Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.985726

57 (2016) 3414–3423
February

Taylor & Francis
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Aerobic granular sludge stabilization in biocathode chamber of newly constructed continue flow microbial fuel cell system treating synthetic and pharmaceutical wastewater

Ping Yang[a], Tingting Chen[a], Hui-qiang Li[a,b,*]

[a]*College of Architecture and Environment, Sichuan University, Chengdu 610065, China, Tel. +86 18602804508; email: yangp_scu@163.com (P. Yang), Tel. +86 13684079119; email: chentingtingscu@163.com (T. Chen), Tel. +86 18980668020; email: lhq_scu@163.com (H.-q. Li)*
[b]*Key Laboratory of Special Wastewater Treatment, Sichuan Province Higher Education System, Chengdu, China*

Received 2 April 2014; Accepted 2 November 2014

ABSTRACT

Relative stable aerobic granular sludge (AGS) formed in a newly constructed continue flow microbial fuel cell (cf-MFC) system. AGS bulking caused by filamentous over-growth was observed in treating simulated wastewater with COD concentration of around 1,000 mg/L after more than 50 d. Addition of pharmaceutical wastewater in the influent of the cf-MFC at COD concentration of around 1,500 mg/L alleviated AGS bulking obviously, with average COD removal efficiency of 82.2%. However, AGS self-collapse happened after influent COD concentration of the pharmaceutical wastewater increased to around 2,500 mg/L, corresponding COD removal efficiency decreased to 58.2% dramatically. High simultaneous nitrification and denitrification performance was obtained in the cf-MFC, which was affected by the addition of pharmaceutical wastewater. Moreover, appearance variations of the AGS under different operation conditions were shown.

Keywords: Aerobic granular sludge; Continue flow microbial fuel cell; Stabilization; Filamentous over-growth; Self-collapse

1. Introduction

Formation of aerobic granular sludge (AGS) is desired in wastewater treatment process, because of its good settling properties, high biomass retention and strong shock load resistant ability [1–3]. The AGS has been reported treating many kinds of urban or industrial wastewaters successfully, which has been a dominant direction for biologically wastewater treatment processes [4–6]. However, instability of AGS under long-term operation condition is the primary problem for AGS utilization [7]. Two reasons have been studied mostly for AGS instability, one is the AGS bulking caused by filamentous [8], the other is self-collapse by the variation of operation conditions, such as the presence of toxic compounds and high pollutant loading rates [9,10].

Filamentous bulking is mentioned mostly when stabilization of AGS is studied and different extents of filamentous growth have been detected during sludge bulking [11–13]. According to the reports on the effect of filamentous growth on AGS stability, suitable

*Corresponding author.

filamentous growth can be used as carriers for adhering microbe, which is in favor of AGS formation. However, the AGS bulking will happen if growth of filamentous is out of control. Then, a series of problems are encountered, such as deterioration of sedimentation performance, AGS disintegration and being washed out, decrease in biomass concentration, pollutant removal performance, and deterioration of effluent wastewater quality. Therefore, extent of filamentous growth must be handled carefully to keep relative stable AGS. On the other hand, study of AGS self-collapse is focused on variation of AGS appearance mostly. First, the difficult granulation floc sludge is washed out from the bioreactor, which induces an insufficient biomass concentration for AGS formation. Second, the formed AGS is dissolved into floc sludge which is washed out from the bioreactor easily. Third, cracks in inner AGS increase with the operation of the bioreactor, which causes decrease in AGS density and is washed out from bioreactors.

Many reasons for filamentous over-growth have been reported, such as, being operated under relative low organic load rate (OLR) level [14], faster grow rate at relative low dissolved oxygen (DO) concentration comparing with most of other microbes, because of the larger specific surface area and lower oxygen saturation constant of the filamentous [15], organic soluble substrate with smaller molecular weight facilitating filamentous growth easier [16]. Two reasons for non-filamentous factors inducing AGS collapse have been reported mostly, one via the limitation of substrates and DO mass transfer with larger AGS size, resulting in the death of microbe at inner AGS and autolysis, the other was inhibition of microbes activity by toxic compounds in the wastewater inducing large number of microbes death and disappear of microbe agglomeration, which caused AGS disintegration. Attempt to recover the bulking AGS can check the listed reasons one by one, which is the method in the following research to analyze possible reasons for AGS bulking.

At present, researches on formation and stabilization of AGS have been reported mostly in sequencing batch reactor (SBR) process. However, to our knowledge, formation and stabilization of AGS in a continue flow microbial fuel cell (cf-MFC) system have been studied little. The MFC process has the characteristics of high conversion rate, simple operating condition, and no secondary pollution [17]. Therefore, a new constructed cf-MFC system was constructed trying to cultivate AGS using glucose as a sole organic substrate, along with investigation of disintegration and recovery of the AGS. Moreover, effect of two kinds of pharmaceutical wastewater on stabilization of the AGS was monitored in the experiments.

2. Materials and methods

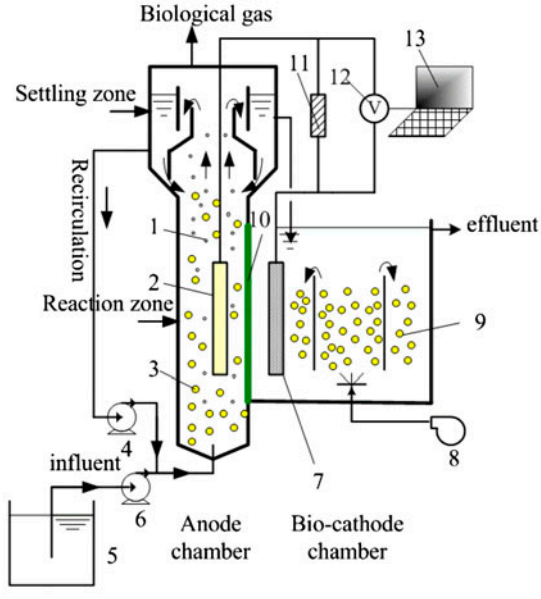
2.1. Wastewater

Three kinds of wastewater were used as influent of the cf-MFC system in the experiments. First, the synthetic wastewater (SW), which was composed by glucose as carbon source, NH_4Cl as nitrogen source, K_2HPO_4 as phosphorus source, and trace elements. The pH value of the SW was controlled from 7.8 to 8.2 using sodium bicarbonate. Influent COD concentration was adjusted by controlling the amount of glucose in the SW. Second, the pharmaceutical wastewater combined by antibiotic wastewater and domestic sewage, which was defined as PW1 below. Third, mixed pharmaceutical wastewater was consisted by residual of pharmaceutical raw material (containing acetone, methanol, biapenem, etc.) and antibiotic wastewater, which was defined as PW2 below.

2.2. Setup and start-up of the cf-MFC system

The experiments were carried out in a dual-chamber plexiglass reactor forming the cf-MFC system, which is shown in Fig. 1.

Anode chamber (AC) of the system was formed by two parts, one was reaction zone with volume of 5.9 L and internal diameter of 100 mm, the other was



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 cf-MFC system.

settling zone with volume of 4.2 L and internal diameter of 160 mm. Wastewater was fed at the bottom of the AC by peristaltic pump, and effluent of the AC flowed into BCC by gravity. The BCC was a 200 (length) × 15 (width) × 320 mm (height) open container with total working volume of 9.6 L. AC and BCC were filled with porous polymer packing by our laboratory for microbes enrichment and filling ratio were 15 and 10%, respectively [18].

Inoculum of the AC was obtained from AC of a dual-chamber cf-MFC with abiotic cathode as reported by Huang et al. [19]. Temperature of the AC was controlled at 30–35˚C during the experiments. The SW with influent COD concentration of around 1,000 mg/L was adopted initially, and relatively good organic pollutant removal performance was obtained in AC after being cultivated for more than one month. Inoculated sludge of BCC was gained from aeration tank of a municipal wastewater treatment plant in Chengdu. BCC started up together with AC. That was, AC effluent flowed into BCC directly after addition of porous polymer packing and 4 L of activated sludge mixed liquor. A month later, sludge concentration in BCC increased obviously and color of suspension sludge was brown. The pH value in BCC was not controlled in the experiments and temperature was controlled at 25 ± 2˚C. Temperature of the system was controlled by thermostat covered outside of the reactors. HRT of the system was controlled at 37.2 h throughout the experiments.

2.3. Analytical methods

During the experiments, samples were taken from influent and effluent of AC and BCC every other day and were analyzed immediately after being filtered through 0.45 μm filter paper. COD, NH_4^+-N, NO_2^--N, NO_3^--N, SV, SVI, and MLSS were analyzed in accordance with monitoring and analytic methods of water and wastewater [20]. The pH value was measured by pHS-2F pH meter. Appearance of the AGS was observed by scanning electron microscope (SEM) and Olympus BX51 electron.

3. Results and discussion

3.1. AGS formation

Generally, AGS formation operated in sequencing batch operated reactor has been reported mostly for the unique characteristics of AGS [21]. Differently, AGS formation was observed in BCC of the cf-MFC system when treating SW, which provided a suitable condition for research of AGS stabilization. Influent COD concentration of the system was kept at around 1,000 mg/L using SW after start-up period. Courses of AGS formation can be shown based on variation of the AGS appearance, which are shown in Fig. 2.

Floc sludge in BCC increased obviously after being operated stably using SW as influent wastewater. At the same time, small quantity of tiny white granular was observed as shown in Fig. 2(b), which had white floss on the surface and the size was below 0.5 mm mostly. Then, the size of white particle increased gradually and AGS of beige color was observed after around 17 d (Fig. 2(c)). Simultaneously, maximal AGS size reached around 3 mm and total sludge concentration (combination of floc and granular sludge) increased from 2.2 to 6.8 g/L with SVI value decreasing from 163 to 73 mL/g. After that, total sludge concentration in the reactor stabilized at around 7 g/L with corresponding SVI value of 54 mL/g, when relative stable AGS was formatted at day 21. As shown in Fig. 2(d), most of the AGS was regular and spherical along with low level of floc sludge concentration. Therefore, the AGS can be formed in the newly constructed cf-MFC system at a relative short period and AGS stabilization was investigated below.

3.2. AGS bulking and recovery

The system was operated continually after AGS formation and sizes of AGS were kept at the range from 1.5 to 3.0 mm. Some filamentous bacteria were observed on the surface of AGS, but the filamentous bacteria were sparse with much small size. Structure of the AGS was relatively stable during this period and total sludge concentration varied from 6.6 to 6.9 g/L with SVI value of around 75 ml/g. Then, obvious flosses were observed on surface of AGS at day 36 (Fig. 3(b)) and settlement performance of AGS deteriorated with average SVI value of 113 ml/g. A week later, total sludge concentration decreased below 6 g/L with SVI value of 142 ml/g. With the prolonging of experiments under the same operation condition at day 56, flosses on the surface of some AGS increased continually. Appearance of AGS became irregular, which is shown in Fig. 3(c). At the same time, some of AGS disintegrated and were washed out from the reactor. Some of AGS were connected by the surface flosses, all of which indicated a serious AGS bulking in the system at this moment.

Plenty of biomass was washed out from the reactor and SVI value increased obviously after AGS bulking happened, which induced deterioration of effluent wastewater quality. The possible reasons for AGS bulking based on the previous mentioned factors were disused as follow: OLR, which was around

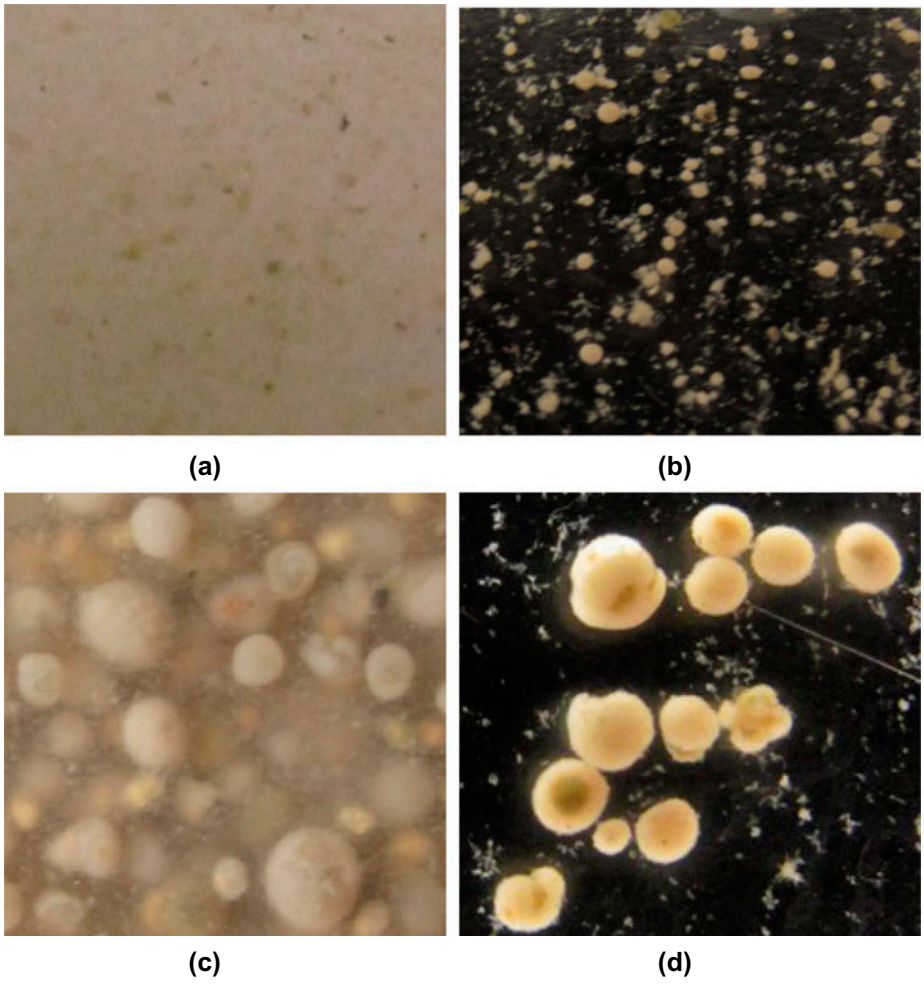


Fig. 2. Appearance variation during AGS formation (a) activated sludge inocula, (b) initial AGS, (c) floc and granular sludge, and (d) mature AGS.

2.3 kg COD/m^3 d and AGS bulking happened rarely at the given OLR level according to the related reports. Substrate type, glucose was used as a sole carbon source for the influent of the system, which was biodegraded easily in the system. The results were also supported by former reports indicating that the AGS have low stabilization if being cultured using a sole substrate, especially using glucose [22,23]. Therefore, AGS bulking was probably due to the influent wastewater quality. Acid environment, pH value in the BCC was always below 7.0 and sometimes below 6.0 because of the easy acidification glucose. Decrease of pH value in the effluent of the AC might induce decline of pH value and promote the AGS bulking in BCC because growth of microbe might be inhibited under acid environment except for the growth of the filamentous. Substrate deficiency, which was not a reason for the AGS bulking because of relative sufficient substrate supplied. Temperature fluctuation was not the possible reason for AGS bulking because temperature control device has been adopted in the system. At last, the bioreactor type, a CSTR-type reactor was adopted in the experiments having much lower substrate concentration gradient level comparing with SBR type, which promoted filamentous growth in BCC, and how to inhibit filamentous growth in a CSTR-type reactor to stabilize AGS is a further investigate problem.

According to the analyses on AGS bulking, operation conditions of the system were adjusted at the following aspects: First, influent of the system was changed from SW into PW1 and influent COD concentration was kept at around 1,000 mg/L as well. Second, DO concentration and shearing force in the bioreactor were enhanced by improving aeration rate. Third, pH value in the bioreactor was handled at the range from 7.5 to 8.0. A week after the variation of the operation conditions, settle-ability of the AGS was

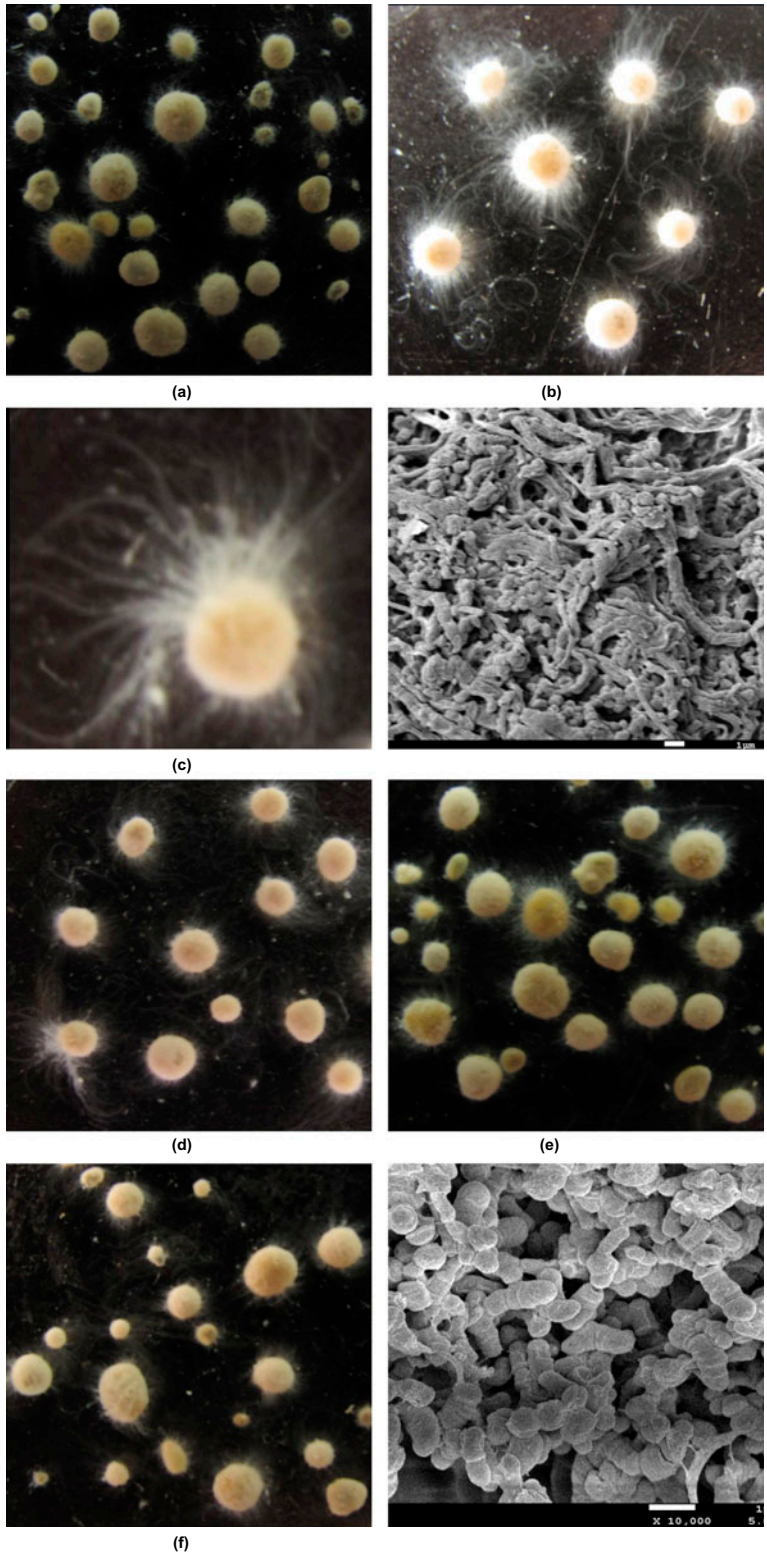


Fig. 3. Evolution of AGS appearance during bulking and recovery period, along with SEM photo of the bulking and normal AGS. (a) stable AGS; (b) bulking in 14 d; (c) bulking in 34 d, SEM photo of bulking AGS; (d) recovery in 7 d; (e) recovery in 12 d; and (f) normal AGS, SEM photo of normal AGS.

improved obviously with smaller flosses on AGS surface (Fig. 3(d)) and corresponding SVI value decreased from 142 to 116 mL/g. SVI value decreased to 78 mL/g further at 25 d, after the variation of the operation conditions and stable at the range from 65 to 80 mL/g with sludge concentration varying from 6.1 to 6.7 g/L. At this moment, flosses on the AGS surface disappeared mostly and outline of the AGS was clear, which are shown in Fig. 3(e). Variation of AGS surface indicated that over-growth of filamentous bacteria can be well controlled by proper adjustment of operation conditions [24,25]. Moreover, SEM photos of the bulking AGS and the recovery AGS were reflected in Fig. 3 as well. Plenty of filamentous bacteria were observed on the surface of bulking AGS, with relative small cocci and bacilli. On the other hand, cocci and bacilli were dominate after the bulking AGS returned to normal AGS, with little filamentous bacteria, which also supported the ahead conclusion that filamentous bacteria over-growth in the AGS was well controlled.

3.3. Variation of AGS in treating PW2

Adaptation performance of the AGS to toxic wastewater was investigated below using PW2. After previous researches on stability of the formed AGS, influent of the system was changed to SW and operated for around one week to stabilize the AGS. Then, influent wastewater was turned into PW1, and corresponding COD concentration was in the range from 1,400 to 2,200 mg/L from day 9 to day 23. At last, PW2 was adopted with COD concentration varying from 1,900 to 2,600 mg/L from day 25 to day 62. Appearances of the AGS varied obviously after influent wastewater was change to PW2, which are shown in Fig. 4.

The relative stable AGS obtained using SW is shown in Fig. 4(a), which had regular shape and smooth surface. Appearances of the AGS varied little after influent of the system was changed to PW1 in 14 d and color and size of the AGS varied a little during this period. After that, influent of the system was shifted into PW2 and color of the yellow AGS deepened obviously at day 28, as shown in Fig. 4(b). At this moment, sizes of some AGS became smaller, but outline of the smaller AGS was still clear and structure was relative holonomic. Surface color of the AGS became much deeper in day 48 comparing with day 28, more like yellowish-brown , as shown in Fig. 4(c). Variation of AGS color might be due to the negative effect of PW2 on the constitution of the AGS. Moreover, cracks appeared on the surface of the AGS, and some of AGS were irregular. Plenty of floc sludge was observed in the bioreactor with a smaller number and size of AGS and shape of the AGS was irregular when the system was operated continually (Fig. 4(d)). Therefore, types of pollutant containing in the wastewater indeed affected AGS performance, which can be reflected by the variation of AGS appearance [26]. In addition, size and sedimentation performance of the AGS were other indicators to weight the variation of AGS performance under the impact of toxic compounds, which are shown in Fig. 5(d), along with the variation of COD, ammonia, NO_2^--N, and NO_3^--N concentrations.

As shown in Fig. 5(d), AGS size decreased gradually with the shift of influent wastewater. More specially, average AGS size was 3.5 mm in treating SW and decreased to 3.2 mm after influent wastewater was shifted to PW1 from day 8 to day 10. Average AGS size declined to around 3 mm at day 14 and decreased continually to 2.6 mm at day 22. Decrease in average AGS size became heavier after the shift of PW2 and the size declined to 1.41 at day 40. After the system was operated for 65 d, most of AGS were below 1 mm and plenty of floc sludge was observed in the effluent of the BCC. Rapid disintegration of the AGS after change of influent wastewater quality probably be due to difficult adaptation of the microbes growth in the AGS to the toxic compounds, although the AGS has been reported having relative strong toxic compounds resistance ability [27]. Activity and growth of microbe in AGS were inhibited extremely by the toxic compounds containing in the PW2, and detrimental effect was enhanced by accumulation effects of toxic pollutants because of the lower down of toxic pollutant biodegradation rates. Therefore, decrease in AGS size and increase in floc sludge were induced by exfoliation of dead or low-activity microbes from AGS. In addition, sedimentation performance of the AGS was undermined with the extension of the experiments. Using glucose as sole carbon source in the SW, average sedimentation velocity of AGS was 115 m/h and decreased to 96 m/h after one week using the PW1, while declined obviously to 32 m/h at day 65. Variation of sedimentation velocity was similar with evolution of the AGS size, which indicated that sedimentation performance of the AGS was undermined with gradual disintegrate of the AGS. Therefore, stability of the AGS was important in treating industrial wastewater, especially plenty of toxic pollutants containing in the wastewater.

On the other hand, variations of COD and ammonium removal performance during the change of influent wastewater was another method to weight effects of different influent wastewaters on AGS performance. COD removal performance of the system during the

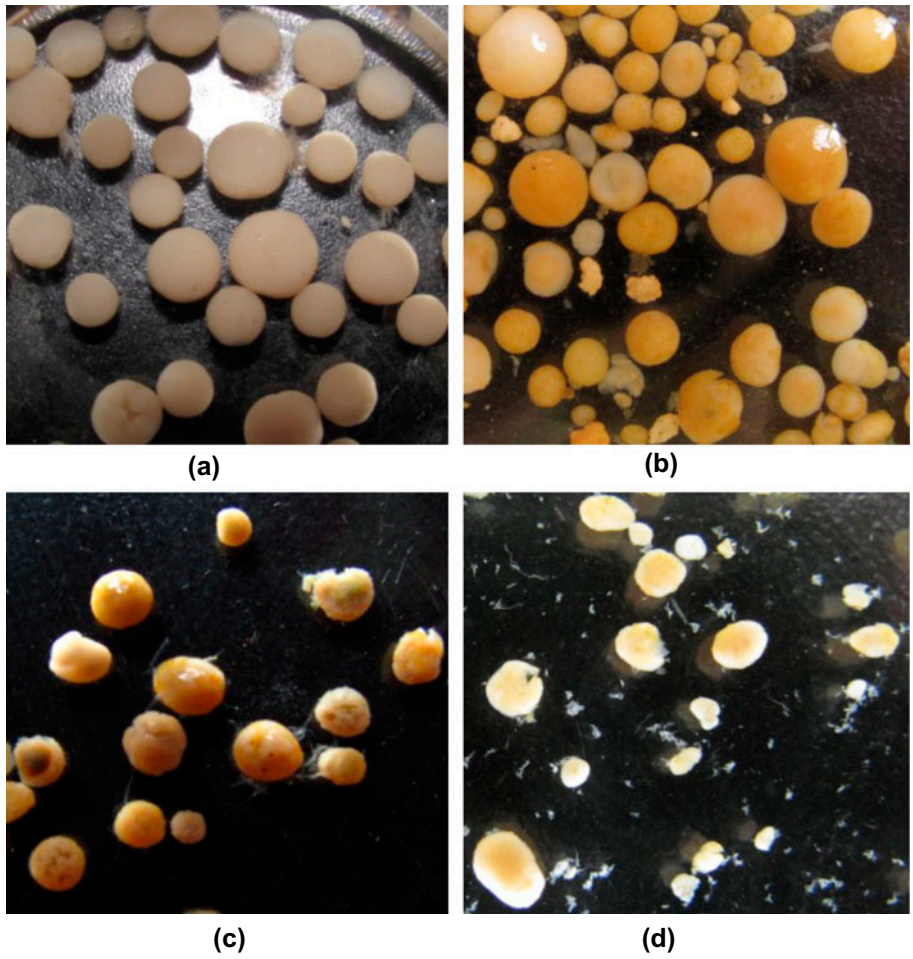


Fig. 4. Variation of AGS appearance in treating PW2 (a) normal AGS, (b) 28 d, (c) 48 d, and (d) 65 d.

shift of influent wastewater is shown in Fig. 5(a). Average influent COD concentration was 980 mg/L using the SW and effluent COD concentration was around 150 mg/L with removal efficiency of about 85%. When PW1 was used with influent COD concentration around 1,500 and 2,000 mg/L, corresponding average COD removal efficiencies were 82.2 and 76.5%, respectively. Therefore, COD removal performance of the system was affected a little, although influent wastewater was change from SW to PW1 and average influent COD concentration increased from 980 to 1,500 mg/L. Simultaneously, COD removal efficiency was still above 75% with influent COD concentration increasing to 2,000 mg/L of further using PW1. The results indicated that AGS formed in the system had relative strong shock load resistance ability to PW1. Average influent COD concentration was controlled at 2,000 and 2,500 mg/L when PW2 was used and corresponding average effluent COD concentrations were 570 and 1,050 mg/L with removal efficiencies of 72.1 and 58.2%, respectively. Effluent COD concentration with influent wastewater varying from PW1 to PW2 increased more obviously than the variation of effluent COD concentration after influent wastewater was changed from SW to PW1. At this moment, AGS appearance was affected badly and total sludge concentration in the BCC decreased obviously which induced the decline of organic pollutant removal performance. Therefore, biotreatment of industrial wastewater containing toxic compounds must be handled carefully, although the AGS has been reported having good toxic compounds resistance ability.

Evolution of ammonium removal in the system using the three kinds of influent wastewaters is shown in Fig. 5(b), along with ammonium removal efficiency. Average ammonium concentration was 90 mg/L in SW with average effluent concentration of 1.8 mg/L. Average ammonium removal efficiency of 98% indicated a good nitrification performance of the BCC.

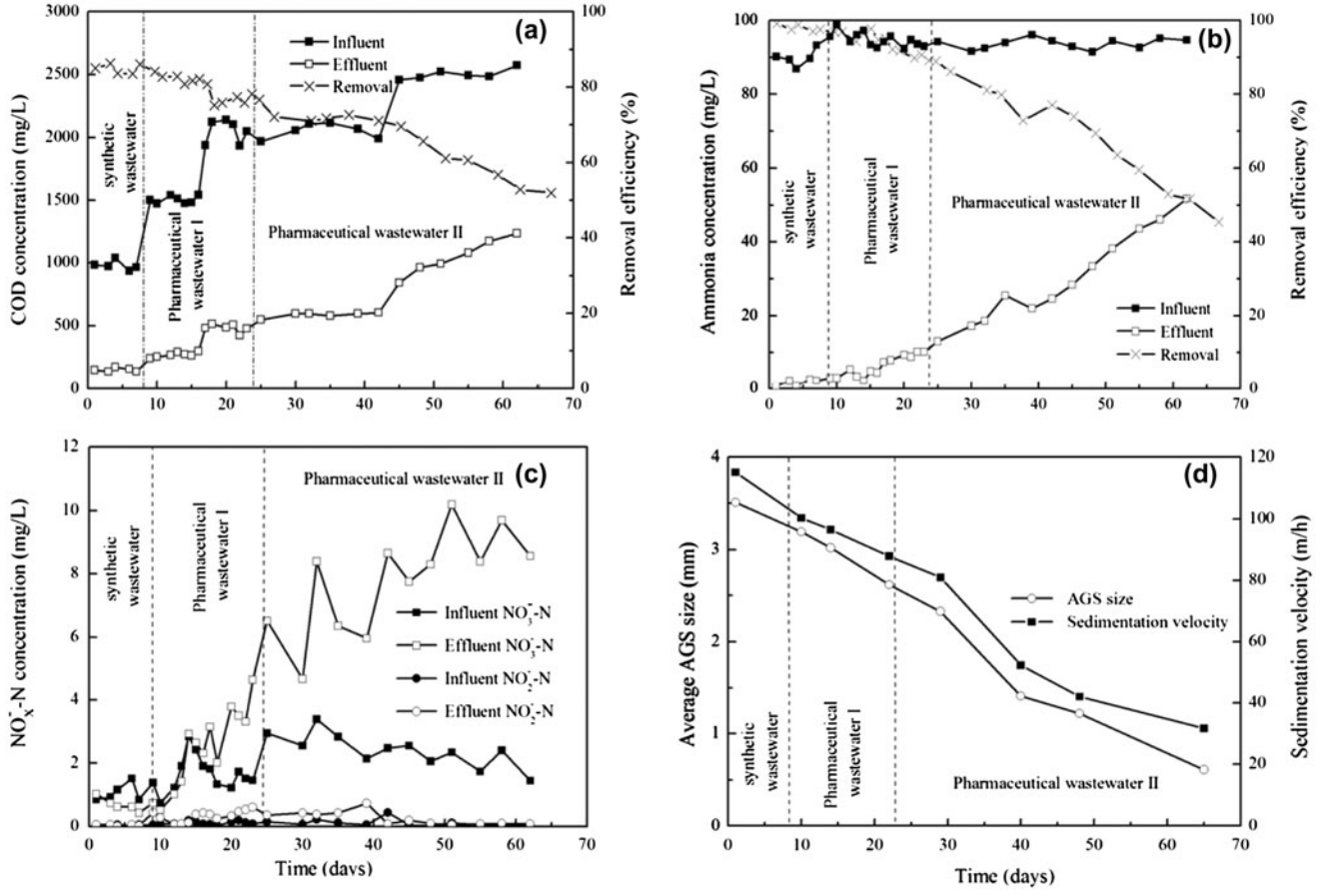


Fig. 5. Variation of COD, ammonia, NO_2^--N and NO_3^--N concentrations using SW, PW1, and PW2 as influent, along with evolution of AGS size and sedimentation performance.

The results also indicated a good nitrifying bacteria retention ability of the formed AGS, with high organic pollutant removal performance simultaneously. Ammonium concentration was around 95 mg/L when PW1 was adopted, and corresponding effluent ammonium concentration varied from 2.3 to 10.2 mg/L with average removal efficiency of 93.8%. The relative lower ammonium removal efficiency using PW1 comparing with SW might be induced by the decrease in AGS size because some of nitrifying bacteria shed from the disintegrative AGS affecting nitrification performance of the AGS. However, ammonium removal efficiency decreased a little, which indicated that the remaining nitrifying bacteria in AGS bore the negative effect of toxic compounds containing in the PW1 mostly. Effluent ammonium concentration varied from 10.2 to 51.7 mg/L with average influent ammonium concentration of 94 mg/L when PW2 was adopted. Ammonium removal efficiency decreased from 89 to 45.4% when the AGS was exposed to PW2. Influent ammonium concentration was similar using the PW1 and PW2 and the sudden increase in ammonium load was not a possible reason for the obvious decrease in ammonium removal rate [28]. Two possible reasons were stated below, one was the extremely negative effect of toxic compounds in PW2 on the activity of nitrifying bacteria, and the other was plenty of nitrifying bacteria being washed out from the bioreactor. Moreover, some studies have been reported to treatment different kinds of PW using the AGS. Shi et al. [29] reported that COD and ammonium removal performance of the AGS declines with the addition of tetracycline in SBR, while COD removal was affected little in treating ofloxacin, norfloxacin, and ciprofloxacin using the AGS in a SBR, as reported by Amorim et al. [30]. The different results obtained above might be due to various substrates and operation conditions. As for the results obtained from this study, COD and ammonium removal were affected differently by addition of PW1 and PW2, which was in accordance with the statement of Shi et al. However, the cf-MFC system would be possible more prevalent than the SBR

process for AGS application in full-scale wastewater treatment process.

In addition, variation of NO_2^--N and NO_3^--N concentrations with the three kinds of wastewaters is shown in Fig. 5(c). Effluent NO_2^--N and NO_3^--N concentrations of the system were always below 1 mg/L using SW as influent wastewater and most of ammonium was removed at this moment. The results indicated that the AGS had a good simultaneous nitrification and denitrification (SND) performance when stable operation condition was achieved. Effluent NO_3^--N concentration increased slowly after influent was changed into PW1 for 5 d, with maximal value of 3.8 mg/L. Average effluent NO_2^--N and NO_3^--N concentrations were 2.3 and 0.3 mg/L after PW1 was adopted, which indicated that SND performance of AGS was affected somewhat, and the most likely reason was that anoxic condition in the inner part of AGS was affected by the reduction in AGS size. Effluent NO_3^--N concentration varied from 4.7 to 10.2 mg/L when PW2 was used and effluent NO_2^--N concentration was relative stable at around 0.3 mg/L. The relative high effluent NO_3^--N concentration would be induced by the disintegration of AGS and the anoxic environment was destroyed, which caused washing out of the denitrification bacteria. Therefore, relative high concentration of NO_3^--N in the effluent of BCC was observed, although nitrification performance of the reactor was limited at this moment.

4. Conclusion

The research studied AGS stabilization in a continued flow bioreactor and variation of the AGS appearance was observed under two conditions, when the AGS stabilization was affected. Moreover, strategy for the AGS bulking affected by filamentous bacteria was stated and verified. The conclusions obtained in the study were listed as follow:

(1) When the AGS bulking by over-growth of filamentous bacteria was observed in the continue flow bioreactor, multi-methods in increasing the OLR, adjusting the pH value, and using relative complicated substrates can be used to inhibit the uncontrolled filamentous bacteria.
(2) Self-collapse of AGS in the experiments was mainly caused by the strong negative effect of toxic compounds in PW2, inducing the death of microbes falling from AGS.
(3) Sedimentation performance of the AGS decreased obviously with the disintegration of AGS, which caused the decline of biomass concentration and pollutant removal performance. The SND disappeared simultaneously in treating PW2 and effective method to recover the self-collapse AGS by toxic compounds needed a further research.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 51308362) and Foundation of Key Laboratory of Special Waste Water Treatment, Sichuan Province Higher Education System (SWWT2014-1).

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