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Structural extracellular polymeric substances determine the difference in digestibility between waste activated sludge and aerobic granules

Hongxiao Guo [a, *], Simon Felz [b], Yuemei Lin [b], Jules B. van Lier [a], Merle de Kreuk [a]

[a] Section Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, Stevinweg 1, 2628, CN, Delft, the Netherlands
[b] Group Environmental Biotechnology, Department of Biotechnology, Faculty of Applied Science, Delft University of Technology, Van der Maasweg 9, 2629, HZ, Delft, the Netherlands

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ABSTRACT

Aerobic granular sludge (AGS) technology is an alternative to conventional activated sludge to reduce the process footprint and energy consumption. Strategies for the efficient management of its produced biomass, that is grown in a granular morphology as well, need further development. Anaerobic digestion (AD) is commonly applied in waste activated sludge (WAS) treatment and is a potential option also for produced AGS treatment. In earlier studies, the biochemical methane potential of AGS was found lower than that of WAS both grown in full-scale municipal wastewater treatment systems. In order to understand this difference, this study aimed to investigate the anaerobic conversion of structural extracellular polymeric substances (SEPS), which is a type of gel-forming biopolymer, being responsible for the aggregation of sludge. Using WAS and AGS as substrates, a comparative AD batch experiment was performed for 44 days during which the SEPS fraction was extracted from both types of sludge. The changes in the SEPS chemical composition was analysed by Fourier transformed infrared spectroscopy and three-dimensional excitation and emission matrix analysis. In addition, the mechanical strength of hydrogels of extracted polymers cross-linked with Ca^{2+} ions was investigated by dynamic mechanical analysis. Results showed that the amount of SEPS was reduced by 26% in AGS ($SEPS_{AGS}$) and by 41% in WAS ($SEPS_{WAS}$), respectively. Polysaccharides and, to a lesser extent, the proteins in the $SEPS_{AGS}$ were more refractory compared to those in $SEPS_{WAS}$. This resulted in a lower loss of the gel stiffness of $SEPS_{AGS}$ than that of $SEPS_{WAS}$ during the AD process. Moreover, the release of SEPS from tightly bound EPS to loosely bound EPS were observed in both types of sludge, but that in AGS exhibited a lower transition rate. The observed properties explain the distinct differences in anaerobic biodegradability, the slower decomposition of the sludge structure, as well as the better dewaterability of AGS as compared to WAS after the AD process.

1. Introduction

Conventional activated sludge (CAS) processes treating municipal wastewater create large quantities of waste activated sludge (WAS), resulting in high costs for sludge processing, i.e. stabilisation, incineration and discharge (Appels et al., 2008). The Nereda® technology (Nereda® is the registered trade name of the aerobic granular sludge (AGS) technology owned by Royal HaskoningDHV) is gaining increasing popularity, due to its lower energy demand and more compact reactor design compared to CAS processes; currently over 70 full-scale Nereda® plants are in operation or under construction worldwide (www.royalhaskoningdhv.com/nereda). Similar to the CAS process, also the AGS process results in the production of waste aerobic granular sludge (WAGS), which asks for proper processing and discharge.

Anaerobic digestion (AD) for WAS stabilisation is commonly applied and is proven to be a cost-effective solution, allowing energy and nutrient recovery (Appels et al., 2008; Luo et al., 2020). To date, the behaviour of WAGS during AD is largely unknown and only few studies have been reported in literature (del Rio et al.,

* Corresponding author. Post: PO Box 5048, 2600, GA, Delft, the Netherlands.
E-mail address: h.guo-2@tudelft.nl (H. Guo).

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2011; del Rio et al., 2014). More insight into the degradation of aerobic granules is needed to be able to design effective WAGS sludge processing reactors (Bernat et al., 2017; Palmeiro-Sanchez et al., 2013). In full-scale Nereda® systems, the WAGS can be two types: the AGS that can be periodically withdrawn from the reactor (mainly granules, 70% particle size > 0.5 mm), and the aerobic granular sludge selection spill (particle size ≤ 0.5 mm), a more flocculent biomass that is wasted every process cycle presenting lower settling velocity than big granules (Pronk et al., 2015). Very recent research has shown that the selection spill was easily biodegradable in AD (Guo et al., 2020) while the AGS showed a biochemical methane potential (BMP) that was significantly lower than WAS (Bernat et al., 2017; del Rio et al., 2011; del Rio et al., 2014). It was demonstrated that the high digestibility of the selection spill was due to the low settle-ability of highly biodegradable cellulose-like fibres. However, the reason for the lower digestibility of AGS compared to WAS remained unclear. Guo et al. (2020) observed that proteins and carbohydrates in AGS were more difficult to degrade than those in WAS, even though the amount of these two fractions was higher in AGS. Since AGS and WAS are mainly composed of cells, (exo-) enzymes and microbial metabolic products, very likely, this difference is related to the microbial organic polymeric matrix. Extracellular polymeric substances (EPS) account for the majority of microbial related carbohydrates and protein fractions in both AGS and WAS (Frolund et al., 1996; Liu and Fang, 2002; Salama et al., 2016). Therefore, analysing the biodegradability of the EPS present in AGS and WAS during AD will help to understand the differences in digestibility between the two sludge types.

Based on the ability to form hydrogels, EPS can be divided into gel-forming and non-gel-forming EPS (Felz et al., 2016; Seviour et al., 2009). The amount of gel-forming EPS or structural EPS (SEPS) can be considerable, i.e. 100-300 mg/g VS sludge, and unlike non-gel-forming EPS, SEPS contributes to the formation of a tertiary network structure within the sludge (Felz et al., 2016; Lin et al., 2010). It is hypothesized that the different concentration, organic composition and ionic gel-forming properties of SEPS extracted from AGS and activated sludge determine the sludge morphology differences of the two (Lin et al., 2013; Sam and Dulekgurgen, 2016). Anaerobic sludge digestion starts with breaking down the sludge structure, resulting in the deterioration of sludge properties (Ye et al., 2014). This enlarges the surface area of the sludge making it more accessible for the hydrolytic enzymes and thus for conversion during AD. Considering the amount of SEPS in sludge and their importance to the sludge structure, the fate of these biopolymers in AD is very likely of crucial importance for the observed differences in digestion performance of AGS and WAS.

As a consequence, in this study, batch mesophilic anaerobic digestion tests were conducted for AGS and WAS under the same experimental conditions. SEPS from AGS ($SEPS_{AGS}$) and WAS ($SEPS_{WAS}$) were extracted during the experiment, and their conversion characteristics regarding overall biodegradability, chemical & gel-forming properties and spatial distribution were investigated and compared. Results led to a schematic representation of the SEPS degradation mechanism.

2. Materials and methods

2.1. Inoculum and substrates

The inoculum for batch tests was taken from the sludge digester of the municipal wastewater treatment plant (WWTP) Harnaschpolder (Den Hoorn, The Netherlands). The WAS was collected from the low loaded, enhanced biological phosphate removal (EBPR) activated sludge tank at the same WWTP. AGS was collected from a full-scale Nereda® reactor treating municipal wastewater (Garmerwolde, The Netherlands). The influent characteristics and operational parameters of these two plants were reported elsewhere (Guo et al., 2020). The volatile solids (VS) concentrations of both types of sludge were manipulated by centrifugation (5 min at 3500 × g) to reach to same sludge concentrations.

2.2. Experimental setup

The anaerobic digestion batch experiments were carried out at 35 ± 1 °C in 2-L flasks for 44 days in an incubator shaker at 120 rpm (Innova 44, Eppendorf AG, Germany). The total volume of the mixture of inoculum and substrate was 1.8 L with an inoculum/substrate ratio of 2 calculated by the dry weight of sludge VS (g) that was dosed into the batch reactor. Phosphorus buffer solution, macronutrients and trace elements were dosed according to the recipes of Zhang et al. (2014). For each type of sludge, three flasks were connected to an Automatic Methane Potential Test System (AMPTS II, Bioprocess Control, Sweden) for real-time online monitoring of the accumulated methane production, while three other flasks were used for periodic collection of an 80 mL sample for further analysis. Prior to the start of the experiment, N_2 was purged into each reactor for 5 min to remove oxygen from the sludge and headspace.

2.3. Analytical methods

2.3.1. EPS extraction

The EPS was extracted from the sludge with a mild temperature-Na_2CO_3 extraction method developed by Felz et al. (2016). In brief: a sludge sample of 3 g (wet weight) was added into a 0.5% (w/v) Na_2CO_3 solution up to 50 mL and subsequently stirred at 400 rpm and 80 °C for 35 min, followed by centrifugation at 4000 × g and 4 °C for 20 min. The organics in the supernatant comprised the total extractable EPS.

In addition, centrifugation and a mild-harsh heat method (Li and Yang, 2007) was applied to extract the stratified EPS: slime, loosely bound-EPS (LB-EPS) and tightly bound-EPS (TB-EPS); a sludge sample of 15 g (wet sludge) was centrifuged at 15,000 × g for 15 min at 4 °C. The produced supernatant was recovered as slime. The solid fraction after decanting the supernatant was re-suspended into a pre-heated 0.05% (w/v) NaCl solution restoring the original weight and reaching a final temperature of 50 °C. After centrifugation at 15,000 × g for 10 min at 4 °C, the organic matter in the supernatant was recovered as LB-EPS. The pellet was re-suspended in a 0.05% (w/v) NaCl solution, restoring the original weight. The mixture was heated to 60 °C in a water bath for 30 min, while steering it at 400 rpm. Hereafter it was centrifuged at 15,000 × g for 15 min at 4 °C. The collected supernatant was regarded as TB-EPS.

2.3.2. SEPS extraction

The SEPS was isolated according to Felz et al. (2016): the aforementioned extracted EPS were dialyzed with a dialysis membrane (3.5K MWCO, SnakeSkin, Thermo Fisher Scientific, USA) for 24 h against 1000 mL MiliQ water. The pH of the dialyzed extracts was slowly adjusted to 2.2 ± 0.05 with 1 and 0.1 M hydrochloric acid. After these extracts were centrifuged at 4000 × g and 4 °C for 20 min, the supernatant was discarded and the gel-like pellet was considered to be the SEPS. A certain amount of SEPS pellet obtained from the total extractable EPS (total extractable SEPS) and from the stratified EPS (stratified SEPS) was used for the VS analysis to measure the content of the SEPS fraction in the sludge (wt %, g/100 g sludge), and the rest was freeze-dried and stored for further analysis.

2.3.3. Physicochemical analyses

VS, total suspended solid (TSS) and volatile suspended solid (VSS) were analysed according to standard protocols (APHA, 2005). To measure the polysaccharides and proteins content of total extractable SEPS, the freeze-dried SEPS from a sludge sample of 3 g (wet weight) was solubilized in 1 M NaOH to 50 mL reaching final pH of 7.5. The polysaccharide concentration was determined by phenol-sulfuric acid assay using glucose (Sigma, USA) as standard (Dubois et al., 1956) while the protein concentration was measured using a modified Lowry method with bovine serum albumin (Sigma, USA) as standard (Frolund et al., 1995). Although methods for quantification of polysaccharides and proteins in SEPS were not fully developed, the aforementioned methods are rapid and widely accepted and function as a relatively good estimator (Felz et al., 2019b). The polysaccharide and protein content in SEPS were then calculated based on the following equation:

$$\text{Content (wt \%, g / 100 g sludge)} = \frac{C_{\text{photometric method}} \times V_{\text{solution}}}{W_{\text{sludge}}} \times 100\% \quad (1)$$

where $C_{\text{photometric}}$ method = concentration of polysaccharides or proteins determined by photometric methods (mg/L); V_{solution} = volume of the solution (50 mL in this study); W_{sludge} = amount of the sludge sample (3 g in the current study).

Changes in dewaterability during AD of AGS and WAS (sludge-inoculum mixture) were detected by a capillary suction time (CST) apparatus (304M, Triton Electronics, England) with CST papers manufactured by the same company. A normalized CST (NCST) method was used to eliminate the influence of solid particles (Lu et al., 2015). Volatile fatty acids (VFAs) were measured by gas chromatography (GC), equipped with a flame ionization detector (FID) (Agilent 7890A, USA). Helium was used as carrier gas with a flow rate of 1.8 mL/min; the column (Agilent 19091F-112) was 25 m × 320 μm × 0.5 μm; injection port and oven temperatures were 240 °C and 80 °C, respectively. The particle size distribution (PSD) of AGS before and after AD process was analysed by a sieving method (Pronk et al., 2015), while that of WAS at Day 0 and 44 was measured by a particle size analyser (Bluewave, Microtrac, Germany).

2.3.4. FT-IR spectroscopy

The original and secondary derivative FT-IR spectra of total extractable SEPS (the freeze-dried solid) in potassium bromide (KBr) pellets were recorded in the 4000-500 cm^{-1} region by a FT-IR spectrometer (Cary 630, Agilent, USA).

2.3.5. 3D-EEM spectroscopy

The prepared solution for polysaccharide and protein measurements (Section 2.3.3) was also used to determine the 3D-EEM spectra of total extractable SEPS. These spectra were measured by a fluorescence spectrometer (Aqualog-UV-NIR-800-C, HORIBA, USA). Wavelength ranges for excitation and emission spectra were 280–550 nm and 240–400 nm, respectively. Both excitation and emission bandwidths were adjusted to 2 nm and the EEM signals were corrected by subtracting a blank (demineralized water). EEM fluorescence spectra were divided into four regions based on the differences of excitation-emission wavelengths of organic matters: aromatic protein-like, fulvic acid-like, soluble microbial by-product-like and humic acid-like regions (Table S1 in Supplementary material) (Sun et al., 2016). The fluorescence regional integration (FRI) technique was utilized to calculate the fluorescent intensities of each region in all EEM spectra (Chen et al., 2003).

2.3.6. Gel formation property

To test total extractable SEPS's gel-forming property, 10% (w/v) freeze-dried SEPS-miliQ solution was extruded into a cylinder mould (1 cm^3) and sealed on both sides with a dialysis membrane (MWCO of 3.5 kDa, Snakeskin, USA). The mould was then submersed into 3% (w/v) $CaCl_2$ solution for 24 h to produce a Ca^{2+}-SEPS hydrogel. The mechanical stiffness of the hydrogel was measured by dynamic mechanical analysis device (DMA7e, Perkin Elmer, USA) under the pressing force rate of 25 mN/min. The Young's modulus represented the mechanical strength of the hydrogel and was calculated by the slope of tensile stress and extensional strain obtaining from the test (Felz et al., 2019a).

2.3.7. Statistical analysis

Linear regression analysis for SEPS degradation was performed using R (R Core Team, Austria). Significant differences in biodegradability of SEPS, FT-IR spectrum and dewaterability between AGS and WAS were based on Student's t-test by means of SPSS Statistics 25 (IBM, USA). The significance level of probability (p-value) was 0.05.

3. Results and discussion

3.1. Batch anaerobic digestion of AGS and WAS

The difference in biodegradability of the sampled WAS and AGS was assessed. The characteristics of the substrate and inoculum are summarized in Table 1. The inoculum sludge was characterised by a specific methanogenic activity of 0.19 g CH_4-COD/g VS/day with acetate as the substrate.

To assess the degradation of the two sludge types, the accumulated methane production, VS and total VFAs were monitored during the anaerobic digestion. The results are presented in Table 2. The average accumulative methane production of AGS and WAS were both in line with prior researches (Guo et al., 2020; del Rio et al., 2014; Palmeiro-Sanchez et al., 2013). Besides, the average VS reduction of AGS and WAS was both in the typical range of 25–45% for sludge digestion (Bolzonella et al., 2005; Mottet et al., 2010). Specifically, the degraded VS was 0.69 ± 0.01 wt % for AGS, and was 0.77 ± 0.02 wt % for WAS, which showed that WAS had a higher degradation extent in VS, approximately 0.08 ± 0.01 wt %, than AGS. VFAs were detected during day 0–6 of the digestion, peaking at day 3 (values shown in Table 2).

The differences in accumulated methane production and the corresponded VS reduction of the two sludge types confirmed their dissimilarity in biodegradability (Table 2). To minimise the impact of surface area limitation of AGS compared to WAS, the biodegradability of crushed AGS and WAS were compared, revealing comparable differences in biodegradability extent (Guo et al., 2020).

It should be mentioned that the inoculum selected in this study was taken from a full-scale anaerobic digester that treats both primary sludge and WAS. However, del Rio et al. (2014) have proved that, after long-term acclimation with AGS as the sole substrate, the main microbial populations present were still those commonly found in digesters treating waste municipal sewage sludge. Thus, to our understanding, also this type of inoculum should be highly active and effective for AGS digestion within a period of 44 days.

3.2. Degradation kinetics of SEPS

Changes in the total extractable SEPS from AGS ($SEPS_{AGS}$) and WAS ($SEPS_{WAS}$) undergoing anaerobic digestion are shown in Fig. 1. The initial average SEPS contents in AGS and WAS (sludge-inoculum mixture) were 0.53 ± 0.01 and 0.49 ± 0.01 wt % respectively

Table 1
Characteristics of the substrate and inoculum.

Parameters	Substrate		Inoculum
	AGS	WAS	Digestate
pH	7.0 ± 0.2	7.1 ± 0.3	8.1 ± 0.4
Median particle size (μm)	1794 ± 121	114 ± 9	50 ± 5
TS concentration (wt %, g/100 g sludge)	5.41 ± 0.10	5.16 ± 0.08	3.30 ± 0.09
VS concentration (wt %, g/100 g sludge)	4.25 ± 0.07	4.14 ± 0.05	2.32 ± 0.03
VS/TS (%)	78.4 ± 0.7	80.0 ± 0.2	70.5 ± 0.2

Table 2
Comparison of the performance of the batch anaerobic digestion of AGS and WAS.

Sludge type (sludge-inoculum mixture)	Accumulated methane production (N-mL/g $VS_{substrate}$)	VS concentration			Maximum total VFA concentration (mg/L)
		Before AD (wt %, g/100 g sludge)	After AD (wt %, g/100 g sludge)	Removal efficiency (%)	
AGS	197 ± 11	2.71 ± 0.02	2.02 ± 0.01	25.4 ± 1.3	329 ± 21
WAS	242 ± 18	2.74 ± 0.01	1.97 ± 0.03	28.1 ± 1.1	388 ± 15

or 196 ± 6 and 179 ± 5 mg/g $VS_{sludge\ mixture}$, respectively. These results are in accordance with previous studies where aerobic granules represented a higher SEPS concentration than activated sludge (Lin et al., 2013; Sam and Dulekgurgen, 2016). However, the total removal efficiency of $SEPS_{AGS}$ (26%, from 0.53 ± 0.01 to 0.39 ± 0.01 wt %) was significantly lower (p-value = 0.038) than that from $SEPS_{WAS}$ (41%, from 0.49 ± 0.02 to 0.29 ± 0.01 wt %). It could be calculated that the difference in degraded SEPS between AGS and WAS (sludge-inoculum mixture), was 0.06 ± 0.01 wt %. Comparing this value with the removed VS content (0.08 ± 0.01 wt %), the result clearly reveals that the SEPS is the major organic matter that differentiates the biodegradability of AGS and WAS. $SEPS_{AGS}$ degradation started with a low rate from day 0–3, followed by a rapid degradation phase from day 3–21, and a slow degradation after day 21. $SEPS_{WAS}$ degradation occurred with a higher rate from the start of the experiment, also followed by a slow degradation phase after 21 days. The degradation rate during the rapid degradation phase of $SEPS_{WAS}$ and $SEPS_{AGS}$ were similar (respectively 0.006 and 0.005 g SEPS/100g sludge/d). Additionally, during the slower degradation phase, the SEPS degradation rates of WAS and AGS differed again, respectively 0.002 and 0.0003 g SEPS/100g sludge/d.

3.3. Variation of chemical composition and gel-forming stiffness of SEPS

In order to explain the differences in degradation rate and extent of the SEPS in WAS and AGS, the chemical and gel-forming properties of the total extractable SEPS fractions were analysed on day 1, 3 and 44 of the AD experiment.

3.3.1. Chemical composition

The composition and degradation of polysaccharides and proteins, within the extracted SEPS, are presented in Fig. 2. These two organic groups form the main constituents of SEPS (84% of $SEPS_{AGS}$ and 82% of $SEPS_{WAS}$). The removal efficiencies of the extracted polysaccharides and proteins in $SEPS_{WAS}$ were 62% and 33%, respectively, which were both higher than that of $SEPS_{AGS}$ (39% and 24%, respectively), during 44 days AD process. Specifically, polysaccharides and proteins in $SEPS_{WAS}$ presented remarkably faster biodegradation than that in $SEPS_{AGS}$ in the first three days. The results reveal that the extracted organic compounds in $SEPS_{WAS}$ can be more easily biodegraded in anaerobic digestion as compared to that in $SEPS_{AGS}$ and thus leads to a higher degradation extent of $SEPS_{WAS}$ (Fig. 1).

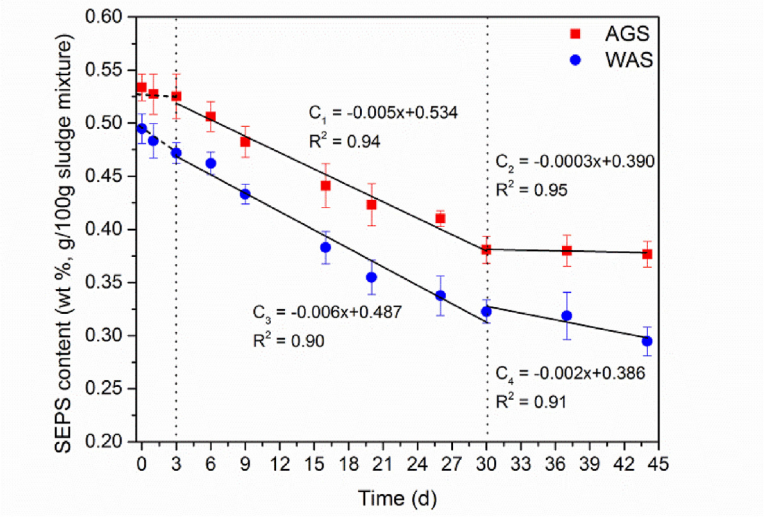


Fig. 1. Degradation kinetics of SEPS during anaerobic digestion of AGS and WAS.

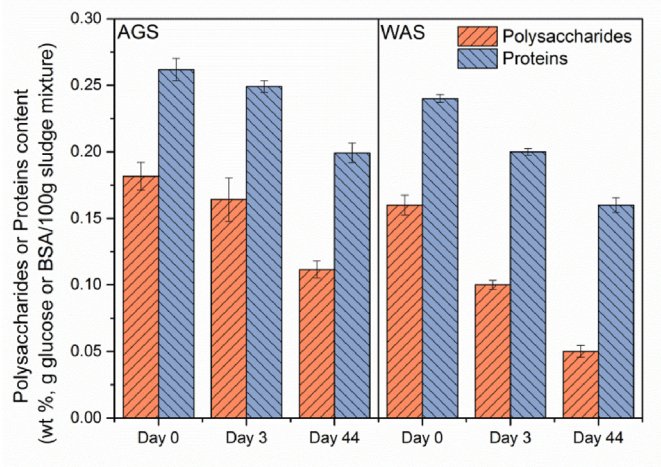


Fig. 2. Polysaccharides and proteins degradation of $SEPS_{AGS}$ and $SEPS_{WAS}$ during anaerobic digestion of AGS and WAS.

3.3.2. FT-IR analysis

To further investigate the changes in chemical structure of the SEPS during AD, FT-IR analysis was carried out (Fig. 3). The FT-IR results at wavenumbers 3000–1000 cm^{-1} for all SEPS samples displayed almost identical spectra with typical functional groups belonging to polysaccharides and proteins (Fig. 3a and Table S2 in Supplementary material) (Badireddy et al., 2010; Guo et al., 2016; Zhang et al., 2015). The biodegradation of polysaccharides and proteins of SEPS extracted from AGS and WAS, that was presented in Figs. 1 and 2, was thus unnoticeable in the FT-IR spectra. The presence of residual polysaccharide and protein fractions after AD, as shown in Fig. 2, resulted in the same FT-IR spectrum as before, since FT-IR only showed the presence of certain molecular structures and not its quantities.

The fingerprint (or anomeric) region at wavenumbers 950-700 cm^{-1} could exhibit a more detailed signal to identify the individual organic compounds of SEPS compared to other regions in FT-IR (Lin et al., 2010). Therefore, a second derivative spectrum of the extracted SEPS of AGS and WAS of this region was made (Fig. 3b). The second derivative spectrum enhances the signal, in order to be able to notice small shifts in the composition of the biomolecules during AD of both sludge types. In general, the change in position and number of the measured peaks of $SEPS_{AGS}$ after the first 3 days of anaerobic digestion was not statistically significant (p-value = 0.75), but a substantial difference (p-value = 0.034) was observed after 44 days of anaerobic digestion. However, for $SEPS_{WAS}$, the positions and number of peaks already changed during the first 3 days (p-value = 0.047). This result is consistent with the data indicating that the overall degradation of polysaccharides and proteins in $SEPS_{WAS}$ started before that in $SEPS_{AGS}$ (Fig. 2).

Particularly, peaks at 960, 770, 737 and 715 cm^{-1} could be assigned to C–C–C, C–O–C, and O–C–O stretching vibrations of glycoconjugates containing glucuronic acids (Tajmir-Riahi, 1984). For both types of SEPS, the position of these peaks remained unchanged during the anaerobic digestion. However, at the end of the digestion period (day 44), the 737 cm^{-1} peak of $SEPS_{AGS}$ was just lower, whereas the peak belonging to $SEPS_{WAS}$ disappeared completely, possibly indicating that the glycoconjugates in AGS were less biodegradable than that in WAS. Several researchers found that uronic acids such as glucuronic acids are important monomers predominating in the glycoconjugates of gel-forming EPS (Seviour et al., 2010; Felz et al., 2019b). For example, they can act as the building blocks in the formation of hyaluronic-like acid, a

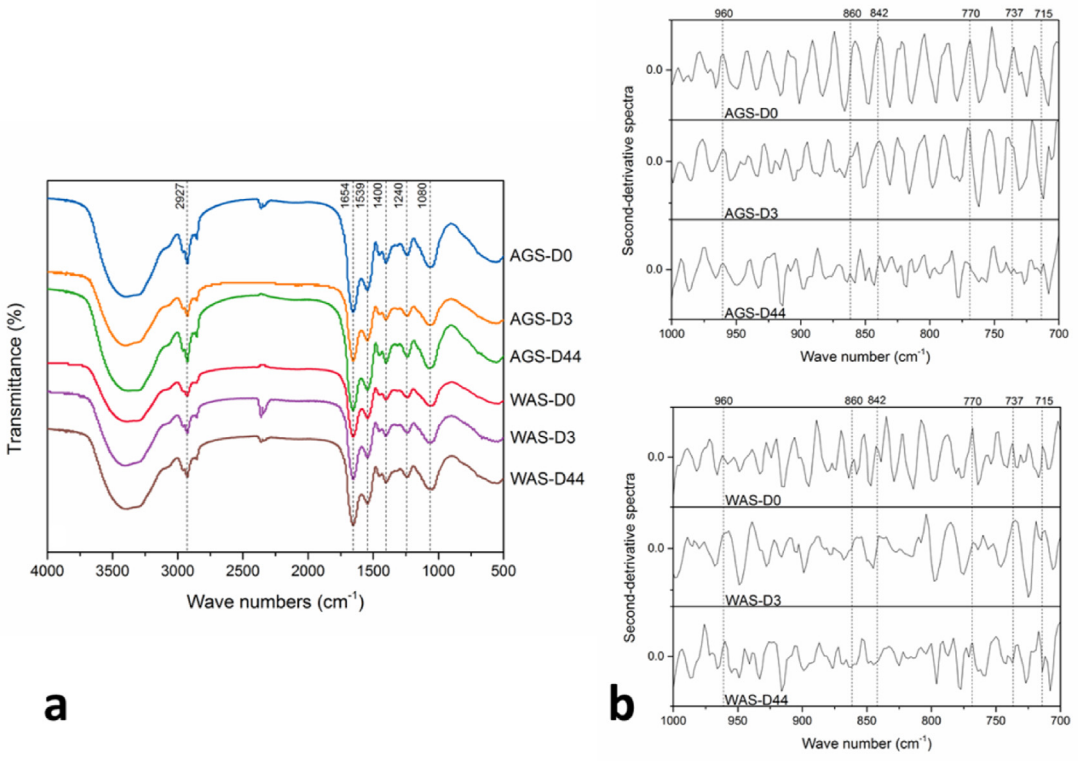
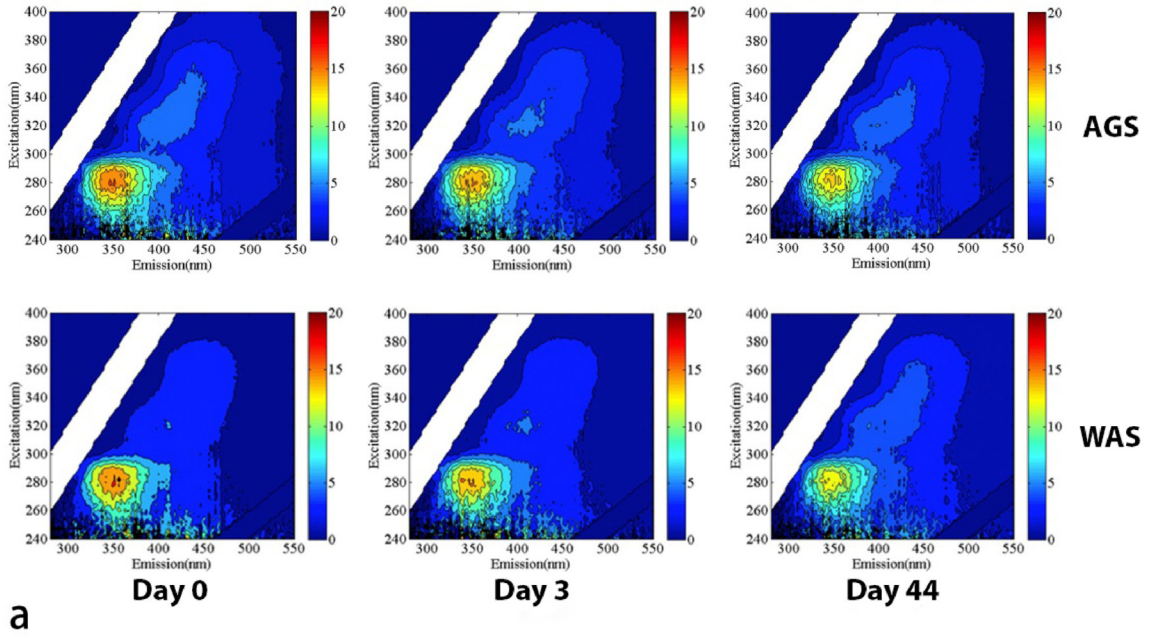


Fig. 3. FT-IR spectra of $SEPS_{AGS}$ and $SEPS_{WAS}$ during anaerobic digestion of AGS and WAS: (a) full-wavelength FT-IR spectra, and (b) second-derivative FT-IR spectra at the fingerprint region.



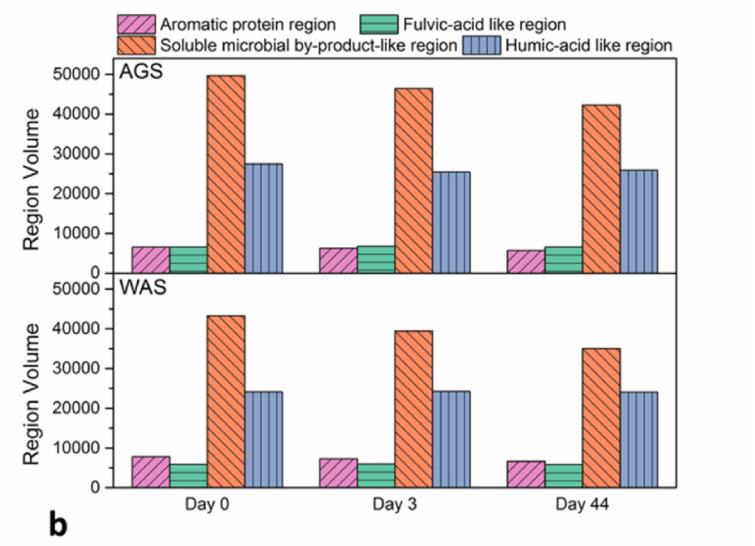


Fig. 4. (a) 3D-EEM spectra and (b) fluorescence intensity of each fluorescence region of $SEPS_{AGS}$ and $SEPS_{WAS}$ during anaerobic digestion of AGS and WAS.

type of extracellular substance that is capable of attracting cations and forming hydrogels (Felz et al., 2020). The characteristics of glucuronic acid-containing glycoconjugates in SEPS in both sludge morphologies are not fully understood: more research is required to understand the precise relation between the presence of glycoconjugates and anaerobic biodegradability of sludge.

Additionally, peaks at 860 and 842 cm^{-1} were observed in all SEPS samples. They could be related to the glycoconjugates containing galactose and mannose (Das et al., 2011; Sardari et al., 2017). Recent studies reported the existence of galactose and mannose in the EPS of AGS, which might contribute to the gel-forming property of AGS (Seviour et al. (2010) and Felz et al. (2019b)). The substantial presence of these monomeric units in the EPS from activated sludge suggested their importance in the aggregation of sludge flocs (Dignac et al., 1998). Interestingly, in this study, the glycoconjugate peaks for the $SEPS_{WAS}$ shifted to low wave numbers after 3 days, while that for the $SEPS_{AGS}$ samples remained unchanged even on day 44, indicating that $SEPS_{AGS}$ was slower degraded than $SEPS_{WAS}$, which implies that the composition of glycoconjugates in $SEPS_{AGS}$ and $SEPS_{WAS}$ is different.

3.3.3. 3D-EEM assays

3D-EEM spectroscopy was applied for characterizing the extracted SEPS at the different days of anaerobic digestion from AGS and WAS (Fig. 4). Compared to conventional photometric methods, EEM provides more information about the presence and concentration of fluorescent organics. These organic matters, such as protein-like substances (i.e., aromatic and tryptophan-like substance), are shown to contribute to the formation of the sludge structure via surface charge adjustment (Zhu et al., 2015). The 3D-

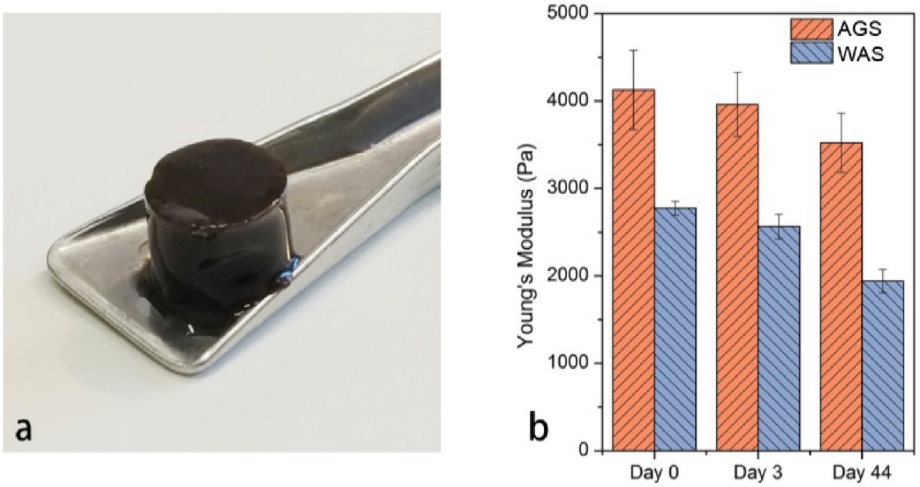


Fig. 5. (a) SEPS hydrogel formed with Ca^{2+}, and (b) changes of gel-forming property of $SEPS_{AGS}$ and $SEPS_{WAS}$ during anaerobic digestion of AGS and WAS.

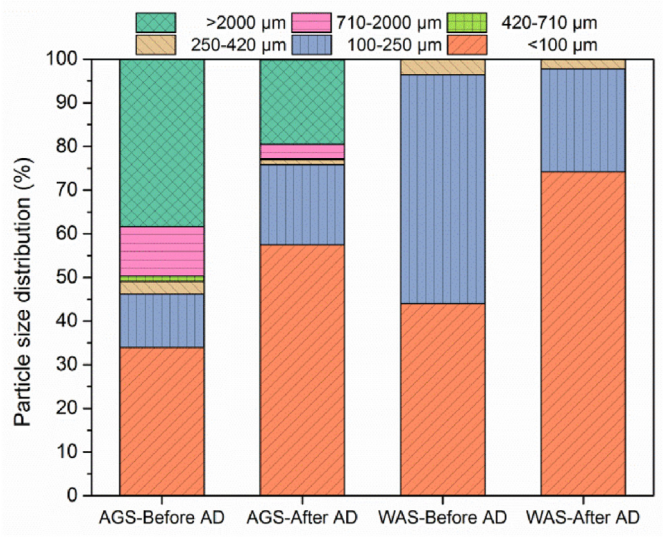


Fig. 6. Particle size distribution of sludge before and after anaerobic digestion of AGS and WAS.

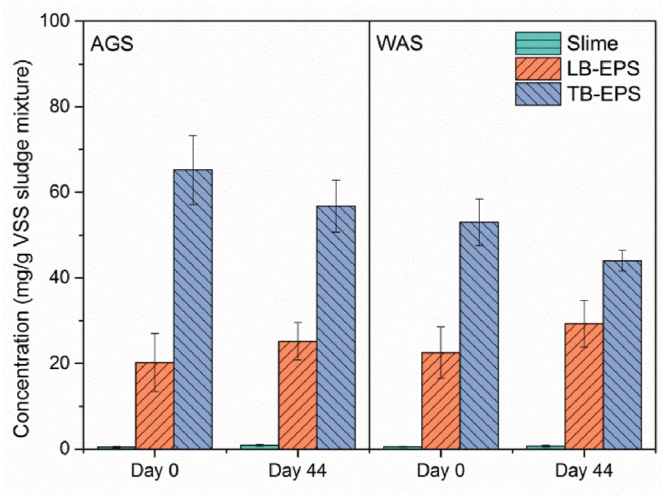


Fig. 7. The content of $SEPS_{AGS}$ and $SEPS_{WAS}$ in slime, loosely and tightly bound EPS before and after anaerobic digestion of both sludges.

EEM plots of four different regions with their corresponding possible substances are given in Table S1 in Supplementary material. Region III (soluble microbial by-products) had the highest fluorescence intensity, with its characteristic peak indicating the tryptophan & protein-like substances, identified at the excitation/emission wavelengths (Ex/Em) of 275–285/325–340 nm. Region IV (humic-like substances) revealed a signal with the characteristic humic-like substances peak observed at the Ex/Em of 345–355/425–440 nm. The results illustrate that irrespective of sludge type, tryptophan & protein-like, as well as humic acid-like substances were dominant among the organic matter with fluorescence characteristics in SEPS. These results were very similar as fluorescence spectra that were determined by Wang et al. (2009) for bound EPS extracted from activated sludge.

During digestion, the characteristic peaks belonging to the protein-like substance regions (Region I and Region III) were reduced, demonstrating degradation of protein-like substances. However, the total calculated reduction ratio of the fluorescence intensity of the protein-like substance regions at day 3 and day 44 compared to day 0 was 6.2 and 14.7% for $SEPS_{AGS}$ and 8.6 and 18.5% for $SEPS_{WAS}$, respectively. This is in accordance with the results obtained by the photometric method (Fig. 3). Reasons for the lower protein degradation efficiency in $SEPS_{AGS}$ can be two-fold. Firstly, the compact structure of AGS results in a lower specific surface area relative to activated sludge (Zheng et al., 2005). It is thus speculated that this may hinder the hydrolysis of structural extracellular proteins by protease. Secondly, as mentioned before, tryptophan & protein-like substances dominated among the protein-like substances. Li et al. (2014) reported that the tryptophan-like group was resistant to anaerobic digestion. In this study, the initial fluorescence intensity of the tryptophan-like substance region of $SEPS_{AGS}$ was higher than that of $SEPS_{WAS}$. The higher content of the tryptophan-like substance in $SEPS_{AGS}$ results in the lower overall degradation of proteins in $SEPS_{AGS}$, which is supported by the lower reduction in fluorescence intensity of Region III for $SEPS_{AGS}$ during AD process. In contrast to protein-like substance regions, the reduction in fluorescence intensity of Region IV (humic-like substances) over time was not observed, suggesting that these substances were refractory to anaerobic biodegradation, which is in line with results reported by Ghasimi et al. (2016).

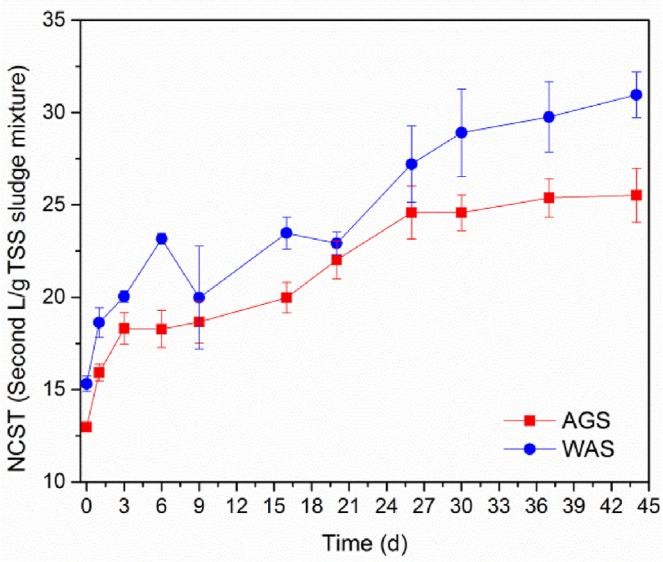


Fig. 8. Changes in normalized CST of sludge during anaerobic digestion of AGS and WAS.

3.3.4. Gel-forming strength

SEPS is known to be capable of forming gels with multivalent cations in a broad range of temperature and pH. The viscous and elastic characteristics displayed by this biopolymer gel when undergoing deformation are regarded as a parameter to indicate the mechanical property of SEPS (Lin et al., 2013). Ca^{2+} is one of the most common cations in wastewater. Ca^{2+}-SEPS (ionic hydrogel) could play an important role in building up the gel matrix structure in both activated sludge and aerobic granules (Felz et al., 2016; Lin et al., 2013). Therefore, to understand the influence of SEPS degradation on the gel stiffness, hydrogels formed from $SEPS_{AGS}$ and $SEPS_{WAS}$ cross-linked with Ca^{2+} were made (Fig. 5a) and subjected to a gel stiffness test. In Fig. 5b the decreased Young's modulus is shown, indicating the loss of gel-forming property and increased susceptibility for destruction of the mechanical structure during the AD process. The Young's modulus of $SEPS_{AGS}$ decreased by 14.6%, from 4126 ± 455 to 3522 ± 338 Pa in average, while the Young's modulus of $SEPS_{WAS}$ showed a much higher reduction of 30.1% (2773 ± 78 to 1939 ± 133 Pa in average), which indicates that the stiffness reduction during AD is much higher for $SEPS_{WAS}$ than for $SEPS_{AGS}$. It has been demonstrated that some functional groups of polysaccharides and proteins, such as carboxyl, hydroxyl, and amino acid groups, can be easily bridged with Ca^{2+} in the sludge matrix, resulting in the gel-forming ability of EPS (Felz et al., 2019b). In the present study, although strong peaks of these functional groups were found in all samples extracted during the AD process of SEPS (Fig. 3), these key organic fractions in $SEPS_{AGS}$ showed higher resistance to anaerobic degradation than that in $SEPS_{WAS}$, which eventually leads to the stronger gel-forming capacity in residual $SEPS_{AGS}$. This finding implies that the granular structure of AGS will likely sustain during digestion.

To prove this assumption, the shifts in particle size distribution of both types of sludge after 44 days of AD were determined and are shown in Fig. 6. Both AGS and WAS experienced deterioration of their particle sizes: the fraction particles larger than 100 μm decreased by 30% in WAS while it decreased only 23% in AGS. The fraction above 250 μm is defined as granules (de Kreuk et al., 2007). Although this fraction in AGS reduced by 30%, going from 54% to only 24% during AD, the fraction of big particles especially larger than 2 mm remained present in AGS after 44 days AD. This observation is in line with the differences in gel-forming properties of $SEPS_{WAS}$ and $SEPS_{AGS}$ as discussed above.

3.4. Structural changes of SEPS in EPS matrix

This study revealed that the structural morphology of AGS and WAS changed during the AD process. Besides the deterioration of sludge dewatering properties due to these structural changes (Lu et al., 2015; Novak et al., 2003), the gel-forming EPS matrix within the sludge also weakened, resulting in degradation and shifts between the different EPS binding strength. Therefore, the content of SEPS in slime, loosely bound-EPS (LB-EPS) and tightly bound-EPS (TB-EPS) before and after AD was determined for both types of sludge. As shown in Fig. 7, initially, the slime fraction of the SEPS was very low. The $SEPS_{TB\text{-}EPS}/SEPS_{LB\text{-}EPS}$ ratio was highest in the AGS, while both sludge types were dominated by TB-EPS, which was in line with previous studies claiming that TB-EPS plays an important role in maintaining the matrix structure of both aerobic granular sludge and activated sludge (Chen et al., 2010; Yuan et al., 2014).

Even though the content of SEPS in TB-EPS remained high, a shift in SEPS from TB-EPS to LB-EPS was observed during AD: The $SEPS_{LB\text{-}EPS}$ fraction increased by 23% and 25% for AGS and WAS respectively, whereas the $SEPS_{TB\text{-}EPS}$ fraction decreased with 13% and 20%, respectively (Fig. 7). This transition is likely due to the loss of binding capacity between SEPS and cations as a result of enzymatic hydrolysis (Yu et al., 2010). In contrast, the slime fraction of SEPS remained very low, indicating that the SEPS that shifted from the LB-EPS to the slime fraction during AD might have been converted to methane during this process.

The presence of LB-EPS and TB-EPS are reported to exhibit different sludge dewaterability (Chen et al., 2010; Yuan et al., 2014): deterioration of dewaterability during AD is associated

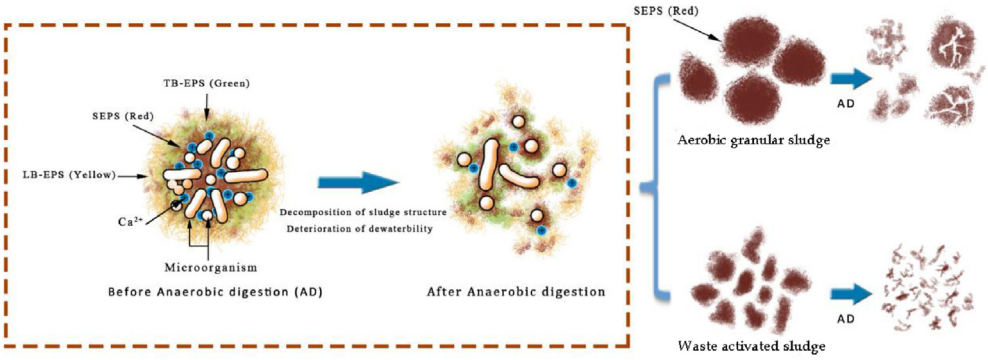


Fig. 9. SEPS degradation mechanism in anaerobic digestion of AGS and WAS.

with a shift of polymers from TB-EPS to LB-EPS (Ye et al., 2014). In the current study, a substantial difference in CST was observed between AGS and WAS at different moments during AD (Fig. 8), which could be explained by the larger SEPS shifts in WAS than in AGS during AD. We used the normalized CST method as an indicative analysis for the sludge dewaterability. Although this method has been accepted by several researchers to characterise dewaterability, it might not be the optimal method to study dewaterability of AGS: the CST of AGS is usually very short (16–19 s) (Basuvaraj et al., 2015; Lotito et al., 2014), because the free water around the granules is loosely bound. However, the water that is captured within the granules is not released during a CST measurement and therefore CST might not represent the overall dewaterability of AGS. In the current study, the AGS and WAS were both mixed with digestate: the latter determined the overall suction time, which was much higher than that of sole AGS and WAS. The dewaterability of digestate is determined by the particle size distribution and the polymer fraction in the matrix. Therefore, in this research it is reasonable to compare the dewaterability of AGS and WAS (sludge-inoculum mixture) by CST as a qualitative parameter.

3.5. SEPS degradation mechanism and implications

In the anaerobic degradation of both sludge types, considering the observed changes in SEPS, the following mechanism is proposed for SEPS degradation (Fig. 9): before the AD process, the SEPS was mainly aggregated in TB-EPS and exhibited a strong gel-forming capacity with cations such as Ca^{2+}. Nevertheless, AD negatively influences the gel-forming strength of the Ca^{2+}-SEPS hydrogel, due to the degradation of key polysaccharides and proteins and related to this, due to the transition of SEPS from TB-EPS to LB-EPS. The lower degradation of $SEPS_{AGS}$ compared to $SEPS_{WAS}$, resulted in a higher residual SEPS fraction in the AGS structure along the entire AD process, leading to a lower overall biodegradability of AGS compared to WAS.

VS removal in both types of sludge during anaerobic digestion was 25–30% and therefore relatively low. Considering that only 25–40% of SEPS can be biodegraded, a possible strategy for enhancing the biodegradability of sludge, especially AGS, could be the acceleration of the decomposition or degradation of the SEPS structure. This could be done through destructive mechanical methods, such as crushing or ultrasound treatment, in combination with the addition of specific enzymes targeting the SEPS polymers. It can be speculated that this pre-treatment would liberate biodegradable compounds of the SEPS matrix, leading to enhanced methane production. Besides, the gel-forming SEPS are useful biopolymers in industrial applications (Lin et al., 2015), and recovery of SEPS from AGS is studied at pilot scale (Kaumera®, Royal HaskoningDHV, The Netherlands). The extraction process for SEPS can be regarded as an effective pre-treatment method to entirely destroy the sludge matrix and remove the non-easily biodegradable SEPS polymers from the sludge simultaneously. The methane production after SEPS extraction and the application potential of "SEPS extraction + AD" for AGS treatment requires further investigation.

4. Conclusions

SEPS isolated from AGS and WAS followed similar degradation steps: (1) degradation of polysaccharides and proteins, (2) reduction of hydrogel stiffness, (3) transition of SEPS from TB-EPS to LB-EPS to slime and (4) further conversion of the slime fraction to methane. $SEPS_{AGS}$ revealed a lower degradation rate and extent than $SEPS_{WAS}$, in combination with a lower reduction in mechanical stiffness upon AD, owing to the lower degradation efficiency of key organic fractions. The perseverance of $SEPS_{AGS}$ resulted in an undisrupted residual structure of AGS after AD. In conclusion, it can be claimed that SEPS is a major constituent that resulted in the distinct digestibility between AGS and WAS in AD.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2020.115924.

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