Reduction of start-up time through bioaugmentation process in microbial fuel cells using an isolate from dark fermentative spent media fed anode

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ABSTRACT

An electrochemically active bacteria *Pseudomonas aeruginosa* IIT BT SS1 was isolated from a dark fermentative spent media fed anode, and a bioaugmentation technique using the isolated strain was used to improve the start-up time of a microbial fuel cell (MFC). Higher volumetric current density and lower start-up time were observed with the augmented system MFC-PM (13.7 A/m³) when compared with mixed culture MFC-M (8.72 A/m³) during the initial phase. This enhanced performance in MFC-PM was possibly due to the improvement in electron transfer ability by the augmented strain. However, pure culture MFC-P showed maximum volumetric current density (17 A/m³) due to the inherent electrogenic properties of *Pseudomonas* sp. An electrochemical impedance spectroscopic (EIS) study, along with matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) analysis, supported the influence of isolated species in improving the MFC performance. The present study indicates that the bioaugmentation strategy using the isolated *Pseudomonas* sp. can be effectively utilized to decrease the start-up time of MFC.

Key words | bioaugmentation, EIS, MALDI-TOF mass spectrometry, single chambered MFC, start-up, *Pseudomonas aeruginosa*

INTRODUCTION

The microbial fuel cell (MFC) is an emerging alternative energy producing technology that can replace high energy intensive conventional wastewater treatment processes (Rozendal et al. 2008). MFCs can directly produce electricity from oxidation of organic waste using electrochemically active bacteria (EAB) (Rabaey et al. 2004). The MFC is becoming a popular approach for wastewater treatment as it couples electricity generation with organics biodegradation, requires less energy for its operation, and generates less sludge compared to the anaerobic digestion process (Oh et al. 2010). However, many improvements are required to make the MFC commercially viable for wastewater treatment (Clauwaert et al. 2008). One of the important aspects that can improve the performance of MFC is the reduction of the start-up time for the process. With the MFC, the start-up time is considered as being the time required for EAB to adapt to the anode. The activities of these microorganisms are one of the critical factors for MFC performance, which is relatively long due to immobilization of the microorganisms under anaerobic conditions (Rinaldi et al. 2008). Bioaugmentation is a promising strategy to accelerate waste removal from hazardous waste sites or bioreactors. Application of bioaugmentation has been previously applied to reduce the start-up time of a bioreactor to enhance reactor performance (Raghavulu et al. 2013). The successful application of bioaugmentation ultimately relies on the identification of appropriate microbial strains and their subsequent survival and activity during waste treatment. Bioaugmentation can be performed in several ways. First, additional substrates can be added since the wastewater strength is typically lower than that used in laboratory systems (usually less than 1 g/L chemical oxygen demand (COD)). Second, the addition of specific alternative electron acceptors can be used to encourage the growth of exoelectrogenic bacteria. Third, efficient microbial consortia or pure culture can be applied to increase the desired product yield.
The effects of the initial pH and the substrate concentration of the spent media on the volumetric power density production were studied using mixed microbial consortia (Pandit et al. 2014). It was observed that the start-up time required for the MFC was relatively high because it took a relatively long time to reach its maximum operating voltage (OV). So, the present study was carried out to reduce the start-up time of the MFC by employing a bioaugmentation process. An EAB was isolated from a spent media fed anode, and the isolated strain was used to augment a previously running mixed culture MFC. The bioaugmented system was compared to an MFC with a mixed culture as inoculum and one with the isolated pure strain as inoculum. The power density, cell resistance, capacitance and Coulombic efficiency (CE) were used as a yardstick to assess the performance of different MFCs.

MATERIALS AND METHODS

Microbial strain, media and growth conditions

An anaerobic mixed consortia obtained from the sludge of a septic tank in IIT Kharagpur was used as the inoculum (pH 7.2) after pretreatment. Spent media obtained after dark fermentation of molasses was fed into the MFCs after dilution. The same media was fed for 14 cycles (36 h/cycle) at ambient temperature (29 ± 2 °C). Prior to feeding, the pH of the wastewater was adjusted to the desired pH. The feed or anolyte for the MFC was prepared as follows: the spent dark fermentation media was initially centrifuged at 5,000 rpm (Sigma 3K30, Osterode am Harz, Germany), and the supernatant was collected. The media was diluted to 3 g/L COD and then the pH of the spent media was maintained at 7.5 using a carbonate buffer.

The isolate was used as the biocatalyst for bioaugmentation purposes in the anode chamber. Colonies of isolate were grown on LB agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) at 37 °C for 24 h. Single colonies were incubated in 50 ml LB broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India) in 100 ml conical flasks, shaken continuously on a rotary shaker (180 rpm) for at least 24 h under aerobic conditions. Ten ml of the culture was used as inoculum for the MFCs.

Three single microbial fuel cells (sMFCs) were operated in multiple batch cycles under close circuit mode. De-watered, pretreated anaerobic sludge of 25.54 g/L volatile suspended solids (VSS; pH 6.7) was used as mixed culture in an sMFC denoted as MFC-M. The same concentration (~25.54 g/L VSS) of pure culture was maintained in another named MFC-P. The augmented system where a mixed culture was blended with a pure culture of EAB (isolate) was denoted MFC-PM. Around 26 g/L VSS of inoculum was used as inoculum by maintaining the ratio of mixed to pure culture 1:1. The anode chamber was kept airtight and filled completely with 100 ml anolyte, with the inoculum added to achieve anaerobic conditions.

MFC assembly construction

Three identical sMFCs were used for the experiments. The MFCs consisted of an anode compartment and a membrane cathode assembly (MCA) placed on opposite sides (Pandit et al. 2013). The anode comprised cuboidal chambers of transparent polyacrylic material with outer dimensions of 7 × 8 × 5.5 cm³ with 110 ml of anolyte capacity. The anode chamber had two ports at the top, one for the electrode terminal and the other for the reference electrode (Ag/AgCl, saturated KCl; +197 mV, EquipTronics, Mumbai, India) and sampling. The additional ports were sealed with clamped tubes to ensure an anaerobic environment.

The anode consisted of a carbon cloth with a working surface area of 12 cm² and a stainless steel wire welded to form the terminal. The MCA was prepared by coating the membrane with catalyst-loaded cathode (0.15 mg/cm² laboratory-prepared manganese cobaltite nanorods (MnCo₂O₄-NRs), dust and carbon black Vulcan XC-72 (0.35 mg/cm²; Cabot India, Mumbai, India)) as described elsewhere (Khilari et al. 2014).

The sMFCs were washed with 70% alcohol and put in the UV chamber for 30 minutes before the experiment. The MFCs were operated in close circuit mode using 100 Ω to determine the COD removal and CE.

Analytical measurements and calculations

The pH and COD values of the anolyte were measured as mentioned earlier (Pandit et al. 2014a). The pH values were monitored using a desktop pH meter (pH510, Cyber-scan, Eutech Instruments Pte Ltd, Ayer Rajah, Singapore). The OV, current density, volumetric and power density with respect to the anodic surface area were measured as described elsewhere (Pandit et al. 2014b).

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), coupled with a mass spectrometer (MS), was used to detect the soluble electron mediator released by the isolate in the chloroform extract of the supernatant of the anolyte. The MALDI-TOF analyses were carried out in
dihydrobenzoic and \( \alpha \)-cyano-4-hydroxycinnamic acids as matrix. Prior to MALDI analysis, the co-crystalline pattern of pyocyanin on the MALDI sample plate was captured directly by a video monitor coupled with MALDI MS using a digital camera. MALDI analysis was performed on an Applied Biosystem Voyager-DE PRO MALDI-TOF MS (Applied Biosystems, Foster City, CA, USA) with a nitrogen laser (337 nm) operated with an accelerating voltage (20 kV). Each spectrum was collected in the positive ion linear mode as an average of 100 laser shots of predetermined or random positions across a slide.

**Scanning electron microscopy**

To examine the surface of the anode (carbon cloth) scanning electron microscopy (SEM) was performed. The sample was prepared as reported earlier [Pandit et al. 2014b].

**Bioelectrochemical measurements**

The electron transfer behavior of the bioanode was studied by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). A three electrode configuration consisting of bioanode, Pt wire and Ag/AgCl as working, counter and reference electrode, respectively, was used for all the electrochemical measurements. CV was recorded in the potential window of \(-0.6\) to \(-0.4\) V at a scan rate of 10 mV/s. The EIS of the bioanode was performed with the same electrode configuration. EIS was performed over a frequency range of 100 kHz to 1 Hz with a sinusoidal perturbation of 5 mV.

**RESULT AND DISCUSSION**

**Isolation of an EAB from dark fermentative spent media fed anode and biochemical characterization**

A bioanode fed with spent media of dark fermentative \( \text{H}_2 \) was used for the isolation of exoelectrogenic bacteria. After the 14th cycle, a small portion of anode carbon cloth was cut into pieces and the surface was scraped off. The cells were then suspended in 0.85% w/v of NaCl sterilized solution. Serial dilutions of \( 10^5 \), \( 10^6 \) and \( 10^7 \) were spread-plated on nutrient agar plates and incubated at 37 °C under anoxic conditions. The isolated colonies were picked from \( 10^6 \) diluted plates and inoculated in acetate ferric citrate (Ac-FC) medium (\( \text{NaHCO}_3 - 2.5 \text{ g L}^{-1} \); KCl - 0.1 g L\(^{-1}\); \( \text{NH}_4\text{Cl} - 1.5 \text{ g L}^{-1} \); \( \text{NaH}_2\text{PO}_4 - 0.6 \text{ g L}^{-1} \); \( \text{CH}_3\text{COONa} - 6.8 \text{ g L}^{-1} \); ferric citrate - 20 mM; vitamins and trace elements; pH adjusted to 7). In the Ac-FC medium, acetate was used as the primary carbon source while ferric citrate was used as the final electron acceptor as described by Lovley and Phillips (1998). The Ac-FC medium was used to enrich the isolates based on reports that the metal reducing bacteria also have the ability to transfer the final electron to the oxidized iron (Lovley & Phillips 1988). A brown colony of cell growth (SS1) was observed in Ac-FC based selective media. After four transfers in Ac-FC medium, it was also inoculated in butyrate Fe-citrate (Bu-Fc) medium and was enriched similarly by sequential transfers. Cultures were simultaneously checked for purity by streaking on nutrient agar plates at every transfer. SS1 was further maintained by streaking on nutrient agar slants. Acetate medium without any \( \text{Fe}^{3+} \) was provided in anolyte since, in an anode chamber of sMFC during ‘bioaugmentation experiment’ to improve start-up, carbon cloth acts as the final electron acceptor and thus if the isolate is able to grow on the anode surface, it would be able to generate electricity.

**Taxonomical characterization of the isolated strain SS1**

After isolation and several sub-culturings of SS1, the agar plate was sent aseptically to Amnion Biosciences Pvt. Ltd (Bengaluru, India) for sequencing. The isolate SS1 was characterized by sequencing its 16S rRNA and analyzing its phylogenetic relationship (Figure 1) with those of the reported bacteria. SS1 showed homology with many genera of *Pseudomonas*. *Pseudomonas aeruginosa* was found to be phylogenetically closest to the isolate, showing 99% similarity with 16S rRNA genes. Most members of the species of *Pseudomonas aeruginosa*, a Gram-negative, facultative anaerobe, represent environmental isolates, which play an important role in bioremediation and the biodegradation of a large number of pollutants like phenol, crude oil, etc. (Zhang et al. 2005). The presence of *Pseudomonas aeruginosa* on an anode was reported by Yong et al. (2011). It is a common electroactive microorganism found in MFCs, which usually produces soluble mediators like pyocyanin, pyoverdin, etc. (Rabaey et al. 2004).

**Biochemical characterization of the isolate**

Morphological tests have been carried out using standard methods. Carbon source utilization tests have been carried out using commercially available biochemical identification kits (Hi25™ identification kit, HiMedia, Mumbai, India). Based on the morphological aspects, the strain SS1 was found to be catalase-positive and Gram-negative. The colony morphology of the strain was found to be slimy
and glossy with green shed on the agar plate. Other features are provided in Table 1.

The MALDI-TOF analysis was performed to examine the ability of an isolated strain to produce mediators for effective electron transfer. Anolyte of the MFC operated with a pure culture of *Pseudomonas* as inoculum was collected and centrifuged 100 ml of supernatant was collected after centrifugation at 5,000 rpm for 10 min; 60 ml of chloroform was added to 100 ml of supernatant and vortexed for 30 s; and 30 ml of the resulting blue layer at the bottom (chloroform + soluble mediator) was transferred to a new tube (Figure 2). MALDI-TOF MS was performed after by condensing it with Eyela rota evaporator (N-1001S-WD). To do so, a dark blue sample was mixed with dihydrobenzoic and α-cyano-4-hydroxycinnamic acids and put onto the matrix. The predominant peak detected was at m/z 211, which corresponded to the protonated form of pyocyanin in the chloroform extracted supernatant. The m/z 224 peak was the product of a thermally promoted intermolecular reaction in the ThermoBeam mass spectral detector (Applied Biosystem, Foster City, CA, USA), which is consistent with the observations of a previous study (Mavrodi et al. 2001).

### Start-up time: voltage development

Three different types of inoculum were used to investigate the influence of *Pseudomonas* bioaugmentation on the overall performance of sMFCs in terms of power output and CE. The sMFCs with three inoculum types: *Pseudomonas*, *Pseudomonas* with mixed culture, and mixed culture as control were used and the OV was monitored. These sMFCs were denoted MFC-P, MFC-PM and MFC-M, respectively. It was
found that the inoculum type can significantly affect the performance of sMFCs in the initial phase. Following inoculation, OV started developing immediately within 48 h in MFC-P and MFC-PM. However, no significant OV was observed even after 3 d (beginning of 3rd cycle) after inoculation in MFC-M. This indicated efficient utilization of *Pseudomonas* for augmentation purposes. A large difference in current generation was found between three MFCs (Figure 3). The maximum volumetric current density generated by MFC-P was 17 A/m³ after the 3rd batch cycle, which was approximately 49% higher than that of MFC-M (8.72 A/m³), while MFC-MP produced a current density of 13.7 A/m³. During the following 15 cycles, the current density for all three MFCs reached a comparable value with 20 A/m³ for MFC-P, 15.1 A/m³ for MFC-M, and 18.09 A/m³ for MFC-MP, respectively. This indicates that *Pseudomonas* had a significant effect during start-up of the MFC.

MFC-P produced voltage almost immediately while MFC-M showed a lag period followed by an enrichment period in the course of the first six cycles. This difference in MFC start-up was likely due to several reasons. The MFC-P inoculum was grown within 3 d in an acid-rich spent medium, so there was a high density of cells in the inoculum, which were able to quickly scavenge any oxygen in the system. Planktonic *P. aeruginosa* IIT BT SS1 had the ability to transfer electrons to the anode using self-produced mediators like pyocyanin and pyoverdin (Mavrodi *et al.* 2001). In addition, *Pseudomonas* has a tendency to form biofilm on the anode. Originally, *P. aeruginosa* IIT BT SS1 was isolated from spent media fed MFC. Therefore, this wild type strain was already acclimatized to the anolyte. Consequently, *P. aeruginosa* IIT BT SS1 in the inoculum utilized spent media containing mostly acetate and butyrate. Mixed cultures often have low CEs (Figure 3). In a case where the ohmic resistances and cathode configurations are the same, the difference in the internal resistance from the MFC-P culture to the mixed culture MFCs is most likely due to the interaction between the microbial communities within the anode. Several reports have shown improvement in MFC performance using a bioaugmentation strategy. Previously, Wang *et al.* showed that it is possible to directly generate electricity from waste corn stover in MFCs through bioaugmentation using naturally occurring bacteria (Wang *et al.* 2009).

### Table 1 | Response of *Pseudomonas aeruginosa* IIT BT SS1 during different biochemical tests

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Response</th>
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<tbody>
<tr>
<td>Gram stain</td>
<td>Negative</td>
</tr>
<tr>
<td>Cellular morphology</td>
<td>Rod</td>
</tr>
<tr>
<td>Indole production</td>
<td>Negative</td>
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<tr>
<td>Methyl red test</td>
<td>Negative</td>
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<tr>
<td>Voges–Proskauer test</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>Negative</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>Negative</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>Positive</td>
</tr>
<tr>
<td>Oxidase activity</td>
<td>Positive</td>
</tr>
<tr>
<td>Maltose</td>
<td>Positive/negative</td>
</tr>
<tr>
<td>Lactose</td>
<td>Negative</td>
</tr>
<tr>
<td>Glucose</td>
<td>Negative</td>
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Figure 2 | Photographs of (a) nutrient agar plate, (b) acetate agar plate of *Pseudomonas aeruginosa* IIT BT-SS1 and (c) Falcon tube showing the chloroform extraction of pyocyanin.

Figure 3 | Volumetric current density and Coulombic efficiency (CE, %) profile of sMFCs having different inoculum in anode after up to 8th cycle. The current density and CE data points are presented as solid and open symbols, respectively.
Polarization study

To determine the difference in the power output between the three MFCs, the polarization study was carried out with a mixed culture containing MFC and compared with a pure culture of *Pseudomonas* and a mixed culture with *Pseudomonas* after completion of the fifth cycle (Figure 4).

The MFC-M produced a maximum power density of 3.7 W/m² (255 mW/m²). The maximum volumetric power density reached 29.5% with MFC-P (5.25 W/m³; 361.25 mW/m²). The MFC-PM showed a maximum power density of 4.1 W/m³ (281 mW/m²). Therefore, the trend suggested that addition of *Pseudomonas* as inoculum has a fairly large influence on CE. The poor performance of the MFC-M might be due to the immature biofilm formation and larger overpotential energy loss during charge transfer from cell to anode. The improved power generation with *Pseudomonas* can be attributed to the biofilm forming ability, which showed efficient electrogenesis within a short period of time. MFCs containing pure culture have previously been reported to be started up within a short period (e.g., < 4 d). However, it has some drawbacks as there is a high risk of microbiological contamination and generally a high substrate specificity as compared to mixed-culture systems. Mixed-culture MFCs generally need a longer time to obtain a stable power output as compared to pure-culture MFCs. Previous studies showed that the start-up stages of two-chambered MFCs varied over a wide range from 4 to 103 days depending on inoculation, anode material, cathode, reactor configuration as well as operational conditions (e.g., temperature, external resistance). Nevertheless, start-up time can be reduced through bioaugmentation.

Microscopic analysis: SEM image of the carbon cloth anode fiber

A SEM study was performed after completion of the 4th batch cycle. SEM micrographs show the morphology of carbon cloth fiber before and after biofilm formation, demonstrating good bacterial adhesion (Figure 5).

Owing to the high affinity of *Pseudomonas* toward anode and biofilm forming capacity, total biomass attached to the *Pseudomonas* inoculated anode was relatively high and morphology of the biofilm was uniform, which facilitated electron generation and transfer. In addition, soluble mediators helped in the anode half-cell voltage development. It was observed that quorum sensing (QS) modulates the current generation of the anode-respiring bacterium *Pseudomonas aeruginosa* because it controls the production of phenazines, which mediate the electron transfer to the anode (Yong et al. 2011). It is reported that *P. aeruginosa* possesses type 4 pili (Chiang & Burrows 2003), which can bind directly with an anode surface. Once bound, they undergo a form of organized movement known as twitching motility (TM). The TM also facilitates in increasing the power density of MFCs (O'Toole & Kolter 1998).

CV and EIS study

CV was performed in all three types of MFC. The cyclic voltammograms recorded with different bioanodes are presented in Figure 6.

Charge harvesting capacity was evaluated from the capacitance of the bioanode, termed as bio-capacitance. Bio-capacitance was calculated with the help of the following equation:

$$ C = \frac{\int_{V_1}^{V_2} \text{IdV}}{A \Delta V (dV/dt)} $$

With the voltammograms of mixed culture inoculum, several oxidation peaks were observed at different potentials (−0.214, −0.04 and 0.265 V), which might be attributed to substrate oxidation by different electrogens. The measured bio-capacitance of the mixed culture based anode was found to be 0.025 F/cm². However, the pure culture (*Pseudomonas*) based anode exhibited a single oxidation peak at
∼ 0.07V, which was consistent with the previously reported literature. Furthermore, the bio-capacitance was significantly improved to 0.071 F/cm². The presence of both mixed culture and \textit{Pseudomonas} increased the electrogenic activity as compared to mixed culture alone. The characteristic oxidation peak for \textit{Pseudomonas} slightly shifted to a more positive potential. This indicates the electrogenic interaction of two different kinds of bacteria. The bio-capacitance of different anodes follows the following order: MFC-P (0.071 F/cm²) > MFC-PM (0.042 F/cm²) > MFC-M (0.025 F/cm²). The study corroborates an improvement in MFC performance with a bioaugmented system during the start-up phase.

To calculate impedance components, impedance data are analyzed by equivalent circuit (EC) modeling. The $R_{ct}$ value directly related to the electron transfer behavior of an electron chemical reaction occurring with the electrode. A significant difference in the semicircle region was observed in the impedance plot of the electrodes (Figure 7).

The $R_{ct}$ value of different MFCs follows the following order: MFC-M (259.4 Ω) > sMFC-PM (212.09 Ω) > sMFC-P (188.86 Ω). The minimum $R_{ct}$ value obtained in a pure culture of \textit{Pseudomonas} catalyzed sMFC indicated the maximum electron transport was due to oxidation of acid-rich spent media, which reduced the anodic voltage losses and increased the current output. The results obtained from EIS support the results obtained from the half-cell polarization study. The internal resistance in MFCs originates from poor biofilm development on the anode surface and electrochemical reactions on the

![Figure 5](https://iwaponline.com/wst/article-pdf/72/1/106/177579/wst072010106.pdf)
anodes. The large drop in voltage in this low current region points to larger activation losses in the MFC-M. The initial voltage drop indicated that a larger amount of energy was lost during the transfer of electrons from the MFC-M to the anode surface than that observed by the transfer of electrons from the MFC-P.

**Power production after start-up**

All MFCs were run for more than 14 weeks, showing that all the MFCs can produce a stable current for extended periods of time. The maximum current density of MFC-PM was 20.9 A/m$^3$, while MFC-M and MFC-M generated 20.72 A/m$^3$ and 18.54 A/m$^3$, respectively, when MFCs were operated across 100 $\Omega$ external resistance. The MFC-P needed 11 days to obtain a similar current output, which was 31 days faster than that of the control MFC operated under 100 $\Omega$ external resistance. When all MFCs were started up, no significant difference in performance was observed. A maximum power output of 4.84 W/m$^3$ (332.6 mW/m$^2$) was achieved in MFC-P, which was approximately 14% higher than that of MFC-M (3.74 W/m$^3$; 258.6 mW/m$^2$), while the maximum power output of MFC-PM (4.96 W/m$^3$; 340.5 mW/m$^2$) was almost on par with MFC-P. After starting up, all the MFCs containing three different inoculum types converged to roughly 20.9 A/m$^3$ after several fed-batch cycles. The augmented system produced slightly higher current output compared to both the mixed culture and the pure culture of *Pseudomonas* at the later phase of operation. The volumetric current density profile of different MFCs showed gradual improvement in the augmented system MFC-PM (Figure 8).

This might be attributed to the improved electron transfer ability of the mixed culture due to the synergistic interaction with exoelectrogenic microorganisms. The CE of MFC-P and MFC-PM varied from 7.1% depending on the current density, which was similar to those obtained in MFC-M (6.88%). The results were almost on par with MFC-P. The results suggest that with long-term operation the current output would be same. This also indicates that the anode biofilm had the same capacity for current generation, regardless of the start-up strategies. This type of evidence was found in an earlier report by Pham *et al.* (2008) where they found *Brevibacillus PTH1* as a dominant species in a microbial community on an anode. However,
the current output by *Brevibacillus* sp. PTH1 was found to be low unless it was co-cultured with *Pseudomonas* sp. or supernatant from an MFC operated with *Pseudomonas* sp. as inoculum (Pham et al. 2008). The present study demonstrated that metabolites produced by *Pseudomonas* sp. enable other bacteria to achieve extracellular electron transfer. Possibly, this bacterial interaction is a key process in the anodic electron transfer of MFC from other anodophiles. *Pseudomonas* species are capable of producing electron shuttles for their own proper shuttling; others may interact with *Pseudomonas* to support its energy metabolism. In another experimental study, Milliken and May demonstrated current generation by several *Desulfitobacterium* strains. It produced bioelectricity only in the presence of electron shuttles like humic acids or their analogs (Milliken & May 2006).

**CONCLUSION**

A bioaugmentation-based approach was explored in MFCs to improve performance from anodic electrogenic biofilms and to accelerate their formation. The isolated *Pseudomonas aeruginosa* produced higher current densities than a mixed consortium. In the bioaugmented system, improved power output was observed along with reduction in start-up time. However, polarization data obtained after long-term operation of all MFCs showed hardly any difference in maximum power densities. Electrochemical impedance spectroscopic analysis of *Pseudomonas aeruginosa* and the mixed culture inoculated MFC revealed a significant difference in charge transfer resistance at the initial phase, indicating better biofilm formation in *Pseudomonas* sp. inoculated MFC. Similar observations were made with SEM, which revealed more compact biofilm formation in MFC-P and MFC-MP compared to MFC-M. Thus, useful remediation of volatile acid rich spent media could be achieved through a bioaugmentation process, which further improves process kinetics.

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