

This document is downloaded from DR-NTU, Nanyang Technological University Library, Singapore.

|           |  |
|-----------|--|
| Title     | Impact of ozone assisted ultrasonication pre-treatment on anaerobic digestibility of sewage sludge   |
| Author(s) | Tian, Xinbo; Trzcinski, Antoine Prandota; Lin, Li Leonard; Ng, Wun Jern  |
| Citation  | Tian, X., Trzcinski, A. P., Lin, L. L., & Ng, W. J. (2015). Impact of ozone assisted ultrasonication pre-treatment on anaerobic digestibility of sewage sludge. <i>Journal of Environmental Sciences</i> , 33, 29-38.  |
| Date      | 2015   |
| URL       | <a href="http://hdl.handle.net/10220/39086">http://hdl.handle.net/10220/39086</a>  |
| Rights    | © 2015 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. This paper was published in <i>Journal of Environmental Sciences</i> and is made available as an electronic reprint (preprint) with permission of The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The published version is available at: [ <a href="http://dx.doi.org/10.1016/j.jes.2015.01.003">http://dx.doi.org/10.1016/j.jes.2015.01.003</a> ]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law. |

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

[www.journals.elsevier.com/journal-of-environmental-sciences](http://www.journals.elsevier.com/journal-of-environmental-sciences)

# Impact of ozone assisted ultrasonication pre-treatment on anaerobic digestibility of sewage sludge

Xinbo Tian<sup>1,3</sup>, Antoine Prandota Trzcinski<sup>1</sup>, Li Leonard Lin<sup>4</sup>, Wun Jern Ng<sup>2,3,\*</sup>

1. Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, Singapore, 637141 Singapore. E-mail: [tian0047@e.ntu.edu.sg](mailto:tian0047@e.ntu.edu.sg)

2. Nanyang Environment and Water Research Institute, Nanyang Technological University, Singapore, 637141 Singapore

3. School of Civil and Environmental Engineering, Nanyang Technological University, Singapore, 639798 Singapore

4. Public Utilities Board, Water Reclamation (Plants) Department, Singapore, 228231 Singapore

## ARTICLE INFO

### Article history:

Received 30 October 2014

Revised 13 January 2015

Accepted 14 January 2015

Available online 11 April 2015

### Keywords:

Sewage sludge

Ultrasound

Ozone

Pre-treatment

Molecular weight

Anaerobic digestion

## ABSTRACT

Impact of ultrasonication (ULS) and ultrasonication–ozonation (ULS-Ozone) pre-treatment on the anaerobic digestibility of sewage sludge was investigated with semi-continuous anaerobic reactors at solid retention time (SRT) of 10 and 20 days. The control, ULS and ULS-Ozone reactors produced 256, 309 and 348 mL biogas/g COD<sub>fed</sub> and the volatile solid (VS) removals were 35.6%, 38.3% and 42.1%, respectively at SRT of 10 days. At SRT of 20 days, the biogas yields reached 313, 337 and 393 mL biogas/g COD<sub>fed</sub> and the VS removal rates were 37.3%, 40.9% and 45.3% in the control, ULS and ULS-Ozone reactors, respectively. ULS-Ozone pre-treatment increased the residual organic amount in the digested sludge. These soluble residual organics were found to contain macromolecules with molecular weights (MW) larger than 500 kDa and smaller polymeric products with MW around 19.4 and 7.7 kDa. These compounds were further characterized to be humic acid-like substances with fluorescent spectroscopy analysis.

© 2015 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

Published by Elsevier B.V.

## Introduction

Ultrasonication (ULS) has been reported to be an effective sludge pre-treatment (i.e. treatment of pre-digestion feed sludge) technology (Tiehm et al., 1997, 2001). Biological flocs in the sludge matrix are mechanically disrupted, resulting in particle size reduction and solubilization of extra/intra-cellular polymeric substances (Bougrier et al., 2005; Wang et al., 2006b). Consequently, methane production and solids removal efficiency during the subsequent sludge anaerobic digestion are improved (Tiehm et al., 1997, 2001). In spite of its advantages, ULS pre-treatment has limitation because it is essentially “single” effect — mechanical disintegration (Khanal et al.,

2007; Lehne et al., 2001). Enhancement of the effectiveness of ULS pre-treatment had been attempted by combining ULS process with chemical pre-treatment methods. Combination of ULS pre-treatment with alkaline (Chiu et al., 1997; Jin et al., 2009; Kim et al., 2010) and acidic pre-treatments (Liu et al., 2008; Sahinkaya, 2014) has been demonstrated to increase sludge disintegration as well as the subsequent anaerobic digestion.

Apart from the aforementioned chemical methods, ozone has also been shown feasible to enhance the ultrasonic pre-treatment (Tian et al., in press; Xu et al., 2010; Yang et al., 2012, 2013). Xu et al. (2010) demonstrated the feasibility of combining ultrasound and ozone to disintegrate waste activated sludge (WAS) and to improve the methane recovery from the

\* Corresponding author. E-mail address: [WJNg@ntu.edu.sg](mailto:WJNg@ntu.edu.sg) (Wun Jern Ng), [tian0047@e.ntu.edu.sg](mailto:tian0047@e.ntu.edu.sg) (X. Tian).

subsequent anaerobic digestion. Yang et al. (2013) observed the combined ultrasound and ozone pre-treatment enhanced the solubilization of amino acids and proteins in WAS. Tian et al. (in press) found that ozone was able to chemically degrade macromolecules solubilized by ultrasound and further increased the sludge anaerobic biodegradability.

These previous studies had focused on characteristics of the solubilized compounds and changes in sludge properties after pre-treatment. Xu et al. (2010) and Tian et al. (in press) did, however, investigate the influence of pre-treatment on sludge anaerobic biodegradability in batch serum bottle tests. Information on the influence of such combined pre-treatment on solid removal efficiency and digested sludge characteristics after anaerobic digestion is not available. Besides, influence of solid retention time (SRT), an important design parameter, on the anaerobic digestion of the combined pre-treated sludge has also not been reported. This work aims to investigate the impact of such pre-treatment on the subsequent anaerobic digestion process with semi-continuous reactors at SRT of 10 and 20 days. Biogas production and solid concentrations in the digested sludge were monitored to assess the possible enhancement with such pre-treatment. Molecular weight (MW) distribution and fluorescent spectroscopy analysis were conducted to provide more information on the soluble residual organics in the digested sludge.

## 1. Materials and methods

### 1.1. Sludge samples

Samples of a mixture of primary sludge and thickened WAS (ratio around 1:1 based on dry solids) were collected from a local municipal wastewater reclamation plant. The characteristics of the sewage sludge samples are as shown in Table 1.

### 1.2. Analytical methods

COD and solid concentrations were measured in accordance with Standard Methods (APHA, 1998). Sludge dewaterability was measured with capillary suction time (CST) as described in Standard Methods (APHA, 1998). Sludge pH was measured with a pH meter (model 3200P, Agilent, Santa Clara, CA, USA). A UV spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) was used in the determination of proteins, carbohydrates and ammonia-nitrogen concentrations. Proteins concentration was determined with the method of Lowry et al. (1951). Carbohydrates concentration was determined colorimetrically

with the phenol-sulphuric acid method (DuBois et al., 1956). Ammonia-nitrogen was measured colorimetrically using Nessler's reagent. VFAs concentration was analyzed with a gas chromatograph (7890A GC system, Agilent, Santa Clara, CA, USA) fitted with a flame ionization detector. The composition of biogas was measured with a gas chromatograph (7890A GC system, Agilent, Santa Clara, CA, USA) with thermal conductivity detectors.

### 1.3. Pre-treatment conditions

The pre-treatment conditions were selected following consideration of the results of a previous study (Tian et al., in press). ULS pre-treatment was performed with an ultrasonicator (Q700, Misonix, Newton, CT, USA) with a frequency of 20 kHz. The temperature was monitored and maintained at 30°C with an ice-water bath during ultrasonication. The specific energy input was 9 kJ/g total solids (TS). Ozonation pre-treatment was performed with an ozone generator (WEDECO GSO 30, Xylem, Herford, Germany). Pure oxygen was used as feed gas and converted to ozone with a high voltage converter. A stone diffuser was used to produce fine ozone bubbles and enhance ozone mass transfer. The applied ozone dosage was 0.012 g O<sub>3</sub>/g TS. Ultrasonication–ozonation (ULS-Ozone) pre-treatment was performed by sequentially applying the ULS and the ozonation treatments at the aforementioned dosages.

### 1.4. Molecular weight distribution

MW distribution was measured in accordance with Tian et al. (in press). A high performance liquid chromatograph (1260 LC system, Agilent, Santa Clara, CA, USA) was used for MW distribution analysis using the PL aquagel-OH 8-μm MIXED-M column. Milli-Q water was used as mobile phase with a flow rate of 1 mL/min. A PL aquagel-OH 8-μm guard column was installed in front of the main column. The sample was first centrifuged at 10,000 r/min for 10 min and then filtered through a 0.2 μm membrane filter before injection. A UV (254 nm) detector was used for the detection of the eluted substances. Corresponding MW of a detected peak was calculated by converting its retention time to the corresponding MW as shown in Eq. (1) (Tian et al., in press):

$$\log(\text{MW}) = 9.8223 - 0.6748(\text{Rt}) \quad (1)$$

where, Rt (min) is the retention time of the detected peaks and MW (Da) is the molecular weights of the compounds detected in the corresponding peaks.

### 1.5. EEM fluorescence spectroscopy analysis

A fluorescence spectrometer (LS 55, Perkin Elmer, Waltham, MA, USA) was used to measure the fluorescence intensity (FI) of the soluble fluorescent products. The measurement procedure was previously described by Wu et al. (2011). Excitation wavelength (Ex) was from 230 to 520 nm with 5-nm intervals. Emission wavelength (Em) was collected from 230 to 550 nm with 5-nm increments. Samples were pre-diluted 10 times with DI water to avoid the measured FI exceeding the maximum level.

**Table 1 – Main characteristics of the untreated sewage sludge.**

| Parameters                      | Value range |
|---------------------------------|-------------|
| Total solids (g/L)              | 14.9–15.4   |
| Total suspended solids (g/L)    | 13.7–14.1   |
| Volatile solids (g/L)           | 12.7–13.2   |
| Volatile suspended solids (g/L) | 11.8–12.0   |
| Total COD (g/L)                 | 17.4–20.0   |
| Soluble COD (g/L)               | 0.82–1.26   |
| pH                              | 5.9–6.2     |

The compounds were identified based on their Ex and Em wavelengths as summarized by Chen et al. (2003). Peaks of simple protein-like substances appeared in the Ex/Em range of Ex < 250 nm, Em < 350 nm. Peaks of soluble microbial product (SMP)-like substances were detected in the Ex/Em range of Ex: 250–280 nm, Em < 380 nm. Fulvic acid (FA)-like substances fell into the Ex/Em range of Ex < 250 nm, Em > 380 nm and humic acid (HA)-like substances were detected in the Ex/Em range of Ex > 250 nm, Em > 380 nm.

### 1.6. Anaerobic digestion tests

Anaerobic digestion was conducted semi-continuously in 1.2 L glass bottles with 1 L working volume at 35°C. Seed sludge was taken from a continuous anaerobic digester with SRT of 28 days from a local reclamation plant. One liter of seed sludge was fed into the reactor before starting the experiment. 100 and 50 mL sludge aliquots were daily removed from the reactors and replaced with the same amount of feed sludge to obtain SRT of 10 and 20 days, respectively. The reactors operating at SRT of 10 days were referred to as Control10, ULS10 and ULS-Ozone10 and these received the untreated, ULS treated, and ULS-Ozone treated sewage sludge as feed, respectively. Similarly, the reactors at SRT of 20 days were referred to as Control20, ULS20 and ULS-Ozone20. Each reactor was run for three SRTs so that process stability may be assumed. Biogas was collected with Tedlar gas bags and volume measured daily with a Gas meter (TG 05, Ritter, Bochum, Germany). Feed sludge in storage was changed every three weeks. Each batch of feed sludge was manually adjusted to keep a consistent TS concentration of around 15 g/L. Daily biogas production was normalized by dividing daily gas production by the amount of COD fed into the reactor.

## 2. Results and discussion

### 2.1. Biogas production and solids removal

Biogas production from the anaerobic reactors is as shown in Fig. 1. Anaerobic biodegradability of sludge was higher at SRT of 20 days with its longer substrate-microbe contact time. At both SRTs, the daily biogas production was higher from the reactors fed with pre-treated sludge than from the control reactor. Average daily biogas production from each reactor is as shown in Table 2. These values were calculated by averaging the daily biogas production in the third SRT. At SRT of 10 days, daily biogas production increased from 256 to 309 (+20.7%) and 348 (+35.9%) mL biogas/g COD<sub>fed</sub> because of the ULS and ULS-Ozone treatments of feed sludge, respectively. At SRT of 20 days, daily biogas production increased from 313 to 337 (+7.7%) and 393 (+25.5%) mL biogas/g COD<sub>fed</sub> due to ULS and ULS-Ozone treatments of feed sludge, respectively. These results indicated that the subsequent ozonation enhanced ULS pre-treatment in terms of biogas production. Nickel and Neis (2007) found that the improvement in biogas production due to the ULS treatment of feed sludge was higher when the reactor was operated at a shorter SRT. For example, biogas production increased by 16% after ULS treatment of feed sludge when the anaerobic reactor was operated at 8 days SRT; while the same ULS treatment condition only resulted in 11% increase in biogas production when the SRT was 16 days. Similar results were obtained in this work. The ULS treatment of feed sludge increased biogas production by 20.7% and 7.7% at SRT of 10 and 20 days, respectively. In addition, results from this work showed that the increase in biogas production after the ULS-Ozone treatment of feed sludge also became more pronounced

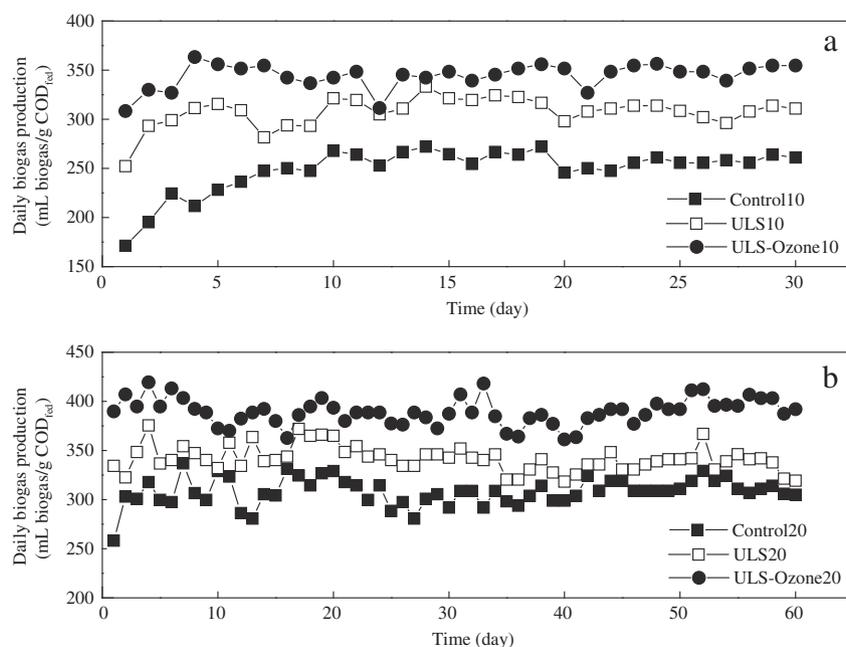


Fig. 1 – Daily biogas production from the reactors at (a) solid retention time (SRT) of 10 days (Control10, ULS10, ULS-Ozone10) (b) SRT of 20 days (Control20, ULS20, ULS-Ozone20) (ULS: ultrasonication, ULS-Ozone: ultrasonication-ozonation).

**Table 2 – Performance of the semi-continuous anaerobic digesters at the assumed steady state.**

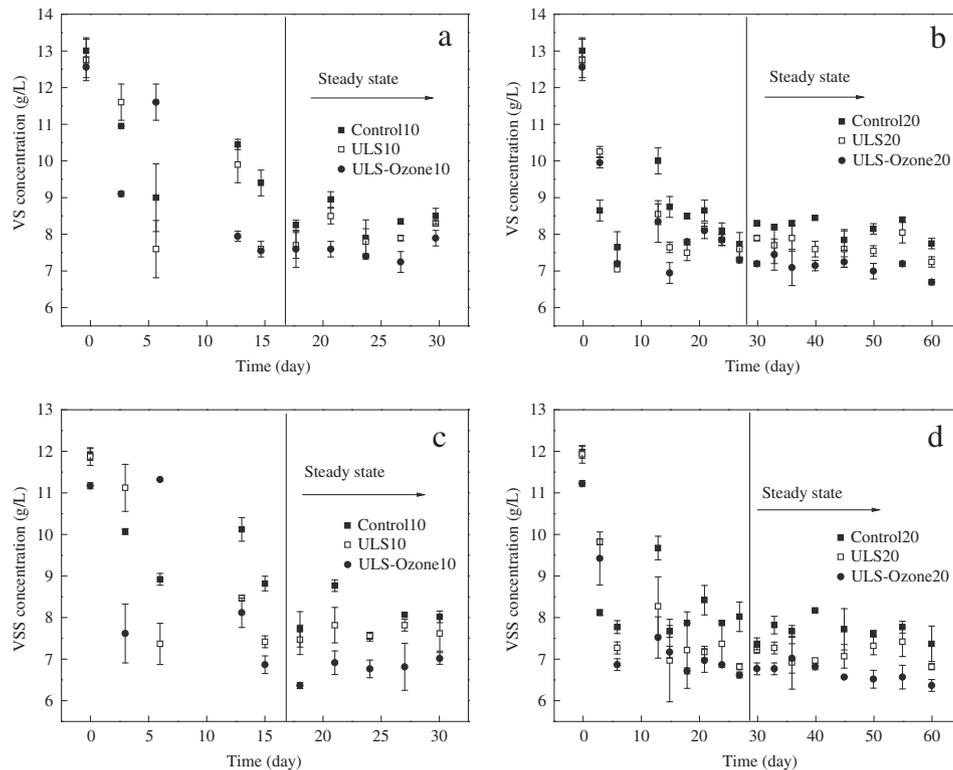
| Reactor  | SRT 10 days     |             |             | SRT 20 days |             |             |
|--|-----------------|-------------|-------------|-------------|-------------|-------------|
|  | Control10       | ULS10       | ULS-OZONE10 | Control20   | ULS20       | ULS-OZONE20 |
| Organic loading rate (g COD/(L·day)) (n = 10 and 20) <sup>a</sup>    | 1.78 ± 0.05     | 1.71 ± 0.04 | 1.62 ± 0.02 | 0.99 ± 0.02 | 0.97 ± 0.01 | 0.93 ± 0.02 |
| Biogas (mL/(day g COD <sub>fed</sub> )) (n = 10 and 20) <sup>a</sup> | 256 ± 5         | 309 ± 6     | 348 ± 9     | 313 ± 7     | 337 ± 10    | 393 ± 12    |
| Methane percentage (%) (n = 5)                                       | 64.2 ± 0.9      | 65.2 ± 0.8  | 65.6 ± 0.6  | 64.9 ± 0.5  | 64.9 ± 1.3  | 65.4 ± 1.2  |
| VS in digested sludge (mg/L) (n = 5 and 8) <sup>b</sup>              | 8340 ± 383      | 7990 ± 344  | 7500 ± 245  | 8125 ± 252  | 7644 ± 253  | 7081 ± 217  |
| VS removal efficiency (%) (n = 5 and 8) <sup>b</sup>                 | 35.6 ± 4.6      | 38.3 ± 4.3  | 42.1 ± 3.3  | 37.3 ± 3.1  | 40.9 ± 3.3  | 45.3 ± 3.1  |
| VSS in digested sludge (mg/L) (n = 5 and 8) <sup>b</sup>             | 8005 ± 469      | 7640 ± 155  | 6760 ± 248  | 7619 ± 258  | 7056 ± 213  | 6606 ± 206  |
| VSS removal efficiency (%) (n = 5 and 8) <sup>b</sup>                | 32.7 ± 3.9      | 35.8 ± 1.3  | 43.2 ± 2.1  | 36.0 ± 2.2  | 40.7 ± 1.8  | 44.5 ± 1.7  |
| Total VFA in digested sludge (mg/L) (n = 3)                          | Nd <sup>c</sup> | Nd          | Nd          | Nd          | Nd          | Nd          |

<sup>a</sup> n = 10 at SRT of 10 days and n = 20 at SRT of 20 days.  
<sup>b</sup> n = 5 at SRT of 10 days and n = 8 at SRT of 20 days.  
<sup>c</sup> Not detectable (<10 mg/L).

when the SRT was shortened from 20 days to 10 days (from 25.5% to 35.9%) which had not been reported previously. In all the reactors, the methane content was relatively stable at around 65%. In addition, no VFA accumulation was observed and pH value remained near neutral throughout the anaerobic digestion test (around 7.0 to 7.2) in all the reactors. This suggested neither ULS nor ULS-Ozone treatment of feed sludge caused stress on the methanogenesis step in the reactors.

Improvement in organic solid removal efficiency during anaerobic digestion was also observed when feed sludge was

treated before anaerobic digestion. The change in volatile solid (VS) and volatile suspended solid (VSS) concentrations in the digested sludge during anaerobic digestion is as shown in Fig. 2. Solid concentration became relatively stable after 18 days and 30 days of operation for reactors with SRT of 10 and 20 days, respectively. After reaching a relatively stable level, the VS and VSS concentrations in the digested sludge were averaged for comparison. The average post-digestion VS and VSS concentrations and the corresponding VS and VSS removal efficiencies against the untreated feed sludge are as



**Fig. 2 – (a) Change in volatile solids (VS) concentration in the digested sludge during the anaerobic digestion at solid retention time (SRT) of 10 days. (b) Change in VS concentration in the digested sludge during the anaerobic digestion at SRT of 20 days. (c) Change in volatile suspended solid (VSS) concentration in the digested sludge during the anaerobic digestion at SRT of 10 days. (d) Change in VSS concentration in the digested sludge during the anaerobic digestion at SRT of 20 days (day 0 stands for feed sludge) (solid concentration was based on at least two replicates).**

shown in Table 2. The control reactor had VS removal efficiency of only 35.6% when it was operated at SRT of 10 days. With the incorporation of ULS and ULS-Ozone treatments of feed sludge, the VS removal rates increased to 38.3% and 42.1%, respectively. Solid removal efficiency was higher at the longer SRT. The VS removal rates of the Control20, ULS20 and ULS-Ozone20 reactors were 37.3%, 40.9% and 45.3%, respectively. Higher VS removal efficiency indicated that more organic matters were digested and converted into biogas. Similarly, the incorporation of the pre-treatment step also improved the VSS removal efficiency as shown in Table 2. The increase in VSS removal efficiency indicated that particulate organics in the treated feed sludge were better hydrolyzed for the subsequent anaerobic digestion process. It has been reported in a full-scale study that ULS treatment of feed sludge was able to slightly decrease the VSS concentration in the digested sludge from 9930 to 9810 mg/L at SRT of 30 days (Xie et al., 2007). However, the improvement in VSS removals after anaerobic digestion due to ULS treatment of feed sludge was more obvious at shorter SRT of 10 and 20 days in this work (e.g. from 8005 to 7640 mg/L at SRT of 10 days). Furthermore, this work demonstrated that ULS-Ozone treatment of feed sludge resulted in a lower VSS concentration in the digested sludge than ULS treatment which had not been reported in previous studies.

T-test at the significance level of 0.05 was conducted to compare the changes in biogas production and post-digestion VS concentration after the ULS and ULS-Ozone treatments of feed sludge (Rivero et al., 2006; Takashima, 2008). As shown in Table 3, the biogas production was significantly higher and the post-digestion VS concentration was significantly lower than the control after the ULS and ULS-Ozone treatments of feed sludge. In addition, the t-test results showed ULS-Ozone treatment of feed sludge resulted in statistically higher biogas production and lower VS concentration in the digested sludge than the ULS treatment. This confirmed application

of ozonation subsequent to ultrasonication could significantly enhance the sludge anaerobic digestion from a statistical point of view. In addition, it was noted that the daily biogas production and solid removal efficiencies of the ULS-Ozone10 reactor were better than those of the Control20 reactor. However, daily biogas production and solid removal rates of the ULS10 reactor were lower than those of the Control20 reactor. This suggested the ULS-Ozone treatment of feed sludge could halve the SRT without affecting digestion performance; whereas, the individual ULS pre-treatment was not able to provide such advantage.

## 2.2. Characteristics of digested sludge

### 2.2.1. Soluble chemical oxygen demand (SCOD) and soluble biopolymers

The SCOD concentration in the digested sludge during anaerobic digestion is shown in Fig. 3a and b. At both SRTs, ULS treatment of feed sludge increased the post-digestion SCOD concentration and the post-digestion SCOD increased further when ULS-Ozone treatment was applied to feed sludge. The increase in SCOD in the digested sludge was also compared statistically with the t-test as shown in Table 3. The statistical results suggested that the SCOD in the digested sludge from the ULS and ULS-Ozone reactors were significantly higher than that from the control reactor. Furthermore, t-test between the SCOD in the digested sludge from the ULS and ULS-Ozone reactors showed that the increase in post-digestion SCOD due to the subsequent ozonation was also statistically significant. As shown in Fig. 3a and b, SCOD in the digested sludge from ULS-Ozone reactors was around 300 and 200 mg/L higher than that from ULS reactors operating at SRT of 10 and 20 days, respectively; while, the SCOD in the ULS-Ozone treated feed sludge was 1200 mg/L higher than the SCOD in the ULS treated feed sludge. This indicated that much of the COD solubilized by the subsequent ozonation treatment of feed sludge was

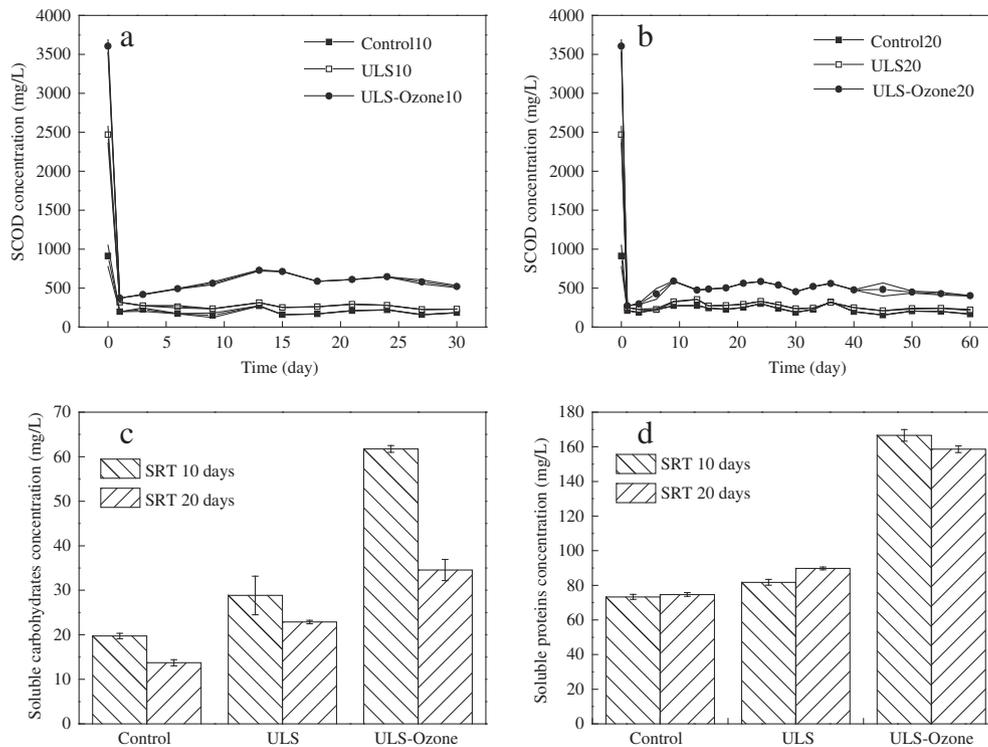
**Table 3 – Statistical analysis of the biogas production, VS concentration in digested sludge and SCOD in digested sludge after ULS and ULS-Ozone pre-treatments at different SRTs.**

|   | ULS                                     | ULS-Ozone                               |
|---|---|---|
| <i>Biogas (mL/(day g COD<sub>fed</sub>))</i>      |   |   |
| Compared to control at SRT of 10 days (n = 10)    | t: 23.398, p: 2.27 × 10 <sup>-9a</sup>  | t: 38.203, p: 2.86 × 10 <sup>-11a</sup> |
| Compared to ULS at SRT of 10 days (n = 10)        | –                                       | t: 16.329, p: 5.39 × 10 <sup>-8a</sup>  |
| Compared to control at SRT of 20 days (n = 20)    | t: 12.709, p: 9.77 × 10 <sup>-11a</sup> | t: 33.657, p: 2.11 × 10 <sup>-18a</sup> |
| Compared to ULS at SRT of 20 days (n = 20)        | –                                       | t: 25.715, p: 3.16 × 10 <sup>-16a</sup> |
| <i>VS concentration in digested sludge (mg/L)</i> |   |   |
| Compared to control at SRT of 10 days (n = 5)     | t: -4.111, p: 0.01473 <sup>b</sup>      | t: -5.126, p: 0.0686 <sup>b</sup>       |
| Compared to ULS at SRT of 10 days (n = 5)         | –                                       | t: -3.642, p: 0.02192 <sup>b</sup>      |
| Compared to control at SRT of 20 days (n = 8)     | t: -7.434, p: 1.45 × 10 <sup>-3b</sup>  | t: -12.166, p: 5.80 × 10 <sup>-6b</sup> |
| Compared to ULS at SRT of 20 days (n = 8)         | –                                       | t: -7.515, p: 1.36 × 10 <sup>-4b</sup>  |
| <i>SCOD in digested sludge (mg/L)</i>             |   |   |
| Compared to control at SRT of 10 days (n = 5)     | t: 8.573, P: 0.00102 <sup>a</sup>       | t: 24.305, p: 1.70 × 10 <sup>-5a</sup>  |
| Compared to ULS at SRT of 10 days (n = 5)         | –                                       | t: 24.331, p: 1.69 × 10 <sup>-5a</sup>  |
| Compared to control at SRT of 20 days (n = 8)     | t: 4.694, P: 0.00222 <sup>a</sup>       | t: 21.649, p: 1.13 × 10 <sup>-7a</sup>  |
| Compared to ULS at SRT of 20 days (n = 8)         | –                                       | t: 18.243, p: 3.68 × 10 <sup>-7a</sup>  |

t is the t statistic value and p is the probability that the two compared values are not significantly different.

<sup>a</sup> The tested value was significantly higher than the reference value.

<sup>b</sup> The tested value was significantly lower than the reference value.



**Fig. 3 – (a) Change in soluble chemical oxygen demand (SCOD) concentration in the digested sludge during the anaerobic digestion at solid retention time (SRT) of 10 days. (b) Change in SCOD concentration in the digested sludge during the anaerobic digestion at SRT of 20 days (SCOD concentration was based on at least two replicates). (c) Soluble carbohydrates concentrations in the digested sludge from different anaerobic reactors ( $n = 3$ ). (d) Soluble proteins concentrations in the digested sludge from different anaerobic reactors ( $n = 3$ ).**

biodegraded and only a relatively small fraction accumulated in the anaerobic reactors.

It is known that biopolymers are a major component of sludge (Rittman and McCarty, 2001). Averaged values of soluble carbohydrates and proteins concentration in the digested sludge during the last three sampling days are compared in Fig. 3c and d. Soluble carbohydrate and protein concentrations in the digested sludge from the ULS-Ozone reactor were much higher than the corresponding concentrations in the digested sludge from the ULS and control reactor at both SRTs. This suggested undigested biopolymers contributed to the higher SCOD in the digested sludge from the ULS-Ozone reactors.

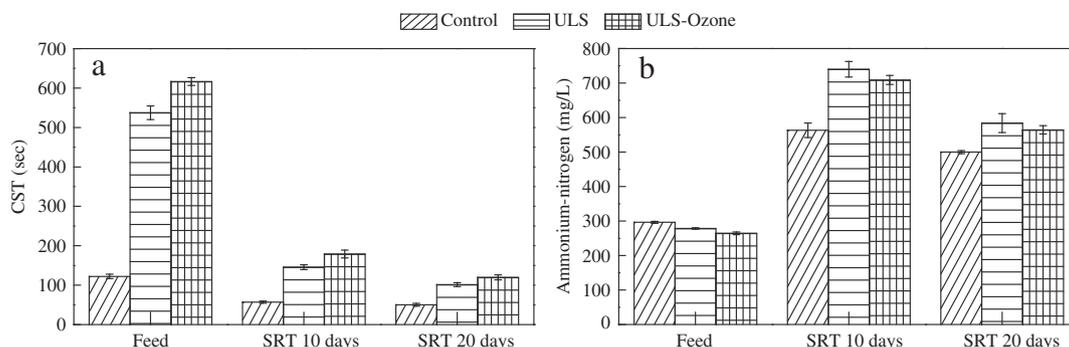
The influence of SRT on the residual carbohydrate and protein concentrations were different. As shown in Fig. 3c, soluble carbohydrate concentrations decreased obviously when all the reactors had longer residence time. For example, the residual soluble carbohydrate concentration decreased from 62 to 35 mg/L when SRT of the ULS-Ozone reactor increased from 10 to 20 days. This is because the solubilized carbohydrates after the treatments of feed sludge were mainly complex polysaccharides from extra- and intra-cellular structures (Tian et al., in press; Wang et al., 2006b). Longer residence time was needed for sufficient degradation. However, the soluble protein concentrations in the digested sludge did not show an obvious difference between SRT of 10 and 20 days for all the reactors as shown in Fig. 3d. The residual proteins were likely to be functional

proteins or enzymes which could not be degraded via microbial utilization (Park et al., 2008). In addition, HAs which were generated during the anaerobic digestion could also be mistakenly detected as proteins with the Lowry's method used.

#### 2.2.2. Dewaterability

The ULS and ULS-Ozone treatments of feed sludge were detrimental to the dewaterability of the digested sludge as shown in Fig. 4a. At SRT of 10 days, CST of the digested sludge from the control reactor, ULS reactor and ULS-Ozone reactor were 56.9, 145.6 and 179.1 sec, respectively. Dewaterability of the digested sludge further improved at a longer residence time. At SRT of 20 days, CST of the digested sludge from the control reactor, ULS reactor and ULS-Ozone reactor were 50.3, 101.4 and 120 sec, respectively. This was because treatment of feed sludge solubilized biopolymers which could bind with free water and worsen the sludge dewaterability (Wang et al., 2006a). Some of these biopolymers were persistent after anaerobic digestion and deteriorated the dewaterability of the digested sludge.

At the same SRT, CST of the digested sludge from the ULS-Ozone reactor was slightly higher than that from the ULS reactor. And, the digested sludge from the control reactor had the lowest CST in comparison to the digested sludge from the ULS and ULS-Ozone reactors. This indicated that the dewaterability of digested sludge was deteriorated by the ULS



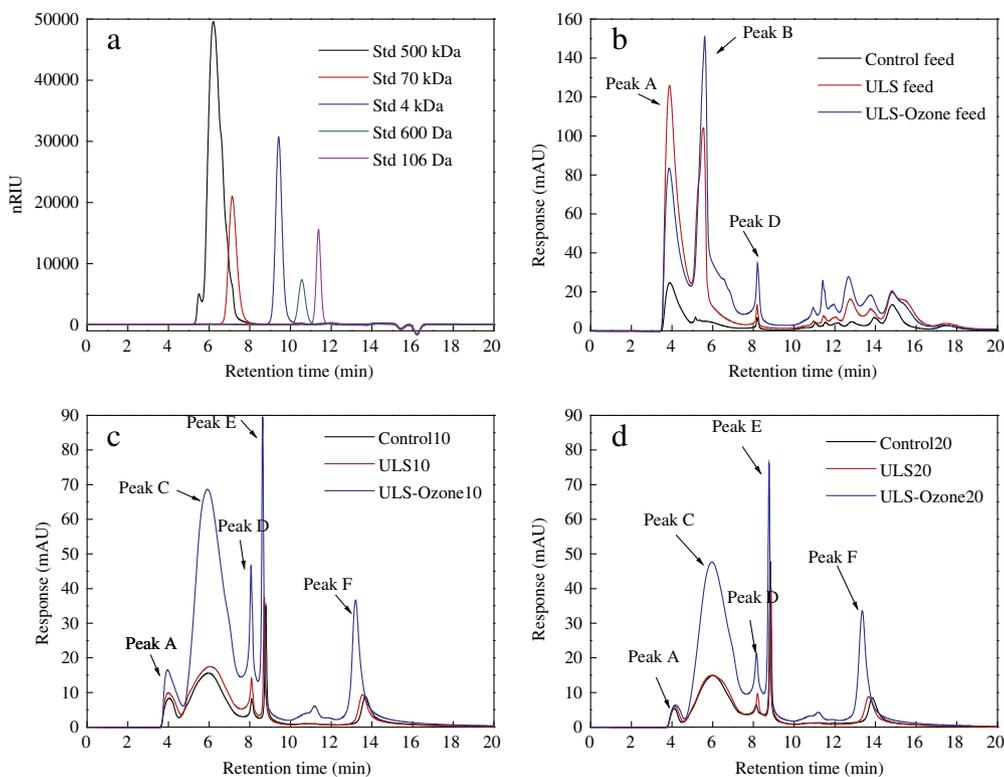
**Fig. 4 – (a) Dewaterability (n = 3) and (b) ammonia-nitrogen (n = 3) in feed sludge and digested sludge from different anaerobic reactors.**

treatment of feed sludge and was further worsened by the subsequent ozonation to ULS pre-treatment. Although influence of ULS-Ozone treatment of feed sludge on the dewaterability of digested sludge has not been reported, results of this work were in accordance with observations in a previous study where individual ULS and individual ozone treatments of feed sludge were found to deteriorate the dewaterability of digested sludge (Braguglia et al., 2012).

2.2.3. Ammonia-nitrogen

Ammonia-nitrogen concentration increased after anaerobic digestion as a result of the degradation of proteinous

compounds and absence of nitrogen removal pathways (Kim et al., 2010). Averaged ammonia-nitrogen concentration in the digested sludge in the last three days of the anaerobic digestion tests are compared in Fig. 4b. Digested sludge from the reactors fed with treated feed sludge had higher ammonia-nitrogen concentration than that from the control reactors. Previous studies indicated the increase of ammonium concentration in the digested sludge could be a drawback of the pre-treatment step because pre-treatment steps released intra- and extra-cellular proteins to be anaerobically degraded (Dogan and Sanin, 2009; Kim et al., 2010). However, it was noted that ULS-Ozone treatment of feed sludge did not have such effect on



**Fig. 5 – Molecular weight distribution chromatograms of (a) standard polymers, (b) supernatant in the feed sludge, (c) supernatant in the digested sludge from reactors operating at solid retention time (SRT) of 10 days and (d) supernatant in the digested sludge from reactors operating at SRT of 20 days.**

ammonia in the digested sludge of the anaerobic digester compared to ULS treatment. This might be due to the oxidative effect of ozone.

### 2.3. Molecular weight distribution

MW distribution chromatograms of the standard polymers are shown in Fig. 5a. MW chromatograms of the soluble substances in the feed sludge, in the digested sludge from reactors operating at 10 days SRT and in the digested sludge from reactors operating at 20 days SRT are shown in Fig. 5b, c and d, respectively. Detected peaks were divided into five groups (A to F) in ascending order of retention time. MWs of the components in these peaks are in the descending order from A to F because larger compounds were retained for a shorter time in the column and eluted earlier.

#### 2.3.1. High molecular weight compounds

Peaks A (Rt: 4.0 min) and B (Rt: 5.6 min) had the most obvious increase after treatments of feed sludge as shown in Fig. 5b. Compounds detected in these peaks were macromolecules with MW higher than 500 kDa because retention time of these peaks was shorter than the retention time of the largest tested standard polymer (MW: 500 kDa, Rt: 6.2 min). Therefore, these compounds were likely to be high MW extra- and intra-polymeric substances released from sludge matrix after the treatments of feed sludge. MW distribution of the digested sludge was shown in Fig. 5c and d. Peak C (Rt: 6.0 min, MW > 500 kDa) instead of peak B was detected in the digested sludge together with peak A. Compounds detected in peak C could be generated from hydrolysis of particulate polymers and higher MW macromolecules (peak A) by hydrolytic bacteria because peak C was detected only after anaerobic digestion. It should be noted that peak C was broader than peak B and covered the retention time of peak B by comparing Fig. 5c, d to b. Therefore, peak B was possibly over-dominated by peak C and thus not detected. As a result, soluble biopolymers released by treatments of feed sludge could also be detected in peak C if remaining undigested.

At SRT of 10 days, responses of peaks A and C in the digested sludge from the ULS-Ozone reactor were significantly higher than the corresponding responses in the digested sludge from the control and ULS reactors as shown in Fig. 5c. This was due to the subsequent ozonation process because such response increase was not observed when only ULS treatment was applied to feed sludge. Similar observations were made on peak D (Rt: 8.2 min) and peak E (Rt: 8.8 min) with MW 19.4 kDa and 7.7 kDa, respectively. These compounds (detected in peaks D and E) were most likely to be intermediate products generated during the anaerobic degradation of macromolecules into monomers because their amounts were significantly lower in the feed sludge than those in the digested sludge.

Responses of peaks A, C, D and E were lower at SRT of 20 days as compared in Fig. 5c and d. This indicated that some of the compounds detected in these peaks were slowly biodegradable compounds. They were not biodegraded at SRT of 10 days but could be digested at SRT of 20 days. Some of these compounds were likely to be carbohydrates because the chemical results determined in Section 2.2.1 showed that

some carbohydrates were complex polysaccharides and were not biodegradable at SRT of 10 days but became biodegradable at SRT of 20 days. At SRT of 20 days, no obvious difference was observed between the MW chromatograms of the digested sludge from the control and ULS reactors as shown in Fig. 5d. However, responses in peaks A, C, D and E were significantly higher in the digested sludge from the ULS-Ozone reactor. This indicated that considerable amounts of polymeric substances remained undigested in the ULS-Ozone reactor even at SRT of 20 days. These residual soluble polymeric compounds in the anaerobic digested sludge were possibly related to solubilization of persistent compounds after the ULS-Ozone treatment of feed sludge (Tian et al., in press; Yang et al., 2013). These results correlated very well with the increase in biopolymers in the digested sludge as observed in Section 2.2.1.

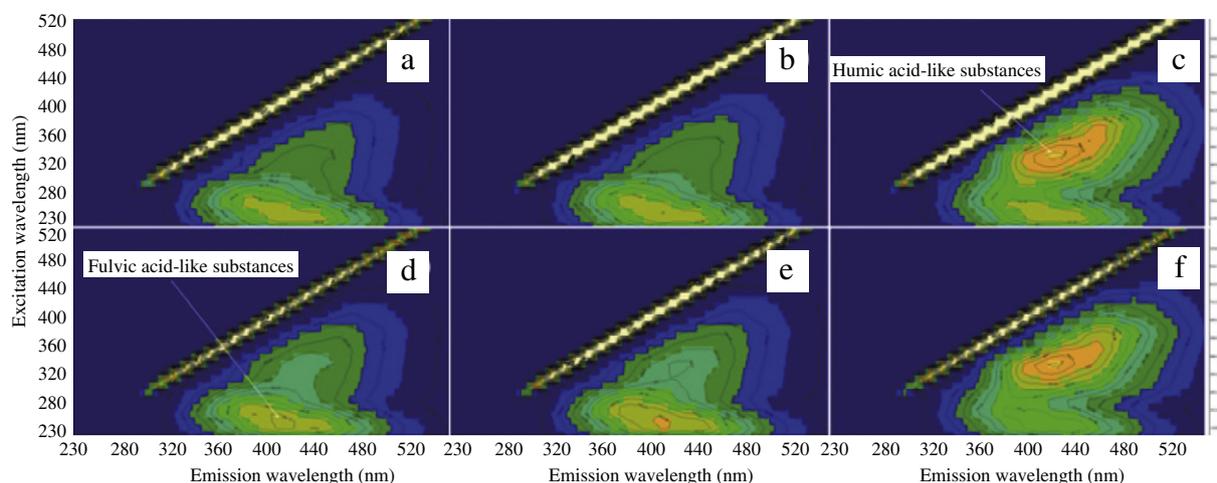
#### 2.3.2. Low molecular weight compounds

Peak F (Rt: 13.3 min, <106 Da) was detected in the supernatant of digested sludge as shown in Fig. 5c and d. The corresponding compounds were not monomers or other easily biodegradable components because they remained undigested at SRT of 20 days. Therefore, they were possibly short chain alkenes or aromatics which are anaerobic digestion by-products and could be detected by the UV 254 nm detector. The formation of these by-products is related to the chemical effects of the subsequent ozonation process because the response of peak F was obviously higher in the digested sludge from the ULS-Ozone reactors. It is possible that the complex polymers in the untreated and ULS pre-treated sludge could only be broken down via biodegradation; while, dosing ozone could provide different degradation pathways by chemically breaking down the high MW biopolymers into smaller fragments (Tian et al., in press). Besides, ozone can convert some refractory compounds into biodegradable ones (Nishijima et al., 2003). These aforementioned factors could generate different substrates and anaerobic digestion of these new substrates could contribute to the accumulation of the detected by-products.

Previous studies had focused on VSS removal and biogas production increase due to the treatment of feed sludge, but insights on the SCOD in the anaerobic digested sludge were not discussed in these studies (Dogana and Sanin, 2009; Tiehm et al., 2001). SCOD in the digested sludge could be attributed to slowly degradable components and recalcitrant anaerobic digestion by-products. MW distribution results allow better realization of the possible sources and categories of the residual components according to their MWs. These would be a good supplementary information to conventional approaches in understanding the influence of treatments of feed sludge on subsequent anaerobic digestion.

### 2.4. Fluorescent product characterization

EEM fluorescence spectroscopy analysis was conducted to measure the FI of fluorescent compounds in the supernatant of digested sludge from the reactors. The EEM spectra of all the samples are shown in Fig. 6. FI of the detected peaks was shown in numbered and colored contour lines for reference. According to the Ex/Em range introduced in Section 1.5, the main peaks were HA-like (as highlighted with a white arrow



**Fig. 6 – Excitation emission matrix fluorescent spectra of the supernatant in the digested sludge from (a) Control10, (b) ULS10, (c) ULS-Ozone10, (d) control20, (e) ULS20 and (f) ULS-Ozone20. (ULS: ultrasonication, ULS-Ozone:ultrasonication-ozonation)**

in Fig. 6c) and FA-like substances (as highlighted with a white arrow in Fig. 6d). These substances were released from the biodegradation of the extracellular polymeric substances, sludge pellets and refractory components (e.g. lignin) in the sewage sludge (Luo et al., 2013). Aside from these two groups, SMP-like substances and simple protein-like matters were also detected in each spectrum. However, their FIs were relatively low and over-dominated by peaks of HA-like and FA-like substances.

#### 2.4.1. Humic acid-like substances

At SRT of 10 days, the FI of HA-like substances were similar in the digested sludge from the control and ULS reactors as shown in Fig. 6a and b. In contrast, the FI of the HA-like substances were significantly higher in the digested sludge from the ULS-Ozone reactor as shown in Fig. 6c. This confirmed some proteins detected with the Lowry's method in Section 2.2.1 were attributed to humic substances. This is because the ULS-Ozone treatment of feed sludge disintegrated the sludge better and solubilized more HA-containing substances in comparison to the ULS treatment. Biodegradation of these solubilized HA-containing substances resulted in a higher concentration of HAs as by-products. These HAs should contribute to the residual polymeric substances in the digested sludge from the ULS-Ozone reactors (e.g., Peak C, in Fig. 5d) as discussed in Section 2.3.1, because HAs are known to be persistent and have high MW (Li et al., 2009; Stevenson, 1994). Such increase in HAs during anaerobic digestion of pre-treated sludge was in good agreement with results obtained by Luo et al. (2013). They observed that anaerobic digestion of enzymatically pre-treated WAS resulted in higher FI of HA-like substances in the digested sludge compared to anaerobic digestion of untreated WAS.

By comparing the EEM spectra in Fig. 6a, b to d, e, FI of the HA-like matters in the digested sludge from the control and the ULS reactors were both found to increase at the longer retention time. This was likely because sludge was better digested at the longer retention time which released more HAs as anaerobic digestion by-products. In contrast, FI of the HA-like substances were similar in the digested sludge of the ULS-Ozone10 and the ULS-Ozone20 reactors as shown in Fig. 6c and f. This indicated

that the HA-containing substances were mostly biodegraded and the HAs were released into the supernatant within 10 days of anaerobic digestion for the ULS-Ozone pre-treated sludge. A longer digestion time did not further increase the FI of the HA-like substances in the anaerobic digested sludge.

#### 2.4.2. Fulvic acid-like substances

FI of the FA-like substances were similar in the digested sludge from the control, ULS and ULS-Ozone reactors at SRT of 10 days as shown in Fig. 6a, b and c. By comparing Fig. 6a, b to d, e, FI of the FA-like substances in the digested sludge of the control and the ULS reactors were both found to increase when the SRT increased to 20 days which was similar to the observations on the HA-like matters. However, the FI of the FA-like substances in the digested sludge of the ULS-Ozone reactor decreased when the SRT increased from 10 days to 20 days, indicating the FA-like compounds became biodegradable at a longer digestion time due to the subsequent ozonation step. This was supported by previous studies which observed the increase of biodegradability of FAs due to ozonation process (Kozyatnyk et al., 2013; Volk et al., 1997). Such increase in biodegradability of FAs is a potential advantage of ozonation treatment of feed sludge and has not been emphasized in previous studies.

### 3. Conclusions

This work investigated the impact of ULS-Ozone pre-treatment on sewage sludge anaerobic digestion. The findings of this work are summarized as follows: subsequent ozonation complemented ULS pre-treatment in improving biogas production and volatile solid removal during the sludge anaerobic digestion. ULS-Ozone treatment of feed sludge could shorten the anaerobic digestion SRT from 20 days to 10 days without an adverse impact on anaerobic digestion performance. Soluble polymeric substances were found to accumulate in the anaerobic digested sludge following the anaerobic digestion of ULS-Ozone treated feed sludge. Such digested sludge had deteriorated dewaterability. Although some of these polymers were

anaerobically degradable at 20 days SRT, most were HA-like substances and persistent. Biodegradability of FA-like substances was improved due to application of ozone.

## Acknowledgments

The authors would like to express their sincere thanks to the Public Utilities Board, Singapore for sponsoring the project and providing sludge samples for analysis and Xylem Water Solutions Herford GmbH for providing the ozone generator.

## REFERENCES

- APHA, 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed. (Washington, D.C.).
- Bougrier, C., Carrère, H., Delgenès, J.P., 2005. Solubilisation of waste-activated sludge by ultrasonic treatment. *Chem. Eng. J.* 106 (2), 163–169.
- Braguglia, C.M., Gianico, A., Mininni, G., 2012. Comparison between onzone and ultrasound disintegration on sludge anaerobic digestion. *J. Environ. Manag.* 95 (S1), 139–143.
- Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. *Environ. Sci. Technol.* 37 (24), 5701–5710.
- Chiu, Y.C., Chang, C.N., Lin, J.G., Huang, S.J., 1997. Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion. *Water Sci. Technol.* 36 (11), 155–162.
- Dogan, I., Sanin, F.D., 2009. Alkaline solubilization and microwave irradiation as a combined sludge disintegration and minimization method. *Water Res.* 43 (8), 2139–2148.
- DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28 (3), 350–356.
- Jin, Y.Y., Li, H., Mahar, R.B., Wang, Z.Y., Nie, Y.F., 2009. Combined alkaline and ultrasonic pretreatment of sludge before aerobic digestion. *J. Environ. Sci.* 21 (3), 279–284.
- Khanal, S.K., Grewell, D., Sung, S., van Leeuwen, J., 2007. Ultrasound applications in wastewater sludge pretreatment: a review. *Crit. Rev. Environ. Sci. Technol.* 37 (4), 277–313.
- Kim, D.-H., Jeong, E., Oh, S.-E., Shin, H.-S., 2010. Combined (alkaline + ultrasonic) pretreatment effect on sewage sludge disintegration. *Water Res.* 44 (10), 3093–3100.
- Kozyatnyk, I., Świetlik, J., Raczyk-Stanisławiak, U., Dąbrowska, A., Klymenko, N., Nawrocki, J., 2013. Influence of oxidation on fulvic acids composition and biodegradability. *Chemosphere* 92 (10), 1335–1342.
- Lehne, G., Müller, A., Schwedes, J., 2001. Mechanical disintegration of sewage sludge. *Water Sci. Technol.* 43 (1), 19–26.
- Li, H., Jin, Y.Y., Nie, Y.F., 2009. Application of alkaline treatment for sludge decrement and humic acid recovery. *Bioresour. Technol.* 100 (24), 6278–6283.
- Liu, X.L., Liu, H., Chen, J.H., Du, G.C., Chen, J., 2008. Enhancement of solubilization and acidification of waste activated sludge by pretreatment. *Waste Manag.* 28 (12), 2614–2622.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193 (1), 265–275.
- Luo, K., Yang, Q., Li, X.M., Chen, H.B., Liu, X., Yang, G.J., et al., 2013. Novel insights into enzymatic-enhanced anaerobic digestion of waste activated sludge by three-dimensional excitation and emission matrix fluorescence spectroscopy. *Chemosphere* 91 (5), 579–585.
- Nickel, K., Neis, U., 2007. Ultrasonic disintegration of biosolids for improved biodegradation. *Ultrason. Sonochem.* 14 (4), 450–455.
- Nishijima, W., Mukaidani, T., Okaga, M., 2003. DOC removal by multi-stage ozonation-biological treatment. *Water Res.* 37 (1), 150–154.
- Park, C., Helm, R.F., Novak, J.T., 2008. Investigating the fate of activated sludge extracellular proteins in sludge digestion using sodium dodecyl sulfate polyacrylamide gel electrophoresis. *Water Environ. Res.* 80 (12), 2219–2227.
- Rittman, B.E., McCarty, P.L., 2001. *Environmental Biotechnology: Principles and Applications*. McGraw-Hill Int., Editions, London.
- Rivero, J.A.C., Madhavan, N., Suidan, M.T., Ginestet, P., Audic, J.-M., 2006. Enhancement of anaerobic digestion of excess municipal sludge with thermal and/or oxidative treatment. *J. Environ. Eng.* 132 (6), 638–644.
- Sahinkaya, S., 2014. Disintegration of municipal waste activated sludge by simultaneous combination of acid and ultrasonic pretreatment. *Process. Saf. Environ. Prot.* 93, 201–205.
- Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Compositions Reactions*. 2nd ed. John Wiley & Sons, New York.
- Takashima, M., 2008. Examination on process configurations incorporating thermal treatment for anaerobic digestion of sewage sludge. *J. Environ. Eng.* 134 (7), 543–549.
- Tian, X.B., Wang, C., Trzcinski, A.P., Lin, L., Ng, W.J., 2014. Interpreting the synergistic effect in combined ultrasonication-ozonation sewage sludge pre-treatment. *Chemosphere* <http://dx.doi.org/10.1016/j.chemosphere.2014.09.007> (in press).
- Tiehm, A., Nickel, K., Neis, U., 1997. The use of ultrasound to accelerate the anaerobic digestion of sewage sludge. *Water Sci. Technol.* 36 (11), 121–128.
- Tiehm, A., Nickel, K., Zellhorn, M., Neis, U., 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water Res.* 35 (8), 2003–2009.
- Volk, C., Roche, P., Joret, J.C., Paillard, H., 1997. Comparison of the effect of ozone, ozone-hydrogen peroxide system and catalytic ozone on the biodegradable organic matter of a fulvic acid solution. *Water Res.* 31 (3), 650–656.
- Wang, F., Ji, M., Lu, S., 2006a. Influence of ultrasonic disintegration on the dewaterability of waste activated sludge. *Environ. Prog.* 25 (3), 257–260.
- Wang, F., Lu, S., Ji, M., 2006b. Components of released liquid from ultrasonic waste activated sludge disintegration. *Ultrason. Sonochem.* 13 (4), 334–338.
- Wu, B., Kitade, T., Chong, T.H., Uemura, T., Anthony, G.F., 2011. Impact of membrane bioreactor operating conditions on fouling behavior of reverse osmosis membranes in MBR-RO processes. *Desalination* 311, 37–45.
- Xie, R.J., Xing, Y.J., Abdul Ghani, Y., Ooi, K.e., Ng, S.w., 2007. Full-scale demonstration of an ultrasonic disintegration technology in enhancing anaerobic digestion of mixed primary and thickened secondary sewage sludge. *J. Environ. Eng. Sci.* 6 (5), 533–541.
- Xu, G.H., Chen, S.H., Shi, J.W., Wang, S.M., Zhu, G.F., 2010. Combination treatment of ultrasound and ozone for improving solubilization and anaerobic biodegradability of waste activated sludge. *J. Hazard. Mater.* 180 (1-3), 340–346.
- Yang, S.S., Guo, W.Q., Cao, G.L., Zheng, H.S., Ren, N.Q., 2012. Simultaneous waste activated sludge disintegration and biological hydrogen production using an ozone/ultrasound pretreatment. *Bioresour. Technol.* 124, 347–354.
- Yang, S.S., Guo, W.Q., Meng, Z.H., Zhou, X.J., Feng, X.C., Zheng, H.S., et al., 2013. Characterizing the fluorescent products of waste activated sludge in dissolved organic matter following ultrasound assisted ozone pretreatments. *Bioresour. Technol.* 131, 560–563.