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Anode Maturation Time for Attaining a Mature Anode Biofilm and Stable Cell Performance in a Single Chamber Microbial Fuel Cell with a Brush Anode

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Abstract: To obtain an accurate and reproducible experimental results in microbial fuel cell (MFC), it is important to know 'anode maturation biofilm' to produce a stable and maximum performance. For this purpose, four single chamber MFCs were tested in this study. The linear sweep voltammetry (LSV) polarization tests illustrated that maximum power densities of three MFCs became stable after 9 weeks. Although there were variations afterwards, such variations were negligible. Average maximum power densities from the 9^{th} to the 17^{th} week were 2,990 mW/m² (MFC-4), 2,983 mW/m² (MFC-2), 2,368 mW/m² (MFC-3) and 837 mW/m² (MFC-1). Polarization resistance shows that MFC-1 had much larger anode resistance (36.6-85.4 Ω) than the other MFCs (1.7-11.6 Ω). Anodic cyclic voltammetry (CV) shows that current production increased over time and MFC-1 had much smaller current production (24.4 mA) than the other MFCs (31.0-34.9 mA) at 17th week. The increased current production indicates anode biofilm became more mature over time, but overall cell performance did not increased accordingly. Possibly due to the bad inoculation, MFC-1 showed the lowest performance. However, its performance was restored to the initial performance and anode resistance was reduced by 47% at 17th week. This study shows that the optimum anode maturation time is 9 weeks and that bioanode performance is a key factor for MFC performance. This study also shows than LSV polarization and CV tests are accurate and non-destructive measurement methods for diagnosing anode performance.

Keywords: anode maturation time; microbial fuel cell (MFC); linear sweep voltammetry (LSV) polarization; cyclic voltammetry (CV)

1. Introduction

Since microbial electrical effects accompanying the decomposition of organic compounds was discovered in 1911 [1], the electrode reaction of *Shewanella* isolates were demonstrated by Kim *et al.* [2, 3]. Several years later, electricity generation using *Geobacter* isolate in microbial fuel cell (MFC) was also discovered [4]. MFC is an environmental technology innovation that converts organic wastewater into electricity with a simultaneous wastewater treatment [5-8]. Due to undivided attention to global climate and energy crisis in recent decades, MFC technology has been increasingly concerned about in environmental and energy fields.

In MFC, anodic microbial biofilm can electrochemically decompose organic compounds. This electrogenic anode biofilm functions as an anodic catalyst, a key part for producing current from waste organic matter. The anode biofilm mainly consists of electrogenic microorganisms that perform extracellular electron transfer reaction [9]. In MFC experiments, a sufficiently mature anode biofilm is very important for producing stable and reproducible data [10]. In routine lab scale MFC tests, the anode is inoculated with wastewater microbial biota. The anodic film is then enriched in a synthetic medium.

However, how long the anode biofilm should be enriched for a stable performance remains unclear. In this study, for a clearer concept, we define anode maturation time as the operation time encompassing the initial inoculation and the biofilm development time until the MFC produces a relatively stable performance.

In a two-chambered MFC with a plain porous carbon paper anode and a Pt-coated plain carbon paper cathode, by using nutrient medium during the development phase, voltage generation increased appreciably after 3 days (70 h), reaching a stable state after 5.2 days (125 h) [11]. In another two chamber MFC study, nine closed-circuit MFCs and three open-circuit MFCs were tested along with three different substrates, in which anode biofilms were enriched for 16 days [9, 12].

In contrast, for simpler MFC configurations (i.e., single-chamber cell or two-chamber cell), the anode maturation times were only a few days. In a single-chamber MFC, a carbon paper anode required about 4-8 days to obtain a stable voltage output. After 2 days of inoculation with a solution including wastewater, nutrient medium and sodium acetate, MFCs could have stable power outputs after 2-3 batches (1-2 days per batch) filled with nutrient medium and acetate [13]. In another single-chamber MFC test with a Pt-coated carbon cloth cathode, a carbon cloth anode were inoculated with full-strength brewery wastewater for four batches. By using only wastewater or the combination of wastewater and PBS, reactors' voltage became stable and reproducible after about 10 days $(230 \pm 30 \text{ h})$ from the initial inoculation. However, its operational voltage continued to increase gradually until 18 days (420 h) [14].

A stacked MFC required a total of 131 days to achieve a stable phase of anode. It seemed the stacked MFC system with a large volume and a complex construction needed a long time for anode to be mature [15].

Anode maturation times reported so far varies a lot depending on many factors, ranging from a few days to several months. It is important to know the appropriate operation time for attaining a mature anode biofilm and stable performance because it allows reliable data production. And it is very important to know it in a most common condition, that is, a single chamber cubic MFC.

In this study, to investigate a representative anode maturation time in a single-chamber cell with a graphite brush anode and an air-cathode, four MFCs were tested to figure out their anode maturation time. This cell configuration has been widely used because of its simple structure, small size, easy operation and high performance [16, 17]. Four replicate MFCs were tested and a series of electrochemical analyses were carried out to explore their performance and electrochemistry. Our results shows that the representative anode maturation time in a single chamber MFC with a brush anode was 63 days (9 weeks).

2. Materials and methods

2.1. Electrode fabrication

Activated carbon based air-cathode using polyvinylidene fluoride (PVDF) as a binder was used in this study [18]. Catalyst mixture contained 10% PVDF solution loading of 1 mL, carbon black (Vulcan XC-72, Cabot Co., USA) loading of 30 mg and activated carbon (Norit SX plus, Norit Americas Inc., TX, USA) loading of 300 mg. This mixture was spread directly on a stainless-steel mesh (60 mesh, SUS 304, a projected cross-sectional area of 7 cm², a total area of 11.3 cm²). Subsequently, the cathode was dipped in distilled water for 15 minutes to induce a phase inversion reaction. It was then dried at room temperature for 8 h. A brush anode (2.5 cm in both length and diameter) was constructed as previously described [19, 20]. A carbon fiber (T700, TORAYCA, Japan) was twisted on a titanium wire (length: 7 cm; 17 gauge; #2 grade; Seoul Titanium, South Korea) to fabricate a brush. The brush anode was heat-treated at 450 °C for 30 minutes in a furnace (FX/FHX, DAIHAN, South Korea) before being inoculated.

2.2. MFC construction and operation

MFC construction

To investigate when an inoculated anode in MFC reached its stable performance, four replicate MFCs designated as MFC-1, MFC-2, MFC-3 and MFC-4 were tested. A polycarbonate single-chamber cubeshaped MFC was used with an anode and a cathode placed on opposite sides. A cubic chamber was 4 cm in length and 3 cm in diameter with an empty cylindrical bed volume of 28 mL [16, 20]. To achieve the maximum power generation, a brush anode was installed at a distance of 12 mm horizontally to the cathode [20]. A titanium plate (10 × 25 mm, G2, Seoul titanium, South Korea) was used as a current collector. A cathode had a projected area of 7 cm² in the medium. A nonwoven fabric was attached onto the projected side of the cathode to prevent a short circuit [21].

Inoculation phase, developing phase and stable phase

For inoculation, domestic wastewater was obtained from Gwangju Wastewater Treatment Plant with a ratio of primary sedimentation to activated sludge of 1:4 [22]. Anodic compartments were inoculated with the mixture wastewater mentioned above and operated with a fixed external resistance of $1,000-\Omega$ at room temperature (30°C). After one week, MFC systems were operated in a fed-batch mode using 50-mM phosphate buffer solution (PBS) as medium. The medium for MFC operation contained 2 g/L of sodium acetate in 50-mM PBS (NaH2PO4, 2.34 g/L; Na2HPO4, 4.33 g/L; NH4Cl, 0.31 g/L; KCl, 0.13 g/L; trace minerals, 1 mL/L and vitamins, 1 mL/L) which was replaced every day [12, 23]. Hence, from day 0 to the 1st week, it was called an inoculation phase. From the 1st week until MFC reaching a stable state, it was called a developing phase. After this time, it was called a stable phase (Figure 1). In the stable phase, MFCs would have the optimum practical performance.

2.3. Electrochemical analysis

MFC voltage was measured every minute using a computer-based multi-meter (3706A, Keithley Instrument, USA). When the voltage declined below 20 mV, fresh medium was fed. All electrochemical measurements were performed after replacing the new medium and after stable cell voltage was achieved. Measurements of LSV polarization test and cyclic voltammetry (CV) were performed using a potentiostat (ZIVE SP1, Wonatech, South Korea). An Ag/AgCl electrode (RE-1B, ALS, Japan; -209 mV vs. SHE) was installed as a reference electrode in all experiments. After every two weeks of operation, all reactors were tested at 3, 5, 7, 9, 11, 13, 15 and 17 weeks.

To observe the maximum power density of MFCs, LSV polarization test was carried out. An MFC reactor was operated in open circuit mode for 90 minutes. It was then connected to the potentiostat with the following programming: scan rate of 1 mV/s and voltage ranging from OCV to 0 V versus Ag/AgCl. The MFC was connected to the potentiostat as a cathode to a working lead, an anode to a counter lead

and an AI+ lead, a reference electrode to an AI- lead.

Cyclic voltammetry measurements were carried out with an electrochemical potentiostat equipped a standard three-electrode system at room temperature (measured between -600 and 100 mV (vs. Ag/AgCl), a scan rate of 1 mV/s). In brief, the anode was used as the working electrode while the cathode and the Ag/AgCl electrode were used as the counter and reference electrodes, respectively.

2.4. Calculation

Power density (*p*, mW/m²) of each MFC system was calculated using the following equation:

$$p = \frac{I.V}{A}$$

 $p=\frac{I.V}{A}$,where I is current (mA), V is voltage (mV) and A is a projected cathode area (7 cm²) [15].

Current density (i, mA/m²) was also calculated based on the cathode projected area with the following equation:

$$i = \frac{I}{A}$$

The optimum external resistance (R_{opt} , Ω) found for each MFC reactor was calculated with the following equation:

$$R_{opt} = \frac{V_{opt}}{I_{opt}} = \frac{p_{max}}{(I_{opt})^2}$$

, where V_{opt} is the optimum cell voltage (V), I_{opt} is the optimum current (A), p_{max} is the maximum power (W). Polarization resistance of electrodes was calculated from linear part of the polarization curve.

3. Results

3.1. Operation cell voltage profile

The average cell voltage was recorded during our study (except for the inoculation phase) (Figure 1). As expected, we filled the synthetic medium after one week, the MFC voltage increased drastically. From the 1st week to the 3rd week, MFC-1, MFC-2, MFC-3 and MFC-4 cells voltage increased from 217 mV to 483 mV, from 216 mV to 477 mV, from 212 mV to 432 mV, from 186 mV to 462 mV, respectively. Cell voltage decreased gradually when we replaced the lower optimum external resistance from the 3rd week. It was found that the actual power generation become stable from the 9th week.

3.2. Power and current production

To evaluate MFC performance at each week, LSV polarization test was performed. The maximum power density (p_{max}), optimum current density (i_{opt}) and maximum current density (i_{max}) of all MFCs were compared. Optimum current density is the current density when an MFC produces the maximum power during an optimum operation. From the initial time to the 7th week, the power density of all reactors had an unstable tendency.

Power overshoots were detected at the 3rd week for all MFCs (Figures 2-A, 2-C, 2-E, 2-G). To prevent this phenomenon, we conducted the optimum external resistance to cells. By performing LSV polarization test, we could determine the optimum external resistance based on LSV polarization data (Table 1).

In the 3^{rd} , 5^{th} , 7^{th} , 9^{th} , 11^{th} , 13^{th} , 15^{th} and 17^{th} weeks, MFC-1 produced the maximum power densities (p_{max}) of 1,229 mW/m², 1,050 mW/m², 557 mW/m², 798 mW/m², 662 mW/m², 732 mW/m², 710 mW/m² and 1,283 mW/m², 700 m mW/m², respectively (Figure 2-A). Its optimum current densities (i_{opt}) were 3,610 mA/m², 3,957 mA/m², 2,559 mA/m², 3,025 mA/m², 2,708 mA/m², 2,946 mA/m², 2,942 mA/m² and 4,747 mA/m², respectively. Its maximum current densities (i_{max}) were 3,918 mA/m², 8,158 mA/m², 5,249 mA/m², 6,579 mA/m², 5,707 mA/m², 6,081 mA/m², 6,104 mA/m² and 9,772 mA/m², respectively (Figure 3 and Table 1).

In the 3rd, 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-2 produced the maximum power densities (p_{max}) of 1,338 mW/m², 2,160 mW/m², 2,642 mW/m², 3,099 mW/m², 2,807 mW/m², 3,137 mW/m², 2,873 mW/m² and 2,997 mW/m², respectively (Figure 2-C). Its optimum current densities (i_{opt}) were 3,774 mA/m², 7,733 mA/m², 9,186 mA/m², 10,040 mA/m², 9,239 mA/m², 9,961 mA/m², 9,838 mA/m² and 9,838 mA/m², respectively. Its maximum current densities (i_{max}) were 3,945 mA/m², 14,633 mA/m², 18,152 mA/m², 20,869 mA/m², 16,575 mA/m², 19,350 mA/m², 19,667 mA/m² and 18,086 mA/m², respectively (Figure 3 and Table 1).

In the 3rd, 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-3 produced the maximum power densities (p_{max}) of 1,029 mW/m², 2,382 mW/m², 2,061 mW/m², 2,527 mW/m², 2,173 mW/m², 2,405 mW/m², 2,362 mW/m² and 2,374 mW/m² respectively (Figure 2-E). Its optimum current densities (i_{opt}) were 3,356 mA/m², 8,498 mA/m², 7,306 mA/m², 8,680 mA/m², 7,566 mA/m², 8,182 mA/m², 7,865 mA/m² and 7,993 mA/m², respectively. Its maximum current densities (i_{max}) were 3,844 mA/m², 16,967 mA/m², 15,431 mA/m², 18,271 mA/m², 15,325 mA/m², 15,342 mA/m², 14,603 mA/m² and 15,567 mA/m², respectively (Figure 3 and Table 1).

In the 3rd, 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-4 produced the maximum power densities (p_{max}) of 1,277 mW/m², 2,327 mW/m², 2,509 mW/m², 2,969 mW/m², 2,684 mW/m², 3,289 mW/m², 3,114 mW/m² and 2,896 mW/m² respectively (Figure 2-G). Its optimum current densities (i_{opt}) were 3,807 mA/m², 8,446 mA/m², 8,622 mA/m², 9,671 mA/m², 8,944 mA/m², 10,591 mA/m², 10,084 mA/m² and 9,547 mA/m², respectively. Its maximum current densities (i_{max}) were 4,188 mA/m², 16,642 mA/m², 18,799 mA/m², 20,393 mA/m², 19,174 mA/m², 19,420 mA/m², 19,174 mA/m² and 18,636 mA/m², respectively (Figure 3 and Table 1).

To sum up, data from actual power production and results from LSV polarization tests showed that all MFCs started to produce relative stable power and current from the 9^{th} week (Figures 1 and 4). Therefore, average values were calculated from the 9^{th} week to the 17^{th} week to evaluate the overall cell performance in this study. Based on actual power production, MFC-4 achieved the highest cell voltage of 222 ± 9 mV, followed by MFC-2 of 217 ± 29 mV, MFC-3 of 209 ± 12 mV and MFC-1 of 193 ± 36 mV. Based on LSV polarization results, MFC-4 also achieved the highest power production with a stable maximum power density of $2,990 \pm 228$ mW/m² and maximum current density of $19,359 \pm 645$ mA/m², followed by MFC-2 ($2,982 \pm 142$ mW/m² and $18,909 \pm 1,638$ mA/m², respectively), MFC-3 ($2,368 \pm 127$ mW/m² and $15,822 \pm 1,417$ mA/m², respectively) and MFC-1 (837 ± 254 mW/m² and $6,849 \pm 1,663$ mA/m², respectively).

3.3. Polarization resistance

Electrodes polarization resistances, internal resistance of the full cell and optimum external resistance for maximum power generation were calculated from electrode polarization curves (i.e., Ran, Rcat, Rint, Ropt, respectively) (Table 1).

In the 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-1 showed anode polarization resistances (Ran) of $52.0\,\Omega$, $70.7\,\Omega$, $61.9\,\Omega$, $85.4\,\Omega$, $58.0\,\Omega$, $68.6\,\Omega$ and $36.6\,\Omega$, respectively. Its cathode polarization resistances (Rcat) were $44.9\,\Omega$, $56.9\,\Omega$, $56.9\,\Omega$, $54.6\,\Omega$, $38.7\,\Omega$, $60.1\,\Omega$, $45.9\,\Omega$ and $41.4\,\Omega$, respectively. Its full cell internal

resistances (R_{int}) were 96.9 Ω , 127.6 Ω , 116.4 Ω , 124.1 Ω , 118.1 Ω , 114.4 Ω and 78.0 Ω , respectively. Its optimum external resistances (R_{opt}) were 96.0 Ω , 126.0 Ω , 125.0 Ω , 129.0 Ω , 120.0 Ω , 117.0 Ω and 81.0 Ω , respectively.

In the 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-2 showed anode polarization resistances (R_{an}) of 5.7 Ω , 3.0 Ω , 5.0 Ω , 2.6 Ω , 2.6 Ω , 3.1 Ω and 3.0 Ω , respectively. Its cathode polarization resistances (R_{cat}) were 45.7 Ω , 42.0 Ω , 39.4 Ω , 44.9 Ω , 41.1 Ω , 37.6 Ω and 41.0 Ω , respectively. Its full cell internal resistances (R_{int}) were 51.4 Ω , 45.0 Ω , 44.4 Ω , 47.4 Ω , 43.7 Ω , 40.7 Ω and 44.0 Ω , respectively. Its the optimum external resistances (R_{opt}) were 52.0 Ω , 45.0 Ω , 44.0 Ω , 47.0 Ω , 45.0 Ω , 42.0 Ω and 44.0 Ω , respectively.

In the 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-3 showed anode polarization resistances (R_{an}) of 5.1 Ω , 6.4 Ω , 7.1 Ω , 7.7 Ω , 10.1 Ω , 11.6 Ω and 9.6 Ω , respectively. Its cathode polarization resistances (R_{cat}) were 41.9 Ω , 47.9 Ω , 41.1 Ω , 45.4 Ω , 42.7 Ω , 42.1 Ω and 42.4 Ω , respectively. Its full cell internal resistances (R_{int}) were 47.0 Ω , 54.3 Ω , 48.3 Ω , 53.1 Ω , 52.9 Ω , 53.7 Ω and 52.0 Ω , respectively. Its optimum external resistances (R_{opt}) were 47.0 Ω , 55.0 Ω , 48.0 Ω , 54.0 Ω , 51.0 Ω , 55.0 Ω and 53.0 Ω , respectively.

In the 5th, 7th, 9th, 11th, 13th, 15th and 17th weeks, MFC-4 showed anode polarization resistances (R_{an}) of 3.7 Ω , 2.0 Ω , 3.3 Ω , 1.7 Ω , 2.0 Ω , 2.7 Ω and 2.7 Ω , respectively. Its cathode polarization resistances (R_{cat}) were 43.0 Ω , 44.4 Ω , 41.7 Ω , 45.3 Ω , 40.7 Ω , 40.7 Ω and 42.4 Ω , respectively. Its full cell internal resistances (R_{int}) were 46.7 Ω , 46.4 Ω , 45.0 Ω , 47.0 Ω , 42.7 Ω , 43.4 Ω and 45.1 Ω , respectively. Its optimum external resistances (R_{opt}) were 47.0 Ω , 48.0 Ω , 45.0 Ω , 42.0 Ω , 44.0 Ω and 45.0 Ω , respectively.

Since anode characteristics were main objects of this study, anode polarization resistance was emphatically indicated. To sum up, MFC-1 showed the lowest anode polarization resistance in the 17^{th} week while MFC-2 showed the lowest anode polarization resistance in the 11^{th} and 13^{th} weeks. MFC-3 and MFC-4 showed the lowest anode polarization resistance in the 5^{th} week and the 11^{th} week, respectively. We selected the 9^{th} to 17^{th} week as the stable time, consistent with actual operation data and LSV polarization results. The stable anode polarization resistance, cathode polarization resistance, full cell internal resistance and optimum external resistance of MFC-1 were $62.1 \pm 17.7 \Omega$, $48.1 \pm 9.0 \Omega$, $110.2 \pm 18.4 \Omega$ and $114.4 \pm 19.2 \Omega$, respectively. Those of MFC-2 were $3.3 \pm 1.0 \Omega$, $40.8 \pm 2.7 \Omega$, $44.1 \pm 2.4 \Omega$ and $44.4 \pm 1.8 \Omega$, respectively. Those of MFC-3 were $9.2 \pm 1.8 \Omega$, $42.8 \pm 1.6 \Omega$, $42.8 \pm 1.6 \Omega$, $42.8 \pm 1.6 \Omega$, respectively. Those of MFC-4 were $2.5 \pm 0.6 \Omega$, $42.2 \pm 1.9 \Omega$, $44.7 \pm 1.7 \Omega$ and $44.8 \pm 2.2 \Omega$, respectively. Those of MFC-4 were $2.5 \pm 0.6 \Omega$, $42.2 \pm 1.9 \Omega$, $44.7 \pm 1.7 \Omega$ and $44.8 \pm 2.2 \Omega$, respectively.

3.4. Cyclic voltammetry test

Anode cyclic voltammetry (CV) experiments were conducted to explore electrocatalytic activities of four reactors. Anode CV was measured in three cycles. Data of the last cycle were used (Figure 5 and Table 2). The value of current production ($I_{production}$) was determined as the difference between the maximum current production (I_{max}) and the minimum current production (I_{min}).

In the 5^{th} , 7^{th} , 9^{th} , 11^{th} , 13^{th} , 15^{th} and 17^{th} weeks, MFC-1 current productions were 11.8 mA, 17.8 mA, 22.0 mA, 14.9 mA, 19.2 mA, 17.9 mA and 24.4 mA, respectively. MFC-2 current productions were 11.1 mA, 14.7 mA, 21.1 mA, 22.0 mA, 25.7 mA, 26.2 mA and 31.9 mA, respectively. MFC-3 current productions were 10.9 mA, 15.6 mA, 23.5 mA, 26.3 mA, 33.5 mA, 31.1 mA and 34.9 mA, respectively; MFC-4 current productions were 14.0 mA, 16.7 mA, 24.2 mA, 23.7 mA, 28.3 mA, 26.6 mA and 31.0 mA, respectively.

With increasing operating time, anode current productions in MFC-2, MFC-3 and MFC-4 also increased. Their cyclic voltammograms showed an S-shape and a current plateau in all measurement time.

However, MFC-1 showed an S-shape and a current plateau in the 5th and 7th weeks. After that, it produced a cyclic voltammogram close to a straight line, resulting in the lowest current production.

4. Discussion

4.1. Analysis of power overshoot

At the early time of anode maturation period, maximum power densities of all MFCs were quite low. An unexpected power overshoot phenomenon was observed in all LSV polarization tests in the 3rd week. This might be because external resistances were too high or because the anode did not have enough time to be mature [24]. Other factors such as poor acclimation of bioanode to the high external resistance [25], high cell internal resistance [26, 27]; and immature bioanode, insufficient organic loading, or insufficient conductivity [28] might have also led to the power overshoot phenomenon. In this study, the power overshoot phenomenon was explored as type D when the power density doubled back to a low current density [25]. According to this previous study, type D power overshoot could be excluded by conducting MFCs with lower external resistance. By using a lower external resistance (i.e., the optimum external resistance in this study), the biofilm displayed higher electrochemical oxidation and improved current production. Using the lower external resistance has been the simplest solution to solve the power overshoot phenomenon. If this method does not help, we could apply other solutions such as cultivate the anode for a longer time because the immature bioanode at that time could lead to this phenomenon [28], or we could supply assistance current to the anode [29].

Based on results of LSV polarization tests in the 3^{rd} week, we changed the external resistance from 1,000- Ω to 100-ohm in MFC-1 and to 50-ohm in MFC-2, MFC-3 and MFC-4. For longer operation time (after 5 weeks), when LSV polarization tests were carried out, the power overshoot situation did not occur again. It indicated that by using the lower external resistance which evidently affected anode biofilm communities and properties, current production increased and electron generation depletion in the 3^{rd} week was eliminated [9].

4.2. Operation cell profile

Actual cell performance is one aspect used to verify three phases of MFCs operation (inoculation phase, developing phase and stable phase). As can be seen from Figure 1, period of 0-1 week was the inoculation phase. After one week, when we used synthetic medium instead of wastewater to have reproducible data, cell voltage showed obvious increases. The duration from when we started to use PBS medium until the time that cell voltage became stable was established as the developing phase. During this phase, although high cell voltage was achieved with PBS medium, high external resistance led to power overshoot and it could adversely affect the anode biofilm. Therefore, the lower external resistance (i.e., the optimum external resistance) was used for the cell as the solution to solve the power overshoot problem. Afterward, cell voltage showed decreases gradually until it reached a stable state from the 9^{th} week, cell performance slightly vacillated (Fig. 1) and stable phase started. On average from the 9^{th} week to the 17^{th} week, actual cell voltages of MFC-1, MFC-2, MFC-3 and MFC-4 were 193 ± 36 mV, 217 ± 29 mV, 209 ± 12 mV and 222 ± 9 mV, respectively.

Figure 6 illustrates practical operation of reactors during one batch in the stable phase. Because of the longer acclimation time with the optimum external resistance than in LSV polarization test, the practical power output MFCs declined gradually. Average data were calculated from the stable stage (~ 10 h) when MFCs operated with the optimum external resistance. MFC-1 showed the lowest performance with an average produced current of 1.8 mA, current density of 2,452 \pm 30 mA/m² and cell voltage of 178 \pm 2 mV. The produced current, current density and cell voltage of MFC-2 were 4.5 \pm 0.1 mA, 6,374 \pm 123 mA/m² and 223 \pm 4 mV, respectively. Those of MFC-3 were 4.2 \pm 0.1 mA, 5,995 \pm 169 mA/m² and 210 \pm 6 mV, respectively. Those of MFC-4 were 4.4 \pm 0.2 mA, 6,346 \pm 250 mA/m² and 222 \pm 9 mV, respectively.

The longer batch but lower power output of MFC-1 could be evidence of a failed inoculation process, which could lead to a lower microbial community and slower substrate consumption than that of other MFCs. There was a sudden drop of power output in the initial time of batch. Based on our lab experience, this phenomenon could be affected by the cathode. During the operation time, a biofilm was created. It attached to cathode and reduced cathode performance. Using a new cathode or removing the biofilm on the cathode surface could solve the drop of the power output.

4.3. Power and current production

MFC systems need certain maturation time to obtain high and stable performances for long-standing operation afterward. LSV polarization test was conducted because it was a simple and reproducible method. By using the same test for all MFC systems with the same operation conditions, performance comparison will become valid and fair [30]. The purpose of LSV polarization test was to: 1) explore the main tendency performance of MFCs through time, 2) establish the stable phase of MFCs, 3) determine the time that MFCs achieve highest performance. All LSV polarization tests were conducted on the same day.

All tested anodes displayed the same operation trend. As shown in figure 4, four MFCs had an unstable maximum power density during maturation time from initial to the 9th week. From the 3rd week to the 9th week, all MFCs except MFC-1 increased promptly in maximum power density, optimum current density and maximum current density. On the contrary, MFC-1 performance declined sharply. From the 9th week, the performances of all MFCs became more stable in general. Performances of all MFCs showed a tendency toward vacillating around average values. These results were consistent with practical cell voltage. Hence, we mainly compared average values of all MFCs from the 9th week to the 17th week depending on the stable phase of these cells. The average performance of MFCs during the stable phase was calculated. Average maximum power density values from the 9th week to the 17th week for MFC-1, MFC-2, MFC-3 and MFC-4 were 837 ± 254 mW/m², 2,982 ± 142 mW/m², 2,368 ± 127 mW/m², 2,990 ± 228 mW/m², respectively. Although there were fluctuations, such changes were negligible. These results indicated that anodes were enriched after mature time so that they could generate stable and reproducible performances subsequently during the stable time. Current values (optimum current density and maximum current density) derived from LSV polarization tests were also evidence for the stable time of anodes. Results generally showed that achieving a stable power production needed at least 9 weeks (Figure 3 and Table 1).

The highest performance of each MFC during the stable time was determined at different time. MFC-1 obtained the highest maximum power density (1,283 mW/m²) in the 17th week, which was 4.4% higher than the initial value in the 3rd week (1,229 mW/m²). MFC-2 obtained the highest maximum power density (3,137 mW/m²) in the 13th week, which was 126% higher than the initial value in the 3rd week (1,229 mW/m²). MFC-3 obtained the highest maximum power density (2,527 mW/m²) in the 9th week, which was 146% higher than the initial value in the 3rd week (1,029 mW/m²). MFC-4 obtained the highest maximum power density (3,289 mW/m²) in the 13th week, which was 158% higher than the initial value in the 3rd week (1,277 mW/m²).

It should be mentioned that in the last week (17th week), the maximum power density of MFC-1 enhanced even higher than its initial one. This change was in good agreement with other reactors. The lack of a significant power output could be explained by a fail inoculation phase of MFC-1 that would lead to the low microbial community on the anode surface. Therefore, the adherent microbial community in the MFC-1 system needed longer time to increase the population and performance. This MFC-1 might achieve better performance in the future. These results were consistent with those of

previous studies while the time for anode performance achieved the highest power density and became stable were different.

4.4. Polarization resistances

Because of the power overshoot problem observed in the 3rd week, there were no electrode potential linear ranges to calculate the polarization resistances. Therefore, polarization resistance were not calculated for the 3rd week.

As shown in Table 1, anode polarization resistance data indicated that anode could affect the full cell performance. Anode polarization resistance seemed to be one explanation for the low power production of MFC-1 and the higher power generation of other MFC reactors. While three-fourths of MFCs with higher power output (MFC-2, MFC-3 and MFC-4) showed that the anode polarization resistance accounted for a minor portion of the full cell internal resistance (3.6 - 21.5 %). MFC-1 with a lower performance showed that anode polarization resistance accounted for a major portion of the full cell internal resistance (46.9 % - 68.8 %). Anode and cathode were connected to each other. Thus, one had a main impact on the other, and vice versa. A high ratio of anode resistance to the full cell resistance of MFC-1 indicated that the anode electrode had a great effect on cathode. This was also clearly shown by polarization curves (Fig. 1-B, 1-D, 1-F, 1-H). In Figure 1-B corresponding to MFC-1, anode polarization curve varied sharply, leading to the variation of cathode polarization. The high anode polarization resistance contributed to a high internal resistance of the full cell and caused a low power production as a result. In contrast, in MFC-2, MFC-3 and MFC-4 reactors, although cathode polarization resistance values were similar to that of MFC-1, they accounted for a major portion of the total internal resistance. In these MFC systems, successful inoculated anode apparently only played a minor role in the overall MFC performance. It was previously evidenced that mature anodes had a small effect on the MFC performance in our test. Previous studies have also indicated that MFC performance is mainly affected by the cathode performance [14]. Hence, making a mature anode is necessary to lower the initial internal resistance.

For MFC-1 case, from the 17^{th} week, results indicated that both cell internal resistance and anode polarization resistance decreased. The cathode polarization resistance gradually became similar with other cathode polarization resistance (45.9 Ω in the 15^{th} week, $41.4~\Omega$ in the 17^{th} week). An intrinsic adaption of microbes could explain such decrease. Since the same MFC construction, electrodes configuration and operation conditions were applied to all MFC, evolution of the microbial community on the anode surface would be one main factor that affected cell internal resistance and anode polarization resistance. In this MFC-1, microbial community required a longer time than in others MFCs to adapt to the environment, resulting in a mature biofilm. Because of the intrinsic heterogeneity of microbes, it will be difficult to predict MFC performance, especially anode. The calculated optimum external resistance of MFC-1 was unstable over time. Remarkably, the optimum external resistance decreased significantly in the 17^{th} week, which was corresponding to a power enhancement. It once more indicated that the microbial community of biofilm in MFC-1 demanded longer time for increment, thus having a strong effect on the anode function.

4.5. Anode cyclic voltammetry test

To observe electrocatalytic activities of MFC systems, anode CV test was performed for reactors. Current production data from CV test enhanced over time and introduced the increasing in anode performance. All reactors in the final week produced the highest current among all periods (Table 2). In the 17th week, current production of MFC-1 was 24.4 mA, which was 106.8% higher than that in the 3rd week (11.8 mA). Current production of MFC-2 in the 17th week was 31.9 mA, which was increased 187.4% from that in the 3rd week (11.1 mA). Current production MFC-3 in the 17th week was 34.9 mA,

which was increased 220.2% from that in the 3rd week (10.9 mA). For MFC-4, current production in the 17th week was 31 mA, which was increased 121.4% from that in the 3rd week (14 mA). Average current production from the 9th week to the 17 week of MFC-1, MFC-2, MFC-3 and MFC-4 were 19.7 mA, 25.4 mA, 29.9 mA and 26.8 mA, respectively, with MFC-2, MFC-3 and MFC-4 showing better anode performance. Our test results indicated that the maturation process had an important effect on anodic current production. An optimum biofilm on electrode surface can promote anode performance and the overall MFC system performance afterward. For instance, MFC-1 with bad biofilm also had lower anodic current production. Measured currents produced by MFC-1 were lower than those produced by other MFCs, consistent with its lower power production and higher full cell internal resistance as mentioned above. The same anode after 17 weeks produced higher current, illustrating an ongoing maturation time. The microorganism community on the anode might become stronger with better efficiency as the time went by.

Although CV anode indicated an enhance in anode performance by time, LSV polarization test showed that all MFCs gradually increased their power output until the highest power was reached (MFC-2 in the 13th week, MFC-3 in the 9th week and MFC-4 in the 13th week) and then decreased slightly afterward. The long-time cathode operation could be the cause for the reduction in cell performance. Therefore, in spite of cathode degradation problem encountered in this study, due to the enhanced anode performance as a reward, we still obtained quite stable cell performance. Results of CV anode tests showed that all anodes at the 17th week kept producing higher current than previous time.

5. Conclusion

In our study, the effect of anode maturation time appeared to be an initial factor for long-term MFC operation afterward. In practice, a brush anode was used to determine how long a single-chamber MFC system could accomplish the maximum and stable performances. One of the worth results was that one of our four anodes might have a failed inoculation process. It meant that the successful inoculation percentage was 75%. An immature anode could lead to a high full cell internal resistance, resulting in a low power production. An ideal anode should have a small effect on a full cell power output while cathode performance has a main impact on MFC performance. Although LSV polarization test implied that after 9 weeks from the initial inoculation, the power generation became stable, CV anode test indicated that anode performance kept increasing after 17 weeks. Due to the correlation between cathode and anode performances, the enhancement of anode performance could compensate the degradation of cathode performance and led to the quite stable overall cell performance. Hence, in our study, an inoculated anode needed more than 17 weeks to attain the fully mature state.

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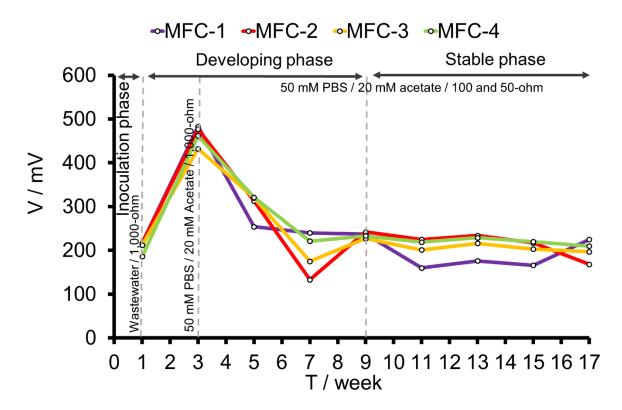


Figure 1. Average voltage of all MFCs operated with different medium and external resistance.

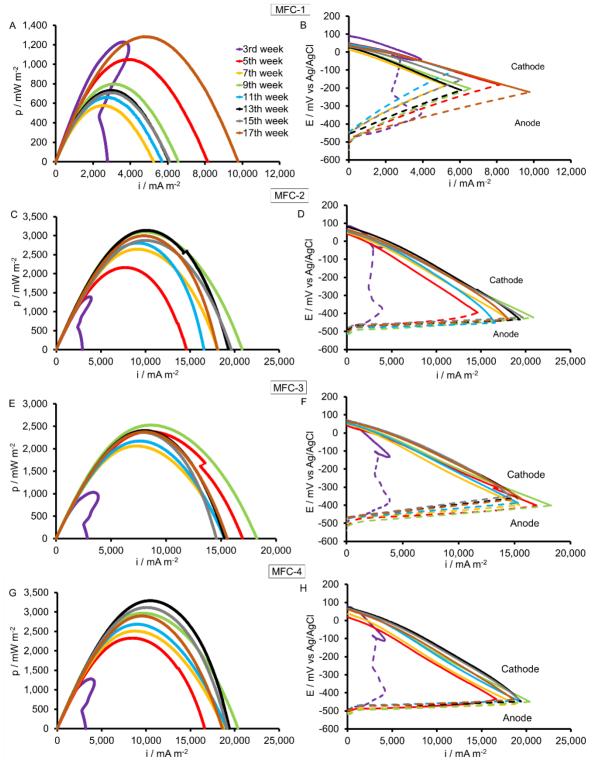


Figure 2. Power density curves and electrodes polarization curves of all MFCs. An LSV polarization test with a scan rate of 1 mV/s was performed at designated time ($3^{\text{rd}} - 17^{\text{th}}$ week)

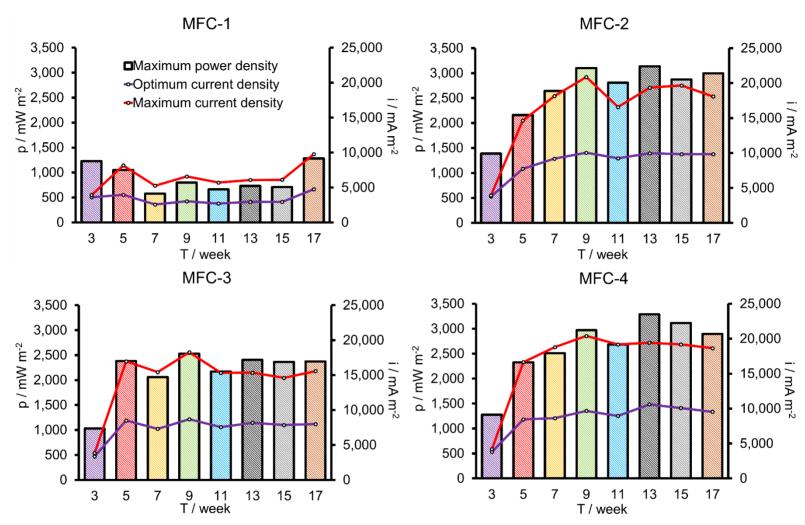


Figure 3. Maximum power density (p_{max}), optimum current density (i_{opt}) and maximum current density (i_{max}) of four MFCs. LSV polarization tests were performed with a scan rate of 1 mV/s.

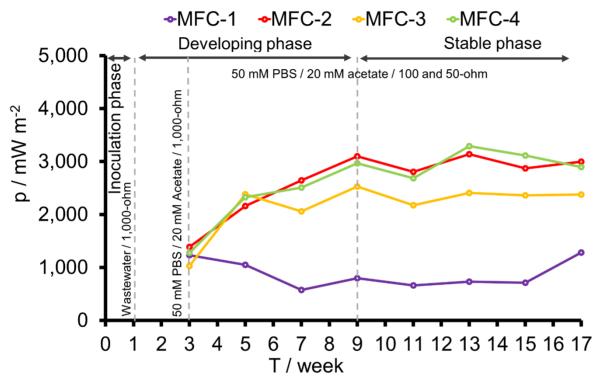


Figure 4. Maximum power density of the four MFCs during the test period.

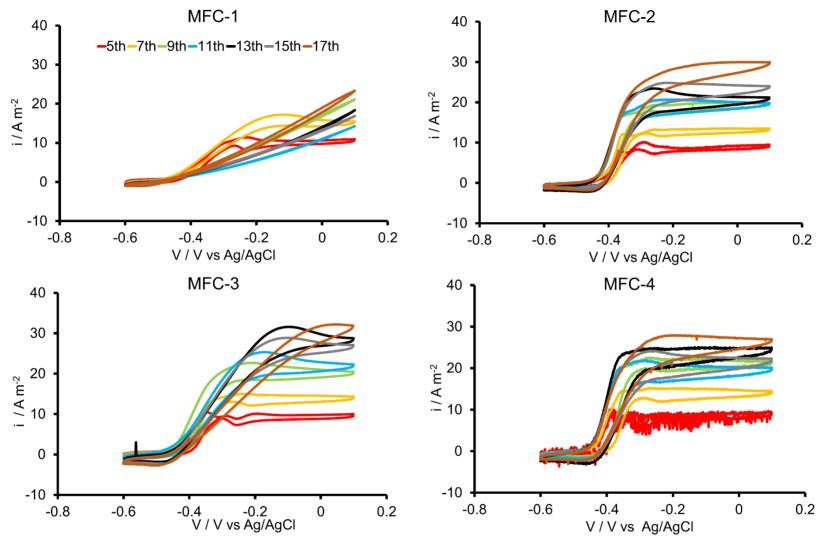


Figure 5. Anodic cyclic voltammograms of the four MFCs. CV tests were performed with a scan rate of 1 mV/s with a scanning range of -0.6 V - 0.1 mV (vs. Ag/AgCl). Arrows illustrate scanning direction of anode CV test.

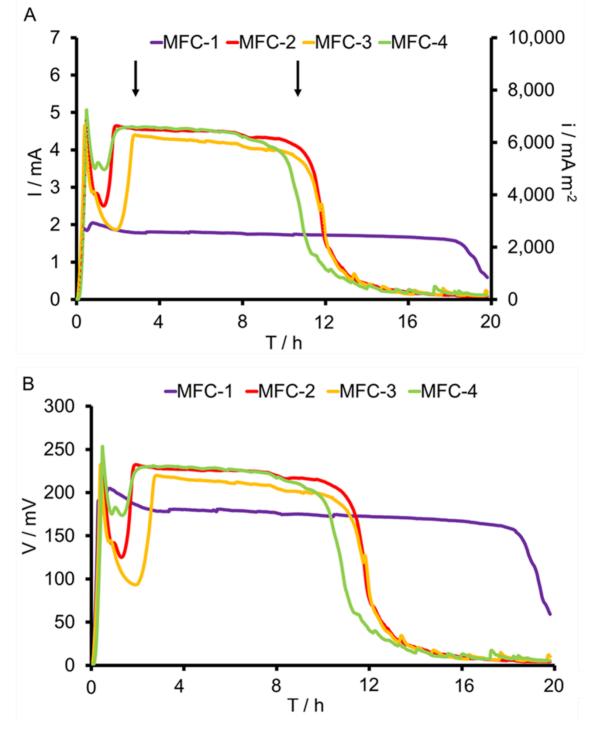


Figure 6. Current (A) and voltage (B) profiles of the four MFCs during a batch operation in the stable phase. Arrows indicate the range that average values are calculated. This batch operation was on the 91^{st} day (12^{th} week) from the initial MFC inoculation.

Table 1. Electrochemical data calculated from polarization data of the four MFCs. Values in brackets illustrate percentages of individual electrode polarization resistance to the total internal resistance.

Time			MI	FC-1			MFC-2							
(week)	<i>p</i> _{max} (mW/m ²)	i _{opt} (mA/m²)	i _{max} (mA/m²)	R _{an} (Ω, %)	R _{cat} (Ω, %)	R _{int} (Ω, %)	R _{opt} (Ω)	p _{max} (mW/m²)	i _{opt} (mA/m²)	i _{max} (mA/m²)	R _{an} (Ω, %)	R _{cat} (Ω, %)	R _{int} (Ω, %)	R _{opt} (Ω)
3	1,229	3,610	3,918	-	-	-	134.8	1,388	3,774	3,945	-	-	-	139.2
5	1,050	3,957	8,158	52.0 (53.7)	44.9	96.9	96.0	2,160	7,733	14,633	5.7	45.7	51.4	52.0
					(46.3)	(100.0)					(11.1)	(88.9)	(100.0)	
7	577	2,559	5,249	70.7	56.9	127.6	126.0	2,642	9,186	18,152	3.0	42.0	45.0	45.0
				(55.4)	(44.6)	(100.0)					(6.7)	(93.3)	(100.0)	
9	798	3,025	6,579	61.9	54.6	116.4	125.0	3,099	10,040	20,869	5.0	39.4	44.4	44.0
				(53.1)	(46.9)	(100.0)					(11.3)	(88.7)	(100.0)	
11	662	2,708	5,707	85.4	38.7	124.1	129.0	2,807	9,239	16,575	2.6	44.9	47.4	47.0
				(68.8)	(31.2)	(100.0)					(5.4)	(94.6)	(100.0)	
13	732	2,946	6,081	58.0	60.1	118.1	120.0	3,137	9,961	19,350	2.6	41.1	43.7	45.0
				(49.1)	(50.9)	(100.0)					(5.9)	(94.1)	(100.0)	
15	710	2,942	6,104	68.6	45.9	114.4	117.0	2,873	9,838	19,667	3.1	37.6	40.7	42.0
				(59.9)	(40.1)	(100.0)					(7.7)	(92.3)	(100.0)	
17	1,283	4,747	9,772	36.6	41.4	78.0	81.0	2,997	9,838	18,086	3.0	41.0	44.0	44.0
				(46.9)	(53.1)	(100.0)					(6.8)	(93.2)	(100.0)	

Time			MI	FC-3			MFC-4							
(week)	<i>p</i> _{max} (mW/m²)	i _{opt} (mA/m²)	i _{max} (mA/m²)	R _{an} (Ω, %)	R _{cat} (Ω, %)	R _{int} (Ω, %)	R _{opt} (Ω)	p _{max} (mW/m²)	i _{opt} (mA/m²)	i _{max} (mA/m²)	R _{an} (Ω, %)	R _{cat} (Ω, %)	Rint (Ω, %)	R _{opt} (Ω)
3	1,029	3,356	3,844	-	-	-	130.4	1,277	3,807	4,188	-	-	-	125.8
5	2,382	8,498	16,967	5.1	41.9	47.0	47.0	2,327	8,446	16,642	3.7	43.0	46.7	47.0
				(10.9)	(89.1)	(100.0)					(8.0)	(92.0)	(100.0)	
7	2,061	7,306	15,431	6.4	47.9	54.3	55.0	2,509	8,622	18,799	2.0	44.4	46.4	48.0
				(11.8)	(88.2)	(100.0)					(4.3)	(95.7)	(100.0)	
9	2,527	8,680	18,271	7.1	41.1	48.3	48.0	2,969	9,671	20,393	3.3	41.7	45.0	45.0
				(14.8)	(85.2)	(100.0)					(7.3)	(92.7)	(100.0)	
11	2,173	7,566	15,325	7.7	45.4	53.1	54.0	2,684	8,944	19,174	1.7	45.3	47.0	48.0
				(14.5)	(85.5)	(100.0)					(3.6)	(96.4)	(100.0)	
13	2,405	8,182	15,342	10.1	42.7	52.9	51.0	3,289	10,591	19,420	2.0	40.7	42.7	42.0
				(19.2)	(80.8)	(100.0)					(4.7)	(95.3)	(100.0)	
15	2,362	7,865	14,603	11.6	42.1	53.7	55.0	3,114	10,084	19,174	2.7	40.7	43.4	44.0
				(21.5)	(78.5)	(100.0)					(6.3)	(93.8)	(100.0)	
17	2,374	7,993	15,567	9.6	42.4	52.0	53.0	2,896	9,547	18,636	2.7	42.4	45.1	45.0
			• •	(18.4)	(81.6)	(100.0)	•				(6.0)	(94.0)	(100.0)	

 p_{max} , maximum power density; i_{opt} , optimum current density; i_{max} , maximum current density; R_{an} , anode polarization resistance; R_{cat} , cathode polarization resistance; R_{int} , internal resistance of the full cell; R_{opt} , optimum external resistance

Table 2. Minimum current production (I_{min}), maximum current production (I_{max}) and current production ($I_{product}$) calculated from anodic cyclic voltammograms. Three CV cycles were performed and data from the last cycle were analyzed and averaged.

Time (recold)	MFC-1 (mA)			MFC-2 (mA)			N	ЛFC-3 (m	A)	MFC-4 (mA)			
Time (week)	I_{min}	I_{max}	$I_{product}$	I_{min}	I_{max}	$I_{product}$	I_{min}	I_{max}	$I_{product}$	I_{min}	Imax	$I_{product}$	
5	-0.4	11.4	11.8	-1.0	10.2	11.1	-0.50	10.4	10.9	-3.7	10.3	14.0	
7	-0.6	17.2	17.8	-1.1	13.5	14.7	-0.6	15.0	15.6	-1.5	15.3	16.7	
9	-0.8	21.1	22.0	-1.2	19.9	21.1	-0.8	22.7	23.5	-1.7	22.6	24.2	
11	-0.7	14.3	14.9	-1.2	20.7	22.0	-1.0	25.3	26.3	-1.8	21.9	23.7	
13	-0.9	18.4	19.2	-2.2	23.5	25.7	-1.8	31.6	33.5	-3.2	25.1	28.3	
15	-1.0	16.9	17.9	-1.3	24.9	26.2	-2.2	28.9	31.1	-2.3	24.2	26.6	
17	-1.1	23.3	24.4	-1.9	30.0	31.9	-2.7	32.2	34.9	-3.1	27.9	31.0	

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