JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2013. Vol. **7**(Spl. Edn.), p. 469-474

Studies on the Characteristics of Aerobic Granular Sludge and the Degradation Dynamics of Organic Substrate Cultivated in Sbr Reactor

Xia Zhao[1,2]*, Jimin Shen[2], Huixia Feng[1], Zhonglin Chen[2], Xiaochun Wang[1] and Yangli Zhao[1]

[1]College of Petrochemical Technology, Lanzhou University of Technology, Lanzhou, China.
[2]State Key Laboratory of Urban Water Resource and Environment, School of Municipal & Environmental Engineering, Harbin Institute of Technology, Harbin, China.

(Received: 03 March 2013; accepted: 14 April 2013)

Aerobic granular sludge was inoculated in SBR reactor and used to treat simulated wastewater after acclimation. The characteristics of the granular sludge during cultivation and the degradation efficiency of the granular sludge were investigated. The matrix degradation dynamics was studied and the dynamic equation was fitted out. The dynamic parameters of granular sludge were compared with those of conventional activated sludge. The results showed that the MLVSS of mature aerobic granular sludge was about 2500mg/L and the SVI was about 30 mL/g, the removal efficiency of COD reached up to 94.52 per cent. Degradation activity of aerobic granular sludge was higher than that of conventional activated sludge according to the dynamic equation. It will provide an important value for the application of aerobic granular sludge technology in high concentration wastewater and water treatment field nowadays.

Key words: Aerobic granular sludge, Substrate degradation, Characteristics, Dynamics.

Granular sludge technology is a great potential for sewage treatment (Adav *et al*., 2009). Aerobic granular sludge is a highly active micro-ecology system with compact structure, excellent settling ability and favorable biological collaborative. And it forms from microbe spontaneous agglomeration and proliferation under the specific environment. Compared with conventional activated sludge treatment process, aerobic granular sludge has high-density biomass (Kreuk *et al*., 2005; Carucci *et al*., 2009; Wan *et al*., 2009). Compared with conventional activated sludge treatment process, aerobic granular sludge can realize simultaneous removal of nitrogen and phosphorus fairly under aerobic condition, and sludge granules can retain [1] biomass highly(Carucci *et al*.,2009; Tu *et al*.,2010), which makes aerobic granulation is widely used in the field of high or low concentration wastewater treatment(Liu *et al*.,2010). The technology does not require additional sedimentation tank for separating mud with water, which will simply the process flow so that the investment and operation cost are reduced.

At present scholars all of the worlds study on the formation of aerobic granular sludge and cultivation condition (Zhao *et al*., 2010). However, the research on matrix degradation dynamic of aerobic granular sludge is less (Adav *et al*., 2009). One of parameters of matrix degradation feature is Vmax, which must almost be measure as the parameters of matrix degradation but it is affected on microbial growth environment and conditions. Furthermore, half-saturation rate constant is an

* To whom all correspondence should be addressed.
Tel:+86 13993184112;
E-mail: hopexia2009@yahoo.cn

expression in the several process of comprehensive dynamic constants, which is called Ks. It has no direct way to measure its value(Zhang, 1984; Hu, 2003;Sun *et al*., 2010). Therefore, it is important and complex part for the study of the degradation dynamic model of aerobic granular sludge to estimate parameters through various means. In this study, aerobic granular sludge technology has obvious advantages and potential compared with traditional water treatment technology through dynamics study.

MATERIALS AND METHODS

Experimental set-up

SBR reactor was made of the glass with diameter of 8cm, the height to diameter ratio of 7.5, the working volume of 3L, and exchange ratio of 50 per cent. The apparent increase in gas velocity was controlled for 0.9~1.66cm / s according to the operation. The water temperature was controlled at 22 ± 3! using electric heating rods, and the operation running implemented the automatic control program with the PLC program controller. System run eight cycles per day, which included 6min water, 140~169min aeration, 30~1min settling time and 4min effluent water in each cycle.

The schematic diagram of the experimental set-up is given in Figure 1.

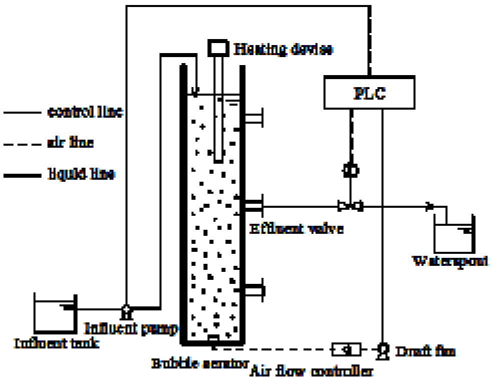


Fig. 1. Schematic diagram of SBR installation

Experimental water

Experimental water was an artificial simulated wastewater with NH_4Cl as the nitrogen source, KH_2PO_4 as the phosphate source, $C_6H_{12}O_6$ and CH_3COONa as an integrated carbon source in the experiment. COD, NH_3-N and TP was about 1100mg/L, 30^60mg/L and 3^5mg/L respectively. Meanwhile, an appropriate amount of magnesium, calcium, iron and other metals were added in wastewater so as to complement every kind of microelement.

Inoculated sludge

Inoculated sludge came from digested sludge of the sewage treatment plant in Lanzhou beer company, which was black and poor activity. After aeration of 2 days, 1L sludge as inoculated sludge. The excessive sludge was used to study matrix degradation dynamic of ordinary flocculent activated sludge. Then a large number of inoculated sludge zoogloea and protozoa in sludge were found under the microscope, and vorticella was dominant among protozoa. MLVSS/MLSS value was 0.54.

Analytical methods

COD, MLVSS, SVI and other indicators were measured according to National Standard Methods (SEPA Chinese, 2002). The shape of sludge particles were recorded by a digital camera. The formation process of granular sludge and microbial community structure were observed through (XSJ-HS type) computer microscope. Microstructure of granular sludge was observed by scanning electron microscope (JSM-5600 type).

RESULTS AND DISCUSSION

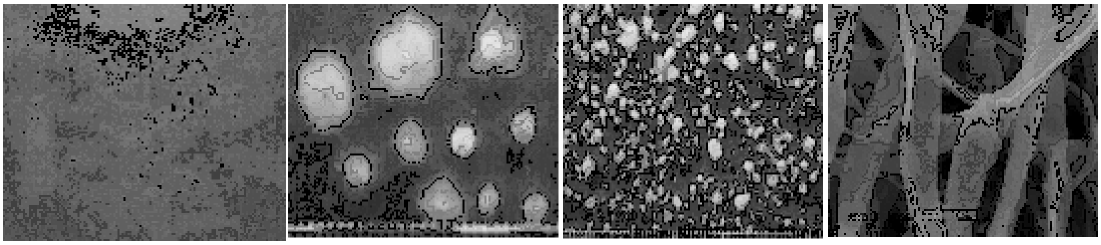
The whole system had been run for 70 days. Firstly, sludge in SBR acclimated 17 days, which was in the stage. Next, the expanded granular sludge appeared in the system, whose stage lasted 20 days. Finally, the size of granules slowly increased and became mature after 33 days in reactor.

Formation of aerobic granular sludge

It could be seen from Figure 2 that variations of granular sludge image in different cultivation stages, and the morphological and structural characteristics physical of the granular sludge were showed in Figure 3.

Inoculated sludge was small dark brown flocculent, which was conventional activated sludge (as shown in Figure 2a).

The system of activated sludge caused selective pressure with the reduction of the settling time, the active biomass in the reactor dropped from the newly inoculated 4950mg/L sharply to



a) Inoculated sludge b)Expanded granular c) Mature granular d) SEM granular sludge (×5000)

Fig. 2. Variations of granule sludge image in different training times

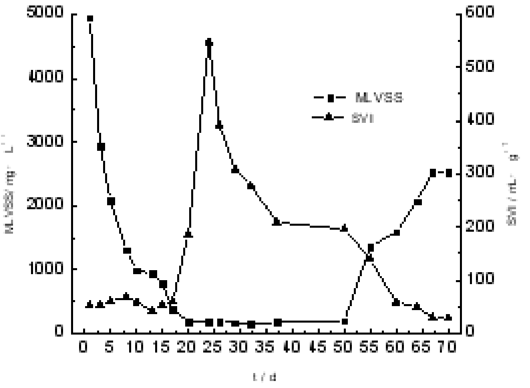


Fig. 3. Variations of MLVSS and SVI during the operation

195mg/L (as shown in Figure 3). The abundant fluffy filamentous sludge began to appear in the reactor with the prolonging of cultivation time. The middle of sludge had small core package and surrounded by radial growth of filamentous micro-organisms (as shown in Figure 2b). But from Figure 3, it can be seen that the settlement performance of granular in this stage was poor and the sludge index(SVI) was up to 548.61 mL/g. Filamentous bulking had been effectively controlled through adjusting operating conditions. After 60 days operation, the sludge bed turned from floc-like sludge to granules. Finally, the sludge in the reactor was nearly completely granulized. Aerobic granular sludge was spherical or elliptical with smooth surface, dense and stable structure (as shown in Figure 2c). As shown in Figure 2d, aerobic granular sludge consisted of the filamentous microbes, which were about 6ìm in diameter under 5000 times magnification. A large number of pores were appeared on particle surface, which were a transport channel of oxygen, substrates and metabolites in granular sludge process. The MLVSS of mature aerobic granular sludge maintained at 2500 mg/L or so, and the SVI was about 30mL/g.

Degradation of organic substrate

The removal efficiencies of COD were studied. The results were given in Figure 4

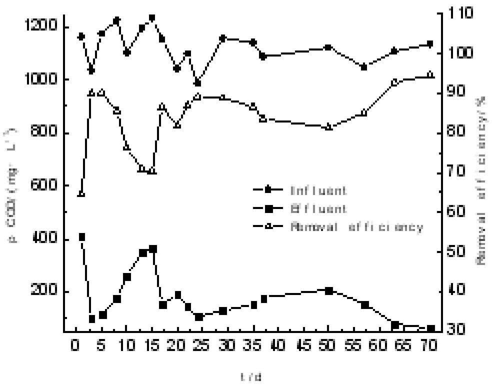


Fig. 4. The removal efficiencies of COD

It can be seen from Figure 4 that degradation of organic was unstable in the initial reaction, which would lead to wave of the COD removal efficiencies. This is due to that the sludge in the reactor was at the acclimation phase. Shorten of the settlement time brought about the loss of the sludge. Then the COD removal efficiencies increased with the formation of particles core. In the period of filamentous bulking, settling characteristics of the sludge were poor due to removal effect of sludge, the COD removal efficiencies had a slight decrease. Lastly, with the formation of granular sludge, the treatment efficiency of COD gradually increased with removal rate of 94.52%. At this point, COD in effluent water was reduced to 100 mg / L or less, which illustrated the greater activity of heterotrophic bacteria in the aerobic granular sludge system. The final VSS/SS was 0.86 and removal of organic was effective.

Matrix degradation Dynamics

The dynamics of matrix degradation was in relation to matrix degradation and matrix concentration, biomass and other factors. The study of the effect factors of substrate degradation rate and their relationship should be described (Li, 2011). Monod who is a French scientist did plenty of continuous cultivation experiments by single biodegradable and soluble limited matrix and pure bacteria in 1940. Subsequently, the Monod equation is introduced into biological wastewater treatment fields and achieves matrix degradation dynamics equation as follows:

$$\mu = \frac{\mu_{\max} S}{K_s + S} \qquad ...(1)$$

In above equation, V is the degradation ratio rate, time^{-1}; V_{max} is the maximum degradation ratio rate, time^{-1}, K_s is half-saturation rate constant, S is matrix concentration, quality·volume^{-1}. Eventually, obtained the equation as follows:

$$\frac{X_t}{S_0 - S_e} = \frac{K_s}{V_{max} S_e} + \frac{1}{V_{max}} \qquad ...(2)$$

In equation (2), S_0 is initial substrate concentration; Se is substrate effluent concentration after t hours X_t is microbial concentration (Zhang,1984;Hu,2003;Li,2011).

In cartesian coordinate, to $X_t/(S_0-S_e)$ the vertical axis, and to $1/S_e$ the horizontal axis, then draw a straight line graph, 1/Vmax is the intercept, K_s/V_{max} is the slope.

In the experiment, 100 mL mature aerobic granular sludge or activated sludge were put in 1000mL beaker respectively. Then to add into 500mL glucose simulated wastewater of the different COD concentration. After 1 h aeration, COD concentration and MLVSS in water were measured. In addition, the matrix degradation required dynamic parameters which could be obtained through graphic method.

Matrix degradation dynamics in aerobic granular sludge

COD concentration of the different influent and effluent and MLVSS concentration after 1 hour were shown in Table 1. And the number column in Table 1 signified the group number of parallel experiments. The required dynamic parameters could be obtained by the graphical method (Figure 5).

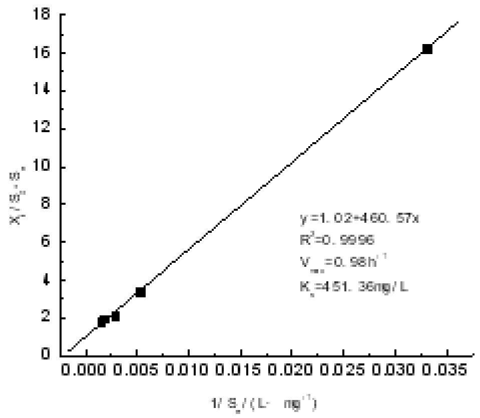


Fig. 5. Degradation dynamics of aerobic granular sludge.

According to Figure 5, fitting a straight line equation could be obtained:

$$y=1.02+460.57x \qquad ...(3)$$

In equation (3), the slope was 460.57, the intercept was 1.02, which could be calculated:

K_s=451.36 mg/L $\quad V_{max}$=0.98h^{-1}

Consequently, dynamics equation of matrix degradation on mature aerobic granular sludge was:

$$V=\frac{0.98S}{451.36+S} \qquad ...(4)$$

Matrix degradation dynamics in activated sludge

Degradation dynamics in activated sludge and parameters fit linear were shown in Table 2 and Figure 6.

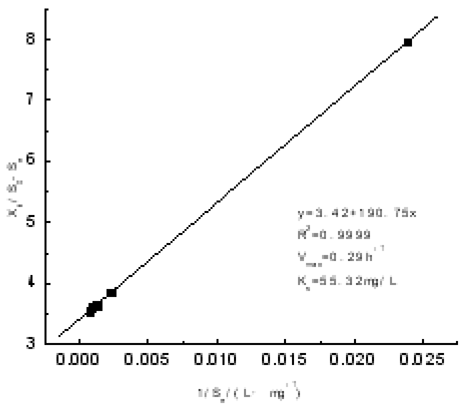


Fig. 6. Degradation dynamics of activated sludge

According to Figure 6, fitting a straight line equation could be obtained:

$$y=3.42+190.75x \quad ...(5)$$

In equation (5), the slope was 190.75, the intercept was 3.42, which could be calculated:

K_s=55.32 mg/L V_{max}=0.29h^{-1}

Therefore, matrix degradation dynamics equation of activated sludge was:

$$V=\frac{0.29S}{55.32+S} \quad ...(6)$$

Compared with dynamic parameters of aerobic granular sludge and conventional activated sludge

Compared to dynamic parameters of aerobic granular sludge and conventional activated sludge on matrix degradation, data showed in Table 3.

As shown in Table 3, the maximum degradation rate of aerobic granular sludge was 3.4 times of conventional activated sludge, it was indicated that the microbial activity of aerobic granular sludge was higher than that of conventional activated sludge. Microbial metabolism was better and degradation rate of organic matter was faster.

Table 1. Degradation dynamics parameters of aerobic granular sludge

Number	S_0, (mg/L)	S_e, (mg/L)	MLVSS,(mg/L)	1/S_e, (L /mg)	X_t/S_0-S_e
1	123	30	1510	0.033	16.23
2	645	189	1560	0.0053	3.42
3	1070	353	1530	0.0028	2.13
4	1300	558	1475	0.0018	1.98
5	1560	672	1590	0.0015	1.79

Table 2. Degradation dynamics parameters of aerobic activated sludge

Number	S_0, (mg/L)	S_e, (mg/L)	MLVSS,(mg/L)	1/S_e, (L /mg)	X_t/S_0-S_e
1	123	42	645	0.0238	7.96
2	645	447	765	0.00224	3.86
3	1070	858	774	0.00125	3.65
4	1300	1078	802	0.00093	3.61
5	1560	1331	812	0.00075	3.55

Table 3. Dynamics parameters

Parameter	Aerobic granular sludge	Conventional activated sludge
V_{max}, h^{-1}	0.98	0.29
K_s, mg·L^{-1}	451.36	55.32

Moreover, the reaction time in aerobic granular sludge process could be effectively shortened so that it could cut down operation cost and energy consumption and kept a high stability in system. Half-saturation rate constant(K_s) was 8.2 times to conventional activated sludge, it was indicated that aerobic granular sludge was more resistant to high concentration wastewater, strong resistance to shock loading and had a good degradation capability compared with conventional activated sludge. It can clearly be seen, microbial biomass and degradation capability of aerobic granular sludge are better than conventional activated sludge under the same operating conditions in SBR. This found has been confirmed in the experimental study and further illustrates aerobic granular sludge technology has a good prospect for the application.

CONCLUSIONS

(1) Aerobic granular sludge was cultivated by $C_6H_{12}O_6$ and CH_3COONa as an integrated

carbon source and activated sludge as inoculated sludge in SBR. When cultivation reached 60 days, aerobic granular sludge became mature granules and filamentous fungi was the main micro-organisms. MLVSS of mature granular sludge remained at 2500 mg/L or so, SVI was about 30mL/g.

(2) The removal efficiency of organic substrate maintained stability on mature aerobic granular sludge. When influent COD concentration was about 1100mg/L, the removal efficiency reached up to 94.52 per cent.

(3) Matrix degradation dynamics in aerobic granular sludge and conventional activated sludge were studied. The dynamics equations of degradation were obtained. Degradation dynamic parameters of aerobic granular sludge K_s of 451.36 mg·/L^{-1} and V_{max} of 0.98h^{-1}. The two dynamic parameters were 3.4 and 8.2 times of conventional activated sludge respectively. It was indicated that microbial biomass and degradation capability of aerobic granular sludge were better than conventional activated sludge process. Aerobic granular sludge technology will play an important role in treatment process of high concentration wastewater and microbial community diversity.

ACKNOWLEDGEMENTS

This work was supported by the Funds for Creative Research Groups of China (NO.51121062), the National Natural Science Foundation of China (NO.5126803), the Research Project for Education Department of Gansu province (NO.1205ZTC032), Excellent Young Teachers Training Project in Lanzhou University of Technology (1005ZCX016).

REFERENCES

1. Alessandra Carucci, Stefano Milia, Giorgia De Gioannis., Acetate-fed aerobic granular sludge for the degradation of 4-chlorophenol. *Bioresource Technology*, 2009; **100**: 68-73.
2. Hu Jicui., The theory and technology of wastewater anaerobic treatment. China Building Industry Press, Beijing, 2003; 83-91.
3. Junfeng Wan, Mathieu Sperandio., Possible role of denitrification on aerobic granular sludge formation in sequencing batch reactor. *Chemosphere*, 2009; **75**: 220-227.
4. Liu, Y.Q., Moy, M., Kong, Y. H.., Formation, physical characteristics and microbial community structure of aerobicgranules in a pilot-scale sequencing batch reactor for real wastewater treatment. *Enzyme and Microbial Technology*, 2010; **46**: 520-525.
5. M.K. de Kreuk, M.Pronk, M.C.M. van Loosdrecht., Formation of aerobic granules andconversion processes in an aerobic granular sludge reactor at moderate and low temperatures. *Water Research*, 2005; **39**: 4476-4484.
6. SEPA Chinese., Water and Wastewater Monitoring Methods. China Environmental Science Publishing House, Beijing, 2002; 96-220.
7. Li Yaxin., The theory and technology of activated sludge. China Building Industry Press, Beijing, 2011; 128-167.
8. Sun Peide, Sun, Yingqi, Wang Ruyi., Kinetic model and numerical simulation Introduction of activated sludge. Chemical Industry Press, Beijing, 2010; 5-12.
9. Sunil S. Adav a, Duu-Jong Lee a, Juin-Yih Lai., Proteolytic activity in stored aerobic granular sludge and structural integrity. *Bioresource Technology*, 2009; **100**: 68-73.
10. Sunil S. Adav, Duu-Jong Lee, Juin-Yih Lai., Treating chemical industries inûuent using aerobic granular sludge. Recent development. *Journal of the Taiwan Institute of Chemical Engineers*, 2009; **40**: 333-336.
11. Tu, X., Zhang, S., Xu, L., Performance and fouling characteristics in a membrane sequence batch reactor(MSBR) system coupled with aerobic granular sludge. *Desalination*, 2010; **261**: 191-196.
12. Zhang, Xiheng., Wastewater treatment. Metallurgical Industry Press, Beijing, 1984; 94-97.
13. Zhao, Xia, Zhao Yangli, Jiang Feng, Feng Huixia Research progress and characteristics of aerobic granular sludge. *Applied Chemical Industry,* 2010; **39**(4): 580-589.