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The integration of methanogenesis with denitrification and anaerobic ammonium oxidation in an expanded granular sludge bed reactor

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Abstract: The integration of methanogenesis with denitrification and anaerobic ammonium oxidation(ANAMMOX) was studied in an expanded granular sludge bed(EGSB) reactor in this work. Experimental results from the continuous treatment of wastewater with nitrite and ammonium, which lasted for 107 days, demonstrated that wastewater with high nitrite and ammonium could be anaerobically treated in an expanded granular sludge bed reactor. More than 91% to 97% of COD were removed at up to about 3.9 g COD/(L·d) of COD volumetric loading rate. More than 97% to 100% of nitrite was denitrified at up to about 0.8 g NO_2^--N/(L·d), which is 16 times higher than that in a conventional activated sludge system with nitrification/denitrification(0.05 gN/(L·d)). No dissimilatory reduction of nitrite to ammonium occurred in the process. However, maximum of about 40% ammonium was found to be lost. Batch tests of 15 days with sludge from the reactor showed that 100% of nitrite was denitrified completely, and about 3% of ammonium was removed when only ammonium (34.3 mg/L) and nitrite(34.3 mg/L) were added into the sludge suspension medium. Furthermore, about 15% of ammonium amounts were lost with organic COD addition. It suggested that the methanogenesis in the system could enhance ANAMMOX because of intermediate hydrogen produced during methanogenesis.

Keywords: methanogenesis; denitrification; ANAMMOX; EGSB reactor

Introduction

The UASB reactor and its various modifications have been successfully applied to the treatment of various kinds of wastewater(high to low strength, industrial and domestic) under psychrophilic, mesophilic and thermophilic conditions (Lettinga, 1995). Modern anaerobic reactors represent high-rate technologies of wastewater treatment because of the high concentration of biomass and the high volumetric loading rate of COD. The recovery of methane makes them also sustainable technologies. However, it is often the case that the effluent of anaerobic digestion contains high nitrogen concentrations with respect to wastewater discharge limits. As a result, further effluent treatment using other technologies is necessary. The biological nitrification/denitrification is the most common and efficient method to remove the nitrogen pollutant in wastewater (Cooper, 1994). Some processes involving aerobic nitrifying reactor coupled with an anaerobic denitrifiying methanogenesis digester have emerged in the past recent years(Akunna, 1994a; b; Tilche, 1994a; b; Hendriksen, 1996; Grauti, 1992; Lin, 1995). Some work has shown that denitrification and methanogenesis could simultaneously occur to remove efficiently nitrogen and carbon from high strength wastewater with high COD/N ratio, in which organic COD and nitrate were used as substrates (Percheron, 1999; Chen, 1993; Hanaki, 1989; Akunna, 1992; 1993; 1994a; b; Hendriksen, 1996). On the other hand, the recent development of nitrification/denitrification research tries to halt the nitrification of ammonium at nitrite, then to denitrify nitrite in order to decrease the energy of aeration for nitrification and to save electron donors of organic carbon for denitrification(Muller, 1995; Bock, 1995; Kuai, 1998; Helmer, 1998; Hippen, 1997; Siegrist, 1998). Hellinga *et al*.(Hellinga, 1997; 1998) developed a novel process——SHARON (single reactor with high activity ammonia removal over nitrite), which is operated in a single, stirred tank reactor without any sludge retention. In the SHARON process nitrite oxidizers are effectively outcompeted through special applications of high temperature(30---40℃), pH(7—8) and short HRT (e.g. 1 day). The results in a stable nitrification with nitrite are as the end product of the process. The SHARON process is suited to waste streams with high ammonium concentrations(Hellinga, 1997; 1998). Furthermore, a complete new pathway of the microbial metabolism of nitrogen, anaerobic ammonium oxidation(ANAMMOX) process, has been established(Strous, 1997a; 1997b; 1999a; 1999b; Jetten, 1997; Schalk, 1998; Mulder, 1995; van de Graaf, 1996), in which the presence of nitrite is a prerequisite. Using organic COD, NH_4^+-N and nitrite as substrates, this work aims to investigate the potential of

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integrating methanogenesis with denitrification and anaerobic ammonium oxidation in an EGSB reactor.

1 Materials and methods

Synthetic wastewater was prepared through the dilution of the stock solution. The synthetic wastewater stock solution composed of sucrose(76 g/L) or glucose (82 g/L), for the continuous treatments of wastewater with more than 120 mg NO_2^--N/L, NH_4Cl(10.2 g/L), $(NH_4)_2SO_4$(3.8 g/L), K_2HPO_4(1.98 g/L), KH_2PO_4(1.52 g/L), contained about 87 g COD/L with the 125 : 5 : 1 of the ratios of COD : N : P. Additional nitrogen sources were provided with $(NH_4)_2SO_4$ and $NaNO_2$ according to experimental requirements. At the same time, 2 g $NaHCO_3$ and 2 ml nutrient solution were added into each litre of wastewater. The mineral solution contained following compounds(g/L): EDTA (5), $ZnSO_4 \cdot 7H_2O$ (2.2), $CoCl_2 \cdot 6H_2O$ (1.6), $MnCl_2 \cdot 4H_2O$ (5.0), $CuSO_4 \cdot 5H_2O$ (1.6), $MgSO_4 \cdot 7H_2O$(5.0), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.1), $CaCl_2 \cdot 2H_2O$ (5.5), $FeSO_4 \cdot 7H_2O$ (5.0).

An UASB reactor of 2.2 L was used in the experiments. It was inoculated with the granular sludge taken from a full-scale UASB reactor treating the wastewater from the processing of potato. The inoculation concentration of the sludge was about 15 gVSS/L (or 18 gTSS/L). Firstly, low-strength wastewater with about 170 mgCOD/L but without any nitrite was treated for about 40 days in the mode of EGSB with the 4 m/h of up-velocity. Then, experiments using wastewater with about 170—700 mgCOD/L, 15—150 mgNO_2^--N/L and 20—40 mg NH_4^+-N/L were carried out. In these experiments, the reactor was operated in the mode of EGSB in order to reduce the potential toxicity of nitrite. The 4 m/h of up-velocity was used for the treatment of wastewater of 170 mgCOD/L with nitrite. For other wastewater with nitrite and ammonium, the up-velocity of 3.5 m/h was used. The mass balance test of the continuous treatment of wastewater with nitrite and ammonium was made for 5 days in the ending period of the experiment 7. The influent was prepared every day. Samples for influent, effluent and biogas were taken and analysed every day. All experiments of continuous treatments of wastewater were made at the temperature of 33℃.

Batch tests were carried out to get the balances of nitrogen and carbon and to verify the ANAMMOX capability of the sludge in the process when all experiments of continuous treatments of wastewater had been completed. The sludge taken from the reactor was washed three times with distilled water to remove the background of COD, NH_4^+ and NO_2^-. It was then stored anaerobically for 15 days in a refrigerator at 4℃. About 10 ml of the washed sludge (about 300 mg TSS) and 60 ml of the medium prepared according to requirements were put in a 120 ml serum bottle. Oxygen in the bottle and the medium was removed by flushing for 15 minutes with helium gas. Serum bottles were sealed with rubber caps and kept at 33℃. Pressure in the head of bottles and concentrations of CH_4, N_2, N_2O, and CO_2 were monitored during the process. The COD, NO_2^--N and NH_4^+-N in the medium were measured at the beginning and the ending of each batch test. Batch tests were run in two series and in triple for each medium. The gas samples in the first series were taken several times for monitoring reactions in the process. The second series was only sampled and analysed at the beginning and ending of each batch test for accurate calculation of C and N balances. Syringes used to take samples from the head of serum bottles were flushed thoroughly with helium gas before sampling. The parallel control tests were also run in two series.

TSS, VSS, COD, NH_4^+-N and (NO_2^- + NO_3^-)-N analyses were performed as described in the APHA standard methods (APHA, 1992). TSS and VSS were measured by the heating method. COD was measured by the potassium dichromate-ferrous ammonium sulphate method. NH_4^+-N and (NO_2^- + NO_3^-)-N were measured with the Kjedahl distillation method. The measured amounts of (NO_2^- + NO_3^-)-N in samples tested were equal to the amounts of NO_2^--N since there was no NO_3^--N in the samples tested. CH_4, O_2, N_2, CO_2 and N_2O were measured with gas chromatography. Chrompack 437A was used for N_2O analysis. Chrompack CP9000 was used for measurements of CH_4, O_2, N_2, CO_2. An activated aluminium column(100—120 mesh, 1.8 m, 1/8 inch) was used for CH_4 measurement with a flame ionisation detector at 50℃ oven, 250℃ detector and 100℃ injector. A CTRI column with activated molecular sieve 5A packed outer and Porapack R mixture packed inner was used for measurements of O_2, N_2 and CO_2 with a thermal conduction detector at 50℃ oven, 250℃ detector and 100℃ injector. A chromosorb column (80—110 mesh, 16 feet and 1/8 inch) was used for N_2O measurement with a flame ionisation detector at 90℃, oven at 300℃ detector and 90℃ at injector.

2 Results

The time-course profile of COD removal from the low-strength wastewater (about 170 mgCOD/L) without nitrite is presented in Fig.1. This experiment lasted about 40 days. The finally stable efficiency of COD removal reached to 91% with less than 15 mg COD/L of effluent under conditions of 7 hours hydraulic retention time (HRT) and 4 m/h upflow velocity (V_{up}).

In the second experimental phase, seven kinds of wastewater with different concentrations of nitrite were continuously treated in the reactor. Fig. 2 indicates the time-course profile of the treatment of the wastewater with about 350 mgCOD/L and about 120 mgNO_2^--N/L. Similar results had been acquired from the treatment of other wastewater with different nitrite concentrations. Generally, the performance of the reactor treating each kind of wastewater with nitrite and ammonium tended to stabilise within 5—10 days since feeding a new wastewater. Table 1 summarised the results of the treatment of 7 kinds of wastewater with nitrite and ammonium.

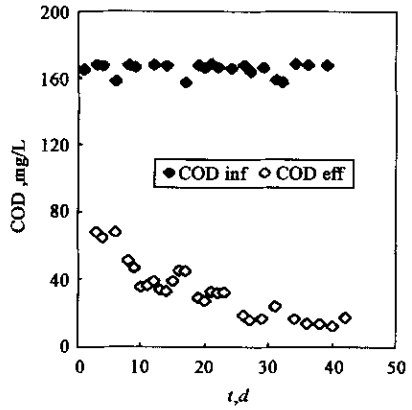

Fig.1 The treatment of low-strength wastewater without nitrite in an EGSB reactor at a volumetric loading rate of 0.6 gCOD/(L·d)

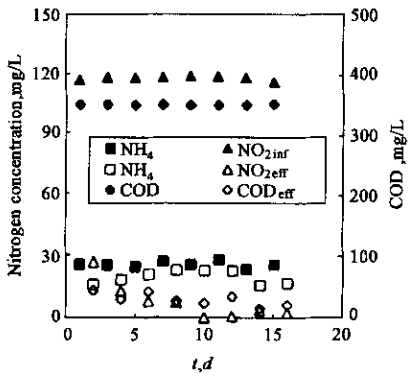

Fig.2 The treatment of wastewater with nitrite and ammonium in an EGSB reactor at the volumetric loading rates of 1.967 gCOD/(L·d), 0.665 gNO_2^--N/(L·d), and 0.147 gNH_4^+-N/(L·d)

Table 1 Operation conditions and treatment efficiencies of the reactor fed wastewater with different concentrations of nitrite and ammonium

Parameter (dimension)	Experiment						
	1	2	3	4	5	6	7
COD_{in}, mg/L	173	353	342	347	346	351	701
NO_2^--N_{in}, mg/L	15	34	51	76	98	119	150
NH_4^+-N_{in}, mg/L	19	25	27	27	26	26	38
VLR_{COD}, g/(L·d)	0.38	0.65	1.02	1.04	1.00	1.97	3.91
$VLR_{nitrite}$-N, g/(L·d)	0.03	0.06	0.15	0.23	0.28	0.67	0.84
$VLR_{ammonium}$-N, g/(L·d)	0.04	0.05	0.08	0.08	0.07	0.15	0.21
V_{up}, m/h	4	3.5	3.5	3.5	3.5	3.5	3.5
$pH_{influent}$	8.0	7.9	7.9	7.8	7.6	7.7	7.7
$pH_{reactor}$	7.8	7.6	7.6	7.8	7.7	7.9	7.7
$pH_{effluent}$	8.1	8.2	8.0	8.6	8.5	8.2	8.2
E_{COD}, %	92	97	97	94	92	95	92
$E_{nitrite}$, %	92	100	97	100	100	97	100
$E_{ammonium}$, %	38	23	23	17	40	37	11

Notes: (1). The continuous treatment of wastewater with nitrite and ammonium was differentiated into 7 experiments according to the concentration of nitrite present in the influent: experiment 1, day 1—18; experiment 2, day 19—27; experiment 3, day 28—38; experiment 4, day 39—47; experiment 5, day 48—63; experiment 6, day 64—79; experiment 7, day 80—107. (2). The parameter values of the operation of the reactor were the average values of each experiment. (3). The efficiencies were the stable values of each experiment

As seen in Table 1, the efficiencies of COD removal varied from 91%—97% with up to about 3.9 gCOD/(L·d) of the COD volumetric loading rate. The efficiencies of nitrite removal except experiment 1 were in 97%—100% with up to the 0.8 gNO_2^--N/(L·d) of the nitrite volumetric loading rate. The maximum efficiency of ammonium removal reached about 40% after the reactor operation became stable for a period of time. These results demonstrated that the wastewater with high nitrite content could be anaerobically treated in an EGSB reactor.

The sludge in the reactor changed its colour to gray-white with yellowish colour from black first at the bottom of the reactor and then upward gradually through the sludge bed since experiment 3. Till to experiment 6, about half sludge in the reactor changed into gray-white colour with yellowish colour. Then, some gray-white sludge returned into black colour once more during experiment 7. No sludge was wasted before experiment 7. Sludge was wasted five times for the amount of about 1.5 litres during the experiment 7. The concentration of the wasted sludge was 60 gTSS/L, which was little lower than that of the seeded sludge(72 gTSS/L).

The mass balance of the continuous treatment of wastewater with nitrite and ammonium was made for 5 days in the experiment 7. Based on the biogas composition(89% N_2, 8% CH_4 and 3% CO_2) and the amount of nitrogen removal, which was presumed to be all changed to nitrogen gas, the expected amount of biogas was calculated and corrected for its dissolution in water. Results showed that the biomass amount collected actually(7.26 L) was very close to the expected amount of it (7.51 L). Only 3.4% of biogas was lost during the tested period. Apparently, the COD was concomitantly used for denitrification and methanogenesis. So, the ratio of COD/N for denitrification from nitrite should be corrected for the production of methane. This came to 4.07 gCOD/g NO_2^--N, which is a normal value for denitrificaton from nitrite.

Table 2 The mass balances in the continuous treatment of wastewater with nitrite and ammonium and batch tests

Type of experiment and items			Input with influent	Output with effluent	Removal efficiency, %
5 days in the end of the experiment 7		COD,	41.37*	3.57	91
		g and mg/L	(701)**	(63)	
		NO_2^--N,	8.87	0.00	100
		g and mg/L	(150)	(0)	
		NH_4^+-N,	2.22	1.97	11
		g and mg/L	(38)	(34)	
Batch tests of 15 days	Sample 1	COD,	27.3	1.47	95
		mg and mg/L	(390)	(21)	
		NO_2^--N,	2.40	0.0	100
		mg and mg/L	(34)	(0)	
		NH_4^+-N,	2.29	1.94	15
		mg and mg/L	(33)	(28)	
	Sample 2	COD,	27.3	1.47	95
		mg and mg/L	(390)	(21)	
		NO_2^--N,	4.08	0.0	100
		mg and mg/L	(58)	(0)	
		NH_4^+-N,	2.29	2.13	7
		mg and mg/L	(33)	(30)	
	Sample 3	COD,	27.3	1.96	93
		mg and mg/L	(390)	(28)	
		NO_2^--N,	5.76	0.0	100
		mg and mg/L	(82)	(0)	
		NH_4^+-N,	2.29	2.01	2
		mg and mg/L	(33)	(29)	
	Sample 4	COD,	–	–	–
		mg and mg/L	–	–	
		NO_2^--N,	2.40	0.0	100
		mg and mg/L	(34)	(0)	
		NH_4^+-N,	2.40	2.32	3
		mg and mg/L	(34)	(33)	

Notes: * The amount of the COD. ** The concentration of COD in the medium

It can be seen in Table 2, 100% of nitrite in 4 samples of batch tests was denitrified. About 93% to 95% of the COD were removed. Even though no COD was added into sample 4, nitrite in it was denitrified completely. This showed that denitrifiers could use some biomass as organic COD to denitrify nitrite and to decrease its toxicity. Some amounts of NH_4^+-N (3%—15%) were always lost in batch tests. About 3% of NH_4^+ – N was lost even in sample 4, where only nitrite and ammonium were added.

N_2 production in batch tests stopped 168 hours after the beginning of the test. It can be seen in Fig.3 that CH_4 production was slow in the initial phase, and became fast after 168 hours of test beginning. This indicates the denitrification took place first and inhibited the methanogenesis at the same time. The methanogenesis became fast after the completion of the denitrification. The maximum of 2×10^{-3} nmol N_2O (data not shown) was found in the gas production of batch tests. It was 10^5 times lower than the N_2 amount produced. Furthermore, a gas product was detected but was not identified when N_2O was measured with GC. The amounts of this gas were high as N_2O amounts were high (data not shown). This might show that the gas unidentified was related to N_2O. Due to the mechanism of denitrification, this unidentified gas might be NO. This is also coincident with the conversion of not 100% NO_2^--N to N_2 as shown in Table 3. Results in Table 3 indicate that about 43%—48% of COD were conversed to CH_4. The ratios of COD/NO_2^--N for denitrification from nitrite varied from 2.49 to 5.64 when decreased nitrite concentration.

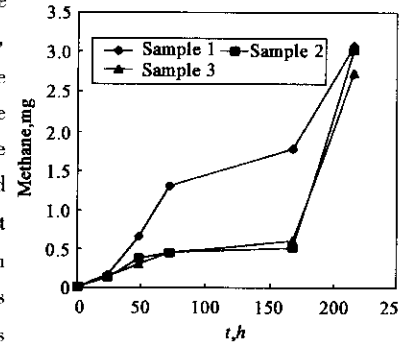

Fig.3 The methane production in the batch tests

Table 3 Characteristics of methanogenesis and denitrification in batch tests

Sample	Input of COD, mg	Input of NO_2^--N, mg	CH_4 production		N_2 production		COD/NO_2^--N for denitrification from nitrite
			Amount, mg	Rate of COD conversion, %	Amount, mg	Rate of NO_2^--N conversion, %	
1	27.30	2.40	3.08	48	2.30	96	5.64
2	27.30	4.08	3.02	47	3.73	92	3.36
3	27.30	5.76	2.74	43	5.31	92	2.49
4	–	2.40	–	–	2.64	110	–

3 Discussion

Kato *et al*. (Kato, 1994; 1997) reported that in the mesophilic condition (30℃), COD removal efficiency was above 80% at up to 7 gCOD/(L·d) of volumetric loading rate (VLR) when the influent of 100 to 200 mgCOD/L was fed. They indicated that the operation of an EGSB reactor should be limited to 7 gCOD/(L·d) VLR and to the V_{up} between 2.5 and 5.5 m/h. However, Rebac *et al*. (Rebac, 1995) used an EGSB reactor to treat low-strength synthetic wastewater of 500—800 mgCOD/L (a mixture of volatile fatty acids) in a liquid upflow velocity of 10 m/h under the psychrophilic conditions (10—12℃). Above 90% of COD removal efficiency was reached at up to 12 gCOD/(L·d). Furthermore, Lettinga *et al*. (Lettinga, 1999) used a staged EGSB reactor to treat low synthetic wastewater (the mixture of 1∶1.5∶1.8 of acetate to propionate to butyrate) under very low temperature (3—8℃). It was reported that about 80% COD removal was reached at 3℃ with 5.5 gCOD/(L·d), and 90% COD removal at 8℃ with 12.5 gCOD/(L·d). Experimental results mentioned above have demonstrated that high upflow velocities generated intense hydraulic turbulence and good sludge bed expansion, enhanced the contact between wastewater and biomass, reduced the resistance of substrate diffusion through the biofilm (granular sludge), as a result, resulted in the decreased apparent affinity (K_s value). Therefore, high upflow velocities used in an EGSB reactor are probably responsible for the high efficiencies of COD removal in low-strength wastewater. In this study 3.5—4 m/h of upflow velocity was used. Here, the treatment of low strength wastewater without nitrite before treating low-strength wastewater with nitrite and ammonium made the sludge adaptive to low-strength wastewater in an EGSB reactor, so that the methanogenesis of very-low-strength wastewater could occur very well with denitrification.

The reactor showed a high capacity of denitrifying nitrite at once as the feed was changed to wastewater with nitrite and ammonium from one without nitrite, revealing that a great amount of denitrifiers were present in the granular sludge in the reactor. Many literatures had also reported that a high denitrifying potential was prominent in anaerobic nitrate-free environment, especially such as deep marine sediment(JΦrgensen, 1989), freshwater sediment(Kasper, 1985) and digested sludge(Kasper, 1981). JΦrgensen and Tiedje (JΦrgensen, 1993) reported that the denitrifier population in the digested sludge was 5.3×10^6 cells/ml, all of which were aerobic, gram-negative bacteria, particularly species of *Pseudomonas* and *Alcaligenes*. They suggested that these aerobes were capable of carrying out the fermentation of organic substrates down to very low residual levels to provide with the energy for their long-term maintenance in the nitrate-free anaerobic environment.

Here, the volumetric loading rate of denitrification from nitrite was much higher, compared with that of denitrification from nitrate in an UASB reactor of methanogesis and denitrification(Hendriksen, 1996a), despite of much higher toxicity of nitrite than that of nitrate(Clarens, 1998; Kluber, 1998; Akunna, 1998) and usage of glucose as carbon source. No dissimilatory reduction of nitrite to ammonium took place in the system used here. More than 97% to 100% of nitrite was removed at 3.9 gCOD/(L·d) and 0.8 gNO_2^--N/(L·d) of volumetric loading rates in an EGSB reactor. Some systems with the potential of methanogenesis and simultaneous denitrification have been reported in the past recent years. Hendriksen and Ahring(Hendriksen, 1996a) reported more than 99% removals of both COD and nitrate at 6.6 gCOD/(L·d) and 0.336 gNO_3^--N/(L·d) of volumetric loading rates in an UASB reactor, which was fed with nitrate and a mixture of acetate, propionate and butyrate in the proportion of 1: 1: 1 of their COD and operated in the recirculation ratio of 6. Hanki and Palprasert(Hanki, 1989) obtained 99.9% N removal and 96% COD removal in an upflow filter fed with methanol and nitrate. Lin and Chen(Lin, 1995) achieved complete removals of both carbon and nitrogen. In the Ananox process, which includes an aerobic nitrification unit as well as the anoxic/methanogenesis step, efficiencies of 63.5% and 80% were found when sewage and piggery waste were treated respectively(Tilche, 1994; Grauti, 1992). The results mentioned above demonstrated that the degrees of both methanogenesis and denitrification depended on substrates and types of reactors used in those studies. Previous researches were principally focused on the effect of different carbon sources on the pathway of nitrate reduction in the systems with methanogenesis and simultaneous denitrification. Akunna *et al*. (Akunna, 1993) showed that with glucose or glycerol as the carbon source, 50% of the nitrogen from reduced nitrate was found as ammonium, whereas 100% of the nitrate was denitrified to nitrogen gas with acetate or lactate acid as the carbon source. Furthermore, Hendriksen and Ahring(Hendriksen, 1996b) demonstrated that denitrifiers preferred butyrate and propionate, which are unfavourable or recalcitrant substrates for methanogens, although acetate could be metabolised. As a result, the growth of syntrophic volatile fatty acid bacteria was suppressed by the denitrifiers in the case of low C/N ratios, with leaving acetate as the major substrate for methanogenesis.

Different configuration system found their application in the study of the methanogenesis and simultaneous denitrification, including completely stirred anaerobic digester(Percheron, 1999), a mixed culture system co-immobilised in gel beads (Chen, 1993; Lin, 1995), anaerobic upflow filter(Hanaki, 1989; Akunna, 1992; 1993; 1994) and Ananox process (Grauti, 1992) and so on. It appeared that an immobilised bacterial system(or biofilm reactor) supported different macro- and micro-environment in it, so that bacteria involved in reactions of methanogenesis and denitrification can then grow and concentrate in different zones favoured to their metabolism in a system. In a co-immobilised mixed culture system of denitrifying bacteria and methanogenic microbes included in polyvinyl alcohol gel beads (Chen, 1993; Lin, 1995), it was demonstrated that methanogens are active inside the beads where nitrate is absent, while denitrifiers grew on the surface of the beads. The denitrification occurred in the bottom of an upflow anaerobic filter, but the methanogenesis happened in the top of the filter(Hanaki, 1989). Phenomena in the former two systems mentioned above could occur in the EGSB reactor used here. The colour change of sludge in the reactor revealed that the community of bacteria in a granular sludge had changed. It might be the case that denitrifers are in the outer part of a sludge granule, but methanogens are in the inner part of the granule as in a mixed culture system co-immobilised in gel beads(Chen, 1993; Lin, 1995). It was demonstrated that denitrifying bacteria also tend to form granular sludge like acidifying and methanogenic bacteria and resulted in the similar colour change of the sludge(van de Hoek, 1987). Hendriksen and Ahring(Hendriksen, 1996a) found the similar colour change of the sludge in their work.

It has been shown that nitrite, NO and N_2O produced during the denitrification, induced a higher redox potential could inhibit the methanogenesis. High nitrate concentration may be also inhibitory to the methanogenesis(Clarens, 1998; Kluber, 1998; Akunna, 1998). It can be seen from Table 4 that the inhibition on nitrogenous compounds to methanogenesis varies largely in a very wide range, and depends on the species of methanogens and their metabolic substrates. However, the inhibition of denitrification to the methanogenesis was reversible as shown in Fig. 3. The methanogenesis would start as soon as the denitrification was completed. The inhibition of N-compounds to methanogenesis seems to be caused by several mechanisms(Kluber, 1998): (1) the toxic effect of N-compounds on members of the methanogenic microbial community; (2) the competition between denitrifiers and methanogens for H_2 substrate; (3) temporary accumulation of sulphate and Fe^{3+} produced during the denitrification, which consequently allow sulphate reducers and Fe^{3+} reducers to become active and also compete for H_2 substrate with methanogens. In the present EGSB reactor, high recirculation would be favourable to decrease the toxicity of nitrite. At the same time, higher pH values(7—8) kept in the reactor were also preferable to lower the toxicity of nitrite.

Table 4 Concentration of N-compounds causing 50% and 100% inhibition of methanogenesis(Clarens, 1998; Kluber, 1998)

Inhibitor concentration	*Methanosarcina mazei* in acetate substrate		*Methanosarcina barkeri* in the H_2/CO_2 substrate		*Methanobacterium bryantii* in the H_2/CO_2 substrate	
in solution	50%	100%	50%	100%	50%	100%
NO_3^--N, mg/L	560	≫980	72	≫720	350	≫420
NO_2^--N, mg/L	1.4	≥2.8	0.7	≥1.4	14	-
NO-N, μg/L	-	-	12.6	≥23.8	4.2	≥11.2
N_2O-N, mg/L	-	≥12.04	3.64	>26.6	0.84	≥2.66

The dissimilatory reduction of nitrate or nitrite to ammonium was principally concerned all the time in the study of methanogenesis and simultaneous denitrification. Some publications reported that the dissimilatory nitrate reduction to ammonium was the main pathway of the nitrate reduction in anaerobic digester(Kasper, 1981; Tiedje, 1988) and in some methanogenic environment(Allison, 1988; Schoten, 1995), which was related to the nature of carbon sources(Akunna, 1993) and higher COD/N ratios(Tiedje, 1988; Akunna, 1992; Hendriksen, 1996b). Yet, Percheron *et al*. (Percheron, 1999) demonstrated that a high COD/N ratio did not favour the dissimilatory reduction of nitrate to ammonium. That is, the dissimilatory reduction of nitrate to ammonium was unrelated to the ratio of COD/N, and denitrification was the main reduction pathway of nitrate at all COD/N ratios tested(9 - 94). The lower COD/N ratios in the range of 3.4—11.4 were used in this work. This would be favourable to the denitrification according to Akunna *et al*. (Akunna, 1992). The results from continuous treatment and batch tests demonstrated that no dissimilatory nitrite reduction to ammonium happened in experiments.

It should be noted that the maximum of about 40% ammonium was be lost in the continuous treatment of wastewater in the reactor. Anaerobic ammonium oxidation (ANAMMOX) might be important one of reasons for this ammonium loss. However, the volatilisation of ammonium and the growth of cells would also contribute to some ammonium loss in the system. ANAMMOX occurs with ammonium as electron donor and nitrite as electron acceptor in the following reaction $NH_4^+ + NO_2^- = N_2 + 2\ H_2O$(van de Graff, 1997; Jetten, 1999). It was found that hydrogen gas enhanced ANAMMOX(Jetten, 1999). Previous studies in very recent years shown that micro-organisms capable of catalysing ANAMMOX possess the diversity in the level of bacterial genus(Schmid, 2000). It has been found that *Brocadia anammoxidans*(Hellinga, 1997), *Candidatas Kueneia Stuttgartiness*(Schmid, 2000) and *Nitrosomonas*(Bock, 1995) can catalyse this novel process. However, the reactive mechanism and enzymology concerned with this process are not completely known(Jetten, 1999). It is not yet known what relation and interaction are present between ANAMMOX and aerobic denitrification(or aerobic ammonium removal or oxygen-limited autotrophic nitrification-denitrification). However, it can be said that prerequisites for ANAMMOX and aerobic denitrification include the presence of both ammonium and nitrite, and anoxic conditions. These two conditions were met in our experiments. Batch test fed medium without carbon source showed that 3% of ammonium removed anaerobically, which indicates that some bacteria capable of catalysing ANAMMOX were enriched in the sludge. Furthermore, results from batch

tests demonstrated that much more NH_4^+-N was lost with organic COD addition than without organic COD addition. It suggested that the methanogenesis in the system enhance ANAMMOX because of intermediate hydrogen produced during methanogenesis.

According to author's knowledge, this work is the first one to study the potential of integrating methanogenesis with denitrification and anaerobic ammonium oxidation. An integration of this process with a process similar to SHARON, which converts ammonium to nitrite, could be used to the treatment of effluents containing high COD and high ammonium. It does not require to adding any external carbon sources and could save 25% oxygen for nitrification compared with a conventional nitrification/denitrification system. Also, alkalinity in the anaerobic digested effluents would compensate the requirement for it during nitrification.

4 Conclusions

This work has demonstrated that wastewater with high concentrations of nitrite and ammonium could be anaerobically treated through the integration of methanogenesis with denitrification and anaerobic ammonium oxidation in an expanded granular sludge(EBSG) reactor. The efficiency of COD removal reached more than 91% to 97% at up to about 3.9 gCOD/(L·d) of COD volumetric loading rate. The efficiency of nitrite removal reached more than 97% to 100% at up to about 0.8 gNO_2^--N/(L·d) of volumetric loading rates. The dissimilatory reduction of nitrite to ammonium did not occurred in the system. At the same time, maximum of about 40% ammonium were removed. It suggested that the methanogenesis could enhance the ANAMMOX.

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