CHAPTER 4

RESULTS AND DISCUSSION

4.1 Process performance

Anammox process was operated at 4-day HRT under anaerobic condition in sequencing batch reactor (SBR) in which initial seed was obtained from Nongkham Municipal Wastewater Treatment Plant. There are two SBR reactors. Experiments of R1(100)-2, R0.75(75) and R0.5(75) were performed in reactor2 and the others in reactor1. Removal of ammonia simultaneously with nitrite as energy reaction results in the final products of nitrogen (N_2) gas and in this work significant amount of nitrous oxide (N_2O) gas. The solution pH was in the range of 7.0 to 7.3. It is important to control pH not less than 6.5 according to higher pH higher anammox growth rate and pH was optimum around 8 [24]. Ammonia and nitrite in influent and effluent of the anammox process were analyzed every day. Concentration profiles of ammonia and nitrite are illustrated below in Figure 4.1 for SBR reactor2 and Figures 4.2 and 4.3 for reactor1.

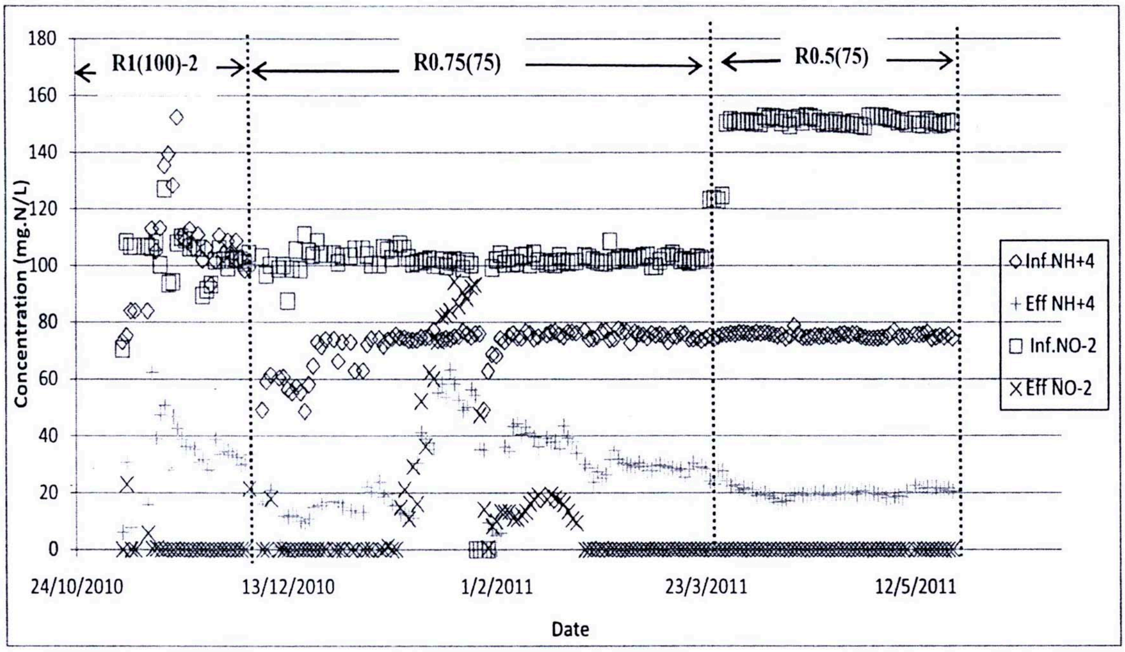

Figure 4.1. The influent and effluent nitrogen concentration from reactor 2 were R1(100)-2, R0.75(75) and R0.5(75)

In reactor2, the experiment of R1(100)-2, R0.75(75) and R0.5(75) with the influent NH_4^+-N to NO_2^--N ratio of 100:100, 75:100 and 75:150 respectively were operated. R1(100)-2 was operated from 4th of November 2010 to 4th of December 2010. At the beginning time, influent concentration to the process was not stable so the effluent concentration of ammonia and nitrite varied. After adjust to stable influent concentrations the process can reach to steady state within 2-3 weeks. At steady state condition of R1(100)-2, the influent ammonia and nitrite were 105.19±5.24 and 101.89±5.81 mgN/L respectively and the effluent ammonia was 33.32±3.29 mgN/L while the nitrite was completely removed.

Experiment of R0.75(75) was started at 7th of December 2010 to 22nd of March 2011 with influent ammonia and nitrite concentration of 102.41±1.64 and 75.12±1.26 mgN/L respectively and steady state effluent ammonia was 28.91±2.19 mgN/L while remaining nitrite was less than 0.1 mgN/L during the day of 4th December to 31st December 2010. However until beginning of January to the end of Febuary, anammox activity in R0.75(75) was decreased because of the drop of temperature during that time (Temp ≈17-22°C). According to Dosta et al. (2008), the application of the anammox process has been usually focused on the treatment of wastewater with temperatures around 30°C in order to operate under optimum conditions [24]. In addition to less anammox activity, nitrite was not used and accumulated in the reactor that caused detrimental effect to the biological process.

From Figure 4.1, it is clear that during January to February, effluent ammonia was increased till there was nearly no more nitrite in the reactor (effluent) due to the stop feeding of nitrite and then the system was reverse to stable again with the NH_4^+-N to NO_2^- -N feeding of 75 mgN/L to 100mgN/L. The experiment R0.5(75) was performed from 23rd of March 2011 to 31st of May 2011 by keeping influent ammonia concentration at 75 mgN/L and gradually increasing influent nitrite concentration from 100 mgN/L to 125 mgN/L and finally 150mgN/L.The influent nitrite at steady state beginning at 7th of April 2011 was 151.05±1.06 mgN/L and the effluent nitrite was completely consumed while ammonia of 19.96±1.40 mgN/L remained.

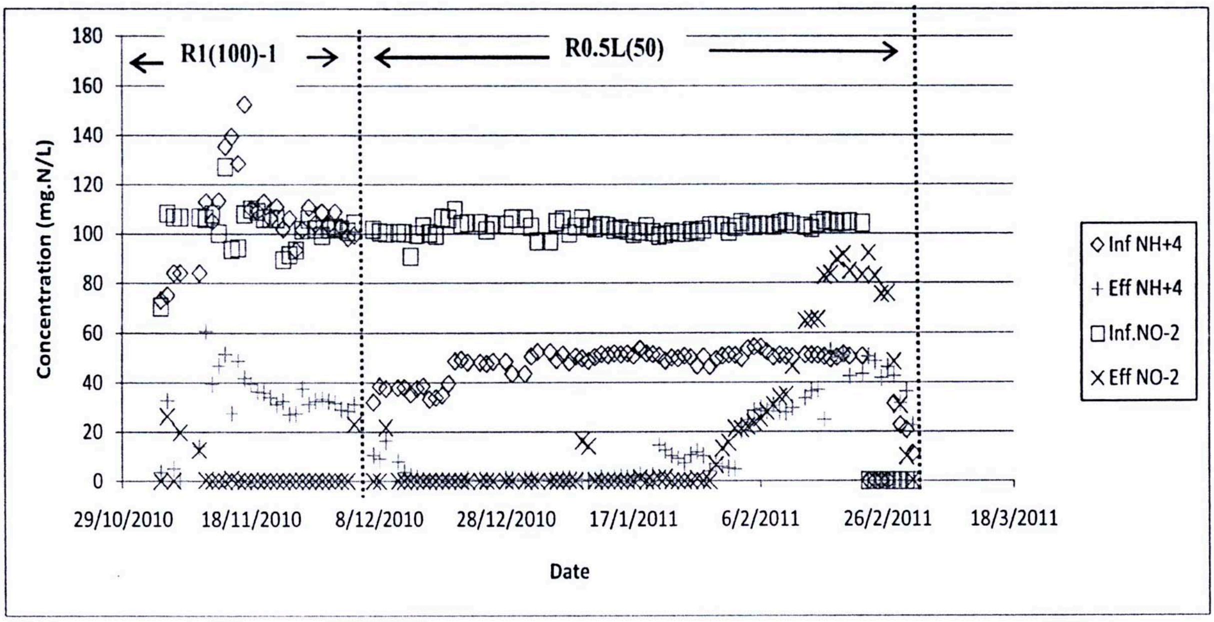

Figure 4.2. The influent and effluent nitrogen concentrations from reactor 1 were R1(100)-1 and R0. 5(50)

In SBR reactor1 (Figure 4.2), the experiment R1(100)-1and R0.5(50) were operated. The R1(100)-1 experiment was run from 4th of November 2010 to 4th of December 2010, nitrite and ammonia in the influent were 101.89±5.81 and 105.19±5.24mgN/L respectively. The steady state effluent ammonia was 32.38±3.51 mgN/L and there was completely consume of nitrite as expected for this NH_4^+-N /NO_2^- -N ratio. At the beginning time, the same as R1(100)-2, the influent concentration to R1(100)-1 was not stable so the effluent concentrations both ammonia and nitrite were fluctuated. After that, it was reached to steady state. The R0.5(50) experiment started from 7th of December 2010 to 2nd of March 2011. At steady state of R0.5(50) (during the middle to end of December), nitrite and ammonia in the influent were 102.96±2.78 and 49.69±2.39 mgN/L respectively and in the effluent, both ammonia and nitrite were completely consumed even nitrite was expected to be remained. Detrimental effect on anammox process due to temperature drop was also observed in R0.5L during January to February .The anammox activity in reactor1 could not recover, finally SBRreactor1 had to be refilled with new anammox microorganism from the stock culture tank.

Figure 4.3 shows the concentration profiles of a restart process in the reactor1. The first run started with the influent NH_4^+-N to NO_2^--N ratio of 100:100 (R1(100)-3 experiment) on 3rd of March 2011. The influent consisted of 101.99±0.67 mgNO_2-N/L and 100.56±1.75 mgNH_4-N/L. At steady state, nitrite was completely consumed and effluent ammonia was

38.71±4.23 mgN/L. The R0.75(100) experiment was run during 23rd of March 2011 to 15th of May in restart reactor1 too after completely monitoring effluent in solution and gas of R1(100)-3. The nitrogen removal process in R0.75(100) was stable since the first day of influent nitrite changed to 134 mgN/L. The influent nitrite and ammonia concentration were 133.21±0.85 and 101.66±0.97 mgN/L respectively and steady state effluent concentration of ammonia was 41.63±2.58 mgN/L while nitrite was less than 0.1 mgN/L. After R0.75(100) experiment has been finished, nitrite influent concentration was adjust to 200 mgN/L to get the new influent NH_4^+-N/NO_2^- -N ratio experiment that is R0.5(100). Rapid increase of influent nitrite concentration from 134 to 200 mgN/L results to the accumulation of both ammonia and nitrite in the reactor. However, after one week, effluent ammonia decreased concurrently with decrease of effluent nitrite and reached steady state concentration. In this period, the influent consisted of 201.15±0.92 mgNO_2-N/L and 101.16±0.87 mgNH_4-N/L and the steady state effluent nitrite and ammonia were 48.41±16.35 and 52.39±5.74 mgN/L respectively.

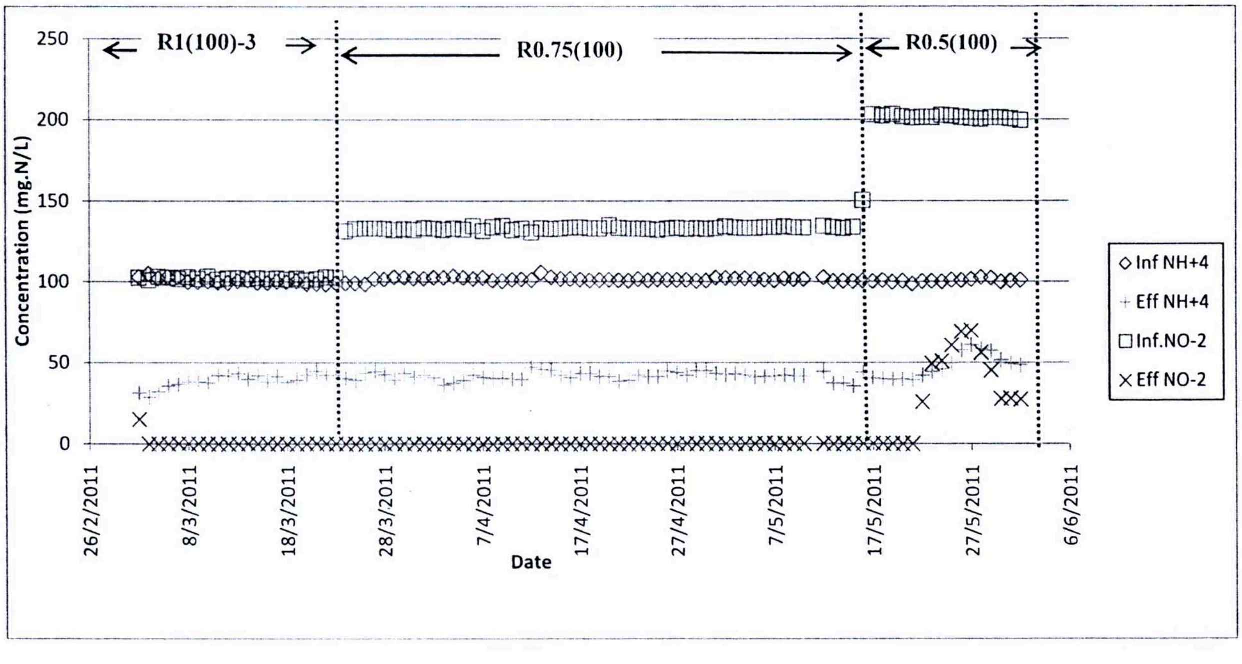

Figure 4.3. The influent and effluent nitrogen concentrations from reactor 1 (2nd run) were R1(100)-3, R0.75(100) and R0. 5(100)

Fluorescence in situ hybridization (FISH) technique was applied to identify *Nitrosomonas* and Anammox microorganisms in primary seed of anammox bacteria in SBR reactors. This primary seed activated sludge obtained from Nongkham Municipal Wastewater Treatment Plant and culturing with anammox specific substrate in stock tank for more than one year. The FISH image of an aggregate from stock culture with probe AMX368 was shown in Figure 4.4a as anammox bacteria. *Nitrosomonas*, usually oxidizes ammonium to nitrite under aerobic however under anaerobic can oxidize ammonia by utilizing nitrite as an electron acceptor and was detected by probe Nsm156 as shown in Figure 4.4b. By comparison between two images, it can be seen that anammox bacteria was much more than *Nitrosomonas*.

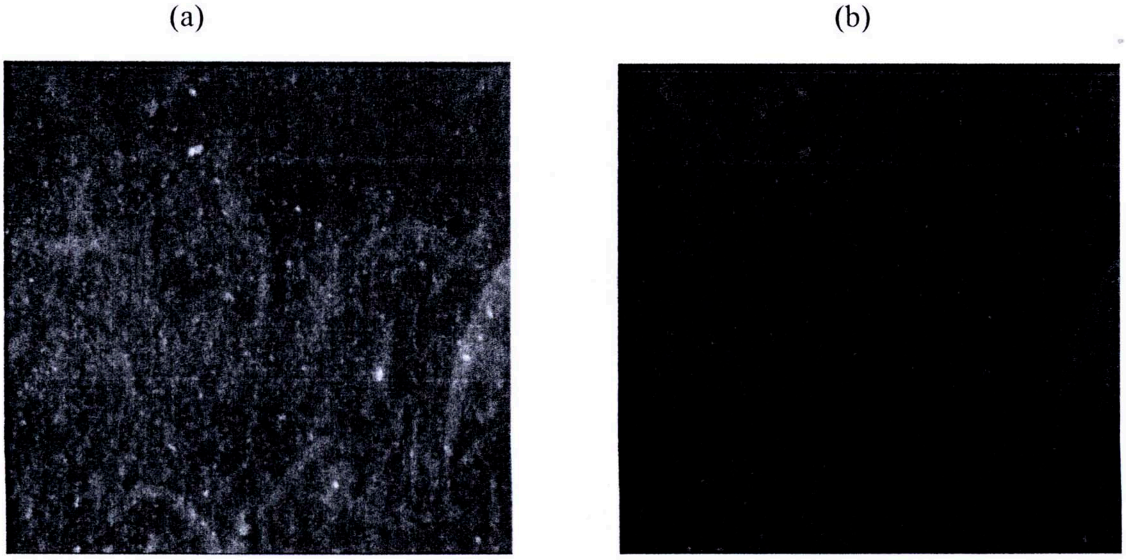

Figure 4.4. Fluorescent in-situ hybridization image of an aggregate from stock culture with (a) AMX368 (all Anammox organism); green colour and (b) Nsm156 (Nitrosomonas); red colour

4.2 Nitrogen removal efficiency

4.2.1 Influent NH_4^+-N to NO_2^- -N ratio of 1:1

The efficiency of nitrogen removal from triplicate experiments that are R1(100)-1, R1(100)-2 and R1(100)-3 for influent NH_4^+-N to NO_2^- -N ratio of 1:1 were shown in Figure 4.5. Effluent nitrite concentrations of all triplicate runs for ratio of 1:1 were less than 0.1 mgN/L, hence all nitrite was completely consumed while effluent ammonia concentration were 32.4±3.4, 33.3±3.2 and 38.7±4.2 mgN/L for R1(100)-1, R1(100)-2 and R1(100)-3 respectively. The influent ammonia to nitrite ratio of 1:1 was higher than the stoichiometric ratio for anammox reaction (0.75:1); therefore, ammonia remaining was expected to be 24 mgN/L (100-(0.75×100)) if nitrite was completely consumed. Higher ammonia than expected one implied the competitive use of nitrite by other denitrifiers. The ammonia removal percentages were 69.2±2.9 %, 68.3±2.8% and 61.4±4.7% for R1(100)-1, R1(100)-2 and R1(100)-3 respectively. Both R1(100)-1 and R1(100)-2 which are concurrent run at the same time gave almost the same ammonia removal while R1(100)-3 operated during the other period which the average temperature was lower, the ammonia removal was a little lower. The consumed nitrite to ammonia ratios of R1(100)-1, R1(100)-2 and R1(100)-3 were 1.38, 1.40 and 1.65 respectively (shown in Figure 4.10) that are nearly close to but still be higher than stoichiometric ratio of anammox reaction (1.32). Thus, there are competiting uses of nitrite with anammox bacteria by other denitrifiers.

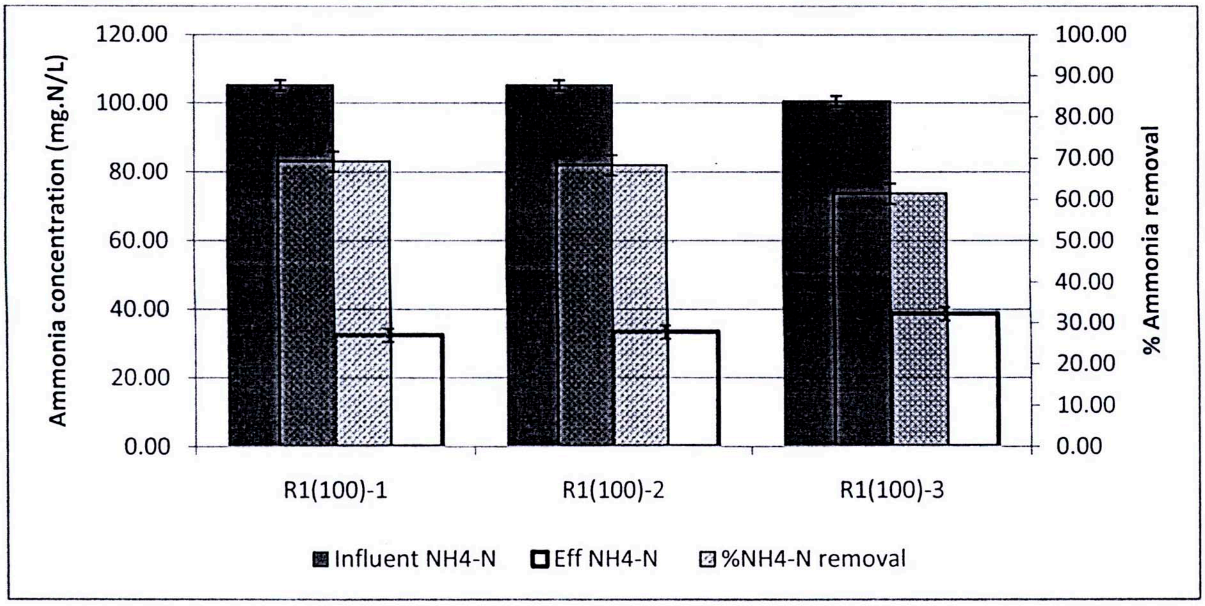

Figure 4.5. Ammonia concentration and nitrogen removal efficiency for influent NH_4^+-N to NO_2^- -N ratio of 1:1

4.2.2. Different influent NH_4^+-N to NO_2^- -N ratios with same NH_4^+-N concentration

4.2.2.1 High ammonia concentration

Anammox bacteria consumed ammonia as electron donor. In case of the same influent ammonia concentration but high concentration of 100 mg NH_4-N/L, the results show that the lower influent NH_4^+-N to NO_2^- -N ratio, the lower ammonia removal efficiency. Nevertheless ammonia remaining concentrations in all of experiments are shown in Figure 4.6 were much more than expected ones. The expected ammonia remaining in R1(100), R0.75(100) and R0.5(100) are 26.2, 0.74 and 0 mgN/L respectively calculated by applying the stoichiometric mole ratio of nitrite to ammonia of 1.32. Even nitrite was left in R0.5(100) as shown in Figure 4.6, anammox in R0.5(100) could not utilize the ammonia as much as that used in R1H when both experiments (R0.5(100) and R1(100)) supplied with same ammonia concentration. This implies that high influent concentration of nitrite in R0.5(100) may cause the inhibition to anammox process especially in batch reactor like SBR. The nitrite concentration of 300 mgN/L was reported to inhibit Anammox [16]. However, considering the consumed nitrite to ammonia ratios that are 3.40, 2.22 and 1.49 for R1(100), R0.75(100) and R0.5(100) respectively, the lower one is the higher ammonia removal percentage. Thus anammox at higher influent NH_4^+-N to NO_2^- -N ratio can more compete with other denitrifiers in using nitrite. For these reasons, R1(100) (100 mgNH_4-N:100 mgNO_2-N) gave highest ammonia removal percentage (66.0±5.1 %) when compare to R0.75(100) (100 mgNH_4-N:134 mgNO_2-N) and R0.5(100) (100 mgNH_4-N:200mgNO_2-N) of which ammonia removal are59.0±2.5% and 48.2±5.4 % respectively as shown in the Figure 4.6.

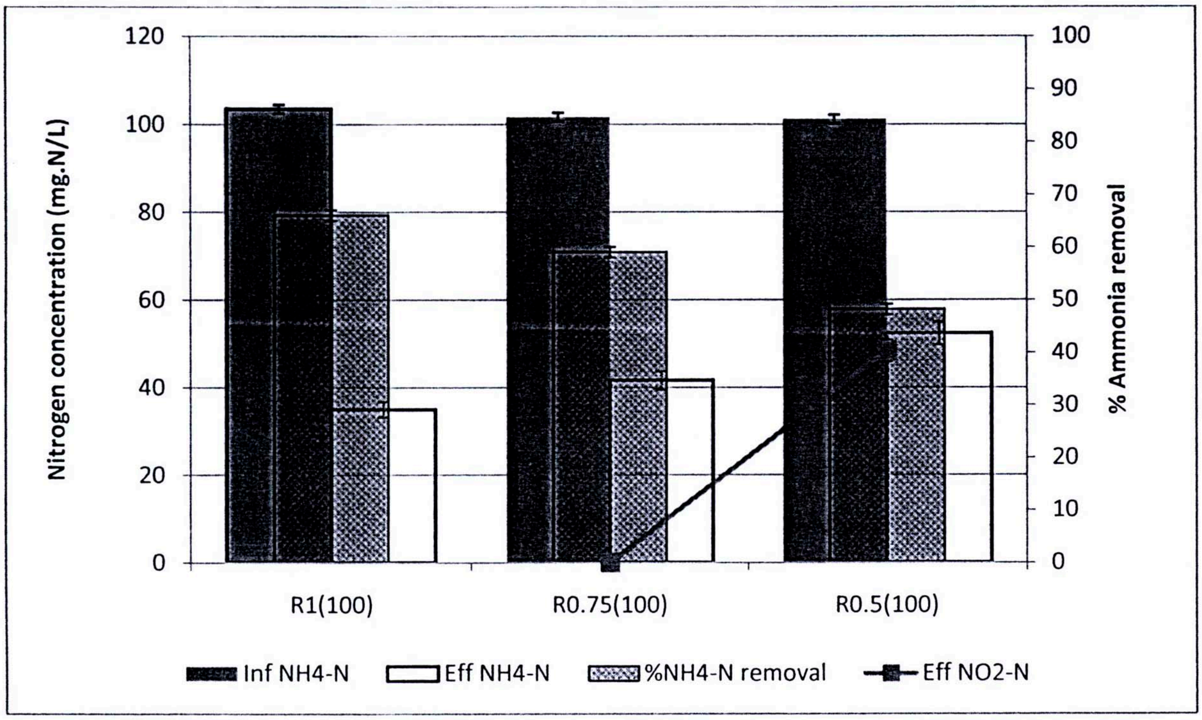

Figure 4.6. Ammonia concentration and nitrogen removal efficiency for different influent NH_4^+-N to NO_2^- -N ratios with the same influent ammonia of 100 mg N/L

4.2.2.2 Low ammonia concentration

Figure 4.7 shows the percentages of ammonia removal in R0.75(75) and R0.5(75). The ammonia removal was 73.5±1.9 % in R0.5(75) which was higher than 61.5±3.0 % in R0.75(75). The results were different from those experiments performed by using high ammonia concentration (Section 4.2.2.1) that is the lower influent NH_4^+-N to NO_2^- -N ratio, the higher ammonia removal efficiency. More influent nitrite concentration more ammonia concentration consumed. The remaining ammonia concentration in both R0.5(75) and R0.75(75) are expected to be 0 mgN/L however ammonia still be left quit high in the effluent 28.9±2.2 and 19.7 ±1.4mgN/L in R0.75(75) and R0.5(75) respectively. High ammonia remained while all nitrite was consumed implies the competitive use of nitrite by other denitrifiers. This competitive use of nitrite by other denitrifiers was shown obviously by comparing consumed nitrite to ammonia ratios of 2.22 and 2.27 for R0.75(75) and R0.5(75) respectively with stoichiometric one (1.32).

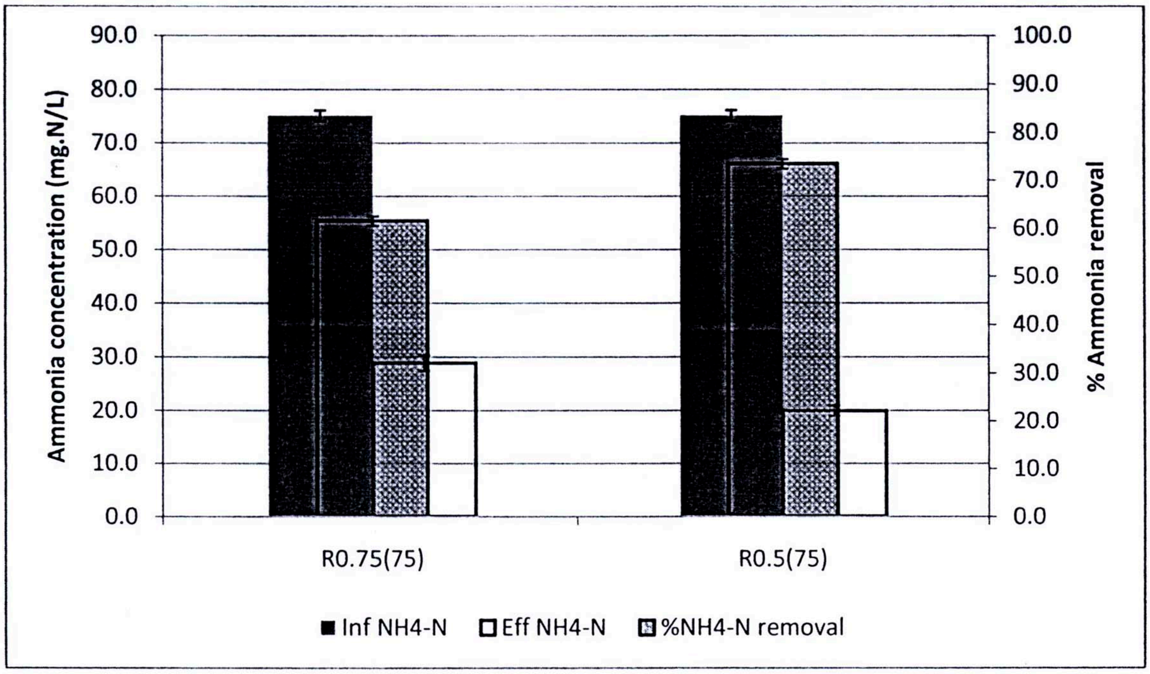

Figure 4.7. Ammonia concentration and nitrogen removal efficiency for different influent NH_4^+-N to NO_2^- -N ratios with the same influent ammonia of 75 mg N/L

4.2.3 Same influent NH_4^+-N to NO_2^- -N ratio with different ammonia concentration

4.2.3.1. Low ratio R0.5

Ammonia is expected to be removed completely in this influent NH_4^+-N to NO_2^- -N ratio of 0.5:1.From Figure 4.8, even the same influent NH_4^+-N to NO_2^- -N ratio of 0.5:1, ammonia removal percentages in R0.5(100), R0.5(75) and R0.5(50) were significant different and depend on influent nitrogen concentration. The results show that the lower influent nitrogen concentration was the more ammonia was removed. The ammonia removal in R0.5L was 98.2±0.7 %, in R0.5(75) was 73.5±1.9 % and in R0.5(100) was 48.2±5.4 %. In both R0.5(75) and R0.5(50), nitrite was completely consumed while in R0.5(100) nitrite still remained at 27.62±0.33 mgN/L. High concentration of both influent ammonia and nitrite can inhibit to anammox activity so ammonia removal in R0.5(100) was lowest. However considering the consumed nitrite to ammonia ratios that are 3.15, 2.74 and 2.09 obtained from R0.5(100), R0.5(75) and R0.5(50) respectively show that high influent nitrogen reduce the anammox activity and the other denitrifiers can competitive use of nitrite better than in reactor applying low influent nitrogen concentration.

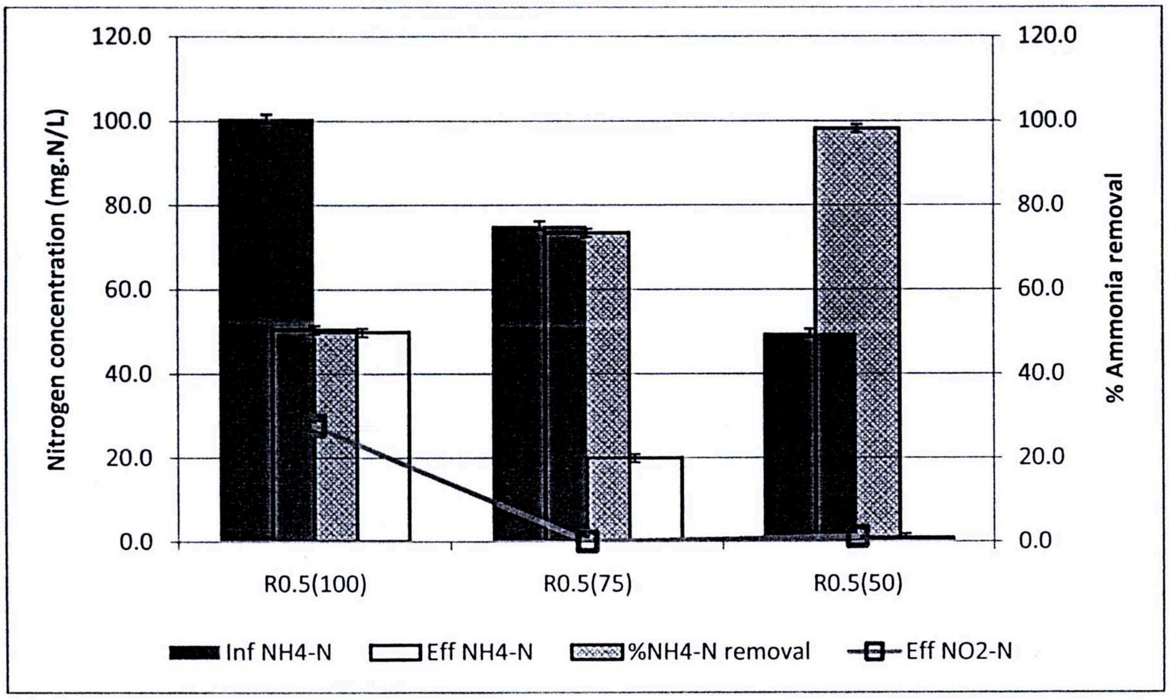

Figure 4.8. Ammonia concentration and nitrogen removal efficiency for different influent ammonia concentration with same influent NH_4^+-N to NO_2^- -N ratio of 0.5:1

4.2.3.2 High ratio R0.75

The tendency of ammonia removal was the same as that applying low influent NH_4^+-N to NO_2^- -N ratio (Section 4.2.3.1) that is the lower influent nitrogen,the higher ammonia removal efficiency as shown in Figure 4.9.

Apparently, influent NH_4^+-N to NO_2^- -N ratio of 0.75 in both R0.75(100) and R0.75(75) is the stoichiometric ratio of anammox reaction and both ammonia and nitrite were expected to be removed completely however ammonia removal efficiencies of R0.75(100) and R0.75(75) in the experiments were only half of that expected. Ammonia removal in R0.75(75) was 61.5±3.0 % slightly higher than percentage ammonia removal of 59.0±2.5 % in R0.75(100). Following this, the consumed nitrite to ammonia ratio of both R0.75(100) and R0.75(75) equals to 2.22 which was higher than anammox stoichiometric one.

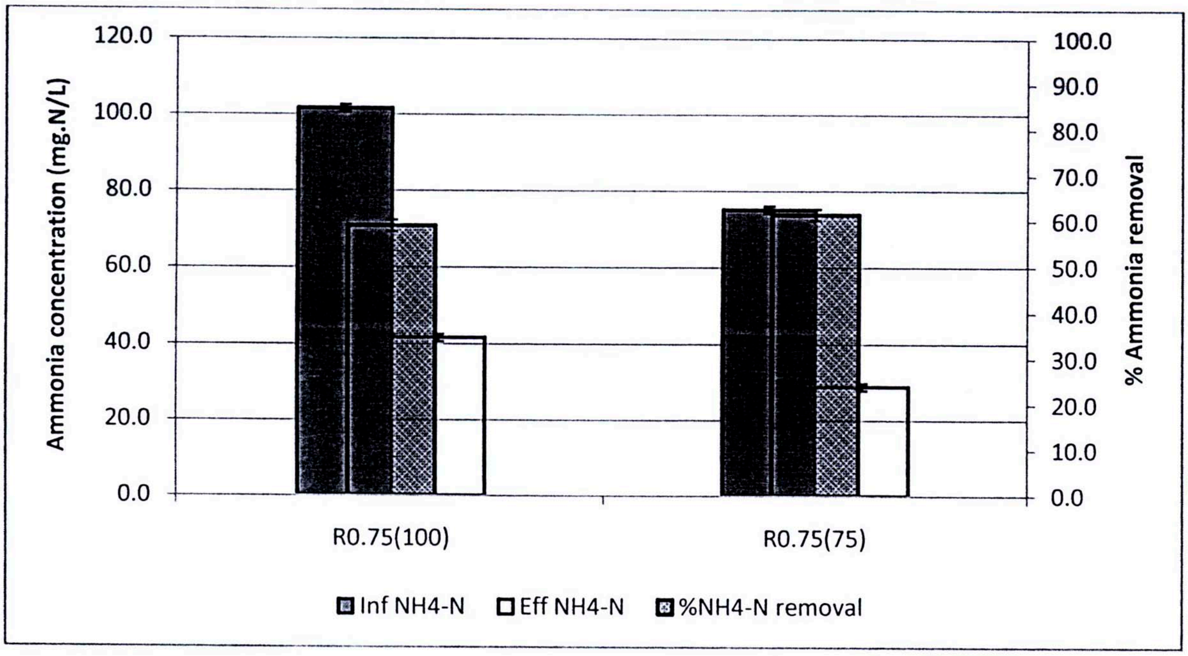

Figure 4.9. Ammonia concentration and nitrogen removal efficiency for different influent ammonia concentration with same influent NH_4^+-N to NO_2^- -N ratio of 0.75:1

4.3 Nitrous oxide (N_2O) production

Table 4.1. The comparison between N_2O concentration and consumed nitrogen of R0.5(75), R0.5(100), R0.75(75), R0.75(100) and R1(100) in SBRs

	R0.5(75)	**R0.5(100)**	**R0.75(75)**	**R0.75(100)**	**R1(100)**
ΔNH_4^+ consumed per day (mg/day)	35.9	33.0	30.0	39.0	44.5
ΔNO_2^- consumed per day (mg/day)	98.2	112.3	66.5	86.6	65.7
$\Delta NO_2^-\big\|_{cal,heter}$ **(mg/day)**	50.8	86.7	26.9	35.1	7.5
N_2O (ppm)	12.63	200.55	0.83	2.04	1.05
N_2O /ΔNH_4^+ (ppm/(mg/d))	0.352	6.072	0.028	0.052	0.024

The measurement values of produced N_2O from R0.5(75), R0.5(100), R0.75(75), R0.75(100) and R1(100) were only comparative ones at operating time of 23 hours in cycle after feed. As shown in Table 4.1, applying anammox stoichiometric ratio, the other denitrifier consumed nitrite ($\Delta NO_2^-\big|_{cal,heter}$) can be calculated. The average of N_2O concentrations in the headspace of SBR were 1.05, 0.83, 2.04, 12.63 and 200.55 ppm for R1(100), R0.75(75), R0.75(100), R0.5(75) and R0.5(100), respectively. The N_2O production increased as NO_2-N consumed in the experiment increased. Similarly, high NO_2-N influent concentration results to high N_2O production possible due to the excess NO_2-N consumed by heterotrophic denitrifiers. Itokawa et al. (2001) [49] and Alinsafi et al. (2008) [50] pointed that high nitrous oxide (N_2O) production was observed at low COD/N or low electron donor to electron acceptor. The calculated NO_2-N consumed by heterotrophic denitrifiers ($\Delta NO_2^-\big|_{cal,heter}$) may be related to produced N_2O as shown in Figure 4.10. The value of the calculated NO_2-N consumed by heterotrophic denitrifiers ($\Delta NO_2^-\big|_{cal,heter}$) increased as N_2O concentration increased. As the nitrous oxide gas was produced from denitrification, Lemair et al. (2006) [51] suggested from the research that N_2O concentration was about 6 ppm. Yang et al. (2009) [52] reported that N_2O production was at approximately 2 ppm under low COD/N ratio (5.3). At R0.5(100), other heterotrophic denitrifers had more strongly competitive use of nitrite with anammox than other ratio experiments indicated by the highest consumed nitrite to ammonia ratio ($\Delta NO_2^-/\Delta NH_4^+$) as shown in Figure 4.11. In addition, high influent nitrite concentration and the possible presence of heterotrophic denitrifiers are two likely explanations to the increasing of nitrite consumption during the process performed. And at R0.5(100), it was the situation of the low electron donor to acceptor ratio that can enhance the production of nitrous oxide.

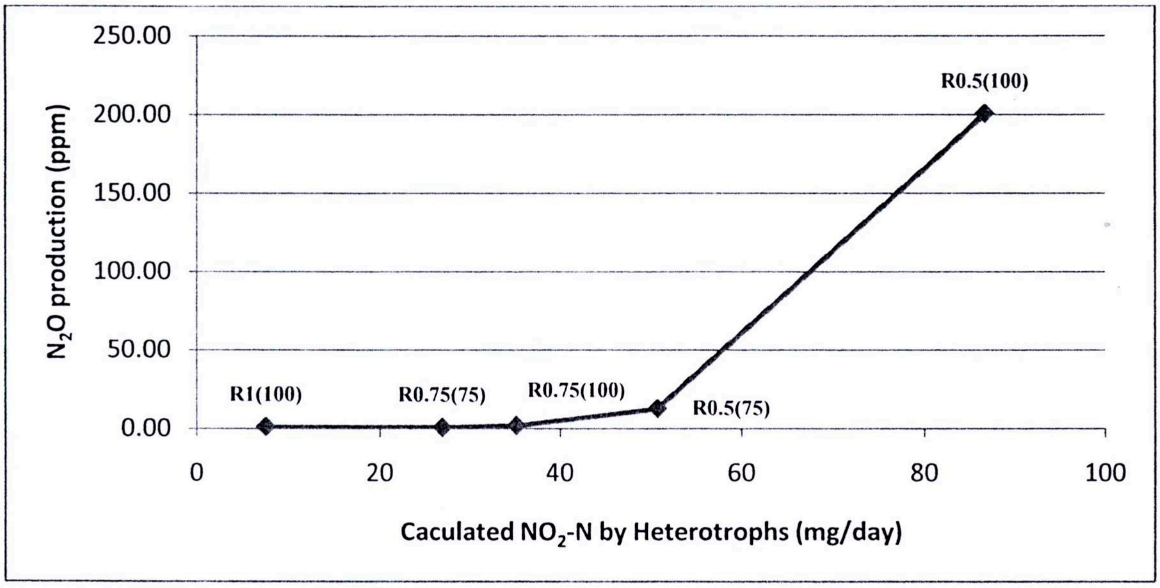

Figure 4.10. Correlation between the N_2O production and calculated consumed NO_2-N by heterotrophic denitrifiers

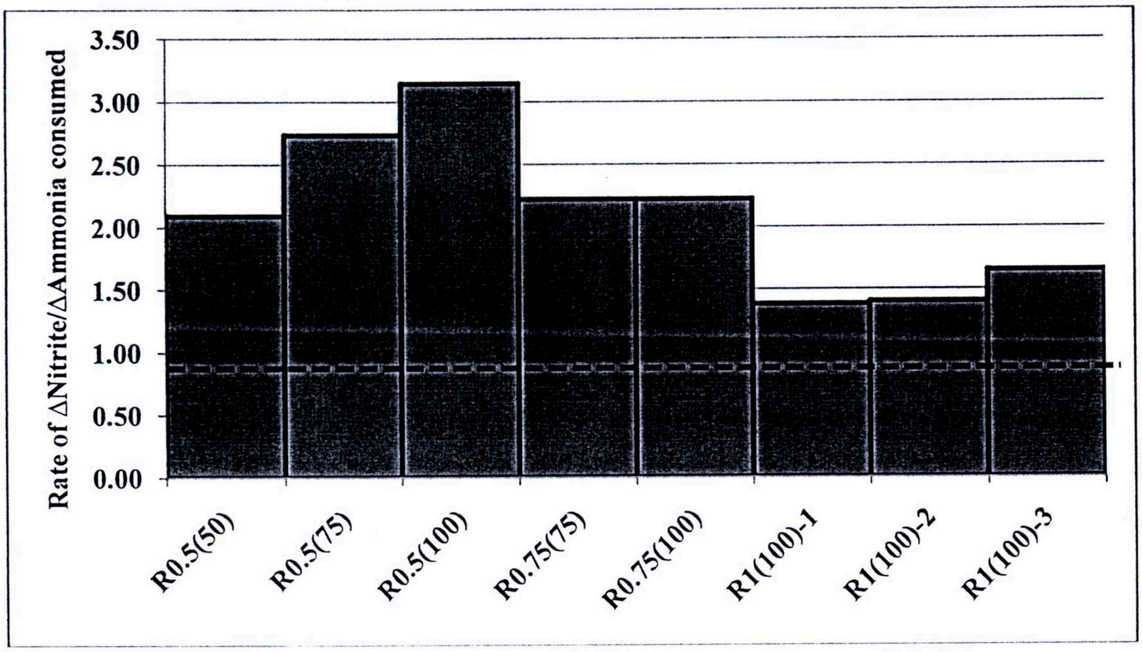

Figure 4.11. Ratios of consumed nitrite to ammonia in SBRs