Journal of Power Sources 191 (2009) 520–527

Contents lists available at ScienceDirect

Journal of Power Sources

journal homepage: www.elsevier.com/locate/jpowsour

Integrating engineering design improvements with exoelectrogen enrichment process to increase power output from microbial fuel cells☆

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ARTICLE INFO

Article history:
Received 9 January 2009
Received in revised form 2 February 2009
Accepted 3 February 2009
Available online 13 February 2009

Keywords:
Microbial fuel cell
Enrichment
Exoelectrogenic
Biofilm-forming
Direct electron transfer
Diversity

ABSTRACT

Microbial fuel cells (MFC) hold promise as a green technology for bioenergy production. The challenge is to improve the engineering design while exploiting the ability of microbes to generate and transfer electrons directly to electrodes. A strategy using a combination of improved anode design and an enrichment process was formulated to improve power densities. The design was based on a flow-through anode with minimal dead volume and a high electrode surface area per unit volume. The strategy focused on promoting biofilm formation via a combination of forced flow through the anode, carbon limitation, and step-wise reduction of external resistance. The enrichment process resulted in development of exoelectrogenic biofilm communities dominated by *Anaeromusa* spp. This is the first report identifying organisms from the Veillonellaceae family in MFCs. The power density of the resulting MFC using a ferricyanide cathode reached 300 W m^{-3} net anode volume (3220 mW m^{-2}), which is about a third of what is estimated to be necessary for commercial consideration. The operational stability of the MFC using high specific surface area electrodes was demonstrated by operating the MFC for a period of over four months.

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1. Introduction

Energy from wastes and renewables is becoming increasingly important as fossil energy increases in cost and environmental impact. Microbial fuel cells produce energy directly from biodegradable matter such as sugars, organic acids and biomass [1,2]. The maximum power density reported for air-cathode MFCs [3,4] is on the order of 100 W m^{-3}, while those using ferricyanide as a catholyte [5] is on the order of 250 W m^{-3}. The maximum power density reported on the basis of projected surface area is about 2700 mW m^{-2} [6]. Most of the MFC power density improvements to date are due to improvements in system design parameters. These include electrode spacing [1,7,8], type of membrane separating the anode and the cathode [9–11], relative areas of the two electrodes [11] and their surface properties [12] related to microbial attachment and electron transfer. The ratio of the electrode surface area to the volume of anode chamber occupied has also been investigated [13]. Maximization of this ratio can result in higher power densities as shown with *Shewanella* biocatalyst [13]; however reports on use of such a design to carry out and characterize the exoelectrogenic enrichments are rare [5]. Logan et al. used a graphite fiber brush anode [3] and Aelterman et al. used a graphite granule-based anode [5] minimizing dead volume, which resulted in power densities up to 258 W m^{-3} or 2600 mW m^{-2}.

The dependence of the power output upon the biology or type of microorganisms is poorly understood [14,15]. The operating parameters known to affect the enrichment of exoelectrogenic organisms and the ability to achieve high rates of electricity production include mode of operation, flow rate, substrate loading and external resistance [16–18]. The circulation of anode liquid (non-continuous vs. continuous flow) dictates whether communities with direct electron-transport or mediated electron transport mechanism inhabit the anode electrode. A non-continuous system is typically enriched with mediator-producing organisms such as *Pseudomonas aeruginosa* [16] with accumulation of pyocyanin. The use of continuous flow systems have potential to enrich microbial communities not relying on mediators. Mediator-based organisms may remain within the electrode even in continuous flow systems depending their affinity to the electrode. Further study is needed to understand the effect of the liquid flow rate on the

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0378-7753/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jpowsour.2009.02.006

enrichment of mediator-based vs. direct electron transfer-based organisms. The substrate loading is another important operating parameter, although studies showing its effect on microbial population are rare. In fed-batch systems, where the carbon source is added periodically, a substantial excess of the carbon source exists initially. This promotes growth of non-exoelectrogenic organisms which convert the available carbon to other byproducts. The applied resistance (load) or poised voltage also affects the enrichment process [17,18]. Improvements in MFCs are possible via incorporation of the existing knowledge about the system design parameters and by exploring less-studied parameters. Investigations which combine efficient MFC designs with use of effective operational regimes to enrich exoelectrogens will result in further improvements.

In this work we report systematic changes to the anode design and the operating parameters that targeted improved MFC anode performance and power density. Previously reported MFC design changes that have been known to improve power density were combined to develop a new design. These included use of high anode electrode surface area [3,11] combined with minimal dead volume in the anode, reducing electrode spacing, essentially to zero, use of a flow-through design for the anode electrode [5,8], similar projected surface areas for anode and cathode and use of a hydrophilic electrode surface. Operating parameters including use of continuous flow [19], reduction of external resistance [20], control of oxygen availability in the anode, which have been reported to improve power output, were also implemented to exert the desired selection pressure. In addition, a forced flow-through anode was used to further enhance exoelectrogenic growth. The improvements in power density and the composition of exoelectrogenic community resulting from this unique integrated design and the use of multi-mode operation are reported.

2. Methods

2.1. MFC construction

The MFC used in this study consisted of an anode chamber (4 cm diameter × 1.27 cm thickness) containing carbon felt (Alfa Aesar, #42107) as the electrode material (0.6 $m^2\,g^{-1}$, estimated specific surface area 45,350 $m^2\,m^{-3}$ of anode volume) and a platinum-coated carbon cathode (Pt/C air-cathode, Fuel cell store, #GDE HT 140W-E) separated by a Nafion-115 membrane (Fig. 1A). The carbon felt was made hydrophilic using a plasma treatment for 20 min [21]. The electrode material completely filled the anode chamber leaving no dead volume. The MFC anode chamber was made up of a piece of 4 cm diameter PVC pipe. The anode, membrane and the cathode were bolted together within two Lexan end plates. The liquid flow through the anode was directed upwards through the carbon felt. A gold wire was used as a current collector for the air-cathode and a graphite rod was used for the anode. The Pt/C air-cathode was used during biocatalyst enrichment (Fig. 1A), while a ferricyanide-cathode was used for determining the maximum power density. Use of air-cathode over long periods resulted in accumulation of salts on the cathode side (due to osmotic pressure), which were washed off with water once a month. The cathode was changed in an anaerobic chamber to minimize exposure to oxygen. The electrode for the ferricyanide-cathode was also made using a pipe (4 cm diameter × 2.54 cm thick) with carbon felt as the electrode material (2.54 cm × 2.54 cm × 0.625 cm). The felt was suspended by a carbon rod in a way that the felt surface was in firm contact with the Nafion membrane. The cathode chamber for the ferricyanide-cathode was sparged with air to mix the contents. The anode projected surface area for the system was 12.56 cm^2 and the anode volume was 15.96 cm^3. A picture of the ferricyanide-cathode MFC is shown in Fig. 1B.

2.2. Inoculation and operation

The nutrient medium (Medium AC-1) used for enrichment consisted of 975 ml of a sterile salts solution and 12.5 ml each of filter sterilized Wolfe's mineral solution and vitamin solution [22]. The sterile salts solution was made up of 0.31 g NH_4Cl, 0.13 g KCl, 4.97 g $NaH_2PO_4{\cdot}H_2O$, and 2.75 g $Na_2HPO_4{\cdot}H_2O$ per liter of nanopure water [23], and was adjusted to pH 7.0 with 1N NaOH prior to sterilization. The nutrient medium AC-1 (200 ml) was placed in a glass bottle reservoir (anode liquid reservoir) and recirculated through the anode chamber at 4–7 ml min^{-1} (Fig. 1A). The medium was deaerated with nitrogen at a flow rate of 5 ml min^{-1} or higher. The MFCs were maintained at 30 ± 1 °C, during the course of the experiment. The MFC components were immersed in water and autoclaved individually and then assembled in a sterile hood.

The anode chamber of the MFC was inoculated with a 1 ml sample of anaerobic digester slurry collected from the Knoxville municipal wastewater treatment plant. The inoculum was added

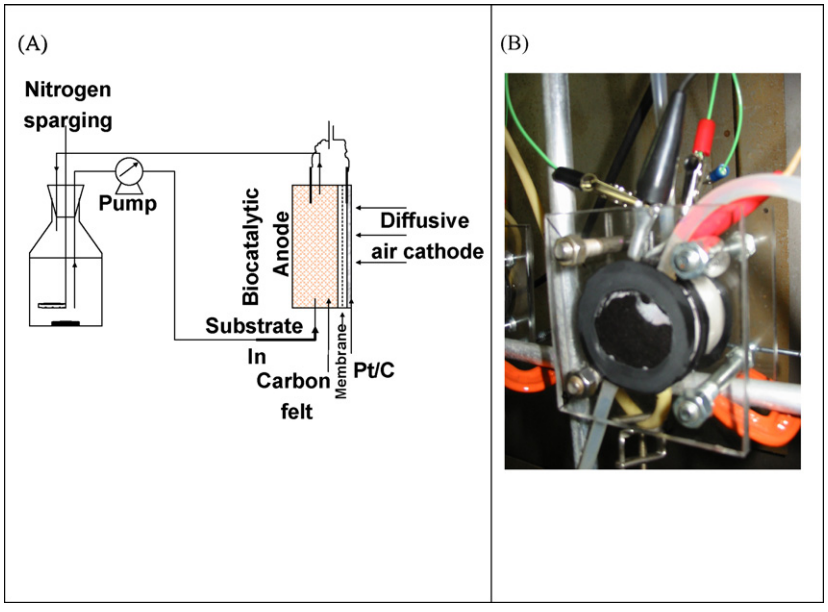

Fig. 1. (A) Schematic of the MFC recirculation set up with an air-cathode and (B) photograph of the MFC.

directly into the flow line entering the anode chamber, carried into the anode chamber by the deaerated recirculating medium. During the enrichment process, the recirculating medium was replaced when the OD_{600} increased above 0.05 units. This was approximately every 3 days. Beginning on day 64, 48 ml of fresh deaerated nutrient medium (3× of the anode volume) was also used to flush out planktonic bacteria from the anode chamber using forced flow through the chamber. A syringe was used to pull freshly added nutrient medium (after deaeration via nitrogen sparging) through the anode chamber to remove mediators and organisms from the pores of the carbon felt. The flushing procedure was carried out at the same frequency as that of the anode medium replacement, beginning day 64 for the rest of the enrichment period. The recirculating medium consisted planktonic bacteria either growing in the anode chamber of the MFC or in the reservoir itself. The enrichment process was targeted to obtain exoelectrogenic biofilm-forming organisms via removal of free-floating cells and any mediators and mediator-based organisms growing in the MFCs.

The enrichment was conducted in three different modes. Mode I was essentially a growth mode, in which the consortia was allowed to grow at a constant load using a given carbon source, but using only the electrode as the electron acceptor. Mode II consisted of a carbon starvation mode, in which the carbon source addition was stopped to enable consumption of carbon source added in mode I. In mode III, the carbon source was restored and the external load was reduced in a step-wise manner from 500 to 250 Ω (day 119) and then to 100 Ω (day 130), to allow higher current to flow between the electrodes.

2.3. Enrichment of the MFC anode biocatalyst

During the start-up of the MFC, a mixed carbon source containing 0.2 g l^{-1} glucose, 0.2 g l^{-1} lactate, and 0.05 g l^{-1} cellulose (final concentration in recirculation medium) was added to the anode solution in fed-batch manner, either when the medium was changed or upon depletion, directly into the recirculation medium. The cellulose was prepared by treating crystalline cellulose (Avicel, FMC, PH105) via a phosphoric acid swelling method [24]. The addition of the carbon source was continued until day 28, at which point the MFC output had stabilized between 0.3 and 0.4 V at a 500 Ω load. Mode II was initiated beginning on day 29. The primary purpose of this mode was to deplete carbon source from the anode. No external carbon source was added thereafter, until day 72, which, in addition to depleting carbon source present in anode, also allowed selection of organisms capable of either using residual cellulose (remaining from the addition during the first 28 days) or those capable of storing carbon internally and then using it subsequently for cellular maintenance needs. Thus, the second phase of enrichment (carbon starvation mode) essentially targeted enrichment of the MFC with organisms capable of storing carbon internally [25] and using it as electron donor and the electrode as electron acceptor.

The addition of the carbon source was restarted on day 73, when a decline in the voltage output was observed. The carbon source added beginning on day 73, contained a mixture of 0.2 g l^{-1} glucose and 0.2 g l^{-1} lactate, but no cellulose. The cellulose was excluded to prevent nonhomogeniety within the anode chamber when making power density and Coulombic efficiency measurements. Glucose and lactate were added into the aqueous anode medium, which was continuously recirculated through the anode chamber at a flow rate of 4–7 ml min^{-1}.

2.4. Power density analysis

The power density analysis for the MFC was conducted by feeding the carbon source in a fed-batch manner. The nutrient medium was completely replaced prior to every analysis, followed by addition of 0.2 g l^{-1} of the carbon source (glucose and lactate, each) into the medium. A 200 mM potassium ferricyanide in 200 mM potassium phosphate buffer was used as the catholyte to determine the power density. The analysis was conducted 60 min after addition of the carbon source, to allow the voltage output to stabilize. A variable resistor ranging from 0 to 5000 Ω was used and the voltage recorded by a Fluke multimeter Model 83. The resistance sweep was conducted at an interval of 5 min. The maximum power density was confirmed by operating the MFC at the particular resistance for at least one hour, following the power density analysis. Multiple measurements of the voltage output at the resistance exhibiting maximum power density were made on different days to determine reproducibility of the power density curve. The results were always found to be within a 10% standard deviation.

2.5. Genetic characterization

2.5.1. 16S clone library

Microbial samples were collected from the anode of MFC on days 113, 126, 133 and day 136. The cells were dislodged from the electrode using a hypodermic needle, followed by withdrawal of the dislodged cells via liquid flow through the anode using a syringe. Genomic DNA was isolated using the standard freeze–thaw procedure, followed by phenol–chloroform extraction [26]. The 16S rRNA genes were amplified using GoTaq Flexi DNA polymerase (Promega, Madison, WI) and Bacteria-specific primers targeting positions 8–27 and 1510–1492 of *Escherichia coli* 16S rRNA [27]. PCR products purified from UltraPure™ Agarose (Invitrogen, Carlsbad, CA) using QIAquick Gel Extraction kit (Qiagen Inc., Valencia, CA) were ligated in pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA), and transformed into TOP10 chemically competent *E. coli* (Invitrogen) according to the manufacturer's instructions. The plasmid DNA released from the positive transformants by heating to 95 °C was amplified by rolling circle amplification (RCA) by the TempliPhi method that utilized bacteriophage ϕ29 DNA polymerase (Amersham Biosciences, Piscataway, NJ). The 16S rRNA genes were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit and TA Forward and TA Reverse primers with priming site on the plasmid. Sequences were determined by resolving the sequence reactions on an Applied Biosystems 3730 automated sequencer.

Clone libraries were checked for presence chimeric sequences using a program Bellerophon (http://foo.maths.uq.edu.au/~huber/bellerophon.pl) [28] and the CHIMERA_CHECK (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU) [29]. Putative chimeras were excluded from further analyses. Sequences were initially aligned against the most similar sequences in the Ribosomal Database Project II (RDP II) and assigned to a set of hierarchical taxa using a Naïve Bayesian rRNA classifier version 1.0 (http://rdp.cme.msu.edu/classifier/classifier.jsp). Closest relatives were retrieved from NCBI GenBank following BLAST search [30]. Multiple sequence alignment was done using the program Clustal X [31]. Phylogenetic 16S rRNA analyses were performed by the neighbor-joining [32] and maximum composite likelihood [33] methods. Bootstrap values were based on 1000 replicates generated using the program Mega 4 [34]. To determine the clone library coverage for each sample, statistical analyses were performed using DOTUR [35]. The population distribution is reported as a percent of the total number of bacterial clones sequenced for each sample. Sequences generated in the present study were deposited in GenBank (accession numbers FJ393061 through FJ393217).

2.5.2. DGGE analysis

DGGE analysis focused on the V3 region of the 16S rRNA gene which was amplified by nested PCR using separate primers to target both Bacterial and Archaeal sequences For Bacteria, the majority of the 16S rRNA gene was initially amplified from genomic DNA,

using the same set of primers as above, employing Phusion DNA polymerase (Finnzymes) in a 50 μl reaction volume. In the nested amplification, primers 518R (ATTACCGCGGCTGCTGG) and GC-341F (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG), containing the GC clamp [36], were used. To examine the benefits of the nested approach, the V3 region was also directly amplified from the genomic templates using primers 518R and GC-341F, directly. For targeting Archaea, the initial amplification used primers A46F: (C/T)TAAGCCATGC(G/A)AGT and A1100r: (T/C)GGGTCTCGCTCGTT(G/A)CC, while nested amplification used primers A340F: CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCCTACGGGG(C/T)GCA(G/C)CAG, containing the GC clamp and A519R: TTACCGCGGC(G/T)GCTG [37].

The products of the PCR reaction were then mixed with 10 μl of DNA loading buffer and separated on a 10% polyacrylamide gel (37.5:1, acrylamide/bisacrylamide) prepared with a denaturing gradient of 35–65% (100% denaturing gradient corresponds to 7 M urea and 40% formamide (Sigma)). Gel electrophoresis was performed at 60 °C, for 20 h at 120 V. The gel was subsequently stained in 1× TAE buffer containing 1:1000 dilution of Cybrgreen I (Invitrogen) for an hour before being photographed and bands excised on a blue light transilluminator.

The excised gel fragments were left overnight to elute in 30 μl of sterile water. One microliter was used as a PCR reaction template as described above using primers 518R and 341F without the GC clamp (ACTCCTACGGGAGGCAGCAG) or ARCH340F and ARCH519R without the GC clamp as appropriate. Fragment termini were adenylated by addition of 0.5 μl of Taq Polymerase (Invitrogen) and incubation at 72 °C for 20 min. They were subsequently cloned in either pCR3.1TOPO (Invitrogen) or pGEM-T Easy (Promega) vectors according to the manufacturers' protocols. Positive clones were identified by PCR with universal primers M13F and M13R (Fermentas) and plasmid DNA prepared using a Qiagen plasmid miniprep kit. Sequencing was performed from the M13F primer at the Genomics Core Laboratory, MRC Clinical Sciences Centre, Hammersmith Hospital, London, UK. Sequence analysis was performed using the programs Bioedit, AlignX (Invitrogen) and Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. Imaging of MFC biofilms

The MFC was transferred to an anaerobic chamber (Thermo Electron Corporation, Model 1025, Marietta, OH) and the electrode chambers was disassembled. Samples of the microbes growing on the carbon felt electrode were removed by using sterile tweezers. The detached carbon fibers from the electrode along with the biofilm/microbes growing on it were transferred on to a glass slide inside the anaerobic chamber itself. The cells were stained with Syto9 at a final concentration of 5 μM (Molecular Probes). Cells were imaged using a Leica TCS SP2 confocal laser scanning microscope equipped with a 488 nm Ar laser and the emission spectrum from 500–535 nm was collected. Z-series optical sections were collected at 0.5 μm intervals through the biofilm and the resulting images were projected as a single 2D image.

3. Results

3.1. Biocatalyst enrichment

The goal of the experiment was to maximize the growth of biofilm-forming, exoelectrogenic organisms in the anode. This was done via careful selection of the anode design and the MFC operating parameters. The MFC was operated in three different modes and in a sequential dual flow regime, which included a continuous recirculation at low flow rate and an intermittant forced flow at higher flow rates enabling removal of mediators and planktonic organisms. The flow-through design used allowed easy removal of non-biofilm-forming organisms via periodic replacement of the anodic fluid (Fig. 1A).

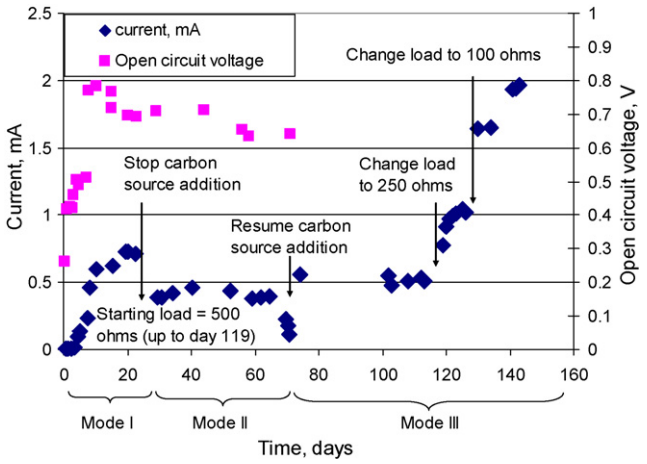

Fig. 2. Current vs. time profile for MFC-A showing the change in current during the various modes of operation.

During the first 28 days (mode I), the carbon source was added in excess of the amount needed for electricity production, based on the voltage measurement on the particular day. This was continued until the voltage output reached 0.4 V at a 500 Ω load. On day 29, mode II was initiated, by halting carbon source addition. It was observed that electricity production continued even after depletion of the carbon source in the nutrient medium, indicating that some carbon was stored internally by the cells. Freguia et al. [25] also reported this storage. Assuming 100% Coulombic efficiency, we estimate that the cellulose added during mode I could produce electricity for 14 days (at 0.2 V output). The MFC was operated in mode II for 44 days, during which time, the current output was stable at ~0.4 mA (Fig. 3). On the 45th day, or 73 days since start

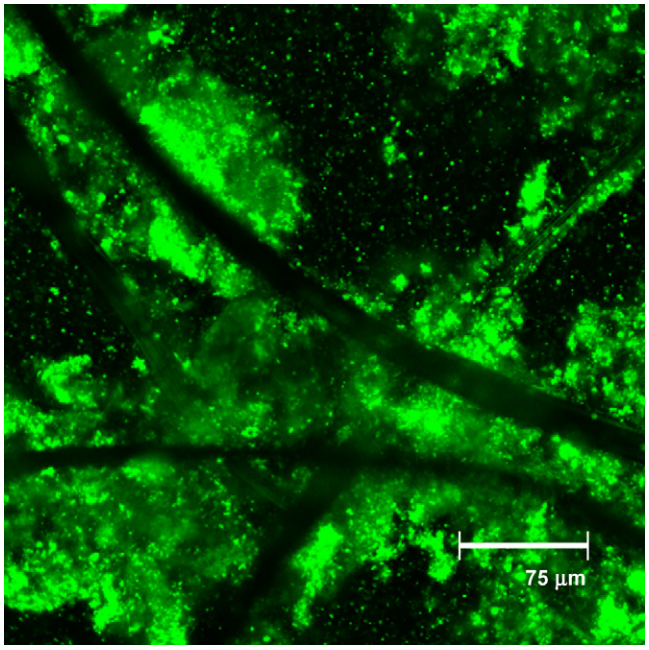

Fig. 3. Image of the microbial consortium inhabiting anode electrode (carbon felt) of the mixed carbon source MFC. The sample was stained with Syto9 (Molecular Probes). The community is dominated by biofilm-forming organisms.

up, current production decreased and the mode II was terminated. The amount of glucose and lactate added during mode I was more than sufficient to produce the observed current for the duration of mode I and mode II, assuming that part of carbon source was added during mode I was stored as internal carbon. The purpose of mode II was two-fold, first to deplete the residual carbon source present in the anode and secondly, to enrich organisms capable of electricity production from internally stored carbon. Such a consortium would be useful in handling process fluctuations (e.g., carbon loading, flow rates). In mode III, reduction in external resistance, which is equivalent to higher availability of an electron acceptor (in this case, the cathode) leads to a higher current between the anode and the cathode and to an opportunity for growth of the exoelectrogenic organisms. The current output increased during this period coinciding with each reduction (Fig. 2). During all these modes, the removal of mediators and free-floating cells was continued via periodic replacement of the anode fluid. During mode III, the forced flow was used in addition to anode fluid replacement to remove the mediators and cells from the anode and enhance biofilm formation.

Fig. 3 shows an image of the microbial community coating the carbon felt fibers from the MFC. A consortium dominated by a biofilm was observed. The image provided strong evidence for formation of a thick biofilm on the electrode fibers. The image shows 4 fibers with up to 100 μm thick microbial growth on each side of the fiber (when observed in 2D). A 3D image observed by taking depth images showed the biofilm surrounding all around the fiber. The presence of planktonic organisms in the bulk liquid shows that they were not completely eliminated from the consortium. The diameter of the biofilm thickness surrounding the electrode fibers in some locations was as much as 150 μm. The time taken for resumption of electricity production after replacement of the nutrient medium (thereby removing planktonic organisms) was less than 15 min, indicating that the electricity production was primarily due to biofilm-based organisms. In MFCs using mediators, hours to days are needed to resume high power output [16]. The maximum power density of the MFC-A using glucose and lactate (0.2 g l^{-1}, each) as the carbon source and using a ferricyanide cathode system was 300 W m^{-3} net anode volume (NAV) (3220 mW m^{-2}). The combination of the different modes of operation and the use of an improved anode resulted in thick biofilm-dominated exoelectrogenic consortia capable of producing electricity at high power densities.

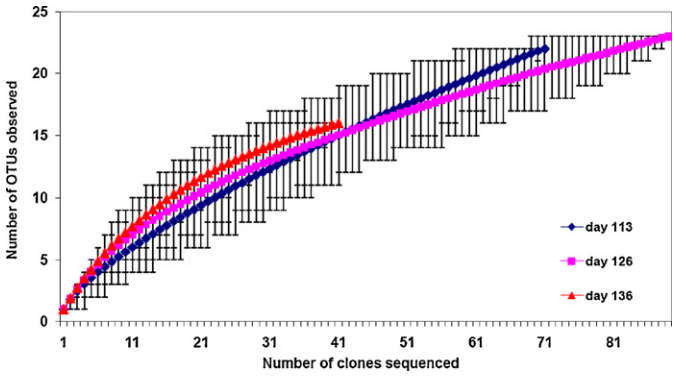

Fig. 5. Rarefaction curves with 95% confidence intervals for the clone libraries from each condition sampled. Number of operational taxonomic units (OTUs) was obtained for every clone library using of the furthest-neighbor approach with a 97% sequence similarity cutoff. The curve leveling indicates saturation in recovering of clones.

3.2. Biocatalyst characterization

3.2.1. 16S rRNA clone library

A 16S rRNA analysis of the anodic consortium from the MFC based on frequency of sequences found in bacterial primer-based clone-library is shown in Fig. 4a. This included 66% Veillonellaceae (25% *Anaeroarcus* spp., 41% *Anaeromusa* spp.), 11% Rhodocyclaceae, 10% γ-Proteobacteria, 8% Bacteroidetes and 4% other Clostridiales. A temporal analysis of the samples collected during mode III indicated that the community was changing slowly during this period. A rarefaction analysis substantiates this observation (Fig. 5). The number of operational taxonomic units obtained and predicted for each time event shows that the community had not reach complete stability by day 136 (Table 1). The dominant genus in the first sample (day 113) was *Anaeromusa*, with *Anaeroarcus* being the second dominant, which subsequently became the dominant genus (Fig. 4b and c). A phylogenetic tree of the microbial community from day 136 is shown in Fig. 6.

3.2.2. DGGE analysis

A DGGE analysis of the samples collected from MFC-A revealed that, on the basis of numbers, mobility and relative intensity of the bands, there was a gradual enrichment of certain species and

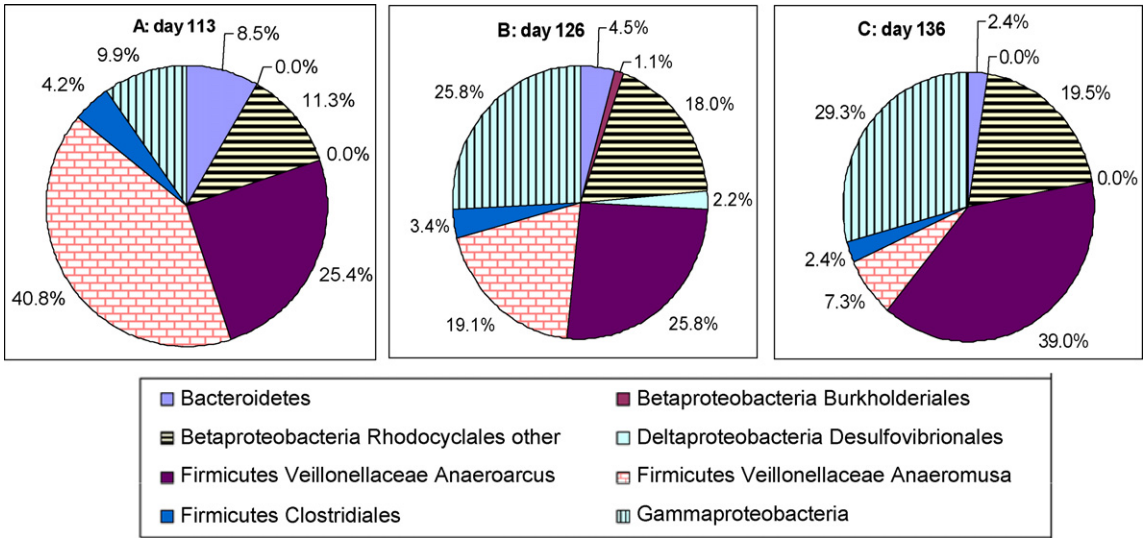

Fig. 4. Composition of microbial consortia from MFC anode: (a) MFC sampled on day 113; (b) MFC sampled on day 126; (c) MFC sampled on day 136.

Table 1
Characteristics and diversity estimates for 16S rRNA gene clone libraries with a 97% sequence similarity cutoff.

Treatment period, days	Number of clones[a]	Number of OTUs obtained[b]	Shannon, H'[c]	Number of OTUs expected[d]
113	71	22	2.19	47
126	89	23	2.50	35
136	41	16	2.49	26

[a]Number of clones analyzed for each treatment. [b]Operational taxonomic units based on partial 16S rRNA gene sequences. [c]Shannon index, the higher number the more evenly species in the population represented. Shannon index does not depend on the total population. [d]Operational taxonomic units expected if we continue to sample bacterial population. [e]Reciprocal of Simpson's index, higher number represents more diversity [35].

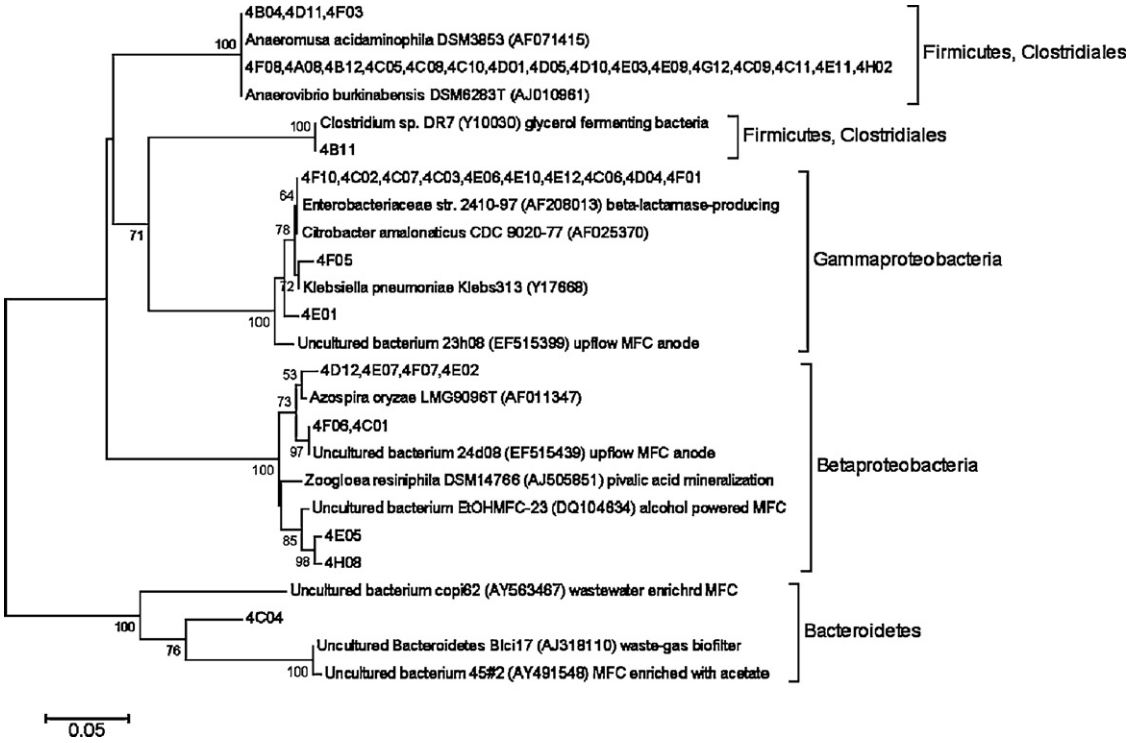

Fig. 6. Phylogenetic tree of the MFC anode consortium analyzed on day 136.

reduction in population diversity between day 113 and day 136 (Fig. 7). Generally, the eubacterial sequences agreed with those described above, with a significant number of sequences indicating members of the family Veillonellaceae (eg *Anaeroarcus*, *Anaeromusa*, *Phascolarctobacterium* spp.). Bands corresponding to *Anaeroarcus*/*Anaeromusa* reduced in intensity over time, while an Enterobacteriaceae band increased. *Azoarcus* and *Azospira* spp. were clearly identified. Nested PCR amplifications done with archaeal specific primers revealed the presence of archaea, with the most frequent sequences being similar to *Methanobacterium* sp. although the most intense band corresponded to a *Methanosaeta* sp. Although the primers used were designed to amplify archaeal 16S rDNA

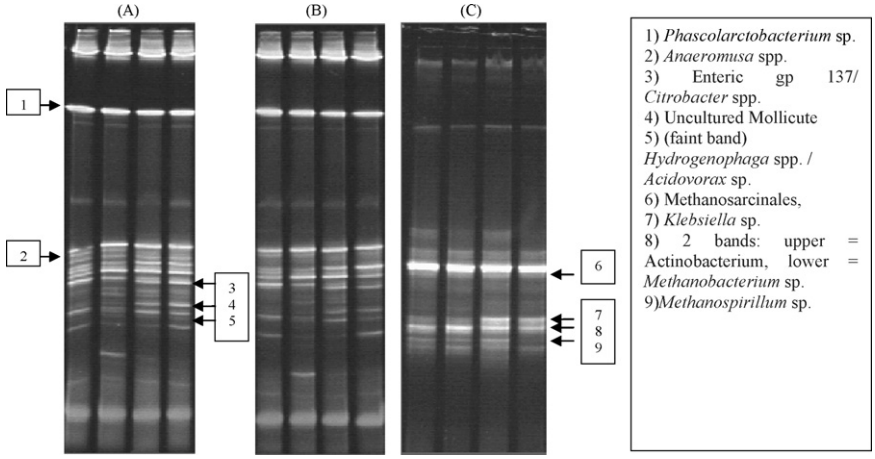

Fig. 7. DGGE analysis of sequences amplified from the V3 region of 16SrDNA amplified from sequential samples (L-R days 113, 126, 133 and 136, in all panels) from MFC anode. Samples in panels (A and B) were amplified with Bacteria selective primers, (A) using nested PCR and (B) amplifying directly with primers 518R and GC-341F. Samples in panel (C) were amplified by nested PCR using Archaea selective primers for both amplifications.

genes, a number of bacterial sequences were also obtained. The relative intensity of the bacterial DGGE bands compared to the archaeal bands, given the expected poor priming efficiency for the former suggested that, while clearly presented, archaea were not abundant.

3.3. Microbial diversity in consortia

Little is known about the two most dominant genera, *Anaeroarcus* sp. and *Anaeromusa* sp., found in the clone-libraries obtained from the anode samples. Both of these genera belong to the family Veillonellaceae, and have been described as amino-acid utilizing organisms [38,39]. This is the first report of the presence of this family in an exoelectrogenic MFC anode community. Fig. 6 shows the phylogenetic tree of the organisms detected in the clone-library of the day 136 sample from the MFC. The tree shows that several of the organisms other than the Veillonellaceae were related to those reported in MFCs. One member of the β-Proteobacteria (4F06) was related to an uncultured bacterium (24d08) from an upflow MFC (99% similarity). The other clones showed 97% or lower similarity to organisms enriched in MFC anodes, indicating that these are different genera. The members from the γ-Proteobacteria class were distinctly related to the uncultured bacterium 23h08 isolated from the same upflow MFC anode (96% similarity). Other clones from the β-Proteobacteria and the Bacteroidetes class show anywhere from 89% to 95% similarity with organisms reported in MFCs in the literature [40,41]. The rest of the community had no similarity to prior MFC-derived organisms, but showed similarity to organisms capable of denitrification and various other organic degrading organisms.

4. Discussion

4.1. Parameters affecting MFC power density

The enrichment of exoelectrogenic biofilm-forming organisms resulted in development of a consortium capable of generating electricity at a high power density (300 W m^{-3} NAV or 3220 mW m^{-2}). This is higher than what has been reported previously for MFCs with three-dimensional anode designs. One estimate suggests this is about a third of the power density needed for commercial consideration of MFCs [42]. High volumetric power densities have been reported in some studies for anode designs using planar electrodes by reducing the anode chamber volume [43,44]. The projected surface area power densities for such designs; however, are typically less than 2000 mW/m^2 and as such are not suitable for practical application. Many of the design and operational parameters used in this study have been studied individually and proven to result in power density improvements. A study using a high surface area anode electrode (graphite fiber brush) reported a maximum power density of 2400 mW m^{-2} with an air-cathode [3]. This electrode had an estimated surface area of 18,200 m^2 m^{-3}, as compared to 45,350 m^2 m^{-3} for the carbon felt used in this study, which potentially contributed to the higher power density observed in this study. Electrode spacing has been shown to have a significant impact on the power density as well. MFCs with negligible space between the electrodes separated by a cation exchange membrane have reported power densities of 1180 mW m^{-2} [45] and 2580 mW m^{-2} [5]. In membrane-free MFCs, the maximum power density as high as 2700 mW m^{-2} was reported [6]. The effectiveness of flow-through systems in improving power densities has also been relatively well established [7,8,19]. A recent study reported use of high shear to develop thick, dense biofilms resulting in power densities of up to 160 W m^{-3} [46]. Operation of the MFCs under dual flow-rate regime was one of the strategies used in this study to improve power density. In terms of the operational stability of MFCs, this study shows that biofilms can be grown in high specific surface area electrodes such as the carbon felt resulting in high power densities while avoiding problems such as clogging. The MFC was operated for almost 5 months without clogging problems. This was possible due to removal of excess growth by periodic flushing of the anode via implementation of a dual flow regime. This study used a combination of techniques to enhance exoelectrogenic growth. Some of these parameters were described recently in a review paper to improve power output from MFCs [4]. Further work is needed in understanding the implications of combining multiple strategies on the microbial community and to separate the effect of biology from design on power density. While there are other issues to be addressed prior to consideration of MFCs for energy production [47], the integrated approach developed in this study resulted in a higher power density and indicated a step in the right direction. This study is unique due to the combined approach of fusing effective engineering design with specific operational parameters leading to development of a microbial community capable of producing high power densities. The new organisms identified in the consortia that lead to formation of thick biofilms on the electrodes that permitted the high power density are evidence of the success of such a strategy. Further improvements in power density will come via further lowering of the applied resistance during enrichment period, complete elimination of methanogens and use of electrodes with better electrical and surface properties.

4.2. Microbial diversity

The microbial consortium enriched in this study was quite diverse and was dominated by Veillonellaceae, which have not been observed in MFCs previously. The mixed carbon source is a potential reason for the high level of diversity. The effect of the carbon source on MFC consortia has been reported in the literature [48,49]. The presence of carbohydrates has been related to presence of fermenting organisms such as Firmicutes in MFCs previously [14,15]. However, whether any of these organisms are exoelectrogens is not clear yet. Further work using pure strains isolated from MFCs is necessary to understand their functional diversity. Methanogens have also been reported to be present in some MFC communities [50]. The use of archaeal primers revealed the presence of a few methanogens in the MFC-A, but at a low abundance. Complete elimination of methanogens from MFC communities is an important need for further development of MFCs, as they represent electron sinks.

While the influence of MFC design and biology on power density is not fully understood, this study shows that combining appropriate enrichment methodologies with proper design can influence the power density and potentially the make-up of the microbial community. In addition to the impact of the MFC operating conditions on enrichment of microorganisms, these parameters can potentially play a large role in maintaining high power output from MFCs during their use as energy production devices, primarily due to the effect of these parameters on the stability of the consortia.

4.3. Achieving higher power densities and sustainable MFC designs

In this work, only the anode chamber and the anode biocatalyst were targeted for improvement. A Pt-based air-cathode was used during the enrichment process, while a ferricyanide-cathode was used for power density analysis. A detailed analysis of the limitations of the MFC using the biocatalysts developed in this study is being reported elsewhere. The air-cathode is a sustainable cathode but it has several limitations for its use in determination of maximum power density. The ferricyanide-cathode is not a sustainable cathode system for practical applications due to the inability

to regenerate ferricyanide effectively. An air-cathode requires protons in addition to electrons for electricity production, while a ferricyanide-cathode only requires electrons. Proton transport from the anode to the cathode limits the overall power output in air-cathode MFCs [51]. Comparing the ability of anode biocatalysts to produce electricity, a cathode which is not a limiting factor in power density determination (e.g. ferricyanide-cathode) should be used. Further work is needed to improve proton availability at the cathode and to achieve simultaneous proton transport at rates equivalent to electron transport without polarization [52]. The use of biological air-cathodes [53,54] in combination with the anode developed in this study and the use of flow patterns in anodes to facilitate proton transport can potentially produce high power densities in a sustainable manner needed for commercialization.

Acknowledgements

The financial support from the Oak Ridge National Laboratory (ORNL) Laboratory Director's Research and Development Program is gratefully acknowledged. ORNL is managed by UT-Battelle, Inc. via a contract #DE-AC05-00OR22725 for the U.S. Department of Energy. The authors would like to thank Mircea Podar, Jonathan Mielenz and anonymous reviewers for helpful suggestions and Lindsey Amason for editing the manuscript.

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