Enhanced total nitrogen removal performance in a modified Orbal oxidation ditch system with internal nitrate recycle

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HIGHLIGHTS

- An Orbal oxidation ditch with nitrate recycle for enhanced TN removal was developed.
- The optimal DO for SND was 0.15–0.25 mg/L.
- The highest TN removal was achieved at internal recycle ratio of 9.4.
- Nitrogen mass balances confirmed SND coupled with pre-denitrification.
- Nitrogen removal was closely related to bacterial community composition.

ARTICLE INFO

Article history:

Received 14 November 2012

Received in revised form 25 April 2013

Accepted 27 April 2013

Available online 7 May 2013

Keywords:

Orbal oxidation ditch

Nitrogen removal

Internal nitrate recycle

Aerated-anoxic

Pre-denitrification

ABSTRACT

A pilot-scale modified Orbal oxidation ditch with internal recycle of the nitrified liquor was developed to significantly enhance total nitrogen (TN) removal through simultaneous nitrification/denitrification (SND) coupled with pre-denitrification under the aerated-anoxic condition. Long-term operational results showed that the highest TN removal efficiency was achieved at very low dissolved oxygen (DO) of 0.15–0.25 mg/L and oxidation–reduction potential (ORP) of 10–40 mV within the outer channel. Appropriate internal recycle ratio played a significant role for increasing nitrates reduction within the outer channel, however, excessively high recycle flow deteriorated nitrogen removal instead because of diluted carbon source. At recycle ratio of 9.4, TN removal efficiency further increased to about 87% at optimal DO and pre-denitrification was responsible for TN removal improvement according to nitrogen mass balances. Molecular biology analysis revealed the correlation between variations in nitrogen removal and changes in bacterial community composition.

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1. Introduction

The oxidation ditch, as a modified activated sludge process, is typically a complete-mix closed-loop bioreactor in which surface aerators provide circulation, mixing and aeration of the mixed liquor. Oxidation ditches have been widely applied in small- to medium-sized municipal wastewater treatment plants (WWTPs) throughout the world [1] mainly due to their abilities to achieve organic removal, almost complete nitrification and partial denitrification with low operational requirements and operation and maintenance costs [2–4]. One of the most commonly used oxidation ditch type is the Orbal process system generally consisting of three concentric round or elliptic channels, originally developed in South Africa in 1970s [5]. Nowadays, more than 500 Orbal oxidation ditches have been used for the domestic wastewater treatment in

North America, Europe, Africa as well as Asia. In China, dozens of Orbal process plants have been put into operation until recently.

Unlike other biological nitrogen removal processes, the mechanism of removing nitrogen in a conventional oxidation ditch is based on both nitrification and denitrification simultaneously occurred due to the alternation of aerobic and anoxic zones along the single aerobic channel [2,3,6]. But in a multi-channel Orbal system, 0–1–2 mg/L dissolved oxygen (DO) profiles are typically created from the outer to inner channel by independently controlling aeration input for each channel [7–9]. The amount of oxygen in the outer channel is usually lower than the oxygen demand for promoting simultaneous nitrification and denitrification (SND) under aerated-anoxic conditions with oxygen deficit [10,11], while the amount of oxygen within the middle and inner channels is higher than the oxygen demand for removing remaining organic matters and ammonia [10]. Due to stratified oxygen levels not only within the each single channel but also across three different channels, nitrogen removal efficiencies in the multi-channel systems

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are much higher and more stable than those in the single-channel systems.

Nitrogen removal performance in the Orbal system is mainly influenced by SND depending on dynamic balance between nitrification and denitrification rates in the outer channel, which is greatly affected by DO [12,13]. However, since DO concentration for optimal SND is generally within a rather narrow range [9,14–16] due to their conflicts of oxygen and organic substrate requirements between nitrification and denitrification [16–18], the balance is unsteadily maintained unless DO can be accurately controlled. Actually, in some practical Orbal oxidation ditches, either partial nitrification owing to insufficient oxygen or reduced denitrification induced by excess oxygen takes place in the first channel while only nitrification is accomplished under high oxygen conditions in the remaining two channels, thus steadily satisfactory TN removal performances via SND solely are not always achieved as expected [9,19,20]. The addition of mixed liquor recycle from aerobic channels to aerated-anoxic channel, similar with anoxic/oxic (A/O) process, seems to be a feasible alternative for enhancing denitrification efficiency [7,10] if SND fails in Orbal processes [19]. Nevertheless, high recycle flow required for effective pre-denitrification [21] would inevitably deteriorate denitrification due to considerable oxygen brought from the aerated channels to anoxic channel [22] and increase energy consumption for internal recirculation. Hence, TN removal efficiency is hard to be further improved in Orbal ditches at such low recycle ratios.

Therefore, a modified pilot-scale Orbal oxidation ditch integrated with internal recycle of the nitrified liquor was developed to considerably enhance TN removal performance under the aerated-anoxic condition. The objectives of this study were firstly to investigate the effect of DO on SND performance to optimize TN

removal within the outer channel of aerated-anoxic oxidation ditch and then determine the optimal internal recycle ratio for enhanced pre-denitrification at the optimum DO within the outer channel of the modified oxidation ditch. Furthermore, dynamic variations in bacterial community structure closely related with nitrogen removal characteristics were analyzed by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE).

2. Material and methods

2.1. System setup

The experiment was conducted in a pilot-scale Orbal oxidation ditch operated for about two years (Fig. 1). Raw domestic wastewater via preliminary treatments such as bar screen and grit chamber was directly pumped into the wastewater tank, and the seed sludge was inoculated from a full-scale WWTP with Orbal process. The total working volume of oxidation ditch is 11.4 m³ with the size ratio of three channels of approximately 48%:33%:19% (outer:middle:inner). Aeration supply and circulation of the mixed liquor within each channel were independently controlled through adjusting rotating speeds of brush aerator via frequency converters. To enhance pre-denitrification, internal recycle of nitrified mixed liquor from the middle channel into the outer channel was implemented in a modified oxidation ditch. A square hole with the size of about 20 cm × 20 cm in the baffle wall between the outer and middle channels was located at one third of the ditch height below the water surface. One rotating door was fixed at the one side of the hole connected through a metal pole controlled by a steering wheel with scale. A certain amount of the mixed liquor

Fig. 1. Schematic diagram of a modified pilot-scale Orbal oxidation ditch.

Table 1
Influent wastewater characteristics of the pilot-scale experiment.

Parameter/Unit	Experiment 1 (114 days)	Experiment 2 (123 days)
pH	7.7 ± 0.3 ^a	7.6 ± 0.3
T °C	24.0 ± 3.5	23.6 ± 2.8
COD mg/L	387 ± 119	363 ± 95
BOD ₅ mg/L	166 ± 54	158 ± 46
NH ₄ ⁺ –N mg/L	47.3 ± 5.9	45.7 ± 3.3
NO ₃ [–] –N mg/L	0.5 ± 0.4	1.0 ± 0.5
NO ₂ [–] –N mg/L	0.02 ± 0.02	0.04 ± 0.02
TN mg/L	52.1 ± 7.8	58.5 ± 3.6
COD/TKN	7.4 ± 1.9	6.7 ± 1.4

^a The values are the average ± standard deviations.

from the middle channel easily controlled by adjusting opening angle of the gate could automatically run back into the outer channel for denitrification due to blocking of the gate without needing any pumping power. The on-line sensors of temperature, DO and ORP were positioned at 15 cm below the water surface at the fixed location of the outer channel.

2.2. Experimental design and wastewater characteristics

The whole pilot experiment mainly included two stages: Experiment 1: efficient TN removal via SND in the outer channel of the Orbal system through precise control of DO and ORP; Experiment 2: enhanced nitrogen removal in a modified Orbal oxidation ditch with internal recycle for pre-denitrification at the optimal DO for SND in Experiment 1. The characteristics of influent wastewater during two experimental stages were summarized in Table 1.

2.3. Operational conditions

Hydraulic residence time (HRT) of the Orbal oxidation ditch was about 18 h, the mixed liquid suspended solids (MLSS) were 2800–3600 mg/L, the returned sludge ratio was 100–120%, and sludge residence time (SRT) was controlled at 20–30 days through discharging the amount of surplus sludge from the secondary settler in terms of the monitoring of MLSS concentrations from the oxidation ditch and the wasted sludge. The organic-loading rate and the nitrogen-loading rate were 0.065–0.075 kgBOD₅/kgMLSS·d and 0.019–0.031 kgTKN/kgMLSS·d, respectively. The settling ability of the sludge throughout the experimental periods was generally better, with the sludge volume index (SVI) between 97 and 129 and the settling velocity (SV) of 35–40%. Both experiments were operated at high and moderate temperatures among 20–27 °C from May to September during two years. DO in the outer channel could be accurately adjusted for enhancing nitrogen removal while DO in the other two channels were kept among 0.7–1.2 mg/L and 1.7–2.5 mg/L, respectively, without intentional oxygen control. The reactor system was operated at different DO concentrations (Experiment 1) and internal recycle ratios (Experiment 2) as listed in Tables 2 and 3.

Table 2
Operational conditions in Experiment 1.

Operation conditions	Run 1	Run 2	Run 3	Run 4
Time (days)	0–28	30–62	64–90	92–114
DO (mg/L)	0.06 ± 0.04	0.20 ± 0.07	0.52 ± 0.05	0.36 ± 0.06
ORP (mV)	–45 ± 11	26 ± 12	72 ± 10	53 ± 13

Table 3
Operational conditions in Experiment 2 (at 0.15–0.25 mg/L DO).

Operation conditions	Run 1	Run 2	Run 3	Run 4	Run 5
Time (days)	0–20	21–43	44–74	75–98	99–123
Opening angle (°)	0	5	15	40	75
Average velocity (m/s)	0	0.017	0.039	0.078	0.102
Returned flow (m ³ /h)	0	2.448	5.616	11.232	14.688
Internal recycle ratio ^a (–)	0	4.1	9.4	18.7	24.5

^a Internal recycle ratio (*R*): the average ratio between the mixed liquor internal recycle flow rate and the influent wastewater flow rate.

2.4. Analytical methods

2.4.1. Chemical analysis

COD was analyzed by a Multi-Function Reactor (DR2800, Euro Tech) and portable spectrophotometers (DR2800, Hach). BOD₅, NH₄⁺–N, NO₃[–]–N, NO₂[–]–N, TN, MLSS and SVI were analyzed according to Standard Methods [23]. pH was measured with a probable pH meter (WTW Multi340i). Temperature, DO and ORP were real-time monitored using on-line sensors (Hach sc100™ controller).

2.4.2. Calculation of SND efficiency

Nitrification rate (*r*_{nitrification}) and denitrification rate (*r*_{denitrification}) as well as the SND efficiency (SND%) achieved in the outer channel were calculated according to formulas as below. [13,24]

$$r_{\text{nitrification}} (\text{mg L}^{-1} \cdot \text{h}^{-1} \text{NH}_4^+) = \frac{\text{NH}_4^+ \text{in} - \text{NH}_4^+ \text{out}}{\text{HRT}_{\text{outer}}}$$

$$r_{\text{denitrification}} (\text{mg L}^{-1} \cdot \text{h}^{-1} \text{NO}_x^-) = \frac{\text{NH}_4^+ \text{in} - \text{NH}_4^+ \text{out} - \text{NO}_x^- \text{out}}{\text{HRT}_{\text{outer}}}$$

$$\text{SND}\% = \frac{r_{\text{denitrification}}}{r_{\text{nitrification}}} \times 100\%$$

where NH₄⁺–N_{in} is the influent NH₄⁺–N; NH₄⁺–N_{out} is the effluent NH₄⁺–N_{in}; (NH₄⁺–N_{in}–NH₄⁺–N_{out}) is NO_x[–] converted by nitrification; NO_x[–] out is the effluent NO_x[–]; HRT_{outer} is HRT in the outer channel.

2.4.3. Measurement of internal recycle flow

Internal recycle flowrate was estimated by cross-sectional area and average velocity based on nine measuring points located at three different heights of the recycle opening (see Fig. 2 and Table 3). Velocity was measured by FlowTracker Handheld-ADV® (SonTek/YSI).

Fig. 2. Measuring points for velocity distributed in an internal recycle hole.

Fig. 3. Nitrogen removal performance in Experiment 1. (a) Profiles of influent $\text{NH}_4^+ \text{-N}$ and effluent $\text{NH}_4^+ \text{-N}$, $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, (b) Variations of nitrogen composition and concentration along the Orbal system.

2.5. PCR-DGGE analysis

The isolation of total DNA was accomplished with DNA Auto-plate (TanBead, Taiwan) by Automatic Platform for Magnetic System-16 (TanBead, Taiwan) and DNA bands were observed by 1.0% agarose gel electrophoresis. F357GC (5'-CGCCGCGCGCGCGCGCGG GCGGGGCGGGGACGCGGGGCTACGGGAGGCAGCAG-3') and

R518 (5'-ATTACCGCGGCTGCTGG-3') were used to amplify the segment of eubacterial 16S rDNA. The PCR amplification reaction was performed using an MJ Research PTC-200 Peltier Thermal Cycler (Bio-Rad, USA) at a final volume of 50 μL . The reaction mixture contained 20 pmol of both primers, 20 μmol of each dNTPs, 5 μL of 10 $\times$ buffer (TaKaRa, Dalian, China), and 1.25 units of Taq DNA polymerase (TaKaRa, Dalian, China). The temperature cycling

conditions were 94 °C for 3 min, followed by 31 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. A final extension at 72 °C for 10 min was used. The PCR product generated from each sample was separated on an 8% acrylamide gel with a linear denaturant gradient increasing from 30% to 60% using the Bio-Rad D-GENE System (Bio-Rad, USA). The DGGE was performed using 30 µl of the PCR product in 1 × TAE buffer at 60 °C and 70 V for 960 min. The resulting gel was visualized with Gel Red (Biotium USA). The digital images were captured by a Fluor-S Multimager (Bio-Rad, USA) and analyzed with Quantity One Software (Bio-Rad, USA).

3. Results and discussion

3.1. Nitrogen removal performance in Experiment 1

Fig. 3a depicts nitrogen profiles in the influent and effluent and nitrogen removal efficiency in the pilot Orbal oxidation ditch without internal recycle. Although the influent NH_4^+-N concentration varied from 35.7 mg/L to 61.9 mg/L, steadily low effluent NH_4^+-N concentration with less than 2 mg/L on average and over 97% ammonia nitrogen removal efficiencies were obtained implying complete nitrification performed in the system during the whole experimental periods regardless of oxygen levels within the outer channel. However, TN removal performances were considerably varying among four operational runs compared with consistently high ammonia removal. Maximum nitrogen removal occurred at DO of about 0.20 mg/L with about 82% TN removal efficiency averagely (Run 2), while 64% and 59% TN removals were obtained at Runs 1 and 4, respectively, while at Run 3 only 42% TN were removed. It was indicated that TN removal efficiency closely depended on oxygen levels within the outer channel.

To further clarify the differences in TN removal performances, nitrogen compositions and concentrations along the triple-channel oxidation ditch under different DO conditions within the outer channel were analyzed (in Fig. 3b). As the figure shown, ammonia nitrogen generally accounted for about 80–85% of the total nitrogen in the influent and the rest represented organic nitrogen and oxidized nitrogen. At extremely low DO of 0.05 mg/L, the first channel just only removed partial influent NH_4^+-N concentration and the remaining of NH_4^+-N were gradually removed through aerobic nitrification within followed two aerated channels while almost complete NH_4^+-N removal was able to be accomplished at above 0.2 mg/L DO within the outer channel. It was likely that full nitrification was successfully performed at rather low oxygen concentration due to longer HRT and SRT of the system [6,25], although nitrification rate at the low DO was much lower than its maximum value at DO of above 2 mg/L. As NH_4^+-N and organic nitrogen were quite low and nitrites were hardly detected in the effluent, NO_3^--N was the main form of nitrogen in the effluent (in Fig. 3a and b) due to complete nitrification at long HRT and SRT. Therefore, TN removal in Orbal oxidation ditch strongly relied on denitrification efficiency of nitrates entirely occurred in the aerated-anoxic channel while ammonification and nitrification were enhanced in the highly aerated channels.

Table 4 summarizes the rates of nitrification and denitrification and SND efficiencies under different DO conditions during the Experiment 1. It was observed that nitrification rates obviously increased while denitrification rates firstly rose and then declined with increased DO within the outer channel implying the great impact of DO on the SND efficiency. At DO of about 0.2 mg/L, nearly equally high rates of nitrification and denitrification led to an optimal SND efficiency of over 65%, whereas, decreased SND efficiencies were caused by either low nitrification rate at extremely low DO or low denitrification rate at quite high DO in the outer chan-

Table 4

Rates of nitrification and denitrification and SND efficiencies in Experiment 1.

Average DO (mg/L)	0.06	0.20	0.52	0.36
r_n (mg $\text{NH}_4^+-\text{N} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$)	2.53	4.77	6.24	5.1
r_{den} (mg $\text{NO}_3^--\text{N} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$)	1.58	3.18	1.17	2.06
Average SND_{eff} (%)	39.5	66.7	18.8	40.4

nel. Thus, TN removal in Orbal process mainly based on the degree of SND in outer channel [9,10,20].

Online monitoring of DO and ORP have been proved to be practical and effective indicators for SND control in nitrogen removal process [26–28]. In this study, on-line ORP measurement for SND optimization in the outer channel was also implemented due to its strong correlation with DO and wider range for process control under very low oxygen condition [26]. Unlike low ORP ranged from –100 mv to –50 mv in non-aerated anoxic zones in A/O process [29,30], ORP value of –50 mv to 50 mv under the aerated-anoxic condition was higher due to the presences of micro-aeration and oxidized nitrogen. The optimal ORP for SND seemed within the range of 10–40 mv at about 0.2 mg/L DO in this study.

3.2. DO concentrations between the outer and middle channels in Experiment 2

Table 5 shows DO concentrations between the outer and middle channels at different internal recycle ratios on the condition of the same total oxygen inputs. It was found that great DO stratification existed between two channels without internal recycle. With an increase of internal recycle in the modified Orbal system, DO in the outer channel rose while gradually declined in the middle channel if aeration supply were kept unchanged. DO differences between both channels became decreased even disappeared at high recirculation flow due to mixing of more high-oxygen liquor returned from the second channel. According to Experiment 1 results, DO should be kept around 0.2 mg/L for optimum TN removal through reducing rotating speeds of the aerated brush in the outer channel.

3.3. Nitrogen removal performance in Experiment 2

Fig. 4a shows the profiles of influent NH_4^+-N and effluent NH_4^+-N , NO_3^--N , NO_2^--N . Rather low NH_4^+-N concentration in the effluent (low than 1.0 mg/L) was obtained during the whole experiment indicating that 0.2 mg/L DO was enough for nitrifiers to remove ammonia whether nitrate recycle was added or not. Besides, compared with Experiment 1, a slight drop of TN removal in Experiment 2 during two experiments was also found perhaps due to lower C/N ratio in the modified oxidation ditch system with zero recycle ratio (see Table 1). Yet, it was more surprising that profile of effluent NO_3^--N experienced a rise firstly and then fall along with increased internal recycle. The similar changing tendency in TN removal (in Fig. 4b) was greatly influenced by nitrates reduction. The experimental results showed TN removal efficiencies at recycle ratios of 4.1 and 9.4 at Runs 2 and 3 were improved by about 6% and 11%, respectively, in contrast with zero recycle at Run 1. This observation concluded that more NO_3^--N from the middle channel by means of recirculation was utilized as electron acceptors to promote pre-denitrification in the aerated-anoxic

Table 5

DO concentrations between the outer and the middle channels under the constant oxygen input in Experiment 2.

Runs	Run 1	Run 2	Run 3	Run 4	Run 5
DO in outer channel (mg/L)	0.21	0.28	0.35	0.46	0.53
DO in middle channel (mg/L)	1.12	0.97	0.82	0.75	0.62

Fig. 4. Nitrogen removal performance in Experiment 2: (a) profiles of influent NH_4^+-N and effluent NH_4^+-N , NO_3^--N , NO_2^--N ; (b) influent and effluent TN and TN removal efficiency.

outer channel. When the ratio was controlled at 9.4, excellent TN removal efficiency as high as 87% with effluent TN < 8 mg/L was acquired. But TN removal performance began to sharply decrease at excessively high recycle ratio of above 10 (Runs 4 and 5). The possible explanation for this was less carbon source for denitrification in the outer channel and more organic matters were used for aerobic degradation rather than for anoxic denitrification due to significantly frequent alternation of the mixed liquor between anoxic and oxic conditions within two channels with further excessively increase in internal recycle ratio. Furthermore, strong diluted effect of the returned liquor on carbon source and high nitrates loading induced by high recycle flow gave rise to insufficient C/N ratios for denitrification, resulting in poor TN removal. Consequently, the suitable control of internal recycle in the modified oxidation ditch system allowed for improved nitrogen removal and reduced oxygen consumption simultaneously.

3.4. Nitrogen mass balances with and without nitrate recycle

Nitrogen elimination in the biological treatment process was mainly attributed to three pathways: ammonia stripping, assimilation, nitrification–denitrification. In the nitrogen balance, ammonia stripping was assumed to be negligible as the pH of the mixed liquor in the ditch was below 7.5 [3]. Nitrogen loss in biomass assimilation through sludge wasting (R_{waste}) can be estimated by the Ref. [22].

$$R_{\text{waste}} \text{ (mgN/d)} = Q_{\text{was}} \times X_{\text{vss}} \times i_{\text{vss}}$$

where Q_{was} is the sludge wasting rate; X_{vss} is the waste sludge MLVSS and i_{vss} represents the nitrogen content by weight of the MLVSS, 7.5% [31].

Nitrogen mass balances in Fig. 5 were compared at recycle ratios of 0 (Day 3) and 9.4 (Day 56) under identical process

Fig. 5. Nitrogen mass balances in the Orbal system at recycle ratios of 0 and 9.4 (figure labels indicate gTN/d).

parameters such as HRT, SRT and influent TN load in Experiment 2. As revealed in the figure, TN in the effluent occupied 23.1% and 13.1% of influent TN (about 880 gTN/d) at the ratios of 0 and 9.4, respectively. The average 9% of the influent TN was removed via assimilation in form of wasted sludge at both ratios. Therefore, denitrification seemed the dominant mechanism of TN removal in the multi-channel process. The great majority of the influent TN was removed via SND in the outer channel, while only a small proportion of about 5% was removed within aerobic channels and final settler. Compared with 63.3% of the influent TN via SND removal without recycle, TN removal was further increased by about 10% at the optimal recycle ratio of 9.4, which strongly implied that pre-denitrification was responsible for TN improvement in the aerated-anoxic system with internal nitrate recycle.

3.5. Variations in microbial community

In this study, the bacteria of different sludge samples from the outer, middle and inner channel of the Orbal oxidation ditch, respectively in Experiment 1 were analyzed by PCR–DGGE to link the relationship between nitrogen removal performance and

bacterial community characteristics. As shown in Fig. 6, La, Lb and Lc (Run 1); Ld, Le and Lf (Run 2); Lg, Lh and Li (Run 3) represented DGGE profiles of different sludge samples from the outer, middle and inner channel of Orbal oxidation ditch, respectively. The number of DGGE bands shows the relative diversity of the bacterial community [32] and the intensity of the specific band represents the relative abundance of the corresponding microbial species [33]. The appearance or disappearance of bands indicates changes in the bacterial community [34].

The dominant microbial population (Band 22) and other mutual populations (e.g. Bands 7, 11, 19 and 20) were commonly found among all lanes by DGGE fingerprints indicating that these bacterial groups were slightly affected by influent wastewater characteristics and operational conditions during the whole experiment.

Fig. 6 also demonstrated similar band patterns under the same operating period while band profiles varied largely among different three runs indicating changes in microbial community mainly associated with operational adjustment rather than spatial distribution of the bio-system. The biodiversities in microbial community at Run 3 were lower than Runs 1 and 2 due to disappearances of several bands (Bands 13–18), despite some differences in bacterial composition at both runs. A few heterotrophic bacteria such as denitrifiers in the outer channel (Lane g) might be inhibited by relatively high oxygen concentration resulting in poor denitrification. For Runs 1 and 2, less similarity in bands pattern between La and Ld from the outer channel, although both had abundant bands. It was more likely that the number and activity of nitrifying bacteria at Run 1 would be greatly decreased owing to extreme absence of oxygen, leading to partial NH_4^+-N removal. With increased aeration supply for the outer channel, intensities of some bands (i.e. Bands 11, 13 and 30) at Run 2 were enhanced indicating that certain functional bacterial populations such as some nitrifying bacteria adapted to low-oxygen environment [35] might be gradually enriched. These bacteria were capable of participating SND within the aerated-anoxic channel in the presence of heterotrophic denitrifying bacteria. As for two other channels, microbial diversities became gradually lower at Runs 2 and 3 while somewhat higher at Run 1, possibly dependent on the quantity of substrates (organic matter and nitrogen) and their uptake rates in the first channel.

These observations implied that variations in microbial diversity and population constitution under different DO conditions

Fig. 6. DGGE profiles of sludge samples from different channels in the Orbal system under different DO conditions (percentages indicate a coefficient similar).

within the outer channel seemed to be responsible for differences in TN removal efficiency of the oxidation ditch system, although specific bacterial identification and quantitative analysis were not conducted.

4. Conclusions

Maximum TN removal efficiency was achieved through accurate control of DO (0.15–0.25 mg/L) within the outer channel in a pilot-scale modified Orbal oxidation ditch with nitrate recycle. TN removal efficiency was further increased to about 87% at the optimal recycle ratio of 9.4 for improved denitrification. Nitrogen mass balances indicated nitrogen removal was enhanced via the coupling of SND and pre-denitrification under the aerated-anoxic condition within the outer channel. Variations in microbial community by PCR–DGGE under different DO conditions in the outer channel might lead to differences in TN removal performance.

Acknowledgements

The authors thank Dr. David Howard for the English revision of this manuscript. This work was financially supported by the Qualified Personnel Foundation of Taiyuan University of Technology (QPFT) (No. tyut-rc201262a) and the National Key Technology R&D Program of China (No. 2006BAC19B02).

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