



# Proof-of-concept for a novel application for in situ Microfluidic Benthic Microbial Fuel Cell device (MBMFC)

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## ABSTRACT

Benthic Microbial Fuel Cells (BMFC) are an environmentally compatible, carbon-neutral energy resource that can operate in marine sediments and provide underwater power. BMFC performance is dependent on both biological factors and engineering materials and design. The biological component, being less predictable in nature, is typically controlled in laboratory settings to optimize fuel cell performance. However, this study seeks to improve the in situ performance of BMFC power production through augmenting engineering design factors. Decreasing the distance between the electrogenic bacteria and the capture electrode could be a solution to improve the BMFC performance for in situ anode devices. To evaluate this, a layered microfluidic elastomeric on quartz chip was fabricated to confine the bacteria within  $\sim 90 \mu\text{m}$  from the chrome microelectrode matrix patterned onto the chip's quartz substrate. The device served as a Microfluidic Benthic anode connected with a carbon cloth cathode to form a Microfluidic Benthic Microbial Fuel Cell (MBMFC). The MBMFC units were placed in sediment under flow-through laboratory conditions and power generation was recorded. Typical membrane-less microbial fuel cells in flow-through seawater laboratories or in situ conditions, have power production ranges  $3\text{--}40 \text{ mW/m}^2$  with steady state power averaging  $8\text{--}10 \text{ mW/m}^2$ . The results from these MBMFC devices demonstrated power density of  $30\text{--}120 \text{ mW/m}^2$  with steady state production levels  $20\text{--}80 \text{ mW/m}^2$ . Conservatively that is 3 times higher than previously recorded BMFC units in sediments from San Diego Bay, and an 8-fold improvement in steady-state production. However, in consideration of the immediate ramp-up time and steady-state power production, it is a marked improvement to traditional in situ BMFC performance. This serves as a proof-of-concept for a scalable in situ microfluidic device that could serve as a future potential power source. The presented approach may offer a testing platform for further optimizations in MBMFC research and development.

## 1. Introduction

Microbial fuel cells (MFC) employ facultative or strict anaerobic bacteria to oxidize organic matter and produce direct electrical current [1,2]. A Benthic MFC (BMFC) uses indigenous bacteria that live in the sediment of the ocean floor [3]. Marine sediment is teeming with microbial activity crucial to marine biogeochemistry [4,5]. As part of their living processes, benthic bacteria expel electrons, which are collected by a capture anode. The charge then passes through a load to a cathode

floating in the seawater column above. The voltage between the anode and the cathode typically ranges from 200 mV to 1 V and it can be used to extract power from the BMFCs [6–11].

BMFCs have been shown to produce power to small, low power sensors, such as to a magnetometer [7] and autonomous sensors [8,9]. However, the average output power density of in-situ units is relatively low, e.g.,  $3\text{--}40 \text{ mW/m}^2$  [6,8–12]. Although, many microbial fuel cells in laboratory studies have demonstrated power generation on the order of  $1000 \text{ mW/m}^2$  and greater, these are usually in fed systems under

**Abbreviations:** MFC, Microbial Fuel Cell; BMFC, Benthic Microbial Fuel Cell; MBMFC, Microfluidic Benthic Microbial Fuel Cell; NPS, Naval Post Graduate School; CMR, Center for Material Research; PDMS, polydimethylsiloxane; MCRD, Marine Corps Recruitment Depot; NAG, N-acetylglucosamine; NIWC, Naval Information Warfare Center.

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optimum conditions [13–17]. For units that are placed in sediments without membranes to better simulate the marine environment, some studies have improved power density by modification of the cathode [18–21], anode [22–24], or both [6,25–27]. Others have explored different electrode spacing, various surface modification of carbon derived materials, such as carbon felt [26,28], and different organic additives, such as acetate, which have been found to boost MFC performance [1,6,27,29–33] and other food sources, such as chitin [12].

Some parameters to optimize BMFC technology may include concentration of the bacteria, the geometry of the capture electrode, and the average spacing between the cells and the electrode [27]. Although a higher cell density is expected to produce higher power output, due to the nature of microbiological systems requiring a balanced environment, beyond a certain concentration the system is likely to experience diminishing returns and perhaps even a decrease in output power [34, 35].

Due to their size, most bacterial biofilms exist wholly within low Reynolds ( $Re$ ) number regimes where viscous forces dominate [36]. The Reynolds number is a dimensionless ratio in hydrodynamics which can indicate whether flow is expected to be laminar or turbulent. For bacteria in marine sediments,  $Re < 1$  [36,37]. Thus, bacterial movement is based on instantaneous forces (i.e. diffusion) within laminar regimes. Elastomeric microfluidic chips provide a platform that offers  $Re < 1$  environments complimentary to the typical microbial inhabitants [38, 39]. They are common in biotechnology [40], used for cell culture work [41], biomedical diagnostics [42], and embedded electric measurements [43].

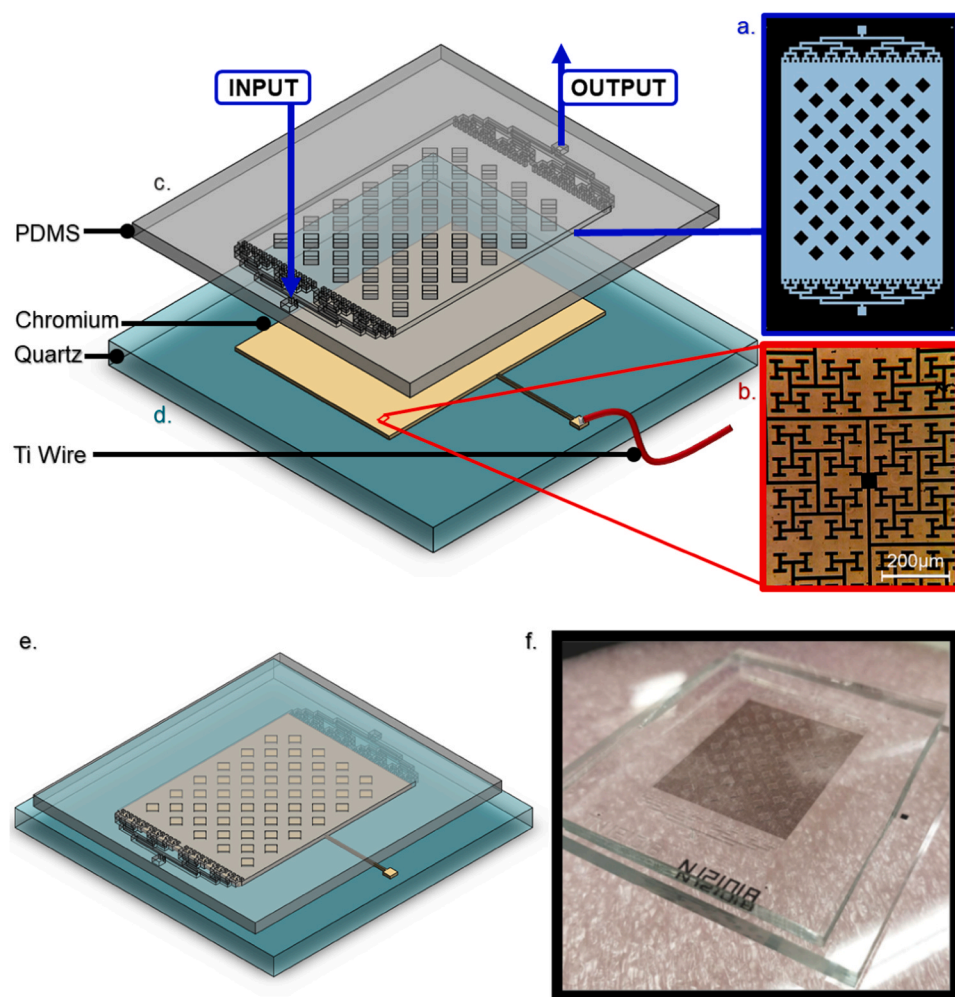
The ability to combine elastomeric microfluidic chips with electrical measurements is well established and has already been shown to increase capabilities toward the characterization of microbial activity [40, 44] through fabricating metal electrodes on glass slides that serve as the substrate layer of the elastomeric chip [43]. These capabilities present an enabling technology for a renewable bioelectrical power generation platform, where fluidic control in a non-toxic bacterial housing within microscale parameters is possible. It was hypothesized that power output may be accordingly improved by shortening the distance between the bacteria and the anode architecture [27,38].

This study describes the design and construction of microchips specifically developed for the microbes present in marine sediment, with the specific goal to improve the capture efficiency of the electrons at the anode. A microfluidic chip was designed and developed that confined the bacteria to within 90  $\mu\text{m}$  of the anode matrix to demonstrate improved BMFC performance utilizing microstructures as a proof-of-concept. The system geometries and experimental conditions may be optimized in future microscale test platforms to later extend to multiple arrays that could power small sensors underwater.

## 2. Materials and method

### 2.1. Microfluidic elastomeric component fabrication

AutoCad 2018 implemented in designing the mold pattern for the elastomeric layer of the MBMFC anode (Fig. 1A). The elastomeric layer was designed specifically for BMFC performance. Briefly, a single input



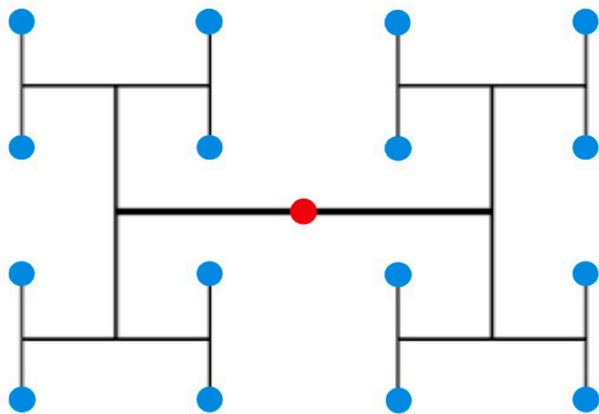
**Fig. 1.** MBMFC components anode chip exploded view, assembled view, and photograph as follows: (a) Schematic of the mold pattern for the PDMS layer showing microchannels/cavities (white) and solid silicone (black), (b) Microscope image of the H-fractal chrome anode patterned on a quartz substrate (c) 3D model of PDMS top layer molded with microchannels and dome cavity, (d) 3D model of Quartz substrate with H-fractal, patterned chrome electrode. (e) 3D model of MBMFC full assembly, (f) Photo of MBMFC device.

for the bacteria was split into a feed without bias through a binary dendritic structure made of 100  $\mu\text{m}$ -wide channels to a central dome of vertical height of  $\sim 90\text{ }\mu\text{m}$ . The dome height confined the bacteria accordingly to within the same distance of the substrate. The square structures in the dome serve as pylons preventing the collapse of the chamber. Standard reagents and laboratory techniques were used in fabrication of the elastomeric microfluidic channels and architecture [45]. Measured on a KLA Tencor Alpha-Step profilometer, the soft-lithography mold for the elastomeric component had height between 88 and 95  $\mu\text{m}$ . To be used in that mold, polydimethylsiloxane (PDMS) was fabricated using a spin speed 1500 rpm mixing stage and 2200 rpm degassing stage. Geometry of the elastomer top is shown in Fig. 1C. The microfabrication was performed in the Naval Postgraduate School (NPS) Center for Materials Research (CMR) clean room in Monterey, CA.

## 2.2. Microelectrode design and fabrication

The substrate of the chip (Fig. 1D) contains a chrome microelectrode matrix, whose pattern follows the fractal H-architecture shown in Fig. 1B. The distance from a centroid to any node in a planar rectangle is made equal through fractal binary splitting (Fig. 2). By alternating reflections along orthogonal bisecting lines, the pattern grows by a factor of  $2^N$ , where  $N$  is the number of recursions, while preserving its basic properties. This architecture removes the location bias in the connectivity of a two-dimensional system [46–48]. In the case of this device, it ensures that the distribution of bacteria across the device would not affect the output power through a bias in the electrical resistivity of different pathways to the output of the anode. As a result, cross-device comparisons would be less affected by variability from the spatial distributions of the bacteria. The electrode matrix had 40  $\mu\text{m}$  scaling (distance between closest nodes of the H-architecture), which compared appropriately with the scaling imposed by the height of the dome. Thus, the developed microfluidic devices were modified to be used for environmental experiments such as energy recovery for BMFC technology.

AutoCAD 2018 was also used to draw the  $4\text{E}-4\text{ m}^2$  H-fractal microelectrode design. The pattern was sent to Photomask Portal Inc. Richardson, TX, for fabrication. This laser-written etch mask was used for the chrome electrode matrix on the quartz substrate to serve as the final microelectrode device for this application. A Pro-4 Four Point Resistivity System was used to determine sheet resistance:  $15\text{ k}\Omega$ , resistivity  $1.5\text{ }\Omega\text{-}\mu\text{m}$ , and  $V/I\text{ }3.4\text{ k}\Omega$ .



**Fig. 2.** H Architecture exhibits fractal binary splitting behavior. The distance from a centroid (red) to any node (blue) in a planar rectangle is made equal by via fractal binary splitting (black), ensuring no bias. The structure can continue to replicate, growing in size while retaining its basic properties. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 2.3. MBMFC anode assembly

The microfluidic PDMS layer and chrome H-fractal microelectrode substrate layers and were aligned and assembled to form the completed MBMFC (Fig. 1E and F), ensuring that the fractal matrix is contained inside the dome structure. The resulting chips were baked at  $80\text{ }^\circ\text{C}$  to improve adhesion. Titanium wires were connected to the chip electrodes using a cold solder conductive silver epoxy (#8331-14G, MG Chemicals) and insulated with electrical tape (Nashua Marine). Because of connectivity issues observed in the first trial; the second trial included the use of liquid electrical tape (Star Brite) over the soldering point between the electrode pad contact and the Titanium wire.

## 2.4. Device solution loading and experimental setup

Before being buried in sediment as shown in Fig. 3, the MBMFC anode chips we injected with various microbial suspension solutions in order to compare their performance. Some devices were only injected with filtered saltwater, while others included nutritional additives of short chain fatty acids comprised of 2, and 4 carbon (C) compounds, and N-acetylglucosamine (NAG), which is a fermentation product of chitin; all are known to be reliable nutritional sources for electrogenic bacteria [1,49]. Prior to chip placement into the beakers, the sediment was allowed to settle for two days allowing any sediment suspensions in the beakers settled to the bottom. Then beakers were placed in a flow-through tank system filled with seawater, to ensure similar environmental conditions for all samples. The tanks were supplied with flowing seawater and two aquarium air-bubbler pumps to provide an influx of fresh seawater and to avoid stagnation. The bubblers that were used in the tanks to simulate the turbulence of marine environments.

Sediment collected from San Diego Bay, Marine Corps Recruit Depot (MCRD) ( $32^\circ 44' 20.65''\text{ N}$ ,  $177^\circ 12' 31.42''\text{ W}$ ) served as the source from which the microbial slurry for the second trial was extracted. Previous laboratory and field experiments showed that MCRD sediment is an ideal environment for the activity of electrogenic bacteria [50]. To prepare this slurry, 1 g of this sediment was placed into a 15 mL conical tube with 10 mL of seawater, secured on an orbital shaker, and shaken at 420 rpm for 30 min, to detach bacteria. The chips were loaded with a slurry and in the second trial, were amended with additional nutrients as described below.

In preparing the suspensions to be injected into the MBMFC anode chips for the second trial, 100 mM stock solutions of the following nutrients were used: Na-acetate (Fisher Scientific)- a 2-C compound, NAG (Sigma-Aldrich)- a 3-C compound, and Na-lactate (Fisher Scientific)- a 4-C compound. These were prepared in seawater filtered with  $0.2\text{ }\mu\text{m}$  pore size to keep the original solute solution with a pH of 8.0. The final concentration of the additives in the microfluidic system was 10 mM and the system was fed in a one-batch mode versus continuous. The solutions were stored at  $4\text{ }^\circ\text{C}$  until used. The volume of liquid required to fill each chip was  $45\text{ }\mu\text{L}$ , based on dome height of  $90\text{ }\mu\text{m}$  and interior surface area. Disposable plastic syringes (1 mL capacity each), and 23-gauge luer-stub adapters, tygon tubing, and 23-gauge deburred steel needles (BD) were used to inject the suspensions into the devices.

After they were prepared, the loaded microfluidic anode chips were left undisturbed and unconnected to allow them to reach open circuit potential for a period of approximately six hours, as in standard practice for marine systems [29,51]. The loaded chips were then inserted into 400 mL beakers containing 300 mL of MCRD sediment (Fig. 3) within a flow-through San Diego Bay seawater system [52]. The anodes were then electrically connected as shown in Fig. 3 to the power management system, the data collector, and linked in series to a single  $15 \times 40\text{ cm}$  carbon-cloth cathode suspended in the above water column. To prevent the cathode limiting power production, the cathodes should be a minimum of  $1.5 \times$  the surface area of the anode [19]. In this experiment, they were twice the size of the anode footprint, to account for the increased surface interactions facilitated by the H-fractal design of the electrodes

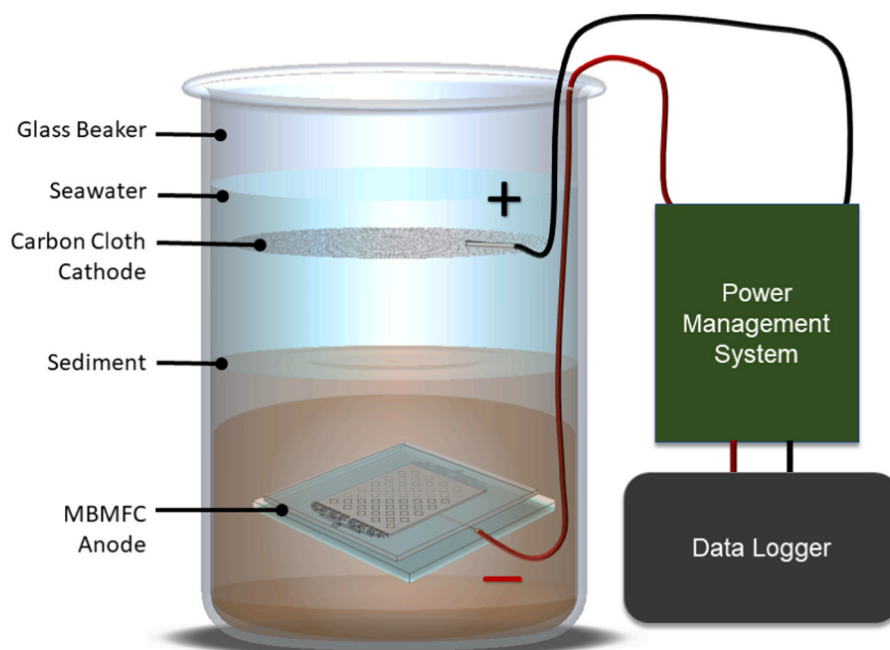


Fig. 3. In-situ experimental set-up of the full MBMFC.

in the MBMFC device.

The power management system, or potentiostat boards, are set to regulate the BMFC voltage to 400 mV (designed by NIWC, patent Navy Case: 108278). This setting has been demonstrated to encompass the maximum power point by marine microbial fuel cells using the MCRD sediment over time [18,51]. The anode and cathode were then connected to current recording data acquisition units (MadgeTech). Periodic readings at 5-minute intervals were taken to determine the BMFC output voltage. From this output voltage drop across the load resistor in the potentiostat board, current is calculated to determine the power produced by the BMFC. Dividing that power produced over the  $4\text{E-}4\text{ m}^2$  surface area of the microelectrode obtains the power density of the device.

### 3. Results and discussion

Two trials were conducted using these MBMFC anodes. The first trial was run as a proof-of-concept to determine if the methodology was feasible and included the use of the microfluidic benthic microbial fuel cell device with a seawater solution. The second trial included a sediment slurry and carbon sources that are more relevant to environmental conditions: acetate, NAG, and lactate which is 2, 3, and 4 carbon compounds, respectively.

#### 3.1. Trial one – a preliminary investigation

The first trial evaluated the capacity of using the MBMFC devices in situ and to determine if it would function with an inoculation of sand-filtered seawater, which contains bacteria and some nutrients, injected

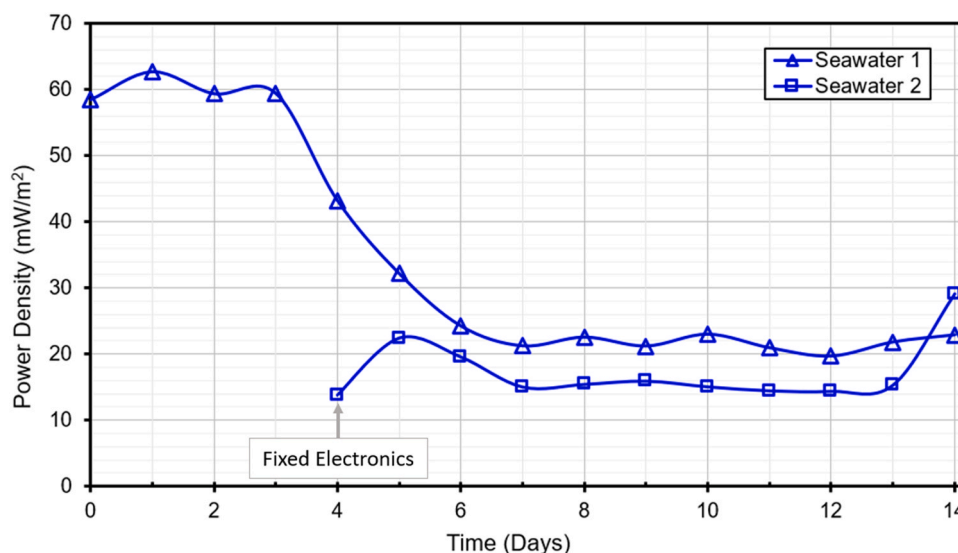


Fig. 4. Power density over time for trial 1. Device 1 showed a high burst average power density over days 1–5 ( $56.65\text{ mW/m}^2$ ) and the steady state average taken days 6–14 ( $22.99\text{ mW/m}^2$ ). Device 2 electronics were disconnected during the sediment burying process, the issue was resolved on day 4 after which the average power density was  $17.34\text{ mW/m}^2$ .



into the anode chip. During experimental set up, device 2 was electrically disconnected and the issue was resolved on day 4 of the experiment. Beyond that point, both seawater anodes developed a steady state power production converging to similar levels, with device 1 coming down to 22.99 mW/m<sup>2</sup> and device 2 averaging 17.34 mW/m<sup>2</sup> (Fig. 4). A notable result is that device 1 recorded over 60 mW/m<sup>2</sup> of power once connected. It was initially assumed that there would be no significant power production in the seawater samples. However, power production was observed and this shows that electrogenic bacteria are present in seawater as the seawater used was only sand-filtered. Chabert et al. have noted that electrogenic bacteria can exist in many ecosystems [53]. This supports the theory that the confining dimensions of the microfluidic anode could facilitate power generation without requiring an established biofilm development, as it typical with in situ BMFC anodes. A separate study also found that currents can be generated by laminar flow of dielectric liquids [54]; and seawater is a dielectric liquid. More recently, flows as small as 1 cm/s of seawater over nanolayers of metals can generate power on the order of  $\mu$ Amps [55]. In these microfluidic devices flows are expected to be laminar, in the 1  $\mu$ m/s to 1 cm/s range as established by previous studies [56]. These could have contributed in power generation by kinetic electricity via oscillatory flow as established by [54,55]. It was noted the edges of the soldering pad showed signs of early corrosion, typical in marine environments. Modifications were made in trial 2 to mitigate those issues, as the second trial was designed to be twice as long as the preliminary evaluation.

### 3.2. Trial 2 – performance with various solution loads

The second trial compared the performance of the MBMFC devices under different cell solution loads. Modifications to the electrical contacts included the addition of liquid electrical tape over the solder point to better adhere the titanium wire to the pad on the quartz, to prevent corrosion in the simulated marine environment over the 30-day trial period. In comparing the seawater data with the first trial, the results are consistent with the results observed in trial 1 with an average power density about 15 mW/m<sup>2</sup> (Fig. 5). Fig. 5 shows that while the higher cell density in the cell slurry without additives in comparison to the seawater doubled the steady state power density to about 30 mW/m<sup>2</sup>, it required a ramp-up time to produce power compared to other in-situ BMFCs. However, it only took 5 days in the MBMFC anode chip, in contrast to

the traditional carbon-cloth anodes in direct contact with marine sediment where typical observed ramp-up to power production takes about two weeks [6,30,31,33]. These carbon-cloth anodes serve as an internal lab reference control electrode (data not shown). In comparison to the carbon-cloth anodes when buried in the same MCRD sediment with a power density of 10 mW/m<sup>2</sup>, this trial shows that the MBMFC anode chips produce 3 $\times$  higher power density. A negative control using an MBMFC anode chip inoculated with seawater filtered using a 0.45  $\mu$ m filter (Millipore) was also used in this experiment, which produced an average power density of 0.57 mW/m<sup>2</sup>. This data is shown in green with diamond markers in Fig. 5, close to the noise floor of the Madgetech data recording device. Lastly, the cell slurry amended with acetate, N-acetylglucosamine, and Na-lactate produced the highest power output among all units (Fig. 5). Added food sourced: acetate, lactate, and NAG are common compounds to stimulate the metabolic activities of electrogenic bacteria [1,2]. Peak power of 130 mW/m<sup>2</sup> was achieved in the first three days. The power density began to drop after the second day and stabilized at an average of 80 mW/m<sup>2</sup> over the next 17 days, with another drop in power after day 20. Further investigations will be conducted to explore the reason for the dramatic drop in power production. It is speculated that saltwater seeped under the liquid electrical tape while the anode was buried in the sediment for testing and became electrically disconnected around day 20. This was also observed on day 29 for the Cell Slurry data. Future designs will consider further mitigation steps in device fabrication.

It was again observed that both the seawater solution and the cell slurry with additives immediately began producing power, whereas the cell slurry without additives required a ramp-up period. This behavior is consistent with power production if cell density overmatches nutritional availability as previously described. By reducing the distance between bacteria and anode, the design and fabrication of these MBMFC device significantly increased power production demonstrating proof of concept.

### 3.3. Capacitive properties in MBMFC device

Aside from reducing biasing, increasing surface interactions with the electrogenic bacteria, and improving power production, the MBMFC anode chip has some capacitive properties due to the H-fractal electrode design and the properties of both seawater and PDMS. Research in

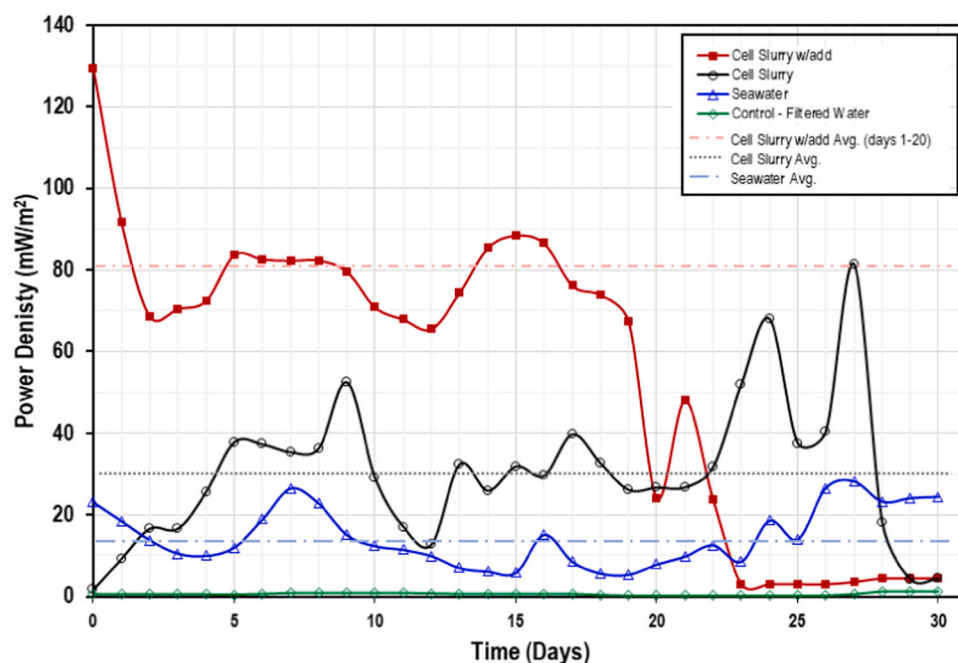


Fig. 5. Power density over time for trial 2. This experiment compared various microbial solutions against a filtered water control. The power density averages are as follows: Cell Slurry w/ additives (for the first 20 days) was 80.04 mW/m<sup>2</sup>, cell slurry was 30.27 mW/m<sup>2</sup>, sand filtered seawater was 14.73 mW/m<sup>2</sup>, and a control of seawater passed through 0.45  $\mu$ m a filter 0.57 mW/m<sup>2</sup> (average line not shown). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

utilizing these biocompatible fractal structures as both electrodes and supercapacitors has been explored for solar cell applications [57]. Furthermore, pseudocapacitance during the 6-h open circuit conditions allowing for charge accumulation on the anode could contribute to the capacitive trends observed in these microfluidic fuel cells. This pseudocapacitive effect has been observed in *Geobacter sulfurreducens* where it has been attributed to bound redox mediators. However, in this example, the MBFMC device both restricts redox mediators from leaving the anode as well as increases power storage capabilities, allowing it to charge and discharge as observed by the high initial power density recorded. This capacitive phenomenon will be further explored. If compatible with scaleup of MBMFC devices, it could provide exciting possibilities for sustainable marine powered microbial cells.

## 4. Conclusion and future development

### 4.1. Conclusion

The presented proof-of-concept MBMFC formed with this microfluidic anode and carbon-cloth cathode could generate power immediately with a high average power density of 80 mW/m<sup>2</sup> for 20 days, which is a significant (~8x) increase compared to previous membraneless systems using natural sediment. Reducing the distance between electrogenic bacteria and the electrode surface, in conjunction with the fractal design of the microelectrode, was responsible for this marked increase in performance. This device also displayed the ability to instantly produce power and store power during the open-circuit phase. The presented MBFMC system is highly suitable for use as a standardized test station to investigate benthic bacterial populations.

### 4.2. Future development

The purpose of optimizing the system parameters, such as cell concentration, media content, microelectrode scaling, and fluidic architecture (e.g., dome height), was to increase power output density from the device. Future iterations would include the examination of the bacteria that are present in the microfluidic device to determine if enriched electrogenic microorganisms can increase power generation. Other energy harvesting strategies may be included in the future to improve the charge and discharge cycle for increased power capture from the device [44]. Furthermore, the final resulting optimized unit can then be super-arrayed to produce larger systems with proportionally larger power generation for small-scale sensors. Future experiments will explore improving power density yield through adjusting device parameters, scaling up the number of device layers to increase surface area, and to improve electrical compatibility of the system with the power generation of electrogenic biofilms. The presented system is also amenable to such a scale-up by a variety of fabrication techniques, such as silk-screening, thin flexible films, and 3D printing.

The initial efforts for this proof-of-concept were challenging with regards to providing a stable contact for electronics to the pad on glass surface. Future considerations would be to increase the area of the connection pad or change the titanium wire to gold wire to improve electrical connectivity and reduce impedance. Furthermore, the potential for this design to serve as a dual electrode and capacitor for microbial fuel cells should be explored. These MBMFCs could enable the manufacture of small automated power stations offering renewable energy for various marine small-scale sensors, or can be used to sustainably power small devices for environmental tagging. The device and techniques presented in this study are a step forward towards renewable power systems for maritime environments and demonstrate the promise of leveraging microfluidics for benthic microbial power production.

### CRedit authorship contribution statement

**Tricia Nguyen:** Fabrication, Experiments, Data Analysis, Manuscript

writing. **Y. Meriah Arias-Thode:** Conceptualization, NIWC lead. **Anna Obratsova:** Experimental work with bacteria. **Angelica Sarmiento:** Data analysis, Figure prep. **Alexander Stevens-Bracy:** Electronics work, Experiments, Data acquisition. **Dragoslav Grbovic:** Fabrication. **Emil P. Kartalov:** Conceptualization, Fabrication, NPS lead.

### Declaration of Competing Interest

The authors declare no conflicts of interest.

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