catalase. The immobilization products obtained with catalase show even after several reuses a

significant activity (see Table 2). The catalase loses 80 - 95 % of its activity. Nevertheless, the integral activity after twenty resp. thirty reuses is much higher than the activity of free catalase, which could only be used once in technical processes. Immobilizing catalase on PA 6.6 using DAP as cross-linking agent gave the best results. The integral activity reaches 366 % after twenty reuses.

Table 1: Load of photochemical immobilized catalase on PA 6.6 and PET.

Carrier material	Cross-linking agent resp. pre-functionalization	Catalase load mg/g carrier]
PA 6.6	DAP	20.8
PA 6.6	CHMV	22.0
PET	DAP	32.2
PET	CHMV	23.9
PET	AGE	70.0

Table 2: Integral activity of photochemical immobilized catalase after twenty resp. thirty reuses in comparison to free catalase.

Carrier material	Cross-linking agent resp. pre-functionalization	Relative activity [%]	Reuses	Integral activity [%]
PA 6.6	DAP	18.3	20	≥ 366
PA 6.6	CHMV	11.3	20	≥ 226
PET	DAP	9.6	20	≥ 192
PET	CHMV	11.3	20	≥ 226
PET	AGE	5.1	30	≥ 153
Free catalase	-	100	1	100

4. Conclusions

Using the photochemical immobilization techniques reported here, catalase can be fixed durably in an easy and rapid way on textile carrier materials. Low-cost synthetic textile fabrics made of polyester and polyamide are alternative carrier materials for the immobilization of enzymes. With a low preparative expense fabrics with a high protein load and a distinct activity can be produced by irradiating the materials with excimer-UV-lamps in the presence of cross-linking agent or by the application of pre-functionalized enzymes. The special construction of fabrics allows an adjustable throughput and a high substrate turn-over. In addition, conventional immobilization products are mostly offered as granulates or pellets, which must be filtered after the enzymatic reactions. Fabrics can be removed very quickly from the reactor without any filtration step and the solution contains no proteinous residues after the enzymatic reaction. Moreover, the photochemical procedure is transferable to many other enzymes or other proteins of biochemical interest. Therefore, the new photochemical immobilization techniques are able to compete with conventional immobilization

procedures and will open widespread applications in bio-catalysis in future. Latest investigations focus the immobilization of organo-metallic catalysts using the same procedures.

5. Acknowledgement

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6. References

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