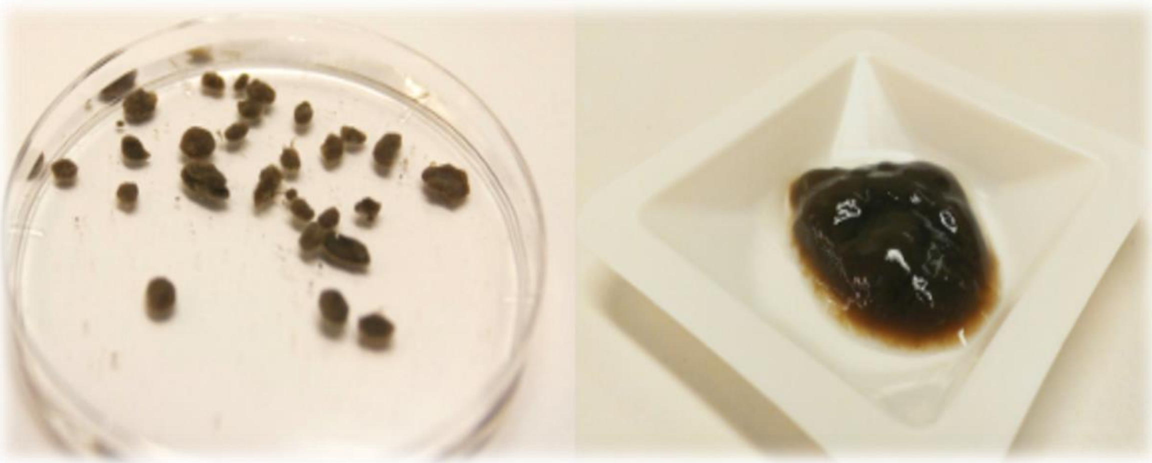
Variation of Chemical and Structural properties of Waste Aerobic Granular Sludge(WAGS) and Flocculent Sludge in Anaerobic Digestion

Additional Master Thesis



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Abstract

A comparative batch experiment as well as a biochemical methane potential(BMP) test were conducted over 44d for aerobic granular sludge (WAGS) and waste flocculent activated sludge(WAS) on sludge anaerobic digestion (AD), to give an insight into the chemical and mechanical properties change of the EPS in different sludge source during AD process, via the analysis of polysaccharides (PS), protein (PN), loosely bound extracellular polymeric substance (LB-EPS), tightly bound extracellular polymeric substance (TB-EPS) change and its gel-forming property with multi-valent ions. The EPS, who play a vital role in forming the compact gel-like sludge structure, was extracted from WAGS and AS and analyzed for several test.

During AD process, WAGS demonstrated a lower hydrolysis rate than AS over the first 6 days and reached a comparable overall methane production potential afterwards, suggesting the necessity of pre-treatment of WAGS to enhance sludge hydrolysis. The isolated SEP was slowly degraded over the AD process with a biodegradability of 31% for WAGS and 39% for WAS and this degradation of EPS mainly contributed from the PS and PN it contained. This EPS degradation led to the loss of gel-like properties for both sludge after AD process. Although the results from fourier transformed infrared (FT-IR) spectra exhibited the similar typical brands within the range of 1000~3000 cm^{-1} in all samples, the second-derivative spectra manifested the occurrence of polysaccharides degradation, especially for homopolymannuronic acid blocks (MM) and homopolyguluronic acid blocks during sludge digestion. Meanwhile, the dewaterability of both sludge was observed to deteriorate with AD ongoing due to the internal transmission from the compact TB-EPS to porous LB-EPS as well as the increase in PN/PS ratio in EPS. Moreover, the mechanical property variation of different sludge was examined by the crosslink of EPS solution with ion to form hydro-bead. Reduction on mechanical strength of EPS formed hydro-beads was seen for both WAGS and WAS but mechanical structure loss of WAGS was less than that of WAS after digestion which can be attributed to higher EPS residues in WAGS. However, no matter for WAGS or WAS, only a limited amount of EPS was degraded during AD process and this provided a possibility of recovery EPS as a coating material from digested sludge.

Key words

waste aerobic granular sludge(WAGS), sludge anaerobic digestion(AD), waste flocculent activated sludge (WAS), extracellular polymeric substance, gel-like structure, gel-forming property

Contents

1. Introduction

Since the world wide application of the conventional activated sludge method for municipal and industrial wastewater treatment, the large amount of excessive sludge generated made it an another problem for completing wastewater treatment. As a consequence, aerobic granular sludge process with aggregation state of sludge and high sedimentation ability is regarded as an emerging and promising biotechnology, having generated great research interest. A treatment technology named Nereda® developed on specific characteristics of aerobic granular sludge, provided with significant lower energy and chemicals, compactness and favorable capital and operational costs, has been verified to a be a mature aerobic granular sludge technology applied at full scSEPS (Giesen, De Bruin, Niermans, & Van Der Roest, n.d.). Although Nereda® system can be considered as a future standard with low excessive sludge production for sewage treatment, there still lack of researches focusing on treatment of aerobic granular sludge.

In order to fulfill the requirements set by the Directive 91/271/EEC for Urban Waste Water Treatment(The Council of the European Communities, 1991) to prevent adverse effects of urban and industrial wastewater discharges, wastewater treatment plants were widely established with huge production of the excessive activated sludge remaining to be treated. Although there are alternative disposal methods for sludge treatment, anaerobic digestion is regarded to be the most popular choice in modern plants with respect to its merits, such as small footprint, possibility of energy recovery from biogas, reducing amount of sludge solids for final disposal as well as killing pathogenic elements in the sludge.(Appels, Baeyens, Degrève, & Dewil, 2008). Except for stabilization for the excessive activated sludge, anaerobic sludge treatment is also verified for treating aerobic sludge in granular state according to the experiments conducted by Ángeles Val Del Río. In the research he found that the anaerobic biodegradability and solids reduction ratio of the raw granular aerobic sludge by anaerobic treatment were similar to those normally reported for waste activated sludge, confirming the feasibility of applying anaerobic treatment as a qualified method for WAGS disposal.(Val Del Río et al., 2014). Although the aggregation of the biomass into the granules seems not to limit the anaerobic treatment, the different morphology of the aggregated granules and open floc structures is predicted to have an impact on the hydrolysis rate in the early stage of the anaerobic treatment. However, studies focus on this part are quite rare to see.

Extracellular polymeric substances(EPS) as a component of sludge secreted by microorganisms, has been associated with physical and chemical contributions to the structural strength and coherence for both activated sludge(AS) and aerobic granular sludge(GAS)(Sam & Dulekgurgen, 2016). EPS are a complex high-molecular-weight mixture of polymers with multifunction such as adhesion, structure and is hypothesized to be responsible for morphology discrepancy between flocculated sludge and granule sludge (Salama et al., 2016). However, even EPS was confirmed to be closely related to sludge morphology, not all EPS functions as structure support material. Among them the polymers that are able to form hydrogels and contribute to the formation of dense structure, as a subset of total EPS, are denoted as structural EPS, which also mentioned as alginate-like exopolysaccharides(SEPS) in some researches (Felz, Al-Zuhairy, Aarstad, van Loosdrecht, & Lin, 2016). Yumei Lin found in her study that alginate-like exopolysaccharides(SEPS) is one the dominant exopolysaccharides in aerobic granular sludge and is suggested to highly involved in WAGS's highly hydrophobic, compact, strong and elastic structure(Y. Lin, de Kreuk, van Loosdrecht, & Adin, 2010). In addition, EPS, as a biodegradable organic mixture consisting of protein (PN), polysaccharides (PS) and little lipids and humic acids, is predicted to be weakened on both contents and chemical structure along with the gradually disintegration of aggregated sludge structure during anaerobic digestion(Salama et al., 2016). Further investigation on the variation of structural EPS is necessary in terms of better understanding sludge deconstruction phenomenon over AD process. However, no study found has assessed the relation between the structural EPS (or SEPS) degradation and WAGS structure and characteristics changes during anaerobic treatment.

In addition to structure variation, evidence also existed that anaerobic digestion had negative impact on sludge dewaterability owing to the stratified EPS changes(Ye, Liu, & Li, 2014). The bounded EPS (insoluble) possesses a dynamic doubled-layer structure and can be stratified into loosely-bound EPS and tightly-bound EPS fractions with regard to the extraction methodology (Yu, He, & Shao, 2009). The observation that sludge after LB-EPS extraction demonstrated the strongest gel-like structure, and the direct experimental evidence that both sludge residue after LB-EPS extraction and TB-EPS exhibited a stronger and more compact structure suggested that TB-EPS played a significant role in gel-like property(Wang, Liu, & Tay, 2005; Yuan, Wang, & Feng, 2014). TB-EPS manifested a stronger flocculating ability comparing to other EPS fractions and was capable for aggregation via the flocculation mechanism of bridging and sweeping(Yu et al., 2009). Since the structural EPS is foreseen to be degraded and deconstructed during anaerobic digestion while dewaterability is an essential parameter to evaluate the quality the quality of treated sludge before disposal, it is of great importance to investigate how different stratified structural EPS influence dewaterability during AD process, especially when the dewaterability change of WAGS during anaerobic digestion still remains unknown so far.

The purpose of this study is to investigate 1) biogas production potential and 2) the degradation changes of structural EPS(SEPS) extracted from WAGS and AS during anaerobic digestion as well as 3) the dewaterability changes of WAGS and AS throughout the whole process. In order to fulfill the goals, kinetics was reveSEPSd by BMP test while dynamic changes of structural EPS were examined by FT-IR, 3D-EEM spectroscopy and mechanical strength test. Capillary suction time (CST) was utilized to reveal dewaterability change of WAGS and AS during anaerobic digestion.

2. Materials and Methods

2.1 Experiment material

In this study, waste activated sludge(WAS)was taken from secondary settling tank in the sewage treatment plant Harnaschpolder (Den Hoorn, Netherlands) as well as aerobic granular sludge from a Nereda® pilot plant (Utrecht, Netherlands) were used as substrates while anaerobic digested sludge collected from digestion tank in Harnaschpolder was applied as inoculum. For WAS, the median size of the selected particles is supposed to below 0.2mm while for WAGS, a screen was used to collect granules with average size above 2.0mm. To make results comparable, the collected WAS and WAGS were both pre-centrifuged to 45±2 gVS/d(volatile solids). The VS of the anaerobic inoculum was 23g/L.

2.2 Experiment set-up

The anaerobic digestion(AD) was conducted together with the bio methane potential (BMP) at the same time. Sludge anaerobic digestion was conducted in duplicate with substrate of WAS and WAGS, respectively. The VS(g) of the inoculated sludge was twice that of the sample sludge to reach an initial total volume of 400ml, placed in a 500ml glass bottle under 35℃ in the shaker. Sludge samples, 30ml each time, were collected periodically from the bottle for consequent SEPS extraction and analysis and the total experiment cycle lasted for 44d. Sampling time interval became longer than the beginning since the reaction rates was predicted to be slower along time. BMP test was performed in triplicate with WAS and WAGS as substrate separately in an Automatic Methane Potential Test System II (AMPTS II , Bioprocess Control, Sweden AB) with the same sample consistent as AD process.

2.3 Analytical methods

Samples collected from AD process is dried under 105℃ for 8h for total solids(TS) test and consequently under 550℃ for 2h to get volatile solids(VS). Total suspended solids(TSS) and volatile suspended solids(VSS) were obtained by filtrating sludge through the 0.45μm glass fiber filter followed by the same steps applied for TS and VS.

Figure 1. Filtrated WAS and WAGS samples for TSS and VSS test

EPS and SEPS extraction were conducted according to the methods proposed by Simon Felz in report(Felz et al., 2016). Protein (PN) in EPS and SEPS was quantified by modified Lowry methods measured under 750nm while polysaccharides(PS) was analyzed by Phenol sulphuric acid method under 490nm adsorption(Fr/olund, Griebe, & Nielsen, 1995; Dubois et al., 1956). Capillary suction time(CST) was obtained by adding a volume of 6.4ml sludge to the Type 304M Capillary Suction Timer . The FT-IR and 3D-EEM spectrum of the structural EPS were tested by utilizing an infrared and a fluorescence spectrometer, respectively. As for the mechanical analysis of SEPS's mechanical property, hydrogels were formed by injecting the 5% (weight/volume) SEPS solution diluted by di-deionized water into a $1cm^3$ cylinder module with dialysis membrane and submerging in the 5% $CaCl_2$ solutions for 24h. The mechanical strength of the formed hydrogels were then tested by a dynamic mechanical analyzer. Duplicates were made for each aforementioned measurements.

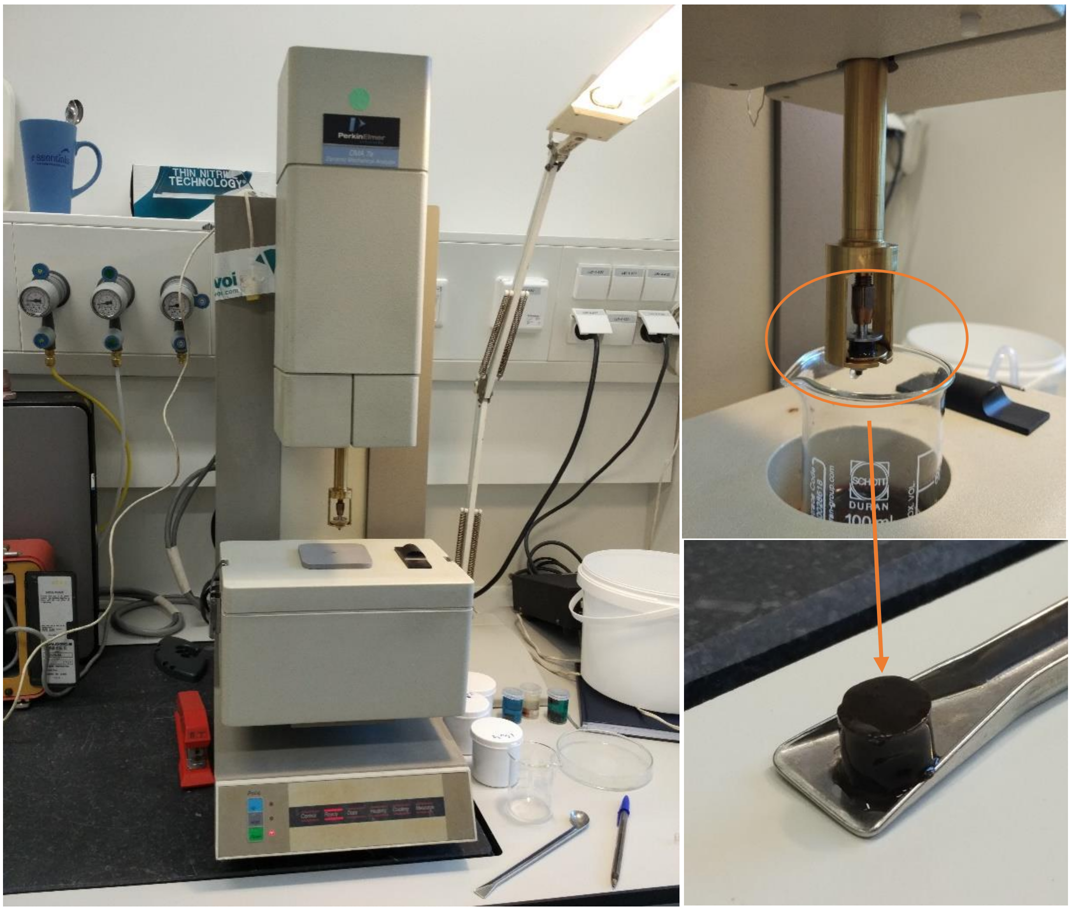

Figure 2. Dynamic mechanical analyzer and the hydrogel formed by cross-link with Ca^{2+}.

3. Results and Discussions

3.1 Biochemical methane production potential of WAGS and WAS

The cumulative biochemical methane potential throughout the 44d is shown as Figure 3. According to the curves, the accumulated methane production amount of WAS (239±8 Nml/gVS substrate) and WAGS(242±8 Nml/gVS substrate) turned out to be the same after 27d while in the first 6d, WAGS showed an obvious slower biogas production rate than WAS. According to the 4 phases of anaerobic digestion, the first 12 days were estimated to fall under the rubric of hydrolysis, indicating a relative slow hydrolysis rate of WAGS. Regarding to the distinct morphology between WAS and WAGS, the possible reason was attributed to the aggregated form of WAGS and the corresponding small specific surface area. The aggregated large granules took more time than the flocs to be penetrated and deconstruct which could be attributed to its relative more compact structure. Since hydrolysis is the speed-limit-step for WAGS digestion, therefore, sludge disintegration such as crush can be considered as a pre-treatment approach to speed up hydrolysis rate and further elevate the reaction rate of AD process.

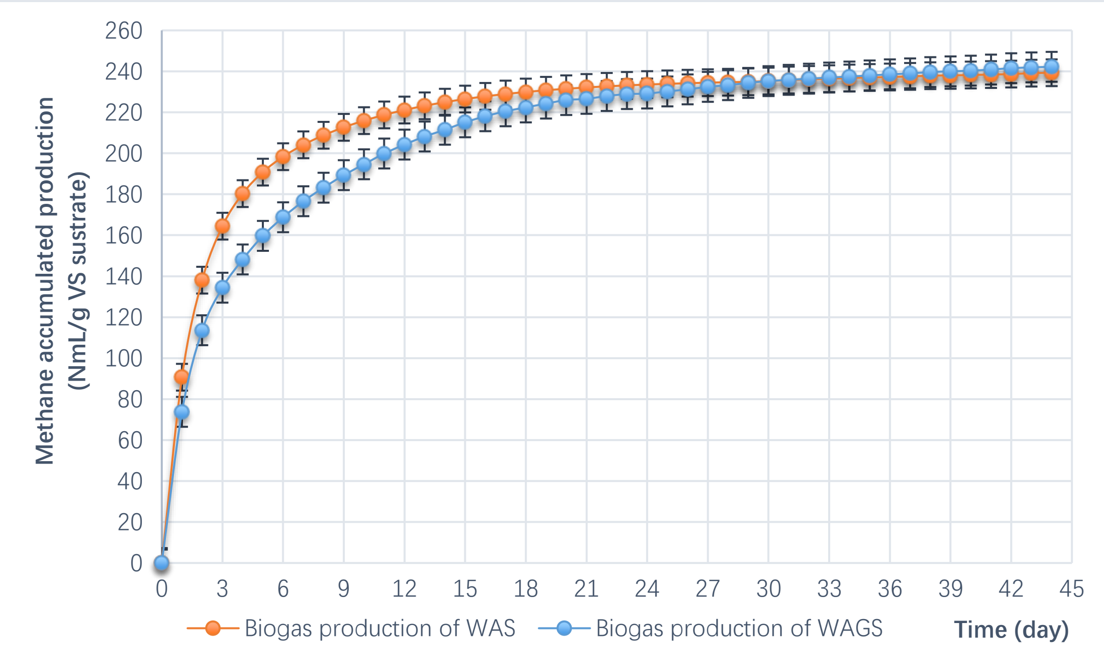

Figure 3. Biomethane production potential of aerobic granule sludge (WAGS) and waste activated sludge (WAS) in BMP test.

3.2 SEPS degradation kinetics in WAGS and WAS during AD process

Figure 4 illustrated the dynamic changes of relative SEPS concentration(mg/gVSS) when sludge was digested anaerobically. Although WAGS always manifested a higher SEPS concentration than WAS throughout the whole process, the variation tendency of WAGS and

WAS was similar. Sludge SEPS concentration increased over the first 3 days followed by a gradual drop down till D44. The raise over the first 3 days seemed to indicate a release of SEPS during hydrolysis step. However, when it came to the absolute SEPS and VS change of both WAS and WAGS, these two parameters both showed decreased trend along with time attributed to organic compounds degradation during AD process. When the VS variation was involved, the cause of relative SEPS concentration can be clearly figure out. This increase not actually attributed to SEPS generation during hydrolysis, but indicating a lower degradation rate of SEPS comparing to VS in sludge which could also be interpreted as retarded biodegradability. In addition, though SEPS was indeed consumed during sludge digestion, it was worth pointing out that the degradation of SEPS was quite incomplete. Experimental data manifested that SEPS in WAS was only reduced by 39% while for that in WAGS was even lower, by 31%, indicating dominate SEPS contents remained in sludge after anaerobic treatment. There is a hypothesis that SEPS plays a significant role in gel-formation property and maintain granular stability for anaerobic granule sludge cultivated by municipal sewage(Y. Lin et al., 2010).Moreover the granular structure of WAGS was not thoroughly deconstructed after 44-days anaerobic digestion. Based on the aforementioned evidences, this could be rationally attributed to the abundant amount of residue SEPS in the sludge functioned as structure supporting material to maintain compact granular.

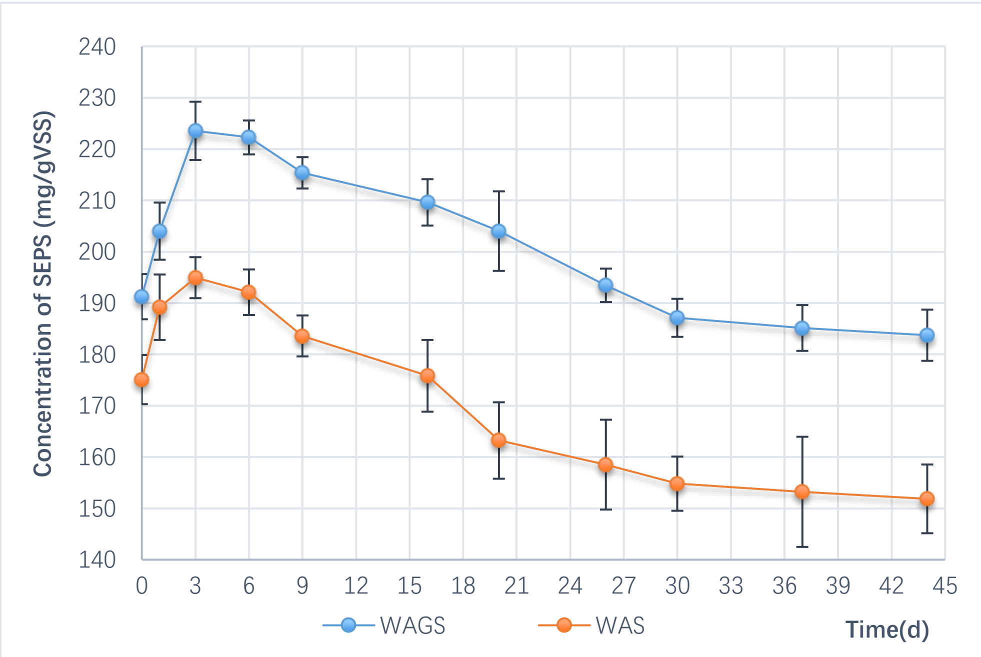

Figure 4. Relative SEPS variation in both WAGS and AS along time.

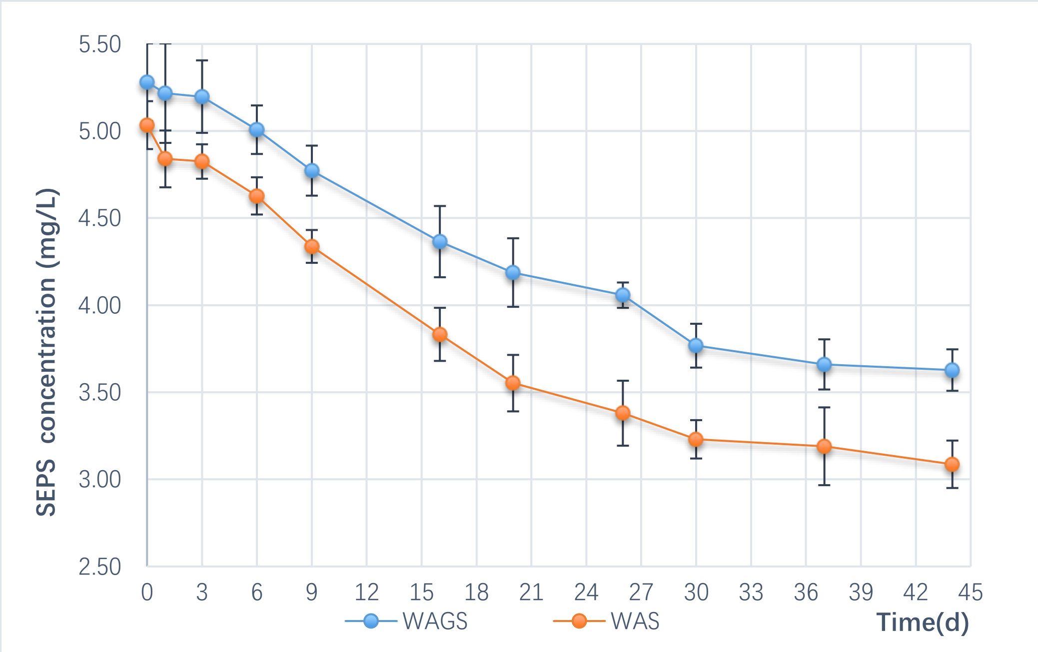

Figure 5. Reduction of absolute SEPS in WAGS and AS against time.

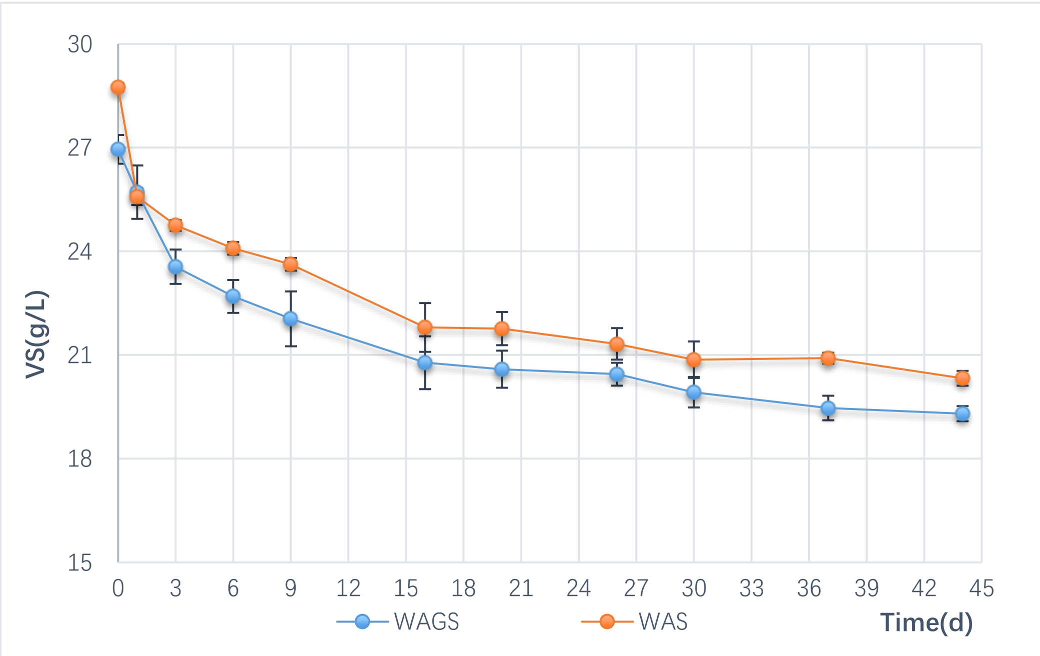

Figure 6. Volatile solids (VS) variation in WAGS and AS with time.

The reduction of SEPS in WAGS differentiated with WAS. Seen from **Figure 5** and Figure 7, WAGS-SEPS was degraded in a slow-fast-slow three stages while WAS-SEPS only demonstrated two stage reduction by a fast-slow pace. According to the linear regression results, a lag stage was seen for WAGS for the first 3 days followed by a rapid degradation period with a degradation rate constant K= 0.0483 d^{-1} from D3 to D21 and then retarded to a relative degradation phase with a K=0.0100 d^{-1} from D21 to D44. As for WAS, it started with a rapid degradation from the first day of anaerobic digestion with a K=0.066 d^{-1} which reduced to 0.0102 d^{-1} after D21. It was noteworthy that the no matter in which phase, WAS always demonstrated a higher degradation rate constant than WAGS, implying WAS degraded prior to WAGS throughout the whole AD process.

Regarding the morphology of WAGS, the compact structure it possessed was likely to pose a restriction to sludge hydrolysis, which could therefore lead to a slow SEPS degradation rate as a consequence.

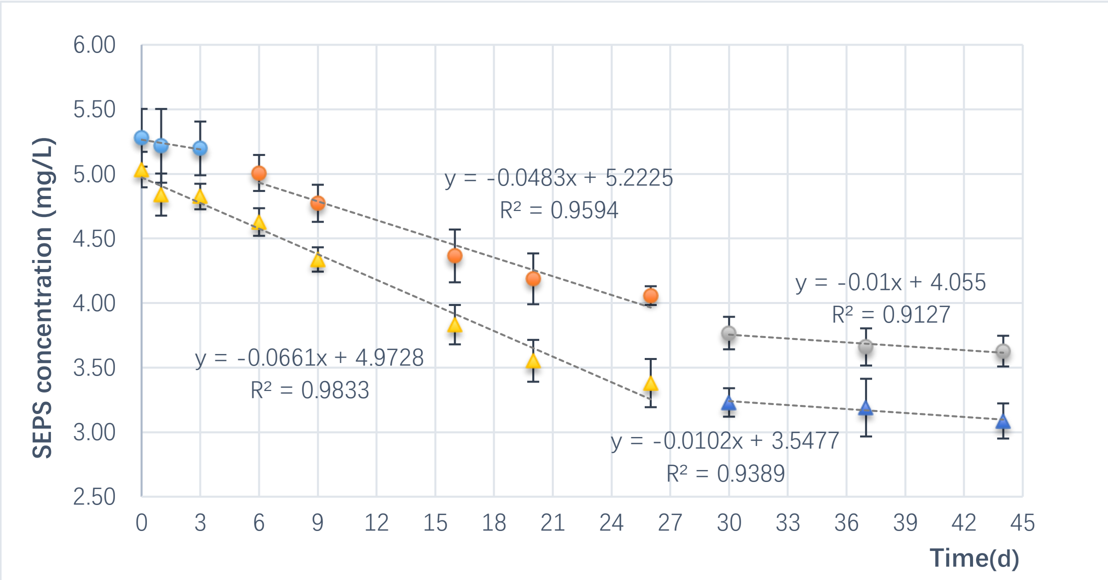

Figure 7. Different phased-reduction of SEPS in WAGS and AS fitted by linear regression.

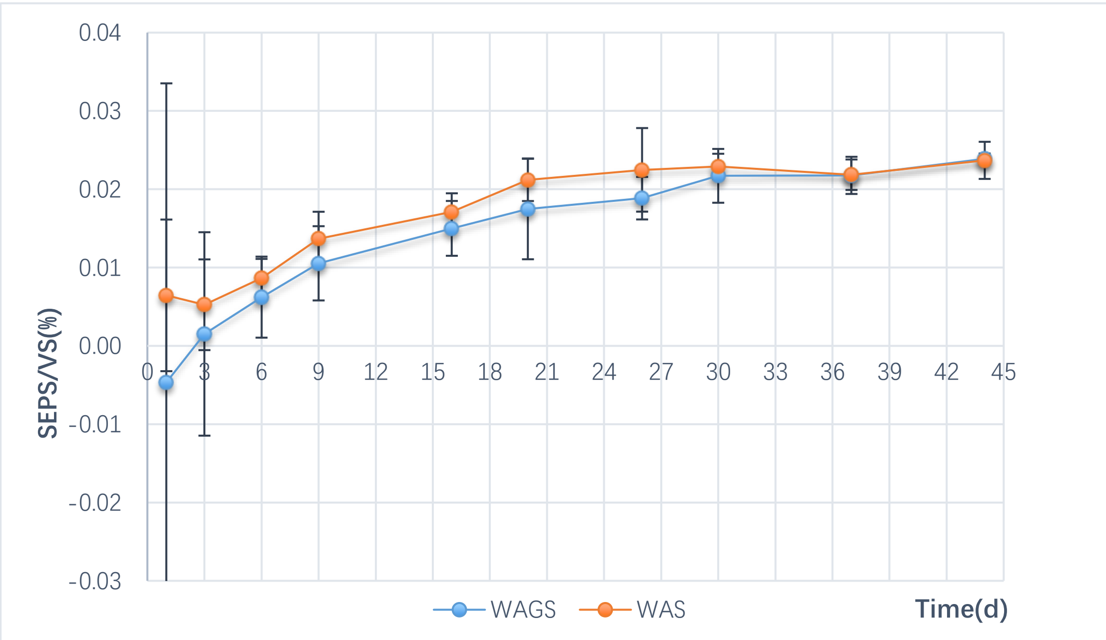

Figure 8. Comparison of SEPS contribution to VS reduction in WAGS and AS.

Though SEPS and VS, as organic constituents, both decreased during sludge digestion, the degradation extent was significantly dissimilar. With respect to low content (comparing to VS), SEPS degradation in WAGS accumulated from 0.06mg/L at D1 until 1.65mg/L on D44 on average while the total VS degradation in the same sludge initially accounted for 1237.50mg/L and reached up to 7556.76mg/L at the end. The situation for WAS was the similar as what occurred in WAGS. On one hand, this significant content gap made SEPS exhibited an extreme low contribution to VS degradation, with maximum 0.23% for WAGS as well as 0.15% for WAS. On the hand, SEPS degradation kept on contributing more for VS reduction suggesting that SEPS was gradually consumed faster than the other organic compounds in VS. It is known that EPS was not evenly dispersed in granule sludge with inner located TB-EPS functioning as "skeleton" to mediate cohesion and adhesion cells and the loose and porous LB-EPS extending from TB-EPS surrounding at the outside(Lu, Zhen, Chen, Kubota, & Li, 2015). It was also reported that TB-EPS could be transferred to LB-EPS during sludge anaerobic digestion(Ye et al., 2014).The observation of slow SEPS degradation at the beginning suggested that SEPS was not an easily biodegradable organic compounds comparing to VS and it might be initially trapped in an inner EPS layer (as TB-EPS) and required time to transfer to the outer layer(as LB-EPS) before it could be degraded. Moreover, higher SEPS/VS in WAGS proved a faster degradation of SEPS in WAGS than WAS that probably originated from higher SEPS content in WAGS. This observation also reached an agreement with the narrowed-down SEPS content difference between WAGS and WAS

3.3 Variation of fine chemical structure of SEPS in WAGS and WAS

Since polysaccharides(PS) and protein(PN) are referred as dominate components in EPS, SEPS, as a kind of EPS, is supposed to contain a quite amount of PS and PN that are prone to impact on sludge property, especially for WAGS. Therefore, changes of PN and PS in WAGS and WAS were investigated and results were shown as Figure 9 and Figure 10. According to the figure, the downward trend of both PS and PN in WAGS and WAS suggested that the SEPS degradation was a joint efforts of PN and PS rather than a single compounds.

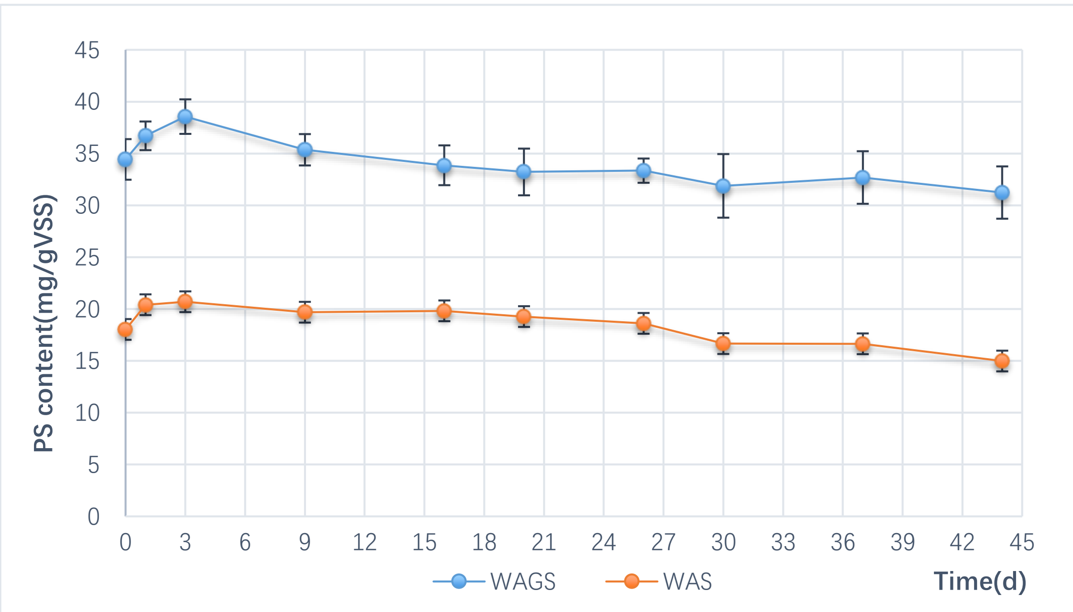

Figure 9. Comparison of polysaccharides (PS) variation in total SEPS between WAGS and AS.

Figure 10. Comparison of protein (PN) variation in total SEPS between WAGS and AS.

PS in WAGS and WAS had the similar developing tendency with a slight increase over the initial 3 days followed by a gradual decrease for the rest of the experiment. The same phenomenon also occurred to PN variation. Since PS and PN were derived from total SEPS as well as the developing trend was in well consistent with relative SEPS variation, thus the increase at beginning could also be attributed to faster VSS degradation than PS and PN generation. Besides, WAGS manifested higher PN and PS contents but lower PN/PS ratio than those in WAS. As PN and PS are main composition of EPS and EPS is proposed highly involved in providing structural support, it was rational that the WAGS with higher PS, PN contents demonstrated a compact "granular" structure with higher strength and mechanical stability. Moreover, no significant change was observed for PS/PN in WAGS but a slight increase with mild fluctuations for AS. PN/PS ratio in EPS plays a vital role in sludge stability which has been proved as higher PN/PS ratio leading to poorer sludge-water separation property. On one hand, this again confirmed the nice settling property possessed by WAGS with regard to its compact structure and high density. On the other hand, the increase of PN/PS ratio in AS indicated even lower density and lower shear force that possibly attributed to the deteriorated dewaterability as a consequence.

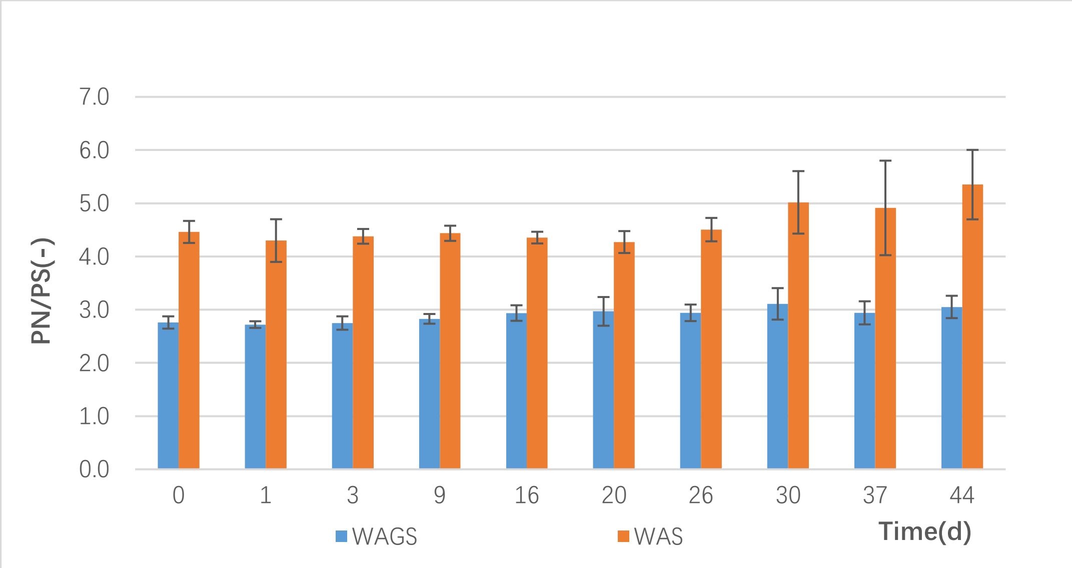

Figure 11. Comparison of PN/PS ratio variation in total SEPS between WAGS and AS.

Apart from the polysaccharides and protein contents, FT-IR and 3D-EEM were also involved to give an insight into the functional groups. Based on the previous studies, SEPS is rather considered as a family of polymers with distinct chemical structures than a single pure polymer, and these chemical structures are considered responsible for SEPS's gel-forming property (Draget et al., 2000). If SEPS is chemically fractionated into single polymers, the obtained components mainly consist of mannuronic acid(M) and guluronic acid(G) units appearing in different proportion of GG blocks, MG blocks and MM blocks irregularly assembled alginate. Interestingly, characteristics of SEPS, especially its gel-forming property, is determined by amount of these 3 different blocks, which is also denoted as the fine chemical structure of alginate polymers(Hampson et al., 2005; Y. M. Lin, Sharma, & van Loosdrecht, 2013).

Table 1. FT-IR spectra band assignment

Frequencies (cm^{-1})	Band assignment
Over 3000	O–H bond stretching vibrations of polysaccharides
2927	C-H bonds associated with polysaccharides
1654	C=O stretching (amide I) and C=N stretching associated with proteins
1539	N–H and C–N stretching in amide II associated with proteins
1400	Symmetric stretching of carboxylic O-C-O vibration for polysaccharides
1240	C-N stretch associated with secondary amides of proteins (amide III)
1080	C–O and C–O–C stretching vibration of hydroxyl in polysaccharides
750-950	Fingerprint (or anomeric) region of carbohydrate

As shown in Figure 12, there was no significant difference among the spectra and all samples demonstrated the typical bands of exopolysaccharides. The FT-IR manifested the occurrence of carboxyl and hydroxyl functional groups of polysaccharides. The spectra of typical polysaccharides are usually manifested as: broad absorption above 3000cm^{-1} assigned to O-H band, absorption peaks at 2800~2974cm^{-1} for C-H band and stretching at 1000~1200cm^{-1} representing C-O-C or C-O bands, which were in good consistent with all SEPS samples despite of their origin. Furthermore, brands located at 1660~1684cm^{-1} and 1440~1414cm^{-1} were considered as the asymmetric and symmetric stretching of - COO vibration in uronic acid residues respectively, confirming this polymer to be salt of uronic acid polysaccharides.

Since the absorption of demonstrated by FT-IR provides no indication of the components' content, it was hard to tell the whether there's degradation of SEPS occurred during the AD process. However, for a specific sludge type, the identical curves peaked at the same wave length reveSEPSd that the initial sludge and the digested residues had no significant differences on composition. These compositions, as shown in the figure, had strong characteristic peaks among all samples, indicating little degradation throughout the AD process. To get further information on the typical bands attributions, especially for those fingerprint region among 900~750 cm^{-1}, which is the most discussed in carbohydrates, a second derivation of the FT-IR was introduced(Tul'chinsky, Zurabyan, Asankozhoev, Kogan, & Khorlin, 1976). This is because the original spectra was manifested along a broad wave length range while fine brand information around/within a certain region was likely to be covered. In this situation, bands falling in the range of 1000~700cm^{-1} were resolved in a second-derivative spectra for WAS and WAGS respectively, to enhance carbohydrate against broad background in structural EPS samples (see **错误!未找到引用源。**). In normal FT-IR spectrum, unobvious brands were deduced and brands located at 890cm^{-1} and 780cm^{-1} were observed WAGS and AS at D0. According to David, the characteristic brand located at around 890cm^{-1} can be assigned to homopolymannuronic acid blocks while peaks around 781cm^{-1} are attributed to homopolyguluronic acid blocks(Leal, Matsuhiro, Rossi, & Caruso, 2008). This evidence proved the appearance of GG and MM blocks, enriched blocks in alginate, in both sludge before aerobic digestion. However, these two peaks gradually shifted down to lower wave length or faded away along time, revealing degradation of GG and MM blocks occurred during AD process. In summary, in spite of sludge origin, the extracted structural EPS is a kind of biopolymer that is not readily degradable and only partial polysaccharides were degraded along sludge digestion.

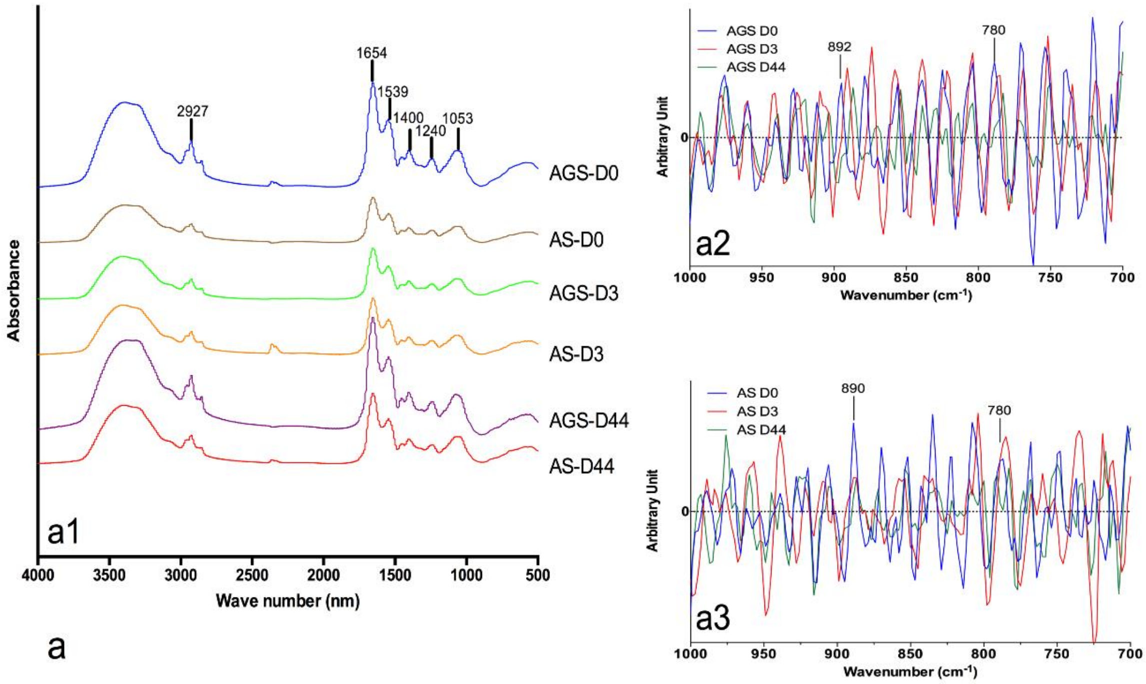

Figure 12. FT-IR spectrum (a) and second-derivative spectra (a2, a3) of WAGS and AS on D0, D3, D44.

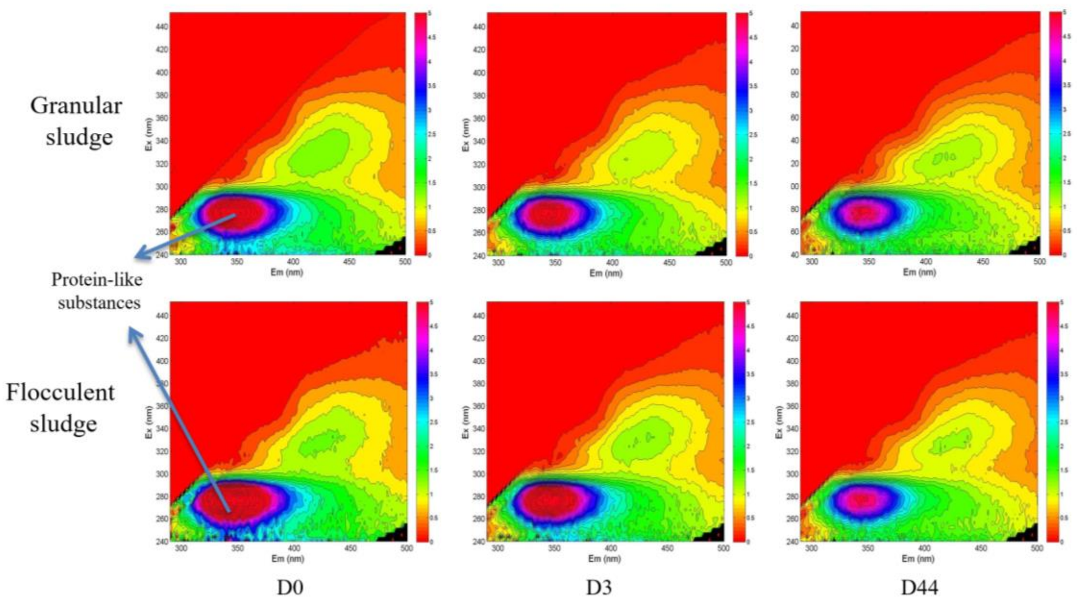

Figure 13. 3D-EEM spectrum for WAGS and AS on D0, D3 and D44.

Since the structural EPS not only consists of polysaccharides, protein is also the main components. Figure 13 illustrated the results of 3D-EEM spectrum in D0, D3 and D44 for AS and WAGS. The tryptophan-like protein representing by the red areas surrounded by blue were observed in different sludge throughout the whole process, but the detected signals tended to shrink as AD undergoing, revealing the degradation of proteins in structural EPS. In other words, the structural EPS degradation was not caused by a single component, but attributed to the joint efforts of the simultaneous PS and PN degradation. From another point of view, structural EPS was incompletely degraded since there was quite protein residues on D44 for both sludge.

Since SEPS, who possessed the property of gel-forming due to abundant GG block contents, was analyzed to be comprised of polysaccharides and protein while the structural EPS(SEPS) was likely to remain in sludge after digestion with regard of quite PN and PS residues. This polysaccharides-based biomaterial had the chain-like structure and amphiphilic property due to containing polysaccharides in dominate proportion and lipid in minor proportion and it was proved to be an emerging environmental-friendly coating material for water resistance with regard to its biodegradability (Y. M. Lin, Nierop, Girbal-Neuhauser, Adriaanse, & van Loosdrecht, 2015). Therefore, this incomplete degradation offered a possibility to recovery SEPS after sludge treatment. Extraction after AD is more preferred rather than before since both biogas production and SEPS source recovery could be achieved. With respect to recovery, relative SEPS enriched WAGS and the predicted wide spread of aerobic granule sludge biotechnology and the consequently produced large amount of excessive WAGS made the granules to be the most preferable source for SEPS extraction. Furthermore, the different performance of SEPS and VS variation in AD also suggested pre-treatments such as pre-hydrolysis are necessary to improve the efficiency of SEPS recovery. Because VS was rather consumed faster than SEPS over the first 3 days in sludge digestion, therefore pre-hydrolysis is able to utilize this mechanism to concentrate SEPS contents(SEPS/VS) in solid phase by enhancing VS degradation during hydrolysis step and made the enriched granule sludges even favorable and economic for recovery.

However, there is a critical point to be considered that, as exhibited in our second-derivative spectra, the fine chemical structure of the EPS tended to be changed, such as some typical brands faded. In this sense, even the structural EPS still remained quite in the digested sludge residues but the properties of the EPS, especially its water-proof ability, were likely to differentiate from the original. The feasibility of SEPS recovery after sludge anaerobic digestion and the applicable extraction methods require further research.

3.4 Variation of dewaterability and SEPS conversion in different EPS layers in WAGS and WAS

Figure 14. Different dewaterability deterioration during anaerobic digestion for WAGS and AS.

Despite of sludge type, the dewaterability deteriorated during AD process as an indicator of sludge destruction by anaerobic digestion. The deterioration of dewaterability for both WAGS and AS shared the same trend with two intersections after half-way of AD. The NCST of AS increased from 15.31±0.42 to 30.88±0.92 s·L/gTSS with an increase rate of 101.67% while the initial NCST of WAGS was 12.98±0.26 and ended up with 31.31 s·L/gTSS, deteriorated by 141.24%. This evidence proved that digestion of sludge had negative influence on dewaterability which was in an agreement with results achieved by Xu who also found out the normalized CST for sludge increased after anaerobic digestion(Xu, He, Wang, Shao, & Lee, 2011), suggesting that anaerobic digestion had a negative effect on sludge dewatering. Apart from dewaterability itself, difference of dewatering property between WAGS and AS also varied. From the figure, WAGS used to display a better dewaterability (lower NCST) than AS at the first 20d but this difference was gradually shrunk and turned out to be almost the same afterwards. This finding figured out that WAGS was likely to loss more dewaterability over time during AD than WAS.

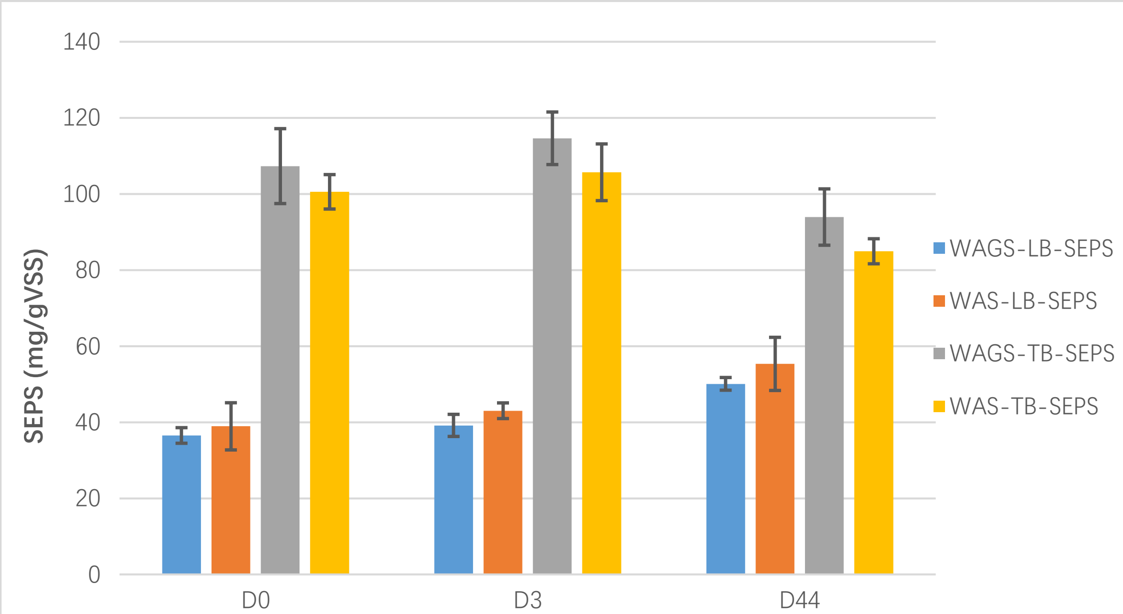

Figure 15. LB-EPS-SEPS and TB-EPS-SEPS contents change is WAGS and WAS against time during AD process.

In order to gain an insight into attribution of the dewaterability deterioration, SEPS in different EPS fractions, loosely bound extracellular polymeric substances (LB-EPS) and tightly bound extracellular polymeric substances (TB-EPS) were extracted from WAGS and AS respectively for analysis and determine the impact of their content and chemical constituents change on dewaterability. Since SEPS is the predominate composition in EPS, in this study, SEPS derived from TB-EPS and LB-EPS were also considered to present the behavior of LB-EPS and TB-EPS in both WAGS and AS. As shown, LB-EPS-SEPS increased by 1.37 and 1,42 for WAGS and AS after sludge was anaerobically digested while TB-EPS-SEPS increased over the initial 3 days and then gradually dropped down for the rest experiment. Prior researchers found that when undergoing anaerobic digestion with flocculent sludge, TB-EPS was possibly transferred from the tight bound layer of the floc into the loose bound layer(Ye et al., 2014). Since in this case WAGS behaved the same as WAS, it was also considered reasonable to apply this finding as explanation for the behavior of LB-EPS-SEPS and TB-EPS-SEPS in WAGS during AD process. Additionally, according to Yuan who reported that TB-EPS played the key role in the gel-like and fractal structures of activated sludge without anaerobic treatment(Yuan et al., 2014). Since TB-EPS had the function of structure-supporting while SEPS is widely accepted as a structural EPS component, it was rational to observe a high SEPS content in TB-EPS at the beginning of AD. Moreover, the high residue contents of LB-EPS-SEPS and TB-EPS-SEPS in sludge after anaerobic digestion also indicated release and solubilization of EPS was not complete.

The observation that sludge after LB-EPS extraction demonstrated the strongest gel-like structure, and the direct experimental evidence that both sludge residue after LB-EPS extraction and TB-EPS exhibited a stronger and more compact structure suggested that TB-EPS played a significant role in gel-like property(Wang et al., 2005; Yuan et al., 2014).Increase in LB-EPS-SEPS content and normalized CST were closely correlated, with Pearson's correlation coefficient R^2=0.9992 and R^2=0.9906 for WAGS and WAS, respectively, which is inconsistent with previous results that LB-EPS-SEPS was positively correlated with NCST(Ye et al., 2014), revealing the increase of LB-EPS-SEPS to be the attribution for sludge dewaterability deterioration. However, there was also a research suggested that the increase of LB-EPS-SEPS was resulted from the reduction of TB-EPS-SEPS (or TB-EPS) during the EPS solubilization which was dissimilar with the results obtained in this study. In this case, LB-EPS-SEPS gradually increased along time while TB-EPS-SEPS increased during 3 days followed by a decrease until the end. In order to take the influence of TB-EPS into consideration, correlation of LB-EPS-SEPS/TB-EPS-SEPS and dewaterability was analyzed and the results showed that dewaterability was well correlated to LB-EPS-SEPS/TB-EPS-SEPS with R^2=0.9974 and R^2=0.9999 for WAGS and AS, respectively. This observation confirmed that LB-EPS-SEPS was supposed to have a negative effect on dewatering property and the component transmission from TB-EPS to LB-EPS was the attribution for dewaterability deterioration

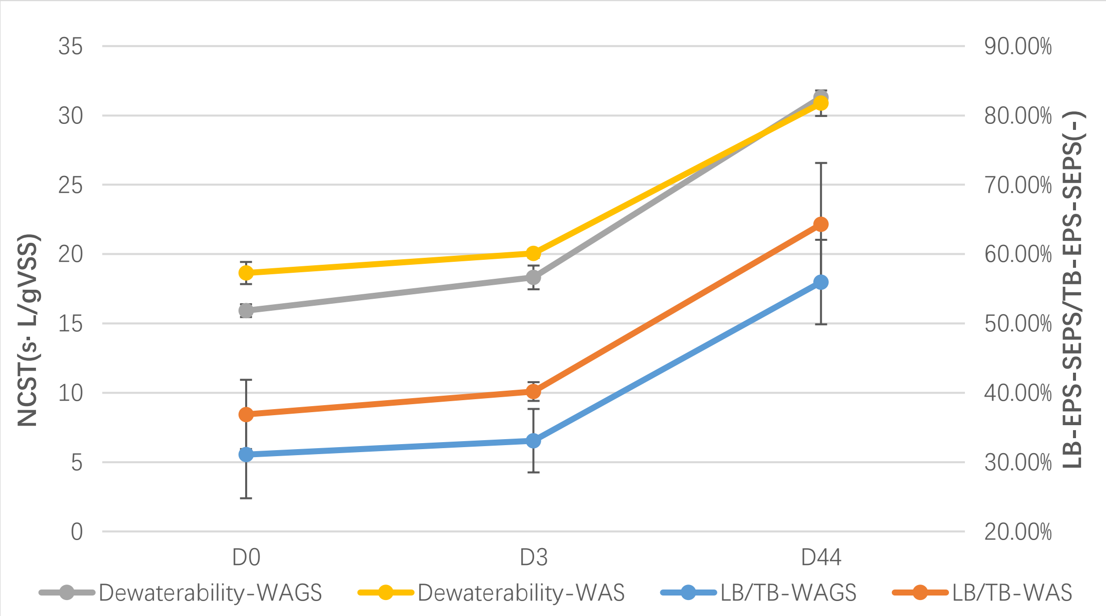

Figure 16. Comparison between the dewaterability deterioration and LB-EPS-SEPS/TB-EPS-SEPS ratio increase for WAGS and AS.

Despite of the stratified layers, EPS, as a gel-like biopolymer, consists of polysaccharides (PS), protein(PN) as dominant components together with some lipid, nucleic and humic-like substances(HS) in low proportion(Yuan et al., 2014). Analysis of a cross-section of literature reveSEPSd that not only EPS but also the components in EPS such as exopolysaccharides and glycosides were confirmed to have contribution to the gel-like properties of aerobic granules as well(Seviour, Pijuan, Nicholson, Keller, & Yuan, 2009).

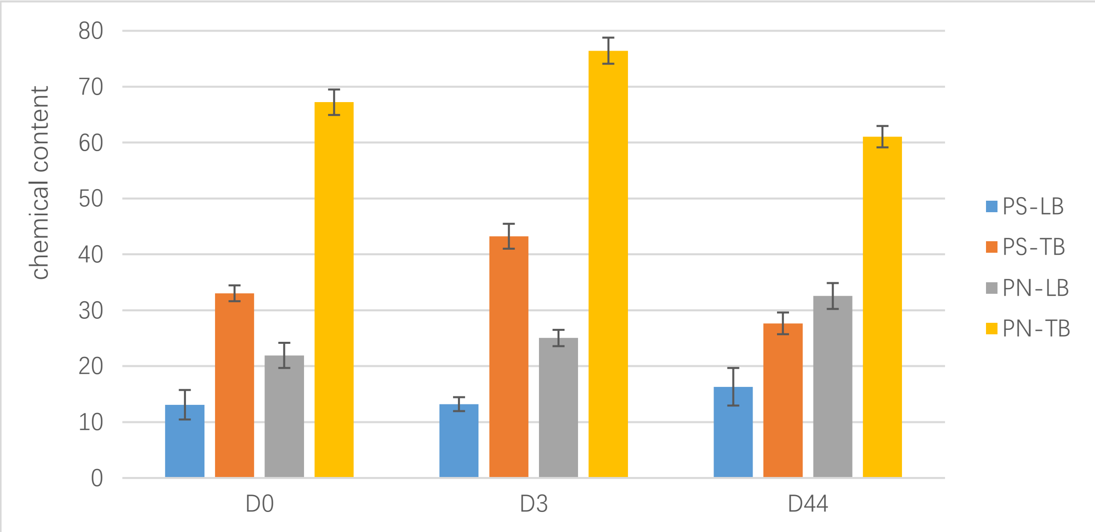

Figure 17. Polysaccharides (PS) and protein (PN) distribution variation in LB-EPS-SEPS and TB-EPS-SEPS in WAGS during AD process.

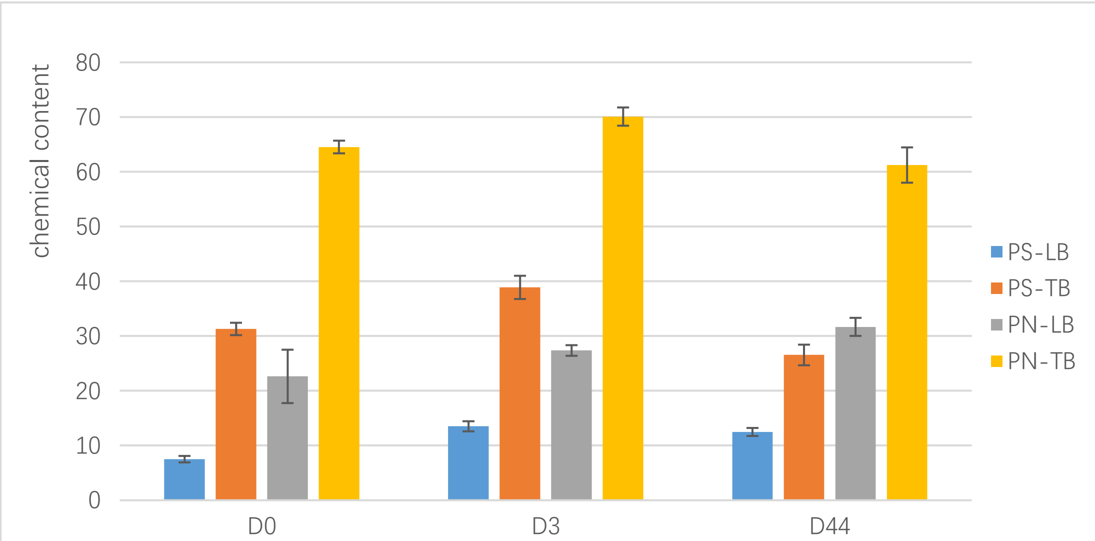

Figure 18. Polysaccharides (PS) and protein (PN) distribution variation in LB-EPS-SEPS and TB-EPS-SEPS in WAS during AD process.

In Figure 17 and Figure 18, PN and PS variations in LB-EPS-SEPS and TB-EPS-SEPS in sludge samples were presented. For both WAGS and WAS, PN and PS were dominantly distributed in TB-EPS-SEPS rather than LB-EPS-SEPS. Over the pioneering stage, PS and PN in TB-EPS-SEPS were approximately three-fold more than those of LB-EPS-SEPS but the discrepancy was narrowed to less than two-fold at the end. It was rational if we took a look at a specific type of sludge, it was observed that both PN and PS in LB-EPS-SEPS increased after sludge digestion while those in TB-EPS dropped down at the meanwhile. PS change in TB-EPS-SEPS and LB-EPS-SEPS shared the same tendency with PN in LB-EPS-SEPS and TB-EPS-SEPS, which reached an agreement with results performed by Ye(Ye et al., 2014). In addition, comparing to PS, PN occupied much more contents in a certain stratified layer, manifesting that PN was likely to decompose more slowly than PS during AD process.

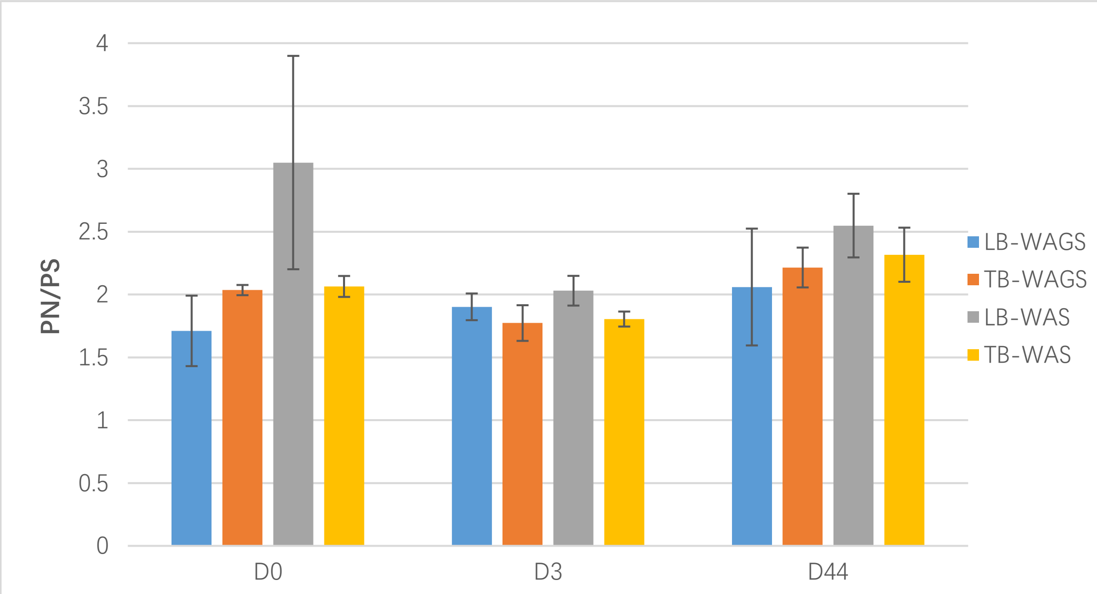

Figure 19. PN/PS ratio variation along time in LB-EPS and TB-EPS in WAGS and AS, respectively.

Furthermore, the ratio of PN/PS in EPS is also considered as key factor for sludge stability. In this case, the obtained results were dissimilar with the aforementioned research. Former researchers reported that the ratio of PN/PS in LB-EPS-SEPS increased dramatically over the first 15 days and remained stable afterwards while not much change was seen for that of TB-EPS-SEPS. However, in this study, regardless of EPS layer and sludge type, PN/PS ratio had no significant variation over time, falling within in the range of 1.7~2.3 for WAGS and 1.8~3.2 for AS. This result was closely approach to the value obtained for the first 104d granules in an UASB(Lu et al., 2015). AS always demonstrated higher PN/PS both in LB-EPS-SEPS and TB-EPS-SEPS than WAGS but the difference between WAGS and AS shrunk as digestion underwent. Biomass with higher PN/PS ratio was more likely to float meaning the sludge a possessed retarded sludge-water separation property(Franco, Roca, & Lema, 2006). It reasonably elucidated the relation between PN/PS and dewaterability observed in our study, since the dewaterability exhibited by WAGS was indeed better than that of AS before sludge digestion but turned out to be almost the same at the end. Besides, no obvious relation was observed between LB-EPS-SEPS and TB-EPS-SEPS in the same sludge.

3.5 Variation of gel-forming property of SEPS derived from WAGS and WAS

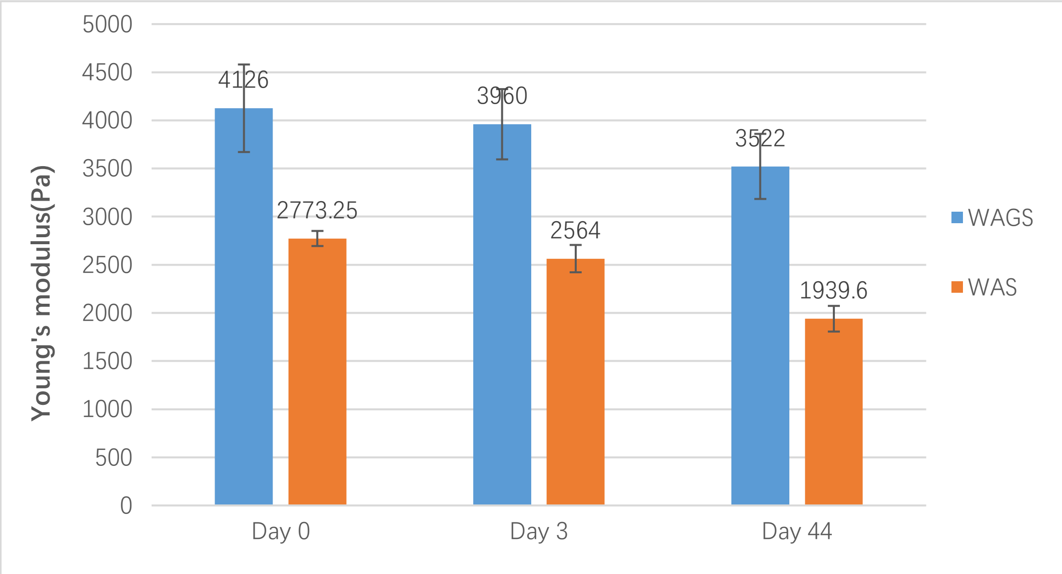

Figure 20. Young's module variation of WAGS and WAS on D0, D3 and D44.

The singularity of structural EPS was characterized by its gel-forming ability with multivalent ions such as Ca2+ under a various of PH and temperature conditions. The formed gel material with high viscous and elastic features. Previous study illustrated that the distinction between gel-forming properties of granule sludge and flocculent sludge was mainly attributed to the ratio of three building blocks: MM, MG and GG blocks in SEPS. SEPS derived from WAGS had significantly higher proportion of GG blocks than GM blocks in chemical structures while AS had equal amount of GG blocks and GM blocks(Y. M. Lin et al., 2013). Since only GG blocks offer the gel-forming capacity, it is predicted that mechanical properties of SEPS extracted from WAGS and AS were distinct. SEPS's uniqueness is that they are capable of forming gels with multivSEPSnt ions such as Ca^{2+} in a broad range of temperature and PH while the viscous and elastic characteristics displayed by this biopolymer when undergoing deformation was be regarded as a parameter to indicate SEPS's mechanical property. Therefore, hydrogels formed by SEPS's cross-link with Ca^{2+} was performed for gel-forming analysis.

From the results, in spite of SEPS origin, downward trend on Young's Module were observed for SEPS throughout sludge digestion. SEPS extracted from WAGS dropped from 4126±454 Pa at D0 to 3522±338 Pa at D44 with reduction rate of 14.6% while for that derived from AS, the reduction rate of Young's Module was twice that of WAGS starting from 2773±78 Pa to 1939±133 Pa at the end. Higher Young's Module corresponds structures that is hard to destroy and its reduction rate indicates the loss of mechanical structure of the sludge. For both sludge type, decreases on Young's Module showed degradation of the structural components in AD process indicating SEPS was biodegradable. The hydrogels formed by WAGS-SEPS always possessed a higher elasticity than that of AS-SEPS. This implied that the mechanical structure of granules were more difficult to be destroyed than flocculent sludge, which is in an agreement with the compact morphology of WAGS. In addition, it was also proposed that this compact and strong mechanical structure of WAGS might lead to a lower hydrolysis rate during sludge digestion.

4. Conclusions and recommendations

Comparison of chemical contents and mechanical structure between WAGS and AS were investigated to figure out the fate of SEPS variation and its consequent influences on sludge properties. Results obtained from biomethane production illustrated the similar biogas production potential of different sludge but a substantial slower hydrolysis rate of WAGS due to its dense and compact structure comparing to AS, suggesting that pre-treatment for WAGS before AD process might be necessary. During anaerobic digestion process, SEPS isolated from WAGS and AS exhibited a biodegradability of 31% and 39%, respectively, but were observed as slowly biodegradable organics comparing to VSS reduction. Both polysaccharides and protein made contributions to structural EPS degradation according to the results from FT-IR and 3D-EEM, but no significant changes observed for overall chemical structures of SEPS. WAGS occupying a higher content in both PS and PN but lower PS/PN ratio than AS indicated a superior sludge-water separation property of WAGS which reached an agreement with function of its dense and compact granule. Meanwhile, the increased ratio of PN/PS in AS predicts a deterioration in sludge-water separation indicating even worsen loose sludge structure of AS after AD. For the dewaterability and stratified EPS, a deterioration in dewaterability was seen for both WAGS and AS after sludge digestion as a consequence of TB-EPS-SEPS increase as well as LB-EPS-SEPS decrease. At the beginning of AD, SEPS mainly enriched in TB-EPS contributing to sludge's gel-like, fractal structures to maintain mechanical strength but with the undergoing transmission from TB-EPS into LB-EPS, that has a negative effect on dewatering property and no structure supporting function, digested sludge gradually lost its compact matrix structure and dewaterability. The degradation of the GG and MM blocks in structural EPS exhibited in FT-IR second-derivative sprectra, were responsible for the decrease on sludge compact structure and gel-like property for AS WAGS.

Since SEPS is proved to be efficient water-proof coating material for industrial use, the results that only a limited partial SEPS was degraded during AD process as well as the mechanical strength loss was less than 20%, provided an opportunity for structural EPS recovery after AD process. However, since the fine chemical structure change of SEPS after sludge digestion is still not clear, the feasibility of SEPS recovery and the extraction methods requires further study.

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