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Observing the Performance of Nitrification with Varying Dilution

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Laboratory Report

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Abstract

In sewage treatment plants, partial mixing of incoming influent with residual supernatant can occur in the reactor. Such dilution may affect the performance of nitrification in the reactor, in which posting a threat to the treatment efficiency. As such, this report aims to investigate the efficiency of nitrification in the wastewater treatment after certain extent of dilution. The study found that with the performance of nitrification decreases with the dilution. However, in real world scenario, it is unlikely that the nitrification performance will be affected significantly as the relative volume of the residual supernatant mixing with the incoming influent is extremely small.

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Nomenclature

SBR	Sequencing batch reactor
STP	Sewage treatment plant
DO	Dissolved oxygen
NH_4-N	Ammonium-nitrogen
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
PO_4-P	Orthophosphate
TN	Total nitrogen
TSS	Total suspended solids
VSS	Volatile suspended solids
TCOD	Total chemical oxygen demand
SCOD	Soluble chemical oxygen demand
TOC	Total organic carbon

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1.0 Introduction

Nitrification is an important biological process in wastewater treatment, converting ammonia to nitrate through the activity of autotrophic microorganisms. In sequencing batch reactor (SBR), the process efficiency depends heavily on operational conditions during each cycle, including the fill, react, settle, and decant phases. Normally, partial mixing of incoming influent with residual supernatant can occur (Martin Jr. & Clark Ii, 2022). Such dilution may affect the performance of nitrification in the reactor. As such, this report aims to investigate the performance of nitrification with varying dilution, in which mimicking the condition where dilution occurs during the fill phase.

2.0 Methodology

2.1 Source of Sewage and Nutrient Solution

Activated sludge and municipal wastewater were sampled in two different sampling points located in Ampang and Pantai, Selangor. For clarity, samples from Ampang will hereafter be referred to as STP (A), and samples from Pantai as STP (B).

To investigate different dilutions factors on affecting the nitrification, two different types of dilution were performed in each sampling, of which are 60% and 30% of the original concentration of ammonia in the municipal wastewater. Raw samples were diluted with nutrient solutions and tap water. **Table 1** outlines the final composition of the nutrient solutions in the batch tests (Ong et al., 2012).

Table 1: Composition of (a) Nutrient Solution, (b) Trace Element Solution

(a)

Chemical	Composition
Potassium dihydrogen phosphate	1460 mg/L
Magnesium sulfate heptahydrate	660 mg/L
Trace Element	3.33 ml/L

(b)

Chemical	Composition (mg/L)
Iron (III) chloride hexahydrate	1.8
Peroxobic acid	0.18
Copper (II) sulfate pentahydrate	0.04
Potassium iodide	0.22
Manganese (II) chloride tetrahydrate	0.14
Sodium molybdate dihydrate	0.07
Zinc sulfate heptahydrate	0.14
Cobalt (II) chloride hexahydrate	0.18
Ethylenediaminetetraacetic acid	12

2.2 Experimental Setup

The incubation of the activated sludge was carried out in a 1 L aerobic reactor **(Figure 2)** operating under continuous supply of air at 28 – 30 °C. The reactor was inoculated with activated sludge. The aerobic conditions were achieved by supplying air at around 0.4 LPM (liter per minute) to the reactor. Dissolved oxygen (DO) probe was installed to monitor the dissolved oxygen throughout the experiment. Sampling was then performed on a 2-hour basis for a total of 6 hours.
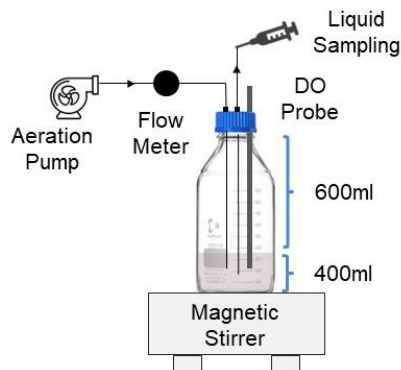
Figure 2: Experimental Setup of Incubation Batch Test

Table 3: Operating Parameters of batch test

Design Parameters	Unit	Value
Reactor Capacity	L	0.4
Room Temperature	°C	27 — 29
Aeration Flow Rate	L/min	0.4
Dissolved Oxygen	mg/L	> 2.0
Initial Biomass Concentration	VSS (mg/L)	2000
Mixing Rate	rpm	100

* VSS = Volatile suspended solid, rpm = Rotation per minute

2.3 Characterization of Influent and Samplings

Influent of municipal wastewater from STP (A) and STP (B) were characterized to obtain their total suspended solids (TSS), volatile suspended solids (VSS), and total nitrogen (TN). Filtration was performed using syringe filters (Minisart NML Plus 0.45μm, Satorius) prior to conducting ion chromatography and total nitrogen analysis.

2.4 Analytical methods

Dissolved oxygen (DO) was monitored using a DO probe meter (Major Science, FS-A-PDO). Total Nitrogen (TN) was analyzed using a Total Organic Carbon (TOC) analyzer (TOC-VSCN, Shimadzu, Japan). Ammoniacal-nitrogen ($NH_4^+ - N$), nitrate ($NO_3^- - N$),

nitrite ($NO_2^- - N$), phosphate ($PO_4^3 - P$) were measured using an Ion Chromatography (IC) (Dionex Inuvion Core IC System, Thermo Fisher Scientific).

Total and soluble chemical oxygen demand (TCOD and SCOD) was determined by using the closed-reflex dichromate method in accordance with APHA Standard Methods 5220 D.

2.3 Specific Ammonia Oxidation Rate

To evaluate the specific ammonia oxidation rate throughout the batch test, the equation below is used.:

$$Specific\ Ammonia\ Oxidation\ Rate = \frac{A_f - A_i}{MLVSS * \Delta t}$$

Where:

A_f = concentration of NH4-N at 0th hour (mg/L)

A_i = concentration of NH4-N at 6th hour (mg/L)

MLVSS = mixed liquor volatile suspended solids (2 g/L)

Δt = duration of batch test (6 hours)

3.0 Results and Discussion

3.1 Overview of Sample Collection

On-site samplings were conducted between 9.30 a.m. to 12.00 p.m., for both STP (A) and STP (B). Wastewater or mixed liquor samples were collected from the influent point after primary treatment, the anoxic tank, the aeration tank, and the final effluent point (Appendix C). The samples were analyzed for TSS (Total Suspended Solids), VSS (Volatile Suspended Solids), COD (Chemical Oxygen Demand), TN (Total Nitrogen), NH4-N, NO2-N, NO3-N, PO4-P, and alkalinity. In addition, on-site measurement of temperature was also carried out.

3.2 Influent Characteristics

The influent samples were collected after the bar screening and grit chamber, and prior to the anoxic tanks.

Table 4 shows the influent characteristics of both STPs. The average temperature of the influents of both STPs is exactly 31 ^oC, as expected for tropical climate. pH values of all influents are around 77, TSS concentrations range from 60 to 250 mg/L, and alkalinity concentrations from 139 to 150 mg $CaCO_3$/L, which are typical characteristics of municipal wastewater (Shi et al., 2015).

The carbon concentrations of the influents expressed in TCOD and SCOD are in the high range: TCOD: 251 – 237 mg/L and SCOD: 97 – 180 mg/L.

Influent NH_4-N concentrations were 24 – 29 mg/L and influent NO_2-N and NO_3-N concentrations were negligible, which are typical concentrations in municipal wastewater (Tchobanoglous et al., 2003). The influent P concentrations are in general low, between 2.38 – 2.68 mg/L, which is also lower than the P discharge limit (5 and 10 mg/L for Standard A and B respectively) of the Environmental Quality (Sewage) Regulations, 2009.

Table 4: Influent Characteristics of STPs

Parameter	STP (A)	STP (B)
pH	7.19 ± 0.02	7.22 ± 0.04
Temperature (^oC)	31 ± 0	31 ± 0
TSS (mg/L)	65 ± 35.36	200 ± 56.57
VSS (mg/L)	45 ± 21.22	100 ± 42.43
TCOD (mg/L)	251.5 ± 0.71	237.5 ± 0.71
SCOD (mg/L)	180.5 ± 0.71	97 ± 1.42
TN (mg/L)	29.13 ± 0.1	29.65 ± 0.33
NH_4^+-N (mg/L)	24.44 ± 0.06	29.6 ± 0.53
NO_2^-N (mg/L)	0 ± 0	0 ± 0
NO_3^-N (mg/L)	0.32 ± 0.02	0.81 ± 0.14
PO_4^{3-}P (mg/L)	2.48 ± 0.07	2.68 ± 0.17
Alkalinity (mg $CaCO_3$/L)	139 ± 1.42	150 ± 1.38

* TSS = Total Suspended Solids, TCOD = Total Chemical Oxygen Demand, SCOD = Soluble Chemical Oxygen Demand, TN = Total Nitrogen

3.3 Nitrogen Profile of STPs

Figure 5 shows the nitrogen profile of each batch test conducted using samples collected from STP (A) and STP (B).
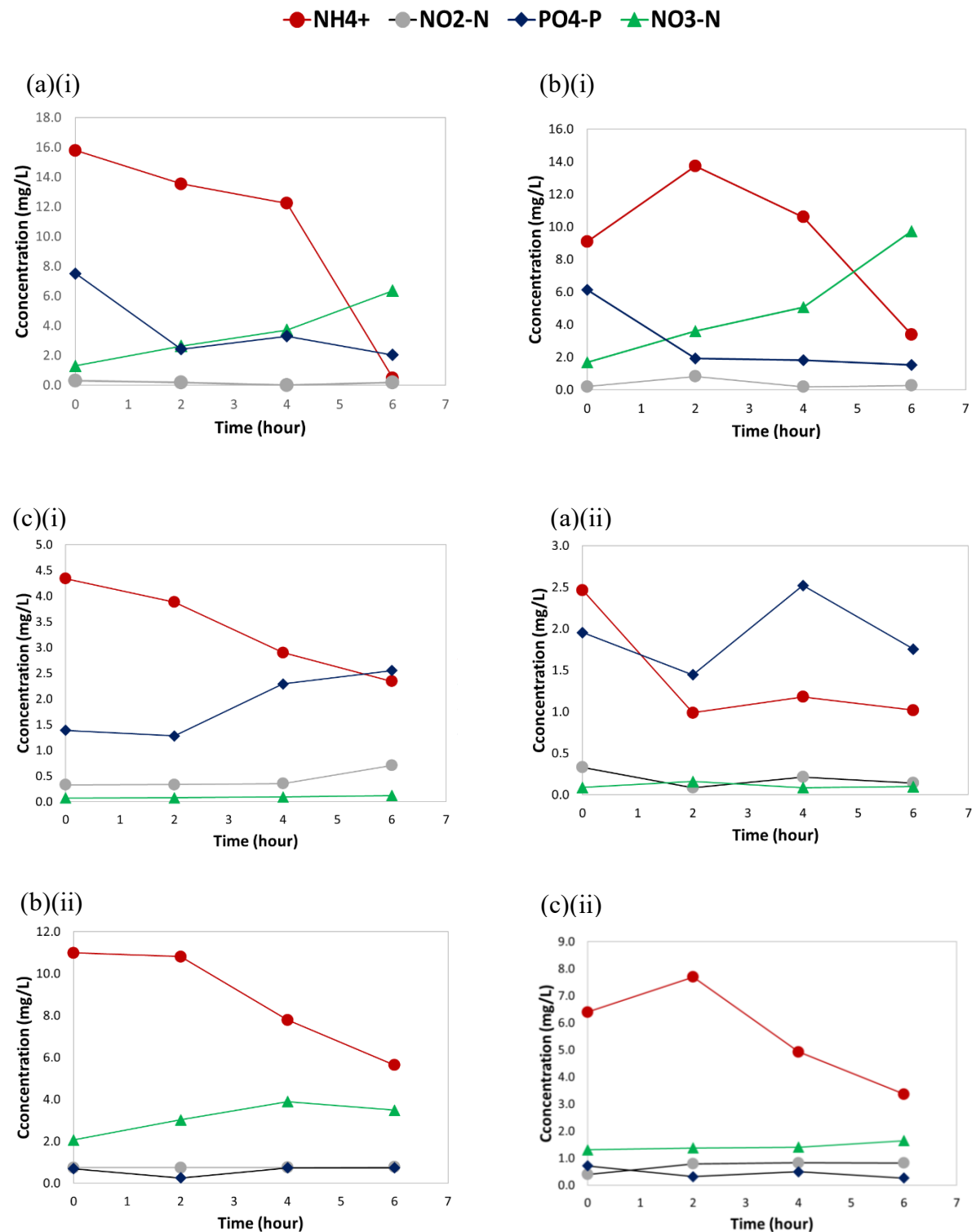
Figure 5: Nitrogen Profile of Batch Test STP A and STP B, (i) STP A, (ii) STP (B)
(a) Original Concentration of NH_4^+, (b) 60% of Original Concentration of NH_4^+.
(c) 30% of Original Concentration of NH_4^+

Based on **Figure 5 (a)(i)**, the NH_4-N dropped steadily in the first 4 hours. A sudden decrease in NH_4-N was observed from the 4th hour to the 6th hour. Similarly, the same trend has been observed for the presence of NO_3-N. The concentration of NO_3-N stays low throughout the batch test, with a maximum concentration of 0.3 mg/L attained at 0th hour.

The steadily decrease in ammonium for the first 4th hours suggests that complete nitrification occurred, with oxidation of 2.3 mg NH_4-N/L and production of 2.4 mg NO_3-N/L. However, the sudden drop of NH_4-N in the following hours implicitly shows that there might be an analytical error as gaseous nitrogen loss is not likely to occur due to the increase in TN for this period. Furthermore, the sludges have been storage for a month, it is likely that partial cell lysis and solubilization of organic nitrogen may have released additional soluble N. Such mineralization processes have been widely reported in aged or starved sludge, where microbial cell lysis releases soluble organic nitrogen that is subsequently converted to ammonium (Batstone & Keller, 2001).

On the other hand, in **Figure 5 (b)(i)**, a sudden increase in NH_4-N was observed in the first 2 hours. Then, the NH_4-N continues to drop steadily until the 6th hour. In contrast, the NO_3-N shows a steady increase trend throughout the 6-hour period.

The sudden increase of NH_4-N in the first 2 hours suggests that there is ammonification happening in the batch test, converting organic nitrogen into NH_4-N. Since the sludge has been stored for a month, it is likely that some biomasses undergo lysis, releasing soluble organic nitrogen that can be converted into NH_4-N. In the following hours, the NH_4-N decreases steadily while NO_3-N increases, suggesting nitrification was ongoing. Owing to the newly released NH_4-N, the net effect is an apparent "excess" of nitrate formation relative to NH_4-N removal when comparing the start and end points. The reduction of PO_4-P is potentially related to the biomass assimilation. This observation is consistent with known nitrogen and phosphorus uptake mechanisms during microbial growth in aerobic systems (Metcalf & Eddy, 2014).

Lastly, in **Figure 5 I(i)**, steady decrease of NH_4-N was observed throughout the batch test. A sudden surge of NO_2-N was observed during the last 2 hours, compared to minimal increase before that period. No distinct increase or decrease of NO_3-N throughout the batch test.

The inconsistency between the concentrations of NH_4-N and NO_3-N suggests that simultaneous nitrification denitrification (SND) may have occurred. Oxygen may not have penetrated the inner core of the flocs despite with high bulk DO (5.12 mg/L), resulting in anoxic zones that favor the denitrification process. Similar oxygen diffusion limitations within microbial aggregates have been observed to create anoxic zones, enabling SND even under high bulk DO (Third et al., 2001). Although the concentration of NO_3-N remained constant in the first 4 hours, a slight increase in NO_3-N was observed afterwards, further implying the possibility of incomplete nitrification as the accumulation of NO_3-N had only just begun.

Three batch tests mentioned above show steadily decrease of PO_4-P. This suggests that biomass assimilation may have occurred. In addition, the batch tests show effective removal of

nitrification, aligning with the acceptable conditions sewage discharge as stated in the Environmental Quality Act 1974.

Based on **Figure 5 (a)(ii)**, the NH_4-N dropped steadily throughout the batch test. In contrast, the increasing trend has been observed for the presence of NO_3-N. The concentration of NO_2-N drops a little throughout the batch test, with an amount of 0.14mg/L at the 6th hour.

The steadily decrease in ammonium for the whole 6 hours suggests that near complete nitrification occurred, with oxidation of 5.2 mg NH_4-N/L and production of 4.8 mg NO_3-N/L. The transient lack of oxygen in the early phase might favor denitrifiers over Nitrite Oxidizing Bacteria (NOB), causing a sudden decrease of NO_2-N in the first 2 hour. However, in the following hours, only a very minimal accumulation of NO_2-N was observed.

On the other hand, in **Figure 5 (b)(ii)**, the concentration of NH_4-N was observed to be decreasing steadily from 0th hour to 6th hour. In contrast, the NO_3-N showed an increase in the first 2 hour, and then with an almost constant trend in the following hours. The concentration of NO_2-N stays low ($\approx$0.75mg/L) throughout the batch test.

Incomplete nitrification was observed throughout the batch test as the conversion of NH_4-N to NO_3-N did not follow the expected stoichiometric trend. A DO of 6.17 mg/L was recorded at the 2nd hour, indicating the presence of strong oxygen gradient that could favor SND within the sludge flocs. Only slight fluctuations of PO_4-P were observed throughout the batch test, suggesting the biomass community is not actively taking up any phosphate which may be owing to the old sludge age.

Lastly, in **Figure 5 (c)(ii)**, a sudden increase in NH_4-N was observed in the first 2 hours. Then, the NH_4-N continues to drop steadily until the 6th hour. In contrast, the NO_3-N shows a minimal increase trend throughout the 6-hour period.

The sudden increase in NH_4-N in the first 2 hours suggests that ammonification may possibly have occurred, whereby organic nitrogen was converted into NH_4-N. As mentioned earlier, the sludge had been stored for an extended period, during which cell lysis may have taken place, releasing soluble organic nitrogen that was subsequently transformed into NH_4-N. The increase in NO_3-N was not sufficient to account for NH_4-N oxidized. This imbalance indicates that SND may have occurred.

Fluctuation of concentration of PO_4-P was observed in all three batch tests mentioned above. Further investigation needs to be done to confirm whether there are metabolic shifts or changes in the environmental conditions happening inside the microbial flocs which leads to fluctuation. Overall, the batch tests show effective removal of nitrification, aligning with the acceptable conditions sewage discharge as stated in the Environmental Quality Act 1974.

3.4 Nitrification Performance of STPs

Figure 6 illustrates the comparison of specific ammonia oxidation rate (sAOR) among the six batch tests. The (sAOR) consistently decreases with increased dilution for all batch tests. This observation scientifically indicates a direct correlation between substrate concentration and the metabolic activity of ammonia-oxidizing microorganisms. The significant decrease in sAOR, particularly from the undiluted "Original" sample to the "30% of Original" sample, suggests that the nitrification process is operating in a substrate-limited regime.
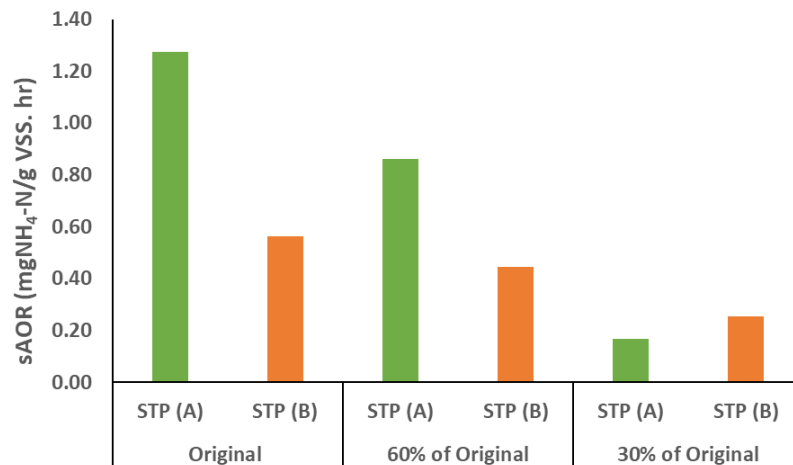
Figure 6: Specific Ammonia Oxidation Rate of each Batch Test

* sAOR = Specific ammonia oxidation rate

4.0 Suggestion and Improvement

Several aspects of this study could be refined to enhance the accuracy and interpretability of the results in future work.

1. Use of Fresh Sludge Inoculum

The sludge used in this batch test had been stored for approximately one month prior to the experiment. Prolonged storage may have caused partial cell lysis and ammonification, releasing organic nitrogen into the medium and influencing the observed nitrogen profiles. To avoid this issue, freshly collected sludge or acclimated biomass should be used in subsequent experiments to better represent active nitrification performance.

2. Continuous Monitoring of Alkalinity and pH

Continuous monitoring of these parameters would provide a clearer understanding of nitrifier activity and system stability. Tracking pH and alkalinity can confirm whether sufficient buffering capacity exists to sustain nitrification.

3. Investigation of Anoxic Zones within Flocs

Although bulk DO was maintained at approximately 5 mg/L for all batch tests, the presence of anoxic regions within sludge flocs could allow simultaneous nitrification–denitrification (SND) to occur. This phenomenon has been demonstrated in several studies investigating oxygen penetration depth and SND behavior in activated sludge (Layer et al., 2020). Future studies could employ microelectrode profiling or fine-scale DO mapping to identify oxygen gradients and confirm whether localized denitrification contributes to nitrogen transformation under high bulk DO conditions.

4. N_2O Monitoring

While N_2O was not measured in this study due to equipment limitations, its monitoring in future work would provide direct insight into intermediate processes such as partial nitrification, nitrite accumulation, or hidden denitrification. This information would be valuable in understanding greenhouse gas emissions associated with the nitrification process.

Implementing these improvements would strengthen the reliability of nitrogen mass balances, reduce experimental uncertainty, and enable a more accurate evaluation of nitrification and potential SND dynamics under varying ammonium concentrations.

5.0 Conclusion

The study revealed that dilution negatively impacts the performance of nitrification, as evidenced by a consistent decrease in the sAOR. Although all tested batch conditions produced effluent concentrations of NH_4-N and NO_3-N that met the Environmental Quality Act 1974's Standard A discharge limits (5 mg/L and 10 mg/L, respectively), these values were approaching the specified thresholds. Despite the significant reduction in sAOR caused by dilution, this effect might not be a major concern in a real-world application like an SBR. In such a system, the relative volume of the residual supernatant from the previous cycle mixing with the new incoming influent is typically small, suggesting that the overall impact on nitrification performance may be limited.

Appendix C: Sampling Point Description

Location of each sampling points are described briefly with accordance to the photo attached. Basically, grab samples obtained were from influent points.

(a) Influent point of STP (A)	(b) Influent point of STP (B)
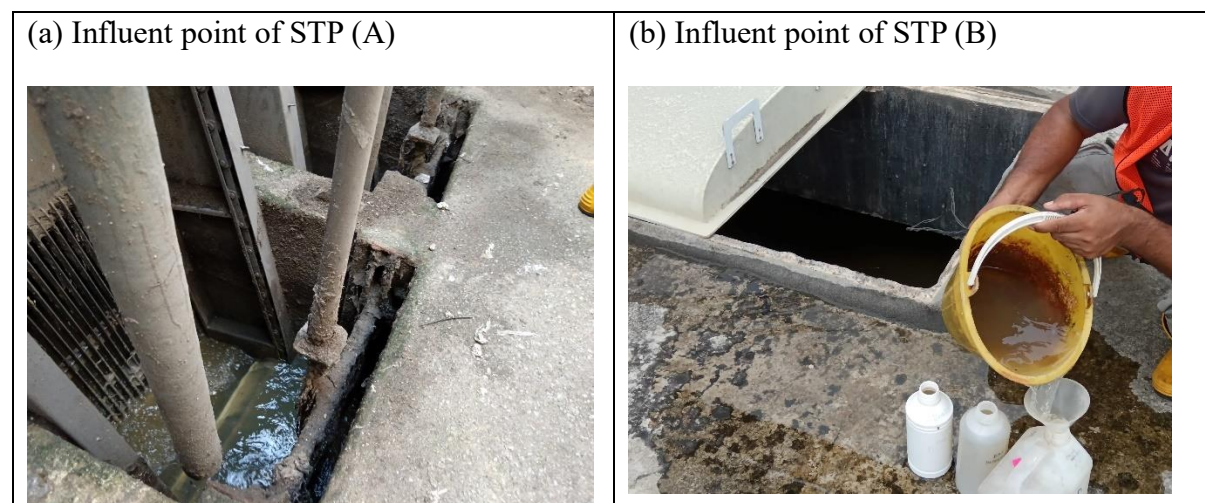
Figure 6: Sampling points of (a) Influent of STP (A); (b) Influent of STP (B).

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