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Start-up of anaerobic ammonia oxidation bioreactor with nitrifying activated sludge

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Abstract: The anaerobic ammonia oxidation(Anammox) bioreactor was successfully started up with the nitrifying activated sludge. After anaerobically operated for 105 d, the bioreactor reached a good performance with removal percentage of both ammonia and nitrite higher than 95% and volumetric total nitrogen removal as high as 149.55 mmol/(L·d). The soft padding made an important contribution to the high efficiency and stability because it held a large amount of biomass in the bioreactor.

Keywords: Anammox bioreactor; nitrifying activated sludge; start-up

Introduction

The anaerobic ammonia oxidation(Anammox) is a novel biological reaction which produces molecular nitrogen with ammonia as electron donor and nitrite as electron acceptor, respectively (Van de Graff, 1996). Anammox process becomes a promising substitute to the conventional denitrifying process because it removes two pollutants (ammonia and nitrite) simultaneously at a low cost (Jetten, 1997). However, Anammox bacteria grow slowly and their cell yield is very small. In order to bring the process into practical use, some measures must be taken to get the inoculum for start-up of the bioreactor and to promote growth of Anammox bacteria(Strous, 1999; Zheng, 2001).

Some aerobic ammonia oxidizers such as *Nitrosomonas eurapaea* and *Nitrosomonas eutropha* show both metabolic diversity and substrate diversity. They oxidize ammonia not only under aerobic conditions but also under anaerobic conditions(Bock, 1995), and carry out denitrification with hydrogen or ammonia as electron donor (Van de Graff, 1996). The metabolic diversity and substrate diversity of ammonia oxidizers lead us to a new strategy to solve the above-mentioned problems, that is, to start up Anammox bioreactor with nitrifying activated sludge. The feasibility of the start-up strategy is investigated in this paper.

1 Materials and methods

1.1 Inoculum and basal medium

The aerobic activated sludge from Hangzhou Shibao Sewage Plant served as inoculum for Anammox bioreactor. Some physical and chemical properties are as follows: total suspended solid(TSS) 39.87 g/L, volatile suspended solid(VSS) 15.6 g/L, VSS/TSS 39.13%, pH 6.94.

The basal medium used in the experiment contained(per liter of water): KH_2PO_4 (0.027 g), $MgSO_4 \cdot H_2O$ (0.300 g), $CaCl_2$ (0.136 g), $KHCO_3$ (0.500 g), and 1 ml/L of trace element solution Ⅰ and Ⅱ. Trace element solution Ⅰ contained(per liter of water): EDTA 5.000 g, $FeSO_4$ 5.000 g. Trace element solution Ⅱ contained(per liter of water): EDTA 15.000 g, H_3BO_4 0.014 g, $ZnSO_4 \cdot 7H_2O$ 0.430 g, $MnCl_2 \cdot 4H_2O$ 0.990 g, $CuSO_4 \cdot 5H_2O$ 0.250 g, $NaMoO_4 \cdot 2H_2O$ 0.220 g, $NiCl_2$ 0.199 g, $NaSeO_4 \cdot 10H_2O$ 0.210 g.

1.2 Reactor system

The laboratory-scale bioreactor was made of grass with working volume of 1.3 l, height of 0.65 m and internal diameter of 0.10 m. Soft padding was filled for bacteria to grow. Three sampling ports were evenly distributed over the height of reactor. The flow diagram of the reactor system is shown in Fig. 1.

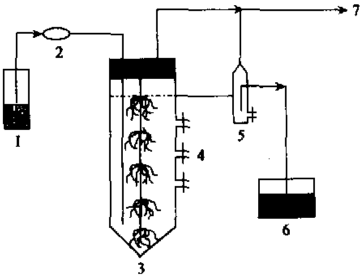

Fig. 1 The Anammox bioreactor system and its diagram
1. influent tank; 2. pump; 3. reactor; 4. sampling port; 5. gas-liquid-solid separator; 6. effluent tank; 7. gas out

The influent was pumped into the bottom of the reactor and flowed upward through the biofilm. The treated 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 bioreactor was covered with a black cloth to eliminate the inhibition of light on Anammox activity.

1.3 Analyses

The concentration of NH_4^+-N, NO_2^--N, NO_3^--N were determined using the standard method issued by the Environmental Protection Agency (EPA) of China. The pH was determined using glass electrodes connected to pHS-9V pH-meter. Dissolved oxygen concentration was determined using YSI MODEL58 DO-meter. The most probable number (MPN) of nitrifying bacteria was determined as described by Chen (Chen, 1998). 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.

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2 Results and discussion

2.1 Enrichment of nitrifying bacteria

The bioreactor was inoculated with 0.7 L of the aerobic activated sludge and was operated at 30℃. In order to enrich nitrifying bacteria, it was run under aerobic conditions for the first 51 d. Oxygen was supplied by an aeration pump at about 6.4 L/min and dissolved oxygen concentration was kept at about 6.5 mg/L. Influent solution was made by adding $(NH_4)_2SO_4$ into the basal medium and its pH value was adjusted to 7—8 with 0.5 mol/L $NaHCO_3$. Hydraulic retention time (HRT) was kept at 1 d, and influent ammonia concentration was raised step by step until 57 mmol/L was reached.

As shown in Fig.2, the performance of the bioreactor was not good with average ammonia removal of 74.79% during the first several days. From day 6 on, its performance improved. Effluent ammonia concentrations went down and then remained at a low level, while effluent nitrite and nitrate concentration gradually went up. On day 51, volumetric ammonia loading rate reached 56.96 mmol/(L · d), and volumetric conversion rate reached 56.85 mmol/(L · d). This implied that the activity of nitrifying sludge was gradually increased.

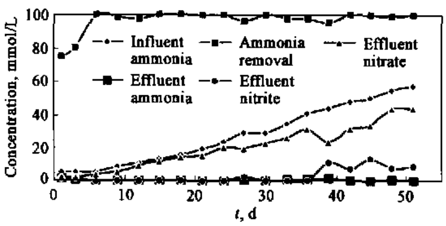

Fig.2 Performance of the bioreactor under aerobic conditions

The results of bacteria counting experiment could give an explanation to the above-mentioned phenomenon. During the same period, the nitrifying bacteria grew quickly. On day 51, their number in the enriched sludge was almost 100 times higher than that in the seeding sludge (Table 1).

Table 1 The number of nitrifying bacteria in seeding sludge and enriched sludge

Number of positive tube	Dilution 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	MPN, cells/ml
Seeding sludge	4	4	3	2	0	0	2.0×10^5
Enriched sludge	4	4	4	3	1	0	1.6×10^7

2.2 Start-up of Anammox bioreactor

When enough nitrifying bacteria were accumulated, the aeration pump was turned off. The bioreactor was sealed by a rubber stopper and was flushed with argon to remove dissolved oxygen. It was then fed with synthetic wastewater (5 mmol/L NH_4^+, 5 mmol/L NO_2^-). After ammonia and nitrite removal was higher than 90%, influent concentration was raised to 20 mmol/L by a step of 3 mmol/L over a period of 31 days, while HRT was kept at 1 d.

As shown in Fig.3, the bioreactor did not work well with average ammonia and nitrite removal as low as 34.79% and 61.04%, respectively. From day 10 on, its performance improved obviously. During the following 20 days, influent ammonia concentration was increased from 4.72 to 19.45 mmol/L and nitrite concentration from 5.02 to 19.82 mmol/L progressively, both ammonia and nitrite removal were more than 90%. This indicated that Anammox bioreactor had been started up successfully.

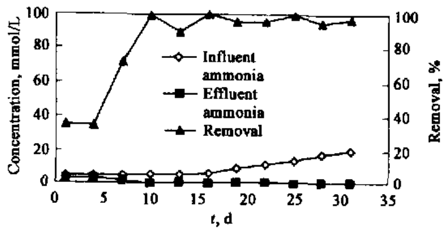

Fig.3 The change of ammonia during the start-up of Anamox bioreactor

It has been proved that there is a relation between aerobic ammonia oxidizers and anaerobic ammonia oxidizers. Anammox bacteria show many common physiological characteristics to nitrifiers. For example, they metabolize ammonia and hydroxylamine aerobically and their conversion product is nitrite (Zheng, 2000; 2001). On the contrary, some nitrifiers like *Nitrosomonas europaea* and *Nitrosomonas eutropha*, which are always associated with oxidizing ammonia to nitrite aerobically, have Anammox activity, and can oxidize ammonia with nitrite as electron acceptor under strictly anaerobic conditions (Strous, 1997). Moreover, a lot of nitrifiers ($9 \pm 5 \times 10^3$ cells/l) have been detected in Anammox sludge (Van de Graff, 1996). The metabolic diversity of ammonia oxidizers offers a possibility for us to take nitrifying activated sludge as seeding sludge of Anammox bioreactor. This experiment has demonstrated that it is feasible to start up Anammox bioreactor with the enriched nitrifying sludge.

Ammonia oxidizers such as *Nitrosomonas* species show substrate diversity as well as metabolic diversity. They are able to carry out denitrification with either ammonia or hydrogen as electron donor (Van de Graff, 1996). The maximum growth rate of the former (0.96—1.92/d) is far higher than that of the latter (0.06/d) (Zheng, 1998; 2000; Strous, 1999). In theory, the substrate diversity of ammonia oxidizers offers another possibility for us to facilitate the growth of nitrifying activated sludge and to shorten the start - up period. The feasibility is under investigation (Fig.4).

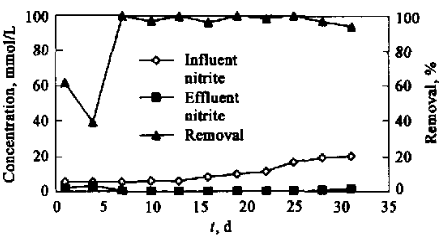

Fig.4 The change of nitrite during the start-up of Anammox bioreactor

2.3 Performance of Anammox bioreactor

In order to test the loading performance of Anammox bioreactor, influent ammonia and nitrite concentration were both set at about 10 mmol/L to avoid inhibition of substrates (Strous, 1998), HRT was shorten from 20.66 to 3.02 h step by step (Table 2). On day 64, volumetric total nitrogen loading rate and volumetric total nitrogen conversion rate reached 179.95 mmol/(L·d) and 142.09 mmol/(L·d), respectively. It is reported that the maximal volumetric conversion rate of A/O process is about 10.0 mmol/(L·d) when sewage wastewater is treated(Liu, 2000), and the maximal volumetric conversion rate of the short nitrifying/denitrifying process is about 84.8 mmol/(L·d) when sodium glutamate production wastewater is treated (Fang, 2001). Compared with these processes, Anammox process is far more efficient.

Table 2 Performance of Anammox bioreactor at different HRT

HRT, h	NH_4^+, mmol/(L·d) Volumetric loading rate	NH_4^+ Volumetric conversion rate	NO_2^-, mmol/(L·d) Volumetric loading rate	NO_2^- Volumetric conversion rate	N_{tot}, mmol/(L·d) Volumetric loading rate	N_{tot} Volumetric conversion rate
20.66	10.51	10.00	11.01	10.79	21.51	20.79
14.75	16.06	15.60	16.82	16.82	32.88	32.42
13.48	19.21	18.58	18.00	18.00	37.21	36.58
12.36	23.11	22.46	21.75	21.75	44.86	44.21
9.39	27.90	26.11	25.32	22.99	53.22	49.09
9.04	31.49	31.49	30.71	30.09	62.20	61.58
7.07	34.44	32.79	35.86	35.86	70.30	68.65
6.37	39.00	39.00	41.17	41.16	80.17	80.17
5.70	43.91	42.86	44.88	44.88	88.80	87.74
4.84	47.66	42.73	50.63	50.61	98.29	93.35
4.80	52.18	51.92	49.83	49.83	102.01	101.75
4.56	55.89	52.49	55.34	55.34	111.23	107.83
4.60	58.35	56.93	60.08	60.08	118.43	117.01
4.06	63.93	51.60	56.03	49.31	119.96	100.91
4.00	64.97	51.70	64.72	64.50	129.69	116.20
3.49	69.04	44.73	70.82	60.14	139.87	104.88
3.45	74.26	60.18	75.70	72.20	149.96	132.38
3.38	79.26	62.70	89.89	83.27	169.15	145.97
3.31	82.39	64.13	83.06	80.13	165.45	144.26
3.03	86.86	63.23	89.58	86.32	176.43	149.55
3.02	89.02	60.03	90.93	82.06	179.95	142.09

The experiment for stability of Anammox bioreactor followed that for loading performance. Influent ammonia and nitrite concentrations were both fixed at about 10 mmol/L. Volumetric conversion rate was set at about 84.8 mmol/(L·d) (reported maximal rate) and its corresponding HRT was about 6 h(Table 2). After the bioreactor was operated under above-mentioned conditions for 10 days, following parameters could be obtained: average ammonia removal 96.89%, average nitrite removal 99.67%, volumetric ammonia loading rate 43.39 mmol/(L·d), volumetric ammonia conversion rate 41.99 mmol/(L·d), volumetric total nitrogen loading rate(including ammonia and nitrite) 84.67 mmol/(L·d), volumetric total nitrogen removal 83.19 mmol/(L·d)(Table 3). As Table 4, the stability of Anammox bioreactor was very good. The difference between maximal and minimal volumetric total nitrogen loading rate was 19.49 mmol/(L·d), and its relative variation was 23.6%. However, the difference between maximal and minimal total nitrogen removal was only 7.2%, and its relative variation was as low as 7.3%. In other words, a large fluctuation in operation parameter led to a small change in efficient parameter.

2.4 Characteristics of Anmmox sludge

Most parts of Anmmox sludge was firmly attached to the soft padding, small parts existed in the form of floc at first and then in the form of granular sludge three months later. Since gas production in the bioreactor was not large enough to promote sludge granulation, the fluid-flow might play a very important role in the formation of granular sludge. As shown in Fig. 5, the effluent sludge concentration was low with average VSS concentration of 0.023 g/L during whole operation.

Table 3 Performance of Anammox bioreactor at HRT 6.32 h

NH_4^+, mmol/(L·d) Volumetric loading rate	NH_4^+ Volumetric conversion rate	NO_2^-, mmol/(L·d) Volumetric loading rate	NO_2^- Volumetric conversion rate	N_{tot}, mmol/(L·d) Volumetric loading rate	N_{tot} Volumetric conversion rate
43.30	37.78	42.78	42.08	86.08	79.86
39.94	37.75	36.16	35.97	76.11	73.92
43.65	41.57	40.54	40.54	84.19	82.10
48.85	47.53	43.31	43.31	92.15	90.84
41.10	40.89	39.51	39.51	80.62	80.41
42.56	41.95	44.94	44.94	87.50	86.89
45.92	45.92	41.00	41.00	86.92	86.92
39.68	38.97	34.61	34.61	74.29	73.58
48.22	46.40	46.01	46.01	94.23	92.41
43.39	41.99	41.20	41.20	84.59	83.19

Table 4 Stability of Anammox bioreactor at HRT 6.32 h

Parameters for stability	Volumetric total *nitrogen* loading rate, mmol/(L·d)	Volumetric total *nitrogen* conversion rate, mmol/(L·d)	*Total nitrogen* removal, %
Minimum	74.29	73.58	92.8
Maximum	94.23	92.41	100.0
Average	82.67	83.01	98.0
Difference	19.94	18.83	7.2
Percentage	23.6	22.7	7.3

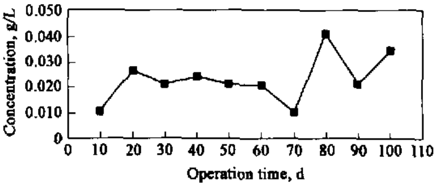

Fig.5 Effluent VSS concentration during whole operation

The color of Anmmox sludge changed from coffee to deep red as its Anammox activity increased. Strous pointed out that Anammox activity and cytochrome c went up and down in a parallel way(Strous, 1998). The increase of cytochrome c content in Anmmox sludge was the reason of color change.

3 Conclusions

The bioreactor was suitable to the development of nitrifying activity and the accumulation of nitrifying activated sludge. After run aerobically for 51 d, volumetric ammonia loading rate reached 56.96 mmol/(L·d), and volumetric conversion rate reached 56.85 mmol/(L·d).

Nitrifying activated sludge could serve as seeding sludge to start up Anammox bioreactor. Anammox bioreactor was highly efficient with total nitrogen volumetric removal rate of 149.55 mmol/(L·d), and its performance was very stable that a large fluctuation(23.6%) of volumetric total nitrogen loading rate only led to a small change(7.3%) of total nitrogen removal.

Soft padding helped to retain Anammox sludge in the bioreactor effectively. The biomass loss from washout was small with average effluent VSS concentration as low as 0.023 g/L.

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