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Nitrogen removal from opto-electronic wastewater using the simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) process in sequencing batch reactor

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ABSTRACT

Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) system was applied to treat the opto-electronic industrial wastewater in a 2.5 L sequencing batch reactor. The characteristics of wastewater were low C/N ratio (~0.2) with 100 mg-COD/L and 567 mgNH_4^+—N/L. The experiment was carried out over 8 months in six different stages, where nitrogen loading rate was gradually increased from 16 g-N/m^3 d in stage I to 230 g-N/m^3 d in stage VI. The COD and nitrogen removal rates by the SNAD system reached to 28 g COD/m^3 d and 197 g NH_4^+—N/m^3 d, respectively in stage VI. These results showed that the SNAD system is suitable to treat wastewater containing high nitrogen pollutants with low COD level. Presence of ammonium oxidizing bacteria and anammox bacteria were confirmed by polymerase chain reaction (PCR). PCR results also indicated that *Candidatus* Kuenenia stuttgartiensis may be one of the dominant species in the reactor.

1. Introduction

Nitrogen removal from wastewater is of extremely important to protect water resources and many countries strive to reduce the emissions of nitrogen compounds to the surface waters. It is necessary to treat nitrogen rich wastewater before discharging it into the environment. Conventionally, biological nitrogen removal is achieved by nitrification followed by denitrification process in two separate reactors. In this process, first ammonia is oxidized to nitrite (NO_2^-) and then to nitrate (NO_3^-) by autotrophic nitrifiers with oxygen as the electron acceptor in aerobic condition, then NO_2^- or NO_3^- gets converted to gaseous N_2 by heterotrophic microorganisms using organic matter as carbon source in anoxic condition. However, conventional nitrification and denitrification process requires an amount of biodegradable and inorganic carbonate source. High energy consumption is another disadvantage of this process. The concept of nitrogen removal model has been changed since the confirmation of the anaerobic ammonium oxidation (anammox) process (Mulder et al., 1995), where autotrophic oxidation process converts ammonia to N_2 using nitrite as the electron acceptor. Anammox is recognized as a cost-effective and sustainable technology for biological nitrogen removal (Siegrist et al., 2008). The stoichiometry of this process is given in Eq. (1), which shows that anammox uses CO_2 as its carbon source to produce biomass ($CH_2O_{0.5}N_{0.15}$) and that NO_2^- not only functions as an electron acceptor for NH_4^+ oxidation, but also as an electron donor for the reduction of CO_2 (Kuenen, 2008):

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad (1)$$

Usually, NO_2^- is absent in the wastewater, but can be generated from partial nitrification. Therefore, anammox is generally combined with other technology such as SHARON (single reactor high activity ammonia removal over nitrite) process, a technology, which control further oxidation of NO_2^-—NO_3^- (Hellinga et al., 1998). The combination of SHARON with anammox process needs two reactors in a sequence to achieve nitrogen removal. The effluent, a 50:50 mixture of NH_4^+ and NO_2^- from first reactor (SHARON) is used as a feed for second (anammox) reactor (Siegrist et al., 1998). Alternatively, several researchers made an attempt to improve the reactor configurations and coupling anammox with other innovative processes. CANON (completely autotrophic nitrogen removal over nitrite) is one such process where aerobic ammonium oxidizing bacteria (AOB) and anammox bacteria can be cultivated in one reactor (Sliekers et al., 2002; Third et al., 2001). However, this process is suitable for the wastewaters containing high ammonium devoid of organic carbon, which could not be removed in this process. Some similar processes also have been developed and studied successfully for nitrogen removal from wastewater, such as: OLAND

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(oxygen-limited autotrophic nitrification–denitrification) (Pynaert et al., 2004) and SNAP (single-stage nitrogen removal using anammox and partial nitrification) (Furukawa et al., 2005).

Simultaneous partial nitrification, anammox and denitrification (SNAD) process was recently developed and successfully used for removal of nitrogen along with organic carbon in a single reactor (Chen et al., 2009; Wang et al., 2010; Lan et al., 2011).

Though, anammox process had been successfully applied for treating creature wastewater, such as urban wastewater (Van Loosdrecht et al., 2004), livestock manure digester liquor (Hwang et al., 2005; Yamamoto et al., 2011), sludge of municipal wastewater treatment (Van der Star et al., 2007), only few studies reported the application of this technology for treating industrial wastewater (Tokutomi et al., 2011). The industrial wastewaters are difficult to treat by this process as they lack nutrients and trace elements necessary for the proper growth of anammox bacteria. Also, most nitrifying and anammox bacteria are chemoautotrophic and a deficit of inorganic carbon could result in a low efficiency of the process (Tokutomi et al., 2010; Kimura et al., 2011).

Therefore, in this study we evaluated the possibility of using the SNAD system to treat opto-electronic industrial wastewater having low C/N ratio in a lab-scale (2.5 L) sequencing batch reactor (SBR).

2. Methods

2.1. Sequencing batch reactor (SBR) and operating condition

This study was carried out in a lab-scale SBR with working volume of 2.5 L. Fig. 1 shows the schematic diagram of reactor. The influent and effluent were introduced using a peristaltic pump. Incubator was used to provide a constant temperature and keep the bacteria away from inhibition of light. During the feeding and reaction stages, a complete mixing inside the SBR was ensured by mixing the reactor content via a stirrer at a suitable rotation rate (~125 rpm). The process parameters such as pH and dissolved oxygen (DO) were monitored online using pH and DO meter, respectively. The DO control system composed of a DO meter, air flow valve and PID controller to maintain DO level at ~0.1 mg/L throughout the experiment.

The reactor was operated in cycles of 24 h. Each cycle had 23.4 h for feeding and reaction, 0.35 h for settling and 0.25 h for withdrawing. The feeding period was over 12 h. The fed batch strategy was adopted in order to avoid a shock loading. During the whole experimental study, the minimum liquid volume of the reactor was 1.5 L and at the end of filling period, the maximum volume was 2.5 L.

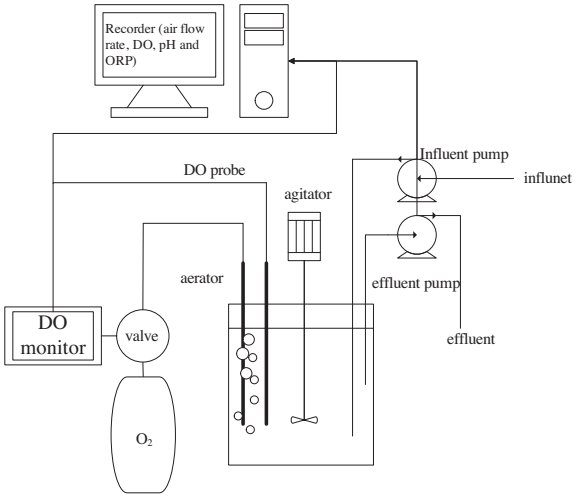

Fig. 1. Schematic diagram of SBR used in the study.

The reactor operation was carried out over six stages (I–VI) with varying nitrogen loading rates (NLR) from 16 to 230 g/m^3 d) either by reducing the hydraulic retention time (HRT) and/or diluting the wastewater with deionized water. The operating temperature was maintained at 37 °C during stages I and II, subsequently the temperature was reduced to 25 °C (from day 87 to the end of the experiment).

2.2. Seed sludge and feeding media

The reactor was inoculated with SNAD seed sludge collected from a full-scale landfill-leachate treatment plant in Taiwan. The presence of anammox bacteria, nitrosomonas-like microorganisms and denitrifiers were verified by the fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) techniques (Wang et al., 2010). The SBR was fed with the wastewater collected from opto-electronic industry located at Tainan, Taiwan. The wastewaters so collected were stored in refrigerator at 4 °C until used. The wastewater was characterized and its characteristics are shown in Table 1, which suggest that it has low C/N ratio (~0.2). As the wastewater used in our study is an effluent mix of several unit processes including a little domestic wastewater, such a low C/N ratio could be anticipated. Table 1 also shows that the wastewater is highly alkaline in nature (pH, 9.1) and the major portion of nitrogen present in the wastewater is inorganic in nature (more than 99%). As opto-electronic industrial wastewater originates from ultrapure water and pure chemicals it lacks nutrients and trace elements necessary for the proper growth of anammox bacteria (Sliekers et al., 2002). Therefore, in this study nutrients and trace elements were supplemented to wastewater for the successful growth of bacteria. In literature, many studies also supply additional nutrient or trace elements to the influent feed for biological treatment (Lei et al., 2010; Tokutomi et al., 2011). Calcium and other polyvalent cations are important for bioflocculation and granulation. The major element of the nutrient is calcium in this study. Graaff et al. (2011) showed that the concentration of calcium was 81 mg/L (or 300 mg/L of $CaCl_2{\cdot}2H_2O$) can obtain sufficient granulation of anammox bacteria. To help the bacteria aggregate, we also supply the additional mineral medium and trace elements. As mentioned trace elements are necessary for the proper growth of anammox bacteria, it was added (1 ml/L) into the mineral medium from the stock solution of trace elements (Table 2). The pH of the wastewater was adjusted to 7.8–8.0 (optimum pH for the anammox bacteria) by 1 M HCl before feeding. Though this wastewater contains high alkalinity, which is due to the presence of high [OH^-], the carbonate or bicarbonate concentration was very low. Therefore, it is necessary to add $NaHCO_3$ in the wastewater to increase the concentration of inorganic carbon.

Table 1
Main characteristics of raw opto-electronic industrial wastewater.

Parameter	Value[a]	n[b]
COD	100 ± 28	3
TKN	572 ± 6.6	3
NH_4^+-N	567 ± 5.8	3
NO_2^--N	–	3
NO_3^--N	7 ± 5.5	3
PO_4^{3-}-P	0.7 ± 0.7	3
pH	9.4 ± 0.1	3
Alkalinity as $CaCO_3$	1260 ± 208	3

[a] All units are in mg/L, except pH.
[b] The number of times wastewater sample analyzed and collected from the industry.

Table 2
Compositions of mineral medium and trace elements supplemented to the wastewater.

Composition of mineral medium		Composition of trace elements	
Component	Concentration[a]	Component	Concentration[a]
KH_2PO_4	25	EDTA	1500
$CaCl_2{\cdot}2H_2O$	300	$ZnSO_4$ $7H_2O$	430
$MgSO_4{\cdot}7H_2O$	200	$CoCl_2$ $6H_2O$	240
$FeSO_4$	6.25	$MnCl_2$ $4H_2O$	990
EDTA	6.25	$CuSO_4$ $5H_2O$	250
$KHCO_3$	1250	$NaMoO_4$ $2H_2O$	220
Trace element	1 ml/L	$NiCl_2$ $2H_2O$	190
		$NaSeO_4$ $10H_2O$	210
		H_3BO_4	14

[a] All units in the table are in mg/L, except trace element.

Also, the anammox and nitrifying bacteria are chemoautotrophic and use CO_2 as the main carbon source, therefore the alkalinity in the reactor was kept at ~1000 mg/L as $CaCO_3$ by adding $NaHCO_3$.

2.3. DNA extraction and polymerase chain reaction (PCR) reaction

To confirm the activities of nitrifiers, anammox and denitrifiers in the SNAD seed sludge PCR analysis of the total genomic DNA was carried out. The total genomic DNA of sample was extracted by using Power Soil DNA Isolation Kit (MO BIO Laboratories, USA). The DNA concentration was determined on a photometer ASP-3700 (ACTGene, USA). PCR reaction was performed in a 96 well Gradient Palm–Cycler (Corbett Research Pty Ltd., Austria). Each reaction was performed in a 25 μL volume containing 1 μL of DNA template (average 30 ng), 1 μL of each primer (10 μM), 9.5 μL of sterile water and 12.5 μL of 2X Taq PCR Master Mix (Genomics BioSd & Tech, Taiwan). Primer set for AOB was amoA-1F with amoA-2R (Rotthauwe et al., 1997), for nitrite oxidizing bacteria (NOB) was nirS-1F/nirS-6R (Braker et al., 1998), for denitrifying bacteria was cnorB-2F/cnorB-6R (Braker and Tiedje, 2003), for anammox bacteria were Brod541F/Amx820R (Penton et al., 2006; Schmid et al., 2000) and AnnirS379F/AnnirS821R (Li et al., 2011). To target specific species of anammox bacteria, primer set KS-qF3/KS-qR3 was used for *Candidatus* Kuenenia stuttgartiensis (KS), while BAqF/BAqR was used for *Candidatus* Brocadia anammoxidans (BA).

The cycling parameters were 3 min at 95 °C and 35 cycles of 30 s at 95 °C, 30 s at 60 °C for Brod541F/Amx820R, KS-qF3/KS-qR3 and BAqF/BAqR, or 53 °C for amoA-1F/amoA-2R, or 51 °C for nirS-1F/nirS-6R, cnorB-2F/cnorB-6R and AnnirS379F/AnnirS821R, and 1 min at 72 °C with finally 5 min at 72 °C. The PCR products were checked by agarose gel electrophoreses and DNA sequencing.

2.4. Analytical methods

The concentrations of nitrogen compounds, suspended solids (SS), volatile suspended solids (VSS), mixed-liquor suspended solids (MLSS), mixed-liquor volatile suspended solids (MLVSS) and alkalinity were measured according to the Standard Methods (APHA, 1998). The NH_4^+-N, NO_2^--N and NO_3^--N concentrations were determined spectrophotometrically, and the organic matter content in the synthetic wastewater was expressed as COD.

3. Results and discussion

3.1. Nitrogen removal from opto-electronic industrial wastewater

The nitrogen removal from opto-electronic wastewater in SBR using SNAD system was monitored over 8 months. Table 3 shows the operation conditions of SBR under various stages investigated in this study. The NLR and organic loading rate (OLR) were calculated by using the Eqs. (2) and (3), respectively, and their values in each stages are also shown in Table 3:

$$\mathrm{NLR} = \frac{\text{Inf. } NH_4^+\text{-N}}{\mathrm{HRT}} \tag{2}$$

$$\mathrm{OLR} = \frac{\text{Inf. COD}}{\mathrm{HRT}} \tag{3}$$

In this system, ammonium would be converted partly to nitrite by AOB (Eq. (4)), and subsequently anammox bacteria would convert ammonia with nitrite to nitrogen gas (Eq. (5)). The overall equation for this process is described as Eq. (6). The ratio of nitrite and nitrate production to ammonium conversion ($Y_{(NO_2^-+NO_3^-)/NH_4^+}$)) was calculated according to (Eq. (7)) and was used to evaluate the performance of the SNAD system. Theoretically, 100% ammonia should produce 88% nitrogen gas with 11% nitrate, which implies the value of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ should be close to 11% for combined partial nitrification and anammox reaction. However, the presence of heterotrophic denitrifiers can reduce the value of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ to below 11% as they will utilize the COD to reduce the nitrate into nitrogen gas.

$$1NH_3 + 1.5O_2^- \rightarrow 1NO_2^- + H_2O + H^+ \tag{4}$$

$$1NH_3 + 1.32NO_2^- + H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \tag{5}$$

$$1NH_3 + 0.85O_2 \rightarrow 0.44N_2 + 0.11NO_3^- + 0.14H^+ + 1.43H_2O \tag{6}$$

$$Y_{(NO_2^-+NO_3^-)/NH_4^+} = \frac{\text{Eff. } \{(NO_2^-\text{-N}) + (NO_3^-\text{-N})\}}{\text{Inf. } (NH_4^+\text{-N}) = \text{Eff. } (NH_4^+\text{-N})} \times 100\% \tag{7}$$

3.1.1. Stage I and II: enrichment at low NLR

Fig. 2 shows the temporal variation of nitrogen compounds (influent NH_4^+ concentration and effluent concentrations of NH_4^+, NO_2^- and NO_3^-) and removal efficiencies of TN and NH_4^+ in the reactor during various stages investigated in this study. In stage I, the NLR and HRT were 16 g N/m^3 d and 5 d, respectively. The system was stabilized after 15 d (3 times of HRT) and the nitrogen removals (%) were in the range of 30–62% during 15–59 d (Fig. 2). The consumption rate of oxygen by microorganisms during the stage I was very low and, therefore, the DO controller was unable to maintain the DO concentration at 0.1 mg/L. The values of DO varied between 0.5 and 5.0 mg/L (Fig. 3) during this stage. Fig. 4 shows the variation of conversion efficiency ($Y_{(NO_2^-+NO_3^-)/NH_4^+}$) at different stages in the reactor and its average value was 59% at stage I. This value is higher than the theoretical conversion efficiency (11%) of the SNAD system. The high concentration of the DO which leads to the growth of NOB in the reactor and inhibit the anammox

Table 3
Operating parameters at different stages in the SBR.

Parameter	Stage					
	I	II	III	IV	V	VI
Temperature (°C)	37	37	25	25	25	25
Inf. NH_4^+-N (mg/L)	78	113	368	400	564	574
Inf. COD (mg/L)	20	20	43	105	99	99
HRT (d)	5	3	3	3	3	2.5
Duration (d)	0–59 (59)	60–86 (27)	87–118 (32)	119–163 (45)	164–191 (28)	192–240 (49)
NLR (g/m^3 d)	16	34	110	120	169	230
OLR (g/m^3 d)	4	6	13	32	30	40

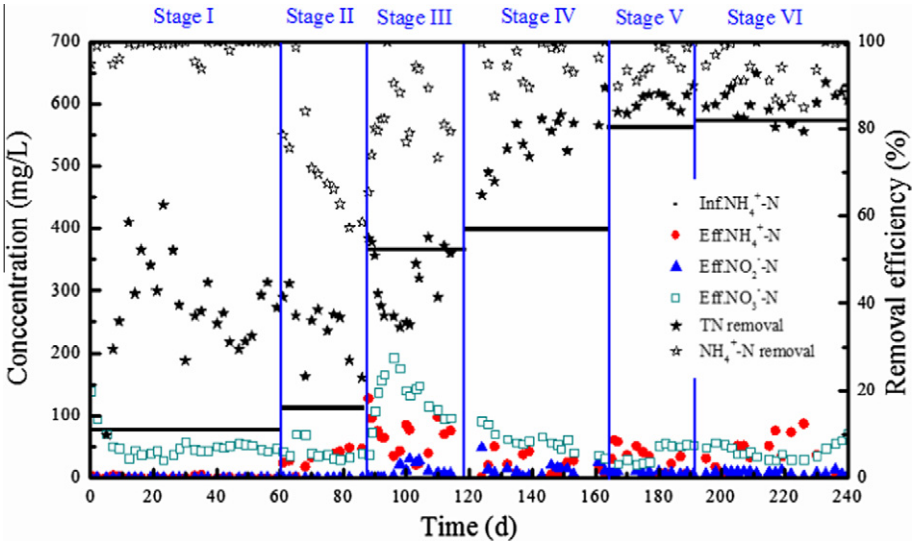

Fig. 2. The temporal variation of nitrogen compounds and removal efficiencies in the SBR.

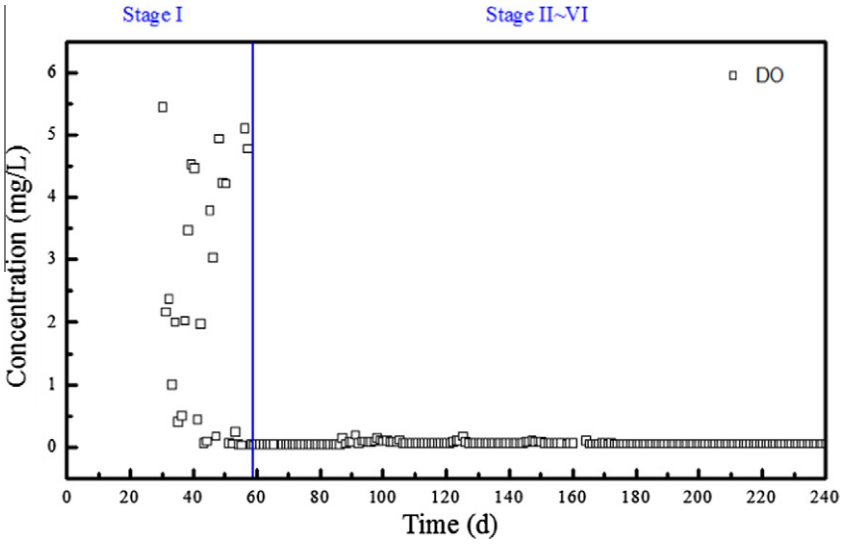

Fig. 3. The temporal variation of DO in the SBR.

bacteria might be the most plausible reason for this high conversion efficiency. Further, the high concentrations of NO_3^- in the effluent (during stage I and II) as shown in Fig. 2 suggested the presence of NOB in the reactor.

In order to avoid the inhibition effect of surplus oxygen on anammox bacteria in the reactor, DO control system was turned off during the stage II. The DO concentration in the reactor came down to 0.1 mg/L (Fig. 3). The NLR was increased to 34 g/m^3 d while the HRT was reduced to 3 d. However, the average conversion efficiency ($Y_{(NO_2^-+NO_3^-)/NH_4^+}$: 52%) during the stage II was almost similar to stage I (Fig. 4). It is apparent from Fig. 2 that the nitration reaction was dominated in the reactor which directly converted NH_4^+—NO_3^- and inhibited the anammox activity, in spite of DO concentration was reduced to 0.1 mg/L.

3.1.2. Stage III and IV: new biomass added and system recovery

In order to improve the anammox activity, new seed was added into the SBR at stage III. Also, the NLR was increased from 34 to 110 g/m^3 d. After the second addition of seed, the DO controller was turned on and now it was able to maintain the DO level at 0.1 mg/L throughout the experiment (from stage III to VI) (Fig. 3). The value of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ gradually increased to 61% from day 87 to day 98 and again gradually dropped after day 98 suggesting the adaptation of the seed sludge in the new conditions and the seed microbes outgrown the NOB from the system. This can be evident from the fact that the nitrite started to accumulate from day 96 onwards in the reactor which indicates the loss of activity of NOB in the system (Fig. 2). At the end of this stage the value of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ was reduced to 34%. The results indicate that the major way of NH_4^+-N removal shifted from complete nitrification to anammox reaction.

Therefore, the NLR was increased to 120 g/m^3 d in the stage IV while the HRT was maintained at 3 d. The performance of the system was improved significantly in this stage as the total nitrogen removal increased from 45% to 80% (Fig. 2). Also, the effluent concentrations of NO_3^--N reduced to below 10% at the end of stage IV. To further improve the efficiency of the SNAD system the NLRs were further increased in the stage V and VI.

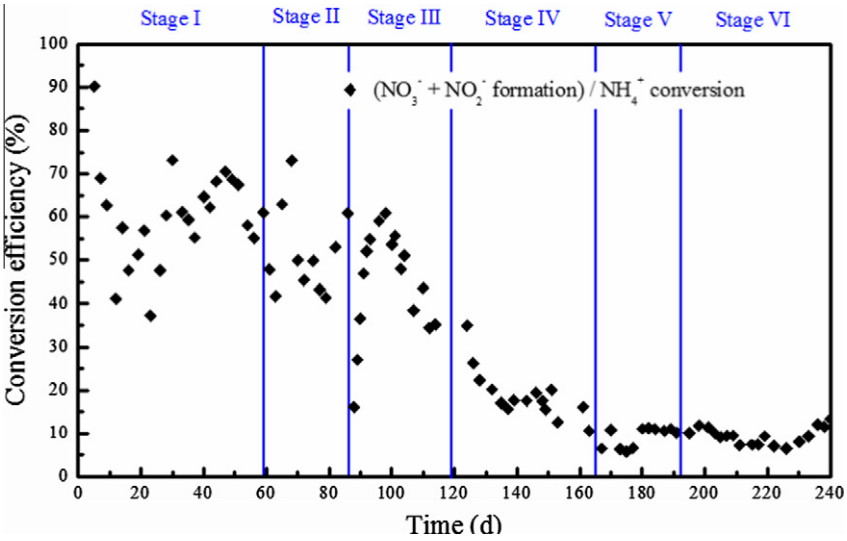

Fig. 4. The temporal variation of conversion efficiency ($Y_{(NO_2^-+NO_3^-)/NH_4^+}$) during stage I–VI in the SBR.

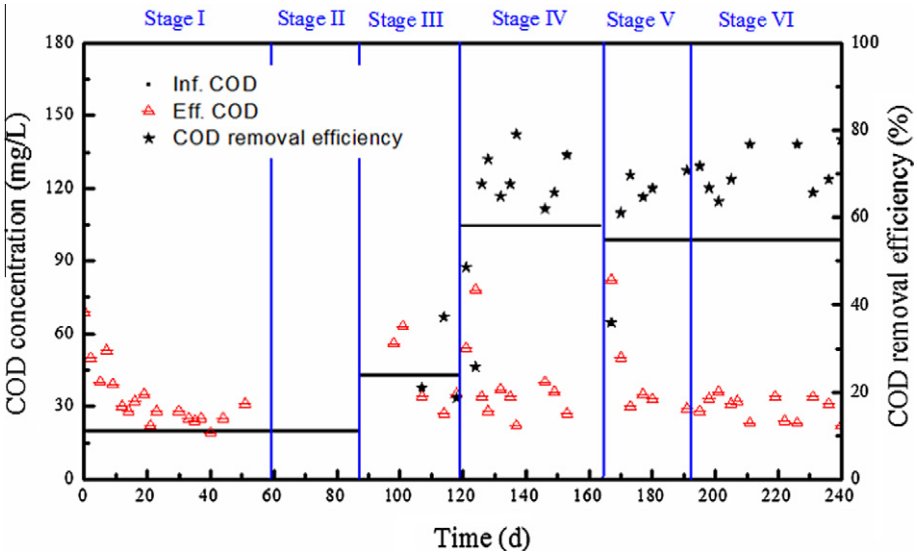

Fig. 5. The temporal variation of COD and removal efficiency during stage I–VI in the SBR.

3.1.3. Stage V and VI: long term stability of SNAD system

Fig. 2 shows that the system was stabilized during the stages V and VI. The NLR and HRT of the system were 169 g/m^3 d and 3 d, respectively during the stage V. In stage VI, the NLR was further increased to 230 g/m^3 d while HRT was decreased to 2.5 d. During these stages (V and VI) the total nitrogen removals were always above 80% (between ~83% and ~93%). It can be seen from Fig. 4 that the values of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ are between ~6% and ~13% from day 167 onwards which suggest the long term stability (74 d) of the SNAD system in the SBR. Also, the value of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ below 11% suggests the presence of denitrification process in the SBR. As the affinity between NO_2^- and anammox bacteria is much higher than that between NO_2^- and denitrification bacteria (Ahn, 2006), in this reactor heterotrophic denitrifier used NO_3^- as electron acceptor over NO_2^-. Thus, at this stage NO_2^- produced by AOB was consumed by anammox bacteria and the NO_3^- produced by the anammox reaction was consumed by denitrification bacteria. Fig. 2 shows that the NO_2^--N concentrations were always below 15 mg/L except the starting days of each stage where the nitrogen loading rates were suddenly increased. This result indicates that anammox could not respond quickly to the sudden increase in NO_2^--N produced by AOB. In final stage (VI) of loading (230 gNH_4^+-N/m^3 d), the percentage removals of TN were in the ranges of 83–93%. At this stage, the average nitrogen removal rate of 197 gNH_4^+-N/m^3 d was obtained. In addition, the average concentrations of SS and VSS were 14 and 7 mg/L, respectively in the effluent. The values of MLSS and MLVSS were found to be 3978 and 2344 mg/L, respectively. The red granules of anammox bacteria were also observed in the reactor.

3.2. COD removal from opto-electronic industrial wastewater

The COD is used by heterotrophic bacteria as carbon and energy sources during denitrification and its removal by the system indicates the presence of active denitrification bacteria in the system (Wang et al., 2010). Fig. 5 shows the performance of percentage COD removal by the SNAD system during different stages. In stages I–III, the influent COD concentration was very low (only 20–43 mg/L) and the COD removal efficiencies were insignificant. The high DO concentration in the reactor during stages I and II, which negatively affects the denitrifying process, could be the most

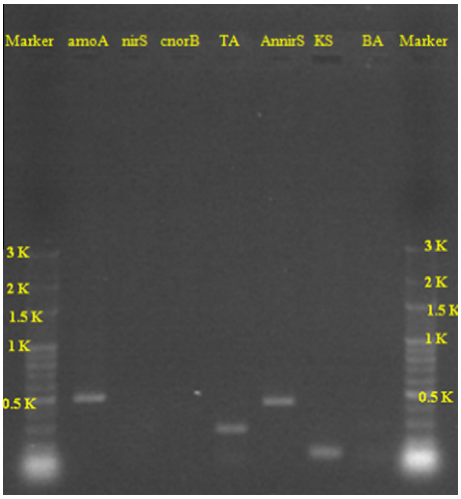

Fig. 6. Results of PCR by performing agarose gel electrophoreses.

probable reason for this low COD removal efficiency. During stage IV–VI the influent COD were almost 100 mg/L and the COD removal efficiencies were above 60%. The maximum % COD removal and average COD removal rate at final stage were found to be 79% and 28 g COD/m^3 d, respectively. This suggests the presence of active heterotrophic denitrifiers in the reactor, which utilized organic matter as carbon and energy sources from the reactor and reduces the COD concentrations in the effluent.

3.3. PCR

To identify the microbial species present in the SBR, PCR experiments were carried out. From Fig. 6, a clear band around 500 bp on the lanes of amoA targeting AOB was observed. While no band around 900 bp exists on the lane of nirS targeting NOB, nor a band near 400 bp appeared on the lane of cnorB targeting denitrifying bacteria. For detecting anammox bacteria, clear bands near 300 bp and 500 bp can be seen on the lanes of TA (Brod541F/Amx820R) and AnnirS (AnnirS379F/AnnirS821R), respectively. AnnirS primer set was designed to mainly target KS and *Candidatus scalindua* genus, while Brod541F/Amx820R primarily targeted *C. scalindua* genus. There is a positive signal on the lane of KS, however, no positive signal for the lane of BA (Fig 6). Therefore, the primary anammox bacteria are considered to be KS and *C. scalindua* genus in this reactor, while KS may be the dominant species. However, further research is needed to identify the heterotrophic denitrifiers present in SNAD system responsible for the COD reduction in the SBR.

4. Conclusions

The SNAD system was successfully applied to treat the optoelectronic industrial wastewater characterized by very low C/N ratio with high ammonium concentration in a lab scale SBR. The SNAD system shows long term stability with very high TN removal efficiency (~93%) at NLR of 230 g/m^3 d. Also, the system was able to remove 79% of COD at final stage. The PCR results confirmed the presence of AOB and anammox bacteria in the SBR where *Candidatus* Kuenenia stuttgartiensis was indicated to be the dominant species of anammox bacteria in the reactor. Overall, this study suggests that the SNAD system can be used for treating other industrial wastewaters having low C/N ratio.

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