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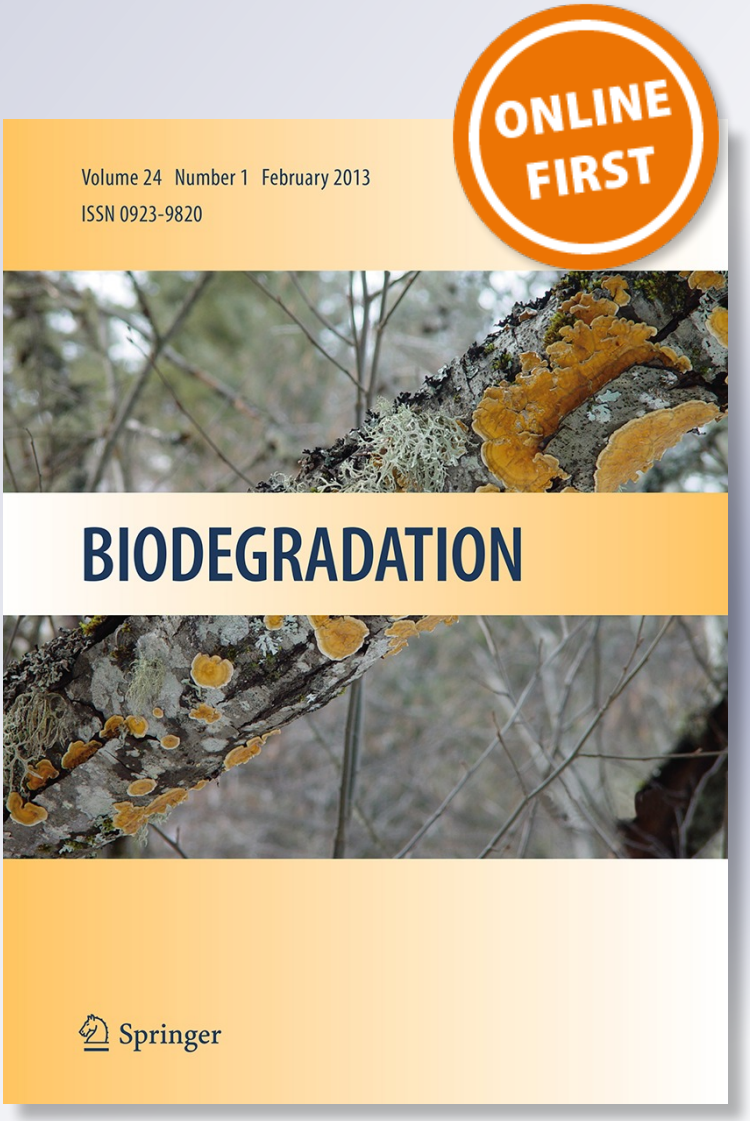

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ORIGINAL PAPER

Biodegradation and proton exchange using natural rubber in microbial fuel cells

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Abstract Microbial fuel cells (MFCs) generate electricity from waste but to date the technology's development and scale-up has been held-up by the need to incorporate expensive materials. A costly but vital component is the ion exchange membrane (IEM) which conducts protons between the anode and cathode electrodes. The current study compares natural rubber as an alternative material to two commercially available IEMs. Initially, the material proved impermeable to protons, but gradually a working voltage was generated that improved with time. After 6 months, MFCs with natural rubber membrane outperformed those with anion exchange membrane (AEM) but cation exchange membrane (CEM) produced 109 % higher power and 16 % higher current. After 11 months, polarisation experiments showed a decline in performance for both commercially available membranes while natural rubber continued to improve and generated 12 % higher power and 54 % higher current than CEM MFC. Scanning electron microscope images revealed distinct structural changes and the formation of micropores in natural latex samples that had been employed as IEM for 9 months. It is proposed that the channels and micropores formed as a result of biodegradation were providing pathways for proton transfer, reflected by the steady increase in power generation over time. These improvements may also be aided by the establishment of biofilms that, in contrast, caused declining performance in the CEM. The research demonstrates for the first time that the biodegradation of a ubiquitous waste material operating as IEM can benefit MFC performance while also improving the reactor's lifetime compared to commercially available membranes.

Keywords Microbial fuel cell · Latex · Biodegradation · Ion exchange membrane · Natural rubber

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Introduction

Microbial fuel cells (MFCs) generate electricity via the extracellular electron transfer of anaerobic microorganisms. The ability to produce power from a range of unwanted or undesirable sources makes the technology highly attractive and to date several end-applications have been proposed. These include: wastewater treatment (Habermann and Pommer 1991),

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direct biosensing of environmental fluctuations (Kim et al. 2003) and robotics (Melhuish et al. 2006).

Despite recent developments, there are several aspects that have prevented the scale-up of the MFC technology, such as: (i) the lack of a clear market (ii) high material costs and (iii) low per-unit power production. Low reactor output can be resolved by developing large stacks of multiple units, thus producing a higher collective power which has been shown to work without catalysts (Ieropoulos et al. 2010a). However, progress is hindered by the cost of materials such as catalysts used for the cathode reaction in the majority of MFC work and the ion exchange membrane (IEM). This study focuses on the IEM, which acts as a separator between the anaerobic anode and aerobic cathode and conducts protons between the two half-cells. Apart from increasing the cost, commercially available IEMs (e.g. Nafion®) are not as suited to MFC operating conditions (i.e. neutral pH and room temperature) than those of traditional chemical fuel cells (Biffinger et al. 2007). This can have a detrimental effect on the microbial community (Cord-Ruwisch et al. 2011) while also increasing the internal resistance of the MFC (Harnisch et al. 2009). A cheap, widely available material, conducive to microbial colonisation and also adaptable, might help boost the progress of the MFC technology.

Various studies have investigated novel alternatives, in particular 'size selective separators' (Li et al. 2011) that rely on the porosity of the material for proton transfer e.g. soil (Takanezawa et al. 2010), microfiltration membranes (Tang et al. 2010), ceramics (Behera et al. 2010) and the incorporation of a J-cloth against the electrode (Fan et al. 2007). The J-cloth proved efficient in a single chamber system by reducing oxygen diffusion to the anode, however the material's tendency to biodegrade has been regarded as a limitation to its practical application (Li et al. 2011). In contrast, the current study looks at exploiting the biodegradability features of natural latex rubber, a readily available waste material, as an efficient medium for proton exchange.

Over the last few decades, there has been an emphasis on improving personal protection both on an industrial level (i.e. scientific, medical, and technological) and on a personal level (infectious diseases). The demand for natural latex products (e.g. condoms, gloves, latex thread) has subsequently risen from 159,000 tonnes in 1983 to 597,000 tonnes in 2003 (Rajan et al. 2006). The majority of products are 'one-use-only' and thus considerable waste is generated. Further to this, up to 15 % of rubber products are rejected following the manufacturing process resulting in disposal problems for the rubber industry (Rajan et al. 2006). Recycling rubber would alleviate some of these problems and a novel form of resource recovery is reported here by employing natural latex (originating from condoms) as an alternative IEM.

Building MFCs from waste materials, for the purpose of treating waste, would add to the technology's appeal, particularly in terms of cost. In addition to waste material recycling, a further benefit in employing biodegradable materials in MFCs is that the reactor will degrade harmlessly into the environment when it reaches the end of its productive life.

Materials and methods

Microbial fuel cell design and operation

Single-chamber cubic MFCs were used as previously described (Ieropoulos et al. 2010b) with duplicates for all experiments. The anodic chamber geometric volume was 25 ml. Anodes were extracted from established MFCs that had been inoculated with activated anaerobic sludge (Wessex Water, Saltford, UK), whereas cathodes were freshly prepared. Both electrodes were constructed from 270 cm^2 untreated carbon veil folded into 3D rectangular cuboids with a geometric surface area of 17 cm^2. The membranes used were either cation or anion exchange (VWR, Leicestershire, UK) or natural rubber latex cut from polyisoprene condoms (Mates, UK). Prior to use, the latex was washed in water and soap to remove surface spermicide. All membranes were 6 × 5 cm and held between the anode and cathode chambers, with neoprene gaskets for water tightness. The open-to-air oxygen-cathode electrodes were held in position using parafilm. For all MFCs, 1 kΩ external resistors were used, and they were fed in batch-mode with tryptone (1 %) yeast extract (0.5 %) once a week, while the cathodes were hydrated daily with tap water.

Data collection

Electrode output was recorded in volts (V) against time by using an ADC-16 Channel Data Logger (Pico Technology Ltd., Cambridgeshire, UK). Recorded

data were processed and analysed using the GraphPad Prism version 4 software package (GraphPad, San Diego, California, USA). Current (I) in amperes (A) was calculated using Ohm's law, $I = V/R$, where V is the measured voltage in volts (V) and R is the known value of the external load resistor in ohms (Ω). Power (P) in watts (W) was calculated by multiplying voltage with current: $P = I \times V$.

Scanning electron microscopy

After 9 months of operation, one of the latex MFCs was taken apart for scanning electron microscopy (SEM), and the biofilm on the anode and cathode sides was observed. Other samples were gently rinsed with water to remove biofilm so that the surface structure could be observed. Samples were fixed in 4 % glutaraldehyde in phosphate buffered saline (PBS) for 1 h at room temperature. The samples were then rinsed in PBS for three 1 h periods before storing at 4 °C overnight. The following day, samples were dehydrated by a graded ethanol series (20, 30, 50, 70, 90 and 100 %; at 5 min stages), then 100 % ethanol/hexamethyldisilazane (HMDS) at a ratio of 2:1 then 2:2 and then 1:2 before three rinses with 100 % HMDS; each stage lasted 5 min. Samples were prepared for microscopy by gold sputter coating using an Emscope SC500 sputter coating unit. Samples were observed and images captured using a Philips XL30 environmental scanning electron microscope (ESEM).

Polarisation experiments

Polarisation experiments were performed after 6 months using manually operated variable resistors (Centrad Boite A Decades De Resistances DR07). Power curves were also produced after 11 months using an automated computer-controlled variable resistor as previously described (Degrenne et al. 2012). In all cases, 59 resistances were connected at 5 min intervals. One day prior to the polarisation sweep, the MFCs were drained and fed with fresh anolyte whereas the cathodes were hydrated immediately before the experiment. Due to the destructive nature of preparation for SEM analysis, only one established latex MFC remained operational (after 9 months) and so data presented (both 6 and 11 months) are based on repeat sweeps of that latex MFC compared to the CEM and AEM MFCs.

Results and discussion

Start-up period

Natural latex rubber MFCs with established anodes produced no output for an initial period of between 1 and 3 weeks, after which a working voltage was measured (Fig. 1). Once the first current was registered the latex MFCs showed a continuous improvement over time.

Power curves

After 6 months of operation, polarisation experiments were performed and Fig. 2 shows CEM to be the most powerful, producing109 % higher power (143 μW) and 16 % higher current (1,453 μA) than the MFC with natural rubber as membrane. On the other hand, the natural rubber MFC outperformed the commercially available AEM, by generating 143 % higher maximum power (68 μW) and 218 % higher maximum current (1,248 μA). After 11 months of operation, the latex MFC continued to improve, outperforming both the AEM and the CEM MFCs (Fig. 3). The maximum power produced by the latex MFC (99 μW) was 12 % higher than the CEM MFC, while the maximum current (1,480 μA) was superior by 54 %. A common problem with the use of proton exchange membranes in MFC is biofouling, an occurrence that can detrimentally affect performance over short periods of time (Xu et al. 2012). This could

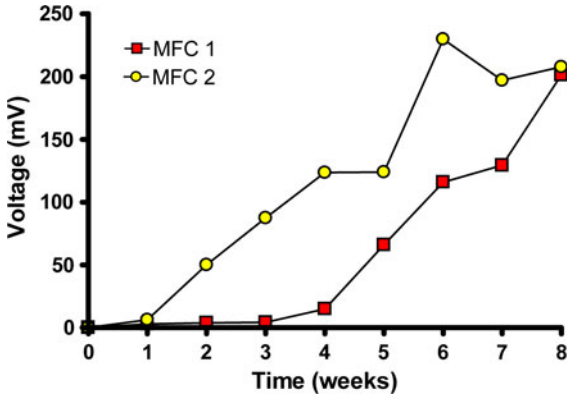

Fig. 1 Average voltage output produced from two MFCs with natural rubber membrane under 1 kΩ during the first 8 weeks of operation

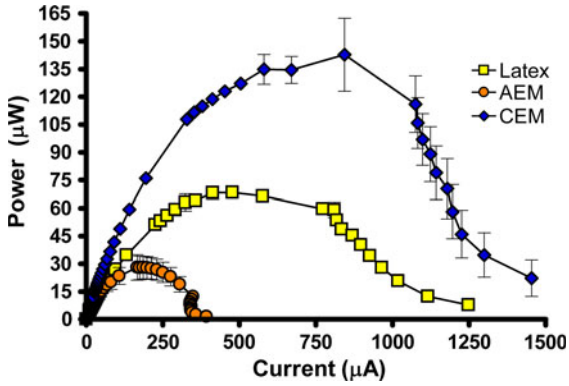

Fig. 2 Power curves produced after 6 months operation for MFCs with natural rubber latex, anion or cation exchange membranes. Error bars indicate mean and standard error of the mean ($n = 2$)

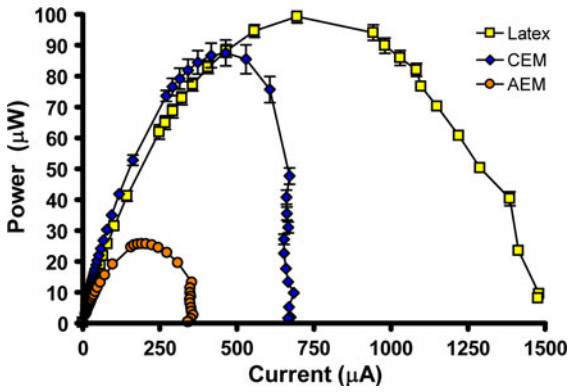

Fig. 3 Power curves produced after 11 months operation for MFCs with natural rubber latex, anion or cation exchange membranes. Error bars indicate mean and standard error of the mean (n = 2)

help explain the drop in power and current in both types of IEM, while latex showed no deterioration despite a considerable biofilm formation on both sides of the material.

MFCs are often described as biological fuel cells because power is generated from the microbial contingent. One of the problems with the technology is that reactor components—such as IEMs—have been built/designed with established technologies in mind, resulting in problems such as biofouling (Xu et al. 2012), pH splitting and increased ohmic resistance (Harnisch et al. 2009). The latex potentially adds a new biological dimension to the MFC. On the one hand the electro-active anodic organisms are required to generate electrons, while on the other the rubber-degrading organisms promote the passage of protons through the membrane.

Biofilm and biodegradation

After 9 months, one of the latex MFCs was taken apart to analyse the latex material. When the membrane was removed, two distinct biofilms were visible on each side. Figure 4a shows the cathodic side and Fig. 4b shows a representative SEM image highlighting the tangle of fungal mycelia produced at a higher magnification from the cathode side. The anodic side (Fig. 4c) was dominated by bacterial growth, however a small proportion of mycelia were also observed (Fig. 4d).

Previous work has shown that the mechanical introduction of a single pore into an impermeable separator (plastic bag) resulted in an immediate burst of current (Ieropoulos 2006). It is by a similar mechanism that we propose the latex to be able to operate as IEM but instead of mechanical, through the microbial introduction of micropores. Figure 5a shows the smooth surface of unused natural latex material. After 9 months inside a MFC functioning as IEM, the latex structure had undergone distinct structural changes as exemplified in Fig. 5b. Here black pores ($\sim$1–2 μm diameter) can clearly be seen. Microorganisms are known to utilise natural rubber (polyisoprene) as a carbon source by the production of mycelial corridors that excrete rubber-decomposing metabolites (Linos and Steinbüchel 2005), and the black pores in Fig. 5b, may have been the result of this mechanism.

A second mechanism of microbial utilisation is through adhesive growth on the rubber material resulting in the production of colony craters, similar to the ones shown in Fig. 5c. A cross section of the latex can be seen in Fig. 5d, where pores and craters extend throughout the material. After 9 months of MFC operation, it was observed that the latex had weakened significantly and could be easily torn, unlike the unused latex that was very elastic and difficult to tear. Future work will investigate further whether the microbial rubber degradation emanated from the anodic side, possibly by anaerobic mycelium-forming actinomycetes (Rose and Steinbüchel 2005), or from the cathodic side that was dominated by fungi (Atagana et al. 1999).

Attempts have previously been made to develop membranes by incorporating polyisoprene into the structure of chemical fuel cells (Sodeye et al. 2011), but also hybrid organic–inorganic membranes are

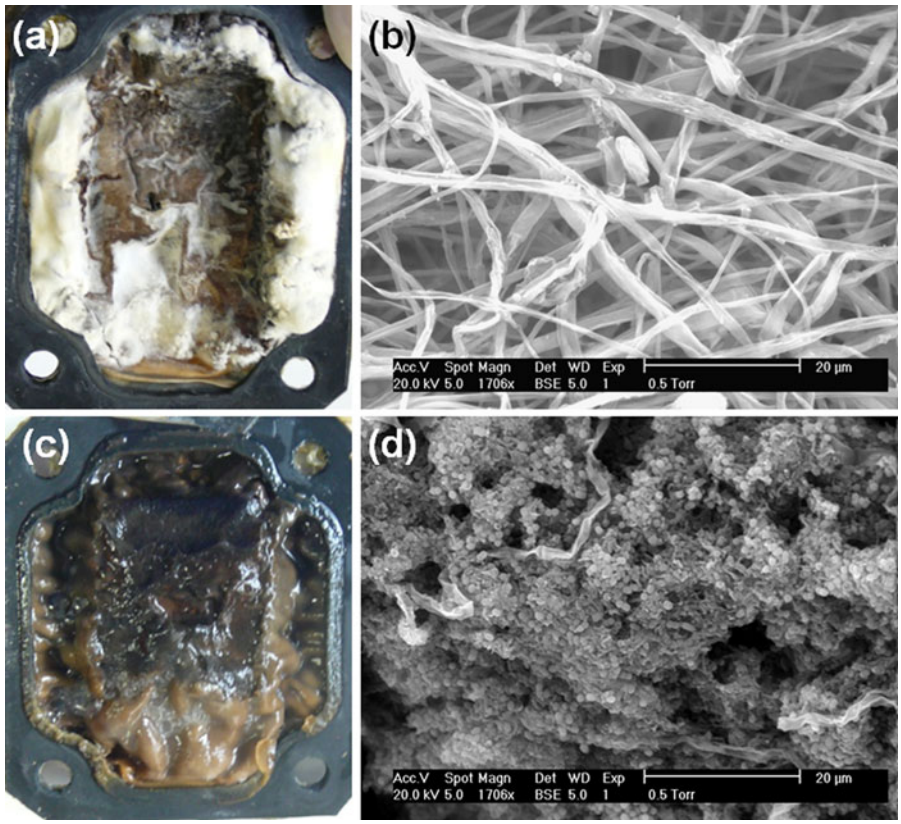

Fig. 4 Biofilm growth on natural rubber membrane; (**a**) photograph showing side exposed to cathode. (**b**) SEM image showing biofilm on cathodic side. (**c**) photograph showing side exposed to anode. (**d**) SEM image showing biofilm on the anodic side

being developed using natural rubber for Polymer Electrolyte Membrane Fuel Cells (Sanchez et al. 2005). To the authors' knowledge, this is the first time that potential 'waste' natural rubber has successfully been employed as IEM in microbial fuel cells.

Slow start-up and temporal improvement

There are various explanations for the slow start up of the natural rubber (condom) MFCs. Firstly, it was thought that residual spermicide could have had a detrimental effect on the microbial community. To verify this, small pieces of washed condom (1 cm^2) were fed into working MFCs with no decline in performance recorded (data not shown). Secondly, even specialised rubber degrading monocultures can require inoculation periods of several months (Rose and Steinbüchel 2005) and so in the present study, where wastewater sludge was used as inoculum, slow microbial latex-degradation might be expected. Furthermore, manufacturing processes can prolong the microbial cultivation period on rubber products such as condoms and latex gloves (Linos et al. 2000). Examples include the addition of antioxidants and microbicidal substances or the removal of potentially allergenic proteins (Rose and Steinbüchel 2005). Methods can be employed to enhance the growth of rubber-degrading strains for example Berekaa et al. (2000) improved the microbial degradation of latex gloves by extracting with organic solvents prior to inoculation. The selection of a condom as the source of latex for the current study was made on the basis of its elasticity, availability, low cost and high quality. However, other forms of natural latex could be more accessible to the microbial consortia, resulting in an accelerated start-up process. Careful selection of the biodegradable material, can dictate the endurance and hence the application of such MFCs.

In order to develop a MFC constructed solely of biodegradable or waste products, further work will

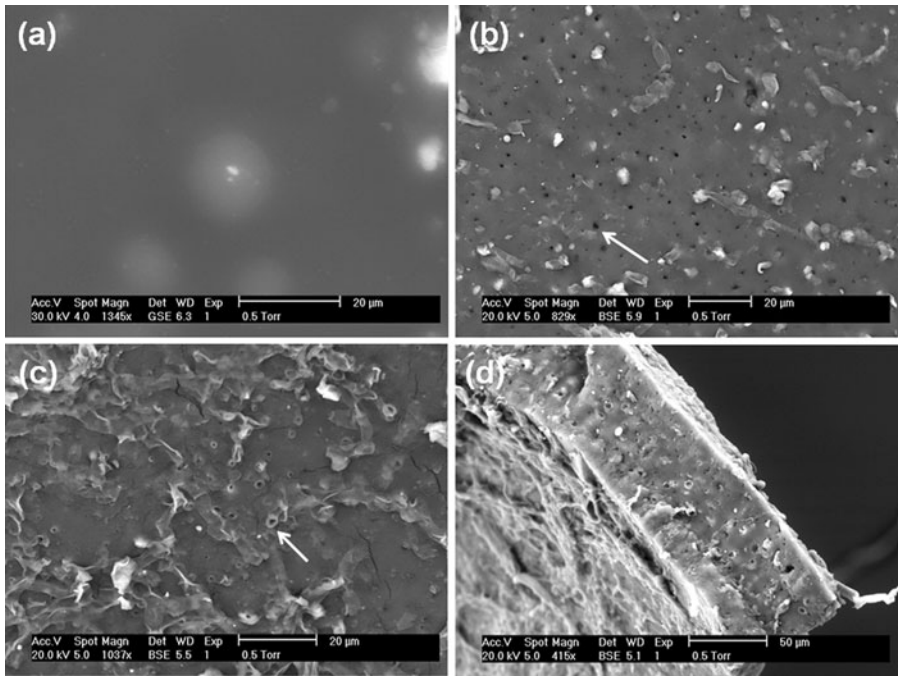

Fig. 5 SEM images comparing clean natural rubber with samples employed as ion exchange membrane (IEM) in a microbial fuel cell for 9 months; (**a**) surface of unused (clean). (**b**) surface after operating as IEM, *arrow* indicates *black* pore. (**c**) surface after operating as IEM, *arrow* indicates *crater*. (**d**) cross section after operating as IEM

need to be carried out in order to identify novel materials that might be used for other reactor components, e.g. electrode and current collection. Some recent examples of alternative low cost materials include graphene covered sponge anodes (Xie et al. 2012) and the production of an efficient anode using recycled tyre crumb rubber coated in graphite paint (Wang et al. 2011). In addition, despite the fact that latex is biodegradable, it also appears to be less prone to the problems that accompany IEMs, such as biofouling and so potentially could offer a longer operational life with less maintenance than conventional non-biodegradable, membranes.

To date the latex MFC has been running for nearly a year without failure, and work will continue to characterise the longevity of the system and whether the material will eventually degrade to the stage that short circuiting occurs. Previous work has indicated that the presence of a mature biofilm can be sufficient to enable proton exchange in the absence of an IEM (Santoro et al. 2012) and so potentially the latex could degrade completely with the remaining biofilm performing the role of proton exchange. Further work will also investigate alternative natural rubber materials to examine differences in the initial start-up stages, power performance and degradation.

Conclusions

In this study we have demonstrated the ability of biodegradable natural rubber to act as an IEM. Initially, MFCs employing the natural latex as IEM took up to 3 weeks to register a working voltage but exhibited gradual improvement thereafter. Following 11 months of operation the latex outperformed both cation and anion exchange membrane MFCs and to date, after almost 1 year, is still operational. SEM images showed pores and craters on the latex surface indicating that biodegradation was responsible for proton exchange. The study demonstrates that the use of biodegradable materials not only presents a cheap alternative but appears to be a legitimate substitute for commercially available IEMs. The longevity of MFCs may be extended because biofouling does not appear to impede performance, and temporal degradation can

even lead to improved performance. Further work will investigate the lifespan of natural latex MFCs and the use of alternative forms of rubber.

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