Identification and evaluation of SND in a full-scale multi-channel oxidation ditch system under different aeration modes

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HIGHLIGHTS

- SND was observed in a multi-channel OD at very low DO of 0.10–0.25 mg L⁻¹.
- SND was identified by micro-environment characteristics within tiny flocs.
- The highest SND occupied 44.1% of influent nitrogen mass by NMB analysis.
- The maximum SND efficiency was 79% with SND rate of 5.09 mg L⁻¹ h⁻¹.

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ABSTRACT

Simultaneous nitrification and denitrification (SND) was evidently observed in a full-scale multi-channel oxidation ditch (OD), especially occurring within the outer channel under microaerophilic conditions. The optimum average dissolved oxygen (DO) concentration for SND was 0.25 mg L⁻¹ with 79% SND efficiency and 5.09 mg L⁻¹ h⁻¹ SND rate, leading to 74.9% overall TN removal efficiency. Small sized flocs consisting of more nitrifying bacteria enhanced nitrification in favor of SND due to less oxygen diffusion limitation inside flocs than larger flocs under micro-aerated conditions. SND was also identified by co-existing of nitrifying and denitrifying bacteria as well as some microaerophilic microorganisms based on molecular biology analysis. Nitrogen mass balances (NMB) quantitatively demonstrated that the highest SND efficiency accounted for 44.1% of influent nitrogen mass. Above valuable findings might deeply reveal SND as a principal mechanism of nitrogen removal in a multi-channel OD from macro- to micro-scale.

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1. Introduction

Biological nitrogen removal (BNR) processes have been widely used in municipal wastewater treatment plants (WWTPs) due to high-efficiency and low-cost for eliminating nitrogen from domestic wastewater to prevent oxygen depletion and eutrophication in receiving water bodies [1,2]. Traditional BNR process is a two-step reaction to remove total nitrogen (TN) involving aerobic nitrification of ammonia (NH₄⁺-N) to nitrite (NO₂⁻-N) and nitrate (NO₃⁻-N) followed by anoxic denitrification of the NO_x⁻-N to nitrogen gas (N₂) [3,4].

An oxidation ditch (OD) is a modified activated sludge treatment system, consisting of a single- or multi-channel configuration within a ring, oval, or horseshoe-shaped basin [5]. Unlike conventional BNR processes, a high internal recycle flow in ODs can subject mixed liquor to alternating spatial aerobic and anoxic zones distinctly created behind and before surface aerators along the single aerated channel by adjusting aeration supply for enhancing nitrogen removal [6]. Hence, compared with other continuous-flow activated-sludge bioreactors, characterization of nitrogen removal in ODs seems to be regarded as simultaneous nitrification and denitrification (SND), suggesting that nitrification and denitrification occur concurrently in a closed-loop channel due to particular spatial inhomogeneity in profiles of flow regime, oxygen transfer and mixing induced by point-aeration devices based on computational fluid dynamics (CFD) simulations [7–10].

Until now, SND phenomena have already been successfully observed in lab- [11], pilot- [11–14] and even full-scale OD

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Nomenclature

N_{inf}	mass of TN in the system influent (kg N d^{-1})	q	average waste sludge flow rate ($\text{m}^3 \text{d}^{-1}$)
N_{eff}	mass of TN in the system effluent (kg N d^{-1})	r	returned sludge ratio (%)
N_{was}	mass of nitrogen in the waste sludge (kg N d^{-1})	X_v	MLVSS of the waste sludge (mg VSS L^{-1})
$N_{c1,nit}$	mass of $\text{NO}_x\text{-N}$ produced through nitrification in the outer channel (kg N d^{-1})	f_N	nitrogen fraction of waste sludge ($\text{mg N mg}^{-1} \text{VSS}$)
$N_{c2,nit}$	mass of $\text{NO}_x\text{-N}$ produced through nitrification in the middle channel (kg N d^{-1})	$\text{NH}_4\text{-N}_{inf}$	$\text{NH}_4\text{-N}$ in the influent (mg L^{-1})
$N_{c3,nit}$	mass of $\text{NO}_x\text{-N}$ produced through nitrification in the inner channel (kg N d^{-1})	$\text{NO}_x\text{-N}_{inf}$	$\text{NO}_x\text{-N}$ in the influent (mg L^{-1})
$N_{c1,denit}$	mass of $\text{NO}_x\text{-N}$ denitrified in the outer channel (kg N d^{-1})	TN_{inf}	TN in the influent (mg L^{-1})
$N_{c2,denit}$	mass of $\text{NO}_x\text{-N}$ denitrified in the middle channel (kg N d^{-1})	$\text{NH}_4\text{-N}_{eff}$	$\text{NH}_4\text{-N}$ in the effluent (mg L^{-1})
$N_{c3,denit}$	mass of $\text{NO}_x\text{-N}$ denitrified in the inner channel (kg N d^{-1})	$\text{NO}_x\text{-N}_{eff}$	$\text{NO}_x\text{-N}$ in the effluent (mg L^{-1})
N_{unacc}	mass of unaccounted nitrogen (kg N d^{-1})	TN_{eff}	TN in the effluent (mg L^{-1})
Q	average influent flow rate ($10^4 \text{ m}^3 \text{d}^{-1}$)	$\text{NH}_4\text{-N}_{c1,out}$	effluent $\text{NH}_4\text{-N}$ from the outer channel (mg L^{-1})
		$\text{NH}_4\text{-N}_{c2,out}$	effluent $\text{NH}_4\text{-N}$ from the middle channel (mg L^{-1})
		$\text{NH}_4\text{-N}_{c3,out}$	effluent $\text{NH}_4\text{-N}$ from the inner channel (mg L^{-1})
		$\text{NO}_x\text{-N}_{c1,out}$	effluent $\text{NO}_x\text{-N}$ from the outer channel (mg L^{-1})
		$\text{NO}_x\text{-N}_{c2,out}$	effluent $\text{NO}_x\text{-N}$ from the middle channel (mg L^{-1})
		$\text{NO}_x\text{-N}_{c3,out}$	effluent $\text{NO}_x\text{-N}$ from the inner channel (mg L^{-1})

systems [15–17]. There were some principal explanations or pre-suppositions for nitrogen removal via SND in oxidation ditches: (1) repeatedly swift alternation is between aerobic (after aerators) and anoxic zones (before aerators) of the mixed liquor in the same aerated channel based on inhomogeneous oxygen distribution [18]; (2) low-oxygen nitrification occurs at low sludge loading rate or long HRT and SRT [14,16,17], despite its nitrification rate is generally maximum value at DO of above 2.0 mg L^{-1} ; (3) anoxic zones are developed inside the activated-sludge flocs under aerobic conditions [14,19]; (4) novel microbes such as aerobic denitrifiers and nitrifiers adapted to low-oxygen conditions [16,20] are capable of performing SND. Nevertheless, behavior and property of SND in ODs were not deeply understood, especially for micro-environment characteristics related to sludge characteristics and bacterial composition for SND. Furthermore, the potentials of SND in oxidation ditches were not effectively assessed yet.

Therefore, the purposes of the present study were to investigate SND performance under different low-oxygen levels and identify SND by floc size distribution and microbial community structure using fluorescence in situ hybridization (FISH) and polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) methods. Additionally, SND efficiency was quantitatively evaluated by nitrogen mass balance (NMB) in our OD and also compared in other types of ODs from related studies. We tried to reveal SND as an underlying mechanism of nitrogen removal in a multi-channel OD.

2. Material and methods

2.1. Oxidation ditch system

A full-scale Orbal OD system with a total volume of $14,916 \text{ m}^3$ is located at a municipal WWTP in Beijing, China. It has a capacity of $80,000 \text{ m}^3/\text{d}$ and operates in two parallel ditches, each $71.3 \text{ m} \times 65.3 \text{ m} \times 4.5 \text{ m}$ (length $\times$ width $\times$ depth) in size with an effective depth of 4.0 m and width of 10 m . The OD has a concentric ring-shape consisting of the outer, middle and inner channel and the size ratio of the three channels is approximately 50%:30%:20% (outer:middle:inner). The separate horizontal shafts across the channels possess a number of aeration discs, which provide oxygen to the mixed liquor in the channels and help prevent the activated sludge from settling in Fig. 1. The characteristics of the influent are listed in Table 1.

2.2. Operational conditions and aeration modes

During experimental periods, all process parameters except DO concentration were almost identical in the multi-channel system. The temperature of the mixed liquor in the oxidation ditch was $20\text{--}25^\circ\text{C}$, the mixed liquor suspended solids (MLSS) were $3000\text{--}3500 \text{ mg L}^{-1}$, the returned sludge ratio was 75–100%, the hydraulic retention time (HRT) was between 10 and 12 h, and the sludge retention time (SRT) were controlled between 12 and 14 days during the period. To determine the influences of DO levels across the three aerated channels on SND in a multi-channel oxidation ditch, four types of aeration modes was designed by independently adjusting the number of surface aerators within the each channel or replacing original discs with high-efficient discs from horizontal shafts in the middle and inner channels. Operational conditions and aeration modes are listed in detail in Table 2.

2.3. SND efficiency and SND rate

The overall nitrogen removal performance via SND in oxidation ditches was estimated by means of SND efficiency (E_{SND}) and SND

Fig. 1. Schematic diagram of a full-scale multi-channel OD system.

Table 1
Influent wastewater characteristics.^a

pH	COD (mg L ⁻¹)	BOD ₅ (mg L ⁻¹)	NH ₄ ⁺ -N (mg L ⁻¹)	NO ₂ ⁻ -N (mg L ⁻¹)	NO ₃ ⁻ -N (mg L ⁻¹)	TN (mg L ⁻¹)
7.6 ± 0.3 ^b	383.0 ± 107.7	176.0 ± 46.1	44.1 ± 9.8	0.1 ± 0.1	0.6 ± 0.5	57.0 ± 10.9

^a 83 samples for each wastewater parameter.^b Average ± standard deviation.**Table 2**
List of 16S rRNA gene-targeted oligonucleotide probes used [23,24].

Probes	Fluorescent label	Sequence (5'–3')	FA (%) ^a	Specificity
Nit3	FITC	CCTGTGCTCCATGCTCCG	40	<i>Nitrobacter</i> spp.
Ntspa662	FITC	GGAATTCGCGCTCTCT	40	Genus <i>Nitrospira</i>
Nrs1156	FITC	CCCGTTCTCTGGGCAGT	30	Genus <i>Nitrospira</i>
Nso190	Cy5	CGATCCCTGCTTTTCTCC	55	Ammonium oxidizing β-proteobacteria
Nso1225	Cy5	CGCGATTGTATTACGTGTGA	35	Ammonium oxidizing β-proteobacteria

^a Formamide concentration.

rate (R_{SND}) at low DO concentration. E_{SND} and R_{SND} were calculated according to equations as below [21]:

$$E_{\text{SND}} (\%) = \left(1 - \frac{\text{NO}_x^- \text{ produced}}{\text{NH}_4^+ \text{ oxidised}} \right) \times 100\%$$

$$R_{\text{SND}} \left(\text{mg L}^{-1} \text{ h}^{-1} \right) = \frac{\text{NH}_4^+ \text{ oxidised} - \text{NO}_x^- \text{ produced}}{\text{time (h)}}$$

where, $\text{NO}_x^- \text{ produced}$ is the sum of nitrites and nitrates produced when ammonium was oxidized; $\text{NH}_4^+ \text{ oxidised}$ is the amount of oxidized ammonium through nitrification; time is the HRT in the single-channel system or HRT of the outer channel in the multi-channel systems.

2.4. 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 and MLSS were analyzed according to Standard Methods [22]. pH was measured with a pH meter (WTW Multi340i). DO was measured using a portable DO meter (WTW Oxi340i). As DO distribution was not uniform in a channel or even within the same cross-section, the oxygen concentration in each channel was the average of the measurements from eight different cross-sections and twelve measuring points at four different heights at each cross-section along the ditch [16].

2.5. Floc size distribution

Floc size distribution was measured with a laser particle size analyzer (Malvern Mastersizer 2000, UK), which enables the measurement of particles in the range of 20–2000 μm. The average size of the sludge flocs was given as the mean based on the volume equivalent diameter.

2.6. FISH analysis

Activated sludge flocs in the outer channel between Modes 2 and 3 were respectively fixed by 4% paraformaldehyde at 4 °C for 12 h for the FISH experiments. The fixed flocs were washed three times with phosphate-buffered saline (pH: 7.2), and suspended in phosphate-buffered saline. 10 μl suspension including ASF was spotted on gelatin-coated glass slides in the form of layer. After air-dried, the flocs were consecutively dehydrated in 50%, 80%, and 100% ethanol. A hybridization solution containing 5 ng/μl of probe was applied to the slide. In situ hybridization was performed

at 46 °C for 3 h. Excess probes were washed at 48 °C for 15 min with washing buffer. Biomass on the slide was stained with DAPI solution. The images of FISH were captured by confocal lasers scanning microscopy (CLSM) and analyzed with LAS AF Lit software. Lists of oligonucleotide probes applied and respective target bacteria are shown in Table 2.

2.7. PCR–DGGE analysis

The isolation of total DNA was accomplished with DNA Autoplate (TanBead, Taiwan) by Automatic Platform for Magnetic System-16 (TanBead, Taiwan) and DNA bands were observed by 1.0% agarose gel electrophoresis. F357GC (5'-CGCCCGCCGCGCGCGGGGCGGGCGGGGACGCGGGGCTACGGGAGGCAGCAG-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× 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 Multilimager (Bio-Rad, USA) and analyzed with Quantity One Software (Bio-Rad, USA).

2.8. Sequencing analysis

Prominent DGGE bands were excised and dissolved in 50 μl of Milli-Q water overnight at 4 °C. The target DNA fragments were reamplified using primer set F357/R518 without the GC-clamp, thus obtaining a pure sample for cloning and sequencing. The PCR products were ligated into the pMD18-T vector (TaKaRa, Dalian, China) and transformed in *Escherichia coli* TOP 10 competent cells following the manufacturer's protocols (Biomed, Beijing, China). Colonies were cultured on LB medium with X-gal, IPTG, Amp. The positive colonies were submitted for sequencing using ABI 3730XL capillary sequencers (Biomed, Beijing, China). Homology searches were conducted using the GenBank server of the National Centre for Bio-technology Information (NCBI) and the

BLAST algorithm. The partial sequences obtained in this study were submitted to the NCBI database under accession numbers.

2.9. Nitrogen mass balances

Fig. 2 shows the multi-channel OD system configuration for the NMB analysis. The mass of nitrogen in the influent leaving in the system is mainly through four fractions [25]: effluent TN, TN of the waste sludge, gaseous nitrogen via denitrification and other unaccounted nitrogen (e.g. ammonia stripping and denitrification from the settler). Therefore, the nitrogen mass balance in Fig. 2 is given by:

$$N_{inf} = N_{eff} + N_{was} + N_{c1,denit} + N_{c2,denit} + N_{c3,denit} + N_{unacc}$$

The mass of TN in the influent (N_{inf}) is given by:

$$N_{inf} = QTN_{inf}$$

The mass of TN in the effluent (N_{eff}) is given by:

$$N_{eff} = (Q - q)TN_{eff}$$

To estimate the mass of N in the waste sludge (N_{was}), the nitrogen fraction in the sludge (f_N) must be assumed [25]. WRC suggests a value for f_N of 0.1 mg N mg⁻¹ VSS over a range of sludge ages [26]. N_{was} is calculated from:

$$N_{was} = qX_v f_N$$

The mass of N denitrified in the system is calculated by the mass balance on nitrite/nitrate (NO_x-N) [6]. To estimate NO_x-N produced in the each single channel, it is assumed that elimination of NH_4^+-N is totally converted to NO_x-N only through nitrification and microbial assimilation of NH_4^+-N is negligible. Therefore, the mass of NO_x-N denitrified in the outer channel ($N_{c1,denit}$) is given by:

$$N_{c1,nit} = QNH_4-N_{inf} + rQNH_4-N_{eff} - (1+r)QNH_4-N_{c1,out}$$

$$N_{c1,denit} = QNO_x-N_{inf} + rQNO_x-N_{eff} - (1+r)QNO_x-N_{c1,out} + N_{c1,nit}$$

Similarly, the mass of NO_x-N denitrified in the middle ($N_{c2,denit}$) and inner channel ($N_{c3,denit}$), respectively, is given by:

$$N_{c2,nit} = (1+r)Q(NH_4-N_{c1,out} - NH_4-N_{c2,out})$$

$$N_{c2,denit} = (1+r)Q(NO_x-N_{c1,out} - NO_x-N_{c2,out}) + N_{c2,nit}$$

$$N_{c3,nit} = (1+r)Q(NH_4-N_{c2,out} - NH_4-N_{c3,out})$$

$$N_{c3,denit} = (1+r)Q(NO_x-N_{c2,out} - NO_x-N_{c3,out}) + N_{c3,nit}$$

The total mass balance of nitrogen in the system is given by:

$$N \text{ balance} = \left(\frac{N_{eff} + N_{was} + N_{c1,denit} + N_{c2,denit} + N_{c3,denit}}{N_{inf}} \right) \times 100\%$$

3. Results and discussion

3.1. Observation of SND during nitrogen removal

Fig. 3a and b depict total removal efficiencies of NH_4^+-N and TN in OD and Fig. 4 shows the profile of nitrogen concentration along

Fig. 3. Overall nitrogen removal performances under different aeration modes. (a) Ammonia nitrogen (NH_4^+-N) removal. (b) Total nitrogen (TN) removal.

the multi-channel OD system under different aeration modes. The effluent ammonia nitrogen concentration was gradually decreased from 44.6 mg L⁻¹ at Mode 1 to 0.8 mg L⁻¹ at Mode 4, corresponding removal efficiency was improved from 8.8% to almost 100%, implying enhanced nitrification due to significantly increasing oxygen levels in three oxygenated channels. In terms of TN removal, however, the highest efficiency of 74.9% was achieved at Mode 3 where DO in the outer channel was higher than Modes 2 and 4, but lower than Mode 4 in the remaining two channels (in Table 3). Consequently, overall nitrogen removal capacities were strongly influenced by different aeration strategies based on each channel of the multi-channel OD system. At Mode 1, almost no NH_4^+-N was removed, since nitrification failed to proceed at extremely low DO level of 0.03 mg L⁻¹ in the outer channel, despite DO

Fig. 2. Schematic diagram showing NMB across the full-scale multi-channel OD system.

Fig. 4. Transformations of nitrogen-form along the multi-channel OD system under different aeration modes.

Table 3

Aeration modes and operational conditions.

Operational conditions	Mode 1	Mode 2	Mode 3	Mode 4
Number of aerators (out-middle-inner)	2-3-3	4-4-4	6-4-4	4-4 ^a -4 ^a
DO in outer (mg L ⁻¹)	0.03 (0–0.06) ^b	0.13 (0–0.38) ^b	0.25 (0.03–0.96) ^b	0.14 (0–0.41) ^b
DO in middle (mg L ⁻¹)	0.14	0.35	0.46	1.47
DO in inner (mg L ⁻¹)	0.76	1.05	1.41	3.10
HRT (h)	11.90	10.80	10.50	10.10
Operating days (d)	70	77	147	70

^a The oxygen transfer capacity of new types of disk in the middle and inner channels is as high as 2.40 kgO₂ h⁻¹ per disk after disk replacement at Mode 4, while that of the original disk is only 1.04 kgO₂ h⁻¹ per disk among other modes.

^b DO concentrations before aerator and after aerator representing the lowest and highest DO concentrations in the outer channel among the aeration modes.

of 0.76 mg L⁻¹ in the inner channel accounting for only 20% of the total volume of the oxidation ditch. Although NH₄⁺-N were partially removed in the outer channel at Modes 2 and 4 with similarly added oxygen, compared with Mode 2, considerably high DO in the followed two channels at Mode 4 triggered much higher removal efficiencies of NH₄⁺-N along with high effluent nitrates which were hardly reduced in Figs. 3 and 4. But at Mode 3, substantial NH₄⁺-N was eliminated, accompanying with only a small portion of nitrates in the effluent, suggesting that noticeable TN removal via SND successfully was completed at about 0.20 mg L⁻¹ DO in the outer channel in Fig. 4. It was concluded that such a relatively low DO concentration was sufficient for nitrification, meanwhile, without inhibitory effects on denitrification at the presence of large macroscopical aerated-anoxic environment of the outer channel [17,27,28]. According to above results, it was likely that obvious SND was efficiently accomplished at a moderately low level of oxygen supply [13,16] in the outer channel which provided continuous alternation between anoxic and micro-aerobic conditions both in space and time to maintain equally high dynamic balances between nitrification and denitrification rates.

3.2. Identification of SND by micro-environment characteristics

Fig. 5 depicts flocs distribution in the outer channel, where remarkable SND was observed, between Modes 2 and 3. Based on the measurement, the average floc diameters were 118 μm for

Mode 2 and 69 μm for Mode 3, respectively, due to different shear force depended on the number of disc aerators under identical operational parameters. Smaller sludge flocs were clearly observed at Mode 3, which facilitated oxygen mass transfer and reduce oxygen diffusion limitation inside flocs for enhancing nitrification [29,30] under oxygen deficit condition within the outer channel, although its rate lower than the maximum under aerobic conditions, while only incomplete nitrification occurred within the larger flocs in Mode 4 due to the decrease of aerobic micro-zone because excessively low DO failed to penetrated through the centre of the large size particle to supply enough oxygen for nitrifiers. Hence, the characteristics of SND in OD systems under low oxygen condition was quite different from biofilm or aerobic granular sludge processes achieving SND under completely aerobic conditions due to sufficient anoxic zones developed within the inner of much more large-sized biological aggregates [31,32].

The assumption was further supported by the FISH analysis in Fig. 6. It was distinctly that compared with big flocs, the micro flocs in activated sludge consisting of more dispersed nitrifying microorganisms such as ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) could significantly improve denitrification due to less oxygen limitation, once they were exposed to large bulk of aerated-anoxic environment [14,33]. According to PCR-DGGE and sequencing analysis (in Table 4), furthermore, these small sized flocs were also constituted by substantial biomass including uncultured bacterium *Bacteroidetes*, *α,ε,δ-proteobacteria*,

Table 4

Sequence identities and characteristics of bacterial clones in twenty-three different bands excised from the DGGE gel.

Band	Closest relatives	Isolation source	Similarity	Group level affiliation
1	Uncultured <i>Bacteroidetes</i> bacterium Z4MB12	Wall biomat sample	99	<i>Bacteroidetes</i>
2	Uncultured bacterium F5K2Q4C04IYVXE	Activated sludge	96	Uncultured bacterium
3	Uncultured bacterium ambient_uncontrolled-65	Anaerobic fermentation reactor with waste activated sludge at ambient and pH uncontrolled	100	<i>Bacteroidetes</i>
4	Uncultured bacterium K1	Nitrogen and phosphorus removal bioreactor	100	<i>Bacteroidetes</i>
5	<i>Arcobacter</i> sp.	Lake	100	ϵ -proteobacteria
6	Uncultured bacterium RBC1-68	Wastewater treatment plant	99	Uncultured bacterium
7	Denitrifying bacterium enrichment culture NOB_2_G8	Ebro River Delta sediment	100	Denitrifying bacterium
8	Denitrifying bacterium enrichment culture NOB_3_F2	Ebro River Delta sediment	100	Denitrifying bacterium
9	<i>Geobacteraceae</i> , IS-22	Iron(II)-rich seep	96	δ -proteobacteria
10	<i>Chloroflexi</i> , TM7	Mesophilic anaerobic digester wastewater sludge	99	<i>Chloroflexi</i>
11	Uncultured bacterium SB4-49	Wastewater treatment plant	100	<i>Bacteroidetes</i>
12	<i>Bacillus</i> sp.	Mangrove soil	100	<i>Firmicutes</i> : <i>Bacillus</i>
13	Uncultured <i>Staphylococcus</i> sp. DH17_95	Anterior nares	100	<i>Firmicutes</i>
14	Uncultured <i>Staphylococcus</i> sp. VA17_13	Anterior nares	100	<i>Firmicutes</i>
15	Uncultured <i>alpha</i> proteobacterium	Aerobic activated sludge	99	α -proteobacterium
16	Uncultured bacterium F5K2Q4C04ICWOM	Activated sludge	100	Uncultured bacterium
17	Uncultured bacterium clone B144	Partial nitrifying-ANAMMOX municipal wastewater reactor	99	Uncultured bacterium
18	Uncultured bacterium clone 13	Denitrification bioreactor	99	Uncultured bacterium
19	Uncultured bacterium clone 13	Denitrification bioreactor	98	Uncultured bacterium
20	Uncultured eukaryote PR-115	Activated sludge	98	Uncultured eukaryote
21	Uncultured <i>Bacteroidetes</i> bacterium	Wastewater treatment plant anoxic basin	100	<i>Bacteroidetes</i>
22	Uncultured <i>Firmicutes</i> bacterium TC9	Cellulolytic bacterial community	98	<i>Firmicutes</i>
23	Uncultured bacterium P1CN04	Sewage sludge	97	Uncultured bacterium

Fig. 5. Floc size distributions in Mode 2 (a) and Mode 3 (b).

Chloroflexi, and *Firmicutes*, as well as enrichment culture bacterium, indicating the co-existing of aerobic, microaerophilic and denitrifying bacteria performing SND capacity [16]. Therefore, it was essential to properly control of low oxygen level and high shear force by surface aerators using real-time online sensors [34,35] such as pH, DO, ORP and flow velocity to accelerate SND within tiny flocs for enhancing TN removal in the outer channel.

3.3. Evaluation of SND by nitrogen mass balances

To evaluate the SND efficiencies responsible for nitrogen removal performance under different aeration patterns, NMB analysis across an entire multi-channel OD system was conducted and the results are summarized in Table 5.

As shown in Table 5, calculated data presented that perfect N balances degrees among all modes were achieved at above 90% according to NMB analysis, demonstrating that unaccounted nitrogen only occupied a small fraction of influent nitrogen mass. The percentages in the total mass of influent nitrogen through the discharge of wasted sludge were ranged 9.8–13.2%; meanwhile, effluent nitrogen accounted for about 24.5–84.3% of the influent nitrogen under different aeration modes depending on TN removal efficiency. Therefore, nitrogen leaving from the system was in the form of the gaseous nitrogen mainly through nitrification and denitrification within each single aerated channel. Based on estimated results, although the lost nitrogen in an OD without internal recycling of mixed liquor was attributed by the sum of SND from three channels, the percentages of removed nitrogen mass via SND in the mass of influent nitrogen gradually dropped from the

Fig. 6. CLSM images of FISH of AOB (a) and NOB (b) in Mode 2 and AOB (c) and NOB (d) in Mode 3 (Eubacterium (blue), AOB (yellow) and NOB (green)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5
Nitrogen mass balances under different aeration modes.

Nitrogen mass	Mode 1		Mode 2		Mode 3		Mode 4	
	kg N d ⁻¹	% in N _{inf}	kg N d ⁻¹	% in N _{inf}	kg N d ⁻¹	% in N _{inf}	kg N d ⁻¹	% in N _{inf}
N _{inf}	1814.4 (241.3) ^a	100.0	1458.6 (187.1)	100.0	1904.0 (260.1)	100.0	2108.7 (321.3)	100.0
N _{eff}	1529.3 (125.0)	84.3	788.5 (72.5)	54.1	467.3 (65.2)	24.5	798.3 (99.5)	37.9
N _{was}	178.4 (22.4)	9.8	192.3 (37.0)	13.2	228.2 (31.3)	12.0	226.7 (35.7)	10.8
N _{c1,denit}	8.3 (4.1)	0.5	296.1 (13.6)	20.3	839.4 (27.0)	44.1	518.8 (17.6)	24.6
N _{c2,denit}	11.6 (5.5)	0.6	78.0 (8.2)	5.3	138.8 (13.9)	7.3	284.4 (9.0)	13.5
N _{c3,denit}	34.9 (12.2)	1.9	26.0 (6.3)	1.8	67.3 (8.8)	3.5	147.0 (7.4)	7.0
N balance		97.1		94.7		91.4		93.8

^a Values are averages (±standard deviation).

outer to the inner channel at each mode except for Mode 1 due to no nitrification along with little produced NO_x⁻-N. The largest aerated-anoxic channel optimized by oxygen supply offered a favorable co-existed environment for both nitrifying bacteria adapted to low oxygen and aerobic denitrifiers for promoting SND [16,20], while higher oxygen had an adverse effect on denitrification in the other channels, causing the failure of SND. In the same outer channel, the highest percentage of influent nitrogen mass via SND was achieved at Mode 3 with 44.1%, about twice as much at Mode 2 or Mode 4. On the other hand, for the same aerated-oxic channels, the ratio of influent nitrogen mass removed via SND at Mode 4 was much higher than two other modes, perhaps because more NO_x⁻-N generated under oxygen-rich conditions allowed to be denitrified within scattered anoxic zones established horizontally (in front of surface aerators) and vertically (at the bottom)

due to limitation of oxygen transfer [7,10] in the aerobic channels where significant nitrification was accomplished.

3.4. Comparison and optimization of SND in full-scale plants

Table 6 summarizes SND performances among different types of oxidation ditches from this study and other related studies. The SND capacity depended on the efficiency and rate of SND occurrence [23], influenced by wastewater characteristics and process parameters [11,37]. From Table 6, the OD systems with very high SND efficiencies (close to 100%) might have quite slow SND rates due to very long HRTs. For multi-channel ODs, SND efficiencies and SND rates greatly varied from 79% to 100% and 0.22 to 5.44 mg L⁻¹ h⁻¹, respectively. Higher SND rate could accelerate nitrification and denitrification to enhance TN removal over a

Table 6

Comparison of SND efficiencies and SND rates among different oxidation ditches.

Ditch type	Organic loading rate (kgCOD kg ⁻¹ MLSS ⁻¹ d ⁻¹)	DO (mg L ⁻¹)	C/N	SRT (d)	HRT (h)	E _{SND} (%)	R _{SND} (mg L ⁻¹ h ⁻¹)	TN removal (%)	Refs.
Passveer	0.11	0.1–0.5	10	44	17.2	100	1.42	97.6	[19]
Passveer	0.04	0.1–0.5	12.1	164	20.5	85	0.65	75.7	[19]
Passveer	0.02–0.21	>1.0	8.1	25–50	60.0	65	0.54	~45	[14]
Carrousel	0.20–0.30	1.0–1.2	5.3	—	7.9–12	67	1.61–2.44	60–70	[11]
Orbal ^a	0.03	0.1	16.1	>50	33.1	81	1.98	76	[36]
Orbal ^a	0.05 ^b	<0.1	6.8 ^c	33	35.9	87–95	0.22–0.24	90.5	[17]
Orbal ^a	—	Near 0	—	10–30	30–43	100	1.06–1.52	87.9	[20]
Orbal ^a	0.06	0.1	2.7	—	16.5	91	5.44	72.1	[12]
Orbal ^a	0.26	0.2	6.7	13	10.5	79	5.09	74.9	This study

^a The outer channel of Orbal oxidation ditch.^b Organic loading rate based on BOD₅.^c C/N based on the ratio of BOD₅/TKN.

shorter retention time. The system HRT and rates of nitrification and denitrification under low DO concentrations played significant roles in SND rate. In our study, considerably high SND rate (5.09 mg L⁻¹ h⁻¹) was obtained, although 79% of SND efficiency was not very excellent due to adverse impacts of high organic loading and low C/N on TN removal [11,12,16], compared with other OD systems.

To establish effective design criteria for plant optimization and retrofitting aiming at higher nitrogen removal efficiency, SND should be optimum based on premise control of oxygen by aeration adjustment [16] to reach the balance between nitrification and denitrification rates owing to their conflicts of oxygen and carbon substrates [11]. From the above comparisons, for oxidation ditches with higher organic rates and short HRT, enough oxygen intensity and tiny flocs are required for organic oxidation and then nitrification under the low-DO condition without the inhibitory effect on denitrification. For those low-organic loading systems, relatively low DO (below 0.1 mg L⁻¹, even almost zero) should be maintained to prevent over-degradation of carbon substrates and improve denitrification at higher C/N ratio, because nitrification is not a limiting factor for those systems with very long HRT and/or SRT.

4. Conclusions

Obvious SND occurred at very low DO concentrations between 0.10 and 0.25 mg L⁻¹ in the outer channel of a full-scale multi-channel OD system. At average about 0.2 mg L⁻¹ DO in the outer channel, the highest SND efficiency and SND rate reached 79% and 5.09 mg L⁻¹ h⁻¹, respectively. Small sized flocs rather than larger ones consisting of more nitrifying bacteria improved nitrification rate under low oxygen level based on floc size distribution and molecular biology analysis. NMB also quantitatively reveals the highest SND efficiency accounting for 44.1% of the total influent nitrogen achieved in the outer channel at DO of 0.25 mg L⁻¹. The observations of nitrogen removal and micro-environment characteristics coupled with mass balance analysis demonstrated SND seemed an underlying mechanism of TN removal in a multi-channel OD system.

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