Study on Microbial Community Shifts Over Time in Anode Biofilm and Its Correlation with Performance of Microbial Fuel Cell

Chao Li*, Jiashun Cao

[a]Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Hohai University, Nanjing 210098, China

[b]College of Environment, Hohai University, Nanjing 210098, China

[c]State Key Engineering Research Center of Efficient Utilization of Water Resources and Engineering Safety, Hohai University, Nanjing 210098, China

*lichao0609@163.com

Abstract- **The microbial community of a MFC anode bio-film was studied in continuous period over the whole operation process, with corresponding MFC performance investigated. Results clearly showed that in the 75 days' operation, the microbial community changed. Different enrichment processes (over the whole operation process) of the found exoelectrogenic bacteria (previous reported) were observed, including: enriched gradually, sharply or constantly, which indicated their various extracellular electrons transfer mechanisms. *Shewanella oneidensis* and *Rhodospirillum rubrum* etc. with gradually increasing enrichment on the anode were finally predominant in the anode biofilm. Correlation analysis showed that the anode microbial community (including both biodiversity and abundance of exoelectrogenic bacteria) and MFC performances (including power generation and coulombic efficiency) have certain correlations, which indicated the anode microbial information can predict the MFC performance. Study of the correlation analysis showed that the abundance of exoelectrogenic bacteria took more responsibility for the coulombic efficiency, comparing to the power generation in MFC.**

Keywords- Microbial Fuel Cells, Anode, Coulombic Efficiency, Biofilm, Microbial Community, Exoelectrogenic Bacteria

I. INTRODUCTION

Microbial fuel cell (MFC) is one of the most promising approaches for anaerobic wastewater treatment [1]. It is commonly known that MFCs couple the oxidation of substrates in anode with the reduction of electron acceptors in cathode, thus producing electricity.

MFC is a complex system which involves biological, chemical, electrical and physical processes so that the performance of MFC is determined by multiple factors, such as the anode bio-film condition [2], substrate (fuel) [3], reactor design [1]etc., which makes it hard to control and predict the MFC performance.

Among these factors, the microbial community (usually forming anode bio-film) is the most important factor since it is responsible for transfer of electrons to anode, and other factors probably influence MFC performance by affecting anode bio-film first. And the potential link between anode microbial community structure and MFC function has been revealed previously, which demonstrated that a close association existed between community structure and MFC performance. A study by Stratford et al.[4] on the relationship, showed it to be significant and promising, since it reflect the condition of MFC systems and can effectively predict MFC performance.

On the other hand, the community composition in MFC anode has earned more and more attention [3]. The calculation and analysis in microbial ecology of biodiversity, such as the Shannon index [5], can be used as a prediction of MFC performance. However, the systematic and successive analysis of the microbial community is still scarce, especial for the variation regular study from MFC start-up to stable operation.

The main objectives of this study were: (1) investigate microbial community shifts and variation regular in MFC anode bio-film over time from start-up to stable operation; (2) correlation analysis between the microbial community (including biodiversity and functional microorganism abundance) and MFC performance (including power generation and CE).

II. MATERIAL AND METHOD

A. MFC System Set-up

An H-type MFCs was assembled, consisting of two cylinder transparent polyacrylic plastic bottles (9 cm×4 cm^2), separated by a proton exchange membrane (Nafion 117, Dupond, USA). Both of the anode and cathode material were carbon felt with projected area 2×7 cm^2.

Each compartment was fed with autoclaved medium (pH adjusted to 7.0) containing 0.1 g/L NH_4Cl, 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 0.1g/L $MgSO_4$ $7H_2O$, 0.015 g/L $CaCl_2$ $7H_2O$ [6], and 1mL/L of trace nutrients solution [7]. In addition, sodium acetate with initial COD 1000 mg/L was added to the anodic feed as sources of carbon.

The anode chamber was inoculated by the anaerobic sludge from secondary sedimentation tank at a sewage treatment plant. All experiments were operated in batch mode at room temperature (25 ± 2 ℃). The external resistance was fixed at 800 Ω during the whole experiments.

The operation cycle was about 5 days according to anodic organic depletion. Therefore, ~120 hours was fixed as one cycle, and then the electrolyte of both anode and cathode chambers were changed at the end of each cycle (subjected to voltage drop, every ~5 days).

B. Electrochemical and Chemical Measurements

The cell voltage was recorded by a digital multimeter. The coulombic efficiency (CE) is calculated as:

$$CE(\%) = \frac{M \int_0^t I dt}{FbV \Delta COD} \times 100\%$$

where M represented the molecular weight of substrate; F is Faraday's constant (96,485 C); b represented number of electrons exchanged per mole of substrate utilized; V was the working volume of MFC.

The value of the Shannon index for our anode microbial communities is calculated as:

$$H' = \sum (Pi \times \ln Pi)$$

where Pi is the fractional abundance of the species. All the results above were averaged from (at least) 3 replicates.

C. Microbial Diversity Comparative Analysis by Integrated PCR-DGGE

The anode attached biofilm samples (150 mg, deposit without drying treatment) for MFC were scratched and collected at each end of the cycle (5 days). The total genomic DNA was extracted from each sample by using the FastDNA kit (Q-Biogene, MP Biomedicals, UK) as described in the manufacturers' instructions.

The 16S rRNA gene-targeted denaturing gradient gel electrophoresis (DGGE) was performed for the total eubacteria investigation with the primers 338f (5'-CCTACGGGAGGCAGCAG-3', with GC-clamp before DGGE) and 518r (5'-ATTACCGCGGCTGCTGG-3'), under the following conditions: 94 ℃/5min denaturation step; 30 cycles of 94 ℃/30 s, 58 ℃/30 s, 72 ℃/45 s; and a final extension step at 72 ℃/10 min.

DGGE was carried out in a denaturing gradient gel electrophoresis system for the PCR products. Polyacrylamide gels (10% (w/v)) were 18 cm×18 cm, thickness of 0.75 mm. The electrophoresis was conduct in 1×TAE buffer at 60 ℃. The condition of each electrophoresis was 100 V, 12.5 h with denaturing gradients of 35-65% for the 16S ribosomal RNA genes. Gels were photographed using Kodak 1D Image Analysis Software after stained by ethidium bromide (EB).

D. Correlation Analysis between MFC Performance and Microbial community

Based on the MFC performance and its anode microbial community, the correlation analysis was further analyzed by Pearson correlation coefficient value, which shows whether the two variables are connected.

The Pearson formula is:

$$r_{xy} = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \sum (y - \bar{y})^2}}$$ (1)

where the x, y refer to the data of MFC performance (voltage or CE) or andoe microbial community (biodiversity or abundance).

III. RESULTS AND DISCUSSIONS

A. MFC Start-up and Performance

The MFC performance was investigated during the whole 75 days' operation process (15 cycles) from startup.
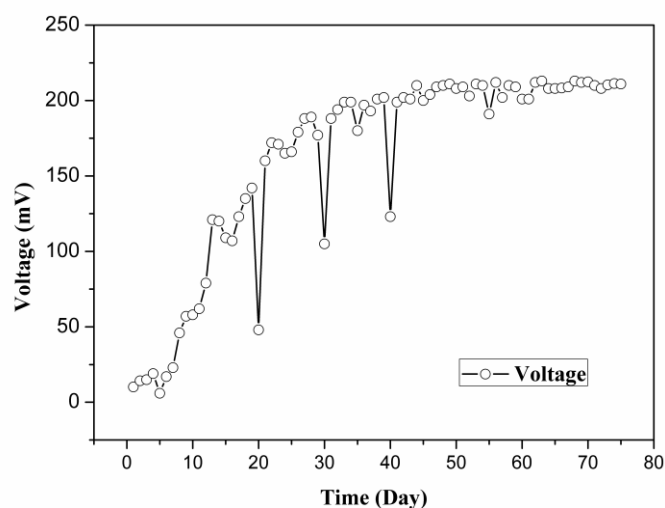
Figure 1 MFC started-up and operated stably in the 75 days' operation. Some sharp drops showed the end of cycle and new feed was added

Figure 1 displayed the whole operation of the MFC. (The sharp decrease in voltage showed when the feed was exhausted and new replaced). After almost the initial 35 days (the 7th cycle), the MFC finished the start-up period and reach the 200 mV (approximate the peak voltage 213 mV). The next 8 cycles keep stable operation at the level of 210 mV, with corresponding power density (versus anode project area) 39.4 m W/m^2. Table 1 showed the MFC performances for all the successive 15 cycle.

TABLE 1 POWER OUTPUT OF EACH CYCLE OF MFC

Cycle No.	Max Voltage (mV)*	Average Voltage (mV)	Max Power Density (mW/m^2)
1	19	12.9±5.0	0.32
2	58	40.2±19.1	3.0
3	121	98.2±26.4	13.1
4	142	111±37.6	18.0
5	172	166.8±4.9	26.4
6	189	167.6±35.4	31.9
7	200	192±8.1	35.7
8	202	183.2±33.8	36.4
9	210	202.4±4.4	39.4
10	211	208.4±2.7	39.8
11	211	204.8±8.3	39.8
12	210	206.8±5.0	39.4
13	213	208.4±4.7	40.5
14	213	210.9±2.1	40.5
15	211	210.1±1.3	39.8

* The external resistance was fixed at 800 Ω during the whole experiments

The average voltage of each cycle during the whole operation was used for correlation analysis, see section 3.

Further, the CE of each cycle was investigated to show the utilization efficiency of the substrate (sodium acetate) in the MFC anode (Figure 2).
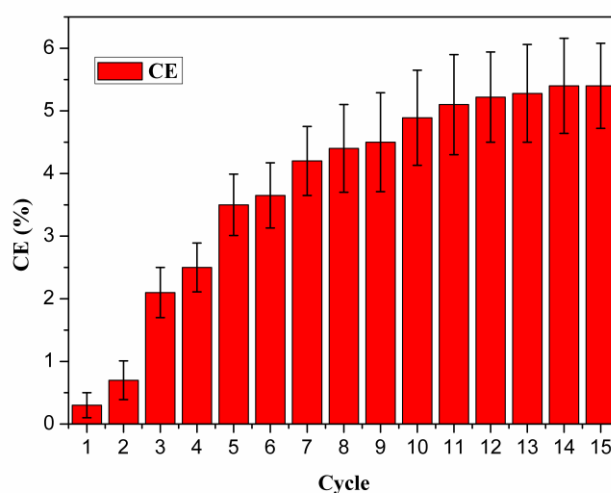
- 425 -

Figure 2 The CE investigation of each cycle during the whole operation

The CEs of MFC usually show a very low values since very little of organics are inclined to be transformed to electric energy [8]. In this study the CE is 0.3% at the beginning, and kept increasing in each cycles until the 14th -15th cycles, reaching the highest 5.4%.

It was noticed that even if the power generation and CE increased gradually (Figure 1 and 2), they were not synchronized. The power generation (voltage) reached the plateau more rapidly (the 35th day, i.e. the 7th cycle), while the CE kept increasing until the 14th cycle, which was an indication that the power generation and CE are not dependent on the same microbial communities in the anode.

Dense selection of samples were conducted to analysis the microbial community shifts, this was done to reveal the relationship between MFC performance (include power output and CE) and shifts in the anode microbial community during the whole study.

B. Analysis of Microbial Community Shifts of the Anode Biofilm

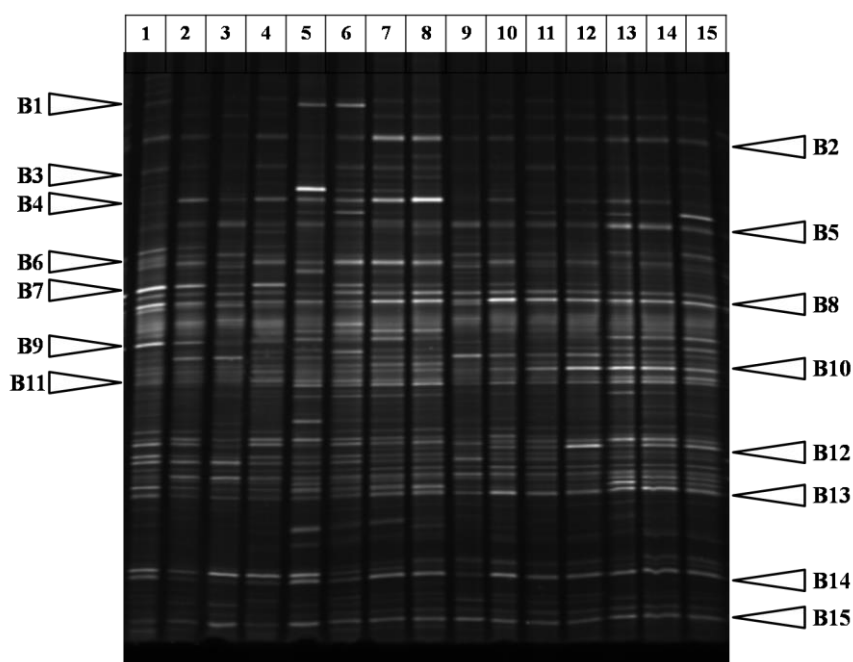PCR-DGGE was used for microbial community analysis; this was done after each operated cycle.

Figure 3 The DGGE fingerprints of the 15 anode biofilm samples (corresponding to the continuous 15 cycles)

According to Figure 3, the large number of detectable bands indicates that our anode biofilm has considerable biodiversity. 15 representative bands were selected for cut and sequencing.

It can also be observed that the microbial community changed a lot from the 1st cycle to 15th and the changes that accoutred in the microbial community (DGGE fingerprints) were very complex.

Some of the bands became more intense, gradually (from a general view), such as B5, B8, B10, B13 (Figure 3), which meant these microorganisms were enriched both during the MFC startup and operation. Some bands appear weaker and weaker over time, such as B7, this indicated that this bacterium was eliminated in the anode. Some other bands changed little, such as B15, or first showed a rise and then a drop, some showed no regular variations, which reflect the irregular shifts in the anode microbial community. These fingerprint variation results showed many similarities to previous experiments by the authors (data not shown), which demonstrated its repeatability.

The Shannon index which evaluated the biodiversity of each anode (for the 15 cycles) was analysed (Figure 4) according to the DGGE fingerprints.
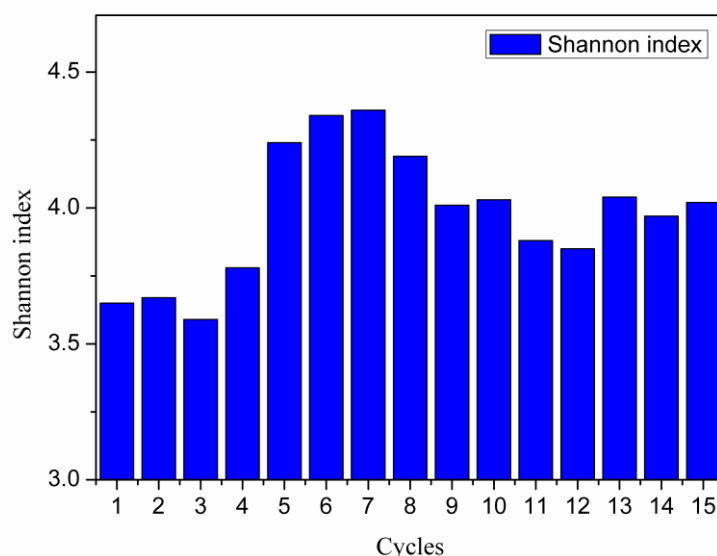

Figure 4 Shannon index comparisons for each cycle during the whole operation process, according to DGGE results

The Shannon index became higher and then showed a bit drop during the whole operation process (in the general view, increased), which indicated more kinds of bacteria enriched over time, with a more mature bacterial community structure. At the 5th -7th cycle there was a rapid increasing compare to the former cycles.

In order to identify these microorganisms and their classification, some representative bands were cut, sequenced and blasted.

TABLE 2 CLOSEST SPECIES AND CLASSIFICATION OF EACH REPRESENTATIVE BAND IN DGGE PROFILE FOR 16S RRNA GENES

No.	Closest species	Similarity*	Classification
B1	*Methylobacterium extorquens* CM4	96%	*Alphaproteobacteria*
B2	*Anabaena cylindrica PCC* 7122	95%	*Cyanobacteria*
B3	*Dehalogenimonas lykanthroporepellens* BL-DC-9	98%	*Chloroflexi*
B4	*Candidatus Desulforudis audaxviator* MP104C	98%	*Firmicutes*
B5	*Rhodoferax ferrireducens* T118	99%	*Betaproteobacteria*
B6	*Clostridium acidurici* 9a	95%	*Firmicutes*
B7	*Flavobacteriaceae bacterium* 3519-10	95%	*Bacteroidetes*
B8	*Shewanella oneidensis* MR-1	98%	*Gammaproteobacteria*
B9	*Methylocella silvestris* BL2	97%	*Alphaproteobacteria*
B10	*Rhodobacter capsulatus* SB 1003	97%	*Alphaproteobacteria*
B11	*Prevotella ruminicola* 23	96%	*Bacteroidetes*
B12	*Bradyrhizobium oligotrophicum* S58	95%	*Alphaproteobacteria*
B13	*Rhodospirillum rubrum* F11	97%	*Alphaproteobacteria*
B14	*Sulfuricella denitrificans* skB26	97%	*Betaproteobacteria*
B15	*Ochrobactrum anthropi* ATCC 49188	96%	*Alphaproteobacteria*

* The similarity was ≥ 95% for all the listed closest species

According to table 2, *proteobacteria, Firmicutes* and *Bacteroidetes* were the dominated communities in our anode biofilm.

The closest species of B1 was *Methylobacterium extorquens* who can perform C1 substrates oxidation, such as methanol dehydrogenase and methylamine [9]. A similar specie is B9 (*Methylocella silvestris*), who was identified as a facultative methanotroph. These bacteria were gradually eliminated under the bioelectrochemical selective condition (Figure 3).

B3 and B4 showed the closest phylogenetic relationship to *Dehalogenimonas lykanthroporepellens* and *Candidatus Desulforudis audaxviator,* who performed dehalogenation and desulfurization metabolic [10,11]. And B14 (*Sulfuricella denitrificans*) was identified as a sulfur-oxidizing autotroph [12]. The band intensity of these kinds of microorganisms displayed at first a rise and then a drop, or irregular variation. This indicated that the potential of special pollutants removal of the anode inoculated sludge gradually reduced during the operation (screening).

B6 and B7 were the representative bands which became faint as the anode operated. They were identified as *Clostridium acidurici* and *Flavobacteriaceae bacterium,* who was found in considerable abundance in MFC anode systems in previous studies [13], even though little reports testified their electrochemical activity.

The bacteria described above were not directly involved in power generation, so most of them tended to be eliminated over time in anode.

B5 (*Rhodoferax ferrireducens*) has been reported as a biocatalyst for MFC startup [14]. B8 (*Shewanella oneidensis* MR-1) was one of the most familiar exoelectrogenic bacteria [15]. Although lactate is the favoured carbon source for the *S. oneidensis,* it has been confirmed to also use acetate with a similar biomass yield as lactate [16]. B10 (*Rhodobacter capsulatus*), a metabolically versatile purple bacteria, was a novel electrochemically active and Fe(III)-reducing bacterium. It has been confirmed by Pham et al., [17] to be feasible for applicability in biofuel cells.

Besides, B13 (*Rhodospirillum rubrum*) and B18 (*Ochrobactrum anthropi*) who were also dominating the microbial communities in our anode biofilm, there were also bioelectrochemical microorganisms according to previous studies [18,19], enriching in the anode bioflim.

The five exoelectrogenic bacteria stated above, who have been confirmed in their bioelectrochemical activities before, in theory, should gradually over time be enriched in our anode system. In order to investigate the enrichment and screening situation of these bacteria, the abundance shifts of them, according to the bands intensity in DGGE, are shown in Figure 5.
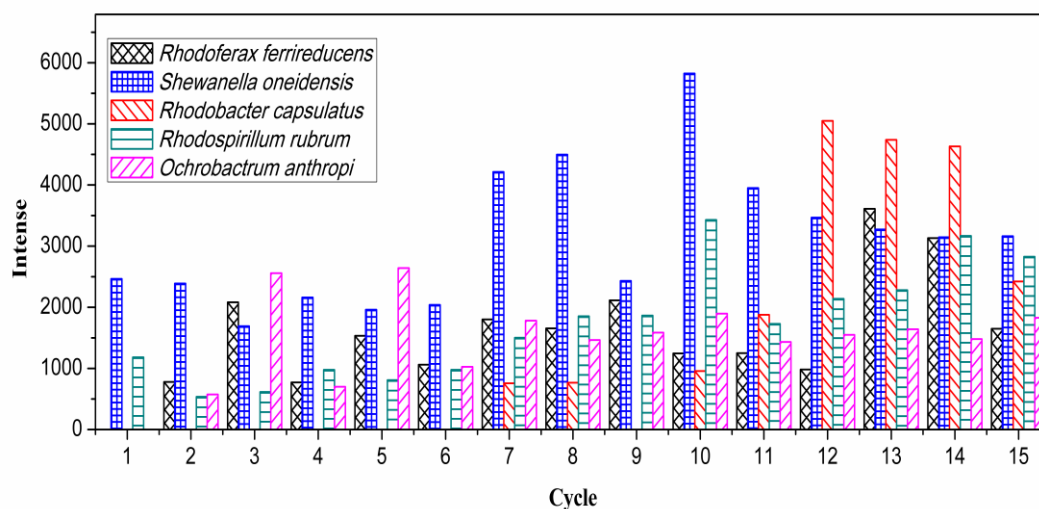

Figure 5 The intensity variation of the identified bioelectrochemical microorganisms (had been definitely reported before) during the whole 15 cycles, according to the DGGE fingerprints

According to the band intensity (corresponding to their approximate abundance) variation in the 5 kinds exoelectrogenic bacteria (Figure 5), different enrichment circumstances were observed, include: enriched gradually, sharply or constantly (all in the general view).

B5 (*Rhodoferax ferrireducens*), B8 (*Shewanella oneidensis* MR-1) and B13 (*Rhodospirillum rubrum*) showed, in general, a gradual increase over the 75 day operation. The abundance of B10 (*Rhodobacter capsulatus*) increased sharply at the 8th cycle. However, B18 (*Ochrobactrum anthropi*) presented a very different enrichment situation from the other 4 exoelectrogenic bacteria, and from the 3rd cycle was (almost) invariable.

The differences were probably due to their different extracellular electrons transfer mechanisms. Some of the exoelectrogenic bacteria, such as B8 (*Shewanella oneidensis* MR-1), rely on their conductive bacterial nanowires produced by

themselves [15]. Others, like B18 (*Ochrobactrum anthropi*), though has been shown to be capable of power generation in MFCs, they do not respire using solid metal iron oxides [15], which indicated *Ochrobactrum anthropi* performed power generation without the requirement of growing and screening on MFC anodes. This explained its invariable enrichment in our study.

C. Relationship between the Performance and Anode Microorganisms

The microorganisms in anode biofilm played the key role in MFC performance, and the relationship between them needs further discussion. In this study, during the whole 15 operation cycles (75 days), the Pearson correlation coefficient (analyzed of the first 8 stable cykles) was first used to investigate the correlation of the average voltage output (Table 1), CE (Figure 2), and biodiversity characterized by Shannon index (Figure 4) (with each other).

Pearson correlation coefficient of average voltage and CE (short for P (voltage, CE)) is 0.98 ($p < 0.01$), which meant they are significant correlated. Obviously, the CE is positively correlated with voltage according to its calculation formula.

The $r_{(voltage, biodiversity)} = 0.63$, showed a moderate correlation between voltage (power generation) and anode biodiversity, while in view of the first 7 cycles (startup period, when voltage keep increasing), the $r_{1-7\ (voltage, biodiversity)} = 0.88$ ($p < 0.01$, significant correlated). It demonstrated that with the voltage rise, the biodiversity became higher, or in other words, the increasing diverse anode microorganisms enable the power output grow of MFC. However, during the enrichment of the microbial community on anode, the power generation can be saturated rapidly, while the microorganism communities would still keep evolving (kept changing from the 8th cycle, Figure 3 and 4).

The $r_{(CE, biodiversity)} = 0.54$, also showed moderate correlated relationship between the CE and biodiversity of anode biofilm.

As discussed above, B5, B8, B10, B13 and B15 were identified as the typical bioelectrochemical microorganisms or had been proved to be able to drive of bioelectrochemical process, which were selected particularly for our further correlation analysis. The total abundance of these bioelectrochemical microorganisms was calculated based on DGGE analysis result (Figure 6).
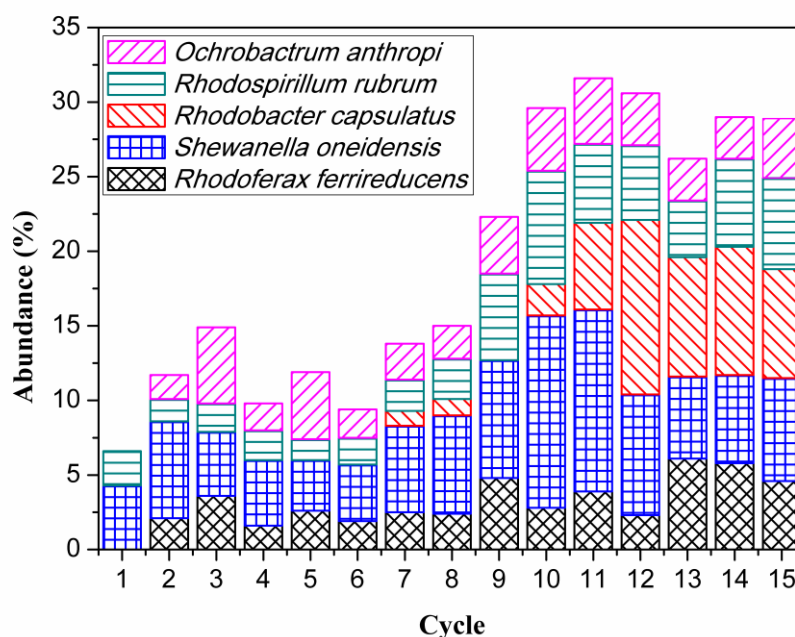

Figure 6 The abundance variation of the identified bioelectrochemical microorganisms (had been definitely reported before) during the 15 cycles

Figure 6 showed that the total abundance of these bioelectrochemical microorganisms kept increasing over the whole operation periods, from the beginning 6.6% to about 30% at final, and the highest is 31.6%. It was noticed that the abundance as mentioned referred to the ratio of (exoelectrogenic bacteria/total eubacteria), the eukarya was not included. The abundance of bioelectrochemical microorganisms was used for further correlation analysis, instead of Shannon index.

The $r_{(voltage, total\ abundance\ of\ bioelectrochemical\ microorganisms)} = 0.73$, the value was higher than $r_{(voltage, biodiversity)}$, 0.63, but they were still non-significant correlated, which meant the bioelectrochemical microbial community determined the power generation, rather than the whole microbial community.

The $r_{(CE, \text{ total abundance of exoelectrogenic bacteria})} = 0.81$ ($p < 0.01$), demonstrating significant correlated between the two. As previous study (Stratford et al., 2014), correlation existed between microbial consortia diversity and electrical output. Further, according to our results, the abundance of bioelectrochemical microbial community was more responsible for the CE than voltage, since these exoelectrogenic bacteria preferred to transfer organics to electrical energy effectively, rather than other anaerobic metabolism.

When comparing the two kinds of performances of MFC (power generation and CE), the power generation was affected by multiple conditions in anode besides microorganisms, such as the anode material and superficial area [20], while the CE was only determined by anode microorganisms metabolism, which was responsible for the higher CE correlation but lower voltage correlation.

The studies of the statistics and correlation between the anode microbial community and MFC performance are very significant and promising during the MFC operation process, including the start-up period, since it not only reflects the situation of the reactor running, but also predict the MFC's working tendency and performance [4] in the next operation period.

In addition，as the technology of modern molecular biology and high throughput sequencing developed, DGGE is not the most effective method to analysis the microbial community. However, it can easily evaluate approximate microbial community shifts during the whole MFC operation, and is very applicative for mass of samples analysis, like the experiment in this study, since the DGGE was visualized and it is also much more economical.

CONCLUSION

The microbial community of MFC anode biofilm obviously shift over time during the whole 75 days' operation. Different enrichment was found for exoelectrogenic bacteria, including: enriched gradually, sharply or constantly, which indicated their various extracellular electrons transfer mechanisms. *Shewanella oneidensis* and *Rhodospirillum rubrum* etc. who showed increasing enrichment on anode gradually over the operation process and were finally predominant in anode biofilm. Correlation existed between MFC performance (including power generation and CE) and anode microbial community (including both biodiversity and abundance of exoelectrogenic bacteria) during the operation period. Further, based on their correlation analysis, the abundance of exoelectrogenic bacteria was more responsible for the CE, comparing to the power generation in MFC.

ACKNOWLEDGMENT

REFERENCES

[1]   B. E. Logan, P. Aelterman, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, et al. "Microbial fuel cells: methodology and technology," *Environmental Science & Technology*, vol. 40, pp. 5181-5192, Sep. 2006.

[2]   Y. Liu, F. Harnisch, K. Fricke, R. Sietmann, U. Schruer, "Improvement of the anodic bioelectrocatalytic activity of mixed culture biofilms by a simple consecutive electrochemical selection procedure," *Biosensors & Bioelectronics*, vol. 24, pp. 1012-1017, Dec. 2008.

[3]   K. J. Chae, M. J. Choi, J. W. Lee, K. Y. Kim, I. S. Kim, "Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells," *Bioresource Technology*, vol. 100, pp. 3518-3525, Jul. 2009.

[4]   J. P. Stratford, N.J. Beecroft, R.C. Slade, A. Grüning, C. Avignone-Rossa, "Anodic microbial community diversity as a predictor of the power output of microbial fuel cells," *Bioresource Technology*, vol. 156, pp. 84-91, Mar. 2014.

[5]   A. D. Steen, K. Ziervogel, C. Arnosti, "Comparison of multivariate microbial datasets with the Shannon index: an example using enzyme activity from diverse marine environments," *Org. Geochem*, vol. 41, pp. 1019-1021, Sep. 2010.

[6]   B. Virdis, S. Read, K. Rabaey, "Biofilm stratification during simultaneous nitrification and denitrification (SND) at a biocathode," *Bioresource. Technology*, vol. 102, pp. 334-341, Jan. 2011.

[7]   H. Lu, A. Oehmen, B. Virdis, J. Keller, Z. Yuan, "Obtaining highly enriched cultures of *Candidatus Accumulibacter phosphates* through alternating carbon sources.," *Water Research*, vol. 40, pp. 3838-3848, Dec. 2006.

[8]   Y. Z. Fan, H. Q. Hu, H. Liu, "Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration," *Journal of Power. Sources*, vol. 171, pp. 348-354, Sep. 2007.

[9]   L. Chistoserdova, S. W. Chen, A. Lapidus, ME. Lidstrom, "Methylotrophy in Methylobacterium extorquens AM1 from a Genomic Point of View, " *Journal of Bacteriology*, vol. 185, pp. 2980-2987, May. 2003.

[10] W. M. Moe, J. Yan, M. F. Nobre, M. S. Costa, F. A. Rainey, "Dehalogenimonas lykanthroporepellens gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater, " *Internatiional Journal of Systematic Evolution Microbiology*, vol. 59, pp. 2692-2697. Jul. 2009.

[11] D. Chivian, E.L. Brodie, E.J. Alm, D.E. Culley, P.S. Dehal, T.Z. DeSantis, "Environmental Genomics Reveals a Single-Species Ecosystem Deep Within Earth," *Science*, vol. 322, pp. 275-278, Oct. 2008.

[12] H. Kojima and M. Fukui, "Sulfuricella denitrificans gen. nov., sp. nov., a sulfur-oxidizing autotroph isolated from a freshwater lake," *International Journal of Systematic Evoluton Microbiology*, vol. 60, pp. 2862-2866, Dec. 2010.

[13] I. Vandecandelaerea, O. Nercessianb, M. Faimalic, E. Segaerta, A. Mollicac, W. Achouakb, et al. "Bacterial diversity of the cultivable fraction of a marine electroactive biofilm," *Bioelectrochemistry*, vol. 78, pp. 62-66, Apr. 2010.

[14] Z. D. Liu, Z. W. Du, J. Lian, X. Y. Zhu, S. H. Li, H. R. Li, "Improving energy accumulation of microbial fuel cells by metabolism regulation using Rhodoferax ferrireducens as biocatalyst," *Letter pf Applied Microbiology*, vol. 44, pp. 393-398, Apr. 2007.

[15] Y. A.Gorby, S. Yanina, J. S. McLean, K. M. Rosso, D. Moyles, A. Dohnalkova, "Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms," *PNAS*, vol. 103, pp. 11358-11363, Jul. 2006.

[16] Y. J. Tang, A. L. Meadows, J.D. Keasling, "A kinetic model describing *Shewanella oneidensis* MR-1 growth, substrate consumption, and product secretion," *Biotechnology & Bioengineering*, vol. 96, pp. 125-133, Jan. 2007.

[17] C. A. Pham, S.J. Jung, N.T. Phung, J.Y. Lee, I.S. Chang, B.H. Kim, et al. "A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell," *FEMS Microbiology Letters*, vol. 223, pp.129-134, Jun. 2003.

[18] Z. He and L. T. Angenent, "Application of Bacterial Biocathodes in Microbial Fuel Cells," *Electroanal*, vol. 18, pp. 2009-2015, Oct. 2006.

[19] B. E. Logan, "Exoelectrogenic bacteria that power microbial fuel cells," *Nature Review of Microbiology*, vol. 7, pp. 375-381, May. 2009.

[20] C. Li, L. B. Zhang, L. L. Ding, H. Q. Ren, H. Cui, "Effect of conductive polymers coated anode on the performance of microbial fuel cells (MFCs) and its biodiversity analysis," *Biosensors & Bioelectronics*, vol. 26, pp. 4169-4176, Jun. 2011.