

Ecotechnologies for Glucose Oxidase-GOx Immobilization on Nonconductive and Conductive Textiles for Heterogeneous Catalysis and Water Decontamination

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Abstract: The need for sustainable and efficient water decontamination methods has led to the increasing use of redox enzymes such as glucose oxidase (GOx). GOx immobilization on textile supports provides a promising alternative for catalyzing pollutant degradation in bio-Fenton (BF) and bio-electro-Fenton (BEF) systems. However, challenges related to enzyme stability, reusability, and environmental impact remain a concern. This communication paper outlines innovative strategies developed to address these challenges, notably the use of ecotechnologies to achieve efficient GOx immobilization while maintaining biocatalytic activity. Plasma ecoprocesses, amino-bearing biopolymer-chitosan, as well as a bio-crosslinker genipin have been used efficiently on conductive carbon and non-conductive polyester-PET nonwovens. In certain cases, immobilized GOx can retain high catalytic activity after multiple cycles, making them an effective biocatalyst for organic dye degradation (Crystal Violet and Remazol Blue) via bio-Fenton reactions, including total heterogeneous bio-Fenton system. Moreover, the conductive carbon felt-based bioelectrodes successfully supported simultaneous pollutant degradation and energy generation in a BEF system. This work highlights the potential of textile-based enzyme immobilization for sustainable wastewater treatment, bio-electrochemical energy conversion, and also for bacterial deactivation. Future research will focus on optimizing enzyme stability and enhancing BEF efficiency for large-scale applications.

Keywords: enzyme immobilization; textile; conductive; ecotechnologies; biopolymers; bio-cross-linkers; bioFenton; bio-electro-Fenton; water depollution



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1. Introduction

The increasing presence of persistent organic pollutants in industrial effluents, particularly from the textile sector, has raised significant concerns regarding water quality and ecosystem health. Conventional treatment methods often rely on high energy input, harsh chemicals, and complex multistep operations, which limit their sustainability and cost-effectiveness [1]. Consequently, biocatalysis, and in particular, the use of redox enzymes such as glucose oxidase (GOx), has emerged as a promising eco-conscious alternative for advanced oxidation processes [1–9].

Glucose oxidase catalyzes the oxidation of β -D-glucose to gluconic acid and hydrogen peroxide, which can serve as a precursor for hydroxyl radical formation in Fenton-based reactions [10–12]. In the bio-Fenton (BF) process, hydrogen peroxide is generated in situ by enzymatic oxidation, reducing the need for external chemical inputs [13–16]. In the bio-electro-Fenton (BEF) process, enzyme-functionalized electrodes can both generate oxidants and serve as bioanodes, enabling simultaneous wastewater treatment and power generation [1,4,5,17]. These methods address some of the major limitations of traditional Fenton systems, such as the need for stored peroxide and issues related to iron sludge formation [1,13,14].

Immobilization of GOx onto solid supports is crucial for practical deployment, as it improves enzyme stability, facilitates recovery, and allows repeated use [1]. Among the available supports, textile materials offer key advantages due to their high surface area, tunable porosity, flexibility, and compatibility with continuous flow systems [6]. In particular, polyester (PET) nonwovens and carbon felts have been identified as attractive platforms [2–9]. However, these substrates are inherently hydrophobic and chemically inert, which limits enzyme loading and long-term stability [2,9].

To overcome these limitations, surface modification techniques are required to introduce functional groups that enhance enzyme binding [6]. Conventional chemical methods often involve toxic reagents and generate undesirable by-products [18,19]. Therefore, the development of safer and more sustainable alternatives is essential [6,20,21]. In this context, plasma treatment has emerged as a powerful, eco-friendly technique to activate the textile surface by introducing hydrophilic and reactive groups (e.g., $-\text{COOH}$, $-\text{OH}$, $-\text{NH}_2$), improving enzyme anchoring and surface energy without compromising the textile's mechanical integrity [1–6,21].

This paper reports studies carried out for advancement in sustainable environmental catalysis by developing eco-friendly strategies for GOx immobilization on both non-conductive PET and conductive carbon nonwoven felts. Building upon the pioneering work of M. Kahoush [1–5] and M. Neaz [7–9], this paper contributes to the field of knowledge in eco-friendly catalytic textile materials for environmental catalysis, particularly for water protection, and provides insights for future advancements.

Plasma treatment is a sustainable technology, as it enables material functionalization in a dry state within a short processing time [22–24]. However, careful selection of plasma activation techniques is critical, as applying dielectric barrier discharge (DBD) plasma to conductive fibers, such as carbon fibers, poses the risk of sparking and potential fire hazards.

Beyond surface activation using plasma, this paper reports the efficient use of bio-based cationic amino-bearing biopolymers such as chitosan, and a novel bio-crosslinker, genipin, as environmentally friendly alternatives to conventional toxic enzyme crosslinkers, like glutaraldehyde [25]. In the past, chitosan was effectively used to immobilize enzymes on electrodes without plasma activation [26]. Genipin with very low toxicity [27] had already been successfully employed as a crosslinker for laccase and GOx immobilization, demonstrating promising potential for sustainable enzyme stabilization [28,29].

This study aims to develop robust and eco-conscious strategies for GOx immobilization on both non-conductive PET and conductive carbon nonwoven textiles using a combination of plasma activation, chitosan functionalization, and genipin crosslinking. We investigate the effects of these treatments on GOx enzyme activity, stability, and reusability, and evaluate the potential of the resulting bio-functional textiles in bio-Fenton and BEF applications. Through this approach, we seek to contribute to the design of multifunctional textile-based biocatalysts for sustainable water treatment and energy generation.

2. Materials and Methods

Figure 1 illustrates the GOx enzyme structure and the two types of fibrous materials used: non-conductive PET and conductive carbon felts, selected for their porosity and functionalization potential.

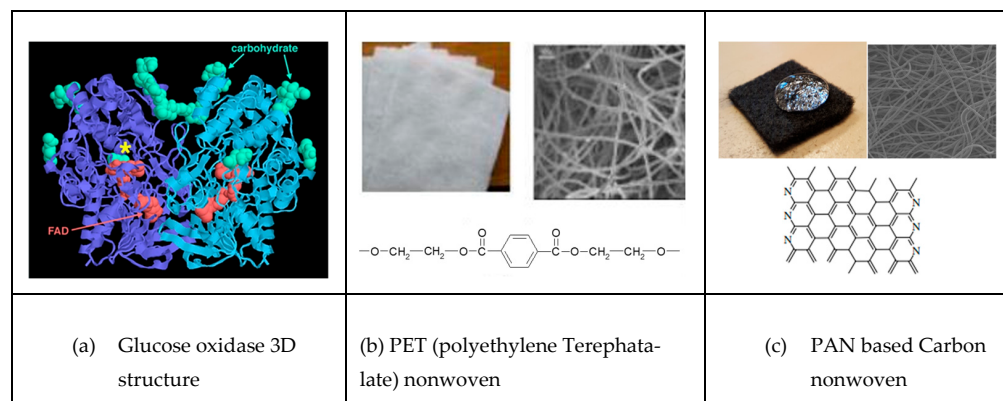


Figure 1. GOx enzyme (a) and supportive materials based on nonconductive PET nonwoven (b) and conductive carbon nonwovens (c).

2.1. Glucose Oxidase (GOx) Enzyme

Glucose oxidase (GOx) is a redox enzyme (EC 1.1.3.4) that catalyzes the oxidation of β -D-glucose into D-glucono-1,5-lactone and hydrogen peroxide using molecular oxygen. First identified in *Aspergillus niger* by Detlev Müller in 1928, GOx is a dimeric protein composed of two subunits, each containing a flavin adenine dinucleotide (FAD) cofactor and an iron atom [30]. The enzyme has a molecular weight of approximately 160 kDa and an average diameter of 8 nm. While GOx remains stable in freeze-dried form, its activity declines at temperatures above 40 °C or outside the pH range of 2–8.

2.2. Textiles as Porous Support Materials for GOx Immobilization

2.2.1. PET Fibrous Nonwoven

A 500 μm thick nonwoven polyester (PET) felt with an areal density of 130 g/m^2 and fiber diameter of 11.5 μm was selected for this study.

The measured water contact angle of 130° confirmed the hydrophobic nature of the PET nonwoven, presenting challenges for enzyme immobilization.

2.2.2. Carbon Fibrous Nonwoven

Carbon fibers, widely used in bio/electrochemical applications and reactors, are integrated into nonwoven structures to form conductive electrodes and bioelectrodes [31–34]. The carbon fibers in the selected nonwoven felt were derived from polyacrylonitrile (PAN) and contained a small percentage of nitrogen, primarily in pyridine ring form. Their hexagonal structure, similar to graphite, aligns parallel to the fiber axis, providing excellent conductivity. A 3 μm thick carbon nonwoven felt with an areal density of 450 g/m^2 and fiber diameter of 20 μm was used. The hydrophobicity of the felt was confirmed by a measured water contact angle of 115°.

2.3. Ecotechnologies for Fiber Surface Activation in GOx Immobilization

To enhance enzyme immobilization while ensuring sustainability, three ecotechnologies were utilized:

- Plasma treatments for fiber surface activation (air-atmospheric plasma and cold remote plasma-see Figure 2);

- Surface modification using amino-based biopolymers such as chitosan;
- Crosslinking with a novel bio-crosslinker, genipin.

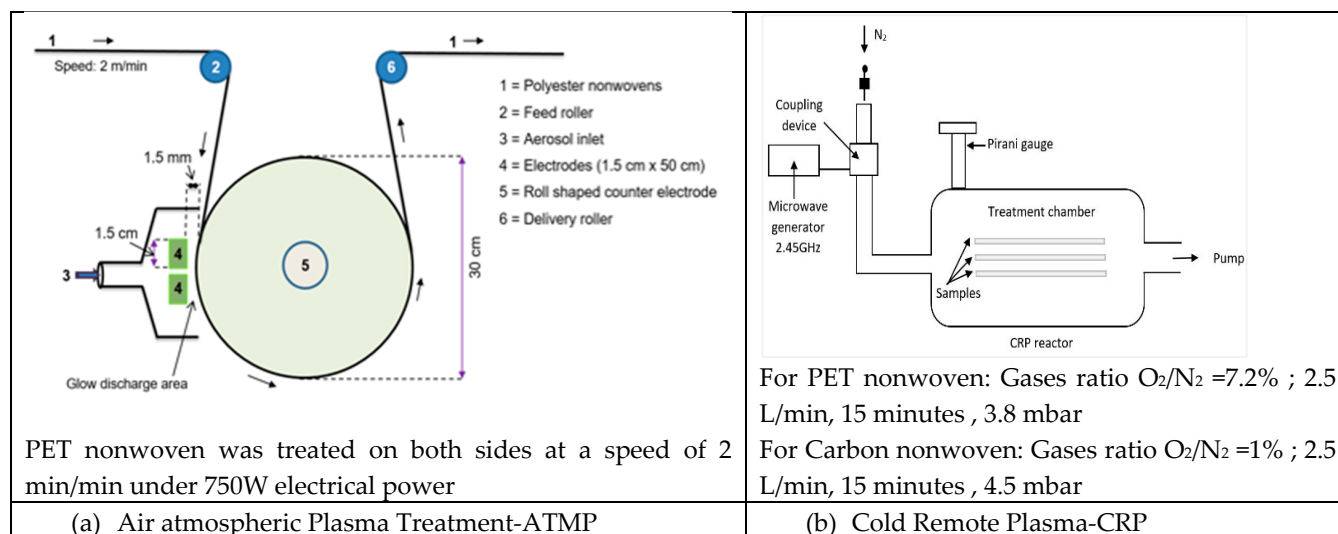


Figure 2. Plasma activation methods used: (a) air atmospheric plasma and (b) cold remote plasma nonwovens.

2.3.1. Plasma Activation of Fibrous Nonwovens (Pre-Treatment)

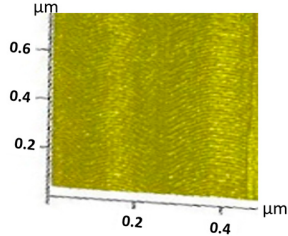
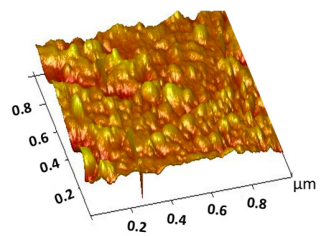
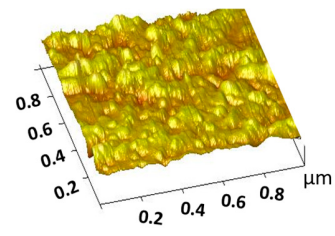
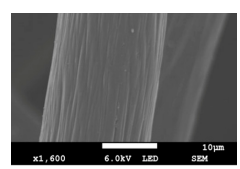
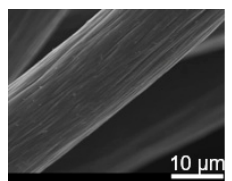
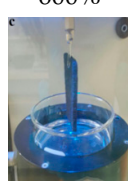
Plasma, the fourth state of matter, is generated by energizing gases to form an ionized medium composed of ions, free electrons, radicals, and UV radiation. Different gases modify surface properties; for example, air, N₂, He, O₂, and Ar enhance wettability, while CF₄ reduces it. By varying gas compositions, specific chemical functional groups can be introduced onto fiber surfaces, while plasma-induced surface micro-roughness improves adhesion. Various plasma techniques—including non-thermal plasma, atmospheric pressure plasma, and dielectric barrier discharge (DBD)—have been explored for textile surface modification to enhance enzyme adhesion [22]. Plasma treatment has also been applied to improve energy efficiency in flow batteries through the integration of functional surface groups [24].

Air Atmospheric Plasma (ATMP)

Air atmospheric plasma is generated by exciting gas molecules between two electrodes under atmospheric conditions. The setup used in this study, installed at ENSAIT, was provided by Ahlbrandt System (Germany). It features a roll-shaped electrode and two counter-electrodes (inter-electrode distance: 1.5 mm) within an atmospheric glow discharge induced by a potential difference, known as dielectric barrier discharge (DBD) plasma (Figure 2a). As described in previous studies [21], the plasma treatment was continuously applied to both sides of the textile at a speed of 2 m/min under 750 W power.

Plasma treatment effectively transforms the hydrophobic PET surface into a hydrophilic one by introducing hydroxyl and carboxyl terminal groups, as confirmed in prior research [21,23] (see Table 1). However, the DBD atmospheric plasma method is unsuitable for carbon fiber felt due to the risk of sparking and potential fire hazards. Therefore, only cold remote plasma (CRP) was used for carbon felts.

Table 1. Physico-chemical characterization of PET and carbon nonwoven textiles before and after plasma treatment: Microscopic images of fiber surfaces, water contact angle, capillary uptake, and ESCA/XPS data.

Sample Description and Fiber Surface Morphology	Water Contact Angle	Capillary Uptake	Chemical Functional Groups (ESCA or XPS Analysis, at Fiber Surface)
PET nonwoven untreated (AFM image) 	141°	0 mg	O/C = 0.3 (ester groups)
PET nonwoven treated with air atmospheric plasma ATMP (AFM image) 	0°	1460 mg	Carboxylic group (COOH) N = 0% O/C = 0.5%
PET nonwoven treated with CRPNO (AFM image) 	0°	1648 mg	Carboxylic group (COOH); amino group (–NH ₂) N = 1.1% O/C = 0.3%
Carbon nonwoven (untreated)-SEM image 	115°	0%	Pyridine Pyridinium groups N = 4.2% O/C = 5.9%
Carbon nonwoven activated with CRPNO-SEM image 	60°	600% 	Pyridone amines and amides N = 4.52% O/C = 24.1%

Cold Remote Plasma (CRP)

The cold remote plasma (CRP) treatment was conducted at Université de Lille (France) using a custom-built system. CRP is particularly effective for conductive materials like carbon felt, as it ensures surface activation without direct contact with plasma discharge. In this setup, the material was placed 0.9 m away from the plasma source, allowing only free radicals to interact with the fiber surface. This method prevents sparking while enabling uniform modification of thick samples on both sides, preparing them for enzyme immobilization.

The system used a ($N_2 + O_2$) gas flow excited by a 2450 MHz microwave discharge within a quartz tube, connected to a Pyrex treatment chamber. By preventing direct electron exposure, this remote plasma configuration is ideal for conductive materials. CRP-treated samples were either used immediately or stored in clean, closed containers to prevent exposure to humidity and light, which could accelerate aging. Different parameters were used for plasma activation of the two nonwovens:

CRP parameters for PET nonwoven: O_2/N_2 ratio = 7.2%; gas flow = 2.5 L/min; duration = 15 min; pressure = 3.8 mbar

CRP parameters for carbon felt: O_2/N_2 ratio = 1%; gas flow = 2.5 L/min; duration = 15 min; pressure = 4.5 mbar

2.3.2. Surface Modification Using Amino-Based Biopolymer (Chitosan)

Chitosan Functionalization for GOx Immobilization

Chitosan (CS), a bio-sourced polymer (CAS No. 9012-76-4), was used for surface modification of plasma-activated polyester nonwoven before enzyme immobilization. Chitosan, a cationic polysaccharide, is biocompatible, biodegradable, and composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit)—see Figure 3a. The presence of amino and hydroxyl functional groups enables strong electrostatic interactions and hydrogen bonding with enzymes, facilitating GOx immobilization. For this study, a 100% deacetylated, low molecular weight chitosan was used.

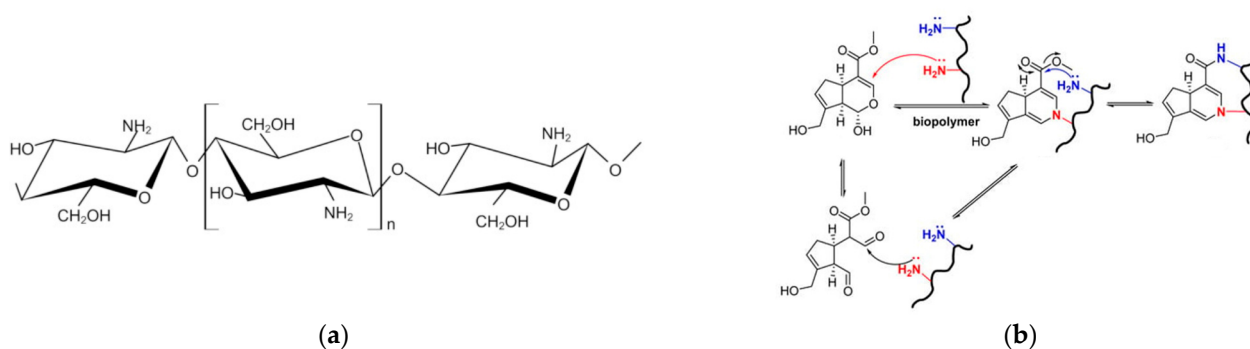


Figure 3. (a) Biosourced chitosan 100% deacetylated low molecular Weight Poly(D-glucosamine). (b) Possible mechanism for the crosslinking of amine-containing biopolymers with genipin [35].

Crosslinking Using Genipin as a Bio-Crosslinker

Genipin is a naturally occurring crosslinker derived from the hydrolysis of geniposide, an active compound extracted from *Gardenia jasminoides* Ellis, commonly used in traditional Chinese medicine. It is significantly less cytotoxic—by a factor of 5000–10,000—compared to conventional glutaraldehyde crosslinking agents [27]. The crosslinking mechanisms proposed by M.F. Butler et al. (2003) [35] involve: (1) Ring-opening of genipin via nucleophilic attack by primary amines on C3, forming covalent bonds with GOx and

(2) Amide group formation through ester substitution between genipin and enzyme amino groups (Figure 3b).

2.4. Total Heterogeneous Bio-Fenton Setup

Figure 4 presents the full heterogeneous bio-Fenton system used for the degradation of crystal violet (CV).

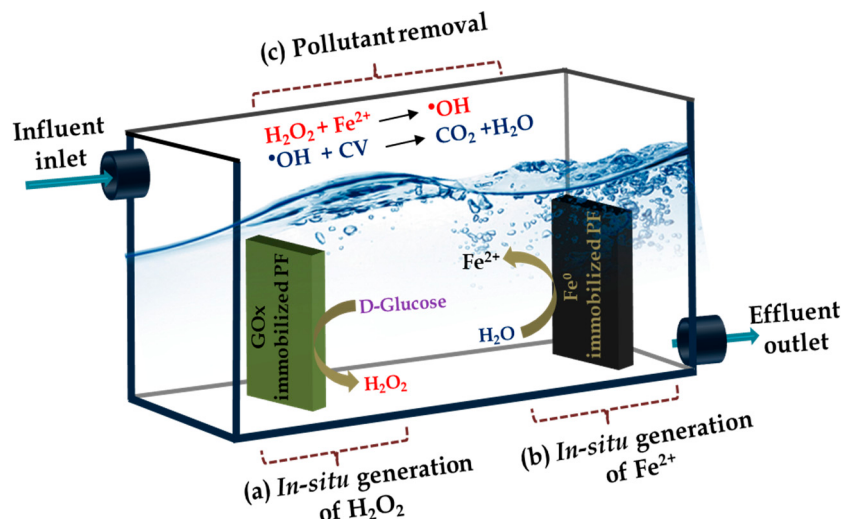


Figure 4. Total heterogeneous bio-Fenton system used for the degradation of crystal violet (CV) dye, using two plasma activated PET nonwovens, one with GOx-immobilized and a second with zero-valent iron (Fe^0) immobilized.

In this configuration, two distinct plasma-treated PET nonwovens were used: one with immobilized glucose oxidase (GOx) and another with immobilized zero-valent iron (Fe^0) [9].

The purpose of this setup is to generate hydroxyl radicals ($\text{OH}\bullet$) through the enzymatic in situ production of hydrogen peroxide by GOx, which then reacts with Fe^0 to activate the Fenton reaction under heterogeneous conditions.

This dual-material approach allows for efficient, localized radical formation and simplified recovery of the catalytic components.

3. Results and Key Findings

3.1. Physico-Chemical Characterization of Plasma Activated Nonwoven Textiles

Water contact angle and capillary uptake measuring were carried by a 3S balance Tensiometer using distilled water with surface tension of 72.6 mN/m. Fiber surface analysis was carried by XPS analysis. Atomic-Force images were made for the fiber surface of PET nonwoven, while SEM pictures show carbon nonwoven fiber surfaces. Table 1 summarizes these data for both PET and carbon nonwovens before and after plasma treatment.

As seen in Table 1, the plasma eco-technology, in particular, the cold remote plasma-CRP (using nitrogen and oxygen gases) is able to activate both PET and carbon fiber surfaces.

Both plasma treatments increased the fiber surface roughness with the appearance of scale-like surface structures in the case of PET nonwoven for both air atmospheric and CRP, whereas the untreated PET fiber surface is smooth.

SEM pictures show that the carbon fibers have grooves and striations appearing in a parallel manner along the fiber length. After CRP there was an increased number of deeper grooves (see image in Table 1).

In addition to morphological changes observed, change in chemical properties attributed to new functional groups on the fiber surfaces are observed: in the case of polyester-PET nonwoven, air atmospheric plasma yields carboxylic terminal groups while the CRPNO yields carboxylic and amino groups (as confirmed by XPS analysis). These additional polar reactive entities present at the fiber surface, improve hydrophilicity of the fiber surface, decrease the water contact and significantly increase the capillary uptake, hence water transport and enzyme penetration into the porous nonwoven structure.

For the carbon fibers, after CRP, XPS analysis (Table 1) show that oxygen percentage (18%) increased more than three times its initial value and the peak intensity at 398.4 eV (pyridine) decreased while a new intense peak at 400.7 eV appeared, probably due to pyridone amines and amides [2].

3.2. Activity of GOx Immobilized on Plasma-Activated PET Nonwovens

This section focuses on the evaluation of the catalytic performance of GOx immobilized on PET nonwoven fabrics activated via two plasma techniques (ATMP and CRPNO), both with and without chitosan modification.

For chitosan grafting, an aqueous solution of chitosan was prepared (4 g/L at pH 4) and stirred for 24 h. Then, the plasma activated nonwoven was padded with the Chitosan solution. The nonwovens were immersed in controlled enzyme solution for 4 h at 4 °C, and then rinsed in buffer solutions. All detailed descriptions are found in a recent thesis [9].

The actual stable and bioactive immobilized GOx was assessed after repeated washing cycles to confirm the robustness of the immobilization. As shown in Table 2, direct sorption on CRPNO-activated PET resulted in 68% active GOx retention, compared to 54% for ATMP-treated PET. The presence of $-\text{COOH}$ and $-\text{NH}_2$ groups is thus favorable for enzyme covalent bonding.

Table 2. Yield of loading and active immobilized GOx on plasma activated PET nonwovens—adapted from [8].

Sample Description	Sample Name	Loading, % pH 5.5	Active Immobilized GOx% (pH 5.5), After 10 Washes
Untreated polyester PET nonwoven	Untreated PET	14	0
CRPNO activated PET + GOx	CRPNO + GOx	20	68
Atmospheric plasma activated PET + sorption of GOx	ATMP + GOx	30	54
Atmospheric plasma activated PET + Padding with chitosan + sorption of GOx	ATMP + Chitosan CS + GOx	46	71

The introduction of chitosan after ATMP treatment further enhanced the enzyme retention, reaching 71% enzyme activity. This improvement is attributed to additional amine- NH_2 functionalities from chitosan, enhancing electrostatic and covalent interactions with GOx.

Importantly, the immobilized enzyme retained approximately 60% of its catalytic activity even after 15 reuse cycles, demonstrating excellent stability under operational conditions.

These results demonstrate that plasma-activated PET nonwovens, especially when combined with biopolymer functionalization, are promising platforms for stable and reusable enzyme immobilization in bio-Fenton applications.

3.2.1. Application of GOx Immobilized PET for BioFenton Reaction

The efficiency of GOx-immobilized PET nonwovens in a heterogeneous bio-Fenton system was evaluated for the degradation of crystal violet (CV) dye. Using β -D-glucose, the system generated highly reactive hydroxyl radicals ($\text{OH}\bullet$) capable of degrading organic pollutants, including crystal violet—a biohazardous triphenylmethane dye with carcinogenic properties [36]. Traditional chemical and biological methods struggle to remove this dye effectively.

Using the immobilized biocatalyst (ATMP + CS + GOx), real-time UV-vis spectroscopy monitoring revealed 88.69% removal of CV (10 mg/L) at a rate of $1.19 \times 10^{-2} \text{ min}^{-1}$, following a pseudo-first-order kinetic model ($R^2 > 97\%$). The immobilized biocatalyst allowed for easy recovery and reusability. Detailed description of this work has been published [8].

Further studies by Morshed [9] explored a complete heterogeneous bio-Fenton system incorporating both GOx-immobilized on PET as well as zero-valent iron (Fe^0) immobilized on plasma-activated PET. More details of the Fe^0 immobilized PET nonwovens are available in a recent paper [37] and in Morshed PhD dissertation [9]. Experiments were conducted using 2 cm^2 of both nonwovens using the experimental setup of Figure 4: one with immobilized bio catalyst (GOx) and the other one with zero valent immobilized iron (Fe^0), immersed in 5 mL of a solution containing 1.0 g/L D-glucose and 10 mg/L CV dye at pH 2 and 35°C . A 93% decolorization of crystal violet was achieved within 3 h.

3.2.2. Enhanced Thermal Stability and Antibacterial Properties

Functionalizing polyester nonwovens with amino-rich polymers such as chitosan significantly improved GOx loading, stability, and resistance to temperature variations. The maximum activity temperature shifted from $\sim 55^\circ\text{C}$ to $\sim 60^\circ\text{C}$ [9].

The antibacterial performance shown in Figure 5 was evaluated using a modified agar diffusion method based on ISO 20645:2004 [38]. Samples were placed on agar plates inoculated with standardized suspensions of *S. epidermidis* (ATCC 12228) and *E. coli* (ATCC 25922). Plates were then incubated at 37°C for 24 h. The zone of inhibition (Figure 5) confirms the additional antimicrobial effect of GOx-functionalized PET, supporting applications in hygiene textiles or for water decontamination through bacterial deactivation.

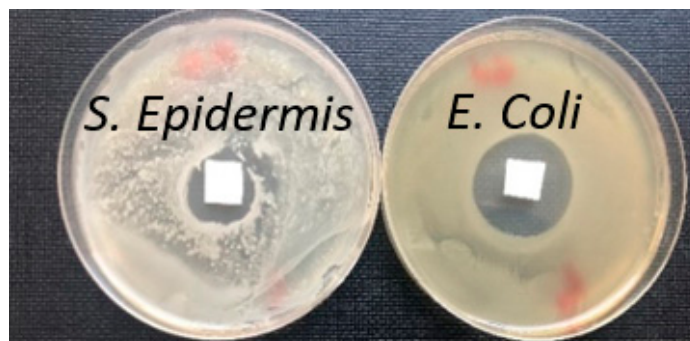


Figure 5. Antibacterial activity against *Staphylococcus epidermidis* and *Escherichia coli*, after GOx immobilization on chitosan modified PET (ATMP + CS + GOx).

These results highlight the potential of GOx-immobilized PET nonwovens as heterogeneous biocatalysts for bio-Fenton reactions, antimicrobial textiles, and sustainable bioremediation applications.

3.3. Activity of GOx Immobilized on CRPNO-Activated Carbon Nonwoven with and Without Genipin

3.3.1. Biocatalytic Activity

This section reports on the catalytic activity and stability of GOx immobilized on conductive carbon felt, which was activated using cold remote plasma (CRPNO).

Direct enzyme sorption on CRPNO-treated carbon fibers led to improved bioactivity compared to untreated samples, due to the emergence of pyridone amines and amides functionalities, as depicted by XPS analysis (Table 1), which facilitates a more stable enzyme immobilization. Figure 6c confirms this enhancement via cyclic voltammetry (CV), showing higher catalytic current responses with increasing D-glucose concentration.

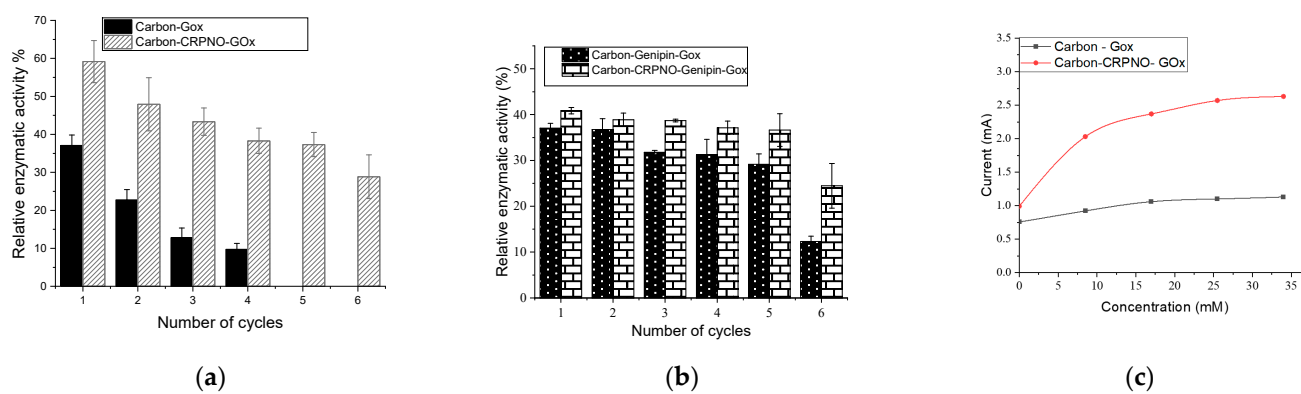


Figure 6. (a) Relative enzymatic activity for GOx immobilized on carbon nonwoven felt before and after CRPNO. (b) Relative enzymatic activity with Genipin bio-crosslinker for GOx immobilized on carbon nonwoven felt before and after CRPNO. (c) Catalytic current vs. D-glucose concentration at E = 0.5 V on carbon felt (without and with CRPNO) with immobilized GOx.

“Relative enzymatic activity” refers to the percentage of catalytic activity retained compared to the initial value (considered as 100%) in repeated use cycles. Repeated use caused gradual loss of activity (Figure 6a), indicating a need for further stabilization. To address this, genipin—a non-toxic, bio-based crosslinker—was applied post-plasma activation. The enzyme-genipin mixture was drop-cast onto the CRPNO carbon felt to enable crosslinking prior to testing. This treatment successfully stabilized GOx, maintaining around 40% of its initial activity over six cycles (Figure 6b), while preserving electrochemical performance. The genipin-crosslinked carbon felts also served effectively in bio-Fenton and bio-electro-Fenton systems for wastewater treatment and power generation.

3.3.2. Applications in Bio-Fenton Wastewater Treatment and Energy Generation

Bio-Fenton Process for Wastewater Treatment

The potential of GOx-immobilized carbon felts in a bio-Fenton wastewater treatment system was assessed using Remazol Blue RR, a bireactive dye with a sulfonated aromatic amine group. This dye is classified as “not fully biodegradable” due to its degradation product (sulfanilic acid) forming recalcitrant byproducts [4,5].

A bio-Fenton system incorporating genipin-crosslinked GOx immobilized on plasma-treated carbon felts and free iron ions achieved 93% color removal within 3 h. This highlights the high efficiency of immobilized GOx in advanced oxidation processes.

Bio-Electro-Fenton (BEF) Process and Power Generation

Beyond wastewater treatment, the GOx-immobilized system was integrated into a bio-electro-Fenton (BEF) reactor, demonstrating dual functionality in both bioremediation and energy generation [5]. The bioanode, consisting of plasma-treated carbon felt with immobilized GOx, achieved a power density of $0.16 \mu\text{W}/\text{cm}^2$, a 3-fold increase compared to the control setup ($0.037 \mu\text{W}/\text{cm}^2$). Simultaneously, the BEF system contributed to 34% COD removal, confirming its efficiency in organic pollutant degradation.

These results demonstrate that CRPNO activation combined with bio-based crosslinker can yield stable and functional GOx-carbon textile electrodes for environmental and bioenergy applications.

4. Conclusions, Challenges, and Future Directions

This study demonstrates that textile-based supports, both non-conductive PET and conductive carbon felt, can be efficiently functionalized with glucose oxidase (GOx) using eco-friendly strategies.

Plasma activation, combined with bio-based additives such as chitosan or genipin, enables stable enzyme immobilization, leading to enhanced biocatalytic activity and operational reusability.

On PET nonwovens, GOx immobilized after CRPNO and chitosan treatment retained up to 71% activity after 10 washes and about 60% after 15 cycles. Dye degradation using immobilized GOx reached over 88% for crystal violet within 3 h.

On CRPNO-treated carbon felts, genipin-crosslinked GOx retained 40% activity after 6 cycles, while supporting bio-electrochemical power generation and 93% dye decolorization using Remazol Blue.

These findings validate the dual potential of the developed enzyme–textile systems for sustainable wastewater remediation and bioenergy production.

Future work will focus on scaling up the immobilization processes, improving enzyme durability under harsh conditions, and integrating textile-based electrodes into modular water purification and energy harvesting systems.

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References

1. Kahoush, M.; Behary, N.; Cayla, A.; Nierstrasz, V. Bio-Fenton and Bio-Electro-Fenton as Sustainable Methods for Degrading Organic Pollutants in Wastewater. *Process Biochem.* **2018**, *64*, 237–247. [CrossRef]
2. Kahoush, M.; Behary, N.; Cayla, A.; Mutel, B.; Guan, J.; Nierstrasz, V. Surface modification of carbon felt by cold remote plasma for glucose oxidase enzyme immobilization. *Appl. Surf. Sci.* **2019**, *476*, 1016–1024. [CrossRef]

3. Kahoush, M.; Behary, N.; Cayla, A.; Mutel, B.; Guan, J.; Nierstrasz, V. Influence of remote plasma on PEDOT:PSS-coated carbon felt for improved activity of glucose oxidase. *J. Appl. Polym. Sci.* **2019**, *137*, 48521. [CrossRef]
4. Kahoush, M.; Behary, N.; Cayla, A.; Mutel, B.; Guan, J.; Nierstrasz, V. Genipin-mediated immobilization of glucose oxidase enzyme on carbon felt for use as heterogeneous catalyst in sustainable wastewater treatment. *J. Environ. Chem. Eng.* **2021**, *9*, 105633. [CrossRef]
5. Kahoush, M. Eco-Technologies for Immobilizing Redox Enzymes on Conductive Textiles, for Sustainable Development. Ph.D. Thesis, Université de Lille, Lille, France, 2019. Available online: <https://pepite-depot.univ-lille.fr/LIBRE/EDSPI/2019/50376-2019-Kahoush.pdf> (accessed on 4 May 2025).
6. Morshed, M.N.; Behary, N.; Bouazizi, N.; Guan, J.; Nierstrasz, V.A. An overview on biocatalysts immobilization on textiles: Preparation, progress and application in wastewater treatment. *Chemosphere* **2021**, *279*, 130481. [CrossRef] [PubMed]
7. Morshed, M.N.; Behary, N.; Bouazizi, N.; Guan, J.; Chen, G.; Nierstrasz, V.A. Surface modification of polyester fabric using plasma-dendrimer for robust immobilization of glucose oxidase enzyme. *Nat. Sci. Rep.* **2019**, *9*, 15730. [CrossRef]
8. Morshed, M.N.; Behary, N.; Guan, J.; Nierstrasz, V.A. Immobilization of redox enzyme on polyester textile: Effect of amine rich textile surface on enzyme stability and bio-catalytic activities. *ACS Sustain. Chem. Eng.* **2021**, *9*, 8879–8894. [CrossRef]
9. Morshed, M.N. Immobilizing Catalysts on Textiles- Case of Zerovalent Iron and Glucose Oxidase Enzyme. Ph.D. Thesis, University of Borås, Borås, Sweden, 2021. Available online: <https://pepite-depot.univ-lille.fr/LIBRE/EDSPI/2021/2021LILUI039.pdf> (accessed on 4 May 2025).
10. Rasmussen, M.; Abdellaoui, S.; Minteer, S.D. Enzymatic biofuel cells: 30 years of critical advancements. *Biosens. Bioelectron.* **2016**, *76*, 91–102. [CrossRef]
11. Tahar, A.B.; Szymczyk, A.; Tingry, S.; Vadgama, P.; Zelsmann, M.; Tsujumura, S.; Cinquin, P.; Martin, D.; Zebda, A. One-year stability of glucose dehydrogenase confined in a 3D carbon nanotube electrode with coated poly-methylene green: Application as bioanode for a glucose biofuel cell. *J. Electroanal. Chem.* **2019**, *847*, 113069. [CrossRef]
12. Yang, D.; Zhao, R. Advanced Oxidation Processes (AOPs) in Wastewater Treatment. *Water Pollut.* **2015**, *1*, 167–176. [CrossRef]
13. Lin, H. Removal of Organic Pollutants from Water by Electro-Fenton and Electro-Fenton Like Processes. Ph.D. Thesis, Université Paris-Est, Créteil, France, 2016.
14. Pushpalatha, M.; Krishna, M.B. Electro-Fenton Process for Waste Water Treatment A Review. *Int. J. Adv. Res.* **2017**, *3*, 439–451. Available online: www.ijariit.com (accessed on 4 May 2025).
15. Elhami, V.; Karimi, A.; Aghbolaghy, M. Preparation of heterogeneous bio-Fenton catalyst for decolorization of Malachite Green. *J. Taiwan Inst. Chem. Eng.* **2015**, *56*, 154–159. [CrossRef]
16. Eskandarian, M.; Mahdizadeh, F.; Ghalamchi, L.; Naghavi, S. Bio-Fenton process for Acid Blue 113 textile azo dye decolorization: Characteristics and neural network modeling. *Desalin. Water Treat.* **2013**, *52*, 4990–4998. [CrossRef]
17. Nasar, A.; Perveen, R. Applications of enzymatic biofuel cells in bioelectronic devices e A review. *Int. J. Hydrogen Energy* **2019**, *44*, 15287–15312. [CrossRef]
18. Bard, E.; Allen, J.; Inzelt, G.; Scholz, F. *Electrochemical Dictionary*; Springer Science & Business Media: Berlin, Germany, 2008.
19. Jain, R.; Jadon, N.; Pawaiya, A. Polypyrrole based next generation electrochemical sensors and biosensors: A review. *Trends Anal. Chem.* **2017**, *97*, 363–373. [CrossRef]
20. Haddaway, N.R.; McConville, J.; Piniewski, M. How is the term “ecotechnology” used in the research literature? A systematic review with thematic synthesis. *Ecohydrol. Hydrobiol.* **2018**, *18*, 247–261. [CrossRef]
21. Arfaoui, M.; Behary, N.; Mutel, B.; Perwuelz, A.; Belhacene, K.; Dhulster, P.; Mamede, A.-S.; Froidevaux, R. Activity of Enzymes Immobilized on Plasma Treated Polyester. *J. Mol. Catal. B: Enzym.* **2016**, *134*, 261–272. [CrossRef]
22. Zille, A.; Oliveira, F.R.; Souto, P.A.P. Plasma treatment in textile industry. *Plasma Process. Polym.* **2015**, *12*, 98–131. [CrossRef]
23. Pasquet, V.; Perwuelz, A.; Behary, N. Environmental Impacts of Chemical/Ecotechnological /Biotechnological hydrophilisation of Polyester fabrics. *J. Clean. Prod.* **2014**, *65*, 551–560. [CrossRef]
24. Nair, L.G.; Mahapatra, A.S.; Gomathi, N.; Joseph, K.; Neogi, S.; Nair, C.P.R. Radio frequency plasma mediated dry functionalization of multiwall carbon nanotube. *Appl. Surf. Sci.* **2015**, *340*, 64–71. [CrossRef]
25. López-Gallego, F.; Betancor, L.; Mateo, C.; Hidalgo, A.; Alonso-Morales, N.; Dellamora-Ortiz, G.; Guisán, J.M.; Fernández-Lafuente, R. Enzyme stabilization by glutaraldehyde crosslinking of adsorbed proteins on aminated supports. *J. Biotechnol.* **2005**, *119*, 70–75. [CrossRef] [PubMed]
26. El Ichi, S.; Zebda, A.; Alcaraz, J.P.; Laaroussi, A.; Boucher, F.; Boutonnat, J.; Reverdy-Bruas, N.; Chaussy, D.; Belgacem, M.N.; Cinquin, P.; et al. Bioelectrodes modified with chitosan for long-term energy supply from the body. *Energy Environ. Sci.* **2015**, *8*, 1017–1026. [CrossRef]
27. Sung, H.W.; Huang, R.N.; Huang, L.H.; Tsai, C.C. In vitro evaluation of cytotoxicity of a naturally occurring cross-linking reagent for biological tissue fixation. *J. Biomater. Sci. Polym. Ed.* **1999**, *10*, 63–78. [CrossRef]
28. Cui, C.; Chen, H.; Chen, B.; Tan, T. Genipin cross-linked glucose oxidase and catalase multi-enzyme for gluconic acid synthesis. *Appl. Biochem. Biotechnol.* **2017**, *181*, 526–535. [CrossRef]

29. Muzzarelli, R.A.A. Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids. *Carbohydr. Polym.* **2009**, *77*, 1–9. [[CrossRef](#)]
30. Hecht, H.J.; Schomburg, D. The 3D structure of glucose oxidase from *Aspergillus niger*. Implications for the use of GOD as a biosensor enzyme. *Biosens. Bioelectron.* **1993**, *8*, 197–203. [[CrossRef](#)]
31. Tiwari, B.R.; Noori, M.T.; Ghangrekar, M.M. A novel low cost polyvinyl alcohol-Nafion-borosilicate membrane separator for microbial fuel cell. *Mater. Chem. Phys.* **2016**, *182*, 86–93. [[CrossRef](#)]
32. Santoro, C.; Arbizzani, C.; Erable, B.; Ieropoulos, I. Microbial fuel cells: From fundamentals to applications. A review. *J. Power Sources* **2017**, *356*, 225–244. [[CrossRef](#)]
33. Du, Z.; Li, H.; Gu, T. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnol. Adv.* **2007**, *25*, 464–482. [[CrossRef](#)]
34. Zhu, X.; Logan, B.E. Using single-chamber microbial fuel cells as renewable power sources of electro-Fenton reactors for organic pollutant treatment. *J. Hazard. Mater.* **2013**, *252–253*, 198–203. [[CrossRef](#)]
35. Butler, M.F.; Ng, Y.F.; Pudney, P.D.A. Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. *J. Polym. Sci. A Polym. Chem.* **2003**, *41*, 3941–3953. [[CrossRef](#)]
36. Mani, S.; Bharagava, R.N. Exposure to crystal violet, its toxic, genotoxic and carcinogenic effects on environment and its degradation and detoxification for environmental safety, in: *Reviews of Environmental Contamination and Toxicology*. Springer **2016**, *237*, 71–104.
37. Morshed, M.N.; Bouazizi, N.; Behary, N.; Vieillard, J.; Thoumire, O.; Nierstrasz, V.A.; Azzouz, A. Iron-loaded amine/thiol functionalized polyester fibers with high catalytic activities: Comparative study. *Dalton Trans.* **2019**, *48*, 8384–8399. [[CrossRef](#)] [[PubMed](#)]
38. *ISO 20645:2004*; Textile Fabrics—Determination of Antibacterial Activity—Agar Diffusion Plate Test. ISO: Geneva, Switzerland, 2004.

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