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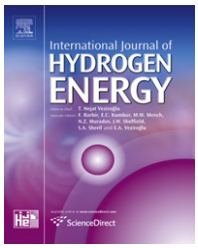

Key factors affecting microbial anode potential in a microbial electrolysis cell for H_2 production

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ARTICLE INFO

Article history:
Received 20 November 2009
Accepted 22 November 2009
Available online 6 September 2010

Keywords:
Microbial anode potential (MAP)
Microbial electrolysis cell (MEC)
Bio-hydrogen
Biofilm

ABSTRACT

In order to optimize operations of microbial electrolysis cell (MEC) for hydrogen production, microbial anode potential (MAP) was analyzed as a function of factors in biofilm anode system, including pH, substrate and applied voltage. The results in "H" shape reactor showed that MAP reflected the information when any factor became limiting for hydrogen production. Commonly, hydrogen generation started around anode potential of −250 mV to −300 mV. While, higher current density and higher hydrogen rate were obtained when MAP went down to −400 mV or even lower in this study. Biofilm anode could work normally between pH 6.5 and 7.0, while the lowest anode potential appeared around 6.8–7.0. However, when pH was lower 6.0 or substrate concentration was less than 50 mg L^{-1} in anode chamber, MAP went up to −300 mV or above, leading to hydrogen reduction. Applied voltage did not affect MAP much during the process of hydrogen production. Anode potential analysis also showed that planktonic bacteria in suspended solution presented positive effects on biofilm anode system and they contributed to enhance electron transfer by reducing internal resistance and lowering minimum voltage needed for hydrogen production to some extent.

1. Introduction

Microbial electrolysis cell (MEC), a device developed from microbial fuel cell (MFC) technology [1], employs exoelectrogenic microbes [2] and small external voltage to generate hydrogen from various biodegradable substrates [3,4]. Although both of them are considered to be work in the same principle as a promising way for bioenergy, MEC system is different to MFC owing to two important conditions. Firstly, the applied voltage is needed in MEC to obtain energy in form of hydrogen, which should affect the formation and evolvement of the microbial anode system, though few studies explain clearly on the effect of applied voltages on microbial communities till now. Secondly, MEC system is a completely anaerobic environment but MFC is a semi-anaerobic system using air-cathode because of oxygen diffusion from cathode area. Hereby, MEC shows some great advantages over MFC on higher efficiency and wider substrates conversion. Over 85% of energy efficiency and 95% of hydrogen recovery from acetate have been reported recently [8,9].

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doi:10.1016/j.ijhydene.2009.11.125

Biofilm anode is the most important part involving electron transfer from exoelectrogens to the circuit. Functional bacteria together with anode form biofilm anode [10,43], and it is the key for improving the performance of MEC system. To well-understand reactor operational factors that control reactor performance, microbial anode potential (MAP) is chosen as a parameter that reflects the effects of operation factors on the system, including pH, substrate concentration, applied voltages and planktonic bacteria as well. MAP has been known in microbial redox reactions from electron generation for energy requirement [11]. The difference between electron donators and acceptors determines the energy obtain according to equation of $\Delta G = -n \times F \times \Delta E$, where $\Delta E = E'_{donor} - E'_{acceptor}$ [12]. Therefore, MAP could be adopted as an indicator that shows the performance of biofilm anode. Some attentions have been paid to anode potential of MFC [5–7], however, there are few reports on microbial electrolysis cell to the authors' best knowledge. More hydrogen could be obtained by optimizing the operation factors that reduce energy transport.

Recently the studies of MFC have been reported on operation factors, such as different pH, temperature, ion strength [14], substrates [8] and different microbial systems [11,15,34]. However, they are intricate and complicated conditions to be handled perfectly for reactor control. A lower anode potential naturally indicates more energy for microbes when the potential is near to NADH reaction potential. According to the principle, it is hypothetically reported that energy potential is based on NADH reaction with E' potential of −320 mV (−520 mV vs. Ag/AgCl reference electrode) under standard condition at pH 7. Actually, E' potential is close related to the ambient factors, and it will become high if oxidants exist around anode, then energy is less for community growth eventually because of lost electrons in narrow energy potential. Lately people have tried to study anode system with fixed anode potential by potentiostat-poised in MFC [6]. By choosing three fixed anode potentials, the research indicated that an optimal anode potential was the lowest potential (−200 mV vs. Ag/AgCl), regulating the activity and growth of bacteria to sustain an enhanced current and power generation [5]. Anode potentials are naturally varying in different situations if there is no external control, however, there exists the lowest point at some optimal conditions for a reactor. Anode potential typically reached −400 to −480 mV (vs. Ag/AgCl) with acetate as organic electron donor in membrane separated MFC [13] and it is considered that higher current density at higher voltage would not affect anodic bacteria in MFC [37] as a result of slight anode potential change with applied voltages. Comparatively, anode potential decreased from −467 mV to 53 mV while cathode potential maintained relative constant when applied voltage ranged from 0 to 1.0 V in two-chamber MEC system [38]. However, anode potential ranged from −470 mV to −350 mV at different applied voltages in single chamber MEC [9]. Although no final answer is got on the relationship of anode potential and reactor performance, it makes sense that a lower anode potential during the reactor operation usually signifies a good energy harvest [5].

Using an MEC system to treat artificial wastewater with low carbon source and weak buffer capacity, the study was carried out to discuss the effects of pH, acetate consumption, applied voltage and suspended solution on anode potential for hydrogen production. MAP was analyzed as a function of operation conditions. Based on potential analysis, optimization of operations would be done for urban wastewater treatment in MEC process.

2. Materials and methods

2.1. Reactor and material

"H" shape reactors were constructed as previously reported [16], separated in two chambers by a proton exchange membrane (12 cm^2, Nafion 117, DuPont, USA). Anode was a plain carbon cloth (64 cm^2, without wet proofing; E-Tek) and cathode was a carbon paper with 0.35 mg cm^{-2} Pt (9 cm^2, E-Tek). Running volume of anode chamber is about 485 mL and electrode distance was 14 cm. Graphite rod (diameter 2 mm) was used to connect electrode and outside circuit. Both anode and cathode chambers were equipped with an Ag/AgCl reference electrode (+0.2 V vs. NHE) to measure the electrode potentials. A power source supplies a steady voltage (0.1–1.0 V) between anode and cathode electrode.

2.2. Startup and measurement

Sewage sludge to inoculate the reactor was collected from the Wenchang wastewater treatment plant for municipal sewage in Harbin, China. The sludge was kept in anaerobic condition for 48 h before mixed with anode medium (50/50, v/v) to inoculate MEC reactor. The reactor was operated at a fixed voltage of 0.6 V immediately for startup after inoculation. Anode medium was prepared in low buffer capacity close to actual wastewater (per 1000 mL): KCl 0.2 g; NH_4Cl 0.4 g; $NaH_2PO_4 \cdot 2H_2O$ 0.6 g; $NaC_2H_3O_2$ 1.0 g; NaCl 2.0 g; Wolfe's vitamin solution 10 mL; Wolfe's mineral solution 10 mL, pH 7.0. Anode medium was not disinfected except for special mention. The cathode medium was autoclaved phosphate buffer solution (10 mM PBS, pH 7.0). pH was adjusted by a series of NaOH solution and monitored by pH meter (pHS-25, Shanghai Precision & Scientific Instrument Co., Ltd., China).

The applied voltage in the study was all supplied by a simple direct current power supply (PS-B202D, Yizhan Electronic Instrument Co., Ltd). Current was measured by multimeters in the circuit for the efficiency calculation. The acetate concentration of anode samples was measured using an ionic chromatograph (5 μL of each sample, Dionex 4500i, USA). The hydrogen volume was determined by draining method and analysis of gas produced with a gas chromatographer (GC122, China) with nitrogen as the carrier gas. All the experiments were done at room temperature of 25°C.

2.3. Experiments

After reactor startup was finished with the repeatable hydrogen production, experiments were going on analysis of pH and substrate under a fixed applied voltage of 0.6 V. To keep suspended bacteria, magnetic stirrer was turned off for 30 min, and then 80% supernatant solution in anode chamber was replaced by fresh anode medium at the end of each cycle.

Cathode solution was completely replaced with fresh PBS. Reactors were sparged with high purity nitrogen (99.998%) for 20 min to removal oxygen in the solution and headspace.

To study the pH effect on biofilm anode system, pH adjustment was done in a batch operation from a low pH around 6.0 in anode chamber. The adjustments were operated evenly with NaOH (0.25 M) step by step till pH 7.2. During the process, anode potential changed as a function of pH in anode chamber. In order to control the abrupt conductivity change, the same dose of NaOH was added into anode chamber each time to lest anode potential jump caused by conductivity related to ion strength change.

A series of applied voltages changed from 0.2 V to 1.0 V stepwise for analysis of external voltage effect in new cycles. Each applied voltage was kept at least 20 min before the data recorded. The same operations were repeated again two times to make sure the stable trend was got. Here to compare the effect of bacteria in suspended solution on anode potential, the suspended solution was removed completely with fresh medium and the same operations were done with applied voltage change.

Community samples were taken from suspended solution and biofilm in Reactors R1 and R2. Reactor R1 was kept working normally for hydrogen production in batch cycle operations around neutral pH in anode chamber; Reactor R2 was incapability of hydrogen production because of acidification (pH $<$ 5.0) for one week. The communities were analyzed using the single-strand conformation polymorphism (SSCP) technology as described before [16]. In order to decrease undesirable nonspecific banding and improve accuracy in SSCP profile, λ-exonuclease (NEB, MA USA) was used to remove the marked DNA chain with 5′ end. Polyacrylamide gel was made using MDE Gel Solution (Cambrex Bio science) mixed with 10 μL enzymatic hydrolysate and 2.7 μL buffer (10 mmol L^{-1} NaOH, 20 mmol L^{-1} EDTA, 0.02% bromophenol blue and 0.02% xylene cyanol FF, 95% deionized formamide). Denaturation at 95°C for 5 min, move the sample in the ice for 10 min and then put the samples at 300 V for 17 h. The obtained gels were silver-stained according to Bassam reported [17] and then got SSCP profile scanned by UMAX Powerlook 2000.

3. Results and discussions

3.1. Hydrogen production as a function of MAP

In MEC system with a fixed voltage of 0.6 V, MAP behaved regular changes in batch cycles. Hydrogen production was recorded as the function of MAP in a typical cycle (Fig. 1) and pH in anode chamber was kept between 6.8 and 7.0 matching that of influent wastewater during this study. Relationship of hydrogen and anode potential was shown in Fig. 1, indicating that MAP was detected from −60 mV to −350 mV (vs. Ag/AgCl) before hydrogen was collected quantitatively in the batch cycle. Hydrogen volume began to increase when MAP decreased to about −360 mV. During the process of continuous hydrogen generation, MAP only concentrated in narrow scope from −360 mV to −440 mV. The highest current density was obtained around anode potential −400 mV, and MAP

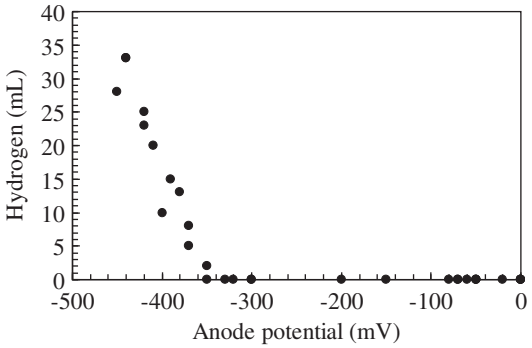

Fig. 1 – Hydrogen production as the function of MAP in a typical batch cycle.

presented the best state of biofilm anode of electron transport for hydrogen production. When it came to the end of the cycle, hydrogen generation rate dropped and anode potential increased in the meanwhile till up to over zero when current was below 0.5 mA.

The lowest anode potential in the study was still far from −520 mV (vs. Ag/AgCl) according to electron transferring reaction in theory [11]. Results hinted that higher hydrogen production needed a lower anode potential for more energy obtained by optimizing operational factors or improving reactor configuration. The following study proved MAP as the comprehensive parameter involving primary condition information in the operations. The factors would affect anode potential and some reports indicated the space for the lowest actual anode potential close to theoretical value under optimal operations [13].

3.2. MAP performance with anodic pH

Now that artificial wastewater was a system with low buffer solution, pH in anode chamber decreased from 7.0 to 6.3 during hydrogen production cycles (Fig. 2). The reason for pH drop was speculated in two-chamber reactors because of inevitable proton accumulation caused by the low transfer efficiency of protons, especially through proton exchange membrane [18,36,38]. MAP went up gradually during the process of pH reduction in anode chamber while pH in cathode chamber was changed slightly in 10 mM PBS. The

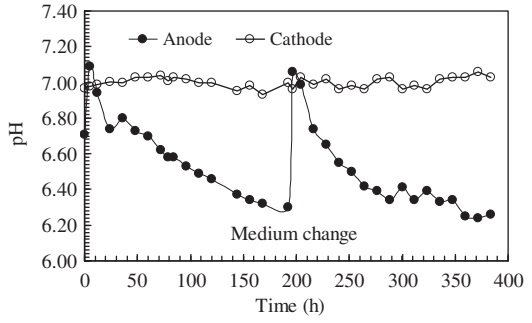

Fig. 2 – pH changes in treating artificial wastewater of two-chamber MEC reactor.

average anode potential was around −400 mV (vs. Ag/AgCl) in the pH range from 6.8 and 7.0 (Fig. 3) in which bacteria were capable to fast produce electrons with more energy generation. Acidification (below pH 6.0) led to obvious drop of anode potential, resulting in low efficiency. During the process that pH was adjusted back to neutral level, anode potential dropped a lot from −250 mV to −350 mV between pH 6.0 and 6.2 (Fig. 4). And current jumped from 0.9 mA to 2.0 mA at the same time. Excluding the interval of 6.0–6.2, anode potential changed slightly and smoothly, indicating that pH adjustment is not the reason for the abrupt change of anode potential or current. And conductivity was not interfered sharply in the process of adjustment by NaOH solution in Fig. 4.

It makes sense that the optimal pH is closely related to the growing conditions of dominant communities. Scope of 6.8–7.0 corresponded with the reasonable pH for most bacteria of *Shewanella* sp. [19–22] and *Pseudomonas* sp. [23–26] as main functional microorganisms [15] in MFC reactor. The community structure was established in this feasible pH range when MEC was inoculated with activated sludge [16]. As a result of proton transfer problem in a membrane reactor, it is inevitable to result in proton accumulation around anode and then low pH and the low system efficiency further [27–29]. It is considered that unsuitable pH inhibited microorganisms' metabolic activities. The increasing anode potential displayed with electron transport reduction from bacteria to anode. Anode potential went up to −300 mV when pH dropped to 6.0, meaning energy reduction of 77 kJ/mol according to $\Delta G = -n \times F \times \Delta E$ (8 electrons per mol acetate) compared with the potential −400 mV during the process of electron transport from exoelectrogens to anode. So energy flow was lower under the low pH suppress in bioelectrochemical system.

During the experiment of the impact of extreme low pH to biofilm anode, pH in anode chamber was reduced to 3.5 from 6.7 with peak current around 1.0 mA under applied voltage 0.6 V. The result showed that anode potential jumped from −370 mV to −70 mV and current dropped to 0.1 mA in step. Then this positive state kept all through the following 24 h. Although anodic pH was adjusted back to neutral and kept for another 24 h, the reactor failed recovering to normal level on current or hydrogen production. Anode potential was still as high as −60 mV and current was still around 0.1 mA, indicating that extreme low pH of 24 h made ruined damage to biofilm anode irreversibly. In contrast, extreme high pH around 12 was also caused anode potential raise and low

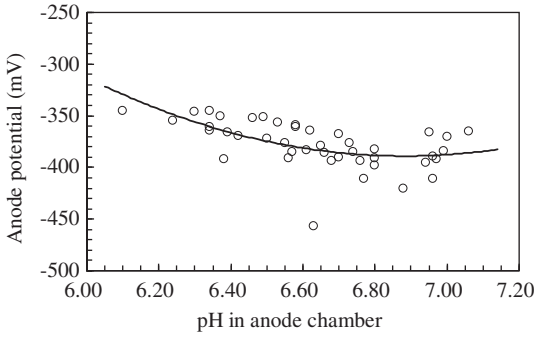

Fig. 3 – MAP as a function of pH in MEC reactors.

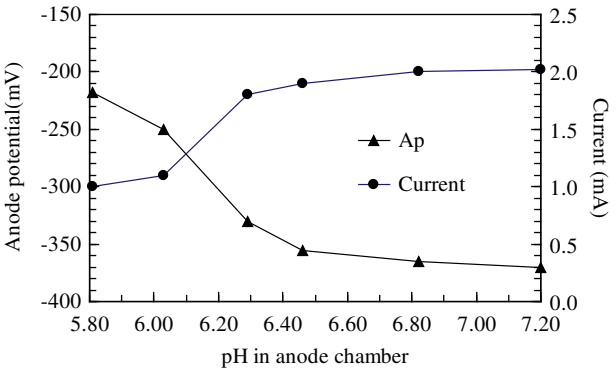

Fig. 4 – pH adjustment in anode chamber.

efficiency. However, the system recovered again for hydrogen production when pH was kept neutral pH back for a period.

3.3. *MAP performance with substrate concentration*

According to the results of pH above, these experiments were finished between the pH 6.5 and 7.0 to avoid pH impacts. Acetate was added into anode chamber when current dropped below 0.5 mA under a fixed voltage 0.6 V. Anode potential immediately went down to around −410 mV. Simultaneously current went up from 0.4 mA to 1.4 mA. Current peak value was over 0.8 mA if there was sufficient acetate in the solution and the peak increased at higher acetate concentration in the range of 100 mg L^{-1}– 600 mg L^{-1} in the study. Anode potential changed slightly from −410 to −400 mV when acetate concentration went down from 600 mg L^{-1} to 150 mg L^{-1} (Fig. 5). But anode potential presented a noticeable jump from −400 mV to −300 mV when acetate was consumed as low as 50 mg L^{-1}. Current dropped sharply at the meanwhile, showing that low substrate contributed to decreases of electron transport which made MAP increasing as a result of reducing hydrogen production rate.

Furthermore, bulk of biofilm fell off anode surface when the system was kept in starvation over one month. Anode potential always maintained positive and no hydrogen generated during the starvation. However, the system was still recoverable for hydrogen production gradually when substrate was fed again. In this way, MAP is much related to

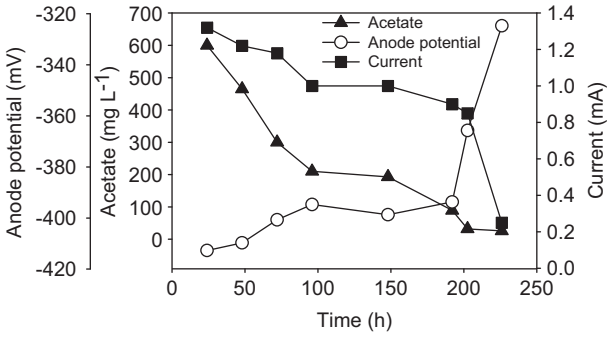

Fig. 5 – Anode potential (vs. Ag/AgCl) and current change with acetate consumption.

the activities of functional microorganisms totally rather than their existence with ability of electron transport. Till now there are few reports on extreme low concentration organic wastewater treatment in MFC and MEC. However, the low substrate situation often happened in actual water treatment process, e.g. oligotrophic wastewater. It was interesting to be concerned as an impact to their functions in bio-electrochemical system in future.

3.4. *Effect of applied voltages on MAP with and without planktonic bacteria*

To evaluate the performance of electrode potential for hydrogen production on different applied voltages, firstly the bioelectrochemical system was recorded by increasing applied voltages stepwise from 0.2 V to 1.0 V after adding acetate for a new batch cycle without discharging suspended solution. Anode potentials (B) went up a little from −410 mV to −399 mV (Fig. 6), while cathode potentials (B) went down significantly from −599 mV to −784 mV during the experiment. The results showed that MAP was not changed much with voltages for hydrogen production during the study, while cathode potentials were affected much responding to applied voltages in MEC. However, the potential difference between two electrodes was increasing in step with applied voltage, and simultaneously current was increasing in MEC system. During the secondly comparing experiment, fresh medium was used to replace suspended solution completely from anode chamber. The results indicated that situation was similar on the trend of electrode potential changes as illustrated above. However, the removal of planktonic bacteria led to a general raise of both anode and cathode potential (A) in comparison to that with planktonic solution (B). Both anode and cathode potentials increased by 30 mV – 40 mV synchronously.

Although there are few conclusions on the essential effect of applied voltages on bacteria or biofilm structure, electrode potentials changed as the responds to different applied voltages. The common results are shown that anode potential went up (less negative) and hydrogen production rate increased with higher applied voltage in the process [9,38]. However, their performances were specific on other factors

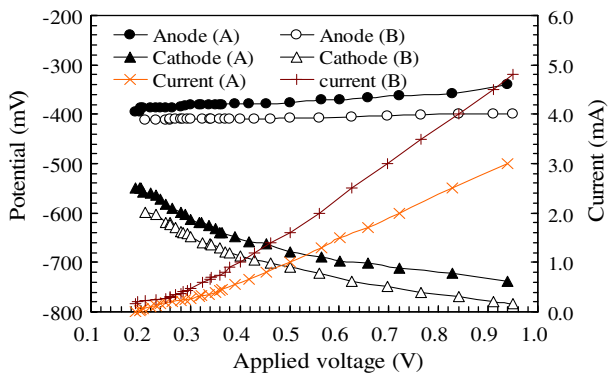

Fig. 6 – Electrode potentials (vs. Ag/AgCl) as a function of applied voltage.

and reactor conditions. In this study, MAP showed a slightly positive trend but cathode potential changed accordingly to external voltages. There was a distinct potential loss between the difference of two electrode potentials and applied voltages. The loss was greater when applied voltage was higher. One of the reasons may be that the higher current led to more voltage loss on the internal resistance of the reactor system and simple DC power supply used in this study. The voltage distribution would be more exact in the circuit using a potentiostat [38]. Moreover, the system may be reacted in some resistance when it shortly responded to continuous increase of applied voltages within a batch cycle. Anode potential increased more to be positive in a higher conductivity solution while the change was gent from −470 mV to −370 mV when applied voltage increased from 0.2 V to 0.6 V in series of cycles and a sharp increase appeared if applied voltage increased further [9].

In Fig. 6, the current and potentials illustrated system resistance change with and without suspended solution, indicating that planktonic bacteria played a part in electron transfer in the system. The current increased higher in the system with suspended solution according to calculation of the slope of current vs. voltage. The current curve slopes changed great at the points around 0.25 V (B) and 0.30 V (A) respectively, showing that planktonic bacteria contributed to lower system resistance around biofilm anode for electron transfer. Moreover, the inflexion voltages showed that planktonic bacteria also reduced the minimum voltage for hydrogen production in MEC, as another part of contributions for electron transfer. However, their contributions did not make a significant part to power generation. Biofilm determined the main performance of the system and anode potential display, which also shown in recent studies on biofilm and suspended solution in MFC [30–32]. Furthermore, it is pointed out that planktonic bacteria and biofilm had close interaction [33,35].

3.5. *Community analysis*

Now that microorganisms in suspended solution contributed to electron transport and then anode potential, the operation factors and conditions made direct effects on community construction and their performance that was reflected by anode potential. According to the community analysis by SSCP (Fig. 7), MAP ranged from −350 to −400 mV normally for hydrogen production. The biofilm was considered to be the significant habitation for functional microbes, while communities were quite similar between suspended solution and biofilm on the dominant bacteria (lane 1 and lane 2). *Pseudomonas* sp. (E3) and *Shewanella* sp. (E9) were detected both in the communities of solution and biofilm in Reactor R1, and they were acknowledged as functional bacteria with exoelectrogenic ability in recent studies. *Stenotrophomonas* sp. (E1) and *Flavobacterium* (E5) were only detected significantly in biofilm communities (lane 2). *Flavobacterium* was found in bioelectrochemical system, which was related to phosphorus accumulating process [39,40] and *Stenotrophomonas* sp. was mentioned in some special substrate degradation MFC reactor recently [41,42]. Besides these microorganisms, some species were also dominant in the communities but not reported to

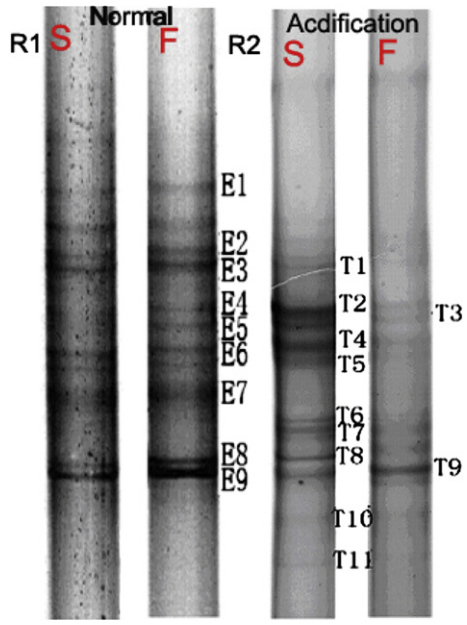

Fig. 7 – SSCP profile of acidification process. S: suspended solution. F: biofilm on the anode.

electron transport, for example, *Agrobacterium* sp., which was reported to be related with denitration or nitrogen fixation, but there was little mention related to electron transfer in MFC or MEC. In this normal situation, exoelectrogenic bacteria, combined to other communities around anode, acted in the main function of bioelectrochemical ability with anode potentials below −300 mV when the system worked in this study.

The anode potential was more positive above 0 mV (vs. Ag/AgCl reference) in Reactor R2. There was no hydrogen production any more because of long-time acidification. *Parabacteroides* sp. (Bands T7, T10) were the rare microorganisms dominantly but they were not found in bioelectrochemical system in recent reports. Most common functional microorganisms were out of the system. The predominant communities were involved in acid production, such as, *Lactococcus* (Bands T1–T3) with ability of spore generation, *Trichococcus* (Bands T4–T6, T9). In comparison to Reactor R1, microorganisms in R2 were much more abundant in suspended solution. The biofilm was thin and scattered on the anode surface in the acidification condition. Few exoelectrogens performed much positive anode potential, indicating that the biofilm was out of work using substrate for extracellular electron generation. Anode potential reflected the status of the system involving all factors that influenced anode biofilm in some way, and the exoelectrogens are important members rather than all microorganisms that contributed to regular anode potential in the bioelectrochemical system.

4. Conclusions

The study demonstrated that MAP reflected important factors that maintained MEC system working optimally during hydrogen production process. MAP analysis indicated that planktonic bacteria played a part of electron transfer in anode system, contributing to reduction of internal resistance and less minimum voltage needed for hydrogen production to some extent. Biofilm anode potential was affected by operation conditions including pH, substrate and applied voltage, especially when any of factors became limiting to electron transport adequately. The lowest anode potential appeared around 6.8–7.0. Acidic environment below 6.0 in anode chamber led to a high anode potential. Substrate became a limiting factor with more positive anode potential when acetate concentration was reduced blow 50 mg L^{-1} in the study. Hydrogen production was normally obtained with higher current density and higher hydrogen rate when anode potential displayed between −360 mV and −440 mV, while MAP of −300 mV above led to low efficiency and low hydrogen production rate.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (NSFC No. 50678049 and No. 50878062), and by the Program for New Century Excellent Talents in University (NCET-2005).

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