Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.23089

129 (2018) 185–193
October

Aerobic granular sludge formation and COD removal in a continuous-flow microbial fuel cell

Ping Yang, Wei Liao, Hui-qiang Li*

College of Architecture and Environment, Sichuan University, Chengdu 610065, China, Tel. +8618980668020; email: lhq_scu@163.com (H.-Q. Li), Tel. +8618602804508; email: yping63@163.com (P. Yang), Tel. +8618200288011; email: lwei0314@163.com (W. Liao)

Received 13 December 2017; Accepted 8 September 2018

A B S T R A C T

A continuous-flow microbial fuel cell (cfMFC) system with bio-cathode chamber (BCC) was designed to improve organic pollutant removal performance, along with electricity production. Aerobic granular sludge (AGS) formation was observed by accident in the BCC of the cfMFC system. Process and mechanism of the AGS formation were stated, and the effect of influent chemical oxygen demand (COD) concentration, hydraulic retention time, cathode aeration rate on organic pollutant removal in the cfMFC was studied. According to the sludge morphology, AGS formation process could be divided into six stages with a total duration of around 80 d. Mechanism of the AGS formation was explained from macroscopic and microscopic point of views. Moreover, COD removal efficiency was always higher than 90% in the cfMFC system after the AGS formation when influent COD concentration was increased from 3,000 to 7,540 mg/L. Maximum output voltage of 91 mV was obtained in the AGS-based cfMFC system.

Keywords: Continuous-flow microbial fuel cell; Bio-cathode; Aerobic granular sludge; Organic pollutant removal; Electricity production

1. Introduction

Microbial fuel cell (MFC) is a relatively novel wastewater treatment technology, which can convert chemical energy contained in the wastewater into electrical energy economically with the metabolism of electricigens. The MFC process has characteristics of wide available substance, high conversion rate, mild operating condition and zero secondary pollution [1]. Configuration of the MFC is usually divided into two types, including single-chamber MFC and dual-chamber MFC, and dual-chamber MFC is used more commonly [2]. Organic pollutants are mostly removed in anode chamber (AC) of dual-chamber MFC under anaerobic condition. However, anaerobic process' organic pollutant removal is not thorough while has no ammonia removal performance. Therefore, traditional MFC is difficult to meet discharge requirement for wastewater treatment.

Our research transformed cathode chamber (CC) in a traditional MFC into a bio-cathode chamber (BCC) aiming at improving organic pollutant removal. Surprisingly, the formation of aerobic granular sludge (AGS) was observed in the BCC of the continuous-flow microbial fuel cell (cfMFC) system after successful start-up of the system. The AGS has great potential in the management of sewage and high concentration organic wastewater treatment, because of good settling properties, abundant microbial population, high sludge concentration and strong shock load-resistant ability [3]. Recently, AGS research was usually in a lab scale, and applications of the AGS in full-scale utilization are rarely reported [4]. Meanwhile, the sequencing batch reactor or its deformation technology was used mostly for the AGS cultivation, and cylindrical reactor with high aspect ratio (the range of H/D from 5 to 15) was adopted [5,6]. However, cylindrical sequencing batch reactor (SBR) process with high aspect ratio is not conducive to be enlarged in practical engineering, and higher aspect ratio will induce greater challenge for aeration equipment, both of which impeded wide application of the AGS process. Therefore, to achieve relative stable AGS

* Corresponding author.

formation in a continuous operation reactor with low aspect ratio is a major strategy to solve the problems.

Six hypotheses about formation mechanism of granular sludge have been proposed mainly, which are microorganisms self-flocculating hypothesis [7], nuclei hypothesis [8], selection pressure driving hypothesis [9], extracellular polymeric hypothesis [10], cell surface hydrophobicity hypothesis [11] and phase formation hypothesis [12]. The quite different formation mechanism of the AGS proposed was due to the variation of reactor type, operating condition and study angle. For example, some scholars proposed the AGS formation hypothesis from microcosmic point of view, such as microbial population structure, physical and chemical characteristics of sludge morphology. Some scholars observed floc sludge granulation process by changing operating parameters of the reactor, proposing the AGS formation mechanism from macro perspective. The hypotheses proposed ahead had their rationality and limitations, but the AGS formation mechanism in a continuous flow reactor has been reported rarely.

In this research, the AGS was observed by accident in the BCC of the cfMFC system. The continuous flow MFC reactor with AGS combined the high efficient anaerobic fluidized bed, AGS and biological cathode. It not only optimized wastewater treatment, but also produced electricity. More importantly, configuration of the BCC adopted in the experiments was cubic which was more common in aerobic wastewater treatment process, and the ratio of height to length was just 1.6 which will be more conducive for application of the AGS in practical engineering. The AGS was found in MFC CC for the first time which provides a new direction for AGS's cultivation technology.

The study focused on studying formation process and characteristic of the AGS in the low aspect ratio BCC-based cfMFC system, and formation mechanism of the AGS in the system was discussed as well. In addition, main advantage of the MFC is to achieve organic pollutant removal and electricity production simultaneously for wastewater treatment. Therefore, the other focus of this study was to investigate organic pollutant removal and electricity production performance in a newly set up cfMFC system after the AGS formation in the BCC, which presented a new research direction for the combination application of the AGS and MFC process.

2. Materials and methods

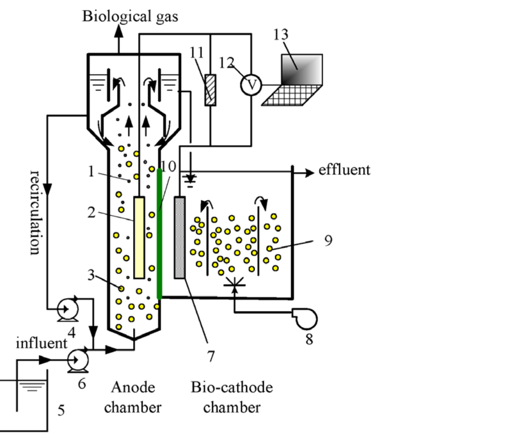
2.1. Wastewater

Synthetic wastewater (glucose as carbon source, NH_4Cl as nitrogen source and K_2HPO_4 as phosphorus source) was used as feed for the cfMFC system, and pH value of the influent wastewater was controlled from 7.8 to 8.2 using sodium bicarbonate. In order to prevent acidification in the anode anaerobic reactor, influent pH should be slightly increased. According to report, the electricity production efficiency of MFC is higher when influent pH is weak alkaline [13]. According to the experimental requirements, influent chemical oxygen demand (COD) concentration was adjusted by controlling the amount of glucose in the synthetic wastewater. In addition, trace elements were added to maintain microbial growth, and detailed components were shown as follow: Zn^{2+}(0.42 mg/L), Cu^{2+}(0.17mg/L),Ni^{2+}(0.21mg/L),Co^{2+}(0.28mg/L),B^{3+}(0.014mg/L), Mn^{2+}(0.85 mg/L), Mo^{6+}(0.20 mg/L), Fe^{2+} (5.0 mg/L). $CaCl_2$ and $MgCl_2$ were added into the synthetic wastewater directly at the concentrations of 75 and 50 mg/L, respectively.

2.2. Set up and start-up of the cfMFC system

The experiments were carried out in dual-chamber bio-reactors which were made of plexiglass. In dual-chamber bio-reactors, the cfMFC system was formed. AC and CC were connected by proton-exchange membrane (DuPont membrane Nafion 117, DuPont company, USA), and schematic diagram of the cfMFC is shown in Fig. 1. AC of the cfMFC system was formed by two parts, one was reaction zone with internal diameter of 100 mm and volume of 5.9 L, and the other was settling zone with internal diameter of 160 mm and volume of 4.2 L. Top of the AC was sealed and installed a three-phase separator. The synthetic wastewater was fed at the bottom of the AC by peristaltic pump, and effluent of the AC flowed into the BCC by gravity. The BCC was a 200 mm (length) ×15 mm (width) × 320 mm (height) open container with total working volume of 9.6 L. AC and BCC were filled with porous polymer packing self-developed by our laboratory for microbe enrichment [14], and filling ratio were 15% and 10%, respectively. Two electrodes in the AC and BCC were connected to a resistor by copper wire, and outer resistance was fixed at 100 Ω during the experiment. In addition, variation of voltage data was recorded automatically by computer.

Inoculum of the AC was obtained from AC of a dual-chamber cfMFC with abiotic cathode as reported by Huang et al. [15]. Influent COD concentration increased from 1,000 to 3,000 mg/L gradually during start-up period, and reflux ratio of improved from 5:1 to 20:1. The AC exhibited relative good organic pollutant removal performance after cultivation for more than 1 month, and gas production was stable. Reflux ratio of the AC in the cfMFC system was controlled at 20:1 after the start-up period. The pH value was controlled at 6.8–7.5 using $NaHCO_3$, and the temperature was controlled at 30°C–35°C. Inoculated sludge of the BCC was gained from aeration tank of a municipal wastewater treatment plant in Chengdu. The BCC of the cfMFC system started up together with the AC. That is, effluent of the AC flowed into the BCC directly after addition of porous polymer packing and 4 L of



1-Biogas 2-Anode 3-Biocarrier 4-Recirculation pump 5-Influent 6-Influent pump 7-Cathode 8-Fan 9-Cathode bio-granular 10-Proton exchange membrane 11-External resistance 12-Voltmeter 13-Computer

Fig. 1. Schematic diagram of the cfMFC system.

activated sludge mixed liquor. The pH value in the BCC was not controlled in the experiment, and the temperature was controlled at 25°C ± 2°C.

2.3. Analytical methods

During the experiment, samples were taken from influent and effluent of the AC and BCC every other day and were analyzed immediately after being filtered through 0.45 μm filter paper. COD, settling velocity, sludge volume index (SVI), mixed liquor suspended solids (MLSS) were analyzed in accordance with Monitoring and Analytic Methods of Water and Wastewater [16]. The pH value was measured by pHS-2F pH meter. Morphology of the AGS was observed by scanning electron microscope (SEM) and Olympus BX51 electron microscope. Load voltage of the cfMFC system was measured by UT70B professional multimeter electronic, and output voltage values were recorded automatically by computer with acquisition interval of 180 s.

3. Results and discussion

3.1. The AGS formation

Generally, oxygen is used as electron acceptor in the CC of conventional MFC, and organic matter is biodegraded by anaerobic microbes in the AC releasing electrons and protons. Electrons were transferred to the CC through external circuit to form a current, and the protons transfer into the CC through the proton-exchange membrane (PEM) forming water with restored oxygen. Oxygen supplied in the CC is achieved by aeration mainly. In the BCC-based MFC system, aeration can stir activated sludge and provide oxygen for microbial activity simultaneously. Floc sludge in the BCC adapted to operation condition of the reactor after start-up period, and MLSS and SVI were around 2 g/L and 150 mL/g. The AGS formation in the BCC underwent more than 80 d, which can be divided into six periods by sludge morphology, as shown in Fig. 2. Division of the AGS formation process would be benefit for understanding formation mechanism of the AGS. In addition, during the AGS formation period, influent COD concentration of the AC was controlled around 3,000 mg/L, and effluent COD concentration of the AC varied from 1,700 to 1,800 mg/L flowing into the BCC directly. Hydraulic retention time (HRT) of the system was 37.2 h with the AC of 19.1 h.

Brown floc sludge in the BCC was observed after start-up period, with loose and irregular shape, and corresponding MLSS concentration and SVI were 1.65 g/L and 167 mL/g. According to previous reports, initial MLSS concentration varied greatly in order to cultivate the AGS, and the range was between 2 and 9 g/L mostly [17–19]. The MLSS concentration in the BCC was at a relative low level comparing with the abovementioned values. Indicating high initial MLSS concentration is not a prerequisite for the AGS formation in the cfMFC system. Floc sludge shape changed significantly after the system ran for around 60 d. Some floc sludge was aggregated together and a small amount of white granular sludge core emerged in the BCC. MLSS concentration in the BCC was raised gradually, which was at the range of 2–3 g/L, and corresponding SVI was 70–80 mL/g. In this period, multiplication rate of microbes in the BCC accelerated after accommodation, which promoted the secretion of the extracellular polymeric substances (EPSs).

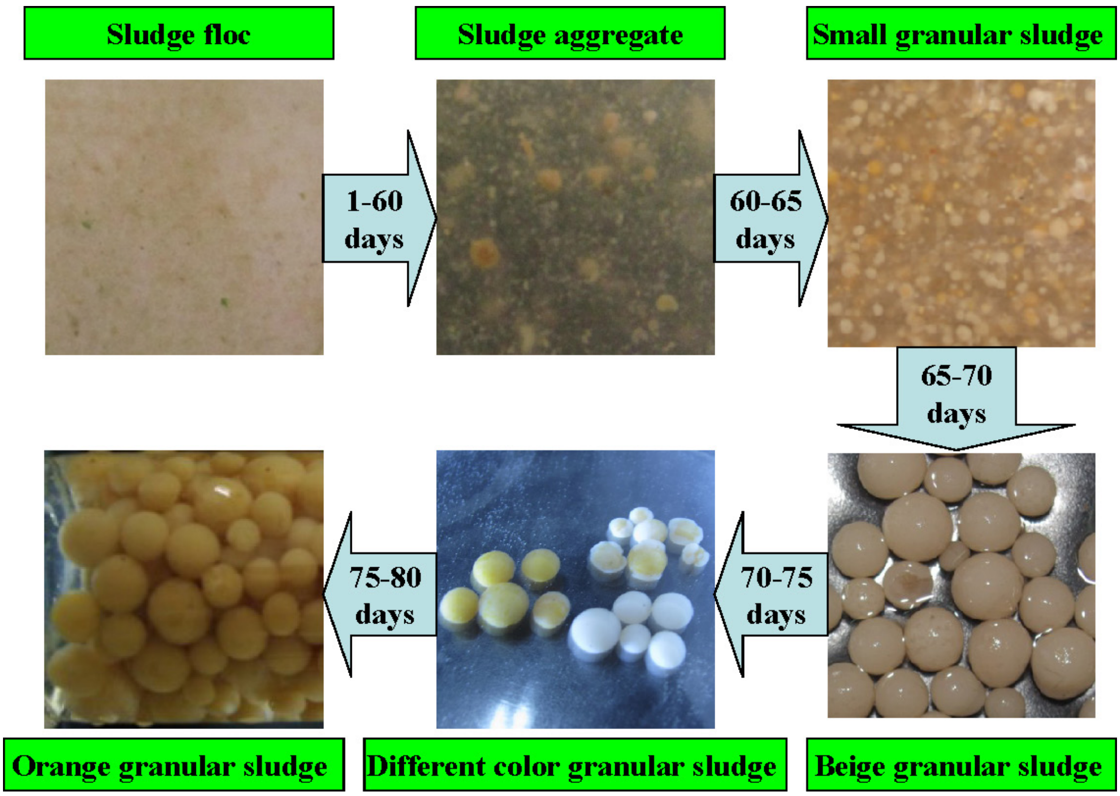


Fig. 2. Sludge morphology variations during the AGS formation.

Microbial cells in the activated sludge were aggregated to small particle cores by the effects of the shear force and the EPS [20]. At around 65 d, the small granular sludge cores increased and the size became larger, and floc sludge was replaced gradually by tiny, white and regular granules with average diameter of 0.50 mm at this period. Subsequently, the small granules increased in size from days 65 to 70 and part of granules increased from 1 to 3 mm with beige color although the white granular sludge in the reactor was still dominant. Total sludge concentration (combination of MLSS and the AGS concentration) in the BCC varied between 6 and 9 g/L, and SVI was 30–50 mL/g. After that, a vary colors of granules including beige, white and pale yellow were observed in the BCC simultaneously at 75 d or so. Smaller granules were white mostly and surface of some white granules became pale yellow as well, and small part of white granules converted to pale yellow completely. On 80 d, orange granules were formed with diameter of 2–5 mm (some granules up to 7 mm), which had smooth and regular spherical or oval shape. The orange granule sludge was identified as mature AGS in the BCC for its morphology and good stability. Wet density of the mature granular sludge was 1.02–1.06 g/cm^3, and sedimentation rate varied along with granules size which was in the range of 83–130 m/h.

Through the AGS's formation course, it can be seen that activated sludge in the BCC has gone the evolution of floc sludge, small white granule, mixed colors granule (exterior white and interior orange) and orange granule. Variation of the AGS appearance was probable due to the following reasons. Distribution of dissolved oxygen (DO) and nutrients was heterogeneous along the axial direction of the AGS with the increase of the AGS size. Then, adaptability of microbes participated in substrate metabolism adjusted according to the type of substrate, concentration of substrate and DO, and so on, which promoted the evolution of microbial community structure and type [21,22]. Therefore, different morphologies of the AGS were observed during the formation of the AGS in the BCC, which can be used to analyze the AGS formation mechanism in the unique environment of the BCC in the cfMFC system. In addition, it took 3/4 of time to achieve the aggregation of floc sludge forming granular sludge core during the AGS formation process. It indicated that the prerequisite for the AGS formation must be the formation of small granular sludge core, which can be used to explain the AGS formation mechanism as well.

3.2. Discussion of the AGS formation mechanism

According to current reports on the AGS formation and characteristics of the system adopted in the experiments, the AGS formation mechanism in the BCC of the cfMFC system was explored by two points of view during different periods. One was the macroscopic aspect, such as reactor operating conditions, and the other was the microscopic aspect, focusing on physical characteristics of the initial forming granules.

From the macroscopic point of view, typical conditions of cycle time, settling time and substance scarcity for the AGS formation in the SBR process which was inexistence in the cfMFC system. Hence, the commonly discussed factors were not determinant elements causing the AGS formation in the system. Hydraulic selection pressure (to improve the liquid shear force) and microbial selection pressure (to improve organic load, shorten HRT or add microbial inhibitors) have been reported facilitating the AGS formation in a continuous flow reactor [23,24]. Operation of the cfMFC system had the following characteristics. First, relative high and stable organic load rate ranging from 2.2 to 2.5 kg COD/(m^3·d) for the BCC was in favor of overcoming the mass transfer resistance affecting the AGS formation, and it has been reported that the AGS can be formed with the organic load rates of 1.5–15 kg COD/(m^3·d) [25]. Second, the internal recycle flow pattern in the BCC generated high water shear force on the sludge, which can improve mass transfer effect and form more regular AGS. Third, small inert carriers (0.56mm of wet particle size) were added into the BCC aiming at supporting the microbe growth, which can be used as "induced nuclear" as well to stimulate the AGS formation. Moreover, proton and other cation passed from the AC to the CC through the PEM, which can reduce the negative charge on the sludge surface and promote aggregation among the bacteria. Through comprehensive effect of the above factors, microbes collided mutually and attracted together forming bacteria group, which grew larger forming the initial irregular small sludge granule and mature AGS was formed finally.

From the microscopic point of view, two types of granular sludge have been reported in the AGS formation process, namely single-core type and multi-core type [26]. Two types of granules were observed simultaneously during the initial formation process of the AGS. Microscopic and SEM examination of these two kinds of granules are shown in Fig. 3. One was white small granule, which had a relative regular shape and was major in the BCC (Fig. 3(a)), the other was pale yellow granule, which had an irregular shape mostly and was minor in the BCC (Fig. 3(b)). A lot of viscous substances were observed on the edge of the white small granule, and protozoa, such as the rotifers, existed around the granule simultaneously (Fig. 3(c)). On the other hand, pale yellow granule was composed of many tiny granules. It can be seen clearly that there were many particle nucleations at the edge and the intermediate of the pale yellow granular, and the gap between the nuclei was filled with viscous material (Fig. 3(d)). As shown in Fig. 3(e), the spherical white small granule had a rough surface and a compact structure, which looked like an integral granule mostly. The pale yellow granule had a rugged surface and a relative loose structure, which was constituted by many condensation cores (Fig. 3(f)). Therefore, AGS formed based on the white small granule was apt to single-core type AGS formation process, and formed based on the pale yellow granule was apt to multi-core type AGS formation process. In addition, the bacteria aggregate formation in MFC is a complicated process. For example, the AIDA adhesin from diarrheagenic *Escherichia coli* would influence cell aggregation and biofilm formation [27]. The EPS in bioflocculation may influence the aggregation of bacteria [28]. The role of bacteria in formation mechanisms of AGS was not studied.

The mature yellow AGS indicated different formation process between white small granule and pale yellow granule formed AGS. Small size of the initial formed white small granule facilitated dissolved oxygen and organic substrate permeating to the granule center, which made a relative uniform microbe distribution forming the white granules. With the increase of the white granule size, dissolved oxygen and organic substrate transfer resistance was enhanced, which promoted the growth of aerobic microbes in the inner of the granule being substituted by anoxic or anaerobic microbes. The variation of microbe constitution might cause color of the white granule turning into light yellow and yellow further. On the other hand, the condensation cores, constituting pale yellow granule, might be formed by the special environment of the cfMFC system. Neighboring condensation cores in the pale yellow granule were filled with a large number of viscous matters. Color shift of the pale yellow granule was not caused by microbe variation, but mainly because the multi-core type granule would have better dissolved oxygen and organic substrate transfer effect than the white small granule. The viscous matters increased with microbe growth, which would absorb more condensation cores. Then, the aggregate condensation cores contacted closely, and merged gradually to form the mature yellow AGS, which was different from formation mechanism of the white small granule formed AGS. Therefore, from microscopic view, two types of AGS formation process existed in the BCC, and the white small granule formed AGS was closer to the AGS formation process shown in Fig. 2. The hypothesized mechanisms for white granule and yellow granule are presented in Fig. 4.

3.3. Variation of the AGS under PW2

The cfMFC system consisted of two relative independent bio-reactors, which was operated at anaerobic and aerobic condition, respectively. On the basis of mature AGS formation, effect of influent COD concentration, HRT and cathode aeration rate on organic pollutant removal and electricity production performance of the cfMFC system was studied.

3.3.1. Organic pollutant removal

COD removal efficiency is an immediate indicator to reflect organic pollutant removal efficiency. Evolution of COD concentration in the influent and effluent of the anode and cathode with the variation of operation conditions are shown in Fig. 5, along with COD removal efficiency. Moreover, detailed operation conditions were as follows. First, cathode

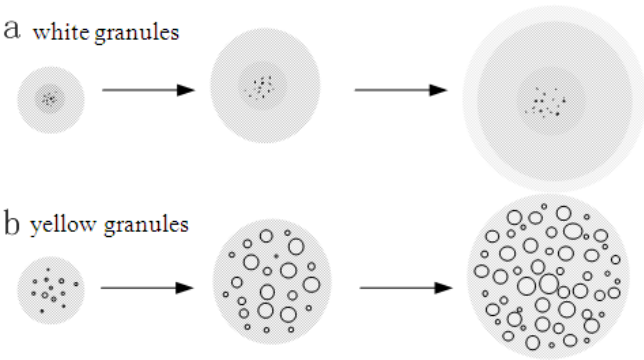


Fig. 4. Two hypothesized mechanisms for granulation.

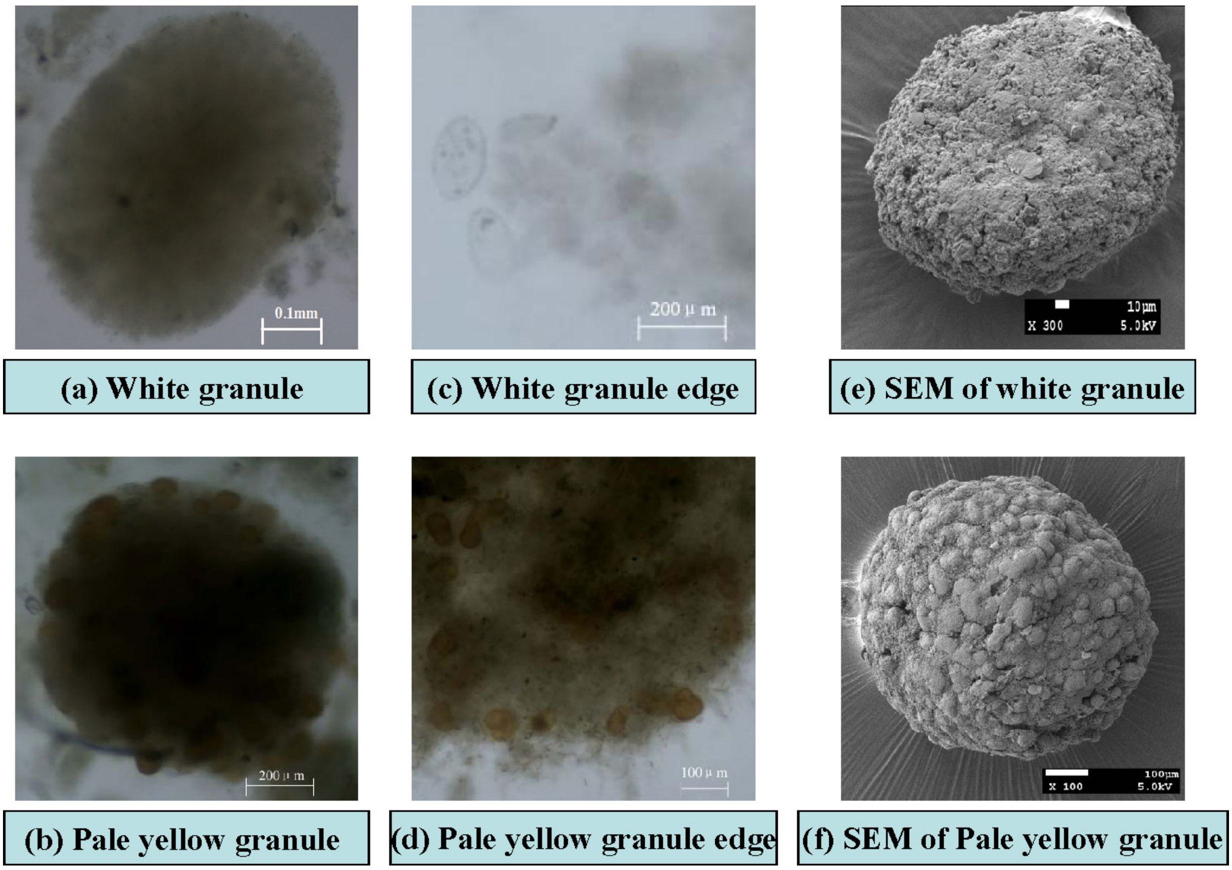


Fig. 3. Appearance and SEM photographs of the initial white and pale yellow color granule.

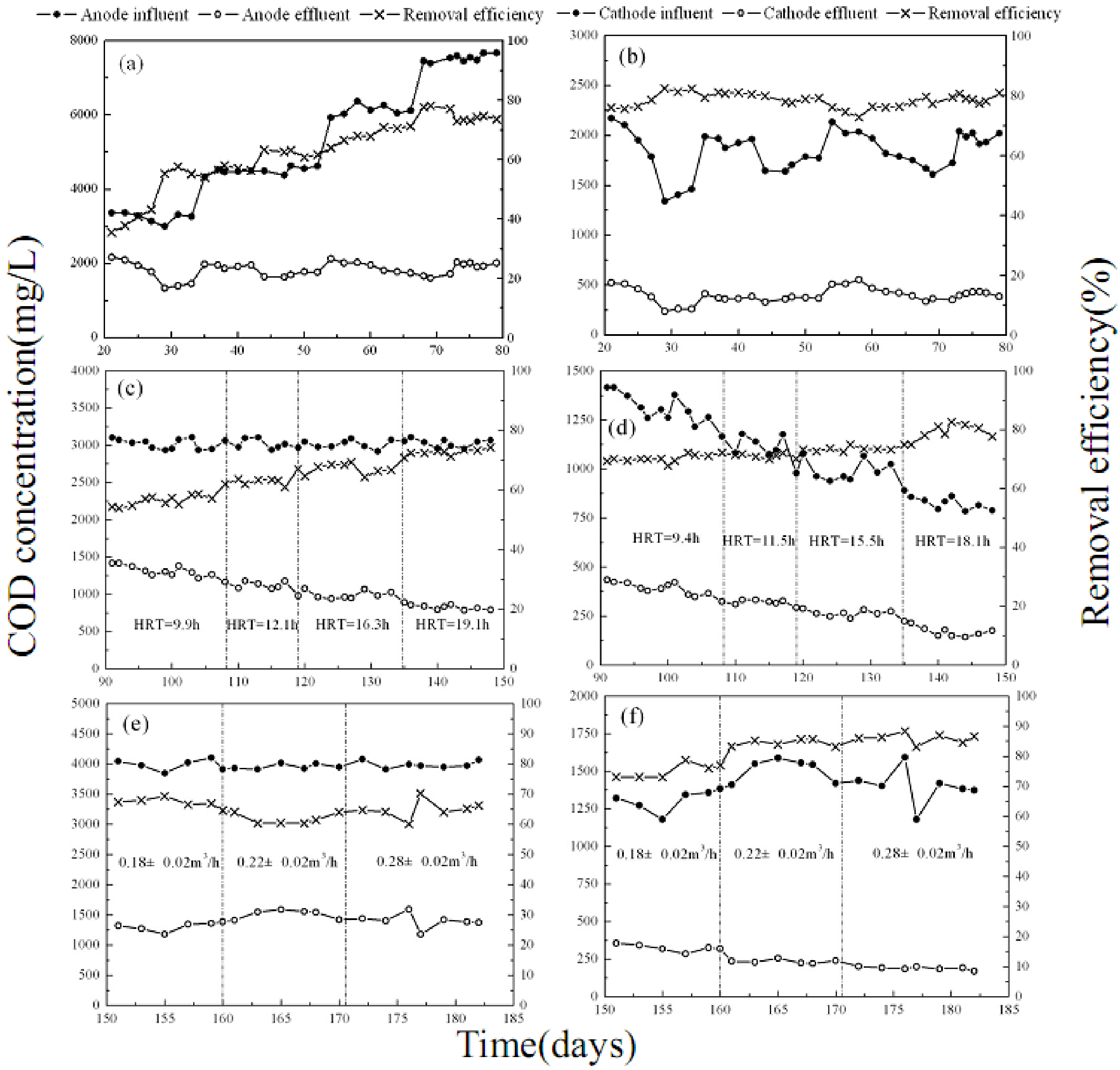


Fig. 5. Evolution of COD concentrations in the influent and effluent of the anode and cathode with the variation of operation conditions along with COD removal efficiency.

aeration rate and HRT of the system was 0.2 ± 0.02 m^3/h and 19.3 h when influent COD concentration increased from 3,000 to 7,540 mg/L (Figs. 5(a) and (b)). Second, HRT of the system improved from 19.3 to 37.2 h with cathode aeration rate of 0.2 ± 0.02 m^3/h, after influent COD concentration decreased to around 3,000 mg/L and operated for around 10 d (Figs. 5(c) and (d)). Finally, cathode aeration rate changed at the range of 0.16–0.30 m^3/h, after influent COD concentration and HRT were adjusted to 4,000 mg/L and 23.6 h and operated for around 10 d (Figs. 5(e) and (f)).

Average COD removal efficiency of the AC increased from 46.5% to 75.1% with the increase of influent COD concentration (Fig. 5(a)). Performance of the AC was not affected obviously with the improvement of organic pollutant load, which was probably due to good adaptability of anaerobes and the addition of porous polymer packing. The high organic pollutant load resistance ability of the pre-anaerobic process would provide a relative stable influent wastewater quality for the post aerobic process, which facilitating the AGS formation. Although influent COD concentration of the BCC varied from 1,340 to 2,170 mg/L after the AGS formation, average COD removal efficiency of the post aerobic process was stable at around 79% (Fig. 5(b)). Therefore, excellent organic pollutant shock load resistance ability was achieved for the large number of the AGS in the BCC, which supported the stable COD removal performance. Then, effluent COD concentration of the AC and BCC turned to normal level (before the improvement of influent COD concentration) after 10 d. When influent COD concentration decreased to around 3,000 mg/L, it indicated a good recovery ability of the cfMFC system. With the extension of HRT from 19.3 to 37.2 h, COD removal efficiency increased from 56.4% to 72.7% in the

AC (Fig. 5(c)) and enhanced from 70% to 82.7% in the BCC (Fig. 5(d)). Organic pollutant removal in the cfMFC system was improved obviously with the increase of the HRT, which indicated that bacteria growth in the porous polymer packing of the AC or in the AGS of the BCC needs a long time to contact with organic pollutant because of the relative low mass transfer rate. Besides, longer HRT is accorded with kinetics of biodegradation process. However, unlimited extension of the HRT is not practical, and a suitable choice between influent COD concentration and HRT (organic load rate) for the cfMFC system is worth studying in subsequent research. After being operated stably for another 10 d at HRT of 23.6 h, the effect of BCC aeration rate on COD removal performance of the cyMFC system was studied, and COD removal efficiency in the AC was affected slightly in this time (Fig. 5(e)). COD removal efficiency in the BCC increased from 75.2% to 86.2% with the improvement of aeration rate (Fig. 5(f)), which was due to the enhancement of oxygen transfer rate in AGS promoting the activity of organic pollutant removal microbe. In addition, granule size (data were not shown) was reduced with the increase of aeration rate, and the SVI decreased from 63 to around 50 mL/g.

Total COD removal efficiency of the AGS-based cfMFC was always higher than 90%, which suggested that organic pollutant removal capacity of the cfMFC system had reached a good level and was relative stable. More importantly, the cfMFC system can generate electricity as well, which was superior to other anaerobic and aerobic combined systems.

3.3.2. Electricity production performance

Electricity production performance is one of main research directions in the MFC process. To produce more electricity on the premise of high wastewater treatment performance is desired. Electrical production performance of the cfMFC system with the variation of operation conditions is shown in Fig. 6. As shown in Fig. 6(a), output voltage increased from 56 to 91 mV with the enhancement of influent COD concentration, which indicated that available organic substrate was important to maintain electricity production in the MFC process. However, increase of the output voltage slowed down obviously with influent COD concentration increased from around 6,000 to around 7,500 mg/L. Therefore, output voltage of the cfMFC approached the limit when the influent organic substrate concentration was too high. The available organic substrate for microbes was improved with the increase of influent COD concentration initially, which promoted generating more electrons in the system and facilitated the increase of output voltage. Concentration of by-product and toxic compounds increased further with continuing enhancement of influent organic pollutant concentration, which would inhibit the activity of microbes, especially the electricigens activity. Hence, higher output voltage was not achieved with the further increase of organic substrate concentration, which was induced by the "saturation effect" [29].

Electricity production performance had close relationship with the HRT of the cfMFC system (Fig. 6(b)). Average output voltage increased from 78 to 91 mV when the HRT was extended from 19.3 to 23.6 h, but decreased to 65.7 mV with further improvement of the HRT to 37.2 h. Maximum output voltage level was achieved at HRT of 23.6 h in the cfMFC system, which indicated that an optimal HRT value or range existed to obtain maximal output voltage in the MFC. The phenomenon was similar with the results reported by Li et al. [30] and Liu et al. [31]. However, the results was contradictory to the results reported by Liu et al. [32] where continuous decrease of electricity production performance was obtained with the increase of the HRT in a cfMFC with single chamber, which might be due to deoxidation handle of influent wastewater and the relative low influent COD concentration. In this experiment, lower output voltage in a shorter HRT could be due to the slower biodegradation rate of organic pollutants and the enhancement of oxidation–reduction potential induced by dissolved oxygen in the influent wastewater. More electrons were generated by efficient biodegradation of organic pollutants with proper extension of the HRT, which promoted the increase of output voltage. The available organic pollutant for electricigens was decreased with further enhancement of the HRT and corresponding electricity

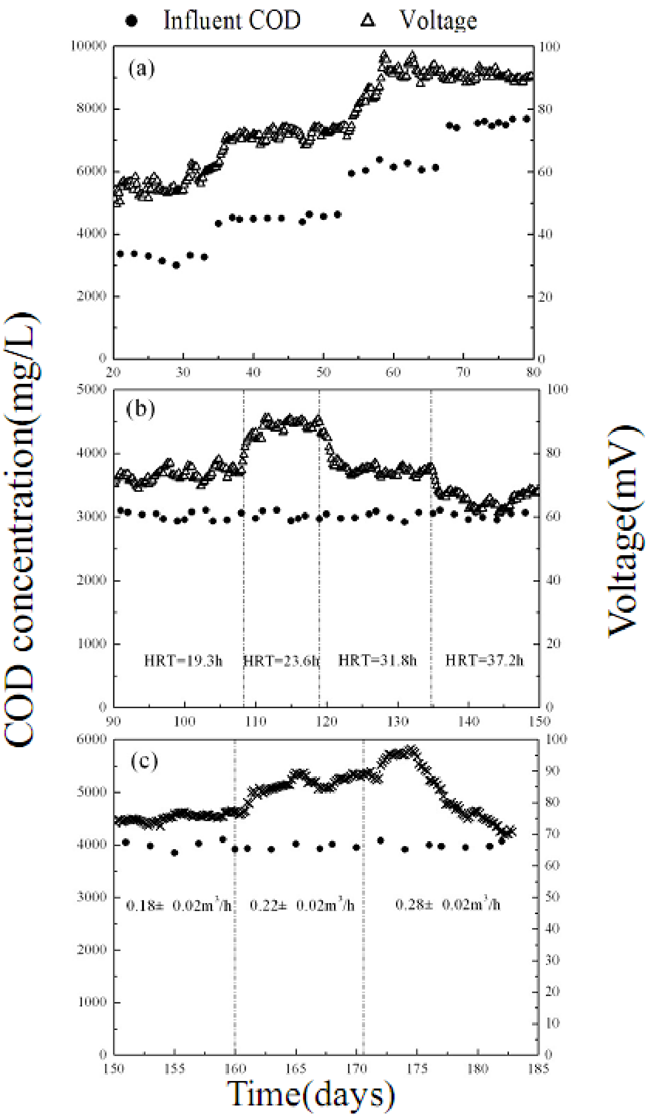


Fig. 6. Evolution of electrical production with the variation of operation conditions.

production performance decreased. In addition, other anaerobic bacteria plundered the limited organic pollutants with electricity production bacteria under longer HRT, and this was the main reason for the continuous elevation of COD removal with the increase of the HRT (see Fig. 5(c)).

Under different aeration rates in the BCC, the output voltage of the system was quite distinct (Fig. 6(c)). Average output voltage rose from75.4 to 86.2 mV after the aeration rate increased from 0.18 ± 0.02 to 0.22 ± 0.02 m^3/h. When the aeration rate was improved to 0.28 ± 0.02 m^3/h further, maximal output voltage achieved 97.1 mV after 4 d, but decreased gradually and stabilized at about 70 mV. Therefore, output voltage of the system can be improved by the enhancement of aeration rate in the BCC at a reasonable range. Most of dissolved oxygen was consumed by aerobic microbes in the BCC to biodegrade remaining organic pollutants in the effluent of the AC when the aeration rate was relative low, which affected the electrons accepting ability generated in the AC and induced the poor electricity production performance. Certainly, dissolved oxygen, main electron acceptor in the BCC, was increased by the enhancement of aeration rate, and the output voltage was heightened subsequently. However, plenty of residual dissolved oxygen would exist at a relative high aeration rate for a long time, part of which might spread into the AC through the PEM. Electricity production performance of the system was affected by the dissolved oxygen passed through the PEM, because the activity of the strict anaerobic electricigens and the quantity of the generated electrons would be affected by the participation of oxygen. And the negative effect of excess aeration on the output voltage would be enhanced by extended aeration at a relative high strength, which induced the decrease of the output voltage.

The results obtained above indicated that electricity production performance of the system can be optimized by adjusting influent COD concentration, HRT and cathode aeration rate appropriately without affecting pollutant removal performance obviously. The maximum output voltage value obtained was 91 mV in the AGS-based cfMFC system which was low. The electrical energy required for the operation of this system could not be covered by the electrical energy produced by this system. However, the purpose of this study was to treat the wastewater meeting the discharge standard, and generation of electrical energy was a unique benefit of this system. Therefore, the energy balance was not considered in this study because the wastewater must be well treated. Output voltage value can achieve 200 mV and even up to 500 mV by optimizing operating parameters in abiotic cathode MFC system [33,34]. The BCC in the cfMFC system was rich in microbes, which affected the reaction in the cathode, resulting in the decrease of output voltage. Configuration of the BCC was designed to ensure a good flow ability in the reactor, which might result in oxygen inadequate near the electrode and affected electricity production performance. The exact reasons for the obvious low output voltage in the BCC need to be demonstrated by following research. In addition, the AGS formed in the CC which had a rectangle shape with a low ratio of height to length. This kind of tank was easy to be found in the domestic wastewater treatment plant which could be used directly after suitable adjustment.

4. Conclusion

The AGS with regular shape was cultivated in the cubic BCC with height to length ratio of 1.6. Formation of the AGS can be divided into six stages according to the variation of the sludge morphology, and mechanism of the AGS formation can be explained by macroscopic view based on hydraulic and microbial selection pressure and microscopic view based on two types of initial formed granular sludge. Organic pollutant removal efficiency was always higher than 90% after the AGS formation. The highest output voltage was 91 mV, which had a large gap comparing with conventional MFC process. In addition, effect of the AGS-based cfMFC system on nitrogenous contaminants removal, such as ammonia and total nitrogen, was not studied during the experiments. The AGS formed in the BCC can provide a suitable environment for nitrogenous contaminant removal because of its special structure. Therefore, subsequent research can strengthen this part of the study to further improve ammonia nitrogen, even total nitrogen removal in the cfMFC system.

Acknowledgment

This research was supported by the National Natural Science Foundation of China (No. 51308362).

References

[1] C.A. Velis, P.J. Longhurst, G.H. Drew, R. Smith, S.J. Pollard, Biodrying for mechanical–biological treatment of wastes: a review of process science and engineering, Bioresour. Technol., 100 (2009) 2747–2761.

[2] D. Li, Y. Hong, M. Xu, H. Luo, G. Sun, Progress in construction of microbial fuel cell, Chin. J. Appl. Environ. Biol., 1 (2008) 147–152.

[3] S.S. Adav, D.J. Lee, K.Y. Show, J.H. Tay, Aerobic granular sludge: Recent advances, Biotechnol. Adv., 26 (2008) 411–423.

[4] Y.Z. Peng, L. Wu, Y. Ma, S.Y. Wang, L.Y. Li, Advances: granulation mechanism, characteristics and application of aerobic sludge granules, Chin. Environ. Sci., 31 (2010) 273–281.

[5] E. Morgenroth, T. Sherden, M.C.M. van Loosdrecht, J.J. Heijnen, P.A. Wilderer, Aerobic granular sludge in a sequencing batch reactor, Water Res., 31 (1997) 3191–3194.

[6] Z. Song, N. Ren, K. Zhang, L. Tong, Influence of temperature on the characteristics of aerobic granulation in sequencing batch airlift reactors, J. Environ. Sci., 21 (2009) 273–278.

[7] J.H. Tay, Q.S. Liu, Y. Liu, Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor, J. Appl. Microbiol., 91 (2001) 168–175.

[8] G. Lettinga, A.F.M. van Velsen, S.W. Hobma, W. de Zeeuw, A. Klapwijk, Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment, Biotechnol. Bioeng., 22 (1980) 699–734.

[9] B. Arrojo, A. Mosquera-Corral, J.M. Garrido, R. Méndez, Aerobic granulation with industrial wastewater in sequencing batch reactors, Water Res., 38 (2004) 3389–3399.

[10] M.C. Veiga, M.K. Jain, W. Wu, R.I. Hollingsworth, J.G. Zeikus, Composition and role of extracellular polymers in methanogenic granules, Appl. Environ. Microbiol., 63 (1997) 403–407.

[11] Y. Liu, S.F. Yang, L. Qin, J.H. Tay, A thermodynamic interpretation of cell hydrophobicity in aerobic granulation, Appl. Microbiol. Biotechnol., 64 (2004) 410–415.

[12] Y. Liu, J.H. Tay, The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge, Water Res., 36 (2002) 1653–1665.

[13] M. Behera, M.M. Ghangrekar, Performance of microbial fuel cell in response to change in sludge loading rate at different anodic feed pH, Bioresour. Technol., 100 (2009) 5114–5121.

[14] H. Wang, P. Yang, Y. Guo, X. Liao, X. Li, L. Wang, Study on property of microbial fuel with porous spheroidal particles, Chin. J. Environ. Eng., 4 (2010) 352–354.
[15] J.S. Huang, P. Yang, Y. Guo, Y. Liu, T. Su, Effects of catholyte and substrate concentration on simultaneous wastewater treatment and electricity production for AFB-MFC, Chin. J. Environ. Eng., 6 (2012) 462–466.
[16] State Environmental Protection Administration of China, Monitoring and Analytic Methods of Water and Wastewater, 4th ed., Environmental Science Press of China, Beijing, China, 2002.
[17] Y. Chen, W.J. Jiang, D.T. Liang, J.H. Tay, Structure and stability of aerobic granules cultivated under different shear force in sequencing batch reactors, Appl. Microbiol. Biotechnol., 76 (2007) 1199–1208.
[18] A.J. Li, X.Y. Li, H.Q. Yu, Granular activated carbon for aerobic sludge granulation in a bioreactor with a low-strength wastewater influent, Sep. Purif. Technol., 80 (2011) 276–283.
[19] S.G. Wang, X.W. Liu, W.X. Gong, B.Y. Gao, D.H. Zhang, H.Q. Yu, Aerobic granulation with brewery wastewater in a sequencing batch reactor, Bioresour. Technol., 98 (2007) 2142–2147.
[20] N.H. Rosman, A.N. Anuar, I. Othman, H. Harun, M.Z. Sulong, S.H. Elias, M.A.H.M. Hassan, S. Chelliapan, Z. Ujang, Cultivation of aerobic granular sludge for rubber wastewater treatment, Bioresour. Technol., 129 (2013) 620–623.
[21] J.H. Tay, V. Ivanov, S. Pan, S.T.L. Tay, Specific layers in aerobically grown microbial granules, Lett. Appl. Microbiol., 34 (2002) 254–257.
[22] S.D. Weber, W. Ludwig, K.H. Schleifer, J. Fried, Microbial composition and structure of aerobic granular sewage biofilms, Appl. Environ. Microbiol., 73 (2007) 6233–6240.
[23] X. Zhang, P.L. Bishop, Biodegradability of biofilm extracellular polymeric substances, Chemosphere, 50 (2003) 63–69.
[24] Y.Q. Liu, W.W. Wu, J.H. Tay, J.L. Wang, Starvation is not a prerequisite for the formation of aerobic granules, Appl. Microbiol. Biotechnol., 76 (2007) 211–216.
[25] J.L. Wang, Z.J. Zhang, W.W. Wu, Research advances in aerobic granular sludge, Acta Scientiae Circumstantiae, 29 (2009) 449–473.
[26] J.J. Barr, A.E. Cook, P.L. Bond, Granule formation mechanisms within an aerobic wastewater system for phosphorus removal, Appl. Environ. Microbiol., 76 (2010) 7588–7597.
[27] S. Orla, A.S. Mark, R. Andreas, K. Per, Novel roles for the AIDA adhesin from diarrheagenic *Escherichia coli*: cell aggregation and biofilm formation, J. Bacteriol., 186 (2004) 8058–8065.
[28] R.B. Appala, C. Shankararaman, L.G. Paul, H.E. Mark, S.L. Alan, M.R. Kevin, Role of extracellular polymeric substances in bioflocculation of activated sludge microorganisms under glucose-controlled conditions, Water Res., 44 (2010) 4505–4516.
[29] S.J. You, Microbial Fuel Cell for Electricity Generation During Organic Wastewater Treatment, PhD Thesis, Harbin Institute of Technology, China, 2008.
[30] Z. Li, L. Yao, L. Kong, H. Liu, Electricity generation using a baffled microbial fuel cell convenient for stacking, Bioresour. Technol., 99 (2008) 1650–1655.
[31] H. Liu, S. Cheng, L. Huang, B.E. Logan, Scale-up of membrane-free single-chamber microbial fuel cells, J. Power Sources, 179 (2008) 274–279.
[32] H. Liu, R. Ramnarayanan, B.E. Logan, Production of electricity during wastewater treatment using a single chamber microbial fuel cell, Environ. Sci. Technol., 38 (2004) 2281–2285.
[33] G. Antonopoulou, K. Stamatelatou, S. Bebelis, G. Lyberatos, Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell, Biochem. Eng. J., 50 (2010) 10–15.
[34] K. Rabaey, W. Ossieur, M. Verhaege, W. Verstraete, Continuous microbial fuel cells convert carbohydrates to electricity, Water Sci. Technol., 52 (2005) 515–523.