國立交通大學

環境工程研究所

碩士論文

結合部分硝化與厭氧氨氧化程序於處理高氮光電廢水之應用

Application of simultaneous partial nitrification and Anammox Process for treatment of high strength nitrogen containing opto-electronic wastewater

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中文摘要

本研究將部分硝化與厭氧氨氧化程序結合於單一反應槽內並針對處理兩股含有高濃度氮氮之光電廢水進行探討。此兩股光電廢水之主要特性略有不同，其分別為僅具有高濃度氨氮 (WW1) 與碳氮比為 0.2 (WW2) 之廢水。為強化培養厭氧氨氧化菌之生長本研究於進流廢水中添加營養鹽與微量元素。WW1 與 WW2 分別以 18 L 與 2.5 L 的序批次反應槽 (SBR-18 & SBR-2.5) 進行處理，並在 16 個月的實驗期間逐漸提升氮負荷。實驗結果顯示於 SBR-18 反應槽的最後階段總氮負荷最高可達到 909 g -N m^{-3} d^{-1} 且平均總氮去除效率可達 90% 並持續維持 1 個月。而在 SBR-2.5 反應槽中的最後階段，總氮負荷與 COD 負荷最高分別可達到 428 g-N m^{-3} d^{-1} 與 89 g-COD m^{-3} d^{-1} 且平均總氮去除效率與 COD 去除效率分別可達 93% 與 79%。最後藉由聚合酵素鏈鎖反應 (PCR) 之菌相鑑定進一步證實了 SBR 內共同存在了氨氧化菌與厭氧氨氧化菌且 *Candidatus* Kuenenia stuttgartiensis 為主要菌種之一。

ABSTRACT

Treatment of two optoelectronic industrial wastewaters (wastewater containing high ammonium concentration (WW1) and wastewater with C/N ratio of 0.2 (WW2)) were achieved using partial nitrification and Anammox processes in a single reactors. 18 L and 2.5 L lab scale sequencing batch reactors (SBR) were used to treat WW1 and WW2, respectively. Essential nutrients and trace elements were added in the influent wastewaters to support the Anammox growth. 18 L SBR (SBR-18) and 2.5 L SBR (SBR-2.5) were run for over 16 months in different stages. Nitrogen loading rate (NLR) was gradually increased from 10 g-N m^{-3} d^{-1} to 909 g-N m^{-3} d^{-1} and 16 g-N m^{-3} d^{-1} to 230 g-N m^{-3} d^{-1} in SBR-18 and SBR-2.5, respectively.

The SBR-18 was successfully run about 1 month (7 times of HRT) to treat WW1 without dilution i.e NLR of 0.9 g-N m^{-3} d^{-1}. The average TN removal was 90% in the high NLR in SBR-18. In the case of SBR-2.5, the system successfully treated 89 g-COD m^{-3} d^{-1} and 428 g g-N m^{-3} d^{-1}, respectively, with 79% and 93% of COD and TN removal efficiencies in later stages. Presence of ammonia oxidizing bacteria (AOB) and Anammox bacteria were confirmed by polymerase chain reaction (PCR) in the SBRs. PCR results also indicated that *Candidatus* Kuenenia stuttgartiensis was one of the dominant species in both SBRs.

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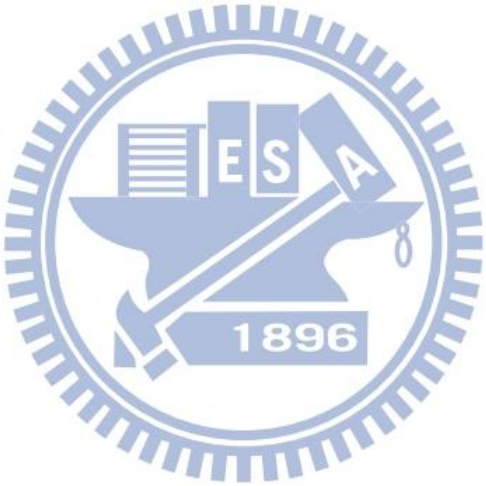


Chapter 1 Introduction

In recent years, the booming in semiconductor and opto-electronic industry has made Taiwan a huge economic growth. However, a number of optoelectronic industries, such as LED (Light-emitting diode) manufacturing plants, produced ammonium-rich wastewater in manufacturing processes.

Under the fast development of high technology industry, the impact of nitrogen pollution discharge brought to the environment has become an important issue. For example, the release of untreated wastewater can result in eutrophication of the receiving water bodies. Along with growing environment awareness, the EPA in Taiwan strives to set stringent standard regulations to control the emissions of nitrogen compounds to the surface waters. For example, an established opto-electronic industry should reduce the discharge limit of ammonium concentration in wastewater to 75 mg/L by July 1, 2012 and 30 mg/L by July 1, 2015.

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 nitrate (NO_3^-) by autotrophic nitrifiers with oxygen as the electron acceptor in aerobic condition, then NO_2^- or NO_3^- converted to gaseous N_2 by heterotrophic microorganisms using organic matter as carbon source in anoxic condition. However, conventional nitrification and denitrification process needs an amount of biodegradable and inorganic carbonate source. High energy consumption is another disadvantage of this process. Therefore, the concept of nitrogen removal model has been changed since the confirmation of the anaerobic ammonium oxidation (Anammox) process [1], which is an autotrophic oxidation process and converts ammonia to N_2 using nitrite as the electron acceptor. Moreover, Anammox reaction is

recognized as a cost-effective and sustainable technology for biological nitrogen removal [2].

In this study, Anammox reaction was used as a main reaction for nitrogen removal. The combination of partial nitrification which control further oxidation of NO_2^- to NO_3^- with Anammox in single reactor can provide enough NO_2^- for Anammox, which uses nitrite as the electron acceptor.

The objectives of this study were (i) Development of partial nitrification/Anammox process in sequencing batch reactor for treating opto-electronic wastewater containing high ammonium concentration. (ii) Evaluation of process performance by chemical analyses of water quality, monitoring of condition parameter and biomass measurements. (iii) Assessment of polyvalent ions effect on the growth of Anammox bacteria by monitoring the Anammox activity through SAA (specific Anammox activity) tests.

Chapter 2 Literature review

2.1 Nitrogen cycle

Nitrogen is constituted 79% by volume of Earth's atmosphere. And it is essential to all organisms, for example nitrogen is used by organisms to produce amino acids, proteins and nucleic acids. However, nitrogen in the atmosphere cannot be used directly by either plants or animals. It must be transformed into other nitrogen species by microorganism. The nitrogen species existed in terrestrial and aquatic ecosystems include N_2, NH_3, NH_4^+, NO_2^-, NO_3^- and organic nitrogen. The transformation of these nitrogen species consisted of several mechanisms including nitrogen fixation, assimilation, ammonification, nitrification and denitrification. These mechanisms related to specific microorganism. Table 1 shows the part of microorganism responsible for these mechanisms.

Table 1 A list of currently sequenced microbial genomes with nitrogen cycle pathways [3]

Species	Pathways or enzymes
Nitrosomonas europaea	Ammonia oxidation
	Dissmilatory nitrite and nitric oxide reductases
Methylomonas sp. 16a	Ammonia oxidation
	Dissimilatory nitrite and nitric oxide reductases
Neisseria menigitidis	Dissimilatory nitrite and nitric oxide reductases
Synechocystis sp. PCC6803	Cytochrome b nitric oxide reductase
Bacillus subtilis strain 168	Dissimilatory nitrate reduction to ammonia
Pseudomonas aeruginosa PAO	Denitrification
Rhodobacter sphaeroides	Denitrification
	Nitrogen fixation
Paracoccus denitrificans ATCC 19367	Denitrification
	Heterotrophic nitrification
P. denitrificans strain SANVA100	Denitrification
Azoarcus tolulyticus Tol-4	Denitrification
Azotobacter adn Rhizobium	Biological fixation

Nitrogen Fixation

Nitrogen fixation is the reaction, which converts the N_2 to nitrogen compound for plants or animals used. And it includes biological, abiotic, or synthetic way. For example, biological way such as bean family (legumes), abiotic way such as lighting strikes and synthetic way such as anthropogenic activities.

Assimilation

Plants absorb nitrogen compound (i.e. NH_4^+ and NO_3^-) from soils through their roots to synthesize vegetable proteins.

Ammonification

Ammonification is the reaction, which decompose organic nitrogen compound back to ammonium nitrogen. For example, when plants or animals dies, or animals expel waste, the abiotic organic nitrogen compounds convert or decompose by bacteria into ammonium.

Nitrification

Nitrification includes both ammonium oxidation reaction and nitrite oxidation reaction. The oxidation of ammonium to nitrite is performed by ammonium oxidizing bacteria (AOB) such as the *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosococcus*. And the oxidation of nitrite to nitrate is responsible by nitrite oxidizing bacteria (NOB) such as *Nitrobacter* and *Nitrococcus*.

Denitrification

Denitrification is the reaction which convert nitrate back to gaseous N_2. This process is performed by bacteria species such as *Peseudomas* in anaerobic conditions.

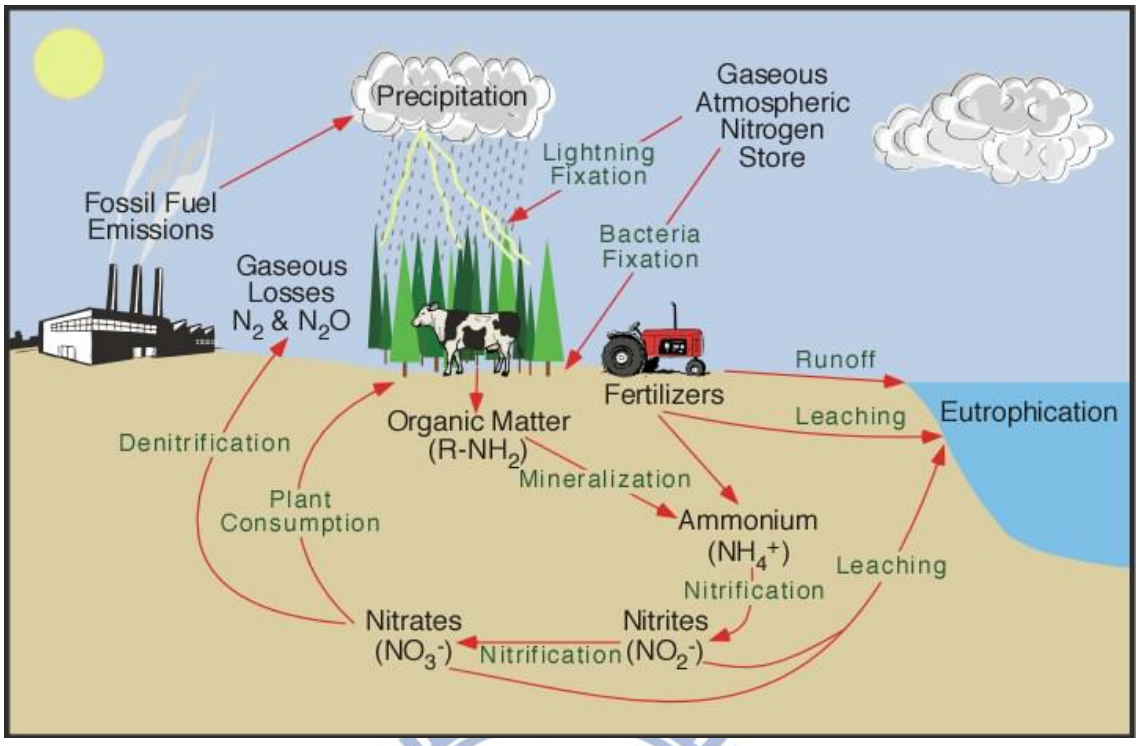


Figure 1 Human activity changing in nitrogen cycle [4]

The nitrogen cycle is a combination of several biological and non-biological processes as shown in Figure 1. However, the growing human population has gradually increased demand for food, energy and essential requirements. With these demands have increased the amount of reactive nitrogen, the primary processes were developed in the past century to convert unreactive nitrogen to reactive nitrogen. These primary processes including combustion of fossil fuels and planting of nitrogen-harnessing croplands resulted in the large acceleration of nitrogen cycle (Figure 2). Furthermore, the excess reactive nitrogen in the environment can lead to pollution. For example, the discharge of ammonium wastewater from anthropogenic activities resulted in the eutrophication of lakes and river. There are some major anthropogenic sources of nitrogenous pollutants enter aquatic ecosystems through point and nonpoint sources originated from human activites described inTable 2.

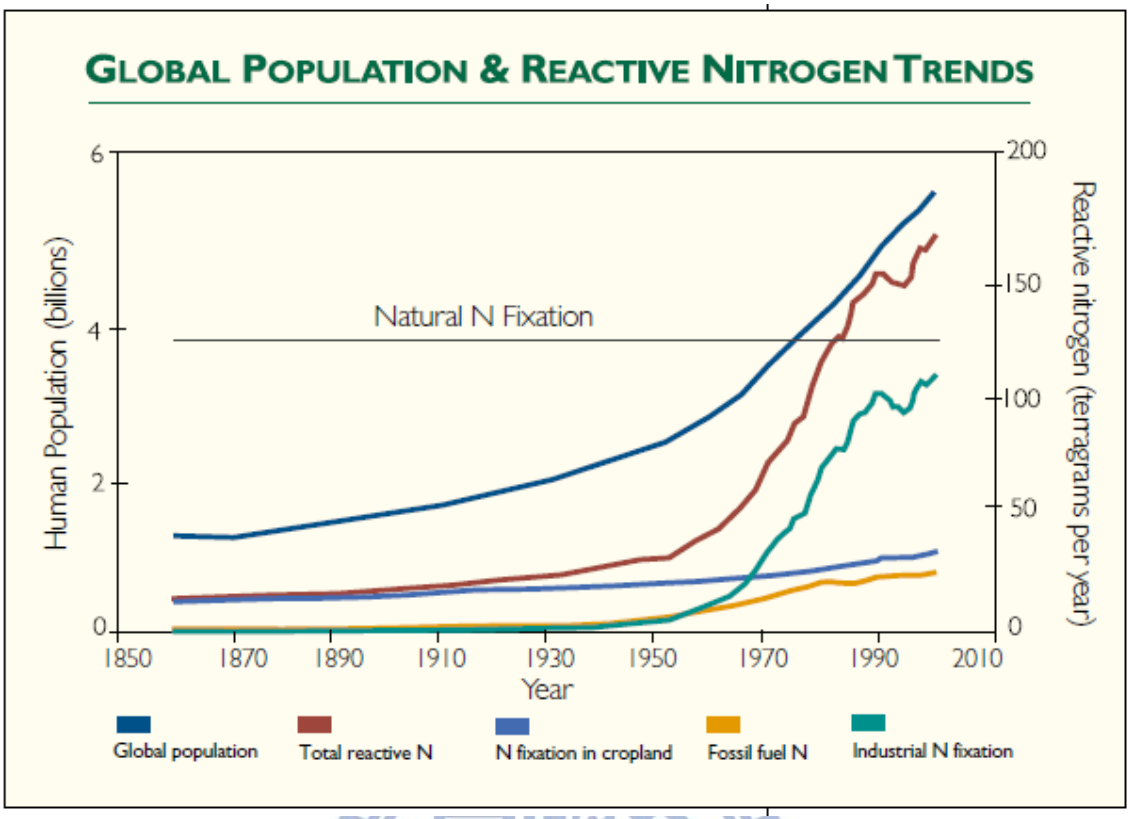


Figure 2 Human activites have increased the production of reactive nitrogen [5]

Table 2 the major anthropogenic sources of nitrogen in aquatic ecosystems [6]

Point sources
- Wastewaters from livestock (cattle, pig, chickends) farming
- N releases from aquaculture (fish,shrimps, spawns) operations
- Municipal sewage effluent (including effluent from sweage treatment plants without tertiary treatments in their facilities)
- Industrial wastewater discharges
- Runoff and infliltration from waste disposal sites
- Runoff from operational mines, oil fields, and industrial sites lacking sewage installations
- Overflows of combined storms and sanitary sewers

Nonpoint sources
- Widespread cultivation of N_2-fixing crops species, and subsequent N moblisation - N loadings to groundwater, ans subsequently, to receving surface water bodies (rivers, lakes, coastal zones) -Urban runoff from sewered and unsewered areas - Septic leachate and runoff from septic systems - Runoff from burned forests and grasslands - Other activities contributing to N mobilization (from long-term storage pools) such as biomass burning, land clearing and conversion, and wetland Drainage

2.2 Biological Nitrogen removal processes

2.2.1 Conventional Nitrification-Denitrification

Conventional biological nitrogen removal process is achieved by nitrification followed by denitrification process in two separate reactors. Nitrification is performed by two-step oxidative stages. First ammonium is converted to nitrite with oxygen as the electron acceptor by ammonia oxidizing bacteria (AOB) in aerobic conditions (Eq. 2.1). The most commonly recognized genus of bacteria for ammonium oxidation is *Nitrosomonas*, *Nitrosopira*, *Nitrosovibrio* and *Nitrosolobus*. In the second stage, nitrite is converted to nitrate by nitrite oxidizing bacteria (NOB) (Eq. 2.2). The most commonly recognized genus of bacteria for ammonium oxidation is *Nitrobactor, Nitrospira, Nitrospina, Nitrococcus and Nitrocystis*. The overall equation for nitrification is Eq. 2.3. Both AOB and NOB are autotrophic bacteria and they use

inorganic carbon as a carbon source [7].

Nitration [8]:

$$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow C_5H_7O_2N + 54NO_2^- + 57H_2O + 104H_2CO_3 \quad (2.1)$$

Nitration [8]:

$$400NO_2^- + NH_4^+ + 195O_2 + 4H_2CO_3 + HCO_3^- \rightarrow C_5H_7O_2N + 3H_2O + 400NO_3^- \quad (2.2)$$

Nitrification (2.1+2.2)

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad (2.3)$$

During the subsequent denitrification process, nitrite (NO_2^-) and nitrate (NO_3^-) are converted to dinitrogen gas. Most of denitrification is performed in anaerobic (or anoxic) condition by heterotrophic denitrifying bacteria. The species of denitrifying bacteria are very wide and the available organic sources for denitrifying are also very wide. The genus of denitrifying includes *Alcaligenes, Pseudomonas, Paracoccus, Thiobacillus, Bacillus* or P*ropionibacterium* and so on [9]. The equation for denitrification using methanol as carbon source is shown in Eq. 2.4.

Denitrification [8]:

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.47N_2 + 0.056C_5H_7O_2N + 1.68H_2O + HCO_3^- \quad (2.4)$$

The Nitrification-Denitrification process is used to treat the wastewater containing COD and ammonium nitrogen. When the COD is insufficient in wastewater for Denitrification process, the additional organic carbon should be dosed. Choi et al. indicated that a C/N ratio over 7 is required for nitrogen removal (above 89%) in intermittently aerated MBR system [10]. Therefore, considering with the operational cost, the Nitrification-Denitrification process is not suitable for treating the wastewater with low C/N ratio.

2.2.2 Innovation technology – Anammox (ANaerobic AMMonium

OXidation)

In 1977, based on thermodynamic calculations, Broda predict the biochemical reaction that the existence of chemolithotrophic bacteria able to oxidize ammonium to dinitrogen gas with nitrate [11]. However, there microorganisms has never been demonstrated in following decade. Until 1995, Mulder et al. found that ammonium and nitrate disappeared at the same time in the fluidized bed reactor which was treating bakery yeast wastewater effluent in the Netherland [1]. This discovery demonstrated the assumption by Broda was correct and the presence of this kind of microorganisms in nature. This novel reaction was named anaerobic ammonium oxidation (Anammox). Initially, Anammox bacteria were considered that nitrate is used as electron acceptor and the stoichiometry of Anammox reaction is shown below (Eq. 2.5). Afterward this stoichiometry has been modified by van de Graff et al. at 1995. The electron acceptor of the Anammox process is nitrite not nitrate and Anammox organisms grow with CO_2 as the carbon source [12] [13]. Subsequently, Strous et al. further formulated the stoichiometry of Anammox reaction (Eq. 2.6) using a sequencing batch reactor (SBR) based on the mass balance [14]. The advantage of the SBR for enriching Anammox bacteria is the efficient retention of sludge. By using an SBR, several important physiological parameters have been determined as Table 3 shown [14]. Compare Anammox with Nitrification, the growth rate of Anammox is really low and the doubling time of Anammox is fifteen times than Nitrification [15]

Table 3 Physiology of Anammox

Parameters	Anammox	Unit

Biomass yield	0.066	mol C (mol ammonium)$^{-1}$
Maximum specific ammonium consumption rate	45	nmol NH_4^+ (mg protein min)$^{-1}$
Maximum specific growth rate	0.0027	h^{-1}
doubling time	11	D

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \quad (2.5)$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066\,CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2.6)$$

Furthermore, suitable operating conditions for growth of Anammox bacteria have been determined. For example, the optimum temperature for Anammox bacterial growth temperature is between 18-40 ^oC while the maximum activity was found at 35-40 ^oC [16]. The optimum range of pH is in between 6.7-8.3 [17]. The affinity of Anammox bacteria towards ammonium and nitrite are to or less 0.1 mg In addition, the potentially negative effects of the compounds present in the wastewater have been studied as summary in Table 4. One of the most critical aspects in the Anammox process stability is substrate. Ammonium and nitrite are nutrients for anammpx, but also potential inhibiting compounds for Anammox. Strous et al. indicated that Anammox is not inhibited by ammonium up to concentration of 980 mg/L [17] however other studies observed that concentrations of ammonium of 700 or 770 mg/L resulting in an activity loss of 50% [18][19]. Because of the coherent results, recently reasearchers consider free ammonia (FA), rather than ammonium, to be the true microbial substrate and inhibitor compound of Anammox [19][20]. Fernandez et al. further investigated the

long-term effects of FA on Anammox in SBR. The results showed a total loss of Anammox activity with long-term exposure to FA levels of 35-40 mg N/L but the performance can be recovered by reduced residual FA in effluent within 1 month [19].

Another inhibiting substrate for Anammox is nitrite. It has been considered that high concentration of nitrite inhibited Anammox activity [21]. The inhibition threshold concentrations of nitrite obtained from various studies were difference (Table 4). It may be attributable to differences in biomass characteristics (Anammox species) and experiment design (operational conditions). Basing on these results, the nitrite exposure concentration below to 80 mg/L was the security concentration for Anammox.

Table 4 Inhibitor factors for Anammox

	Concentration	Unit	Effect	Substrate	Type	Reference
NH_4^+-N	980	mg N/L	No effect	Synthetic	Batch test	[17]
	770	mg N /L	-50 % activity	Synthetic	Batch test	[18]
	700	mg N/L	-50 % activity	Synthetic	Batch test	[19]
FA (NH_3-N)	38	mg N/L	-50 % activity	Synthetic	Batch test	[19]
	20-25	mg N/L	-25 % activity	Synthetic	SBR	[19]
	35-40	mg N/L	-100 % activity	Synthetic	SBR	[19]
NO_2^--N	80	mg N/L	-80 % activity	Synthetic	Fixed-bed reactor	[22]

	100	mg N/L	-100 % activity	Synthetic	Batch test	[17]
	140	mg N/L	-70% activity	Synthetic	Batch test	[19]
	280	mg N/L	-100 % activity	Synthetic	Batch test	[21]
	350	mg N/L	-50 % activity	Synthetic	Batch test	[18]
NO_3^--N	980	mg N/L	No effect	Synthetic	Batch test	[17]
	630	mg N/L	-50 % activity	Synthetic	Batch test	[18]
Chloride	50	mM	No effect	Synthetic	Batch test	[13]
	200	mM	-50% activity	Synthetic	Batch test	[18]
Phosphate	1	mM	No effect	Synthetic	Batch test	[13]
KH_2PO_4	5 or 50	mM	-100 % activity	Synthetic	Batch test	[13]
	1	mM	No effect	Synthetic	Batch test	[13]
$KHCO_3$	21	mM	-50 % activity	Synthetic	Batch test	[18]
	20 or 40	mM	No effect	Synthetic	Batch test	[13]
Sulphide	1 or 5	mM	Increase activity	Synthetic	Batch test	[13]
	0.3	mM	-50 % activity	Synthetic	Batch test	[18]

2.2.3 Single reactor High activity Ammonia Removal over Nitrite (SHARON)

The single reactor high activity ammonia removal over nitrite (SHARON) process, a technology, control further oxidation of NO_2^- to NO_3^- [23]. Compared with conventional nitrification-denitrification processes, the advantage is needless oxidation of nitrite to nitrate results in the lower aeration cost and the dosage of organic carbon in denitrification process.

The strategies for controlling SHARON process include temperature, dissolved oxygen level, free ammonia (FA) concentration, pH and alkalinity. The maximum growth rate of AOB is higher than NOB at the operational temperature of 35°C [23]. By controlling operational temperature at 35°C and a proper sludge retention time, the AOB can retain in the reactor and NOB wash out. Because oxygen affinity of AOB is higher than NOB, the low dissolved oxygen level (0.4 mg/L) limits the growth of NOB [24]. The concentrations of free ammonia (FA) that inhibit AOB are greater than that inhibit NOB. The range of FA concentrations that begin to inhibit for AOB is 10-150 mg N/L while for NOB is 0.1-1 mg N/L [25]. Therefore keeping the higher influent ammonium concentration could inhibit NOB growth. Moreover, according to the chemical equilibrium of ammonium and ammonia in water the FA can increase by increasing the pH value (Eq.2.7 and Eq.2.8).

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \tag{2.7}$$

$$[NH_3 - N]_{free} = \frac{TAN \times 10^{pH}}{K_a / K_w + 10^{pH}} \tag{2.8}$$

where TAN is total ammoniacal nitrogen = ammonium + free ammonia

$K_a K_w = \exp [6334 / (273+t)]$

K_a is ionisation constant for ammonium (e.q., Ka at 20°C = $10^{-9.24}$)

K_w is ionisation constant for water (e.q., Kw at 20°C = 0.69 10^{-14})

t is temperature in°C If the wastewater contains high ammonium and low COD, the combination of SHARON and Anammox is an appropriate system for ammonium removal. The first full-scale SHARON-Anammox reactor in the world was started in Rotterdam (NL). The effluent, a 50:50 mixture of NH_4^+ and NO_2^- from SHARON reactor is used as a feed for following Anammox reactor [26].

2.2.4 The combination of partial nitrification and Anammox in single reactor

The combination of partial nitrification and Anammox in a single reactor is also called several names such as CANON (Completely Autotrophic Nitrogen Removal over Nitrite) [27], [28], OLAND (Oxygen-Limited Autotrophic Nitrification – Denitrification) [29] and SNAP (Single-stage Nitrogen removal using Anammox and Partial nitrification) [30]. These operational condition and nitrogen removal modes are similar. The partial nitrification / Anammox process consist of AOB and anammox bacteria. When the presence of ammonium, AOB oxidize ammonium to nitrite, consume oxygen and so create anoxic conditions the anammox bacteria need. Compared with SHARON / Anammox in series, this process needs only one reactor. This implies that the less investment costs. The key for the successful partial nitrification / Anammox process is the suppression of nitrite oxidation and avoid high toxic nitrite. The performances of partial nitrification / Anammox process in single reactor from various literatures are summaried in Table 5.

Table 5 Conversions for partial nitrification / Anammox process in single reactor

Reactor type	Volume	NVRR[a] (kg N $m^{-3}d^{-1}$)	Medium	Reference
SBR	2 L	0.075	Syntheses	[27]
SBR	1 L	0.08	Syntheses	[31]
SBR	2 L	0.12	Syntheses	[28]
Gas-life	1.8 L	1.5	Syntheses	[32]
Up-flow	1.25 L	0.77	Syntheses	[33]
RBC	44 L	1.06	Syntheses	[34]
SBR	1400 m^3	0.43	Sewage sludge digestate	[35]
SBR	300 m^3	0.3	Sewage sludge digestate	[35]
SBR	160 m^3	0.32	Sewage sludge digestate	[35]
MBBR	2.1 m^3	0.24[b]	Sewage sludge digestate	[35]
SBR	500 m^3	0.6	Sludge liquor	[36]
SBR	400 m^3	0.4	Sludge liquor	[37]
RBC	240 m^3	1.7	leachate	[38]
Moving Bed	102 m^3	1	Sludge liquor	[39]

[a] Nitrogen Volumetric Removal Rate.

[b] Not reported, value estimated based on a biofilm surface of 250 $m^2 m^{-3}$.

Chapter 3 Materials and methods

In this research work, two sequential batch reactors with working volume of 18 L and 2.5 L, respectively, were operated for treating two types of opto-electronic wastewater, from LED manufacturing plants located in Tainan, Taiwan. One of the wastewater only contains high ammonium (abbreviated as WW1), but the other wastewater contains both ammonium and COD with C/N ratio of ~0.2 (abbreviated as WW2). Studies on WW1 treatment were carried out in 18 L SBR (abbreviated as SBR-18), while studies on WW2 were carried out in 2.5 L SBR (abbreviated as SBR-2.5). Detail characteristics of opto-electronic wastewaters used in the study are given in below sections.

3.1 Sequencing batch reactor with carriers (SBR-18) for treating ammonium-rich wastewater

In order to investigate the possible application of partial nitrification and Anammox process for treating ammonium rich opto-electronic wastewater, SBR-18 was used. The details of seed sludge and feeding media used, reactor configuration and modes of operation are given below.

3.1.1 Seed sludge and feeding media used in SBR-18

Seed sludge

The original sludge in the reactor were the same with our previous study [40], which is used to treat synthetic wastewater and reported to have Anammox bacteria, Nitrosomonas-like microorganisms and denitrifiers. The concentration of VSS in the reactor was 1370 mg/L at day 0. On day 94 the reactor was inoculated with new seed sludge collected from a full-scale landfill-leachate treatment plant in Taiwan, to

increased VSS concentration in the reactor up to 2800 mg/L. Anammox bacteria, nitrosomonas-like microorganisms and denitrifiers were reported in this landfill-leachate treatment plant sludge [41]. Furthermore, a part of biomass (16 g) from the reactor was discharged and 31 g of new seed sludge was introduced in to the reactor on day 306. The initial concentration of VSS after discharge and recharge biomass was 2589 mg/L.

Feeding media

The feeding wastewater (WW1) was collected from opto-electronic industry located at Tainan, Taiwan. The collected samples were stored in a refrigerator at 4°C until used. WW1 was produced from the local scrubber where residual ammonia gas from etching process was washed. WW1 was characterized and its characteristics are shown in Table 6. As WW1 was originated from ultrapure water and pure chemicals, it was supplemented with mineral medium [27] as nutrients (Table 7). Moreover, the additional $NaHCO_3$ was added to the wastewater for providing the inorganic carbon source which Anammox and nitrifying bacteria needed in the lack of inorganic carbon condition. The pH of the wastewater was adjusted to 7.8-8.0 with HCl before introducing it to the reactor.

Table 6 Main characteristics of raw opto-electronic wastewater (WW1)

Parameter	Value[a]	n[b]
COD	13.5 ± 0.7	2
TKN	3799 ± 9	2
NH_4^+-N	3712 ± 120	2
NO_2^--N	-	2

NO_3^--N	-	2
PO_4^{3-}-P	-	2
pH	9.7±0.1	2
Alkalinity as $CaCO_3$	5785±3341	2

a: all units are in mg/L, except pH

b: The number of times wastewater sample analyzed and collected from the industry

Table 7 Compositions of mineral medium and trace elements supplemented to WW1 & WW2

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

3.1.2 Experimental set-up and reactor system

Reactor configuration

A sequencing batch reactor (SBR) with working volume of 18 L (SBR-18) was

established for treating WW1 and the carrier of polyurethane spheres (diameter is 3 cm, total of 100) was employed as a bacterial support. Figure 3 shows the set-up of SBR-18 and the configuration of carriers. The carriers were stuffed in the bottom of the reactor at day 0. However, the reactor with carriers in the bottom had poor diffusion of sludge and nutrients. The poor diffusion will be a limiting factor for the reaction rate of bacteria at high loading rate condition. Therefore, the carriers were arranged in a hollow center of circle in the reactor on day 45 till the end of experiment.

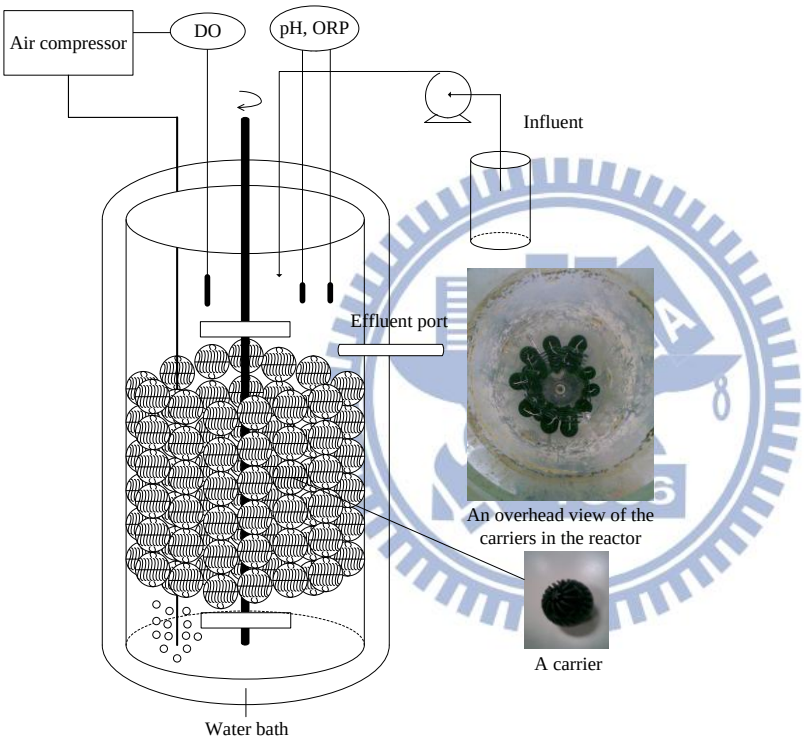


Figure 3 Configuration of SBR-18

Operation strategy of SBR-18

SBR-18 was operated in cycles of 24 h. Each cycle consists of 23.4 h for feeding and reaction, 0.45 h for settling and 0.15 h for decanting as shown in Figure 4 . The feeding period was 12 h. The fed batch strategy was adopted in order to avoid a shock loading.

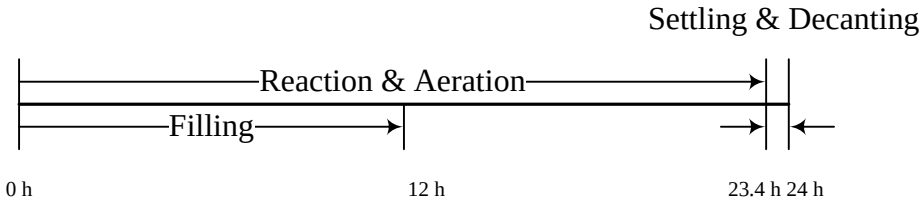


Figure 4 Operated cycle of SBR-18.

Experimental conditions

The experimental conditions were classified into 4 stages and each stage consists of several phases as summarized in Table 8. During stage I, the configuration of carrier in the reactor had been switched the bottom to the side. During stage II and IV, the loading rates were increased stepwise. However the loading rates were decreased gradually in stage III. The loading rates were determined by reducing the hydraulic retention time (HRT) and/or diluting the wastewater with deionized water. The alkalinity concentration in the reactor was controlled by dosing bicarbonate ($NaHCO_3$) in influent wastewater to keep the alkalinity of effluent at ~850 mg/L as $CaCO_3$ during stage II~ IV, while the alkalinity concentration was not controlled in stage I. The operating temperature was maintained at 37°C during stage I, subsequently the temperature was reduced to 25°C (from day 95 to 199). Because of the broke down of the water bath the temperature was out of control on day 200, and subsequently the reactor was operated under ambient temperature. The literature shows that Anammox bacteria can successfully grow between 18~30°C, and only when temperature higher than 45°C or lower than 15°C, the Anammox bacteria will lost its activity [16]. The temperature in the reactor was in ambient until the cold current come and result in temperature dropped to 17 °C. Therefore, the water bath kept the temperature at 25°C during day 418~445. During the experiment period, the sludge retention time (SRT) was maintained at

infinitive except the first and second addition of sludge.

Table 8 Operating parameters at different stages in the SBR

Stage	Phase (d)	Temperature [a]	Inf. NH_4^+-N (mg/L)	HRT (d)	NLR ($g/m^3 \cdot d$)	Remark
I	0~83	37	183	18	10	From day 44 on, carriers were arranged in a hollow center of circle.
	95~164	25	200	6	33	New seeding was introduced to the reactor at day 95.
II	165~199	25	400	4	100	Nitrogen loading rate increased stepwise.
	200~216	32.7±0.9	800	4	200	
	217~234	33.5±1.4	1600	4	400	
III	235~247	34.1±0.6	2400	4	600	Reduced Inf. NH_4^+-N stepwise and fixed NO_2^--N in effluent at ~50 by controlling aeration rate. New seeding was introduced to the reactor again and part of sludge was discharged.
	253~262	34.7±0.4	1600~800		400~200	
	263~305	33.2±1.8	434		109	
	306~373	28.0±3.0	434		109	
IV	374~391	22.0±1.1	922	4	230	Nitrogen loading rate increased stepwise.
	392~417	21.4±1.2	1329		332	
	418~423	25	1787		447	
	424~433	25	2192		548	
	434~442	25	2454		614	
	443~453	25	3181		796	
	445~487	25	3636		909	

[a]: Temperature was controlled by using a water bath or in ambient.

3.1.3 Measurements in SBR-18

For getting a grip on the reactor performance, ammonium, nitrite, nitrate, COD,

alkalinity, suspended solids (SS), volatile suspended solids (VSS), MLSS, MLVSS were monitored twice or thrice per week. The process parameters such as pH, ORP and DO were monitored using pH, OPR meter (Suntex PC3200, Taiwan) and DO meter (Insite IG model 1000CE, America), respectively. Moreover, process parameters were recorded manually in the end of the SBR cycle.

3.2 Sequencing batch reactor (SBR-2.5) for treating the wastewater with low C/N ratio

In order to investigate the possible application of partial nitrification and Anammox process for treating opto-electronic wastewater with very low C/N ratio, SBR-2.5 was used. The details of seed sludge and feeding media used, reactor configuration and modes of operation are given below.

3.2.1 Seed sludge and feeding media for SBR-2.5

Seed sludge

The reactor was inoculated with SNAD seed sludge collected from a full-scale landfill-leachate treatment plant in Taiwan as mentioned above for SBR-18. As mentioned above, 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 [41].

Feeding media

The feeding wastewater (abbreviated as WW2) was also collected from opto-electronic industry located at Tainan, Taiwan. The collected samples were stored in a refrigerator

at 4°C until used and its characteristics are shown in Table 9. WW2 is a comprehensive wastewater which is mixed with several unit processes, moreover the sources of COD are cleaning solvents used in manufacturing processes such as isopropyl alcohol (IPA), acetone or acetic acid. The source of ammonium is from ammonia gas used in etching process.

Additional medium supply to WW2

Metal ion is important for growth of microorganism. For example, part of enzyme needs some molecules to form complete enzymes and this kind of enzymes called holoenzyme. Metal ions can be one of the molecules as cofactor and it can assist enzymes during the catalysis of reactions. Furthermore, many studies also provided the synthesis wastewater with metal for inoculums of Anammox bacteria. To ensure Anammox bacteria can successful growth without lacking of trace element, additional medium was supplemented with mineral medium to WW2. In addition, the present of polyvalent metal cations can help the formation of boiflocculation causing more efficient of sludge settling. The compositions of the additional mineral supplied to WW2 were the same with supplied to WW1 as shown in Table 7. Moreover, the additional $NaHCO_3$ was also added to the wastewater. The pH of the feeding wastewater was adjusted to 7.8-8.0 by HCl before introducing it to the reactor.

Table 9 Main characteristics of raw opto-electronic wastewater (WW2)

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

3.2.1 Experimental set-up and reactor system

Reactor configuration

This study was carried out in a SBR with working volume of 2.5 L (SBR-2.5) for treating WW2. Figure 5 shows the schematic diagram of the 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 via a stirrer at a suitable rotation rate (~ 125 rpm).

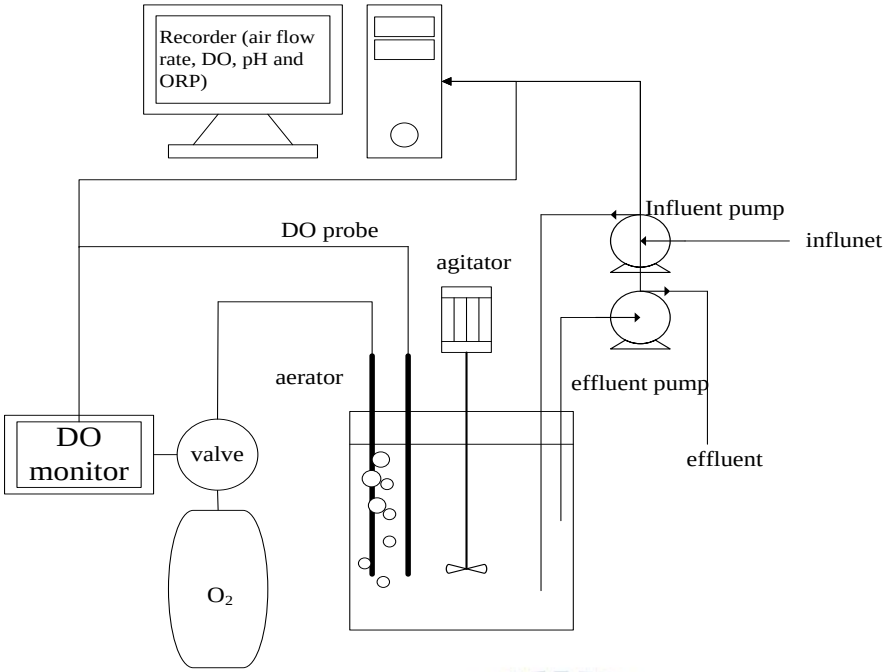


Figure 5 Configuration of SBR-2.5

Operation strategy of SBR-2.5

The operating mode of SBR-2.5 was the same as used in SBR-18 shown in Fig. 4.

Experimental conditions

The reactor operation was carried out over eight stages (stage I to VIII) with varying NLR from 16 to 428 ($g/m^3 \cdot d$). The organic loading rate (OLR) was also increased with as NLR increased. The loading rates were determined by reducing the hydraulic retention time (HRT) and/or diluting the wastewater with deionized water. The operating temperature was maintained at 37°C during stage I and II, subsequently the temperature was reduced to 25°C (from day 87 to the end of the experiment). In view of reducing operation cost, the influence of additional medium on enrichment of Anammox bacteria was also studied in this case study. During stage VIII, supply of Ca^{2+}, Mg^{2+} and Fe^{2+} to the WW2 were stopped at the period of day 472 to day 497.

NO.	Chemical analysis[1]

Table 10 Operating parameters at different stages in the SBR-2.5

Parameter	Stage							
	I	II	III	IV	V	VI	VII	VIII
Temperature (°C)	37	37	25	25	25	25	25	25
Inf.NH_4^+-N (mg/L)	78	113	368	400	564	574	531	765
Inf. COD (mg/L)	20	20	43	105	99	99	107	159
HRT (d)	5	3	3	3	3	2.5	1.7	1.7
Duration (d)	0-59	60-86	87-118	119-163	164-191	192-288	289-415	416-497
	(59)	(27)	(32)	(45)	(28)	(97)	(127)	(82)
NLR ($g/m^3 \cdot d$)	16	34	110	120	169	230	297	428
OLR ($g/m^3 \cdot d$)	4	6	13	32	30	40	60	89

3.2.2 Measurements in SBR-2.5

Similar to SBR-18, ammonium, nitrite, nitrate, COD, alkalinity, suspended solid (SS), volatile suspended solid (VSS), MLSS, MLVSS were monitored twice or thrice per week. The process parameters such as pH, ORP and DO were monitored using pH, OPR meter (Suntex PC3200, Taiwan) and DO meter (MACH sc100, Germany), respectively. Process parameters were recorded manually in the end of the SBR cycle. Specific Anammox activity (SAA) test were also performed in order to monitor the variation of Anammox activity during day 465 to day 497. The concentrations of Ca^{2+}, Mg^{2+} and Fe^{2+} in sludge were analyzed through Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

3.3 Analytical methods

All chemical analyses were performed according to the Standard Methods [42] as shown in Table 11.

Table 11 Water quality analytical method

Item	Method
Alkalinity	Method 2320 B
TSS & SS & MLSS	Method 2540 B & D
VSS & VS & MLVSS	Method 2540 E
COD	Method 5520 B
TKN	Method 4500-Norg C
NH_4^+-N	Method 4500 NH_3-F
NO_2^--N	Method 4500 NO_2^-B
NO_3^--N	Method 4500 NO_3^-B
PO_4^{3-}-P	Method 4500-P E

The nitrogen loading rate (NLR) and organic loading rate (OLR) were calculated by using the Eq. (3.1) and (3.2) , respectively.

$$NLR = \frac{\text{inf. } NH_4^+ - N}{HRT} \quad (3.1)$$

$$OLR = \frac{\text{inf. COD}}{HRT} \quad (3.2)$$

3.4 Biological activity analyses

3.4.1 Specific Anammox activity (SAA) test

Anammox is the reaction where Anammox bacteria convert both ammonium and nitrite to nitrogen gas. According to the characteristics of gas production and ideal gas law, specific Anammox activity (SAA) tests were invented to assess the reaction rate of Anammox [18].

SAA tests were performed at the experimental period VIII of SBR- 2.5. In this period SAA were performed to assess Anammox activity per unit VSS inside the reactor. With the measurement of VSS inside the reactor and SAA of sludge, the nitrogen volumetric removal capacity (NVRC) was calculate by using equation (3.1) and it represents the total Anammox activity inside the reactor. SAA tests were performed once per 3-5 days, and each test needs 150 ml of sludge. The experimental procedure is introduced in the following paragraphs.

Nitrogen volumetric removal capacity =

$$NVRC(gN_2 - Nm^{-3}d^{-1}) = SAA(gN_2 - N(gVSS)^{-1}) \times VSS(gVSSm^{-3}) \quad (3.3)$$

SAA tests procedure

The tests were performed as described by Dapena-Mora et al [18]. The chemical solutions used in this test include (1) NH_4Cl solution (2300 mg N/L) (2) $NaNO_2$ solution (2300 mg N/L) (3) Phosphate buffer solution (0.14 g/L KH_2PO_4, 0.75 g/L K_2HPO_4 and 0.5 g $KHCO_3$ g/L. All of these chemical solutions were purged with N_2 gas for 10 min before use.

The serum bottles with a total volume of 67 ml and rubber caps were used for SAA tests. The sludge was washed twice with phosphate buffer solution before it introduced to serum bottle. Each serum bottle was filled with the sludge of 53.6 ml, NH_4Cl solution of

1.7 ml and $NaNO_2$ solution of 1.7 ml. Therefore, the total volume of liquid phase was 57 ml and the gas phase was 10 ml. Moreover, the concentrations of NH_4^+-N and NO_2^--N inside the bottles were 70 mg N/L. The headspace was gasified with N_2 to remove the oxygen. The initial pH value was always about 7.7. The serum bottles were placed in a thermostatic shaker, at 125 rpm and 25℃. The initial pressure was equalized to the atmospheric one. The pressure in the headspace was monitored by using a pressure meter (Copal Electronics model PG-100N) with a needle. The duration of the test was 4 hours and the measurement of pressure was carried out at every hour. All of the tests were performed in triplicate.

Calculations for SAA tests

Specific Anammox activity (SAA)

The N_2 gas production rate was calculated from the slope of the curve describing the pressure increase in the bottle along the time

$$\frac{dN_2}{dt} = \frac{\alpha \times V_G}{R \times T} \quad , \text{ mol } N_2 \text{ hr}^{-1} \tag{3.4}$$

where V_G is volume of gas phase (0.01 L), R the ideal gas constant 0.0820575 (atm l mol^{-1} K^{-1}), T the temperature (K) and α the slope of pressure increase in the bottle along the time (atm).

The SAA is calculated from the N_2 gas production rate divided by the VSS concentration inside the bottle.

$$SAA = \frac{\frac{dN_2}{dt} \times 28}{X \times V_L} \times 24, \text{ g } N_2\text{-N (g VSS)}^{-1}\text{d}^{-1} \tag{3.5}$$

where 28 is molecular weight of N_2 (g N/mol), 24 the unit conversion factors from hour to days (24 hr/day), X the biomass concentration inside the bottle (g VSS/l) and V_L the

volume of liquid phase in the bottle (0.057 L).

3.4.2 Nitrate Uptake Rate (NUR) test

To verify the presence of denitrification reaction in the reactor, the NUR test was carried out to assess the activity of denitrifying bacteria. The NUR tests were performed in a serum bottle with a total volume of 250 ml and gas-tight rubber caps. The sludge was taken from reactor and washed twice with mineral medium before it introduced to serum bottle. Nitrate (as sodium nitrate) and COD (as glucose) were added to a final concentration of about 90 mg N/L and 85 mg O_2/L, respectively. The composition of the mineral medium was: $KHCO_3$ 1.25, KH_2PO_4 0.025, $CaCl_2 \cdot 2H_2O$ 0.3, $MgSO_4 \cdot 7H_2O$ 0.2, $FeSO_4$ 0.00625 and EDTA 0.00625. The serum bottles were made anaerobic by flushing with pure nitrogen gas and were placed in a thermostatic shaker, at 125 rpm and 25℃. Nitrate, nitrite and COD were measured over time during 24 hours.

3.5 Polymerase chain reaction (PCR)

To confirm the activities of nitrifiers, Anammox and denitrifiers in the sludge of SBR-18 and SBR-2.5, 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 sterilized 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 [43], for

nitrite oxidizing bacteria (NOB) was nirS-1F/nirS-6R [44], for denitrifying bacteria was cnorB-2F/cnorB-6R [45], for Anammox bacteria were Brod541F/Amx820R [46], [47] and AnnirS379F/AnnirS821R [48]. 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 checked by agarose gel electrophoreses and DNA sequencing. For qPCR analysis, primers BACT1369F/PROK1492R [49] and Amx809F/Amx1066R [50] were used to detect the eubacteria and most 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 30s at 95℃ and 40 cycles of 5s at 95℃, 5s at 54℃ for BACT1369F/PROK1492R, or 58℃ for Amx809F/Amx1066R followed by a dissociation stage (95℃ for 15 seconds, 65℃ for 15 seconds, followed by a slow ramp to 95℃). The melt curve appeared no detectable peaks that were associated with primer-dimer artifacts and no other nonspecific PCR amplification products were observed. Specificity of the qPCR products were also checked by agarose gel electrophoreses.

Chapter 4 Results and Discussion

4.1 Sequencing batch reactor with carriers (SBR-18) for treating ammonium-rich wastewater

4.1.1 Characteristics of opto-electronic industrial wastewater

The characteristics of optoelectronic industrial wastewater are shown in Table 6. It is evident from the Table that the wastewater is highly alkaline in nature (pH, 9.7), very rich with inorganic nitrogen as the concentrations of NH_4^+-N and TKN are almost similar, and depleted with organic carbon source. Very high NH_4^+ ion concentration (3795 mg/l) without COD suggesting the wastewater is very complex in nature.

4.1.2 Nitrogen removal performance

SBR-18 was used to study the ammonium rich wastewater treatment. The overall study was divided into different stages viz (Table 8). Stage I – Reactor start-up (day 0-164); Stage II – increasing nitrogen loading (days 165-234); Stage III – Inhibition and recovery of reactor performance (235-373 d); Stage IV – Reactor performance at very high nitrogen loading rate (374-487). The concentrations of NH_4^+-N in influent, HRT and NLR in each phase along with the duration of each phase is mentioned in Table 8.

4.1.2.1 Stage 1: Reactor start-up (days 0-164)

The reactor used in this study was previously used to develop a SNAD process using synthetic wastewater [40]. The synthetic wastewater was replaced with the real world opto-electronic wastewater. The carriers were also introduced into the reactor to support the microbial growth. The reactor was started with NLR and HRT of 10 $g/m^3/d$ and 18 d, respectively. The DO level was always maintained at 0.3-0.4 except day17~day 19 (Figure 6).The high DO resulted in high nitrate level in effluent and inhibited Anammox

activity, which resulted in the decreasing TN removal efficiency (Figure 7). However, Anammox activity recovered itself when DO level was maintained at low level (day 25-44). Strous et al also indicated that high DO is a reversible factor for Anammox activity [51].

In order to improve the diffusion of sludge and nutrients, the configuration of carriers was changed at day 45. However, TN removal efficiency reduced dramatically while nitrate concentration in the effluent increased sharply during days 50-88, this phenomenon was similar to the previous condition. The shock due to DO contamination during the period of the change in configuration of carriers might be the probable reasons for this decrease activity of the reactor. Moreover, the ratio of nitrite and nitrate production to ammonium conversion ($Y_{(NO_2^- + NO_3^-)/NH_4^+}$) in this stage were always higher than theoretical value in this stage (Figure 8), which indicates that partial nitrite was further oxidized to nitrate and suggests the enrichment of nitrite oxidizing bacteria (NOB) in the reactor .

Therefore, to improve the reactor performance fresh seed sludge was inoculated in the reactor on day 95 and NLR increased to 33 $g/m^3/d$, while HRT reduced to 6 d. The reactor performance started to recover and TN removal and NH_4^+-N removal increased to 60% and 80%, respectively on day 122 (Figure 7). After day 150, the TN removal and NH_4^+-N removal were sharply decreased to 40% and 80%, respectively. Low NLR (less than 100 $g/m^3/d$) during this start-up period might be the reason for poor and unstable performance of the system. As below this NLR, NOB such as nitrite-oxidizing *Nitrobacter* and *Nitrospira* species start developing in the system [28]. 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. The average concentration of NO_3^--N in the reactor during this period (days 15-80) was

found to be 75 mg/L. This high concentration of NO_3^+-N in the reactor suggested the presence of NOB in the reactor. Therefore, the NLR was further increased to 100 g/m^3/d on day 165 and reactor performance at increasing nitrogen loading rates was studied. Figure 9 shows the profiles of pH and alkalinity in the effluent. The DO was maintained below 0.5 mg/L for better growth of Anammox bacteria. The pH inside the reactor was maintained between 7.0 and 8.0 by adding the alkalinity in the reactor.

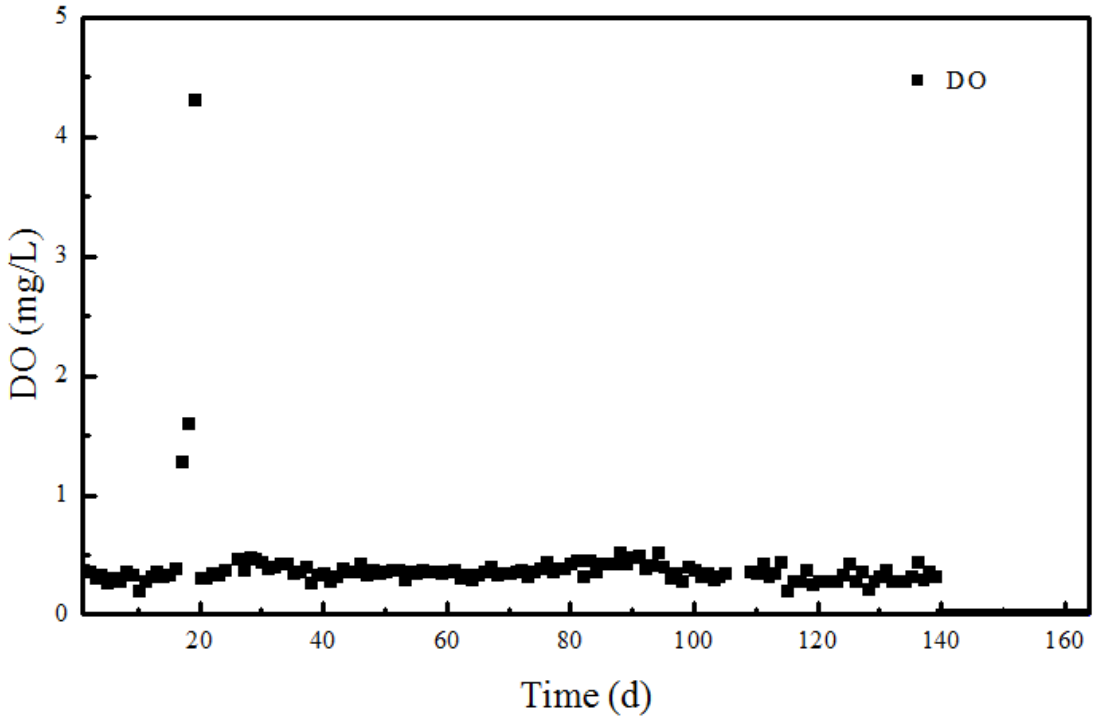


Figure 6 Profile of dissolved oxygen in SBR-18 during stage I.

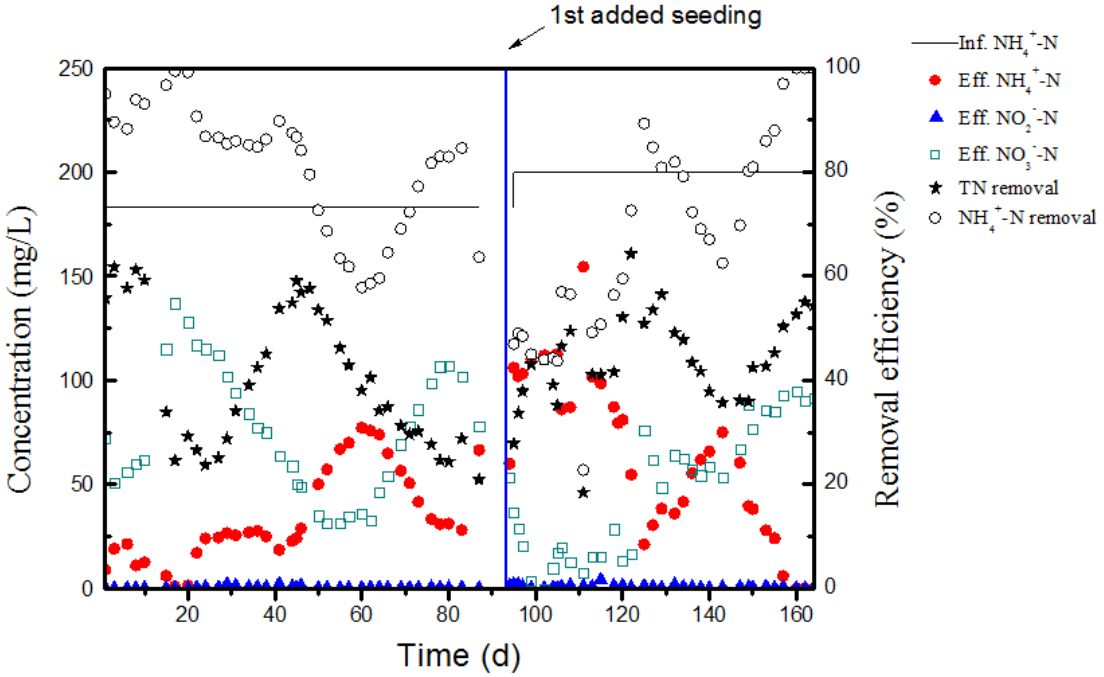


Figure 7 Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies in stage I of SBR-18.

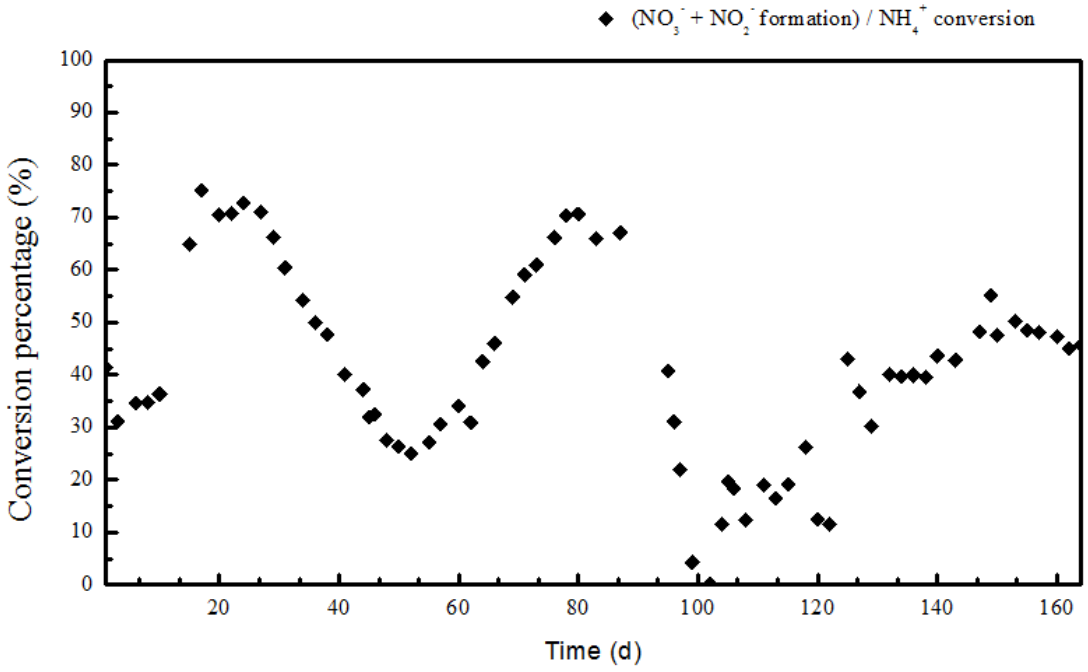


Figure 8 The temporal variation of conversion efficiency ($Y_{(NO_2^- + NO_3^-)/NH_4^+}$) during stage I of SBR-18.

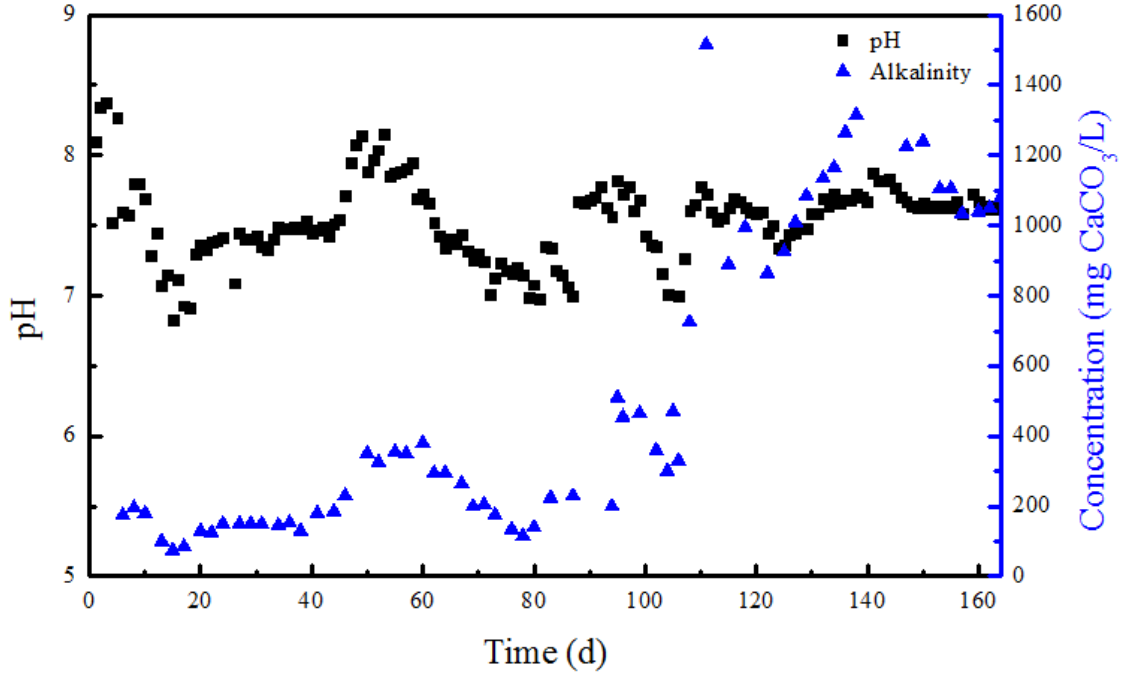


Figure 9 Time profiles of pH and alkalinity in effluent during stage 1 of SBR-18.

4.1.2.2 Stage II: Increasing nitrogen loading (days 165-234)

During this period the NLR was increased stepwise from 100 to 400 g m^{-3} d^{-1}, which represents 1600 mg/L NH_4^+-N concentration in influent. The HRT was reduced from 6 d to 4 d and kept constant at this value throughout the experiment. From day 165 onwards, the nitrite was gradually accumulated in the reactor (Figure 10) and the value of $Y_{(NO_2^- + NO_3^-)/NH_4^+}$ was gradually decreased (Figure 11). This result indicated that NOB did not consume all available nitrite produced by AOB and the nitrite was used by Anammox bacteria. In addition, this accumulated level of nitrite (~35 mg N/L) in this stage is not inhibiting Anammox reaction according to Strous et al [17]. The TN removal and NH_4^+-N removal increased exponentially to 90% and ~100%, respectively during this stage and maintained at these values for more than four times of HRT (Figure 10). Similar to stage I, DO was maintained below 0.5 mg/L and pH was maintained between 7.0 and 8.0 by adding the alkalinity in the reactor (Figure 12).

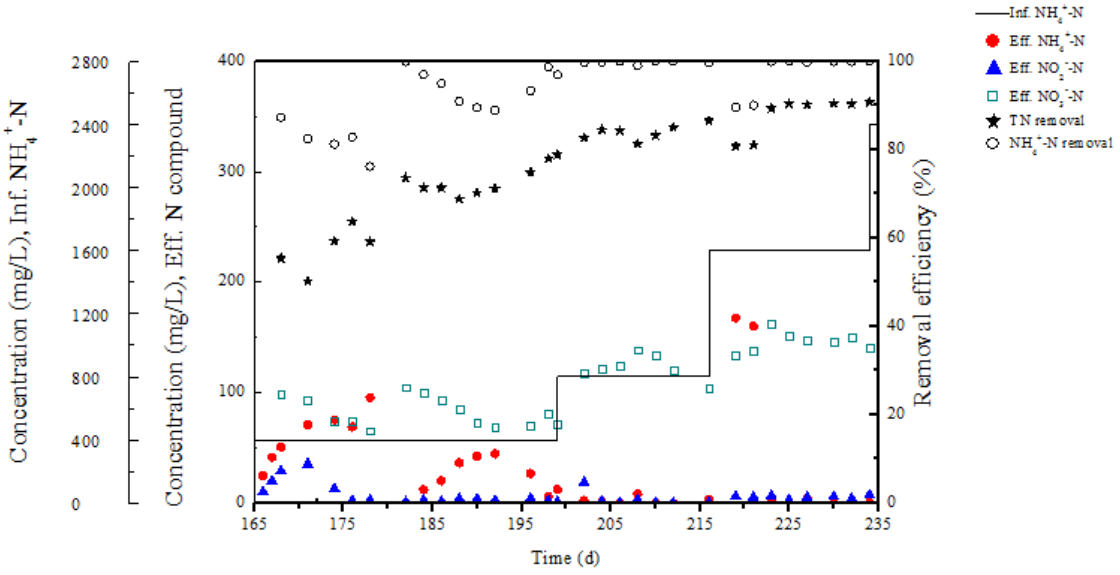


Figure 10 Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies in stage II of SBR-18.

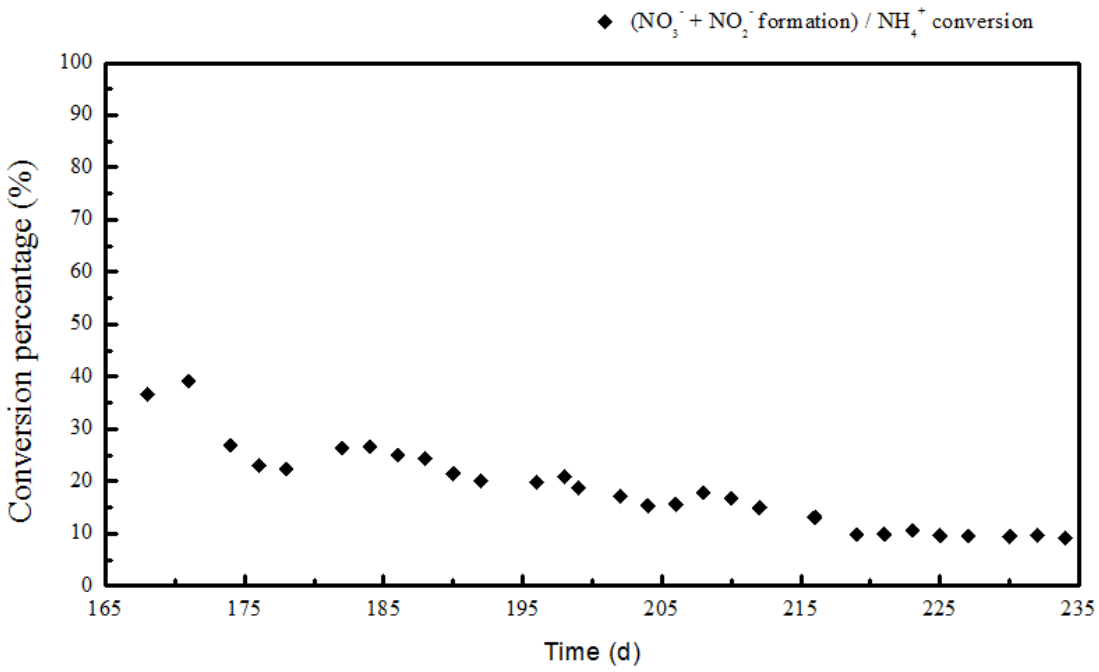


Figure 11 The temporal variation of conversion efficiency ($Y_{(NO_2^- + NO_3^-)/NH_4^+}$) during stage II of SBR-18.

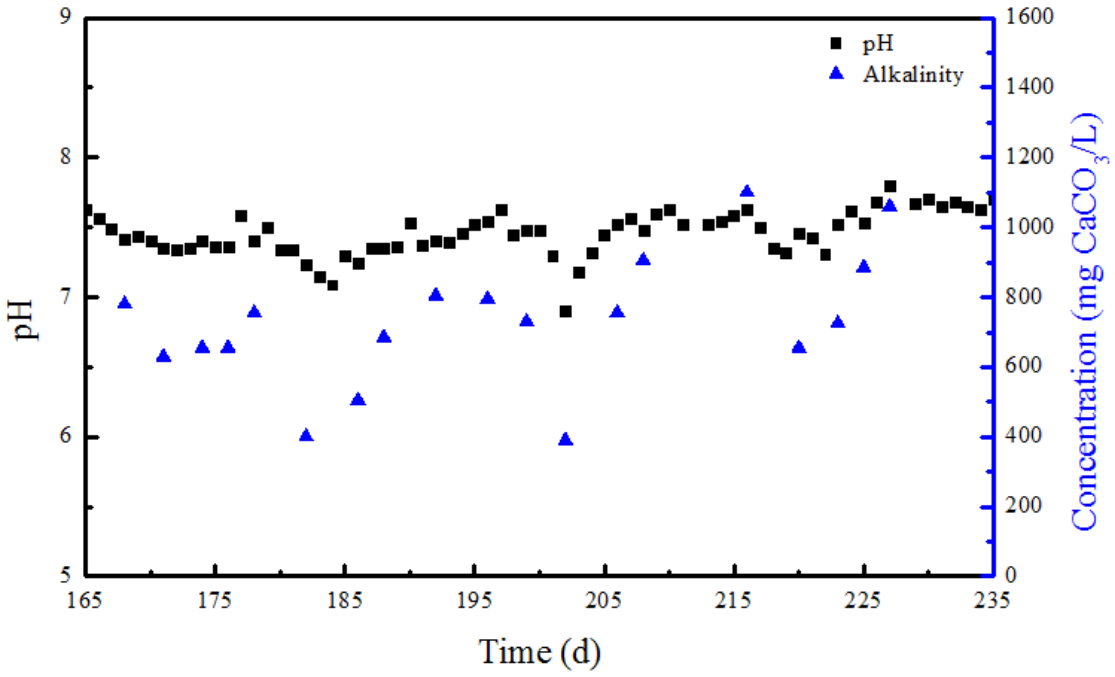


Figure 12 Time profiles of pH and alkalinity in effluent during stage II of SBR-18.

4.1.2.3 Stage III: Inhibition and recovery of reactor performance (235-373 d)

The system successfully treated wastewater with influent NH_4^+-N concentration of 1600 mg/L i.e NLR of 400 g m^{3-1} d^{-1}. However, as soon as the influent NH_4^+-N concentration increased to 2400 mg/L (NLR of 600 g m^{3-1} d^{-1}) and the aeration rate also increased from 0.4 NL/min to 0.6 NL/min, sharp decrease in reactor performance was observed. The accumulation of high nitrite concentration in the reactor was observed and the highest NO_2^--N concentration was 166 mg/L. Sudden increase in aeration rate (0.6 L/min) and insufficient Anammox activity could be the most probable reason for this. After day 247, feeding and aeration into the reactor was stopped until the nitrite level decreased to 10 mg/L. The NLR was decreased stepwise to 200 g m^{3-1} d^{-1} and aeration at 0.5 L/min was introduced into the reactor. The effluent concentrations of NH_4^+-N, NO_2^+-N and NO_3^+-N were increased to 1000 mg/L, 100 mg/L and 40 mg/L, respectively, between day 253-262 (Figure 13). Therefore, NLR was further decreased (on day 263) and maintained between 100-125 g m^{3-1} d^{-1} for 43 days (more than 10 times of HRT).

The reactor performance was not improved at this NLR, in spite of nitrite in effluent level fixed at ~50 mg N/L. The TN and NH_4^+-N removal were in range of 5-15%, respectively, between days 263-305. The average concentrations of MLSS and MLVSS were 3300 mg/L and 1700 mg/L between days 235-305. On day 306, part of original sludge (16 g) was discharged and fresh seed sludge (31 g) was added into the reactor and the NLR was kept to 109 g m^{-3} d^{-1} for 68 days (17 times of HRT). The cumulate level of nitrite (92 mg N/L) was observed again, while this time Anammox perform temporary negative effect. The performance of reactor recovered as we observed 90% and 100% of TN and NH_4^+-N removal, respectively between days 340-373. Similar to pervious stage, pH was maintained between 7.0 and 8.0 by adding the alkalinity in the reactor (Figure 14).

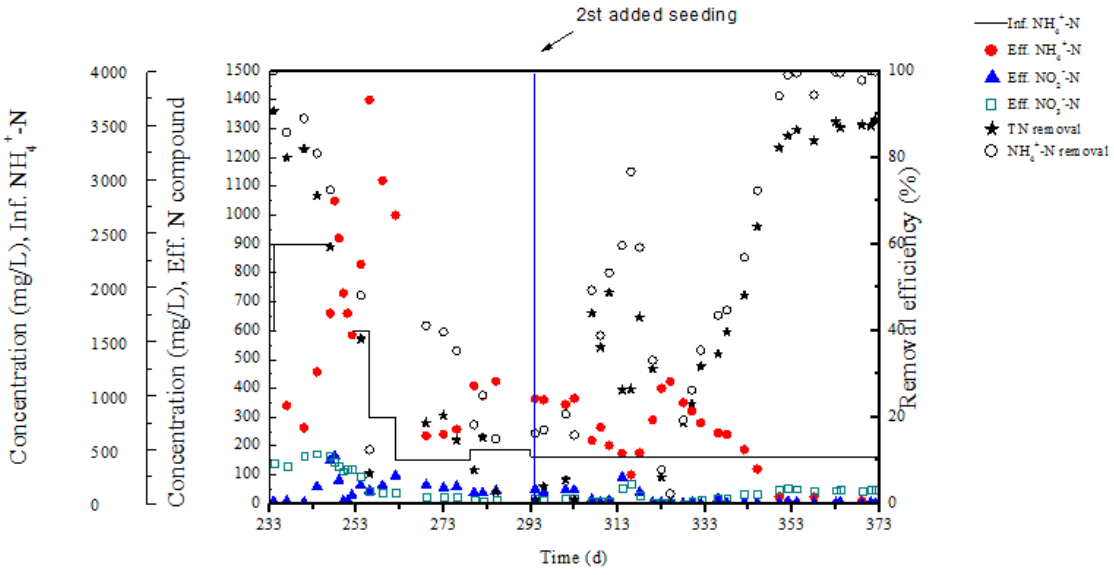


Figure 13 Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies in stage III of SBR-18.

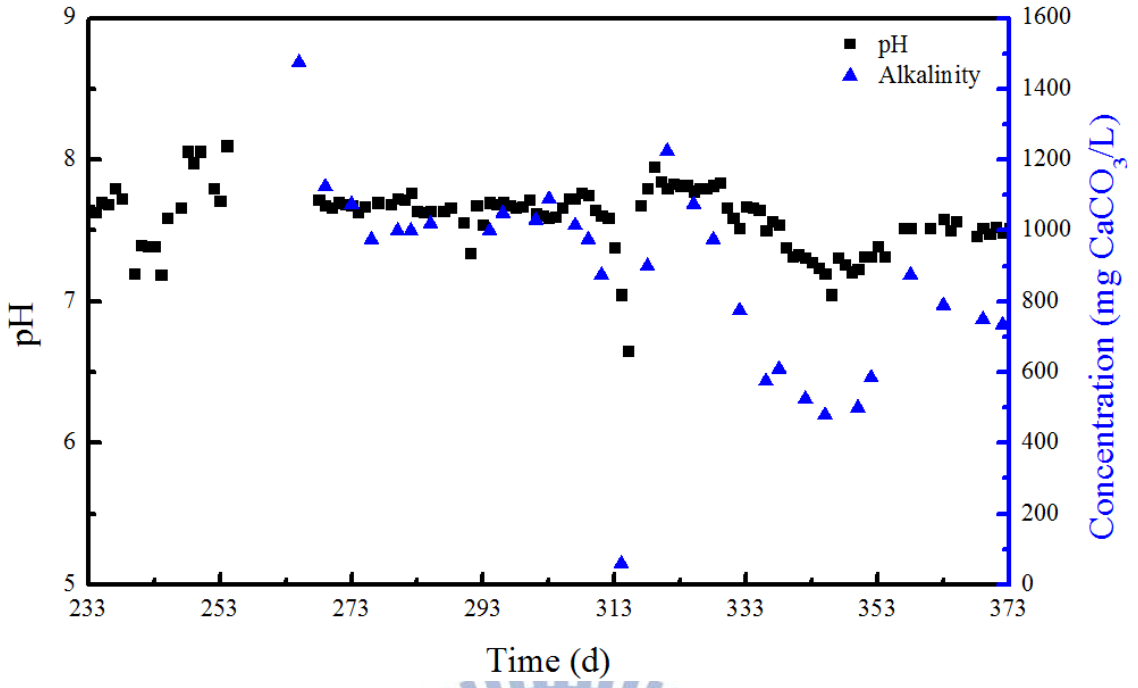


Figure 14 Time profiles of pH and alkalinity in effluent during stage III of SBR-18.

4.1.2.4 Stage IV: Reactor performance at very high nitrogen loading rate (days 374-487)

The strategy of controlling nitrite level was very important for Anammox. In order to avoid nitrite accumulation because of insufficient Anammox activity, the nitrite level was monitored in following 4 hours of the feeding phase and aeration rate adjusted gradually for different NLR. In this phase, the NLR was increased exponentially from 109 to 909 g m^{3-1} d^{-1} (the maximum possible NLR). At NLR of 909 g m^{-3} d^{-1}, the influent NH_4^+-N concentration was 3636 mg/L, which represented the concentration of NH_4^+-N in the real world optoelectronic industrial wastewater used in this study. The SBR was run about 1 month (7 times of HRT) successfully to treat this high NLR (0.9 Kg m^{3-1} d^{-1}). The average TN removal and NH_4^+-N removals were 90% and 100%, respectively (Figure 15), in this phase. The average effluent concentrations of NH_4^+-N and NO_2^+-N were less than 50 mg/L and 15 mg/L, respectively. MLSS and MLVSS increased gradually in this stage and stabilize at concentration of 9500 mg/L and 6500

mg/L, respectively (Figure 17).

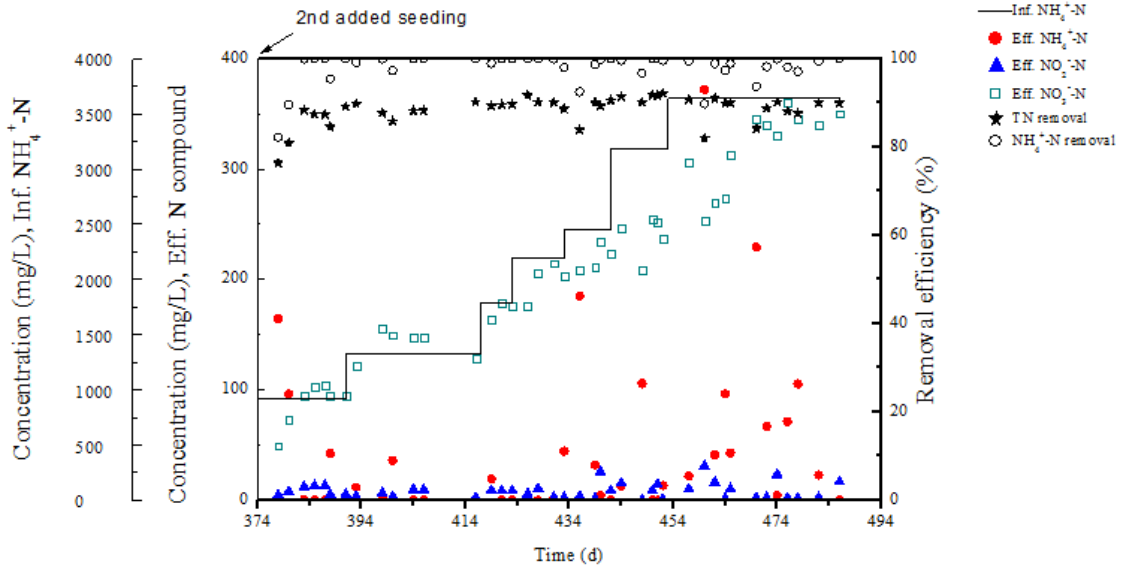


Figure 15 Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies in stage IV of SBR-18.

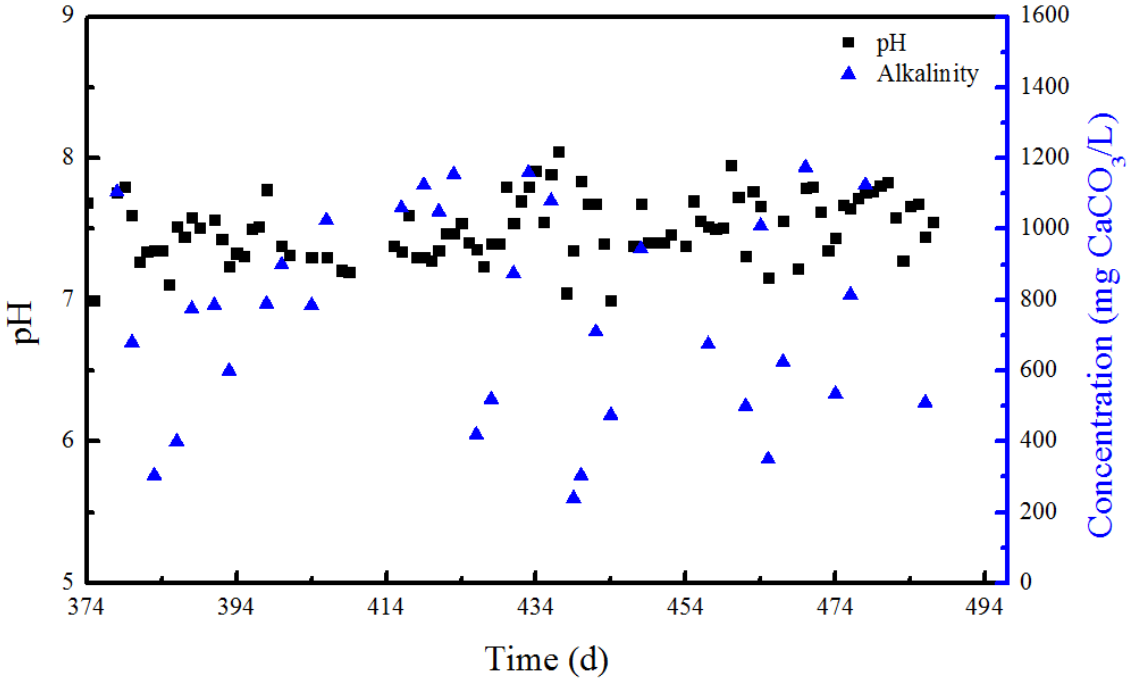


Figure 16 Time profiles of pH and alkalinity in effluent during stage III of SBR-18t.

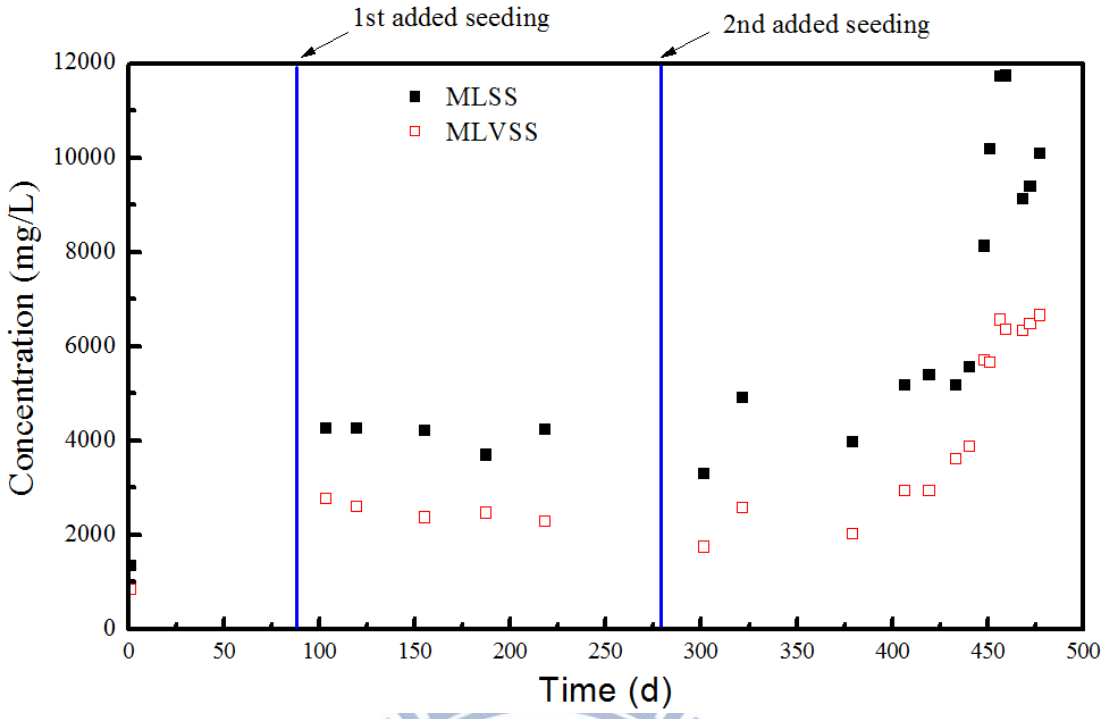


Figure 17 Profiles of MLSS and MLVSS in SBR-18.

4.1.3 Results from PCR

To know the microbial community present in the reactor, PCR experiments were carried out using specific primers for AOB, Anammox bacteria (including primers for Kuenenia stuttgartiensis (KS) and Brocadia anammoxidans (BA)) and NOBs. Fig. 18 shows the PCR results of samples taken on day 229 from SBR-18. Bright DNA bands (around 500 bp and around 900 bp) exist on the lanes of amoA targeting AOB and on the lane of Nitro and NSR targeting NOB, respectively. For detecting most anammox bacteria and specific anammox species - KS and BA, bands near 500 bp, 100 bp and 300 bp can be seen on the lanes of AnnirS, KS, and BA, respectively. These results suggest that AOB, NOB and Anammox (BS and KS) bacteria were present in the reactor on day 229. However, the active population of NOB must have been very low in the reactor since the value of η was 10% on day 229.

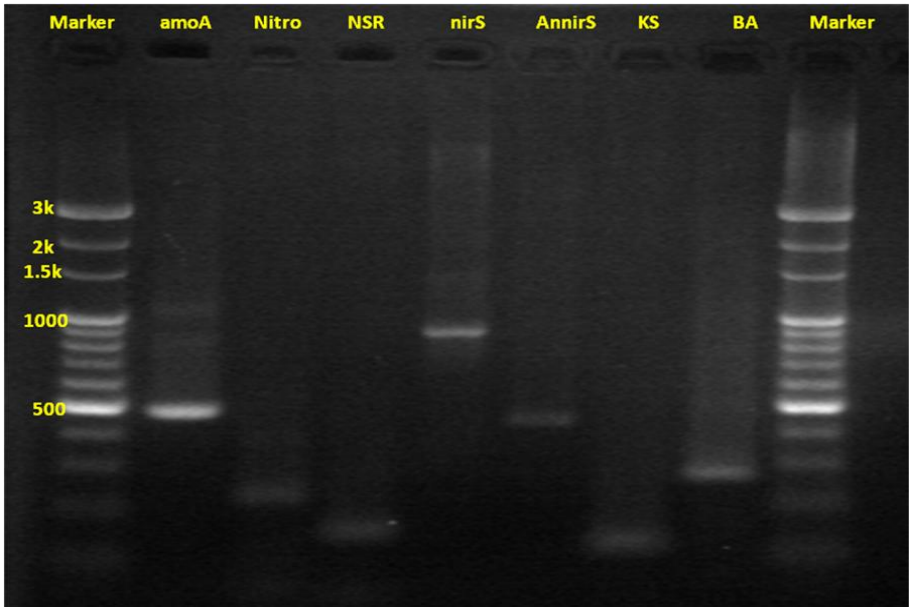


Figure 18 Results of PCR by performing agarose gel electrophoreses. The sample was taken on day 229 from SBR-18.

Fig. 19 shows the PCR results of sample taken on day 304 from SBR-18. Presence of AOB and NOB (the lane of nirS) in the reactor was evident from the Fig. 19. A very faint band in the lane of TA (total Anammox) in Fig. 19 compared to Fig. 18 suggests that Anammox activity is very little in the reactor during this period and therefore, reactor performance was inhibited during days 263-305 of stage III in the SBR-18.

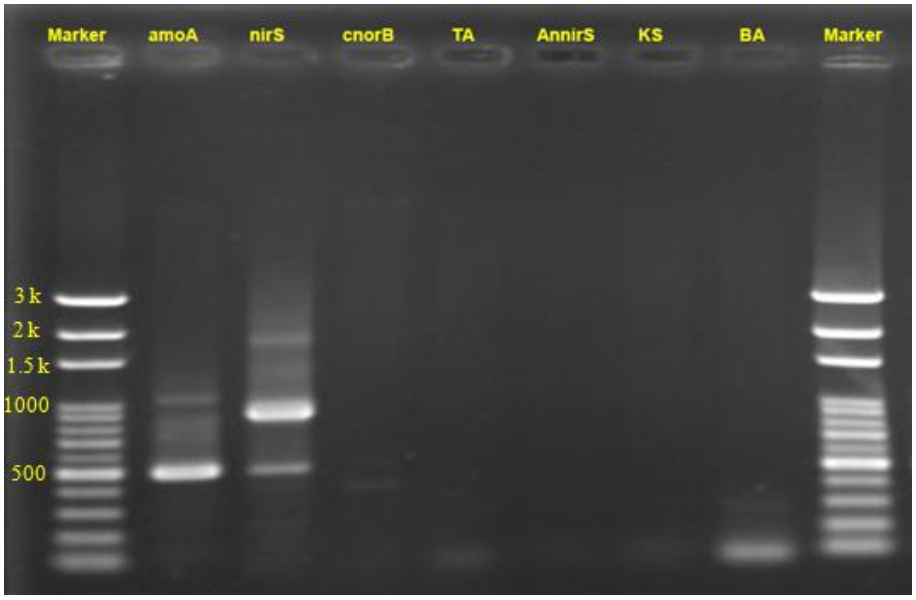


Figure 19 Results of PCR by performing agarose gel electrophoreses. The sample was

taken on day 305 from SBR-18.

PCR results of sample taken on day 487 (steady state condition under highest NLR) confirmed the co-existence of AOB and anammox bacteria in the reactor (Figure 20). Besides, results from relatively quantitative analysis of qPCR showed the cell number of eubacteria changed from 1.1×10^8 to 3.4×10^9 cells/μg DNA and most anammox bacteria changed from 4.3×10^6 to 3.1×10^8. The percentages of anammox to eubacteria were 1.8% and 9.0% on 229 d and 487 d, respectively. These results showed anammox bacteria were enriched in the reactor under steady state condition, and the ratio of anammox to eubacteria increased 5 times.

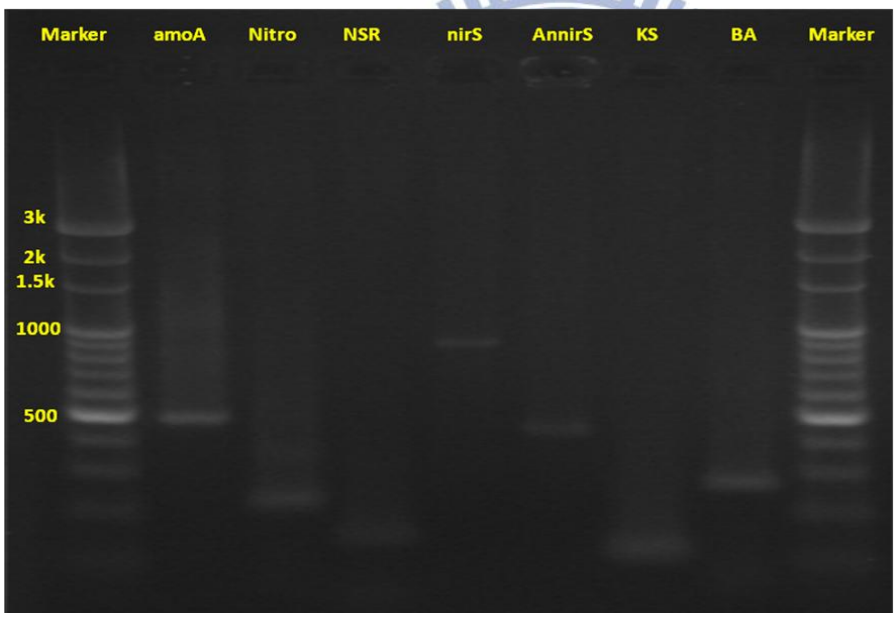


Figure 20 Results of PCR by performing agarose gel electrophoreses. The sample was taken on day 487 from SBR-18.

487

4.2 Sequencing batch reactor (SBR-2.5) for treating the wastewater with low C/N ratio

The nitrogen removal from opto-electronic wastewater with low C/N ratio was monitored over 16 months in SBR-2.5. As small amount of COD is present in the wastewater and the seed sludge used for start-up of SBR-2.5 contains AOB, Anammox and denitrifier, it is expected that SNAD system will develop in the SBR-2.5. Table 3 shows the operation conditions of SBR under various stages investigated in this study.

In this system, ammonium would be converted partly to nitrite by AOB (Eq. (4.1)), and subsequently Anammox bacteria would convert ammonia with nitrite to nitrogen gas (Eq. (4.2)). The overall equation for this process is described as Eq. (4.3). The ratio of nitrite and nitrate production to ammonium conversion ($Y_{(NO_2^- + NO_3^-)/NH_4^+}$) was calculated according to Eq. (4.4) and was used to evaluate the performance of the SNAD system in the reactor. Theoretically, 100% ammonia should produce 88% nitrogen gas with 11% nitrate. This suggests that 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.

$$1\ NH_3 + 1.5\ O_2^- \longrightarrow 1\ NO_2^- + H_2O + H^+ \quad (4.1)$$

$$1\ NH_3 + 1.32\ NO_2^- + H^+ \longrightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2\ H_2O \quad (4.2)$$

$$1\ NH_3 + 0.85\ O_2 \longrightarrow 0.44\ N_2 + 0.11\ NO_3^- + 0.14\ H^+ + 1.43\ H_2O \quad (4.3)$$

$$Y_{(NO_2^- + NO_3^-)/NH_4^+} = \frac{\text{Eff. } \{(NO_2^- - N) + (NO_3^- - N)\}}{\text{Inf. } (NH_4^+ - N) - \text{Eff. } (NH_4^+ - N)} \times 100\% \quad (4.4)$$

4.2.1 Nitrogen removal performance

4.2.1.1 Stage I and II: Enrichment at low NLR

Figure 21 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 days, respectively. The system was stabilized after 15 days (3 times of HRT) and the nitrogen removals (%) were in the range of 30 to 62% during 15 to 59 d (Figure 21). 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 (Figure 22) during this stage. Figure 23 shows the variation of conversion percentage ($Y_{(NO_2^-+NO_3^-)/NH_4^+}$) at different stages in the reactor and its average value was 59% at stage I. This value is higher than the theoretical conversion percentage (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 bacteria might be the most plausible reason for this high conversion percentage. Further, the high concentrations of NO_3^- in the effluent (during stage I and II) as shown in Figure 21 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 (which provide the oxygen gas into the reactor) was turned off during the stage II. The DO concentration in the reactor was come down to 0.1 mg/L (Figure 22). The NLR was increased to 34 g/m^3·d while the HRT was reduced to 3 d. However, the average conversion percentage ($Y_{(NO_2^-+NO_3^-)/NH_4^+}$: 52%) during the stage II was almost similar to stage I (Figure 23). It is quite visible from Fig. 3 that the nitration

reaction was still existed in the reactor which directly converted NH_4^+ to NO_3^- and inhibited the Anammox activity, in spite of DO concentration reduced to 0.1 mg/L.

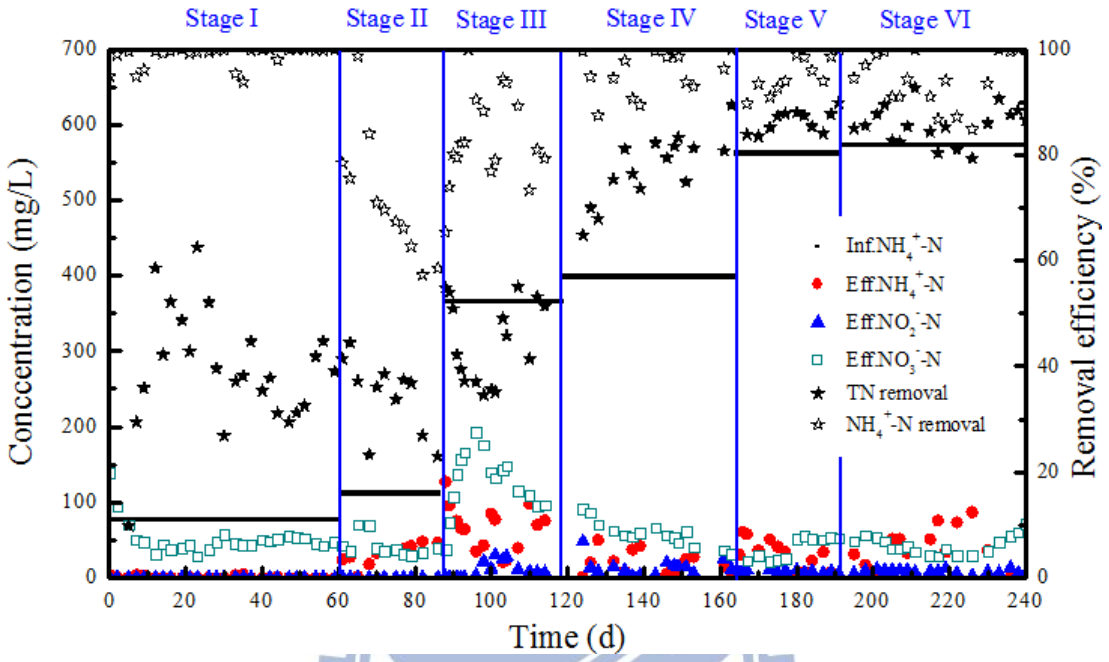


Figure 21 Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies during stage I-VI of SBR-2.5.

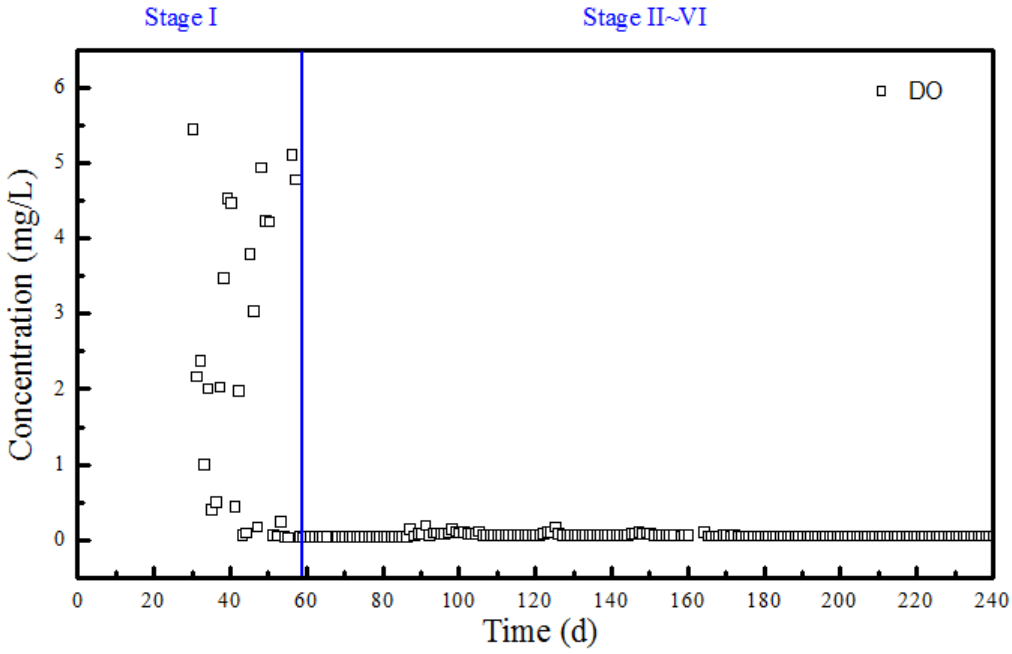


Figure 22 Profile of dissolved oxygen in SBR-18 during stage I-VI.

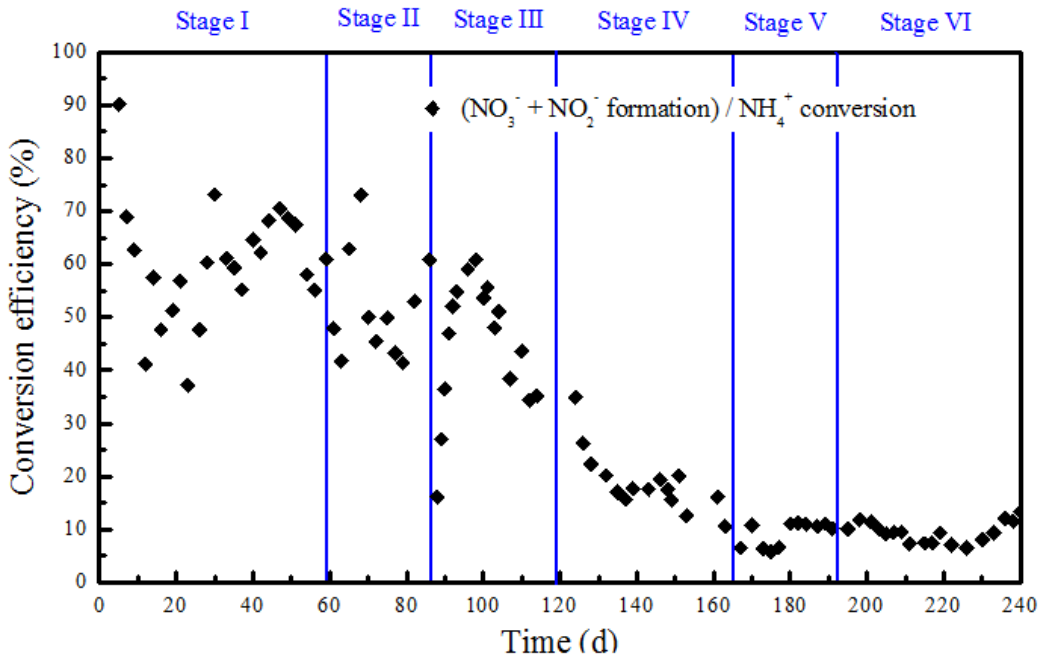


Figure 23 The temporal variation of conversion efficiency ($Y_{(NO_2^- + NO_3^-)/NH_4^+}$) during stage I-VI of SBR-2.5.

4.2.1.2 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 $g/m^3 \cdot d$ at stage III. After the second addition of seed, the DO controller was turned on and it could maintain the DO level at 0.1 mg/L throughout the experiment (from stage III to VIII) (Figure 22). The value of $Y_{(NO_2^- + NO_3^-)/NH_4^+}$ increased gradually to 61% during day 87 to day 98 but decreased gradually after day 98 suggesting that the seed sludge had adapted the new conditions and the seed microbes outgrown the NOB in the system. This can be evident from the fact that the nitrite was started to accumulate from day 96 onward in the reactor which indicates the loss of activity of NOB in the system (Figure 21). 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 was shifted from complete nitrification to Anammox reaction. Therefore, the NLR was increased to 120 $g/m^3 \cdot d$ 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% (Figure 21). 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 system the NLRs were further increased in the stage V and VI.

4.2.1.3 Stage V ~ VIII: Long term stability on nitrogen performance

Figure 21 shows that the system was stabilized during the stage V and VI. The NLR and HRT of the system were 169 $g/m^3 \cdot d$ and 3 d, respectively during the stage V. In stage VI, the NLR was further increased to 230 $g/m^3 \cdot d$ while HRT was decreased to 2.5 d. The ammonium level in effluent closed to 0 at the end of these stages (V&VI) and TN removal also closed to 90%. Therefore, NLR was further increased to 297 $g\ m^{-3}\ d^{-1}$ in stage VII and increased to 428 $g\ m^{-3}\ d^{-1}$ in stage VIII, respectively. Table 12 shows the summary of functional indicators of SBR-2.5. During stage V-VIII, the high nitrogen

removal efficiency (above 85%) and stable single stage partial nitrification / Anammox process were achieved. The average nitrogen removal rate of 383 g NH_4^+-N m^{-3}·d was obtained in stage VIII. It can be seen from the Table 12 that the average values of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ are in between of 7 to 10% during stage V-VIII. Also, the value of $Y_{(NO_2^-+NO_3^-)/NH_4^+}$ below 11% suggests the presence of denitrification reaction in the SBR. On the other hand, the NO_2^--N concentrations were below 15 mg/L except the starting days of each stage where the nitrogen loading rates were increased suddenly. This result also indicates that Anammox could not respond quickly to the sudden increase in NO_2^--N produced by AOB. In addition, the red granules of Anammox bacteria were also observed in the reactor. Figure 24 shows many red granules in the sludge while size of the biggest granule is below 1 mm.

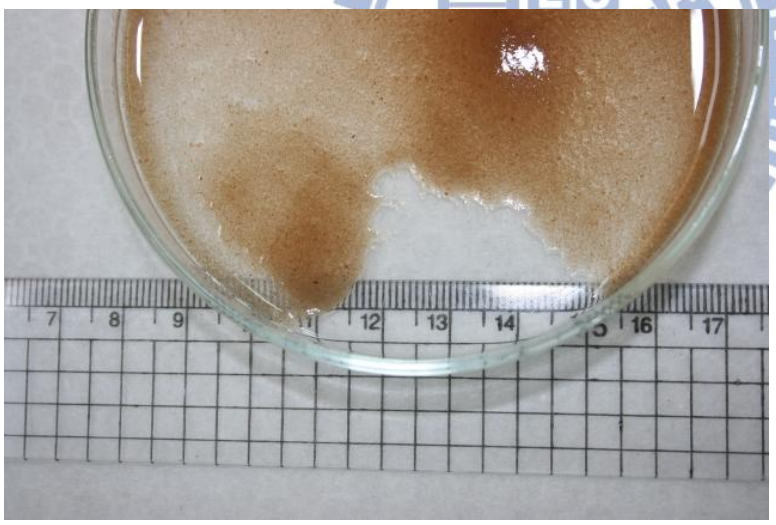


Figure 24 Sludge in SBR 2.5 at day 497.

Table 12 Summary functional indicators of SBR-2.5

Parameter	Stage							
	I	II	III	IV	V	VI	VII	VIII
Temperature (°C)	37	37	25	25	25	25	25	25
Inf.NH_4^+-N (mg^{-1})	78	113	368	400	564	574	531	765

Inf. COD (mg L^{-1})	20	20	43	105	99	99	107	159
HRT (d)	5	3	3	3	3	2.5	1.7	1.7
Duration (d)	0-59	60-86	87-118	119-163	164-191	192-288	289-415	416-497
	(59)	(27)	(32)	(45)	(28)	(97)	(127)	(82)
NLR (g m^{-3}·d)	16	34	110	120	169	230	297	428
OLR (g m^{-3}·d)	4	6	13	32	30	40	60	89
TN removal (%)	39±11	34±6	45±8	77±7	86±2	86±4	91±3	90±4
COD removal (%)	-	-	26±16	38±15	60±18	73±7	83±4	79±4
MLVSS (mg VSS L^{-1})		1822	2478	1809±295	2108	2405±87	3261±644	4582±940
$Y_{(NO_2^- + NO_3^-)/NH_4^+}$	60±11	52±10	44±12	17±7	9±2	10±2	8±2	7±2

4.2.1.4The effect of changing metal ion's concentrations on the system during stage III

In order to reduce overall cost of the system, studies on reduction of dosage supply to WW2 were carried out during last days of stage VIII. The concentrations of divalent metal ions Ca^{2+}, Mg^{2+} and Fe^{2+}, were very high in the medium shown in Table 3. Moreover, the concentrations of these three kind of metal ions were also present in WW2 (check this) while Fe^{2+} is relatively low to others as shown in Table 13. Table 14 shows the different concentrations of metal ions (Ca^{2+}, Mg^{2+} and Fe^{2+}) in the feeding wastewater. During this period, nitrogen compounds in effluent, metal ions (Ca^{2+}, Mg^{2+}, Fe^{2+}) in sludge and SAA were analyzed. The SAA tests were performed to assess and quantify the change in Anammox bacterial in the reactor. Figure 25 shows that there are no specific effects on the system performance after changing the concentrations of metal ions. The result from SAA tests (Figure 26) shows the sudden decrease in SAA at day 486, however no particular tendency toward increasing or decreasing in SAA was observed by changing the concentrations of the metal ions in the system. Since, part of

sludge was used to carry out the SAA tests, the decrease in VSS concentration was observed. By calculating the nitrogen volumetric removal capacity (NVRC) using equation (3.1), we can assess total Anammox activity inside the reactor as shown in Figure 27. The NVRC in black were calculated based on previously calculated NVRC in red with the assumption of no biomass production. For example, the NVRC was 340.8 (g N_2 m^{-3} d^{-1}) at day 491, when 150 ml of sludge was taken for SAA tests, the residual NVRC will be 320 (g N_2 m^{-3} d^{-1}). The results are summarized in Figure 27. The figure shows the increment of NVRC during day 472-497 except on day 486, however no such tendency was observed before day 472.

In conclusion, the metal ions (Ca^{2+}, Mg^{2+} and Fe^{2+}) have no effect on Anammox activity while the concentration of metal ions in the sludge was decreased as shown in Table 15. It might be the possible reason for the formation of tiny granules (Figure 24).

$$340.8 \times (2500 - 150) / 2500 = 320.3 \quad (4.5)$$

Table 13 the metal ions (Ca, Mg, Fe) in WW1

	Value	n[a]
Ca (mg/L)	29.4 ± 8.9	3
Mg (mg/L)	7.8 ± 1.5	3
Fe (mg/L)	0.02 ± 0.01	3

a: The number of times wastewater sample analyzed and collected from the industry

Table 14 Different medium compounds (Ca, Mg, Fe) using in stage VIII

	A (before day 472)	B (during day 472-497)
Ca (mg/L)	114.4	26.5
Mg (mg/L)	29.2	8.1
Fe (mg/L)	1.4	0.01

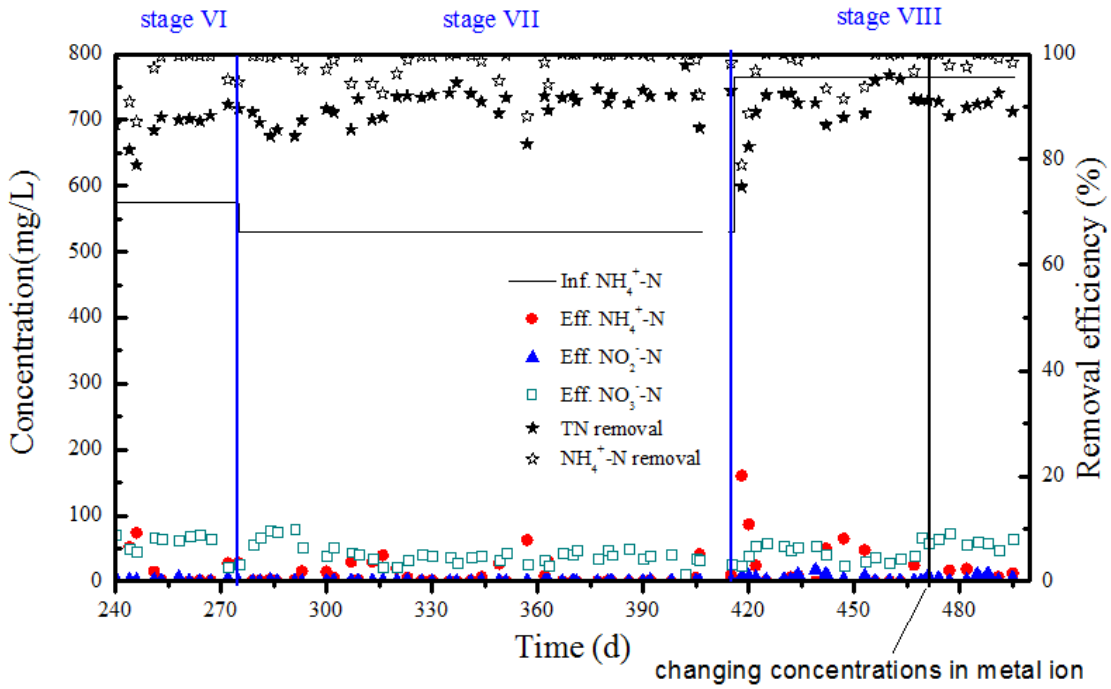


Figure 25 Profiles of nitrogen compounds in influent and effluent, and nitrogen removal efficiencies during stage VI-VIII of SBR-2.5.

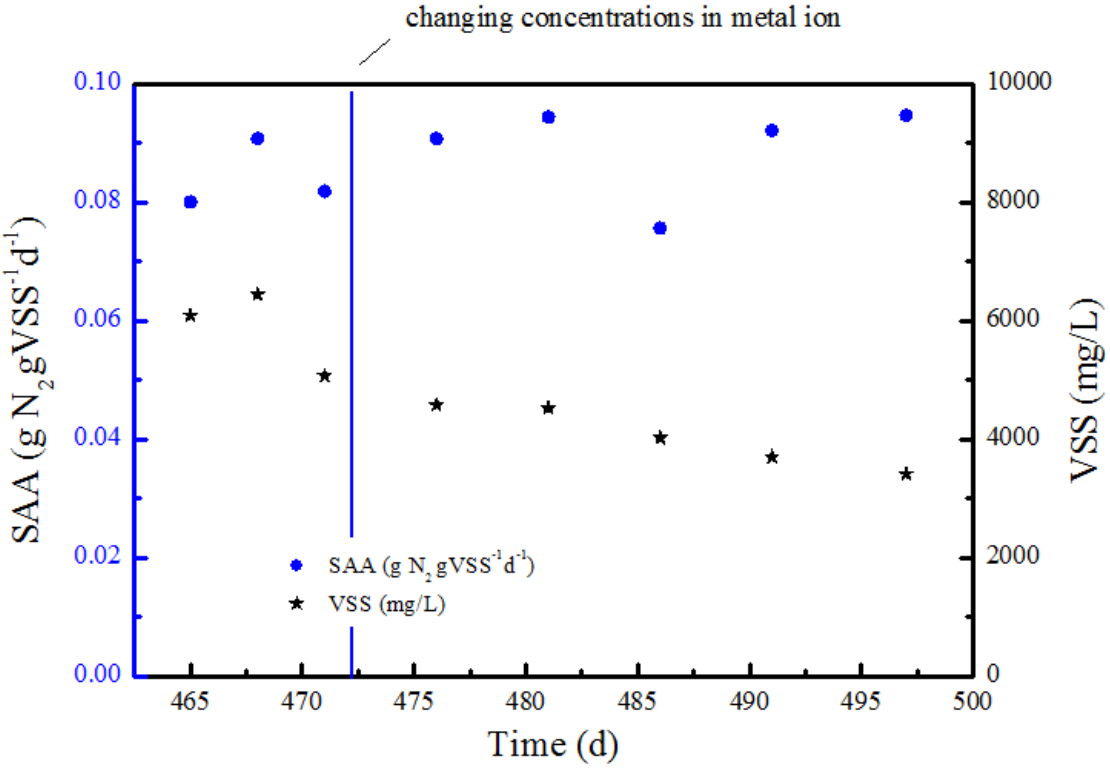


Figure 26 Activity of Anammox bacteria in sludge

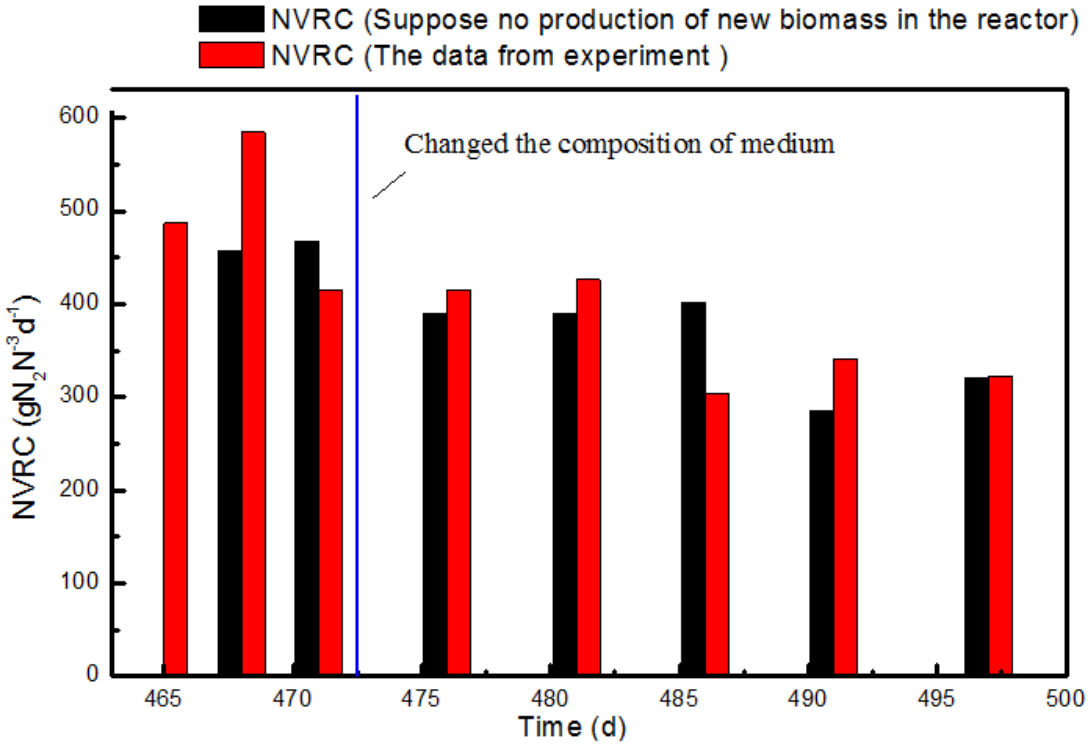


Figure 27 Profiles of Nitrogen volumetric removal capacity (NVRC) during day 465-497 of SBR-2.5.

Table 15 Concentrations of metal ions in the sludge

	Conc. In sludge (before day 472)	Conc. In sludge (during day 472-497)
Ca (mg/g)	68.7±1.2 (2)	51.4±1.4 (3)
Mg (mg/g)	0 (2)	0 (3)
Fe (mg/g)	25.9±0.7 (2)	21.1±1.4 (3)

4.2.1 Operational parameters for pH, Alkalinity, MLSS and MLVSS

During stage I and II, the alkalinity provided from mineral medium of the feeding wastewater was enough to maintain the pH in effluent at the range of 7~8. However, during initial days of stage III of NLR of 110 (g m^{-3}·d^{-1}), a too low alkalinity in wastewater caused a pH drop down to ~6.5 (due to acidification from nitrification process) and very low alkalinity of the liquid in effluent was observed as shown in Figure 28. This result indicted that the alkalinity in feeding wastewater was not enough for NLR of 110 (g m^{-3}·d^{-1}). In order to provide a constant pH, the additional bicarbonate ($NaHCO_3$) was dosed in feeding wastewater to keep the alkalinity of effluent at ~1000 mg/L as $CaCO_3$.

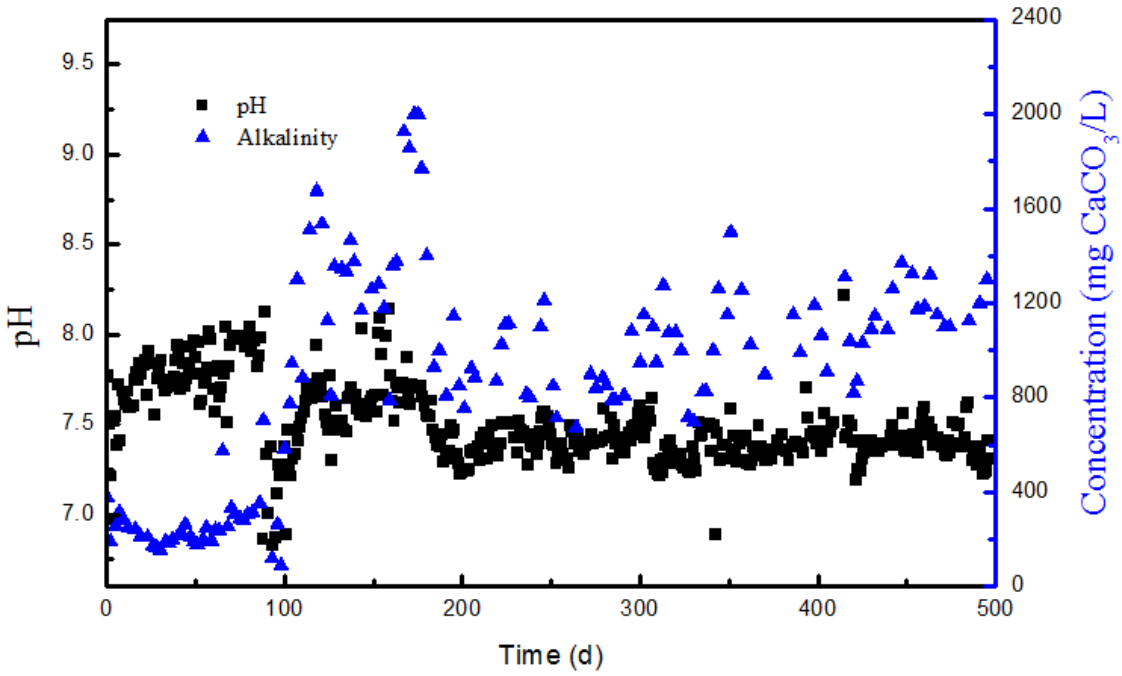


Figure 28 pH and Alkalinity concentration in effluent

To enrich the Anammox bacteria with very low doubling time, sludge retention time (SRT) was kept infinite during stage I-VI. However, the higher biomass production rate results in the increasing MLVSS from stage VII onwards (Figure 29).

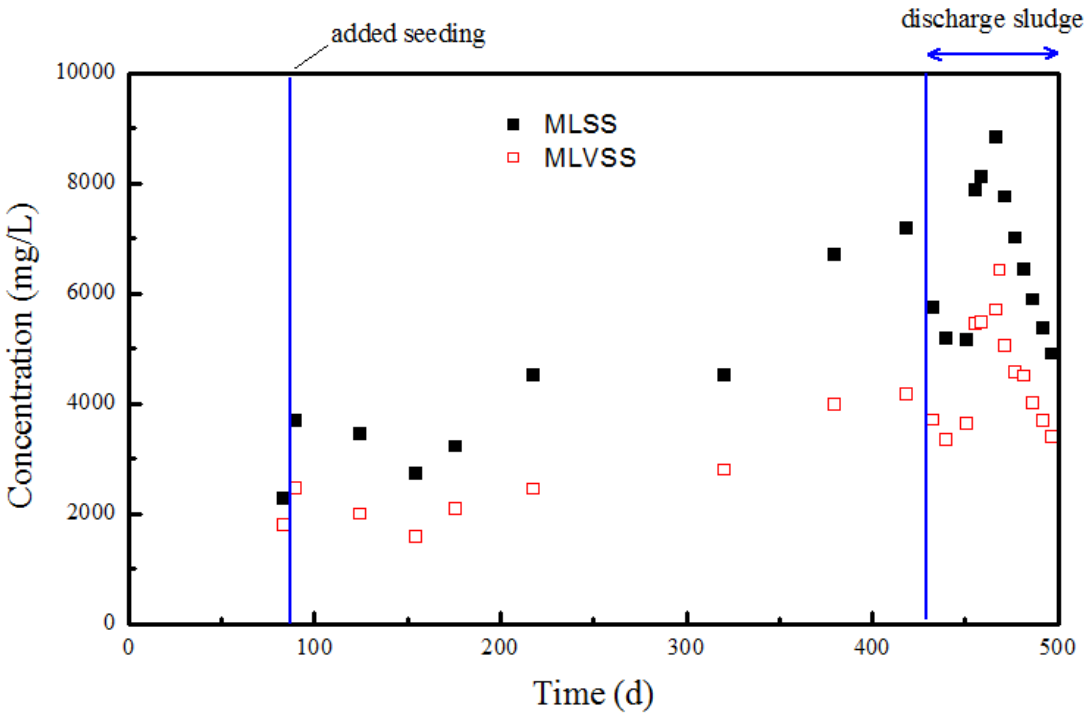


Figure 29 Profiles of MLSS and MLVSS during stage VI-VIII of SBR-2.5.

4.2.2 COD removal from opto-electronic industrial wastewater

Figure 30 and Figure 31 shows the performance of percentage COD removal by the system during different stages. In stage I to III, the influent COD concentrations were very low (only 20-43 mg/L) and the COD removal efficiencies were not significant. The high DO concentration in the reactor during stage I and II, which affects denitrifying process negatively, could be the most probable reason for this low COD removal efficiency. And the other probable reason is the change in feeding wastewater (from landfill-leachate to opto-electric wastewater) resulted in the dead biomass. During stage IV to 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 stage VI were found to be 79% and 28 g COD $m^{-3} \cdot d^{-1}$, respectively. Furthermore, with the increasing in organic loading rate (OLR), the efficiency of COD removal also increased. This suggests the presence of active heterotrophic denitrifiers in the reactor

which utilized organic matter as carbon source from the reactor and reduces the COD concentrations in the effluent.

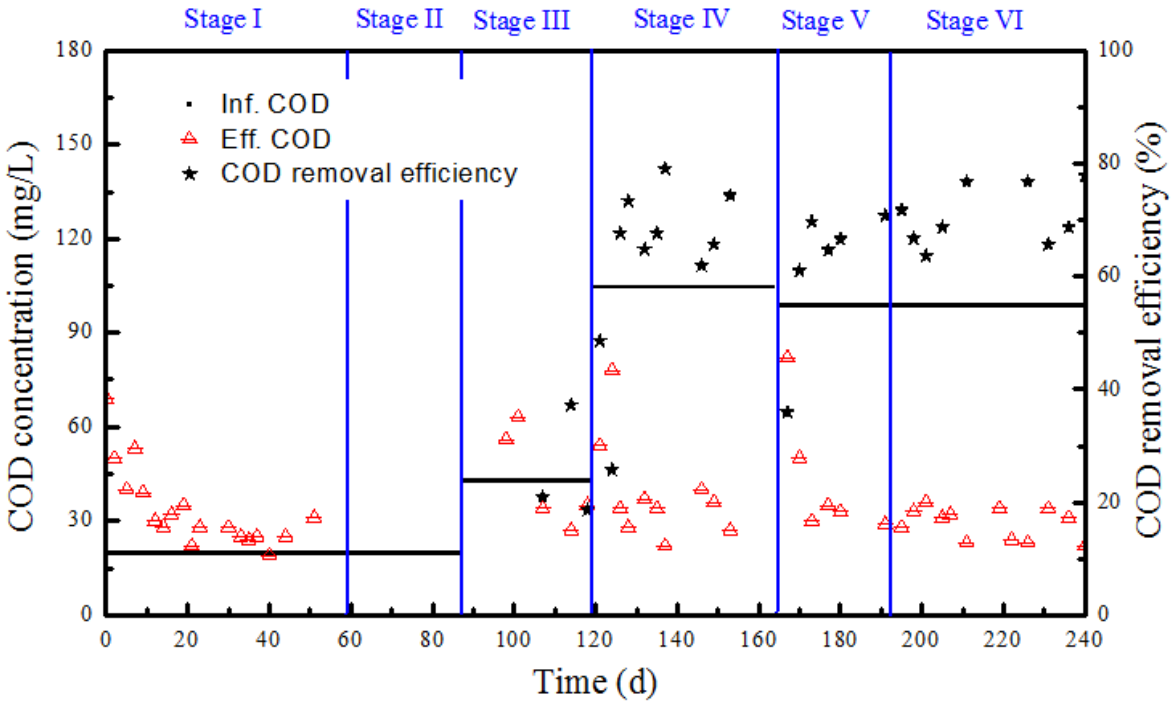


Figure 30 Profiles of COD in influent and effluent, and COD removal during stage I-VI of SBR-2.5.

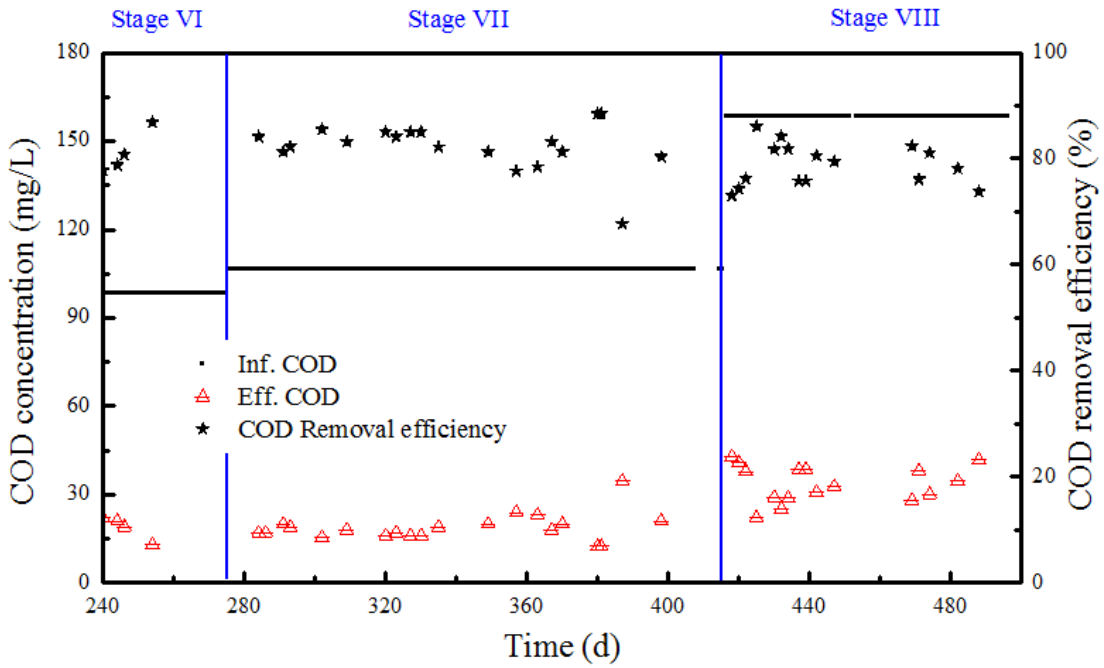


Figure 31 Profiles of COD in influent and effluent, and COD removal during stage VI-VIII of SBR-2.5.

4.2.1 Result from NUR

On day 381 the NUR test was performed to verify the presence of denitrification in the reactor. The sludge was taken directly from the reactor and the concentration of VSS in batch bottle was 993 mg/L. Figure 32 shows that both COD and nitrate were consumed. This indicated that denitrifying bacteria were present in the sludge.

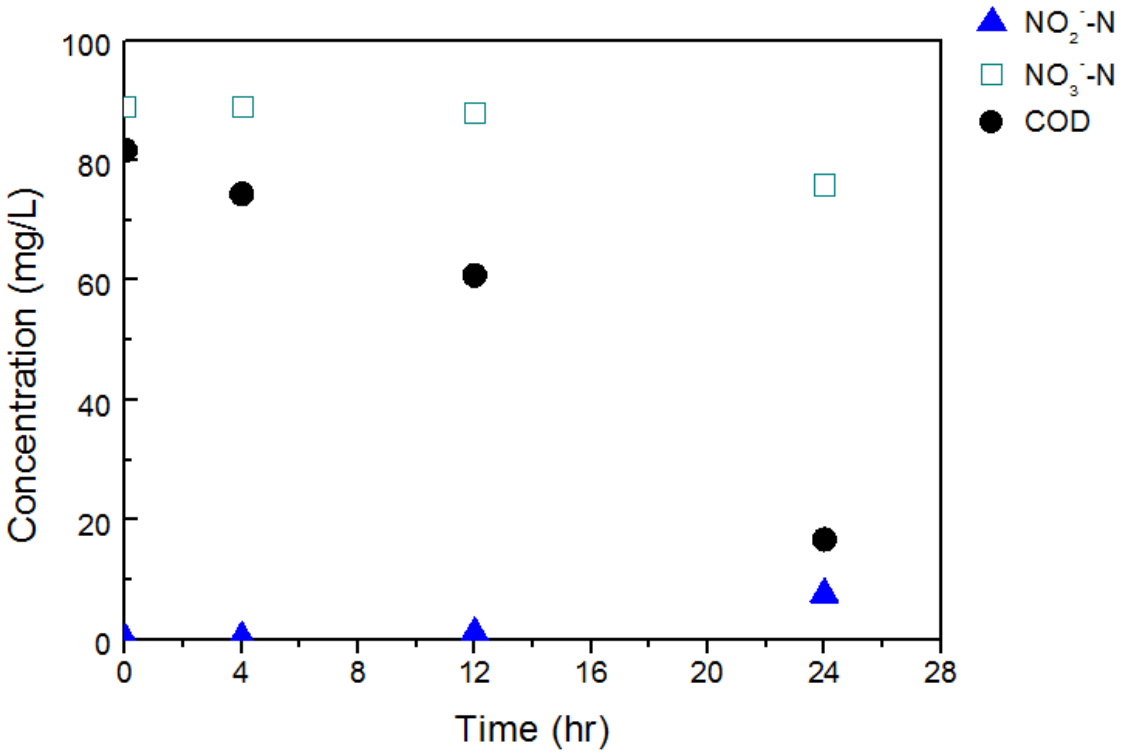


Figure 32 Consumption of COD and nitrate by denitrifiers present in the sludge of SBR-2.5.

Overall this study shows the applicability of SNAD (simultaneously partial Nitrification, Anammox and Denitrification) system for nitrogen and COD removal from optoelectronic industrial wastewater characterized by low C/N ratio.

4.2.2 Results from PCR

To identify the microbial species present in the SBR, PCR experiments were carried out. From Figure 33, a visible band around 500 bp exists on the lanes of amoA targeting AOB, while no band around 900 bp exists on the lane of nirS targeting NOB, nor a band near 400 bp appears 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 *Candidatus Scalindua* genus. There is a positive signal on the lane of KS, however, no positive signal for the lane of BA (Figure 33). Therefore, the dominant species of Anammox bacteria is considered to be KS in this reactor.

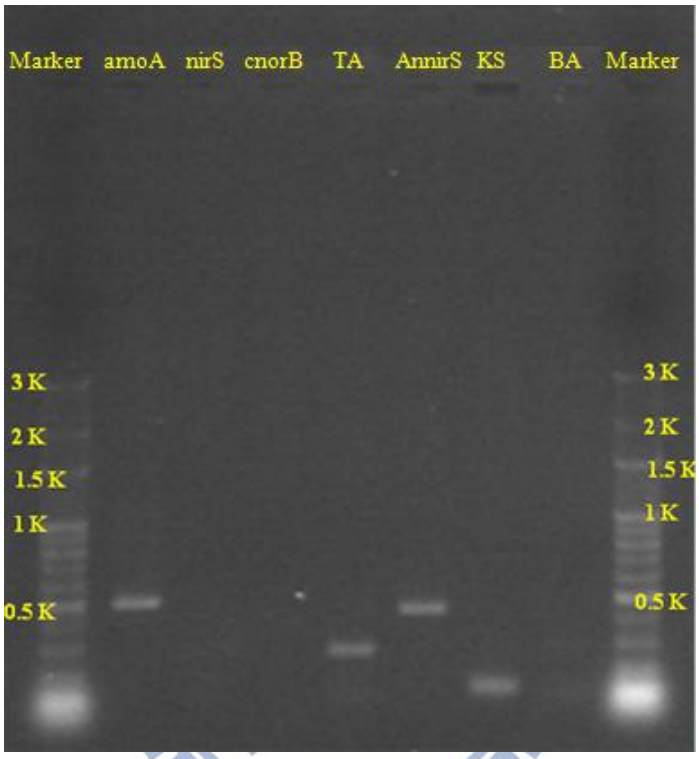


Figure 33 the PCR results at day 229

Chapter 5 Conclusion

The conclusion from SBR-18

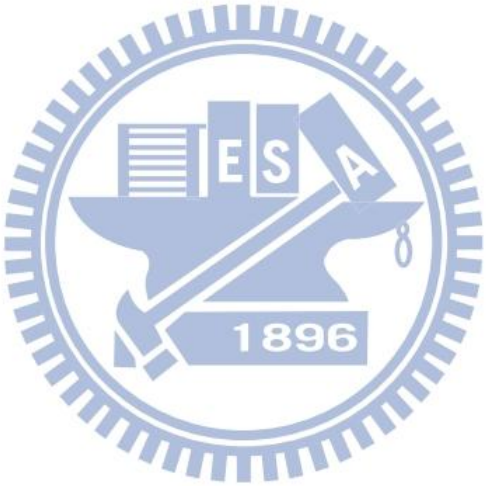
An ammonium rich optoelectronic wastewater was successfully treated in 18 L SBR by partial nitrification and Anammox process in a single reactor (CANON system). High aeration rate and nitrite concentration negatively affected the Anammox bacteria in SBR-18 and the reactor activity was not able to recover for about 2 months even after maintaining the nitrite concentration at low levels, and a fresh seed biomass was needed to recover the reactor performance. This toxic effect of nitrite can be overcome by online monitoring of the nitrite concentration in the reactor. However, the system successfully treated ammonium (3636 mg/L of NH_4^+-N) rich optoelectronic wastewater without diluting it with an NLR of about 0.9 Kg m^{-3} d^{-1} in later stage. The results of PCR confirmed the presence of AOB and Anammox bacteria in the reactor. Denitrifiers and NOB were also found in the reactor, but these microbes were not active in the reactor.

The conclusion from SBR-2.5

During the experiment period, the further oxidation of nitrite results in the low TN removal. However, the NOB lost its activity by inoculation of seeding with NLR of 110 $g/m^3 \cdot d$ and low DO level. The results of NUR test showed that the COD in wastewater was removed by denitrifying bacteria, which resulted in high TN removal (above 91%). The additional metal ion's Ca^{2+}, Mg^{2+} and Fe^{2+} were no effect on Anammox activity. In the last stage of the treatment process, the system was able to treat ammonium rich optoelectronic wastewater with low C/N ratio at an NLR of 0.4 Kg m^{-3} d^{-1} and OLR of 0.09 Kg m^{-3} d^{-1}.

Overall, this study suggested that the difficult to treat optoelectronic wastewaters (both

high ammonium concentration and low C/N ratio) can achieved by partial nitrification and Anammox processes in a single reactor.



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