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Novel strategy of nitrogen removal from domestic wastewater using pilot *Orbal* oxidation ditch

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Abstract: A pilot-scale *Orbal* oxidation ditch was operated for 17 months to optimize nitrogen removal from domestic wastewater of average COD to total nitrogen ratio of 2.7, with particular concern about the roles of dissolved oxygen (DO), mixed liquor suspended solids (MLSS) and return activated sludge (RAS) recycle ratio. Remarkable simultaneous nitrification and denitrification (SND) was observed and mean total nitrogen (TN) removal efficiency up to 72.1% was steadily achieved, at DO concentration in the out, middle and inner channel of 0.1, 0.4 and 0.7 mg/L, respectively, with an average MLSS of 5.5 g/L and RAS recycle ratio of 150%. Although the out channel took the major role in TN removal, the role of middle channel should never be ignored. The denitrification potential could be fully developed under low DO, high MLSS with adequate RAS ratio. The sludge settleability was amazingly improved under low DO operation mode, and some explanations were tried. In addition, a series of simplified batch tests were done to determine whether novel microorganisms could make substantial contribution to the performance of nitrogen removal. The results indicated that the SND observed in this *Orbal* oxidation ditch was more likely a physical phenomenon.

Keywords: nitrogen removal; simultaneous nitrification and denitrification; low DO; MLSS; novel bacterial

Introduction

Recent research on nitrogen removal was mostly oriented either towards improvement of efficiency and energy saving in traditional pathway or towards identification of new process (some cases with novel microorganisms) able to convert ionized nitrogen into harmless forms. All efforts which endeavor to innovative processes must satisfy the following requirements: high removal efficiency; a minimum of interference with the existing facilities (i.e., low investment); and simple technologies (low operating costs). Simultaneous nitrification and denitrification, which means that both processes occur in the same time and space, takes the advantage of saving not only tank volumes but also simplified process.

Conventional activated sludge processes for nitrogen removal always include several reactors with different dissolved oxygen (DO) concentration, or only in one reactor in which alternating aerobic/anoxic phase is achieved subsequently. In the case of non-conventional processes, DO concentration is controlled to obtain situations in favor of behaviors of both nitrifying and denitrifying bacteria (Bertanza, 1997). It has been demonstrated that denitrification could occur in aerobic conditions and nitrification could also be possible at low levels of DO concentration (Rittmann and Langeland, 1985; Lee *et al.*, 2001). However, the nitrogen losses in non-conventional ways have long been attracting the attentions. The phenomena of simultaneous nitrification and denitrification (SND) process have been observed in many fields both from lab to field scale plants (Munch *et al.*, 1995; Collivignarelli and Bertanza, 1999; Slikers *et al.*, 2003).

An oxidation ditch (OD) is a modified activated sludge biological treatment process that utilizes long solids retention time (SRT), and may also be operated to achieve partial denitrification. The main advantage of OD is the ability to achieve nitrogen removal performance objectives with low operational requirement and operation costs. In the past two decades, more than one hundred of them have been built up in China for municipal wastewater treatment.

So far, there are two main explanations for the mechanism of SND: firstly, the physical explanation, that SND occurs as a consequence of DO gradients in sludge floc or the existence of macro-anoxic zone caused by reactor configuration and agitation pattern (Furukawa *et al.*, 1998; Daigger and Littleton, 2000); secondly, the biological explanation, that the proved existence of aerobic denitrifiers and heterotrophic nitrifiers (Robertson *et al.*, 1995; Muller *et al.*, 1995), anaerobic ammonium oxidation (Mulder *et al.*, 1995, Sliekers *et al.*, 2003) or denitrification by autotrophic nitrifiers (Bock *et al.*, 1995; Schmidt and Bock, 1997), could partially, if not fully, explain the undefined nitrogen loss in certain systems. Recently, a range of new microbial processes to get nitrogen removal has been described and investigated in the laboratory. Nevertheless, there are still many problems in field-scale application of those new processes, especially for the rigorous environment for the microorganisms responsible for the biochemical ways

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recently known to us.

This study was conducted to develop practical and economical methods to get high nitrogen removal efficiency in a pilot *Orbal* oxidation ditch. The specific objectives were: (1) to determine the effect of DO, mixed liquor suspended solids (MLSS) concentration and return activated sludge (RAS) recycle ratio on the performance of total nitrogen (TN) removal, and (2) to evaluate the role of the novel microorganism to TN removal in this system by simple batch tests, and further to discuss the mechanism of SND.

1 Materials and methods

1.1 Pilot-scale *Orbal* oxidation ditch

A typical *Orbal* OD with three closed-loop reactors was promoted. Fig.1 shows a schematic of the reactor to perform the experiments. The influent and return activated sludge were pumped by the vermicular pumps firstly into the out channel, and then flowed to the middle and inner ones in sequence by gravity though the submerged ports set near the bottom before they entered the secondary clarifier, which volume was 100 L. Each of the three channels was a complete mixed reactor having an endless mixed liquor flow circuit forced by recirculating pumps immerged in each channel. The horizontal velocity of the flow was control at average 0.01 m/s with the help of the broad with hole, which was previously measured by tracer test in pure water examination. Mixers were used to prevent the mixed liquor from settling down. Fine air diffusers were installed in the bottom of each reactor as point source aeration instrument, coupled with the submerged pumps to stimulate the point-aeration mode carried out by disc aerators in full-scale plant. The working volume of this pilot plant was 300 L, and the out, middle and inner channel occupied 155 L, 83 L and 62 L, respectively. The hydraulic retention time (HRT) was controlled at 16.5 h, without internal recycle flow from the inner channel to the out channel. On-line detection of DO was done by WTW 340i (Germany), and the membrane of the DO probe (CellOx 325, WTW) was daily cleaned and revised every 2 weeks. The DO regulation was carried out by manual adjustment of airflow rate meter. Separate air pipelines were set to different channels, and the airflow rate to each channel was adjusted according to corresponding DO set value. The reactor was operated at room temperature between 20 to 26℃, and no specific action was taken to control the reactor temperature except when the influent temperature was lower than 18℃.

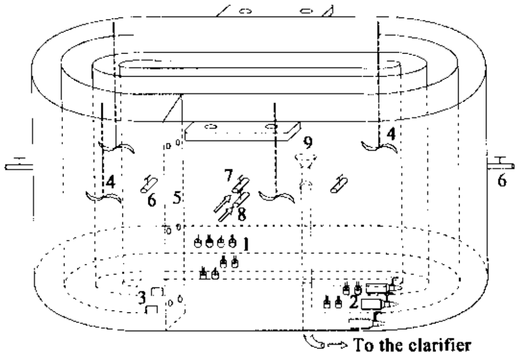

Fig. 1 Schematic diagram of *Orbal* oxidation ditch
1. air diffuser; 2. recirculating pumps; 3. submerged connecting ports; 4. mixer; 5. board with holes, to control the horizontal velocity; 6. sample point; 7. influent point; 8. returned sludge point; 9. effluent point

1.2 Feeding and seeding

The domestic wastewater from a septic tank in a residential area was impressed high ammonium concentration (average of 81 mg/L), meanwhile low COD (average of 220 mg/L). Every morning between 8:00 to 9:00 a.m., the wastewater was collected from the septic tank to keep the influent quality as constant as possible. The characteristics of the wastewater are listed in Table 1.

Table 1 Characteristics of the raw domestic wastewater

Parameters	NH_4^+	TN	PO_4^{3-}	BOD_5	COD
Min. to max., mg/L	58—108	59—110	5—9.5	100—165	160—310
Mean, mg/L	80	81	6.8	115	220

The reactor was inoculated with sludge from the nearby Jiuxianqiao Municipal Wastewater Treatment Plant in Beijing, China, which exhibit both biological nitrogen and phosphate removal. The reactor was acclimated to domestic wastewater for three weeks before the experiment started.

1.3 Batch tests for autotrophic denitrification and heterotrophic nitrification

Practical batch tests were done, based on the known biochemistry of bacteria that could exhibit autotrophic denitrification and heterotrophic nitrification/aerobic dentirification, to determine whether the activity of such organisms was sufficiently large so that it represented a substantial nitrogen removal mechanism that must be considered in the analysis of nitrogen removal in the pilot-scale system. Autotrophic denitrification organisms were examined by a 2 by 2 factorial anoxic batch test, while heterotrophic nitrification/aerobic dentirification organisms were also examined but under aerobic conditions (Littleton *et al.*, 2002).

1.4 Analytical methods

Sludge samples were immediately centrifuged at 3000 r/min for 10 min to remove microorganism from liquid medium and tested. Daily analyses of COD, NH_4^+-N, NO_3^--N, NO_2^--N, MLSS and sludge volume index (SVI) were conducted according to the standard methods (APHA, 1995).

2 Results and discussion

2.1 Overall performance

The experiment was carried out during May 2004 to September 2005; and 10 periods (I–X) have been characterized by different operating conditions, as reported in Table 2.

Table 2 Operating conditions (average values)

Parameter	I	II	III	IV	V	VI	VII	VIII	IX	X
DO, mg/L (out channel)	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DO, mg/L (middle channel)	1.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
DO, mg/L (inner channel)	3.0	1.0	0.7	0.5	0.7	0.7	0.7	0.7	0.7	0.5
MLSS, g/L	3.5	3.5	3.5	3.5	4.5	5.5	5.5	5.5	5.5	5.5
RAS recycle ratio, %	100	100	100	100	100	100	120	150	200	200

Because of the high oxygen utilization rate by microorganisms that consumed the oxygen and relatively high organic load in the out channel, the average DO still was very low although substantial oxygen supply was provided. The air supply to the out channel would make nitrification possible, while the low DO concentration also made simultaneous denitrification to occur. This oxygen-depleted condition could make more effective oxygen exploitation and this energy efficient operation also results in reduced energy costs.

TN removal efficiency and nitrogen species both in influent and effluent versus operation period are summarized in Fig.2. Throughout the experiment no nitrite accumulated with effluent concentration always lower than 0.2 mg/L. The data were collected after the system achieved a quasi-steady state. Under period I to III, DO was gradually decreased from the typical used 0.3, 1.5 and 3 mg/L to 0.1, 0.4 and 0.7 mg/L in the corresponding channels, and there did not show any detrimental effects on nitrification, while nitrate concentration in the effluent was reduced, indicating the enhancement of denitrification. Nitrogen removal efficiency climbed from 36% under period I to 53.4% under period III. When the DO concentration lowered further to 0.5 mg/L in the inner channel, ammonium of 8.5 mg/L appeared in the effluent and nitrogen removal efficiency draw back to 44.6%.

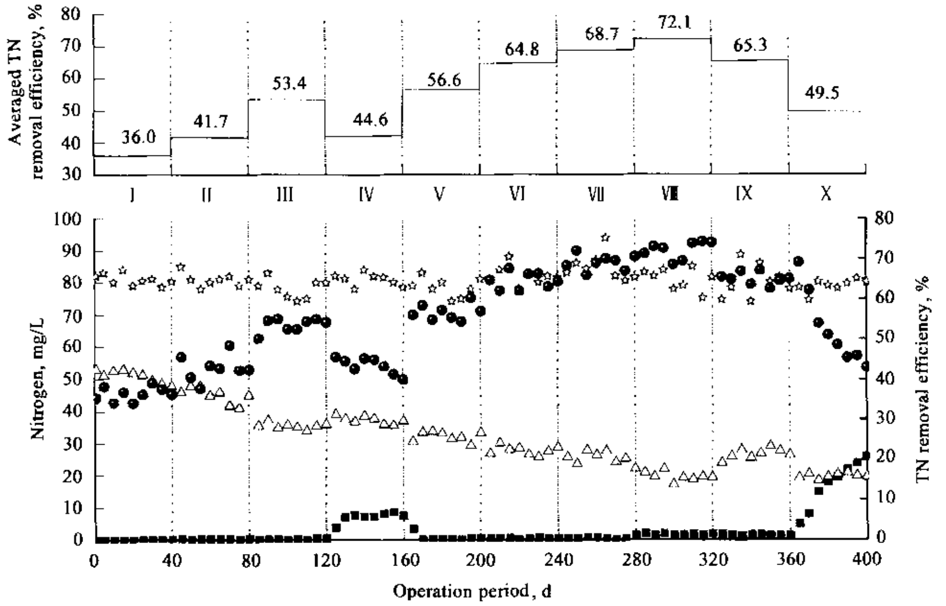

Fig.2 Nitrate (△) and ammonium (■) in the effluent, TN in the influent (☆), and TN removal efficiency (●) as function of time

By contrasting period III, V and VI, as it could be seen from Fig.2, under the same DO level, enhancing the MLSS could prominently enhance nitrogen removal. Nitrate in the effluent decreased gradually from about 36 mg/L under condition III to about 25 mg/L under condition VI. Ammonium concentration was kept lower than 1 mg/L. Although under low DO concentration, long HRT could still ensure nitrification, in other word, nitrification would still happen even at low DO. Enhanced denitrification using endogenous carbon source seemed to better explain the high TN removal efficiency at high MLSS condition.

Return activated sludge (RAS) recycle ratio is believed to be another important factor to maximize the nitrogen removal efficiency. Under the same DO level of 0.1, 0.4 and 0.7 mg/L in the sequential channel, TN removal efficiency increased 3.9% from

64.8% to 68.7% with RAS recycle ratio from 100% increased to 120% ; further increased RAS recycle ratio to 150%, less increase of 3.4% could be got. RAS recycle ratio of 200% did not contribute to nitrogen removal, with the TN removal efficiency fell back to 65.3%. However, it was apparent that when more nitrate with RAS was returned to the reactor, denitrification process could be enhanced to a certain extent. But once the dentrification potential had been fully exploited, too much RAS would make the reactor much more as a completely mixed reactor and destroyed the anoxic zone formed in the out channel. Under period X, further reducing the DO level in the inner channel to 0.5 mg/L, ammonium concentration in the effluent rose dramatically to over 15 mg/L, followed by TN removal efficiency drop down to less than 50%.

It had been demonstrated that skilful operation using low DO, high MLSS, and an increasing RAS recycle ratio could be practical, especially when the influent was of low influent COD/TN ratio, to realize high nitrogen removal. COD and nitrification did not be affected but TN in the effluent was significant reduced. Low DO operation to some extent could notably save the aeration costs. Denitrification using endogenetic carbon source was believed to account for the high TN removal performed. No sludge was wasted under MLSS of 5.5 g/L, and little sludge was observed remaining in the clarifier.

Fig.3 depicts COD and nitrogen (TN, ammonium and nitrate) profiles under condition VIII. In order to investigate the nitrogen removal mechanism in *Orbal* process, a small contact tank, which HRT was 5 min, was temporarily added to mix the influent and RAS before they entered the out channel. The dramatic decrease of TN concentration from 81.1 mg/L of influent to 44.6 mg/L after mixed with the returned sludge was mainly because of dilution effect. Besides dilution effect, sludge adsorption could be another reason for COD reduction from 200 of influent to 51 mg/L after mixed with the returned sludge.

Fig.3 shows that after the mixed liquor entered the out channel, COD concentration dropped to 40 mg/L. Due to the oxidation by heterotrophic bacteria and dilution effect of the reactor, it was obvious that denitrification using carbon source from raw influent would be limited. COD showed little changes thereafter from middle channel on, for the biodegradable substances were already depleted in the out channel after over 8 h mean retention time.

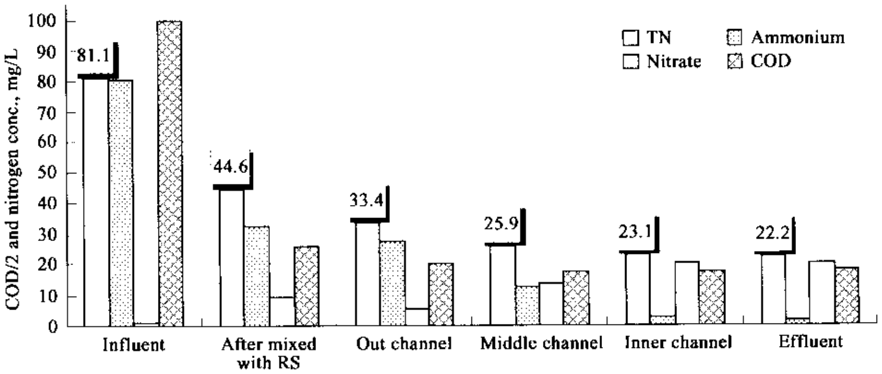

Fig.3 COD and nitrogen profiles in different sample points under condition VIII

It was found 11.2, 7.5 and 2.8 mg/L total nitrogen loss in the out, middle and inner channel, respectively. This indicated that out channel contributed the most to the nitrogen elimination. Low DO concentration and the present of organic substances explained this phenomenon. It is of particular important to enhance the denitrification potential of middle channel. In this situation, DO in the middle channel was lower to 0.4 mg/L and specific HRT over 5 h, and denitrification used endogenetic carbon source was believed to occur at MLSS of 5.5 g/L, compared with DO of 1 mg/L, MLSS of 3 g/L and HRT of 2 to 3 h in typical *Orbal* process where no significant nitrogen removal was observed. In other words, the denitrification potential of middle channel should never be ignored to optimize TN removal performance. Although inner channel only accounted for only 2.8 mg/L TN removal, ammonium removal was guaranteed here, and the existence of inner channel permitted the previous ones to carry out low DO without considering much about nitrification.

2.2 Sludge volume index (SVI) variation

The low F/M and low DO level experienced in this study risked the process of filamentous bulking. The SVI and TN removal efficiency are shown in Fig.4. With the decrease of DO level and increase of TN removal efficiency, SVI showed a tendency to decrease, from about 170 ml/g initially to about 115 ml/g in the optimized nitrogen removal performance, condition VIII.

It is amazing that low DO could improve the sludge settling characteristics, which was conflicted with many present reports. Some explanations were tried. Firstly, it is commonly accepted that filamentous

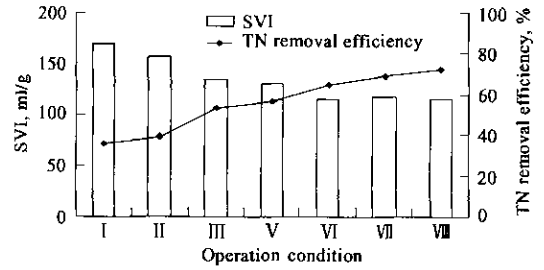

Fig.4 Relation of sludge volume index (SVI) variation with TN removal efficiency under different operation conditions

bacteria have low kinetic constant (μ_{max} and K_s) and could take the competitive advantage over floc-forming bacteria under low DO condition and cause filamentous bulking. However, in the *Orbal* process, although substantial amount of oxygen was inputted into the out channel, high oxygen uptakes by microorganisms still resulted in oxygen-limiting condition, and the point aeration style could make the existence of prominent part of anoxic zone (i.e., DO concentration is zero with the presence of nitrate). In the absence of O_2, facultatively aerobic and anaerobic organisms (most of the flocculent microbes) could also obtain energy by fermentation or anaerobic respiration using nitrate (which came from both nitrification in out channel and RAS) as terminal electron acceptors. However, most of the chemo-heterotrophic filamentous bacteria described in the literature appeared to have a strictly respiratory metabolism, and only could use O_2 as their terminal electron acceptor. As a result, filamentous bacteria were selected inhibited in the out channel under very low DO concentration with prominent existence of anoxic zone. Secondly, although the horizontal velocity made each channel of completely mixed pattern, the in-series arranged type of *Orbal* process could take the advantage of the plug-flow reactor in overcoming the filamentous bacteria. Thirdly, it is hypothesized, and there are some supportive data, that flocculent microbes have high rates of excess substrate accumulation and storage than the filamentous organisms. The long HRT and SRT in this experiment could, as a result, allow a sufficiently long period of endogenous respiration to regain their full substrate accumulating capacity, and gave the competitive advantage of flocculent microorganisms over filamentous ones.

2.3 Batch tests of novel bacteria on nitrogen removal

Some batch tests were carried out to examine whether the novel bacteria, which could exhibit heterotrophic nitrification or aerobic denitrification, were large sufficiently to account for a substantial SND mechanism that should not be ignored during the analysis of the pilot-scale plant. All the experiments were done under room temperature (23—25℃). Fresh mixed liquor was taken from the inner channel of the pilot *Orbal* process to carry out the batch tests, with only about 20 mg/L nitrate (ammonium and nitrite could be well lower than 1 mg/L) and 40 mg/L COD.

2.3.1 Anoxic batch test assay for autotrophic denitrification

It has been observed that when ammonia and nitrite are present, at the same time dissolved oxygen and available organic substances are both absent, some bacteria can use ammonia as the electron donor and nitrite as the electron acceptor (Equation (1)). Bock *et al.* (1995) had firstly employed this reaction in the Anammox process, even the yield efficiency of the microorganisms is very low. In the *Orbal* process, the out channel firstly takes in the influent and always adopts low DO environment, coupling with the long SRT, which might allow this reaction to take place.

$$NH_4^+ + NO_2^- = N_2 + 2H_2O \qquad (1)$$

Sample of fresh sludge from the inner channel was distributed to four sealed glass vessels (labeled No.1 to 4), each of which has a working volume of 2 L with a single sample port. Ammonium chloride and sodium nitrite, with the former added to Reactor 3 and 4 and the latter to Reactor 2 and 4 (Table 3), were added to the reactor to get the ammonium and nitrite concentration each to 20 mg/L as nitrogen. Reactor 1 added neither to achieve the control of anoxic test.

Table 3 Batch tests scheme for novel bacteria

Anoxic test					Aerobic test				
Reactor	No.1	No.2	No.3	No.4	Reactor	No.5	No.6	No.7	No.8
NH_4^+	-	-	+	+	NH_4^+	-	-	+	+
NO_2^-	-	+	-	+	COD	-	+	-	+

Notes: "-". means the absence; "+". means presence; all present nitrogen species added were 20 mg/L as nitrogen, while COD were 100 mg/L

Magnetic stirrers were used to provide adequate mixing during the react period. The original pH of anoxic tests was around 7.00 to 7.20, and with the proceeding of the reaction pH gradually increased and reached 7.25 to 7.50 at the end of reaction. No action was taken to manipulate the pH during the experiment. Samples were taken twice in the first hour and then once an hour for 6 h with 40 ml each time, and the length of the reaction was calculated approximately on the volumetric nitrogen reduction rate observed in the out channel of the pilot plant, to be able to observe about 50% of the nitrogen reduction in the set time.

2.3.2 Aerobic batch test assay for heterotrophic nitrification/aerobic denitrification

Some heterotrophs, such as *Thisphaera*, can convert ammonia to nitrite under aerobic condition, with the consumption of energy, and furthermore, they can also carry out both aerobic and anaerobic denitrification. Heterotrophic nitrification and aerobic denitrification by *Thisphaera* may be explained by a bottleneck in electron transfer to oxygen. When electron transfer to oxygen is rate limiting, the excess electrons flow to nitrate and nitrite then denitification occurs (aerobic denitrification). Nevertheless, if nitrate and nitrite are both absent, energy is used to oxidize ammonia (heterotrophic nitrification) with the present of oxygen. This reaction occurs when both ammonia and readily biodegradable substrate are present under aerobic conditions.

The same reactors as anoxic tests were used in aerobic test but not sealed. Compressed air was pumped into the reactors to maintain the DO concentration higher than 6 mg/L. During aerobic tests no action was done to regulate pH value, which varied from 7.00 to 7.18 from the beginning and 6.85 to 7.05 in the end. Ammonium chloride were added to Reactor 7 and 8 to get ammonium concentration to 20 mg/L, and sodium acetate were added to Reactor 6 and 8 to get COD concentration to about 100 mg/L (Table 3). Reactor 5 added neither to achieve the control of aerobic test. The reaction time was 3 h.

2.3.3 Batch tests data analysis and discussions

The results of anoxic tests are listed in Fig.5. In Reactor 1, nitrate took the main part of the total nitrogen and showed a tendency of decrease with time elapsed and this could be due to denitrification of the traditional heterotrophic bacteria. In the other three reactors, nitrate maintained almost constant, even a slight increase. In Reactor 2 nitrite decreased from 20.31 to 9.6 mg/L also leading to the decline in the curve of total nitrogen, and in Reactor 4 nitrite showed the same tendency of decrease as in Reactor 2, from 20.49 to 9.42 mg/L. It is believed that the traditional heterotrophic microorganisms prefer to spent nitrite than nitrate to denitrification, which resulted in the consumption of nitrite. Besides, some nitrite was believed to transform to nitrate, due to the incomplete anoxic zone during sampling. And it should be announced that no inert gas was pumped into the reactors, so it could also give the chance for oxygen to incursion. The evident increase of nitrate in the present of nitrite could support the above assumption. In Reactor 4 only 2.59 mg/L ammonium was consumed, even less than that of Reactor 3 of 3.17 mg/L ammonium. Heterotrophic growths could be a satisfied explanation to the consumption of ammonium.

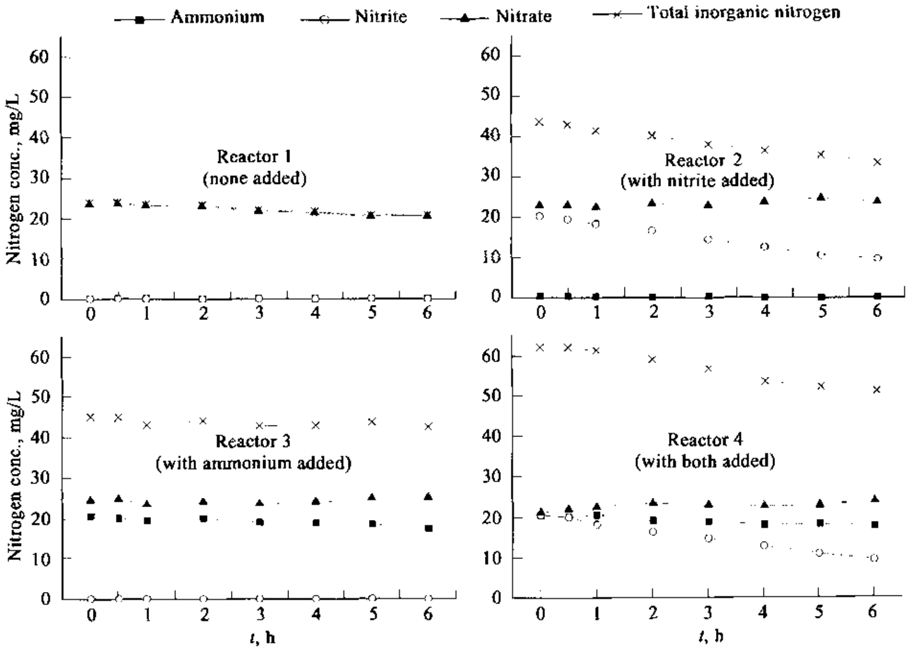

Fig.5 Anoxic batch tests results for autotrophic denitrification

By contrasting Reactor 2 to 4 for nitrite consumption and Reactor 3 to 4 for ammonium consumption, no evidence convinced the substantial existence of the microorganisms which could utilize ammonium and nitrite to exhibit autotrophic denitrification.

The results of aerobic tests are shown in Fig.6. In Reactor 5 there was almost not any change in the nitrogen species, but in Reactor 6 the addition of acetate resulted in a slight decrease of nitrate of 1.96 mg/L nitrogen, from 24.73 mg/L down to 22.77 mg/L. In Reactor 7, with the present of ammonium nitrification occurred and there was also inorganic nitrogen loss of 2.50 mg/L from 46.78 to 44.28 mg/L. In Reactor 8, with both ammonia and acetate additions, total inorganic nitrogen lost 3.81 mg/L from 48.05 to 44.24 mg/L. Contrasting Reactor 7 to 8, nitrification both happened and the latter showed even slower nitrification rate with the addition of acetate. Taken the control tests of Reactor 6 and 7 into

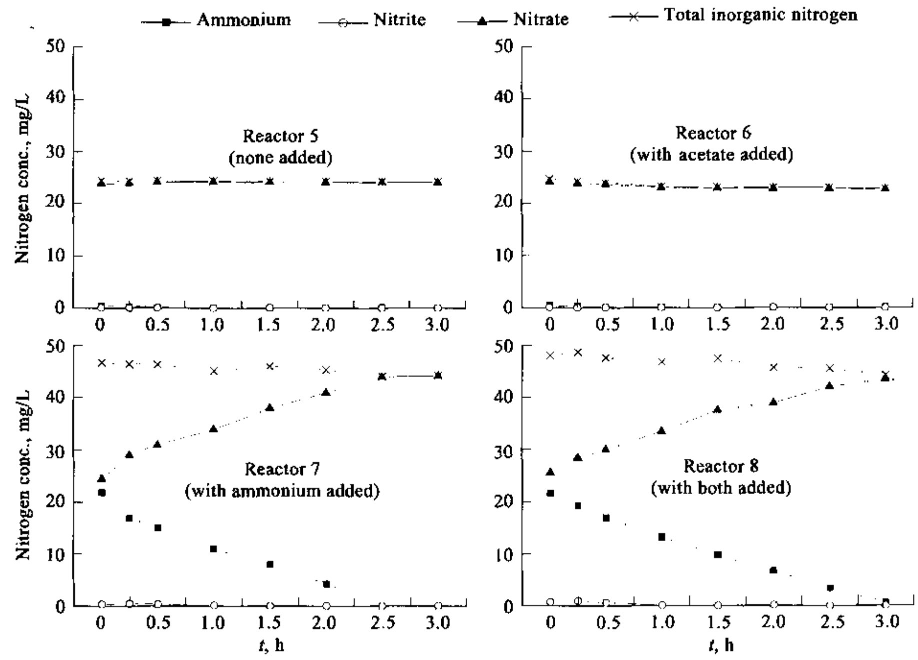

Fig.6 Aerobic batch tests results for heterotrophic nitrification/aerobic denitrification

consideration, no significant data demonstrated substantial role of heterotrophic nitrification/aerobic denitrification happened in Reactor 8.

In general, batch tests under both anoxic and aerobic conditions cited above did not give convincing data that could strongly support the existence of novel bacteria, and the roles of these bacteria to nitrogen removal could be ignored in this *Orbal* process.

3 Conclusions

The SND process has been demonstrated to be significant energy savings, incorporating with existing facilities without new construction. Well-performed effluent quality, especially total nitrogen could be possible with skillful operation. SVI control was also enhanced. Novel strategy based on DO, MLSS and RAS recycle ratio was developed in a pilot-scale *Orbal* oxidation ditch, with long-term reliable experiment data, and was proved to effectively enhance the TN removal performance, especially in treating influent of low COD/TN ratio. By simplified batch test assays, it is believed that SND was rather a physical phenomenon than a microbiological one, which was caused by reactor configuration and aeration style.

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