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

**Xinbo Tian[1,3], Antoine Prandota Trzcinski[1], Li Leonard Lin[4], Wun Jern Ng[2,3*]**

1. Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

Email: tian0047@e.ntu.edu.sg

2. Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

3. School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

4. Public Utilities Board, Water Reclamation (Plants) Department, 40 Scotts Road, #15-01 Singapore 228231, Singapore

**Abbreviations:** ***CST*:** Capillary suction time (second); ***Em***: emission wavelength (nm); ***Ex***: excitation wavelength (nm); ***FA***: fulvic acid; ***HA***: humic acid; ***MW***: molecular weight (Da); ***OLR:*** organic loading rate (g COD/L day); ***Rt*:** retention time (min); ***SMP***: soluble microbial products; ***SRT:*** solids retention time (day); ***ULS***: ultrasonication; ***ULS-Ozone***: ultrasonication-ozonation; ***WAS***: waste activated sludge;

**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 SRTs of 10 and 20 days. The control, ULS and ULS-Ozone reactors produced 256, 309 and 348 mL biogas/g $COD_{fed}$ and the 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/g $COD_{fed}$ 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 organics 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.

**Keywords: Sewage sludge; ultrasound; ozone; pre-treatment; molecular weight; anaerobic digestion**

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** Corresponding author Email: WJNg@ntu.edu.sg*

## 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; Tiehm et al., 2001). Biological flocs in the sludge matrix would be 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 is improved (Tiehm et al., 1997; Tiehm et al., 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 pretreatments (Liu et al., 2008; Sahinkaya, 2014) have 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., 2014; Xu et al., 2010; Yang et al., 2012; Yang et al., 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 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. (2014) found 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. (2014) 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 solids removal efficiency and digested sludge characteristics after anaerobic digestion is not available. Besides, influence of solids 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 solids 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 solids 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 (Agilent, model 3200P). A UV spectrophotometer (Shimadzu, UV-1800) was used in the determination of proteins, carbohydrates and ammonia-nitrogen concentrations. Proteins concentration was determined with Lowry's method (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 analysed with a gas chromatograph (Agilent Technologies 7890A GC system) fitted with a flame ionization detector. The composition of biogas was measured with a gas chromatograph (Agilent Technologies 7890A GC system) 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., 2014). ULS pre-treatment was performed with an ultrasonicator (Misonix, Q700) with ultrasound frequency of 20 kHz. The temperature was monitored and maintained at about 30 °C with an ice-water bath during ultrasonication. The specific energy input was 9 kJ/g TS. Ozonation pre-treatment was performed with an ozone generator (WEDECO, GSO 30). 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 $O_3$/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. (2014). A HPLC (Agilent Technologies 1260 LC system) was used for MW distribution analysis using the

PL aquagel-OH 8μm MIXED-M column. Milli-Q water was used as mobile phase with a flow rate of 1 mL/min. A PL aquagel-OH 8μm 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 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., 2014):

$$Log(MW) = 9.8223 - 0.6748(\mathrm{Rt}) \tag{1}$$

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, 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.

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 litre of seed sludge was fed into the reactor before starting the experiment. 100 and 50 mL sludge aliquots were daily removed daily 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 (Ritter, 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 normalised by dividing daily gas production by 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. 1a and 1b**. 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 $COD_{fed}$ 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 $COD_{fed}$ 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 increasing biogas production. Nickel and Neis (2007) found the improvement in biogas production due to 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 the increase in biogas production after the ULS-Ozone treatment of feed sludge also became more pronounced 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 VFAs 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 treatments of feed sludge caused stress on the methanogenesis step in the reactors.

Improvement in organic solids removal efficiency during anaerobic digestion was also observed when feed sludge was treated before anaerobic digestion. The change in VS and VSS concentrations in the digested sludge during anaerobic digestion is as shown in **Fig. 2a-d**. Solids 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 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. Solids 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 more organic matters were digested and converted into biogas. Similarly, incorporation of the pre-treatment step also improved the VSS removal efficiency as shown in **Table 2**. The increase in VSS removal efficiency indicated particulate organics in the treated feed sludge were better hydrolysed 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 9,930 to 9,810 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 8,005 to 7,640 mg/L at SRT of 10 days). Furthermore, this work demonstrated 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 the daily biogas production, solids removal efficiencies of the ULS-Ozone10 reactor were better than those of the Control20 reactor. However, daily biogas production and solids 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. SCOD and soluble biopolymers

The SCOD concentration in the digested sludge during anaerobic digestion is shown in **Fig. 3a and 3b**. 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 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 the increase in post-digestion SCOD due to the subsequent ozonation was also statistically significant. As shown in **Fig. 3a and 3b**, SCOD in the digested sludge from ULS-Ozone reactors were 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 1,200 mg/L higher than the SCOD in the ULS treated feed sludge. This indicated much of the COD solubilized by the subsequent ozonation treatment of feed sludge was 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 3d**. Soluble carbohydrates and proteins 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 carbohydrates and proteins concentrations were different. As shown in **Fig. 3c**, soluble carbohydrates concentrations decreased obviously when all the reactors had longer residence time. For example, the residual soluble carbohydrates 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., 2014; Wang et al., 2006b). Longer residence time was needed for sufficient degradation. However, the soluble proteins concentrations in the digested sludge did not show 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, HA 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 s, 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 s, 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 the dewaterability of digested sludge was deteriorated by the ULS 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 were 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 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, 5c and 5d**, 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 MW compounds

Peak 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 5d**. 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, 5d to 5b**. 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 peak 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 around 19.4 kDa and 7.7 kDa, respectively. These compounds (detected in peak 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 peak A, C, D and E were lower at SRT of 20 days as compared in **Fig. 5c and 5d**. This indicated 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 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 peak A, C, D and E were significantly higher in the digested sludge from the ULS-Ozone reactor. This indicated 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., 2014; 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 MW compounds

Peak F (Rt: 13.3 min, < 106 Da) was detected in the supernatant of digested sludge as shown in **Fig. 5c and 5d**. 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. 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., 2014). 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 treatment of feed sludge, but insights on the SCOD in the anaerobic digested sludge were not discussed in these studies (Dogan 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 good supplementary information to conventional approaches in understanding the influence of treatments of feed sludge on subsequent anaerobic digestion.

## 2.4. Fluorescent products characterization

EEM fluorescence spectroscopy analysis was conducted to measure the fluorescence intensity (FI) of fluorescent compounds in the supernatant of digested sludge the reactors. The EEM spectra of all the samples are shown in **Fig. 6a-f**. FI of the detected peaks was shown in numbered and coloured 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 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 6b**. 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 treatments 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 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 (Luo et al., 2013).

By comparing the EEM spectra in **Fig. 6a, 6b** to **Fig. 6d, 6e**, 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 were 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 6f**. This indicated 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, 6b and 6c**. By comparing **Fig. 6a, 6b** to **Fig. 6d, 6e**, 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 treatment of pre-digestion feed sludge on sludge anaerobic digestion. The findings of this work are summarized as follows:

- Subsequent ozonation complemented ULS treatment in improving biogas production and volatile solids removal when the feed sludge was treated before anaerobic digestion.
- ULS-Ozone treatment of feed sludge could shorten the anaerobic digestion SRT from 20 days to 10 days without adverse impact on anaerobic digestion performance.
- Soluble polymeric substances were found to accumulate in the anaerobic digested sludge following 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.

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## List of Tables:

**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 |

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

| | SRT 10 days | | | SRT 20 days | | |
|---|---|---|---|---|---|---|
| Reactor | Control10 | ULS10 | ULS-Ozone10 | Control20 | ULS20 | ULS-Ozone20 |
| Organic loading rate (g COD/L.day) (n=10 and 20)[a] | 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 $COD_{fed}$) (n=10 and 20)[a] | 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)[b] | 8340 ± 383 | 7990 ± 344 | 7500 ± 245 | 8125 ± 252 | 7644 ± 253 | 7081 ± 217 |
| VS removal efficiency (%) (n=5 and 8)[b] | 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)[b] | 8005 ± 469 | 7640 ± 155 | 6760 ± 248 | 7619 ± 258 | 7056 ± 213 | 6606 ± 206 |
| VSS removal efficiency (%) (n=5 and 8)[b] | 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[c] | Nd | Nd | Nd | Nd | Nd |

[a] n =10 at SRT of 10 days and n= 20 at SRT of 20 days

[b] n =5 at SRT of 10 days and n= 8 at SRT of 20 days

[c] not detectable (< 10 mg/L)

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

t is the *t statistic* value and P is the probability that the two compared values are not significantly different

[a] The tested value was significantly higher than the reference value

[b] The tested value was significantly lower than the reference value

**List of figures:**

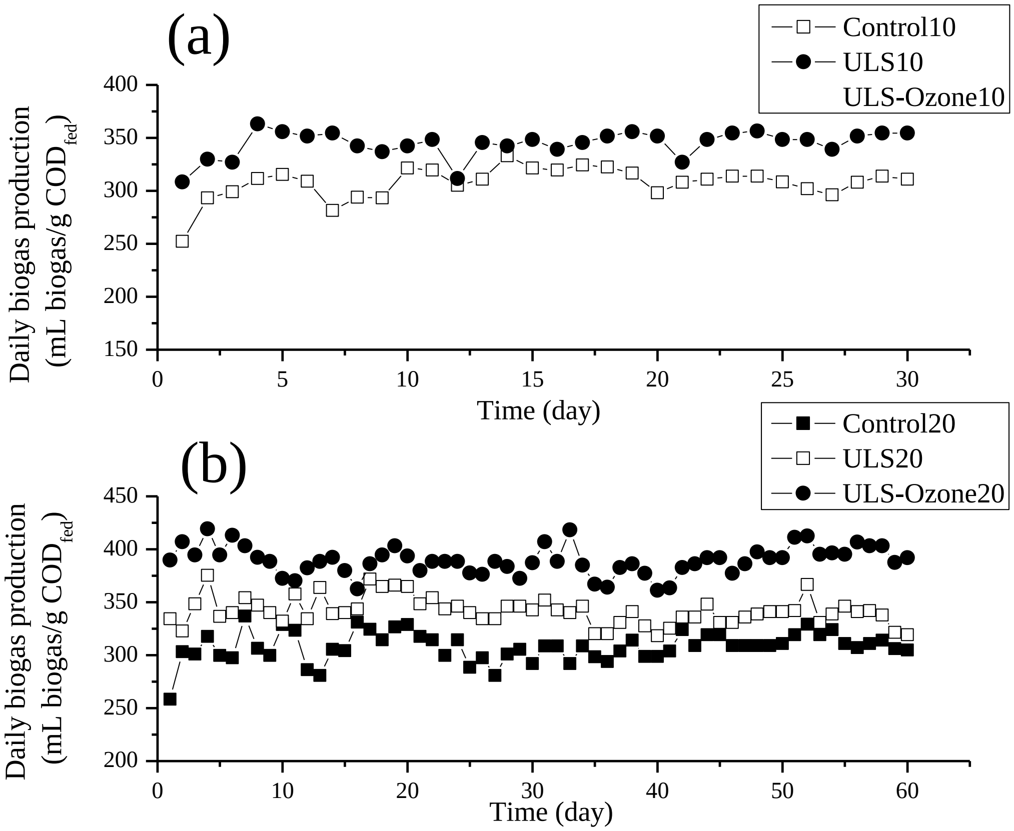

**Fig.1 Daily biogas production from the reactors at (a) SRT of 10 days (Control 10, ULS10, ULS-Ozone10) (b) SRT of 20 days (Control 20, ULS20, ULS-Ozone20)**

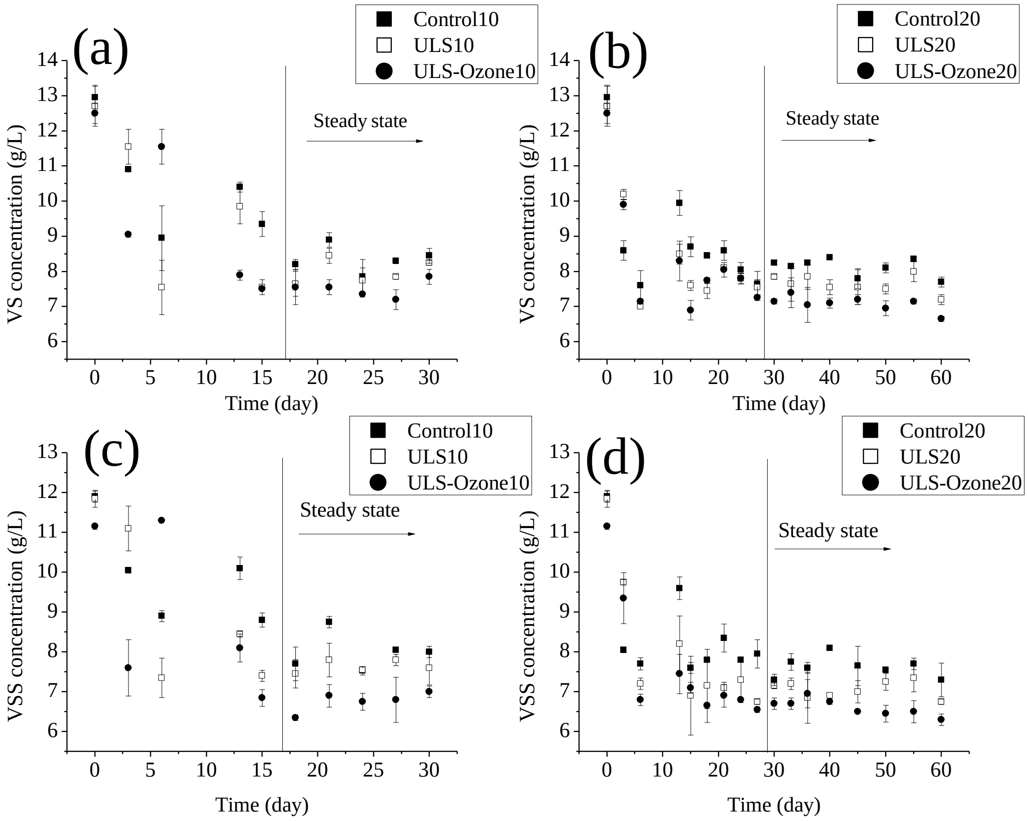

**Fig. 2 (a) Change in VS concentration in the digested sludge during the anaerobic digestion at SRT of 10 days (b) Change in VS concentration in the digested sludge during the anaerobic digestion at SRT of 20 days (c) Change in VSS concentration in the digested sludge during the anaerobic digestion 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)**

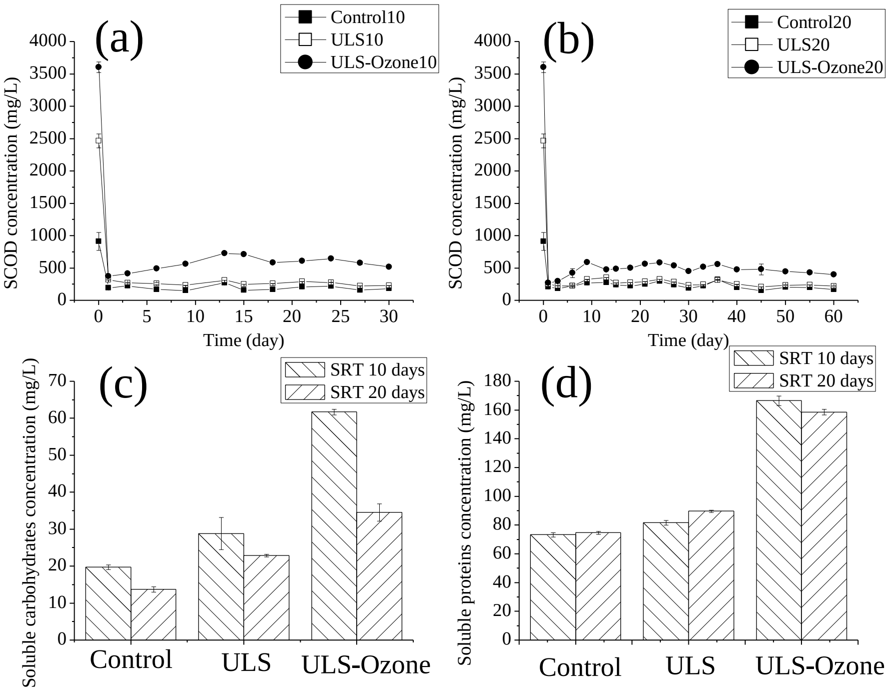

**Fig. 3 (a) Change in SCOD concentration in the digested sludge during the anaerobic digestion at SRT of 10 days (b) Change in SCOD concentration in the digested sludge during the anaerobic digestion at SRT of 20 days (SCOD concentration was based on at least two replicates) (c) Soluble carbohydrates 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)**

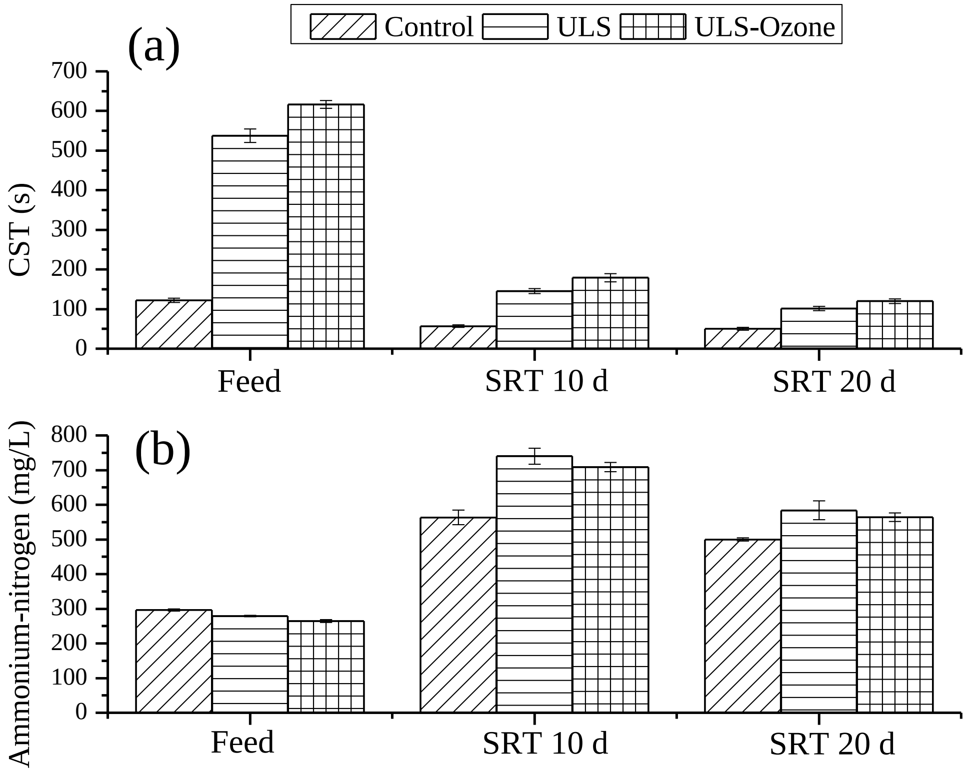

**Fig. 4 (a) Dewaterability (n=3) (b) Ammonia-nitrogen (n=3) in feed sludge and digested sludge from different anaerobic reactors**

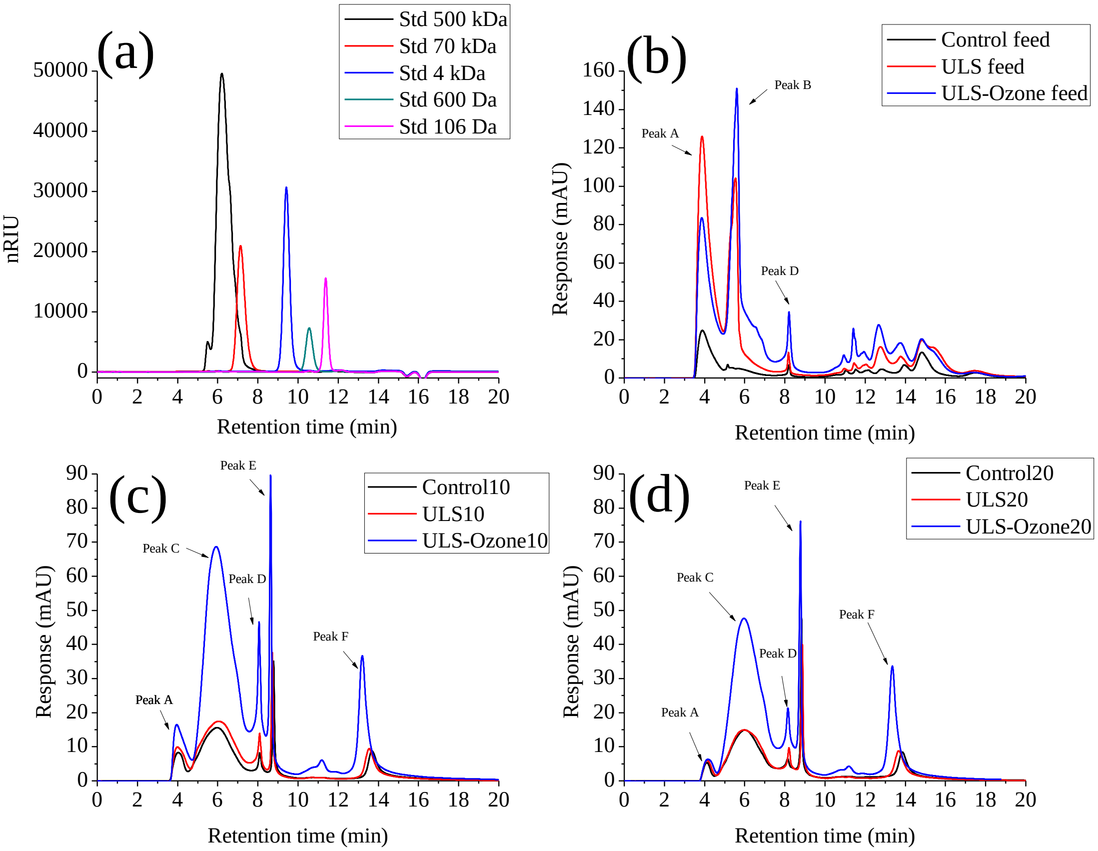

**Fig. 5 Molecular weight distribution chromatograms of (a) standard polymers (b) supernatant in the feed sludge (c) supernatant in the digested sludge from reactors operating at SRT of 10 days (d) supernatant in the digested sludge from reactors operating at SRT of 20 days**

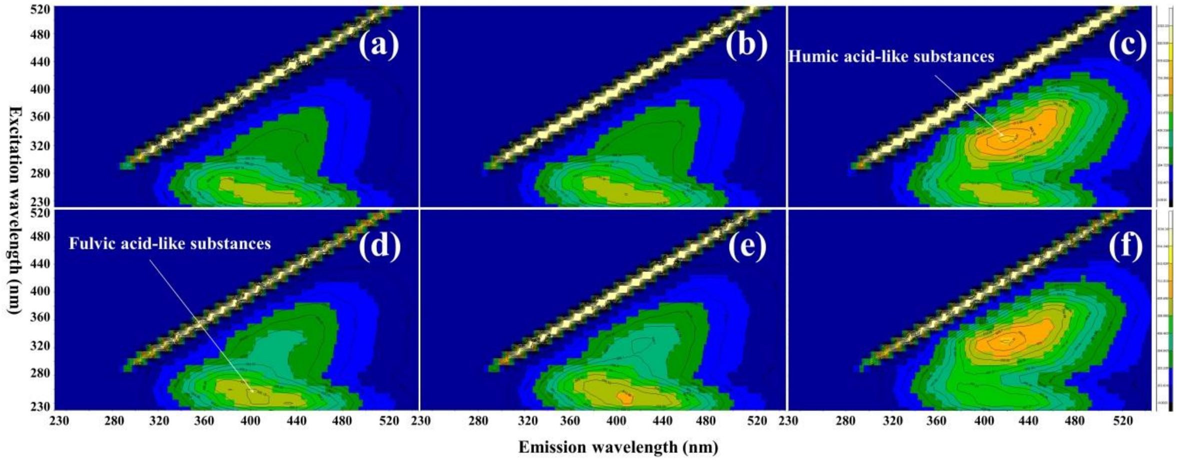

**Fig.6 Excitation emission matrix fluorescent spectra of the supernatant in the digested sludge from (a) control10 (b) ULS10 (c) ULS-Ozone10 (d) control20 (e) ULS20 (f) ULS-Ozone20**