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Partial nitrification and anammox process: A method for high strength optoelectronic industrial wastewater treatment

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Abstract

Completely autotrophic nitrogen removal over nitrite (CANON) process was employed in an 18 L sequencing batch reactor (SBR) for treatment of optoelectronic industrial wastewater containing high strength ammonium nitrogen (3712±120 mg NH_4^+-N L^{-1}). About 89% of total nitrogen and 98% of NH_4^+-N removal efficiencies were observed at the loading rate of 909 g N m^{-3} d^{-1} and the HRT of 4 d. A profound variation in the performance of CANON process was experienced at high DO exposure (above 1 mg L^{-1}) and high nitrite concentration (above 100 mg L^{-1}). Inhibition due to high DO exposure was found to be reversible phenomenon whereas the synergistic inhibition of nitrite, free ammonia and free nitrous acid was irreversible. The fluctuation of reactor temperature between 17 and 37°C did not affect the performance of CANON system. The CANON process was stably controlled at high nitrogen loading rate for more than one month. The co-existence of aerobic and anaerobic ammonium oxidizing bacteria in the reactor was detected by The PCR analysis. About 5 fold increase in amount of anammox bacteria over a period of 258 days was confirmed from the results of qPCR on day 487.

Keywords: Optoelectronics wastewater; Anammox; Partial nitrification, Sequencing batch reactor

1. Introduction

Nitrogen removal from wastewater is gaining a lot of attention due to its potential threat to environment. In general, biological processes like nitrification-denitrification and anaerobic ammonium oxidation (anammox) are used to remove nitrogen from wastewater. Among these processes anammox reaction has become more popular as it is the shortcut of ammonia removal cycle, which directly converts ammonium to nitrogen gas using nitrite as an electron acceptor (van Graaf et al., 1996). Application of anammox reaction over conventional process (nitrification followed by denitrification) to remove nitrogen from wastewater has many advantages as it is a single reactor anaerobic-autotrophic system, consume less energy and there is no need to add extra carbon source (Siegrist et al., 2008; van Graaf et al., 1996). However, this process is dependent of availability of nitrite, which is not common in real wastewaters. On the other hand nitrite can be generated by the partial nitrification process. Therefore, combining anammox reaction with partial nitrification in a single reactor, which harbours both anammox bacteria and aerobic ammonia oxidizing bacteria (AOB), can overcome the shortcoming of anammox process. This process is known as completely autotrophic nitrogen removal over nitrite (CANON) (Sliekers et al., 2002; Third et al., 2001). In this process, first ammonia is converted partially to nitrite (Eq. (1)) by AOB under low oxygen concentration and subsequently, anammox bacteria convert ammonia and nitrite to nitrogen gas (Eq. (2)). The overall stoichiometry of CANON process is shown in Eq. (3) (Strous, 2000).

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

$$1NH_3 + 1.3NO_2^- + H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \tag{2}$$

$$1NH_3 + 0.85O_2 \rightarrow 0.11NO_3^- + 0.44N_2 + 1.43H_2O + 0.14H^+ \qquad (3)$$

Due to the slow growth rate of anammox bacteria, different reactor configurations such as fluidized bed reactor (van Graaf et al., 1996), sequencing batch reactor (Strous et al., 1998), membrane bioreactor (Trigo et al., 2006) has been investigated. Among these, sequencing batch reactor (SBR) is considered to be the most suitable reactor for the growth of anammox bacteria due to complete biomass retention, which effectively reduce the doubling time (from 30 d in fluidized bed reactor to 11 d in SBR) (Strous et al., 1998; van Graaf et al., 1996).

In recent past, high-technology industries such as optoelectronics and semiconductor industries have gone through a rapid development to meet the ever increasing demand of electronic devices all over the world and it plays a vital role in Taiwan economics. The wastewater generated from such industries is complex and hazardous in nature and need to be treated properly in order to meet the effluent standards before discharging into the environment (Kumar et al., 2012). The situation is aggravated by the fact that the industrial wastewater lacks essential trace elements and nutrients, which are otherwise, necessary in biological nitrogen removal. In literature, only few studies have been reported on biological nitrogen removal from high-technology industrial wastewaters (Chen et al., 2003; Daverey et al. 2012, Kumar et al., 2012). To the best of our knowledge, no study has been reported in literature on the treatment of optoelectronic industrial wastewater containing high strength ammonium nitrogen by CANON process. Therefore, the present study focused on nitrogen removal from the high strength optoelectronic industrial wastewater by CANON process in 18 L laboratory scale SBR.

2. Materials and Methods

2.1. Experimental set-up of sequencing batch reactor (SBR) and operating condition

A SBR with working volume of 18 L, which previously established to treat synthetic wastewater (Lan et al., 2011) was used to study the nitrogen removal from optoelectronic industrial wastewater. Fig.1 (a) shows the schematic representation of SBR set-up. Polyurethane spheres (biocarriers) of 3 cm diameter were inserted in reactor to enhance biomass retention. The carriers (total 100 in number) were placed as a single layer on the reactor wall surface above the air diffuser and the mixer. The total volume of carriers placed in the reactor was 750 cm^3. However, the total volume of liquid medium used in the reactor was 18 L. The SBR was operated in a 24 h cycle with 23.4 h of feeding and reaction phase, 0.45 h of settling phase and 0.15 h of decanting phase as shown in Fig. 1 (b). The feeding period was extended to 12 h in order to avoid shock load. A peristaltic pump and an effluent port were used to feed wastewater into the reactor and to discharge the supernatant, respectively. DO level was maintained below 0.5 mg L^{-1} with a DO controller system (Insite IG model 1000CE, LA), which equipped with DO meter and needle air flow valve. The temperature of the reactor was maintained at 37°C during initial 94 days and at 25°C (from days 95 to 199 and days 416 to 487) by using a thermostatic water jacket. The reactor was run at ambient temperature (17.5 to 36°C) between days 200 and 415. The alkalinity of effluent was controlled at ~ 850 mg L^{-1} as $CaCO_3$ by dosing bicarbonate ($NaHCO_3$) in the influent wastewater.

2.2. Seed sludge and feeding media

Sludge from reactor treating a synthetic wastewater (Lan et al., 2011) was used as seed for developing CANON process in the SBR. Initially (day 1), the concentration of mixed liquor volatile suspended solids (MLVSS) in the reactor was 1,370 mg L^{-1}. On day 94, the reactor was inoculated with new seed sludge collected from a full-scale landfill-leachate treatment plant in Taiwan, to increase MLVSS concentration in the reactor to 2,800 mg L^{-1}. Anammox bacteria, nitrosomonas-like microorganisms and denitrifiers were reported in this landfill-leachate treatment plant sludge (Wang et. al., 2010). Moreover, a part of biomass (16 g) from the reactor was discharged and new seed sludge (31 g) added into the reactor on day 306 to recover the reactor performance, which was inhibited due to accumulation of nitrite. The concentration of MLVSS after this sequential discharge and recharge of biomass was found to be 2,589 mg L^{-1}. The feeding wastewater was collected from optoelectronic industry located at Tainan, Taiwan and stored in a refrigerator at 4°C until used. The characteristics of the wastewater are shown in Table 1. It is evident from the Table that the wastewater is highly alkaline in nature (pH of 9.7), very rich in inorganic nitrogen as the concentrations of NH_4^+-N (3,712 ± 120 mg L^{-1}) and TKN (3,799 ± 9 mg L^{-1}) are almost the same. The wastewater was supplemented with mineral medium and trace elements (Sliekers et al., 2002) as nutrients for proper aggregation and growth of anammox bacteria. The main components of the mineral medium (in mg L^{-1}) were $KHCO_3$, 1,250; KH_2PO_4, 25; $CaCl_2 \cdot 2H_2O$, 300; $MgSO_4 \cdot 7H_2O$, 200 and $FeSO_4$, 6.25. Moreover, $NaHCO_3$ was supplemented to the wastewater as an inorganic carbon source for anammox and nitrifying bacteria. The pH of the wastewater was adjusted to 7.8-8.0, which is optimal for anammox bacteria before introducing it to the reactor.

2.3. Microbial analysis

To identify microbial community and quantify anammox bacteria, polymerase chain reaction (PCR) and quantitative PCR (qPCR) of completely mixed suspended biomass samples withdrawn from effluent port of the reactor on 229 d (before inhibition of reactor performance), 304 d (during inhibition of reactor performance) and 487 d (high nitrogen removal rate under steady state condition) from the reactor were carried out. PCR experiments were performed as reported earlier (Daverey et al., 2012). The total genomic DNA of samples was extracted by using Power Soil DNA Isolation Kit (MO BIO Laboratories, USA). The DNA concentration was determined on a photometer Gene Quant pro (Amersham Biosciences, Pittsburg, PA, 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 (about 50 ng), 1 µl of each primer (10 µM), 9.5 µl of sterilized water and 12.5 µl of 2X Taq PCR Master Mix (Genomics BioSd & Tech, Taiwan). Primer set used for AOB was amoA-1F/amoA-2R (Rotthauwe et al., 1997), for nitrite oxidizing bacteria (NOB) were Nitro-1198f/Nitro1423r (Knapp and Graham, 2007) and NSR-1113f/NSR-1264r (Dionisi et al., 2002), for denitrifying bacteria was nirS-1F/nirS-6R (Braker et al., 1998), and for anammox bacteria was 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). Results of PCR were ensured by agarose gel electrophoreses and DNA sequencing. For qPCR analysis, primers BACT1369F/PROK1492R (Suzuki et al., 2000) and Amx809F/Amx1066R (Tsushima et al., 2007) were used to detect the eubacteria and most of the anammox, respectively. Each reaction was performed in a 10 µl volume containing 1 µl of DNA template (about 5 ng), 0.5 µl of each primer (10 µM), 3 µl of sterilized water and 5 μl of fluorescent dye SsoFast™ EvaGreen® Supermix (BIO-RAD, USA). The cycling parameters were as follows: denaturation for 30s at 95°C, followed by 40 cycles of 5s at 95°C, annealing for 5s at 54°C for BACT1369F/PROK1492R, or 58°C for Amx809F/Amx1066R, followed by a dissociation stage (95°C for 15 seconds, 65°C for 15 seconds, followed by a slow ramp to 95°C). The melt curve showed no detectable peaks that were associated with primer-dimer artifacts and no other nonspecific PCR amplification products. The R^2 values were always greater than 0.99 for all of the standard curves of qPCR (see supplementary Fig. S2). Specificity of the qPCR products were also ensured by agarose gel electrophoreses.

2.4. Analytical methods

The concentrations of nitrogen compounds, TKN, suspended solids (SS), volatile suspended solids (VSS), mixed-liquor suspended solids (MLSS), MLVSS and alkalinity were measured twice or thrice per week according to the Standard Methods (APHA, 1998). The influent and effluent concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were determined spectrophotometrically. The process parameters such as pH, ORP and DO were monitored using pH, ORP meter (Suntex PC3200, Taiwan) and DO meter (Insite IG model 1000CE, America), respectively.

3. Results and Discussion

3.1 Nitrogen removal from optoelectronic industrial wastewater

CANON is a single reactor process for NH_4^+-N removal under oxygen limited conditions. In this study, NH_4^+-N removal by CANON process in SBR was studied over a long period

(487 d). This nitrogen removal study is divided into four different phases: reactor start-up (1-164 d), SBR performance study (165-235 d), DO, nitrite, free ammonia and free nitrous acid inhibition study (233-373 d), and high nitrogen loading rate (NLR) study (374-487 d). The concentrations of NH_4^+-N in influent, HRT and NLR in each phase along with the duration of each phase are mentioned in Table 2. To evaluate the performance of the CANON system nitrite and nitrate residual to ammonium ratios (η) were calculated, as reported earlier (Daverey et al., 2012). According to CANON process stoichiometry (Eq. (3)), the value of η should be close to 11%. A higher value indicates the presence of active NOB in the reactor, which competes for the nitrite substrate with the CANON system.

3.1.1 Reactor start-up (days 1-164)

The SBR (18 L) was used to study the treatment of optoelectronic wastewater by CANON process. The carriers were introduced into the reactor to support the microbial growth. The reactor was started with NLR and HRT of 10 g m^{-3} d^{-1} and 18 d, respectively. Fig. 2 (a) shows the profiles of ammonia, nitrite, and nitrate in influent and effluent, and nitrogen removal efficiencies during start-up period. After 90 days of experiment NLR was increased to 33 g m^{-3} d^{-1}, while HRT decreased to 6 d. The DO level was indented to maintain below 1 mg L^{-1} (Figure 3). The DO of 1 mg L^{-1} or above resulted in high nitrate levels in effluent which could inhibit anammox activity (Fig. 3). The TN removal efficiency was decreased to approximately 20% during this period (day 1 to 24). However, anammox activity could be recovered by maintaining DO at a lower level and TN removal efficiency was improved to 60% from day 25 to 44. Strous et al (1997) also indicated that loss of anammox activity due to high DO is a reversible phenomenon.

On day 45, the reactor was opened for repositioning the bio-carriers. TN removal efficiency reduced drastically while nitrate concentration in the effluent increased sharply to over 100 mg L^{-1} at day 88 (Fig. 2 a). The shock of DO contamination during the period of the change in configuration of carriers might be the reason for this decrease in activity. Moreover, the value of η in this stage was higher than the theoretical value (11%) (Fig. 4), which indicates that part of nitrite was further oxidized to nitrate by NOB in the reactor. Therefore, to improve the reactor performance fresh seed sludge was supplemented in the reactor on day 95 and NLR increased to 33 g m^{-3} d^{-1}, while HRT decreased to 6 d. The reactor performance started to recover and the efficiencies of TN removal and NH_4^+-N removal were enhanced to 60% and 80%, respectively on day 122 (Fig. 2 a). However, the steady state condition in the reactor was not achieved even after two months (from day 95 to 164) at NLR of 33 g m^{-3} d^{-1}. The TN removal and NH_4^+-N removal efficiencies were varied between 40-60% and 60-95%, respectively. There was not enough selection pressure during this start-up period to wash out NOB due to low NLR. NOB such as nitrite-oxidizing *Nitrobacter* and *Nitrospira* species remained active in the system. These NOBs disturbed the stoichiometry of CANON process by oxidizing NO_2^--N into NO_3^--N and inhibit the anammox reaction due to unavailability of NO_2^--N in the reactor (Third et al., 2001). Fig. 2 (a) shows that the NO_3^--N concentration increased from 20 mg L^{-1} to ~100 mg L^{-1} during day 120-164 and the average concentration of NO_3^--N in the reactor during this start-up period (days 1-164) was found to be 61 mg L^{-1}. This high concentration of NO_3^--N in the reactor suggested the presence of NOB in the reactor. Therefore, the selection pressure was further increased by shortening HRT and raising substrate concentration on day 165. Reactor performance was studied at increasing NLRs of 100 g m^{-3} d^{-1}.

3.1.2 SBR performance study (days 165-234)

In between 165-234 days the NLR was increased stepwise from 33 to 400 g m^{-3} d^{-1}, which represents NH_4^+-N concentration of 1,600 mg L^{-1} in the influent, to minimize the activity of NOB and increase anammox activity. The HRT was decreased from 6 d to 4 d and maintained throughout the experiment. From day 165 onwards, the nitrite was gradually accumulated in the reactor (Fig. 2 b). However, this accumulated level of nitrite (~35 mg L^{-1}) at this stage was not inhibitory for anammox reaction (Strous et al., 1999). The value of η decreased gradually from 50% to 10% in this phase (Fig. 4) and the average value of η was 10% (near to theoretical value) between days 205 and 234. This result indicated that nitrite produced by AOB was mainly utilized by anammox bacteria. The TN removal and NH_4^+-N removal increased to ~90% and ~100%, respectively and maintained at these values for more than 4 times of HRT (Fig. 2 b).

3.1.3 DO, nitrite, free ammonia and free nitrous acid inhibition study (235-373 d)

The CANON system was able to treat the wastewater successfully with influent NH_4^+-N concentration of 1,600 mg L^{-1}, which was at NLR of 400 g m^{-3} d^{-1}. However, as soon as the influent NH_4^+-N concentration and aeration rate increased to 2,400 mg L^{-1} (NLR of 600 g m^{-3} d^{-1}), and 0.6 L min^{-1}, respectively, sharp decline in reactor performance was observed (Fig. 2 c). The high value of η (>90%) suggests the inhibition of CANON reaction (Fig. 4) due to the accumulation of nitrite (at concentration of 166 mg L^{-1}) coupled with increased levels of free ammonia (FA, 146 mg L^{-1}) and free nitrous acid (FNA, 6.7 μg L^{-1}) in the reactor (Fig. 2 c and Fig. S1 of supplementary file). The high concentrations of nitrite (above 100 mg L^{-1}), FA (above 20 mg L^{-1}) and FNA (above 0.5 μg L^{-1}) have been reported as inhibitor to anammox bacteria (Strous et al., 1999; Fernandez et al., 2012). Strous et al. (1999) found that anammox process completely inhibited when NO_2^--N in the medium increased above 100 mg L^{-1} and resulted in increased levels of NO_3^--N in the reactor. Similar results were observed in our study, as it could be seen from Fig. 2 (c), effluent concentrations of NH_4^+-N and NO_3^--N increased in SBR during days 235-246. The overload of NLR with insufficient anammox activity and sudden increase in aeration rate from 0.4 to 0.6 L min^{-1} along with the inhibitory levels of FNA and FA (Fig. S1) were the main reasons for this rise in nitrite concentration. After day 247, feeding and aeration into the reactor were stopped until the nitrite level decreased to 10 mg L^{-1}. The NLR was decreased stepwise to 200 g m^{-3} d^{-1} and aeration at 0.5 L min^{-1} was introduced into the reactor. However, the effluent concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were increased to 10,00 mg L^{-1}, 100 mg L^{-1} and 40 mg L^{-1}, respectively, between days 253-262 (Fig. 2 c). Therefore, NLR was further decreased (on day 263) and maintained between 100-125 g m^{-3} d^{-1} for next 43 days (more than 10 times of HRT). The reactor performance could not be improved at this NLR, though the effluent concentrations of FA and nitrite were less than 20 mg L^{-1} and 50 mg L^{-1}, respectively. Both the TN and NH_4^+-N removals were in the range of 5-15%, between days 263-305 (Fig. 2 (c)). This suggests that high nitrite concentration (>100 mg L^{-1}) in presence of high concentrations of FA and FNA has irreversibly inhibited the anammox activity. The average concentrations of MLSS and MLVSS were 3,300 mg L^{-1} and 1,700 mg L^{-1} between days 235-305. To recover the anammox activity, on day 306, part of the original sludge (16 g) from the reactor was replaced with fresh seed sludge (31 g). The NLR was kept to 109 g m^{-3} d^{-1} for next 68 days (17 times of HRT). The accumulation of nitrite was again observed and its concentration reached to 92 mg L^{-1}, which negatively affects anammox process temporarily. Between days 340 and 373, the performance of reactor was recovered and ~90% of TN and ~100% NH_4^+-N removals, were observed (Fig. 2 (c)). Also, the value of η decreased to 11%, which suggests that CANON reaction was dominant in the reactor between days 340 and 373. Similar to pervious stage, pH was maintained between 7.0 and 8.0 by adding the alkalinity in the reactor (Fig. 3).

3.1.4 High nitrogen loading rate study (days 375-487)

In this phase, the NLR was increased exponentially from 109 to 909 g m^{-3} d^{-1} (the maximum possible NLR). At NLR of 909 g m^{-3} d^{-1}, the influent NH_4^+-N concentration was 3,636 mg L^{-1}, which represented the concentration of NH_4^+-N in the real world optoelectronic industrial wastewater used in this study. The SBR was able to treat wastewater successfully at this high NLR (909 g m^{-3} d^{-1}) for more than 1.5 months (~11 times of HRT). The value of η was always near to 11% (Fig. 4) suggesting that CANON was the prevailing reaction in the reactor during days 375-487. Fig. 2 (d) shows the profiles of nitrogen compounds in this phase and Table 3 shows the average values of different parameters under steady-state condition (days 454-487) of CANON process in SBR. The average nitrogen removal efficiencies were very high (TN and NH_4^+-N removals were ~89% and above 98%, respectively) under steady-state conditions (Fig. 2 (d) and Table 3). The average effluent concentrations of NH_4^+-N and NO_2^--N were less than 90 mg L^{-1} and 10 mg L^{-1}, respectively. The average concentrations of FA and FNA in the reactor were less than 2 mg L^{-1} and 1 µg L^{-1}, respectively. The MLSS and MLVSS gradually increased and stabilized at 9,500 mg L^{-1} and 6,500 mg L^{-1}, respectively. The maximum nitrogen removal rate (NRR) in this phase was found to be 825 g N m^{-3} d^{-1}. In literature, many authors have been reported the high NRR by partial nitrification-anammox process but most of those studies were carried out using synthetic wastewaters or digested liquor and/or using two step processes (Cho et al., 2011; Joss et al., 2009; Sliekers et al., 2002; Third et al., 2001, 2005). In contrast, this study suggested the application of partial nitrification coupled with anammox process in a single reactor to successfully treat the ammonium rich industrial wastewaters.

3.2 Effect of temperature on the CANON

Temperature is one of the most important physical parameter for the growth of microorganisms and the optimum value varies from one species to another. In this study, effect of temperature on the performance of CANON process was evaluated. During the start-up period for optimum growth of anammox bacteria, temperature of the reactor was maintained at 37°C. Subsequently, the reactor temperature was reduced and maintained at 25°C during 95 to 199 d. The reactor was run at ambient temperature during 200 to 415 d, where it varied between 17 and 36°C (Fig. 5). However, to avoid the further decrease in reactor temperature (below 17°C) in winter, it was maintained at moderate temperature (25°C) after day 416. The change in temperature had no significant effect on reactor performance, except days 235 to 340, where the reactor performance was poor due to the inhibitory effects of nitrite on the anammox bacteria. This result is in good agreement with the study conducted by Dosta et al. (2008), where anammox bacteria has been reported to grow successfully between 30-18°C and lost its activity at temperature higher than 45°C due to cell lysis or lower than 15°C due to nitrite accumulation in the system. The optimum temperature for achieving partial nitrification i.e. for the growth of aerobic ammonia oxidation bacteria (AOB) is also believed to be in between 30-35°C (Gu et al., 2012). However, AOB can also grow properly at low (11 to 16°C) and mid (20 to 25°C) temperature ranges (Guo et al., 2009; Gu et al., 2012). In addition, Table 4 summarizes the effects of temperature on various parameters at different phases in the SBR. The specific anammox activity (SAA) at 33.5°C (0.153 g N g VSS^{-1} d^{-1}) was found to be higher than 25°C (0.132 g N g VSS^{-1} d^{-1}). However, it did not affect the reactor performance as it could be seen from Table 4 and Fig. 2. The NRRs were higher at 25°C than 33.5°C and the removals of TN and NH_4^+-N were similar at both temperatures. This study further confirmed that temperature between 17 and 35°C is not a critical factor for growth of anammox bacteria, as it can actively grow in a wide range of temperature without affecting its performance.

3.3 Microbial community analysis

To identify the microbial community present in the reactor, PCR experiments were carried out using specific primers for AOB, NOB (both *Nitrobacter sp.* and *Nitrospira sp.*), denitrifiers and anammox bacteria (including primers for KS and BA) with the samples drawn at different stages of reactor operation. Fig. 6 (a) shows the PCR results of sample taken on day 229 from SBR (stage 2). Clear bands around 500 bp and 900 bp on the lanes of amoA and nirS confirmed the presence of AOB and denitrifiers, respectively. Faint bands near 200 bp in lanes of Nitro and NSR ensured the presence of NOB. Existence of anammox bacteria and specific anammox species - KS and BA were confirmed by the presence of bands near 500 bp, 100 bp and 300bp on the lanes of AnnirS, KS, and BA, respectively (Fig. 6 a). These results suggest that AOB, NOB, denitrifires and anammox bacteria (BA as well as KS) all were present in the reactor on day 229 (stage 2). However, lack of COD in the influent suggests that denitrifier were not active in the reactor. Whereas the active population of NOB must have been very low in the reactor as the value of η was 10% on day 229. Sliekers et al. (2003) also observed the small population of NOB in their CANON system. Therefore, only AOB and anammox bacteria were most active in the reactor on day 229 (stage 2). Fig. 6 (b) shows the PCR results of sample taken on day 304 from SBR (inhibition period, stage 3). Presence of AOB and NOB in the reactor was evidenced from the Fig. 6 (b). A very faint band in the lane of TA (total anammox) in Fig. 6 (b) compared to Fig. 6 (a) suggests that anammox activity is very less in the reactor during this period and therefore, reactor performance was inhibited during days 263-305 in the SBR. PCR results of sample taken on day 487 (stage 4) also confirmed the co-existence of AOB and anammox bacteria in the reactor (Fig. 6 c). Besides, results from quantitative analysis of qPCR showed the cell number of eubacteria changed from 3.2×10^7 to 9.5×10^8 cells/μg DNA and most anammox bacteria changed from 1.2×10^6 to 8.5×10^7. The percentages of anammox to eubacteria were 1.8%, 3.7% and 9.0% on 229 d, 304 d and 487 d, respectively. These results depicted that anammox bacteria were enriched in the reactor under steady state condition, and 5 times increase in the ratio of anammox to eubacteria was observed when reactor temperature was reduced to 25°C from 35°C.

4. Conclusion

CANON process was successfully developed in 18 L SBR to treat the NH_4^+-N rich real world optoelectronic industrial wastewater without dilution. At highest possible influent

NH_4^+-N concentration (3,636 mg NH_4^+-N L^{-1}) the reactor was able to remove 89% and ~98%, of TN and NH_4^+-N, respectively at a HRT of 4 d consistently for more than one month. Results of PCR revealed the co-existence of AOB, NOB, denitrifires and anammox bacteria in the reactor. The amount of anammox bacteria in the reactor increased about 5 times at the end of steady state. Based on this study, following measures can be taken to develop a successful CANON process in SBR: (1) for fast start-up of CANON process, NLR should be higher than 100 g m^{-3} d^{-1} to avoid the unnecessary growth of NOB, which negatively affects the anammox reaction; (2) high DO (above 1 mg L^{-1}) inhibits the activity of anammox bacteria reversibly whereas high nitrite accumulation (above 100 mg L^{-1}) along with high FA (>20 mg L^{-1}) and FNA (>1 µg L^{-1}) in the reactor affects them irreversibly; (3) temperature (between 17 and 37°C) has no effect on the performance of CANON reaction.

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Figure Captions:

Fig.1. Schematic representation of (a) 18 L sequential batch reactor (SBR) along with the configuration of carriers, and (b) distribution of operation cycles in SBR used for the treatment of optoelectronic industrial wastewater.

Fig. 2. Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies in SBR.

Fig. 3. Time profiles of DO in the reactor, and pH and alkalinity in effluent of SBR.

Fig. 4. Temporal variation of nitrite and nitrate production to ammonium conversion efficiency (η) by the CANON system in SBR.

Fig. 5. Time profile of temperature in the reactor.

Fig. **6.** Results of PCR by performing agarose gel electrophoreses of sludge samples taken from SBR on (a) day 229, (b) day 304 and (c) day 487.

Table1 Main characteristics of raw optoelectronic industrial wastewater.

Parameter	Value[a,b]
COD	13.5 ± 0.7
TKN	3799 ± 9
NH_4^+-N	3712 ± 120
NO_2^--N	-
NO_3^--N	-
PO_4^{3-}-P	-
pH	9.7±0.1
Alkalinity as $CaCO_3$	5785±3341

a: all units are in mg L^{-1}, except pH

b: two times wastewater sample collected from the industry and analyzed

476 **Table 2** Operating parameters of SBR at different stages.

Stage	Phase (d)	Inf. NH_4^+-N (mg L^{-1})	HRT (d)	NLR (g m^{-3} d^{-1})	Duration (d)
1: Start-up	1~83	183	18	10	83
	95~164	200	6	33	70
2: Increasing nitrogen loading rate	165~199	400	4	100	35
	200~216	800	4	200	17
	217~234	1600	4	400	18
3: Inhibition and recovery of reactor	235~247	2400	4	600	13
	253~262	1600~800	4	400~200	12
	263~305	400~500	4	100~125	43
	306~373	434	4	109	68
4: Reactor performance at very high nitrogen loading rate	374~391	922	4	230	18
	392~417	1329	4	332	26
	418~423	1787	4	447	6
	424~433	2192	4	548	10
	434~442	2454	4	614	9
	443~453	3181	4	796	11
	445~487	3636	4	909	43

477

Table 3 Average values of different parameters under steady-state condition of CANON process in SBR (days 454-487).

Parameter	Value
Influent NH_4^+-N concentration (mg L^{-1})	3636
Influent NO_2^--N concentration (mg L^{-1})	0
Influent NO_3^--N concentration (mg L^{-1})	0
NLR (g m^{-3} d^{-1})	909
DO (mg L^{-1})	<0.1
HRT (d)	4
pH	7.6±0.2
Alkalinity (mg $CaCO_3$ L^{-1})	868±526
Effluent NH_4^+-N concentration (mg L^{-1})	90±108
Effluent NO_2^--N concentration (mg L^{-1})	10±10
Effluent NO_3^--N concentration (mg L^{-1})	320±36
Conversion ratio (η) of nitrite and nitrate production to ammonium consumption (%)	9.3±0.8
TN removal efficiency (%)	89±3
NH_4^+-N removal efficiency (%)	98±2.9

Table 4 Effects of temperature on MLVSS, specific anammox activity (SAA) and nitrogen removal rates (NRR) at different phases in the SBR.

Phase[1] (d)	MLVSS (mg L^{-1})	NLR (g m^{-3} d^{-1})	NRR (g m^{-3} d^{-1})	SAA[2] (g N g VSS^{-1} d^{-1})	Temperature (°C)
217~234	2300	400	352	0.153	33.5[3]
418~423	2956	447	400	0.136	25
424~433	3633	548	494	0.136	25
434~442	3900	614	541	0.139	25
443~453	5706	795	724	0.127	25
454~487	6496 (879)[4]	909	791	0.122 (0.108)	25

[1] Selected phase had average TN removals above 85%.

[2]Specific anammox activity (SAA) was only estimated with MLVSS in the reactor. Both attached and suspended biomass was existed in the reactor. However, measurement of the attached biomass on carriers was carried out to accurately estimate the volatile solids in the reactor at final phase. The values in parenthesis are considered with attached biomass on carriers. The SAAs of both attached biomass and suspended biomass were almost equivalent. This suggests that the anammox bacteria were equivalently active in attached and suspended cell aggregates.

[3] Temperature varied between 31.9~34.7°C and average value is 33.5°C.

[4] The MLVSS of the suspended biomass (6496 mg/L) was more than 7 times of the attached biomass (849 mg/L), which suggests that the major portion of bacteria was present as suspended cell aggregates.

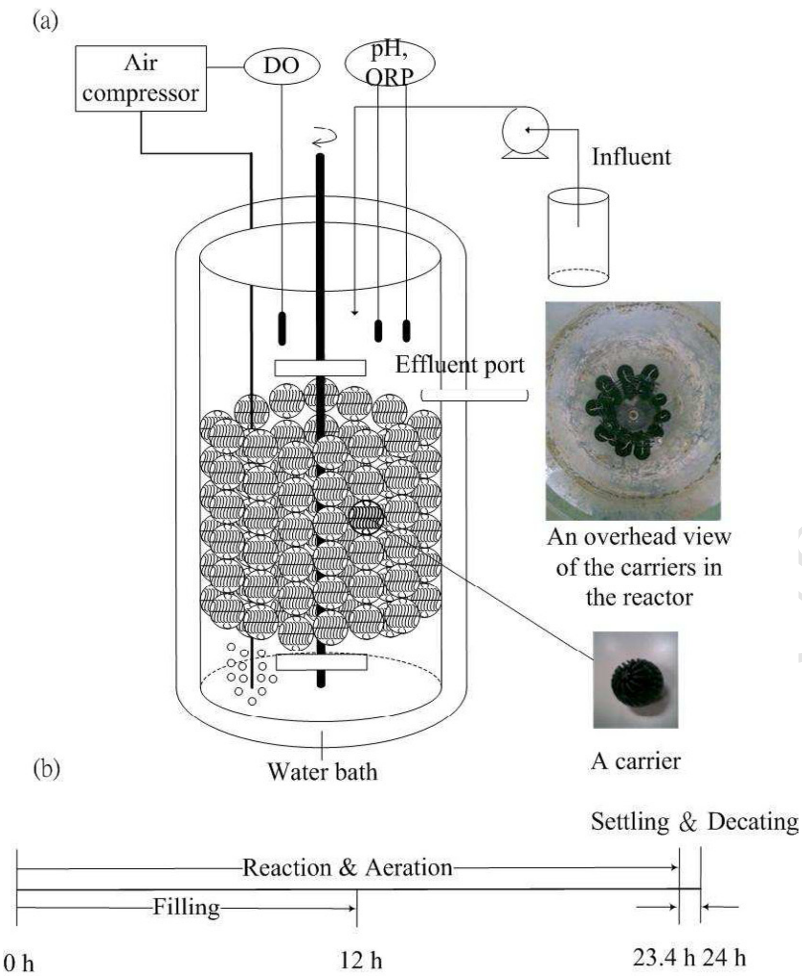

(a)
Air compressor
DO
pH, ORP
Influent
Effluent port
An overhead view of the carriers in the reactor
A carrier
(b)
Water bath
Settling & Decating
Reaction & Aeration
Filling
0 h
12 h
23.4 h 24 h

Fig.1

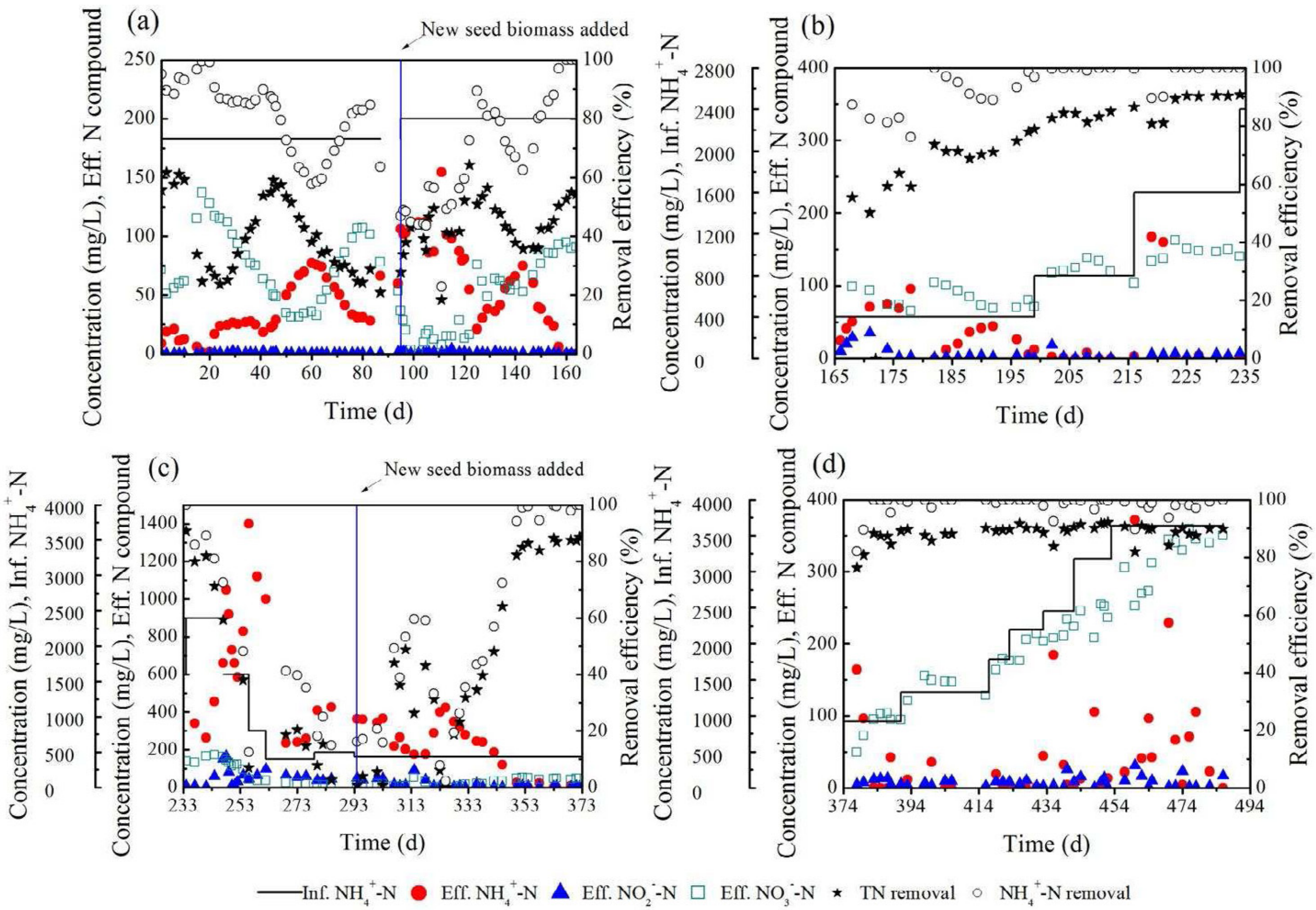

(a)
New seed biomass added
Concentration (mg/L), Eff. N compound
Removal efficiency (%)
Time (d)
(b)
Concentration (mg/L), Inf. NH_4^+-N
Concentration (mg/L), Eff. N compound
Removal efficiency (%)
Time (d)
(c)
New seed biomass added
Concentration (mg/L), Inf. NH_4^+-N
Concentration (mg/L), Eff. N compound
Removal efficiency (%)
Time (d)
(d)
Concentration (mg/L), Inf. NH_4^+-N
Concentration (mg/L), Eff. N compound
Removal efficiency (%)
Time (d)
Inf. NH_4^+-N
Eff. NH_4^+-N
Eff. NO_2^--N
Eff. NO_3^--N
TN removal
NH_4^+-N removal

Fig. 2

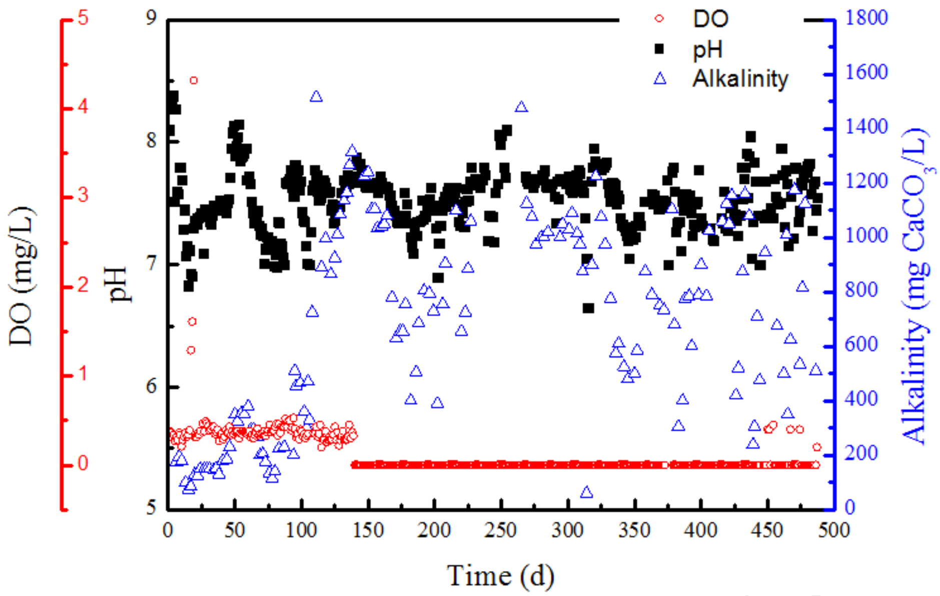
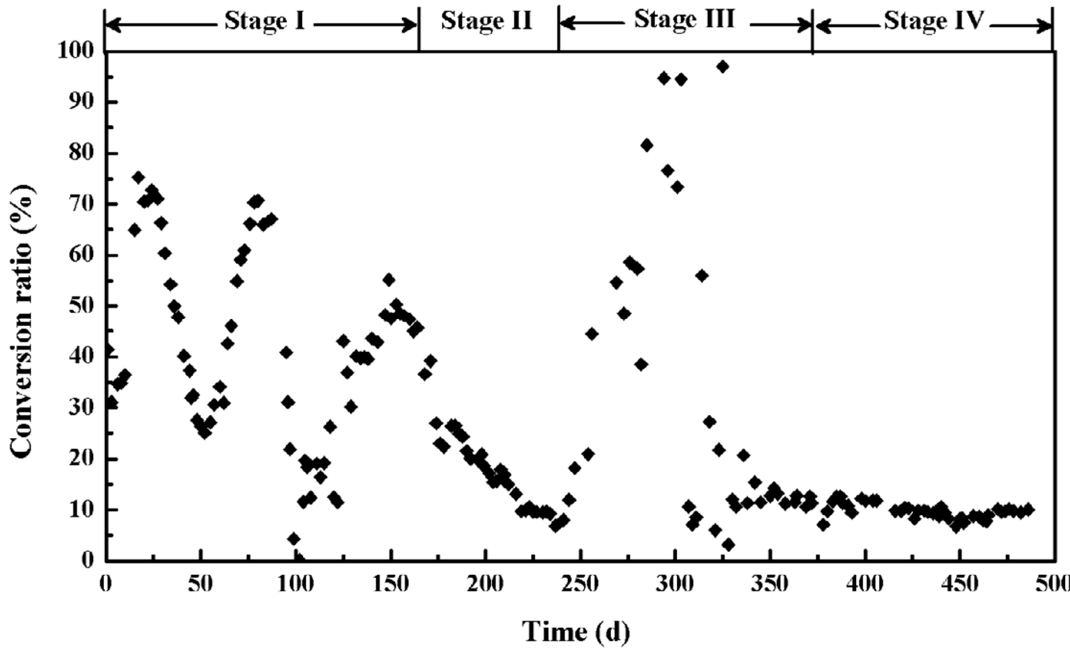
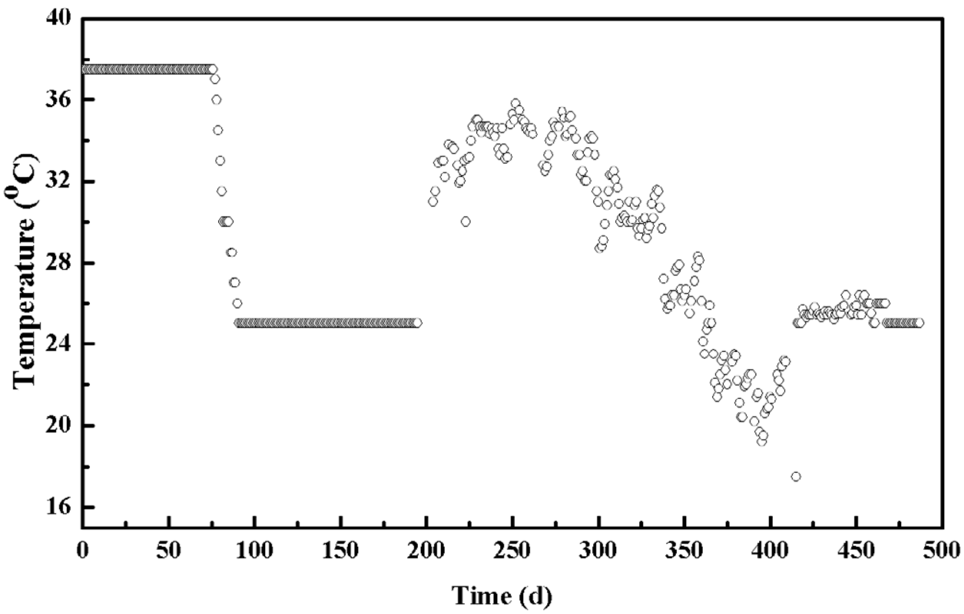

DO
pH
Alkalinity
DO (mg/L)
pH
Alkalinity (mg CaCO$_3$/L)
Time (d)
0
50
100
150
200
250
300
350
400
450
500
5
6
7
8
9
0
1
2
3
4
5
0
200
400
600
800
1000
1200
1400
1600
1800

Fig. 3



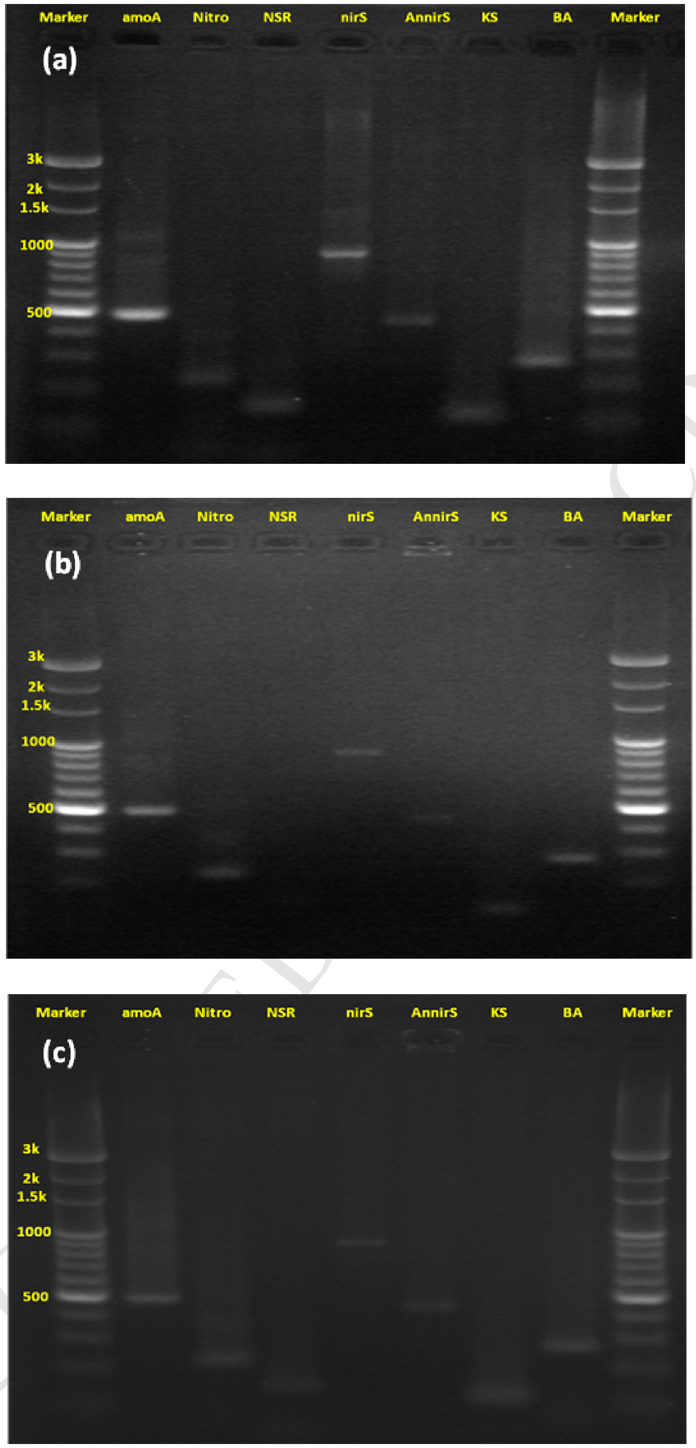
Stage I
Stage II
Stage III
Stage IV
Conversion ratio (%)
Time (d)
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450
500

Fig. 4



Temperature (^{0}C)
40
36
32
28
24
20
16
0
50
100
150
200
250
300
350
400
450
500
Time (d)

Fig. 5



Marker amoA Nitro NSR nirS AnnirS KS BA Marker
(a)
3k
2k
1.5k
1000
500
Marker amoA Nitro NSR nirS AnnirS KS BA Marker
(b)
3k
2k
1.5k
1000
500
Marker amoA Nitro NSR nirS AnnirS KS BA Marker
(c)
3k
2k
1.5k
1000
500

Fig. 6

Highlights

- CANON process is developed to treat the NH_4^+-N rich optoelectronic wastewater
- High TN (~89%) and NH_4^+-N (~98%) removal achieved at NLR of 909 g N m^{-3} d^{-1}
- Long term stable performance of CANON with high NLR was achieved
- Co-existence of AOB and Anammox bacteria confirmed by PCR
- Temperature (between 17 and 37°C) had no effect on the CANON process