

# MEDICAL WASTE TO ENERGY: EXPERIMENTAL STUDY

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## SUMMARY

**Objective.** Although waste is traditionally assessed as a pollutant which needs to be reduced or lessened, its management is certainly necessary. Nowadays, biological fuel cells, through the direct conversion of organic matter to electricity using biocatalysts, represent a technology able to produce sustainable energy by means of waste treatment. This study aims to propose a mean to generate energy from blood and saliva, that are common risk-infectious medical waste.

**Materials and methods.** Material employed (purchased by Sigma-Aldrich) were: Glucose oxidase (GOx), Nafion perfluorinated resin solution at 5% in a mixture of lower aliphatic alcohols and water, Polyethylene oxide. Stock solutions of D (+) glucose were prepared in a 0.1 M phosphate buffer solution and stored at 4 °C for at least 24 h before use. Carbon cloth electrode ELAT HT 140 E-W with a platinum loading of 5 gm<sup>-2</sup> was purchased by E-Tek. Electrospun Nafion fibers were obtained as follows. Scanning electron microscopy was used to characterize the electrode morphologies.

**Results.** In order to develop an effective immobilization strategy of GOx on the electrode surface, Nafion fibers (a fully fluorinated ion conducting polymer used as a membrane material in enzymatic fuel cells - EFC) were selected as immobilizing polymer matrix. In this work, exploiting the nafion fibers capability of being able to catalyze Gox activity, we have tried to produce an enzymatic fuel cell which could produce energy from the blood and the saliva within medical-dental waste.

**Conclusions.** Medical waste refers to all those materials produced by the interaction among doctor and patient, such as blood and saliva. During our research we will try to complete an EFC prototype able to produce energy from blood and saliva inside the risk-infectious medical waste in order to contribute to the energy requirements of a consulting room.

**Key words:** medical waste, enzymatic fuel cells, electrospun nafion fibers.

## Introduction

Although waste is traditionally assessed as a pollutant that needs to be reduced or lessened, its management is certainly necessary. More than reducing waste, sustainable development requires recycling it or, even better, assessing it as an energy resource. Waste contains a significant energy amount stored in the chemical bonds in its components; its conversion into a reusable form could provide a mean to supply clean energy and

to contribute to solve the waste global problem. Dental surgery produces dangerous and infectious-risk waste requiring costly disposal processes. The use of such waste as fuel for a bio-energy production device would help reducing the cost of waste-management, thus leading to an overall significant environmental.

Biological fuel cells are an innovative technological solution for a "sustainable" global economy. Such a technology allows indeed reaching at once both the goals of sustainable energy production and waste treatment, through the direct conver-

sion of organic matter to electricity using biocatalysts (1, 2). Among biological fuel cells, enzymatic fuel cells (EFCs) have attracted increasing interest in the last years due to their applicability as power sources for portable electronics, and in particular implantable medical devices (3).

EFCs are based on the conversion of chemical energy into electricity through the catalytic action of enzymes which require a fuel (i.e. a substrate) which can be readily oxidized. Medical waste are consisting on materials which cannot be easily employable for energy production with the exception of blood and saliva. These biological fluids contain glucose, which, on the contrary, can be exploit in producing energy amounts. Blood and saliva are, in fact, interesting candidates as possible fuel sources. Among the blood and saliva components, glucose – a carbohydrate that has a major role in the bioenergetics of animals – can be quickly oxidized, therefore can be considered as the primary source of electrical energy in dentistry waste. The biocatalyst most suitably designated for the conversion of glucose is glucose oxidase (GOx), an oxidoreductase-type enzyme commonly found in many fungus and bacteria, which catalyzes the oxidation of  $\beta$ -D glucose into  $\beta$ -D-glucono lactone (4, 5).

This paper reports on the development of materials and strategies to design GOx-based EFC devices to produce energy from the oxidation of glucose coming from dentistry waste. In particular, aim of the present research activity is to develop effective immobilization strategy of glucose oxidase on electrode and to test its suitability for fabricating bioanodes to be used in EFC devices.



## Materials and methods

Glucose oxidase (GOx, EC 1.1.3.4, type VII from *Aspergillus niger*), Nafion perfluorinated resin solution at 5 wt.% in a mixture of lower aliphatic alcohols and water, Poly-ethylene oxide (PEO, MW 8000 kg/mol) were purchased by Sigma-Aldrich. Stock solutions of D (+) glucose (5 mM-

1 M) were prepared in a 0.1 M phosphate buffer solution (PBS pH 7) and stored at 4°C for at least 24 h before use. Carbon cloth electrode ELAT HT 140 E-W with a platinum loading of 5 gm<sup>-2</sup> (henceforward named as CC-Pt) was purchased by E-Tek. Electrospun Nafion fibers were obtained as follows. Nafion solutions were prepared by dissolving PEO of three different molecular weights (100, 2000, 8000 kg/mol) in the commercial Nafion solution under gentle heating at 40°C and stirring at 600 rpm overnight, in order to obtain a PEO concentration of in the 0.005-3 wt.% range. A 5 cm<sup>3</sup> plastic syringe (B-D Plastipak) equipped with a stainless steel needle (n° 18;  $\Phi_{int}$ =0.838 mm) was filled with the obtained solutions. The syringe was placed on an automatic pump (R-99, Razel) and grounded. A stainless steel needle was connected to a high voltage power supply (model SPELLMAN, SL150). The solutions were electrospun in the following conditions: applied voltage 12 kV, needle-target distance 6 cm, flow rate 0.4-0.1 mL/h, the target being CC-Pt electrode. Afterward GOx was immobilized onto the Nafion fibers modified electrode surface by physisorption. 150  $\mu$ L GOx solution (3 mg/mL in phosphate buffer 0.1 M, 7 pH) was cast onto the modified electrode and the surface was dried overnight at room temperature. Then, it was washed thoroughly with a buffer solution (0.1 M, 7 pH) and dried at room temperature again. 2  $\mu$ L of 0.5 wt.% Nafion solution was pipetted onto GOx/Nafion fibers modified electrode and was dried for approximately 2 h at room temperature. The resulted modified electrode was stored in a refrigerator at 4°C before use.

The resulting sample was labeled as N-fibers-GOx. Scanning electron microscopy was used to characterize the sample morphologies. The SEM apparatus used for obtaining high resolution images is a field emission scanning electron microscopy (FE-SEM, LEO mod. Supra 35), which consists of a cold cathode field emission gun (7-20 kV range). The average diameter dimensions of samples were evaluated by Image-J software. FT-IR spectra were acquired by using a Perkin-Elmer Spectrum 100 spectrophotometer in the range 4000-450 cm<sup>-1</sup> at room temperature (16 scans at a resolution of 4 cm<sup>-1</sup>), on 3 mg of N-fibers-GOx

samples pelleted with 300 mg KBr (spectroscopic grade). All electrochemical experiments in this work were performed using a multichannel potentiostat VMP3 (Princeton Applied Research) interfaced to a computer-controlled EC-Lab® V 10.02 electrochemical analyzer software.

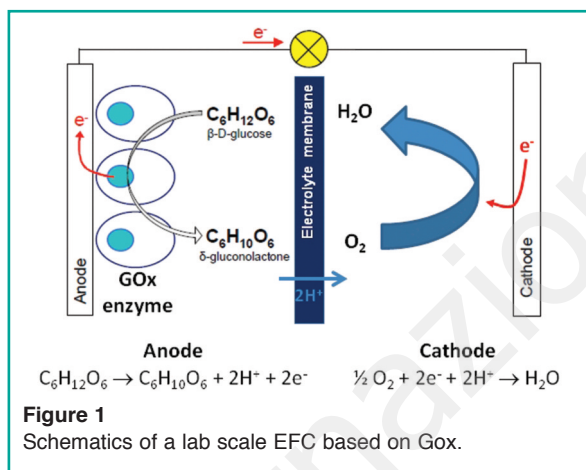
The electrochemical cell, placed in an oven at 30°C, incorporated a conventional three electrode system, using a saturated calomel reference electrode and a platinum counter electrode. The working electrode consisted in carbon cloth modified with N-fibers-GOx. CV curves were recorded in the 0-1.0 V range, at a scan rate of 25-100 mV/s range. CA curves were recorded at the oxidation potential of 0.5 V, as a function of time with glucose 5 mM in solution.

The cell contained 30 mL of KCl 0.1 M in PBS 0.1 M, pH 7 and was degassed with N<sub>2</sub> for 15 minutes prior to recording the voltammograms.

## Results and discussion

Figure 1 shows a schematics of a EFC devices based on GOx as biocatalyst and glucose as fuel. In the anode compartment, GOx catalyzes the oxidation of glucose, releasing two electrons and two protons. The protons permeate across the electrolyte to the cathode, while the electrons flow through an external circuit and provide power. Oxygen is supplied to the cathode and this combines with the electrons and the protons to produce water (Fig. 1).

On the other hand, the electric communication between enzyme and electrode surface is made difficult due to the deeply buried redox center of the enzyme. Therefore, immobilization of enzyme is the key to promote direct electron transfer from Gox to the electrode surface. To develop effective immobilization strategy of GOx, Nafion – a fully fluorinated ion conducting polymer commonly used as a membrane material in polymer electrolyte fuel cells (6, 7) – was selected as immobilizing polymer matrix. In this work, we explored the possibility to prepare fibrous Nafion matrices since they are expected to be optimal candidates as immobilizing matrix



due to high surface to volume ratio that can promote GOx catalytic activity and high porosity for easy access on fiber surfaces where enzyme can be entrapped.

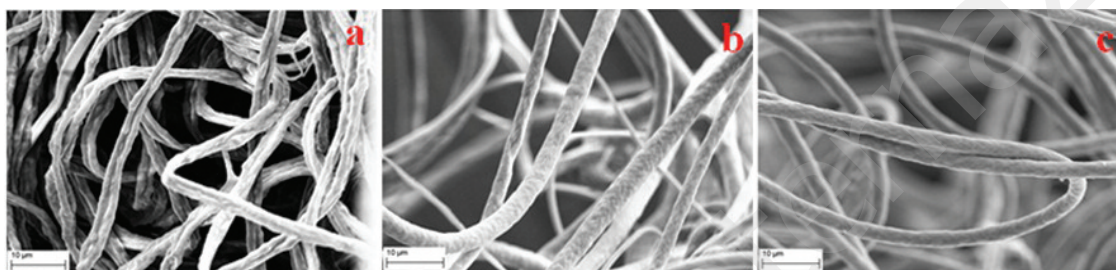
Nafion fibers were prepared by electrospinning, a technique producing nanoscale to microscale sized polymer fibres by the application of strong electric field to a polymer solution (8, 9) (Tab. 1). However, the low viscosity and the formation of aggregate in Nafion solutions prohibited fiber formation during electrospinning due to the lack of sufficient polymer chain entanglement (Fig. 2). For this reason, Nafion solutions containing a carrier polymer consisting of poly-ethylene oxide (PEO) were prepared. Their composition and samples labeling is given in Table 1, while Figure 2 shows the FE-SEM micrographs resulting from the different samples.

The addition of PEO to Nafion solution results in increased viscosity, suppressed aggregate formation, and ionic interactions between the sulfonic acid groups in Nafion (10), allowing to produce almost pure (99.90 wt.%) Nafion fibers (Fig. 1c). The average fiber diameter was 2.8 ± 0.1 μm. To test the suitability of Nafion fibers as immobilizing matrix, GOx was physisorbed on the fibers as described in the experimental. Figure 2 shows the electrospun Nafion matrix loaded with GOx (N-fiber-GOx).

GOx covers the porous matrix of Nafion, being distributed on fiber surfaces as well as in the inner portion of the matrix.

**Table 1** - Composition of the solutions used for the electrospinning process and sample labeling.

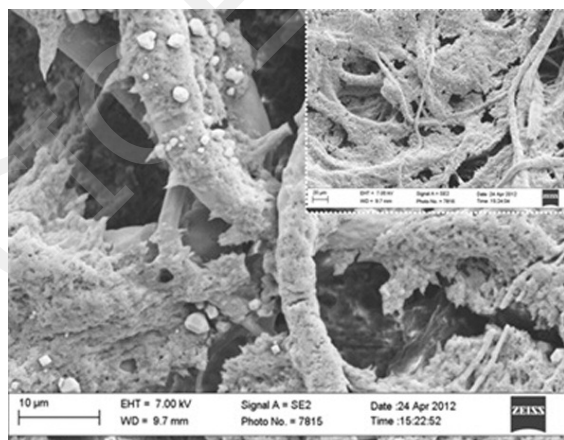
Figure 1	PEO-8000 wt.% in solution	PEO wt.% in fiber	Nafion wt.% in fiber
(a)	0.05 wt. %	1%	99.00%
(b)	0.0125 wt. %	0.25%	99.75%
(c)	0.005 wt. %	0.1%	99.90%

**Figure 2**

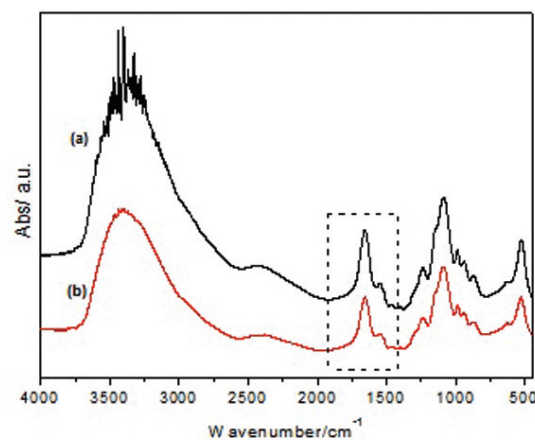
SEM micrographs of electrospun Nafion fibers: a) Nafion/PEO-8000 0,05 wt.%; b) Nafion/PEO-8000 0,0125 wt.%; c) Nafion/PEO-8000 0,005 wt.%.

Inset in Figure 3 shows a magnification of the image emphasizing the homogeneous coverage of the Nafion-fibers electrode surface by GOx. The possible leaching of GOx from Nafion matrix was investigated by FT-IR. Figure 4 shows the spectra of N-fibers-GOx sample before and after several cycles of rinsing in pure water.

Both spectra displayed a band at  $1540\text{ cm}^{-1}$ , due to N-H bending of of GOx amide groups, the intensity being similar in the two cases. In particular, the intensity of the band is decreased by only 15% due to the rinsing treatment, thus indicating that GOx leaching is limited as desired for EFC applications (Fig. 4).

**Figure 3**

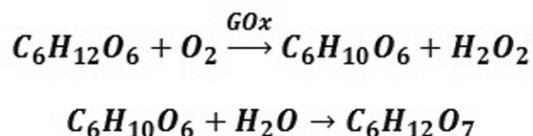
SEM images of N-fibers-GOx.

**Figure 4**

FT-IR spectra of N-fibers-GOx (a) and N-fibers-GOx after the rinsing treatment (b).

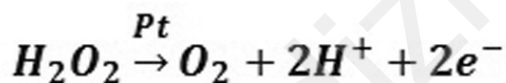


The effect of the immobilization procedure on the enzyme biocatalytic activity was monitored by cyclic voltammetry. GOx catalyzes the oxidation of  $\beta$ -D-glucose into  $\beta$ -D-gluconolactone which then hydrolyzes to gluconic acid, according to the following reactions (11) (Fig. 5):



**Figure 5**  
Oxidation reaction of  $\beta$ -D-glucose into  $\beta$ -D-gluconolactone.

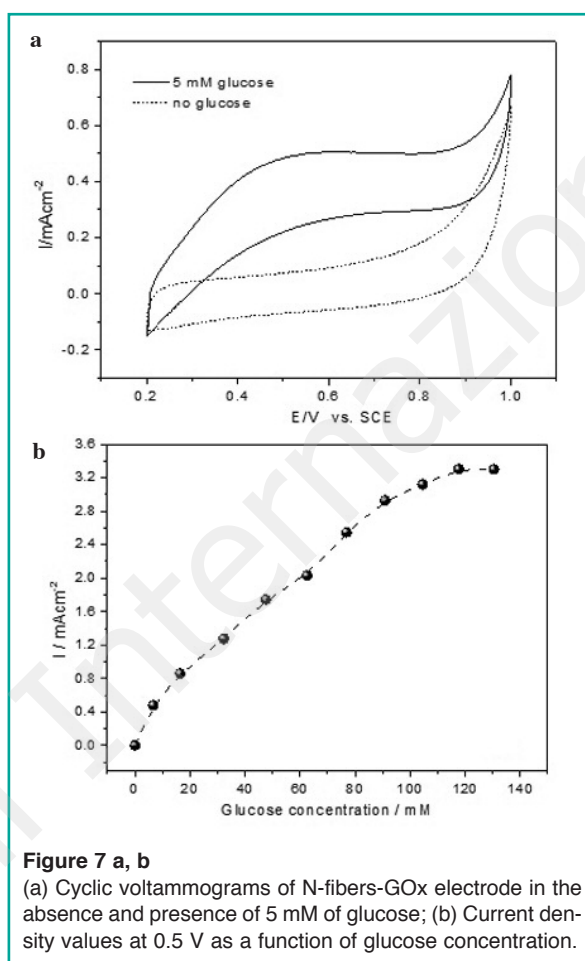
The amperometric detection of the hydrogen peroxide, a co-product formed during the enzymatic oxidation of glucose, is a versatile strategy to verify the catalytic activity of immobilized GOx. The production of  $\text{H}_2\text{O}_2$  by the enzymatic reaction can be monitored at the surface of platinum electrodes, according to the following reaction (Fig. 6):



**Figure 6**  
Amperometric detection of hydrogen peroxide through its oxidation to oxygen.

The produced current is directly proportional to the amount of hydrogen peroxide and, hence to the amount of glucose oxidized in the enzymatic reaction of GOx and, therefore, can be used as indication of GOx bioelectrocatalytic activity (12).

Figure 7a shows the cyclic voltammetry graphs of N-fibers-GOx-f in absence and in the presence of 5 mM glucose concentration. Whereas no electrocatalytic current was recorded in the absence of glucose, the voltammogram of N-fiber-GOx sample in the presence of 5 mM of



**Figure 7 a, b**  
(a) Cyclic voltammograms of N-fibers-GOx electrode in the absence and presence of 5 mM of glucose; (b) Current density values at 0.5 V as a function of glucose concentration.

glucose showed an oxidation peak centered at 0.5 V due to the oxidation of  $\text{H}_2\text{O}_2$  produced by the enzyme-catalyzed glucose oxidation (5) (Fig. 7).

The intensity of the oxidation peak linearly increases with increasing glucose concentration up to 90 mM, and then it reaches a saturation threshold (Fig. 7b).

These electrochemical features demonstrate that GOx maintains its catalytic activity after the immobilized procedure on the Nafion fibers, opening the possibility to exploit it for the oxidation of glucose in a bioelectrochemical device.

By virtue of its ease of fabrication and catalytic properties, the N-fiber-GOx system can be considered as a promising bioanode to be used in an EFC device, fueled with dentistry waste.

## Conclusions

Medical waste refers to all those materials produced by the interaction among doctor and patient, such as blood and saliva. Because of the problems concerning transmissible diseases, such as Hepatitis C and AIDS, there was a public concern increase about medical waste management. The medical waste are consisting on materials which cannot be easily employable for energy production with the exception of blood and saliva. These biological fluids contain glucose, which, on the contrary, can be exploit in producing energy amounts. The conversion of the stored chemical energy into electricity via a suitable electrochemical device such an enzymatic fuel cell requires a substrate which can be promptly oxidized. Glucose is the animal energetic chain pre-eminently carbohydrate. It can be quickly oxidized and it is considered as a primary energy resource inside dentistry special waste.

In this study we have successfully obtained a bioanode suitable for energy production, which represents the first step towards the fabrication of an EFC prototype able to get energy from blood the saliva contained in risk-infectious medical waste. In fact, our study will be carried on with the composition of the required EFC prototype. The final aim is not only to ease waste management but also to contribute to satisfy the energy requirements of a consulting room.

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