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Review Article

Microbial community structure in the biofilm anode of MFC fed with landfill leachate

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ABSTRACT

This study describes the kinetics and diversity of anode respiring bacteria (ARB) in a two-chambered microbial fuel cell (MFC) with a Ti-TiO_2 electrode and continuously fed with young or old landfill leachate at varying organic strength and hydraulic retention time. With increasing organic loading, current generation increased, although the Coulombic efficiency decreased. The maximum current densities for young and old leachates were 11 and 6 A/m^2, respectively. We observed maximum current densities (J_{max}) in kinetics modeling 12.0 A/m^2 and 8.0 A/m^2 for young and old landfill leachates corresponding to low anode potential losses (η) of 0.25 V. A sequencing analysis of anode biofilm community after PCR-DGGE showed that the *Deltaproteobacteria* family was predominant on anode surface, especially with young leachate.

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INTRODUCTION

While all leachate contains high concentrations of organic matter and ammonium nitrogen, its composition depends upon the landfill age, the quality and quantity of waste, biological and chemical processes took place during disposal, rainfall density, and water percolation rate through the waste in the landfill [1]. "Young" leachate is generally produced is the first 1 or 2 years of the landfill's life, and its organic matter has relatively low molecular weight, such as from volatile organic acids, a high COD concentration (> 10 g/L), and a BOD_5-to-COD > 0.6 [2]. "Old" leachates generally come from a landfill after about 10 years; its organic matter has relatively high molecular weight, such as from humic and fulvic substances, a relatively low COD concentration (< 5 g/L), and a BOD5-to COD ratio < 0.3 [2].

Up to now, for an effective leachate treatment, many researchers around the world have intensively focused on the combination of biological and physico-chemical treatment systems that require usage of high energy [3]. After 2000s years, it has been focused on the development of

Highlights

- MFC with Ti-TiO2 electrodes emerges as a promising technology for sustainable electricity generation from landfill leachate.
- The age of leachate establishes a crucial link between microbial ecology, kinetics, and current density output.

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innovative technologies to realize energy efficiency in the wastewater treatment sector. To a certain extent, this expenditure using anaerobic digestion can be minimized through energy recovery processes in wastewater treatment plants due to more suitable for concentrated leachate streams, lower operating costs, the production of a useable biogas product (for a review see Renou, et al. [4]). Nowadays, there is ample scope for further innovation in energy recovery processes. For example, a novel technology possibility to use in leachate treatment is microbial fuel cell (MFC) that marries microbial catalysis to electrochemistry [5], but it needs substantially improved the power density to become commercially feasible.

However only a handful of reports have investigated the power output in MFC fed with landfill leachate. Several scientists have previously demonstrated the feasibility of using the MFC technology for simultaneous leachate treatment and energy generation [6–10]. In our knowledge there is no report on power generation from leachate in MFC depending on landfill age, which is an important point out that young leachate is a mixture of the most common fermentation products reflecting a real fermentation effluent and old leachate contains humic acids able to be used as artificial electron mediators to carry electrons from inside the cell to an external electrode [11]. We used young (<1 year) and old leachate (>10 years) for generating electricity from continuously flow MFC with Ti-TiO_2 anode and cathode electrode to evaluate the effect of substrate concentration on current density by varying concentration of leachate in the reactor. The steady state MFC data was used to estimate important parameters that represent the **kinetics for substrate utilization of biofilm anode**. Previously, a biofilm model on Nernst-Monod Equation developed by Marcus et al. [12], describing the anode potential losses of anode respiring bacteria that transfer electrons through a solid conductive matrix, which can be facilitated by mathematical models (e.g. Nernst-Monod equation) for understanding and designing of biofilm reactors having the complex inter-related phenomena [13]. That's why we linked up between kinetics and microbial ecology to explain performance in the MFCs of young and old landfill leachate.

Observed high current densities in MFCs generally give low coulombic efficiency because of a three-way syntropy among ethanol fermenters, acetate-oxidizing anode respiring bacteria and a hydrogen scavenger, a hydrogen oxidizing methanogens. On the other hand, some indicator microbes grow at high anode potential and many of which are known to respire the anode using electron shuttles and produce redox mediators [14], which contribute high current output. Therefore, there is a strong linkage between microbial community in MFCs and current density output. Namely, high current density and low anode potential losses, which are the keys of Nernst-Monod equation used in our work, can be only explained by the biofilm anode communities, which are able to transfer electrons from reduced substrate to a solid electrode.

The biofilm anode community can be quite diverse, but MFC must contain anode-respiring bacteria (ARB), which are able to transfer electrons efficiently to the anode by extracellular electron transport (EET) [15]. Many studies that characterized the microbial community of the biofilm anode in MFCs showed a high bacterial diversity in the biofilm anode, but with a general enrichment of Proteobacteria (mainly α-, γ- and δ- proteobacteria), Firmicutes, Bacteriodetes, and Actinobacteria [16–19], as well as Geobacteraceae in some of studies [20] used as inoculum of sediment. Using pyrosequencing targeting the 16S rRNA genes, we analyzed the community structure of **biofilm anodes** from the continuously fed MFCs to explore if certain microbial types are associated with leachate type or with better vs poorer performance.

MATERIAL AND METHODS

Landfill leachate

Leachates were collected from separate drainage pipelines coming from young (<1 year of operation) and old (>10 years of operation) cells of the Odayeri Municipal Landfill in Istanbul. Samples were transferred immediately to the laboratory and kept at 4°C until used for MFC experiments. The leachates were assayed every 15 days for water-quality parameters, and they did not change during storage for 30 days. All water-quality analyses were performed according to *Standard Methods* [21]. The young leachate contained COD 49,500±500 mg/l, BOD5 28,500±1000 mg/l, TOC 16,800±1000 mg/l, pH 5.5±0.5, conductivity 26±1 ms/cm, nitrate-N 12±2 mg/l, sulfate 900±50 mg/l, ammonia-N 860±50 mg/l, Fe 32 mg/l, Zn 3.5 mg/l, and Ni 1.85 mg/l.

Old leachate contained COD 5000±500 mg/l, BOD 1200±500 mg/l, TOC 4200±500 mg/l, pH 7.8±0.2, conductivity 24±1 ms/cm, nitrate-N 8±2 mg/l, sulfate 45±5 mg/l, ammonia-N 920±20 mg/l, Fe 10 mg/l, Zn 6.7 mg/l, and Ni 2.1 mg/l.

Microbial Fuel Cell

The MFC setup, similar to Ozkaya et al. [22], is shown in Figure 1. The active volumes of the anode and cathode sections were 275 mL, and the two compartments were separated by CMI– 7000 cation exchange membrane (Membrane International Inc. from USA) that was conditioned by boiling it in 30% H_2O_2 for 15 minutes and then rinsing it with deionized water. Any remaining H2O2 on the membrane surface was cleaned using 0.5 M H2SO4 before rinsing with deionized water. Anode and cathode had the same dimensions with an active electrode area (wet) of 7.5 cm^2: Each electrode was a titanium rod coated with mixed metal oxide including titanium dioxide, niobium oxide, ruthenium oxide and mangan oxide by electro-catalytic coating method (Akat Co. from Turkey). Electrodes were washed with ethyl alcohol and rinsed with distilled water to remove impurities prior to MFC operation.

The liquid in both chambers was completely mixed by a magnetic stirrer at 350 rpm. The cathode chamber was filled with distilled water and continuously aerated by a simple air pump. The anode chamber was sparged with nitrogen gas to remove oxygen. The anode was inoculated only from the original leachate. The potentials of the anode and cathode were measured by Ag/AgCl reference electrodes (BASi Reference Electrode from USA, model: MF-2079).

The external resistance (R) was set at 10 Ω, and the current (I) was calculated from the measured voltage drop across the resistor (V) according to Ohm's Law (I = V/R) and then normalized by the wetted surface area of anode (7.5 cm^2) or the volume of liquid media in the anode chamber (275 cm^3). The voltage (V) across an external resistance (10 Ω) in the MFC circuit was monitored on-line at 5-min intervals using a four-channel precision multimeter (Fluke 8846A) connected to a personal computer. The coulombic efficiency is defined as a ratio of coulombs recovered (C_p) to total coulombs in substrate (C_{max}). Coulombic efficiency (C_E=C_p/C_{max}) was calculated according to Logan [23] and Sleutels et al. [24]:

$$C_E = \frac{M_s\, I}{F\, b_{es}\, q\, \Delta C}$$

where M_s: 32 g/mol for the molecular weight of O_2, I: steady-state current output (A), F (Faraday constant): 96,485 Coulomb per mol e^-, b_{es} : 4 mol e^- per mol substrate for the number of electrons exchanged per mole oxygen, q: flow rate (l/s), and ΔC: substrate utilization based COD ($C_{inf.}$-$C_{effl.}$) (g/l).

Molecular Techniques

At the end of continuous operation, biofilm was removed from an anode with a pipette tip. 0.25 g of biofilm was put into a bead tube provided by the MOBIO Powersoil DNA extraction kit, and a Power Soil DNA isolation kit (MoBio laboratories, Carlsbad, CA, USA) was used for DNA extraction of anodic biofilm samples. DNA extracts were stored at -20 °C and then thawed for use as templates for the polymerase chain reaction (PCR) to amplify the 16S rDNA using a primer set of GC-BacV3f and reverse 907r [25]. PCR amplification was conducted in an automated

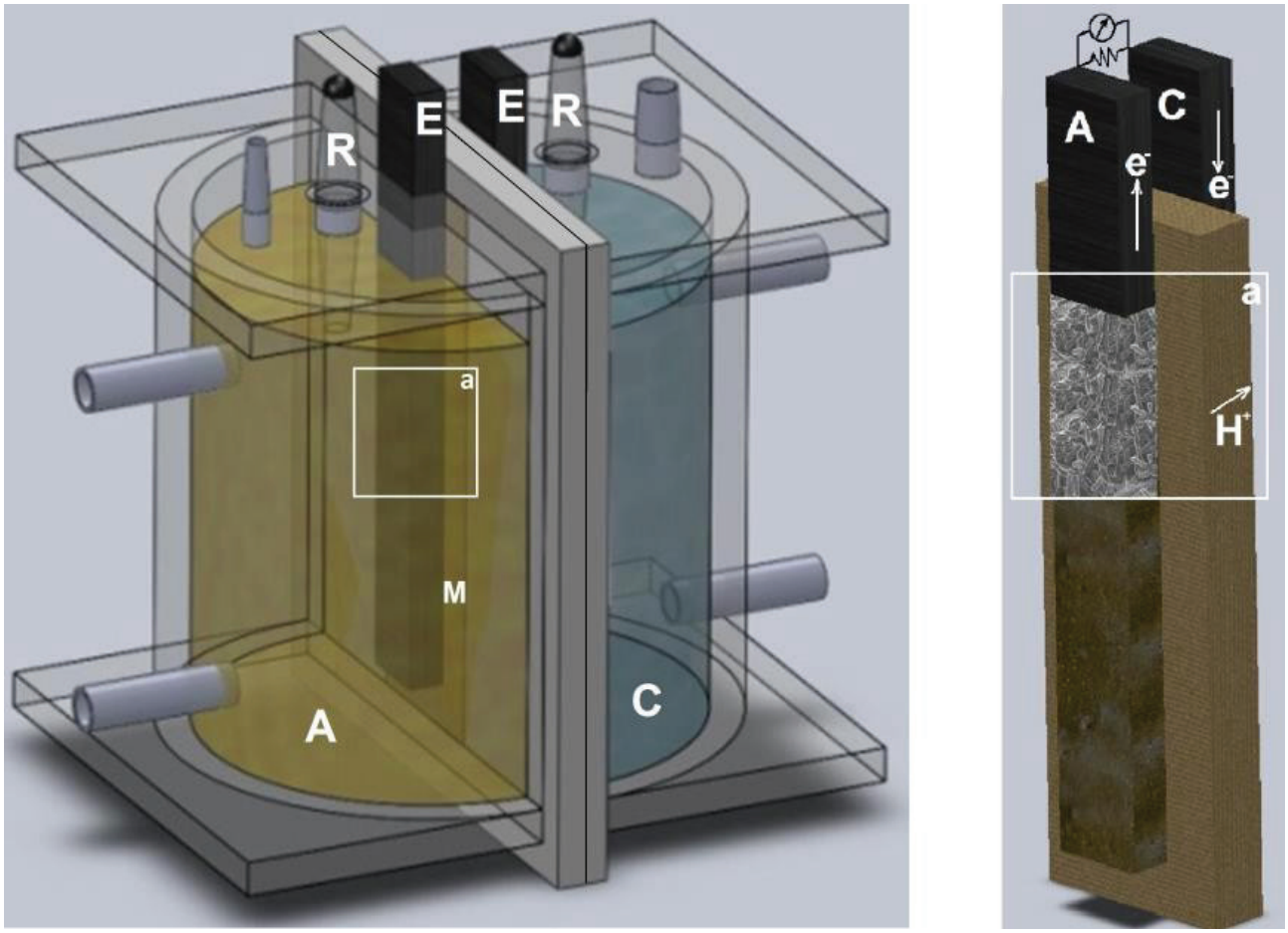

Figure 1. Microbial fuel cell (left) and membrane-electrode couple (right). A: Anode, C: Cathode, R: Reference electrode, E: Electrodes and M: CMI 7000 Membrane.

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, extension for 1 min at 72 °C, and final extension for 7 min at 72 °C.

Denaturating gradient gel electrophoresis (DGGE) was performed with an INGENYphorU 2x2 system (Ingeny International, Goes, The Netherlands) using 8% polyacrylamide gels with a denaturing gradient from 30% to 70% (100% denaturing solution contains 7 M urea and 40% formamide) in 1xTAE at a constant temperature of 60 °C for 18 hours. The gel was stained with Sybr-Gold (1000× concentration) for 1 hr and visualized on an UV transilluminator. Bands in the DGGE gel were carefully cut out with a razor blade under UV illumination and eluted in 25 µL of sterile H2O overnight. DNA sequences were determined after re-amplification following the same PCR protocol, except that the primer did not have a GC-clamp. Sequence data were analyzed by database searches in GenBank using the BLAST program. A phylogenetic tree was constructed by the neighbor-joining method using the Unipro UGENE v.1.9.1.

RESULTS AND DISCUSSION

MFC performance

The operational conditions during the experimental periods of the MFCs fed with young and old age leachate are summarized in Figure 2. Old and young leachate running MFC have five and six running periods with different substrate concentration and various HRTs. The MFCs were operated in continuous mode for around two months at increasing OLRs achieved by changing hydraulic retention time (HRT) or the feed COD concentration.

Figure 3A and Figure 3B show OLRs during the experimental periods of the MFC system. The OLR was increased up to maximum value of 200 gCOD/l-d for young- and 20 g/l-d for old-landfill leachate.

Figure 3C and Figure 3D show how the progressions of current density. In order to compare directly the performance with young versus old leachate, the MFCs fed with young or old leachate were operated with a constant influent COD of 1000 mg/L and an HRT of 2 d, corresponding to organic loading rate (OLR) of 0.5 g COD/d. While current generation was negligible during first one week, it steadily increased to 2 A/m^2 and 0.5 A/m^2 at 18 days for young and old leachate, respectively. During this period, the COD removals were 5% at one week and 15-20% by 20 days for the MFC fed with old age leachate. The COD removal rose from 5% at one week up to around 20% at 15 days in MFC fed with young landfill leachate.

The MFCs were then operated in continuous mode for two months at increasing OLRs achieved by changing hydraulic retention time (HRT) or the feed COD concentration. As the feed COD concentration increased, the current density increased, reaching 9 A/m^2 for the young leachate. Decreasing the HRT from 1 d to 0.75 d in MFC with young leachate, corresponding to OLR of 67 g COD/d, did not adversely affect the COD removal efficiency, which remained between 25-30% (Figure 3E and Figure 3F). Similar observation was found in old age leachate and HRTs were decreased 1 d to 0.75 d, corresponding OLR of 6.7 g COD/d. Then, COD removal efficiency had a little change with the value of 15-20% (Figure 3E). The current increased to around 7 mA and 1 mA during this period for MFC fed with young and old age landfill leachates, respectively. Recorded maximum current densities were 11 A/m^2 and 6 A/m^2 for young and old landfill leachate (Figure 3C and Figure 3D). Thereafter,

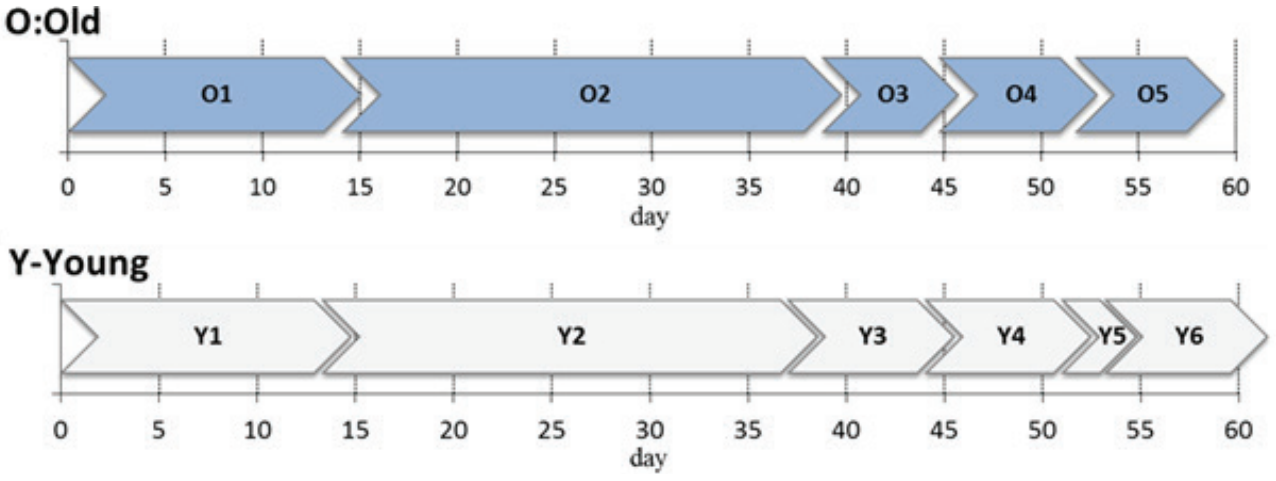

Figure 2. Operational conditions during the five (O1-O5) and six (Y1-Y6) experimental periods of the MFC system. HRTs in old and young leachate MFCs are O1: 2 day, O2: 1 day, O3: 0.75 day, O4: 0.5 day, O5: 0.25 day and Y1: 2 day, Y2: 1 day, Y3: 0.75 day, Y4: 0.5 day, Y5: 0.25 day, Y6: 0.5 day.

HRT values of both reactors were decreased to 0.5 d corresponding OLR of 100 g COD/d for young leachate and 10 g COD/d for old leachate. The COD removal efficiency averaged around 30% and current increased slightly around 7.7 mA in MFC fed with young leachate, corresponding current density of around 11 A/m^2 (Figure 3C). Hence, the reactor performed well even at OLR of 100 g COD/d. Our observation was similar for MFC fed with old age leachate and current density were rinsed 2.2 A/m^2 when HRT decreased to 0.5 day (Figure 3D).

System performance was adversely while further decreasing HRT to 0.25 day and COD removal efficiency sharply decreased to below 5% and current decreased sharply close to the zero in MFC fed with young leachate (Figure 3E and Figure 3F). Thereafter, HRT was increased back to 0.5 d to recover the process performance. In this case, COD removal efficiency and current density increased again to around 25-30% and 10 A/m^2, respectively when HRT of MFC decreased to same value of 0.25 d, the performance of MFC fed with old leachate was not much affected as in young leachate; but its performance decreased with the decreasing rate of 50% (Figure 3D). The initial COD concentrations were 50 g/L and 5 g/L, respectively, for young and old leachate fed MFCs, and these corresponded net COD removal efficiencies of 30% and 20%. Our observations showed that young leachate is the better substrate for MFC than old leachate considering removal efficiency and high current output, but in our case and reported values by other MFC researchers shows that MFCs may require post treatment before effluent discharge to receiving water.

Coulombic efficiencies of all runs varied around 2-13% for young leachate and 5-20% for old leachate, whereas three data had high CE of 30-50% for low organic loads. It was observed that the coulombic efficiency decreased with increasing the OLR, but current output increased. Much higher current density values (11 A/m^2) were reached at influent COD of 100 g/L. When we loaded high organic matter to MFCs, CE sharply dropped to minimum value (0.5-5%) especially in young leachate MFC. Thus high current density correlated negatively to high CE. Although COD removal efficiency has constant value during the steady state conditions, CE dropped to low level with incremental OLR. We observed constant COD removal efficiency except for extremely high OLR (100 g/L-d) with young landfill leachate. Although removal efficiency was stable depending on increasing OLR, extremely high OLR significantly affected both current density and COD removal efficiency (Figure 3G and Figure 3H).

The reason of constant COD removal but low CD for both reactor is that methane production may not be avoidable in MFCs fed by fermentable substrates, which are inevitable with complex wastewater inputs like landfill leachate besides agricultural residues, activated sludge and anaerobic digestion sludge [26] in case H2 poorly utilized by anode respiring bacteria [27]. During fermentation H_2 will be a considerable electron sink and H_2 competition between anode respiring bacteria and other H2 consumers can have a strong impact on the CE. Therefore, methanogenesis is always a possibility with MFC oxidation of complex organic compounds like landfill leachate. Despite all of that observed current density values in this study are among the highest values recorded in the current works (10-15 A/m^2; e.g. Catal et al. [28], Torres et al., [29, 30]).

Landfill bioreactor works as a real anaerobic digester and produced leachate, especially in initial phase of landfill, includes fermentation products such as complex organic substrates, alcohols, simple acids, and acetate. In addition, fermentation is an important process for the utilization of these substrates. However, significant drops were recorded when fermentable substrates were used at the anode of MFCs [31, 32] and methane was a major electron sink accounting for the decrease in CE. In this study, we had very low CE, especially in MFC fed with young leachate giving high current density. When we consider observed highest current densities MFCs fed with young- (11 A/m^2) and old-landfill leachate (5 A/m^2), the currents correspond to 950,400 Coulomb/m^2 (9.85 eeq/m^2) and 482,400 Coulomb/m^2 (5 eeq/m^2), respectively. Thereafter, the removed COD per anode surface area by anode respiring bacteria can calculate to be 80 g COD/m^2 and 40 g COD/m^2. The removed COD per anode surface area under steady state condition giving high current densities were 58,000 g $COD_{removed}$/m^2 and 3,600 g $COD_{removed}$/m^2 in MFCs fed with young- and old-landfill leachate, which indicated that a very low amount of COD were removed by anode respiring bacteria at a rates of %0.15 and %1.1, respectively. The young landfill leachate had a very low CE at high organic loads because of a real fermentation substrate from landfill bioreactor. In addition, young leachate feed may contain possible methanogenesis domain but not identify in this work and no inhibitor was added to inhibit methanogens. Therefore, H_2 may route to CH_4 by H_2 oxidizing methanogens and homo-acetogens became a channel for electron" flow from H_2 to current through acetate. In a study, Parameswaran et al. [19] compared the microbial community structure developed in the biofilm anode of two microbial electrolysis cells fed with ethanol one where methanogenesis was allowed and another in which it was completely inhibited. They found that H_2 was routed to CH_4 by H_2 oxidizing methanobacteriales, which resulted in decreasing rate of a CE from 84% to 60%.

Another possible way for electron flow is the presence of other electron acceptors such as sulfate and nitrate. The young leachate included around 1000 mg/L sulfate and 12 mg/L nitrate and old leachate had 50 mg/L sulfate and 10 mg/L nitrate, which can be calculated as the COD/SO_4^{2-} ratio of 50 and 100 for the percent electron flow to sulfate reduction, respectively.

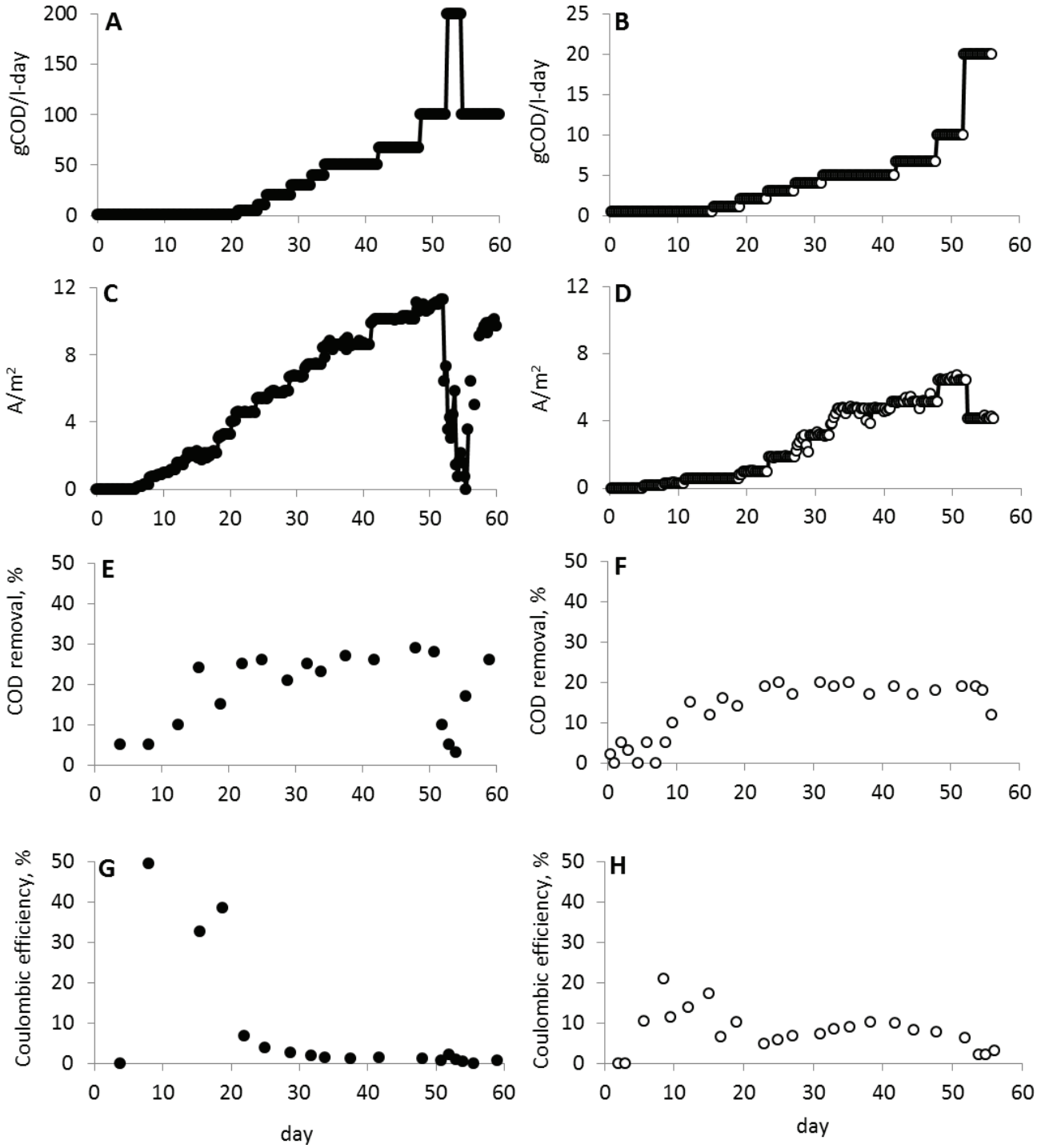

Figure 3. OLRs (A and B), current density outputs (C and D), COD removal efficiency (E and F) coulombic efficiency (G and H), left side and right side of figure show young- and old-landfill leachate, respectively.

Microbial community profiles

The microbial community profiles works covered a comparison of medium of MFC under different HRTs and biofilm anode after completing MFC works. The changes in DGGE profiles of microbial community in MFC anode chamber and in biofilm on anode electrode are presented in Figure 4 and the closest relatives of each band are given in Table 1. The first 8 lanes represent the microbial diversity at various HRTs for both young (Figure 4A) and old (Figure 4B) leachate, while Figure 4C and Figure 4D show the bacterial community in biofilm. Microbial dynamics in the figure indicated that abundance of bacteria was considerable depended on the operational conditions. Bands 1 to 6 were observed in all operational conditions

of anodic chamber and biofilm. Uncultured *Clostridium sp.* (Band 8) was only present in the liquid medium at start-up period then it disappeared during the subsequent operation courses while it was also detected in biofilm. Similarly, *Pseudomonas sp.* (Band 25) and Uncultured bacterium clone (Band 28) were dominant in biofilm and in anode liquid at start-up period while they were not detected at lower HRTs. Conversely, *Clostridium sp.* (Band 9) was not present at start-up period while it was dominant at lower HRTs and biofilm. *Shewanella putrefaciens* (Band 10) and Uncultured *Firmicutes* bacterium (Band26) were only present at HRT 2 days and then they were washout at lower HRTs. Those strains might have no ability to live attached since they were not detected in biofilm. Subramaniam [33] indicated that *S. putrefaciens* needs time to adapt to the landfill leachate treatment by MFC. The disappearance of *Shewanella putrefaciens* in our study was probably due to the fact that the operation duration at each HRT was less than its adaptation time and it was easily wash out the MFC reactor. Similar to *Clostridium sp.* (Band 9), Uncultured *Clostridiales* bacterium (Band 11), Uncultured *Clostridium* (Band12), Uncultured *Clostridium sp.*(Band 13) and *Geobacter sp.*(Band 14) were not present at HRT 2 days while they were dominant at lower HRTs. Uncultured gamma proteobacterium (Band 15), Uncultured *Geobacter sp.* (Band 16), Uncultured *Clostridium sp.* (Band 17) and Uncultured *Clostridiales* bacterium clone (Band 18), Uncultured *Pseudomonas* (Band 22) and Uncultured *Geobacter sp.* (band 23) were dominant in all HRTs and biofilm community. This result indicated that those strains were not affected by the changes in operational conditions. The distribution of bacterial community indicated that MFC anode and biofilm was mainly dominated by *Shewanella* and *Geobacter* species. *Shewanella* and *Geobacter* species were commonly reported to possess the capability to generate electricity. *S. Putrefaciens* is a metal reducing bacterium and can produce its own mediators such as soluble qinones during MFC operation [34], [35]. Galvez et al. [8] reported that *S. Putrefaciens* can consume substrates of lactate and pyruvate while it can not convert complex organics during the electricity production in an MFC. *Geobacteraceae* can directly transfer electrons to electrodes using electrochemically active redox enzymes, such as cytochromes on their outer membrane [36]. Both young and old landfill leachate did not detected sulphate reducing microbes, and therefore, the electrons did not flow to another electron acceptors, which confirmed with community structure experimental results both in biofilm anode and reactor mixture. The strong way of electron flow signed methanogenesis species considering community structure.

Figure 4C and Figure 4D show the DGGE patterns of partial 16 sRNA genes for biofilm anode. We detected relatively more bands representing different communities developed in the biofilm anode of MFC fed with old age leachate. Based on sequencing analysis biofilm samples in MFC fed with the young leachate, all of the total bacterial 16S rRNA genes belonged to the phylum *Proteobacteria*, of which 33% were *Gammaproteobacteria*, 33% *Firmicutes,* 27% *Deltaproteobacteria* and 7% *Betaproteobacteria* (Table 1 and Figure 4). Figure 4 shows phylogenetic tree for anodic biofilm in MFCs. The bacterial population in the biofilm of the old leachate MFC revealed that the almost all of patterns *Proteobacteria* phylum was a diversity, being distributed among *Deltaproteobacteria* (20% *Proteobacteria*), *Gammaproteobacteria* (44% of *Proteobacteria*) and *Firmicutes* (31% *Proteobacteria*). *Deltaproteobacteria* was mostly represented by the family of *Geobacteraceae* representing *Uncultured Geobacter sp*. with the different accession number in

GenBank and band label in DGGE pattern of 2, 7, 9, 12 and II, XI, XVIII (Table 1). The phylum *Firmicutes* in MFCs accounted for 33% and 25% of the total bacteria in biofilm from young and old leachates, respectively (Figure 4). Compared to anode biofilm in young leachate MFC, the fraction of *Geobacter sp.*, which is a known anode respiring bacteria, was much higher than in biofilm of old leachate MFC; even so this family was among dominant species in both reactors. This explains the true ecological advantage for anode respiring bacteria. The *Firmicutes* 16s rRNA genes in young leachate MFC were much higher than old leachate MFC. Ishii et al. [37] reported that the origin of *Firmicutes* characterized filamentous biofilm community in cellulose fed MFC. We found same culture with the DGGE pattern numbers of 3, 4, 6, 8 and VIII, X, XIII. *Clostridiales sp.* was found in only one band (VI Band) belong to *Acetobacterium* species. With DGGE analysis, *Pseudomonas sp.* was detected, as well as *Geobacter sp.* as a family of *Gammaproteobacteria*. Only old leachate MFC contained *Shewanella sp.* (HM589853) belong to family of *Shewanellaceae* and class of *Gammaproteobacter* with the band no of III in DGGE pattern (Figure 4). This species transfers electrons to the electrode surfaces via electron transferring proteins, our observation with the accession number of HM589853 in GenBank. The results of community analysis from various studies show that there is no single specific microorganism in the bacterial populations that develop on the anode. *Deltaproteobaceria* family group found in young landfill leachate biofilm had more fraction than old leachate. This group has been identified as the major bacterial family in acetate enriched MFCs and believed to be responsible for the direct electron transfer to electrode [38], which might be the main reason when considered that young landfill leachate contains more fermentation product like acetate during initial degradation phase of organic fraction of municipal wastes in landfill. We mainly detected that anode respiration was a probable role of predominant bacterial genera in biofilm samples from two MFC reactors fed with young and old leachate.

Observed kinetics responses of biofilm anode community gave high current density and low anode potential losses (η=0.24) with low anode potential (~ -400mV vs

AgAgCl) in steady-state conditions. Therefore, we should expect anode respiring bacteria in biofilm anode according to these observations. Our anode potential was enough low to healthy grow anode respiring bacteria, but DGGE patterns included some *Pseudomonas sp.* especially in old landfill leachate, which had more band than young leachate MFC. This strain may grow at lower anode potential during start-up period (~ -120 mV vs AgAgCl) or existed in old landfill leachate. Because old or stabilized leachate used in this study had over ten years old and may contain more dissolved oxygen because of air diffusion on the surface of landfill. Although the dominant group of bacteria were Gammaproteobacteria (~ 35%), but this group contains mainly members of Pseudomonadeledes. On the other hand, Pseudomonas sp. was found in DGGE pattern of both leachates with more band in old leachate, which grows

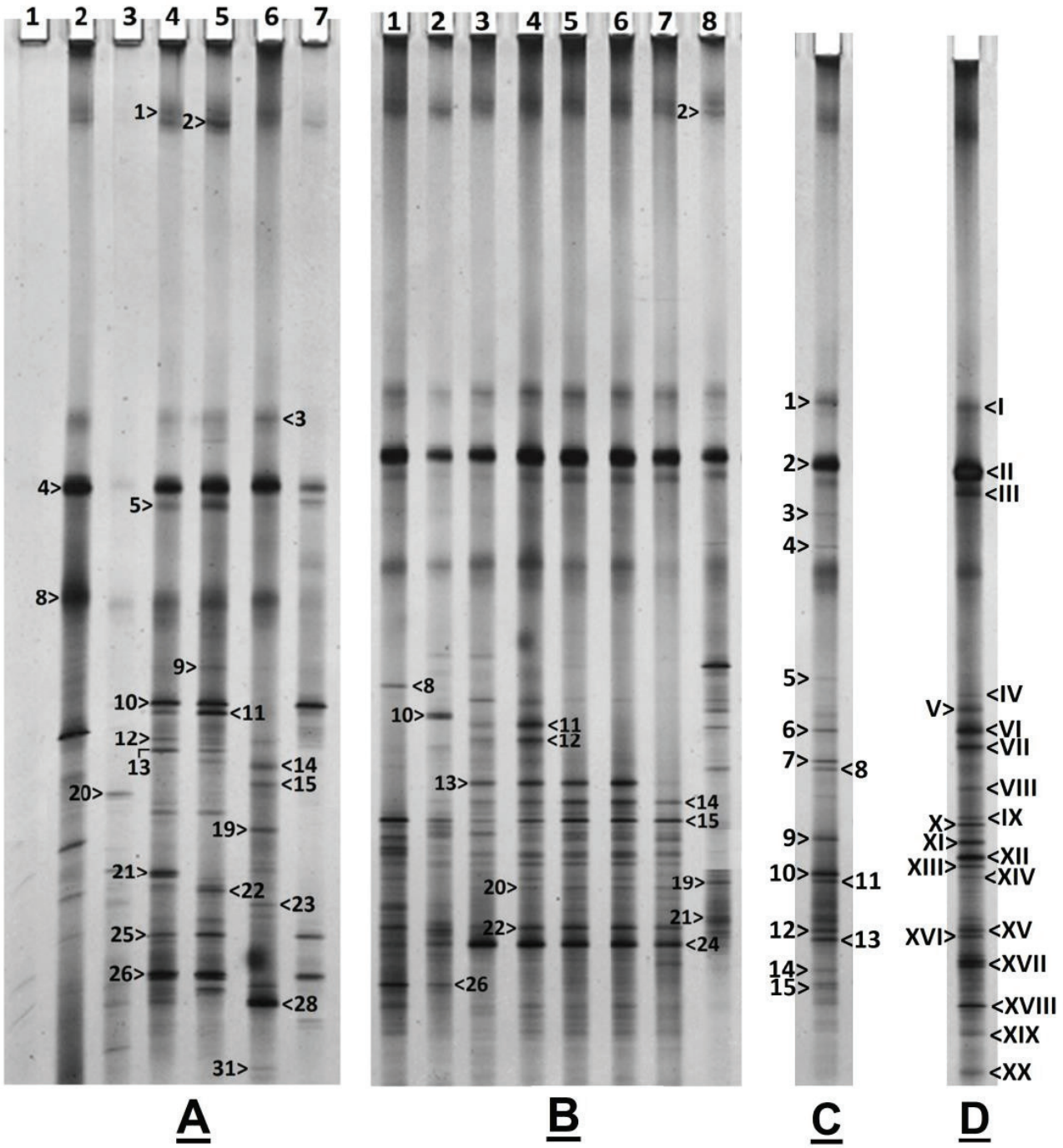

Figure 4. DGGE fingerprint of reactor medium under different conditions (A: young leachate, B: old leachate medium) and anode biofilm (C: young, D: old) see Table 1 for the labeled bands.

Table 1. Selected band identities and affiliations of the anode biofilms from DGGE conducted with young leachate samples amplified with the bacterial specific PCR primers

BN[1]	SeqL[2]	%3	Affiliation (ACN[4])	Class / Family
Young and old leachate MFC medium (Fig 4A and 4B)				
1	397	97	*Uncultured Geobacter sp. clone* (JF817819)	Deltaproteobacteria
2	387	99	*Uncultured Geobacter sp. clone* (JF817819)	Deltaproteobacteria
3	365	100	*Citrobacter sp.* (HQ845373)	Gammaproteobacteria
4	395	100	*Uncultured Geobacter sp*. (JF817819)	Deltaproteobacteria
5	387	90	*Shewanella sp.* (HM589853)	Gammaproteobacteria
6	435	100	*Arcobacter butzleri* (AP012047)	Epsilonproteobacteria
7	423	100	*Arcobacter butzleri* (AP012048)	Epsilonproteobacteria
8	471	100	*Uncultured bacterium clone* (GU992384)	Unknown / Unknown
9	357	97	*Uncultured Clostridium sp.* (AB478910)	Firmicutes
10	383	100	*Uncultured Clostridium sp.* (AB286220)	Firmicutes
11	368	100	*Clostridium sp.* (JN873174)	Firmicutes
12	382	97	*Uncultured Firmicutes bacterium clone*(EU638686)	Firmicutes
13	383	100	*Uncultured Geobacter sp.* (JF817428)	Deltaproteobacteria
14	382	99	*Uncultured Clostridiales bacterium* (FJ393201)	Firmicutes
15	395	100	*Uncultured Clostridium sp.* (AB478911)	Firmicutes
16	378	99	*Uncultured Clostridium sp.* (AB288647)	Firmicutes
17	391	100	*Uncultured Geobacter sp.* (FJ393123)	Deltaproteobacteria
18	398	100	*Pseudomonas anguilliseptica* (HM103328)	Gammaproteobacteria
19	384	98	*Uncultured Clostridium sp.* (AB286238)	Firmicutes
20	393	100	*Uncultured Clostridium sp.* (AB478911)	Firmicutes
21	415	100	*Uncultured beta proteobacterium clone* (GU202941)	Betaproteobacteria;
22	375	99	*Uncultured Geobacter sp.* (JF817455)	Deltaproteobacteria
23	362	100	*Uncultured Clostridium sp.* (AB478910)	Firmicutes
24	387	100	*Uncultured gamma proteobacterium* (AB286293)	Gammaproteobacteria
25	381	90	*Uncultured beta proteobacterium* (AB286282)	Betaproteobacteria
26	392	100	*Uncultured Geobacter sp.* (JF818012)	Deltaproteobacteria
27	381	99	*Microbial fuel cell bacterium* (AY483174)	Alphaproteobacteria
28	370	100	*Uncultured Pseudomonas sp.* (JF736640)	Gammaproteobacteria
29	361	100	*Uncultured Firmicutes bacterium gene* (AB478911)	Firmicutes
30	366	100	*Uncultured Geobacter sp*.(JF817997)	Deltaproteobacteria;
31	358	100	*Uncultured bacterium clone* (AY491586)	Unknown / Unknown
Young leachate anode biofilm (Fig 4C)				
1	388	100	*Citrobacter sp.* (HQ845373)	Gammaproteobacteria/ Enterobacteriaceae
2	301	100	*Uncultured Geobacter sp.* (JF817819)	Deltaproteobacteria/ Geobacteraceae
3	379	98	*Uncultured Clostridium sp.* (AB286220)	Firmicutes/ Clostridiaceae
4	375	98	*Uncultured Clostridium sp.* (AB286234)	Firmicutes/ Clostridiaceae
5	385	97	*Uncultured Clostridium sp.* (AB478910)	Firmicutes/ Clostridiaceae
6	405	98	*Uncultured Clostridium sp.* (AB286238)	Firmicutes/ Clostridiaceae
7	325	90	*Uncultured Geobacter sp.* (JF817471)	Deltaproteobacteria/ Geobacteraceae
8	372	100	*Uncultured gamma proteobacterium* (AB286293)	Gammaproteobacteria/ Unknown
9	349	99	*Uncultured Geobacter sp.* (JF817455)	Deltaproteobacteria/ Geobacteraceae
10	372	98	*Uncultured gamma proteobacterium* (AB286293)	Gammaproteobacteria/ Unknown
11	379	100	*Uncultured beta proteobacterium clone* (GU202941)	Betaproteobacteria/ Unknown
12	372	99	*Uncultured Geobacter sp.* (JF817455)	Deltaproteobacteria/ Geobacteraceae
13	391	100	*Uncultured Clostridium sp.* (AB478910)	Firmicutes/ Clostridiaceae

14	381	100	*Uncultured Pseudomonas sp.* (JF736640)	Gammaproteobacteria/ Pseudomonadaceae
15	391	96	*Pseudomonas sp.* (GQ463725)	Gammaproteobacteria/ Pseudomonadaceae
			Old leachate anode biofilm (Fig 4D)	
I	388	100	*Citrobacter sp.* (HQ845373)	Gammaproteobacteria/ Enterobacteriaceae
II	301	100	*Uncultured Geobacter sp.* (JF817819)	Deltaproteobacteria/ Geobacteraceae
III	384	90	*Shewanella sp.* (HM589853)	Gammaproteobacteria/ Shewanellaceae
IV	353	98	*Uncultured bacterium clone* (GU591545	Unknown / Unknown
V	375	90	*Pseudomonas sp. (*HM103333)	Gammaproteobacteria/ Pseudomonadaceae
VI	395	99	*Uncultured Clostridiales bacterium* (FJ393201)	Firmicutes/Clostridiacea
VII	384	100	*Uncultured Clostridium sp.* (AB478911)	Firmicutes/ Clostridiacea
VIII	405	98	*Uncultured Clostridium sp.* (AB286238)	Firmicutes/ Clostridiaceae
IX	379	100	*Uncultured beta proteobacterium clone* (GU202941)	Betaproteobacteria/ Unknown
X	349	100	*Uncultured gamma proteobacterium* (AB286293)	Gammaproteobacteria/ Unknown
XI	372	99	*Uncultured Geobacter sp.* (JF817455)	Deltaproteobacteria/ Geobacteraceae
XII	391	100	*Uncultured Clostridium sp.* (AB478910)	Firmicutes/ Clostridiaceae
XIII	405	98	*Uncultured Clostridium sp.* (AB286238)	Firmicutes/ Clostridiaceae
XIV	369	100	*Bacterium enrichment culture clone* (FJ624397)	Unknown / Unknown
XV	381	100	*Uncultured Pseudomonas sp.* (JF736640)	Gammaproteobacteria/ Pseudomonadaceae
XVI	391	96	*Pseudomonas sp.* (GQ463725)	Gammaproteobacteria/ Pseudomonadaceae
XVII	391	96	*Pseudomonas sp.* (GQ463725)	Gammaproteobacteria/ Pseudomonadaceae
XVIII	369	100	*Uncultured Geobacter sp.* (JF817997)	Deltaproteobacteria/ Geobacteraceae
XIX	362	100	*Uncultured bacterium clone* (AY491586)	Unknown / Unknown
XX	362	100	*Uncultured bacterium clone* (AY491586)	Unknown / Unknown

1:Band numbers in Figure 4, **2:**Sequence length, **3:**Similarity, **4:**Closest species in GenBank database with an accession number

at high anode potential and many of which are known to respire the anode using electron shuttles and Pseudomonas species are known to produce redox mediators as pyocyanin [14], which contribute high current output in our works.

We used Nernst-Monod based model as the baseline to distinguish intracellular potential losses from anode potential losses (η). Thus, deviations from the Nernst-Monod model will help us determine extracellular potential losses due to interphase electron transfer and extracellular electron transfer of anode respiring bacteria [30]. Electron transport a solid conductive matrix can explain with the high current densities and low potential losses. The η value shows anode potential losses. We observed this parameter as 0.25 V ($\eta=E_{anode} - E_{KA}$) with the high current density output of 11 A/m^2 without any large any potential loss. Direct contact of anode respiring bacteria confirmed in our community structure analysis provided to achieve high current densities in MFCs.

Microbial composition in MFC system changed relative to substrate composition and operating conditions. DNA extraction from the biofilm anode and suspended microbial consortia were independently analyzed from these two sources from young and old leachate, and population differences were observed between the biofilm and suspended cultures. Interestingly, Deltaproteobacter in biofilm anode of young landfill leachate was more dominant phylum than old leachate biofilm anode. Sulphate concentration in young landfill leachate have higher concentration than old leachate because of low pH and solubility of sulphate and other ion. The reason of Deltaproteobacter (Geobacter sp. and Desulfuromonadales) dominancy is that this phylum has ability to reduction of sulphate. The delta- and gamma-proteobacter play an important role during anodic electron transfer in young leachate MFC; however, Gammaproteobacter more active culture in biofilm anode of old leachate MFC not Deltaproteobacter.

CONCLUSION

Based on the current outputs, microbial community and kinetics performance of microbial fuel cell with Ti-TiO_2 electrode, is a promising candidate for electricity generation, the following conclusions can be drawn:

1) The highest current density is around 11 A/m^2 in MFC fed with young age landfill leachate at the hydraulic retention time of 0.5 day. Current density is almost 6 A/m^2 for old age leachate with the same HRT.
2) Dominated bacterial groups in anode biofilm are *Deltaproteobacteria* and *Gammaproteobacteria*. *Deltaproteobacteria* family group found in leachate fed MFC.

3) The kinetic parameters for young age leachate are computed as η = 0.25 V, Ks=3.4 g sCOD/L, and J_{max} = 12 A/m^2 and computed values are η = 0.25 V., Ks=1.3 g sCOD/L, and J_{max} 8 A/m^2 for old leachate substrate.

In conclusion, a high current density output achieved with MFC fed with landfill leachate with the low anode potential and potential losses confirming kinetics parameters.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

REFERENCES

[1] Taskan E, Hasar H. Effect of different leachate/acetate ratios in a submerged anaerobic membrane bioreactor (SAnMBR). Clean (Weinh) 2012;40:487–492. [CrossRef]

[2] Zhang H, Choi HJ, Huang C. Landfill leachate treatment by Fenton's reagent. The variation of leachate characteristics. Fresenius Environ Bull 2005;14:1178–1183.

[3] Ozkaya B, Demir A, Bilgili MS. Enhanced stabilisation and methane potential of MSWs in a field-scale landfill with leachate recirculation. Int J Environ Pollut 2004;21:277–292. [CrossRef]

[4] Renou S, Givaudan JG, Poulain S, Dirassouyan F, Moulin P. Landfill leachate treatment: Review and opportunity. J Hazard Mater 2008;150:468–493. [CrossRef]

[5] Rittmann BE. Opportunities for renewable bioenergy using microorganisms. Biotechnol Bioeng 2008;100:203–212. [CrossRef]

[6] You SJ, Zhao QL, Jiang JQ, Zhang JN, Zhao SQ. Sustainable approach for leachate treatment: electricity generation in microbial fuel cell. J Environ Sci Health A 2006;41:2721–2734. [CrossRef]

[7] Zhang JN, Zhao QL, You SJ, Jiang JQ, Ren NQ. Continuous electricity production from leachate in a novel upflow air-cathode membrane-free microbial fuel cell. Water Sci Technol 2008;57:1017–1021. [CrossRef]

[8] Gálvez A, Greenman J, Ieropoulos I. Landfill leachate treatment with microbial fuel cells; scale-up through plurality. Bioresour Technol 2009;100:5085–5091. [CrossRef]

[9] Greenman J, Gálvez A, Giusti L, Ieropoulos I. Electricity from landfill leachate using microbial fuel cells: comparison with a biological aerated filter. Enzyme Microb Technol 2009;44:112–119. [CrossRef]

[10] Puig S, Serra M, Coma M, Cabré M, Balaguer MD, Colprim J. Microbial fuel cell application in landfill leachate treatment. J Hazard Mater 2011;185:763–767. [CrossRef]

[11] Lovley DR, Fraga JL, Coates JD, Blunt-Harris EL. Humics as an electron donor for anaerobic respiration. Environ Microbiol 1999;1:89–98. [CrossRef]

[12] Kato Marcus A, Torres CI, Rittmann BE. Conduction-based modeling of the biofilm anode of a microbial fuel cell. Biotechnol Bioeng 2007;98:1171–1182. [CrossRef]

[13] Wanner O, Ebert HJ, Morgenroth E, Noguera D, Picioreanu C, Rittmann BE, et al. Mathematical modeling of biofilms. IWA Scientific and Technical Report No.18 IWA Task Group on Biofilm Modeling, 2006.

[14] Rabaey K, Verstraete W. Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 2005;23:291–298. [CrossRef]

[15] Torres CI, Marcus AK, Lee HS, Parameswaran P, Krajmalnik-Brown R, Rittmann BE. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. FEMS Microbiol Rev 2010;34:3–17. [CrossRef]

[16] Logan BE, Regan JM. Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol 2006;14:512–518. [CrossRef]

[17] Kim JR, Jung SH, Regan JM, Logan BE. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour Technol 2007;98:2568–2577. [CrossRef]

[18] Torres CI, Krajmalnik-Brown R, Parameswaran P, Marcus AK, Wanger G, Gorby YA, et al. Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization. Environ Sci Technol 2009;43:9519–9524. [CrossRef]

[19] Parameswaran P, Torres CI, Lee HS, Krajmalnik-Brown R, Rittmann BE. Syntrophic interactions among anode respiring bacteria (ARB) and Non-ARB in a biofilm anode: electron balances. Biotechnol Bioeng 2009;103:513–523. [CrossRef]

[20] Bond DR, Lovley DR. Electricity production by Geobacter sulfurreducens attached to electrodes. Appl Environ Microbiol 2003;69:1548–1555. [CrossRef]

[21] American Public Health Association. Standard Methods for the Examination of Water and Wastewater. American Water Works Association, Water Environmental Federation. 21st ed. Washington, DC: American Public Health Association; 2005.

[22] Ozkaya B, Akoğlu B, Karadag D, Acı G, Taksan E, Hasar H. Bioelectricity production using a new electrode material in microbial fuel cell. Bioprocess Biosyst Eng 2012;35:1219–1227. [CrossRef]

[23] Logan BE. Microbial Fuel Cells. Hoboken, New Jersey: John Wiley & Sons; 2008.

[24] Sleutels TH, Darus L, Hamelers HV, Buisman CJ. Effect of operational parameters on Coulombic efficiency in bioelectrochemical systems. Bioresour Technol 2011;102:11172–11176. [CrossRef]

[25] Muyzer G. Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA-a new molecular approach to analyse the genetic diversity of mixed microbial communities. Mol Microbial Ecol Manual 1996:344-1.

[26] Kim GT, Hyun MS, Chang IS, Kim HJ, Park HS, Kim BH, et al. Dissimilatory Fe (III) reduction by an electrochemically active lactic acid bacterium phylogenetically related to Enterococcus gallinarum isolated from submerged soil. J Appl Microbiol 2005;99:978–987. [CrossRef]

[27] Torres CI, Kato Marcus A, Rittmann BE. Kinetics of consumption of fermentation products by anode-respiring bacteria. Appl Microbiol Biotechnol 2007;77:689–697. [CrossRef]

[28] Catal T, Xu S, Li K, Bermek H, Liu H. Electricity generation from polyalcohols in single-chamber microbial fuel cells. Biosen Bioelectron 2008;24:849–854. [CrossRef]

[29] Torres CI, Marcus AK, Rittmann BE. Phosphate and Bicarbonate Buffers as Proton Carriers Inside the Biofilm of Anode-Respiring Bacteria in Microbial Fuel Cells. In ECS Meeting Abstracts (No. 7, p. 239). Bristol: IOP Publishing; 2008. [CrossRef]

[30] Torres CI, Kato Marcus A, Rittmann BE. Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria. Biotechnol Bioeng 2008;100:872–881. [CrossRef]

[31] Lee HS, Parameswaran P, Kato-Marcus A, Torres CI, Rittmann BE. Evaluation of energy-conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. Water Res 2008;42:1501–1510. [CrossRef]

[32] Min B, Logan BE. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environ Sci Technol 2004;38:5809–5814. [CrossRef]

[33] Subramaniam PK. Microbial transport and the use of microbial fuel cell technology to prevent iron release in landfills nearby northwest Florida. Dissertations Thesis. The Florida State University, 2011.

[34] Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH. A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme Microbial Technol 2002;30:145–152. [CrossRef]

[35] Liu H, Ramnarayanan R, Logan BE. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 2004;38:2281–2285. [CrossRef]

[36] Ghangrekar MM, Shinde VB. Wastewater treatment in microbial fuel cell and electricity generation: A sustainable approach. In 12th International Sustainable Development Research Conference (Vol. 8, p. 201). Princeton, New Jersey: Citeseer; 2006.

[37] Ishii SI, Watanabe K, Yabuki S, Logan BE, Sekiguchi, Y. Comparison of electrode reduction activities of Geobacter sulfurreducens and an enriched consortium in an air-cathode microbial fuel cell. Appl Environ Microbiol 2008;74:7348–7355. [CrossRef]

[38] Lee J, Phung NT, Chang IS, Kim BH, Sung HC. Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. FEMS Microbiol Lett 2003;223:185–191. [CrossRef]