Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.834272

53 (2015) 27–35
January

Taylor & Francis
Taylor & Francis Group

Cultivation and characteristics of micro-aerobic activated sludge with weak magnetic field

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Received 6 April 2013; Accepted 6 August 2013

ABSTRACT

Dynamic experiments were carried out in two reactors with anaerobic sludge as the seed sludge under low dissolved oxygen concentration (lower than 1.0 mg/L) condition, one reactor with magnetic particle and the dosage being 4 g/L determined by the static experiments and the other as the blank control. The synthetic glucose solution was used to culture the micro-aerobic sludge. The results indicated that the reactor with magnetic particles had a higher chemical oxygen demand removal efficiency and mixed liquid suspended sludge than the blank in which no magnetic particles existed. The sludge under the influence of weak magnetic field had better settling property with the average value of sludge volumetric index (SVI) lower than 100 mL/g, while in the reactor without magnetic particles, the sludge had a loose structure, poor setting property, and a SVI value higher than 200 mL/g. The influence of the weak magnetic field on the flocculation capability of the sludge was investigated through the testing of the indexes such as the total dose of extracellular polymers, the ratio of protein and polysaccharides, flocculating ability, relative hydrophobicity, and surface charge. The results showed that the existence of magnetic particles had greatly increased flocculating performance of the sludge.

Keywords: Weak magnetic field; Micro-aerobic; Activated sludge; Cultivation; Magnetic particles

1. Introduction

Aerobic activated sludge process has been widely used in sewage and industrial wastewater treatment for its flexible operation, low operation cost, and no secondary pollution, and so on [1,2]. Through decades of practice, activated sludge process has become a more perfect and mature biochemical technology. But a vexing problem in the wastewater treatment by activated sludge is activated sludge bulking. According to the investigation, there are problems of activated sludge bulking either in the traditional activated

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sludge system or in the biological nitrogen and phosphorus removal process. The reasons for activated sludge bulking are various. One of the most common reasons is excessive growth of filamentous bacteria due to the lack of dissolved oxygen (DO). So in the operating parameters of the process, the control of the DO concentration is critical. It not only affects the sewage treatment effect, but also affects the physical and chemical properties of the activated sludge, especially for the settling characteristics of sludge [3].

Studies have shown that DO concentration should be controlled above 2.0 mg/L for normally activated sludge. Below this value, filamentous bacteria would grow excessively, resulting in poor settling property of the sludge, and more severe, activated sludge bulking would occur [4]. The physicochemical properties such as compressibility, filterability, and settleability got worsen, and turbidity in the effluent increased under low DO concentration [5,6]. Even in the process of denitrification in a bench-scale sequencing batch reactor, it was found that low DO concentration was not only disadvantageous to nitrification, but also disadvantageous to denitrification process [7]. When DO concentrations were lower than 0.09 mg/L, denitrification process was severely inhibited for very low NO_3^- or NO_2^- concentration produced by nitrification process at very low DO concentration. But low DO concentrations were favorable for macromolecules of fat to be degraded to the soluble small molecules, and improved the effluent quality [8].

Generally, in the operated activated sludge, high DO concentration is needed, which not only increases operating costs, but also requires exact aeration devices [9]. And compared with the low DO concentration, in the high DO concentration, the complex organic molecules cannot be degraded with chain scission effectively. However, there is problem of activated sludge bulking in low DO concentrations.

As the integrated use of interdisciplinary technology, magnetic biological effects used in the treatment of wastewater have developed to be a new wastewater treatment technology. When the magnetic field is generated by magnetic Fe_3O_4, the activated sludge settling ability can be improved significantly. The study by Hattori et al. showed that large amounts of magnetic powders enhanced the settleability of activated sludge [10]. Sakai et al. showed that when the dosage of magnetic powders was 2,000 mg/L, the sedimentation rate of activated sludge was 36 times faster than that of without magnetic powder [11], and the liquid-solid separation performance increased [12,13].

Magnetic materials not only have physical effects, but also the magnetic field produced by it has a biological effect. In the appropriate magnetic field intensity, the activity of the activated sludge improved, thus enhanced the pollutant treatment effect in wastewater [14]. But high strength of magnetic field had a negative impact. The studies of Yavuz et al. and Ji et al. showed the same conclusion that the removal efficiency of organic matter increased with the magnetic field intensity first and then decreased [15,16].

Stimulation of the magnetic field on activated sludge mainly showed two aspects, one was the effect of the magnetic field on the growth of microorganisms in the sludge and the other was the effect on the enzyme activity of microorganisms. There had been many reports about the effects of magnetic field on microbial growth, but the unanimous conclusion had not been obtained, mainly because the effects of magnetic field on microbial growth were not only related with the strength and frequency of magnetic field, but also related with the strains [17–19]. The growth rate and activity of majority microbe increased at the appropriate strength and frequency of magnetic field or magnetic dosage [20].

Activation of magnetic field on enzyme has been confirmed by many studies. Compared with no magnetic field, the activities of enzymes such as dehydrogenase, Na, K-ATPase cytochrome oxidase of the microbes increased under low-frequency magnetic field, so COD removal efficiencies improved during the wastewater treatment [21–24]. Even for the immobilized enzymes with magnetic powder, the activities and stabilities kept more higher than that of without magnetic powder [25,26].

In this paper, activated sludge was cultured under micro-aerobic conditions (DO concentration less than 1.0 mg/L), which could be not only reduce the power consumption of the process, saving operating cost, but also make complex organic molecules be effectively degraded. At the same time, the introduction of weak magnetic field produced by Fe_3O_4 not only solved the problem of activated sludge bulking under low concentration of DO, but also increased the activity of the sludge, thereby enhanced the treatment effect of the wastewater.

2. Material and methods

2.1. Materials

Anaerobic sludge from a beer factory was inoculation sludge, and the sludge properties were as follows: moisture content was 90.89%, sludge volume index (SVI) was 38.74 mL/g, mixed liquid suspended sludge (MLSS) was 12.37 g/L, the ratio of volatile suspended sludge and total suspended sludge VSS/TSS was 0.54, the ratio of proteins and polysaccharides (PN/PS) in extracellular polymers (ECP) was 0.19. Domestication of inoculation sludge under micro-aerobic conditions lasted around three weeks using synthetic wastewater whose composition was as follows: glucose 1,600 mg/L, $(NH_4)_2SO_4$ 369 mg/L, KH_2PO_4 140 mg/L, $MgSO_4$ 67 mg/L, $CaCl_2$ 133 mg/L, Na_2CO_3 521 mg/L and the trace elements solution 0.7 ml. The composition of trace elements solution was as follows: $FeCl_3{\cdot}6H_2O$ 1.5 g L^{-1}, H_3BO_3 0.15 g L^{-1}, $CuSO_4{\cdot}5H_2O$ 0.03 g L^{-1}, KI 0.03 g L^{-1}, $MnSO_4{\cdot}H_2O$ 0.10 g L^{-1}, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.065 g L^{-1}, $ZnCl_2$ 0.057 g L^{-1}, $CoCl_2{\cdot}6H_2O$ 0.15 g L^{-1}, $Ni(NO_3)_2$ 0.15 g L^{-1}.

The magnetic powder used in the experiment was Fe_3O_4 of 100 mesh, magnetized before using.

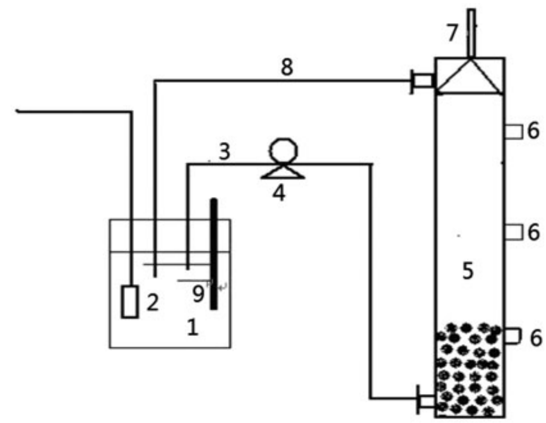

Fig. 1. Experimental equipment diagram. (1) Aeration tank; (2) air diffuser ball; (3) inlet pipe; (4) pump; (5) reactor; (6) sampling valve; (7) three-phase separator; (8) effluent pipe; (9) heater.

2.2. Determination of microbial growth curve

The dosage of magnetic powder had a great influence on the growth of microorganisms and the treatment of wastewater. In this paper, the dosage of magnetic powder was determined by the effect of magnetic powder on the growth curve of microorganisms and the removal efficiency of chemical oxygen demand (COD_{Cr}).

The inoculated sludge domesticated for the activity of anaerobic sludge was low in the environment with sufficient oxygen. And the activity of microorganisms was improved by continuous aeration. Synthetic glucose wastewater containing nutrient salt was added into the inoculated sludge by gradually increased concentration for the purpose of making the inoculated sludge be adapted to wastewater with high COD_{Cr} levels and this process lasted for three weeks.

In the measurement of biological growth curve, the supernatant of domesticated activated sludge was inoculated in the conical flasks with fluid nutrient medium, different amounts of magnetic powders were added into each conical flask. After the bottlenecks were sealed with gauze, the conical flasks were shaking in the constant temperature oscillator with 30℃. The OD600 values with different culture time were determined to construct the microbial growth curve. The composition of the fluid nutrient medium was as follows: beef paste 5 g/L, peptone 10 g/L, NaCl 5 g/L, pH = 7.0–7.2.

2.3. Micro-aerobic activated sludge cultivation experiments

The micro-aerobic activated sludge cultivation experiments were carried on in the apparatus shown in Fig. 1. Two sets of equipments were used in the experiments and magnetic powder was added into one of them, the other one used as blank control. The experimental apparatus was mainly comprised of aeration tank and the reactor made by organic glass. The inner diameter and the height of aeration tank were 250 and 350 mm, respectively, and that of reactor were 60 and 700 mm, respectively. The inoculated sludge volume was 10% of the reactor volume (v/v). The wastewater was first fed into the aeration tank in which aeration was carried out via a porous air diffuser ball, and then pumped into the reactor from the bottom. A solid-liquid-gas separator was placed on the top of the reactor to prevent the outflow of suspended sludge. The wastewater recycled into the aeration vessel from the top of the reactor, and pumped into the reactor again, at last discharged through the effluent valve. The DO concentration was controlled within 1.0 mg/L, the temperature was 25 ℃, and pH was 7.2. The treatment cycle was 24 h.

2.4. Analytical methods

The pH was measured by pH analyzer of Sension 6 (HACH, US); DO concentration was measured by DO analyzer of Sension 6; COD_{Cr} was measured by portable water quality analyzer (DR2700, made by HACH company, US). SVI, TSS/VSS (total suspended solids/volatile suspended solids) were measured using standard methods [27]. A phenol-sulfuric acid method was used to quantify PS and PN were measured by Folin spectrophotometry [28].

Surface charge (SC) was determined by colloid titration method [29]. 1 mL sludge sample was taken into the conical flask and diluted to 100 mL, then 5 mL of poly condensate amine was added, 0.001 N polyvinyl alcohol potassium sulfate (PVSK) was used for titration with toluidine blue as an indicator. The titration ended when the color from blue to pink when electric neutrality reached. Here the consumption of PVSK volume was marked as *A*, and PVSK consumption of the bland replacing polybrene with same volume of distilled water was signed as *B*.

SC (ueq/L) = (A − BN (1,000))/1 where N was equivalent charge of the PVSK.

Relative hydrophobicity (RH) was determined by means of the method in the reference documents [30]. Two sludge samples of 30 mL were taken, one was washed by Tris buffer solution (pH = 7.1, 0.05 mmol/L), and was shaken for 2 min with ultrasound (50 W) in 4℃ water bath to make sludge flocs disperse into single cell. Then the uniform suspended sludge turbid liquid was transferred to a separating funnel together with 15 mL n-hexadecane. The two-phase of solid and liquid was separated after 30 min of shaking, and then the aqueous phase was cleared away, the concentration of the remaining solid phase was measured signed as MLSSe. Another sludge sample was untreated and its concentration was marked as MLSSi. So:

$$\mathrm{RH}(\%) = (1 - \mathrm{MLSSe}/\mathrm{MLSSi}) \times 100\%$$

Flocculation ability (FA) was determined by means of the method in the reference documents [31]. About 80 mL sludge sample of 4 g/L was taken into the beaker, shaking for 15s by ultrasound vibration (50 W) in the ice bath. Then 10 mL sludge was taken for centrifugation with 1,200 r/min for 2 min, and the absorbance of supernatant at 650 nm was measured and signed as A. The remaining sludge in the beaker was placed on a magnetic stirrer and mixed for 10 min at low speed, then static settled for 15 min, the absorbance of supernatant at 650 nm was measured marked as *B*. So:

$$\mathrm{FA}(\%) = (1 - B/A) \times 100\%$$

3. Results and discussion

3.1. The appropriate dosage of magnetic powder

3.1.1. Effect of the dosage of magnetic powder on the growth curve of microorganisms

Effect of magnetic powder on the microorganisms growth curves was shown in Fig. 2. Fig. 2 shows that the microorganisms without magnetic powder achieved the logarithmic growth phase at the 9th hour, and the microorganisms achieved the logarithmic

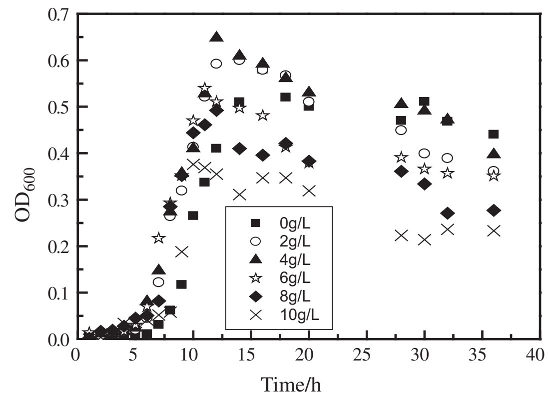

Fig. 2. The growth curves of microorganisms at different magnetic powder dosage.

growth phase at the 6th, 5th, 7th, 8th hour, respectively when the magnetic powder dosage was 2, 4 and 6, 8, 10 g/L. It was indicated that the lag phase was shortened with magnetic powder addition. The OD_{600} value of the microorganisms reached the maximum value (0.648) at the 12th hour when the magnetic powder dosage was 4 g/L. When the magnetic powder dosage was lower than 4 g/L, the maximum OD_{600} value increased with the increasing magnetic powder dosage for the benefit of the magnetic powder to the microorganisms growth. When the magnetic powder dosage was higher than 4 g/L, the maximum OD_{600} value decreased with the increasing magnetic powder dosage, indicating a slight inhibition to the microorganisms growth. Fojt et al. [32] found that the colony forming units of bacteria (*E.coli*, *L. adecarboxylata* and *S. aureus*) increased obviously with an appropriate magnetic field intensity, which indicated that magnetic field had a stimulative effect on the growth of *E.coli*, *L. adecarboxylata*, and *S. aureus*. That was consistent with our research conclusions. So, the results indicated that 4 g/L was the optimal for the microorganisms growth.

3.1.2. Effect of the dosage of magnetic powder on the removal efficiency of COD_{Cr}

The appropriate dosage of magnetic powder should be further revised by COD_{Cr} removal efficiency. Effect of magnetic powder dosage on COD_{Cr} removal efficiency was shown in Fig. 3.

Seen from Fig. 3, COD_{Cr} removal efficiency increased to a maximum value and then decreased with increasing magnetic powder dosage. The maximum value of 83.53% was reached when magnetic powder addition was 4 g/L. According to Figs. 2 and 3, the magnetic powder dosage was determined to be 4 g/L.

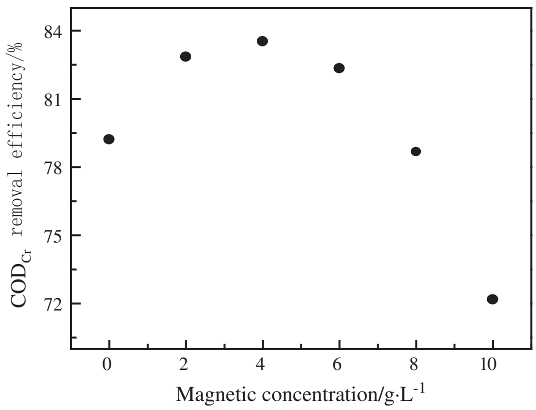

Fig. 3. The removal efficiency of COD_{Cr} at different magnetic powder dosage.

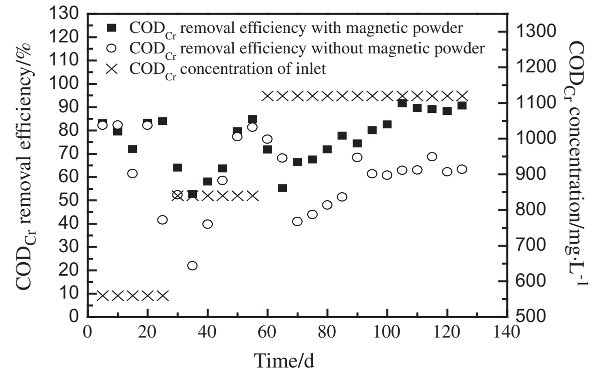

Fig. 4. Changes of COD_{Cr} removal efficiency during cultivation.

3.2. The cultivation of micro-aerobic activated sludge

3.2.1. The operation of the reactor

Anaerobic sludge should be domesticated when it entered into a micro-aerobic environment for its low shock resistance against COD_{Cr}. The concentrations of sludge inoculated in two reactors (experimental group and controlled group) were the same (10 g/L), but the experimental group was added with magnetic powder (4 g/L) and the controlled group was without magnetic powder. During the domestication process, the pH value was controlled at the range of 6.5–8, the concentration of DO was controlled at 0.6 mg/L and the synthetic wastewater with low concentrations was used. The color of the sludge became brown from dark black which indicated that the anaerobic sludge was changed to be micro-aerobic activated sludge after 5 days, and the wastewater concentration gradually increased from the 6th day on, until the glucose concentration reached 1600 mg/L at the 50th day.

Three layers appeared in the reactor with magnetic powder during the cultivation due to the force of the up-flow fluid and gravity. The floc was big and compact in the lower layer, loose in the middle layer was and small in the upper layer that was easy to be washed out from the reactor. Separation of sludge and water was appeared at the initial period in the reactor without magnetic powder, the floc became loose and pale yellow and easy to be washed out from the reactor with the culture time.

3.2.2. Changes of COD_{Cr} removal efficiency during the cultivation

The changes of COD_{Cr} removal efficiency with time were shown in Fig. 4. It showed that the COD_{Cr} removal efficiency in the reactor with magnetic powder was higher than that of the reactor without magnetic powder. The COD_{Cr} removal efficiency in the reactor without magnetic powder decreased faster than that of the reactor with magnetic powder with time. The COD_{Cr} removal efficiencies decreased to 21.86 and 52.59% in the reactors without and with magnetic powder, respectively on the 35th day when the influent concentration of COD_{Cr} was 840 mg/L.

When the concentration of COD_{Cr} was 560 mg/L at the initial period, the COD_{Cr} removal efficiencies in the reactors with and without magnetic powder were all relatively high in the early 10 days due to the adsorption of organics by sludge. The COD_{Cr} removal efficiency in the two reactors decreased, and that of the reactor without magnetic powder decreased especially faster with the culture time. The COD_{Cr} removal efficiencies in the two reactors increased and that of the reactor with magnetic powder increased especially faster for the increasing numeral of the aerobic microorganisms as well as the improvement in the activity of microorganisms. The microorganisms experienced another shocked-adapt-stable stage from the 26th day on when the concentration of COD_{Cr} became 840 mg/L, the COD_{Cr} removal efficiency decreased first and then increased, consequently. The COD_{Cr} removal efficiency in the reactor with magnetic powder was stable and kept at 90%, while that of in the reactor without magnetic powder was 60% after the concentration of COD_{Cr} became 1120 mg/L on the 55th day.

All of the aerobes, facultative anaerobes and anaerobes existed in the reactors for both anaerobic and aerobic microenvironments in the micro-aerobic sludge. Under anaerobic conditions, organic acids were produced by the fermentation of glucose and then methane and hydrogen sulfide were formed by the function of methanogen and sulfate reduction bacteria. Under aerobic conditions, pyroracemic acid was produced by the fermentation of glucose and then entered into the tricarboxylic acid cycle. Dehydrogenase took an important role during the degradation of glucose, no matter under anaerobic or aerobic conditions. It was reported that suitable magnetic field strength could improve the activity of dehydrogenase. Ma et al. [26] reported that the dehydrogenase activity of PSB improved about 30% when the magnetic powder dosage was 1.2 g/mL, temperature was 30–40℃, and pH was 8. And the dehydrogenase activity improved about 20% even after it was kept at 4℃ for 45–21 days. On the other hand, magnetic powder improved the growth of microorganisms. Those were the reasons why the COD_{Cr} removal efficiency of the reactor with magnetic powder was higher than that of the reactor without magnetic powder.

3.2.3. Changes of the physicochemical characteristics of sludge during the cultivation

The DO concentration should be higher than 2.0 mg/L to avoid activated sludge bulking in the culture of aerobic microorganisms. The effect of magnetic powder on sludge bulking phenomenon was studied under the condition that the concentration of DO was lower than 1.0 mg/L. Fig. 5 showed the changes of SVI which reflected the degree of sludge bulking with the culture time. It showed that SVI increased with the culture time. The maximum value of SVI in the reactor with magnetic powder was 93.21 mL/g which indicated the sludge had a good performance of sedimentation. The maximum value of SVI in the reactor without magnetic powder was 267.12 mL/g which indicated a phenomenon of sludge bulking.

Only the sludge with a good performance of sedimentation could guarantee the treatment efficiency of the activated sludge. Magnetic powder could absorb the pollutants and zoogloea in the wastewater for its large superficial area. The concentration of sludge in the reactor with magnetic powder was higher than that in the reactor without magnetic powder for the benefit of magnetic powder to the growth of microorganisms and preventing microorganisms from being washed out from the reactor for its absorption property. Also magnetic powder improved the settling performance of the sludge and prevent the sludge bulking [10,11]. Filamentous organisms grew rapidly which caused the sludge become loose in structure and weak in settling performance in the micro-aerobic and without magnetic powder environment [4].

The concentration of sludge was an important indicator for the activated sludge in the reactor. Fig. 6 shows the changes of the MLSS during the culturing. It showed that MLSS increased as a whole in the reactor with magnetic powder and decreased as a whole in the reactor without magnetic powder. Those tendencies might be caused by two reasons, one was the absorption of magnetic powder; the other was that the benefit of magnetic powder to the growth of microorganisms. The activity of microorganisms in the reactor without magnetic powder would be lower and viscous polysaccharide would be less when the sludge was shocked by more and higher concentration wastewater, so the sludge flocs in the reactor without magnetic powder were easy to be washed out from the reactor.

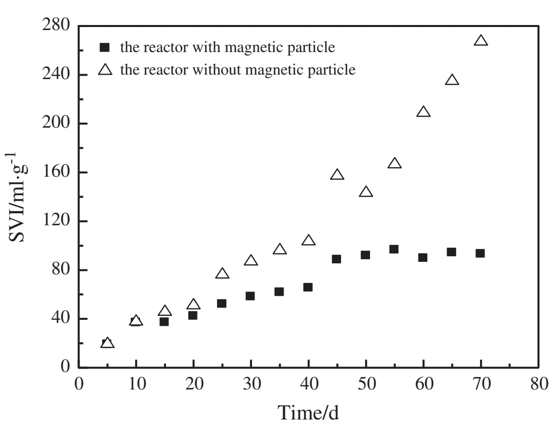

Fig. 5. Changes of SVI during the cultivation.

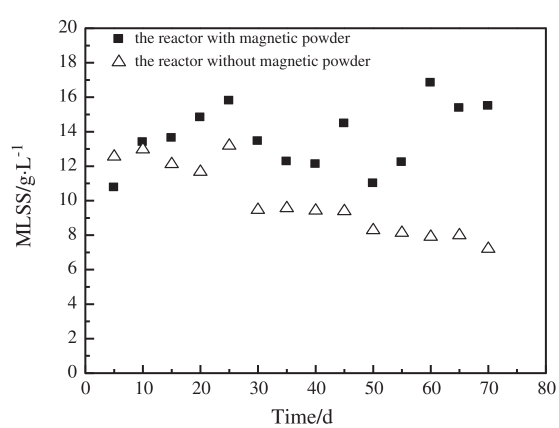

Fig. 6. Changes of MLSS during the cultivation.

3.2.4. Changes of ECPs during the cultivation

PN and PS were the main components of ECPs which could reflect the flocculation performance of sludge [33]. Fig. 7 shows the effect of magnetic powder on the amount of extracellular polymers. It showed that in the reactor with magnetic powder, the amount of ECPs increased from 18.48 to 23.91 mg/L along with the culture time. ECPs, which could be affected by magnetic powder, were the substances secreted by microorganisms. The amount of ECPs changed little in the reactor without magnetic powder.

The value of PN/PS affected the surface property and physicochemical properties of sludge [34]. Fig. 8 showed the changes of PN/PS during culturing. It showed that in the reactor with magnetic powder, the values of PN/PS increased rapidly and finally kept stable. While in the reactor without magnetic powder, the values of PN/PS increased first and then decreased. The values of PN/PS in the reactor with magnetic powder were all larger than those in the reactor without magnetic powder.

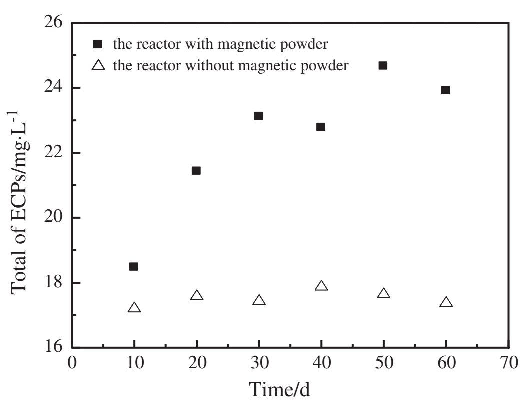

Fig. 7. Changes of the total of extracellular polymers during the cultivation.

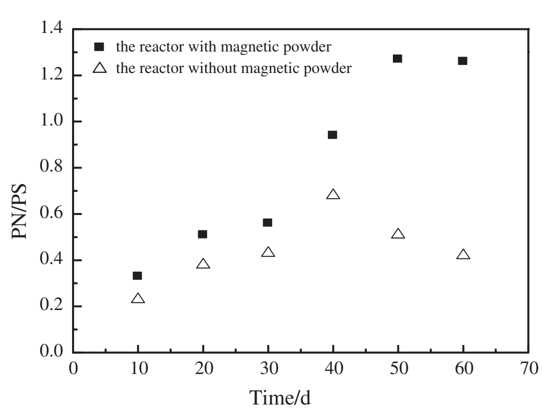

Fig. 8. Changes of PN/PS during the cultivation.

It was reported that the hydrophobic property of protein was stronger than that of polysaccharide, and it was the increase of protein that caused the decrease of detained water in sludge and the decrease of SVI values. Proteins have a stronger ability to bond with cation than polysaccharide, so proteins could form a three-dimensional structure through the bridging action of cation, which can benefit for the stability of sludge [35–37]. It was deduced that the improvement of sludge in sedimentation property and flocculation property was caused by the increase of protein content in ECPs under the effect of magnetic powder.

3.2.5. Changes of the surface property of sludge during the cultivation

It was reported that the flocculation property of sludge was not only connected with ECPs but also connected with the surface property of sludge, such as SC and RH [38].

A high RH was good for the flocculation property of sludge and the separation of sludge and water. Fig. 9 shows the changes of RH during the culturing. It showed that in the reactor with magnetic powder, RH gradually increased with the culture time. While in the reactor without magnetic powder, RH increased first and then decreased at the 40th day.

Activated sludge was electronegative because of the anionic groups such as –COOH and –OH on the surface of sludge. According to the DLVO theory, the increase of SC caused increase in the electrostatic repulsion, so the flocculation property of sludge was weak for its large value of SC. Fig. 10

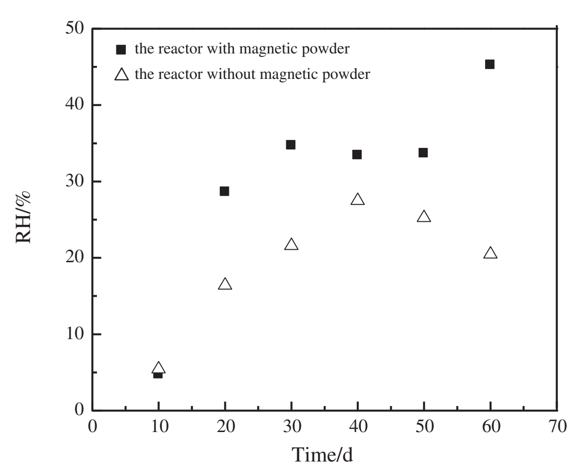

Fig. 9. Changes of RH during the cultivation.

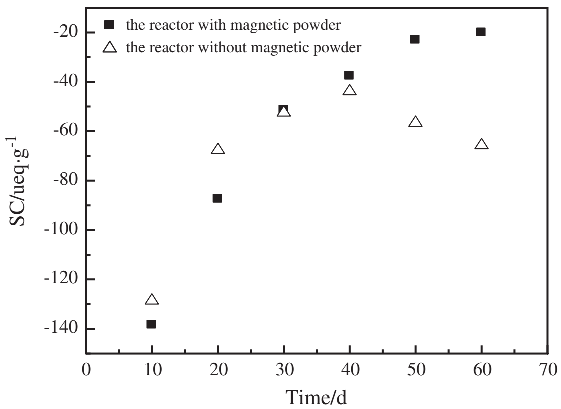

Fig. 10. Changes of SC during the cultivation.

shows the changes of SC during the culture process. It showed that in the reactor with magnetic powder, the negative charge on the surface of sludge decreased rapidly and finally kept stable with the culture time. While in the reactor without magnetic powder, the negative charge on the surface of sludge decreased first and then increased with the culture time.

Fig. 11 showed the changes of FA during culturing. It showed that the values of FA increased with the culture time and the value of FA was 79.42% on the 60th day in the reactor with magnetic powder. The values of FA increased first and then decreased, and the value of FA was 38.47% on the 60th day in the reactor without magnetic powder. It indicated that magnetic powder could improve the FA of sludge obviously.

Collectively, the SC decreased, the RH increased, and the FA of sludge improved by the addition of the magnetic powder.

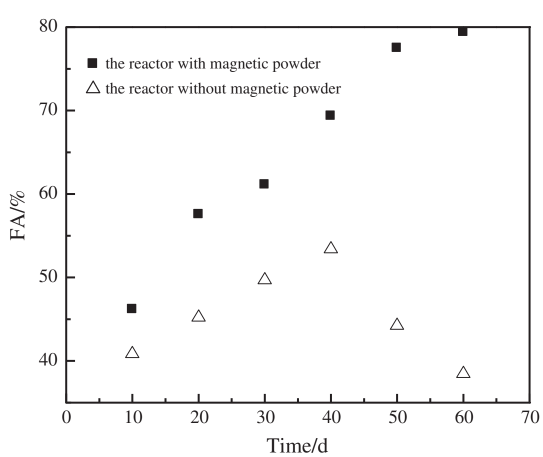

Fig. 11. Changes of FA during the cultivation.

4. Conclusions

(1) In the static experiments, the optimal magnetic powder dosage for the micro-aerobic activated sludge was 4 g/L, under which the stimulation to the growth of microorganisms occurred and the COD_{Cr} removal efficiency reached to 83.53%.
(2) In the dynamic experiments, the COD_{Cr} removal efficiency of the reactor with magnetic powder (90%) was much higher than that of the reactor without magnetic powder (60%).
(3) The magnetic powder was a benefit for the sedimentation property of sludge. The values of SVI in the reactor with magnetic powder were lower than 100 mL/g and the values of SVI in the reactor without magnetic powder were about 200 mL/g. Under the micro-aerobic condition, the sludge kept good settling ability during the whole cultivation by the addition of magnetic powder, and no sludge bulking phenomenon occurred, while in the blank control reactor, the sludge structure was loose, the settling ability was poor, and serious sludge bulking occurred. The values of MLSS and VSS/TSS in the reactor with magnetic powder were obviously higher than those in the reactor without magnetic powder.
(4) The surface property, flocculation property, and sedimentation property of the sludge improved by the addition of the magnetic powder.

Acknowledgments

This work was supported by the Open Fund of Jiangsu Provincial Key Laboratory of Pulp and Paper Science and Technology (201314), the Qingdao Municipal Science and Technology Plan Project (12-1-4-3-(28)-jch), the Industry-university-research Cooperation Project of Guangdong Ministry of Education (2012B091100496) and Dongguan Municipal Science and Technology Plan Project (2011108102005).

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