






Characterization of slowly-biodegradable organic compounds and hydrolysis kinetics in tropical wastewater for biological nitrogen removal

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ABSTRACT


Many developing countries, mostly situated in the tropical region, have incorporated a biological nitrogen removal process into their wastewater treatment plants (WWTPs). Existing wastewater characteristic data suggested that the soluble chemical oxygen demand (COD) in tropical wastewater is not sufficient for denitrification. Warm wastewater temperature (30 °C) in the tropical region may accelerate the hydrolysis of particulate settleable solids (PSS) to provide slowly-biodegradable COD (sbCOD) for denitrification. This study aimed to characterize the different fractions of COD in several sources of low COD-to-nitrogen (COD/N) tropical wastewater. We characterized the wastewater samples from six WWTPs in Malaysia for 22 months. We determined the fractions of COD in the wastewater by nitrate uptake rate experiments. The PSS hydrolysis kinetic coefficients were determined at tropical temperature using an oxygen uptake rate experiment. The wastewater samples were low in readily-biodegradable COD (rbCOD), which made up 3–40% of total COD (TCOD). Most of the biodegradable organics were in the form of sbCOD (15–60% of TCOD), which was sufficient for complete denitrification. The PSS hydrolysis rate was two times higher than that at 20 °C. The high PSS hydrolysis rate may provide sufficient sbCOD to achieve effective biological nitrogen removal at WWTPs in the tropical region.

Key words | COD fractionation, denitrification, low COD/N, particulate settleable solids, readily-biodegradable COD

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INTRODUCTION

Nitrogen pollution in freshwater is a serious issue faced by many developing countries, many of which are situated in the tropical region (United Nations 2019). The largest point source of nitrogen pollution is inadequately treated domestic wastewater (Department of Environment 2017). United Nations World Water Assessment Programme (2017) estimated that 80% of wastewater was discharged without proper treatment in developing countries. Point-source control of nitrogen input from wastewater treatment plants (WWTPs) will effectively prevent nitrogen pollution from threatening freshwater resources (United Nations World Water Assessment Programme 2017). To ensure a safe and sustainable freshwater supply, many developing countries

have imposed nitrogen discharge limits in their wastewater effluent quality standards. For example, new regulations in Thailand limit the total Kjeldahl nitrogen (TKN) concentration in the wastewater effluent to 35 mg/L. Malaysia also enforced discharge limits on ammoniacal nitrogen (NH₄-N) and nitrate nitrogen (NO₃-N) to 5 mg/L and 10 mg/L, respectively, in the most stringent standard. Consequently, the WWTPs have integrated biological nitrogen removal into their design and operation to comply with the new regulations.

The conventional biological nitrogen removal process involves two steps. Ammonium (NH₄⁺) is oxidized sequentially into nitrite (NO₂⁻) and nitrate (NO₃⁻) in the

nitrification step, followed by a denitrification step that reduces the NO_3^- and NO_2^- into nitrogen gas (N_2). The denitrification step is carried out by heterotrophic bacteria, which require sufficient biodegradable organics for their metabolism. The theoretical ratio of biodegradable organics in terms of chemical oxygen demand (COD) consumed per $\text{NO}_3\text{-N}$ reduced ranges from 3.5 to 4.5 g COD/g $\text{NO}_3\text{-N}$ (Matějů *et al.* 1992). In full-scale WWTPs, Henze *et al.* (1994) recommended a wastewater COD-to-nitrogen ratio (COD/N) of 6 to 11 g COD/g $\text{NO}_3\text{-N}$ for complete denitrification. Several studies on wastewater characteristics in the tropics found that the wastewater soluble COD/N (3.0–6.0) were lower than the required ratios (6–11) for denitrification (Lee *et al.* 2010; How *et al.* 2018). These low COD/N tropical wastewaters may not have sufficient biodegradable organics to achieve complete biological nitrogen removal.

When sufficient biodegradable organics are not available in the wastewater, endogenous decay of the sludge may provide a source of biodegradable organics for denitrification (Sedlak 2018). WWTPs may use long solids retention time (SRT) to promote endogenous decay in warm wastewater temperatures (Sedlak 2018). Sedlak (2018) reported that using endogenous source of biodegradable organics for denitrification may reduce the effluent total nitrogen (TN) below 3 mg/L. However, Cao *et al.* (2008) found that the denitrification rate using an endogenous source of biodegradable organics was two to 16 times lower than that using exogenous biodegradable organics at tropical temperature due to slow endogenous decay of the sludge, which was the rate-limiting step. The lower denitrification rate may result in a larger anoxic tank and plant footprint. A common method to improve the denitrification performance in treating low COD/N wastewater is by dosing external carbon sources, such as glycerol, ethanol and methanol (Guerrero *et al.* 2012; Guo *et al.* 2016). An external carbon dosage system requires a costly control mechanism to prevent carbon breakthrough in the effluent (Shen & Wang 2011; Wang *et al.* 2017).

Apart from the soluble biodegradable organics in wastewater, known as readily-biodegradable COD (rbCOD), and an endogenous source of biodegradable organics, Sophonsiri & Morgenroth (2004) and How *et al.* (2019) showed that slowly-biodegradable COD (sbCOD) present in particulate settleable solids (PSS) in the wastewater made up 45% to 50% of the total COD. To utilize sbCOD for denitrification, PSS must be hydrolyzed into soluble compounds (Henze *et al.* 1987; Morgenroth *et al.* 2002). Henze *et al.* (1987) and Morgenroth *et al.* (2002) described the hydrolysis process of PSS in Activated Sludge Model 1 (ASM1). The

hydrolytic bacteria first hydrolyze PSS in the wastewater into soluble biodegradable organics, which could be oxidized by heterotrophic bacteria for growth. The hydrolysis rate of PSS in warm wastewater temperature ($30 \pm 2^\circ\text{C}$) may be higher when compared with that at 20°C because the hydrolysis process is catalyzed by enzymes secreted by hydrolytic bacteria (Morgenroth *et al.* 2002). The higher PSS hydrolysis rate in tropical temperatures may provide sbCOD as a potential source of biodegradable organics for denitrification in the low soluble COD/N tropical wastewater (How *et al.* 2019). However, most of the wastewater characteristics data in the tropics have focused on general chemical parameters, such as soluble COD (sCOD), total COD (TCOD) and $\text{NH}_4\text{-N}$ (Lee *et al.* 2010; How *et al.* 2018). We lack sufficient wastewater characteristic data on sbCOD from different sources of low soluble COD/N tropical wastewater to justify that sbCOD is a propitious source of biodegradable organics for denitrification.

Our study aimed to characterize the different fractions of COD as the sources of biodegradable organics for denitrification in low soluble COD/N tropical wastewater. We characterized the wastewater from six WWTPs across different locations in the Greater Kuala Lumpur region, Malaysia, for 22 months. We performed nitrate uptake rate (NUR) experiments to quantify the fractions of rbCOD and sbCOD in the wastewater samples. We determined the PSS hydrolysis rate constants at warm wastewater temperature (30°C) using oxygen uptake rate (OUR) experiments.

METHODS

Sampling of activated sludge and wastewater

Grab samples of return activated sludge (RAS) and raw wastewater after preliminary treatment were collected from six WWTPs in the Greater Kuala Lumpur region from July 2017 to April 2019. These WWTPs' locations, configurations, population equivalent (PE) ranges, hydraulic retention time (HRT) and SRT are listed in Table 1. WWTP 1, 2, 4, 5 and 6, operating in biological nitrogen removal configurations, have sludge acclimated for nitrification/denitrification. Kuala Lumpur has a tropical climate with daily temperature between 24°C and 34°C during the sampling period. This region has a separate sewer system catering mainly for municipal wastewater. Industries are required to treat their wastewater on site or in industrial WWTPs before discharge into the environment. Activated

Table 1 | Details of the six WWTPs in the Greater Kuala Lumpur region

Plant	Location	Plant configuration	PE range	HRT (hr)	SRT (d)
WWTP 1	Petaling Jaya	EA with pre-anoxic tank	10,000–30,000	18–24	>20
WWTP 2	Puchong	EA with pre-anoxic tank	10,000–30,000	18–24	>20
WWTP 3	Puchong	CAS	<10,000	6–16	5–10
WWTP 4	Puchong	Oxidation pond	10,000–30,000	18–24	>20
WWTP 5	Pantai Dalam	A ₂ O	>50,000	18–28	5–10
WWTP 6	Titivangsa	SBR with anoxic selector	>50,000	18–24	10–30

A₂O: Anaerobic-anoxic-oxic.
 CAS: Conventional activated sludge.
 EA: Extended aeration.
 SBR: Sequencing batch reactor.

sludge and raw wastewater samples from WWTP 1 was collected weekly from July 2017 to October 2018 ($n = 66$). We collected two samples of activated sludge and raw wastewater from WWTPs 2 to 6 consecutively every 2 weeks from January 2019 to April 2019 ($n = 10$). Sampling activity for WWTP 1 was carried out over a longer period as the wastewater was collected for a long-term reactor study utilizing sbCOD to enhance denitrification performance (How *et al.* 2019). The wastewater characteristics will be discussed later in the Results and discussion section. The preliminary treatment in all the WWTPs includes a bar screen and aerated grit chamber. The samples were stored at 4 °C prior to use.

Chemical analyses

We analyzed the total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the wastewater samples according to the standard method (APHA/AWWA/WEF 1998). We filtered the wastewater samples through a 0.2- μm membrane filter for NO₂⁻, NO₃⁻ and NH₄⁺ analysis using an 861 Advanced Compact Ion Chromatograph (Metrohm, Switzerland). We filtered aliquots of wastewater samples through a 0.45- μm membrane filter prior to TN analyses using a TOC-V CSN total organic carbon analyzer coupled with a TNM-1 nitrogen measuring unit (Shimadzu, Japan). In addition, we analyzed unfiltered samples and samples filtered through a 0.45- μm membrane filter for TCOD and sCOD, respectively, using a high range COD test kit with a DRB 200 COD digester (Hach, USA).

Nitrate uptake rate experiment

We conducted NUR experiments to determine the concentrations of rbCOD and sbCOD in the wastewater samples

based on the method described by van Loosdrecht *et al.* (2016). We used a 500-mL working volume beaker in the NUR experiments. We mixed the washed RAS from each WWTP, potassium nitrate (KNO₃) solution, allylthiourea solution and raw wastewater from each WWTP to form the reaction mixture for each wastewater sample. The initial composition of the reaction mixture was NO₃-N 100 mg/L, allylthiourea 20 mg/L and VSS 3000 mg/L, as adopted from How *et al.* (2019). Allylthiourea was used to suppress nitrification activity that may be induced by stirring during the experiment. The batch experiments were carried out under room conditions (28 ± 1 °C). Mixed liquor samples were taken regularly for anion analysis (NO₂⁻ and NO₃⁻) and the pH readings were recorded periodically from an 827 pH Lab with Primatrode (Metrohm, Switzerland). Two sets of the NUR experiments were conducted using wastewater samples collected on two different days from each WWTP listed in Table 1.

We calculated the rbCOD and sbCOD in the wastewater samples using Equations (1) and (2) (van Loosdrecht *et al.* 2016),

$$rbCOD = \frac{2.86}{1 - Y_{OHO,ax}} (a_{rbCOD} - a_{sbCOD}) \quad (1)$$

$$sbCOD = \frac{2.86}{1 - Y_{OHO,ax}} (a_{sbCOD} - a_{endo}) \quad (2)$$

where $Y_{OHO,ax}$ is the anoxic heterotrophic yield coefficient; we adopted the value of 0.4 g biomass COD/g substrate COD from How *et al.* (2019). The coefficient 2.86 g COD/g NO₃-N represents the oxygen equivalence term for NO₃⁻. a_{rbCOD} , a_{sbCOD} and a_{endo} are the y-intercepts of the rbCOD degradation, sbCOD degradation and endogenous respiration lines on the NO_x-N time profile, respectively.

The $\text{NO}_x\text{-N}$ was calculated by summing 0.6 times the nitrite nitrogen ($\text{NO}_2\text{-N}$) and $\text{NO}_3\text{-N}$ ($\text{NO}_x\text{-N} = 0.6 \cdot \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$). The rbCOD degradation, sbCOD degradation and endogenous respiration lines were fitted to the $\text{NO}_x\text{-N}$ time profile by linear regression.

Oxygen uptake rate experiment

We determined the PSS hydrolysis kinetic coefficients using the OUR experiment (van Loosdrecht et al. 2016). The OUR experiment setup consisted of two compartments, a 500-mL aerated vessel and 100-mL closed respiration cell. Mixed liquor was circulated between the aerated vessel and closed respiration cell. The reaction mixture in the aerated vessel was continuously aerated to maintain the dissolved oxygen (DO) concentration above 4 mg/L. We cut off the circulation of mixed liquor at a regular interval to measure the DO drop in the closed respiration cell for 10 minutes. We prepared the reaction mixture by mixing raw wastewater, allylthiourea solution and washed sludge from each WWTP in Table 1. The initial composition of the reaction mixture was 100 mg allylthiourea/L and 3000 mg VSS/L. The OUR experiment was conducted at room temperature ($28 \pm 1^\circ\text{C}$). Mixed liquor samples were taken regularly for cation analysis (NH_4^+) to confirm negligible nitrification activity. We conducted two sets of OUR experiments for each WWTP.

We subsequently estimated the hydrolysis kinetic constants by fitting ASM1 to the OUR profiles from the OUR experiments (Henze et al. 1987). ASM1 is a widely accepted mathematical model to simulate processes in an activated sludge system. We included three processes, which include hydrolysis of PSS, heterotrophic bacteria growth and endogenous decay in the model fitting (Table 2). The decay of heterotrophic biomass was modelled using the endogenous respiration concept in Activated Sludge Model No. 3 (ASM3) (Gujer et al. 1999). The combined model was adopted from Benneouala et al. (2017) and Wu et al. (2014), where ASM3 decoupled the degradation of endogenous decay products from the hydrolysis of PSS in the wastewater. The decoupling allowed quantification of hydrolysis rate constants for PSS in batch experiments where slow endogenous respiration do not produce a significant amount of biodegradable organics (Wu et al. 2014; Benneouala et al. 2017). We simulated the activated sludge models using AQUASIM (Reichert 1994). The process matrix and nomenclatures are provided in Table 2. The detailed derivations for equations describing the rate of change of each component in Table 2 is described in the Supplementary Materials. The initial concentrations of the rbCOD and sbCOD were determined from the NUR experiments. The kinetic parameters for aerobic growth and decay of heterotrophic biomass were fixed at temperature-corrected default values at 30°C , as listed in Table 2 (Henze et al. 1987; Gujer et al. 1999).

Table 2 | Process kinetics, stoichiometry, nomenclatures and adopted values for the OUR experiment (Henze et al. 1987; Gujer et al. 1999)

Processes	Components					Reaction rate
	S_o	S_s	X_H	X_s	X_I	
Hydrolysis of X_S		1		-1		$k_H \frac{X_S/X_H}{K_X + X_S/X_H} X_H$
Aerobic growth of X_H	$1-1/Y_H$	$-1/Y_H$	1			$\mu_H \frac{S_S}{K_S + S_S} X_H$
Endogenous respiration of X_H	f_p-1		-1		f_p	$b_H X_H$

b_H = Aerobic endogenous respiration of heterotrophic organisms (0.0125 h^{-1}).

f_p = Fraction of non-biodegradable decayed biomass (0.08).

K_S = Saturation constant for rbCOD (20 mg/L).

Y_H = Heterotrophic growth yield (0.67 g COD/g COD).

μ_H = Maximum heterotrophic growth rate (0.49 h^{-1}).

K_H = Maximum hydrolysis rate (parameter to be estimated).

K_X = Hydrolysis saturation constant (parameter to be estimated).

S_o = DO (state variable).

S_s = rbCOD (state variable).

X_H = Heterotrophic organisms (state variable).

X_I = Inert decayed biomass (state variable).

X_S = sbCOD (state variable).

RESULTS AND DISCUSSION

Wastewater characteristics

The tropical wastewater samples in our study has a temperature between 28 °C and 33 °C (Table 3). The pH of the wastewater samples was at near-neutral condition (6.5–7.5) as shown in Table 3. The wastewater TSS ranged from 70 mg/L to 297 mg/L. The NH₄-N and TN concentrations of the tropical wastewater in our study (Table 3) were characteristics of a moderate-strength wastewater (20–30 mg/L for NH₄-N, 35–50 mg/L for TN) (Henze *et al.* 1997). The NO₂-N and NO₃-N in the wastewater samples were less than 0.5 mg/L. Besides, Table 3 shows that the tropical wastewater samples had low sCOD concentrations. The sCOD of the wastewater ranged from 55 to 170 mg/L, while the TCOD ranged from 160 to 640 mg/L. The sCOD in our wastewater was significantly lower than the concentrations (180–210 mg/L) found in colder regions (Henze *et al.* 1997). Cao *et al.* (2008) reported a similar range of sCOD values (123 mg/L) for wastewater in Singapore. The tropical temperature (24–34 °C) experienced in Malaysia and Singapore may have enhanced the biodegradation of sCOD in the sewer network (Cao *et al.* 2008). The sCOD-to-TN ratio (sCOD/TN) of the tropical wastewater was between 3 to 6 (Table 3), which was lower than the recommended ratio of between 6 and 11 for complete denitrification (Henze *et al.* 1994). The TCOD-to-TN ratio (TCOD/TN) was significantly higher than the recommended

ratio for complete denitrification. Based on the sCOD and TCOD data, a significant fraction of the biodegradable organics may be present in the PSS as sbCOD to supplement biodegradable organics for denitrification in low soluble COD/N tropical wastewater. We further performed NUR experiments to quantify the rbCOD and sbCOD to support the hypothesis.

We adopted a value of 0.4 g biomass COD/g substrate COD for $Y_{OHO,ax}$ of the sludge (How *et al.* 2019). The $Y_{OHO,ax}$ of tropical sludge determined in our previous study by How *et al.* (2019) was similar to the $Y_{OHO,ax}$ values (0.42–0.53 g biomass COD/g substrate COD) in the literature (Muller *et al.* 2003; van Loosdrecht *et al.* 2016). We determined the rbCOD and sbCOD based on the NO_x-N uptake rate profiles obtained from the NUR experiments (Figure 1). The change in NO_x-N during rbCOD and sbCOD degradation were used to quantify the rbCOD and sbCOD using Equations (1) and (2), respectively.

The rbCOD and sbCOD concentrations in the wastewater of each WWTP are listed in Table 3. The rbCOD made up 3–40% of the TCOD, while the sbCOD constituted 15–60% of the TCOD (Figure 2(a)). We calculated the biodegradable COD (bCOD) by summing rbCOD and sbCOD. The bCOD relative to TCOD was 30–90%. Samples from WWTP 2 and WWTP 3 had a lower bCOD than the rest of the WWTPs as these plants were situated close to an industrial estate. The influent TSS of WWTP 2 and WWTP 3 were also lower (70–84 mg/L) when compared with the rest of the WWTPs (138–297 mg/L), which contributed to

Table 3 | Mean wastewater characteristic data for each WWTP

Plant	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5	WWTP 6
Temperature (°C)	29 ± 1	29 ± 1	31 ± 1	31 ± 2	29 ± 1	31 ± 2
pH	7.2 ± 0.2	7.1 ± 0.4	6.8 ± 0.9	6.8 ± 0.2	7.1 ± 0.3	7.2 ± 0.4
TSS (mg/L)	297 ± 169	70 ± 14	84 ± 28	145 ± 109	138 ± 12	251 ± 106
NH ₄ -N (mg/L)	21 ± 4	23 ± 1	23 ± 5	19 ± 4	18 ± 1	29 ± 2
TN (mg/L)	26 ± 4	30 ± 1	32 ± 2	24 ± 5	23 ± 1	33 ± 2
sCOD (mg/L)	85 ± 39	171 ± 2	105 ± 1	104 ± 20	81 ± 5	89 ± 4
TCOD (mg/L)	492 ± 133	373 ± 82	215 ± 81	188 ± 16	270 ± 55	413 ± 97
rbCOD (mg/L)	53 ± 33	34 ± 8	18 ± 8	69 ± 32	50 ± 9	39 ± 23
sbCOD (mg/L)	250 ± 66	78 ± 44	78 ± 16	83 ± 15	143 ± 23	159 ± 23
sCOD/TN	3.2 ± 1.0	5.7 ± 0.3	3.2 ± 0.2	4.4 ± 0.1	3.6 ± 0.1	2.7 ± 0.1
TCOD/TN	20 ± 9	13 ± 3	7 ± 2	8 ± 1	12 ± 2	13 ± 4
rbCOD/TN	2.0 ± 1.0	1.1 ± 0.2	0.5 ± 0.2	2.8 ± 0.8	2.2 ± 0.3	1.2 ± 0.6

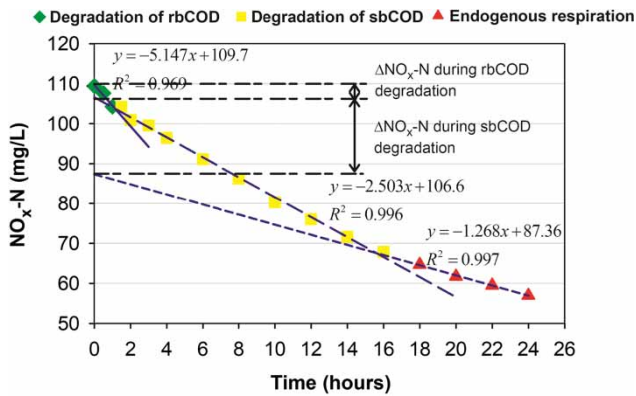


Figure 1 | A representative $\text{NO}_x\text{-N}$ uptake profile from the NUR experiment of wastewater sample from WWTP 6 to illustrate the steps for quantifying rbCOD and sbCOD.

the lower bCOD content. WWTP 4 operating in CAS configuration may have a lower sbCOD from the NUR experiments as the sludge was not acclimated for biological nitrogen removal and the WWTP has a shorter SRT (5–10 days) than the other WWTPs (>20 days). Thus, we found that the tropical wastewater samples had low soluble biodegradable organics (rbCOD) and most of the biodegradable organics was present in PSS (sbCOD).

Interestingly, the warm wastewater temperature approximately at 30°C did not significantly degrade the sbCOD in the sewer network as observed for rbCOD. A probable explanation is that sbCOD degrades more slowly than rbCOD in the sewer network as bacteria need to colonize the surface of PSS in the sewer before hydrolysis (Drewnowski & Maki-*nia* 2013; Benneouala et al. 2017). The Greater Kuala Lumpur region has a high density of WWTPs of 2021 plants in an area of 2793 km^2 , which may result in a short sewer HRT (Malaysian Water Association 2017). The short HRT in the sewer may have prevented most of the sbCOD from biodegradation. However, other areas with warm temperature and long sewer HRT may observe significant biodegradation

and change in the compositions of biodegradable organics (Yun et al. 2013; Li et al. 2019b).

The rbCOD-to-TN ratio (rbCOD/TN) ranged from 0.5–3.5, which was much lower than the theoretical COD required for denitrification (Figure 2(b)). Thus, the soluble biodegradable organics in the wastewater were insufficient for denitrification. By considering both rbCOD and sbCOD in the tropical wastewater, the bCOD-to-TN ratios (bCOD/TN; 3.0–15.0) were significantly higher than the rbCOD/TN. If sbCOD could be utilized for denitrification, the denitrification performance of the WWTPs would be enhanced.

From the NUR experiments, we found that our tropical wastewater had low rbCOD content. The low rbCOD concentration was insufficient to achieve complete denitrification. sbCOD might improve the denitrification performance if PSS could be hydrolyzed at a high rate. The warm wastewater temperature in the tropical region ($30 \pm 2^\circ\text{C}$) may enhance the PSS hydrolysis rate as PSS hydrolysis is an enzymatic reaction that occurs faster at higher temperature (Morgenroth et al. 2002). The higher PSS hydrolysis rate may allow sbCOD to be utilized for denitrification. In the following section, we further determined the PSS hydrolysis rate constants at tropical temperature.

Particulate settleable solids hydrolysis kinetic coefficients

We determined the PSS hydrolysis kinetic constants in tropical temperatures from the OUR profiles (Figure 3). The modified ASM1 fitted the OUR data well, the p -values were above the level of significance of 0.05 in the Chi-Square (χ^2) Test (Figure 3). We excluded the first point of each OUR profile in the χ^2 Test due to systematic error during the measurement of the first OUR value. In the

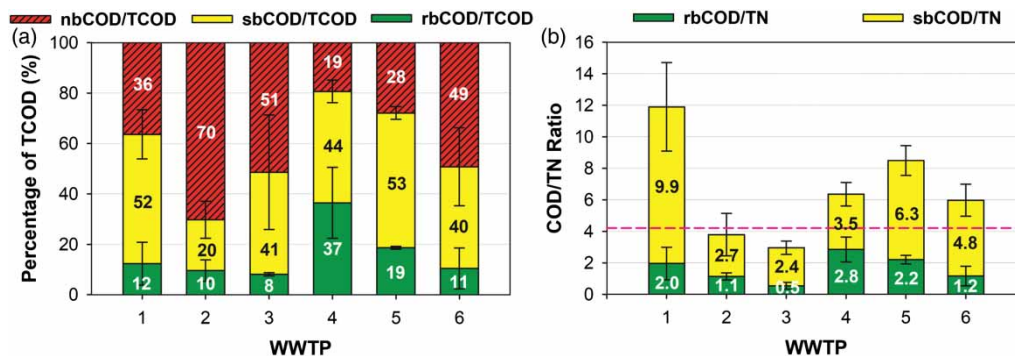


Figure 2 | COD fractions from wastewater fractionation experiments. (a) Fraction of rbCOD, sbCOD and bCOD relative to TCOD and (b) COD/TN ratios, the horizontal dashed (–) line corresponds to theoretical COD consumption per $\text{NO}_3\text{-N}$ reduced ($4.2\text{ g COD/g NO}_3\text{-N}$).

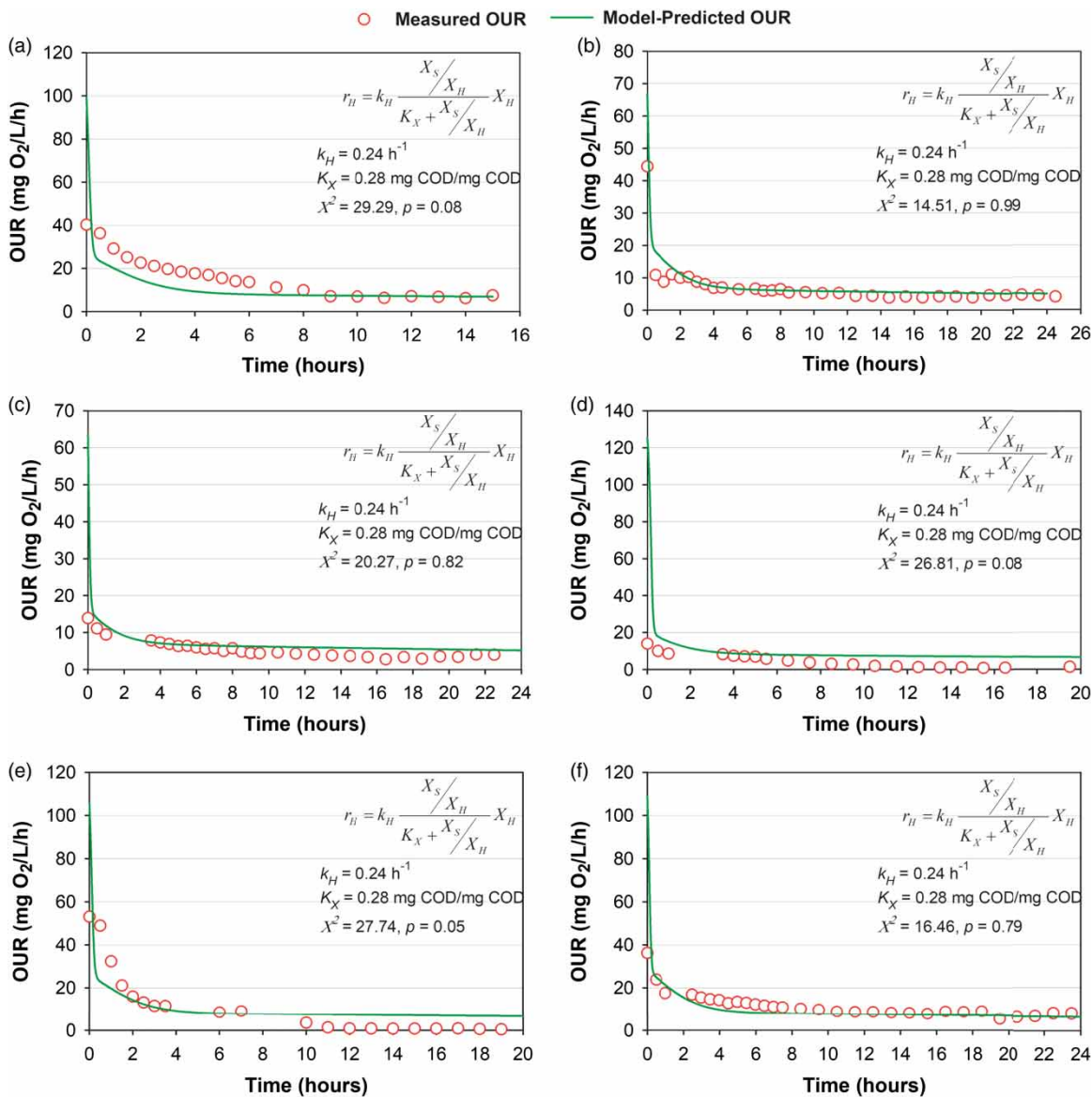


Figure 3 | Measured and fitted OUR profiles from PSS hydrolysis kinetic parameter determination experiment. (a) WWTP 1, (b) WWTP 2, (c) WWTP 3, (d) WWTP 4, (e) WWTP 5 and (f) WWTP 6. r_H represents the PSS hydrolysis rate.

PSS hydrolysis reaction rate equation (Table 2), k_H represents the maximum PSS hydrolysis rate. The value of k_H was 0.24 h^{-1} , which was two times higher than the literature values ($0.10\text{--}0.15 \text{ h}^{-1}$) from similar ASM1 simulations of PSS hydrolysis at 20°C (Henze et al. 1987; Drewnowski & Makinia 2013; Wu et al. 2014). K_X is the affinity constant of hydrolytic bacteria for PSS, which should be independent of temperature. The estimated value for K_X ($0.28 \text{ mg COD/mg COD}$) was approximately twice the default value ($0.15 \text{ mg COD/mg COD}$) in ASM1 (Henze et al. 1987). The higher k_H here implied that PSS hydrolysis rate was accelerated in warm wastewater temperature. Our k_H

value was also 1.3 times higher than the temperature-corrected k_H value (0.19 h^{-1}), which implied that the Arrhenius temperature correction factor may not be valid for PSS hydrolysis in our tropical systems. Long SRT operation of the WWTPs above 20 days may have promoted higher populations of hydrolytic bacteria (Li et al. 2019a), which may result in a higher k_H than the value predicted by Arrhenius relationship. We need to determine the hydrolysis kinetic parameters experimentally to model the PSS hydrolysis rate in ASM1 accurately at tropical temperature. On the other hand, the K_X value obtained here ($0.28 \text{ mg COD/mg COD}$) was two times higher than the default value

(0.15 mg COD/mg COD at 20 °C) (Henze *et al.* 1987). The higher K_X value in our work implied that the bacteria in our sludge had a lower affinity for PSS than that predicted by ASM1. The higher k_h and K_X values of our sludge were characteristics of *r*-strategist organisms in the hydrolysis process. *r*-strategist organisms proliferate in an environment where substrate is not limiting because of their high maximum growth rate but low substrate affinity. Oppositely, *k*-strategist organisms tend to grow in substrate-limiting conditions as they have higher substrate affinity but lower maximum growth rate than *r*-strategists (Arnaldos *et al.* 2015). Arnaldos *et al.* (2015) suggested that exposing a bacteria community under high substrate concentration for a prolonged period may favor the proliferation of *r*-strategist organisms. Thus, the high abundance of sbCOD in our wastewater may have promoted the growth of *r*-strategist hydrolytic organisms. The higher PSS hydrolysis rate suggested that sbCOD in different sources of low soluble COD/N wastewater could be a propitious source of biodegradable organics. The sbCOD could significantly enhance the biological denitrification performance in a tropical wastewater treatment process (How *et al.* 2019).

Implications for the design and operation of wastewater treatment plants

From the results of NUR experiments, we found that sbCOD was the largest fraction of bCOD in different sources of low soluble COD/N tropical wastewater. The PSS hydrolysis rate at tropical temperatures was also two times higher than the default value at 20 °C, which suggested that sbCOD may be utilized by heterotrophic bacteria as a source of biodegradable organics in the denitrification process. How *et al.* (2019) suggested that utilizing sbCOD in a biological nitrogen removal process significantly enhanced the denitrification performance. Using sbCOD as a source of biodegradable organics for denitrification may reduce the operating cost of the process when compared with conventional external carbon dosage systems. The traditional external carbon dosage systems require additional procurement cost for organic compounds, such as glycerol, methanol and ethanol (Shen & Wang 2011). Complex carbon dosage control systems must be installed to ensure the optimal carbon dosing rate (Wang *et al.* 2017). Using sbCOD as the source of biodegradable organics in the denitrification step will eliminate these additional operating costs for an external carbon dosage system.

Using sbCOD for denitrification requires longer anoxic HRT to allow adequate time for the hydrolysis of PSS in

the wastewater, which is the rate-limiting step (Tas *et al.* 2009; Drewnowski & Makinia 2013). PSS hydrolysis rate under anoxic conditions is also known to be 2.5 times lower than the rate under aerobic conditions (Henze *et al.* 1987). The NO_x-N uptake profile in Figure 1 shows that the specific denitrification rate during sbCOD degradation (0.9 mg N/g VSS·hr) was two times lower than that during rbCOD degradation (1.8 mg N/g VSS·hr). Similar magnitude of reduction in the specific denitrification rate was observed in other NO_x-N uptake profiles (Figure S1 in the Supplementary Materials). For WWTPs operating in EA with pre-anoxic configuration, a potential solution to accommodate the longer anoxic HRT is to modify part of the aerobic tank into an anoxic tank. The WWTPs' operators may cut off aeration at the middle and end of the aerobic tank to induce an anoxic zone for denitrification. A previous study by How *et al.* (2019) found that complete nitrification can be achieved with an aerobic HRT of 8 hours in a tropical biological nitrogen removal process; the high nitrification activity was attributed to a long SRT operation of 20 days to promote nitrifiers' growth. Many EA plants with a pre-anoxic tank in Malaysia are operating with a long SRT of 20 days and an aerobic HRT of 12–16 hours as recommended by Malaysian Sewerage Industry Guidelines (MSIG) (National Water Services Commission 2009). The excess aerobic HRT suggested for EA plants with a pre-anoxic tanks in MSIG resulted in merely the heterotrophic consumption of sbCOD. We may modify the latter part of the aerobic tank into an anoxic tank to promote degradation and utilization of sbCOD for denitrification. Considering the similar wastewater characteristics of our tested samples, other WWTPs treating low soluble COD/N tropical wastewater may apply this strategy to encourage utilization of sbCOD for denitrification.

CONCLUSION

This study successfully characterized the fractions of rbCOD and sbCOD as the sources of biodegradable organics for denitrification in treating low soluble COD/N tropical wastewater. The tropical wastewater samples had low rbCOD content. sbCOD was the largest fraction of bCOD in the wastewater. The concentration of bCOD in the wastewater was sufficient to achieve complete denitrification. The PSS hydrolysis rate at tropical temperature (30 ± 2 °C) was approximately twice the rate at 20 °C. In principle, sbCOD in the wastewater can be utilized for denitrification at a high PSS hydrolysis rate. A larger anoxic zone in WWTPs

may be required to utilize sbCOD as a source of biodegradable organics for denitrification. Utilizing sbCOD as a source of biodegradable organics for denitrification is potentially a more cost-effective way to reduce the effluent NO_3^- concentration in WWTPs than using external carbon dosage systems. We recommend investigating the feasibility and long-term performance of utilizing sbCOD for the biological nitrogen removal process at tropical temperature ($30 \pm 2^\circ\text{C}$) in a future pilot-scale reactor study. A pilot-scale biological nitrogen removal reactor mimicking full-scale operation enables identification of the trade-offs between lower operating cost and higher capital cost for larger anoxic tanks.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at <https://dx.doi.org/10.2166/wst.2020.077>.

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