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Justin C. Biffinger

US Naval Research Laboratory, justin.biffinger@nrl.navy.mil

Bradley R. Ringeisen

Naval Research Laboratory, Bradley.ringeisen@nrl.navy.mil

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Engineering Microbial Fuels Cells: Recent Patents and New Directions

Justin C. Biffinger* and Bradley R. Ringeisen

US Naval Research Laboratory, 4555 Overlook Ave. SW., Code 6113, Washington DC., 20375, USA

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Abstract: Fundamental research into how microbes generate electricity within microbial fuel cells (MFCs) has far outweighed the practical application and large scale development of microbial energy harvesting devices. MFCs are considered alternatives to standard commercial polymer electrolyte membrane (PEM) fuel cell technology because the fuel supply does not need to be purified, ambient operating temperatures are maintained with biologically compatible materials, and the biological catalyst is self-regenerating. The generation of electricity during wastewater treatment using MFCs may profoundly affect the approach to anaerobic treatment technologies used in wastewater treatment as a result of developing this energy harvesting technology. However, the materials and engineering designs for MFCs were identical to commercial fuel cells until 2003. Compared to commercial fuel cells, MFCs will remain underdeveloped as long as low power densities are generated from the best systems. The variety of designs for MFCs has expanded rapidly in the last five years in the literature, but the patent protection has lagged behind. This review will cover recent and important patents relating to MFC designs and progress.

Keywords: Microbial fuel cell, electricity, energy harvesting, waste water treatment, biological fuel cell.

1. INTRODUCTION

A fuel cell is an electrochemical device that is capable of coupling oxidation of a desired fuel in the anodic chamber (H_2 , methanol, ethanol, formic acid) with the reduction of a sacrificial electron acceptor (primarily oxygen) in the cathodic chamber. These two chambers are divided by a membrane or separator that is capable of transporting protons to balance the charge generated by the fuel cell while maintaining a separation between the fuel supplies. In the last twenty years, the variety of fuel cell systems and potential fuels has rapidly expanded to meet the ever growing need for energy independence from fossil fuels. Systems that take advantage of bioenergy and renewable biomass are going to ultimately be preferred over fossil fuel supplies for fuel cells because they can be continuously produced.

Microbial fuel cells (MFCs) are a unique subset of fuel cells that take advantage of microbial metabolism to either generate fuels for commercial fuel cells (e.g., generate H_2 gas) or electricity directly. Most microbes are capable of generating a current if reduction/oxidation (redox) active mediators are added to the system or immobilized on the electrode. However, the addition of exogenous mediators creates systems that have defined life-times. Therefore, practical systems are designed with microbes that can either self-mediate or perform direct electron transfer to the anode through membrane-anode contact (extracellular electron transfer through membrane proteins and/or bacterial nanowires) [1,2]. A comparison between standard PEM fuel cell technology and MFCs are presented in Fig. (1). The benefits of using MFC technology include the use of unpurified waste or biomass as fuels, operation under ambient

Fig. (1). Schematic comparison between H_2/O_2 fuel cells and MFCs.

conditions, and no requirement of excess heat for activation or operation.

Several excellent reviews have been published in the last three years that cover almost every aspect of microbial fuel cell engineering and research. The most encompassing review was written by Logan and collaborators [3] and a recent book expands significantly on subjects from that review [4]. In addition, a recent review by Du and co-workers is an excellent source for MFC engineering and principles [5]. Since the subject of this review is focused on MFCs and ideas that have been patent protected, a review of metal reducing bacteria and the molecular biology surrounding them will not be addressed (the authors refer the reader to several relevant reviews on these subjects [2,6-10]). This review will also only focus on MFC systems that generate electricity directly from the bacteria (extracellular electron transfer) and not the bacterial generation of fuels for conventional fuel cells [11].

*Address correspondence to this author at the US Naval Research Laboratory, 4555 Overlook Ave. SW., Code 6113, Washington DC., 20375, USA; Tel: 202-767-2398; Fax: 202-404-8119; E-mail: justin.biffinger@nrl.navy.mil

2. PATENTS FOR MICROBIAL FUEL CELLS PRIOR TO 1987

The considerable interest in designing MFCs in the last decade was preceded by two international and US patents filed between 1967 and 1987. The very first patent describing MFCs was issued to John Davis from Mobil Corporation in 1967 [12]. This patent describes an externally mediated (addition of methylene blue) MFC using *Nocardia salmonicolor* or environmental bacteria isolated from sludge that oxidized hydrocarbons (ethane, *n*-propane, *n*-butane) to alcohols, aldehydes, and carboxylic acids while generating a current from a fundamental alkaline fuel cell. A current of 0.05 mA was generated from these alkaline MFC experiments with the addition of mediator and linear alkanes of a varying length (CH_4 - C_{20}) as the sole electron source. From the data presented, the concentration of the methylene blue redox mediator dictated the open circuit potentials (the potential difference between the cathode and anode electrodes when no current is collected) for the fuel cell. *Nocardia* sp. are still used for oxidizing alkanes [13], but they have not been used in MFCs since the 1967 work.

Fig. (2). Fundamental design from first MFC design patent [12].

A very similar patent was published that same year by John Davis using the same general MFC design in Fig. (2) but with the addition of aerobic pre-treatment of the microbial component for the anodic reaction [14]. While oxygenated, alkanes of varying length would be oxidized by the microbes. The oxidized alkanes and the microbial culture were then purged with nitrogen and pumped into the MFC design to generate power. Unlike the first patent that used only *Nocardia* sp., this patent was the first to utilize *E. coli* under anaerobic conditions with glucose. The *E. coli* containing fuel cell (using methylene blue as the redox mediator) generated an open circuit potential of approximately 600 mV corresponding to 0.5 mA. At this point of MFC development and considering the fundamental nature of the MFC design, 0.5 mA is outstanding by even present day standards.

3. RECENT PATENTS ON MICROBIAL FUEL CELLS (1987-PRESENT)

3.1. General MFCs and Scalable Designs

Following the original patent on MFC designs, there were few actual patents for fundamental MFCs because most

concepts and designs were disclosed in publications [3]. Overall, the advantages of scaling up (by stacking) several similar but smaller MFCs should result in less efficiency loss than scaling up the actual size of the MFC itself (single reactor). Very promising short circuit currents (maximum current possible) were generated (425 mA) using six stacked MFCs connected in parallel with a ferricyanide catholyte [15]. This system used granular carbon electrode materials that are common in some of the up-flow MFCs described in section 3.2 and highlights the potential impact MFCs will have in the near future.

The stacking of MFCs clearly shows that practical power outputs and currents can be obtained and is an impetus for continuing to improve MFC designs and characteristics. Several stackable single chamber designs have recently been disclosed in the Chinese patent literature [16-18]. There are three two-chamber designs for MFCs Fig. (3) that have been patented in the last 5 years that are scalable and stackable. Chiao and co-inventors fabricated a microliter volume flow cell using microchannels and Baker's Yeast with methylene blue as the redox mediator [19]. This device was patented as an implantable MFC for micro-electro-mechanical (MEM) and nano-electro-mechanical (NEM) sensors but only generated 0.5 W/m^3 , Fig. (3A).

A MFC design that does generate large power densities is the Ringeisen and co-workers miniature MFC (mini-MFC) that was patented in 2006 [20]. This device is a flowing MFC that maximizes the ratio of electrode surface area to volume of the device Fig. (3B). Using the MFC volume of 1.2 mL, power densities of 500 W/m^3 are commonly generated. Since the bulk of the bacterial culture is external to the operating volume of the MFC, the mini-MFC has generated significant power from aerated analytes [21].

The mini-MFC shows promise both as a power source for aerobic environments and as a device to evaluate current production from bacteria under a wide variety of aerobic and anaerobic growth conditions [22]. Power in the presence of air is possible because the bulk of the anolyte (50 mL) is external to the operating void volume ($< 500 \mu\text{L}$) within the fuel cell. The high bacterial cell counts and surface area-to-chamber volume ratio ($> 500 \text{ cm}^{-1}$) enable rapid and efficient shuttling of electrons to the anode surface and optimal utilization of *Shewanella* sp. metabolism. Such conditions allow the *Shewanella* sp. to efficiently scrub oxygen from the fuel supply while maximizing the current generated in nominally aerobic conditions.

Bruce Logan has also recently protected a stacked MFC design incorporating nanoporous membranes as a protective layer for the cathode [23]. By protecting the cathode in this fashion Fig. (3C), the tubular brush anode can be stacked within the MFC chamber. The use of this design was not only for creating electricity from microbes, but also as a method to generate and isolate hydrogen gas from bacterial cultures.

3.2. Upflow Microbial Fuel Cells

MFCs can be characterized by the mechanism of electron transfer to the anode. This transfer can be either mediated by artificial redox active molecules added to the anode chamber or self-mediated (or mediator-less) where the bacteria

Fig. (3). Patented two-chamber scalable MFC designs from A) Chiao, *et al.*, [19], B) Ringeisen, *et al.*, [20] and C) Logan, *et al.*, [23].

produce mediators to aid in exoelectron transfer. The first mediator-less MFC was protected by Byung Hong Kim, *et al.*, in 2003 [24]. This MFC design was the first of three upflow MFCs protected in the last five years Fig. (4). Preceding the Kim upflow MFC, all MFCs consisted of two defined chambers separated with a polymeric ion exchange membrane. Since this MFC was designed for waste water treatment, a membrane was not included in the design but rather the cathode and anode chambers were separated with glass wool and glass beads. Controlling the distance between the two electrodes dictates the total deleterious internal resistance losses in the device. The actual effects of modulating the distance between the anode and cathode in a membrane-less MFC were later followed-up by Logan and co-workers [25].

The MFC shown in Fig. (4A) is interesting for a variety of reasons. This was the first membrane-less MFC, which is important considering the cost and overall effectiveness of cation exchange membranes at neutral pH [26]. This patent was also the first to protect the concept of using a MFC with no additional artificial mediators to aid in exoelectron transfer from the microbe to the electrode surface. This design was also on a larger scale than most other MFCs (total volume 7.8L). It is reasonable to assume that because there was a current generated from this design that bacterial contamination of the cathode was prevented by the glass separator. However, eventual biofouling of the cathode seems inevitable and would most likely limit the long term survivability of this design. The glass beads also resulted in a high internal resistance which limited the current generated.

A second upflow MFC design (total internal volume 0.39 L) was disclosed by Rabaey and Verstraete in 2005 [27]. Their device was tubular but designed using a modified two chamber framework where the cathode surrounds a tubular granular anode separated by a robust cation exchange membrane Fig. (4B). The catholyte for this design was ferricyanide, which is not practical for autonomous deployment considering it is a sacrificial electron acceptor. However, this device could easily incorporate oxygen reduction cathodes if desired. The Rabaey and Verstraete MFC is an elegant alternative to the Kim, *et al.* upflow MFC because the cathode is not directly in the path of the influent water for the waste water treatment. Degradation of the cathodic catalyst with time will limit most MFC systems and designing the cathode as a second flowing chamber around the outside should increase the duration of operation. The

Logan lab also fabricated a tubular single chamber MFC for wastewater treatment, but the anode was oriented around an internal cathode unlike the Rabaey and Verstraete MFC [28]. More specifically, the anode rods encircle the cathode chamber/tube separated by a Nafion membrane. Even though the Logan and co-workers system could utilize high rates of waste water flow, the device was complex to build, maintain, and produced low power density (26 mW/m^2) per electrode surface area. Interestingly, two Chinese patents describe devices very similar to Logan tubular single chamber device [29, 30].

The final upflow MFC that has been disclosed for patent protection was submitted by the Angenent and He in 2006 [31]. There were two devices protected within this patent application. The first resembled a two chamber flow reactor and was used as their initial prototype, and the second could be classified as an upflow MFC device Fig. (4C). The Angenent and He MFC is similar to the Rabaey and Verstraete device because of the use of a granular anode and is similar to the Logan and co-workers tubular MFC because of the internal cathode. Unlike the Logan and co-worker device, the Angenent and He cathode is U-shaped and is formed using a PEM membrane. The first Angenent and He prototype device was the most efficient from a power density per electrode surface area metric (170 mW/m^2) but unfortunately resembles an efficient two chamber system more than an upflow reactor for large scale wastewater treatment. The second device with a U-shaped cathode would most likely be used for high flow rates applications.

3.3. Benthic MFCs

Benthic unattended generators (or BUGs) are one of the only MFCs that have been deployed to power environmental sensors for greater than one year [8]. These power sources are composed of graphite electrodes that are either buried into the sediment (anode) or supported above the sediment to catalyze the reduction of oxygen (cathode). This device was issued as a full patent in 2005 [32] to Tender and co-workers for the natural diffusion of nutrients to the anode and protected again in 2006 for the active transport of nutrients to the anode [33]. The primary focus of the patent was to describe a device that could be used to generate power from the natural voltage gradients established between microbial activity in the anoxic sediment and the surrounding aerobic water column Fig. (5).

Fig. (4). Three independent upflow MFCs from A) Kim and coworkers [24], B) Rabaey and Verstraete [27], and C) Angenent and He [31].

When the anode and cathode are connected through a load, current can be collected. This device is simple (in concept) and utilizes environmental microbes and substrates for operation. This type of device was also modeled effectively in the laboratory using fish tanks and collected sediment samples. Analysis of the microbes that colonize functioning BUGs has lead to the isolation and identification of several new isolates for microbial energy harvesting applications [34]. MFCs similar to BUGs have advantages over batteries and photovoltaic panels because they are generating power by utilizing the natural nutrients surrounding the device and are capable of continuous power output during prolonged periods of darkness.

Fig. (5). Schematic representation of the benthic fuel cell [33].

3.4. Alternative Membranes to Cation Exchange Membranes

Most standard fuel cells consist of two chambers that are commonly separated by a polymer electrolyte membrane (PEM). However, common drawbacks for the use common PEM's are cost (for example, Nafion™ is a perfluorosulfonic acid membrane which costs about \$0.22/cm²), high internal resistances using other materials (example: glass beads) and long term stability in the presence of high salt concentrations. There is significant interest in utilizing new membranes that could replace Nafion™ and yet have the same physical properties. The scientific interest in the biological fuel cell community is based almost entirely on scaling up these systems, mainly to satisfy power requirements of portable electronics, sensors, or communication devices. Two patent applications were submitted in 2007 containing the concept of using nanoporous membranes for MFCs directly [23,35].

The purpose of using nano- and micro-porous membranes is to create a scalable (micrometer to meter in diameter) electrochemical power generation device based on biological metabolic function that includes three unique features [23,35]. First, the use of a nanoporous membrane will isolate the anode chamber from the environment, reducing potential contamination within the anode chamber by sequestering the metal reducing microbes. These membranes could also be used to isolate the electrochemically active bacteria from the rest of the cell while allowing the flow of nutrients to diffuse freely between the two electrode chambers. Gases can also be collected from microbes more easily if porous membranes are used. Secondly, sequestering the active microbes around the anode is the only requirement for power production. Therefore, any disconnection between the two electrodes by a nanoporous membrane could replace the need for an expensive polymer electrolyte membrane in biological fuel cells. Finally, the use of a size selective membrane could provide a significant advantage over other microbial fuel cells using proton exchange membranes or no membrane at all by inverting the flow of nutrients through the cathode to the anode. This inverted flow could enable a true single chamber device with the oxygen reduction reaction at the cathode acting as a method to scrub oxygen from anolyte prior to exposure to the anode [35].

If microbial fuel cells are to be used in natural aquatic environments, the ability to sequester the electrochemically active bacteria (EAB) at the anode will be imperative considering the likelihood that biofilms formed by bacteria such as *Geobacter* sp. and *Shewanella* sp. would dissipate or be out-competed in an open system located at the water/air interface. The use of microporous membranes and nanoporous membranes in biological fuel cells will lead to inexpensive designs, realistic power generation in real world scenarios, and increase columbic efficiencies without relying on creating stable biofilms on the anodes.

The use of micropillar polydimethyl sulfide (PDMS) coatings in channeled MFCs was patented by Siu and Chiao in 2007 [36]. Their work focused on a bio-compatible and flexible MFC using a yeast species and methylene blue as the redox mediator. The ultimate goal of their system was to create better power sources for NEMs and MEMs. The

device with micro-pillar channels generated 29.1 W/m^3 from glucose using a mediator. This power density is impressive considering the size of the device, but the use of mediators biases the results for practical applications.

3.5. Biosensing Using MFC Technology

The development of sensors for chem/bio agents or toxins in water remains of high priority for homeland security and defense related applications. Fluorescent microbes, water fleas, and even whole fish have been used to detect the release of toxins in water streams [37, 38] with varying success. Even though MFCs could be used to power a water toxin sensor, there is only one patent that uses a MFC as the detector for water toxins. This patent (filed in 2003) for the detection of toxins monitors large current decreases from an operating microbial fuel cell [39]. The MFC sensor operates around the principle that when a toxin is introduced into the MFC anode chamber, the microbes producing electricity die and the current from the MFC drops in correlation with toxin concentration.

Sensing toxins directly with a microbial death response is an interesting divergence from using whole fish and certainly could be easily quantified by using the continuous voltage signal from the MFC under ideal operating conditions. The MFC toxin sensing patent claims a sensitivity of around 0.03 ppm for mercury, lead, and phenol. However, other compounds or biological agents (ricin, *B. anthracis*, *F. tularensis*) might prove difficult to sense by this technology. Microbial adaptation and bacterial specificity for certain toxins severely limits the potential applications for a MFC type of sensor.

3.6. Bacterial Focus within MFCs

Manipulation of electrochemically active bacteria (EAB) in MFCs will result in the highest potential impact on power output. There are any number of growth variables (temperature, O_2 concentration, concentration levels of nutrients) and mechanisms of extracellular electron transfer that can be manipulated to generate significant changes in current. Bennetto and co-inventors patented in 1987 the concept of lowering the concentration of the food source as a method to increase current densities from MFCs [40]. Within that patent they also described a MFC system that would contain sensors that could maintain the maximum level of food to create a sustained current. This mechanism of nutrient control over MFC power would certainly be applicable when a microbe is identified that produces practical current densities, but this system is better suited for developing a sensor system using MFCs as the detector similar to the work discussed in section 3.5.

Several microbial species and families have been used in MFC architectures. Of these species, strains from the *Shewanella* and *Geobacter* families comprise the bulk of MFC work in the last 20 years [10]. However, other species are slowly being integrated into MFC research. The standard use for microbes in MFCs is to catalyze the oxidation of carbon electron sources and generate electrons in the anodic chamber. However, a recent patent application was filed using *Acidithiobacillus ferrooxidans* in the cathode chamber [41]. The MFC containing *A. ferrooxidans* was designed with a standard hydrogen gas oxidation as the anodic reaction and

reduction of Fe^{3+} as the cathodic reaction. The microbial component in this fuel cell system would be used to regenerate Fe^{3+} instead of driving current from the fuel cell anode [41]. A simplified schematic from the *A. ferrooxidans* MFC is presented in Fig. (6). Oxygen reduction cathodes will be preferred for autonomous power supplies but would most likely not be necessary for on-grid applications if Fe^{3+} can be regenerated efficiently.

Fig. (6). Operational characteristics of the *A. ferrooxidans* MFCs [41].

A. ferrooxidans is categorized as an extremophile because of the pH required for optimal growth ($\text{pH} < 2$) [42]. Operational ionic strength and temperature are two variables that could significantly affect MFC performance [25]. Nafion™, a membrane commonly used with MFCs, was designed for temperatures above 50°C and below $\text{pH} 2$ [43]. So, the use of microorganisms that can withstand these conditions would be of interest for using Nafion™ effectively. A MFC modified for higher temperatures was recently disclosed, but no specific microbes were described for use within it and the difference between the design for the thermophilic MFC [44] and standard flowing MFC designs was negligible. In one case, the inventors of the thermophilic MFC describe a porous sand barrier being used between the anode and cathode chambers. However, this sand barrier will result in the same high internal resistances encountered within the Kim, *et al.*, upflow MFC discussed in section 3.2 and will ultimately prove problematic for practical applications.

The use of extremophiles is a new direction for MFCs considering the significant effort has been focused on using *Geobacter* sp. and *Shewanella* sp. in MFCs. There were two patents that discussed *Shewanella* and *Geobacter* specifically for MFC designs. One patent describes the use of these bacteria and the enhancement of microbial growth within a H-cell MFC [45]. However, the one unique claim within their application was the identification of adenosine triphosphate (ATP) usage for determining increased power and not the use of the bacteria. Another inventor filed a

patent to protect the use of MFCs with biofilms pre-formed on the anode and/or cathode as a way to increase MFC efficiency using hydrogen gas and oxygen as the fuel sources [46]. The increase of biofilm formation and activity for this purpose is a very active research area with some recent success reported using ammonia treated graphite felt [47].

4. CURRENT & FUTURE DEVELOPMENTS

MFCs will continue to be developed into a practical alternative energy source as long as their impractical power outputs are increased. MFCs benefit greatly from more efficient designs and electrode materials. Biological hydrogen production from bacteria could also be a pathway to generate high power densities, but limitations on the rate of microbial hydrogen generation will severely limit its applicability. In general, MFCs have yet to find their ideal application. Creating a practical autonomous power source is challenging, but the benefits for both society and national security would be tremendous. Compared to photovoltaics, MFCs generate an order of magnitude less power with the same surface area device. However, MFCs are designed to operate in the dark and can also bioremediate waste to simplified components while generating power. These attributes make MFCs useful technologies for light-limited applications. The ability to harvest energy from waste (industrial, food, municipal) and generate electricity as a by-product will likely be the most suitable niche in the next decade for MFCs and progress is already being made in this direction.

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