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► To cite this version:

May Kahoush, Nemeshwaree Behary, Aurélie Cayla, Brigitte Mutel, Jinping Guan, et al.. Surface modification of carbon felt by cold remote plasma for glucose oxidase enzyme immobilization. Applied Surface Science, 2019, 476, pp.1016-1024. 10.1016/j.apsusc.2019.01.155 . hal-03140653

HAL Id: hal-03140653

<https://hal.science/hal-03140653>

Submitted on 21 Oct 2021

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1 **Title page**

2

3 **Surface modification of carbon felt by cold remote plasma for glucose oxidase**
4 **enzyme immobilization**

5

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34 **Abstract**

35 Despite their chemical inertness and poor hydrophobicity, carbon-based materials are widely used in
36 electrochemical applications due to their robustness, good electrical conductivity and corrosion
37 resistance. The purpose of the work carried was to increase the wettability of nonwoven carbon fiber
38 felts for improved efficiency in bio/electrochemical applications. Virgin Carbon Felt (VCF) was first
39 treated with cold remote plasma (CRP) using a mixture of nitrogen and oxygen (1 or 2 %) as plasma
40 gas. Bio-functionalization of the carbon felts with glucose oxidase (GOx) enzyme was then carried
41 using physical adsorption method. FTIR and XPS analysis showed an integration of new oxygenated
42 functional groups (C-O and C=O) as well as amines and amides on the surface of VCF treated by the
43 CRP treatment, which improved the wettability of the samples. Capillary uptake increased from
44 around 0 % (for VCF) to nearly 750 % with 2 % oxygen in plasma gas. GOx enzyme showed higher
45 activity after immobilization at pH 5.5 on the CRP treated samples, maintaining up to 50 % of its
46 initial enzymatic activity after six cycles while with the VCF, no enzymatic activity was observed
47 after the fourth cycle. These obtained felts can be used as electrodes in sustainable bioprocesses.

48

49 **Keywords:** Carbon felt; Cold remote plasma; Surface modification; glucose oxidase immobilization;
50 enzyme activity

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59 **1. Introduction**

60 Carbon-based textile materials are well known for being chemically stable, and resistant to corrosion
61 in various mediums including aqueous or solvent-based solutions. These properties make them a
62 good choice when a robust, conductive, relatively cheap material is required for many applications
63 including bio/electrochemical setups [1–3]. One of the most important applications for carbon-based
64 textiles is the production of electrodes. These electrodes are used widely nowadays to produce
65 biosensors for medical or environmental purposes, biofuel cells and wastewater treatment reactors.

66 However, their chemical stability renders these materials inert, with low surface energy and
67 hydrophobic tendencies, causing technical issues such as low affinity towards aqueous solutions, and
68 poor adhesion with polymers and resins during production and performance [4].

69 To overcome these difficulties, many studies have been conducted to modify the surface of different
70 carbon materials using various methods. Approaches including: physical, chemical or thermal
71 methods have been used to increase the surface energy of these materials, and to facilitate treatments
72 for different purposes. These treatments were efficient in surface modification and functionalization,
73 in addition to increasing adhesion in composite materials, improving mechanical properties of
74 nanocomposites, and increasing the power efficiency in flow batteries [5–18].

75 Plasma treatment, among these methods, is considered as an effective and dry approach for surface
76 modification. Unlike wet-chemical processes, it does not require solvents and it can be completed in
77 a relatively short time, using different gases and elements according to the required functions. In
78 recent years, different types of plasma have been used to treat carbon materials using a variety of
79 gases to increase their wettability, improve fiber-matrix adhesion in composite materials or to
80 improve the energy efficiency in flow batteries, through the integration of functional groups on the
81 treated surfaces [4,15–24].

82 As mentioned, the obtained plasma-treated carbon materials possess higher surface energy than their
83 initial state, and thus can be further bio functionalized with enzymes. The combination of carbon

84 with custom modified surfaces via plasma, and the immobilized enzymes provide the opportunity to
85 produce bio-electrodes for applications including medical biosensors, self-powered implants,
86 pollution detection biosensors, and power generation from bio-sourced materials such as bio-ethanol
87 and sugars.

88 Recently, researchers have worked on bio-functionalization of carbon materials (carbon nanotubes,
89 carbon nanoballs, carbon-graphite rods and graphene) with enzymes using different types of plasma
90 including low temperature plasma, atmospheric pressure plasma jet and downstream oxygen plasma,
91 using gases like CH₄, N₂ and O₂ [25–28]. These treatments produced high performance bio-
92 electrodes with improved enzymatic activity. Nevertheless, these electrode materials possess less
93 specific surface area than the carbon nonwoven felts. Nonwovens from microfibers like the carbon
94 felts have a higher surface area to immobilize higher quantity of enzymes, and allow capillary uptake
95 and diffusion of substrate and products, after plasma treatment, enabling more efficient bio-
96 processes.

97 However, the efficiency of cold remote plasma in modifying the surface of carbon felt-based
98 materials and improving their wettability and bio-functionalization with enzymes has not been tested
99 to the best of our knowledge. This type of plasma, out of the discharge zone, is well controlled, due
100 to the ability of customizing the type and percentage of gases used according to the desired obtained
101 functions. CRP activates the carbon fiber surface without causing hazardous sparking. Indeed, if the
102 conductive sample is in the vicinity of the dielectric barrier discharge used in the plasma treatment,
103 hazardous sparking will occur (as in the case of air atmospheric plasma treatment). For the CRP, the
104 discharge is around one meter from the conductive nonwoven samples. Free radicals produced by the
105 dielectric barrier discharge are the only reactive species reaching the carbon felt. The CRP is thus
106 able to activate surface of electrically conductive materials, which are attractive for smart and
107 functional textiles.

108 Furthermore, the design of the instrument of the CRP used allows the treatment of samples without
109 limitation on the thickness, unlike some other plasma setups, which require certain maximum
110 thickness for the film or fabric, and that limits the treatment of 3D structures like the bulky felts. In
111 addition, both sides of the samples can be treated at the same time if desired using CRP setup, which
112 saves both energy and time.

113 In this paper, the ability of a far cold remote (nitrogen + oxygen) plasma to modify surface properties
114 of a nonwoven commercial Virgin Carbon Felt as received (VCF) was investigated. The modified
115 felts were then bio-functionalized with glucose oxidase enzymes classified as EC (1.1.3.4) using
116 adsorption method, and the activity of immobilized enzyme was evaluated. Surface modifications
117 were monitored using different techniques, including Scanning Electron Microscopy (SEM), Fourier
118 Transform Infrared (FTIR), X-ray Photoelectron Spectroscopy (XPS), water contact angle and
119 capillary uptake measurements. Enzymatic activity assays and cyclic voltammetry scans were carried
120 out to evaluate the efficiency of enzyme immobilization methods and more specifically those leading
121 to least enzyme denaturation and to increased re-usability.

122

123 **2. Materials**

124 **2.1. Carbon Felt**

125 Commercial poly acrylonitrile (PAN) based carbon felt (Supplementary data Fig.1 (a)) was
126 provided by Ceramaterials (USA). The carbon content >97%, thickness of 3 mm, bulk density of
127 0.15 g.cm^{-3} , relative surface area is between $10 - 30 \text{ m}^2.\text{g}^{-1}$, average fiber diameter of $20 \mu\text{m}$. The
128 carbon nonwoven felt, baked at $1200 \text{ }^\circ\text{C}$ during manufacturing, was free of residual organic
129 material or resins. Hence, no cleaning was required before use. The high specific surface area of the
130 felt makes it a good candidate for an enzyme immobilization process. Moreover, the porous carbon
131 felt has a low-pressure drop and can enable easy diffusion of reaction mixture.

132 **2.2. Glucose oxidase enzyme**

133 Glucose oxidase from *Aspergillus Niger* type X-S G7141 with EC (1.1.3.4) and potassium
134 dihydrogen orthophosphate were provided by Sigma-Aldrich (Germany). Glucose oxidase activity
135 kit (K-GLOX 11/16) from Megazyme (Ireland) was used to assess the activity of free or
136 immobilized enzyme. In addition, phosphate buffered saline (PBS) and Ferrocenecarboxylic acid
137 (FCA) used in cyclic voltammetry test were also provided by Sigma-Aldrich (Germany).

138

139 **3. Methods**

140 **3.1. Cold remote plasma treatment (CRP)**

141 The experimental setup shown in (Supplementary data Fig. 2) is available in the University of Lille
142 in France. The ($N_2 + O_2$) mixture was excited by an electrodeless microwave discharge (2450 MHz –
143 800 W) in a fused silica tube (30 mm diameter) which was coupled to the treatment chamber (15
144 liters). The N_2 flow, regulated by mass flow controllers Alphagaz was fixed at 2500 sccm (standard
145 cubic centimeters per minute), while O_2/N_2 ratios were ranging from 1 to 2 %. Total working
146 pressure was equal to 8.5 mbars. By a continuous pumping (primary rotary pump provided by
147 Pfeiffer), the reactive excited species flow from the discharge to the treatment chamber located 0.9 m
148 from the discharge where the CRP appears as a bluish-white afterglow. This zone, far from the
149 discharge, is free of charged particles or UV radiations and its temperature is around the ambient.
150 Main reactive species are atomic nitrogen, vibrationally and/or electronically excited nitrogen
151 molecules and atomic oxygen. The long lifetime of nitrogen atoms due to a re-dissociation of
152 nitrogen molecules allows getting a large volume expansion of this remote plasma on condition that
153 O_2 percentage remains lower than 5 %. The VCF were settled in the treatment chamber, and were
154 treated during 15 min with the selected $N_2 + O_2$ mixture.

155 The treated samples were used directly after treatment, or stored in clean closed containers until use.
156 This minimizes the access of light and humidity, which may accelerate ageing. Table 1 summarizes
157 the nomenclature of each sample studied in this work.

158

159

Table 1 Nomenclature of each sample

Without plasma treatment	With plasma CRP (N ₂ + O ₂) Ratio of O ₂ /N ₂ =1%	With plasma CRP (N ₂ + O ₂) Ratio of O ₂ /N ₂ =2%
VCF	CF 1%	CF 2%

160

161 3.2. Enzyme immobilization

162 The method used for immobilization of glucose oxidase enzyme was physical adsorption. The
163 different carbon-based samples (dimension 1 cm²) were each placed in 3 mL of phosphate buffer
164 solution containing glucose oxidase (1 mg.mL⁻¹ , with 0.0858 U.mg⁻¹) at 4 °C for 24 hours.

165 Immobilization using adsorption was carried out at two different pH values 5.5 or 7 for untreated and
166 CRP treated carbon felts. It is worth mentioning that the samples treated with plasma were placed in
167 the enzyme solution directly after treatment. The bio-functionalized carbon felts were then rinsed
168 twice with phosphate buffer (pH 7) and stored at 4 °C till use. The rinsing process was efficient
169 enough to remove any unfixed enzyme; and this was confirmed using the GOx kit assay on the
170 rinsing buffer solution: no pink coloration was observed (see section 3.5).

171

172 3.3. Surface Analysis

173 3.3.1. FTIR, SEM and XPS analyses

174 All FTIR analyses were performed in transmission mode (expressed in arbitrary units) with Nicolet
175 NEXUS 670 FTIR in the range of wavenumbers between 400 - 4000 cm⁻¹ with 4 cm⁻¹ resolution, and
176 a correction for CO₂ from air was performed. SEM images were taken using field emission scanning
177 electron microscope JSM-7800F (LED detector, 6 KV accelerating voltage).

178 To obtain the XPS spectra, Kratos Axis Ultra DLD spectrometer with monochromatic Al K α X-ray
179 source (h ν = 1486.6 eV) working at 120 W (10 mA, 12 kV) was used. The base pressure of the
180 analysis chamber was 2 \times 10⁻¹⁰ mbar. Survey scans were taken at analyzer pass energy of 160 eV over

181 1200 to -5 eV binding energy range with 1 eV step and a dwell time of 1sec. High-resolution spectra
182 were collected using an analysis area of $\approx 300 \mu\text{m} \times 700 \mu\text{m}$ and a 40 eV pass energy. Spectra were
183 charge corrected to give the adventitious carbon C 1s spectral component C- (C, H) a binding energy
184 of 285 eV. The XPS spectra were fitted using OriginLab[®] software, and the accumulated peaks are
185 shown without noise for convenience.

186 **3.3.2. Water contact angle and capillary uptake measurements**

187 For measuring water contact angle greater than 90° , sessile drop method using “Digidrop” from
188 GBX Instrument (France) was used (Supplementary data Fig.1 (b)). However, for lower water
189 contact angle ($< 90^\circ$), the porous felt immediately absorbed the water drop, and so a wicking test
190 (Supplementary data Fig. 1(c)) was performed with 3S scale tensiometer from GBX instruments, to
191 measure the water contact angle and capillary uptake. A rectangular piece of felt was used, its top
192 area connected to the tensiometer at the weighing position, and the bottom area was progressively
193 brought into contact with the surface of distilled water placed in a container. On immediate contact
194 with water surface, a sudden increase in weight (W_m) was measured due to meniscus weight
195 formation of the nonwoven carbon felt, and the water contact angle then calculated using Eq. 1.

$$196 \quad W_m \times g = \gamma_L \times \cos \theta \times P \quad \text{Eq.1}$$

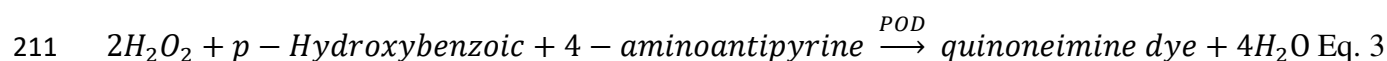
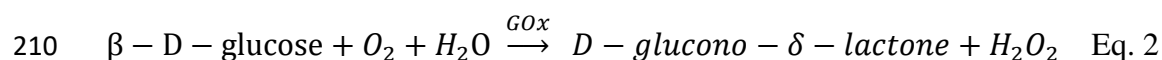
197 Where W_m is the meniscus weight (g), γ_L is the surface tension of the liquid ($\text{mN}\cdot\text{m}^{-1}$), P is the
198 sample perimeter that is in contact with the liquid (m), and θ is the water contact angle of the carbon
199 felt ($^\circ$).

200 Each sample was $(3 \times 5) \text{ cm}^2$, and the time of the contact with the water was 3 min. Capillary weight
201 was measured after removal of fabric from water surface. More details about this method can be
202 found in previous work [29].

203 **3.4. Method to quantify activity of GOx**

204 **3.4.1. Glucose oxidase activity Assay**

205 Glucose oxidase activity was measured according to the enzyme's ability to catalyze the oxidation
206 reaction of β -D-glucose. Hydrogen peroxide is released as a by-product of this reaction (Eq. 2 and 3).
207 The produced H_2O_2 enters in another reaction with p-hydroxybenzoic acid and 4-aminoantipyrine in
208 presence of peroxidase enzyme (POD), which results in a pink colored complex (quinoneimine dye
209 complex) at 25° C and pH 7.



212

213 The absorbance intensity of the colored complex was monitored at 510 nm, using a JascoV-350 UV-
214 VIS spectrophotometer. For the assay, absorbance intensity at $t = 20$ min was recorded when 2 mL of
215 POD mixture added to 0.5 mL of glucose (90 g.L^{-1}), and then 0.5 mL of enzyme solution (1 mg.mL^{-1}
216 with 0.0858 U.mg^{-1}) added to the mixture. A calibration curve was thus plotted, which allowed to
217 quantify the number of enzyme unit (mU) per 0.5 mL of enzyme solution.

218 Indeed, one unit of glucose oxidase is the amount of enzyme that oxidizes $1.0 \mu\text{mole}$ of β -D-glucose
219 to D-gluconolactone and H_2O_2 per minute at pH 5.1 at 35 °C.

220 **3.4.2. Method to quantify the activity of immobilized Gox**

221 In order to quantify the activity of immobilized enzyme on one cm^2 of nonwoven felt, each sample
222 was rinsed twice after immobilization, and then was immersed in the assay solution (2 mL of POD
223 mixture + 0.5 mL of glucose (90 g.L^{-1})). Moreover, absorbance values were measured after 20 min,
224 using the UV spectrophotometer to assess the immobilized enzyme activity (deduced from the
225 standard calibration curve provided by Megazyme (Supplementary data Fig. 3)). This method allows
226 to quantify the activity of immobilized enzyme (U.cm^2) and not the amount (in units) of enzyme
227 protein immobilized.

228 CH Instruments electrochemical workstation was used for Cyclic Voltammetry (CV) measurements.

229 The cell has a standard three-electrode configuration. CH1650D software was used to collect the

230 curves and OriginLab[®] was used in plotting. The carbon felt samples were used as the working
231 electrode (WE) with 1 cm² immersed surface area, Ag/AgCl as the reference electrode (RE), and
232 platinum wire was used as a counter electrode (CE). The measurements were taken in a (0.01 M)
233 PBS buffer solution at pH 7 and room temperature, the scan window was -0.4 – 0.6 V (-0.2 – 0.8 V
234 vs. Standard Hydrogen Electrode SHE). FCA was used as a mediator (30 mM), and D-glucose was
235 added to the medium as a substrate of the enzyme. The scan rate of 0.01 V.s⁻¹ was used unless it is
236 mentioned otherwise, the current densities were normalized according to immersed surface area of
237 the electrode (1 cm²).

238 **3.4.3. Method to quantify re-usability of immobilized Gox**

239 The enzymatic activity assay was performed for multiple cycles for each sample as described
240 previously, where one cycle refers to performing the activity assay for 20 min, followed by rinsing
241 the sample twice with a buffer solution and restoring the sample at 4 °C for at least 24 hours until the
242 next cycle.

243

244 **4. Results**

245 **4.1. Surface modification characterization**

246 **4.1.1. SEM images**

247 SEM images in Fig. 1 show the different morphological changes occurring before and after CRP
248 plasma treatment of the carbon fibers of the felt. The VCF have rough surface with striations and
249 grooves running parallel to the fiber length. After CRP treatment, whatever gas mixtures (N₂ + 1 %
250 or 2 % O₂), there is slight modification of the carbon fiber surface with an increased number of
251 grooves showing higher depths. This observation is in agreement with a previous study concerning
252 the use of CRP plasma on a woven carbon fabric [4].

253 **4.1.2. FTIR spectra**

254 The FTIR spectra after CRP of the VCF sample with both gas mixtures are illustrated in Fig. 2. The
 255 spectra confirm the integration of new functional groups on the surface of both plasma treated carbon
 256 fiber surfaces. New integrated groups, mostly oxygenated and amino groups appeared on the plasma
 257 treated samples. Functional groups detected by different peaks are described. The weak broad peak
 258 appeared at 3345 cm^{-1} was attributed to N-H stretching vibration of secondary amine [30]. Presence
 259 of the peak at 1650 cm^{-1} was assigned to C=O stretching vibration of amide group [31], and the peak
 260 between $1550\text{-}1640\text{ cm}^{-1}$ was attributed to N-H stretching vibration, indicate the presence of amide
 261 groups [32]. Double peaks at 2850 and 2920 cm^{-1} were assigned to stretching vibration of =C-H of
 262 an aldehyde and a peak at 1733 cm^{-1} was assigned to stretching vibration of C=O, indicate the
 263 presence of aldehyde groups [32]. The strong peak at $1030\text{ -}1155\text{ cm}^{-1}$ was attributed to stretching
 264 vibration of C-O of ether group [30].

265 4.1.3. XPS analysis

266 The typical survey spectra by XPS of the VCF felt and of plasma treated CF 1% and CF 2% were
 267 conducted. The main photopeaks are labeled C 1s, N 1s and O 1s in the untreated VCF felt and
 268 plasma treated felt. C 1s, O 1s are predominant, while N 1s is also present in all the cases. Table 2
 269 gives a more quantified data (in %) of different atomic contents on the different carbon felt fiber
 270 surfaces.

271 Table 2: Atomic content (%) as measured by XPS

	C 1s	C-C	C-O	C=O	O 1s	N 1s
VCF	90	90	10		5.36	4.22
CF 1%	76	56.2	29.8	14	18.37	4.52
CF 2%	75	56.2	30.9	12.9	19.65	4.44

273
 274 Table 2 shows that more than three times the initial percentage of oxygen (18%) was present on the
 275 fiber surface after CRP treatment with 1% of oxygen. Nevertheless, further increase in oxygen
 276 content in the gas mixture (to 2%) did not increase significantly oxygen content of the CF 2%

277 sample. Deconvolution of the C 1s photopeak (Fig. 3) shows for the CF 1% sample a peak at 284.8
278 eV which is related to graphitic carbon C-C, a second peak at 286.2 eV related to C-O and a third
279 peak at 288.6 eV related to carbonyl C=O group. Meanwhile, for the VCF, the spectrum showed a
280 sharp peak for the C 1s related to graphitic carbon C-C, with only 10 % of C-O content. CRP
281 treatment increases significantly both C-O and C=O functional groups. FTIR indeed showed intense
282 double peak between 2920 and 2850 cm^{-1} due to stretching vibration of aldehyde groups, and so the
283 increase in C=O is due to aldehyde groups and possibly amides detected by FTIR. The C-O increase
284 would be due to ether formation.

285 The VCF surface is composed of at least 3- 4% of nitrogen atoms. Table 2 reveals very little increase
286 in nitrogen content after the CRP treatment whatever the gas mixture used. Deconvolution of the N
287 1s photopeak (Fig. 3) shows a modification of the XPS spectra after the plasma treatment. On the
288 VCF, the N 1s spectra show two main peaks related most probably to pyridinium at 401.5 eV, and to
289 pyridine at 398.4 nm. The ratio of pyridine with respect to pyridinium is almost 1: 1.4 in the VCF.

290 With CF 1%, the intensity peak at 398.4 eV (pyridine) decreases abruptly, and a new intense peak
291 between 400.1 eV and at 400.7 eV probably due to pyridone amines and amides appears [33]. With
292 CF 2%, the areal ratio of this last slightly lower.

293 Both pyridinium and pyridone are amine and amide bearing groups. Indeed, FTIR (Fig. 2) also
294 indicates presence of secondary amine (at 3345 cm^{-1}) and of C=O of amide (at 1650 cm^{-1}) after
295 plasma treatment.

296 **4.1.4. Water contact angle measurements**

297 The water contact angle of the VCF as received, using sessile drop method and distilled water was
298 $116 \pm 1^\circ$ at room temperature (Supplementary data Fig. 1(b)). This hydrophobic tendency affects the
299 affinity of the felts towards aqueous enzyme solutions, which are used for enzyme immobilization by
300 physical adsorption. Thus, a reduction of the water contact angle is expected to lead to higher affinity
301 with those mediums in order to facilitate the bio-functionalization treatments. One of the

302 conventional ways to increase the wettability of carbon materials is treatment with hydrogen
303 peroxide using 10 % H₂O₂ solution at 90 °C for 3 hours [12]. This treatment was efficient in reducing
304 the water contact angle of carbon-based felts similar to ours, and in our study, it reduced the water
305 contact angle by half. Nevertheless, this method has many disadvantages since it consumes both
306 energy and long time due to constant heating for several hours. That makes plasma treatment a better
307 candidate to achieve the same goals within a short time and without using heated chemical solutions.
308 To compare the effect of cold remote plasma on VCF carbon felts with the traditional hydrogen
309 peroxide treatment, water contact angle and capillary uptake values of the samples were measured
310 immediately after plasma treatment, and four weeks later, in order to study the ageing effect.
311 The variation of water contact angle capillary uptake values without and after plasma treatment is
312 illustrated in Fig. 4(a) and Fig. 4(b). CRP was efficient in reducing the water contact angle of the
313 VCF from 116° to around 57-59°, and increasing the capillary uptake from almost 0 % to 650 -700
314 %, in a short time and without using any added chemicals for both gas mixtures used. Higher
315 wettability of the carbon felt was achieved with plasma gas mixture containing 2 % oxygen, with
316 more than 700 % capillary uptake. However, after 4 weeks, a very small decrease in wettability was
317 observed, in terms of both increase in water contact angle and a decrease in capillary uptake. The
318 decrease in wettability was higher for the CF 2% compared to the CF 1%, with water contact angle
319 increasing to 70°± 5 and capillary uptake decreasing to 570 %.

320 Indeed, water contact angle measurement is a measure of wettability of fiber surfaces at the outer felt
321 surface, whereas, the capillary uptake is related to that of the fiber surfaces inside the carbon felt.
322 This would mean that ageing of oxidized species takes place both in the inside and the outer faces of
323 the plasma treated carbon felt. However, compared to other types of plasma used to activate carbon
324 fiber surface (e.g. active screen plasma [34]), the ageing effect with CRP (N₂ + O₂) plasma is very
325 slow.

326

327 4.2. Characterization after enzyme immobilization

328 4.2.1. SEM images

329 The SEM images (Fig. 5) illustrates the felt samples bio-functionalized with GOx enzymes. On the
330 CF 2% plasma treated samples, enzymes seem adsorbed directly onto the carbon fiber surface, as if
331 they were on the upper extremities of the parallel grooves.

332 4.2.2. Immobilized Enzyme activity

333 The immobilized enzymatic activity was measured for all the bio-functionalized carbon felt samples,
334 using the glucose oxidase activity kit as described in the section 3.4.1. As previously mentioned, the
335 activity test was conducted at pH 7 and 25 °C, according to the protocol provided by the producer.
336 However, for the adsorption of enzymes on 1 cm² carbon felt in 3 mL of enzyme solution, two
337 different pH values were used (pH 5.5 and 7). The activity of immobilized enzyme on 1 cm² was
338 compared to the total activity of enzyme (0.2574 U) present in 3 mL, and the “relative enzyme
339 activity %” after immobilization was defined in Eq. 3.

$$340 \text{ Relative enzyme activity efficiency (\%)} = \frac{\text{Immobilized Gox activity } \left(\frac{\text{mU}}{\text{cm}^2}\right)}{\text{Free Gox activity (mU/0.5ml)} \times 6} \text{ Eq. 3}$$

341 As can be seen (Fig. 6) on untreated carbon (VCF) felt, only 35 % of the total free enzyme activity
342 was reached after immobilization at pH 7 or pH 5.5, while CRP treatment allowed to reach enzyme
343 activity as high as 55 to 60 % during the first cycle. Plasma treated samples (CF 1% and CF 2%)
344 gave higher relative enzymatic activity values in most cases compared to VCF samples.

345 When enzymatic activities are compared for enzymes sorbed at pH 5.5 and pH 7, the pH 5.5 value
346 (Fig. 6(b)) gave higher enzymatic activities after immobilization process, for all three felt samples
347 (VCF, CF 1 % and CF 2 %)

348 Furthermore, the stability and reproducibility of these samples were better at pH 5.5 than at pH 7. It
349 is worth mentioning that the enzymatic activity of free enzymes in 3 mL the buffer solution used for
350 enzyme sorption on 1 cm² of carbon felt, was around 0.08 U.mL⁻¹. Enzyme immobilization via

351 adsorption, maintained part of enzymatic activity after immobilization, since not all free enzymes
352 would be immobilized, and all immobilized enzyme do not necessarily maintain their biocatalytic
353 activity after immobilization.

354 The cyclic voltammetry scans for the CF 1% samples in the absence and presence of different
355 concentrations of D-glucose (8.5, 17 and 25.5 mM) are illustrated in Fig. 7. An increase of the
356 biocatalytic current occurred due to the increase of the glucose added to the medium, which indicates
357 that the GOx maintained an enzymatic activity after the immobilization and the obtained felts are
358 bioactive.

359 It is worth mentioning that, above the concentration 30 mM of glucose, no further increase in the
360 currents, a plateau was reached at around 3.5 mA and a potential of around 0.5 V (0.7 V vs. SHE).
361 This was applicable in the case of CF 2% as well.

362 To estimate the parameters of the enzyme kinetics, Michaelis–Menten equation was used [35],
363 Lineweaver-Burk method was used to estimate the apparent Michaelis constant (k_m) and the
364 maximum current response (I_{max}) for the different samples (Supplementary data Fig.4, Fig. 5). The
365 maximum current I_{max} reached 1.22, 2.92 and 3.8 mA, while the apparent $k_m = 2.7, 3.8$ and 5.2 mM
366 for VCF, CF 1% and CF 2%, respectively ($R^2 = 0.998, 0.996, 0.998$, respectively).

367 **4.2.3. Reusability of the bio-functionalized felts**

368 The enzymatic activity of all samples decreased with the number of repeated cycles. The samples
369 with no plasma treatment (VCF) showed no enzymatic activity after the fourth cycle. The plasma
370 treated bio-functionalized samples CF 1%, maintained better enzymatic activity after a higher
371 number of repeated cycles, with only 50 % of activity loss after the sixth cycle (Fig. 6 (a) and (b)).

372

373 **5. Discussion**

374 The purpose of this study was to investigate the efficiency of cold remote ($N_2 + O_2$) plasma to
375 activate Virgin Carbon Felt (VCF), for optimized enzyme GOx immobilization.

376 CRP treatments used here, revealed to be an efficient method to activate the surface of conductive
377 carbon fiber surfaces. The CRP leads to physical and chemical modification of the carbon fiber
378 surfaces. Our results agree with literature when using reactive plasma gas mixtures like oxygen and
379 nitrogen, the main dominant effects are expected to be etching and surface functionalization [36].

380 In CRP, the carbon fiber samples are treated far from the discharge, in a reactive zone free from
381 ions, electrons and UV radiations. This approach was efficient in treating the conductive felts used in
382 this study without direct exposure to dielectric discharges. The etching effect is thus limited.

383 In accordance, SEM images showed slight topographical changes of VCF after the CRP treatment.

384 Both FTIR and XPS characterizations confirmed the integration of new functional oxygenated
385 groups (such as C-O and C=O) as well as amino groups, after CRP treatment with both plasma gas
386 mixtures. The percentage of oxygen atoms on the carbon fiber was multiplied more than three times,
387 with the introduction of aldehyde, ether, and amide groups. No further significant increase in oxygen
388 or nitrogen atom content was observed when the 2% of oxygen was used instead of 1% in the plasma
389 gas mixture.

390 A significant ratio of nitrogen atom (4%) was already present on the untreated carbon fiber, contrary
391 to a previous study [4], and this may be explained by the fact that the carbon fiber felt used in this
392 study has been obtained by the oxidation of nitrogen containing polyacrylonitrile (PAN). After CRP,
393 very small variation in nitrogen atom content was observed. FTIR showed peaks due to secondary
394 amine and amide groups after CRP, while XPS revealed that on the virgin carbon, almost 50% of the
395 nitrogen atoms were from pyridine groups, which were gradually converted by CRP into pyridone.
396 Fig. 8 shows the possible functional groups on the virgin carbon fiber produced from
397 polyacrylonitrile and the possible functional groups created by the CRP ($N_2 + O_2$) treatments used,
398 this figure is adapted from [37].

399 Most of these new functional oxygenated groups should be formed on fiber surfaces both at the outer
400 surface and inside the felt, resulting in increase in wettability both in terms of decreased water

401 contact angle and increased capillary uptake. Hydrophobic recovery due to ageing was indeed very
402 slow.

403 The enzymatic activity of GOx deviated when pH 7 was used during the immobilization process,
404 compared with the activity values for adsorption at pH 5.5, which showed to be slightly better and
405 more stable. This might be due to the optimal pH value for glucose oxidase activity (EC 1.1.3.4).
406 While each enzyme functions within a specific range of pH, for each enzyme there is an optimal pH
407 value, at which the enzymatic activity can be maximal if the other conditions such as temperature are
408 maintained [38]. For the glucose oxidase, the pH range is reported to be broad and it can be active
409 between pH 4 and 7, but the optimal activity can be obtained in mediums with pH 5.5 [39]. In
410 general, plasma treated samples gave better enzymatic activity, re-usability and shelf life when
411 compared to the non-treated VCF samples. This can be explained by better bonding between the
412 surfaces and the immobilized enzymes due to the integration of groups such as carbonyl groups and
413 amino groups on the surface of the samples after plasma treatment for both gas mixtures. These
414 groups help to create the hydrogen bonding with the adsorbed enzymes and possible covalent
415 bonding with the amino acids of the enzymes, and this leads to less leaching of the enzyme into the
416 reaction medium, and consequently an extended reusability even when immobilized in a neutral
417 buffer solution. Furthermore, the conformation of the enzyme at the fiber surface allowed the
418 substrate to access the active site of the enzyme, which resulted in maintaining enzymatic activity
419 and reusability. The I_{max} values estimated from the CV scans show that the plasma treated felts gave
420 higher currents (for one cycle scan), in accordance with the colorimetric assay for the enzyme. Since
421 I_{max} is attributed to the quantity of the active units of enzyme, thus the plasma treatment improved the
422 adsorption of the enzyme on the surface of the treated felts, in comparison with the VCF. However,
423 K_m values indicate that a slight decrease in the enzyme's affinity towards its substrate occurred after
424 the immobilization on the plasma treated felts, which might be due to the increased hydrogen and
425 covalent bonding between the new integrated functions on the carbon fibers and the enzyme. That

426 requires only a slight increase in the substrate's concentration to reach the maximum enzymatic
427 activity. Nevertheless, this small reduction in the affinity was compensated by the higher quantity of
428 active units adsorbed on these samples which resulted in higher currents and activity overall.

429 It can be noticed from the results, that the increased percentage of oxygen used in the gas mixture to
430 2% did not have a proportional influence on the treated samples when compared to 1%. However,
431 the more rapid loss in enzymatic activity during the 6th cycle, for sorption at pH 5.5, with CF 2%,
432 may be explained by the more rapid ageing effect of the CF 2% compared to CF 1%. Hence, further
433 studies on the different percentages of the gas mixture can be beneficial in determining the optimal
434 mixture for better wettability of the carbon-based textile materials.

435

436 **6. Conclusion**

437 It can be concluded based on this study, that the cold remote ($N_2 + O_2$) plasma treatment allowed
438 successful functionalization of the conductive carbon fibers, with carbon fiber enriched with
439 hydrophilic functional groups (carbonyl, amino, amides, etc.) leading to a significant increase in
440 wettability of the carbon-based samples. Consequently, it improved the GOx enzyme
441 immobilization process on the conductive supports, with improved enzymatic activity.

442 Furthermore, the stability and reusability of the bio-functionalized felts were improved for the
443 plasma treated samples overall. Hence, it is shown that cold remote ($N_2 + O_2$) plasma is an
444 interesting non-destructive dry method for surface modification of conductive carbon-based felts as
445 pre-treatment before enzyme immobilization processes. These bio-functionalized felts can be used
446 as electrodes in biofuel sustainable systems like biosensors or biofuel cells.

447 **Acknowledgment**

448

449 This work was financially supported by Erasmus Mundus joint Doctorate Program – Sustainable
450 Management and Design for Textiles (SMDTex), grant number [2015-1594/001-001-EMJD].

451 Chevreul Institute (FR 2638), Ministère de l'Enseignement Supérieur et de la Recherche, Région

452 Haut de France, FEDER and Dr. Pardis Simon are acknowledged for supporting the XPS
453 experiments of this study. The authors would like to thank Mr. Christian Catel and Miss Mariza
454 Mone as well, for their technical assistance.

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575 **FIGURES LEGENDS**

576

577 Fig. 1. SEM images of VCF carbon felt without plasma (a) and (b), and after CRP treatment CF 2%
578 (c)

579 Fig. 2. FTIR spectra of virgin carbon felt (VCF) before and after cold remote plasma with two
580 different gas mixtures (CF 1%, CF 2%)

581 Fig. 3. Deconvolution of N 1s and C 1s photopeaks for the virgin carbon fibers VCF, and plasma
582 treated carbon fibers (CF 1% and CF 2%)

583 Fig. 4. Wettability in terms of water contact angle (a) and capillary uptake (b) of virgin carbon felt
584 VCF and plasma treated CF 1% and CF 2%

585 Fig. 5. SEM images of plasma treated CF 2% carbon fiber functionalized with GOx enzyme

586 Fig. 6. Relative enzyme activity (%) versus the number of cycles for enzyme immobilized on VCF,
587 CF 1% and CF 2%, at pH 7 (a) and pH 5.5 (b)

588 Fig. 7. Cyclic voltammograms of CF 1% felt in 0.01 M PBS in the absence and presence of different
589 glucose concentrations (mM) vs. Ag/AgCl

590 Fig. 8. Possible chemical structure of carbon fiber, and possible chemical modification after CRP
591 plasma treatment - adapted from [37]

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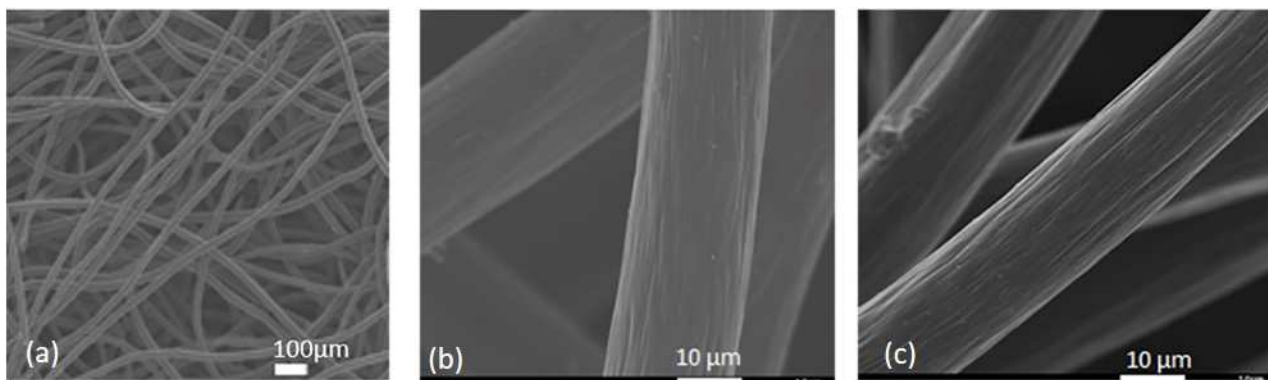
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608 **Fig. 1.** SEM images of VCF carbon felt without plasma (a) and (b), and after CRP treatment CF 2% (c)

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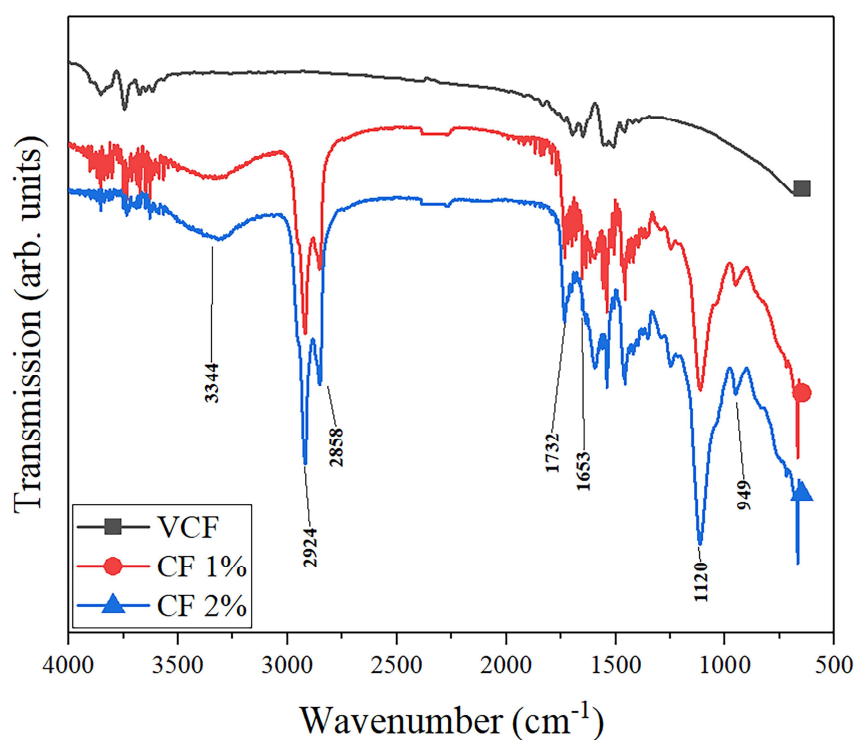
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630 **Fig. 2.** FTIR spectra of VCF before and after cold remote plasma with two different gas mixtures
631 (CF 1%, CF 2%)

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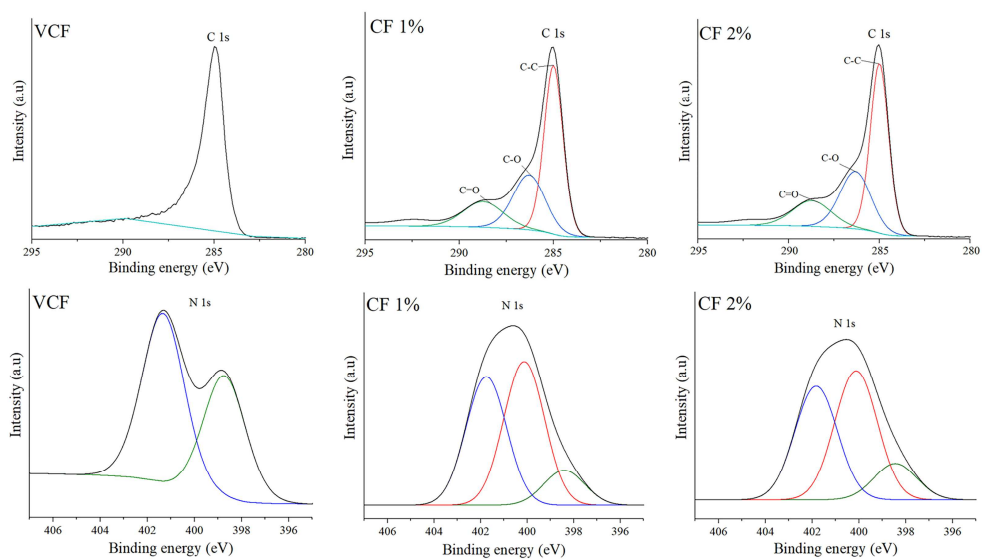
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646 **Fig. 3.** Deconvolution of N 1s and C 1s photopeaks for the virgin carbon fibers VCF, and plasma
647 treated carbon fibers (CF 1% and CF 2%)

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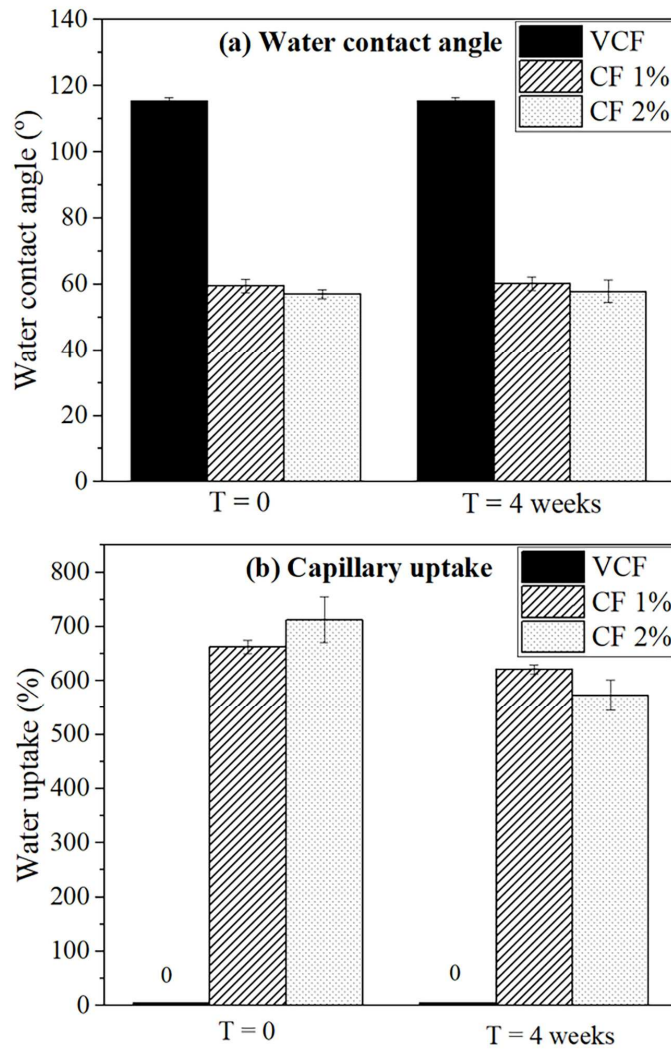
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667 **Fig. 4.** Wettability in terms of water contact angle (a) and capillary uptake (b) of virgin carbon felt
668 VCF and plasma treated felts CF 1% and CF 2%

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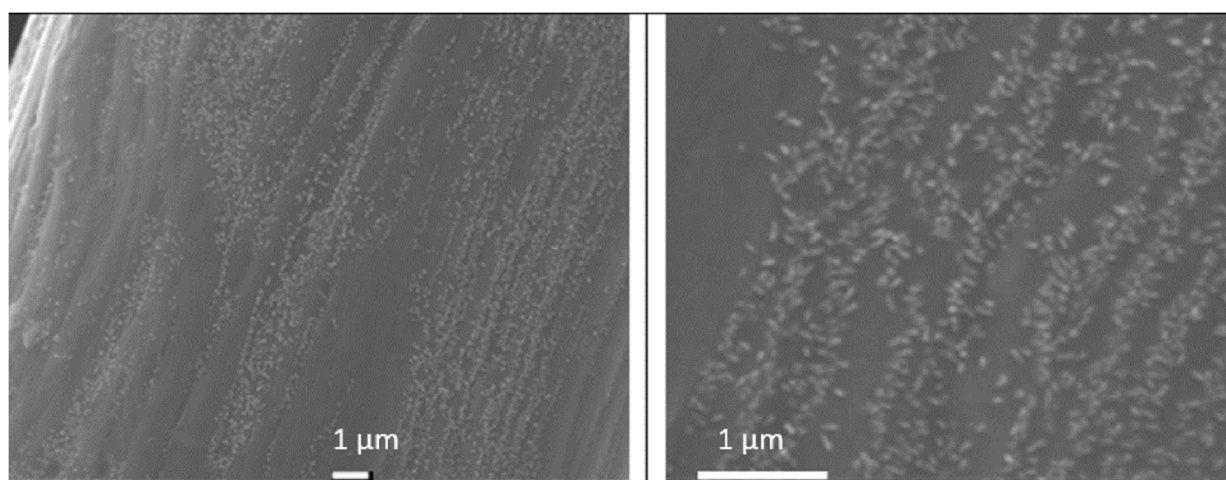
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680 **Fig. 5.** SEM images of plasma treated CF 2% carbon fiber functionalized with GOx enzyme

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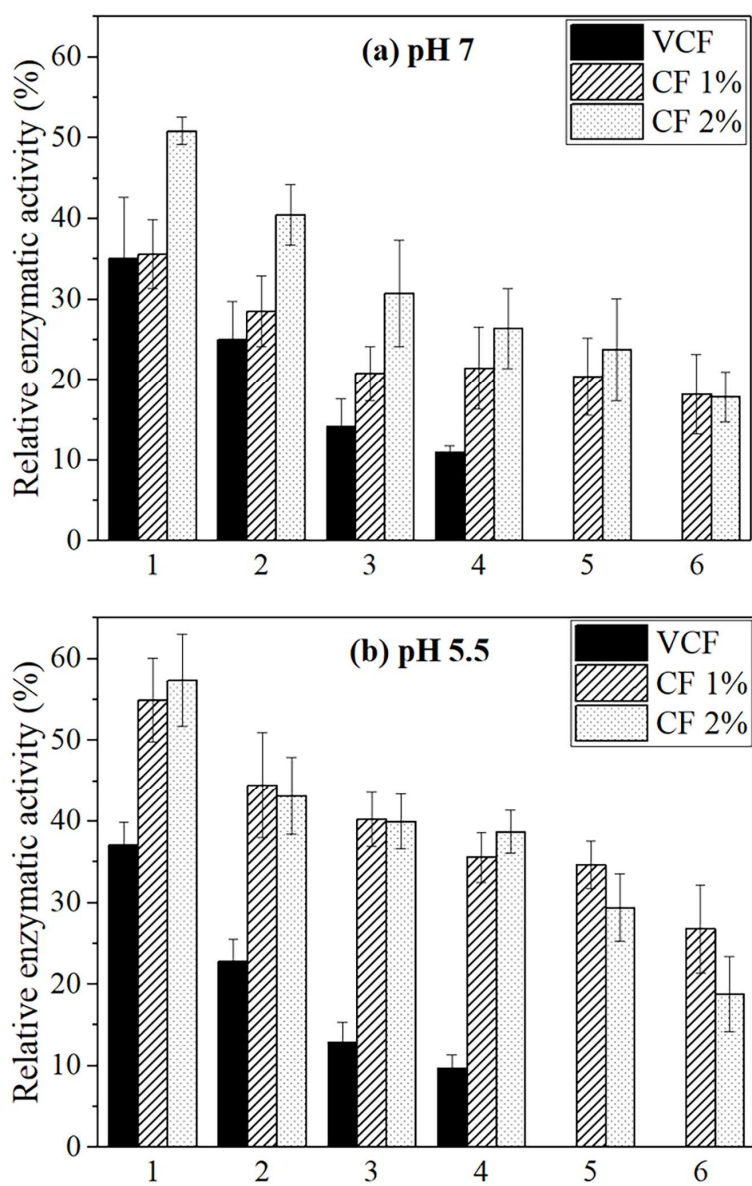
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701 **Fig. 6.** Relative enzyme activity (%) versus the number of cycles for enzyme immobilized on VCF,
702 CF 1% and CF 2%, at pH 7 (a) and pH 5.5 (b)

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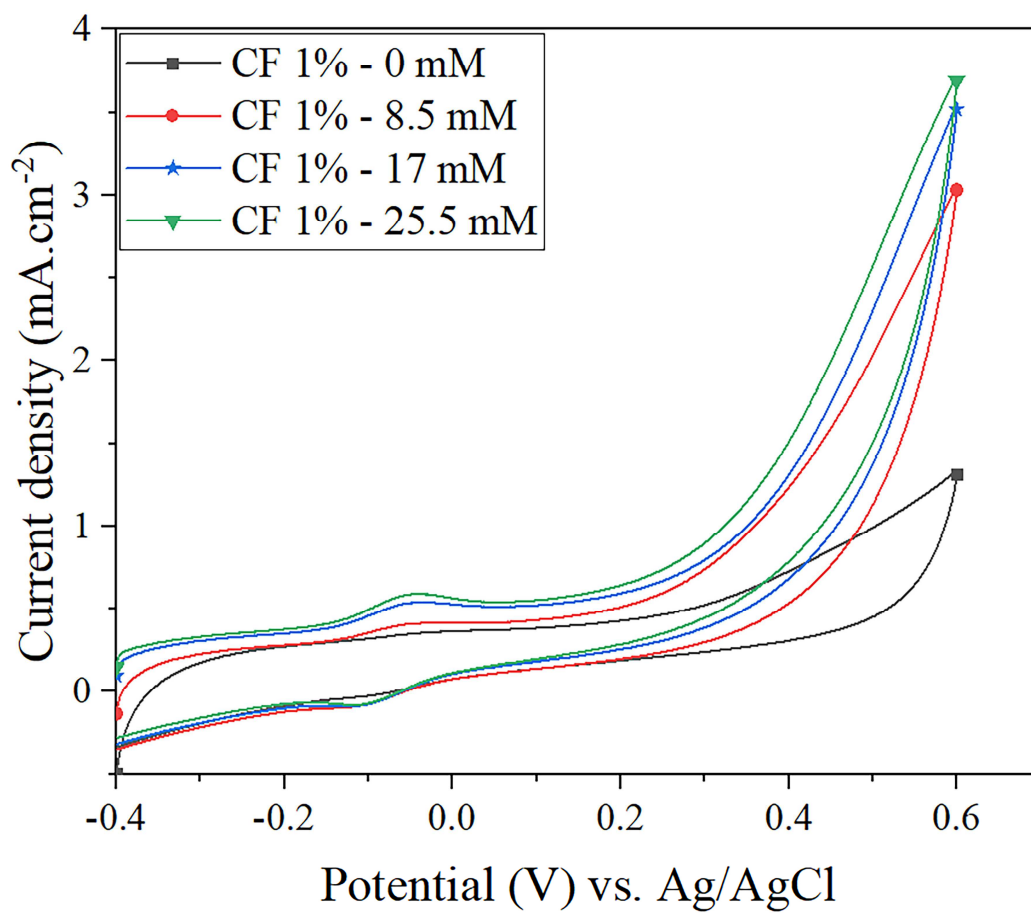
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711 **Fig. 7.** Cyclic voltammograms of CF 1% felt in 0.01 M PBS in the absence and presence of different
712 glucose concentrations (mM) vs. Ag/AgCl



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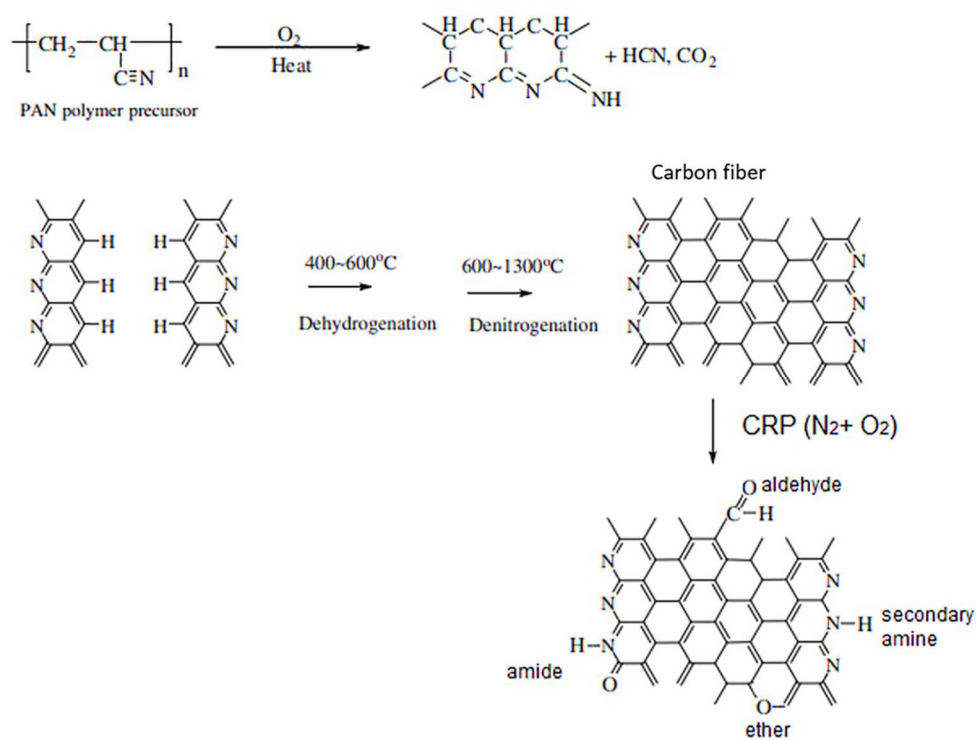
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725 **Fig. 8.** Possible chemical structure of carbon fiber, and possible chemical modification after CRP
726 plasma treatment - adapted from [37]

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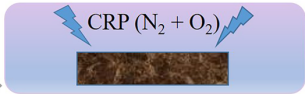
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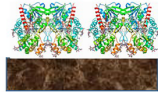
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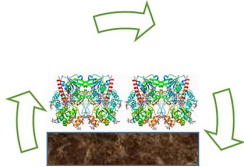
Virgin carbon felt
Water contact angle $\approx 116^\circ$



New functional groups
Water contact angle $< 60^\circ$



Bio-functionalized carbon
felts with glucose oxidase
redox enzyme



Sustainable bio-processes