Journal of Fiber Bioengineering and Informatics 15:1 (2022) 45–54

Cite as: M. Kahoush et al.
doi:10.3993/jfbim00397

Eco-Technologies for Immobilizing Redox Enzymes on Conductive Textiles, for Sustainable Development [⋆]

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Abstract

The objectives of this work are to investigate the use of different eco-technologies as strategies for immobilization of redox enzymes on conductive carbon-based felts, to produce bio-functionalized textiles for a future use in sustainable applications while maintaining low environmental impact. Methods using cold remote plasma, electrically conductive biocompatible coating (PEDOT:PSS) and natural crosslinker for the enzyme (genipin) were tested and showed to be efficient in the intended applications. The enzymatic activity of the used glucose oxidase was maintained for multiple number of uses, and showed potential in sustainable wastewater treatment applications in bio-Fenton and bio-electro-Fenton setups.

Keywords: Eco-technology; Glucose Oxidase Enzyme; Sustainable Wastewater Treatment; Plasma

1 Introduction

Appropriate methods for enzyme immobilization on conductive materials are necessary to improve the bio-catalytic activity of enzymes for use in applications where electrochemical response is of prime importance, such as in bioelectrodes, biosensors, or biofuel cells.

This field is getting more attention in recent years with applications used on a daily basis at different levels. Glucose biosensors for domestic use facilitate the lives of diabetes patients, while other biosensors make easy and reliable detection of cholesterol, alcohol and heavy metals,

[⋆]Project supported by Erasmus Mundus joint Doctorate Program for Sustainable Management and Design for Textiles (SMDTex) [grant number 2015-1594/001-001-EMJD], and Chinese Scholarship Council (CSC) [grant number 2018CEU013907].

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Mar. 2022

to improve safety and control diseases and pollution. Furthermore, power generation from bio-sourced enzyme materials is of great interest for more sustainable processes and services.

Many enzyme immobilization techniques are widely used nowadays, like immobilization on membranes and entrapment in gels or matrix. However, numerous of these methods can be improved in terms of pretreatment requirements and the use of several chemicals or energy consumption.

In this work, the main objective was to immobilize redox enzymes using eco-friendly approaches in attempt to reduce the amount of added chemical and power consumption, in addition to minimize waste production within the frame of sustainable development.

Eco-technologies and products such as plasma as dry treatment, biodegradable and biocompatible conductive polymer coatings, and low toxicity bio-based crosslinking reagent, have been used in this study to achieve the objectives. Cold remote plasma has been used to activate the surface of carbon textile for immobilization of glucose oxidase enzyme. The immobilization was carried out using either direct physical adsorption method, or bio-based naturally occurring crosslinker (Genipin), which is known for its low toxicity in comparison with conventional enzyme crosslinking agents such as glutaraldehyde. The bioactivity and bio-electro-chemical response of the immobilized enzyme were assessed primarily for a future use as bio-anode in bio/bio-electro-Fenton process, for degradation of organic pollutants. A model pollutant Remazol Blue RR reactive dye has been chosen for these treatments, since it is extensively used in textile industry worldwide, and it is known to be persistent and hard to treat. The sustainable applications mentioned show to have advantages over the traditional wastewater treatment methods in regards to environmental impact and safety in work place.

The interest of enzyme immobilization comes from the need of improving the stability and reusability of the enzymes when compared to their free state. Reusability of enzymes also contributes to the reduction of process costs [1]. In addition, the immobilization process reduces the risk of contamination of the products with the residues of the enzyme and hence, reduces the risk of allergies and other undesirable side effects [1]. Another advantage of immobilization of enzymes is the ability of extracting the enzymes from media when desired, to stop the reaction resulting in better control of the process, and less additional steps for deactivating or processing the residual enzymes in the media.

The main methods used in literature are covalent bonding, physical adsorption, cross-linking, entrapment in a matrix and encapsulation bonds [2-5].

Carbon fibers are used intensively in both research and industry nowadays in different forms (yarns, woven and nonwoven structures), to produce composite materials, and to produce electrodes and bioelectrodes for bio/electrochemical applications and reactors [6]. The use of carbon felts was more distinguished in the bio/electrochemical reactors for microbial or enzymatic fuel cells, and in wastewater treatment [7-10]. They possess high specific surface area, which helps immobilization of higher amounts of bioactive materials such as microbial populations and enzymes [11, 12].

2 Problem Formulation

The main motivation behind this work was to investigate milder and more eco-friendly methods to immobilize redox enzymes such as glucose oxidase on conductive textiles, and test their potential uses in applications such as bio-Fenton and bio-electro-Fenton processes. As previously mentioned, the conventional methods have some drawbacks despite their efficiencies, requiring large consumption of power or hazardous toxic chemicals, and producing wastewaters in big amounts.

Carbon felts are hydrophobic structures, their surface energy is low, which causes technical problems when introduced to aqueous media. Since the glucose oxidase enzyme is contained in water-based buffer solution, this will hinder the immobilization process remarkably. Thus, pretreatment of the fibers should be conducted to increase the wettability of the felts and increase their surface energy.

Usually, this process is conducted using strong chemicals at high temperatures for a long duration of treatment.

Hence, the proposed methods are focused on resource-efficient approaches to minimize material and energy consumption, produce less waste, prevent health and environmental hazards, and consequently reduce the costs of operations. Taking into account these criteria, the main objectives are:

- To use eco-friendly methods to obtain enzyme-functionalized textile-based felts/electrodes, with maintained enzymatic activity and reusability.
- To investigate the possibility of using these felts in sustainable treatment of wastewaters from textile industry.

The main methods used in this study were 1- Cold remote plasma as a dry method to modify the chemical and physical structure of the treated textile material, instead of wet chemical processes as pretreatment before enzyme adsorption. 2- Dip-coating with biocompatible polymer mixture in ambient conditions to improve electrical conductivity while enhancing the affinity between textile and enzymes. 3- Crosslinking the enzyme with naturally derived agent "genipin" to reduce toxicity of conventional crosslinkers while omitting the use of matrix to encapsulate the enzyme. Characterization methods were used to identify changes after each step: Techniques including scanning electron microscopy (SEM) micrographs, X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR), capillary uptake measurements, and water contact angle goniometry were used in order to evaluate the changes after CRP treatment. Furthermore, electrical and electrochemical methods such as bulk resistivity and cyclic voltammetry (CV) were carried to assess the electrical conductivity changes of the studied samples. Enzymatic activity colorimetric assays were performed to check the success of the enzyme immobilization method and the reusability of the treated samples. The efficiency of the used methods was evaluated for both color removal and the level of pollutant degradation post-treatment, using UV-Vis spectroscopy and Chemical Oxygen Demand (COD), since color removal does not accurately reflect the level of (RB) degradation, thus COD removal will be a better indicator.

3 Problem Solution

3.1 Cold remote plasma

As previously mentioned in the methods paragraph, the first approach to solve the wettability issue of carbon is Cold Remote Plasma (CRP) with gas mixture of N_2 and O_2, as a dry nondestructive method, was used to modify the surfaces of bare carbon nonwoven felts without added chemicals and within a short time. Immobilization of glucose oxidase (GOx) redox enzyme via physical adsorption followed CRP. Virgin Carbon Felt (VCF) was first treated with cold remote plasma (CRP) using a mixture of nitrogen and oxygen (1 or 2%) as plasma gas. Biofunctionalization of the carbon felts with glucose oxidase (GOx) enzyme was then carried using physical adsorption method. FTIR and XPS analysis showed an integration of new oxygenated functional groups (C-O and C=O) as well as amines and amides on the surface of VCF treated by the CRP treatment (Fig. 1), which improved the wettability of the samples. Capillary uptake increased from around 0% (for VCF) to nearly 750% with 2% oxygen in plasma gas. GOx enzyme showed higher activity after immobilization at pH 5.5 on the CRP treated samples, maintaining up to 50% of its initial enzymatic activity after six cycles while with the VCF, no enzymatic activity was observed after the fourth cycle. These obtained felts can be used as electrodes in sustainable bio-processes.

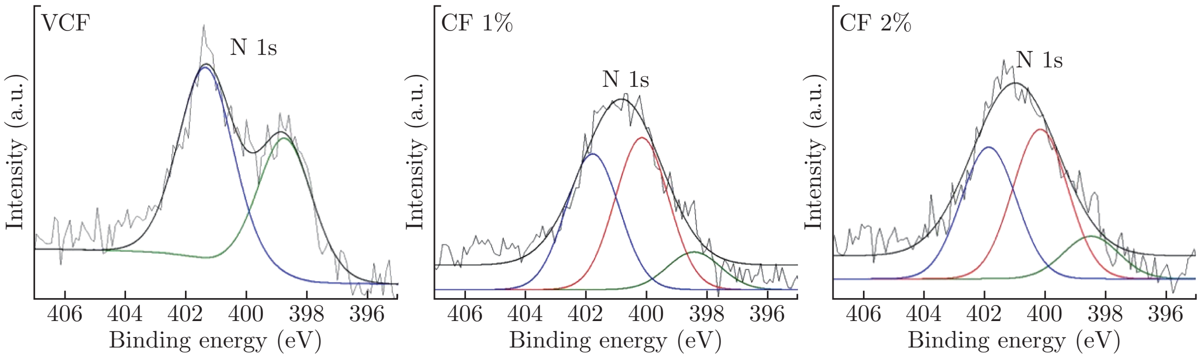

Fig. 1: High-resolution N 1s XPS spectra for bare carbon samples before and after CRP

3.2 Dip-coating with PEDOT:PSS

The second approach used was using dip-coating with biocompatible and biodegradable conductive polymer blend PEDOT:PSS followed by simultaneous CRP for both carbon and coating, to improve surface characteristics followed by immobilization of GOx via physical adsorption. The plasma improved coverage of fibers with coating since it has been appearing as random dispersed patches of polymer on the fibers before plasma, but seemed smoother and more uniformly coating the fibers after CRP treatment due to ionic bonds between PSS (-) and hydrophilic regions of carbon after plasma (+) (Fig. 2). Furthermore, electrical conductivity, and capillary uptake were improved due to oxidation of both carbon and coating, which lead to better capillary uptake and improved the mass transfer process. Consequently, this method improved the immobilization process, activity and reusability of the obtained felts. VCF was dip-coated with PEDOT:PSS, then it was submitted to CRP, using two gas mixtures of $N_2 + O_2$, followed by enzyme immobilization. Surface micrographs showed better uniformity and coverage of individual fibers with the coating after the CRP, compared to irregular patches of coating on the felt prior to the CRP.

Capillary uptake was improved after CRP and the oxidation of both carbon and coating was confirmed by surface analysis. Spectra of N 1s and O 1s, confirmed the integration of carbonyl and ether groups as well as amide and amine groups on the bare carbon fiber surface, while on the PEDOT:PSS coated fibers, carbonyl groups were pre-dominant. S 2p spectra also confirmed the oxidation of the PEDOT:PSS coating with reduction of the sulfur atom that is bonded to carbon (S^-) compared to sulfonate (SO_3^-) group. GOx immobilized on the different samples showed the highest activity in the case of PEDOT:PSS coating subjected to a CRP with 2% O_2,

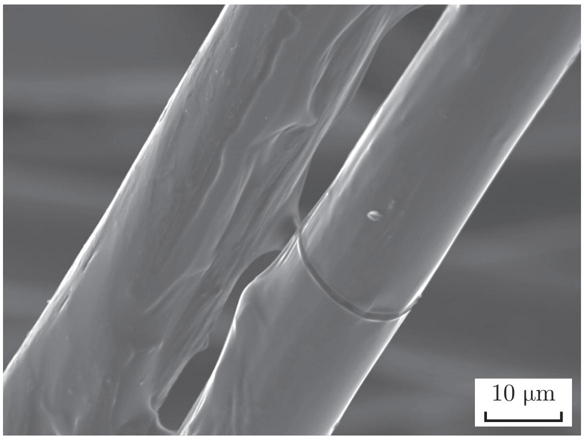

Fig. 2: SEM of PEDOT:PSS deposition on carbon fibers after CRP 2% oxygen with magnification of X1600

maintaining up to 60% after immobilization, and 37% of its enzymatic activity after six cycles for some samples. Enzymes immobilized on samples without CRP were no longer active after the fourth use-cycle (Fig. 3).

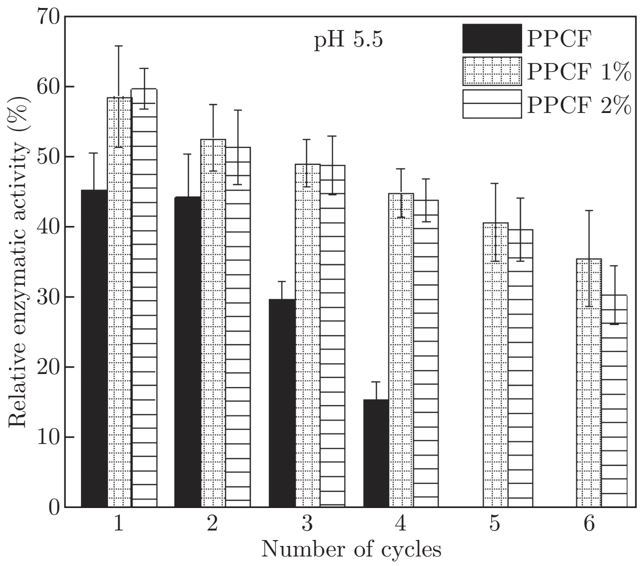

Fig. 3: Relative enzymatic activity PPCF, PPCF 1% and PPCF 2%, respectively

3.3 Crosslinking with Genipin

The third approach was the use of naturally occurring crosslinking agent (genipin) to immobilize GOx. It was reported that it is less cytotoxic than glutaraldehyde in about 5000 - 10000 times [13]. Genipin showed to be an effective anti-inflammatory agent, in addition to its use as medicine to treat the type (II) diabetes in Chinese traditional medicine over the years [14]. It is a biocompatible material, which makes it a good candidate in many environmental and medical applications. Its median lethal dosage LD_{50} i. v. is 382 mg/kg in mice which makes it so much less toxic than glutaraldehyde [15]. Recently, it is used extensively in research for crosslinking of materials like chitosan and gelatin for applications regarding medical and tissue engineering, details about these applications are provided elsewhere [16]. It was reported that only the primary amines can react with genipin to form blue pigments (Fig. 4), and that oxygen is essential for the blue pigment formation [17].

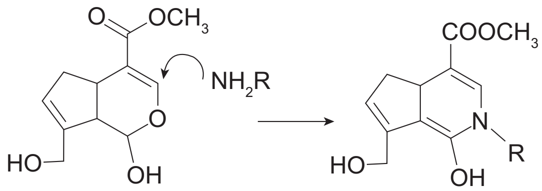

Fig. 4: Genipin reaction with primary amines

The immobilization process took place over the carbon-based samples, which helped to stabilize the obtained aggregates and significantly improved the stability of enzymes due to bonding between the felts and the crosslinked GOx. The results confirmed the formation of blue pigment that indicates the formation of bonds between genipin and the primary amino groups within the enzyme [18].

FTIR spectra of genipin and GOx before and after crosslinking confirmed the formation of aromatic amines at 1642 cm^{-1} and C-N at 1400 cm^{-1} after the reaction, therefore, the crosslinking reaction occurred and a ring opening reaction took place to for these amines (Fig. 5). In addition, the peak of primary amines of GOx at 1560 cm^{-1} did not appear after crosslinking for 24 h, which can be due to the disappearance of the primary amine groups during the crosslinking process.

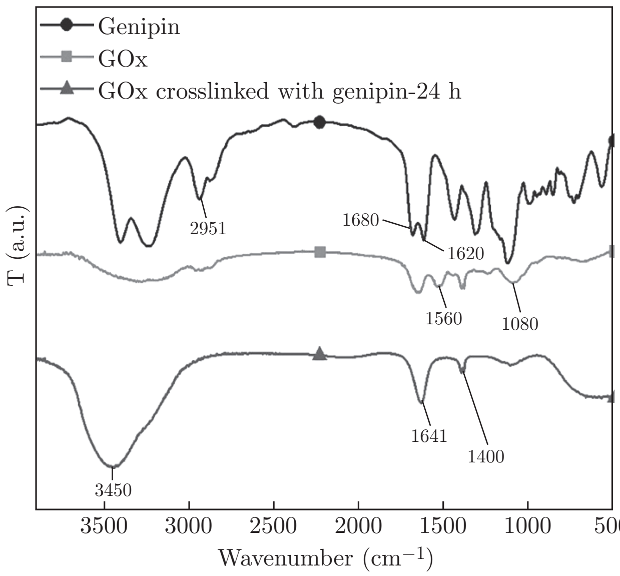

Fig. 5: FTIR of genipin, GOx and their mixture after crosslinking for 24 h (from top to bottom)

The mechanism of crosslinking between enzymes and genipin can be explained by opening the ring of genipin due to the reaction of primary amines of GOx with carbon number 3 in genipin, and/or the formation of amide by ester substitution with the amino groups of GOx [18]. Besides those, the reaction with secondary amines may occur in an intermediate stage before the formation of the aromatic amines.

3.4 Application in Sustainable Wastewater Treatment

The bio-functionalized textiles obtained were assessed for possible applications in wastewater treatment. Since textile industry contributes remarkably to production of wastewater, sustainable methods were proposed to use the obtained textile samples in the treatment process and the primary obtained results were presented.

The first approach was Bio-Fenton (BF), which was useful in evaluating the enzymatic bioactivity of the immobilized GOx. This approach was efficient in color removal and partial COD removal, which indicates the partial degradation of dyestuff of Remazol Blue RR. The bare carbon samples with crosslinked enzymes resulted in better degradation overall and stayed partially efficient in removing color in the second use.

CF 1% - G samples resulted in up to 93% discoloration of Remazol Blue for the first use as observed in the UV-Vis spectra of the solutions before and after treatment, the color was almost entirely removed and the peak at 605nm disappeared post-treatment after 3h (Fig. 6).

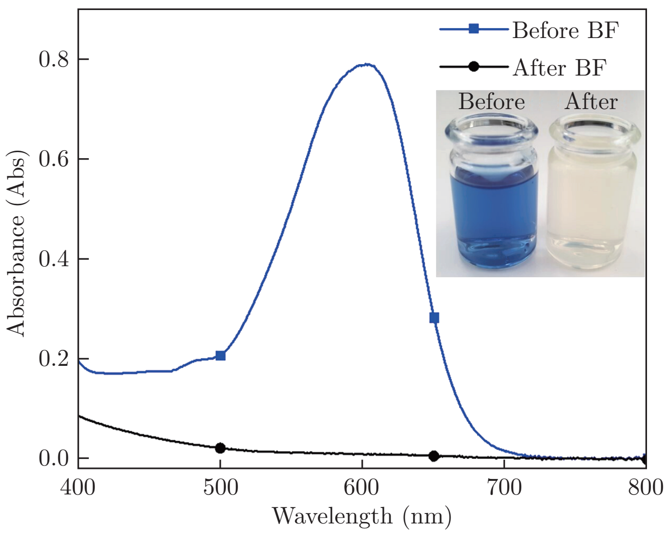

Fig. 6: UV-Vis absorbance of Remazol Blue RR solution before and after BF treatment using CF 1% - G (Inset: the corresponding samples)

For CF 1% with GOx immobilized via adsorption, 83% of the dye discoloration occurred while for GOx immobilized directly on VCF almost 60% of discoloration occurred in the same conditions. When these mentioned samples were reused for a second time in the same process, discoloration values of Remazol Blue decreased to 82% for the CF1% - G, to 57% for the CF 1% and 34% for the VCF, in the same used conditions and for 3h.

Meanwhile, the second possible application Bio-Electro-Fenton (BEF) was useful in evaluating the bioelectrical activity of the obtained samples as bio-anodes. The bio-anodes were responsible for generating power to stimulate the degradation of the dyestuff in BEF reactor. All the obtained bio-anodes resulted in improving of power output of the cell when compared to the control. However, bio-anodes with crosslinked GOx resulted in better color removal, COD degradation and higher power density generated overall.

Color removal showed to be close for both anodes with immobilized GOx with genipin, with color removal efficiency reaching 31% and 34% for CF 1% - G and PPCF 1% - G, respectively. For bio-anodes produced via physical adsorption of enzymes, discoloration was close to 20%.

Furthermore, the COD removal was in accordance with the color removal results, and the efficiency obtained reached up to 36% for genipin-based anodes, and 27% for anodes with enzyme adsorption.

With external resistance varied between 1 - 10000 Ω, a maximum power density of 0.5 $\mu W \cdot cm^{-2}$ was obtained at a current density of 15 $\mu A \cdot cm^{-2}$ in the case of PPCF 1% - G bio-anode, and the power was stable until reaching the current density of 30 $\mu A \cdot cm^{-2}$. Meanwhile, when CF 1% - G was used as bio-anode the power density generated reached up to 0.16 $\mu W \cdot cm^{-2}$ at a current density of around 10 $\mu A \cdot cm^{-2}$.

These two cases resulted in better power output of the reactor in general than the bio-anodes obtained via physical adsorption, while the control cell with VCF with GOx immobilized via adsorption resulted in only 0.037 $\mu W \cdot cm^{-2}$ at a current density of 1 $\mu A \cdot cm^{-2}$.

4 Conclusion

Hence, it can be concluded based on results and observations obtained in this work that [19]:

(1) Cold remote plasma is an efficient eco-technology for the treatment of conductive textile materials like carbon, without the use of added chemical, destructive effects, hazardous sparkling or long treatment time. It can be used with customized gas blends according to the functionality required, with possibility of treating big batches of samples with different thickness on both sides simultaneously. Consequently, it facilitated bio-functionalization with enzymes in this study and improved stability and activity when compared to untreated samples.

(2) PEDOT:PSS as a coating for conductive textile materials showed to improve conductivity, biocompatibility and surface properties of the samples. The oxidation of this coating via CRP further improved these desirable properties and enhanced the activity and reusability of immobilized enzymes. As a biodegradable eco-friendly conductive coating, PEDOT:PSS blend has a great potential in the wide spectrum of applications.

(3) The gas mixture of nitrogen with 1% oxygen was sufficient to integrate functional groups such as C-O and C=O as well as amino groups, which resulted in improved hydrophilicity and maintained enzymatic activity. However, the increase of oxygen percentage to 2% did not show a significant proportional effect on the treated samples.

(4) Genipin as natural crosslinker with low toxicity showed to be a good candidate for directly crosslinking GOx enzymes without the use of polymer matrix. Its low toxicity and biocompatibility improved the stability of crosslinked GOx remarkably and prevented leaching phenomenon better than physical adsorption.

(5) Within the theme of sustainable development, the obtained materials from this study were primarily evaluated for wastewater applications that consume less energy, less added chemicals, reduce wastes and help prevent health and environmental hazards. The primary results showed the feasibility and significant potential of BF and BEF using immobilized GOx on carbon-based textiles for degradation of persistent dyestuff like sulfonated reactive dyes. The color removal efficiency was around 40% and 90% for BEF and BF, while COD removal reached up to 30% and 36%, respectively.

Furthermore, in BEF setup, a synchronized power generation was estimated to reach 0.5 $\mu W \cdot cm^{-2}$ for bio-anodes with crosslinked GOx.

(6) These obtained carbon-based textiles maybe used in variety of applications related to power

generation and pollution control.

(7) In order to be able to scale-up these setups to be used in industry, overcoming several challenges must be achieved. The cost of the materials used in constructing BEF setup need to be reduced especially for the membranes used (cheaper GORE TEX® might be a good option). The power generation efficacy must be optimized to achieve better level of discoloration and degradation of pollutants by reducing the ohmic over potential of the electrical setups. In the case of BF, protection of the enzyme via a membrane or hydrogel might be beneficial to avoid denaturation of GOx via Fenton reaction.

Acknowledgement

This work was supported by Erasmus Mundus joint Doctorate Program for Sustainable Management and Design for Textiles (SMDTex) [grant number 2015-1594/001-001-EMJD], and Chinese Scholarship Council (CSC) [grant number 2018CEU013907].

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