JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Civil Engineering

Research Article

Effect of Organic Loading and DO on Stability of Hypersaline Aerobic Granular Sludge

Miaomiao Dong[1*], CHEN Yao[2], HUANG Peng-cheng[1], ZHANG Zhi-min[1], YU Gang[1]

[1]*School of River and Ocean Engineering, Chongqing Jiaotong University, Chongqing, 400074, China.*

[2]*National Inland Waterway Regulation Engineering Research Center, Chongqing Jiaotong University, Chongqing, 400074, China*

**Corresponding author: Miaomiao Dong, Chongqing Jiaotong University, No.66 Xuefu Avenue, Nan'an District, Chongqing, China, Tel: 13618386050; Email: dmm201309@sina.com*

Received: 07-31-2015

Accepted: 08-12-2015

Published: 08-15-2015

Abstract

In order to investigate the effect of organic loading and dissolved oxygen (DO) on the characteristics of hypersaline aerobic granular sludge in some sequencing batch reactors (SBR), the indicators of sludge were analyzed such as the sludge volume index (SVI), minimal sedimentation rate, dehydrogenase activity, extracellular polymeric substances (EPS) component and size distribution of granule. Results indicate that the effect of organic loading and DO on the stability of aerobic granular sludge is significant. The organic loading which is over low or over high does not go against the stability of granular sludge. Over high organic loading can inhibit the growth of granular sludge and decrease the dehydrogenase activity and the content of extracellular proteins (PN), which cause granular sludge disintegrate and worse stability of granular sludge. The content of EPS in granular sludge achieves maximum under the organic loading of 5.4 kgCOD•(m3•d)-1, and the EPS is given priority to with extracellular proteins, resulting in the larger of average particle size, uniform of size distribution, thus the granular sludge displays a good stability. Over low DO can easily trigger filamentous bacteria breeding, and which can cause that granular sludge is unstable, increasing DO concentration can promote the growth of granular sludge, and the size distribution can be more concentrated, but over high DO can affect the stability of the system, because providing over high hydraulic shear force can lead to granular sludge disintegrating. When the reactor is controlled under DO of 6mg•L-1, the EPS is mainly with extracellular polysaccharide.

Keywords: hypersaline; aerobic granular sludge; stability; organic loading; dissolved oxygen

Introduction

Aerobic granular sludge is a special kind of biofilm, which is formed by cell self-immobilization in aerobic conditions. It shows good settle ability, which indicates that using aerobic granular sludge can reduce the volume of reactor, shorten the process operation cycle and improve the processing efficiency, especially in sequencing batch reactor. It has high biomass, so the process has higher capacity in resistance to shock loading and can bear higher organic loading. It is regarded as the ideal subject to realize the integrated treatment of biological nutrient in wastewater for its abundant microbe phase and diversified forms in metabolism [1]. Therefore, aerobic granular sludge is particularly suitable for treating high concentration industrial wastewater, and it has shown good stability and high treatment efficiency when applied in industrial wastewater disposal such as malt wastewater, dairying wastewater, brewery wastewater, slaughter wastewater and other industrial wastewater [2]. However, aerobic granular sludge is often prone to instability in the reactor operation process because of changes in operating conditions and which can also become the technical bottlenecks of engineering application of aerobic granules in industrial wastewater treatment. The main influence factors on the formation and stability of aerobic granular sludge are organic loading [3], extracellular polymeric substances (EPS) [4], dissolved oxygen concentration [5,6], carbon source and the ratio of C/N [7], temperature [8], shear stress [9], etc.

The hypersaline wastewater is one of the main sorts of industrial wastewater, which is hard to treat with the general physical and chemical methods, and the treatment cost of which is huge, so it is hard to treat in wastewater treatment field [10]. High salinity has a stronger inhibition on the growth of the general microbiology, mainly the high osmotic pressure destroys the cell membrane of microbiology and the enzyme in the thallus in hypersaline environment, thus the physiological activities of microbiology may be destroyed, and which may inhibit and poison the biological treatment [11]. So it is of great significance to explore an economic and efficient biological treatment process in hypersaline wastewater treatment field.

For now, research on application of aerobic granular sludge in hypersaline wastewater treatment has been rarely reported, and the key problem in engineering application which must be solved is how to control the stability of aerobic granular sludge during operation under high salt stress. Therefore, the treatment of hypersaline mustard tuber wastewater (salinity 3%, calculated in NaCl, the same as below) was investigated in SBRs inoculating with hypersaline aerobic granular sludge which cultivated successfully in the laboratory, the purpose of this experiment is to investigate the effect of organic loading and DO on the characteristics of hypersaline aerobic granules in some SBRs, and establish foundation for the study of the treatment of hypersaline mustard tuber wastewater which comes from mustard tuber industrial plants, the indicators of sludge were analyzed such as the SVI, minimal sedimentation rate, dehydrogenase activity, EPS component and size distribution of granules.

Materials and Methods

Experimental setup

Four identical SBR reactors with a working volume of 1.9 L were used in the experiment, which made of polyvinyl chloride (PVC), namely R1, R2, R3 and R4. The internal diameter of the reactor was 4.8 cm, the working height was 105 cm, and the working H/D was about 22. The volume exchange rate was identified by the requirement of influent loading. The Aeration device, fed by compressor, was set at the bottom of the reactor. And the aeration rate was decided by different needs of dissolved oxygen (DO) in different experimental phase. The reactor operation cycle was 12 h, including 2 min of influent filling, 5 min of settling, 3 min of effluent discharging and remainder of aeration. The reactor temperature was controlled in (25±1) ^{0}C by heating rod.

Water quality and seed sludge

A synthetic analogue hypersaline wastewater with the following composition was used in experiments [12,13]: salinity, 3%; COD, 4500 mg·L^{-1}; NH_4^+-N, 225 mg·L^{-1}; PO_4^{3-}-P, 25 mg·L^{-1}; Ca^{2+}, 100 mg·L^{-1}; Mg^{2+}, 130 mg·L^{-1}; $FeSO_4·7H_2O$, 0.10 mg·L^{-1}; $ZnCl_2$, 0.10 mg·L^{-1}; $MnCl_2·4H_2O$, 0.14 mg·L^{-1}; molybdate, 0.09 mg·L^{-1}; $CuSO_4·5H_2O$, 0.03 mg·L^{-1}; and pH, 7.0~7.5.

Experimental seed sludge was hypersaline aerobic granular sludge, which was formed in 3% salt stress concentration with flocculent sludge and stored in low temperature before finally recovered by slowly increasing the influent organic loading. The characteristic of sludge is shown in table 1.

Table 1. Key indicators of seed sludge.

Project	indicators
appearance	deep yellow
average particle size/（mm）	0.99
SVI /（mL·g^{-1}）	34.2
minimal sedimentation rate /（m·h^{-1}）	3.83
dehydrogenase activity /（ugTF·g^{-1}SS·h^{-1}）	38.1

Experimental methods

The experiment was divided into two stages, and parallel test was used in the two stages. Aerobic granular sludge can realize graining when the influent organic loading is in the range of 2.5 to 15.0 kgCOD·(m^3·d)$^{-1}$, and the organic loading which is over low or over high does not go against the stability of granular sludge. So the organic loading of R1, R2, R3 and R4 were controlled at 3.6, 4.5, 5.4 and 6.3 kgCOD·(m^3·d)$^{-1}$ respectively through controlling volume exchange rate in the first stage, and the stability of granular sludge was investigated. Under the best organic loading conditions identified in the first stage, the stability of granular sludge was investigated in different DO, namely 4.0, 5.0, 6.0 and 7.0 mg·L^{-1} respectively. The indexes of sludge characteristics were measured during the experiment, such as SVI, the minimal sludge sedimentation rate, EPS content and components, sludge dehydrogenase activity and granular sludge particle size distribution.

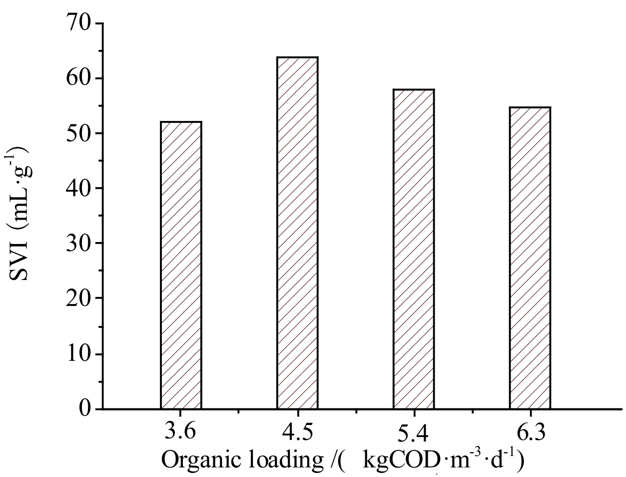
Analytical methods

The morphology of granular sludge was observed by BA210 microscope, and the distribution of particle size was described by Sauter formula [14]; the dehydrogenase activity of sludge was measured by TTC reduction; EPS was extracted by ion exchange resin [15], and it's protein (PN) and polysaccharides (PS) was measured by Folin-phenol reagent method and anthrone reagent method respectively; DO was measured by using HACH HQ30D dissolved oxygen meter, and other indexes were measured according to standard methods.

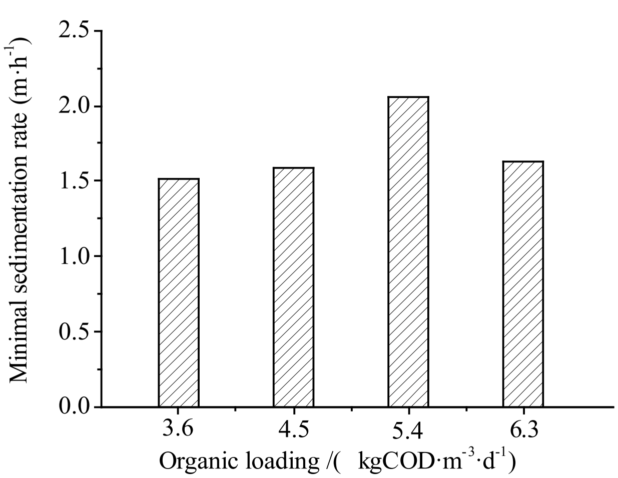
Results and Discussion

Organic load impact on the stability of aerobic granular sludge

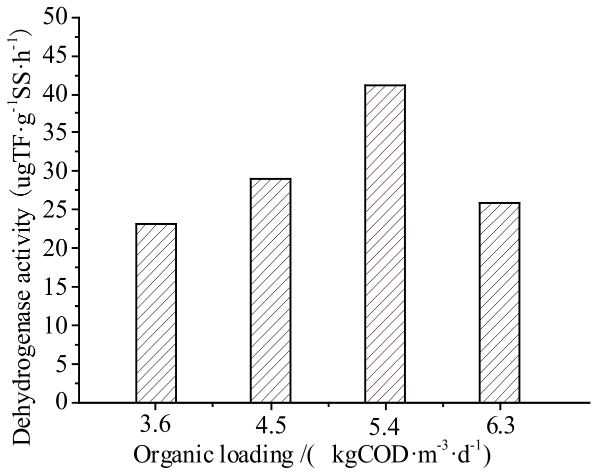
The indexes of the characteristics of sludge are shown in Fig 1 when the reactor ran stably under the condition of different organic loading.



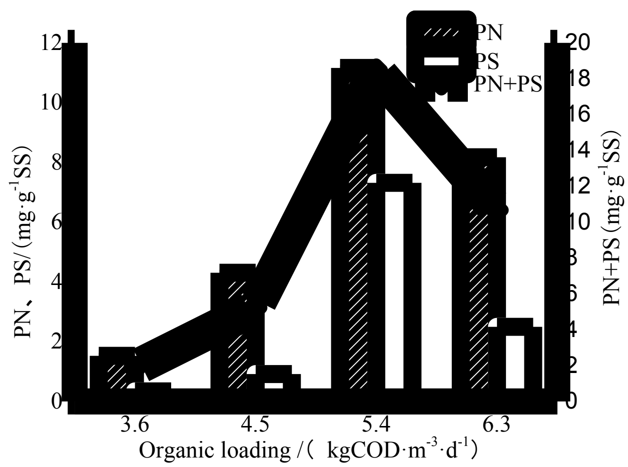
(a) SVI



(b) Minimal sedimentation rate



(c) Dehydrogenase activity



(d) EPScomponent

Figure 1. Effect of organic load on sludge characteristics.

As shown in Fig. 1 (a), (b) and (c), the settle ability and the dehydrogenase activity of sludge first increased and then decrease with the increase of organic loading. Analysis of the results believed that the microorganisms in the granular sludge grew and reproduced quickly with the increase of organic loading, and the particle size increased constantly. Meanwhile, high organic loading could help to overcome the mass transfer resistance, so as to show the settle ability and the dehydrogenase activity of sludge increased. However, when the organic loading is over high, the over high growth rate of the microorganisms could lead to the particle size over large increase, the substrate mass transfer was affected, the particles kernel began to break down, and these could lead to the particle density and mechanical strength lower. Meantime under the condition of the same concentration of DO, over large particle size could result in appearing anaerobic area in the particles which could produce gas, finally the granular sludge disaggregated. So it is shown that the settle ability and the dehydrogenase activity of sludge gradually decreased when the organic loading increased from 5.4 to 6.3 kgCOD·(m3·d)$^{-1}$.

As shown in Fig 1. (d), the total EPS content increases along with the increase of organic loading, which indicates that EPS was the key factor of effecting the stability of aerobic granular sludge. Shen et al [16] found that with the increase of organic loading, microorganism in sludge could not timely use the entire carbon source to maintain their growth and metabolism when reactor under such relatively high organic loading, and the excess carbon source was transferred to intracellular for storing or to EPS result in increasing the EPS content. Meanwhile, the characteristics of microbial metabolic were shown as producing more EPS to connect the floc-bacteria in the granular sludge for maintaining the structure of sludge particles under higher organic loading. And it could prompt the increase of sludge surface hydrophobicity when the content of PN in the EPS increased from 1.53 to 11.16 mg·g^{-1}, and the granules were more stable. Although the content of PS also increased from 0.34 to 7.32 mg·g^{-1}, related studies showed that hydrophobic part of EPS consisted of extracellular protein, and had nothing to do with polysaccharide [4,16]. The sludge might disintegrate for over larger particle size under over high organic loading, which might lead that the content of PS and PN in EPS decreased, especially the PS content decreased significantly. The reason for this phenomenon is that the polysaccharide EPS was the articulated skeleton of aerobic granular sludge [17], and the content of PN decreases could result the skeleton gradually disappearing, which shown that the organic loading was over high and granules disintegrated. Meanwhile, under the condition of high loading, the reducing of the secretion of PN could reduce the surface hydrophobicity of sludge, and then affected the stability of structure of aerobic granular sludge.

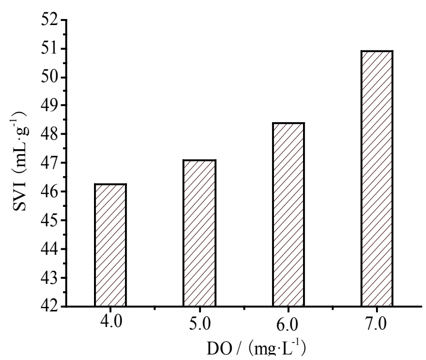
The sludge particle size represents the activity and stability of granular sludge, and which has a significant impact on the sludge sedimentation ability, mass transfer characteristics and biological activity. Under the condition of different organic loading, the result of the particle size distribution of granular sludge showed that R1 had smaller particle diameter in the whole particles, the average particle diameter was 0.89 mm, and 52% was less than 1mm; average particle diameter of R2 was 0.99 mm, 63% mainly distributed between 0.77 and 1.2 mm; R3 and R4 had relatively large particle diameter, the average particle diameter were 1.28 mm and 1.3 mm respectively, the particle diameter of R3 was mainly concentrated between 0.99 and 1.53 mm, accounting for 65%, and 20% between 1.5 and 2 mm, the particle diameter of R4 was mainly distributed between 0.95 and 1.3 mm, accounting for 64%, and 10% between 1.5 and 2 mm. It can be seen that the distribution of particle diameter has some differences under different organic loading. The particle size distribution of granular sludge obeyed normal distribution under stable running, the dispersion of particle and average particle diameter showed a positive correlation. It was analyzed that the proliferation rate of granular sludge could increase with the increasing of organic loading in a certain range, and which caused the particle diameter increasing accordingly. But when the influent loading increased from 5.4 to 6.3 kgCOD·(m^3·d)$^{-1,}$ the average particle diameter increased slowly, and then the largest particle diameter proportion declined. Aday et al. found that this was because the high microbial growth rate lead particle diameter increasing too much which affected the substrate mass transfer, and then the sludge disintegrated [18].

Effect of DO concentration on the stability of aerobic granular sludge

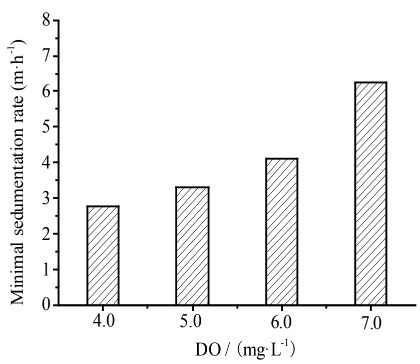
The indexes of the characteristics of sludge were shown in Fig 2 when the reactor ran stably under the condition of different DO.

As shown in Fig. 2 (a) and (b), the settle ability of sludge became better along with the increasing of DO concentration. The value of SVI increased from 46.26 to 50.89 mL·g^{-1}, and the minimum sedimentation rate increased from 2.77 to 6.25 m·h^{-1}. It indicated that over low DO could make oxygen supply in short supply in sludge, which easily lead to the appearing anaerobic metabolism inner particles and the propagation of filamentous bacteria, and then lead to granular sludge unstable [19]. At the same time, the increase of DO concentration was conducive to the propagation of microbes in the aerobic granular sludge in a certain range, and the growth and propagation of filamentous bacteria could be inhibited under higher concentration of DO. The expansive and small surface density fungus was easily eluted out of reactor under high hydraulic shear stress produced by aeration, remaining granular sludge with large density and good settle ability. Fig. 2 (c) indicated that the dehydrogenase activity of granular sludge increased with the increase of DO concentration, from 23.3 to 45.5 ugTF·(gSS· h)$^{-1}$, because the higher concentration of DO promoted the growth of the microorganisms. But over high concentration of DO will cause the needs of power consumption increasing, at the same time the high shear stress could cause granular sludge disintegration. Therefore, the control of DO concentration should be considered synthetically.

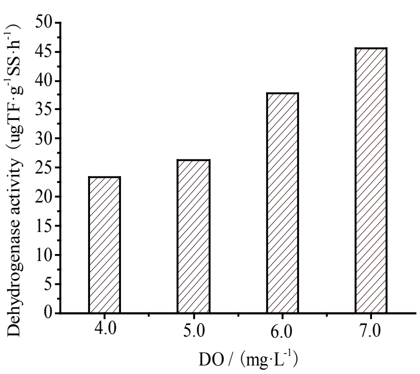
Fig. 2 (d) indicated that the different concentration of DO has little effect on the content of PN in EPS, which maintained at about 3.5 mg/g; and the effect on the content of PS content was remark significant, with the increase of DO, the content of PS increased from 2.87 to 4.36 mg·g^{-1}. At the same time, the relationship between the content of EPS was positively correlated with the concentration of DO, and the EPS content increased from 6.48 to 8.13 mg·g^{-1}. Results showed that different DO concentration in granular sludge and the different growth state of microorganism could directly affect the content and composition of the secretion of EPS. Verawaty et al. [20] found that the hydraulic shear stress could change the contents and compositions of EPS, and which was directly affected by the concentration of DO. The protein in EPS mainly played a role of the surface modification [17], and the polysaccharide in EPS as the basic framework of aerobic granular sludge. To maintain high shear stress generated under the condition of high DO, microbial secreted more PS to format the basic skeleton of aerobic granular sludge and maintain its granular structure.



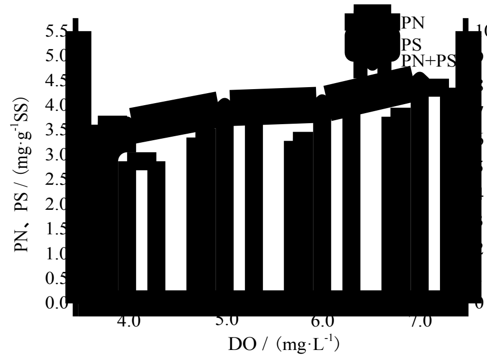
(a) SVI



(b) Minimal sedimentation rate



(c) Dehydrogenase activity



(d) EPScomponent

Figure 2. Effect of DO on sludge characteristics.

Results of the particle size distribution indicated that R1 had smaller particle diameter in the whole particles, the average particle diameter was 0.894 mm, and 60% was less than 1mm; the average particle diameter of R2 was 1.14 mm, the particle size distribution was more concentrated in 0.75 and 1.5 mm, accounting for 74%; the average particle diameter of R3 was 1.38 mm, the particle size was mainly distributed in 1 and 1.8 mm, accounting for 78%, and the particle size distribution showed a comparative balanced ratio before and after the average particle diameter; the average particle diameter of R4 was 1.32mm, particles larger than the average particle diameter only accounted for 20%, lower than the proportion of the same particle size in R3. Results showed that the concentration of DO has a certain impact on the particle size, and high hydraulic shear stress powered by high DO in R4 made some larger particles begin to decompose or disintegrate. From the results of the normal fitting of the particle size distribution of aerobic granular sludge in 4 reactors, the higher fitting degree was 0.96 of R3, which indicated the particle size distribution is more concentrated and system is more stable with the concentration of DO increasing in a certain range.

Conclusions

Results indicate that the effect of organic loading on the stability of aerobic granular sludge is remark significant. The organic loading which is over low or over high is all not propitious to the stability of granular sludge, over high organic loading can inhibit the growth of granular sludge, decrease the dehydrogenase activity and reduce the content of extracellular proteins, which may cause granular sludge worse stability and disintegrating. The organic loading should be controlled at 5.4 kgCOD·(m^3·d)$^{-1}$, at this point , the value of SVI is 57.89 mL·g^{-1} the minimal sedimentation rate is 2.05 m·h^{-1}, the dehydrogenase activity is 41.2 ugTF·(gSS· h)$^{-1}$, and the average particle diameter of aerobic granular sludge is 1.28 mm, mainly distributed between 1mm and 1.5mm, which is related to the content of

EPS in granular sludge achieves maximum under organic loading the organic loading of 5.4 kgCOD·$(m^3·d)^{-1}$ and the EPS is given priority to with extracellular proteins.

It indicates that the effect of DO on the stability of aerobic granular sludge is fairly significant. Over low DO can easily trigger filamentous bacteria breeding, and which can cause granular sludge unstable. Increasing DO concentration can promote the growth of granular sludge, and the size distribution can be more concentrated, but over high DO can affect the stability of the system, because providing over high hydraulic shear force can lead to granular sludge disintegrating. When the reactor is controlled under DO of 6mg·L^{-1}, the value of SVI is 48.39 mL·g^{-1}, the minimal sedimentation rate is 4.10 m·h^{-1}, the dehydrogenase activity is 37.8ugTF·$(gSS·h)^{-1}$, and the value of average particle size is 1.37 mm, mainly distributed between 1mm and 1.8mm,which is related to the EPS is given priority to with extracellular polysaccharide under DO of 6mg·L^{-1}.

Acknowledgements

This work was financially supported by Science and Technology Research Foundation of Chongqing Education Commission (KJ110403).

References

1. Liu Y, Wang Z, Tay J H. A unified theory for upscaling aerobic granular sludge sequencing batch reactors. Biotechnol Adv, 2005, 23(5): 335-344.

2. Wang J L, Zhang Z J, Wu W W. Research advances in aerobic granular sludge. Acta Scientiae Circum stantiae. 2009, 29(3): 449-473.

3. Song Z W, Liang Y, Ren N Q. Influence of organic loading on characteristics of aerobic granular sludge. Environ Sci Technol. 2008, 31(10): 128-131.

4. Zhu L, Lv ML, Dai X. Role and significance of extracellular polymeric substances on the property of aerobic granule. Bioresour Technol, 2012, (107): 46-54.

5. Chen H. Progress in effect of dissolved oxygen on the aerobic granular sludge. Sichuan Environ. 2010, 29(2): 109-112.

6. Liu Y Q, Tay J H, Moy B Y P. Characteristics of aerobic granular sludge in a sequencing batch reactor with variable aeration. Appl Microbiol Biotechnol. 2006, 71: 761-766.

7. Wei D, Qiao Z M, Zhang Y F. Effect of COD/N ratio on cultivation of aerobic granular sludge in a pilot-scale sequencing batch reactor. Appl Microbiol Biotechnol. 2013, 97: 1745-1753.

8. Yang X, Li Z H, Tan Z Q. Effect of temperature on formation and stability of aerobic granular sludge. China Water & Wastewater, 2014, 30(1): 10-17

9. Wang C, Zheng X Y. Effect of shear stress on morphology, structure and microbial activity of aerobic granules. Environ Sci. 2008, 29(8): 2235-2241.

10. Lin B, Wang X G, Tang Y B. Review on the application granular sludge in treatment of hypersaline organic wastewater and the research progress of the salt-tolerant mechanism of aerobic granular sludge. Environ Sci and Technol. 2013, 26(1): 66-68.

11. Xu J, Tian J B, He Y. An engineered project of hyper-saline mustard tuber production wastewater treatment. Technol of Water Treatment. 2013, 39(7): 116-117.

12. Chen Y, Zeng C Y, Long T R. Selection of aerobic biological process for treatment of integrated mustard tuber wastewater. China Water & Wastewater. 2009, 25(15): 21-24.

13. Chen Y, Zhou J, Gan C J. Effect of DO and aeration mode on the process of phosphorus removal by phosphate reduction. Industrial Water Treatment. 2011, 31(10): 31-34.

14. Zhang D H, Yue Q Y, Wang S G.. Characteristics of aerobic granular sludge in sequencing batch reactor. China Water & Wastewater, 2006, 22(1): 80-83

15. Long X Y, Long T R, Tang R. Study on extraction of Extracellular polymeric substance from activated sludge using cation exchange resin. China Water & Wastewater. 2008, 24(3): 29-33, 38.

16. Shen Z G, Zhang L L, Chen J M. Effect of organic loading on characteristics of aerobic granule. Environ Pollution & Control, 2008, 30(8): 69-72

17. Gao B Y, Zhu X B, Xu C H. Influence of extracellular polymeric substances on microbial activity and cell hydrophobicity in biofilms. Journal of Chemical Technol and Biotechnol. 2008, 83: 227-232.

18. Adav S S, Lee D J, Lai J Y. Potential cause of aerobic granular sludge breakdown at high organic loading rates. Appl Microbiol Biotechnol. 2010, 85: 1601-1610.

19. Tang C C, Jian M P, Liu M, et al. Research advances in aerobic granule stability enhancement. Chemical Industry and Engineering Progress. 2013, 32(4): 919-924.

20. Verawaty M, Pijuan M, Yuan Z. Determining the mechanisms for aerobic granulation from mixed seed of floccular and crushed granules in activated sludge wastewater treatment. Water Research. 2012, 46(3): 761-771.