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Comparison of Nitrogen Removal and Full-Scale Wastewater Treatment Plant Characteristics in Thailand and Japan

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Abstract

Four full-scale systems wastewater treatment plants (WWTPs) were used as study sites. All of these WWTPs were designed and operated for biological nitrogen removal (BNR) by using nitrification-denitrification processes. In general, the WWTPs in Thailand operated at higher values of temperature, HRT and SRT. Influents and effluents from these sites are compared and discussed in terms of BNR, dominant nitrifying microorganisms and WWTP design. Nitrogen removal was observed in all the sites and correlated to the influent total N (TN) to BOD ratio. Polymerase chain reaction coupled with denaturing gradient gel electrophoresis was used to identify dominant bacteria involved in nitrogen transformations: ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and nitrate reducing bacteria (NRB). AOB *Nitrosomonas* sp. was found only in Thailand where aerobic HRT was ≥ 4 hours and SRT was ≥15 days. Furthermore, AOB *Nitrosospira* sp. were found only in Japan at aerobic HRT ≤ 4 hours and SRT≤ 13 temperature (21-27°C). NOB *Nitrospira* sp. was found at aerobic HRT ≥ 4 hours and SRT ≥ 6 days. Interestingly, *Nitrotoga* sp. was found in the aerobic tank one in Thailand and one in Japan and co-occurred with NRB *Burkholderia denitrificans*. The higher wastewater temperature and lower influent nitrogen concentration in Thailand appear to promote a different AOB and NOB community structure than in Japan. The most important factor affecting TN removal was the influent TN to BOD ratio.

Keywords: nitrifying bacteria; nitrogen removal; long and short HRT and SRT

1. Introduction

Domestic wastewater is a source of nitrogen (N) water pollution. Different forms of nitrogen can have deleterious effects on human health, aquatic life, and environment. For example, ammonia (NH_3) is toxic to fish and many other aquatic organisms. Nitrate (NO_3^-) in drinking water is a significant potential public health hazard posing the risk of methemoglobinemia (blue baby syndrome) in infants. Nitrogen is the major nutrient that enhances eutrophication of freshwater, lakes, estuaries, and oceans. Domestic sewage, agriculture, and industries are sources of N, but domestic sewage is the major source of this nutrient in Thailand (Noophan *et al*., 2007). In order to control excessive discharge of the nutrient, high efficiency treatment systems have been developed but these are expensive to build and operate. Biological nitrogen removal (BNR) from sewage commonly relies on the conventional nitrification-dentrification process (Strous *et al*., 1999).

The conventional approach for nitrogen removal in wastewater involves nitrification of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-). Two groups of microorganisms are involved in the nitrification process: ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Bai *et al*., 2012). This process is followed by denitrification from nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and then to the nitrogen gas (N_2) end product. The process of nitrification followed by denitrification is well known and is widely used for the treatment of municipal wastewater (Tchobanoglous *et al*., 2003).

In general, WWTPs in Japan report higher nitrogen removal than WWTPs in Thailand. However, there is little information available to elucidate the factors responsible for the lower nitrogen removal efficiency of WWTPs in Thailand (humid tropical climate) relative to the WWTPs in Japan (humid subtropical climate). Potentially important differences include climate, influent wastewater characteristics, WWTP design and dominant bacteria that oxidize or reduce nitrogen.

1.1 Municipal wastewater treatment plants, Bangkok and Phuket province, Thailand

Bangkok, capital of Thailand is home to approximately 15 million people. Currently, eight WWTPs are operated in the Bangkok area (Si Phraya, Rattanakosin, Nongkhaem, Thung Kru, Chongnonsi, Jatujak, Dendaeng, and Bang Sue, which began operating in mid-2014). Most wastewater treatment plants in Bangkok are capable of managing larger organic loading but are not all designed to significantly removal nutrients (Noophan *et al*., 2009). The lack of land area for wastewater treatment infrastructure has led to more compact system designs. For this reason, one WWTP (an average of 120,000 m^3/day) operated with step-feed at long HRT and SRT and with BNR from Bangkok was selected as study site. Another WWTP was selected from Phuket area because this plant is also operated with long HRT and SRT, higher influent BOD but with a different design. The Phuket plant was designed to treat (an average of 28,000 m^3/day) domestic wastewater. This system was designed for biological nitrogen removal (BNR). Its many advantages include the capability to remove both organic matter and nutrient and the production of small volumes of biosolids. The primary disadvantage of the system is its requirement for a large area. This system would not be feasible in large Thai cities such as Bangkok, where large areas are not available.

1.2 Municipal wastewater treatment plants in southern Japan

The WWTPs in Fukuoka and Saga cities were selected for study because influent characteristics and operations are different than those of the Bangkok and Phuket WWTPs. The Fukuoka and Saga WWTPs, Japan are each designed to treat (an average of 145,300 m^3/day and 15,000 m^3/day, respectively) of domestic wastewaters. The treatment systems in Japan also use biological nitrogen removal (nitrification and denitrification processes). The Saga WWTP includes growth media in the aerobic zone to create anoxic microenvironments without explicitly operating an anoxic zone. The Japan WWTPs were designed to operate at lower HRT and SRT that the ones in Thailand. In addition, influent organic and nitrogen concentrations are higher in Japan.

1.3 Scope of this investigation

Linking influent, design, temperature and microbial characteristics to nitrogen removal at full scale domestic WWTPs is not widely available. The goal of this research study was to identify the dominant ammonia oxidizers (AOB) and nitrite oxidizers (NOB) in full scale domestic WWTPs with BNR and relate to the nitrogen removal of performances of WWTPs with influent and design characteristics. The TN/BOD ratio and other nitrogen species from influent and effluent of tropical humid and humid subtropical WWTPs were analyzed and discussed.

2. Material and Methods

2.1 Quality of influent and effluent of wastewater quality

Influent and effluent wastewater quality was determined according to Standard Methods for the Examination of Water and Wastewater (APHA *et al*., 1995). Samples were collected every day for one year from the end of 2014 through the end of 2015. All samples were kept at 4°C until analysis. Wastewater quality was determined by measuring biochemical oxygen demand (BOD), organic nitrogen, ammonium, nitrate, and total nitrogen and phosphorus. Temperature and pH were immediately measured in the field. The wastewater influent and effluent data from the WWTPs were used to determine the efficiencies of nitrogen removal. The schematic diagram of facility in each WWTP is shown in Fig. 1. All the samples from points from each WWTP (Fig. 1) were taken in duplicate.

2.2 DGGE analysis and sequencing of DGGE fragments

For DNA analysis WWTP samples from Bangkok and Phuket were collected twice in 2015 (in February and November). WWTP samples were collected twice in Fukuoka (also in February and November 2015) but in Saga only once in November 2015. All the samples of DNA analysis from this work were only collected in the aerobic basins because of focus on nitrification.

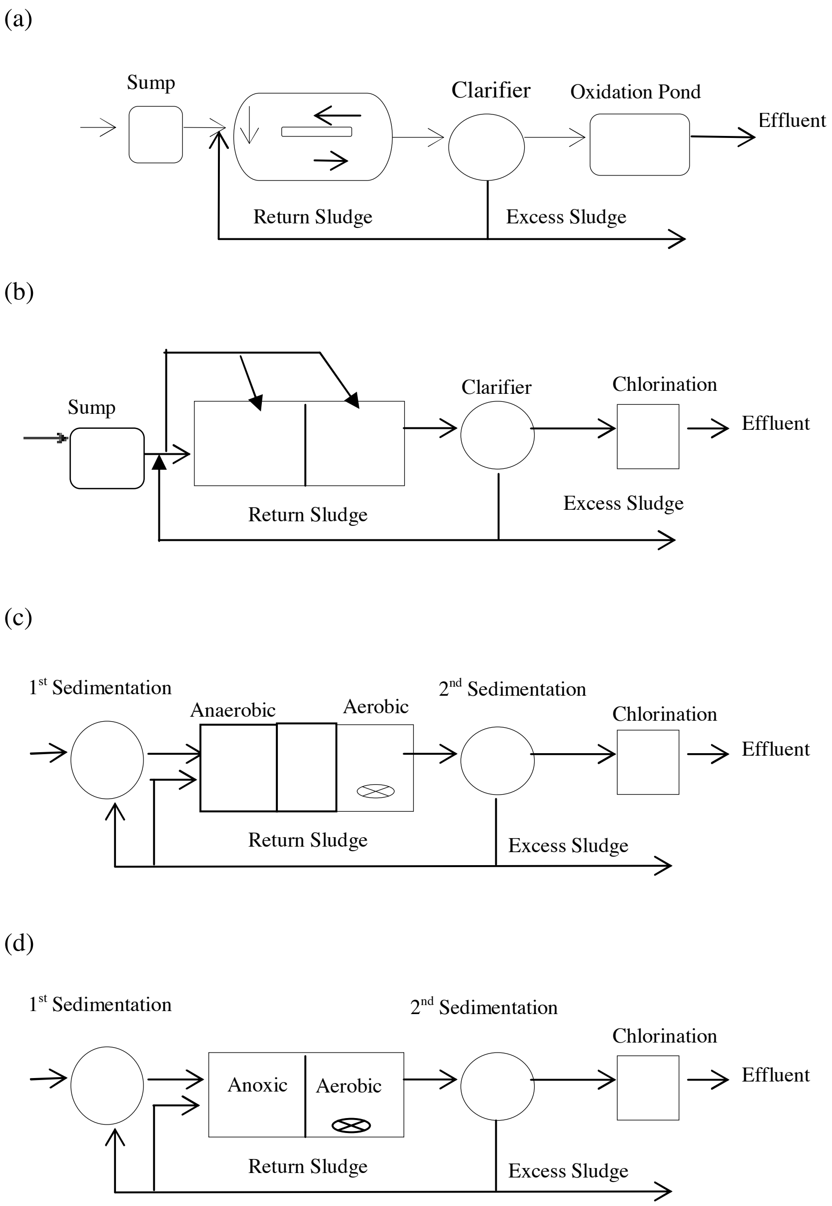

Figure 1. The schematic of facility in each WWTP (a) Oxidation ditch system in Phuket, (b) activated sludge step feed in Bangkok, (c) A_2O (Anaerobic, anoxic, and aerobic) (A_2O) in Fukuoka and (d) LE (Ludzack-Ettinger) in Saga.

PCR amplification of bacterial 16S rRNA gene fragments of bacterial group was performed using primers Bac338F-GC/Bac805R as described previously (Yu *et al*., 2005), see Table 1. The 338F*-GC/805R primer set was also attached GC-clamp (Muyzer *et al*., 1993). Cycle conditions for the touchdown PCR amplification were denaturation at 94°C for 10 min; 20 cycles consisting of denaturation at 94°C for 30 s, annealing at 65 to 55°C (reducing the temperature by 0.5°C per cycle) and extension at 72°C for 1 min; additional 15 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and final extension at 72°C for 7 min.

DGGE was performed using the DCode™ Universal Mutation Detection System (BioRad, Hercules, CA, USA). Samples containing approximately equal amounts of PCR amplicons were loaded onto 1mm thick vertical gels containing 8% (w/v) polyacrylamide and a linear gradient of denaturants: urea and formamide. A denaturing gradient of 30-55% was applied to separate16S rRNA fragments (100% denaturant is defined as 5.6 M urea and 40% (v/v) formamide). The gels were prepared in 0.5X TAE buffer (20 mM Tris, 10 mM acetic acid, 0.5 mM ethylenediamine tetraacetic acid (EDTA), pH 8.0), which was also used as the electrophoresis buffer.

Table 1. List of PCR primers for the amplification

Specificity group	Primer name	Sequence (5'–3')	Annealing T (°C)	References
All bacteria	Bac338F*	ACTCCTACGGGAGGCAG	52	Yu *et al*., 2005
	Bac805R	GACTACCAGGGTATCTTATCC	48	

*attached GC-clamp (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3') (Muyzer *et al*., 1993)

Electrophoresis was run at 58°C, 60 V 150 mA at 16 h at a constant voltage. After electrophoresis, the gels were stained with SYBR Gold nucleic acid (1:10,000 dilutions) for 40 min followed by destaining in distilled water for 20 min (Boon *et al*., 2002). Images were acquired using UV transilluminator UVCI-1100 (Major Science, New Taipei, Taiwan).

3. Results and Discussion

3.1 Design and operational parameters for the WWTPs

Key average design and operational parameters of Bangkok, Phuket, Fukuoka, and Saga WWTPs are shown in Table 2.

These WWTPs were selected as study sites because each WWTP has biological nutrient removal (BNR) (nitrification and denitrification process) and had been designed and similar operation for municipal treatment system. These WWTPs have similar volume capacities and other design criteria, also see Table 2. However, a notable difference between Thailand and Japan would be influent water temperature and BOD concentrations. Average physical and chemical characteristics of influent and effluent of WWTPs are shown in Table 3.

Several factors may contribute to the low influent BOD to the Bangkok and Phuket WWTPs as compared the influent BOD for Fukuoka and Saga WWTPs. First, each house or hotel, condominium, apartment, etc., in Bangkok and Phuket areas have a primary treatment system (such as septic tank, grease trap). Theoretically, the septic systems are able to remove about 40-50% of organic matter and BOD (Crites and Tchobanoglous, 1998; U.S. EPA, 2002). Second, infiltration and inflow could dilute the sewage. Third, no food or garbage disposal is to be dumped into Bangkok and Phuket wastewaters. Finally, sewage pipes and storm water pipes are combined. However, in Phuket WWTP the influent BOD is a little bit higher than the influent BOD in Bangkok WWTP because of many luxury hotels, apartments, condominiums, guest houses, and many tourists throughout the year. These factors are significantly major contributors of the influent BOD in Phuket wastewater.

Efficient treatment for N is attributed to insufficient carbon sources at Phuket and Bangkok (tropical humid climate) of WWTPs. It is noted that the nitrogen removal efficiency in the Phuket and Bangkok WWTPs was lower (11-16 mg N/L removed) than that in Fukuoka and Saga (23-25 mg N/L removed) WWTPs. It is thought that the

Table 2. Key average design and operational parameters for the WWTPs in Bangkok, Phuket, Fukuoka, and Saga

Parameter	WWTPs in Thailand		WWTP in Japan	
	Phuket	Bangkok	Fukuoka	Saga
	Oxidation ditch	Activated sludge step feed	Activated sludge A^2/O	Activated sludge LE
HRT (hours)				
Anaerobic	No	No	1-2	No
Anoxic	1-2	1-2	1-2	1-2
Aerobic	8-12	4-8	3-4	1-3
SRT (days)	20-30	15-20	6-13	2-5
DO (mg/L)				
Anoxic	0.2±0.1	0.2±0.1	0.3±0.1	0.3±0.1
Aerobic	0.9±0.5	0.9±0.5	1.4±0.3	1.4±0.3

Table 3. The average influent and effluent characteristics of the WWTPs in Bangkok, Phuket, Fukuoka, and Saga

Parameter	WWTP in Thailand				WWTP in Japan			
	Phuket		Bangkok		Fukuoka		Saga	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Avg. flow rate(m^3/day)	28,000		120,000		145,300		15,000	
pH	6.8-7.4	6.9-7.2	7.2-7.6	7.2-7.8	7.3-7.8	7.0-7.5	6.7-6.9	7.2-7.4
Temp (°C) in summer	28.5±1.5	NR	26.5±1.5	NR	20.5±2.5	NR	20.5±2.5	NR
Temp (°C) in winter	23.5±2.5	NR	21.5±2.5	NR	19.6±0.6	NR	18.6±0.6	NR
TSS (mg/L)	300±80	8	46.3±5	8	210±90	3±1	230±60	5
BOD (mg/L)	80±20	3±1	54±2	4±2	200±70	3±2	120±10	3±1
TN (mg N/L)	25	9	20	9	39±8	14±4	30	7
NH_4^+ (mg N/L)	16±3	3±1	11±2	2±1	30±6	8±3	16±3	2±1
NO_3^-(mg N/L)	0.4±0.1	3.2±1.5	0.4±0.1	6.5±1.5	0.2±0.1	3.8±1.4	1.3±0.3	4±1
TP (mg P/L)	5.6±0.4	1.3±0.6	4.6±0.6	1.3±0.6	5.4±1.2	0.3±0.1	4±1	0.3±0.2

NR = not reported

Samples from each WWTP from tropical humid and subtropical climate were averaged from the whole data in one year: (average±S.D.)

difference is linked to insufficient carbon source for denitrification process. Influents to the Phuket and Bangkok WWTPs have low BOD but high nitrogen. Nitrogen removal is hindered by biological treatment because the influent ratio between BOD and N is low (high N/BOD ratio). Fig. 2 shows a good correlation ($R^2=0.83$) between TN removal and N/BOD ratio for the 4 WWTPs. The most practical solution for nutrient removal from influent with low BOD:N ratio (high N/BOD) is by adding an external carbon source (e.g. methanol, molasses, sucrose, and acetate) to support more complete denitrification (Tchobanoglous *et al*., 2003).

However, in Thailand, not only is wastewater pollution a significant problem, but water supply is also a huge issue. The supply of sufficient fresh water and the disposal of wastewater are mutually dependent. Until wastewater treatment problems are solved, water quality will be insufficient for reuse. For this reason, the new nutrient removal and water reuse standards in tropical humid climates like Thailand could be reconsidered in the near future to augment fresh water supplies. In addition, large population centers in Thailand such as Bangkok and Phuket threaten fresh water resources with huge volumes of domestic wastewater. The most practical solution for nutrient removal in the tropical humid climate WWTPs is by adding an external carbon source for the denitrifying bacteria.

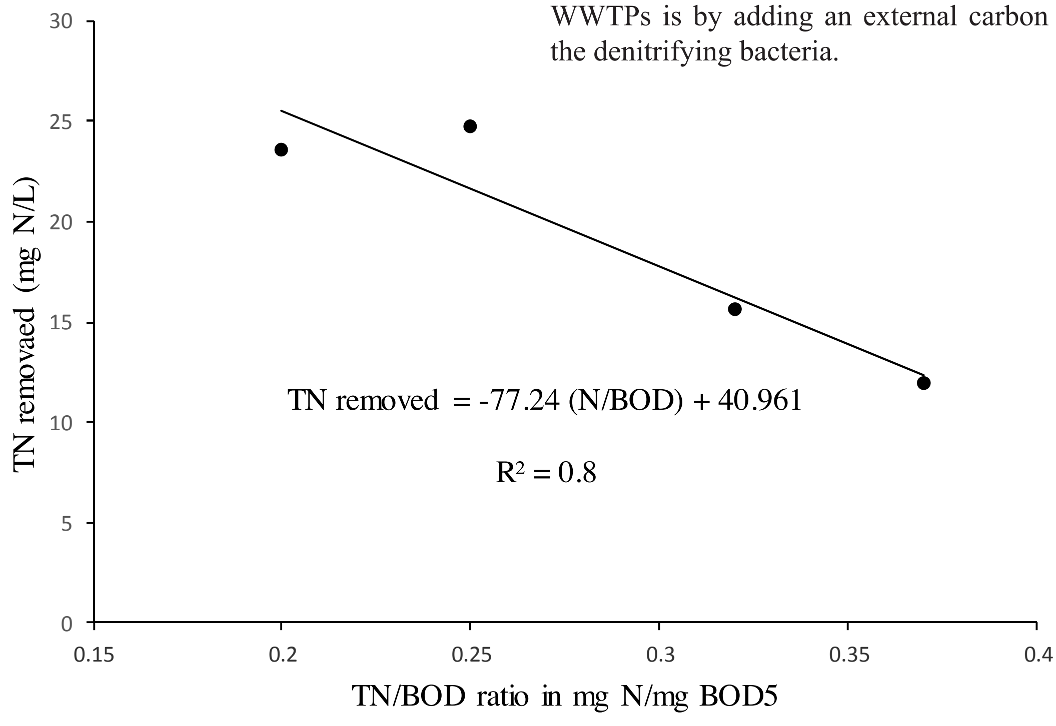

Figure 2. Total nitrogen removal relative to influent TN to BOD_5 ratio

Table 4. Dominant nitrogen transforming microorganisms for the four WWTPs in aerobic zones of Phuket, Bangkok, Fukuoka, and Saga

Type of Microorganism	Tropical WWTP in Thailand		Subtropical WWTP in Japan	
	Aerobic Phuket	Aerobic Bangkok	Aerobic Fukuoka	Aerobic Saga
AOB				
Nitrosomonas sp.	Yes	Yes	No	No
Nitrosospira sp.	No	No	Yes	Yes
Nitrosovibrio tenuis	No	No	No	Yes
NOB				
Nitrospira sp.	Yes	Yes	Yes	No
Nitrotoga sp.	No	Yes	No	Yes
NRB				
Burkholderia denitrificans	No	Yes	No	Yes

3.2 Microbial characteristics

PCR-DGGE was used to characterize the dominant microbial community members in the 4 WWTPs. Known microbial species related to nitrogen transformation are summarize from both first and second survey in Table 4. In the Bangkok and Phuket WWTPs, ammonia oxidizing bacteria (AOB) include *Nitrosomonas* sp. and the nitrite oxidizing bacteria (NOB) include *Nitrospira* sp. In the Fukuoka WWTP, AOB *Nitrosospira* sp. and NOB *Nitrospira* sp. were identified, while in the Saga WWTP, the AOB *Nitrosospira multiformis* was observed but *Nitrotoga* sp. was the NOB present. *Nitrosomonas* sp. was not found in aerobic tanks of both Fukuoka and Saga WWTPs but *Nitrosospira* spp. were found, in contrast to the Thai WWTPs that contained *Nitrosomonas* but not *Nitrosospira*. In general, *Nitrosomonas* sp. has an optimum temperature over 30°C (Mota *et al*., 2005; Pester *et al*., 2014). For this reason, in Fukuoka and Saga WWTPs only *Nitrosospira* sp. were found in the samples collected in February and November

Furthermore, bacteria species of *Nitrospira* spp. were found in aerobic tank of WWTPs in tropical humid climate such as Bangkok and Phuket than humid subtropical climate such as Fukuoka and Saga. Normally, *Nitrospira* sp. is the most widespread and key nitrifier in natural and engineered ecosystems (such as WWTP with BNR) (Pester *et al*., 2014). Daims *et al*. (2001) demonstrated that *Nitrospira* sp. were able to grow in an aerated bioreactor with lower nitrite and oxygen concentrations. Maintaining high dissolved oxygen concentrations in the aerobic reactor could be costly at the high temperature of tropical humid climates compared with to that in humid subtropical climates. For this reason, most plant operator at WWTPs in tropical humid climate want to keep as low as or minimum dissolved oxygen for surviving for microorganisms. Mota *et al*. (2005) postulated that if bacteria species of *Nitrospira* spp. are found in the aerobic basins, the environment of those places are low dissolved oxygen concentrations at long period of aeration.

Moreover, Mota *et al*. (2005) reported that during all aeration conditions for the nitrification process resulted in significant populations of *Nitrosomonas* sp. but this was for intermittent aeration cycles. Mota *et al*. (2005) found *Nitrosospira* sp. were found at regimes with the shortest aeration cycle. The appearance *Nitrosospira* sp. in the lower HRT aeration tanks of Saga and Fukuoka WWTP is consistent with Mota's observation. The Saga plant operator uses a short period of aeration at high dissolved oxygen concentrations to save cost of energy. Tchobanoglous *et al*. (2003) reported that 70% of total operation cost in the WWTP comes from aeration in the aerobic tank. For this reason, short aeration period would be good thing to operate in the WWTP of subtropical climate (Fukuoka and Saga). In the tropical humid climate such as Bangkok and Phuket, using high dissolved oxygen concentrations could cause a high cost for operation. For the application in the tropical humid climates to keep as low as dissolved oxygen concentrations in the aeration tank is desirable.

The results from this work could be applied in the field of wastewater treatment of tropical climate WWTP is that keeping as low as dissolved oxygen concentrations for nitrifying bacteria survive in the aeration tank could be possible. Although plant design and operating procedures for WWTP recommend high dissolved oxygen concentrations in the aeration tank, it could be very expensive for operation cost of WWTP in the tropical climate. However, the results of PCR-DGGE for this work show that both *Nitrosomonas* sp. and *Nitrospira* sp. are found in the aerobic tank and water quality data demonstrate that their nitrification activities are significant. Sufficient carbon source for denitrification process would be keys for nutrient removal in the BNR WWTP.

4. Conclusions

Influent ratios of BOD:N in Phuket and Bangkok WWTPs are 3.2:1 and 2.7:1, respectively. For this reason, the lowest amount of nutrient removal was observed at the Bangkok WWTP because carbon source was insufficient for denitrification. AOB (*Nitrosomonas* sp.) were found when aerobic HRT and SRT were 4-12 hr. and 15-30 days, respectively. AOB (*Nitrosospira* sp.) were also found when aerobic HRT was 1-4 hr. and/or temperature (21-27°C). NOB (*Nitrospira* sp.) were found in the conditions of aerobic HRT 4-12 hr. and SRT 3-6 day, when the aerobic tank has quite low DO (< 1.0 mg/L), WWTP in Thailand and one in Japan found *Nitrospira* sp. Not present in Japan system with HRT 1-3 and SRT 2-5 days. NOB (*Nitrotoga* sp.) was found when in the aerobic tank concurrently with the DNR *Burkholderia* denitrificans. Saving for energy in the aerobic tank at tropical humid climate WWTP could be possible because the result from this work is shown that the microorganisms in nitrification process are still active.

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