Evaluation of inoculation method and limiting conditions on bacterial activity in microbial electrochemical cells

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Keywords:
Microbial bioelectrochemical cell
Startup
Mixing
Salinity
Electron donor
Electron acceptor

Abstract

There is growing interest in the potential of microbial electrochemical systems (microbial electrochemical cells – MXCs) for sustainable wastewater treatment and energy production, and extensive research has been undertaken to improve their power production. To optimize MXCs, their performance under technical and operational deficiencies should also be characterized. Using experiments with fed-batch reactors, this study investigated the effects of inoculation method, electron donor and acceptor limitations, mixing, salinity, and substrate concentration on performance.

The MXCs required 0–8 days for current generation depending on the inoculum source; the most rapid generation was achieved with attached electrogenic bacteria. When the electrogenic bacteria were exposed to air for 3 h, the current production was deferred for 5 h. The bacteria could handle the lack of an electron donor for at least 3 days, and the lack of a solid electron acceptor for at least 5 days, which would facilitate long distance delivery. A 1.54-fold increase in electron donor concentration contributed to a 1.7-fold enhancement in peak current. The addition of 75 mM NaCl increased the power density from 1.64 mW m⁻² to 2.16 mW m⁻², whereas optimal mixing increased the power from 0.613 mW m⁻² to 1.786 mW m⁻². Thus, electrogenic bacteria may endure some unfavorable conditions, but optimization of operational conditions is necessary to maximize MXC performance.

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Introduction

Freshwater is critical for life and is in short supply, and water pollution intensifies water deficiency problems. Wastewaters pose a serious threat to water bodies. The release of untreated wastewater to water resources is considered a consequence of the technical and economical requirements of conventional wastewater treatment processes. Microbial electrochemical cells (MXCs) are potential sustainable technologies that can be used for wastewater treatment, bioremediation, and renewable energy production. MXCs extract energy during wastewater treatment, which is an energy consuming process [1,2], and biotransformation of organic matter results in electron and proton release [3]. Technically, the ability of bacteria to transfer electrons to an insoluble electron acceptor (anode electrode), a process known as extracellular electron transfer, differentiates these systems from conventional biological methods. It is therefore essential that soluble acceptors are separated from bacteria, which require anaerobic conditions [4]. Bacteria known as electrogenic or anode-respiring bacteria (ARBs) [5] transfer electrons to the anode electrode through direct contact [1,6], from nanowires originating from their membranes [7,8], or from natural electron shuttles synthesized by the bacteria themselves [9,10]. After taken by the anode electrode, electrons are conducted to the cathode through an external circuit. This flow of electrons gives rise to electrical energy [11], but can also help hydrogen gas production with an extra energy [12,13]. Migration of electrons toward the cathode leaves the anode unbalanced. A membrane usually separates anode and cathode and exchanges charges in order to neutralize protons.

MXC performance is influenced by many factors, including reactor configuration [14–18], bacteria species [19], substrate composition [20], and operational conditions [21,22]. Attempts to enhance performance in terms of power output led to a 10,000-fold improvement in less than 10 years [23]. The use of materials with a high specific area as electrodes facilitates this process [24]; reductions in electrode spacing also reduce internal resistance and increase power [25]. Separators between electrodes play a key role in charge balancing and in subsequent energy production. Compared to cation or proton exchange membranes, anion exchange membranes (AEM) are much more successful [26]. Not all substrates act the same in MXCs, and substrates with simpler metabolism had been better electron donors [27]. For given reactor design and wastewater composition operational conditions are determinant factors.
Before a system can produce any energy, it has to go through a start-up period during which a sufficient bacterial population has to build up for efficient energy production. Operators of wastewater treatment plants are concerned about how fast they can start new systems or repair damaged ones. High temperatures have assisted with the adaption phase [28], but heating a large volume of wastewater is not cost effective, and is at times even impossible. Moreover, if the wastewater temperature is raised just for the start-up step, altering the temperature during standard operation might have an adverse effect on the well-adapted microbial community. However, increasing the strength and conductivity of the wastewater has not improved start-up [29]. Because MXCs require bacteria with special capabilities, culturing methods might significantly prolong or shorten the start-up phase. However, currently, there is no comprehensive explanation for bacteria assimilation in new systems. Laboratory experiments expect to lead to practical implementations, and several points should be considered with respect to real-life wastewater treatment. First, neither the quality nor the quantity of the wastewater remains consistent throughout the day. Second, most industries do not produce wastewater continuously. Moreover, operational or technical accidents are always possible. Prior to scale-up, the response of the process to these variations should be clearly understood. Some variations, such as temperature and anodic pH, have been targets in previous studies [30], but several other operational factors remain to be addressed.

This study was designed to evaluate the effect of different inoculums on the start-up period and to identify the consequences of several possible operational failures. It also investigated the effect and importance of several operational conditions on current generation.

Materials and methods

MXC configuration and operation conditions

Sandwich configuration MXC was used in the current study. Cylindrical anode and cathode chambers established working volumes of 290 and 120 mL, respectively. Electrons in the anode chamber were collected by carbon fibers united in a stainless steel frame. Carbon fibers were put onto acetic acid, ethanol, and nitric acid solutions, each for one day, to be activated and prepared for installation in the MXC reactors. A stainless steel mesh acted as cathode electrode and received electrons to produce hydrogen gas. Anion exchange membrane (AMI-7001, Membranes International Inc., USA) separated two chambers so that created contact area of 32 cm² between cathode and anode compartments. AEM allows anion migration from the cathode to the anode. The MXC systems were operated in the batch mode and a multi position stirrer at 150 rpm mixed liquid in chambers. The potential of working electrode was fixed at −0.4 V vs. Ag/AgCl reference. A potentiostat (BioLogic, VSP, Canada) set this potential and recorded current and cumulative current at every 120 s to a connected computer. The reactors were placed under a plastic cover in which temperature was controlled at 25 ± 1 °C. Fig. 1 depicts a schematic diagram of the applied reactor.

Medium was composed of acetate as electron donor, phosphate buffer, and trace elements. Different acetate concentrations were used. For 25 mM acetate, 50 mM of phosphate and one ml of metal solution were used. Base on the acetate concentration, other chemicals changed to maintain the same proportion in the mentioned composition. Medium for 25 mM acetate included: C₆H₁₂O₆ 2.05 g L⁻¹, KH₂PO₄ 2.27 g L⁻¹, Na₂HPO₄·12H₂O 11.68 g L⁻¹, MgCl₂·6H₂O 0.025 g L⁻¹, NH₄Cl 0.037 g L⁻¹ and 1 mL of a mineral solution.

While anolyte was acetate medium, milli-Q water with resistivity of 18.2 MΩ cm acted as catholyte. In order to create anaerobic condition in the bioreactor, at the startup of each run, a mixed gas of 80% N₂ and 20% CO₂ (v/v) was sparged for an hour.

Inoculum sources

When the aim was assessing the effect of inoculum methods, different inoculums were used to culture microorganisms in the MXCs, including activated sludge, MXC suspension, attached electrogenic bacteria and anaerobic digested sludge. MXC effluent was taken from an MXC working in the batch mode for three months and producing a steady state current density of 6.84 A/m².

To inoculate MXCs, 30% of reactor volume was filled with this inoculum. Attached bacteria grew on the carbon fibers within a MXC system. The stainless steel frame was taken and placed in the new reactor. Activated sludge was collected from a municipal wastewater treatment plant, Easton Avenue Treatment Plant (Waterloo, Canada), and retained in the refrigerator under anaerobic condition for one week. Anaerobic digester, in which glucose was used as the substrate, was another source for taking inoculum. This digester was operating under both 20-day solid and hydraulic retention times.

For other purposes, inoculation was done by mixed liquor from the working MXC reactor.

Measurements and data analysis

Acetate available in fresh substrates and effluents was measured by Gas Chromatography with Flame Ionization Detector (GC-FID). GC-FID was equipped with Nukol fused-silica capillary column. The initial temperature of the column was 110 °C and increased with a constant rate to the final temperature of 195 °C within 9.5 min. The carrier gas for GC-FID operation was helium. Samples required acidification before acetate detection and were acidified by phosphoric acid to pH 2. All samples were analyzed in duplicate and the average was reported.

The MXC performance was evaluated with respect to current production and columbic efficiency. Current and cumulative current were directly recorded by EC lab software. Columbic efficiency (CE) considers electron balance between consumed electron donor and total energy output: CE = Qe/Qout.

In which Qe: energy recovered (mmol electron) as current; Qout: total available energy in the consumed substrate (mmol electron).
Pure culture MXCs

Several experiments were performed using a MXC reactor with a *Pseudomonas aeruginosa* (PAO1) culture with uncontrolled potentials. A potentiostat measured and recorded voltage output across a 330-ohm resistor. The catholyte was 100 mM potassium ferrocyanide and the anolyte was a substrate that contained acetate and lactate as electron donors. The substrate composition was selected according to the experimental literature [31]. The anode electrode was a 2 cm × 3 cm graphite plate, and the cathode electrode comprised 50-cm carbon fibers wrapped around a stainless steel wire. Copper wire conducted the electrons from the anode to the cathode. The anode and cathode compartments were identical cubic chambers with a 700 ml working volume. A Nafion 117 membrane, a proton exchange membrane, was placed between the anode and the cathode to separate anolyte from catholyte and let proton transfer. Figure shows schematic of the pure culture reactor. The power (W) was calculated by \( P=V^2/R \) where \( V \) is voltage (V) and \( R \) is resistance (Ω) normalized to the membrane surface area of 46.5 cm² (Fig. 2).

To prepare PAO1 for MXC reactors, bacteria colony was grown on a plate were first aerobically cultured in Luria Broth (LB) media containing 10 g/L tryptone, 5 g/L yeast extract and 10 g/L sodium chloride. Bottles were put into the incubator rotating at 200 rpm speed and holding a temperature of 37.5 °C. Aerobic bacteria then transferred to acetate-lactate medium to grow under anaerobic condition. MXC reactors used these anaerobic bacteria for startup phase. Anode chamber was sparged with nitrogen gas during operation.

Results and discussion

Substrate injection method, current production

Fed-batch reactors are fed intermittently. They are filled with wastewater, discharged after treatment, and then fed again with new wastewater to start a new cycle. In this study, the performance of a new cycle was evaluated in the presence of treated wastewater. When the MXCs were out of electron donors and the current had decreased to almost zero, the substrate was replaced through two different methods. In one reactor (control), the treated wastewater was fully discharged; the reactor’s suspension liquid was completely removed using nitrogen sparging, centrifuged, and the sludge was returned to the reactor. The second reactor was partially discharged and some of the wastewater was left in the reactor for the next cycle. The substrate was added to the reactor from the bottom as the old treated wastewater was removed from the top. After feeding, about 10% (v/v) of the reactor contained treated wastewater.

Refeeding the reactors immediately restored the current, but the current pattern was highly dependent on the feeding method. Fig. 3 clearly demonstrates this dependency. Injecting a new substrate into treated wastewater lacking an electron donor could have led to this mixing with and therefore diluting the feed. However, this type of feeding was followed by a big jump in current immediately after injection; the current was increased up to 12 mA in 1 h. In contrast, feeding the whole substrate to the empty reactor over a short period provided a rich source of electron donors for ARBs. This control reactor however needed more than 10 h to achieve the same current generation. All of the reactors that retained some effluent produced a higher current than the reactors without effluent, even if the reactor was completely discharged and effluent from a different reactor was added in. Since the communities of suspended and attached bacteria did not change and the substrate was identical, it appears that dissolved materials were involved in current generation.

Some electrogenic bacteria are able to produce dissolved mediators that shuttle electrons between donors and collectors. These dissolved mediators need time to be synthesized, and are washed in batch reactors as the effluent leaves the reactor [32]. These remain in the treated wastewater and are likely to participate in current enhancement during the next run. Obviously, the faster the electrons are shed, the faster the organic matter is removed from the wastewater.

Effluent from a MXC reactor, even if it did not contain any microorganisms (suspended solids), increased the performance of the reactor, highlighting the benefit of effluent recirculation in bioelectrochemical systems.

The effect of inoculation method on start-up period

The start-up period is important in setting up a new system, but it also determines the recovery time of an impaired system. The time required for starting a new bioelectrochemical system varies from several minutes to several months [29]. The present study determined the effect of seeding methods on start-up time using different inocula sourced from 20 ml anaerobic digested sludge, 80 ml MXC effluent, 20 ml activated sludge, a mixture of 20 ml digested sludge plus 65 ml MXC effluent, attached ARBs, and attached ARBs plus MXC effluent. The corresponding reactors, and the first sign of current generation by these reactors, are depicted in Table 1.
When attached ARBs were combined with MXC effluent, a current was generated immediately after set-up and this combination was ranked as the best choice for inoculation. The prepared ARBs did not require any time to adapt to the new system. When the attached ARBs were added to a system without effluent, a current was also generated immediately but this was lower than the current generated by the combination of ARBs and MXC effluent due to the presence of mediators in the effluent as discussed in the former section.

Mixed cultures of anaerobic sludge and MXC effluent were also used for starting up a new MXC system. Electron acceptors in the digester are typically soluble and so the bacteria did not use solid acceptors. Once transferred to the MXC, some bacteria gradually gave electrons to the anode, which resulted in current generation by day three. When anaerobic digested sludge was used without MXC effluent, and extra day was required for current generation.

The presence of mediators in the mixed culture could have facilitated electron transfer. Taking electrons away from the donor could have helped further electron release, thereby accelerating the growth of electrogenic bacteria and shortening the start-up period. An inoculum of MXC effluent alone did not accelerate the start-up time compared with anaerobic sludge as it contained an insufficient number of ARBs for substrate consumption and electron production, although many mediators were present. It took approximately 8 days to generate a sufficient number of ARBs for current generation. Activated sludge was also fed to culture bacteria in the new MXC. This did not contain any mediators or ARBs, but after 5 days of operation, the bacteria adapted and initiated electron transfer to the anode.

These data indicate that the inoculation method significantly influences the start-up time. In previous studies adding acetate, an ideal electron donor in MXCs, to domestic wastewater did not shorten the start-up period [29]. Other additives, such as a phosphate buffer or glucose, could even delay the generation of a stable current in microbial fuel cells [29]. In contrast, increasing the wastewater temperature to 30 °C accelerated the start-up period [28]. Without adding any increased costs, MXC inoculums, if available, can eliminate the lag time for the set-up of new treatment plants.

**Effect of substrate concentration on current generation**

The substrate acts as an electron donor for the bacteria, and 20 mM and 30.8 mM acetate concentrations were used in the present study. The higher concentration resulted in greater energy production, but not at the same ratio (Fig. 4). The peak current was also based on the acetate concentration. A 1.69-fold increase in the peak current was observed after a 1.54-fold increase in the electron donor concentration. The peak current was 21.5 mA with 20 mM acetate and 36.3 mA with 30.8 mM acetate. The higher substrate concentration yielded a cumulative current almost 1.8-fold higher than that of the lower concentration in 100 h (Fig. 5). The currents produced at this time matched the use of 74% and 80% of the initial substrate, respectively, for the low and high concentrations of acetate.

Regardless of their common definition in bioelectrochemical systems as biocatalysts, bacteria use part of the available substrate for growth and self-maintenance, and this proportion of the substrate is not available for energy production. Since the bacterial population was probably the same in both cases, the proportion of the substrate dedicated to maintenance would be higher at the lower concentration. In addition, the higher concentration experiment was performed after the lower concentration experiment, and this could have increased the population of ARBs. Therefore, the better performance of the MXC at the higher substrate concentration might have been a result of a better bacterial population as well as due to less percentage of the substrate being used for microbial growth.

Despite these differences, both current curves showed the same pattern with a sharp rise to a peak, a plateau, and a final decrease in current. With the same reactor configuration and electrode surface areas, the MXCs reached the lowest current at the same time. In a separate study aimed at reducing the hydraulic retention time (HRT) during a cycle in a pure *P. aeruginosa* MXC, the concentrations of the electron donors, 20 mM acetate and 20 mM lactate, were reduced to 3/4 and 1/4. The potential trend showed that HRT would not be influenced, but the potential was affected (Fig. 6). With a resistance of 330 Ω, the potential peak fell from 416.2 mV at the higher concentration to 328 and 117 mV, respectively, for the 3/4 and 1/4 concentrations. It should be pointed out the anode surface area relative to the reactor volume was relatively low at 6 cm² to 700 cm². The diminishing potential was linearly related to the reductions in the feed concentration. Since each cycle lasted for a long time, these did not

**Table 1**

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Start-up time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>5</td>
</tr>
<tr>
<td>Anaerobic digested sludge</td>
<td>4</td>
</tr>
<tr>
<td>MXC effluent</td>
<td>8</td>
</tr>
<tr>
<td>MXC effluent and Anaerobic sludge</td>
<td>3</td>
</tr>
<tr>
<td>Attached ARBs plus MXC effluent</td>
<td>0</td>
</tr>
<tr>
<td>Attached ARBs</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. 4.** Current generation pattern of two concentrations of electron donor in MXC.

**Fig. 5.** Cumulative current production of two different concentrations of electron donor in MXC.
run until the end. The correlation between concentration and current output in MXCs represents their possible use as biosensors [35].

Higher concentrations did not lengthen the treatment process, but did increase the rate of current production. This eliminates the need for higher volume reactors for stronger wastewater.

**Effect of mixing**

Reactors were mixed using a stirrer and a stir bar. The experiments were performed after the potential peak had been reached. Mixing speed was changed through 7 steps. To ensure stable condition, each speed was applied over a certain period time during which a slight, gradual decrease in power was observed due to slight substrate limitation. Hence, for two series phases, the power at the end of one phase was compared to the power at the beginning of the next phase. Power was enhanced as the speed of mixing increased. The reactor produced a power density of 1.58 mW/m² at 100 rpm, but 2.32 mW/m² at 350 rpm, which is a 46.9% improvement (Fig. 7). 350 rpm was the minimum speed at which the maximum power density was obtained, and so called the optimum mixing rate. Power changes within the range 350–1500 rpm were negligible. There was a substantial difference in power between the optimum mixing and non-mixing conditions.

Switching from no mixing to optimum mixing resulted in a 191% improvement in power density (an increase from 0.613 mW/m² to 1.786 mW/m²).

Qi et al. found a 28.9% increase in power density when they used a biometric mixer rather than no mixer with a pure culture of Escherichia coli [34]. The higher effect of mixing in the current study might be a result of the type of bacteria used or the mixer itself. PAO1 is thought to produce electron shuttles that move electrons to the anode [15], which is necessary for circuit formation in the reactor. Mixing could have facilitated this electron transfer. It would also homogenize the substrate in the reactor, increasing its availability to bacteria, and therefore reducing concentration overpotential. Therefore, mixing enhances contact between electron donors and bacteria as well as between electron acceptors and electron shuttles. Therefore, a failure in mixing would lead to a substantial decrease in energy production.

**Effect of salinity on power output**

The initial substrate contained 0.5 g l⁻¹ (8.5 mM) NaCl; if we assume this amount as zero, another 9–75 mM was added to the anode of a pure culture MXC in order to evaluate the effect of salinity on power efficiency. Salt was added through six phases; the first phase involved the addition of 9 mM and the subsequent phases 13 mM each. A period time was given for each phase to reach a stable power. Upon the addition of NaCl, the potential increased, which led to a 31.8% enhancement of power generation (Fig. 8). The first 9 mM had the largest effect: power increased from 1.64 mW/m² to 1.85 mW/m², a 12.7% increase. The remaining additions more slowly increased the power, and after 49 mM the power was almost unchanged.

Compared to mixing, salinity had a much lower effect on power. NaCl was added to the reactor under optimal mixing conditions and long after it had passed the voltage peak where bacteria had most likely already produced the maximum number of shuttles. It thus appears that these conditions ensure good electron transfer and reduce the requirement for extra facilitators or salinity. No great changes in power were observed, and salinity was not a limiting factor for bacteria in the stated range. Mohan et al., observed a positive effect on power of MXCs cultured with Enterobacter cloacae after the addition of 10 mM NaCl, but a negative effect when this exceeded 15 mM; the reactor stopped working after the addition of 100 mM NaCl [35]. The ionic strength of 100–400 mM NaCl has, however, improved the power and cumbolic efficiencies of mixed-culture MXCs [36].

It is worth mentioning that the control reactor, reactor with no power production, did not show any power following the addition of 100–400 mM NaCl. Therefore, the effect of salinity on power generation comes back to its role in electron transfer. It increases anolyte conductivity and decreases the internal resistance of the system.
Effect of ARB exposure to air

The problem with anaerobic methods is the possibility of air leakage. In anaerobic digesters, these leaks decrease methane production [37]. Therefore, the sensitivity of ARBs to air should be determined for improved handling. Since MXC performance can easily be measured by tracing energy output, the consequences of air leakage, if any, can rapidly be determined by measuring the current. Hence any leak is most likely to last for just a few hours; a period of 3 h was presumed in this study.

Two reactors running simultaneously under the same operational conditions produced a similar current. When both reactors were at the tail end of the current production curve, they were disconnected from the potentiostat, and one was emptied by filling it with nitrogen/CO\(_2\) gas (control), whereas the other was emptied by opening it and exposing it to the air (reactor #2). Whereas the control reactor was closed and maintained in an anaerobic condition, the stainless steel frame (including the carbon fibers with the attached bacteria) of reactor #2 was exposed to environmental air for 3 h. After reassembling the open reactor, both reactors were fed with a new substrate and sparged with nitrogen gas. Current generation in the control reactor was immediate, but there was a 5–6 h delay in the reassembled reactor #2 (Fig. 9).

Bacteria pass electrons to the anode in a MXC when they cannot find a dissolved electron acceptor. Oxygen can act as a dissolved acceptor, but when bacteria use oxygen they do not usually externalize electrons. The possibility of a reaction between oxygen and electrons externalized by ARBs leading to water production is low. Since oxygen was replaced by nitrogen gas at the beginning of the run, electrons could not have been consumed in this way. Second, this reaction would need a catalyst (such as platinum), and no such catalyst was available in the anode compartment.

Exposure to oxygen deferred electron generation as bacteria had selected it as their electron acceptor. The effect of oxygen may not be limited to its oxidation properties; it might be undesirable for some anaerobic bacteria such as electrogenic bacteria. All of the above are possible reasons for the loss of current in the reactor exposed to the air. Clearly this exposure did not seriously damage the bacteria or a new inoculation would not have been required.

Inactivation of the solid electron acceptor and the behavior of ARBs

An open circuit voltage mode was placed between two closed-circuit voltage modes to investigate the stability of the electrogenicity of bacteria lacking their own electron acceptor. Two MXC reactors that had been operating under closed-circuit voltage for one month were switched to an open circuit for 5 days and then back to a closed circuit. Organic matter was removed (35–40%) in the absence of solid electron acceptors, and so dissolved acceptors must have picked up the electrons. The switch did not alter the ability of ARBs to restore the current when returned to the closed-circuit condition. After resting in the open circuit condition, even without any MXC effluent both reactors began producing a current when connected to the potentiostat and switched back to closed-circuit voltage. With a CE of ~70% (which was the case in the previous closed-circuit runs), ARBs could not have chosen to use the dissolved acceptors. During closed-circuit modes, 70–90% of organic matters were removed over a 5-day period, which is rather large compared to 30–40%, indicating limitation of dissolved acceptors during open-circuit. Well-adapted ARBs were possible to use some dissolved acceptors, which might have been oxygen leaked from the cathode, but returned to using the solid acceptor once it was available. If dissolved acceptors had been oxygen, then small leakage did not have severe effect on the process. As previously indicated; non-assimilated bacteria required around 3–8 days before they were able to pass electrons to the anode, and a 3-h exposure to air postponed current for 5 h.

Resistance of ARBs to starvation

Food is essential for bacterial growth, maintenance, and energy production. To determine whether ARBs were able to live without food, they were starved for 80 h. Electron donors were withheld for two series of runs. Treated wastewater was not emptied, but was instead held in the reactor during this period. Adding electron donors reactivated ARBs and they returned to normal functioning (Fig. 10). The figure shows current restoration once the suspension had been cleansed from the reactor and a clear substrate added; a rapid increase in power was observed. This finding shows that ARBs endured the lack of an electron donor, and reflects the ability of MXCs to treat intermediate wastewaters, the flow of which can be interrupted temporarily. ARBs could overcome starvation of 3 days duration. Keeping the treated wastewater in the reactor protected ARBs from desiccation.

The start-up data indicated that ARBs grown on carbon fibers did not require any lag period to start up a new system. Electrogenic bacteria were also found to not lose their electrogenic ability when prevented from accessing a solid electron acceptor for days: they became reactivated upon exposure to an anode electrode. In addition, we observed that the bacteria survived the absence of an electron donor for at least half a week. The potential of bacteria to survive under conditions where electron donors and acceptors are limited is such for a long period facilitates their long distance delivery. Therefore, start-up and establishment of new wastewater treatment plants employing a bioelectrochemical method would be rapid provided there is a source of electrochemically active bacteria from which inoculums could
be derived. Since materials such as carbon fibers are flexible and do not occupy a large space, they would not be costly to transport or require many precautions when used.

Conclusions

Different sludge or effluent sources were used to create ARBs capable of energy production in MXC systems. Attached bacteria that developed in a MXC could convert the substrate into a current immediately after the installation of a new system. Sludge sources taken from municipal wastewater treatment plants or from a sludge digester required 3–8 days before they could give up electrons to the solid electron acceptor. During operation, the speed of current production in a batch cycle started in the presence of 10% (v/v) treated wastewater from the preceding cycle increased up to 10–12 times faster than the control.

Bioelectrical treatments or energy extraction from different concentrations of electron donors in a given reactor were performed with similar hydraulic retention times. Furthermore, food limitations were not a serious threat to ARBs, and placing an open-circuit voltage cycle between two closed-circuit runs did not cause well-adapted ARBs not to externalize electrons to the anode.

Growth and salinity influenced the power density of pure-culture MXCs. Mixing had a much larger effect and improved power density by a factor of 2.91 compared to non-mixing, whereas salinity improved power by a factor of 1.32. Increasing the mixing speed 4.3-fold above the optimum and increasing the salinity 25 mM above the optimum did not have any effect on performance.

Acknowledgement

The authors are grateful for founding by UNESCO/Keizo Obuchi Research Fellowships Program (UNESCO/Japan Young Researchers’ Fellowships Program). Cycle 2011. Sakineh Haddadi also would like to thank the staff of Waterloo Environmental Biotechnology Lab for supporting her during her stay in Waterloo, Canada.

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