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ORIGINAL PAPER

Bioelectricity production using a new electrode in a microbial fuel cell

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Abstract Electrode materials play a key role in enhancing the electricity generation in the microbial fuel cell (MFC). In this study, a new material (Ti-TiO_2) was used as an anode electrode and compared with a graphite electrode for electricity generation. Current densities were 476.6 and 31 mA/m^2 for Ti-TiO_2 and graphite electrodes, respectively. The PCR-DGGE analysis of enriched microbial communities from estuary revealed that MFC reactors were dominated by *Shewanella haliotis*, *Enterococcus* sp., and *Enterobacter* sp. Bioelectrochemical kinetic works in the MFC with Ti-TiO_2 electrode revealed that the parameters by non-linear curve fitting with the confidence bounds of 95% gave good fit with the kinetic constants of η (difference between the anode potential and anode potential giving one-half of the maximum current density) = 0.35 V, K_s (Half-saturation constant) = 2.93 mM and $J_{max} = 0.39\,A/m^2$ for $T = 298$ K and $F = 96.485$ C/mol-e^-. From the results observed, it is clear that Ti-TiO_2 electrode is a promising candidate for electricity generation in MFC.

Keywords MFC · Ti-TiO_2 electrode · Kinetics · Microbial community

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Introduction

Dual-chambered microbial fuel cells (MFCs), consisting of anode and cathode compartments, have been used most commonly for the conversion of chemical energy in organic matter to electricity via catalytic reaction of microorganisms. In addition to CO_2, electrons and protons are generated through the anaerobic oxidation of organic matter in the anodic chamber. Anodic and cathodic chambers are separated by a proton exchange membrane (PEM), through which the released protons in the anodic chamber under anaerobic conditions diffuse. Electrons are transferred to the anode electrode by bacteria using mediator chemicals or nanowires [1]. Electricity is produced through the transfer of electrons from anode to cathode electrodes through an external circuit; eventually, water is produced when electrons combine with protons and oxygen in the cathode chamber.

Many researchers have reported that the efficiency of electricity production in an MFC is dependent on several factors such as pH, temperature, initial concentration, external resistance, substrate composition, microbial type

and the performance of anode electrode [1–6]. In particular, the anode electrode plays the most crucial role in high current production [2, 5]. The material and structure of the anode electrode directly affect bacteria attachment, electron transfer and substrate oxidation [7]. For a sustainable MFC operation, the anode electrode should be noncorrosive, conductive, biocompatible to microbial growth and made of electrochemically inert material [8, 9]. In general, carbon-based materials such as carbon cloth and graphite have been conventionally used due to lower cost. Carbon electrodes have been reported to be less effective due to lower conductive and surface properties. On the other hand, various metals such as Pt, Mn, Co and Ti have also been used as anode electrode [10]. However, some researchers have reported a decrease in the current production because of the thin metal oxide layer on metal electrodes at the positive potentials [9, 11]. Another restrictive reason for the usage of metal electrodes is their high cost. In order to improve power output through optimization of anode material, Sond et al. [12] increased the power density up to 100 mW/m^2 via improving anode materials such as 1,6-disulfonic acid (AQDS)-modified graphite or graphite power containing Mn^{2+} and Ni^{2+}.

Chemical and physical modification methods have been frequently applied to overcome the negative effects of conventional carbon and metal electrodes and to enhance the performance of electrodes. Zhu et al. [13] modified the anode surface by applying nitric acid and shortened the start-up time to achieve maximum voltages by 51%. With a modified anode electrode, the power density increased by 58% due to changes in the surface functional groups on the anode electrode. Lowy et al. [14] constructed a modified electrode by using Fe_3O_4 and a combination of Ni_2 and Fe_3O_4, by causing 1.5- and 2.2-fold greater kinetic activity compared to plain graphite. Coating of the graphite anode with manganese ions led to a power increase by 510-fold [15]. Utilization of gold as a support material for the anode electrode produced 47% more electricity compared to carbon-based anode [5]. Recently, modification of carbon- and metal-based anodes with conductive polymers has been used as a practical approach to improve MFC performance [16]. Schröder et al. [11] increased the current density by covering a platinum electrode with polyaniline (PANI). Heijne et al. [9] compared the uncoated and coated anodes and reported a higher electricity production with Pt-coated titanium electrode. Morris et al. [17] reported a fourfold improvement of power output and 50% reduction in cost per unit of power compared to that obtained with uncoated Pt cathodes by coating Pt with PbO_2. Similarly, Heijene et al. [9] significantly improved the MFC performance by coating the surface of a titanium electrode with Pt. As briefly explained above, the encouraging results from previous studies have focused on the modification of conventional electrodes with various coating materials to enhance the electricity production from wastewater by using MFC.

TiO_2 is one of the attractive electrode materials due to some properties such as being biocompatible, stable and environmentally friendly; however, there is little knowledge related to the use of TiO_2 in MFC applications. As far as we know, only one study on the application of TiO_2 anode has been reported [2]. They obtained a higher surface area with different PANI/TiO_2 ratios, and a composition of 70% TiO_2 and 30% PANI provided twofold higher power density.

In the present study, the performance of TiO_2-coated anode electrode has been investigated for electricity production in a dual chamber MFC operated under room temperatures. Enriched bacteria from the deep sludge of Golden Horn in Istanbul, Turkey, were used as inoculum. The current production of TiO_2 electrode was evaluated against the graphite-based electrode. Moreover, in addition to the definition of microbial ecology, a bioelectrochemical kinetics model was applied to the experimental data.

Materials and methods

Inoculum preparation

The inoculum culture was enriched using a bulk sediment sample taken from a deep sludge of Golden Horn in Istanbul, Turkey. The enrichment was conducted using a 250-ml serum bottle under anaerobic conditions. The enrichment medium was composed of synthetic wastewater with the following composition (amount in 1 l deionized water): 9 g glucose, 4 g yeast extract, 4 g $NaHCO_3$, 0.6 g NH_4Cl, 9.3 g $NaH_2PO_4{\cdot}H_2O$, 3.2 g $Na_2H_2PO_4$, 0.125 g $K_2HPO_4{\cdot}3H_2O$, 0.1 g $MgCl_2{\cdot}6H_2O$, 0.11 g $CaCl_2{\cdot}2H_2O$, 3.92 g $NaHCO_3$ and minor amounts of metal ions (Fe, Zn, Co, Cu and Ni) and vitamins [18]. The medium pH was 6.7 and 0.5 g/l cysteine was added to keep the medium in anaerobic conditions; 100 ml of the medium was filled into bottles and flushed with nitrogen gas for 5 min to remove air, capped with a rubber stopper and stirred at 150 rpm with a magnetic stirrer. Each enrichment cycle continued for 2 days at room temperature (25 °C). After the second transfer, the enriched culture was inoculated to the anode chamber.

Microbial community and phylogenetic analysis

DNAs from anaerobic MFC bacteria were extracted using a PowerSoil DNA isolation kit (MOBIO Laboratories) and then stored at −20 °C prior to the polymerase chain reaction (PCR). PCR was applied for the amplification of 16S rRNA fragments of the extracted DNAs using a primer set

of GC-BacV3f (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and reverse 907r (5′-CCG TCA ATT CMT TTG AGT TT-3′). The amplification was conducted in an automated thermal cycler (TECHNE© from UK) using the following protocol: initial denaturation for 5 min at 94 °C, 30 cycles of denaturation for 1 min at 95 °C, annealing for 30 s at 55 °C, and extension for 1 min at 72 °C, followed by a final extension for 7 min at 72 °C. The profiles of the PCR-amplified DNA were obtained by denaturing gradient gel electrophoresis analysis (DGGE), which was performed using 8% polyacrylamide gels with denaturing gradient from 30 to 70% (100% denaturing solution contains 7 M urea and 40% formamide) in 1× TAE at a constant temperature of 60 °C for 22 h. The gel was stained with Sybr-Gold (1,000 × concentration) for 1 h and visualized on a UV transilluminator. The bands in DGGE gel were cut and eluted in 25 μL of sterile H_2O overnight. DNA sequences were determined by means of re-amplification of bands following similar PCR protocol, with the exception of primer and without GC-clamp. Sequence data were analyzed by database searches in GenBank using BLAST program. A phylogenetic tree was constructed by the neighbor-joining method using the Unipro UGENE v.1.9.1.

MFC configuration

Two dual-chambered MFC reactors, fabricated as two cylindrical chambers using plexiglass material, were operated in a batch mode to compare the modified electrode and graphite electrode for bioelectricity production. The chambers were identical with a length of 12 cm and volume of 300 ml, and separated with a cation exchange membrane (CEM) from the Ultrex Company (Ultrex™ CMI7000, Membranes International Inc., USA). CEMs used in MFCs were sandwiched between anode and cathode electrodes. The electrode supplied from Akat Engineering Company in Turkey was a mixed metal oxide titanium (Ti-TiO_2), currently used in a different field for cathodic protection of pipelines. Same electrodes were used in both the anode and cathode compartments. Titanium-based composite electrodes included an electrocatalyst coated with titanium oxide. Electrocatalytic coating was carried out with thermal decomposition of mixed metal salts sprayed on titanium. As physical and chemical properties, it has a crystal structure, density of 6−12 g/cm^3, resistivity of 0.00001 Ω × cm and a large surface area and a B.E.T. surface of 20–50 m^2/g with a surface area of 10 cm^2.

The Ti-TiO_2 coating electrodes and the fine grade graphite electrodes were located into MFC-1 and MFC-2, respectively. All electrodes have the same dimensions, 10-cm height, 1-cm long and 0.2-cm thick, with an effective surface area of 10 cm^2 for biofilm growth, because one side of the electrode in the anode compartment was in contd with the membrane surface. Electrodes were connected through a fixed resistance of 10 Ω. The oxidation–reduction potentials of anode and cathode compartment were recorded with regard to the Ag/AgCl reference electrode. Measurements were recorded for each 3 min online using a digital multimeter (Fluke-8846) with 6.5 digits. The current was calculated via the voltage on 10 Ω and 1 W metal film resistor connected to the anode and cathode ends. It can be formulated as;

$$I = V/R \quad (1)$$

where I is the current produced in MFC, V is the voltage recorded in MFC and R is the resistance, 10 Ω. Resistance was verified before using the digital multimeter as nearly 10 Ω. In this study, an open circuit voltage in MFC was carried out by measuring the voltage between the anode and cathode ends with a fixed resistance of 10 Ω. The voltage measurement system has been described in a previous study [19]. Schematic views of the MFC reactor and voltage monitoring system are shown in Fig. 1.

MFC operation

MFC reactors were operated in fed-batch mode and stirred continuously at 150 rpm using a magnetic stirrer at room temperature (25 ± 2 °C); 250 ml of the anode chamber was filled with the nutrient composition and then sparged with nitrogen to make anaerobic conditions. Subsequently, the anaerobic anode reactor was inoculated with 50 ml of enrichment culture. The cathode chamber was filled with distilled water and aerated continuously using a small air pump. Once per day, 30 ml of liquid medium was taken from the anode chamber and then 30 ml of the tenfold concentration of nutrient medium was replaced in the reactor using a syringe. At the same time, the water in the cathode chambers was refreshed daily. During the experiments, no pH adjustment was done in the anode and cathode medium.

Bioelectrochemical kinetics

Anode respiring bacteria (ARB) use the anode as their terminal electron acceptor. Because the anode is a solid, it does not have a concentration that controls the kinetics of respiration; instead, the ARB respond to the anode potential [20].

ARB kinetics as a function of the anode potential with the Nernst–Monod equation is represented as (Marcus et al. [21] and Lee et al. [20]):

$$J = J_{\max} \times f(\text{biological factor}) \times f(\text{electrochemical factor}) \quad (2)$$

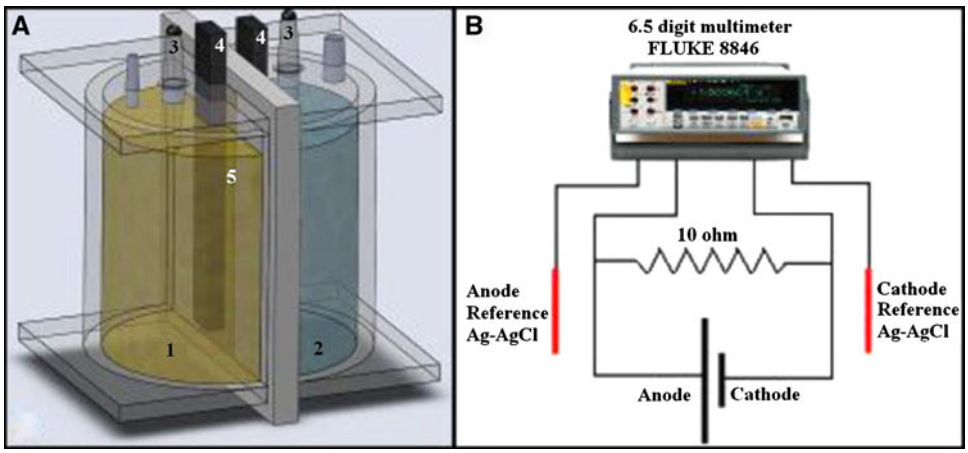

Fig. 1 Schematic view of dual-chambered MFC (**a**) and voltage monitoring system (**b**). (*1* Anode chamber, *2* cathode chamber, *3* reference electrodes, *4* electrodes, *5* membrane)

where, J is the current density in A/m^2 equivalent to the subtrate utilization rate; J_{max} is the maximum current density (A/m^2), which equals to [$J_{max} = 0.14\, f_e\, q_{max}\, X_f\, L_f$] (0.14 is a conversion constant for changing g COD/m^2-d into A/m^2 (0.14 A = 1 g COD/day); q_{max} is the maximum rate of substrate utilization (g COD/g VSS-day); X_f is the density of ARB in the biofilm (g VSS/m^3); L_f is the biofilm thickness (m); and f_e is fraction of electron equivalents removed from the donor and turned into current.

($S_d/S_d + K_{sd}$) equation, mentioned in Monod, stands for normal biofilm modeling related to kinetics occurring from soluble substrates. Although the biofilm anode cannot not be oxidized, reduced and dissolved, electrons are transferred to maintain electrical–potential gradient. In this way, anode and biofilm anode can be admitted as an "anodic electron acceptor". The anodic electron acceptor (EA) is related to EA concentration, using Nernst equation. This equation is represented in Eq. (3):

$$E_{Anot} = E_A^0 - \frac{RT}{nF}\ln\left(\frac{S_a^0}{S_a}\right) \tag{3}$$

where S_a^o = a standard anodic–EA concentration (1 mmol—EA cm^{-3}), E_A^0 = standard reduction potential for the anodic EA (V), R = ideal gas constant (8.3145 j mol^{-1} K^{-1}), F = Faraday constant (96,485 Coulomb per mol-e$^-$), T = temperature (298.15 K) and η = number of electrons transferred to the anodic E_A.

In the part of electrochemical factor,

$$\eta = E_{Anode} - E_{KA}(V) \tag{4}$$

where E_{Anode} = anode potential (V) and E_{KA} = anode potential giving one-half of the maximum current density (V).

The biological factor could be established with Monod equation [$S/(K_s + S)$].

where S = donor substrate concentration (g COD/m^3) and K_s = half-maximum rate concentration (g COD/m^3).

Hence, the Nernst–Monod based equation for bioelectrochemical kinetics of microbial fuel cell could be expressed as:

$$J = J_{max}\left(\frac{S}{K_S + S}\right)\left(\frac{1}{1 + \exp\left(-\eta\frac{F}{RT}\right)}\right) \tag{5}$$

In our study, kinetic constants (J_{max}, K_s and η) given in Eq. (5) were fitted to growth data by nonlinear regression with a Trust-Region Reflective Newton algorithm with the help of MATLAB. Trust-Region Reflective Newton algorithm is a search method to minimize the sum of the squares of the differences between the predicted and measured values. The model results and coefficients were calculated with 95% confidence interval.

Coulombic efficiency

The coulombic efficiency (CE) is defined as the ratio of total Coulombs transferred to the anode from the substrate, to maximum possible Coulombs if all substrate removal produced current. In another sense, CE describes how many electrons can be abstracted from the substrates via the electrodes. Coulombic efficiency is effected by the configuration of MFC, physical and chemical operating conditions and types of microbe in the anodic chamber. The total Coulombs obtained is determined by integrating the current over time, so that the Coulombic efficiency for an MFC run in fed-batch mode, evaluated over a period of time, is calculated as:

$$\varepsilon_C = \frac{M\int_0^{t_b} I\,dt}{Fbv_{An}\Delta COD} \tag{6}$$

where $M = 32$, the molecular weight of oxygen, F is Faraday's constant, $b = 4$ is the number of electrons exchanged per mole of oxygen, v_{An} is the volume of liquid in the anode compartment and ΔCOD is the change in COD over time t_b [22].

Results and discussion

Electricity generation

During the first 2 days, cathode and anode potentials were around 50 and −50 mV versus Ag/AgCl. The potentials in MFC-1 began to increase sharply at the end of the 2nd day and then reached −200 mV in the anode compartment and +200 mV in the cathode compartment versus the Ag/AgCl reference electrode (Fig. 2). The potential of the anode remained stable in the range 175 to −100 mV versus Ag/AgCl during the periods of 5th and 30th days.

The MFC-1 reactor was operated in batch mode with a total period of 45 days. Under batch mode of operation, at feed cycle time of 24 h, the MFC-1 took a period of 2 days to reach stable conditions with the enrichment culture from an estuary sediment. After start-up, the current density started increasing with duration of operation and reached the maximum value of 290 mA/m^2 with respect to the anode surface area on the 2nd day. Afterward, the current density reached a maximum value of 476.6 mA/m^2 on the 5th day. Then, MFC was fed in each day with different concentrations of glucose changing between 25 and 75 mM. Each concentration was run three times for obtaining representative current densities (J) and a low standard deviation. Afterward, kinetic constants for MFC performance were calculated with each current density and each concentration.

When a stable current was generated, MFC was run at varying concentrations of 25–75 mM for kinetic studies, in the period between the 4th and 23rd days. Current generation started on the 3rd day and fluctuated depending on substrate consumed (Fig. 3).

Figure 4 indicates the generation of current and power densities for MFC-1 and MFC-2. Current and power densities of the two reactors were different and current MFC-1 had higher density than MFC-2. The maximum current density was 476.6 mA/m^2 in MFC-1 with Ti-TiO$_2$, and 31 mA/m^2 in MFC-2 with graphite electrode. During the operation in fed-batch mode, MFC was run for at least three batches at each concentration to ensure repeatable current output. A maximum current density of 476.6 mA/m^2 was generated by the MFC-1 with a glucose concentration of 50 mM and an effective reactor volume of 300 mL. The current increased between the glucose concentrations of 0–25 mM. However, no significant increase in the current was observed when the glucose concentration was raised from 25 to 75 mM. At this fuel feeding rate, the current density was between 281 and

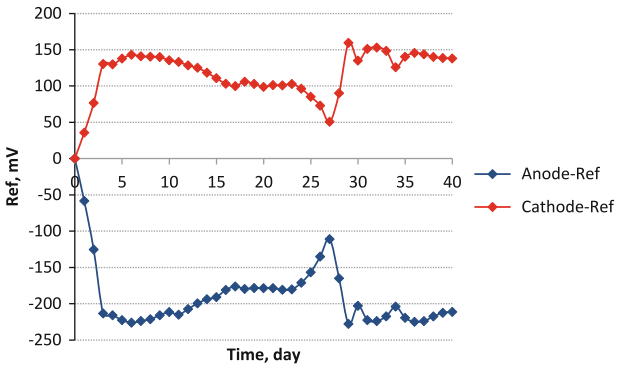

Fig. 2 Reference electrode output in MFC-1 (Ti-TiO$_2$)

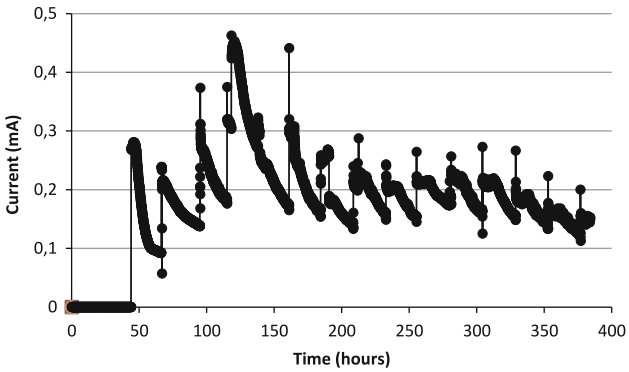

Fig. 3 Current values of MFC with Ti-TiO$_2$ electrode

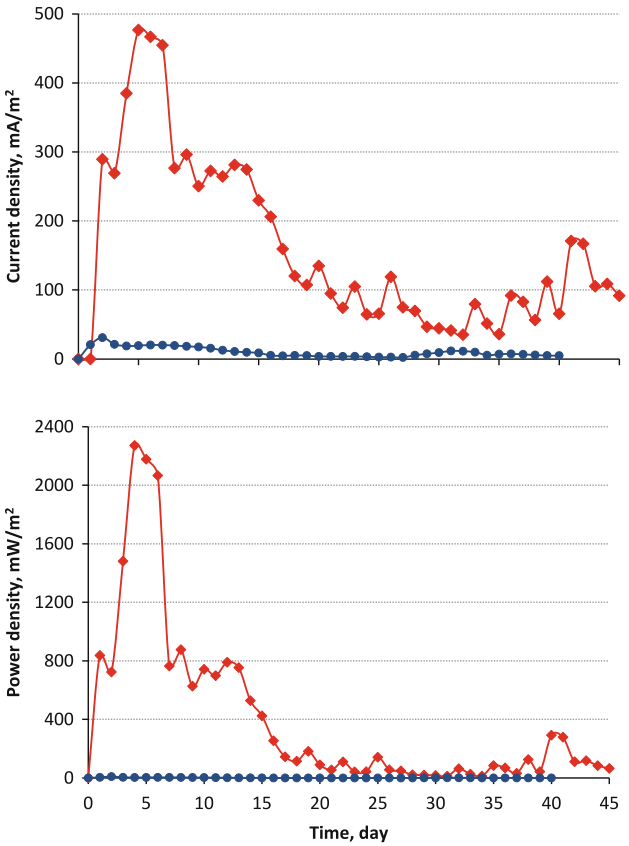

Fig. 4 Power and current densities of Ti-TiO$_2$ and graphite MFCs (circle data: MFC-1; diamond data: MFC-2)

75 mA/m^2. The maximum current density was 476.6 mA/m^2 in MFC-1. Hu [23] used both anaerobic sludge and glucose as fuel for electricity generation in the MFC and reported that the maximum power output was very low because anaerobic sludge had very limited substrate, whereas it was 161 mW/m^2 with glucose.

As shown in Fig. 4, power and current densities decreased after the 15th day. This result indicates the cation occupation of sulfonate function groups of membranes. Membrane biofouling also affected the MFC performance, but cation occupation had much influence on the reactor performance than the biofouling [24]. These factors increased the electrical resistance of the membrane and thus decreased the anode performance.

Chemical cleaning of CEM and regeneration of the functional groups of CEM are more effective methods to remove biofilms than physically removing the biofilm from the CEM [24].

Lu et al. [25] tested the generation of electricity from starch-processing wastewater as a substrate, using air cathode single-chamber MFC. They generated a maximum current density of 893.3 mA/m^2 with a minimum external resistance (120 Ω). Diluted cheese whey is another type of wastewater that is used in an MFC as a substrate [26]. In this study, lactose and glucose were also investigated. Teflon-treated carbon fiber paper was used as anode electrode and carbon cloth coated with a Pt catalyst as cathode electrode. According to those studies, the generated maximum current densities are 80 mA/m^2 (external load 100 Ω), 72,5 mA/m^2 (2,000 Ω) and 76,8 mA/m^2 (2,000 Ω) for cheese whey, glucose and lactose, respectively. He et al. [27] studied electricity generation using sucrose in an upflow dual-chamber microbial fuel cell. They used reticulated vitreous carbon (RVC) as anode and cathode electrodes. They obtained a maximum current density of 516 mA/m^2. As a result of these studies, it can be said that Ti-TiO_2 electrode has comparable performance for electricity generation in MFC.

The Coulombic efficiency was changed between 15 and 42% during MFC runs. In a study, Chae et al. [28] operated four microbial fuel cells (MFCs) that were inoculated with anaerobic sludge and fed with four different substrates for over 1 year. Their results showed that acetate-fed MFC showed the highest CE (72.3%), followed by butyrate (43.0%), propionate (36.0%) and glucose (15.0%). Glucose resulted in the lowest CE because of its fermentable nature, implying its consumption by diverse non-electricity-generating bacteria.

Kinetic results

The voltage output initially increased with the glucose concentration; however, further increases above a certain level (0.35 mM) did not improve electricity generation (Fig. 5). Substrate concentration of 0–75 mM glucose (S) versus current density in A/m^2 equivalent to the substrate utilization rate (J) is shown with the fitting parameters by non-linear curve fitting at confidence bounds of 95%. The experimental results revealed that the current density (J) increased up to around 0.4 A/m^2 after a glucose concentration of 20 mM (Fig. 5). The observed data gave a reasonably good fit to the bioelectrochemical kinetics described in Eqs. 2–5 with R^2 value of 0.98. The kinetic parameters for electricity production obtained by using an enrichment estuary culture in MFC with Ti-TiO_2 electrode were as follows: η (number of electrons transferred to the anodic E_A) = 0.35, K_s (half-saturation constant) = 2.93 mM and J_{max} (maximum current density in A/m^2) = 0.39 A/m^2 for the constant temperature of 298 K, ideal gas constant of 8.3145 j mol^{-1} K^{-1} and Faraday constant of 96.485 Coulomb per mol-e$^-$. Hence, the current density is as follows using the observed kinetic parameters (K_s and J_{max}):

$$J = 0.39\left(\frac{S}{2.93+S}\right)\left(\frac{1}{1+\exp\left[-0.35\frac{96485}{8.3145\times 298}\right]}\right) \quad (7)$$

Kim et al. [29] observed a power density of 488 ± 12 mW/m^2 with ethanol and investigated the kinetics as a function of ethanol concentration in dual-chambered MFC with a half-saturation constant (K_s) of 4.86 mM. They did not observe a significant generation of electricity in the usage of methanol as a substrate. Kim et al. [30] generated

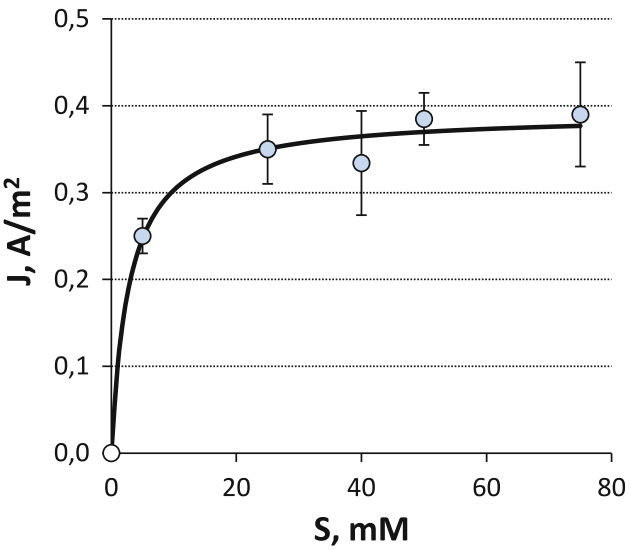

Fig. 5 Substrate concentration in mM glucose (S) versus current density in A/m^2 equivalent to the substrate utilization rate (J); with the fitting parameters of F: 96.485; R: 8.3145; T = 298 K; η = 0.35; 95% confidence bounds by non-linear curve fitting using MATLAB© (R2009b). Goodness of fit; standard squared error: 0.09166, R-square: %98, Adjusted R-square: %98, root mean squared error: 0.018

a maximum power density of 23.7 W/m^3 at a half-saturation constant (K_s) of 4.42 mM with lactate in dual anode-chambered MFC. They obtained the half-saturation constants of 2.4 mM and 8.36 mM by setting the maximum power density to 20 and 30 W/m^3, respectively. Huang et al. [31] exhibited a maximum power density of 6.3 mW/m^2 and half-saturation constant (K_s) of 0.29 mM at a xylose concentration below 9.7 mM, and K_s of 3.0 mM with an increase in the substrate concentration up to 9.7 mM. Catal et al. [32] investigated the power generation from 12 monosaccharides in MFCs and found the maximum voltage in the range of 0.26–0.44 V at external resistance of 120 Ω and a half-saturation constant (K_s) range from 110 to 725 mg/L. They received the highest maximum voltage (0.39 V) with the K_s value of 637 mg/L using glucose as a substrate. Li et al. [33] investigated electricity generation with the baffled single-chambered MFC using glucose as a substrate. They generated a maximum power density of 164 mW/m^2 with a fixed external resistance (100 Ω) and half-saturation concentration (K_s) 259 mg/L. In another study Min et al. [34] studied the production of electricity from swine wastewater using two-chambered MFC for the first time. They reported a maximum power density of 225 mW/m^2 (fixed 1,000 Ω resistor) and half-saturation concentration of $K_s = 1{,}512$ mg/L. In a study, direct electricity generation from six polyalcohols using single-chambered air-cathode MFCs was examined [35]. They produced the maximum power density ranging from 1,490 ± 160 to 2,650 ± 10 mW/m^2 using galactitol as a carbon source. The calculated maximum voltage ranged between 0.24 and 0.34 V with half-saturation kinetic constants varying from 298 to 753 mg/L.

In the present study, the maximum voltage and maximum power density were 5 mV and 2,272 mW/m^2, respectively, at external resistance of 10 Ω with the K_s value of 2.93 mM (527 mg/L).

Microbial community

The microbial community structure has been proved to be a useful tool for MFC applications. Figure 6 shows the DGGE patterns of the amplified partial 16 sRNA genes and the phylogenetic tree. The estuary sediment culture from a bulk sediment sample taken from the deep sludge of Golden Horn was enriched before MFC run. The enrichment was conducted using a serum bottle under anaerobic conditions. The DGGE pattern number of 5 in Fig. 6 shows bands of enrichment culture. In this figure, bands numbered 4, 5 and 6, searched in GenBank using BLAST program, characterize *Vagococcus fluvialis* (FN997619.1), *Shewanella haliotis* (FN997626.1) and *Uncultured bacterium clone* (AY483171.1), respectively. *Vagococcus fluvialis* (Band No. 4) and *Shewanella haliotis* (Band No. 5) are a consortium of bacterial biofilms enriched from estuarine sediments in a microbial fuel cell and its microbial communities from Zhang et al. (unpublished in GenBank blast program). *Uncultured bacterium clone* (Band No. 6) is a consortium of biofuel cells selected for microbial consortia that self-mediate electron transfer [36]. But, these bands

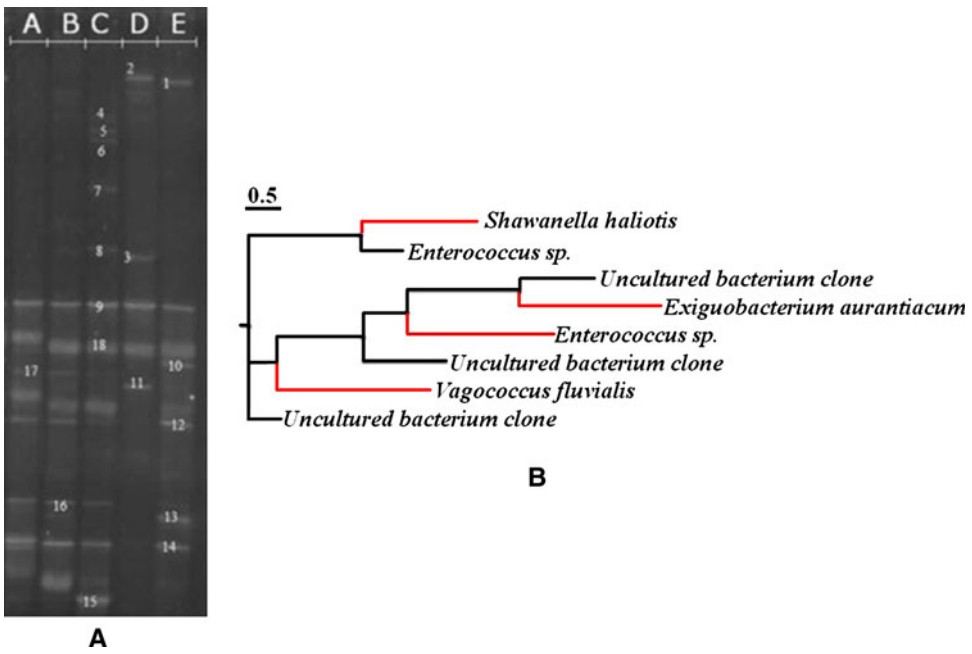

Fig. 6 **a** DGGE fingerprint gel view (*A* graphite anode, *B* TiO_2 anode, *C* Haliç En-2, *D* TiO_2 anode, *E* TiO_2 anode; **b** phylogenetic tree

disappeared during MFC batch runs fed with glucose. MFC culture was characterized with Band Nos. 1, 2, 3, 9, 14, 15 and 17. Band Nos. 1 and 2 show the same culture as *Uncultured bacterium clone (*AY483165.1) from Rabaey, et al. [36] and Band No. 3 appears with only DGGE pattern of D in Fig. 6, characterizing *Exiguobacterium aurantiacum (*FN997628.1) from Zhang et al. [37]. Band Nos. 9, 11, 13 and 18 characterized *Enterococcus* sp. (AY489118.1) from Rabaey et al. [36], *Enterococcus* sp. (AY489118.1) from Rabaey et al. [36], *Enterococcus* sp. (FN997607.1) from Zhang et al. [37] and *Enterococcus* sp. (AY489118.1) from Rabaey et al. [36]. Band Nos. 14 and 15 were the same culture as *Shewanella haliotis* (FN997626.1) from Zhang et al. [37], and Band Nos. 16 and 19 were *Uncultured bacterium clone* (EU704562.1) from Chae et al. [28] and *Enterobacter* sp. (FN997607.1) from Zhang et al. [37]. Similarly, Rabaey et al. [36] isolated *Enterococcus* sp. from a glucose-fed MFC reactor inoculated with methanogenic granular sludge and reported that the metabolic activity of *Enterococcus* sp. changed notably in the presence of an electron-accepting anode in an MFC compared to a serum flask, which was confirmed in this study with the DGGE pattern of C (serum flask) and other patterns (A, B, D and E).

This study and many researchers have focused on the microbial ecology in MFCs fed with different carbon sources. The results of community analysis from various studies show there is no single specific microorganism in the bacterial populations that develop on the anode. As a result of several bacterias are convenient for electricity generation and the operating conditions, system architectures, electron donors and electron acceptors [38]. For example, Kim et al. [29] determined anode biofilm and suspension from a dual-chambered MFC fed with ethanol. They reported that bacteria with sequences similar to *Proteobacterium* Core-1 (33.3%), *Azoarcus* sp. (17.4%) and *Desulfuromonas* sp. M76 (15.9%) were significant members of the anode chamber community. Catal et al. [35] investigated the microbial community of the anodic biofilms with different polyalcohols as carbon sources in a single-chamber MFC using denaturing gradient gel electrophoresis (DGGE). Results showed that microbial community varied with different substrates. Mixed culture MFCs have produced higher power densities than pure culture MFCs. Because of synergistic interactions within the anode communities and participation of strains and mechanisms, MFC reactors have diverse microbial communities [39]. Choo et al. [40] examined the microbial community of MFCs fed with glucose and glutamate. They reported that *Alfaproteobacteri* and *Firmicutes* were dominant bacterias. Varied bacterial communities enriched under different conditions indicate that electrochemical activity is not restricted to a few groups of bacteria. The differences in microbial communities between the enriched cultures may also be due to the types of fuel cells used for the enrichment [40]. In a study, the bacterial communities in MFCs were investigated by using different electron donors like sodium acetate, lactate and glucose [39]. They indicated that all anode communities included sequences closely affiliated with *Geobacter sulfurreducens* and uncultured bacterium clone in the *Bacteroidetes* class. Betaproteobacterial sequences were found in the acetate- and the lactate-fed MFCs. Spirochaetales and Firmicutes were found in the acetate and glucose-fed MFCs. *Shewanella* spp, the most common bacteria found in MFCs, were not detected in any of the anode biofilms. A few phylogenetically diverse bacteria which include dissimilatory iron-reducing *Geobacter* sp. [41], *Shewanella* sp. [42], *Pseudomonas aeruginosa* [36] and *Enterecossus gallinarum* [43] are known to generate electricity in MFCs [36].

Conclusions

The results showed that Ti-TiO_2 can be used as an alternative electrode to enhance the power generation in MFCs. The power generation in MFC with Ti-TiO_2 electrode was approximately 250 times higher than that in MFC with graphite electrode. MFC were dominated by *Shewanella haliotis*, uncultured bacterium clone and *Enterococcus* sp. The observed bioelectrochemical kinetic constants were: number of electrons transferred to the anode, $\eta = 0.35$; $K_s = 2.93$ mM; $J_{max} = 0.39$ A/m^2 for $T = 298$ K, $R = 8.3145$ j mol^{-1} K^{-1} and $F = 96.485$ C/mol-e$^-$.

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References

1. Cheng S, Logan BE (2007) Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochem Commun 9:492–496
2. Qiao Y, Bao SJ, Li CM, Cui XQ, Lu ZS, Bao J (2008) Nanostructured polyaniline/titanium dioxide composite anode for microbial fuel cells. ACS Nano 2:113–119
3. Pant D, Bogaerta GV, Dielsa L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543
4. Jadhav GS, Ghangrekar MM (2009) Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresour Technol 100:717–723
5. Sun M, Zhang F, Tong ZH, Sheng GP, Chen YZ, Zhao Y, Chen YP, Zhou SY, Liu G, Tian YC, Yu HQ (2010) A gold-sputtered carbon paper as an anode for improved electricity generation from a microbial fuel cell inoculated with *Shewanella oneidensis MR-1*. Biosens Bioelec 26:338–343

6. Zhang BG, Zhou SG, Zhao HZ, Shi CH, Kong LC, Sun JJ, Yang Y, Ni JR (2010) Factors affecting the performance of microbial fuel cells for sulfide and vanadium (V) treatment. Bioprocess Biosyst Eng 33:187–194
7. Li CM (2007) Advanced microbial fuel cell development, miniaturization and energy and power density enhancement. School of Chemical and Biomedical Engineering Nanyang Technological University, Singapore
8. Kim IS, Chae KJ, Choi MJ, Verstraete W (2008) Microbial fuel cells: recent advances, bacterial communities and application beyond electricity generation. Environ Eng Res 13:51–65
9. Heijne A, Hamelers HVM, Saakes M, Buisman CJN (2008) Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. Electrochim Acta 53:5697–5703
10. Du Z, Li H, Gu T (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Advan 25:464–482
11. Schröder U, Niessen J, Scholz F (2003) A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. Angew Chem Int Ed 42:2880–2883
12. Song TS, Yan ZS, Zhao ZW, Jiang HL (2011) Construction and operation of freshwater sediment microbial fuel cell for electricity generation. Bioprocess Biosyst Eng 34:621–627
13. Zhu N, Cheni X, Zhang T, Wu P, Li P, Wu J (2011) Improved performance of membrane free single-chamber air-cathode microbial fuel cells with nitric acid and ethylenediamine surface modified activated carbon fiber felt anodes. Bioresour Technol 120:422–426
14. Lowy DA, Tender LM, Zeikus JG, Park DH, Lovley DR (2006) Harvesting energy from the marine sediment–water interface II Kinetic activity of anode materials. Biosens Bioelec 21:2058–2063
15. Park DH, Zeikus JG (2002) Impact of electrode composition on electricity generation in a single-compartment fuel cell using *Shewanella putrefaciens*. Appl Microbiol Biotechnol 59:58–61
16. Watanabe K (2008) Recent developments in microbial fuel cell technologies for sustainable bioenergy. J Biosci Bioeng 106:528–536
17. Morris JM, Jin S, Wang JQ, Zhu CZ, Urynowicz MA (2007) Lead dioxide as an alternative catalyst to platinium in microbial fuel cells. Electrochem Commun 9:1730–1734
18. Karadag D (2011) Anaerobic H_2 production at elevated temperature (60°C) by enriched mixed consortia from mesophilic sources. Int J Hydrogen Energy 36:458–465
19. Ozkaya B, Karadag D, Akoglu B, Acı G, Taskan E, Hasar H (2010) Electricity generation in MFC using selective membrane, pp 1222–1227, IWA regional conference and exhibition 18–22 October 2010 on membrane technology and water reuse Istanbul-Turkey
20. Lee HS, Vermaas WFJ, Rittmann BE (2010) Biological hydrogen production: prospects and challenges. Trends Biotechnol 28:262–271
21. Marcus AK, Torres CI, Rittmann BE (2007) Conduction-based modeling of the biofilm anode of a microbial fuel cell. Biotechnol Bioeng 98:1171–1182
22. Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K (2006) Microbial Fuel Cells. Methodol Technol Environ Sci Technol 40(17):5181–5190
23. Hu Z (2008) Electricity generation by a baffle-chamber membraneless microbial fuel cell. J Power Source 179:27–33
24. Choi MJ, Chae KJ, Ajayi FF, Kim KY, Yu HW, Cw Kim, Kim IS (2011) Effects of biofouling on ion transport through cation exchange membranes and microbial fuel cell performance. Bioresour Technol 102:298–303
25. Lu N, Sg Zhou, Zhuang L, Jt Zhang, Jr Ni (2009) Electricity generation from starch processing wastewater using microbial fuel cell. Biochem Eng J 43:246–251
26. Antonopoulou G, Stamatelatou K, Bebelis S, Lyberatos G (2010) Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell. Biochem Eng J 50: 10–15
27. He Z, Minteer SD, Angenent LT (2005) Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ Sci Technol 39:5262–5267
28. Chae KJ, Choi MJ, Lee JW, Kim KY, Kim IS (2009) Effect of different substrates on the performance, bacterial diversity and bacterial viability in microbial fuel cells. Bioresour Technol 100:3518–3525
29. Kim JR, Jung SH, Regan JM, Logan BE (2007) Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour Technol 98:2568–2577
30. Kim MH, Iwuchukwu IJ, Wang Y, Shin D (2011) An analysis of the performance of an anaerobic dual anode-chambered microbial fuel cell. J Power Source 196:1909–1914
31. Huang L, Zeng RJ, Angelidaki I (2008) Electricity production from xylose using a mediator-less microbial fuel cell. Bioresour Technol 99:4178–4184
32. Catal T, Li K, Bermek H, Liu H (2008) Electricity production from twelve monosaccharides using microbial fuel cells. J Power Source 175(1):196–200
33. Li Z, Yao L, Kong L, Liu H (2008) Electricity generation using a baffled microbial fuel cell convenient for stacking. Bioresour Technol 99:1650–1655
34. Min B, Kim JR, Oh SE, Regan JM, Logan BE (2005) Electricity generation from swine wastewater using microbial fuel cells. Water Res 39:4961–4968
35. Catal T, Xu S, Li K, Bermek H, Liu H (2008) Electricity generation from polyalcohols in single-chamber microbial fuel cell. Biosensor Bioelec 24:849–854
36. Rabaey K, Boon N, Siciliano SD, Verhaege M, Verstraete W (2004) Biofuel cells select for microbial consortia that self-mediate. Appl Environ Microbiol 70:5373–5382
37. Zhang J, Zhang E, Scott K, Burgess JG. (Unpublished) Electricity production by consortium bacterial biofilms enriched from estuarine sediments in microbial fuel cell and its microbial communities
38. Logan BE, Regan JM (2006) Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol 14:512–518
39. Jung S, Regan JM (2007) Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors. Appl Microbial Cell Physiol 77:393–402
40. Choo YF, Lee JY, Chang IS, Kim BH (2006) Bacterial communities in microbial fuel cells enriched with high concentrations of glucose and glutamate. J Microbiol Biotechnol 16(9):1481–1484
41. Bond DR, Lovley DR (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 69(3):1458–1555
42. Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ish S, Logan B, Nealson KH, Fredrickson JK (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis strain MR-1* and other microorganisms. Proc Natl Acad Sci USA 103(30):11358–11363
43. Kim GT, Hyun MS, Chang IS, Kim HJ, Park HS, Kim BH, Kim SD, Wimpenny JW, Weightman AJ (2005) Dissimilatory Fe(III) reduction by an electrochemically active lactic acid bacterium phylogenetically related to *Enterococcus gallinarum* isolated from submerged soil. J Appl Microbiol 99(4):978–987