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Highlights

- Soft materials tested for the first time as feedstock in MFCs
- Higher output from gelatin fed MFCs after constant feeding stopped
- Detrimental effect of liquid Nafion to the microbial community
- Potential implementation of the proof of concept to EVOBOT platform for 3D printed MFCs

GELATIN AS A PROMISING PRINTABLE NUTRIENT FEEDSTOCK FOR MICROBIAL FUEL CELLS (MFC)

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Abstract

The Microbial Fuel Cell (MFC) is an energy transducer technology that can directly produce electricity from bacterial oxidation of organic matter. MFCs consist of two reaction chambers (anode and cathode) separated by a semipermeable membrane. This study describes the work carried out towards the optimization of critical MFC components, with potential 3D fabricated materials. The response of the optimized fuel cells, which were fed with soft materials such as gelatin, alginate and Nafion™, is also reported. The optimized components were the membrane and the cathode electrode. A traditional Nafion membrane was substituted with a custom made terracotta sheet and the electrode used was a single sheet of carbon veil coated with an activated carbon paste. The results showed that among the soft materials tested within the anodic chamber, gelatin performed the best; it also revealed that even after a 10-day starvation period gelatin demonstrated better longevity. These results show that MFCs have the potential to be 3D-printed monolithically using the EVOBOT platform.

Keywords MFC, EVOBOT, Gelatin, 3D-printing, Nutrient feedstock

1. Introduction

The microbial ability to decompose organic matter and liberate electrons as part of their metabolic pathways has been proven beneficial for the development of microbial fuel cells (MFCs), which emerged more than 100 years ago [1]. Microbial fuel cells (MFCs) can be defined as energy transducers, which convert chemical energy stored in organic matter into electricity through bacterial utilisation [2]. The electrical output from MFCs is due to the bacterial catalytic conversion, which occurs in the anode. MFCs consist of two compartments, the positive cathode and the negative anode; each compartment has its own electrode which acts as the electron sink (anode) and electron acceptor (cathode) of the cell [3]. The two parts are separated by a semi-permeable membrane allowing the protons generated in the anode, from the bacterial oxidation of the organic molecules, to flow through to the cathode. Electrons flow through an external connecting wire or circuit, which facilitates current flow. Both electrons and protons are re-combined at the cathode side with dissolved oxygen to form H_2O as the by-product [4]. Due to these distinct characteristics MFCs are often described in the literature as biological batteries or bio-batteries [5]. MFCs

have a great potential as a new green source of energy, since many types of organic substrates can be utilised; use of MFCs in wastewater treatment processes can potentially reduce treatment costs and pollution, as well as generate on site electricity.

The 3D printing technology was first proposed in the 1980s [6] and since then it is driving major innovations in many sectors including food industry [7], cell biology [8] and pharmaceuticals [9]. MFC technology and 3D printing (rapid fabrication) were interlinked in 2010 where compartments of MFCs were fabricated and tested over Perspex material showing the advantages of 3D printed compartments, not only in accelerating the assembly process but also reducing the internal resistance of MFCs [10]. Many improvements have been achieved in the field of MFCs due to 3D printing including the fabrication of Nanocure® MFCs and their implementation on autonomous robots [11] as well as the emergence of new designs for easy assembly, such as twist n' play MFCs [12]. The era of 3D printing opened new roads for the improvement of MFCs' core materials such as 3D printed, ion exchange membranes [13] and electrodes [14].

It is aimed to develop monolithically 3D fabricated MFCs through the EVOBOT platform [15]. To facilitate the monolithic 3D fabrication process of the MFC, essential MFC components such as membrane, electrodes and even feedstock need to be optimised. The cells employed in this study had open-to-air cathodes with micro porous layer (MPL) and coated carbon veil as the cathode electrode. MPL is an activated carbon paste which is able to be extruded from the platform. A custom made, potentially extrude-able, single layer of terracotta clay was used as a semi-permeable membrane.

The materials tested were selected based on their properties, as these are critical at ensuring that maximum growth conditions are maintained within the MFCs, which will result in maximum power output performance levels. The ideal substratum has to have (a) the appropriate porosity, which will facilitate both access to the electrode surface for the microbes and free percolation of the liquid medium to reach all the colonized parts and (b) the appropriate conductivity, in order to encourage optimum surface reactions, between the microbial cells and the electrode surface. This is the key mechanism that maintains a fixed thickness biofilm on a given surface area of electrode material, since this direct conductance of electrons (charge transfer) is the primary mechanism of bacterial survival, under anaerobic conditions. The electrode surface acts as the end-terminal electron acceptor, which the microbes need (instead of e.g. oxygen) to anaerobically respire. The material must also be biocompatible, chemically inert, long-life and with a good structural integrity.

This study presents the results from MFCs' fed for the first time with soft materials (gelatin, alginate as a nutrient feedstock) and Nafion® as a negative control. The aim of the study was to test the power output response and the behaviour of the MFCs by feeding them with these different soft materials, which can all be potentially extruded from the EVOBOT platform.

2. Materials and Methods

2.1 MFC design

Twelve analytical size, cubic MFCs were employed for this experiment. The MFC chambers were laser cut from Polymethyl methacrylate (Perspex) sheets **(Fig. 1A)**. The cathode chamber was removed completely, in order to form an oxygen cathode MFC, and replaced with a Perspex framework, which sandwiched the 6 x 5 cm terracotta membrane (2mm thickness) with the 25 mL anode chamber **(Fig. 1B)**. A thick layer of silicone was deposited between the anode chamber and the cathode framework, which acted as a 'cushion' for the ceramic membrane, when bolted together. The screws used for the assembly where 5 mm nylon studding and nuts. The whole cells were then partially wrapped with Parafilm® M Sealing Film to ensure that the moisture was retained in the open-to-air cathode side (not shown in the figure).

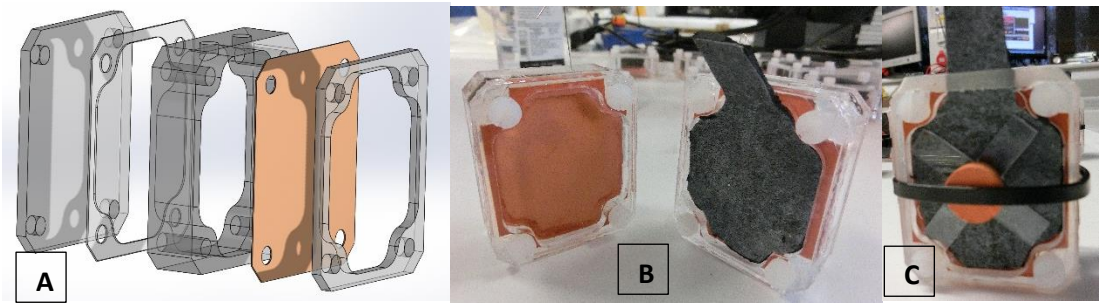

Fig. 1 – A. Computer aided design (CAD) of the optimised MFCs. B. Clay membrane and the attached MPL electrode. C. Open to the air cathode construction

2.2 Electrode Materials

Anode electrodes were constructed from untreated (catalyst free) carbon veil fibre, with 30g/m^2 carbon loading (PMF Composites, Dorset, UK) and a total surface area of 270 cm^2. The anode electrode was folded down five times, until the projected surface area was 8.45 cm^2 and was able to fit into the anodic half-cell (30 cm^2). A piece of nickel wire, approximately 10 cm in length, was pierced through the electrode to provide the connection point with the data logger and external load crocodile clip. The cathode electrode was made of two layers; a gas diffusion layer (GDL) and a microporous layer (MPL). The GDL was a single sheet of the same carbon veil material used for the anode electrode but coated with 30% polytetrafluoroethylene (PTFE) (Sigma Aldrich, UK). The sheet was left to dry for 24 hours in room temperature, and once the GDL dried the activated carbon paste was applied on top to form a thick layer of MPL (2 mm). The MPL was a mixture of activated carbon powder (G.Baldwin & Co., London, U.K.) blended with PTFE in a 4:1 ratio and deionised water (120 mL). The activated carbon paste was then hot pressed, using a household iron [16], and subsequently heated for 15 minutes to 200 ^{o}C to allow MPL liquefaction. The cathode electrode sheet was directly attached onto the exposed membrane but in order to ensure the electrode-membrane contact, a thin (0.5 mm) Perspex cross was pressed against the electrode using a cut-to-shape cork **(Fig. 1C)** that was tightened with a cable tie.

2.3 Membrane preparation

Red terracotta earthenware clay was used for the membrane fabrication. The terracotta was worked with a pastry roller in order to remove air bubbles and until it reached 5 mm of thickness **(Fig.2)**. Then, it was processed using a pasta making machine until it reached 2.5 mm thickness. The flat sheet was then cut to size (6 x 5 cm) and placed between two sheets

of wood to absorb the moisture and dried for 12 hours. The membranes were then kilned at a temperature of 1070 ^{o}C, which cured the materials through structural bonding of the clay.

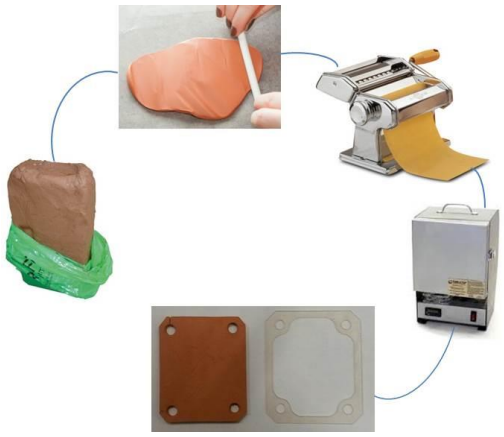

Fig. 2 – In-house preparation technique for custom made clay membrane

2.4 Inoculation process and load condition

Activated sludge, which was supplied by the Wessex Water Scientific Laboratory (Saltford, UK) was used as the initial inoculum. The sludge (25 mL) was injected manually into the sterile chamber and the experiment initially started in batch mode, but then turned into continuous flow. Three sludge exchanges occurred in the first three days of the experiment by emptying the chamber and re-filling it with fresh inoculum. For the next three batch mode feedings the inoculum used was sludge with tryptone (1%) and yeast extract (0.5%) (TYE) as a background nutrient solution, fed at a final concentration of 1:10. Due to inherent absorption/evaporation processes, the experiments turned into continuous flow on the 18th day of the experiment. The flow rate of the constant pumping was 0.5 rpm (4.2 mL.h^{-1}) and the hydraulic retention time (HRT) was 7.77 h. The feeding regime was full strength (1.5%) TYE.

One hour after the first inoculation, once the open circuit cells performance reached a plateau, an external load of 2.7 kΩ was connected and this remained unchanged until the end of the experiment.

2.5 Feeding regime and process

After 18 days of sole 1.5% TYE feeding the triplicates of MFCs were fed with water-soluble pork-derived gelatine powder (240 Bloom Type A, MM ingredients, UK) , sodium alginate (pure powder, Minerals Water, UK) and as a negative control, liquid Nafion® perfluorinated resin solution (Sigma Aldrich, UK). TYE (1:10) was also used as a background solution in all experiments. To ensure that the same weight of nutrients was added, the final concentration of the target material into the solution was 2%. This concentration was selected after testing different ratios in order to obtain a liquid state with sufficient low viscosity that will enable it to be pumped through the tubes without causing blockage. The control triplicate was fed with neat human urine and 1:10 TYE background solution, where pooled urine was obtained from anonymous healthy individuals. The cells were fed in continuous flow and this was maintained using a 16-channel peristaltic pump (205U, Watson Marlow, Falmouth, UK) with a flow rate of 0.5 rpm (4.2 mL.h^{-1}).

2.6 Data recording and analysis

For the data collection, MFC output was recorded in millivolts (mV) against time using Agilent Keysight 34970A Data Acquisition / Data Logger Switch Unit (Keysight Technologies, UK) with a 3 min sample rate. Data were processed and analysed using MS Office Excel and GraphPad Prism® version 5.01 software package (GraphPad, San Diego, California, U.S.A).

Current (I) in amperes (A) was calculated using Ohm's law, I = V/R, where V is the measured voltage and R is the known value of the external resistive load in ohms (Ω). Power (P) in watts (W) was calculated by multiplying voltage with current: P = I x V [17].

2.7 Polarization Experiment

Polarization experiment was performed using a resistorstat device, by sweeping 37 resistor values covering the range of 3.74 Ω - 30 kΩ [18]. The resistance load was changed every 3 minutes; however data recording occurred every 30 seconds (6 readings per load value). Polarization curves were generated from the collected data.

3. Results

3.1 Batch mode inoculation period and continuous flow operation

The power output obtained from the microorganisms' inoculation procedure with neat sludge, is highlighted in **Fig. 3A**. The loaded cells were able to develop a visibly dense biofilm over the electrode, which gave approximately 20μW of power output. After the inoculation and colonisation phase, once the electrode biofilm was exposed to sludge in 1.5% TYE (1:10), the power output was increased by three-fold **(Fig.3B)**. Although the performance of the cells was consistent and repeatable, a high evaporation loss caused the anode chamber to dry within 96 hours, leaving behind semi solid sediment at the bottom of the chamber, and having a deteriorating effect on the performance of the cells. An almost zero power performance was recorded after the anode chamber was left to dry out completely **(Fig. 3C).** As can be seen from **Fig. 3D**, once the cells turned to continuous flow operation, the power output increased by 0.4-fold. **Fig. 3** shows the consistency of the twelve cells' behaviour when all of them were fed with the same feedstock at the same flow rate.

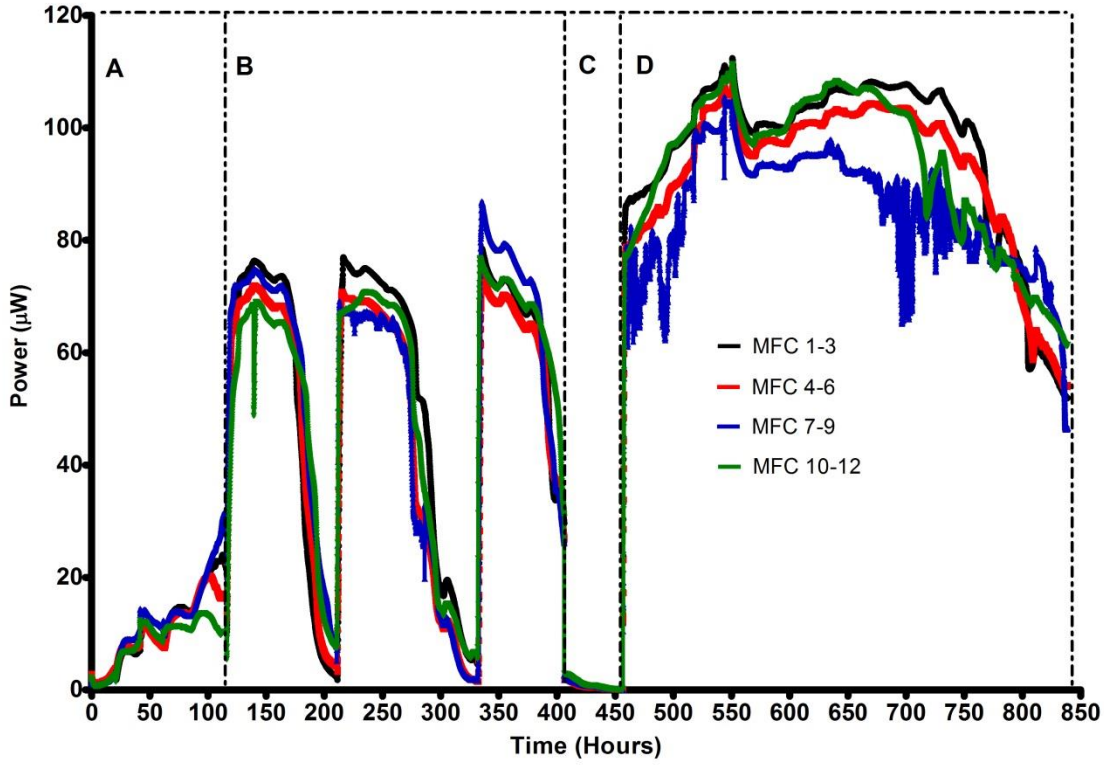

Fig.3 - Power profile of the inoculation process and initial feeding of the twelve MFCs Area A represents the batch mode inoculation period of the fuel cells with neat sludge. In area B the cells were fed with TYE and sludge (1:10). The power decrease was related with the absorption loss of anolyte liquid, due to the clay membrane. Area C highlights the total dry period of the cells while area D shows the behavior of the cells after turned into continuous flow.

3.2 Initial Response of cells fed with soft materials

After feeding the 12 MFCs with TYE for 18 days, the two triplicate groups, namely MFCs 4-6, and MFCs 7-9, were fed with the target polymeric feedstock substrates gelatin and alginate, respectively, whereas and MFCs 10-12 were fed with the negative control, Nafion. MFCs 1-3 were fed with the positive control urine medium. Even though the feedstock switching had a slight decreasing effect on the MFC power output for the first 10 hours, after this period the performance levels began to diverge **(Fig. 4B)**. The profile of the first 5 days showed that the urine fed MFCs' performance improved, compared with the soft material fed MFCs whose performance decreased. Similar decreasing profiles were identified from alginate and gelatin, with the only difference being that gelatin was more than two-fold higher in power performance than alginate. A possible explanation for the superiority of gelatin over alginate is the difference in the calorific value of the two substances (gelatin: 329 kCal/100g – alginate: 248 kCal/100g). As stated in section 2.5 the dilution of the compounds was standardised based on their viscosity and not their calorific value. As shown in **Fig.4**, the performance from the Nafion fed MFCs deteriorated rapidly over time.

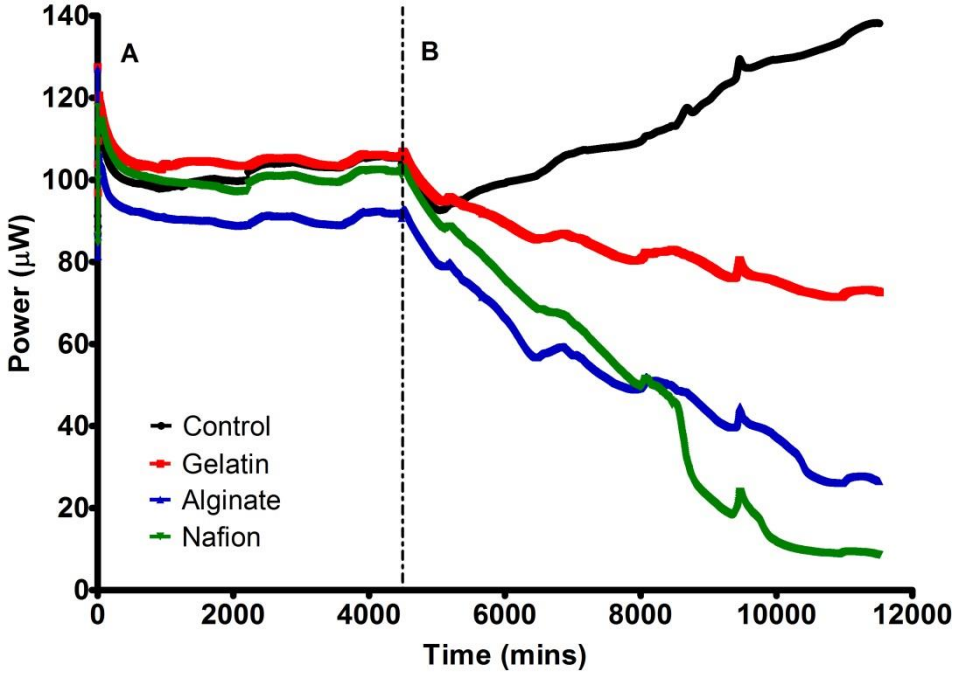

Fig. 4 - Time profile of MFC's response after feeding with soft materials for the first time.

The last three days of the 18-day period is presented in the graph (area A) followed by the response once the feedstock changed (area B). The fuel cells were fed with 1.5% TYE for the first 18 days, and then target soft materials added. Gelatin outperformed the other soft materials ($p < 0.0001$).

3.3 Overall performance and starvation period of the cells

The average power production of the MFCs fed with different soft materials is shown below **(Fig. 5)**. The data were consistent with the initial response to the change of feedstock. The urine fed control MFCs remained the highest in power output with the maximum being 149.23 µW; gelatin followed as the second best with a maximum power at 111.26 µW. The performance is represented also as area under curve shown in the graph of **Fig. 6A**.

While constant pumping was supplied to the MFCs the output was stable over time, however, when the feeding paused for ten days and cells left to starve, a different behavior was observed. Gelatin fed cells appeared to have better longevity as their performance gradually declined, and even for the first four days they had stable performance **(Fig. 5 - inset)**. The rate of decrease of the positive control (urine) cells was the fastest among all the others with a decreasing trend of 0.73 $\mu W.h^{-1}$. In all cases Nafion was consistently close to zero. The area under curve of the starvation period is presented in **Fig. 6B**.

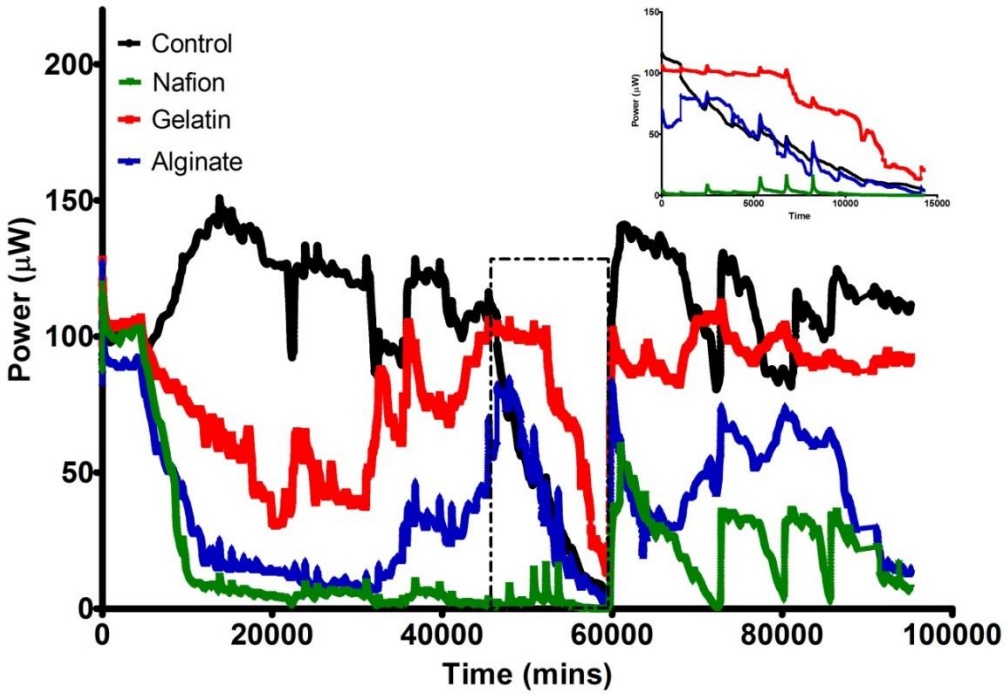

Fig. 5 - Average power production of MFCs after feeding with different soft materials. The highest absolute power output for the control was 149.23 µW and for the gelatin 111.26 µW. Starvation period (total 10 days) is highlighted in the dotted box, a magnification of which is shown as the inset graph. Gelatin fed MFCs decreased at the slowest rate, which was the reason for its higher power output.

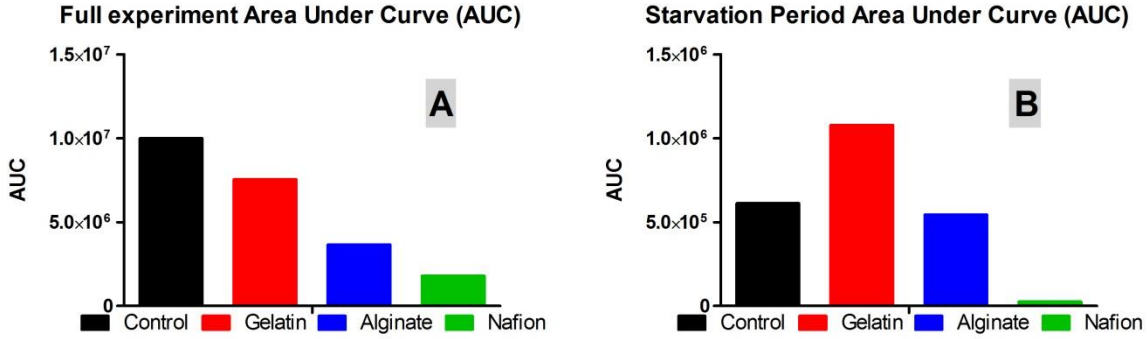

Fig. 6 – Area under curve (AUC) of the full experiment (A) and the starvation period (B).

3.4 Polarization Experiment

The polarization experiment was conducted two months after the start of the experiment. By this time the biofilm community was already well established and developed based on the 2.7 kΩ load. Polarization power curves **(Fig. 7)** are consistent with the power output data **(Fig. 6)**.

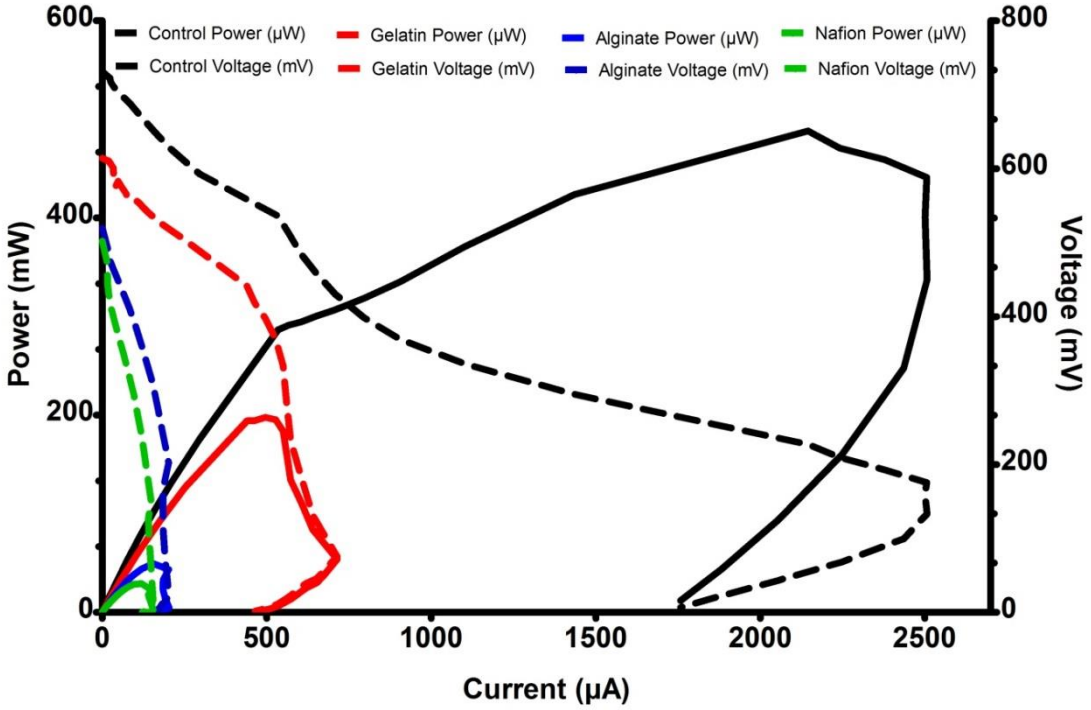

Fig. 7 – Power curves produced after two months operation on a fixed load of 2.7 kΩ

4. Discussion

4.1 Clay membrane complications in batch mode

Clay membranes possess a great advantage over the conventional proton exchange membranes (PEM) (eg. Nafion) because of their beneficial porosity, low cost, durability, as well as their ability to be 3D printed [19] [20]. On one hand, as the early results showed, the use of ceramic membrane in an open to the air batch mode fed MFC allow a significant percentage of water to be absorbed leaving the anode chamber almost dry having a detrimental effect on the performance. On the other hand, the always hydrated clay membrane in continuous flow offers a higher rate of proton/cation transfer [21] reflected by the higher output. Clay membranes have the potential to be extruded layer by layer from the Evobot platform, thus the three first steps of the custom made preparation of the membranes can be skipped.

4.2 Polarization experiment

The polarization experiment performed in this study was at the latter stage of the experiment, and cells were already operated at a stable resistance load. Studies suggest that early and regular polarisation experiments can determine the ideal resistance for maximum power production and by switching to that ideal load value the best power performance is achieved [22]. However other studies indicated that changing the external resistance does not improve the power output, as different combinations of microbial communities are developed based on each load that lead to comparable power outputs, showing the flexibility and resilience of MFC systems [23]. Thus, in this study it is believed that the unchanged load did not have a limiting effect on the MFCs' performance.

Nevertheless, an overshoot phenomenon was in fact observed in the polarization curves [17]. The overshoot phenomenon occurs when there is either a delay in or a prevention of charged molecules (ions and electrons) transfer, which results in decreasing the current at the same time as the voltage. This overshoot may have therefore occurred due to the complex nature of the substrates used (as well as the molecular weight/size) in conjunction with the flow rate (resultant HRT), which appear to have resulted in high mass-transfer (kinetic) losses. The power output recorded during the polarization experiments was much higher (>3-fold) compared to the levels recorded in the real-time temporal curves. This might be due to the short time of sampling (3 mins) during the polarization experiment, suggesting that the period was not sufficiently long to reach steady-state conditions for identifying the optimum resistance value for long-term experiments with a fixed load [17].

4.3 Selection of target materials

In this experiment gelatin, alginate and Nafion were tested as substrates with the prospect of being used in the future as bacterial substrata or membranes for the 3D printed MFCs. Each of these materials was selected because of its distinct properties. Gelatin and alginate are biodegradable and all the materials tested are biocompatible. It is quite evident that gelatin is a material that can be employed as both a substratum (3D extrude-able) and as a substrate (microbially utilise-able), and this forms part of future work. Nafion was only used as a negative control, due to its excellent ion-exchange properties, and the data show that if employed in an EVOBOT line of work, it will need to be supplemented with a carbon-energy substrate for sustaining bacterial growth.

4.3.1 Alginate and gelatin

Alginate or gelatin as well as pectin can be mixed with food proteins to be incorporated into the 3D printing process [7]. For these reasons we tested alginate and gelatin as possible printable feedstocks for the MFCs' bacterial community. This was due to the potential for being 3D printed and blended with carbon energy sources, as well as immobilising bacterial cells on an electrode surface and allowing their accumulation as a digesting biofilm.

Alginate is a polysaccharide and the second most abundant biopolymer in the world next to cellulose [24], and it is composed of mannuronic and glucaronic acid residues which are cross linked by calcium acids and form the ionotropic gel [7]. Alginate is derived from seaweed and has been used as a useful cell-immobilising (entrapment) technique in biotechnology due to its biocompatible properties as well as its ability to form heat-stable gels that can be developed and set in room temperature [24]. Some species of bacteria can hydrolyse alginate into cell transportable sugars with subsequent fermentation into short-chain fatty acids [25].

Gelatin is an animal derived protein which has been known to be used as gelling agent in early bacteriological media as a source of growth promoting substance [26]. However over the years, agar based media proved more suitable for bacterial cultivation than gelatin based media as gelatin cannot remain solid in temperatures above 37 ^{o}C (optimal condition for pathogen growth) and it can be digested by many bacteria. Bacteria possessing the enzyme gelatinase can break down gelatin into amino acids by hydrolyzing it [27]. Apart from their biochemical and physiological characteristics, both gelatin and alginate powders are considerably inexpensive substances (approximately £4-5/kg).

4.3.2 Nafion

Nafion is the main component of the commercially available proton exchange membranes (PEM) for MFCs as it offers excellent thermal and mechanical stability as well as conductivity. Nafion's high cost (liquid: £100/ 25 mL) though makes it an obstacle for MFCs scale up and practical applications. In addition Nafion membranes require activation/hydration prior to use and cannot be 3D printed, however the Nafion liquid mixed with polymers, can be deposited from the EVOBOT platform into a solid layer and form a thin layer of membrane. Even though, it is well known that Nafion is not a carbon energy source, in this experiment it was used as feedstock for the purpose to identify if a jelly form nafion membrane will cause biofouling [28] which is a common effect observed in Nafion membranes (anode side) or have a detrimental effect on the bacterial community.

4.4 Gelatin as 3D printable feedstock

The long-chain polymer composition of gelatin and chitin renders the feedstock to be longer lasting than monomeric substrates, which wash through the system or are quickly utilized. Whereas proteolytic enzymes (such as gelatinase) are commonly encountered amongst many different species of microorganism, the enzyme to hydrolyze chitin is thought to be relatively rare, but encountered more in marine species. Gelatin's outperformance over the other soft materials, and also its viscous characteristics make it a suitable material to be employed into the 3D process towards the aim of monolithically fabricated MFCs which can provide nutrients during a starving period or act as an endogenous store of fuel. The material employed in the 3D process can be any soft material which can be easily extruded using a RepRap EVOBOT machine and can also be used as a structural material.

4.5 EvoBot and 3D printing

The key to make MFCs more accessible is by simplifying the construction of MFCs through the use of 3-dimensional (3D) fabrication techniques. The 3D printed/extruded MFCs will not only speed up the manufacture of individual units, but can also help in automating the production process of many units for scale-up. This will benefit the electrical power output as rapidly fabricated multiple units can be stacked together to increase voltage or current output [29]. It is envisaged that the EVOBOT machine will add a layer of nutrient agar – in the case of flat surfaces – or continuously supplying nutrient broth – in the case of chambers – for microbial growth and maintenance. Both, the nutrient agar on flat surfaces as well as the nutrient broth for chambers, can be easily modified and supplied to the microbial communities to test a wide variety of conditions, with the energy being the response of selective pressure. The conductive element can be (initially) manually deposited, to allow the bacteria to conduct electrons and then they can be extruded from the 3D printer as well. The gelatin, as a feedstock and activated carbon paste as an electrode, serves the aim of the experiment as a suitable alternative printable substratum and electron acceptor. The optimised clay membrane can be extruded from the EVOBOT machine however at present the kilning process required for the membrane to become durable and functional, is prohibitive; other clay materials are currently being investigated.

4.6 Future work

This experiment can be optimised by feeding the fuel cells with feedstock that has been standardised based on calorific value (even urine), rather than based on utilizable-energy

concentration. This may provide a clearer picture between alginate and gelatin. In addition, alternatives for extrude-able membranes which do not require firing can be employed in order to have the membrane ready to be used in the platform after extrusion. A next step can be the extrusion of the materials through the EVOBOT syringe to form layer by layer a 3D printed MFC prototype.

5. Conclusion

This experiment shows for the first time that entirely 3D printed MFCs have the potential to be developed using the EVOBOT platform. Gelatin seems to be a promising soft material that can be 3D printed and can be used as a feedstock for MFC operation. Flexible materials such as ceramic clay used as a membrane, and activated carbon paste used as a cathode electrode can be used in analytical type MFCs with the potential to be 3D-printed. Further work will investigate different material combinations suitable for MFC fuel and compartments, which could be used as part of an entirely 3D printable fuel cell.

Acknowledgments

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