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

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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 seeding 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.

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.

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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

Configuration and operation conditions of mixed culture MXC

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 acetone, 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 cm^2 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: $C_2H_3O_2Na$ 2.05 g l^{-1}, KH_2PO_4 2.27 g l^{-1}, Na_2HPO_4-$12H_2O$ 11.68 g l^{-1}, $MgCl_2$-$6H_2O$ 0.025 g l^{-1}, NH_4CL 0.037 g l^{-1} and 1 mL of a mineral solution.

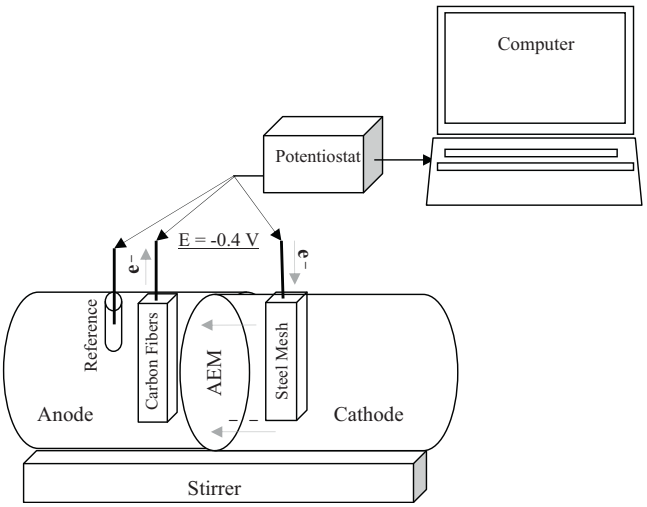

Fig. 1. Schematic of reactor configuration.

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% N_2 and 20% CO_2 (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/m^2. 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: $CE = Q_r/Q_{tot}$.

In which Q_r: energy recovered (mmol electron) as current; Q_{tot}: total available energy in the consumed substrate (mmol electron).