



# Long solid retention time (SRT) has minor role in promoting methane production in a 65 °C single-stage anaerobic sludge digester

Yun Chen<sup>a,b</sup>, Keke Xiao<sup>a</sup>, Xie Jiang<sup>a</sup>, Nan Shen<sup>d</sup>, Raymond J. Zeng<sup>b</sup>, Yan Zhou<sup>a,c,\*</sup>

<sup>a</sup> Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

<sup>b</sup> CAS Key Laboratory of Urban Pollutant Conversion, Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, People's Republic of China

<sup>c</sup> School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

<sup>d</sup> School of Environmental Engineering and Science, Yangzhou University, 196 West Huayang Road, Yangzhou, Jiangsu 225127, People's Republic of China

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## ABSTRACT

In this study, a thermophilic (65 °C) single-stage wasted activated sludge (WAS) digester was established and the effects of solid retention time (SRT) on the reactor performance were investigated. The result showed that the optimum SRT was 6 days with methane yield of 186.16 mL/g VS. It was found that SRT had little effect on the hydrolysis and volatile solids (VS) destruction, and the high temperature employed seemed sufficient to achieve maximum hydrolysis and VS destruction performance. Longer SRT, however, promoted the release of recalcitrant compounds and impaired acidification, leading to the low methane yield. The microbial community analysis revealed that the dominant pathway for methane production was through syntrophic activity of acetate oxidizing bacteria and hydrogenotrophic methanogens while acetoclastic methanogens were absent in the system.

## 1. Introduction

Sludge is a by-product of wastewater treatment process, and sludge management is now becoming a serious issue all over the world (Yang et al., 2015). Anaerobic digestion is a well-known process which can convert the sewage sludge into biogas that can be applied for electricity and heat production in a combined heat and power (CHP) plant (Kleerebezem et al., 2015). In general, anaerobic sludge digestion includes four major steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Chen et al., 2017a). Hydrolysis is the rate-limiting step due to the wasted activated sludge's (WAS) complex floc structure and hard cell wall of microbes. To accelerate the hydrolysis and improve the efficiency of anaerobic digestion, various pretreatment technologies have been developed (Zhen et al., 2017).

Temperature phased anaerobic digestion (TPAD), compared with a single-stage mesophilic or thermophilic digestion, has been shown to be an effective treatment method for increasing methane production and volatile solids (VS) destruction. For example, Bolzonella et al. (2007) reported that 30–50% higher methane yield was obtained in TPAD than the mesophilic and thermophilic single-stage systems. Skiadas et al. (2005) found a TPAD system (70 °C in first phase and 55 °C in second

phase) had 43% and 6% increase in VS reduction for primary and secondary sludge digestion respectively, as compared to those achieved in the single-stage thermophilic (55 °C) anaerobic digesters.

Usually, TPAD consists of a hydrolysis stage operated under thermophilic temperature (50–70 °C) with short hydraulic retention time (HRT; 1–4 days), followed by a methanogenesis stage operated at mesophilic or thermophilic temperature with a longer HRT (Ge et al., 2010). Although increases in the biogas yield between 20% and 50% have been reported in TPAD system, the technology has not been widespread due to the high construction and operation costs of the second digester (Peces et al., 2013). Furthermore, enhanced volatile fatty acids (VFAs) production in the first stage can inhibit the methanogenesis in the second stage if the process is not properly controlled (Ho et al., 2014; Leite et al., 2016). Thus, single-stage thermophilic anaerobic digestions (ADs) (50–55 °C) are still commonly applied in large scale biogas plants (Ferrer et al., 2010; Gagliano et al., 2015; Tezel et al., 2014). Furthermore, a higher extent of pathogen reduction was achieved at 55 °C, resulting in Class A biosolids (Tezel et al., 2014). One of the drawbacks of single-stage thermophilic AD (55 °C) is the lower hydrolysis efficiency as compared to TPAD system (Leite et al., 2016). Hence, a more efficient system to improve hydrolysis should be

\* Corresponding author at: Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore.

E-mail address: [ZhouYan@ntu.edu.sg](mailto:ZhouYan@ntu.edu.sg) (Y. Zhou).

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developed. Recently, [Chen et al. \(2017b\)](#) demonstrated a single-stage thermophilic sludge digestion system operated at 65 °C, and that system could eliminate conventional thermophilic pretreatment step and achieve similar treatment performance as TPAD. However, operating conditions were not optimized in that study.

Solid retention times (SRTs) have direct influence on treatment costs, with respect to initial capital investment ([Ferrer et al., 2010](#)). For more complex and non-soluble substrates, such as sewage sludge, the hydrolysis rate of the sludge determines the designed SRTs. SRTs should be long enough to allow for optimal hydrolysis and fermentation of the sludge. However, longer SRT may also increase the capital and operational cost ([Arslan et al., 2016](#); [Zhang et al., 2015](#)). Hence, the effects of SRT on the single-stage system performance should be investigated. Usually, a minimum retention time of 15 days is required at 35 °C to keep the key trophic groups and maintain balance between the faster-growing bacteria and the slower-growing groups treating WAS. The theoretical SRT may be reduced to 5–8 days at 55 °C because of the growth rates of thermophilic methanogens are 2–3 times higher than those of mesophilic homologues ([Ferrer et al., 2010](#)). [Ho et al. \(2014\)](#) described VFAs were accumulated at HRT of 4 days with 65 °C pretreatment step. Thus, the minimum SRT of 6 days was chosen in this study to avoid VFA accumulation. There is no relevant study so far to investigate the optimum SRT at 65 °C for methane production. The main objective of this study was to study the effect of the SRT on methane production in a 65 °C single-stage system.

## 2. Materials and methods

### 2.1. Feedstock preparation and fermenter setup

The feed sludge (WAS) used for this work was obtained from local water reclamation plant (Singapore). WAS was collected weekly and concentrated by settling at 4 °C for 24 h and then the thickened WAS was stored at 4 °C until further use. The total solids (TS), volatile solids (VS), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD) of the concentrated sludge were  $14.41 \pm 0.25$ ,  $11.52 \pm 0.25$ ,  $17.54 \pm 0.90$ , and  $0.67 \pm 0.16$  g/L, respectively. The pH of WAS was 6.3. The inoculum was collected from a thermophilic anaerobic digester (65 °C) which described in [Chen et al. \(2017b\)](#).

A commercial fermenter (Major Science, USA) with 3 L working volume was operated in semi-continuous mode for 82 days at 65 °C. The overhead mixer speed was controlled at 100 rpm throughout the entire fermentation period. Fermenter was firstly subjected to 5 days SRT during the initial operation stage, however, significant amount of VFAs were accumulated (data not shown). Hence, the SRT was prolonged to 6 days. 0.5, 0.4, and 0.3 L of treated sludge was withdrawn and replaced with the corresponding volume of WAS daily resulting in different SRTs in the following three phases. SRT of 6 days and organic loading rate (OLR) of 1.86 g VS/L/d was operated in phase I, SRT was 7.5 days and 10 days in phase II and III, resulting in the OLR of 1.53 g VS/L/d and 1.15 g VS/L/d, respectively. The temperature of fermenter was controlled by circulating hot water in the water jacket of the reactor. pH was not controlled. The detail schematic diagram of the fermenter can be found in [Chen et al. \(2017a\)](#).

### 2.2. Analytical methods

TS, VS, and tCOD of sludge were measured according to the standard methods ([APHA, 2005](#)). The sludge samples were collected daily and immediately filtered through 0.45 µm membrane to analyse VFAs, sCOD, NH<sub>4</sub><sup>+</sup>-N, alkalinity, soluble carbohydrate and protein. Soluble carbohydrate was measured by the phenol–sulfuric acid method with glucose as standard ([Xiao et al., 2016](#)). Soluble protein was determined by the modified Lowry–Folin method using the protein assay kit (ThermoFisher, USA) with bovine serum albumin as standard. Ammonium was measured with the Nessler method kit (TNT832, Hach, USA).

Alkalinity was measured using colorimetric method kit (TNT870, Hach, USA). VFAs and ethanol were analyzed using a 7890A GC (Agilent, USA) with flame ionization detector and equipped with DB-FFAP fused-silica capillary column.

The volume of biogas produced from the reactor was measured by a wet gas meter (TG 05 Model 5, Ritter, Germany) daily, while its composition was further analyzed by a customized 7890A gas chromatography (GC; Agilent, USA) equipped with dual thermal conductivity detectors ([Maspolim et al., 2015](#)).

Dissolved organic matters (DOMs) distributions at different SRTs were quantified based on the molecular weight of organic compounds with a size-exclusion chromatography, in association with organic carbon and nitrogen detection (LC-OCD-OND, DOC-LABOR, Karlsruhe, Germany). The detailed method can be found in [Xiao et al. \(2017\)](#) and [Chen et al. \(2017a\)](#). Injection volume of samples was 1000 µL.

The extent of hydrolysis and acidification, VS destruction efficiency and methane yield were calculated according to [Chen et al. \(2017a\)](#). Methane yield denotes the potential volume of methane production from 1 g VS of sludge (mL/g VS); biogas production rate means the potential volume of biogas production daily (L/d).

### 2.3. DNA extraction and Illumina high-throughput sequencing

The biomass for DNA extraction were collected from day 25, 53, and 76 corresponding to the steady state operating conditions of SRT 6 days, 7.5 days, and 10 days. DNA extractions were performed using FastDNA Spin kits for Soil (MP Biomedicals, Singapore) according to the manufacturer's instructions. The concentrations of each eluted DNA were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE). The extracted DNA was immediately frozen at –20 °C before further analysis ([Gagliano et al., 2015](#)). The primers to target the V3 + V4 regions of both bacterial and archaeal 16S rRNA genes were the primers 341F (5'-CCTAYGGGRBG-CASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') ([Chen et al., 2016a](#)). Sequencing was subsequently determined on an Illumina MiSeq platform by Novogene (Beijing, China) and then the OTUs were classified into phylum, family, and genus using GreenGenes database. Sequence data reported in this study were uploaded to the Sequence Read Archive (SRA) under BioProject accession number PRJNA379031.

## 3. Results and discussion

### 3.1. System performance at different SRTs

The effects of SRT on sludge digestion performance are shown in [Fig. 1](#). In phase I (0–25 d), CH<sub>4</sub> and CO<sub>2</sub> in the headspace increased sharply to 60% and 30%, respectively, after a few days acclimation. This biogas composition maintained till the end of the experiment regardless of the SRT applied. The biogas production rate increased from 1.45 L/d to 1.87 L/d and maintained at  $1.87 \pm 0.05$  L/d for the rest of phase I. High VFAs concentration was observed at day 0 due to the carry-over VFAs from SRT 5 days operation before day 0. Once the SRT was increased to 6 days, propionate concentration decreased drastically from 6 mmol/L to 0.3 mmol/L during the initial 20 days, while acetate was maintained below 3 mmol/L during the whole period. In phase II (26–53 d), the biogas production rate decreased from 1.87 L/d to 1.36 L/d. Acetate and propionate concentrations were always below 1.5 mmol/L during this period. In phase III (54–81 d), the biogas production rate was declined quickly from 1.36 L/d to 0.95 L/d. Propionate concentration was slightly fluctuated but always below 3 mmol/L. Little acetate was accumulated in phase III. Meanwhile, hydrogen was undetectable in the headspace during the whole operating period. Ammonium, which is an intermediate of protein degradation, was slightly increased with the increased SRT.

High alkalinity in the digesters is important to maintain stable pH ([Zamanzadeh et al., 2016](#)). As shown in [Table 1](#), the near-neutral pH

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