

STRIVE

Report Series No.114

From Doughnuts to Energy: Miniature Enzyme driven Biofuel Cells

STRIVE

Environmental Protection
Agency Programme

2007-2013

Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

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- Office of Environmental Enforcement
- Office of Environmental Assessment
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet several times a year to discuss issues of concern and offer advice to the Board.

EPA STRIVE Programme 2007–2013

From Doughnuts to Energy: Miniature Enzyme driven Biofuel Cells

Investigation of Microfluidic Platforms for Enzymatic Biofuel Cells

(2009-ET-MS-10-S2)

STRIVE Report

Prepared for the Environmental Protection Agency

by

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The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive summary

The ever-growing demand for energy, combined with global socio-political parameters, has sparked a quest for alternative energy sources. Energy conversion is a strategic research theme of the EPA, whose outcomes will have a major impact on global ecology and economy. Among novel alternative sources of energy, energy conversion based on electrochemical means has attracted very strong interest.

Currently there is substantial research on the development of fuel cells. This technology is based on the conversion of chemical energy into electrical energy via redox reactions. Among the different fuel cell categories, biofuel cells use enzymatic biological systems for energy conversion. They are among the energy sources being investigated for the powering of portable devices, remote sensor platforms deployed out of the laboratory and implantable or swallowable diagnostic devices. There is also a large demand for smaller, lighter, simpler, cleaner and less expensive power sources. Microfluidic biofuel cells have the potential to offer these technological improvements. They have zero pollution emission and use ambient compounds (e.g. glucose) as fuel and oxygen as an oxidant. Although still in its infancy, the development of microfluidic biofuel cells is of utmost significance for the realisation of a new generation of biofuel cells with improved performance.

The aim of this EPA-funded STRIVE project is to develop an all-plastic microfluidic enzymatic biofuel cells that will act as

alternative energy sources for portable devices. This objective has been achieved by confining enzymatic biofuel cells in a microfluidic environment. The use of biological catalysts such as enzymes allows simplification of the overall structure of the fuel cells. Enzymatic biofuel cells reproduce naturally occurring electrochemical processes and are alternative energy sources that can operate without the use of precious catalysts such as platinum. Unlike the noble metals, enzymes are environmentally friendly and biocompatible, and can selectively and efficiently catalyse the conversion of fuels such as carbohydrates, alcohols and organic pollutants found in industrial waste water into electrical energy. The research will enable improvements in the design of microfluidic devices for energy conversion. The innovative designs of the microfabricated electrodes and plastic microfluidic chips realized in this project will help overcome the problems of diffusion layer thickness and of depletion of the fuel in the microfluidic channel. Miniaturization of the biofuel cells and their fabrication using plastic materials will allow the stacking of several chips, thus reducing the volume occupied.

It is envisaged that this technology will be able to provide energy to sensor platforms deployed in the environment. The research undertaken offers great prospects for the development of miniaturized energy sources that can be used to power autonomous sensor modules de-localized within the environment for monitoring purposes. Such autonomous sensor devices are under

development within various research groups both in Ireland and abroad. However, the energy to run such systems is reliant on commercially available batteries, which create post-consumer waste harmful to the environment.

This project has achieved its goals and demonstrated how environmentally friendly and efficient devices harnessing biochemical sources of energy may be developed in a miniaturized fashion in a way that could be easily integrated with autonomous sensor modules.

It has also extended the scientific knowledge on energy conversion in microfluidic devices and has created the basis for the development of innovative energy devices. Carefully designed and fabricated microfluidic devices are now possible which can lead to improvements in the quantity of energy converted that go beyond the current state of the art. The knowledge obtained during the project also has significant interest for allied sciences and technologies including microfluidic, surface chemistry, electrochemical and biosensors.

1. Objectives

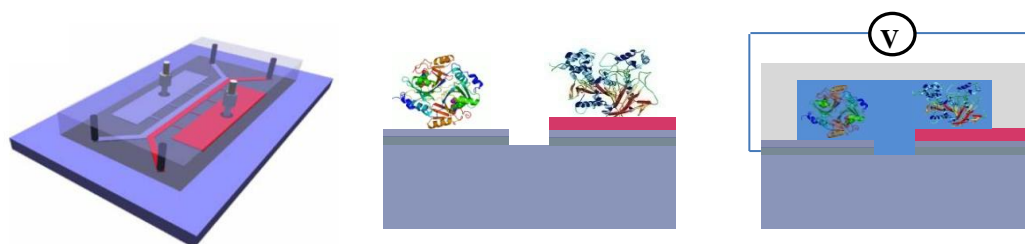
In biofuel cells the enzymes are immobilized onto electrodes within microfluidic channels that enable laminar flow of the fuel over the electrodes. The advantage of this approach is that the implementation of a semipermeable membrane to maintain the separation of different substrates in the cell is no longer required, greatly increasing the efficiency of the reactions.

Additional benefits include simplified and miniaturized devices relative to traditional designs, lowering fabrication costs and complexity.

This research offers good prospects for the development of miniaturized energy sources that can be used to power autonomous modules such as mobile sensors for monitoring purposes.

The abovementioned work on enzymatic biofuel cells covers three main aspects:

- 1: Modelling the enzyme kinetics within the microchannels;
- 2: Fabrication of the microfluidic devices; and
- 3: Electrochemical characterization of assembled biofuel cells (see Fig. 1).



Microfluidic device + Enzyme immobilization = Enzymatic biofuel cell

Figure 1 Assembly of microfluidic platforms into biofuel cells.

2. Modelling studies

Modelling studies involve looking into mass transport of species in the microfluidic channel, investigating the fuel and oxidant consumption at the electrodes and generating the current from redox reactions on the anode and cathode. The purpose of the simulation work was to get the best recommendation for the design of our microfluidic devices and to be able to optimize the working conditions in terms of the best fuel distribution and maximum power efficiency.

2.1 Modelling of the flow in the channel

Preliminary modelling studies focused on the physics of the flow in the channel and its dependency on different channel geometries and flow rates. Some results of these investigations are shown in Figs 2A and 2B. As one can see, in order to provide good fluid separation, a compromise between the flow rate and the channel dimensions has to be made.

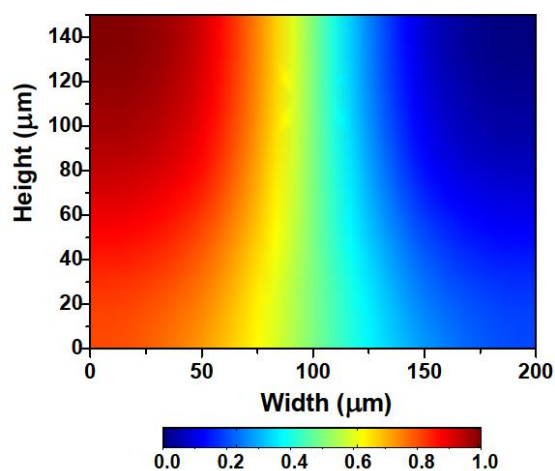


Figure 2A Diffusion of oxygen in a $200\ \mu\text{m}$, wide channel at a $1.5\ \text{mm/s}$ velocity. Red: oxygen concentration is $1\ \text{mM}$; blue: oxygen concentration is $0\ \text{mM}$.

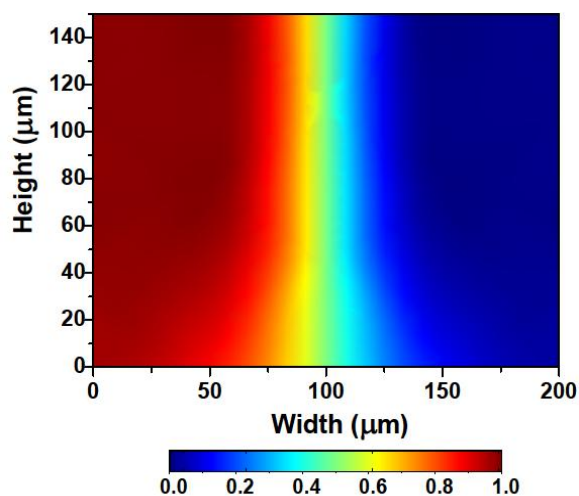


Figure 2B Diffusion of oxygen in a $200\ \mu\text{m}$, wide channel at a $10\ \text{mm/s}$ velocity. Red: Oxygen concentration is $1\ \text{mM}$; Blue: Oxygen concentration is $0\ \text{mM}$.

Different inlet shapes shown in Fig. 3 have been investigated as well in terms of the best fuel separation. It can be seen that that Y-shape of the channel inlet provides lower levels of intermixing.

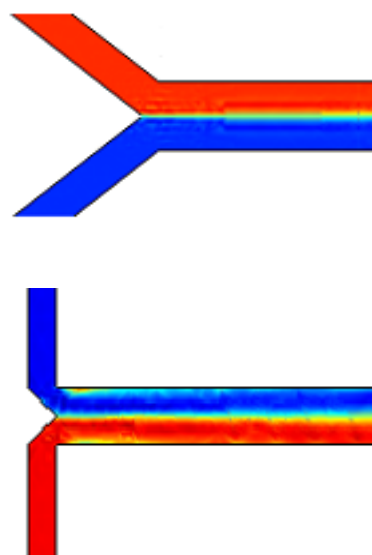


Figure 3 Glucose concentration profile for a microfluidic device with a Y-shaped (top) and a T-shaped (bottom) inlet. $w = 200\ \mu\text{m}$, $u = 1.3\ \text{mm/s}$, $[\text{Glucose}]_{\text{Inlet}} = 2\ \text{mM}$.

Concentration profiles of glucose and oxygen as the fuel and oxidant respectively were studied at set points across the channel (Fig. 4) to determine the degree of mixing and diffusion of species. As one can see, further down the channel the profiles show elevated cross-mixing between the oxidant and the fuel.

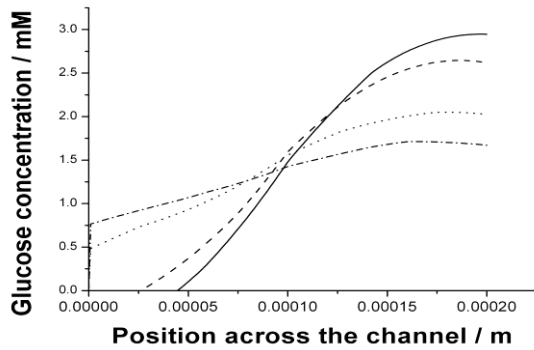


Figure 4 $[\text{Glucose}]_{\text{inlet}} = 2.5 \text{ mM}$ at different positions in the channel: $x = 0.002 \text{ m}$ (solid line); 0.004 m (dashed line); 0.010 m (dotted line) and 0.014 m (dashed–dotted line). u is 1.3 mm/s and w is $200 \text{ }\mu\text{m}$.

Consumption of the fuel and oxidant at the electrodes was at first considered for an ideal scenario where as soon as the species reaches the surface of the electrode, it was completely consumed by the reaction. In this situation enzyme kinetics wasn't taken into account. Modelling has been done using numerical COMSOL Multiphysics software, and sample boundary and initial conditions are shown in Fig. 5.

In this schematic we are showing the boundary conditions that have been used for the simulations of the fuel and oxidant consumption at the surface of the electrodes located at the bottom of the microfluidic channel.

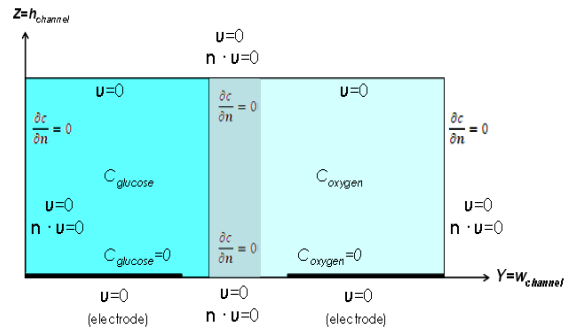


Figure 5 Boundary conditions applied for the simulations of the fuel and oxidant consumption within the channel.

A couple of electrode layouts have been considered, including two main designs where there was only one electrode all along the channel's length and when it was broken down into a series of smaller electrodes (see Fig. 6).

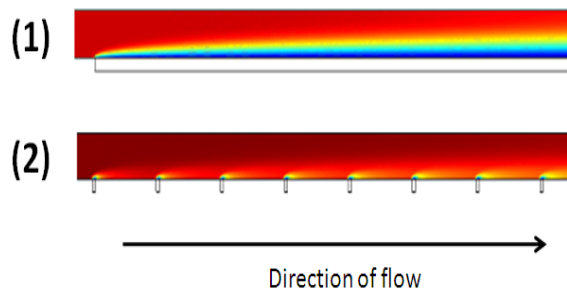


Figure 6 Glucose concentration profiles for two case studies: one electrode along a channel (1); electrode broken down into series of small electrodes (2).

As can be seen from the above results, better distribution of the fuel is obtained in the second case, where the species can easily access the gap between the electrodes and enter the reaction surface.

Based on this, a series of electrode layouts has been considered to study the influence of the flow rate and the channel dimensions on the species consumption using 3D models (Figures 7A and 7B).

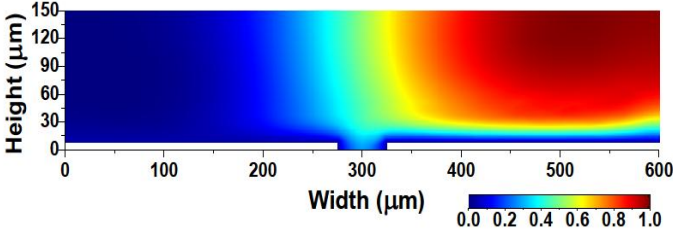


Figure 7A Concentration profile for glucose at the electrode located 5 mm into the microfluidic channel of a width of 600 μm and height of 150 μm , 1 mm/s velocity.

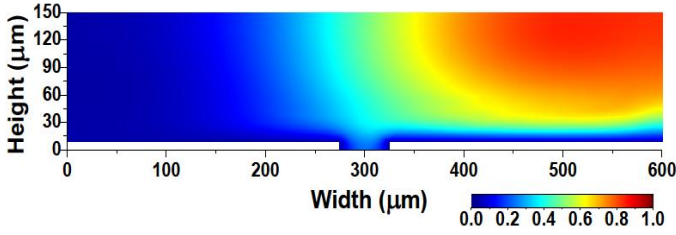
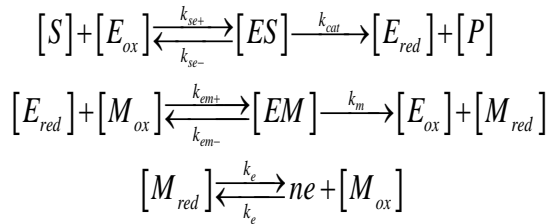


Figure 7B Concentration profile for glucose at the electrode located 11 mm into the microfluidic channel of a width of 600 μm and height of 150 μm , 1 mm/s velocity.

2.2 Mathematical model on enzyme kinetics

Following the simulations of the mass transport and species consumption, enzyme kinetics was studied at the electrodes. Based on Michaelis-Menten reaction scheme incorporating the mediator molecules (1) a set of corresponding rate equations was obtained (2).

(1)



(2)

$$\begin{aligned}
 \frac{d[S]}{dt} &= -k_{cat}[ES] = -\frac{d[P]}{dt} \\
 \frac{d[E_{ox}]}{dt} &= -k_{cat}[ES] + k_m[EM] = -\frac{d[E_{red}]}{dt} \\
 \frac{d[M_{ox}]}{dt} &= k_m[EM] - k_e[M_{red}] = -\frac{d[M_{red}]}{dt}
 \end{aligned}$$

A mathematical model to describe the catalytic system in the enzyme/mediator tailored bioelectrodes has been defined based on the Michaelis-Menten kinetics (1) and the appropriate differential equations (2) using the proposed model of the reaction layer as shown in Fig 8.

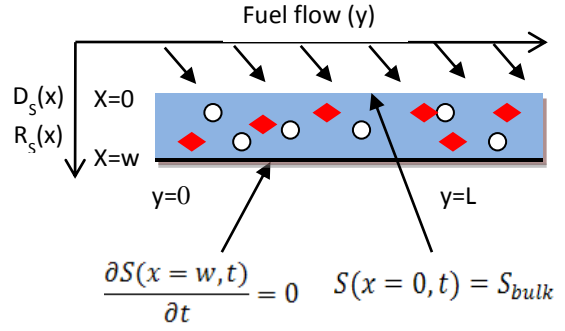


Figure 8 Model of the reaction layer including the initial conditions used for modelling purposes.

The model is composed of an electrode (length L) on top of which a layer of a conductive polymer has been placed (width w) where the enzyme and mediator molecules are immobilized and are not free to physically move. The substrate for the reaction diffuses across the membrane towards the electrode where the reaction takes place.

The concentration of the substrate in¹ the reaction layer is governed by three factors:

- 1: The supply of the species by the flow
- 2: Their diffusion into the polymer film and
- 3: The reaction within the membrane, which can be described by the following relationship (3):

(3)

$$\begin{aligned}
 \text{Substrate } (x, t) &= \\
 \text{Flow } (y, t) + \text{Diffusion } (x, t) - \text{Reaction } (x, t)
 \end{aligned}$$

Because the system is considered under its steady state condition, there is no overall change in the substrate concentration and the above can be rewritten as (4):

$$(4) \quad \text{Flow } (y,t) + \text{Diffusion } (x,t) = \text{Reaction } (x,t)$$

Assuming a very fast flow rate of the fuel at the outer boundary of the polymer film, we can neglect the change of the substrate with the flow of the fuel and simplify the equation in the steady state further on (5):

$$(5) \quad \text{Diffusion } (x,t) = \text{Reaction } (x,t)$$

For the steady state $\frac{\partial S(x,t)}{\partial t} = 0$

The catalytic reactions at the electrodes were then studied using numerical software called *Mathematica* to obtain recommendation for the system design and optimization of the working conditions. Two cases were considered; 1: Static, when the fuel was supplied only once, and 2: Dynamic, when the substrate was constantly flowing into the electrodes. A complete mathematical model composed of eight highly coupled differential equations has been proposed to describe the behaviour of all the system components (6):

$$(6) \quad \begin{aligned} \frac{d[S]}{dt} &= k_{se-}[ES] - k_{se+}[S][E_{ox}] \\ \frac{d[ES]}{dt} &= -(k_{se-} + k_{cat})[ES] + k_{se+}[S][E_{ox}] \\ \frac{d[E_{ox}]}{dt} &= k_{se-}[ES] - k_{se+}[S][E_{ox}] + k_m[EM] \\ \frac{d[E_{red}]}{dt} &= k_{cat}[ES] - k_{em+}[E_{red}][M_{ox}] + k_{em-}[EM] \\ \frac{d[M_{ox}]}{dt} &= k_{em-}[EM] - k_{em+}[E_{red}][M_{ox}] + k_e[M_{red}] \\ \frac{d[EM]}{dt} &= -(k_{em-} + k_m)[EM] + k_{em+}[E_{red}][M_{ox}] \\ \frac{d[M_{red}]}{dt} &= k_m[EM] - k_e[M_{red}] \\ \frac{d[P]}{dt} &= k_{cat}[ES] \end{aligned}$$

2.2.1 Simulation results and simplified model

Based on the model, concentration profiles of the substrate and reaction products were obtained for an isolated point in the polymer membrane, for the system in equilibrium (see Fig. 9).

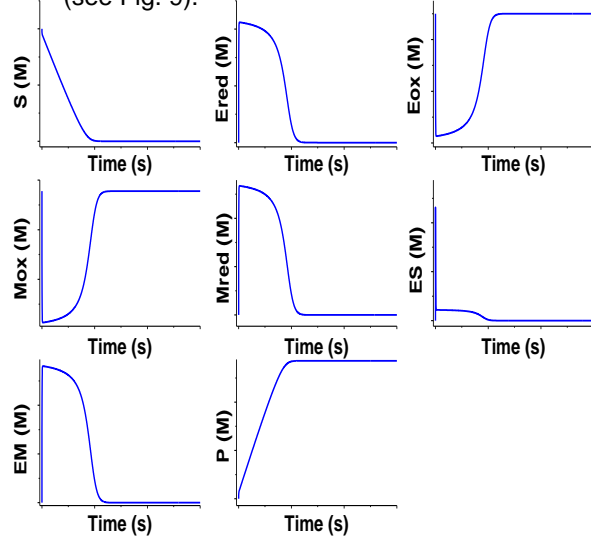


Figure 9 Concentration profiles of system components in a single point of the membrane in equilibrium. Complete mathematical model.

The influence of the rate coefficients on the profiles of all species was also studied and a sample result is presented. (see Fig 10).

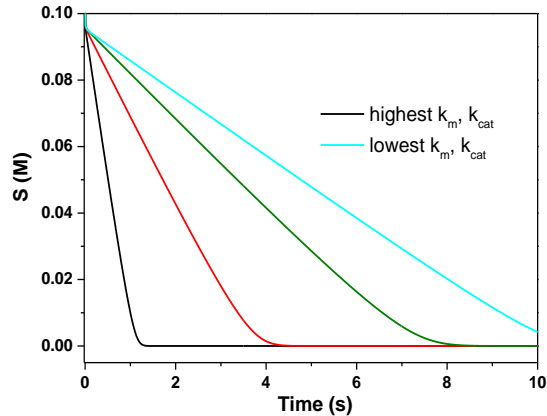


Figure 10 Influence of rate coefficients on the [S] profile. Complete mathematical model.

Based on the complete mathematical model, using an assumption of the steady state concentrations of ES and EM complexes as well as the mass balance for both the enzyme and mediator (7), a simplified system described by only three rate equations has been defined and proposed. For simplicity only two rate equations are shown (7).

(7)

$$\begin{aligned} \frac{d[ES]}{dt} = \frac{d[EM]}{dt} &= 0 \\ [E_o] &= [E_{ox}] + [E_{red}] + [EM] + [ES] \\ [M_o] &= [M_{ox}] + [M_{red}] + [EM] \end{aligned}$$

$$\frac{d[S]}{dt} = \frac{-k_{cat}}{K_{ES} + [S]} \left\{ [E_o] - \left[1 + \frac{[M_o] - [M_{red}]}{K_{EM} + [E_{red}]} \right] [E_{red}] \right\} [S] = -\frac{d[P]}{dt}$$

$$\frac{d[M_{red}]}{dt} = k_m \frac{([M_o] - [M_{red}])}{K_{EM} + [E_{red}]} [E_{red}] - k_s [M_{red}] = -\frac{d[M_{ox}]}{dt}$$

Concentration profiles of the substrates and reaction products in time at the single point of the polymeric membrane were obtained using *Mathematica* (see Fig.11).

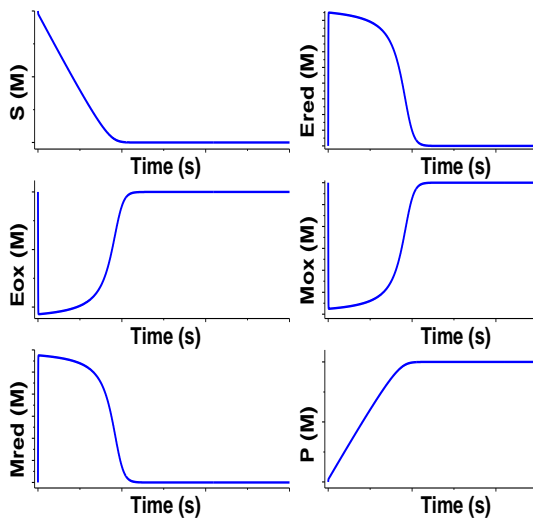


Figure 11 Concentration profiles of system components in a single point of the membrane in equilibrium. Simplified mathematical model.

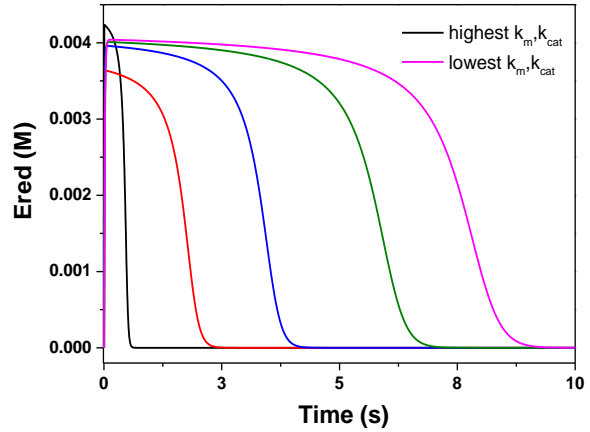


Figure 12 Influence of rate coefficients on the $[E_{red}]$ profile for simplified mathematical model.

The influence of the rate coefficients on the reaction progress has been investigated as well (Fig. 12), and illustrates how the time of reaction can be affected by the rate parameters.

Results of the numerical modelling for both the complete and simplified mathematical models were compared, and not only showed the expected behaviour of the enzymatic system but were in very good agreement (see Figs. 13A and 13B).

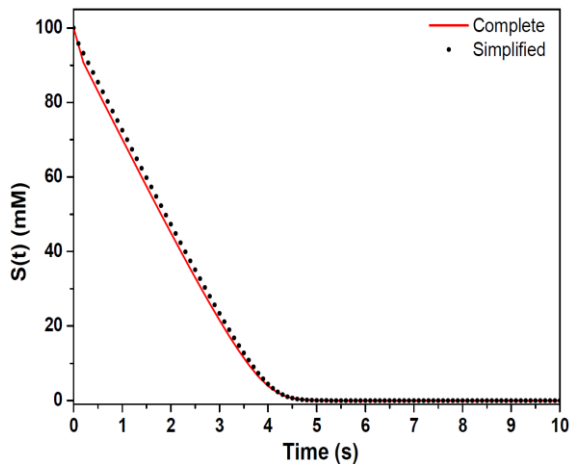


Figure 13A Comparison of the concentration profiles for S obtained for the two models.

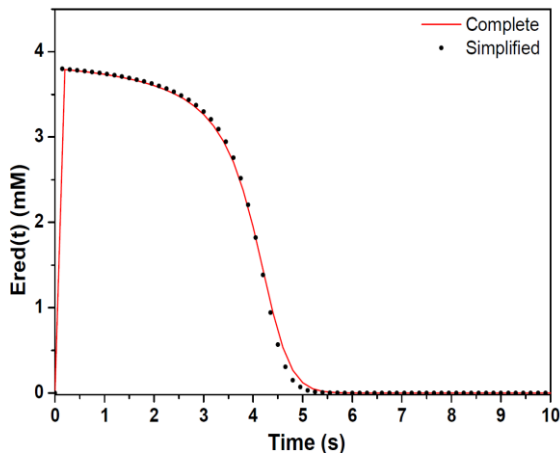


Figure 13B Comparison of the concentration profiles for E_{red} obtained for the two models.

2.2.2 Enzymatic diffusion-reaction system

After initial studies of the enzyme kinetics in the static environment, diffusion-controlled catalysis was considered. Both the complete and simplified models were supplemented with the diffusion terms (8), and the time-dependent concentration profiles of substrate and products were modelled across the membrane as described by the system of equations (6). Sample results are presented in Figure 14.

$$\frac{\partial[S(x,t)]}{\partial t} = D_S \frac{\partial^2[S(x,t)]}{\partial x^2} + k_{ss-}[ES(x,t)] - k_{ss+}[S(x,t)][E_{ox}(x,t)] \quad (8)$$

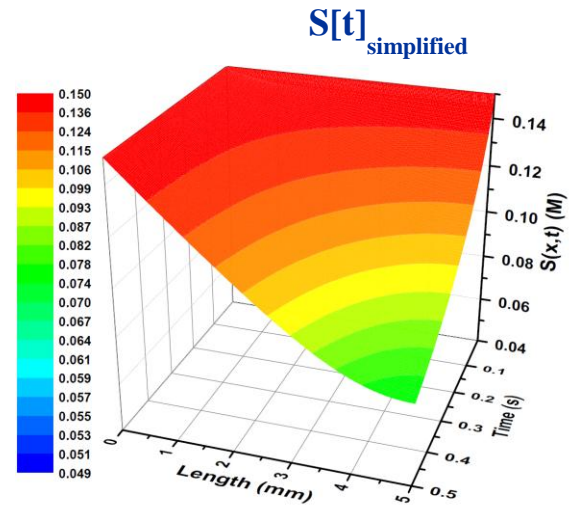
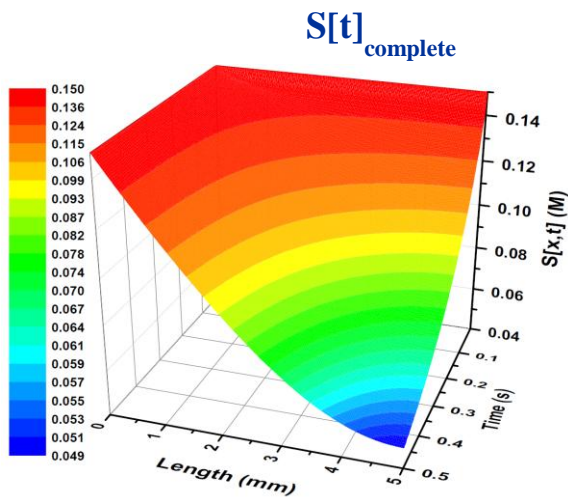


Figure 14 Steady state concentration profiles of the substrate across the polymer membrane obtained using both simplified and complete mathematical models.

Results of the numerical modelling for the complete and simplified mathematical models were compared (Figure 15) and showed some discrepancies, most likely due to high complexity of diffusion-reaction systems.

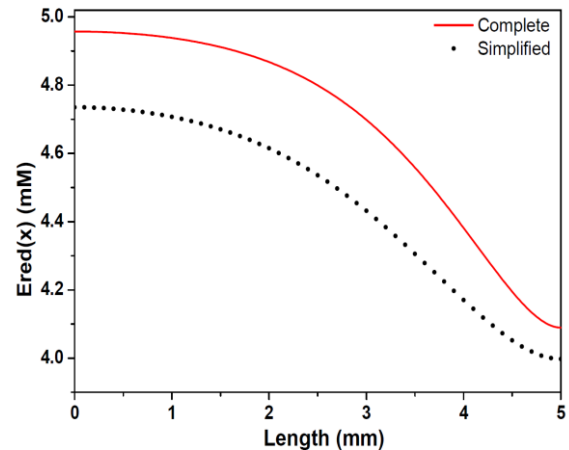
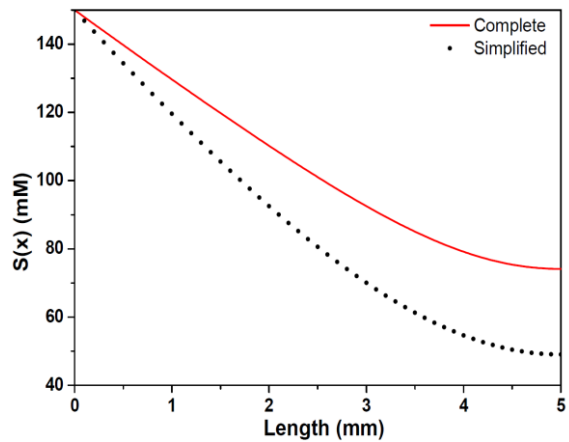


Figure 15 Comparison of the concentration profiles for S and E_{red} obtained for the two models.

3. Fabrication of microfluidic devices

Microfluidic devices for the biofuel cell applications are composed of two polymer-based substrates. The top slide carries a microfluidic channel and the bottom part has metal electrodes (see Fig.16).

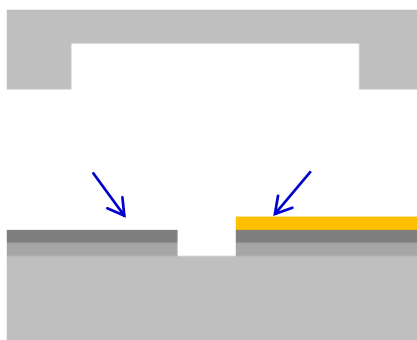


Figure 16 Schematic illustrating the top channel part and bottom Au and Pt electrodes.

The two are assembled together to create a watertight seal as shown in Fig. 17.

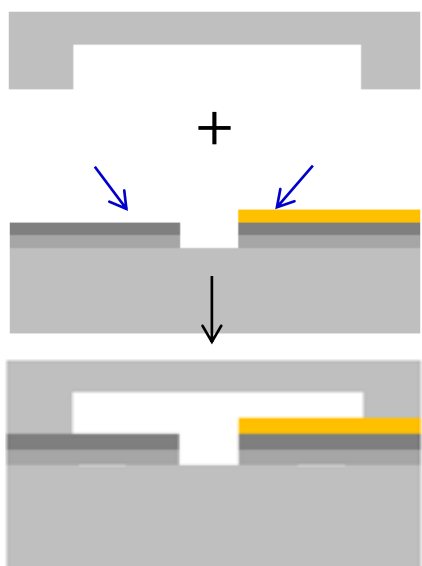


Figure 17 Assembly of the top polymer part with channels and the bottom substrate carrying Au anodes and Pt cathodes.

A solution containing the enzyme is then loaded into the channel of an assembled device and, by application of a voltage, the enzyme becomes immobilized onto the surface of the electrodes. Gold and platinum have been chosen as anodes and cathodes, respectively and their layouts are represented in Fig. 18.

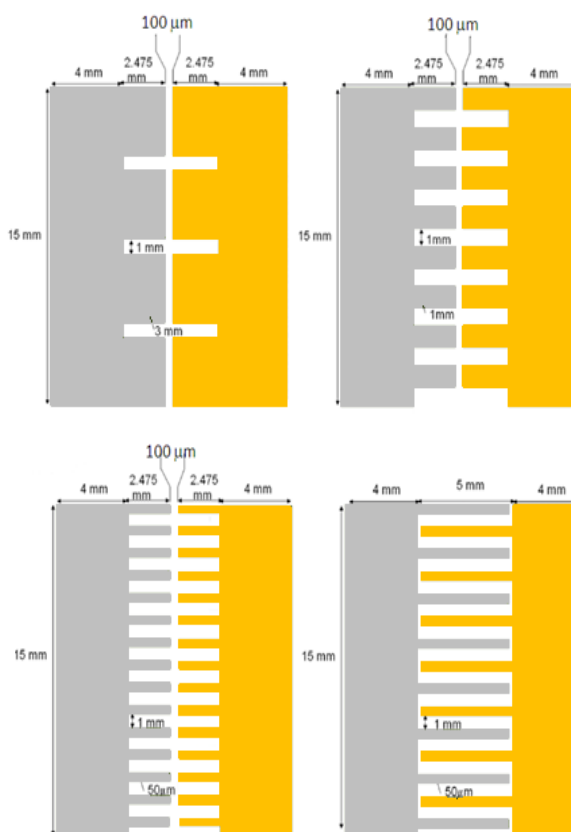


Figure18 Designs of Au and Pt electrode layouts with appropriate dimensions.

Multiple designs of the microfluidic channels (see Fig. 19) have been used to pattern the top substrate.

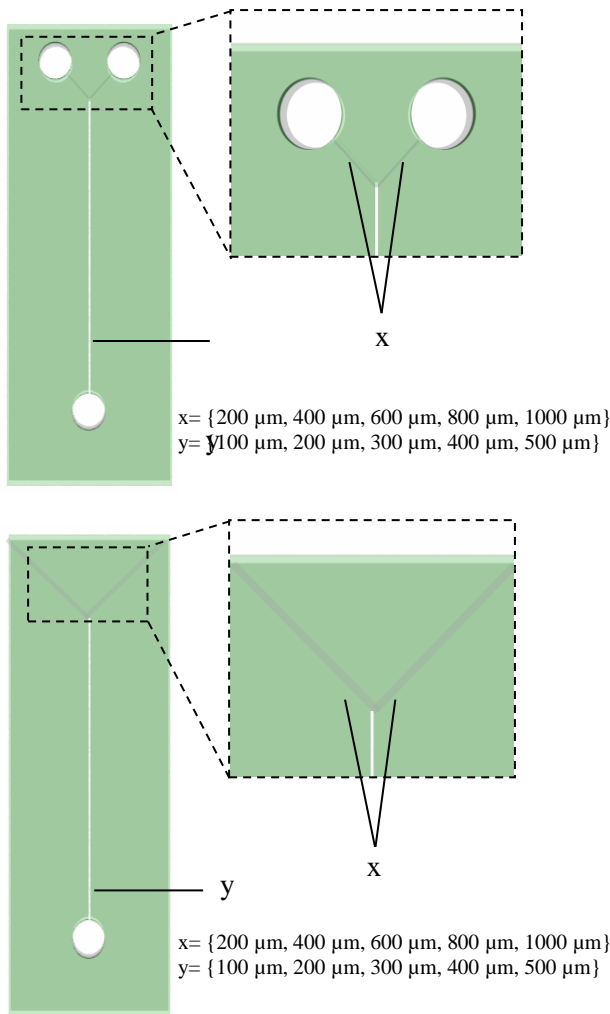


Figure 19 Designs of the microfluidic channels including dimensions.

In the original proposal, ITO was mentioned; however, deposition of ITO is problematic as it is an ion assisted evaporation technique and leads to heating of the chamber. As a result, the plastic is distorted, inducing cracks in the ITO layer and loss of conductivity. Cyclo-olefin-copolymer (COC) has been chosen as the material for both substrates as a more durable polymer. Fabrication of microfluidic channels in the top Zeonor slides is done by a technique called *hot embossing*, where both high temperature and pressure are applied to transfer a pattern of the channel from the silicon master onto the Zeonor substrate.

Silicon stamps for the fabrication of the top parts were designed and prepared by *deep reactive ion etching* (DRIE) technique, using a mixture of gases (see Fig. 20).

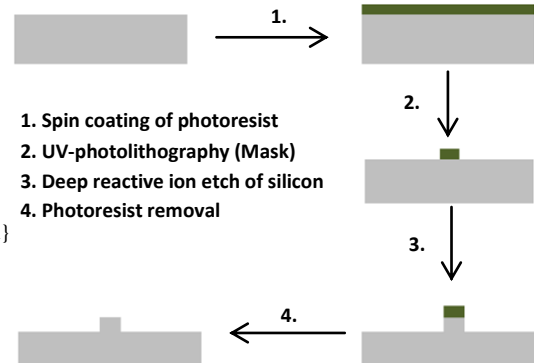


Figure 20 Fabrication of silicon masters with patterns of the channels using DRIE.

Once the stamp is fabricated, it is used to imprint its features by applying pressure and temperature to the substrate (see Fig. 21).

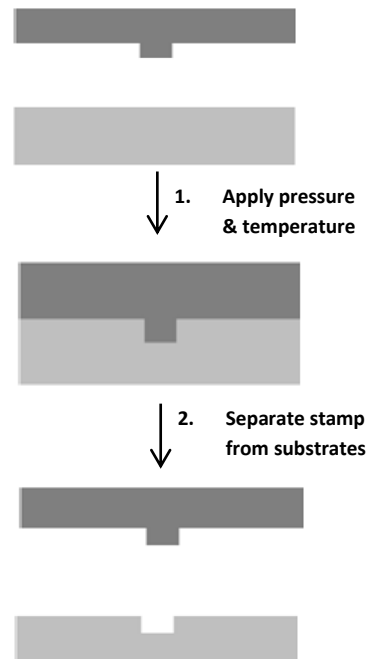


Figure 21 Fabrication of the channels using the hot embossing technique.

The efficiency of the process has been confirmed by the scanning electron microscopy (SEM) images of hot-embossed reversed patterns of the channels (Fig. 22).

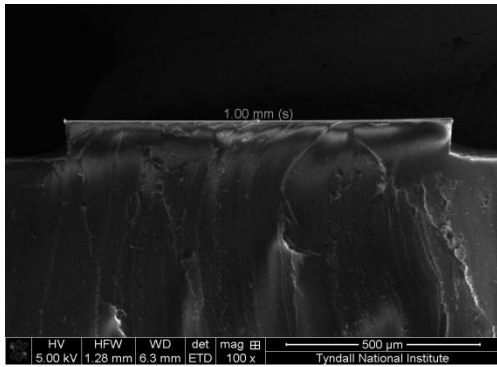


Figure 22 SEM image of a polydimethylsiloxane (PDMS) relief of a microfluidic channel hot embossed in Zeonor substrate.

Separate templates for the deposition of electrodes on the bottom substrate were used to lay out Pt and Au electrodes in a process called *UV lithographic lift off*. Following the two separate fabrication steps (see Fig. 23), binding of both substrates is required.

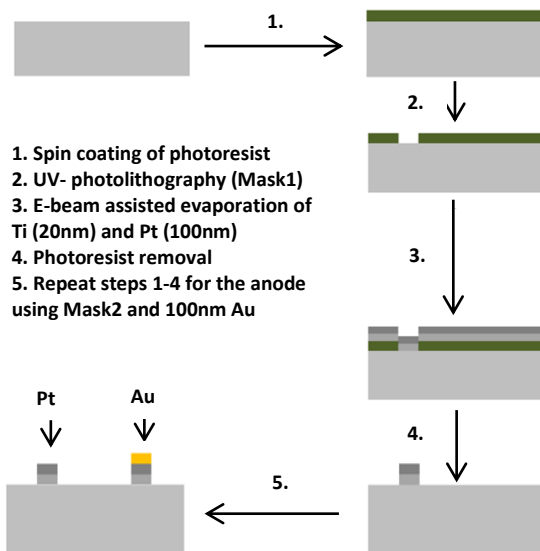


Figure 23 Deposition of metal electrodes using UV lithographic lift off process.

Gold anodes and platinum cathodes were then investigated under the microscope (see Fig. 24).

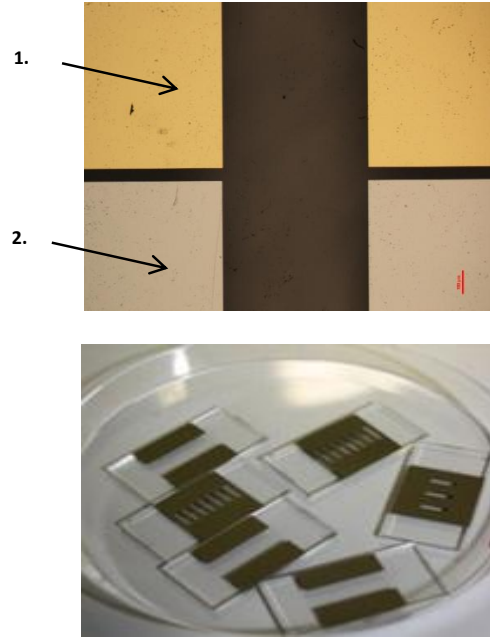


Figure 24 Microscopic image of Au (yellow) and Pt (grey) electrodes for design 4 of the layout (top). Picture of fabricated Zeonor slides with metal electrodes (bottom).

Most common binding techniques involve oxygen plasma (1) and UV activation (2) or solvent bonding (3), where the substrate is usually exposed to cyclohexane vapours (see Fig. 25).



Figure 25 Common bonding techniques: (1) oxygen plasma activation; (2) UV bonding and (3) solvent bonding using hexane.

Due to difficulties with the Zeonor–Zeonor bonding in the presence of metal electrodes, a commercially available elastomer, polydimethylsiloxane (PDMS), has been used for the microfluidic channels. PDMS is composed of repeating monomers of silicon oxide and a methyl group, $\text{SiO}-(\text{CH}_3)$. Its intrinsic hydrophobic nature prevents easy adhesion to other materials, which can be achieved only when the polymer is made hydrophilic. In order to make PDMS temporarily hydrophilic, limited exposure to oxygen plasma can be applied. Oxygen plasma activation acts as an oxidizing agent. It removes the hydrogen atoms from the surface of the PDMS, subsequently creating free carbon radicals, which on contact with oxygen molecules give functional hydroxyl groups (see Fig. 26).

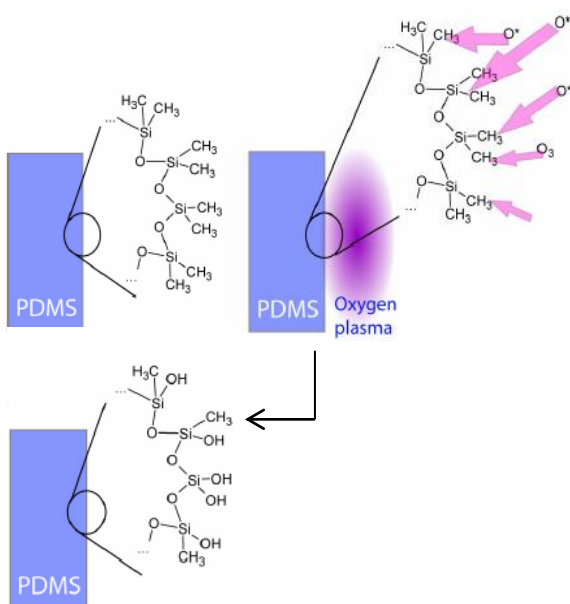


Figure 26 Activation of the surface of PDMS by the oxygen plasma treatment via formation of reactive hydroxyl groups. www.ims.ut.ee/~alar/microtech

This surface modification of the polymer strongly enhances permanent adhesion of PDMS to glass or silicon substrates. However, achieving a stable bonding with non-glass and non-silicon, metal-containing

surfaces can be problematic. Many approaches have been investigated, including varying plasma conditions and commercial glues. In all cases, neither of the bonding procedures was efficient enough to create strong and long-term adhesion, in many cases leaving the channels blocked. A solution has been found in a form of an adhesive Sylgard prime coat (Dow Corning). It has been reported (Kim et al., 2007) that dilutions of adhesive in heptane (10–100% of adhesive glue by volume) create an irreversible bond between the above materials and PDMS. Overnight heating of the assembly greatly increases the strength of the bonding.

Another concept to overcome the binding problem with PDMS is to implement a microfluidic holder. Two approaches have been considered.

1. Commercial holder available at *Micronit Microfluidics* (see Fig. 27)
 - Solid performance
 - Can be custom-made but at a very high cost

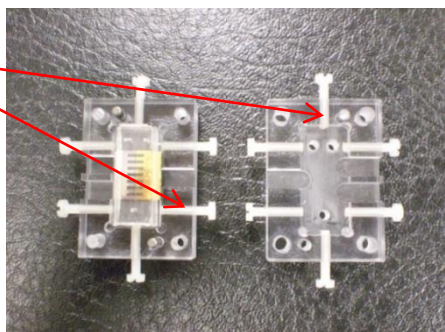


Figure 27 Microfluidic holder kit from *Micronit Microfluidics*.

2. In-house designed and fabricated holder (see Fig. 28)
 - Device-tailored
 - Can be fabricated at low cost from cheap materials

- Provides openings for metal electrodes
- Pockets for the electrical connectors (e.g. crocodile clips) available
- Additional features for the best alignment of the top and bottom substrates

Aligning features



Pockets for electrical connectors

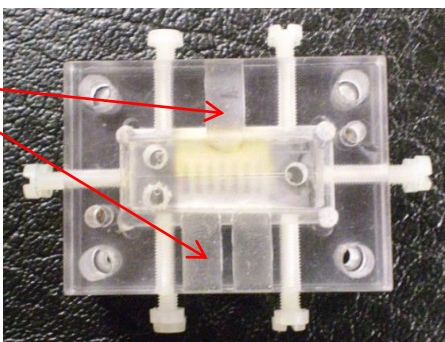


Figure. 28 Images of a PMMA holder for the assembly of a microfluidic device. Horizontal and vertical screws provide accurate alignment of top PDMS and bottom Zeonor substrates.

As part of the fabrication work, package gold anodes were modified with nanoporous gold structures (NPG). Deposition has been carried out from a solution of potassium gold cyanide $[KAu(CN)_2]$ and potassium silver cyanide $[KAg(CN)_2]$ in a 0.18:0.82 Au:Ag molar ratio. Deposition of the gold–silver alloy was carried out for 600 s at 1.2 V vs. Ag/AgCl as the reference electrode. To remove Ag from the deposit, dealloying in 30% nitric acid for 1.5 h was found to be optimal. The cyclic voltammetry of the alloy

has been studied in 0.1 M NaOH and compared to a planar Au electrode response in the same solution to prove successful deposition (see Fig. 29).

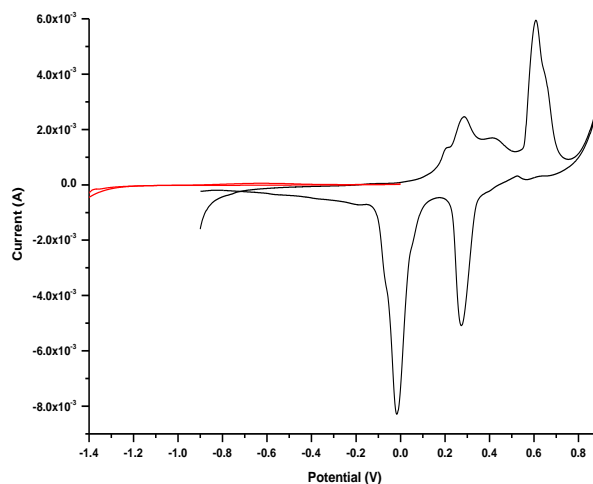


Figure 29 Au anode before (red line) and after (black line) deposition of NPG, design 4. CV in 0.1 M NaOH vs. Ag/AgCl, 200 mV/s. Electrode area 28 mm².

SEM measurements of the modified electrodes confirmed the presence of a deposit of 200 μm thickness with a pore size of approximately 20 nm in diameter (Fig. 30).

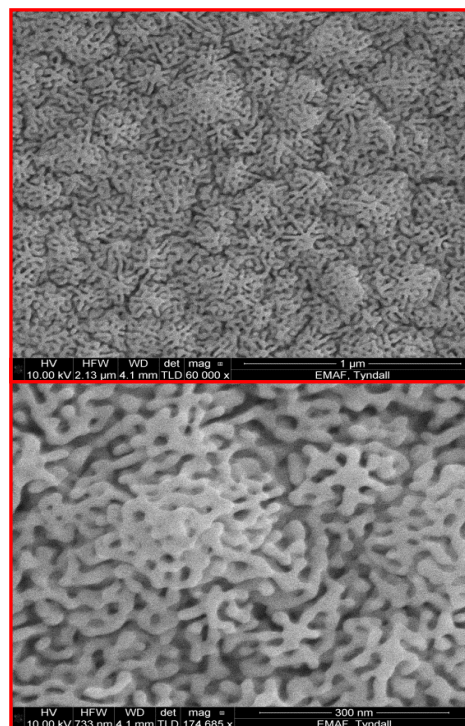


Figure 30 SEM images of the nanoporous gold structures on Au anodes shortly after the deposition.

Electrochemical characterization of NPG electrodes was performed in static conditions by cyclic voltammetry measurements in 0.1 M H₂SO₄ and 0.01 M PBS solutions containing glucose. Increased current values have been obtained for the NPG electrodes in comparison with planar Au electrodes, due to enhanced active surface areas of the electrodes (see Fig. 31).

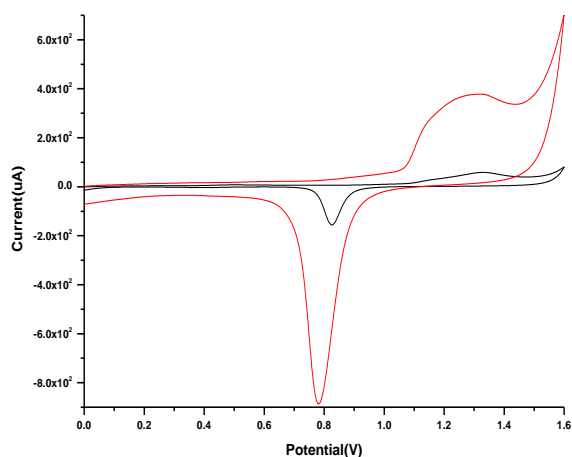


Figure 31 CV of planar Au (black line) and NPG electrode (red line) for design 4 in 0.1 M H₂SO₄, 50mV/s, signal vs. Ag/AgCl. Electrode area 28 mm².

Electrochemically active surface areas of NPG electrodes have been calculated and compared with their geometric values for all designs. Subsequently roughness factors for NPG electrodes were obtained, confirming a significant porosity of the modified electrodes (Table 1). Thus, NPG electrodes due to their high porosity can provide an accommodating and stabilizing environment for the biocatalyst molecules (Scanlon et al., 2012).

Table 1 Summary of theoretical and experimental active surface areas as well as roughness factors for planar Au and NPG electrodes. Designs 3 and 4, microelectrodes.

Design 3	A _{geometric} [cm ²]	A _{electrochem} [cm ²]	fr	fr _{NPG} /fr _{planar}
planar Au	5.22	0.531	0.102	13.836
NPG	5.22	7.340	1.406	
Design 4	A _{geometric} [cm ²]	A _{electrochem} [cm ²]	fr	fr _{NPG} /fr _{planar}
planar Au	5	0.342	0.068	8.001
NPG	5	2.736	0.542	

4. Electrochemical characterisation

Once fabricated, planar electrodes were characterised electrochemically using a technique called cyclic voltammetry (CV). CV response of 5 mM $K_3[Fe(CN)_6]$ in 0.1 M phosphate buffer saline has been studied in static conditions, for different electrode designs, for both Au (Fig. 32) and Pt (Fig. 33) as working electrodes, confirming a good electrochemical response of both metals.

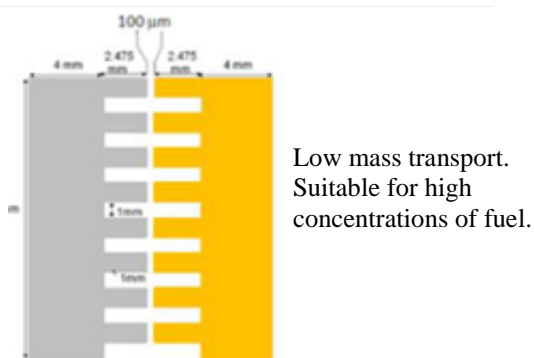
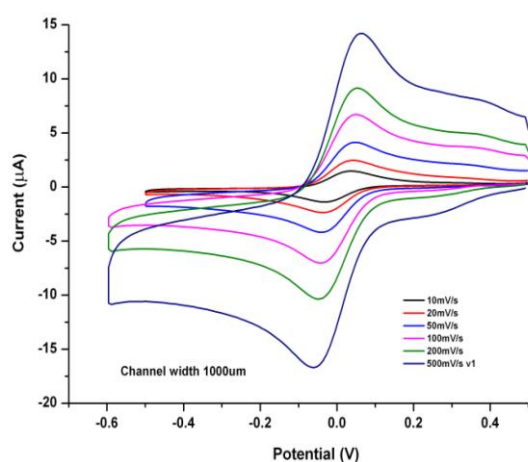


Figure 32 CV of 5 mM $K_3[Fe(CN)_6]$ in 0.1 M PBS on Au electrode, vs. Ag/AgCl. Design 4_channel design 5, electrode area 3.6 mm^2 . Response vs. Ag/AgCl.

As can be seen from Fig. 32, linear diffusion (macroelectrodes of design 4) is characterised by a diffusive shape of the electrochemical signal, provides low mass transport of species and is suitable for high concentrations of fuel.

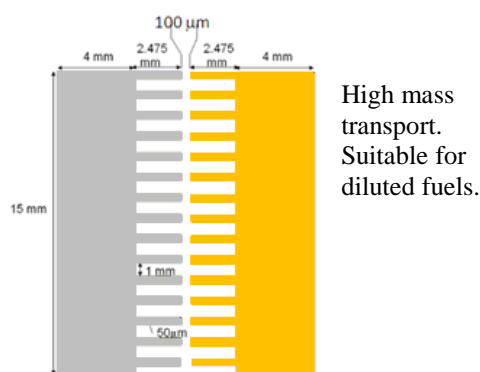
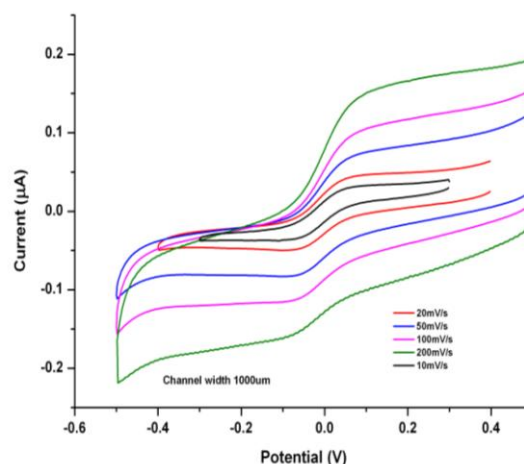


Figure 33 CV of 5mM $K_3[Fe(CN)_6]$ in 0.1M PBS on Pt electrode, vs. Ag/AgCl. Design 1_channel design 5, electrode area 0.34 mm^2 . Response vs. Ag/AgCl.

Radial diffusion (microelectrodes of design 1), on the other hand, is characterised by a sigmoidal shape of the electrochemical signal and by providing high mass transport of species is suitable for low concentrations of fuel. In general, electrode arrays result in higher current densities per surface area and provide more uniform fuel distribution throughout the channel. This is especially important if the reaction is catalysed by enzyme molecules, immobilized on the surface of the electrodes at the bottom of the channel, as in our case.

Electrochemical characterization of NPG electrodes was performed in static conditions by cyclic voltammetry measurements in 0.1 M H₂SO₄. Increased current values have been obtained for the NPG electrodes in comparison with planar Au electrodes, due to enhanced active surface areas of the electrodes (Fig. 34).

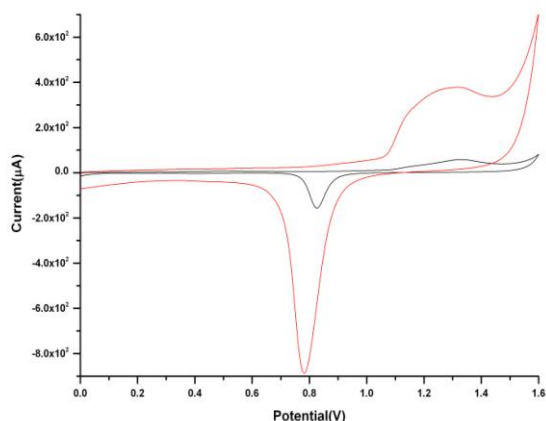


Figure 34 CV of 0.1 M H₂SO₄ vs. Ag/AgCl for planar Au (black line) and NPG electrode (red line), 100 mV/s. Design 4, electrode area 28mm².

To provide the best set-up for the enzymatic biofuel cell, efficient immobilization of the catalyst onto the surface of the electrodes is usually required. Effective attachment to the electrode's surface maintains the protein's biological activity, which is a key feature for the successful and stable operation of a biofuel cell. Different techniques have been developed throughout the years to enable efficient confinement of biomolecules in close proximity to metal electrodes. These include non-covalent adsorption or covalent attachment to an insoluble particle, enzyme entrapment within a semipermeable membrane or the encapsulation of a catalyst within an insoluble matrix (see Fig. 35).

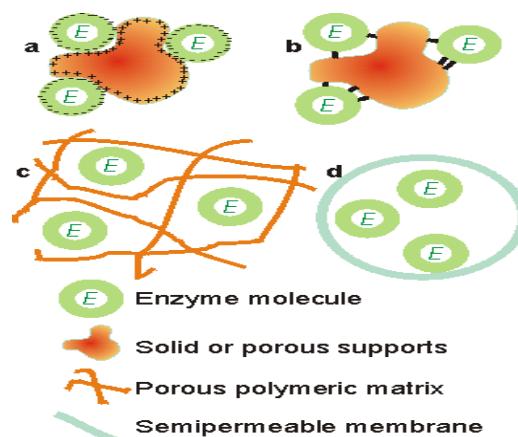


Figure 35 Enzyme immobilization systems: (a) non-covalent adsorption to an insoluble particle, (b) covalent attachment to an insoluble particle, (c) confinement within a semipermeable membrane and (d) entrapment within an insoluble matrix.

Bio-immobilization can be done through either drop coating of the enzyme solution or electrochemically assisted deposition. Drop-coating provides a convenient way to immobilize bio-catalysts on the surface of disk electrodes. Nevertheless, when working with microelectrodes precise control of the enzyme location is required, and the only method that can grant that type of accuracy is electrodeposition (see Fig. 36).

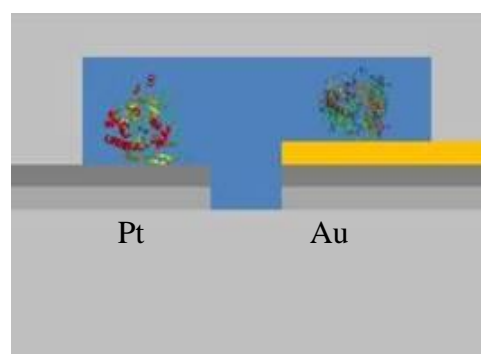


Figure 36 Enzyme molecules immobilized in a controlled manner onto the surface of Pt cathode (left) and Au anode (right).

Typical methods for enzyme bio-immobilization of molecules are not generic and in most cases can be used only for a limited range of biological species and applications.

Methods such as non-covalent adsorption or microencapsulation into polymer microspheres and hydrogels pose risks of leaching, enzyme denaturation and difficulties in obtaining the correct spatial orientation of the catalyst [3, 4]. Every time a new biomolecule is introduced, the system has to be re-optimized and its components readjusted according to the individual requirements of the species incorporated, turning the procedure into a time- and labour-consuming protocol.

Recent studies show growing interest in the application of inorganic silicate matrixes as materials for the encapsulation of biological species. So-called sol-gels have been known as inorganic glasses for over 160 years (Ebelman, 1846), but it wasn't until the mid-1950s that the first encapsulation of biological entities was reported (Wyllie, 1954). Sol-gel materials are prepared under ambient conditions using mild reagents. They are porous substrates with a tuneable pore size for stable immobilization of large biomolecules and a good permeability to smaller substrates, providing an accommodating environment for enzyme catalysis. Inorganic characters and neutral preparation techniques retain the biological activity of species, which is essential when working with proteins. These properties of the sol-gel matrix overcome the flaws of the existing microencapsulation approach, thus this method has been selected for enzyme immobilization in biofuel cell applications.

One of the European leaders in the field of sol-gel-based bio-composites is a team of scientists in Nancy (France) led by Dr Alain Walcarius. Implementation of the advanced technique for enzyme immobilization into sol-gels developed in Nancy can significantly reduce time and provide state-of-art results. Thus, two months' research has been conducted within the group to study the method of enzyme encapsulation in silicate matrixes and to be able to apply this approach in the area of enzymatic biofuel cells.

4.1 Enzyme encapsulation in sol-gel matrix

For the purpose of the research carried out in Nancy, enzyme encapsulation in porous tetraethyl orthosilicate (TEOS) sol-gel was carried out by means of drop-coating and electrodeposition using a procedure optimized by the Walcarius group (Urbanová et al., 2013). Two enzymatic systems were considered for our biofuel cell devices. The enzyme of choice for the bioanode was D-sorbitol dehydrogenase (DSDH). Nicotinamide adenine dinucleotide-3-glycidoxypyltrimethoxysilane (NAD⁺-GPS) has been used as a cofactor necessary for the activity of the enzyme, and the system was supported by a mediator regenerating enzyme, diaphorase (DI). Mediated electron transfer (MET) was studied where the oxidation of the substrate D-sorbitol has been carried out in the presence of a mediator molecule, ferrocene-dimethanol (FDM) (see Fig. 37).

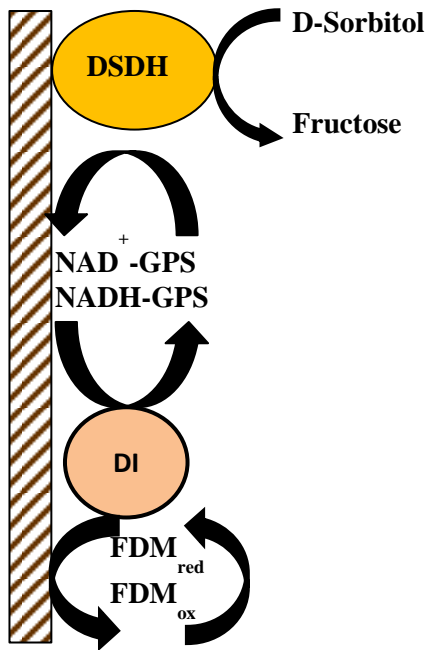


Figure 37 Enzymatic system on bioanode. D-sorbitol is oxidized by DSDH, forming fructose. Electrons are shuttled to the surface of the electrode through a series of mediating molecules: cofactor (NAD⁺-GPS) and FDM with a subsequent regeneration of the mediator molecule by DI.

The catalyst for the biocathode was bilirubin oxidase (BOD), an enzyme capable of a catalytic reduction of molecular oxygen to water. This reaction has been studied in the presence of osmium polymer as a mediator (Os) (see Fig. 38).

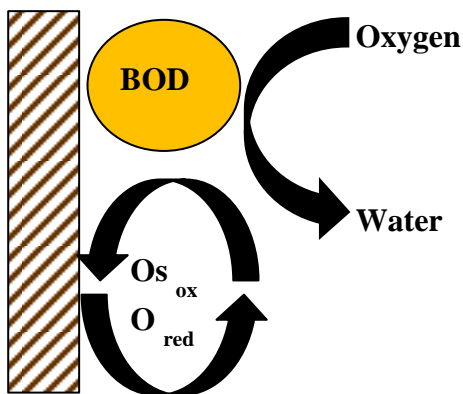
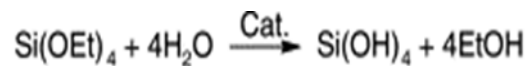


Figure 38 Enzymatic system for biocathode. BOD reduces oxygen into water, while the electrons are being transported to the electrode by osmium polymer.

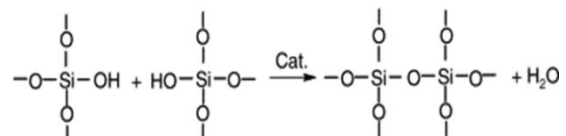
TEOS solutions were prepared overnight and mixed with buffered enzyme solutions containing polymethylenimine (PEI) and polydiallyldimethylammonium chloride (PDDA) as polyelectrolytes. PEI and PDDA are cationic polymers and due to their positively charged amine groups can stabilize negatively charged protein molecules. The first step in the sol-gel formation is the hydrolysis of TEOS in acidic environment, resulting in production of silicic acid and alcohol (9).

(9)

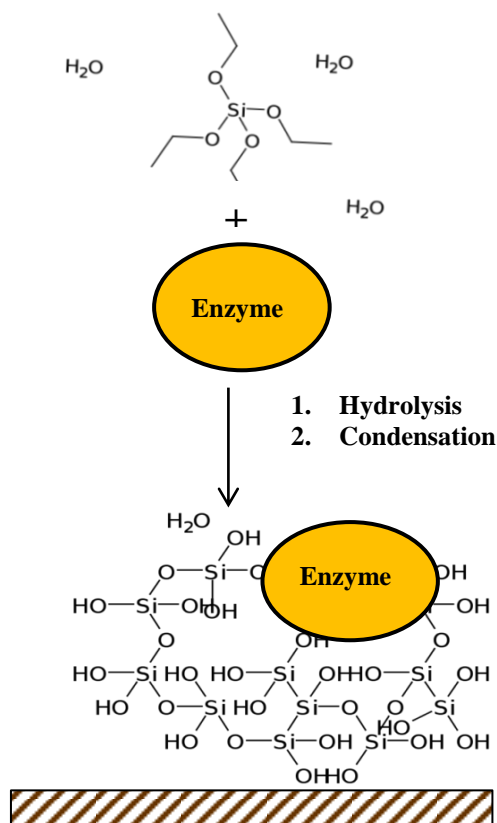


Increase in pH due to applied voltage or natural formation of reactive hydroxyl groups causes subsequent polycondensation of the TEOS matrix and, in the presence of enzyme molecules, their incorporation into a growing chain of siloxane polymer (10 and 11) (Wyllie, 1954).

(10)



(11)



Drop coating and electrodeposition are two methods by which we can condense the TEOS matrix. While the first is suitable for macroelectrodes, the only way in which we can precisely control the location of the enzymes on the microelectrodes is electrodeposition. Electrochemically assisted encapsulation of catalysts in TEOS matrix has been carried out by applying a negative voltage (-1.2 V vs. Ag/AgCl) for sufficient time (usually 60 s). Enzymatic response with respect to increasing concentrations of substrate has been studied in buffer solutions in the presence of a mediator. Gold, platinum and glassy carbon were used as the materials for the electrodes.

4.2 Experimental results: bioanode

Electrochemical characterisation using cyclic voltammetry of the electrodes with encapsulated enzyme was performed to evaluate its biological activity. No catalysis was initially found when the electrochemical response of D-sorbitol dehydrogenase (10 mg/ml in H_2O) to increasing concentrations of the substrate, D-sorbitol was studied (see Fig. 39).

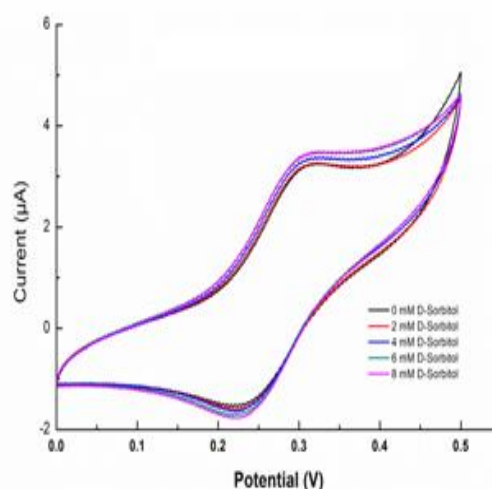


Figure 39 Response of Au rod electrode (3 mm) drop-coated with DSDH/ NAD^+ -GPS/DI/TEOS film to increasing concentrations of D-sorbitol in 0.1 M Tris-HCl buffer pH 9.0 containing 0.1 mM FDM. Response vs. Ag/AgCl, 5 mV/s.

The ratio of NAD^+ to GPS used was 0.67:1 (w/w). Addition of NAD^+ to the solution confirmed the activity of D-sorbitol dehydrogenase. The maximum catalytic efficiency was detected at D-sorbitol concentrations of approximately 6 mM (see Fig. 40).

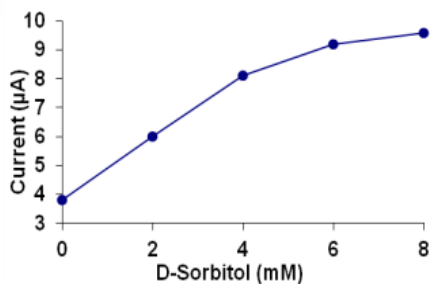
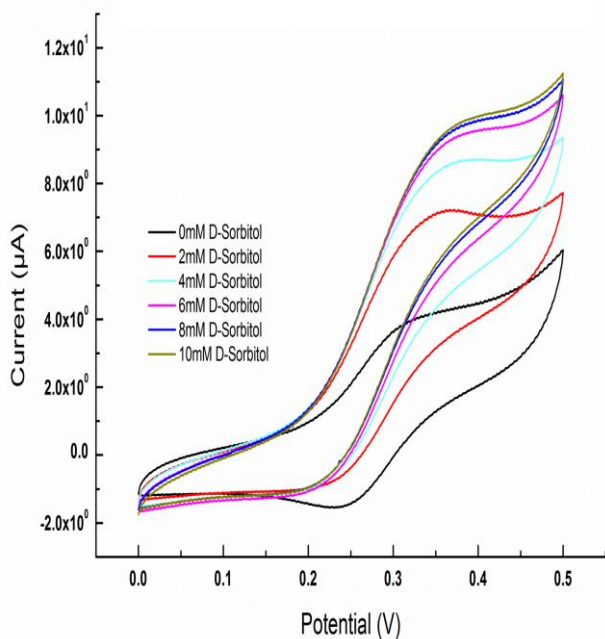


Figure 40 Response of Au rod electrode (3 mm) with DSDH/NAD⁺-GPS/DI/TEO films to increasing concentrations of D-sorbitol in 0.1 mM FDM 0.1M Tris-HCl buffer pH 9.0 (top). Addition of 1 mM NAD⁺ to solution (left). Dependency of the current on the concentration of substrate (bottom). Response vs. Ag/AgCl, 5 mV/s.

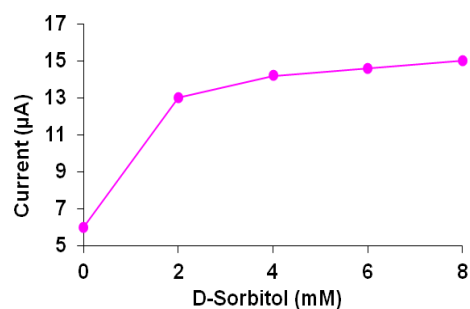
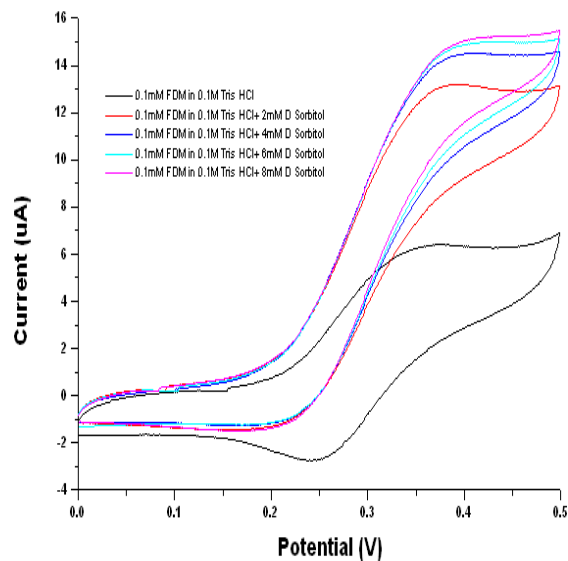


Figure 41 Increased response of Au rod electrode (3 mm) coated with DSDH/NAD⁺-GPS/DI TEOS when NAD⁺-GPS ratio 1:6.25 (top). Dependency of the current on the concentration of substrate (bottom). Response vs. Ag/AgCl, 5 mV/s.

Decreasing the ratio of NAD⁺ to GPS to 1:6.25 (w/w) provided better stabilization of the cofactor molecule and resulted in higher catalytic currents with the substrate saturation at merely 2 mM (see Fig. 41).

Catalytic responses of DSDH systems when the cofactor was freely dispersed in the buffer solution and encapsulated within the TEOS film have been compared. Four times' increase in the oxidation current was noticeable when NAD^+ was co-immobilized within the sol-gel matrix (see Fig. 42). That suggests that the encapsulation of the cofactor in the film stabilizes it and enhances the transfer of electrons between the active site of the enzyme and electrode, improving the current output.

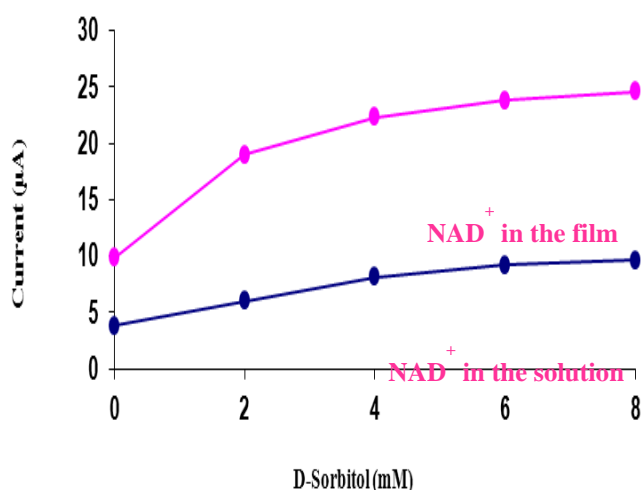


Figure 42 Comparison of the current outputs when NAD^+ dissolved in solution (blue) and co-immobilized in the TEOS matrix (pink).

4.3 Experimental results: biocathode

Both direct (DET) and mediated electron transfer (MET) have been investigated in the examination of the ability of bilirubin oxidase to reduce molecular oxygen. MET has been studied in the presence of mediator molecules: osmium incorporated polymer, single walled carbon nanotubes modified with carboxyl groups (SWCNT-COOH) and with osmium polymer (SWCNT-Os).

Gold, platinum and glassy carbon have been used as electrode materials. The surface of Au and Pt has been additionally activated by treatment with 3-mercaptopropyl-trimethoxy silane (MPTMS) to provide better covalent attachment of the TEOS films (see Fig. 43).

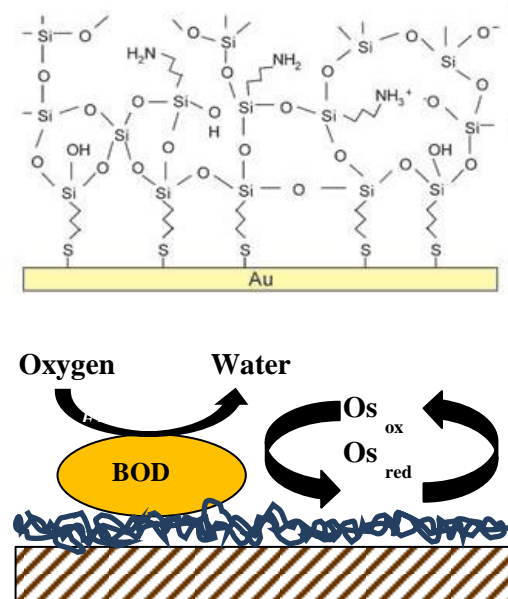


Figure 43 Schematic of the biocathode system containing BOD and osmium polymer on Au electrode after the modification with MPTMS.

TEOS solutions containing BOD were drop coated on the disk electrodes and left at room temperature for further condensation of the matrix. Electrochemical measurements were performed in 0.1 M PBS buffer pH 7.0, in the absence and presence of molecular oxygen to study the influence of the mediator molecules on the catalytic activity of bilirubin oxidase. Direct electron transfer of BOD encapsulated in TEOS hasn't been confirmed on any of the electrodes tested (Fig. 44).

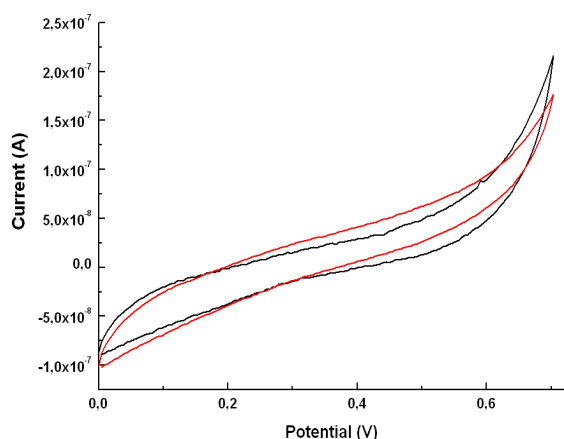


Figure 44 Response of BOD/TEOS/PEI films on GCE rod electrode (5 mm) in the presence (red line) and absence of oxygen (black line). Response vs. Ag/AgCl, 5 mV/s.

Catalytic activity of the enzyme was only noticeable on glassy carbon in the presence of a stabilizer, PEI, and with osmium polymer co-immobilized in the film (Fig. 45). Neither SWCNT-COOH nor SWCNT-Os successfully shuttled the electrons between the active centre of BOD and the surface of the electrode

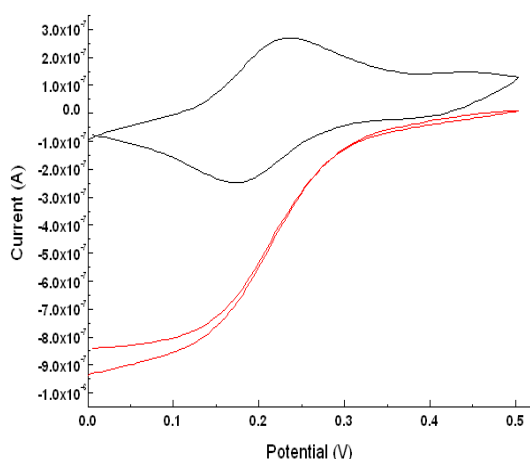


Figure 45 Response of BOD/TEOS/Os/PEI films on GCE rod electrode (5 mm) in the presence (red line) and absence of oxygen (black line). Response vs. Ag/AgCl, 5 mV/s.

Experiments in the presence of osmium polymer and PEI were repeated using Au and Pt. No catalysis was observed on gold or platinum even in the presence of an additional layer of SWCNT mediator. Modification of the electrode surface by treatment with MPTMS was investigated to mimic the properties of a glassy carbon electrode and to counteract the poor adhesion and the lack of enzymatic activity on Pt and Au. Unfortunately no catalysis has been shown on either of the metals (see Fig. 46).

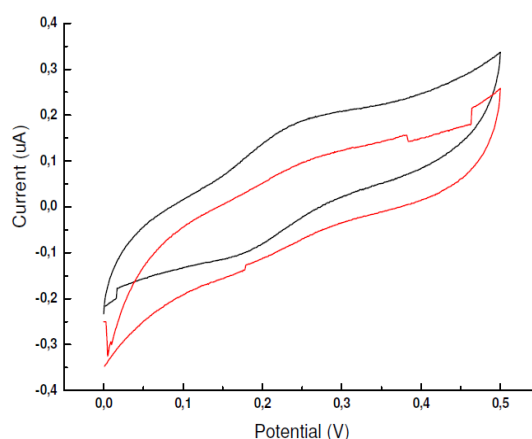
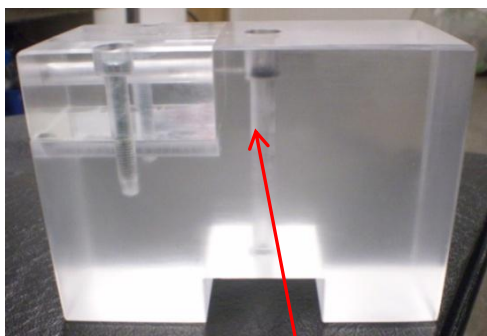
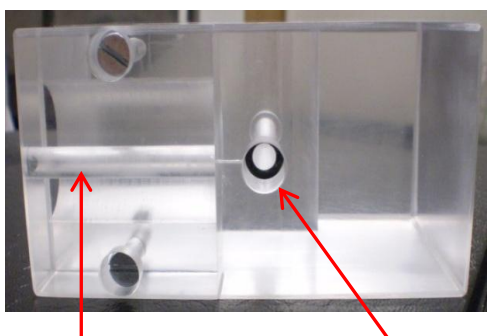


Fig. 46 Response of BOD/TEOS/PEI/Os films on MPTMS treated Pt rod electrode (3 mm) in the presence (red line) and absence of oxygen (black line). Response vs. Ag/AgCl, 5 mV/s.

Special holders for both micro- and macro-electrodes have been designed and fabricated at the Tyndall National Institute to assist the electrochemically assisted deposition of enzyme and mediator based sol-gel films onto metal electrodes (Figs 47A and 47B).



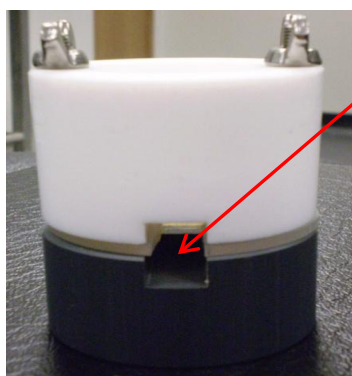
Cavity to accommodate working electrode



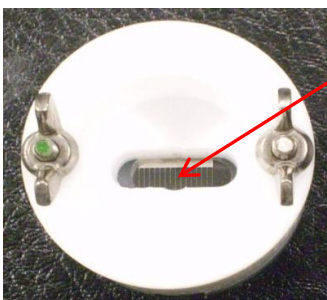
Cavity for Pt wire as counter-electrode

Chamber for the enzyme and mediator sol-gel solution

Figure 47A Images of a PMMA holder for the electrodeposition of sol-gel films on disk electrodes.



Pocket for electrical connectors



Pocket for Zeonor substrate

Figure 47B Top and side view of a holder for the electrodeposition of sol-gel films on microelectrodes embedded in the channels of microfluidic

4.3.1 Chitosan modified films

Properties of chitosan as a potential matrix for enzyme immobilization have been investigated and compared to TEOS films. While TEOS turned out to be more rigid and stable in solution, the chitosan matrix gave a better electrochemical response in the presence of biocatalysts (Fig. 48).

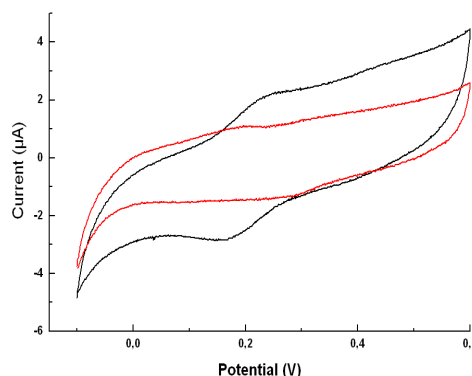


Figure 48 Comparison of the electrochemical response of TEOS (red line) and chitosan (black line) films.

Combination of tetramethyl orthosilicate (TMOS) and chitosan films (Urbanová et al., 2013) has been also studied to provide a both stable and accommodating environment for laccase, an enzyme reducing oxygen to water. As a natural, biodegradable and biocompatible polymer, chitosan is a promising material for enzyme encapsulation [8].

TMOS on the other hand has been reported repeatedly as a much better reagent due to its less harmful effect on enzyme molecules when compared to TEOS [9, 10]. 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was used to mediate the transfer of electrons between the surface of cathode and the substrate. TMOS/chitosan solutions have been prepared in the presence of methanol catalyst and drop-coated on a previously applied layer of buffered laccase

solution (50 mg/ml in 0.1 M PBS) containing 10% PEI. The catalytic signal has been studied in oxygen-deprived and saturated 0.1 M PBS pH 7.0 solutions containing 0.5 mM concentration of ABTS. Glassy carbon, gold and platinum rod electrodes were plasma treated prior to electrochemical measurements. Removal of oxygen was possible by purging the solution with pure nitrogen. Cyclic voltammetry of the biocathode system shows increased current values in the presence of oxygen (see Fig. 49).

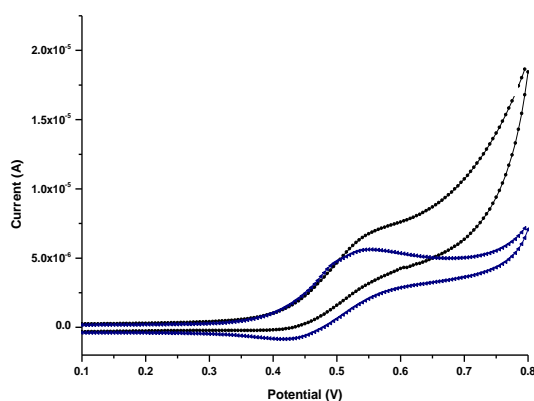
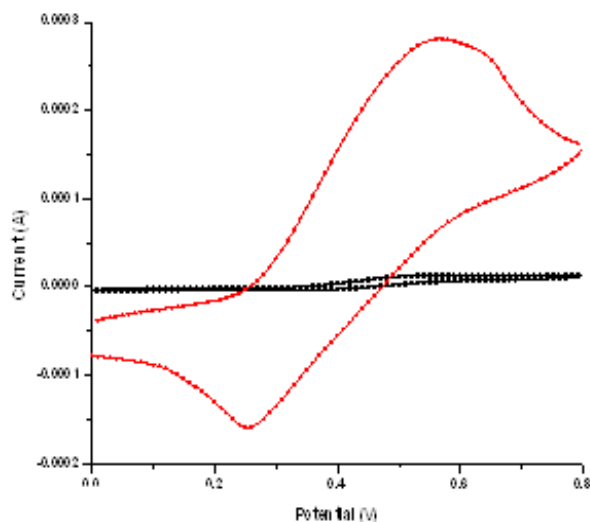


Figure 49 Response of laccase/PEI/TMOS/ chitosan films on GCE disk electrode in the presence (black line) and absence of oxygen (blue

Preliminary experiments to co-immobilize ABTS in the TMOS matrix have been



undertaken. Cyclic voltammetry of the modified electrodes shows significant change in the catalytic response of the mediator when the substrate is present (see Fig. 50).

Figure 50 Response of ABTS/PEI/TMOS/chitosan films on Pt disk electrode in the presence (red line) and absence of oxygen (black line)

5. Miniature enzymatic biofuel cell

To prove the concept of enzyme-driven devices, a small biofuel cell utilizing two types of redox enzymes has been constructed using simple elements (see Fig. 51). Laccase for the catalytic conversion of the oxygen to water was immobilized on the Pt cathode, while glucose oxidase on the Au anode was responsible for the oxidation of glucose to gluconolactone and production of hydrogen ions. Two solutions providing the substrate and

mediator have been used, phosphate buffer containing 20 mM glucose and 5 mM $K_3[Fe(CN)_6]$ and acetate buffer containing oxygen and 5 mM ABTS for GOX and laccase respectively. The two compartments have been separated by an insulating, semiporous membrane permeable only to protons. The application of both enzyme catalysts produced much higher power and current densities in comparison to enzyme free or single enzyme systems.

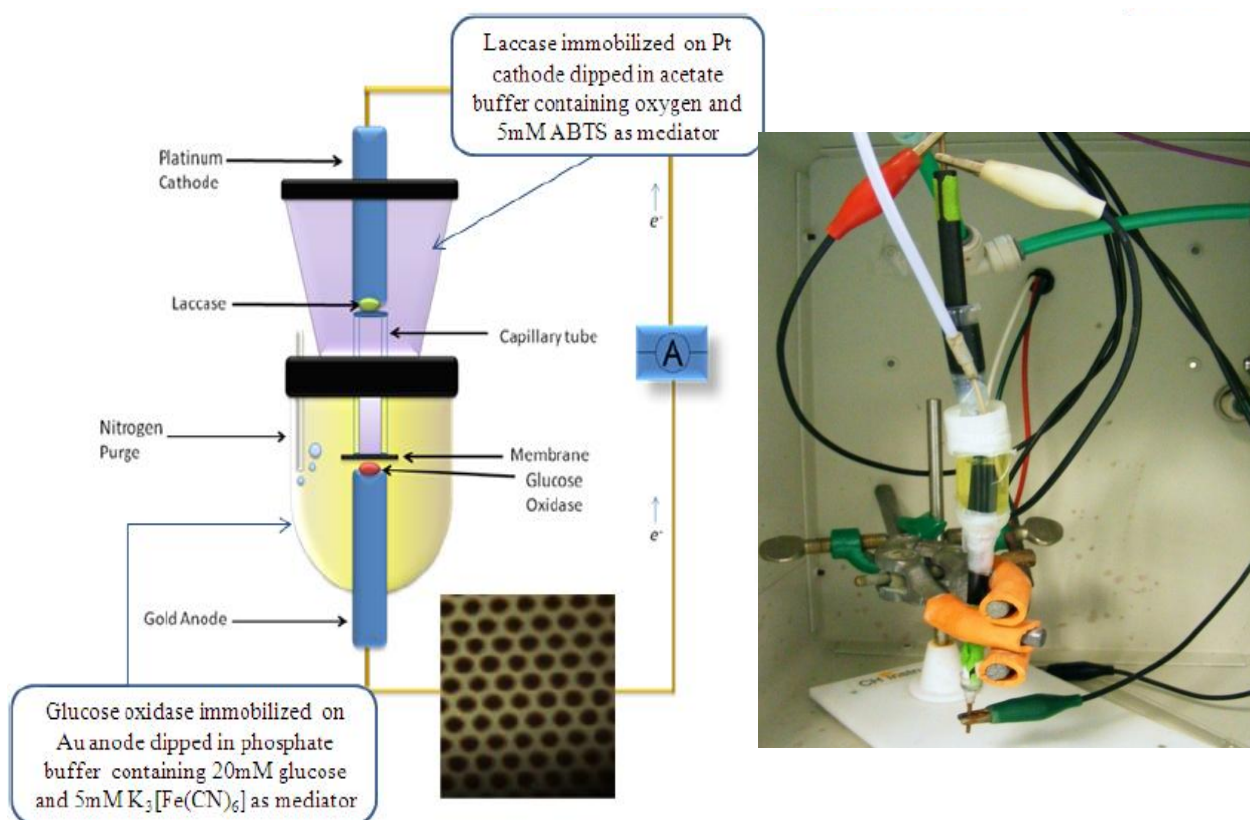


Figure 51 Fully assembled enzymatic biofuel cell utilizing anodic GOX for efficient glucose oxidation and cathodic laccase for the reduction of oxygen to water. Enzyme buffered solutions separated by a semiporous membrane.

6. Summary of overall project

Project outputs

The output of this project includes new methods, new technologies and processes in the field of alternative sources of energy capable of harvesting fuel from the surrounding environment in an environmentally friendly way. The results obtained also have essential importance for a new generation of the sensing system, especially for sensor-on-chip and lab-on-chip systems for environmental, health, food and security applications. The project has built a capacity for research in environment and in related fields such as health, food and security.

New methods

Mathematical models have been defined to study the behaviour of the flow and the characteristics of the enzymatic reactions within the microfluidic channel of the suggested biofuel cells. Based on the modelling studies, optimum designs of the electrode and channel layouts have been proposed and fabricated to achieve the maximum efficiency of the fuel and oxidant conversion.

New technologies and processes

Polymer substrates for the microfluidic biofuel cells have been successfully designed and fabricated using facilities at the Tyndall National Institute, Cork. Gold anodes have been modified with nanoporous gold structures which significantly increased the active surface area of the electrodes. A novel microfluidic holder has been manufactured incorporating the alignment marks and additional features to accurately

control the position of Zeonor and PDMS parts. This set-up creates a tight seal between the two substrates. Providing a uniform pressure should prevent one of the everlasting problems in microfluidics – the possible leakage of the fuel – and hence can be used for future experiments in static and flowing conditions.

Planar and nanoporous gold modified electrodes of all designs have been tested electrochemically in static conditions using the cyclic voltammetry technique.

D-sorbitol dehydrogenase and bilirubin oxidase, which were selected for implementation in the biofuel cell device, were successfully immobilized within the TEOS matrix, and mediated electron transfer has been confirmed by cyclic voltammetry measurements. Further studies need to be carried out to improve the quality and stability of the silicate-based films, looking primarily at the electrodeposition of biological species onto microelectrodes of the microfluidic channel. Co-immobilization of laccase and ABTS requires further optimization. Glucose dehydrogenase and glucose oxidase will be used as enzymes for bioanodes while work with bilirubin oxidase will be continued in the cathodic half-cell focusing on direct electron transfer.

Holders for the electrodeposition of catalytic silicate based matrixes onto both disk electrodes and microelectrodes are essential tools to obtain reproducible results in terms of film quality, stability and precise control of location on the electrode. These have been

successfully designed and fabricated from polymeric substrates and are currently being tested for the successful immobilization of laccase and ABTS on the cathode. Sol-gel encapsulation of anodic catalysts such as glucose oxidase (GOD) and dehydrogenase (GDH) or cellobiose dehydrogenase (CDH) may also be investigated to provide stable entrapment of the enzyme and efficient electron control from/to the electrode.

Electrodes bearing catalytic silicate films can be easily aligned with the PDMS channels to form ready-to-use enzymatic biofuel cells. A miniature enzymatic biofuel cell was constructed incorporating glucose oxidase and laccase as enzyme catalysts. Power and current output produced by the cell has proved the concept of the enzyme-driven devices.

Capacity building

The project has provided postgraduate and project-management training and experience to four individual scientists building the

capacity for environmental research and environmental protection in Ireland. The project constitutes an important part of Tyndall National Institute's work in the new environmental field, which is green energy generation capable of harvesting fuel from the surrounding environment in an environmentally friendly way.

The project also provided an important contribution to the FP7 Guardian Angels FET Flagship proposal. The EPA STRIVE project helped to promote awareness of the relationship between energy and the environment at various conferences and workshops where the research was disseminated.

Partnerships for Environmental Research

A positive relationship has been developed between Tyndall and EU groups working in the field of alternative sources of energy, which offers considerable potential for future environmental research and participation in EU and national environmental projects.

7. Conclusions and Future Research

Fabrication of device compartments has been accomplished and the tools for the assembly of the microfluidic platforms have been defined. Stable attachment of the cell has been achieved using a microfluidic holder supported by an oxygen plasma activated adhesive.

Numerical studies on mass transport and enzyme kinetics within the channel were carried out with an insight into the device optimisation. Preliminary results on the current generation were obtained based on the rate equations but further work needs to be done to obtain the best estimate of the cell's output generated as a result of enzymatic reactions. Theoretical data needs to be supported by experimental findings.

Immobilization of enzyme catalysts in a sol-gel matrix was investigated on both half-cells proving to be a stable and non-degenerative entrapment method to study enzyme chemistry. Due to high dependency of the sol-gel system on working conditions and the

individual properties of the protein catalysts a need for a general immobilization protocol for various biological species exist. Use of alternatives to sol-gel matrices such as other conductive polymers which can undergo electrodeposition should be considered. Various enzyme molecules are known to catalyse conversions of different organic biofuels and these should be taken into account as a possible choice of active species for biofuel cell applications.

Miniature enzymatic biofuel cell have been built from simple components and characterised in static conditions confirming the operability of enzyme driven cells.

Initial characterisation of microfluidic platforms in static and flowing conditions have been carried out but future research requires stable assembly of both half-cells incorporating enzyme modified electrodes and electrochemical characterization in the flowing environment.

8. Recommendations for implementation and uptake of research findings

1. There is an essential need for alternative sources of energy capable of harvesting fuel from the surrounding environment in a friendly way. In this regard microfluidic enzymatic biofuel cells, which can produce electrical energy from carbohydrates, alcohols and organic pollutants found in industrial waste, represents a very promising solution of the problem.
2. The results obtained in the project proved the possibility of the creation of an enzymatic biofuel cell. At the same time they show the complexity of the problem involving a number of state-of-art technologies such as microfluidics, silicon fabrication, surface and enzyme chemistries, and mathematical modelling.
3. Further development of research and development of microfluidic enzymatic biofuel cell for green harvesting energy from the surrounding environment should be supported.
4. Further advances in the technologies developed in this study (surface and enzyme chemistries, electrode modification, microfluidic system, microfabrication processes) and the effective transfer of these findings into related technologies i.e. sensing systems, especially sensor-on-chip and lab-on-chip system, should be also considered.

Table 2 on the following page provides a summary of recommendations for the implementation and uptake of these research findings.

Table 2 Recommendations for implementation and uptake of research findings.

Issue	Recommendation	Target	Time Frame
Awareness of importance of alternative sources of energy capable of harvesting energy from the surrounding environment in a friendly way	Environmental awareness shows perceptiveness of using microfluidic enzymatic biofuel cell for green harvesting energy.	SFI / EI Technology Innovation Development Award (TIDA)	Short term and medium term
Microfluidic enzymatic biofuel cell	Implementation of developed technologies in EU and national environmental projects.	Horizon 2020, EI, EPA	Short term and medium term
Surface chemistries, electrode modification, enzyme chemistries, microfluidic system, microfabrication processes	Implementation of developed technologies in EU and national projects on sensing system for environmental, health, food and security applications.	Horizon 2020, EI	Short term and medium term

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2. Kim, L., Toh, Y.-C., Voldman, J. and Yu, H., 2007, A practical guide to microfluidic perfusion culture of adherent mammalian cells. *Lab on a Chip* **7**: 681–694.
3. Scanlon, M.D., Salaj-Kosla, U., Belochapkine, S., MacAodha, D., Leech, D., Ding, Y. and Magner, E., 2012, Fabrication and characterisation of nanoporous gold electrodes for biosensor and biofuel cell applications. *Langmuir* **28**: 2251–2261.
4. Urbanová, V., Etienne, M. and Walcarius, A., 2013, One step deposition of sol-gel carbon nanotubes biocomposite for reagentless electrochemical devices. *Electroanalysis* **25**: 85–93.
5. Wyllie, M.R.J., 1954, Ion-exchange membranes. I. Equations for the multi-ionic potential. *Journal of Physical Chemistry* **58**: 67–73.

Research outputs

Conference presentations

Oral presentations

1. G. Herzog at the Eirelec'11 Conference (Adare, Ireland, 16–18 May 2011): *Microfabricated electrochemical devices for sensing and energy applications.*
2. G. Herzog at the Guardian Angels workshop (Helsinki, Finland, 15–16 September 2011): *Energy harvesting from glucose and oxygen. Can biofuel cells power up smart autonomous systems?*
3. C. Ó Mathuna at the Tyndall Technology/Analog Devices Forum (Limerick, 20 October 2011): *Wireless sensors and energy harvesting.*
4. M. Zygowska at the 12th Topical Meeting of the International Society of Electrochemistry & XXII International Symposium on Bioelectrochemistry and Bioenergetics of the Bioelectrochemical Society (Bochum, Germany, 17–21 March 2013): *Electrochemically-assisted deposition of chitosan and sol-gel enzyme bio-composites for microfluidic biofuel cell applications.*
5. M. Zygowska at the Tyndall Internal Conference (Cork, Ireland, 25 April 2013): *From doughnuts to energy. Miniature enzyme driven biofuel cells.*

Poster presentations

1. M. Zygowska at the EPA National Conference (Dublin, Ireland, 23 June 2010): *Design and fabrication of microfluidic systems for efficient energy bioconversion.*
2. M. Zygowska at the 8th EPA Postgraduate Conference (Dublin, Ireland, 11 November 2010): *Simulation studies for the optimization of microfluidic designs for biofuel cell applications.*
3. M. Zygowska at the Tyndall Postgraduate Poster Competition (Tyndall National Institute, Cork, 23 July 2010): *Microfluidic systems for efficient energy bioconversion* (poster no. 23, p. 7 of Book of Abstracts).
4. M. Zygowska at the XXI International Symposium on Bioelectrochemistry and Bioenergetics (Krakow, Poland, 8–12 May 2011): *Fabrication and characterisation of microfluidic devices for efficient energy bioconversion* (poster no. PS3-17, p. 33 of Conference Programme).

5. M. Zygowska at NanoBio-Europe (Cork, Ireland, 21–23 June 2011): *Fabrication and characterisation of microfluidic devices for efficient energy bioconversion* (poster no. 67, p. 112 of Book of Abstracts).
6. M. Zygowska at the Tyndall Postgraduate Poster Competition (Tyndall National Institute, Cork, 22 July 2011): *Fabrication and characterisation of microfluidic devices for biofuel cell applications*
7. M. Zygowska at the 9th EPA Postgraduate Conference (Dublin, Ireland, 17 November 2011): *Fabrication, characterisation and modelling of microfluidic devices for efficient energy bioconversion* – 2nd Prize for poster presentation (p. 8 of Book of Abstracts).
8. M. Zygowska at the ENVIRON 2012 conference (Dublin, Ireland, 7–9 March 2012) Our Environment: Integrating Today's Research with Tomorrow's Actions. The 22nd Irish Environmental Researcher's Colloquium: *Microfluidic devices for enzymatic biofuel cell applications. Fabrication, characterisation and modelling studies* (p. 148 of Book of Abstracts).
9. M. Zygowska at the Lab-On-A-Chip conference (Edinburgh, Scotland, 28–29 March 2012): *Enzymatic Biofuel Cells on chip: Fabrication, characterisation and modelling studies*
10. M. Zygowska at the 2012 Postgraduate Student Poster Competition (Tyndall National Institute, Ireland, 20 July 2012): *Mathematical model for the catalytic system of the Enzymatic Biofuel Cells*
11. M. Zygowska at the Conference on Analytical Sciences in Ireland, CASi (Cork, Ireland, 1–2 July 2013): *Development and investigation of miniature enzyme driven biofuel cells*

Other

1. Section on enzymatic biofuel cells in the Guardian Angels. *Energy Benchmarking & Roadmaps* proposal.

An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntimid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomhnithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil, Pobal agus Rialtais Áitiúil.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreal;
- scardadh dramhuisce;
- dumpáil mara.

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí chomhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRSCIÚ AR AN GCOMHSHAOIL

- Monatóireacht ar chaighdeán aer agus caighdeáin aibhneacha, locha, uisce taoide agus uisce talaimh; leibhéal agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntí a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Cainníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdeán aer agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstíúrthóir agus ceithre Stíúrthóir.

Tá obair na Ghníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar inní iad agus le comhairle a thabhairt don Bhord.

Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.



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