

Simultaneous Removal of Nitrogen, Phosphorus, and Organic Matter from Slaughterhouse Wastewater Using AnA²/O² SBR and Its Economic Benefits

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Abstract

In this study, 20 L anaerobic/anoxic/oxic/anoxic/oxic sequencing batch reactor (AnA²/O² SBR) process was operated at a cycle time of 12 h and performed under an anaerobic static fill and four alternating anoxic/oxic conditions (1.5/2.5/2.5/1.5 h) to evaluate the performance of slaughterhouse wastewater treatment using a large-scale and to investigate the feasibility of upgrading the operating process up to the full-scale level. The results showed that the AnA²/O² SBR process has been highly successful in the treatment of slaughterhouse wastewater with average COD removal efficiency of more than 90%. While the average removal efficiency of TKN and TP was up to 90-99% and 93-97%, respectively. In accordance with those of the small scale (5 L) of the AnA²/O² SBR process in the previous studies under similar operating conditions. The results confirmed the efficiency and stability of organic matter and nutrients removals at the scale-up reactor, demonstrating the feasibility of their application in the real operating conditions. As the results of low concentrations of all pollutants in effluent and high nutrient content in excess sludge, it is quite clear that this process has revealed the high potential for environmental and economic benefits, including the energy and cost savings. AnA²/O² SBR process could be, therefore, concluded that it is one of the most effective treatment alternatives used for biological nutrient removal of the slaughterhouse wastewater.

Keywords: AnA²/O² SBR; Biological Nutrient Removal (BNR); Slaughterhouse Wastewater; Environmental and Economic Benefits

1. Introduction

The meat processing industry is growing rapidly due to increasing demand for consumption in parallel with the growth of the population. As a result of increasing both the amounts and capacity of slaughterhouse facilities, it appears that an expected higher volume of slaughterhouse wastewater needed to be treated prior to discharging it into the water bodies (Bustillo-Lecompte and

Mehrvar, 2017). Since it contains a high quantity of diluted blood, protein, urine, feces, fat, colloidal, and suspended solids, it is then certain that a large concentration of biodegradable organics and nutrients (nitrogen and phosphorus), as well as total suspended solids are often found in large quantities in slaughterhouse effluents (Massé and Masse, 2000; Seif and Moursy,

2001; Liu and Haynes, 2011; Kundu *et al.*, 2013; Amini *et al.*, 2017). If it is discharged into water bodies without any treatments, it can cause eutrophication and algal blooms leading to a decrease of dissolved oxygen until insufficiency for the survival of aquatic organisms in streams. Thus, it is necessary to effectively treat these combined organic matters and nutrients in wastewater prior to discharging into the stream with an appropriate and effective biological nutrient removal system.

Sequencing batch reactors (SBRs) is one of the attractive solutions for slaughterhouse wastewater treatment (Warodomrungsimun and Fongsatitkul, 2009; Kundu *et al.*, 2013), which is a well promoted and proved alternative with distinct advantages over conventional activated sludge (CAS) process. Due to it operates continuously with a timed control sequence in a series of five steps includes fill, react, settle, draw (decant), and idle in a single tank, this leads to gain a low capital investment and operating costs which reduce more than 60% of the operating expenses required for a typical process (Gürtekin, 2014; Dutta and Sarkar, 2015). In addition, it is widely recognized in the biological treatment of industrial wastewater since it is easy to be used and flexible in operating conditions by adjusting the sequences of anaerobic, anoxic, and aerobic (oxic) conditions to achieve high removal efficiencies of organic carbon, nitrogen, and phosphorus in the biological nutrient removal (BNR) process.

An anaerobic/anoxic/oxic/anoxic/oxic sequencing batch reactor (AnA²/O² SBR) is one of the novel technologies in BNR process that operates with a series of anaerobic-anoxic-oxic-anoxic-oxic conditions which can be modified to provide a suitable condition for microorganisms. This allows the reactors to perform a series of reactions, for example; organic oxidation, nitrification, denitrification, phosphorus release, and phosphorus uptake in the treatment system (Fongsatitkul *et al.*, 2004; Fongsatitkul *et al.*, 2008). In previous studies on slaughterhouse wastewater treatment using AnA²/O² SBR process, they were found that this process could achieve a highly effective capability with the removal efficiencies of COD, TKN, and TP over 90%

(Boonfruang, 2003; Kasawayut, 2004; Warodomrungsimun and Fongsatitkul, 2009; Buayoungyuen *et al.*, 2016; Saikomom *et al.*, 2017; Pinvattanachai *et al.*, 2019). These results suggest that this process is one of the most important for industrial wastewater treatment with high concentrations of organic matter and nutrients such as slaughterhouse wastewater. However, all studies were conducted at lab-scale with small reactors and very few have reported considering process scaling to handle large- or full-scale process efficiency. This does not provide complete background information on some knowledge factors, including the confirmation of the design procedures. These affect the effectiveness of the treatment system in actual operating condition and pose a risk to investment and operating cost. The problems mentioned above reflect the challenges of large-scale operations that require further investigation in evaluating the efficiency of slaughterhouse wastewater treatment with AnA²/O² SBR process on a large scale to pave the way for future application.

This research aims to evaluate the performance of slaughterhouse wastewater treatment using AnA²/O² SBR in a large scale and to investigate the feasibility of operating an AnA²/O² SBR process intended to apply it in the future at the full-scale application.

2. Materials and Methods

2.1 Seed sludge and Slaughterhouse wastewater

The bioreactor was seeded with sludge biomass obtained from a portion of the returned sludge line in the activated sludge system of the Din-Daeng Domestic Wastewater Treatment Plant in Bangkok (the capital city of Thailand), to achieve an initially mixed liquor suspended solids (MLSS) concentration of around 3,500 mg/L. Then it was acclimatized under ambient conditions with slaughterhouse wastewater collected from a pork processing factory located in the Nakhon Pathom province. The characteristics and composition of raw slaughterhouse wastewater were as follows: pH 6.79 - 7.95, SS 280.0 - 690.0 mg/L, COD 3,120.0 - 3,557.7 mg/L, TKN 330.5 - 457.8 mgN/L, NH₃-N 110.6 - 299.6 mgN/L, and TP 22.5 - 73.0 mgP/L.

2.2 Bioreactor set-up and operation

Experimental studies were carried out in a laboratory-scale reactor, made of a clear acrylic cylinder reactor with a working volume of 20 L. At the top of the reactor, it was equipped with an electric stirrer linking with a slowly revolving motor (30 rpm), and an axial and paddles were connected to a motor. The air outlet was on the upper part and the sludge drain port was on the under part of the reactor. While the influent and effluent ports, as well as air inlets, were connected at the side of the reactor. The system consisted of the influent container, effluent container, excess sludge container, feeding pump, air pump, air diffusers, and controller timers, as shown in Figure 1.

Operating procedures of the AnA²/O² SBR process was initiated by seeding and acclimatizing of seed sludge step by step with the slaughterhouse wastewater, starting from 25% and then progressively increasing to 50%, 75%, and finally 100% of the total volume capacity. The ratios of feed volume to volume after supernatant withdrawal and the ratio of feed volume to the total active volume of the reactor was 1:1 and 1:2, respectively, under the working volume of 10 L for the influent feed

and the effluent withdrawn in each cycle (Warodomrunsimun *et al.*, 2019). The system was operated continuously under the sequence of operating times of AnA²/O² SBR process until reaching the steady condition (at least 80% COD removal with no more than 10% variation in the removal values) prior to starting the actual designed experiments.

A set of operating times of the process was performed on a 12 h cycle time, consisting of 0.5 h filling, 1.5 h on the first anoxic time (anoxic I), 2.5 h on the first oxic time (oxic I), 2.5 h on the second anoxic time (anoxic II), 1.5 h on the second oxic time (oxic II), 0.5 h settling, 0.25 h drawing, and 2.75 h on the idle time, as shown in Figure 2. While the solids retention time (SRT) of the system was maintained at 20 and 25 d as a result of the optimum conditions from the small scale of AnA²/O² SBR process in the previous studies (Warodomrunsimun and Fongsatitkul, 2009; Buayoungyuen *et al.*, 2016; Pinvattanachai *et al.*, 2019). This was controlled by removing excess sludge daily during the idle phase. The experiments were conducted under the ambient condition (26.8 - 31.0°C) and the dissolved oxygen (DO) concentration in the aeration periods was controlled not less than 2 mg/L (2.0 - 3.5 mg/L).

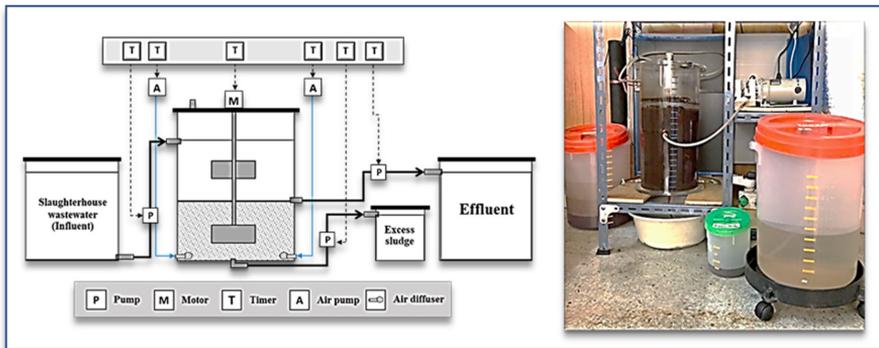


Figure 1. Schematic diagram and real photograph of the AnA²/O² SBR process

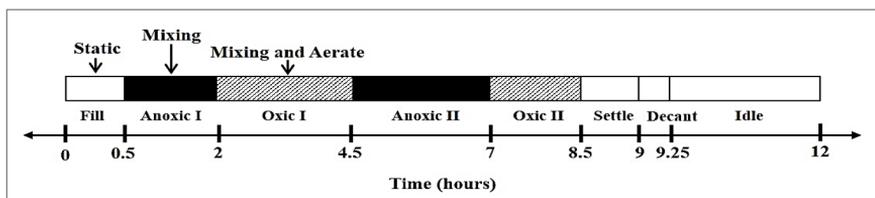


Figure 2. Operation pattern of AnA²/O² SBR

2.3 Analytical methods

Influent and effluent samples were collected two to three times per week and analyzed for chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH₃-N), total phosphorus (TP), nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N), and suspended solids (SS), along with the excess sludge which was collected and analyzed for nutrient content to evaluate the performances of the treatment system. Analysis of each parameter was conducted in accordance with the procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). While the reactor MLSS, DO, and pH, they were measured on a daily basis. The MLSS was determined by filtering the samples through a 0.45 μm membrane filter and then drying in an oven at 103°C until constant weight (APHA, 2012). For the DO and pH were measured using a commercially available membrane and glass electrodes of YSI 550A and YSI pH1200, respectively.

After that, all data were analyzed and tested for the statistically significant difference (0.05 level of significance) with the one-way analysis of variance (ANOVA) using the software package of SPSS version 18. The analysis in terms of environmental benefits was then analyzed through the quality of the effluent and waste sludge received. For the economic benefits, it was analyzed through the calculation of the energy consumption and electricity costs (Vera et al., 2013; Jafarinejad, 2017; Capodaglio and Olsson, 2020).

3. Results and Discussion

3.1 Performance of the slaughterhouse wastewater treatment using AnA²/O² SBR at the large scale

An acclimation period in the AnA²/O² SBR process lasted for about 30 d, the results illustrated that increasing each feed-step of wastewater can achieve in the wastewater treatment with more than 80% of COD removal efficiency, as shown in Figure 3. It is observed that the adaptation of microorganisms to the new feed material under controlled conditions and their ability to decompose organic matters in slaughterhouse wastewater.

All experimental results are presented in Table 1, illustrating the organic matter and nutrient concentrations in the effluents of both SRTs were lower than those in the influent wastewater. While the concentration of nitrite and nitrate (NO₂-N and NO₃-N) nitrogen were still high in the effluent of both measuring conditions. This is due to the high ammonia concentration in influent affecting the C:N ratio in the system which trended to decrease until it is insufficient for denitrification reaction. According to the study results of Ribeiro et al. (2018), who illustrated that NH₄⁺ shock loading was one of the stress conditions in the system that may cause limited DO conditions and nitrite accumulation. Additionally, Dutta and Sarkar (2015), who also found that a low COD/NH₄⁺ ratio can cause the simultaneous nitrification-denitrification (SND) process to be unbalanced.

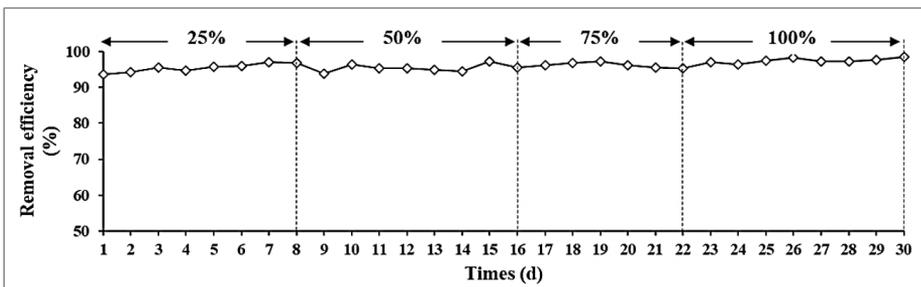


Figure 3. COD removal efficiency during AnA²/O² SBR acclimatization period

Table 1. Characteristics of the influent and effluent from slaughterhouse wastewater treatment using AnA²/O² SBR at the large scale

Parameter	Unit	SRT 20 d		SRT 25 d	
		Influent*	Effluent*	Influent*	Effluent*
pH	-	7.17 ± 0.27	6.86 ± 0.37	7.00 ± 0.15	7.29 ± 0.19
SS	mg/L	196.0 ± 17.8	5.90 ± 3.30	177.3 ± 18.0	6.45 ± 2.45
COD	mg/L	1066.6 ± 17.7	39.65 ± 9.75	1087.8 ± 16.5	33.57 ± 3.68
TKN	mg N/L	152.7 ± 18.6	1.49 ± 0.75	127.73 ± 8.70	5.05 ± 4.16
TP	mg P/L	17.67 ± 4.26	1.22 ± 1.40	12.02 ± 2.52	0.44 ± 0.35
NH ₃ -N	mg N/L	120.6 ± 17.3	0.90 ± 0.75	88.73 ± 4.69	4.69 ± 4.00
NO ₃ -N	mg N/L	8.20 ± 2.25	11.02 ± 6.85	7.18 ± 1.36	9.00 ± 6.49
NO ₂ -N	mg N/L	0.40 ± 0.07	21.18 ± 5.66	0.33 ± 0.04	5.48 ± 2.91

Note: *average ± S.D. (n = 15)

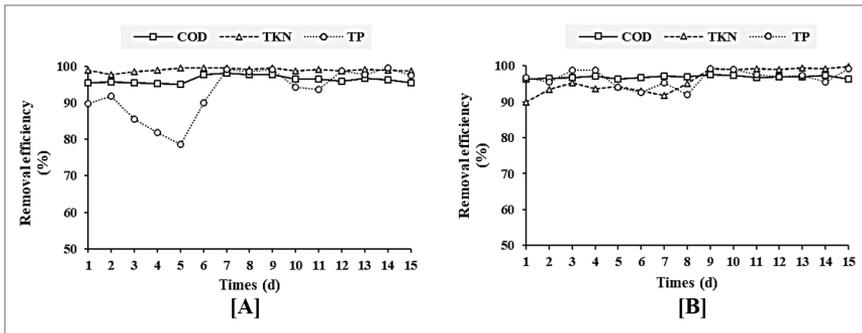


Figure 4. Performance of the AnA²/O² SBR treating slaughterhouse wastewater; [A] SRT 20 d and [B] SRT 25 d

Furthermore, the study also found a decline of TP during certain periods of operation (as shown in Figure 4[A]). This is due to a decrease in the concentration of organic matter in the reactor at a lower influent C:N ratio, which resulted in the competition for organic substrates between denitrifying bacteria and phosphorus accumulating organisms (PAOs) (Mulkerrins *et al.*, 2004; Xu *et al.*, 2014). In the study of Zeng *et al.* (2011), it was described that an insufficient amount of carbon sources would affect the release of phosphorus and the synthesis of poly-hydroxyalkanoates (PHAs), leading to the limited efficiency of phosphorus uptake in anoxic and aerobic conditions.

However, the final effluent concentrations of the interested parameters (pH, COD, TKN, TP, and SS) of both SRTs were greatly reduced and in line with the industrial effluent standard of Thailand (Department of Industrial Works, 2017), reflecting the removals of organic matter and nutrients in the treatment system using AnA²/O² SBR process. The results showed that more than 90% of the removal efficiencies of COD, TKN, and TP were achieved, as shown in Figure 4. These results revealed

that this process was an extremely effective process for eliminating organic matter and nutrients in wastewater. Hence, it is an important process that allows for the reduction of pollutant concentrations and also improves the effluent quality to meet regulatory standards prior to discharging to the receiver.

From the experimental results, the removal efficiencies of organic matter and nutrients obtained in this study were in accordance with a small-scale AnA²/O² SBR process performed in previous studies, which were observed under similar features and conditions in the wastewater treatment system, for example; the operating pattern, the type of wastewater, and SRT (Buayoungyuen *et al.*, 2016; Pivattanachai *et al.*, 2019). The results are illustrated in Figure 5, revealing similar removal efficiencies of all parameters over 90% in both the small- and large-scale reactors. According to the statistical analysis, it was found that there were no significant differences ($p < 0.05$) between two lab-scale reactors, indicating a good consistency in the performance of AnA²/O² SBR process as the process was converted from small-scale to large-scale.

Therefore, these results indicate that this process on a large scale has successfully eliminated both organic matter and nutrient concentrations in wastewater, which ensures the high and stable removal efficiencies as the reactor is expanded and demonstrates a high potential to be applied at a larger scale.

3.2 Performance of the AnA²/O² SBR process

All results obtained under different operating conditions from both the larger reactor of AnA²/O² SBR process in this study and the small reactor of AnA²/O² SBR process in the previous studies, were shown in Table 2. It pointed out that the results were achieved with removal efficiencies of more than 90% for organic matter and nutrients from slaughterhouse wastewater. It demonstrated the stability of the reactor as it was scaled up illustrating clearly the feasibility of using the SBR process at the full-scale application.

Overall, the application of AnA²/O² SBR process appears to be one of the best alternatives for slaughterhouse wastewater treatment due to the ease of operation and flexibility in operating conditions of the SBR process. It allows to have an effective process control to achieve the desired quality of treatment (Dutta and Sarkar, 2015; Alagha et al., 2020). While the alternate cycling of anaerobic, anoxic, and oxic conditions during the fill and react phase can stimulate the growth of microbes, especially autotrophs, heterotrophs, and PAOs, allowing the simultaneous removal of very high biological organic carbon and nutrients (Zuthi et al., 2013; Daigger and Littleton, 2014; Jaafari et al., 2017). It is due to the anaerobic and anoxic condition occurred during the shutting down aeration would support the growth of denitrifying bacteria for denitrification reaction and the growth of PAOs to take up volatile fatty acids (VFAs) and store it

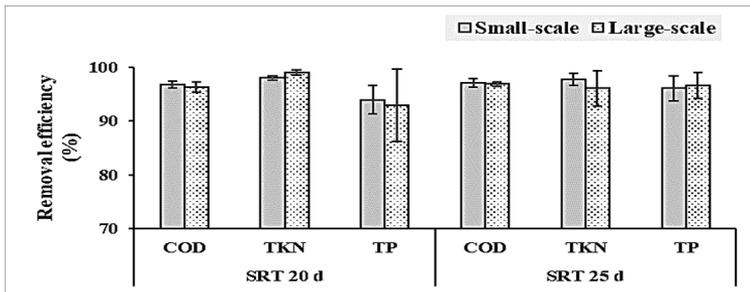


Figure 5. Mean efficiency removal and standard deviation (SD) values of organic matter and nutrients in slaughterhouse wastewater using AnA²/O² SBR under SRT 20 and 25 d in small- and large-scale

Table 2. The operation of AnA²/O² SBR process

Reference	Wastewater type	Removal efficiency (%)			Cycle time (h)	SRT (d)	Reactor volume (L)
		COD	TKN	TP			
Kasawayut, (2004)	Slaughterhouse (Raw wastewater + Anaerobic filter effluent)	98.2	98.2	98.9	12	70	5
Warodomrungsimun et al. (2009)	Slaughterhouse	97.9	97.7	95.5	12	60	5
Buayoungyuen et al. (2016)	Slaughterhouse	96.4	98.1	92.1	12	20	5
Pinvattanachai et al. (2019)	Slaughterhouse	95.0	96.6	84.9	12	20	5
This study	Slaughterhouse	96.3	99.0	93.0	12	20	20
		96.9	96.1	96.6	12	25	20

as PHAs within their cells. While the oxic (aerobic) condition occurred in the aeration portion to stimulate the growth of nitrifying bacteria for improving nitrification reaction and PAOs to metabolize stored organic matter and growth. In addition, the second of anoxic and oxic conditions in the react phase increase the efficiency of the system for nutrient removal in which the denitrifying bacteria used nitrate under the second anoxic condition without external substrate in the endogenous denitrification process. Then the excess COD removal, excess nitrification, N₂ stripping, and prevention of secondary phosphorus release could be occurred under the second oxic condition (Warodomrungsimun *et al.*, 2009).

Moreover, it is important to note that the SRT is a key operational factor for both the removals of organic matter and nutrients from wastewater and the improvement of growing microorganisms in the operating of AnA²/O² SBR process. Even though, the results in previous studies showed that the short SRT (below 10 d) allowed to achieve a substantial carbon removal with low energy demands and operation costs. But it may cause dispersion of suspended growth in the bioreactor due to a high organic loading or F/M ratio in the system, resulting in the lost sludge biomass and finally the system failed. While the extended SRT resulted in an additional time for bacteria to acclimate to potentially toxic or inhibitory compounds and to adjust to any carbon compounds that were difficult to degrade, which allowed to maintain more active sludge in the system. These led to an increase in the

endogenous respiration and predation of microbes and a reduction in the sludge yield (Warodomrungsimun *et al.*, 2009; Kundu *et al.*, 2013; Zuo and Ji, 2013; Buayoungyuen *et al.*, 2016). Consequently, the longer SRT is required to achieve a high level of nitrification reaction for the nitrogen removal and more accumulated phosphorus in the biomass. But under extremely prolonged SRT (above 30 d) conditions, it had the effect of a reduction in nitrogen removal due to the loss of bacterial growth rate. Similarly, this is still the cause of poor phosphorus removal performance due to the reduction of excess sludge wasting (Li *et al.*, 2008; Lee *et al.*, 2013).

However, several studies show that the best operational SRT was obtained from a long SRT of 10 - 20 d or during 20 - 25 d of SRT, which allowed the enrichment of slowly growing bacteria and suitable for carbon oxidation and nitrification reaction in the treatment system (Wu *et al.*, 2011; Kundu *et al.*, 2013; Zeng *et al.*, 2013; Bustillo-Lecompte and Mehrvar, 2015). A similar trend had been observed in the results of this study, which was found that both SRT (20 and 25 d) were sufficient for microbial growth under anaerobic, anoxic, and aerobic conditions. Also, it was suitable for the biomass accumulation in the bioreactor, leading to an increase in permanence for long-term operation. Where the comparison results were found that the operating condition at SRT 25 d was the optimal condition of the AnA²/O² SBR process in a large scale with the removal efficiency of COD, TKN, and TP of 96.9%, 96.1%, and 96.6%, respectively.

Table 3. Concentration of phosphorus in treated effluent and excess sludge

References	P discharge (mg P/L)	P content (mg P/g sludge)	Cycle time (h)	SRT (d)	Reactor volume (L)
<i>Buayoungyuen et al.</i> (2016)	0.27 - 5.12	15.5 - 38.0	12	10	5
	0.17 - 3.59	16.0 - 36.5	12	20	5
	0.27 - 4.17	16.0 - 30.5	12	30	5
<i>Pinvattanachai et al.</i> (2019)	0.04 - 16.5	8.72 - 31.5	12	20	5
	0.03 - 6.27	10.9 - 28.6	12	25	5
	4.82 - 23.1	6.37 - 25.7	12	60	5
<i>This study</i>	0.10 - 4.40	16.5 - 20.5	12	20	20
	0.10 - 1.20	15.7 - 23.2	12	25	20

Meanwhile, the results also indicating that a large amount of phosphorus content accumulated in the excess sludge was obtained from the AnA²/O² SBR process, as shown in Table 3. These results indicated a highly effective of assimilation occurred in metabolism and growth as nucleic acids, phospholipids, and nucleotides, including phosphorus uptake into the cell of PAOs to storage as polyphosphate. As a result of the AnA²/O² SBR operation, it has improved the effluent quality and phosphorus removal efficiency via the release and uptake phosphorus process by cycling anaerobic/anoxic and oxic conditions (Paul *et al.*, 2001). It is obvious that this process not only improved the quality of the nutrient concentrations in treated effluents but also preserved a high concentration of nutrients in sludge. These could be helpful for sustainable nutrient recovery in the future (Ndegwa *et al.*, 2008; Yuan *et al.*, 2009).

3.3 Feasibility of environmental and economic benefits

Based on the evidence of this study, it can be stated that the AnA²/O² SBR process was found to be more environmentally feasible benefits than the CAS process for treating slaughterhouse wastewater due to it could reduce the pollutant load in effluent over a long-term operation. The removal efficiencies of COD, TKN, TP, and SS were more than 90%, these results were consistent with those of several studies. These results indicated that the SBR system was highly capable of removing nitrogen and phosphorus concentrations. It was also able to eliminate the growth of filamentous bacteria and improve the settling properties of activated sludge, resulting in high biomass and no sludge bulking in the system (Abdelkader, 2009; Kaur *et al.*, 2014; Dutta and Sarkar, 2015; Capodaglio and Olsson, 2020).

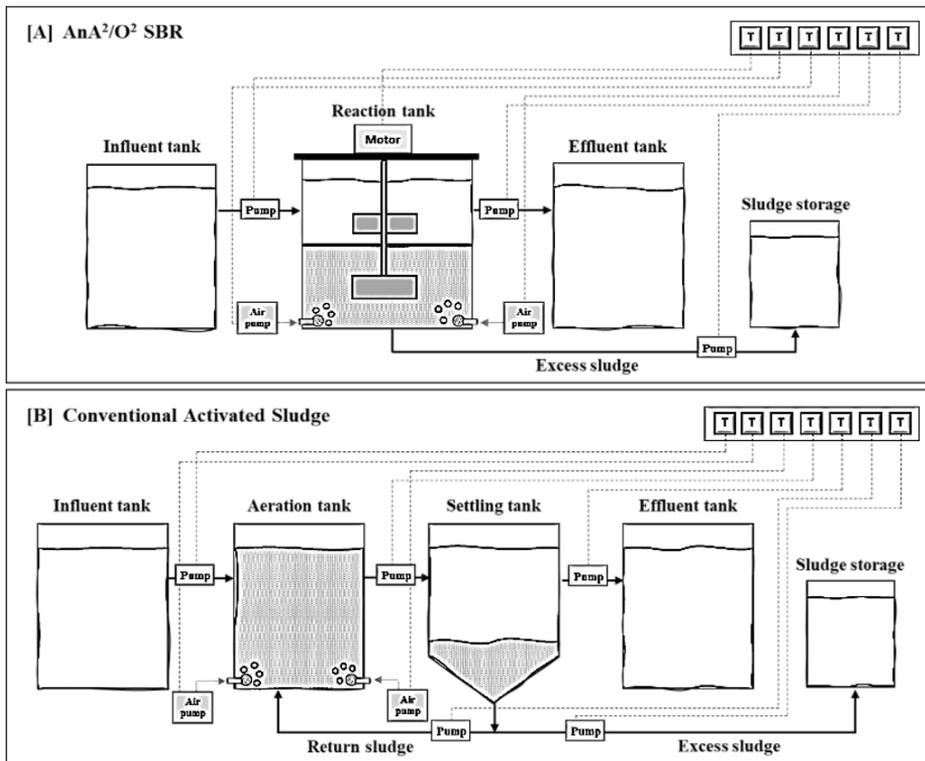


Figure 6. Layout of wastewater treatment plant; [A] AnA²/O² SBR process and [B] CAS process

In Addition, the experimental results showed that this process can be preserved a high nutrient concentration in excess sludge. The concentration of phosphorus accumulated in excess sludge obtained from the small reactors in a previous study and the large reactors in this study were 18.3 - 22.8 mg P/g sludge and 18.0 - 19.0 mg P/g sludge, respectively. Therefore, this can be utilized further in the recovery of nutrients for being reused as fertilizer in agriculture or soil conditioner for land application (Mtshali *et al.*, 2014; Gordon, 2015). Obviously, the process still had great potential economic benefits as well.

This process has a high potential for energy and cost savings. The operational process and energy consumption of AnA²/O² SBR process in this study and CAS process are modified from the literature (Metcalf and Eddy, 2003; Vera *et al.*, 2013; Jafarinejad, 2017; Capodaglio and Olsson, 2020), were depicted in Figure 6 and Table 4. It was found that the electric energy requirement for equipment used in AnA²/O² SBR process was about 42.65 kWh/m³ of wastewater corresponding to 0.039 kWh/g of COD, which was equivalent to the cost of wastewater treatment of approximately

Table 4. Comparison of measured electrical energy consumption in a wastewater treatment plant

AnA ² /O ² SBR process			
Equipment	Power (W)	Working times (h/d)	Energy (kWh/d)
Fill phase			
Pump _{feed}	22	1.0	0.022
Timer _{feed}	3	1.0	0.003
React phase			
Stirrer	34	16.0	0.544
Air pump	10	8.0	0.080
Air pump	10	8.0	0.080
Timer _{stirrer}	3	16.0	0.048
Timer _{air}	3	8.0	0.024
Timer _{air}	3	8.0	0.024
Settle phase			
-			0.000
Decant phase			
Pump _{decant}	22	0.5	0.011
Timer _{decant}	3	0.5	0.002
Idle phase			
Pump _{excess}	22	0.25	0.006
Stirrer	34	0.25	0.009
Timer _{excess}	3	0.25	0.001
Timer _{stirrer}	3	0.25	0.001
Total energy consumption (kWh/d)			0.853
Costs of process:			
Total cost = 3.334 THB (0.100 USD)			
Electricity cost = 0.167 THB/L (0.005 USD/L)			

CAS process			
Equipment	Power (W)	Working times (h/d)	Energy (kWh/d)
Influent tank			
Pump _{feed}	22	24.0	0.528
Timer _{feed}	3	24.0	0.072
Aeration tank			
Air pump	10	24.0	0.240
Air pump	10	24.0	0.240
Timer _{air}	3	24.0	0.072
Timer _{air}	3	24.0	0.072
Settling tank			
-			0.000
Effluent tank			
-			0.000
Return sludge			
Pump _{decant}	22	24.0	0.528
Timer _{decant}	3	24.0	0.072
Sludge storage			
Pump _{excess}	22	0.25	0.006
Timer _{excess}	3	0.25	0.001
Total energy consumption (kWh/d)			1.830
Costs of process:			
Total cost = 7.154 THB (0.214 USD)			
Electricity cost = 0.358 THB/L (0.011 USD/L)			

Note: exchange rate = 33.432 Thai Baht (THB) to 1 US Dollar (USD)

0.167 THB/L (or equal to about 0.005 USD/L) of wastewater flow in the process. These results pinpointed that the required energy was approximately 50% lower than that of the CAS process due to the reduction in the aeration system during operation. According to the related studies, it was found that the aeration process accounts for 45 - 75% of total energy consumption in an activated sludge process. While the SBR process can save 30 - 40% of energy consumption through the aeration control (Luccarini *et al.*, 2013; Vera *et al.*, 2013; Ekici, 2017; Capodaglio and Olsson, 2020; Lazic *et al.*, 2019). Similarly, this process was performed through the operation of an automatic timer switch, allowing it to control the oxygen transfer and avoid excessive aeration for mixing and biological activity, which may affect the flocculation and cause the solids to be transferred into the effluent.

Moreover, the results showed that AnA²/O² SBR process was less energy intensive than the CAS process since the SBR process was operated without a continuous flow of influent or effluent and aeration. It can be performed without return activated sludge (RAS) pipes and pumps like those involved in an activated sludge process (US.EPA, 1999; Au *et al.*, 2013; Marnier *et al.*, 2016). This has resulted in a decrease in energy consumption during the operation, causing the costs of operation in this process were simultaneously lower than other processes. Since the system was a combination of all treatment steps and processes in a single reactor without the use of primary and secondary settling tanks, it then results in a reduction in less space need for operations. It was also operated without a sludge return system and adding equipment or chemicals into the system that made it less equipment to maintain. Likewise, the automated processes of operation allowed to reduce the need for less workforce. The observations made it apparent that the process can save money by reducing electricity costs, operational costs, and staff working hours (NEIWPC, 2005; Luccarini *et al.*, 2013; Piotrowski *et al.*, 2019; Alagha *et al.*, 2020).

4. Conclusion

In this study, an AnA²/O² SBR reactor is replicated to evaluate the performance of the process in a large scale and to investigate the operational feasibility of the process to be used at the full-scale application. The experimental results can be concluded as followed:

- AnA²/O² SBR process has been highly successful in the treatment of slaughterhouse wastewater with the removal efficiencies of organic matter and nutrients above 90%, which were consistently in both a small-scale of AnA²/O² SBR process in the previous studies under similar operating conditions. Obviously, this process ensures the efficiency and stability of the removals of organic carbon, nitrogen, and phosphorus as the reactor is expanded and demonstrated the feasibility of implementation in the real operating conditions.

- The SRT has a direct influence on bioprocess performance in which it is associated with the growth of microorganisms and equilibrium in the treatment system. Also, it indicated that the removal efficiencies of organic matter and nutrients in wastewater at the extended SRT were better than that short SRT. Therefore, the operating condition at SRT 25 d was the optimum condition of the AnA²/O² SBR process in a large scale with the average removal efficiencies of COD, TKN, and TP were 96.9%, 96.1%, and 96.6%, respectively, and 19.0 mg P/g of phosphorus concentration was accumulated in excess sludge.

- From the experimental results, it was found that this process can reduce the pollutant load in effluent over a long-term operation. Also, the process can recover essential nutrients and turning them into a valuable product. These were evident in both the environmental and economic benefits of AnA²/O² SBR process. For the economic benefits, the results were found that the process could save the expenses due to the reduction of electricity costs, operational costs, and staff working hours. This study clearly pinpoints the energy and cost saving potential for the innovative wastewater management.

AnA²/O² SBR process is therefore one of the good alternatives for the wastewater treatment system. However, in the full scale of WWTP operation, the energy consumption from the actual equipment used, especially the aerators and pumps should be ensured because it is quite different from the laboratory scale.

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