



## Performance and Kinetics of the Treating Slaughterhouses Wastewater Using Sequencing Batch Reactor

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### Abstract

This research investigated the processes of simultaneous biological removal of organic carbon, nitrogen, and phosphorus from slaughterhouse wastewater and determined the bio-kinetics of the organic carbon and nutrient removal process under long sludge age (solids retention time 60 days) using a sequencing batch reactor (SBR). The reactor processed the wastewater by varying the solids retention times (SRTs) (10, 20, 30 and 60 days) under a 12 h cycle, which included a 0.5 h anaerobic static fill period and a 0.5 h settle period, while the remaining reaction time consisted of Anoxic I/Oxic I/Anoxic II/Oxic II (1.5/2.5/2.5/1.5 h). The result of the cyclic study revealed that the majority of the chemical oxygen demand (COD) removal occurred in the first anoxic period with the reaction of the denitrification process and the anaerobic phosphorus release process. The concentration of total kjeldahl nitrogen (TKN) in the reactors decreased, and the percentage of oxidation was greater than 80%, at the end of oxic I period. The resulting biomass composition was that the VSS/SS ratio, at 60 days SRT, was lower than at the 30 days SRT. The fraction of PHA ( $f_{PHA}$ ) at the 60 days SRT was lower than at 30 days SRT. Prior to the study of the kinetics parameters, sensitivity analysis was conducted to evaluate the most important parameters. The results of the sensitivity analysis showed that the  $\mu_{H,S}$ ,  $\eta_{NO_2}$ ,  $\eta_{NO_3}$ ,  $k_{STO}$ ,  $Y_{H,S}$ , and  $Y_{H,STO}$  had an influence on the denitrification process, and the  $\mu_{AOB}$ ,  $\mu_{NOB}$ ,  $Y_{AOB}$  and  $Y_{NOB}$  had an influence on the nitrification process. Also, the  $q_{PO_4}$ ,  $q_{PP}$ ,  $Y_{PHA}$ ,  $K_{max,PAO}$  had an influence on the biological phosphorus removal. The result of kinetic parameter estimation of  $q_{PHA}$ ,  $Y_{PO_4}$ ,  $q_{PP}$ , and  $Y_{PHA}$  were  $1.33 \text{ day}^{-1}$ ,  $0.18 \text{ mgP/mg COD}$ ,  $0.13 \text{ day}^{-1}$ , and  $0.19, \text{ mgP/mg COD}$ , respectively. The results of the kinetics studies and evaluation of the systems performance can be used to justify the operational and design for the effectiveness of SBR.

**Keywords:** Slaughterhouses Wastewater, Sequencing Batch Reactor, Solids Retention Time (SRTs), Biological Nutrient Removal

### Introduction

Population growth and economic expansion in Thailand has lead an expansion of Thai food industries in both number of producers and production capacity. According to the report of the Department of Industrial Work in the Ministry of Industry, there were 1,327 registered food industries in 2015 (Ministry of Industry, 2017). Also, the 2017 report from the Bureau of Livestock Standards and Certification showed that 17,079 tons of swine products were produced in 2015, which represented an increase of 18.5% over the 2012 levels of production (Ministry of Agriculture and Cooperatives, 2017).

This increase in the growth of the meat processing industry has generated a significant increase in the amount of high-strength organic wastewater, generated during slaughtering and cleaning operations. The constituents of concern in this wastewater are bio-degradable organic matter and plant nutrients (nitrogen and phosphorus). The organic matter in the meat processing wastewater are residual blood, fat, oil and grease



(from skin), which are desorbed during the process of feather removal and immersion chilling. Additionally, residual blood, urine, manure, cleaning and sanitizing compounds are also notable sources of nitrogen and phosphorus (Lawrence, Yung-Tse, Howard Lo, & Constantine, 2006; U.S. EPA, 2002). Discharging of organic matter and plant nutrients (N and P) were major causes of eutrophication (algae bloom) and increase in growth of aquatic plant. This result can lead to adverse effect to water body and induce to rise of water pollutions (Sawyer, McCarty, & Parkin, 2003).

Biological nutrient removal (BNR) systems are processes used for simultaneous removal of carbonaceous organic matter, nitrogen, and phosphorus from wastewater. These processes are based on the ability of microorganisms (autotrophs, heterotrophs, and phosphorus accumulating organisms (PAOs)) under different environmental conditions (anaerobic, anoxic, and aerobic) in the treatment processes (Comeau, Hall, Handcock, & Oldham, 1986; Brdjanovic et al., 1998; Mino, Van Loosdrecht, & Heijnen, 1998).

However, full-scale BNR plants have two significant drawbacks: they require large areas of land, and are operationally complex. Sequencing Batch Reactor (SBR) is a technology that can serve as a viable alternative solution, solving the land availability problem, and providing flexibility and simplicity of operations (Artan, Wilderer, Orhon, Morgenroth, & Ozgur, 2001; Hopkins, Lant, & Newell, 2001; Randall, Barnard, & Stensel, 1992; Metcalf & Eddy/Aecom, 2014).

SBR systems allow for the selection of a sequence of oxygen tensions or electron acceptors in the operating cycle, providing alternation of anaerobic, anoxic, and oxic conditions during a single cycle in a reactor without requiring a recycling line or clarifiers. This system has shown highly effective performance for carbonaceous organic matter and nutrient (nitrogen and phosphorus) removal during the treatment of a variety of wastewaters. (Fongsatitkul, Wareham, & Elefsiniotis, 2008; Sathian, Rajasimman, Radha, Shanmugapriya, & Karthikeyan, 2014; Cassidy & Belia, 2005). Additionally, the operations of the SBR depend on the solids retention time (SRTs) or sludge age (Kargi & Uygur, 2002). SRT is the main driver that governs the active biomass and the effluent quality of the wastewater treatment plants.

Our work was carried out with the objectives:

- (1) to assess the performance of the treatment process, using the SBR at different SRTs, for simultaneous removal of COD and nitrogen and phosphorus nutrients (N and P) from slaughterhouse wastewater, and
- (2) to determine the bio-kinetics of the organic carbon and nutrient removal process under long sludge age (SRT 60 days).

The result of our kinetics studies can be used to justify the operational and design effectiveness of SBR as an alternative to biological nutrient removal systems.

## Methods and Materials

### 2.1 Bio-reactor Setup, Sludge Acclimatization and Wastewater Characteristics

The currently employed treatment systems were based on biological processes, using three identical sequencing batch reactor (SBR) systems, operating in parallel. The reactor was controlled by a microprocessor, and each reactor consisted of a 5 L acrylic cylinder with a diameter of 14.3 cm and length of 38 cm, with both ends of the cylinder sealed. At the top of the cylinder, a slowly revolving motor (30 rpm) was installed and connected to a rotating axle, to which two stainless steel paddles were attached, for aeration



and agitation, one halfway down the cylinder, the other near the bottom of the cylinder (Figure 1). Aeration and agitation were provided by using an air pump / air diffuser and stirred by the paddles linked with the slowly axle, driven by the revolving motor at 30 rpm.

The total liquid volume of the reactor was 5 L, the volume in the reactor after effluent discharge (supernatant withdrawal) was 2.5 L per cycle, or the ratio of  $V_F:V_O$  is 1:1 and the ratio of  $V_F:V_T$  is 1:2, where  $V_F$  is volume of the feed,  $V_O$  is remaining volume after supernatant withdrawal and  $V_T$  is the total active volume of the reactor. Prior to start of the sequencing batch reactor operation, the seeding sludge for make up the initial concentration of active biomass collected from the returned activated sludge line of Rajavithi Hospital Wastewater Treatment Plant. The initial seed concentration of active biomass (Mixed Liquor Suspended Solid (MLSS)) was controlled about of 3,000 mg/L for each reactor. The SBR systems start-up with acclimatization period to allowed the active biomass to get acclimatized to the new condition. The operating liquid volume of 2.5 L was an initial starting of the reactors. A step by step wastewater feeding approach was followed during acclimation. Each reactor allowed achieving at least 80% of COD removal in each operating cycle of the system before a further increase the volume of feeding about 0.25 L step by step. In this period, the system operating without any liquid and sludge wastage until reaching the target liquid volume of about 5 L.

The wastewater was collected from the Pork Traders Co-operative of Bangkok Limited located in Klongtoei District, Bangkok. The characteristics of influent wastewater were presented in Table 1.

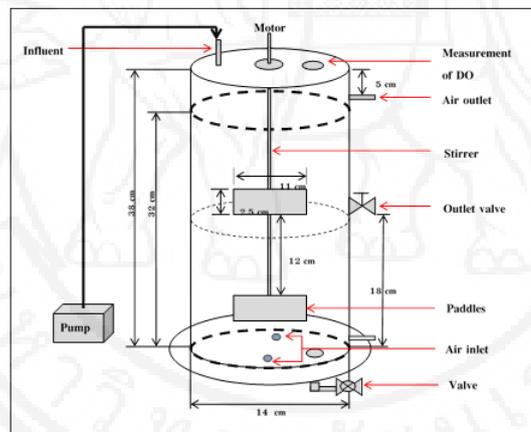


Figure 1 Schematic diagram of AnA<sup>2</sup>/O<sup>2</sup> SBR

Table 1 Characteristics of influent wastewater

Parameters	Average	Range
pH	7.3 ± 0.2	7 - 8
TCOD <sup>*</sup> (mg/L)	862.0± 128.4	672 - 1,090
SCOD <sup>*</sup> (mg/L)	669.6± 92.8	452 - 818
TKN (mg/L)	84.2± 11.9	63 - 119
Total Phosphate (mg/L)	14.7± 3.3	9 - 22
NH <sub>3</sub> -N (mg/L)	42.5± 11.4	23 - 60
NO <sub>2</sub> -N (mg/L)	0.08± 0.04	0.04 - 0.21
NO <sub>3</sub> -N (mg/L)	7.0± 1.9	4 - 12
Suspended Solids (mg/L)	303.0± 118	150 - 680

\*Remarks; TCOD = total chemical oxygen demands; SCOD = soluble chemical oxygen demands



## 2.2 Experimental Procedures and Analytical Methods

The operational patterns of the SBR were performed by varying the SRTs (10, 20, 30, and 60 days) under a 12-hour cycle, which including a 30 min anaerobic static fill period and a 30 min settle period. The remaining reaction time consisted of anoxic I, oxic I, anoxic II, and oxic II (1.5/2.5/2.5/1.5 hours) and the DO concentration in the reactor was controlled at not less than 2 mg/L during the oxic phase. Raw wastewater (influent) and effluents were measured to ascertain out the performance of the SBR system. After the system reached the steady-state, samples were collected during the anoxic and oxic phases of the SBR process in order to determine the cyclic profile of COD, TKN, TP, nitrite and nitrate concentrations, prior to estimating the kinetic parameters with the AQUASIM 2.0 software. (Reichert, 1998)

System monitoring studies sampled for COD, TKN, phosphorus, and nitrate analysis. Dissolved oxygen (DO) concentration was analyzed by a YSI DO meter (electrode methods), COD, TKN, phosphorus, nitrate, MLSS, and MLVSS also followed the Standard Methods (APHA, 2012). The concentrations of internal storage polymers (polyhydroxyalkanoates; PHA) were measured using the methanolysis-GC method according to Chinwetkitvanich, Randall, and Panswad (2014). Sludge-biomass collected from the batch systems was centrifuged and dried at 100°C for at least 24 h. The dried sludge-biomass was extracted with chloroform and sulfuric-methanol solution. The extracted layers were analyzed by gas chromatograph (Agilent 6890N) equipped with a flame-ionization detector and capillary column (INNOWAX 19095N). The internal standard for the determination of PHA content was benzoic acid.

## 2.3 Kinetic Parameter Estimation and Sensitivity Analysis

The Activated Sludge Model 2d (Henze, Gujer, Mino, & van Loosdrecht, 2000) was used for developing the four reaction phases, anoxic I, oxic I, anoxic II, and oxic II SBR (AnA<sup>2</sup>/O<sup>2</sup> SBR model). The ASM2d was a model for bio-P removal with the concept of nitrification and denitrification (biological nitrogen removal) in the activated sludge processes. This model expanded the process to include the activity of the phosphorus accumulating organisms (PAOs) under anoxic condition. The model in this study was modified from ASM2d plus the concepts of 2-step nitrification and 2-step denitrification, for describing the phenomenon of AnA<sup>2</sup>/O<sup>2</sup> SBR.

The estimation and sensitivity analysis of kinetic parameters were performed and calculated by AQUASIM 2.0 with a mass balance kinetics model concept (the Activated Sludge Model bases). Before estimating the parameters, the effects of the uncertainties in the model parameters were taken into the calculation by sensitivity analysis. A result of low sensitivity indicates that it is difficult to give coefficient values to a unique parameter. The parameter estimation procedure was a process of adjusting the coefficient values of the kinetic model, so that the results calculated by the model with these coefficients fitted with a group of measured data. The selection of the parameters for estimation is mainly based on the results of the sensitivity analysis. The kinetic and stoichiometric parameters of the active biomass were calculated at room temperature.



## Results and Discussion

### 3.1 Systems performance

System performance would help to visualize the picture of the operational benefits of AnA<sup>2</sup>/O<sup>2</sup> SBR. The cyclic profile of organic carbon (COD) and nutrients in the reactor represented the transformation and movement of COD, nitrogen, and phosphorus concentration in the reaction periods (Anoxic I, Oxidic I, Anoxic II, and Oxidic II), as illustrated in Figure 2. The results of the cyclic study revealed the majority of COD removal occurred in the first anoxic period with the reaction of denitrification process and anaerobic phosphorus release process. It can be seen that very high COD removal was achieved throughout this period. During the first oxidic period, the concentrations of TKN in the reactors decreased and the percentage of oxidation was greater than 80% at the end of oxidic I period. In addition, phosphorus was taken up during this period which was demonstrated by the decreased concentration of phosphorus. Conversely, the concentration of nitrite and nitrate increased during the first oxidic period through nitrification process. After that, both nitrite and nitrate slightly decreased during the second anoxic period.

The effluent concentrations of COD, TKN, TP, and NH<sub>3</sub>-N were decreased gradually in accordance with the SRTs. It indicated that the shorter SRT (10–20 days) conditions could remove organic and nutrients efficiently, according to that reported by Ge, Batstone, and Keller (2013), who found that the SBR process at low SRT (2–3 days) can effectively treat the slaughterhouse wastewater, achieving more than 80% reduction in COD and phosphorus, and approximately 55% reduction in nitrogen. However, this study also demonstrated that the SRT 30 and 60 days had larger trends, showing a greater removal than those of the SRT 10 days due to the detection of NO<sub>2</sub>-N during the aerobic or oxidic condition. Li and Wu (2014) found that at low SRT, the growth of nitrifiers was restricted, resulting in NO<sub>2</sub>-N existing in the effluent under the shorter SRT (5 days).

In order to obtain the details of the reactor operation with active biomass concentration, F/M ratio was represented as the rate of bacterial metabolism. The F/M ratio of the SBR operated on the concerned SRTs is presented in Table 2, which shows that the SBR operating at high SRT (60 days) was average in the range 0.09–0.17. The result of the low F/M ratio indicated a low rate of bacterial metabolism, or the system could reach the endogenous phase of bacterial growth, which is a characteristic of low-rate similar to an extended aeration system (Marcos von Sperling and Carlos Augusto de Le Chernicharo, 2005).

### 3.2 Sludge Biomass Composition

Average values of biomass composition in the cyclic operation of AnA<sup>2</sup>/O<sup>2</sup> SBR at 30 and 60 days of sludge age are presented in Table 3. The VSS/SS ratio at the initial time was 0.71–0.76. The average PHA content at the end of Anoxic I was 10–11% MLSS, and at the end of Oxidic II was 0.7–0.8% MLSS. The changes in the VSS/SS ratio was caused by the fraction of storage polymers (such as PHAs, Poly-P, etc.) in the biomass. The result of the biomass composition was that the VSS/SS ratio at 60 days SRT was lower than at 30 days SRT. The fraction of PHA ( $f_{\text{PHA}}$ ) at 60 days SRT was lower than at 30 days SRT. Analysis of the sludge biomass composition revealed the change in biomass composition through the operation periods of AnA<sup>2</sup>/O<sup>2</sup> SBR. The low VSS/SS and  $f_{\text{PHA}}$  at 60 days SRT was caused by the function of bacteria accumulating bio-polymers such as PAOs and non-PAOs in the MLVSS. This is consistent with the study by Brdjanovic et al. (1998), who reported that the low VSS/SS ratio in a BPR system indicated a high poly-p in



the biomass. In addition, Wentzel, Ekama, Loewenthal, and Marais (1988) found that the percentage of phosphorus increased due to the function of the PAOs in the increased MLVSS. This phenomenon may be at least partially attributed to the lower decay rate of the PAOs compared to non-PAO at longer SRTs, where a proportionally larger part of the active biomass would consist of PAOs. Then, the increase SRT was the fraction of phosphorus increased.

### 3.3 Kinetics of COD, nitrogen and phosphorus removal

Kinetic parameters were obtained from the biochemical activity of the microorganisms (Heterotrophs, Autotrophs, and phosphorus accumulating organisms) in the SBRs. The kinetic coefficient was studied on the optimum operating condition for treating slaughterhouse wastewater at 60 days SRT.

Prior to the kinetics parameters estimation, sensitivity analysis was conducted to evaluate the most important parameters. The result of the sensitivity analysis revealed that the Anoxic yield coefficient for storage ( $Y_{STO}$ ), Yield coefficient for growth of Heterotrophic biomass on COD ( $Y_{H, S}$ ), Yield coefficient for growth of Heterotrophic biomass on internal storage material ( $Y_{H, STO}$ ), and Saturation constant for substrate utilization ( $K_s$ ) had an influence on the COD removal. The concentration of nitrate (anoxic condition) was sensitive to the anoxic reduction factor ( $\eta_{NO_3}$ ). In the phase of ammonium oxidation or nitrification, the concentrations of nitrite and nitrate were sensitive to the Yield coefficient for growth of Ammonium oxidizer bacteria ( $Y_{AOB}$ ), and the yield coefficient for growth of Nitrite oxidizing bacteria ( $Y_{NOB}$ ). As well, the concentration of phosphate was sensitive to the Maximum storage rate of biomass ( $q_{PHA}$ ), the Aerobic yield for poly-P formation ( $Y_{PO_4}$ ), the Rate constant for storage of Poly-phosphate ( $q_{PP}$ ), and the Yield coefficient for growth of Phosphate accumulating organisms ( $Y_{PAO}$ ). The values of the bio-kinetic coefficients for carbon oxidation, nitrification and denitrification are presented in Table 3.

The yield coefficient for anoxic growth on the external substrate and stored material in this study were analogous to the value reported by Ni and Yu (2008). The maximum anoxic growth rate on the external substrate ( $\mu_{H,S}$ ) and stored material ( $\mu_{H, STO}$ ) were different from the values suggested by Henze, Harrenmoes, Jansen, and Airvin (2002), Sin (2004) and Iacopozzi, Innocenti, Marsili-Libelli, and Giusti (2007), which was 0.03 day<sup>-1</sup>. This is because that growth was partially attributed to the heterotrophic anoxic growth on the stored material rather than on the external substrate.

The result of yield coefficients and specific growth rates of the Ammonium Oxidizing Bacteria (AOB) was higher than that of the Nitrite Oxidizing Bacteria (NOB). In addition, the yield coefficients and specific growth rates were similar to the values reported by Pai (2007), who studied the nitrification kinetics in anaerobic, anoxic, and oxic processes (A2/O process). Additionally, the maximum specific growth rates of the AOB and NOB for activated sludge were in the range of 0.019–0.092 hr<sup>-1</sup> and 0.012–0.06 hr<sup>-1</sup>, respectively (Jubany, Baeza, Carrera, & Lafuente, et al., 2005; Moussa, Hooijmans, Lubberding, Gijzen, & Van Loosdrecht, et al., 2005). The results of kinetic parameter estimation showed that  $q_{PHA}$ ,  $Y_{PO_4}$ ,  $q_{PP}$ , and  $Y_{PHA}$  were 1.33 day<sup>-1</sup>, 0.18 mgP/mg COD, 0.13 day<sup>-1</sup>, and 0.19, mgP/mg COD, respectively. These values were slightly different from the kinetic coefficient for Bio-P removal of activated sludge model 2 (ASM2) ( $q_{PHA} = 3$  day<sup>-1</sup>,  $Y_{PO_4} = 0.4$  mgP/mgCOD,  $q_{PP} = 1.5$  day<sup>-1</sup>, and  $Y_{PHA} = 0.2$  mgP/mgCOD)



(Henze et al., 2000). As well, these kinetic results were different from the values reported in the ASM and other authors because the differences of the kinetics values might be the reason for such a difference in the kinetics model structure. In this model, substrate electrons were diverted to microbial growths, maintenance and biomass storage products simultaneously, which were subsequently by the growth on cell internal stored materials rather than on the external soluble substrate directly.

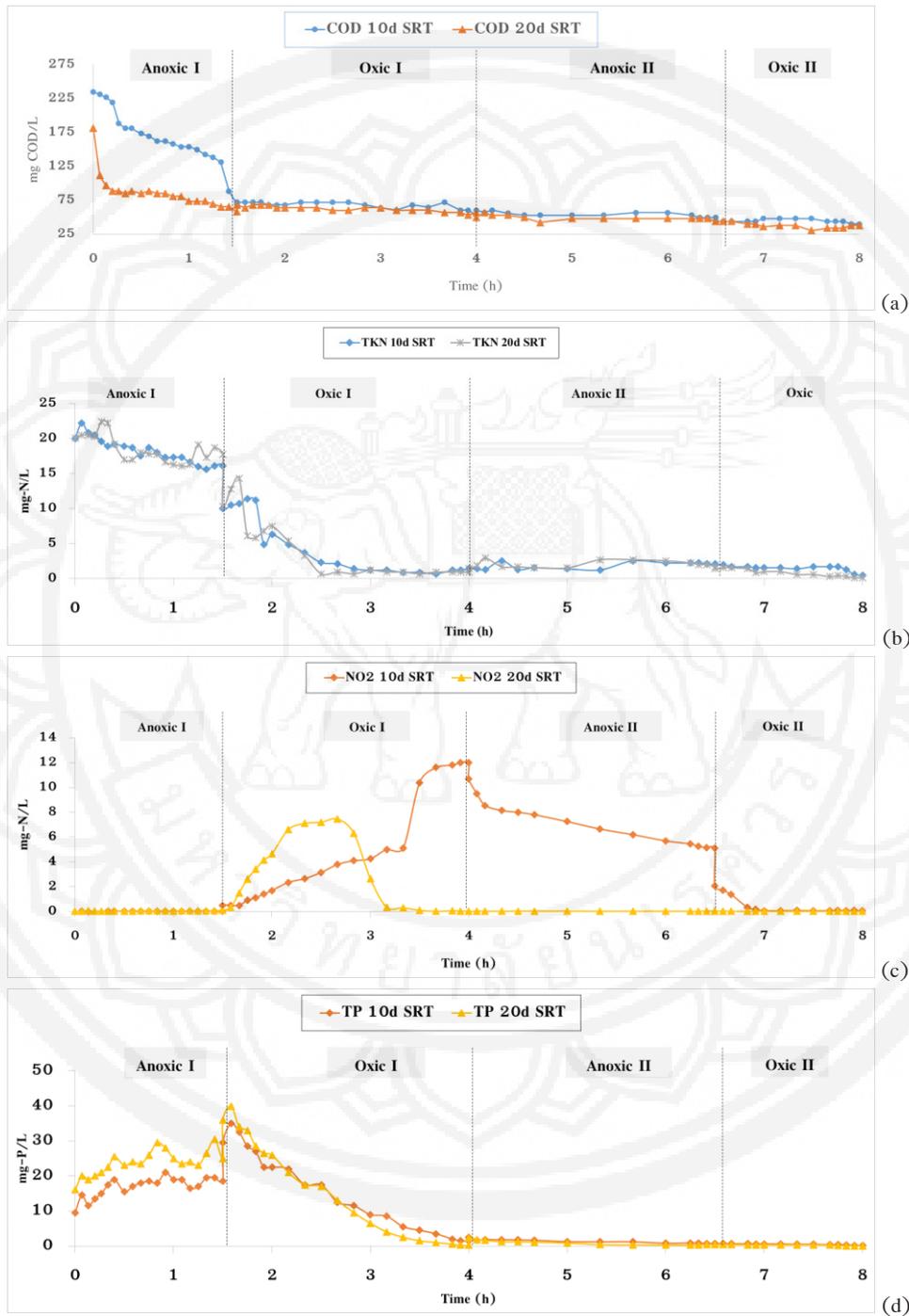


Figure 2 The cyclic profile of nitrogen and phosphorus in the AnA2/O2 SBR; (a) COD at 10 and 20 days SRT, (b) TKN, at 10 and 20 days SRT, (c) NO<sub>2</sub><sup>-</sup> at 10 and 20 days SRT, (d) TP at 30 and 60 days SRT



**Table 2** Food to Microorganisms ratio

SRT (days)	F/M (kgCOD/kg MLSS.d)
10	0.17–0.18
20	0.14–0.16
30	0.10–0.20
60	0.09–0.17

**Table 3** Biomass composition during the experiment

Parameters	Unit	Initial Condition		End Anoxic I		End Oxidic I		End Anoxic II		End Oxidic II	
		30	60	30	60	30	60	30	60	30	60
SRT	days	30	60	30	60	30	60	30	60	30	60
MLSS	mg /L	5667	7810	5767	7930	5762	7966	5780	7968	5789	7970
MLVSS	mg /L	4307	5545	4672	6265	4322	5635	4226	5480	4226	5499
VSS/SS		0.76	0.71	0.81	0.78	0.75	0.70	0.73	0.69	0.73	0.69
Ash	mg /L	1360	2265	1095	1665	1440	2331	1554	2488	1563	2471
PHA	mg /L	63.1	72.4	634	880	175	159	51.2	47.5	49.0	62.6
$f_{PHA}$	g PHA/g MLSS	0.011	0.009	0.109	0.110	0.030	0.019	0.008	0.005	0.008	0.007

**Table 4** Kinetics of Heterotrophs

Parameters	Unit	Value (estimated)
$Y_{STO}$	gCOD/gCOD	0.82
$Y_{H,S}$	gCOD/gCOD	0.67
$Y_{H,STO}$	gCOD/gCOD	0.71
$\eta_{NO3}$		0.25
$K_S$	gCOD/m <sup>3</sup>	20
$\mu_{H,S}$	hr <sup>-1</sup>	0.04
$\mu_{H,XSTO}$	hr <sup>-1</sup>	0.01
$Y_{AOB}$	g COD/g N	0.28
$Y_{NOB}$	g COD/g N	0.09
$\mu_{AOB}$	hr <sup>-1</sup>	0.09
$\mu_{NOB}$	hr <sup>-1</sup>	0.02

### Conclusion

The results of this study demonstrated that the sequencing batch reactor (SBR) is an efficient biological method for treating slaughterhouse wastewater. The effluent concentrations of COD, TKN, TP and NH<sub>3</sub>-N were decreased over the period of the SRTs, which show that the shorter SRT (10 days) conditions could remove organic and nutrients efficiently. However, this study also demonstrated that an SRT of 60 days had larger trends, showing a greater removal than was shown in the SRT of 10 days. In the 10-day SRT, the growth of nitrifier was restricted while the lysis rate of nitrite oxidizers bacteria (NOB) was accelerated, resulting in the detection of NO<sub>2</sub>-N in the effluent. In addition, the using of a high SRT with low volume of wasted sludge from the reactor leads to a high biomass sludge accumulation.

In our cyclic study of the biomass composition, the ratio of VSS/SS of AnA<sup>2</sup>/O<sup>2</sup> SBR operated at 30-day SRT was higher than that of 60-day SRT. The storage polymers were increased due to the increased function



of the PAOs in the MLVSS. This phenomenon may be at least partially attributed to the lower decay rate of the PAOs compared to that of non-PAO, at longer SRTs. A proportionally larger part of the active biomass would consist of PAOs. Then, the increased SRT was the fraction of phosphorus increased.

Estimation of the sensitivity analysis revealed that the  $\mu_{H,S}$ ,  $\eta_{NO_2}$ ,  $\eta_{NO_3}$ ,  $k_{STO}$ ,  $Y_{H,S}$ , and  $Y_{H,STO}$  had an influence on the denitrification process, the  $\mu_{AOB}$ ,  $\mu_{NOB}$ ,  $Y_{AOB}$ , and  $Y_{NOB}$  had an influence on the nitrification process, and the  $q_{PP}$ ,  $q_{PHA}$ ,  $K_{MAX}$ ,  $Y_{PHA}$  and  $Y_{PAO}$  had an influence on the biological phosphorus removal process.

The microbial kinetic results were different from those values reported in the original activated sludge model (ASM) since the difference of kinetics values might be the main reason for such a different in model structure and concept and operational procedure. Furthermore, different microbial communities, reactor operating conditions, and wastewater characteristics may also be responsible for the difference of bio-kinetic model.

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