

A SIMPLE BIOFUEL CELL BASED ON CHITOSAN FILMS FOR LAB ON A CHIP APPLICATION

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Abstract: There are several methods for the construction of biofuel cells. For low cost and low power applications enzymatic biofuel cells are an alternative to those with noble metal catalysts. The lack of power density and a short lifetime are no obstacle for these applications. The presented biofuel cell is constructed to fulfill these requirements. It is based on layers made from Chitosan from crab shells and made conductive by the addition of carbon nanotubes (CNTs). Chitosan serves as a material to immobilize the Enzymes and pure Chitosan without CNTs also works as an isolator between the conductive layers. This enables a metal free, thin, flat and scalable energy source.

Key words: Chitosan, Carbon Nanotubes, Biofuel cell, Glucose dehydrogenase, Bilirubin oxidase

1. INTRODUCTION

The first enzyme based fuel cell for Glucose/O₂ which operated at neutral pH was presented by Yahiro et al. in 1964 [1]. It was intended to power an artificial heart [2]. Over the years the focus of the applications switched from the artificial heart to other implantable medical devices like cardiac pacemakers [3]. In animal experiments sufficient power to drive a pacemaker was generated. But the live time of up to 150 days, the stability and the power density of the fuel cell could not compete with the power capacity of the Li/SOCl₂ batteries for example [4].

In the 1980s and 1990s the focus shifted again. Power plants of huge dimensions were planned [5]. Big fuel cells should convert processed organic waste into clean energy. This was intended to power remote dwellings. But because of the size and the costs of these power plants, the power density is one thousand times smaller than conventional generators, the concept failed to establish in the market. The efforts to develop microbial biofuel cells for large scale energy harvesting continued till 2003[6]. It was tried to convert organic waste from biomass of municipal or agricultural sources into electrical energy.

Today the research focuses again on medical applications. The main attention is drawn to small and low power applications, where biofuel cells are of advantage, because biofuel cells can be kept very simple and cost effective by leaving out parts which are not needed and decreasing the number of components [7]. There is no need for case seals or membranes for example. Only the anode and the cathode are needed for power generation. Depending on the application the size of the electrodes can be shrunken to volumes below 1mm³ to supply energy to implanted devices, which operate autonomous for a

certain period of time [8].

Not only implants but lab on a chip (LOCs) applications benefit highly for simple, small, cost efficient and disposable biofuel cells. In this case the size and the total volume are not as crucial as with implantable sensors.

Lab on a Chip system is where our research interests lie. We want to provide cheap biofuel cells with enough power for an analytical application.

With both electrodes based on enzymatic reactions no noble metal is necessary to catalyze the electrochemical reactions. This results in an one hundred percent recyclable energy source. At the anode the enzymes Glucose Dehydrogenase (GDH) and Diaphorase (Dp) from *Clostridium kluyveri* enable the production of free electrons as described by Sato et al. [9]. The excess Protons and the electrons react at the cathode with oxygen catalyzed by Bilirubin Oxidase (BOD) and water is generated as described by Tsujimura et al. [10].

2. EXPERIMENTAL

2.1 Material

As basic material for the organic film electrodes middle-viscous Chitosan from crab shells was chosen. It was used as 1 % solutions at a pH of 4.5. Chitosan was purchased from Fluka and used without further purification.

All used enzymes were purchased from Sigma-Aldrich and used without purification. At the anode Glucose Dehydrogenase from *Pseudomonas sp.* was used for the processing of the sugar and the release of the electrons. The needed co-factor NAD⁺ was purchased from Carl Roth. The transport of the electrons from NADH to the electrodes is done by Diaphorase from *Clostridium kluyveri* with the

mediator Menadione sodium Bisulfate. Menadione Sodium Bisulfate also known as water soluble Vitamin K3 was purchased from Sigma.

The cathode of the fuel cell consisted only of the enzyme Bilirubin Oxidase from *Myrothecium verrucaria*, which catalyses a four electron reaction of oxygen with protons from the surrounding solution to water.

For the treatment of Chitosan films in order to change their mechanical properties glutardialdehyde was chosen, because Krajewska [11], showed that the permeability of Glucose is only three times less than that of water in the highest tested concentrations of glutardialdehyde.

Current and voltage were recorded with a meilhaus red lab analog digital converter with an operational amplifier. The setup was controlled by a Lab-View program and the recorded data stored on a computer.

2.2 Methods

The Chitosan solution was prepared with 0.05 M hydrochloric acid and sonification with 100 Watts ultra sound, two times 10 min with a break to cool the solution back to room temperature. Afterwards the pH was set to 4.5 with sodium hydroxide [12].

The basic solution was made conductive with Carbon Nanotubes [12]. The ratio of Chitosan to CNTs is 10:1 dry weight. The Chitosan films were produced by the drying method. On a 9 cm diameter Petri dish were 14 ml of the 1% Chitosan solutions cast, either with or without CNTs. After complete evaporation of the water only a thin film, with a weight per area of 2,2mg/cm², remains in the Petri dish. The film does not stick to the surface and can be pulled out of the dish easily. With a normal pair of scissors the desired shape of the electrode can be cut out.

The electrodes were functionalized by coating the active area with a mixture of Chitosan/CNT solution and the enzymes and mediators needed for the generation of electrical currents. The composition of the coating consists of Chitosan, CNTs and BOD at the anode. The cathode coating consists of Chitosan CNTs, the enzymes GDH and Dp and the Mediators NADH and Menadione Sodium Bisulfate. It was used one unit of each enzyme to coat the electrodes.

The coating solution is spread on the Chitosan CNT films and is dried at room temperature until all water has evaporated. These layers are assembled with an isolating film of pure Chitosan to get a functional biofuel cell. The surface of the membranes gets sticky after wetting and the conductive layers can be glued to the isolating membrane.

Tensile testing was done in dry and wet conditions. The membrane was fastened vertically between to clips. The weight of the lower mobile clip

of 12 gr. is pulling down on the membrane. It was surveyed, if the membrane patch can stand the stress.

2.3 Measurements

The measurement of the electrical current of the fuel cell is done with 10mM Glucose buffered at pH 7.5 and at room temperature. All other necessary chemicals are included in the coated electrodes.

Measurement procedure:

- Connect the instrument to the BFC, simple alligator clips are sufficient.
- Start measurement Program (the values were taken every second)
- Add 20mM Phosphate buffer pH 7.5 to the BFC with a Glucose concentration of 10mM
- Record the voltage and current values 4 minutes with open circuit and 1 Minute with a load of 7 k Ω . 5-6 repeats.

The tensile measurements were done with strips of membranes 1 cm wide and 2 cm long. These were fastened with a clip with a smooth edge to avoid ripping the sensible membrane. On the other end of the strip was a weight of 12 gr. with a similar clip added. The weight was released and it was observed if the dry Chitosan patches could hold the weight.

After that the membrane patch was sprayed with 100mM Phosphate buffer pH 7.5, till the whole patch was wet. It was noted if the wet membrane can also withstand the tensile force.

3. RESULTS AND DISCUSSION

To verify the function of the fuel cell design a mediated electron transfer to the anode was chosen. It may not be the most efficient design for enzyme electrodes, but the fact that it is simple to setup, modest in its requirements on the surface preparations and that all components are purchasable by well known companies fit perfectly in the project. The enzymes are most likely to work and the design concept of the electrodes can be investigated. At the anode two glucose molecules are metabolized to glucono- δ -lactone. Four Protons and four electrons are released. At the cathode two water molecules are formed using oxygen O₂ and the four electrons and four protons from the anode (fig.1).

All components needed for the chemical reaction and the material of the foils, are water soluble. They can be intermixed. The foils consist of Chitosan or Chitosan CNTs and are just dried at room temperature. The electrode mixture, Chitosan CNTs enzymes and mediator, was used to coat prepared membranes, resulting in an operational electrode. After drying the

enzyme layer can't be removed from the electrode. The prepared electrodes need to be separated by an electrical isolating layer, which is permeable for protons and glucose to allow the diffusion of the necessary substances. This way it is possible to coat the surface of a micro fluidic device with the three layers of the fuel cell. The oxygen electrode to the outside and the glucose electrode placed directly on the surface of the device.

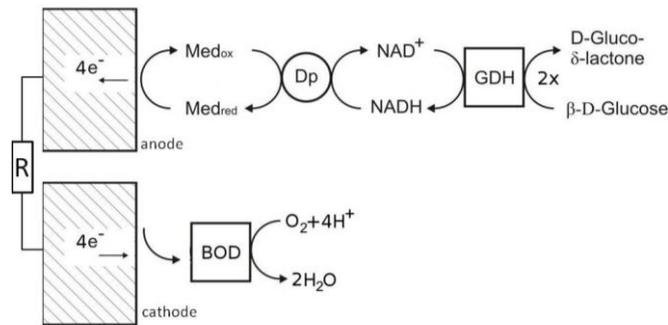


Fig. 1 Reaction scheme of enzymatic anode and cathode. Anode reaction with GDH (glucose dehydrogenase) and Dp (diaphorase) is described in Sato et al [9]. Two protons and two electrons are cleaved of the Glucose molecule. The electrons are transported by NADH to the Dp enzyme where the electrons are transferred to a mediator which releases the electrons at the electrode. The cathode reaction with BOD (bilirubin oxidase) is described at Tsujimura [10]. The BOD catalyzes a transfer of four electrons, released by GDH at the anode to O₂. The four electrons from the anode, four protons and oxygen O₂ form two water molecules.

Fig. 2 shows an example of an assembled fuel cell. The lowest membrane was placed damp on glass coverslip and sticks to it. It even stays in place when the biofuel cell is completely covered with Glucose solution. The assembly of the other layers works just like that. The layer on the coverslip is sprayed with water and the next Chitosan film is placed on top and taped lightly to enhance the adhesion. The single layers stay together even if completely covered by glucose solution.

The alligator clips connect the fuel cell to the measuring instrument.

A typical measurement of a Chitosan/CNT bio fuel cell (fig.3). The open circuit voltage reached -0.4 V and the current at the end of the one minute interval with a load of 7kΩ did not rise below -2μA .

To maximize the power output of the cells the load has to be adjusted. The optimal power output is reached when the operating voltage is half of the open circuit voltage.

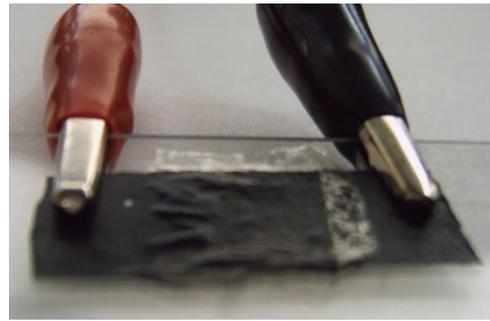


Fig. 2. Schematic illustration of a layered bio fuel cell. The Black foils are conducting Chitosan CNT composites coated with an enzyme mediator mixture. The transparent foil between the two electrodes is an isolator film made of pure Chitosan.

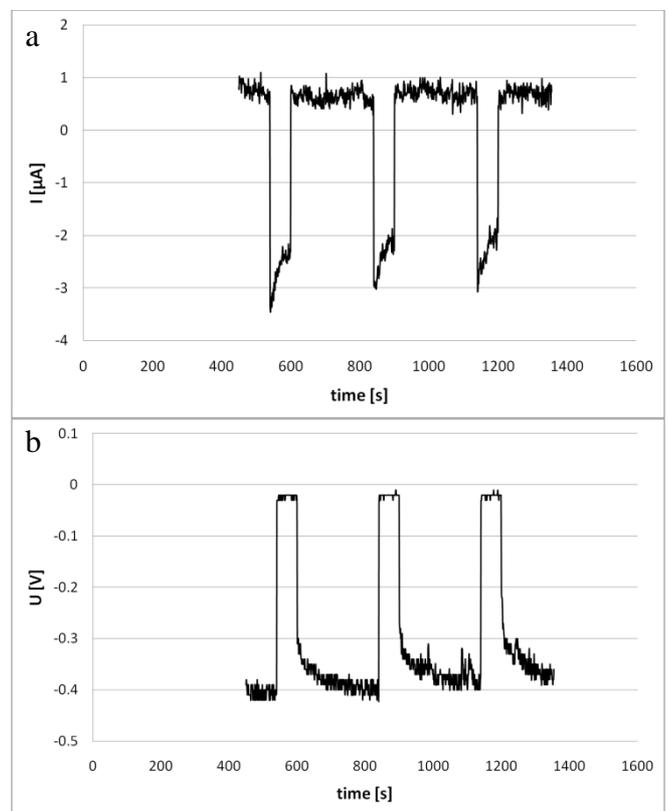


Fig. 3 Measuring results of the layered fuel cell. a: Current over time b: Voltage over time. Both measurements were done simultaneously. Four minutes open circuit and following one minute with a load of 7 kΩ.

The well established enzyme system on the electrodes is working in this fuel cell design very well. With the addition of only Phosphate buffer no significant current is recorded (data not shown). But the mechanical stability of the fuel cell in wet conditions is not sufficient. In Glucose solution the membranes (Chitosan foils) are swelling and getting very soft.

To get an idea of this process 12 gr. were

suspended by one centimeter wide membrane patches. In dry conditions all patches could withstand the weight. After wetting the membranes were torn apart by the weight. The tearing happened very quickly. Handling of wet membranes or fuel cells is not possible without destruction of the fuel cells.

Another problem is a significant swelling. The wet membranes swell and expand in all directions and the layers crumple. This could block small channels when the membranes are used in a microfluidic device.

Preliminary results indicate that the swelling in water can be reduced by cross linking the chitosan molecules with glutardialdehyde. The draw-backs, of this method is, that the permeability of the membranes for Glucose or smaller molecules is reduced and the electrical resistance is increased.

The permeability decrease for Glucose and smaller molecules is still tolerable. The mobility of larger molecules, like the enzymes, is stronger affected, which enhances the immobilization of the enzymes on the electrodes [11]. The increasing resistance is more problematic for the output of the fuel cell.

4. CONCLUSION

Enzymatic bio fuel cells can be reduced in their number of parts [7]. If you leave a gap between the electrodes, to avoid short circuit the minimal number of components is two, both electrodes and nothing else. The presented biofuel cell is quite near the optimal value. It consists of three parts; three different foils are produced and assembled. But on the other hand one only has to incorporate a single foil in the devices to power, because the membranes are fused together.

Another feature of this design is the high variety of shapes the membranes can be produced in. Many different silhouettes are possible and that means the fuel cell can be easily adapted to the needs of the device.

The drawbacks of this device are the susceptibility to water and the electric resistance of about 10-20 k Ω . Preliminary results show that the softening of wet Chitosan foils can be overcome by chemical cross linking. On the other this procedure increases the resistance even more up to the range of M Ω . Further efforts have to be taken to solve these problems.

Last but not least the simple, noble metal free and flat enzymatic fuel cell is likely to be suited for efficient reel to reel production, which decreases the costs.

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