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Simultaneous nitrification and denitrification in step feeding biological nitrogen removal process

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Abstract

The simultaneous nitrification and denitrification in step-feeding biological nitrogen removal process were investigated under different influent substrate concentrations and aeration flow rates. Biological occurrence of simultaneous nitrification and denitrification was verified in the aspect of nitrogen mass balance and alkalinity. The experimental results also showed that there was a distinct linear relationship between simultaneous nitrification and denitrification and DO concentration under the conditions of low and high aeration flow rate. In each experimental run the floc sizes of activated sludge were also measured and the results showed that simultaneous nitrification and denitrification could occur with very small size of floc.

Key words: biological nitrogen removal; dissolved oxygen; floc size; simultaneous nitrification and denitrification; step feeding process

Introduction

Because of the stringent nutrient levels being required in the effluents to protect lakes and other natural water from eutrophication, removal of nitrogen from discharged wastewater has been required in many wastewater treatment plants. The step-feeding anoxic/aerobic biological nitrogen removal process is characterized by high total nitrogen removal efficiency and unnecessary internal recycle. During the last decade, many researchers have put much attention to this process and drawn many valuable conclusions (Larrea *et al*., 2001; Zhu *et al*., 2005). Moreover, nitrogen loss and simultaneous nitrification and denitrification in step feeding process were also reported by researchers (Gorgun *et al*., 1996; Zhu *et al*., 2007a).

Simultaneous nitrification and denitrification (SND) implies that nitrification and denitrification occur concurrently in the same reaction vessel under identical overall operating conditions. SND is of particular interest in saving anoxic volume and in treatment wastewaters with low C:N ratio (Zhu *et al*., 2007b). The mechanism and explanation for SND can be divided into two broad categories. The physical and conventional explanation is that SND occurs as a consequence of DO concentration gradients within microbial flocs or biofilms due to diffusional limitations. The biological explanations for SND are in contrast to the traditional "engineering" conception of nitrification and denitrification. Microbiologists have reported the existence of aerobic denitrifiers as well as heterotrophic nitrifiers (Kim *et al*., 2005; Zhu *et al*., 2007b).

Previous researches showed that three principle factors, carbon supply, oxygen concentration and floc size, predominantly influenced SND (Zhu *et al*., 2007b). But there were still some unresolved problems. The large floc parameter was likely to promote the SND due to diffusional limitation of oxygen in the floc, whether small size parameter is adverse to SND was still not reported. In addition, it has not been reported in literature that denitrification reaction could be accomplished under high DO concentration condition and the relationship of DO concentration and SND. In the article, the SND in step feeding process was investigated. The main objective was to explain the relationship of DO concentration and SND.

1 Material and methods

1.1 Reactor system

A four stages step feeding biological nitrogen removal process made of plexiglass with a working volume of 80 L (800 mm × 220 mm × 500 mm) was used in this study (Fig.1). The volume of four stages was the same and the volume ratio of anoxic to oxic in each stage

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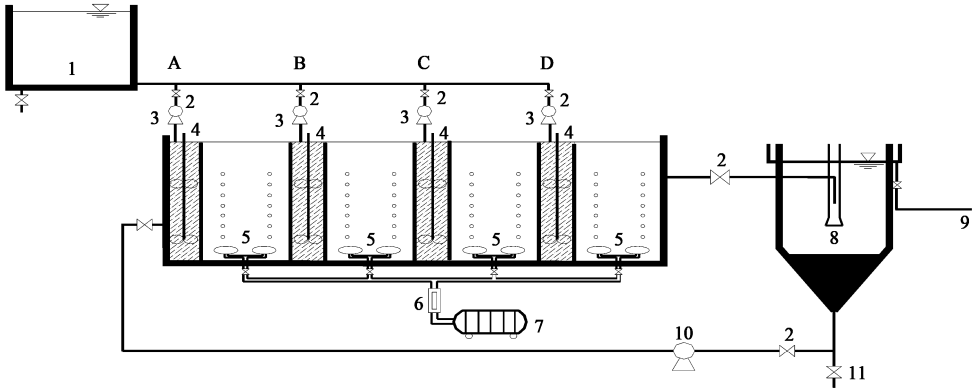

Fig. 1 Schematic diagram of step feeding biological nitrogen removal process. (1) influent tank; (2) check value; (3) feeding pump; (4) mechanical mixer; (5) diffuser; (6) air flow meter; (7) air compressor; (8) secondary clarifier; (9) effluent; (10) return sludge pump; (11) waste sludge.

was maintained at 1:3.5. A mechanical mixer was used in anoxic zone to provide liquid mixed well. A number of outlets for sample analysis were emplaced with the distance of 20 cm from reactor bottom in each anoxic and aerobic zone. An air compressor with micropore diffuser was used for aeration. An air flow meter was used for controlling the airflow rate. The type of final clarifier was upright clarifier with a working volume of 30 L.

1.2 Experimental operating procedure

The reactor feeding consisted of synthetic wastewater with characteristics similar to those of domestic wastewater (Watnabe *et al*., 1995). It was prepared by using tap water, dechlorinated by the use of sodium thiosulfate, and the addition of dosages of chemicals, as indicated in Table 1. During the experimental period, the solid retention time (SRT) was controlled at 18 d using hydraulic controlling approach (Zhu *et al*., 2007c).

The total influent flow rate was 240 L/d and each stage was set at 60 L/d, respectively, which was controlled by four same peristaltic pumps (Model ESBN4, Iwaki Cop. Japan). The sludge return ratio was set at 50% of influent flow rate by a peristaltic pump. The experimental operating procedure is shown in Table 2. During each experiment the step feeding process was operated for more than two sludge ages (36 d).

1.3 Samples and analytical procedures

The parameters measured included floc size, temperature, DO, COD, NH_4^+-N, NO_2^--N, NO_3^--N, TN and alkalinity. Samples were prepared by filtering with 0.45 μm Whatman filter papers. The DO measurements were conducted using YSI Model 58 DO meter. The floc size was measured using Liquid Particle Counting System Model 9703 (HIAC Royco) with the sensor HRLD-400. All analyses were performed according to the Standard Methods (APHA, 1995).

Table 1 Composition of synthetic wastewater

Compound	Concentration (mg/L)
Brewery wastewater	1080
NH_4Cl	114.6
$NaHCO_3$	900.0
KH_2PO_4	105.0
$MgSO_4{\cdot}7H_2O$	150.0
NaCl	110.0
$CaCl_2$	85.5
$ZnSO_4$	90.0

Table 2 Experimental operating procedure

Lab	Influent TKN (mg/L)	Influent COD (mg/L)	Aeration flowrate (m^3/h)
E1	40	268	0.6
E2	40	345	0.6
E3	55	272	0.6
E4	55	348	0.6
E5	46	278	1.2
E6	46	340	1.2
E7	46	450	1.2

2 Results and discussion

2.1 Simultaneous nitrification and denitrification

The original experimental observed mass of ammonia loss and NO_x-N formation in seven experiments are shown in Table 3. It shows that the mass of NH_3-N decay was obviously larger than the mass of NO_x-N formation, especially in the former stage of the step feeding process. But this phenomenon of nitrogen loss could not definitely be attributed to the simultaneous nitrification and denitrification. In step feeding process suspended solids gradient along the reactors was formed because of influent step feeding. The large amounts of biomass in the former stage grew and reproduced rapidly with abundant organic substrate. It is well known that nitrogen is also needed in the process of metabolisms of activated sludge microorganisms. So it is necessary to make certain that the nitrogen loss should be attributed to the assimilation of activated sludge microorganisms or simultaneous nitrification and denitrification.

In steady state of activated sludge process the mass of sludge discharged is equal to the mass of microorganism growth and formation. The nitrogen consumption because of cell assimilation would be calculated with the cell formula ($C_5H_7NO_2$) (Bruce and Perry, 2001), in which N is accounted for about 12%. The calculated results of nitrogen loss for assimilation during different experimental

Table 3 Observed mass of ammonia loss and NO_x-N formation during different experimental operating conditions

Lab	Stage	NH_3-N (mg/L)		Mass of NH_3-N decay (g/d) (1)	NO_x-N (mg/L)		Mass of NO_x-N formation (g/d) (2)
		Start of aerobic zone	End of aerobic zone		Start of aerobic zone	End of aerobic zone	
E1	A	14.2480	0.8220	2.4168	0.0000	7.8330	1.4100
	B	10.0830	0.0000	2.4199	0.0000	5.9300	1.4232
	C	8.0010	0.0000	2.4002	0.0000	4.9950	1.4986
	D	6.1650	0.0000	2.2195	0.0000	4.1150	1.4815
E2	A	12.9000	2.6500	1.8451	0.0000	5.5420	0.9977
	B	9.8900	1.8550	1.9284	0.0000	4.2190	1.0126
	C	8.7200	1.3000	2.2260	0.0000	4.3500	1.3051
	D	6.0950	0.2650	2.0988	0.0000	3.5310	1.2713
E3	A	18.0200	6.3600	2.0988	0.0000	6.5420	1.1777
	B	14.5750	3.1800	2.7348	0.0000	6.8440	1.6426
	C	13.2500	4.2400	2.7031	0.0000	5.5950	1.6786
	D	11.3950	4.7700	2.3851	0.0000	4.3750	1.5751
E4	A	14.1750	6.0000	1.6214	0.0000	3.3750	0.6074
	B	12.0000	5.5000	1.5300	0.0000	2.6020	0.6245
	C	9.2000	5.2000	1.3200	0.0000	2.2800	0.6840
	D	8.5000	3.0000	1.9800	0.0000	3.0730	1.1064
E5	A	11.4800	0.0000	2.2664	0.0000	8.7920	1.4826
	B	10.7700	0.0000	2.5848	0.0000	9.0470	2.1713
	C	8.7700	0.0000	2.7311	0.0000	7.4100	2.2231
	D	7.2400	0.0000	2.6964	0.0000	6.3850	2.1987
E6	A	9.0200	0.0000	1.8236	0.0000	6.5830	1.0849
	B	6.8900	0.0000	1.6536	0.0000	6.3750	1.253
	C	6.0950	0.0000	1.7686	0.0000	5.4000	1.262
	D	5.0350	0.0000	1.8127	0.0000	4.4060	1.2862
E7	A	11.3950	0.7950	1.9080	0.0000	6.7920	1.2226
	B	7.9500	0.0000	1.9080	0.0000	6.4690	1.5526
	C	8.2150	0.0000	2.4646	0.0000	6.3450	1.9034
	D	6.6250	0.0000	2.3851	0.0000	5.1040	1.8374

Lab	Stage	Mass of N loss (1)–(2) (g/d)	Total N loss in system (g/d)	Discharge sludge conc. (mg/L)	Discharge rate (mg/d)	N loss for assimilation (g/d)	Average MLSS (mg/L)
E1	A	1.0068	3.6432	7350	13.230	1.5876	4900
	B	0.9967					3680
	C	0.9017					2960
	D	0.7380					2450
E2	A	0.8474	3.5116	7530	13.554	1.6265	4976
	B	0.9158					3778
	C	0.9209					2977
	D	0.8275					2510
E3	A	0.9211	3.8479	7440	13.392	1.6070	4918
	B	1.0922					3736
	C	1.0246					2933
	D	0.8100					2480
E4	A	1.014	3.4291	7680	13.824	1.6783	4995
	B	0.9055					3790
	C	0.636					3050
	D	0.8736					2590
E5	A	0.7838	2.203	7518	13.532	1.6563	4947
	B	0.4135					3772
	C	0.508					2995
	D	0.4977					2556
E6	A	0.7387	2.1724	7830	14.094	1.6731	4977
	B	0.4006					3780
	C	0.5066					3001
	D	0.5265					2582
E7	A	0.6854	2.1496	7596	13.673	1.7107	5050
	B	0.3554					3905
	C	0.5611					3150
	D	0.5477					2640

operating conditions are shown in Table 3. From Table 3 it was obvious the mass of nitrogen loss in the system were larger than the mass of nitrogen loss for assimilation. So it could reasonably be speculated that there must be simultaneous nitrification and denitrification in the reactor.

The occurrence of SND was also verified with alkalinity variation in the reactor. The observed rates of alkalinity loss due to nitrification during different experimental operating conditions are shown in Table 4. From Table 4 the calculated results of alkalinity loss were all below the theoretical value of 7.14 mg/L (Bruce and Perry, 2001), average of 6.247 mg/L, of alkalinity as $CaCO_3$ per mg

Table 4 Observed alkalinity consumption, rates of ammonia loss and NO_x-N formation, DO concentration during different experimental operating conditions

Lab	Stage	Alkalinity consumption (mg/H)	$\frac{\Delta Alkalinity}{\Delta NH_3\text{-}N}$ (1)	$\frac{\Delta Alkalinity}{\Delta NO_x\text{-}N}$ (2)	Alkalinity loss ratio (1):(2)	Rate of NH_4 decay ($kgNH_4$/(kg MLSS·d))	Rate of NO_3 formation ($kgNO_3$/(kg MLSS·d))	$\frac{\Delta NO_x\text{-}N}{\Delta NH_3\text{-}N}$	Average DO (mg/L)
E1	A	675.00	6.7034	11.4898	0.5834	0.0322	0.0188	0.5839	0.3900
	B	625.00	6.1986	10.5397	0.5881	0.0430	0.0253	0.5890	0.8500
	C	562.50	5.6243	9.0090	0.6243	0.0533	0.0333	0.6250	0.9700
	D	565.05	6.1103	9.1543	0.6675	0.0592	0.0395	0.6670	1.9700
E2	A	468.75	6.0976	11.2776	0.5407	0.0246	0.0133	0.5390	0.1670
	B	560.00	6.9695	13.2733	0.5251	0.0343	0.0180	0.5262	0.2630
	C	625.00	6.7385	11.4942	0.5863	0.0495	0.0290	0.5850	0.5460
	D	525.00	6.0034	9.9122	0.6057	0.0560	0.0339	0.6050	0.8460
E3	A	581.25	6.6467	11.8466	0.5611	0.0280	0.0157	0.5600	0.3600
	B	675.01	5.9237	9.8627	0.6006	0.0486	0.0292	0.6000	0.6000
	C	656.25	5.8269	9.3834	0.6210	0.0601	0.0373	0.6200	0.9360
	D	675.00	6.7925	10.2858	0.6604	0.0636	0.0420	0.6600	1.4800
E4	A	266.25	5.7490	10.5185	0.5466	0.0148	0.0081	0.5490	0.1670
	B	290.00	6.4444	11.1452	0.5782	0.0192	0.0111	0.5780	0.2630
	C	350.00	7.0000	12.2807	0.5700	0.0267	0.0152	0.5710	0.5460
	D	525.00	6.3636	11.3895	0.5587	0.0528	0.0295	0.5580	0.7680
E5	A	431.25	5.0087	6.5400	0.7659	0.0276	0.0211	0.7660	2.1630
	B	750.00	6.9638	8.2901	0.8400	0.0460	0.0386	0.8400	4.0900
	C	625.01	5.7013	6.7477	0.8449	0.0585	0.0494	0.8440	3.9300
	D	449.99	4.1436	4.6985	0.8819	0.0695	0.0613	0.8818	4.5630
E6	A	468.75	6.9290	9.4941	0.7298	0.0216	0.0158	0.7326	2.4630
	B	475.00	6.8940	7.4509	0.9253	0.0294	0.0272	0.9266	4.3800
	C	475.00	6.2346	7.0370	0.8860	0.0406	0.0360	0.8866	4.4700
	D	337.50	4.4687	5.1067	0.8751	0.0483	0.0423	0.8750	4.1600
E7	A	562.50	7.0755	11.0424	0.6408	0.0254	0.0163	0.6430	1.7830
	B	540.00	6.7925	8.3476	0.8137	0.0339	0.0276	0.8130	3.7400
	C	660.00	6.4273	8.3216	0.7724	0.0548	0.0423	0.7720	3.6800
	D	705.00	7.0943	9.2084	0.7704	0.0636	0.0490	0.7710	3.8260

of ammonia oxidized. The low alkalinity consumption rates could be attributed to the occurrence of simultaneous nitrification and denitrification, because alkalinity would also be affected by two processes occurring at the same time.

The theoretical rate of alkalinity production during denitrification is 3.57 mg of alkalinity per mg of NO_x-N reduced. Theoretically, the ratio of observed alkalinity destruction rates with respect to ammonia loss and NO_x-N formation is corresponded to the ratio of observed rates for NO_x-N formation and ammonia loss. Fig.2 presents a comparison of the data shown in column 6th and column 9th of Table 4. The production of alkalinity due to simultaneous nitrification and denitrification would have the effect of decreasing the observed rate of alkalinity loss and NO_x-N formation per mg of ammonia oxidized. The ratio of NO_x-N formed to NH_3-N consumed is expected to be near the theoretical value after accounting for NH_3-N consumed in synthesis. This ratio would also be reflected in the kinetic rates ammonia consumption and NO_x-N formation depicted in Table 4. From the figure the data were also consistently less than 1, which suggested that nitrification and denitrification occurred simultaneously.

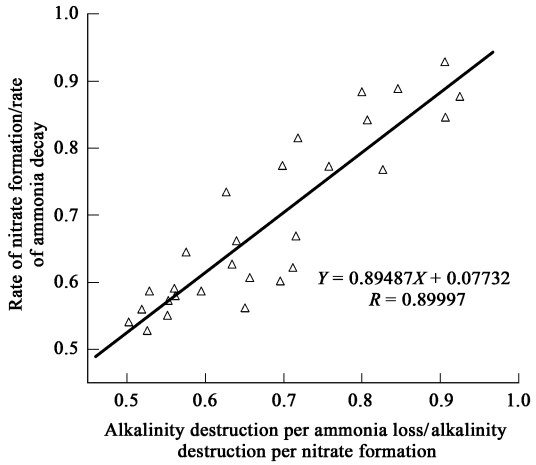

Fig. 2 Comparison of the ratio of alkalinity loss with respect to NH_3-N decay and NO_x-N formation with the ratio of NO_x-N formation to NH_3-N decay.

2.2 Dissolved oxygen concentration

Experiments were performed under low and high aeration flow rate condition to evaluate the influence of DO concentration on the SND. The results are shown in Table 4. With the experimental data in column of 9th and 10th of Table 4, the dependency of the ratio of NO_x-N formation rate to NH_3-N decay rate on DO could be depicted in Fig.3. From Fig.3 the following equations were drawn:

low DO concentration :

$$\frac{\Delta NO_x\text{-}N}{\Delta NH_3\text{-}N} = 0.07502DO + 0.53455 \quad (1)$$

high DO concentration :

$$\frac{\Delta NO_x\text{-}N}{\Delta NH_3\text{-}N} = 0.07555DO + 0.54036 \quad (2)$$

From the figure the dependency of the ratio on DO under low and high DO concentrations respectively was linearity.

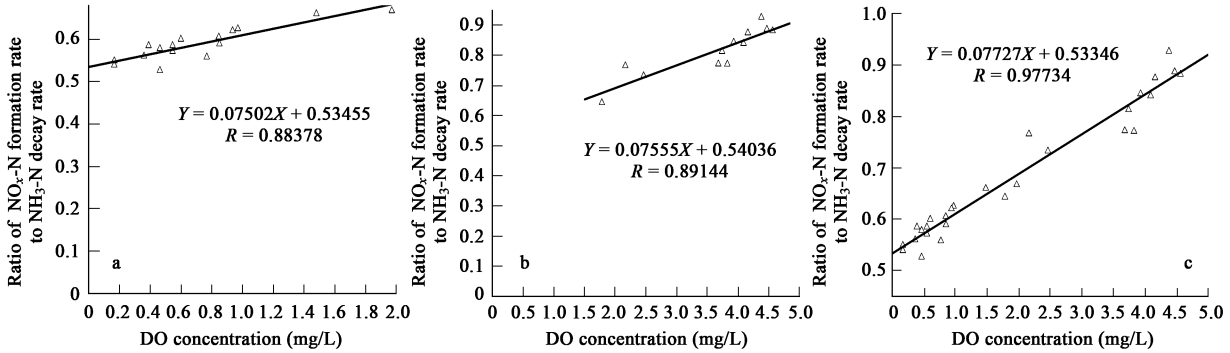

Fig. 3 Relationship of DO concentration and the ratio of the NO_x-N formation rate to the NH_3-N decay rate. (a) low aeration flow rate; (b) high aeration flow rate; (c) over all.

The DO concentration showed greatly effects on SND. The overall dependencies of the ratio on DO under both low and high DO concentrations was drawn and expressed as:

$$\frac{\Delta NO_x\text{-N}}{\Delta NH_3\text{-N}} = 0.07727DO + 0.53346 \quad (3)$$

Equation (3) shows that there is an obvious linear relationship between DO concentration and simultaneous nitrification and denitrification. The rate of NO_x-N formation was almost equal to that of ammonia decay when the DO concentration was 6.05 mg/L. At this DO concentration simultaneous nitrification and denitrification did not happen in the reactor. When the DO concentration was 0.5 mg/L the rate of NO_x-N formation was 0.57 times than that of ammonia decay, which was corresponded well with the results of the Munch *et al*. (1996). In this research the specific relationship of DO concentration and SND was proposed.

2.3 Floc size

To test the hypotheses of physical explanation on SND, the activated sludge floc sizes of different experimental conditions were measured and shown in Fig.4. The average floc size of this study was 7.754 μm, which was significantly smaller than the mean floc diameter reported by Bakti and Dick (1992) of 32 μm for nitrifying suspended growth biological reactors, the value of 80 μm reported by Beccari *et al*. (1992). Small floc diameter should be attributed to the influent characteristic. Compared with the real municipal sewage the synthetic wastewater is more simplex in constitute and content. The large floc parameter is likely to promote the SND due to diffusional limitation of oxygen in the floc. But in such small floc size it is impracticable to produce DO concentration gradients within microbial flocs leading to denitrification in this area (Hisashi *et al*., 2003; Li and Bishop, 2004), which remarkably clarify the incorrectness of physical explanation standpoint.

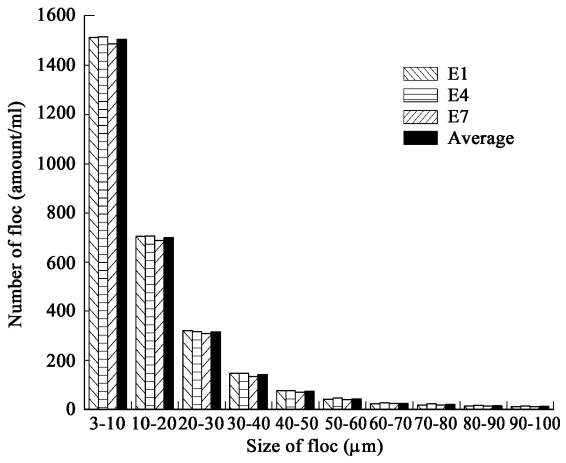

Fig. 4 Floc size under the experimental condition of E1, E4 and E7.

3 Conclusions

The laboratory pilot scale studies were conducted to evaluate the simultaneous nitrification and denitrification in step feeding biological nitrogen removal process. The results demonstrated that the biological occurrence of simultaneous nitrification and denitrification was obvious in step feeding process, even under high DO concentration. There was a distinct linear relationship between the ratio of ammonia decay rate to NO_x-N formation rate and DO concentration. The mathematical expression of SND with DO concentration was proposed to be: $\frac{\Delta NO_x\text{-N}}{\Delta NH_3\text{-N}} = 0.07727DO + 0.53346$. In addition, it is verified that the phenomenon of simultaneous nitrification and denitrification should not solely attribute to physical matter.

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