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2 **High Rate Nitrogen Removal in an Alum Sludge-Based Intermittent**
3 **Aeration Constructed Wetland**

4

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12

13 **ABSTRACT:** A new development on treatment wetland technology for the purpose of
14 achieving high rate nitrogen removal from high strength wastewater has been made in
15 this study. The laboratory scale alum sludge-based intermittent aeration constructed
16 wetland (AIS-IACW) was integrated with pre-denitrification, intermittent aeration and
17 step-feeding strategies. Results obtained from 280 days' operation have demonstrated
18 extraordinary nitrogen removal performance with mean TN removal efficiency of 90%
19 under high N loading rate (NLR) of $46.7 \text{ g N m}^{-2}\cdot\text{d}^{-1}$. This performance was a substantial
20 improvement compared to the reported TN removal performance in literature. Most
21 significantly, partial nitrification and simultaneous nitrification denitrification (SND) via
22 nitrite was found to be the main nitrogen conversion pathways in the AIS-IACW system
23 under high dissolved oxygen concentrations ($3\text{-}6 \text{ mg L}^{-1}$) without specific control. SND

1 under high DO brings high nitrogen conversion rates. Partial nitrification and SND via
2 nitrite can significantly reduce the demand for organic carbon compared with full
3 nitrification and denitrification via nitrate (up to 40%). Overall, these mechanisms allow
4 the system to maintaining efficient and high rate TN removal even under carbon limiting
5 conditions.

6

7 **INTRODUCTON**

8 Although constructed wetlands (CWs) have been applied successfully in pollutants
9 removal from various wastewaters, their capacity and efficiency is still low with regard to
10 nutrient removal. With typical configurations (un-aerated surface flow and subsurface
11 flow), removals of total nitrogen (TN) and total phosphorus (TP) remain around 50% in
12 most cases under N loading rate (NLR) of 0.6-2 g N m⁻² d⁻¹ and P loading rate (PLR) of
13 0.02-0.2 g P m⁻² d⁻¹.¹⁻³ This is mainly because that the most effective nutrient removal
14 processes, such as enhanced biological phosphorus removal (EPBR) and biological
15 nitrogen removal (BNR)⁴ are limited in the conventional CWs.

16 Recently, a so-called alum sludge based-CWs (AIS-CWs) has been developed at
17 University College Dublin, Ireland, which adopts dewatered alum sludge (DAS) as the
18 main wetland medium.^{5,6} DAS is an inevitable by-product from drinking water treatment
19 process when aluminium sulphate is used as the primary coagulant. Conventionally, DAS
20 is regarded as a waste with unknown reuse value. However, this largely and locally
21 available “waste” has been demonstrated as a low-cost adsorbent for P immobilization
22 with high P retention capability.^{7,8} Accordingly, intensive efforts have been made by the
23 authors’ research group to develop the AIS-CWs for nutrient removal. The results from

1 laboratory and field trials have proven successful in P removal, ^{5, 6} thus making TN
2 elimination remaining as the only challenge in AIS-CWs.

3 Complete TN elimination relies firstly on the complete nitrification. Most
4 conventional CWs fail to fulfill this first step due to insufficient oxygen supply.⁹⁻¹¹ Hence,
5 oxygen supply is the key issue to enhance nitrogen removal in CWs, and artificial
6 aeration is the most effective alternative to guarantee sufficient oxygen supply ¹²⁻¹⁷. In
7 particular, when dealing with high strength wastewater, artificial aeration seems to be the
8 only option to achieve complete nitrification. However, with the enhanced oxygen supply
9 denitrification usually becomes the limiting step for effective TN elimination due to the
10 lack of effective anoxic zone and the depletion of the influent carbon source.
11 Consequently, TN removal might still be not desirable even if nitrification has been well
12 conducted.^{12, 14, 16} Hence, alternative wetland configuration and operational strategies are
13 requested for efficient denitrification in aerated CWs.

14 A pre-denitrification stage is the most common adaptation in practice to make
15 efficient utilization of the influent carbon source. And Batch mode is the most effective
16 way to create such a pre-denitrification stage by keeping a certain stationary volume from
17 the previous cycle and starting with an un-aerated phase. Moreover, multiple alternate
18 anoxic/aerobic (An/A) phases and step-feeding can be easily employed in batch mode by
19 intermittent aeration and introducing raw wastewater into each anoxic phase to further
20 enhance the denitrification potential.¹⁸ Significantly, simultaneous nitrification
21 denitrification (SND) and partial nitrification denitrification have been well studied for
22 the purpose of a better nitrogen removal.¹⁹⁻²² It has been noted that both processes are

1 likely to occur in a biofilm system.^{19, 21} Therefore, they may also play an important role in
2 nitrogen conversion in CWs.

3 Thus, the primary goal of the current study was to achieve high rate nutrient removal
4 from high strength wastewater with a single stage alum sludge-based intermittent aeration
5 CW (AIS-IACW) system. Since efficient P removal was guaranteed by DAS, the main
6 focus was placed on TN elimination. Artificial aeration was employed to ensure complete
7 nitrification under high NLR. Intermittent aeration, pre-denitrification and step-feeding
8 were integrated to achieve efficient denitrification, which is a common problem limiting
9 TN removal performance in aerated CWs.^{12, 14, 16} Furthermore, special attention was paid
10 on SND and partial nitrification/denitrification, which were expected to be the main
11 mechanisms on N removal in the AIS-IACW system.

12

13 **MATERIALS AND METHODS**

14 **System configuration and operation**

15 The laboratory scale AIS-IACW system was constructed with plexiglass column
16 (diameter 9.3 cm) (Fig. 1(a)). 10cm-depth gravel was filled into the bottom as the support
17 medium and 60cm-depth air dried DAS (2 kg, moisture content 74%, particle size 1-3 cm)
18 was filled as the main wetland medium layer, which gives a total volume of 4.75 L with
19 initial porosity of 42% (2 L liquid contained). The source and characteristics of the DAS
20 can be found from the authors' previous study.²³ Common reeds, *Phragmites australis*,
21 were planted on the top of the column. Influent was introduced into the column from the

1 top while effluent was drained from the bottom by peristaltic pumps. Aeration was
2 supplied with a diffuser placed on the support layer and controlled with an air flow meter.

3 The system was operated in batch mode with 3 cycles per day. Incorporated pre-
4 denitrification, intermittent aeration and step feeding was designed as illustrated in Fig.
5 1(b). Initially, each cycle started with a pre-denitrification stage (An1) by maintaining a
6 stationary volume (V_S) from the previous cycle. V_S serves as $\text{NO}_X\text{-N}$ ($\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$)
7 pool to form the first anoxic condition after filling. The degree of pre-denitrification is
8 limited by the available $\text{NO}_X\text{-N}$, which is determined by the volume exchange ratio (VER)
9 defined as V_S/V_F (V_F , the total filling volume per cycle). The higher the VER, the more
10 $\text{NO}_X\text{-N}$ is provided for pre-denitrification.¹⁸ However, the hydraulic loading rate (HLR)
11 is decreased if a high VER is adopted since the V_F has to be reduced for a certain total
12 reactor volume ($V_T=V_F+V_S$). To obtain a high degree of pre-denitrification, a high VER
13 of 9 was adopted with V_S of 1.8 L and V_F of 0.2 L, which results in a daily flow rate of
14 0.6 L d^{-1} and a HLR of $0.09 \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1}$. Air was supplied intermittently with on/off
15 control of the air compressor by a programmable timer. Three alternate An/A sub-cycles
16 in sequence were then created, which provide post denitrification steps. Wastewater was
17 step-fed into the system during the start of each anoxic phase for effective use of carbon
18 substrate for denitrification, with distribution ratios of 60%, 30% and 10% for each
19 feeding.

20

21

[Fig. 1]

22

1 The system was seeded with activated sludge collected from a local municipal
2 wastewater treatment plant for two weeks before it was formally operated. The AIS-
3 IACW was then operated for 280 days under room temperature, which can be divided
4 into three periods according to the distribution of the An/A phases and the air flow rate.
5 In general, air supply was increased from period 2 by extending the aerobic phases and/or
6 increasing the air flow rate (Table S1).

7

8 **Wastewater**

9 Piggery wastewater, which is a typical representation of high strength wastewater, was
10 used as the influent source. The raw wastewater was collected from an animal farm at
11 Newcastle, Co. Dublin, Ireland. After collection, the raw wastewater was diluted 4-6
12 times with tap water to obtain appropriate loading rates and then served as the influent.
13 The targeted influent range was set as COD 3000-6000 mg/l and TN 250-700 mg/l, which
14 is the typical range after common pre-treatment (sedimentation & anaerobic digestion)
15 and generally used in other studies. The characteristic of the influent and the loading rate
16 for different periods were summarized in Table 1. The general trend was that the NLR
17 increased while the organic loading rate (OLR) and the COD/TN ratio decreased over the
18 operation periods.

19

20

[Table 1]

21

22 **Overall performance and cyclic profile**

1 Grab samples of the influent and effluent were collected 1-2 times per week and analyzed
2 for COD, BOD₅, TN, NO₂-N, NO₃-N, NH₄-N, TP, PO₄-P, SS and pH. The overall
3 performance in organic matters (OMs) and nutrient removal was evaluated with
4 pollutants removal efficiency. In this case, nitrogen removal through plant uptake was
5 considered to be neglectable due to the high NLR applied (32.5-46.7 g N m⁻² d⁻¹, Table
6 1), which was far beyond the N uptake rate by plant of 0.03 g N m⁻² d⁻¹ ²⁴.

7 To investigate the profiles of carbon and nutrient within a cycle, cyclic study was
8 conducted on day 181 in period 3 when the system was considered in steady state which
9 was indicated by the stable influent and effluent between day 175 and 200. Liquid
10 samples were collected over time and analyzed for soluble COD (SCOD), PO₄-P, NH₄-N,
11 NO₂-N and NO₃-N. DO was monitored online with an oxygen probe placed at the middle
12 of the CW throughout the cycle. As the raw wastewater was introduced from the top of
13 the column and it was not well mixed after the first filling, there was significant
14 difference in concentrations among the different locations in the first subcycle (highest at
15 the top and decreased downwards). Hence composite liquid samples were taken for the
16 first subcycle. Then the following aeration provided strong mix and it was justified that
17 the concentrations were more or less the same after the first aeration period. Therefore,
18 only grab liquid samples from the middle were taken for the remaining sub-cycles.

19

20 **SND efficiency**

21 To estimate the SND efficiency, aerobic batch tests were conducted at the end of this
22 study under room temperature. Representative DAS samples were obtained from the AIS-
23 IACW and pretreated by washing several times with distilled water one day before the

1 tests to remove the residual substrates and eliminate storage effect. Then three 100 mL
 2 pretreated DAS samples were mixed with 50 mL piggery wastewater in 250 mL beakers.
 3 The beakers were then filled to 200 mL with distilled water. Thereafter, glucose or
 4 ammonium chloride was added to adjust the initial COD/N ratio. The initial conditions
 5 were summarized in Table S2. In particular, the initial COD/N ratio was set at 6.3, 9.4
 6 and 13.7, respectively, to reflect the influent variety during the system operation. Air was
 7 supplied with three diffusers placed at the bottom of the beakers, and DO was maintained
 8 between 4.7-6.3 mg L⁻¹. For each beaker, 15 liquid samples (3 mL each) were taken and
 9 analyzed for NH₄-N, NO₂-N and NO₃-N over a period of 35 hrs (minimum interval 1 h).
 10 At 24 hrs, inorganic carbon (IC) was supplemented to each beaker by adding 0.5 mL of
 11 NaHCO₃ solution (7.2 g C L⁻¹). The overall SND efficiency is defined as the amount of N
 12 denitrified divided by the amount of NH₄-N oxidized and expressed as a percentage (Eq.
 13 (1)). Specific rate of nitrification, NO_x-N production and denitrification is calculated
 14 according to Eq. (2) to Eq. (4), respectively, and expressed as mg N LDAS⁻¹ h⁻¹.

15

$$E_{SND}(\%) = 100 \times \frac{NH_{4Oxi.} - (NO_2 + NO_3)_{Acc.}}{NH_{4Oxi.}} \quad (1)$$

$$r_{Nitri} = \frac{V_T \times (NH_{4,t1} - NH_{4,t0})}{V_{DAS} \times (t_1 - t_0)} \quad (2)$$

$$r_{NOx} = \frac{V_T \times (NO_{X,t1} - NO_{X,t0})}{V_{DAS} \times (t_1 - t_0)} \quad (3)$$

$$r_{Deni} = r_{Nitri} - r_{NOx} \quad (4)$$

16 Where V_T is total volume, 0.2 L; V_{DAS} is the volume of the DAS sample, 0.1 L.

1

2 **Nitrogen conversion rates**

3 Nitrogen conversion pathway is the main focus to explore in this study. There are two
4 main pathways of nitrogen conversion, namely conventional nitrification/denitrification
5 and partial nitrification/denitrification. When SND is significant, both conversion ways
6 can occur concurrently in one reactor under aerobic condition.²⁵ The final route will be
7 determined by three conversion rates: rate of ammonia oxidization (nitritation, r_{AOB}), rate
8 of nitrite oxidization (nitrataion, r_{NOB}) and rate of denitrification over nitrite ($r_{\text{Deni-NO}_2}$).
9 To determine these three rates, aerobic activity tests were conducted at the end of the trial
10 under room temperature. Pretreated DAS samples (described in section “SND efficiency”)
11 of 100 mL were added into 250 mL beakers with different substrates ($\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ or
12 COD). Buffering solution²⁶ was supplied in r_{AOB} and r_{NOB} tests to avoid IC limitation. As
13 such, ATU (Allylthiourea) and Azide²⁷, which are selective inhibitors for AOB
14 (ammonia oxidation bacteria) and NOB (nitrite oxidation bacteria), respectively, were
15 added in $r_{\text{Deni-NO}_2}$ determination to ensure that all the $\text{NO}_2\text{-N}$ reduction is caused by
16 denitrification. The beakers were then filled to 200 mL with distilled water. pH was
17 adjusted to 7.8 ± 0.1 . DO was maintained between 5.8 and 7.0 mg L^{-1} . The detailed
18 experimental conditions were summarized in Table S3. Liquid samples were taken every
19 15 min over a period of 2.5 hrs and analyzed for $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$. Rates were
20 determined as the slopes of the substrates reduction over time via linear regression, and
21 expressed as $\text{mg N LDAS}^{-1} \text{ h}^{-1}$. DAS samples were prepared in duplicate.

22

23 **Analysis**

1 COD, NH₄-N, NO₂-N, NO₃-N, PO₄-P and SS were analyzed using a Hach DR/2400
2 spectrophotometer according to its standard operating procedures. BOD₅ was measured
3 with a Hach BODTrak instrument. TP and TN were determined with ascorbic method
4 and persulfate method, respectively.²⁸ pH was measured with a pH meter (Orion 920 A+,
5 Thermo). DO was monitored with a microprocessor oximeter (Oxi 325, WTW).

6

7 **RESULTS**

8 **Overall performance**

9 **Organic matters degradation:**

10 Although this study is focused on the nitrogen removal, reduction of OMs in terms of
11 BOD₅ and COD are highly related to nitrogen removal behavior. During period 1, both
12 the COD and BOD₅ degradation were not well achieved with average removal of 74%
13 and 82%, respectively (Figure S1). This is probably due to the insufficient oxygen supply,
14 indicated by the high effluent BOD₅ (average 192 mg L⁻¹) and low DO (< 0.8 mg/l) at the
15 end of A3. Air supply was accordingly increased at the beginning of period 2 (day 58) by
16 extending the aerobic phases to 250 min in total and increasing the airflow rate to 3.16
17 mL h⁻¹ L⁻¹ (Table S1). Nine days after the action, effluent SCOD and BOD₅ decreased
18 substantially with average of 223 and 45 mg L⁻¹, respectively. High COD and BOD₅
19 removal of 89% and 94% on average were obtained. The DO at the end of A3 was
20 increased up to 4.3 mg/l accordingly. A drop in removal efficiency on day 145 was
21 observed due to the air diffuser blockage, indicated by the visible decrease of air bubble
22 and low DO (< 1 mg L⁻¹) during aeration. The air flow rate was further increased to 4.42
23 mL h⁻¹ L⁻¹ in period 3. Good performance was recovered steadily and nearly complete

1 BOD₅ removal (average 97%) was obtained with the enhanced air supply from day 175
2 (Figure S1). However, the COD removal (89%) remained more or less the same as period
3 2 regardless of the further increase of air flow. The low effluent BOD₅ (21 mg L⁻¹) and
4 high residual SCOD (261 mg L⁻¹) suggested that there was significant part of inert SCOD
5 in the influent, which could not be biologically degraded²⁹. Consequently, the COD
6 removal could not be further increased by increasing the air supply. Another drop in
7 treatment efficiency was observed on day 220. The reason was identified as serious bed
8 clogging. Action (backwash) was thus taken and treatment performance had been
9 resumed very soon thereafter. Overall, a total aeration time of 250 min with air flow rate
10 of 3.16 mL h⁻¹ L⁻¹ seems to be adequate to obtain effective OMs removal under OLR of
11 0.32 kg COD m⁻²·d⁻¹.

12

13 **Nitrogen removal:**

14 Overall nitrogen removal performance is presented in Fig. 2. Nitrification in period 1 was
15 quite poor. Only 56% NH₄-N removal (Fig. 2a, solid circle) was obtained on average. It
16 was quickly increased to 85% with effluent NH₄-N of 51 mg L⁻¹ (on average) after the
17 enhancement of air supply in period 2 (Fig. 2b, solid diamond). Extreme nitrification was
18 recorded in period 3 with average NH₄-N removal of 97% and effluent NH₄-N of 12 mg
19 L⁻¹. Overall, a total aeration time of 250 min with air flow rate of 4.42 mL h⁻¹ L⁻¹ could
20 guarantee nearly complete nitrification under NLR of 46.7 g N m⁻²·d⁻¹ (Period 3). Similar
21 to the removal of OMs, diffuser blockage and bed clogging seriously decreased the
22 nitrification performance (Fig. 2a), which could be the major scale up issues. Diffuser
23 blockage has been reported by Nivala et al.,¹⁴ while bed clogging is common problem in

1 all CWs practice³⁰. Total suspended solids (TSS) accumulation and biofilm development
2 have been identified as the major causes for bed clogging,³⁰ which could be the main
3 reasons for diffuser blockage as well. Hence, proper design and operation strategy are
4 requested for further study.

5 TN removal was mainly dependent on nitrification and the availability of carbon
6 source for denitrification. During period 1, incomplete nitrification showed as the limiting
7 factor for TN elimination. The major N component in the effluent was NH₄-N (average
8 128 mg L⁻¹) (Fig. 2b, solid diamond). Effluent NO_x-N (NO₂-N and NO₃-N) was
9 neglectable (average 1.7 mg L⁻¹) (Fig. 2b, open circle and triangle). After effective
10 nitrification was achieved from period 2, carbon deficiency became the key limiting
11 factor for TN removal, and NO₃-N accounted for the major part of the effluent TN (Fig.
12 2b, triangle). It has been found that the influent COD/TN ratio controlled the NO_x-N
13 accumulation (NO_x-N/NH₄-N_{Oxi}) (Figure S2). When this ratio was around or below 5,
14 there was great chance of substantial NO_x-N accumulation. If the ratio was above 6, both
15 the NO₂-N and NO₃-N accumulation rarely exceeded 4%.

16 Overall, effective TN removal was achieved after sufficient oxygen (total aeration
17 time of 250 min with air flow rate of 4.42 mL h⁻¹ L⁻¹, Table S1) was supplied in the
18 proposed system. TN removal up to 97% with mean removal efficiency of 90% (from
19 528 mg L⁻¹ to 52 mg L⁻¹, NLR 46.7 g N m⁻²·d⁻¹) was obtained in period 3 (Fig. 2a). Even
20 under carbon limiting conditions (influent COD/N around 3), TN reduction was still
21 maintained above 80%. This performance is a substantial improvement in both treatment
22 efficiency and capacity (NLR) compared with the reported TN removal efficiency of 51%
23 under NLR of 6 g N m⁻²·d⁻¹¹² and 67% under NLR of 3.3 6 g N m⁻²·d⁻¹¹⁵ in the literature,

1 which properly reflected the effectiveness of the enhanced aeration and process design of
2 the proposed system.

3

4

[Fig. 2]

5

6 **Cyclic nitrogen profile**

7 A cyclic nitrogen profile is illustrated in Fig. 3. The nitrogen profile was quite different
8 with the typical profiles in BNR processes. Generally, $\text{NH}_4\text{-N}$ is oxidized in aerobic
9 phase. Accordingly, buildup of $\text{NO}_x\text{-N}$ is expected along with the decrease of $\text{NH}_4\text{-N}$.
10 The reduction of $\text{NO}_x\text{-N}$ (denitrification) can only be expected in the un-aerated phases ^{4,}
11 ¹⁸. However, no notable $\text{NO}_x\text{-N}$ buildup (circle and triangle) was observed throughout the
12 whole cycle in this study regardless of the substantial decrease of $\text{NH}_4\text{-N}$ (square) and the
13 high DO (red line) during the aerobic phases. This suggests that most of the oxidized N
14 was denitrified once produced, i.e. a very effective SND process in the AIS-IACW
15 system. The effective SND has a great impact on the process design. As stated in section
16 2.1, a high VER of 9 was adopted at the cost of a low HLR. This adoption was based on
17 the assumption that SND was not significant. In such case, the degree of pre-
18 denitrification determines the extent of the overall denitrification process. ¹⁸ However,
19 such a design rationale was confounded by the effective SND, because the major part of
20 the oxidized N had been denitrified already during the aeration periods and thus the pre-
21 denitrification stage lost its significance. For this reason, the treatment capacity of AIS-

1 IACW could be extended by increasing HLR, i.e. by adopting low VER without notable
2 decline of the overall nitrogen removal performance.

3

4 **[Fig. 3]**

5

6 **DISCUSSION**

7 **Controlling factors for SND**

8 The profiles of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ under aerobic condition are shown in Fig. S3.
9 Specific rates of nitrification, $\text{NO}_x\text{-N}$ production and denitrification during different
10 reaction phases are summarized in Table 2. The overall SND efficiencies are compared
11 with other studies in Table 3. Fig. S3 and Table 3 demonstrate that the SND performance
12 was controlled by carbon substrate. Under low COD/N ratio (6.3), substantial $\text{NO}_x\text{-N}$
13 accumulation (up to 22 mg L^{-1}) was observed along the $\text{NH}_4\text{-N}$ decrease (Fig. S3),
14 resulting in a relative low SND efficiency (72.9%, Table 3). With the increase of COD/N
15 ratio (9.4 and 13.7), $\text{NO}_x\text{-N}$ accumulation decreased (Fig. S3), and a higher SND
16 efficiency was achieved (80.8% and 89.4% respectively, Table 3). These results are
17 consistent with the previous studies (Table 3). It is believed that a high COD/N ratio can
18 promote SND efficiency mainly through three mechanisms: (1) to provide sufficient
19 reducing power for denitrification;^{36, 37} (2) to reduce the oxygen penetration depth under
20 certain oxygen supply, thus to create more micro anoxic zone for denitrification;³⁴ (3) to
21 lower the nitrification rate due to the competition for DO between nitrifiers and
22 heterotrophic organisms, therefore, to bring more balanced nitrification and

1 denitrification rates.^{31, 32, 34} In this case, the COD/N played its role mainly through the
2 mechanism (3), which is revealed by the decrease of nitrification rate along the increase
3 of COD/N (Table 2). Accordingly, a more balanced equilibrium between nitrification and
4 denitrification (higher $r_{\text{Deni}}/r_{\text{Nitri}}$ ratio) was obtained under high COD/N situation (Table 2).
5 This led to the less accumulation of $\text{NO}_x\text{-N}$ and a more effective SND (Fig. S3 and Table
6 3).

7

8

[Table 2]

9

10

[Table 3]

11

12 DO is another controlling factor for SND.^{34, 35} The main cause of SND is the
13 stratification of the biofilm/floc due to the oxygen diffusion limitation.³⁴ In the outer
14 layer of the biofilm/floc, nitrification takes place. DO decreases along the penetration
15 depth and finally micro anoxic zone exist inside the biofilm/floc, where denitrification
16 occurs. Obviously, the degree of SND relies on the size of the micro anoxic zone, which
17 decreases with the increase of the bulk DO.^{38, 39} That is why low DO favors SND (Table
18 3). To achieve a high SND efficiency, low DO ($0.5\text{-}1\text{ mg L}^{-1}$) is generally recommended
19 at the cost of a lower SND rate.^{20, 34} However, comparable SND efficiency was obtained
20 under much higher DO ($4.7\text{-}6.3\text{ mg L}^{-1}$) in this study. A high oxygen transfer resistance
21 in the biofilm system (this study) could be the explanation. Under the same bulk DO
22 concentration, thick biofilm/large floc could promote the formation of a larger anoxic

1 zone (Table 3).^{32, 36} The relative high substrate loading rate applied in this study might
2 result in a thick biofilm (indicated by the clogging phenomenon),⁴⁰ which allowed the
3 system to achieve the effective SND under high DO. This is of great significance for
4 practice, since high SND efficiency and high SND rate can be obtained simultaneously.

5 It's also very interesting to explore the SND characteristics during different reaction
6 phases (Table 2). Nitrification rate of the first two hours was much higher than that of the
7 later phases, which induced the most serious imbalance between nitrification and
8 denitrification ($r_{\text{Deni}}/r_{\text{Nitri}}$, Table 2) and thus high $\text{NO}_x\text{-N}$ accumulation (Fig. S3).
9 Thereafter, nitrification rate decreased markedly and a more balanced equilibrium was
10 established and maintained in phase 2. Denitrification rate could even exceed the
11 nitrification rate in this phase, such as at 11-18 hrs for COD/N of 6.3, 7-18 hrs for
12 COD/N of 9.4 and the whole phase 2 for COD/N of 13.7 (Table 2). This suggests that the
13 organic substrate can be preserved for a long period to support SND. Third et al.³⁴ and
14 Zeng et al.³⁵ attribute this phenomenon to the storage of soluble organic substrate in the
15 form of polyhydroxybutyrate (PHB), which is degraded more slowly than the soluble
16 form and thus preserves reducing power for denitrification. These characteristics provide
17 good evidence that a more delicate aeration scheme should be considered to bring achieve
18 more efficient SND. For example, air flow rate in the first aerobic phase can be reduced
19 to bring more balanced nitrification and denitrification rates.

20

21 **Partial nitrification and SND via nitrite**

22 During the SND batch tests, transient nitrite buildup was observed especially with low
23 COD/N ratio (Figure S3). It implies that the rate of ammonia oxidization is faster than the

1 nitrite oxidization, i.e. partial nitrification might be the major conversion way of
2 ammonia oxidization. The degree of partial nitrification is usually evaluated by nitrite
3 accumulation ratio (NAR, effluent $\text{NO}_2\text{-N}/\text{NO}_x\text{-N}$).⁴¹ However, this is not adequate if
4 SND is significant because the major part of the $\text{NO}_2\text{-N}$ could be reduced via SND
5 already. A comparison of r_{AOB} , r_{NOB} and $r_{\text{Deni-NO}_2}$ can give a better assessment of the
6 nitrogen conversion pathway (Table 4). The results revealed that nitrification
7 ($r_{\text{AOB}}=14.67\pm 1.24$ mg N LDAS⁻¹ h⁻¹) was much faster than nitrification ($r_{\text{NOB}}=4.47\pm 0.82$
8 mg N LDAS⁻¹ h⁻¹). If there is no nitrite reduction via SND, substantial nitrite buildup
9 after complete nitrification could be expected. However, this phenomenon was not
10 observed in the aerobic batch tests (Fig. S3). This was because that the denitrification rate
11 via nitrite was comparable to nitrification (Table 4), so that the major part of nitrite was
12 denitrified once produced. The percentage of nitrite denitrified via SND can be estimated
13 by the ratio of $r_{\text{Deni-NO}_2}/(r_{\text{NOB}}+r_{\text{Deni-NO}_2})$, and the result is illustrated in Fig. 4. It shows that
14 65% of nitrite was removed through SND under high COD/N (14) condition. Even with
15 low COD/N of 6, the proportion of nitrite reduction via SND was still above that of
16 nitrification. Hence, a conclusion that partial nitrification and SND via nitrite (instead of
17 nitrate) is the main nitrogen conversion pathway in the AIS-IACW can be drawn
18 accordingly.

19 Typically the rate of nitrification is faster than the rate of nitrification and nitrite is rarely
20 accumulated during the BNR processes unless specific process control is adopted. The
21 advantages of BNR via nitrite, such as lower oxygen consumption, higher denitrification
22 rate and particularly significant reduction (40%) of COD demand, aroused persistent
23 efforts to achieve and maintain stable partial nitrification.²² Yoo et al.²⁵ summarized the

1 controlling factors for partial nitrification, which include high free ammonia (FA); high
2 temperature (30-36 °C); lag-time during the transition from anoxic to aerobic; low DO
3 and free hydroxylamine (FH). They claimed to achieve SND via nitrite by controlling DO
4 concentration, DO increase rate, length of aeration and FA level. In this study, however,
5 partial nitrification has been achieved without any specific controlling actions listed
6 above. On the contrary, the operational condition is actually adverse to partial
7 nitrification with the high DO (3-6 mg L⁻¹). Instead, the partial nitrification that occurred
8 in this study might be an outcome of long-term competition between nitrification and SND
9 via nitrite. Because of the effective SND via nitrite, NOB consistently lost its substrate
10 (nitrite) and was out-competed after long-term operation. By keeping this in mind, it
11 seems that effective SND via nitrite is the cause of partial nitrification, rather than the
12 result of partial nitrification.

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[Table 4]

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[Fig. 4]

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20 Department of Agriculture, Fisheries and Food under the Research Stimulus Fund
21 (project no. RSF 07-529).

22

1 **Supporting information**

2 System operation periods and conditions; Initial conditions for the SND efficiency tests
3 and nitrogen conversion rates determination; Overall performance in organic matter
4 removal; Effects of influent COD/TN ratio on NO_x-N accumulation; Nitrogen profiles
5 under aerobic tests with different initial COD/N ratio. These materials are available free
6 of charge via the internet at <http://pubs.acs.org>.

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8 **References**

- 9 (1) Verhoeven, J. T. A.; Meuleman, A. F. M. Wetlands for wastewater treatment:
10 opportunities and limitations. *Ecol. Eng.* **1999**, 12 (1-2), 5-12.
- 11 (2) Sundaravadivel, M.; Vigneswaran, S. Constructed wetlands for wastewater treatment.
12 *Crit. Rev. Env. Sci. Technol.* **2001**, 31 (4), 351-409.
- 13 (3) Vymazal, J. Removal of nutrients in various types of constructed wetlands. *Sci. Total*
14 *Environ.* **2007**, 380 (1-3), 48-65.
- 15 (4) Wiesmann, U.; Choi, I. S.; Dombrowski, E. M. *Fundamentals of biological*
16 *wastewater treatment*. Wiley-VCH: Weinheim, Germany, **2007**.
- 17 (5) Zhao, Y. Q.; Babatunde, A. O.; Zhao, X. H.; Li, W. C. Development of alum sludge-
18 based constructed wetland: An innovative and cost-effective system for wastewater
19 treatment. *J. Environ. Sci. Health A*, **2009**, 44 (8), 827-832.
- 20 (6) Zhao, Y. Q.; Babatunde, A. O.; Hu, Y. S.; Kumar, J. L. G. Zhao, X. H. Pilot field-
21 scale demonstration of a novel alum sludge-based constructed wetland system for
22 enhanced wastewater treatment. *Process Biochem.* **2011**, 46 (1), 278-283.

- 1 (7) Yang, Y.; Tomlinson, D.; Kennedy, S.; Zhao, Y. Q. Dewatered alum sludge: A
2 potential adsorbent for phosphorus removal, *Water Sci. Technol.* **2006**, 54 (5), 207-213.
- 3 (8) Mortula, M. M.; Gagnon, G. A. Alum residuals as a low technology for phosphorus
4 removal from aquaculture processing water. *Aquacult. Eng.* **2007**, 36 (3), 233-238.
- 5 (9) Reed, S. C.; Brown, D. Subsurface flow wetlands - a performance evaluation. *Water*
6 *Env. Res.* **1995**, 67 (2), 244-248.
- 7 (10) Green, M.; Safrai, I.; Agami, M. Constructed wetlands for river reclamation-
8 Experimental design and start-up. *Bioresour. Technol.* **1996**, 55 (2), 157-162.
- 9 (11) Tanner, C. C.; Kadlec, R. H. Oxygen flux implications of observed nitrogen removal
10 rates in subsurface-flow treatment wetlands. *Water Sci. Technol.* **2003**, 48 (5), 191-198.
- 11 (12) Cottingham, P. D.; Davies, T. H.; Hart, B. T. Aeration to promote nitrification in
12 constructed wetlands. *Environ. Technol.* **1999**, 20 (1), 69-75.
- 13 (13) Ouellet-Plamondon, C.; Chazarenc, F.; Comeau, Y.; Brisson, J. Artificial aeration to
14 increase pollutant removal efficiency of constructed wetlands in cold climate. *Ecol. Eng.*
15 **2006**, 27 (3), 258-264.
- 16 (14) Nivala, J.; Hoos, M. B.; Cross, C.; Wallace, S.; Parkin, G. Treatment of landfill
17 leachate using an aerated, horizontal subsurface-flow constructed wetland. *Sci. Tot.*
18 *Environ.* **2007**, 380 (1-3), 19-27.
- 19 (15) Ong, S.-A.; Uchiyama, K.; Inadama, D.; Yamagiwa, K. Simultaneous removal of
20 color, organic compounds and nutrients in azo dye-containing wastewater using up-flow
21 constructed wetland. *J. Hazard. Mater.* **2009**, 165(1-3), 696-703.

- 1 (16) Maltais-Landry, G.; Maranger, R.; Brisson, J.; Chazarenc, F. Nitrogen
2 transformations and retention in planted and artificially aerated constructed wetlands.
3 *Water Res.* **2009**, 43 (2), 535-545.
- 4 (17) Zhang, L. Y.; Zhang, L.; Liu, Y. D.; Shen, Y. W.; Liu, H.; Xiong, Y. Effect of
5 limited artificial aeration on constructed wetland treatment of domestic wastewater.
6 *Desalination.* **2010**, 250 (3), 915–920.
- 7 (18) Artan, N.; Orhon, D. *Mechanisms and design of sequencing batch reactors for*
8 *nutrient removal.* IWA Scientific and Technical Report no. 19, IWA Publishers: London,
9 **2005**.
- 10 (19) Çeçen, F. Investigation of partial and full nitrification characteristics of fertilizer
11 wastewaters in a submerged biofilm reactor. *Water Sci. Technol.* **1996**, 34 (11), 77-85.
- 12 (20) Münch, E. V.; Lant, P.; Keller, J. Simultaneous nitrification and denitrification in
13 bench-scale sequencing batch reactors. *Water Res.* **1996**, 30 (2), 277–284.
- 14 (21) Helmer, G.; Kunst, S. Simultaneous nitrification/denitrification in an aerobic biofilm
15 system. *Water Sci. Technol.* **1998**, 37 (4-5), 183-187.
- 16 (22) Peng, Y. Z.; Zhu, G. B. Biological nitrogen removal with nitrification and
17 denitrification via nitrite pathway. *Appl. Environ. Microbiol.* **2006**, 73 (1), 15-26.
- 18 (23) Babatunde, A. O.; Zhao, Y. Q.; Burke, A. M.; Morris, M. A.; Hanrahan, J. P.
19 Characterization of aluminium-based water treatment residual for potential phosphorus
20 removal in engineered wetlands. *Environ. Pollut.* **2009**, 157 (10), 2830-2836.
- 21 (24) Kadlec, R. H.; Wallace, S. D. *Treatment Wetlands*, 2nd, ed.; CRC Press: Boca Raton,
22 Florida, USA, **2008**; pp 285.

- 1 (25) Yoo, H.; Ahn, K. H.; Lee, H. J.; Lee, K. H.; Kwak, Y. J.; Song, K. G. Nitrogen
2 removal from synthetic wastewater by simultaneous nitrification and denitrification (SND)
3 via nitrite in an intermittently aerated reactor. *Water Res.* **1999**, 33 (1), 145-154.
- 4 (26) Windey, K.; De Bo, I.; Verstraete, W. Oxygen-limited autotrophic nitrification–
5 denitrification (OLAND) in a rotating biological contactor treating high-salinity
6 wastewater. *Water Res.* **2005**, 39 (18), 4512-4520.
- 7 (27) Ginestet, P.; Audic, J. M.; Urbain, V.; Block, J. C. Estimation of nitrifying bacterial
8 activities by measuring oxygen uptake in the presence of the metabolic inhibitors
9 allylthiourea and azide. *Appl. Environ. Microb.* **1998**, 64 (6), 2266–2268.
- 10 (28) Clesceri, L. S.; Greenberg, A. E.; Eaton, A. D. *Standard methods for the*
11 *examination of water and wastewater*, 20th, ed.; American Public Health Association:
12 Washington, DC., **1998**.
- 13 (29) Hu, Y.S.; Kumar, J.L.G.; Akintunde, A.O.; Zhao, X.H.; Zhao, Y.Q. Effects of
14 livestock wastewater variety and disinfectants on the performance of constructed
15 wetlands in organic matters and nitrogen removal, *Environ Sci. Pollut. R.* **2011**, 18(8),
16 1414-1421.
- 17 (30) Zhao, Y.Q.; Sun, G.; Allen, S.J. Anti-sized reed bed system for animal wastewater
18 treatment: a comparative study. *Water Res.* **2004**, 38(12), 2907-2917.
- 19 (31) Xia, S. Q.; Li, J. Y.; Wang, R. C. Nitrogen removal performance and microbial
20 community structure dynamics response to carbon nitrogen ratio in a compact suspended
21 carrier biofilm reactor. *Ecol. Eng.* **2008**, 32 (3), 256-262.

- 1 (32) Wang, J. F.; Wang, X.; Zhao, Z. G.; Li, J. W. Organics and nitrogen removal and
2 sludge stability in aerobic granular sludge membrane bioreactor. *Appl. Microbiol.*
3 *Biotechnol.* **2008**, 79 (4), 679-685.
- 4 (33) Meng, Q. J.; Yang, F. L.; Liu, L. F.; Meng, F. G. Effects of COD/N ratio and DO
5 concentration on simultaneous nitrification and denitrification in an airlift internal
6 circulation membrane bioreactor, *J. Environ. Sci.* **2008**, 20 (8), 933-939.
- 7 (34) Third, K. A.; Burnett, N.; Cord-Ruwisch, R. Simultaneous nitrification and
8 denitrification using stored substrate (PHB) as the electron donor in an SBR. *Biotechnol.*
9 *Bioeng.* 2003, 83 (6), 706-720.
- 10 (35) Zeng, R. J.; Lemaire, R.; Yuan, Z. G.; Keller, J. Simultaneous nitrification and
11 denitrification and phosphorus removal in a lab-scale sequencing batch reactor.
12 *Biotechnol. Bioeng.* **2003**, 84 (2), 170-178.
- 13 (36) Pochana, K.; Keller, J. Study of factors affecting simultaneous nitrification and
14 denitrification (SND). *Water Sci. Technol.* **1999**, 39 (6), 61-68.
- 15 (37) Chiu, Y. -C.; Lee, L. -L.; Chang, C. -N.; Chao, A. C. Control of carbon and
16 ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch
17 bioreactor. *Int. Biodeterior. Biodegr.* **2007**, 59 (1), 1-7.
- 18 (38) de Kreuk, M. K.; Heijnen, J. J.; van Loosdrecht, M. C. M. Simultaneous COD,
19 nitrogen and phosphate removal by aerobic granular sludge. *Biotechnol. Bioeng.* **2005**, 90
20 (6), 761-769.

- 1 (39) Mosquera-Corral, A.; de Kreuk, M. K.; Heijnen, J. J.; van Loosdrecht, M. C. M.
2 Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor,
3 *Water Res.* **2005**, 39 (12), 2676-2686.
- 4 (40) Peyton, B. M. Effects of shear stress and substrate loading rate on *Pseudomonas*
5 *aeruginosa* biofilm thickness and density. *Water Res.* **1996**, 30 (1), 29-36.
- 6 (41) Kim, D. J.; Lee, D. I.; Keller, J. Effect of temperature and free ammonia on
7 nitrification and nitrite accumulation in landfill leachate and analysis of its nitrifying
8 bacterial community by FISH. *Bioresour. Technol.* **2006**, 97 (3), 459-468.

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Tables

Table 1 Influent wastewater characteristic (Average \pm SD)

	Period 1 (0-58 days)	Period 2 (59-145 Days)	Period 3 (146-280 Days)
COD (mg L ⁻¹)	4007 \pm 2337	3644 \pm 2738	2823 \pm 1060
TN (mg L ⁻¹)	368 \pm 102	431 \pm 97	528 \pm 142
NH ₄ -N (mg L ⁻¹)	312 \pm 106	358 \pm 111	435 \pm 136
TP (mg L ⁻¹)	27 \pm 15	35 \pm 25	56 \pm 26
SS (mg L ⁻¹)	2141 \pm 1612	1979 \pm 2651	941 \pm 954
pH	7.9 \pm 0.2	8.0 \pm 0.4	7.7 \pm 0.4
OLR (kg COD m ⁻² ·d ⁻¹)	0.35	0.32	0.25
NLR(g N m ⁻² ·d ⁻¹)	32.5	38.1	46.7
PLR(g P m ⁻² ·d ⁻¹)	2.4	3.1	4.9
COD/TN	11	9	5

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2 Table 2 Rate of nitrification, NO_x-N production and denitrification during SND with
3 different initial COD/N ratio (Unit: mg N LDAS⁻¹·h⁻¹)

COD/N	Phase 1 (0-2 hrs)			Phase 2 (3-18 hrs)			Phase 3 (24-35 hrs)		
	6.3	9.4	13.7	6.3*	9.4	13.7	6.3	9.4	13.7
r _{Nitri}	22.3	7.7	9.0	8.1	2.5	2.0	4.2	1.5	1.6
r _{NOx}	19.8	6.8	4.9	2.1	0.5	-0.2	2.3	0.6	0.6
r _{Deni}	2.5	0.9	4.1	6.1	2.1	2.2	1.9	0.9	1.0
r _{Deni} /r _{Nitri}	0.11	0.12	0.46	0.75	0.81	1.10	0.44	0.61	0.63

4 *Phase 2 for COD/N=6.3 is 3-9 hrs.

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Table 3 Comparative evaluation of SND performance

System	COD/N	DO (mg L ⁻¹)	Particle size (mm)	E _{SND} (%)	Controlling factors	Reference
Biofilm (AS-IACW)	6.3			72.9		This study
	9.4	4.7-6.3		80.8	COD/N ratio	
	13.7			89.4		
Biofilm (Suspended carrier)	3			83.3		31
	5	1.5-2.5		84.5	COD/N ratio	
	10			87.2		
Biofilm (Aerobic granular)			<0.18	≈0		32
	5.8	2-3	0.18-0.45	19.1	Particle size	
			0.45-0.9	31.9		
			>0.9	49.3		
Suspension (AIC-MBR)	4.77			53.6		33
	10.04	1		98.2	COD/N ratio	
	15.11			97.4		
Suspension (SBR)		0.5		78		34
	10.4	1.0		61	DO	
		1.5		55		
		5.0		31		
Suspension (SBR)		0.5		98		35
	10	1.5		62	DO	
		2.5		51		
Suspension (SBR)	10.4	0.3-2.5	0.04-0.05	21	Particle size	36
			0.08	52		

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2 Table 4 Rate of nitrification (r_{AOB}), nitrification (r_{NOB}) and denitrification via NO_2 -N (r_{Deni-}
3 NO_2) under aerobic condition (Unit: mg N LDAS⁻¹·h⁻¹)

	No.1	R ²	No.2	R ²	Mean	S.D.
r_{AOB}	13.79	0.938	15.55	0.966	14.67	1.24
r_{NOB}	5.05	0.788	3.89	0.807	4.47	0.82
$r_{Deni-NO_2}$, COD/N 6	4.66	0.967	4.49	0.968	4.57	0.12
$r_{Deni-NO_2}$, COD/N 14	8.11	0.941	8.18	0.969	8.15	0.05

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Figure caption:

Fig. 1 Laboratory alum sludge-based IACW (a) System set-up (b) Aeration and feed scheme within a cycle

Fig. 2 Overall performance of nitrogen removal (a) Influent $\text{NH}_4\text{-N/TN}$ and $\text{NH}_4\text{-N/TN}$ removal (b) Effluent N and influent COD/TN ratio

Fig. 3 Cyclic nitrogen and DO profiles (Influent COD/TN=8. Data: An1/A1 – average of top, middle and bottom of the reactor; others – middle of the reactor)

Fig. 4 Nitrogen conversion pathway determined by COD/N ratio during SND

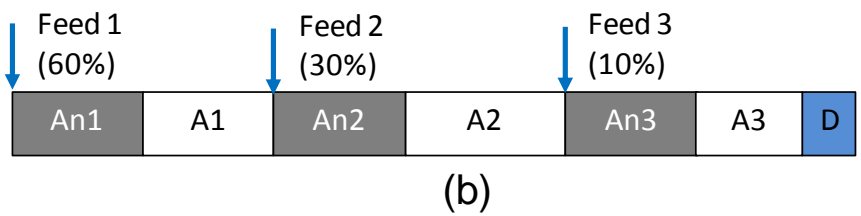
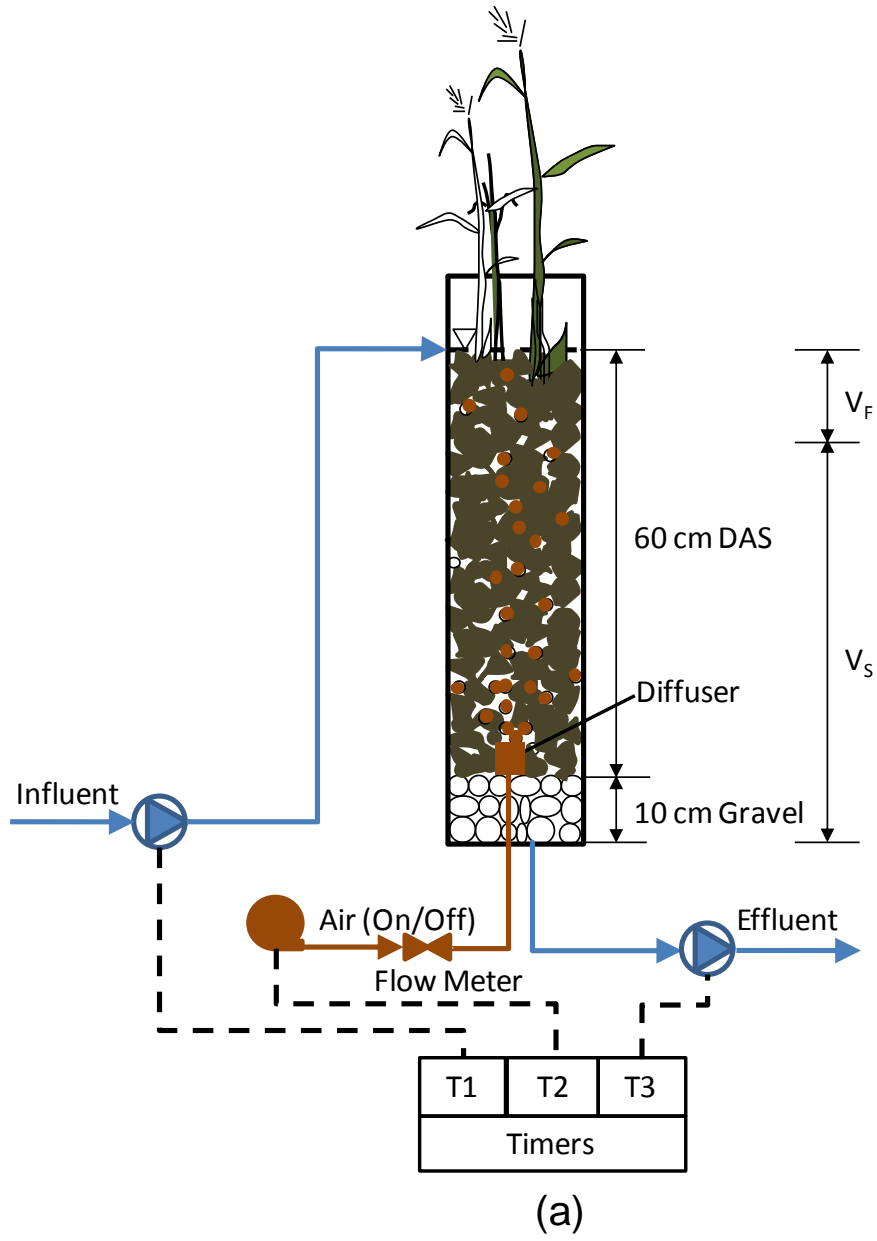


Fig. 1

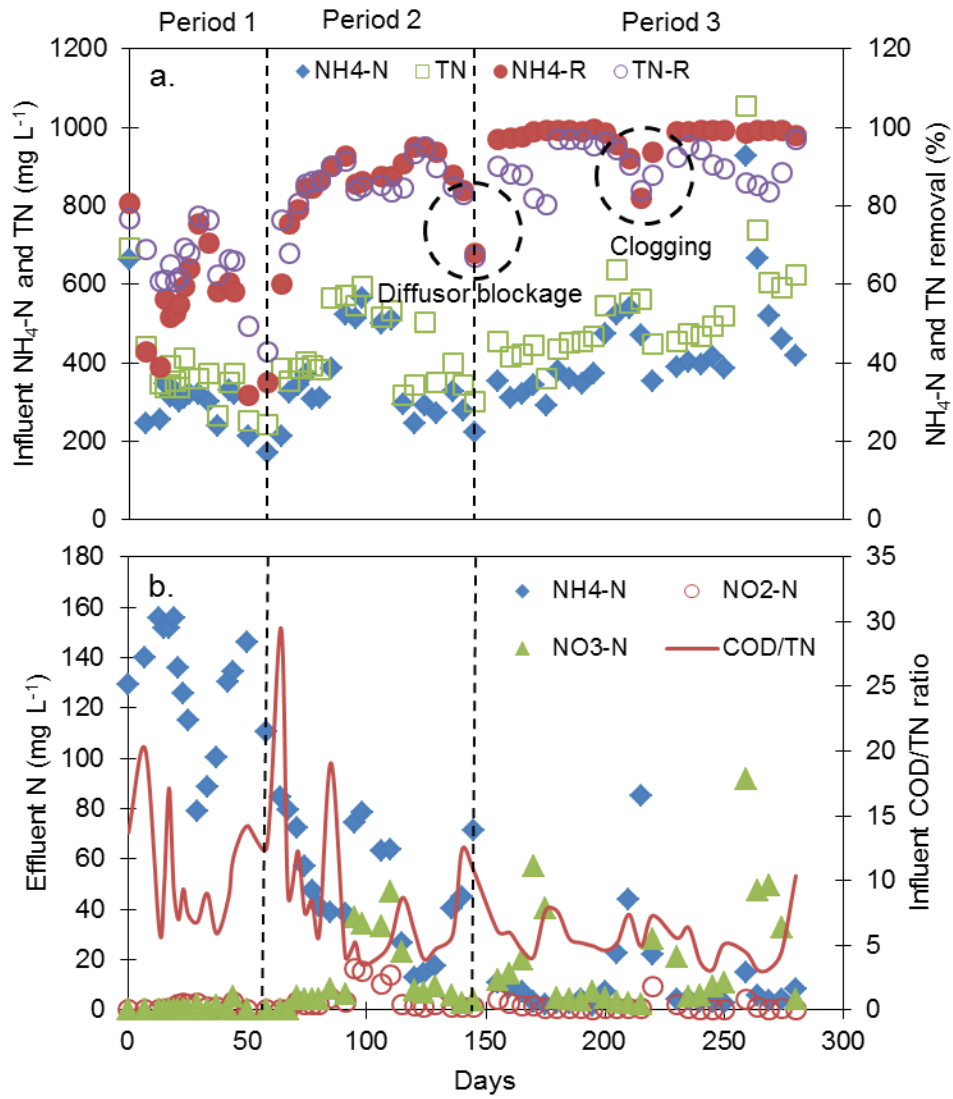


Fig. 2

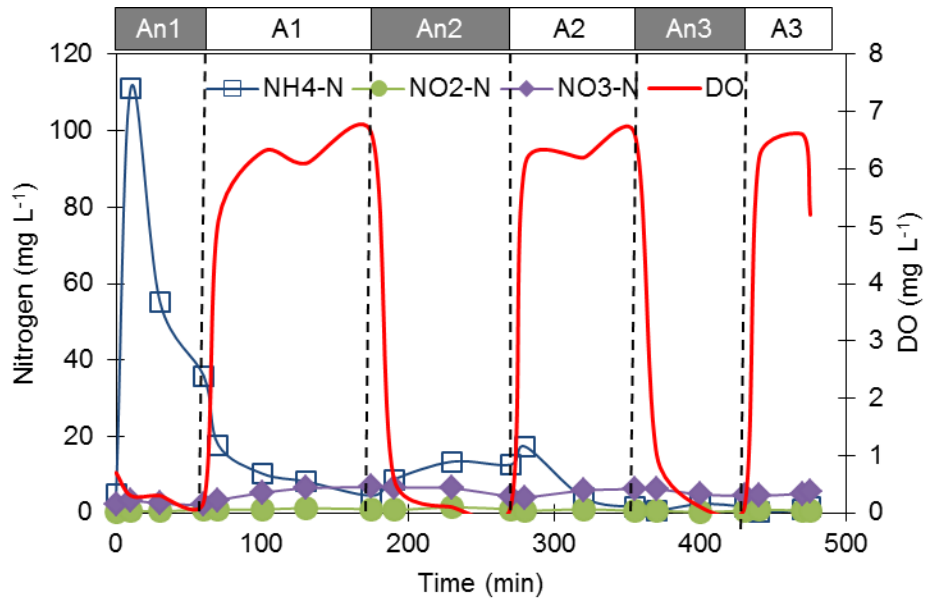


Fig. 3

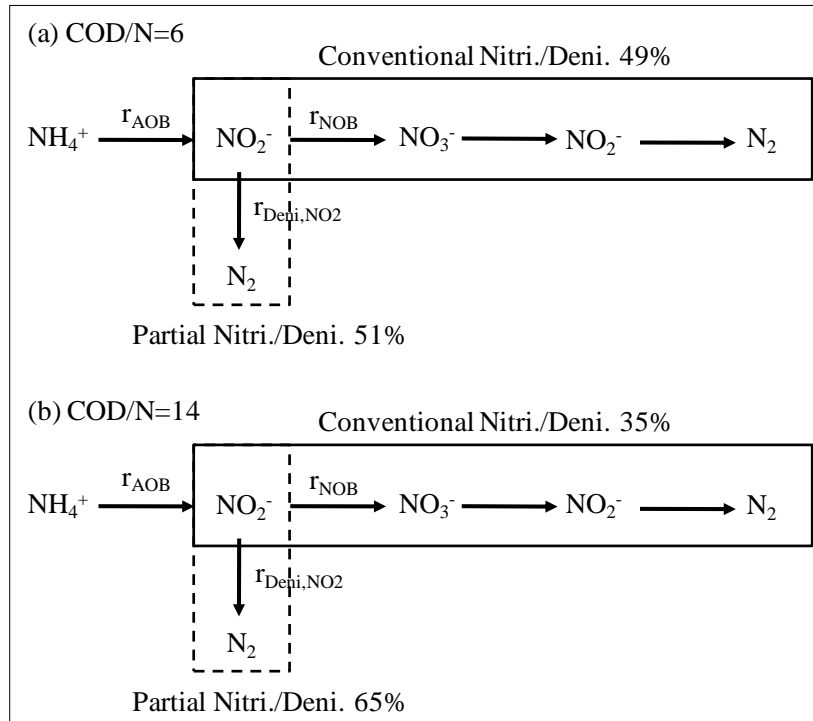


Fig. 4