ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)02-0339-04 **CLC number**: X53 **Document code**: A

Performance of Anammox granular sludge bed reactor started up with nitrifying granular sludge

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Abstract: The anaerobic ammonia oxidation(Anammox) granular sludge bed reactor was started up successfully with nitrifying granular sludge. During the operation, the nitrifying granular sludge was gradually converted into Anammox granular sludge with good settling property and high conversion activity. The Anammox reactor worked well with the shortest HRT of 2.43 h. Under the condition that HRT was 6.39 h and influent concentration of ammonia and nitrite was 10 mmol/L, the removal of ammonia and nitrite was 97.17% and 100.00%, respectively. Corresponding volumetric total nitrogen loading rate and volumetric total nitrogen conversion rate were 100.83 mmol/(L·d) and 98.95 mmol/(L·d). The performance of Anammox reactor was efficient and stable.

Keywords: nitrifying granular sludge; anaerobic ammonia oxidation (Anammox); granular sludge bed reactor; performance

Introduction

The anaerobic ammonia oxidation(Anammox) is a novel biological reaction that produces molecular nitrogen with ammonia as electron donor and nitrite as electron acceptor, respectively (van de Graff, 1995; 1996). Anammox process has been shown to be a promising way of removing nitrogen at a low cost from wastewater(Jetten, 1997; Zheng, 1998). However, the growth rate of Anammox bacteria is extremely slow and the doubling time of Anammox bacteria has been reported to be as long as 11 d (Strous, 1998; 1999). Because the performance of Anammox reactor depends largely on the amount of biomass, the slow growth rate has become one of the most important factors affecting the efficiency and stability(Strous, 1998; Zheng, 2001). In order to bring the process into practical use, some measures must be taken to accumulate the biomass in Anammox reactor.

It has been proved that sludge granulation is a successful method for cell immobilization and biomass retention in the upflow anaerobic sludge blanket (UASB) reactor(Lettinga, 1995; Schmidt, 1996). The aim of this experiment is to investigate the feasibility of improving the performance of Anammox reactor by formation of granular sludge.

1 Materials and methods

1.1 Inoculum and synthetic wastewater

The nitrifying granular sludge was taken from an airlift nitrifying granular sludge bed reactor(Lin, 2002) and was used as inoculum for Anammox reactor. Some physical and chemical properties of the nitrifying granular sludge are as follows: total suspended solid (TSS) 39.08 g/L, volatile suspended solid (VSS) 18.78 g/L, VSS/TSS 48.06%, pH 7.6.

The composition of synthetic wastewater is listed in Table 1(Graff, 1996).

Table 1 Composition of the synthetic wastewater

Component	Concentration, g/L	Component	Concentration, g/L
KH_2PO_4	0.027	$(NH_4)_2SO_4$	Added as need[a]
$MgSO_4 \cdot H_2O$	0.300	$NaNO_2$	Added as need[a]
$CaCl_2$	0.136	Trace elements solution Ⅰ	1 ml/L[b]
$KHCO_3$	0.500	Trace elements solution Ⅱ	1 mL/L[c]

Notes: a. the molar ratio of NH_4^+ and NO_2^- is 1:1; b. trace elements solution Ⅰ (g/L): EDTA 5.000, $FeSO_4$ 5.000; c. trace elements solution Ⅱ (g/L): EDTA 15.000, H_3BO_4 0.014, $ZnSO_4 \cdot 7H_2O$ 0.430, $MnCl_2 \cdot 4H_2O$ 0.990, $CuSO_4 \cdot 5H_2O$ 0.250, $NaMoO_4 \cdot 2H_2O$ 0.220, $NiCl_2$ 0.199, $NaSeO_4 \cdot 10H_2O$ 0.210

1.2 Reactor system

The laboratory-scale reactor was made of glass with total volume of 1.5 L, height of 0.65 m and internal diameter of 0.10 m. Three sampling ports were evenly distributed over the height of reactor. The flow diagram of the reactor system is shown in Fig.1. The influent was pumped into the bottom of the reactor and flowed upward through the granular sludge bed. The effluent passed the gas-liquid-solid separator and was then collected in the effluent bottle. The nitrogen gas was led off by gas-tube. The reactor was covered with a black cloth to avoid the inhibition of light on Anammox activity.

1.3 Analytical procedures

The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were determined by the standard methods (Environmental

Foundation item: The National Natural Science Foundation of China(No. 30070017); Zhejiang Provincial Natural Science Foundation of China; the Excellent Young Teachers Program of MOE, P.R.C.; * Corresponding author

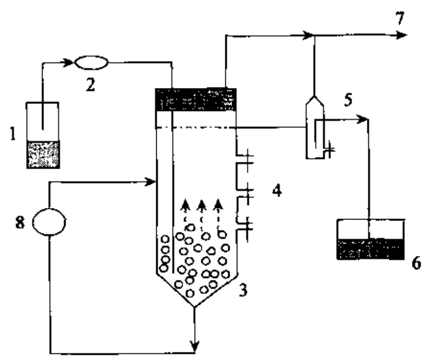

Fig.1 Anammox granular sludge bed reactor system and its diagram
1. influent tank; 2. influent pump; 3. granular sludge bed reactor; 4. sampling port; 5. gas-liquid-solid separator; 6. effluent tank; 7. gas out; 8. circulation pump

Protection Bureau of China, 1997). The pH was determined using glass electrodes connected to pHS-9V pH-meter. Dissolved oxygen concentration was determined using YSI Model58 DO-meter. TSS was determined by drying the sample at 105℃ for at least 24 h. After burned at 550℃ for 1 h, the ash was measured. The difference between TSS and ash was termed VSS.

The average diameter of sludge granule was measured as follows: take 50—100 sludge granules from Anammox reactor at random, measure the diameter of every particle with Nikon microscope and calculate the average diameter according to Sauter equation(Eq.1).

$$D = \frac{1}{n}\sum(ab^2)^{\frac{1}{3}}. \quad (1)$$

Where D is the average diameter(mm); a is the length of granule(mm); b is the width of granule(mm); n is the number of granule.

The settling velocity of sludge granule was determined as follows: put 50—100 sludge granules taken from Anammox reactor into a cylinder(with height of 0.5 m) filled with water, record the maximum and minimum settling time and calculate the settling velocity by dividing the height of cylinder by the average settling time.

The biomass concentration of sludge granules (gVSS/L granule) was determined as follows: put sludge granules onto filter paper to remove free water, transfer sludge granules into 10 ml measuring cylinder filled with 5 ml water, measure the volume of sludge granules by the volume increment of mixed liquid (water plus granules), determine VSS of sludge granules as above-mentioned and calculate the biomass concentration by dividing the VSS by the volume of the sludge granules.

The microscopic picture was taken with electron microscope (Philips XL30-ESEM) and the pretreatment method was made as described by Lin and Jin(Jin, 1989).

2 Results and discussion

2.1 Start-up

Anammox reactor was inoculated with 700 ml nitrifying granular sludge from an airlift nitrifying granular sludge bed reactor(Lin, 2002), operated with synthetic wastewater(5 mmol/L NH_4^+, 5 mmol/L NO_2^-) at 30℃. During start-up, the hydraulic retention time(HRT) was kept at 1 d. When both ammonia removal and nitrite removal was higher than 90%, their concentrations were raised by step of 3 mmol/L until they reached 30 mmol/L.

As shown in Fig.2 and Fig.3, Anammox reactor did not work well in the first 3 days with average ammonia and nitrite removal as low as 48.15% and 46.93%, respectively. From day 4 on, its performance improved obviously. The ammonia removal and nitrite removal reached 98.43% and 100% separately. During the rest of start-up period, influent ammonia concentration was increased progressively from 4.95 to 30.94 mmol/L and nitrite concentration from 5.43 to 31.98 mmol/L. Both ammonia removal and nitrite removal were more than 85%. This means that Anammox reactor was successfully started up.

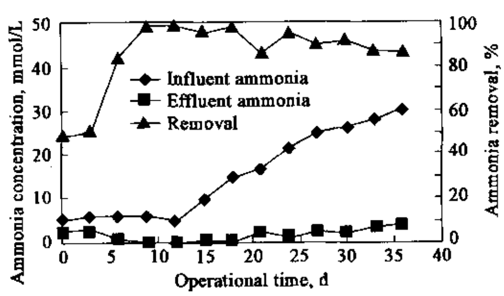

Fig.2 Ammonia conversion during start-up of Anammox reactor

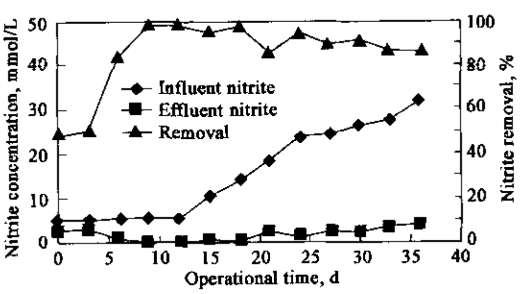

Fig.3 Nitrite conversion during start-up of Anammox reactor

The inoculum is very important for the start-up of Anammox reactor. Without proper inoculum, Anammox process can not be put into practical use. Although some Anammox bacteria have been isolated and identified(Strous, 1999), nobody can culture them even for research purpose, let alone for industrial uses(Kuenen, 2001). Fortunately it has been reported that some ammonia oxidizers such as *Nitrosomonas europaea*, *Nitrosomonas eutropha* and the nitrifying activated sludge have Anammox activity (Bock, 1995; Zheng, 2000; Hu, 2001), they provided us an alternative way to obtain inoculum for Anammox reactor. The success of this experiment demonstrates that it is feasible to start up Anammox reactor with the nitrifying granular sludge.

2.2 Potential

After Anammox reactor was started up with nitrifying granular sludge, HRT was shortened step by step from 24.02 h to 2.43 h for testing the loading potential of the reactor. During this period, influent ammonia concentration and nitrite concentration were both fixed at 10 mmol/L to avoid possible inhibition of substrates on the Anammox activity (Strous, 1998; 1999). The circulation pump had been working to mix substrates and biomass.

The performance of Anammox reactor is shown in Table 2. As long as HRT was longer than 4.97 h, ammonia removal and nitrite removal were higher than 84% and 90%, respectively. When HRT was further shortened, the removal efficiency decreased. At HRT of 3.69 h, the reactor reached its highest volumetric total nitrogen conversion rate (151.22 mmol/(L·d)) in this experiment. After HRT was shortened to 2.43 h, volumetric total nitrogen loading rate reached its maximum (194.82 mmol/(L·d)), but volumetric total nitrogen conversion rate decreased to 136.21 mmol/(L·d).

Table 2 Performance of Anammox granular sludge bed reactor at different HRT

HRT, h	NH_4^+			NO_2^-			Total nitrogen	
	Volumetric loading rate, mmol/(L·d)	Volumetric conversion rate, mmol/(L·d)	Removal, %	Volumetric loading rate, mmol/(L·d)	Volumetric conversion rate, mmol/(L·d)	Removal, %	Volumetric loading rate, mmol/(L·d)	Volumetric conversion rate, %
24.02	13.76	13.49	98.04	14.91	14.91	100	28.67	28.40
20.16	15.77	15.05	95.43	16.41	16.26	99.09	32.18	31.31
18.99	19.39	18.10	93.35	21.67	20.29	93.63	41.05	38.39
13.45	23.69	21.08	88.98	23.82	21.59	90.64	47.51	42.67
11.28	27.59	27.59	100	30.50	29.76	97.57	58.09	57.35
11.09	30.47	30.47	100	30.11	29.47	97.87	60.58	59.94
8.72	35.74	32.83	91.86	30.56	28.27	92.51	66.30	61.10
8.54	38.32	34.23	89.33	43.16	40.43	93.67	81.49	74.67
8.02	42.92	39.04	90.96	41.46	38.92	93.87	84.38	77.97
7.82	46.44	42.73	92.01	43.99	41.16	93.57	90.43	83.89
6.89	51.82	48.12	92.86	53.52	52.57	98.22	105.34	100.70
6.10	54.92	46.64	84.92	46.93	43.13	91.90	101.84	89.77
5.11	61.70	56.35	91.33	63.05	62.04	98.40	124.75	118.39
4.97	63.17	52.73	83.47	67.64	59.18	87.49	130.81	111.91
4.21	67.61	50.71	75.00	68.16	48.60	71.30	135.77	99.31
4.15	72.81	55.12	75.70	67.81	52.74	78.53	140.62	107.86
3.99	74.95	56.62	75.54	77.71	64.23	82.65	152.65	120.84
3.88	79.14	65.06	82.20	75.05	63.55	84.68	154.20	128.60
3.85	83.05	56.34	67.84	88.83	72.76	81.91	171.88	129.09
3.69	86.82	68.86	79.31	94.43	82.36	87.22	181.25	151.22
2.43	92.31	61.67	66.81	102.52	74.54	72.71	194.82	136.21

2.3 Stability

Following the test of reactor conversion potential, HRT was prolonged to 12 h to test the reactor stability. The influent concentration of substrate (ammonia and nitrite) was set at about 10 mmol/L. After operation for 7 d, both ammonia and nitrite removal went up to 90%. From day 8 to day 13, the ammonia and nitrite removal was higher than 95%. Thereafter, HRT was shortened to around 6.39 h that is often adopted in the sewage treatment plant (Liu, 2000) and the reactor was run on the steady condition for 30 d. Table 3 shows that when the volumetric total nitrogen loading rate was about 100.83 mmol/(L·d), the volumetric total nitrogen conversion rate reached 98.95 mmol/(L·d), the average removal of total nitrogen was as high as 98.61% (the average removal of ammonia and nitrite was 97.17% and 100%, respectively). The working performance of Anammox reactor was very stable.

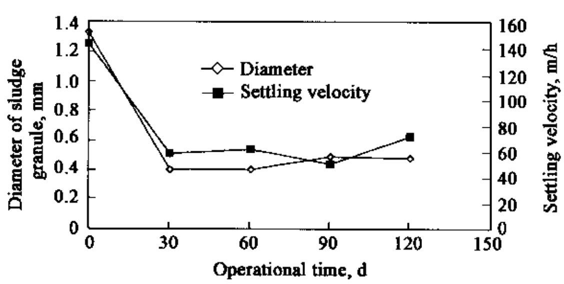

Fig.4 The change of diameter and settling velocity of granular sludge during operation

2.4 Biomass

Anammox reactor was run under anaerobic condition since it was inoculated with nitrifying granular sludge. In order to observe the change of biomass, samples were taken every month and the physical and chemical properties were determined. As shown in Fig.4, the sludge granules (with average diameter of 1.33 mm) used as inoculum broke up into smaller particles because of the transition from aerobic to anaerobic condition. On day 30, the average diameter of sludge granules was 0.40 mm. The settling velocity of sludge granules slowed down from 143.28 m/h to 58.14 m/h. From day 30 on, the sludge granules stopped breakup, the average diameter was relatively stable during the rest of operation. From day 90 to day 120, the settling velocity sped up from 58.14 m/h to 71.80 m/h, which belongs to a good property (Jin, 1998).

The good settling characteristics of Anammox granules alleviated the washout of biomass effectively. The effluent VSS concentration was as low as 0.0243 g/L during the whole experiment except the days with HRT shorter than 4.97 h. The VSS concentration increased from 13.25 g/L on day 30 to 31.60 g/L on day 120. The volumetric total nitrogen conversion rate rose from 6.72 mmol/(L·d) on day 30 to 120.85 mmol/(L·d) on day 120 (Fig.5).

Table 3 Performance of Anammox granular sludge bed reactor at HRT 6.39 h

NH_4^+			NO_2^-			Total nitrogen		
Volumetric loading rate, mmol/(L·d)	Volumetric conversion rate, mmol/(L·d)	Removal, %	Volumetric loading rate, mmol/(L·d)	Volumetric conversion rate, mmol/(L·d)	Removal, %	Volumetric loading rate, mmol/(L·d)	Volumetric conversion rate, mmol/(L·d)	Removal, %
47.15	44.77	94.95	52.33	52.33	100.00	99.47	97.10	97.62
49.36	47.48	96.19	55.32	55.32	100.00	104.67	102.80	98.41
46.34	46.34	100.00	53.34	53.34	100.00	99.68	99.68	100.00
50.11	50.02	99.82	51.39	51.39	100.00	101.50	101.42	99.92
53.60	49.67	92.67	51.53	51.53	100.00	105.13	101.20	96.26
51.53	48.96	95.01	47.10	47.10	100.00	98.63	96.06	97.39
47.04	47.04	100.00	50.31	50.31	100.00	97.35	97.35	100.00
45.74	45.10	98.60	50.80	50.80	100.00	96.53	95.90	99.35
48.46	46.85	96.68	52.46	52.46	100.00	100.92	99.31	98.40
53.19	51.97	97.71	46.70	46.70	100.00	99.89	98.67	98.78

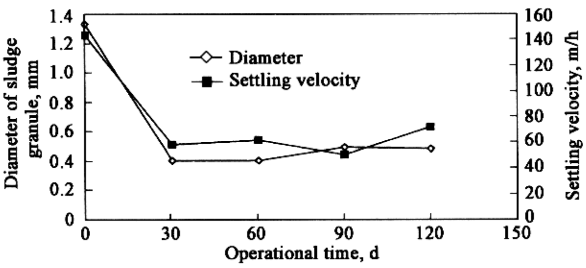

Fig.5 The change of activity and amount of granular sludge during operation

Compared with the sludge granule on day 1(Fig.6 left), the sludge granule on day 120 (Fig. 6 right) was more compact and the inner microstructure was also changed. The color gradually changed from brown to red, which implied the increase in cytochrome content and improvement of activity of biomass(Jetten, 1999).

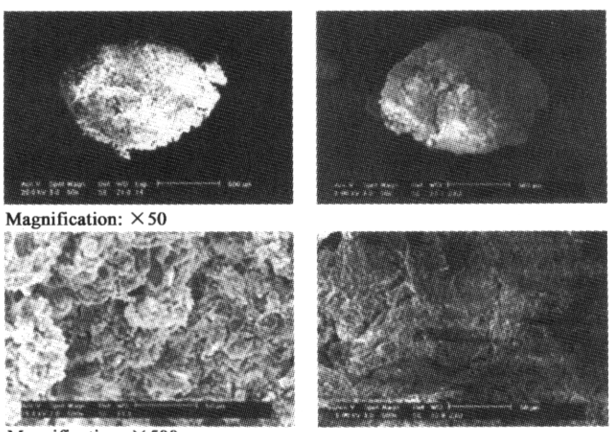

Fig.6 Comparison between granular sludge on day 1(left) and on day 120 (right) conclusions

3 Conclusions

Nitrifying granular sludge could serve as seeding sludge to start up Anammox reactor. During the operation, the nitrifying granular sludge was gradually converted into Anammox granular sludge with good settling property and high conversion activity. The Anammox granular sludge could be efficiently held in the reactor and the effluent VSS concentration was as low as 0.0243 g/L.

The potential performance of Anammox reactor started up with nitrifying granular sludge was good with the shortest HRT of 2.43 h, maximum volumetric total nitrogen loading rate of 194.82 mmol/(L·d) and maximum volumetric total nitrogen conversion rate of 151.22 mmol/(L·d).

On the condition that the HRT was 6.39 h and the influent concentration of ammonia and nitrite was 10 mmol/L, the removal of ammonia and nitrite was 97.17% and 100.00%, respectively. Corresponding volumetric total nitrogen loading rate and volumetric total nitrogen conversion rate were 100.83 mmol/(L·d) and 98.95 mmol/(L·d). The working state of Anammox reactor was efficient and stable.

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(Received for review March 3, 2003. Accepted March 26, 2003)