Interpreting the synergistic effect in combined ultrasonication-ozonation sewage sludge pre-treatment

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Abstract

The sequential combination of ultrasonication and ozonation (ULS-Ozone) as sewage sludge treatment prior to anaerobic digestion was investigated. Synergistic volatile suspended solids (VSS) solubilization was observed when low energy ultrasonication ($\leq$ 12 kJ/g TS) was followed by ozonation. 0.048g O_3/g TS ozonation had induced the maximum VSS solubilization of 41.3% when the sludge had been pre-ultrasonicated at 9 kJ/g TS. The same ozone dosage applied without prior ultrasonication had only induced 21.1% VSS solubilization. An additional level of analysis based on size exclusion chromatography (SEC) was conducted to determine the changes in molecular weight (MW) distribution of soluble components in the sludge samples. High MW components (MW > 500 kDa) were found to be the main solubilized products when sludge was only ozonated. However, solubilized products by ozone were mainly in the form of low MW components (MW < 27 kDa) when sludge had been pre-ultrasonicated. The high MW products generated by ultrasound were effectively degraded in the subsequent ozonation. Sludge biodegradability was found to have been enhanced after ULS-Ozone pre-treatment. A biodegradability increase of 34.7% was observed when ultrasonication (9 kJ/g TS) and ozonation (0.036 g O_3/g TS) were applied sequentially. This was 7.0% more than the sum of the biodegradability increase if ultrasonication (9.9%) and ozonation (17.8%) had been applied individually and without the sequence of application identified.

Keywords: Sewage sludge; ultrasound; ozone; pre-treatment; size exclusion chromatography (SEC); anaerobic digestion.

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1. Introduction

Sludge produced in wastewater treatment plants needs to be stabilized before it can be safely disposed of. This is because of its high organic as well as pathogen content (Bitton, 2005). Anaerobic digestion is generally accepted as an appropriate way to stabilize a sludge's organic components and reduce the final amount of solids requiring disposal as well as produce methane gas as a means of energy recovery (Zhang et al., 2007). However, anaerobic digestion is a slow process and its performance is typically limited by hydrolysis of the particulate organic matters in the sludge (Pavlostathis and Giraldo-Gomez, 1991). Therefore, sludge is often pre-treated before it is fed into an anaerobic digester so as to accelerate hydrolysis and so enhance the overall anaerobic process.

Sludge pre-treatment technologies can be categorized into mechanical, thermal and chemical. The aim of sludge pre-treatment is to solubilize the particulate organics and so make them more accessible for subsequent microbial action (Tiehm et al., 1997). Conventional pre-treatments such as thermal, alkaline and thermo-alkaline pre-treatments have been reported since late 1970s (Stuckey and McCarty, 1978, Stuckey and McCarty, 1984, Ray et al., 1990, Li and Noike, 1992). Compared to these conventional pre-treatments, ultrasound and ozone pre-treatments are relatively newer technologies and have only been widely reported in the last decade. Ultrasonic sludge disintegration had been preferred at lower ultrasonic frequency (Tiehm et al., 2001). The predominant sludge disintegration mechanism in low frequency ultrasonication, a mechanical method, is the hydro-mechanical shear force caused by collapse of cavitation bubbles (Wang et al.,

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2005). Ultrasound readily reduces biological floc sizes, rupturing microorganism cells as well as significantly increasing sludge biodegradability in the subsequent anaerobic digestion (Tiehm et al., 1997, Lehne et al., 2001, Zhang et al., 2007). Ozonation has also been reported as an effective sludge pre-treatment technology but with a different disintegration mechanism. Ozone chemically reacts with sludge and destroys microorganism cell components (Chu et al., 2009, Yan et al., 2009). Ozone also attacks the extracellular polymeric substances and breaks down the complex macromolecules in soluble phase (Yan et al., 2009). In addition, ozone is able to convert refractory organic matters into biodegradable form (Volk et al., 1993, Nishijima et al., 2003). Most importantly sludge biodegradability is reported to be remarkably improved after ozonation (Weemaes et al., 2000, Goel et al., 2003).

Ultrasound and ozone acted differently on sludge (Bougrier et al., 2006). Comparison between sludge ultrasonication and ozonation have been conducted by previous researchers (Bougrier et al., 2006, Braguglia et al., 2012). Bougrier et al. (2006) found that ultrasonication and ozonation resulted in different physical and biochemical characteristics in the same sludge. Due to the supplementary effects of these two pre-treatments, combination of ultrasonication and ozonation has been suggested for a larger impact (Xu et al., 2010, Yang et al., 2012, Yang et al., 2013). However, information in the published literatures on such combination is relatively scarce and the synergistic mechanisms between the two pre-treatment are still ambiguous. Eskicioglu et al. (2006) have indicated the molecular weight (MW) of the solubilized substances is an important indicator of the sludge pre-treatment performance. However, the size of the solubilized

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substances by ultrasound, ozone and their combination has not been reported previously. Given the information discussed above, this work aims to investigate the interaction between the two pre-treatment processes and seek explanations for the synergistic effects. The optimum combination sequence was determined and changes in the sludge characteristics after individual and combined pre-treatments were measured. In addition to the conventional parameters such as SCOD for measurement of sludge solubilization, size exclusion chromatography (SEC) measurement was conducted to determine the size of the solubilized substances. Batch anaerobic digestion tests were then conducted in this study to evaluate the effectiveness of the pre-treatment in terms of increasing sludge biodegradability.

2. Materials and Methods

2.1. Sludge samples

Samples of a mixture of primary sludge (PS) and thickened waste activated sludge (WAS) (ratio around 1:1 based on dry solids) were collected from a local municipal wastewater reclamation plant. Properties of the sludge used in this study are listed in Table 1.

Table 1 Sludge characteristics

Parameter	Value range
Total solids (g/L)	16.2-17.2
Volatile solids (g/L)	12.6-13.4
Total suspended solids (g/L)	15.5-15.9
Volatile suspended solids (g/L)	12.4-13.3
Total COD (mg/L)	19,500-25,000
Soluble COD (mg/L)	700-1,200

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Soluble Protein (mg/L)	<100
Soluble Carbohydrate (mg/L)	<50

2.2. Analytical methods

The total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), soluble COD (SCOD) and total COD (TCOD) were measured as described in Standard Methods (APHA, 1998). COD solubilization was quantified as SCOD+ which was the SCOD difference before and after pre-treatment shown as below:

$$SCOD+ = SCOD_{after} - SCOD_{before}$$

Solids solubilization was calculated as follows (Salsabil et al., 2009):

$$VSS \text{ solubilization} = (VSS_b - VSS_a)/VSS_b \times 100\%$$

Where VSS_b stands for the VSS concentration before each pre-treatment; VSS_a stands for the VSS concentration after each pre-treatment.

Protein concentration was determined with the Lowry's method (1951) using bovine serum albumin as standard and a UV spectrophotometer (Shimadzu, UV-1800) against the blank at a wavelength of 750 nm. As the precise chemical formula of the proteins detected was not determined, the percentage of SCOD represented by protein had to be estimated by assuming a stoichiometric conversion factor of 1.5 which was derived from the typical formula of proteins ($C_{16}H_{24}O_5N_4$) as presented by Rittmann and McCarty (2001). Carbohydrate concentration was determined with the sulfuric-phenol method using D-Glucose as standard (DuBois et al., 1956). To convert into equivalent COD, 1g carbohydrates assumed as $C_6H_{12}O_6$ was taken as equivalent to 1.07 g COD (Rittman and

McCarty, 2001). Sludge pH was measured with a pH meter (Agilent, model 3200P) with an accuracy of 0.001. Buffer solution of pH4, pH7 and pH10 (Fisher, UK) were used to calibrate the pH meter every time before measurement.

2.3. Pre-treatment conditions

Ultrasonication was performed with an ultrasonicator (Misonix, Q700). The ultrasound frequency was 20 kHz and the maximum power input was 700 Watts. The power input was around 130 Watts and the treated sludge volume was 200 mL. Ultrasonication energy was quantified in terms of specific energy input and the calculation was shown as follows (Lehne et al., 2001):

$$\text{Specific energy input} = (P \times t) / (V_{\text{sludge}} \times TS)$$

Where P is power input of the ultrasonicator (W), t is the time of ultrasonication (s), V_{sludge} is volume of treated sludge (L) and TS is the total solids concentration of treated sludge (g/L). During ultrasonication the temperature was monitored and maintained at about 30 °C with an ice-water bath. The maximum specific energy input was 21 kJ/g TS which can be alternatively expressed as 5.83 kWh/kg TS.

Ozonation 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. The power input of the ozone generator was 180 Watts. The applied ozone dosage was quantified according to the potassium iodide method (Konsowa, 2003). A stone diffuser was installed to produce fine ozone bubbles and to enhance ozone mass transfer. The applied ozone was quantified in terms of ozone dosage (g O_3/g TS). The maximum applied ozone dosage was 0.12 g O_3/g TS.

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Optimum combination sequence of ultrasonication and ozonation was determined before commencing the main experiment. The Ultrasonication-Ozonation (ULS-Ozone) sequence was performed by applying ozonation after ultrasonication and the Ozonation-Ultrasonication (Ozone-ULS) sequence was conducted by dosing ozone prior to ultrasonication. The maximum tested specific energy input and ozone dosage were 12 kJ/g TS and 0.12 g O_3/g TS in the sequence determination experiment, respectively. The main combination pre-treatment tests were carried out in the sequence of ULS-Ozone sequence and this was based on the results of the proceeding sequence determination experiments. The maximum tested specific energy input and ozone dosage were 21 kJ/g TS and 0.048 g O3/g TS, respectively.

2.4. Molecular weight distribution

An HPLC (Agilent Technologies, 1260 LC system) was used for the 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. Both refractive index (RI) detector and UV (254 nm) detector were used for the detection of the eluted substances. Calibration was done using polyethylene glycol and polyethylene oxide standards with molecular weight of 500 kDa, 70 kDa, 4 kDa, 600 Da and 106 Da with the RI detector. A linear relationship was derived between the log value of MW (Da) and retention time (Rt: min) with a correlation coefficient of 99.2%:

$$Log (MW) = 9.8223 - 0.6748(Rt)$$

A PL aquagel-OH 8μm guard column was installed in front of the main column. The sample was first centrifuged at 10,000 rpm for 10 minutes. The supernatant was then

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filtered through a 0.2μm membrane filter before injection. Larger molecules tend to have shorter residence time in the column and so eluted faster, while smaller molecules have longer residence time and eluted later.

2.5. Anaerobic biodegradability

Biochemical methane potential (BMP) assays were conducted according to Owens et al. (1979) in 120 mL serum bottles to quantify the sludge anaerobic degradability. 10 mL of substrate sludge and 30 mL of degassed inoculum (VS: 9.9 g/L) were added to serum bottles. A mixture of 20% CO_2 and 80% N_2 was used to purge each bottle for three minutes to create an anaerobic environment. All bottles were incubated in an orbital shaker at 35°C. The biogas volumes were regularly measured using a wetted glass syringe. The biogas composition was determined with gas chromatography (Agilent Technologies, 7890A GC system).

3. Results and Discussion

3.1. Individual pre-treatment

The SCOD+ increase was different during ultrasonication and ozonation. For ultrasonicated sludge, SCOD+ increased linearly with specific energy input as shown in Fig. 1a. The SCOD+ was 3,450 mg/L after 21 kJ/g TS ultrasonication and values did not plateau. However, for ozonated sludge, SCOD+ increased linearly with ozone dosage up till only around 2,700 mg/L as shown in Fig. 1b. Thereafter, the increase in SCOD+ became less and plateaued at 3,700 mg/L with 0.08 g O_3/g TS ozonation. Proteins and carbohydrates were responsible for around 80% of the COD solubilization measured for both pre-treatments. The equivalent COD of the solubilized proteins and carbohydrates

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after 21 kJ/g TS ultrasonication were 2,370 mg/L and 450 mg/L, respectively. The equivalent COD of the solubilized proteins and carbohydrates were 2,500 mg/L and 560 mg/L after 0.08 g O_3/g TS ozonation, respectively. These are consistent with the past results, showing that ultrasound and ozone solubilized the extra-cellular and intra-cellular biopolymers in the sludge (Wang et al., 2006, Zhang et al., 2009) .
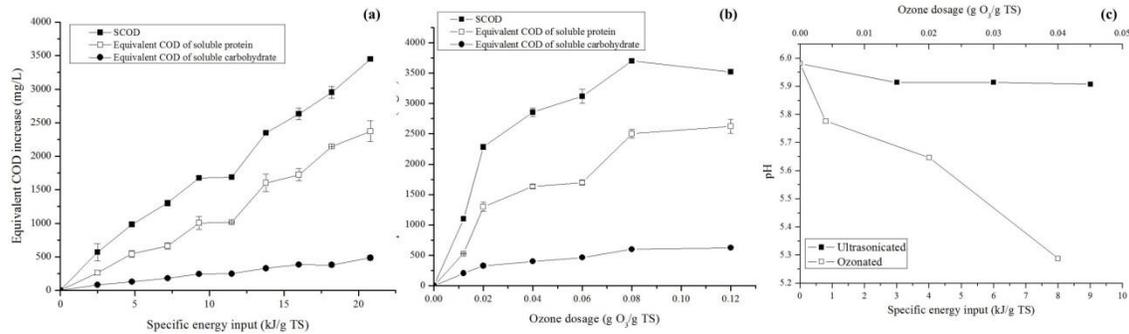
Figure 1 (a) Increase of COD, equivalent COD of proteins and carbohydrates in soluble phase with specific energy input in individual ultrasonication (b) Increase of COD, equivalent COD of proteins and carbohydrates in soluble phase with ozone dosage in individual ozonation (c) Change in pH with specific energy input and ozone dosage

In addition, ultrasound and ozone resulted in different change in pH value. Sludge pH remained relatively constant at around 5.9 with increasing specific energy input as shown in Fig. 1c. However, sludge pH decreased obviously with increasing ozone dosage. Sludge pH dropped from 5.9 to 5.2 after 0.04 g O_3/g TS ozonation. These results were consistent with past results and indicated that ultrasound and ozone did not disrupt the sludge in the same way (Bougrier et al., 2006). The pH drop during ozonation was due to the formation of acidic compounds. Yasui and Miyaji (1992) observed the formation of carboxylic acids during human waste ozonation. Bougrier et al. (2006) suggested that the pH decrease was due to the formation of volatile fatty acids from the degradation of lipid compounds in sludge. Yan et al. (2009) found that the lactic acid concentration increased

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after ozonation due to the oxidation of the soluble macromolecules. This difference in the products of the pre-treatment process used would have impact on subsequent anaerobic degradation.

In order to illustrate the difference in the solubilized substances between ultrasonication and ozonation, the MW distribution chromatograms of the raw sludge, the ultrasonicated sludge and ozonated sludge are compared in Fig. 2b and 2c. High MW compounds (Rt < 6 min) were found to be the main solubilized compounds after individual ultrasonication and ozonation as shown in both RI and UV signals. The corresponding MWs of these compounds were over 500 kDa according to the chromatograms of the standard polymers shown in Fig. 2a. These high MW compounds were mainly biopolymers from cell fragments and extracellular polymers in WAS with MW as high as 10^5 kDa (Pavoni et al., 1972, Namkung and Rittmann, 1986, Schiener et al., 1998, Aquino et al., 2006). Besides, the retention times of the detected peaks were slightly different between ultrasonicated sludge and ozonated sludge, indicating solubilization of different compounds. It should be noted that RI is a universal detector which detects most of the eluted substances if these are present in sufficient concentrations, while the UV 254 nm detector provides good sensitivity towards aromatic compounds (Trzcinski et al., 2011). For both ultrasonicated and ozonated samples, the detected peaks had similar retention times in both the RI and UV detector signals as shown in Fig. 2b and 2c, suggesting that most of the solubilized high MW compounds were possibly aromatic compounds. Yang et al. (2013) reported that tryptophan proteins were the main solubilization products after ultrasonication while humic acids and fulvic acids were the primary solubilization products after ozonation.

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In addition, low MW compounds (8 min < Rt < 13.5 min) were also released in both pre-treated sludge but not as obvious as the high MW ones. The MWs of these polymers were lower than 27 kDa according to the calibration curves. The peak with retention time longer than 13.5 minutes stands for compounds which are smaller than 106 Da (chromatograms of the standard polymers shown in Fig. 2a). Concentration of such compounds was lower in ozonated sludge than in raw sludge as shown in Fig 2c. This decrease in concentration of the simple organic compounds was because of the mineralization effect induced by ozone (Weemaes et al., 2000, Ahn et al., 2002). Simple organic compounds were oxidized into carbon dioxide.

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(a)

(b)

(c)
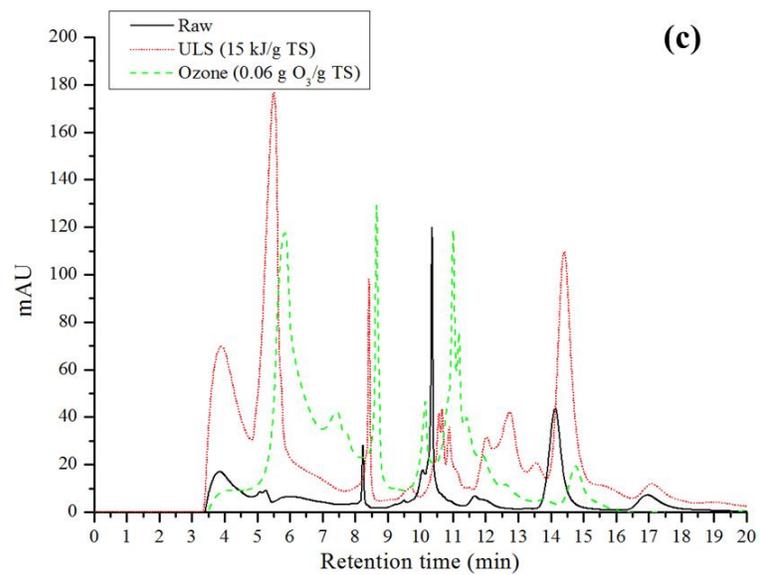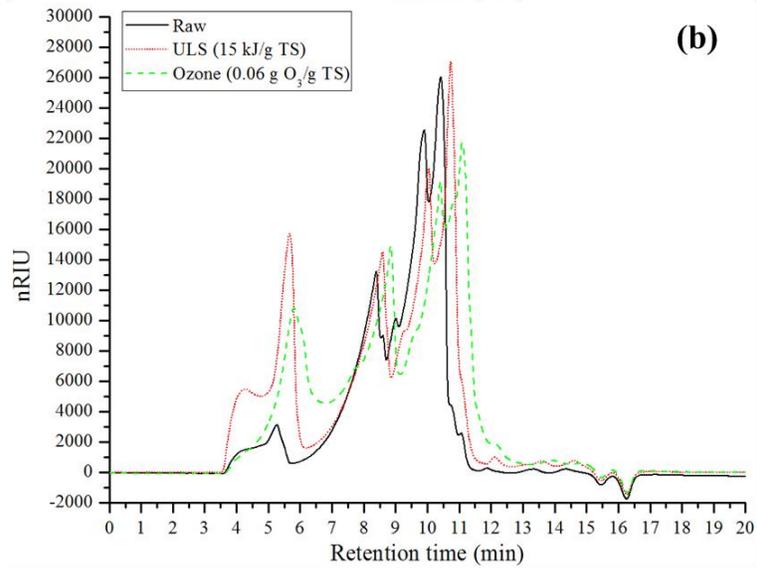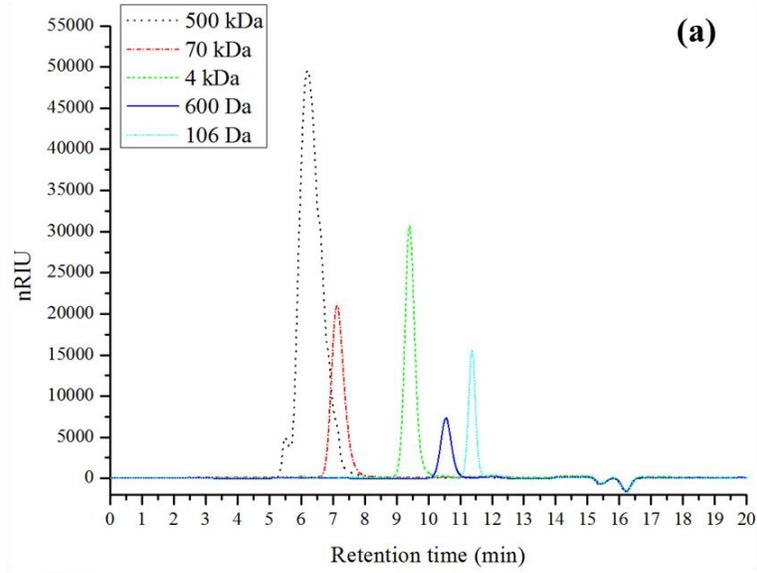
Figure 2 (a) MW distribution chromatograms of the standard polymers in RI signal (b) MW distribution chromatograms of the soluble substances in raw sludge, ultrasonicated sludge (15 kJ/g TS) and ozonated sludge (0.06 g O₃/g TS) in RI signal (c) MW distribution chromatograms of the soluble substances in raw sludge, ultrasonicated sludge (15 kJ/g TS) and ozonated sludge (0.06 g O₃/g TS) in UV signal (254 nm)

3.2. Sequential combination of ultrasonication and ozonation

3.2.1. Sequence determination

In order to investigate the interaction between ultrasound and ozone, various sequences were tested. The SCOD+ changes in each sequence are shown in Fig. 3a and 3b. The maximum SCOD+ values obtained in the ULS-Ozone and Ozone-ULS sequences were around 4,000 and 3,800 mg/L, respectively showing an improved COD solubilization compared to the individual treatment. However, the Ozone-ULS sequence did not appear to be substantially advantageous because the SCOD+ increase was marginal during the subsequent ultrasonication step, especially at high prior ozone dosage (e.g. 0.12 g O₃/g TS). This is because ozone significantly solubilized the solids in sludge and fewer solids were available for the ultrasonic mechanical disruption. Therefore, the ULS-Ozone sequence was chosen for subsequent experiments.
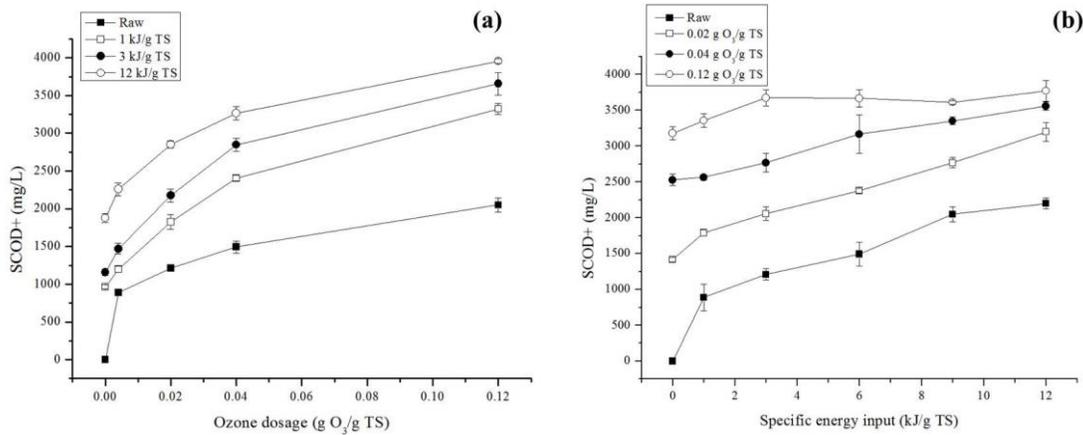
Figure 3 (a) Change in SCOD+ with ozone dosage for raw and ultrasonicated (at various specific ultrasonic energy inputs) in sequence of ULS-ozone (b) Change in

SCOD+ with specific energy input for raw and ozonated sludge (at various ozone dosages) in sequence of Ozone-ULS

3.2.2. Sludge solubilization

Prior ultrasonication did not enhance the COD solubilization induced by ozone. The COD solubilized by ozone decreased with the prior ultrasonication energy as shown in Fig. 4a. For example, the SCOD+ induced by 0.048 g O_3/g TS ozonation was 2,600 mg/L for raw sludge but was only 1,500 mg/L and 400 mg/L for sludge which had been pre-ultrasonicated at 9 kJ/g TS and 21 kJ/g TS, respectively. This is because changes in SCOD concentration can be due to organic solubilization but also degradation of organics to oxidized species such as CO_2. Previous works have shown that mineralization and degradation of the soluble organics due to ozone oxidation resulted in SCOD concentration decrease (Ahn et al., 2002, Erden and Filibeli, 2011).

Foladori et al. (2010) suggested that the VSS can be used as an alternative to represent the particulate organics in sludge sample. The VSS solubilization induced by ozone for both raw and pre-ultrasonicated sludge is shown in Fig. 4b. In contrast with the SCOD results, a greater VSS solubilization due to ozonation was obtained when the specific energy input was lower than 12 kJ/g TS. The highest VSS solubilization induced by ozone was 41.3% when 0.048 g O_3/g TS ozonation was applied after 9 kJ/g TS ultrasonication. The same ozone dosage applied without prior ultrasonication only induced 21.1% VSS solubilization. This implied that ultrasound made the organic solids easily disrupted by ozone. Agglomerations of particulates would have become smaller in size after ultrasonic dispersion resulting in a higher specific surface area, and therefore affording a greater probability of contacting with ozone. This is important because the

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half-live of ozone is only 30 minutes, and is likely much lower in practice as it may react with none target materials before it has the chance to do so with the target organic solids. Besides, smaller particles are more readily ozonated than bigger ones because of a lower mass transfer resistance (Cesbron et al., 2003). Similar synergistic solids solubilization was reported in previous study. Yang et al. (2013) reported that combined ultrasonication and ozonation pre-treatment solubilized 6.1% more solids than ultrasound and ozone had been applied individually. This confirms the positive interaction between ultrasound and ozone pre-treatments.

VSS solubilization induced by ozone started to decrease when specific energy was higher than 12 kJ/g TS. This was possibly due to the reaction between ozone and solubilized organics released by the prior ultrasound. Cesbron et al. (2003) showed that the soluble and particulate organics competed for ozone in a sludge ozonation system. There have more organics released after high energy ultrasonication (> 12 kJ/g TS). The reaction between soluble organics and ozone became significant and less ozone was therefore available for the organic solids. This negated the positive effect of the prior ultrasonic dispersion resulting in the decrease of VSS solubilization induced by ozone.

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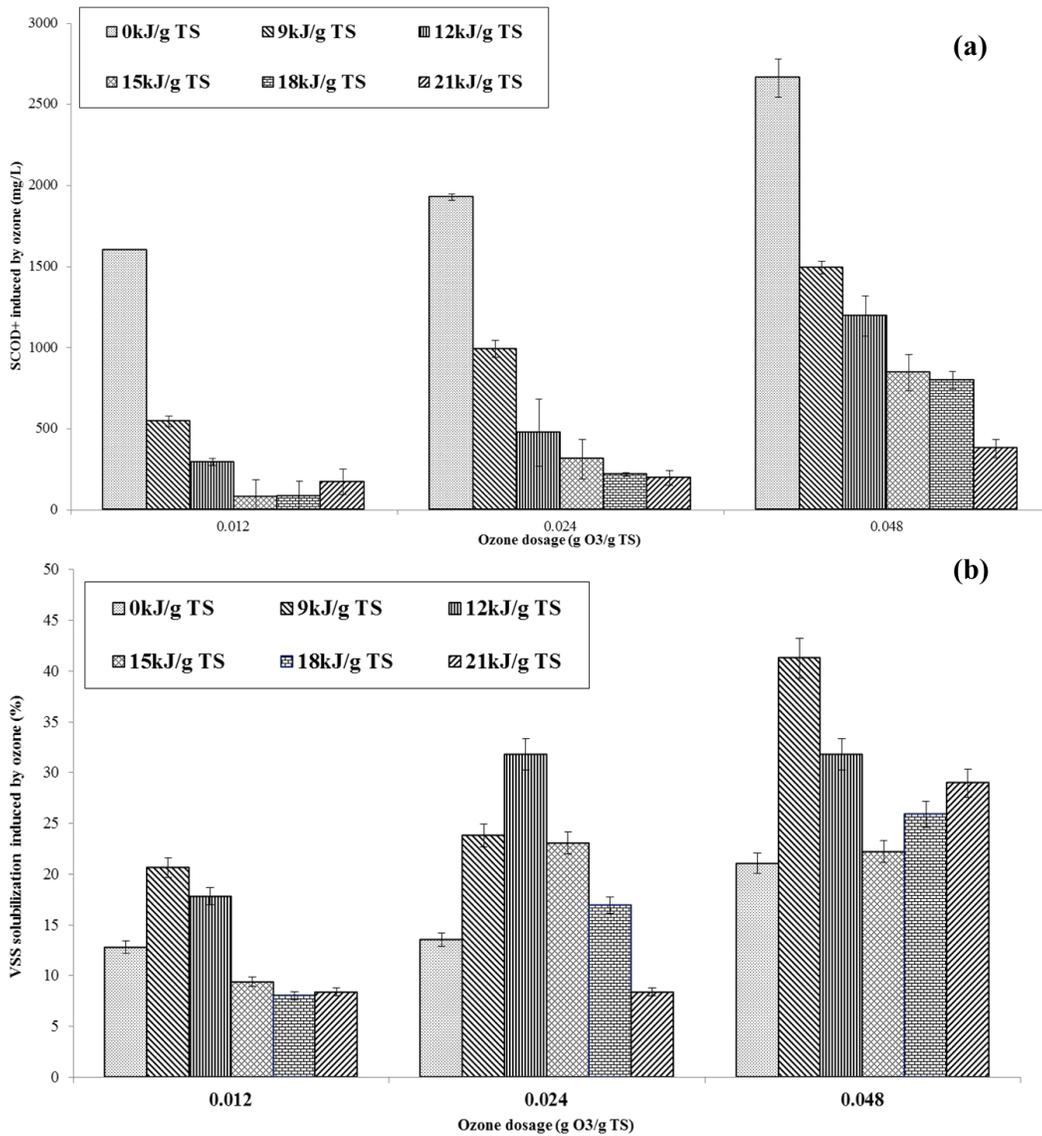
Figure 4 (a) SCOD+ induced by different ozone dosages for raw sludge and ultrasonicated sludge (at various specific energy inputs) (b) VSS solubilization induced by different ozone dosages for raw sludge and ultrasonicated sludge (at various specific energy inputs)

3.2.3. Molecular weight distribution change

In order to evaluate the size of the solubilization products by ozone when sludge was pre-ultrasonicated, the MW chromatograms of the ultrasonicated sludge and the ultrasonicated sludge with subsequent ozonation are shown in Fig. 5a and 5b. Organic solids were mainly solubilized in the form of low MW components (8 min< Rt < 13.5 min) as shown by the arrows in Fig. 5a. In addition, the concentration increase in high MW compounds was not as marked as was observed when sludge was only ozonated (as shown in Fig. 2c).

Some of the high MW components released by ultrasound were found to be very sensitive to ozone attack. The peak which represented high MW components (Rt: ~4 min) disappeared after ozonation was applied as shown by an arrow in Fig. 5b. This suggested prior ultrasound released more organics which could be ozone scavengers. Consequently, the reaction between these soluble organics and ozone became more pronounced compared to the situation when ozone was directly applied to the raw sludge. It should be borne in mind that organic solids solubilization by ozone was also enhanced in ultrasonicated sludge ($\leq$ 12 kJ/g TS) as shown in Fig. 4b. This meant that the prior ultrasonication step enabled better utilization of ozone by the soluble and particulate organics in sludge. This is important in a sludge ozonation system since ozonation performance would be assessed in terms of applied ozone dosage as well as utilization of the applied ozone (Chu et al., 2008, Manterola et al., 2008).

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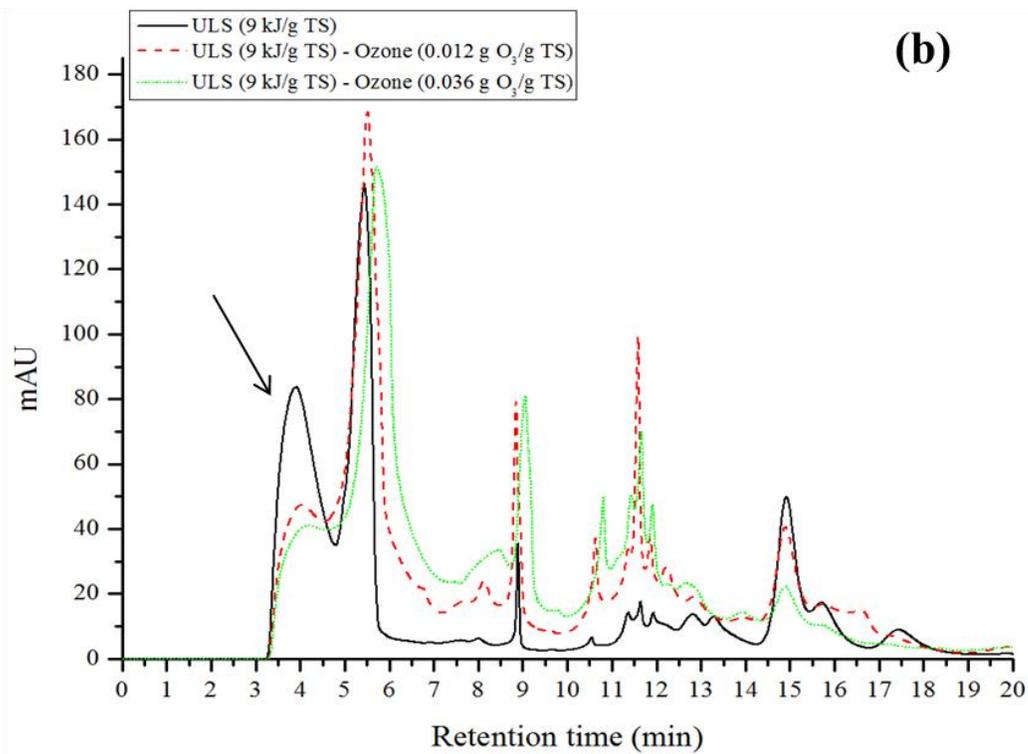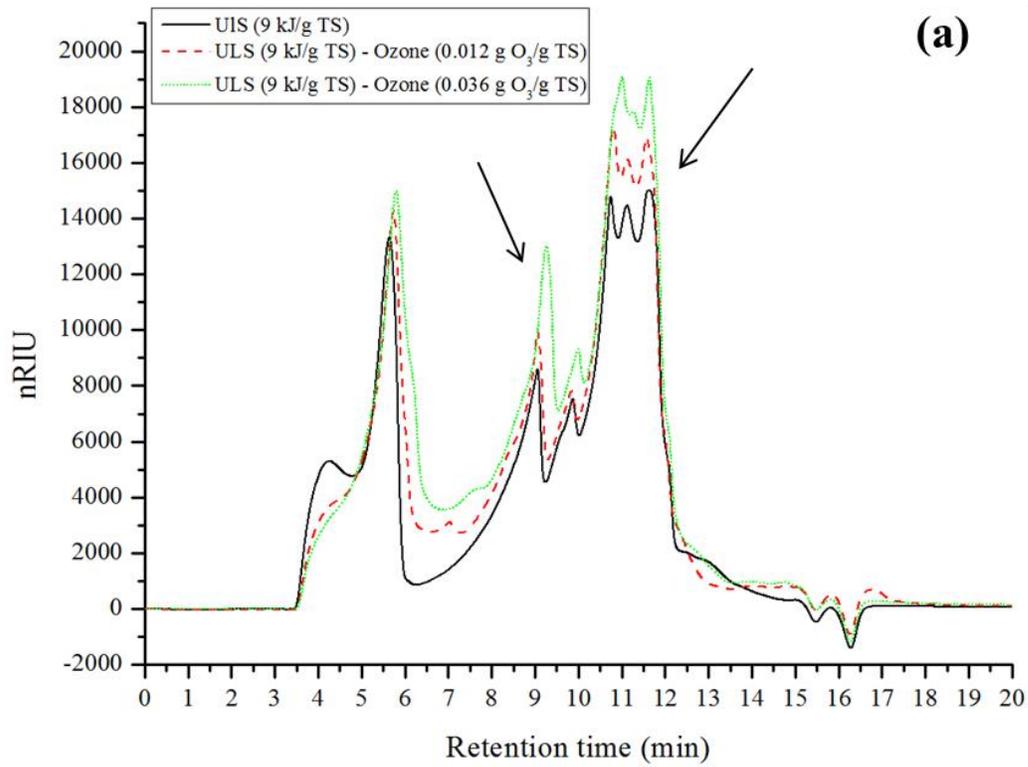
Figure 5 MW distribution of the soluble substances in ultrasonicated sludge (9 kJ/g TS) and ultrasonicated sludge (9 kJ/g TS) with various subsequent ozone dosages (a) RI signal (b) UV signal (254 nm)

The aforementioned advantage of combined pre-treatment could only be observed when ozonation was applied after ultrasonication. It can be seen from Fig. 6a that in the ULS-Ozone sequence ozone further hydrolyzed the organics generated by ultrasound. However, in the Ozone-ULS sequence, ultrasound was not efficient in hydrolyzing the components generated by ozone as shown in Fig. 6b. The subsequent ultrasonication could only increase the concentration of high MW compounds, but was ineffective at generating low MW compounds. This confirmed the ULS-ozone sequence as the more interesting one.
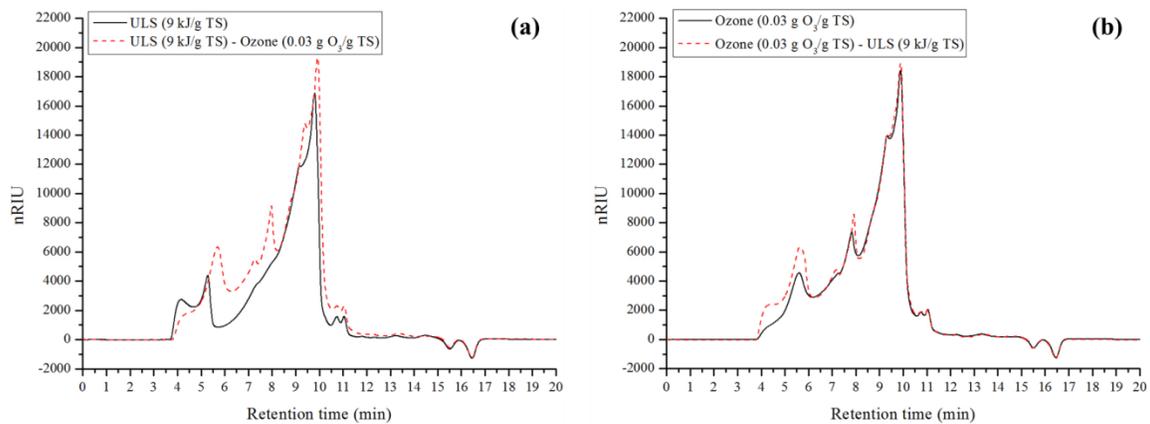
Figure 6 Comparison of the molecular weight distribution chromatograms in RI signal under different combination sequences (a) ULS-Ozone (b) Ozone-ULS

3.3. Biodegradability

The batch anaerobic digestion results of raw sludge, ozonated sludge, ultrasonicated sludge, and ultrasonicated-ozonated (ULS-Ozonated) sludge are shown in Fig. 7. Biodegradability increase was observed for the ultrasonicated sludge in the first four days of anaerobic digestion. However, a lag phase was observed for both ozonated sludge and the ULS-ozonated sludge in the first four days of the anaerobic digestion and the

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biodegradability of the ozonated sludge and the ULS-ozonated sludge were lower than the raw sludge. This was because the oxidized species generated by ozone had inhibited the methanogens as these are known to thrive at very low redox potentials (Weemaes et al., 2000). After day 7, the biodegradability of the ozonated and the ULS-ozonated sludge increased significantly compared to the raw and ultrasonicated sludges. For all the tested samples, the increase in biodegradability became insignificant after 15 days of anaerobic digestion and reached plateau values after 22 days.
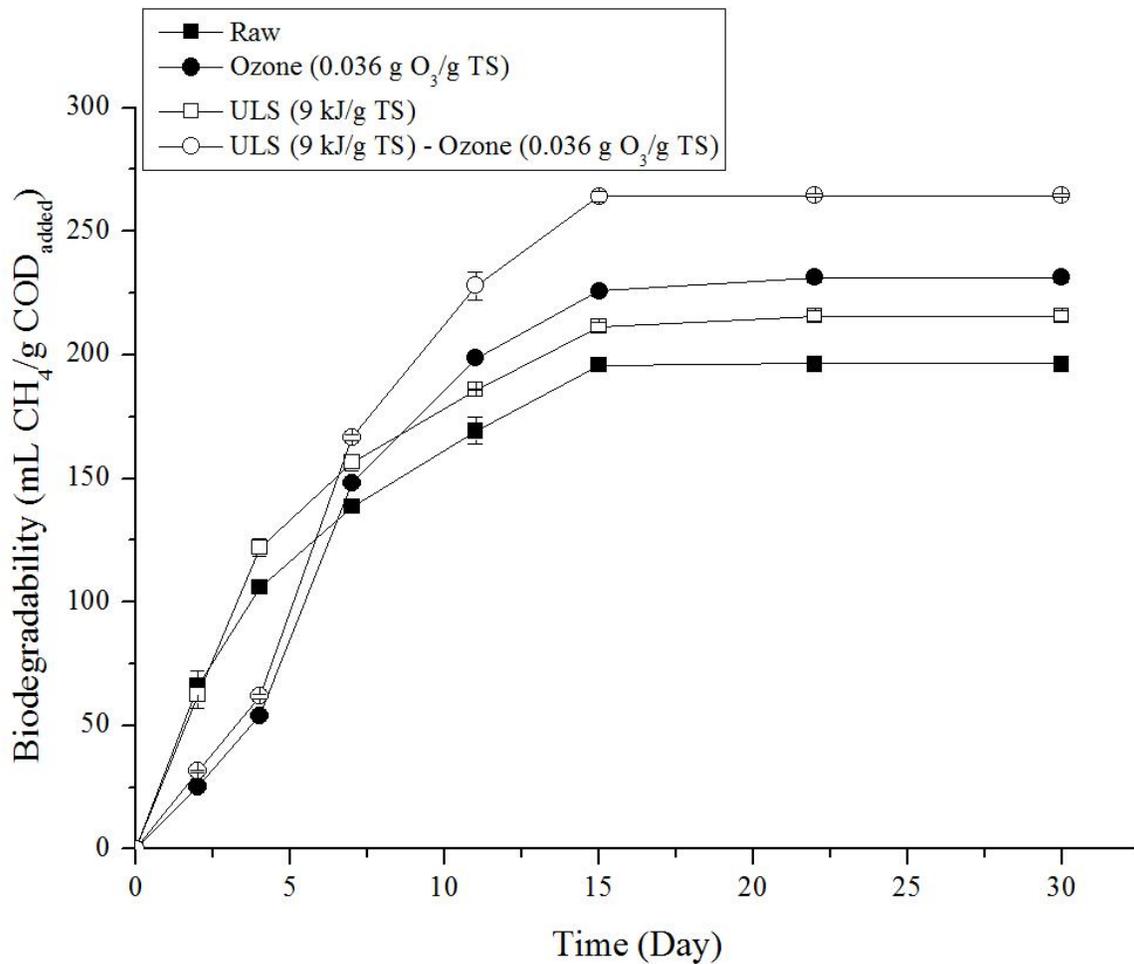
Figure 7 Results of BMP assays for raw sludge, ozonated sludge, ultrasonicated sludge and ULS-ozonated sludge

The ultimate biodegradabilities of all the sludges are shown in Table 2. The combined ULS-ozonation pre-treatment resulted in a biodegradability of 265 mL/g COD_{added} which

was 34.7% more compared to the raw sludge. A previous study had found that sludge biodegradability had improved when ultrasonication and ozonation were applied simultaneously (Xu et al., 2010). However, this work's results showed better increase in biodegradability with the ULS-Ozone sequence. This was 7.0 % more than the sum of biodegradability increase had ultrasonication (9.9%) and ozonation (17.8%) been applied on their own. This synergistic increase in biodegradability had not been reported in previous literatures before. Eskicioglu et al. (2006) indicated that the macromolecules with MW higher than 300 kDa were mostly complex cell fragments and humic acids which were harder to degrade anaerobically than the smaller ones. In this work, the soluble high MW components (MW > 500 kDa) released by ultrasound were effectively degraded by ozone and most of the organic solids were solubilized to low MW components (MW < 27 kDa) which benefited the subsequent anaerobic digestion.

Table 2 Summary of ultimate biodegradability of raw and pre-treated sludge

Sludge	Raw	Ultrasonicated	Ozonated	ULS-Ozonated
		(9 kJ/gTS)	(0.036 g O_3/g TS)	(9kJ/g TS - 0.036 g O_3/g TS)
Biodegradability (mL CH_4/g COD_{added})	196	216	231	265
Biodegradability Increase (%)	N/A	9.9	17.8	34.7

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4. Conclusions

Ultrasound and ozone were found to disrupt the sludge differently and induce synergistic effects when sequentially combined. The ULS-Ozone sequence was shown to be advantageous compared to the Ozone-ULS sequence in terms of increasing soluble COD and reducing size of the solubilized substances. The ULS-Ozone sequence pre-treatment had worked as follows:

- Prior ultrasonication helped disperse the biological flocs and enhance the reaction between ozone and the organic solids.

- The resulting organic solids were then solubilized by ozone in the form of low MW organics (MW < 27 kDa).

- The concentration increase of high MW compounds (MW > 500 kDa) during the ozonation had then also not been significant.

- Ozone utilization by the ultrasonicated sludge was better compared to non ultrasonicated sludge. Some of the high MW compounds solubilized by ultrasound were likely ozone scavengers and hence effectively degraded by ozone.

- Synergistic sludge biodegradability increase was observed due to the aforementioned actions.

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