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Insights into anaerobic transformation of key dissolved organic matters produced by thermal hydrolysis sludge pretreatment

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Abstract

The detailed dissolved organic matters (DOMs) profile by thermal hydrolysis pretreatment and their transformation during anaerobic digestion (AD) were investigated. Among the temperature tested, 172 °C treatment showed the best sludge solubilization and the maximum methane production. The study revealed that high temperature sludge pretreatment mainly improved the release of low molecular weight (LMW) proteins, LMW neutrals and LMW polysaccharides. Notably, the effluent from thermal treated sludge digesters contained more DOMs residues. The predominant residual DOMs were humic substances, LMW proteins and LMW neutrals. At the molecular level, over 50% of the residual LMW components were slowly biodegradable or nonbiodegradable steroid-like compounds and aromatics. Further profiling of the higher MW compounds detected the recalcitrant or inhibitory compounds, e.g. benzenoids, flavonoids, pyridines and their derivatives. It is recommended that

polishing step should be considered to further reduce the refractory residues in AD liquor.

Keywords

Anaerobic digestion; Dissolved organic matters; Refractory compounds; Thermal hydrolysis pretreatment

1. Introduction

Sewage sludge generated from the wastewater treatment plants (WWTPs) has been a big issue due to the limited treatment techniques and strict environmental regulations for sludge disposal (Eshtiaghi et al., 2013). The operation cost for sludge treatment is up to 50% of the total investment in WWTPs (Hii et al., 2014; Kroiss, 2004). To compensate the high cost, anaerobic digestion (AD) has been implemented to stabilize sludge and recover energy (Coelho et al., 2011). As an environmentally friendly method, AD can effectively remove the odor, pathogens and organics, as well as generate biogas energy. However, the rate-limiting step of hydrolysis introduces the inevitable drawbacks of the process, which needs very long sludge retention time (large reactor volume) and thus hinders the application of AD technology (Ruiz-Hernando et al., 2014).

To accelerate the hydrolysis efficiency thus improve the subsequent AD performance, various pretreatment methods prior to AD have been studied, such as thermal hydrolysis, mechanical, chemical and biological pretreatment (Kim et al., 2010; Park and Kim, 2015; Wilson and Novak, 2009). Among them, thermal hydrolysis pretreatment (THP) has been successfully installed at the full scale for more than a decade (Pilli et al., 2014). Generally, THP can be categorized into high temperature hydrolysis pretreatment and low temperature thermal pretreatment. It is reported that AD performance and sludge dewaterability were likely to be enhanced with the elevated high temperature from 100

to 180 °C (Li et al., 2017a). One of the well-known high temperature THP processes is CAMBI, where sludge is heated up to around 170 °C by direct steam injection (Han et al., 2017). It has various inherent merits in sludge treatment, e.g. higher volatile solids (VS) reduction, enhanced biodegradability, improved dewaterability and reduced viscosity (Carrere et al., 2008; Ennouri et al., 2016; Li and Noike, 1992). Temperature above 180 °C is not favorable which may produce more refractory compounds and reduce the sludge biodegradability (Abe et al., 2013; Bougrier et al., 2008; Stuckey and McCarty, 1984). Meanwhile, the low temperature pretreatment (below 100 °C) has also been developed considering the concerns of huge energy consumption (Appels et al., 2010). Certainly, different temperature will have different treatment mechanisms on sludge. It was proposed that solubilization of exo-polymeric substances (EPS) may be dominant under the lower temperature (< 121 °C) while liberation of intracellular components may become prevalent under high temperature (> 121 °C) with the elevated percentages of cells rupture (Ennouri et al., 2016). However, the true mechanisms and the profiles of solubilized organic matters under different temperature have not yet been confirmed. This study selected low, medium and high temperature (70, 121 and 172 °C) thermal treatment methods for comparison.

THP is particularly effective in treating waste activated sludge (WAS), which consists of considerable not easily biodegradable bacterial cells (Barber, 2016). These cells have a sturdy structure of EPS which protects the cells from the hydrolytic enzyme and other attacks from outside (Toreci et al., 2009). The major components of EPS, i.e. proteins (PN), polysaccharides (PS) and humic acid (HA), amount differently in different sludge flocs layers, and EPS in these layers can be characterized as soluble EPS (SB EPS), loosely bound EPS (LB EPS) and tightly bound EPS (TB EPS). Yu et al.

(2008) reported that the location of the dominate EPS components in different EPS fractions will affect their accessibility and biodegradability.

Dissolved organic matters (DOMs) of sludge are directly accessible to bacteria in comparison with particulates. It is known that DOMs generated with different sludge pretreatment methods may have different distributions and fate. However, previous studies mainly focused on either qualitative or semi-quantitative characterization of the overall DOMs in EPS matrix, without a comprehensive analysis of the specific key compounds. Further, the transformation of solubilized DOMs during thermal pretreatment and subsequent AD process is not clear.

Hence, this study aimed to analyze the generation of DOMs with low, medium and high temperature thermal pretreatment, investigate the transformation of these compounds during AD process, and identify the residual compounds after AD process. The solubilization and biodegradability of WAS with different thermal treatments were compared and discussed. Characterization of DOMs was carried out using size exclusion chromatography (SEC) coupled with organic carbon detection and organic nitrogen detection (LC-OCD-OND) (DOMs sub-fractions analysis), Gas Chromatography–Mass Spectrometry (GC-MS) (volatile low molecular weight (LMW) components analysis), and Ultra Performance Liquid Chromatography–Mass Spectrometry (UPLC-MS) (unbiased comprehensive untargeted analysis of the higher MW organics). This study provided fresh insights of thermal pretreatment prior to AD with respect to the fate and transformation of DOMs in sludge treatment process.

2. Materials and Methods

2.1 Sludge source

The WAS from a local wastewater treatment plant in Singapore was collected as the feed sludge. It was concentrated and stored at 4 °C before use. Anaerobic sludge from

the same plant was used as the seed sludge for AD. The properties of sludge samples are presented in Table 1.

2.2 Reagent and chemicals

Acetone, n-hexane, chloroform and dichloromethane (GC-MS grade) were purchased from Merck KGaA (Germany). Isopropanol, acetonitrile, methanol and water (LC-MS grade) were purchased from Sigma-Aldrich (US). Ammonium formate and formic acid were purchased from Sigma-Aldrich (US). The alkane standard mixture of C10 – C40 (50 mg/L) was purchased from Sigma-Aldrich (US). Ultrapure water was obtained from a Milli-Q water process (Millipore Advantage A10, Merck, France).

2.3 Sludge pretreatment

The pretreatment at 70 °C was carried out in a water bath for 30 min. The pretreatment at 121 °C was carried out in a vertical pressure steam sterilizer (HG-50, Hirayama Manufacturing Corporation, Japan) for 30 min. The pretreatment at 172 °C was carried out in a pressure reactor (Model 4534, Parr Instrument Company, US), where the temperature was controlled at 172 ± 3 °C for 30 min. All the pretreated sludge was cooled down to the room temperature before DOMs characterization and use in AD. Triplicates were conducted for each temperature pretreatment and each replicate was characterized separately. The three replicates were then mixed together and used as feed sludge for AD.

2.4 Anaerobic digestion process

An automatic methane potential test system (AMPTS II, Bioprocess Control Company, Sweden) was used to carry out AD experiments. $1.5 \text{ g tCOD}_{\text{feed}}/\text{g VSS}_{\text{seed}}$ was chosen as the optimal organic loading. Each reactor had a working volume of 400 mL and a headspace of 150 mL. All reactors with sludge were purged with nitrogen gas for 10 min and sealed immediately to induce the anaerobic environment. The AD

reactors were all operated at 35 °C for about 30 days with the semi-continuous stirring. Control reactors were conducted with raw sludge as the feed sludge. Blank reactors with only seed sludge and deionized water were prepared for determination of the background biogas generation. Other three types of thermally treated sludge were used as pretreated sludge feed. The net gas production was obtained by subtracting the biogas from the blanks. Four reactors were set-up for each type of feed sludge. Two reactors were sampled at 0, 2nd, 5th, 10th, 16th and 30th day respectively to monitor DOMs. The samples from the other two reactors were collected on the 30th day for the residual DOMs analysis.

2.5 Kinetics analysis

The digestion of DOMs produced from pretreatment was fitted to the First-Order kinetics model using Eq (1) to evaluate the degradation rate constants (k) (Li et al., 2017b).

$$\ln M = -k * t + b \quad (1)$$

where M is the concentration of soluble organics, b is the constant, k is the first-order degradation constant (day⁻¹), and t is the digestion time.

2.6 Analytical methods

2.6.1 Characterization of feed sludge and anaerobic sludge

For the measurement of soluble substances in the raw or treated sludge, the supernatant was collected after centrifuging sludge samples at 10000 rpm for 10 min, then filtered through 0.45 µm nylon membrane filters. The measurements of sCOD, tCOD, TS, VS, TSS and VSS were based on standard methods (APHA, 2005). pH value was determined with a pH meter (Model S220, Mettler-Toledo).

2.6.2 EPS extraction and analysis

The EPS of sludge samples was extracted and collected as SB EPS, LB EPS and TB EPS fractions separately according to the methods reported in Xiao et al. (2017a). Prior to the measurement of soluble organics, the extracted supernatants were filtered through 0.45 μm nylon membrane filters. Soluble PS was determined by phenol-sulfuric acid method (Dubois et al., 1956). Soluble PN and HA were measured by modified Lowry-Folin method (Le et al., 2016). Dissolved organic carbon (DOC) was determined with a total organic carbon (TOC) analyzer (Shimadzu, Japan).

2.6.3 SEC coupled with LC-OCD-OND analysis

SEC associated with LC-OCD-OND system (DOC-LABOR, Karlsruhe, Germany) was carried out to separate the specific groups of major DOMs in terms of their different MW and chemical properties in the pretreated and digested sludge samples. The detailed procedure was reported in Xiao et al. (2016).

2.6.4 GC-MS analysis

Prior to GC-MS, organic compounds of samples were extracted using both solid phase extraction (SPE) and liquid-liquid extraction (LLE) according to the methods reported in Kunacheva et al. (2017). Then samples extracted were analyzed using GC-MS (GCMS-QP2010ULTRA, Shimadzu, Japan) successively. The detailed description of operation and analysis process can be found in Kunacheva et al. (2017). Briefly, samples extract was injected into an RTX-5MS (30 m $\times$ 0.25 mm ID, Restek) column for identification of compounds. Splitless injection was used at 280 $^{\circ}\text{C}$ with carrier gas (helium) at 1 mL/min. The program was designed as follows: 50 $^{\circ}\text{C}$ for 7 min, increase 7 $^{\circ}\text{C}/\text{min}$ to 325 $^{\circ}\text{C}$, hold for 14 min. The MS was operated with the ion source temperature at 230 $^{\circ}\text{C}$, and mass spectra was acquired from m/z 30 to 580. The chromatographic peaks were identified by analysis of the mass spectrum, retention time

(RT) and compared with the NIST11 library (National Institute of Standards and Technology, Gaithersburg, MD, USA, <http://www.nist.gov/srd/mslist.htm>). Each compound was semi-quantified based on the alkane with the closest RT.

2.6.5 UPLC-MS analysis

Since GC-MS analysis could only work on relatively volatile and thermo-stable compounds ($MW < 580$ Da) effectively, untargeted analysis of sludge samples using UPLC-MS approach was conducted to profile and identify more compounds with higher MW (< 2 kDa).

First, the filtered samples were lyophilized, from which the fractions of polar metabolites and non-polar lipids were extracted following the steps in Tiphara et al. (2017). Then the extracted metabolites and lipids were separately injected into ACQUITY UPLC (Waters, USA) coupled with a Xevo G2-XS QToF mass spectrometer (Waters, U.K.) with an electrospray ionization (ESI) source. The detailed description and analysis of UPLC-MS operation were reported in Tiphara et al. (2017). During data processing, the software of EZinfo (version 3.0.3.0, Umetrics) was used for multivariate analysis of compounds in raw WAS, 172 °C thermal pretreated sludge, digested sludge samples with and without 172 °C pretreatment respectively. The most significant compounds were selected by orthogonal projection to latent structures discriminant analysis (OPLS-DA) and then exported to the software of Progenesis QI for further identification and analysis. The selected biomolecules were identified based on (1) HMDB (<http://www.hmdb.ca/>), LIPID MAPS (<http://www.lipidmaps.org/>), ChEBI (<https://www.ebi.ac.uk/chebi/>) and MetaScope by searching compounds against structural databases (SDFs) for *E. coli* (<http://ecmdb.ca/>) and (2) Chempider by searching compounds against the KEGG database (<http://www.genome.jp/kegg/>).

2.7 Statistical analysis

The significance of DOMs composition difference was evaluated by analysis of variance (ANOVA) and $p < 0.05$ was considered to be statistically significant. The software of SPSS version 19.0 was used for the statistical analysis.

3. Results and Discussion

3.1 Effects of thermal pretreatment on sludge content

3.1.1 Effects of thermal pretreatment on EPS distribution and compositions

The changes of fractionized EPS, PN, PS and HA with different temperature thermal treatments are displayed in Figure 1. Clearly, all the thermal pretreatment contributed to the concentration increase of total DOC. The maximum concentration of 266.7 ± 8.5 mg DOC/g VS was obtained with the temperature of 172 °C, which was more than doubled amount compared to the other pretreatment. In addition, the EPS compositions were significant different ($p = 0.019 < 0.05$) in 172 °C pretreated sludge compared to raw sludge, whereas no significant difference was found among the lower temperature (70, 121 °C) pretreated sludge and raw sludge (Figure 1), indicating the different mechanisms occurred during DOMs solubilization.

From Figure 1a, the majority of organics were locked up in TB EPS for raw sludge. After thermal pretreatment, the concentration of organic compounds increased in SB EPS and decreased in TB EPS, which implies the solubilization of particulates and shift of these substances from inner layers of EPS to outside layers. PN, PS and HA are the main components in EPS of WAS (Wilén et al., 2003). The changes of these compounds in respective EPS layers show the similar trend with thermal pretreatment. The concentration of PN, PS and HA increased greatly in SB EPS and LB EPS, while they decreased in TB EPS fraction. The results confirm that pretreatment can improve the accessibility of these organics to anaerobic microorganisms during AD.

Among the three temperature treatments, the highest concentrations of PN (386.7 ± 11.0 mg/g VS), PS (234.5 ± 5.8 mg/g VS) and HA (258.0 ± 4.4 mg/g VS) were obtained under the pretreatment temperature of 172 °C (Figure 1). The released soluble PN was more than the soluble PS, and this is reasonable that more than half of the organic substances in microorganisms is PN (Li and Noike, 1992). In addition, the net production difference of PS between 172 and 121 °C pretreatment was far less than that between 121 and 70 °C pretreatment (38.2 v.s. 159.4 mg PS/g VS), implying that temperature higher than 121 °C may not necessarily help on the extracellular PS solubilization. The minor increase of PS content with high temperature may be also related to the Maillard reaction where some released sugars reacted with amino compounds under high temperature (> 150 °C) (Dwyer et al., 2008). The concentration of HA increased significantly after thermal pretreatment, which may have adverse effects on the downstream process. Azman et al. (2015) also reported that part of cellulose in the cell walls could be converted to humic substances during the thermal treatment process. Overall, PN was remarkably increased with high temperature pretreatment (172 °C), followed by PS and HA.

3.1.2 Effects of thermal pretreatment on released DOMs compositions

More specific fractions of DOMs were investigated and the results are shown in Figure 2. According to the different functional groups properties and MW, the observed DOMs were mainly classified into hydrophobic dissolved organic carbon (HO DOC), biopolymers (> 20 kDa, including high molecular weight (HMW) PS, HMW PN and amino-sugars), humic substances (HS), building blocks (300 - 500 Da, breakup products of HS), LMW neutrals (< 350 Da, including aldehydes, alcohols, mono-oligosaccharides and ketones) and LMW acids (< 350 Da). HO DOC was determined by deducting hydrophilic dissolved organic carbon (HI DOC) from the total DOC (Xiao et al., 2017b).

From Figure 2, most of the fractions (except HMW PN, LMW neutrals and LMW acids) had a marked increment with the ascending temperature. LMW PN, LMW neutrals and LMW PS composed the majority of the observed DOMs after 172 °C treatment. This may be attributed to the disruption of chemical bonds of HMW substances at high temperature. Interestingly, in contrast to the fact that there is higher PN content in biomass (Li and Noike, 1992), the concentration of HMW PS was higher than HMW PN after 172 °C treatment.

The distribution of HMW PN was highly different when the treatment temperature was different. The concentration of HMW PN was the highest with 121 °C treatment compared to the other temperature pretreatment. Correspondingly, there was a great deal of denatured HMW PN with double binds and benzene ring structures detected in biopolymers. It has been reported that protein size would be reduced when the temperature was higher than 150 °C (Wilson and Novak, 2009). Thus, in this study, much more LMW PN other than HMW PN were detected at 172 °C. Further, at the high temperature, the Maillard reaction of amino acids with reducing sugars could produce HMW heterogeneous polymers (> 10 kDa), which contributed to the significant amount of HS (Bougrier et al., 2008; Dwyer et al., 2008). Therefore, it should be noted that the concentrations of both HS and building blocks increased greatly at the higher temperature. These compounds are able to attach to the active groups of enzymes, leading to the inhibition on AD (Azman et al., 2017; Fernandes et al., 2014).

3.2 Effects of thermal pretreatment on methane production in anaerobic digestion

Figure 3 shows the measured methane production from control and THP sludge anaerobic digesters. The biogas production increased rapidly at the beginning and gradually stabilized, which in turn indicated the utilization of available biodegradable organics. It is clear that all the experimental reactors had better methane production

performance with thermal treated sludge as feed. Such performance can be explained by the improved sludge disintegration and solubilization mentioned in Section 3.1. The best performance of methane production was obtained with 172 °C pretreated sludge (212.1 ± 8.2 mL CH₄/g tCOD_{feed}), which is significantly higher compared to other thermal pretreated sludge. In this study, 70 °C and 121 °C pretreated sludge digesters (referred as 70 °C digesters and 121 °C digesters hereafter) achieved the similar methane production (178.8 ± 7.5 mL CH₄/g tCOD_{feed}), with an increase of 25.6% compared to control digesters. Additionally, 172 °C pretreated sludge reactors (referred as 172 °C digesters hereafter) produced 142.4 ± 5.3 mL methane per g tCOD_{feed} on 5th day while untreated sludge reactors took 30 days to achieve the same amount, implying thermal pretreatment could remarkably reduce solid retention time (SRT) in AD.

3.3 DOMs transformation during anaerobic digestion

As 172 °C treatment provided the best performance in terms of sludge solubilization and biogas production, samples collected from 172 °C digesters were analyzed to investigate the transformation of key DOMs during AD. In order to compare, samples from control reactors were also analyzed. In the control digesters, the removal rate of organics was limited by the availability of the readily biodegradable organic matters, leading to the minor reduction of the monitored soluble substances (Figure 4). The concentration of LMW neutrals even increased at the early stage of digestion due to the degradation of other HMW substances, like HMW PN and HMW PS. The remaining organics may consist of insoluble particulates which are not accessible to bacteria or some soluble nonbiodegradable substances.

On the other hand, thermal treatment released abundant readily usable carbon sources which can be digested directly. In the early stage of 172 °C AD, almost all such components were consumed rapidly, especially the easily biodegradable substances like

LMW PN, LMW PS and LMW neutrals (Figure 4). Interestingly, LMW PN and LMW PS were the major organic matters transformed during AD (consumed by 727.5 ± 10.6 and 239.4 ± 6.8 mg/L $-C$ respectively), suggesting that they were more associated with the total methane production than other DOMs fractions. According to the concentration profile, the depletion of DOMs was fitted to the first-order kinetics (Li et al., 2017b). Statistical indicators (R^2) were also considered to evaluate the soundness of the model (Table 2). The degradation rate constant of LMW PS was higher than that of LMW PN (0.337 v.s. 0.088 day $^{-1}$), implying the faster utilization of LMW PS.

A rapid reduction of LMW neutrals ($k = 0.081$ day $^{-1}$) was also observed, since the smaller molecules are thought to be less resistance for contacting bacteria. HS was partially degraded within the first 2 days, and decreased slightly onwards. It suggests most of the humic-like substances, mainly associated with the inherent aromaticity and complexity of the polycyclic components, were hardly biodegradable. The HMW DOMs, including HMW PN and HMW PS, only changed slightly during digestion, which suggests that HMW DOMs released by the higher temperature treatment (172 °C) were more recalcitrant to digestion. Li et al. (2016) concluded that the rate-limiting step for HMW PN degradation was the rupture of aromatic moieties other than the breakup of peptide bonds.

3.4 Chemical compositions and characterization of residual DOMs after AD

At this stage, the study found out that the thermal pretreatment released both easily biodegradable DOMs and some relatively recalcitrant substances. Thus, it is reasonable that the concentration of residual DOMs in thermal pretreated AD was higher than that in control anaerobic digesters (Figure 4). For instance, the residual DOMs in 172 °C digesters was 3-fold more than that in control digesters. Besides, the distribution of

residual DOMs was distinctly different with different temperature pretreatment (Figure 5).

In control digesters, the residual DOMs were dominated by HS, LMW PN and HO DOC, accounting for 24.4%, 19.2% and 15.6% of total residues, respectively. Part of HO DOC was reported to belong to hydrophobic HS (Penru et al., 2013). Thus, these three types of residuals were refractory to digestion and stayed in the effluent. With the higher temperature (172 °C) sludge pretreatment, almost all the residual components were more compared to that in control and other temperature digesters. Thus, the discussion on residues will mainly focus on 172 °C digesters below.

It was found that HO DOC, HS, LMW PN and LMW neutrals constituted the majority of the residues in 172 °C digesters (Figure 4). The concentration of residual HO DOC from 172 °C digesters was 3.9 times higher than other digesters. The remaining HO DOC may contain lots of complex Maillard products, which were nonbiodegradable due to their heterogeneous polycyclic structures (Dwyer et al., 2008). Residual HS-like matters (including HS and building blocks) were almost three folds higher in 172 °C digesters compared to control, due to the higher production under the higher temperature pretreatment (Figure 2) and their nonbiodegradability. The significant amount of LMW PN residues was also observed, which may contain the breakup products of aromatic PN, e.g. tryptophan-like PN.

Generally, LMW substances were more likely to be degraded by microorganisms owing to low mass transfer resistance (Li et al., 2016). However, this study reveals large amounts of LMW PN, LMW PS and LMW neutrals, which should be consumed and transformed to biogas, were still present at the end of AD process. Therefore, it would be interesting to know the compositions of those LMW compounds. In this study, LMW (< 580 Da) specific compounds in digested sludge samples were identified and

semi-quantified by GC-MS. The results imply that some released components by thermal hydrolysis treatment were removed in AD and new compounds were also produced during AD with 70 identified compounds reduced to 60 compounds.

The identified LMW DOMs were classified into alkanes, esters, acids (compounds with carboxyl groups), aromatics (mostly compounds with benzene rings), steroid-like matters, N-compounds (nitrogenated compounds, mostly amino acids and amide compounds) and others (alcohols, aldehydes and others) (Figure 6). It should be noted that alkane with the closest RT was used as standards to quantify the LMW organic matters. As the retention index is different for standards and actual compounds, the concentration derived is only an indication of the abundance of each compound, while the actual concentration of identified compound may be varied.

The concentrations of residual aromatics and N-compounds increased with the elevated treatment temperature and it can be concluded that 172 °C sludge pretreatment released the highest amount of both compounds (Figure 6). The aromatic organics, probably the breakup products of tryptophan-like PN, HS-like matters and other substances, increased by a factor of 7.1 compared to other lower temperature pretreatment. N-compounds were likely from the breakup products of PN and other intracellular N-containing substances. The results suggest that 172 °C could certainly disrupt the bacterial cells as well as the bio-macromolecules, achieving the best performance of hydrolysis. But the high concentration of soluble N, ammonia in particular, may lead to inhibition on anaerobic processes (Barber, 2016).

The LMW residues after digestion in all thermal treated sludge AD reactors were higher than those in control reactors, which may be associated with the higher release of DOMs during pretreatment. The highest amount of steroid-like matters residues was found in the 70 °C digesters other than 172 °C digesters. That is reasonable that

steroid-like matters are likely to decompose under the higher temperature (evidenced by Figure 6a) thus were lower in 172 °C reactors. With the elevation of pretreatment temperature from 70 °C to 172 °C, the percentage of residual steroid-like matters decreased from 54.7% to 26.9%.

For control reactors, the majority of the residual LMW DOMs were steroid-like matters (29.2%) and alkanes (23.7%) (Figure 6b). However, in thermal pretreated AD reactors, the compounds of steroid-like matters (26.9%) and aromatics (22.9%) dominated the residues. Minor steroid-like matters were consumed during AD, indicating such polycyclic compounds produced by pretreatment were relatively nonbiodegradable. The steroid-like matters, e.g. cholesterol and coprostanol observed in this study, are reported to be the degradation products of cholesterol by the mediation of anaerobic bacteria during digestion (Jarde et al., 2005; Leeming et al., 1996). Alkanes were considered as slowly biodegradable compounds as they require a longer retention time for digestion under both aerobic and anaerobic conditions (Rojo, 2009). Aromatics may also require a longer retention time. For example, the intermediate products phthalates (94% -similarity index) were found to reduce in 172 °C digesters, indicating that phthalates can be gradually but slowly degraded. In addition, compounds like squalene (87% -similarity index) and erucyl amide (86%) were observed only in digested samples while absent from both raw WAS and treated sludge samples. It is likely that these compounds were produced as by-products of substrate utilization by microorganisms.

UPLC-MS was employed to further investigate the higher MW (< 2 kDa) compounds before and after 172 °C pretreatment and subsequent AD. By the relative abundance profiles, the significant DOMs before and after 172 °C sludge pretreatment, as well as the key DOMs from control digesters and 172 °C digesters were putatively identified.

The results demonstrate that thermal pretreatment mainly assisted in the release of amino acids, dipeptides, fatty acids, purine nucleotides, pyrimidine bases, benzenoids, sugars, glycerophospholipids, steroids and steroid derivatives, which were primarily the breakup products of proteins, polysaccharides, nucleotides and other substances from extracellular, cytoplasm, lysosome, endoplasmic reticulum, nucleus and cell membrane, respectively. Besides, the presence of nucleotides, such as pyrimidine bases, pyrimidine nucleosides and purine nucleosides confirms that the extent of damage by high temperature pretreatment (172 °C) reached the microbial cell nuclei.

Lots of residual DOMs were detected in the digested samples, including polar metabolites (benzenoids, peptides, long chain fatty acids (LCFA), pyrrolidines, pyridines and their derivatives) and lipids (flavonoids, steroids and their derivatives). It is known that organics like benzenoids, pyrrolidines, pyridines and their derivatives were more recalcitrant or even inhibitory to cell growth and methanogenesis (Fernandez et al., 2017; Pham et al., 2013). 2-acetylpyrrolidine (classified as pyrrolidines) was observed in both thermal treated and digested sludge. This reveals high temperature pretreatment will produce nonbiodegradable compounds. LCFA (mainly metabolites of lipids) was also considered as an inhibitor by adsorption onto cell walls, of which the digestion is possible but needs longer retention time (Salminen and Rintala, 2002).

It is noteworthy that the majority of the residues contained monocyclic or polycyclic structures, implying they were relatively recalcitrant. For instance, most of the residual amino acids and peptides were confirmed to be the breakup products of aromatic PN, like tyrosine and tryptophan over other aliphatic PN. A considerable abundance of thiophene, which is the product of Maillard reaction, was found in 172 °C digesters. Flavonoids compounds, which are supposed to be refractory with multiple complex benzene rings and double bonds, were also present in the thermal AD effluent. This

study found out that some of the residual organic compounds that were produced during thermal treatment or AD process can be regarded as environmentally hazardous. Further polishing steps should be considered before final disposal.

4. Conclusions

This study provided novel insights into the transformation of the key DOMs during THP and AD process. Their impacts on biogas generation were also investigated. Regardless of the improved sludge biodegradability, DOMs (e.g. HO DOC, HS, LMW PN and LMW neutrals) remained more in the effluent of thermal pretreated digesters. At the molecular level, steroid-like matters and aromatics were proved to dominate the LMW DOMs residues. Further study of the higher MW compounds detected the refractory or inhibitory compounds. Overall, THP WAS prior to AD improved the performance with a tradeoff of higher residual DOMs, which requires further consideration.

Supplementary material

E-supplementary data for this work can be found in online version of the paper.

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Figure Captions

Figure 1. Effects of different pretreatment temperature on the concentration of DOC (a), PN (b), PS (c) and HA (d).

Figure 2. Quantification of DOMs (mg/L -C) after different thermal pretreatment.

Figure 3. Methane production in the different AD reactors fed with control and thermal pretreated sludge.

Figure 4. Transformation of sub-fractions (mg/L -C) of observed organics in control and 172 °C digesters during AD.

Figure 5. Distribution of residual DOMs (mg/L -C) sub-fractions from control and 172 °C digesters.

Figure 6. (a) Semi-quantification of LMW DOMs (< 580 Da) after 172 °C temperature pretreatment and control. (b) Distribution of residual LMW DOMs (< 580 Da) from control and 172 °C digesters.

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Tables Captions

Table 1. Characteristics of raw sludge and seed sludge.

Table 2. Degradation kinetics of DOMs during AD using First-Order kinetics.

Table 1. Characteristics of raw sludge and seed sludge.

	Units	Seed sludge	Raw sludge
Total solids (TS)	g/L	15.02 ± 0.25	13.13 ± 0.15
Volatile solids (VS)	g/L	11.24 ± 0.13	10.65 ± 0.21
Total suspended solids (TSS)	g/L	14.12 ± 0.20	12.27 ± 0.12
Volatile suspended solids (VSS)	g/L	10.60 ± 0.21	10.21 ± 0.16
Total chemical oxygen demand (tCOD)	g/L	15.17 ± 0.23	14.37 ± 0.29
Soluble chemical oxygen demand (sCOD)	g/L	0.26 ± 0.11	0.24 ± 0.10
Protein (PN)	mg/L	96.24 ± 2.47	89.91 ± 3.43
Polysaccharide (PS)	mg/L	54.43 ± 1.12	45.06 ± 1.75
pH	1	7.44 ± 0.02	6.46 ± 0.02

Table 2. Degradation kinetics of DOMs during AD using First-Order kinetics.

	k (day ⁻¹)	b	R ²
LMW PN	0.088 ± 0.021	6.527 ± 0.097	0.887
LMW PS	0.337 ± 0.060	5.518 ± 0.076	0.956
LMW Neutrals	0.081 ± 0.017	5.653 ± 0.086	0.906

Highlights

- High temperature sludge treatment mainly improved the release of LMW matters
- LMW DOMs after THP were closely correlated with biogas generation
- The residual DOMs after AD were mainly HS, LMW PN and LMW neutrals
- Steroids and aromatics were dominant in residual LMW refractory compounds