

National Textile Center

FY 2003 (Year 12) Continuing Project Proposal

Project No. C02-AE02

Competency: Chemistry

Biomimicking of Enzymes for Textile Processing

Project Team:

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Objective:

The increased acceptance of enzyme technology in the textile industry worldwide shows that innovative approaches are needed to solve problems concerning sky-rocketing energy costs, the use of toxic chemicals and their impact on the environment. The objective of this project is to find simple biomimics that simulate the active sites of oxidoreductase enzymes. First we are focusing on compounds as mimics for glucose oxidase and peroxidase enzymes, aiming to achieve equally good wet finishing results on cotton as with the actual enzymes. The work will be extended to include biomimics for lignin oxidases and other oxidoreductases with the goal of improving the retting process of flax or other bast fibers. The proposed project builds on the knowledge obtained by a previously funded project.

Progress Statement:

Enzymes are large high-molecular weight protein structures with relatively small three-dimensional active sites within their molecules that perform the actual biocatalytic work. In many cases, the molecular size of the enzyme by far exceeds the size of its substrate, one example being glucose oxidase and its substrate glucose. This research investigates whether simpler compounds can be found that mimic the active site and the behavior of these biocatalysts without all or part of the bulk of the protein structure. These compounds should also significantly increase the reaction rate, facilitate the catalytic process and significantly decrease costs without adverse effects on the fiber material. At the same time a better understanding of how the enzymatic process works will be achieved.

Enzymatic Oxidative Reactions

In the first project phase experiments were performed that involved the actual enzymes to prepare control samples and institute working conditions. Biobleaching experiments using glucose oxidase were carried out based on our previous experience. As reported, whiteness indices (WI) for cotton of approx. 65 can be achieved with our process (conventional bleaching with H₂O₂: WI 68-70).

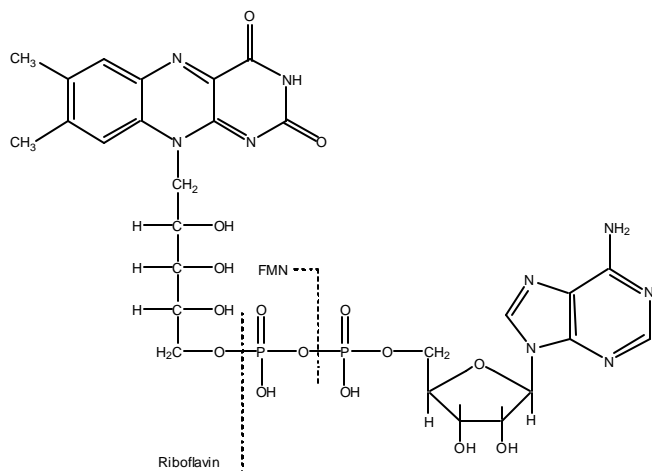
Simultaneously, trials were performed with manganese peroxidase in presence of oxygen on desized, unbleached cotton. The dosage of manganese peroxide and the pH of the solution were varied with the goal of establishing a control sample using this type of enzyme. The bleaching effect still needs to be enhanced for more satisfactory results. So far, an WI increase of about 50% over the untreated control could be achieved.

A third series of experiments was concerned with the use of laccases which have been applied for pulp bleaching. Laccases are fairly un-specifically acting enzymes. Thus, mediators, such as violuric acid and 1-hydroxybenzotriazole (HBT) are commonly added during pulp bleaching to aid the enzymatic reaction. For our initial experiments we adopted the mediator concept and varied both enzyme and mediator concentrations as well as mediator types, focusing on the most environmentally benign compounds.

Biometric Approach

In Year 1 of this project we first focused on mimics for glucose oxidase since this enzyme showed to be very effective for biobleaching (see previous NTC project, C99-A07). Glucose oxidase is a flavin containing enzyme. During the

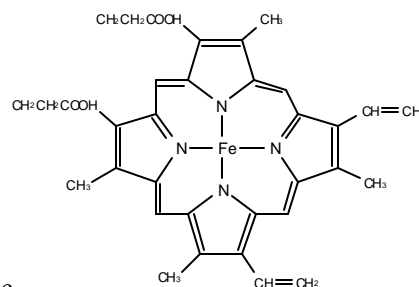
bleaching process it reacts with β-D-glucose to D-gluconolactone. Single electrons are transferred to molecular oxygen and hydrogen peroxide for bleaching by this enzyme and flavoquinone is formed and subsequently reduced via two one-electron transfers. For the biomimetic approach it is necessary to consider the pH-dependency of the enzyme's active site and thus its redox-potential, the oxygen donor, possible mediators or cofactors, such as metal ions, and generally the ionic strength of the treatment solution. The reaction conditions and the type of mimicking compound, of course, are of importance as well.



Experiment series involved unbleached desized 100% cotton fabric that was treated with 1×10^{-4} molar solutions of mimics including flavin mononucleotide (FMN) and riboflavin in buffer (see scheme on the left: FAD, flavin-adenine-dinucleotide). The solutions were made at three different pH values to coarsely include the pH dependency of the active site. Oxygen was mainly supplied in gaseous form. After sufficient reaction time the samples were rinsed and air-dried. Next, experiments with glucose present with varying concentrations but identical conditions otherwise were conducted. For all samples, weight loss and CIE $L^*a^*b^*$ color coordinates were determined. Whiteness increased to a certain extent in some cases, however, the level of biobleached samples could not yet be achieved.

Preliminary experiments were also performed to mimic lignin- and manganese peroxidases, both hemoproteins with protoporphyrin as prosthetic group. A series of trials was performed with hemin added at various dosages and at different pH values. It appears that with lower pH values better results can be achieved. In all other cases a slight increase in yellowness was observed.

Compounds with metalloporphyrin-based structure (see ferri-protoporphyrin IX, hemin, scheme on right-hand side) and similar to phthalocyanine found in dyes have been reported as effective mimics for bleaching of pulp model compounds. Their usefulness for our system is currently being explored.



Polyphenol oxidases (laccases) are capable of both depolymerization and polymerization reactions depending on the experimental conditions. We are in the process of establishing conditions that would limit polymerizations and direct specific oxidations of materials such as flavons, aromatic phenols, etc., which might be responsible for the yellowish tint of unbleached cellulose. Such conditions will then be applied to a biomimetic system also. It appears that the combination of different mediators would allow some control over the system.

Next Year's Goals:

We plan to continue to work on parallel routes, one involving enzymes, the other compounds that mimic the active site of the enzymes since the achieved results so far need more research work. We will continue to work with unbleached cotton, however move on to fibers that contain more noncellulosic impurities, such as flax (linen), etc.

- Oxygen Source

A major part of the next phase will be to modify the oxygen supplying source since the form and availability of oxygen plays an important role in enzymatic processes (so far, we used gaseous oxygen). We will include enzymatically in-situ generated oxygen, hydrogen peroxide, per-salts (for example, perborate, persulfate), peracetic acid, and others.

- Ionic Strength

Enzymes are sensitive to the ionic strength in the treatment solution. Some metal ions have catalytic effects, some are inhibiting, depending on the nature of the enzyme and the metal ion. We will further investigate this effect.

- Sensitivity to Light

We will investigate the role of light and specific wavelengths for enzymatic and biomimetic reactions since some of the mimetic substances have light-induced sensitization effects, one example being riboflavin.

- Effect of Biomimetic Compounds on Fiber Properties

The effect of the biomimetic reactions on the fiber material will be assessed by measuring the degree of polymerization, the mechanical properties and surface related changes, in addition to whiteness and weight loss. The redox potential of the mimics will be determined and correlated to the observed effects.

- Application to Model Compounds

The exact chemical make-up of yellowing pigments and lignins in cellulosic fibers is still not known, however, some basic structures have been suggested in literature. We are planning to apply the enzymes as well as the biomimics to such compounds and study their effect.

Approach:

We will pursue the approach as detailed in “This Year’s Goals” section. Enzyme reactions, though very effective for textile materials, still leave many fundamental questions open. We are expecting to gain major understanding in how the enzyme performs its catalytic action by step-wise separating major parts of the active site from the bulk of the enzyme structure and applying them to the substrate. We will also perform the reactions on model compounds to further break-down the enzymatic reaction. Important connections between the active site and other factors in the enzymatic system (role of metal ions, co-factors, mediators, etc.) are expected to become more transparent.

Outreach to Industry:

The textile industry is always interested in simple cost-effect processes that can be applied without major modification of existing equipment. It is thus not surprising that enzymatic processes in non-traditional areas are continuously emerging. With this project we go one step further on our endeavor to create a pathway to “green chemistry” and simplification of processes. Close cooperation with the textile and the enzyme manufacturing industries will be established and the results of this new technology disseminated through company visits and presentations at national and international conferences.

New Resources Required:

For our planned experiments no new equipment is required. Support for graduate students is needed.