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Digestibility of waste aerobic granular sludge from a full-scale municipal wastewater treatment system

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ABSTRACT

Full-scale aerobic granular sludge technology under the trade name Nereda® has been implemented for municipal, as well as industrial wastewater treatment. Owing to the operational reactor procedures, two types of waste aerobic granular sludge can be clearly distinguished: 1) aerobic granular sludge selection discharge (AGS-SD) and 2) aerobic granular sludge mixture (AGS-RTC). This study systematically compared the anaerobic biodegradability of AGS-SD and AGS-RTC under mesophilic conditions. Results were further compared with the anaerobic conversion of waste activated sludge (WAS) as well as primary sludge (PS) from full-scale municipal wastewater treatment plants. Analysis showed similar chemical characteristics for AGS-SD and PS, which were both characterized by a high carbohydrate content (429 ± 21 and 464 ± 15 mg glucose/g VS sludge, respectively), mainly cellulose fibres. Concurrently, AGS-RTC exhibited chemical properties close to WAS, both characterized by a relatively high protein content, which were individually 498 ± 14 and 389 ± 15 mg/g VS sludge. AGS-SD was characterized by a high biochemical methane potential (BMP) (296 ± 15 mL CH4/g VS substrate), which was similar to that of PS, and remarkably higher than that of AGS-RTC and WAS. Strikingly, the BMP of AGS-RTC (194 ± 10 mL CH4/g VS substrate) was significantly lower than that of WAS (232 ± 11 mL CH4/g VS substrate). Mechanically destroying the compact structure of AGS-RTC only accelerated the methane production rate but did not significantly affect the BMP value. Results indicated that compared to WAS, the proteins and carbohydrates in AGS-RTC were both more resistant to anaerobic bio-degradation, which might be related to the presence of refractory microbial metabolic products in AGS-RTC.

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1. Introduction

Conventional activated sludge wastewater treatment systems (CAS) have been widely applied in the treatment of many types of wastewater for decades. However, large quantities of waste sludge, i.e. primary (PS) and waste activated sludge (WAS), are being produced during this process, which is regarded problematic owing to its environmental risk and high cost for treatment and disposal (Appels et al., 2008). A relatively new biological treatment for wastewater is the aerobic granular sludge (AGS) or Nereda® technology which is applied at full scale since 2005 (Giesen et al., 2013). Currently, there are over than 70 Nereda® wastewater treatment plants in operation or under construction worldwide (https://www.royalhaskoningdhv.com/nereda). It’s main advantages, i.e. a smaller process footprint, quicker sludge settling and reduced energy demand, resulted in a rapid market acceptance from promising innovation to a mature technology, capable of competing with established conventional wastewater treatment technologies (de Kreuk et al., 2007; Pronk et al., 2015). The biomass yield, represented by the mass of sludge produced over the mass of organic matter (COD or BOD) consumed, appears to be similar for aerobic granules and activated sludge (AS) given the same conditions (Nancharai and Reddy, 2018). As granular sludge processes are typically operated at relatively long solid retention times (SRTs), the sludge production of this process would be lower than that of CAS in this sense. However, the waste sludge of AGS installations is not yet separately processed. With the increasing number and size of Nereda® installations, strategies for the efficient management of generated waste sludge from AGS systems (WAGS) are required.

Anaerobic digestion (AD) has been commonly used in the treatment of PS and WAS with the purpose of organics reduction and energy recovery in the form of biogas. Similarly, AD is a potential option for WAGS treatment. However, to our best
knowledge, the anaerobic digestion of WAGS has been limitedly studied (Bengtsson et al., 2018). The anaerobic biodegradability of WAGS with large granules (larger than 1.6 mm) has been investigated in batch and continuous AD systems (Bernat et al., 2017; del Rio et al., 2011; del Rio et al., 2014; Palmeiro-Sanchez et al., 2013). Most studies merely reported the biochemical methane potential (BMP) in mL CH\textsubscript{4} per g VS substrate of WAGS, but lack information on hydrolysis rate coefficients or degradation efficiencies of its key organic fractions. Therefore, a direct comparison of the biodegradation kinetics of WAGS with the better understood WAS degradation is not yet possible. Furthermore, the BMP results of WAGS are often inconsistent between studies, which can be attributed to the highly biodegradable, often synthetic or industrial, influent used to grow these granules in a well-controlled laboratory or pilot scale system. The operational conditions of AGS systems and its feeding characteristics significantly influence the physiochemical and morphological properties of WAGS, and thus may affect their degradation behaviour in AD (Bernat et al., 2017; de Kreuk et al., 2010).

So far, the granular sludge based Nereda\textsuperscript{®} technology has been mainly applied in municipal wastewater treatment (https://www.royalhaskoningdhv.com/nereda). Municipal wastewater typically has a much lower chemical oxygen demand (COD) concentration than industrial wastewater and has more complex substrates and a higher suspended solids concentration than synthetic influent used in laboratory experiments (Moy et al., 2002). Therefore, there is a great interest about the actual anaerobic biodegradability of the WAGS produced in full scale AGS systems treating municipal sewage. Two types of WAGS can be distinguished: (1) sludge that is removed every cycle, which we call the aerobic granular sludge selection discharge, or selection spill (AGS-SD). This is the more flocculent sludge with a lower settling velocity than the aerobic granules. By removing this sludge, a biological selection pressure is applied towards faster settling granules. Because it is removed every cycle, this waste aerobic granular sludge has had a lower retention time than the granules (Ali et al., 2019); (2) The excess granular sludge that originates from biomass growth and that is removed to avoid too high biomass concentrations in the reactor, the so called solid retention time control of the AGS (AGS-RTC).

Therefore, this study systematically assessed (1) the characteristics and anaerobic biodegradability of AGS-SD and AGS-RTC as well as (2) the differences in physicochemical property and biodegradability of these fractions with PS and WAS. BMP tests were conducted under mesophilic conditions to compare solids reduction, methane yield, hydrolysis rate coefficient, as well as the biodegradability of carbohydrates, proteins, lipids and lignocelluloses in all types of sludge. These results lead to an improved understanding of the biodegradation of WAGS in AD and are helpful to design and effectively operate AD systems for WAGS treatment at full-scale implementations.

2. Materials and methods

2.1. Substrates and inoculum

AGS-SD and AGS-RTC were collected from a full-scale municipal wastewater-fed Nereda\textsuperscript{®} system (Garmerwolde, The Netherlands), which has a treatment capacity of 91,583 population equivalent (p.e.). At the time when both types of sludge were sampled, the Nereda\textsuperscript{®} reactor was operated with a process cycles of approximately 6 h; 4 h of aeration, 1 h of settling, 1 h of anaerobic feeding/ simultaneous effluent withdrawal, and 15 min of excess sludge discharge. The excess sludge was stored in a sludge buffer tank before being transported to the on-site sludge treatment facilities. AGS-RTC was withdrawn at the end of the aeration phase under fully mixed conditions, whereas AGS-SD was collected from the sludge buffer tank. Wastewater treatment plant (WWTP) Garmerwolde has 6 mm screens, followed by grit removal, where after the influent is stored for 3.4 h in a mixed influent buffer. There is no primary settler (Pronk et al., 2015) and the influent from the buffer tank is directly fed to the Nereda\textsuperscript{®} tanks. WAS and anaerobic inoculum were sampled from an enhanced biological phosphate removal (EBPR) activated sludge tank under fully aerated and mixed conditions and an anaerobic sludge digester at WWTP Harnaschpolder (Den Hoorn, The Netherlands), respectively. The latter WWTP is designed for a 1.3 million p.e., and is equipped with 6 mm screens, sand and grit removal, followed by a primary clarifier. Primary sludge (PS) was sampled from this primary clarifier. The inoculum characteristics were pH 8.1 ± 0.4, total solids (TS) 3.3 ± 0.09 wt% and volatile solids (VS) 2.32 ± 0.03 wt%. All types of sludge were stored at 4°C for a maximum of 24 h to prevent acidification. Cellulose (microcrystalline powder, Sigma Aldrich, USA) was used as model substrate for the positive control of the BMP tests. Wastewater characteristics and operational parameters of both WWTPs are shown in Table 1.

2.2. Anaerobic batch BMP test

Batch BMP tests of sludge were conducted in quadruplicates by using an automated methane potential test system (AMPTS) (Bio-process Control, Sweden) with 500 mL serum bottles. One of the serum bottles was sacrificed for VFA concentration measurements. The recipes and dosages for phosphorus buffer solution, macronutrients, and trace elements were according to Zhang et al. (2014). The volume of the mixture of inoculum and substrate was 300 mL. The ratio of VS (g) of inoculum to VS (g) of substrate was 2 in the batch bottles. VS concentrations of different types of sludge were manipulated by centrifugation (5 min at 3,500 × g). In order to explain whether the morphology of the sludge affect the biodegradability, the structures of AGS-RTC and WAS were destroyed by crushing the sludge with a household blender (HR2052/90, Philips, The Netherlands) for 5 min at 10,000 RPM and 450 W.

2.3. Biochemical methane potential tests modelling

The hydrolysis rate coefficient (k) and biochemical methane potential (B\textsubscript{0}), two key parameters associated with methane production from the sludge (Gonzalez et al., 2018), were used to evaluate and compare methane production kinetics and BMP values between different types of sludge. To analyse the data, a two-substrate model, consisting of a rapidly biodegradable substrate and slowly biodegradable substrate, developed by Rao et al. (2000) was used:

$$B_t = B_{0,\text{rapid}}\left(1 - e^{-k_{\text{rapid}}t}\right) + B_{0,\text{slow}}\left(1 - e^{-k_{\text{slow}}t}\right)$$

where $B_{0,\text{rapid}}$ = biochemical methane potential of the rapidly biodegradable substrates (mL CH\textsubscript{4}/g VS substrate); $k_{\text{rapid}}$ = hydrolysis rate coefficient of the rapidly biodegradable substrates (1/d); $B_{0,\text{slow}}$ = biochemical methane potential of the slowly biodegradable substrates (mL CH\textsubscript{4}/g VS substrate); $k_{\text{slow}}$ = hydrolysis rate coefficient of the slowly biodegradable substrates (1/d).

The simulation of accumulated methane production by the two-substrate model was implemented in MATLAB R2016b (MathWorks, USA).
Injection temperature was 240°C and Helium was used as carrier gas with a flow rate of 1.8 mL/min. A digital microscope (VH-Z20UR, Keyence, Belgium) was used to identify the morphology of the sludge. Principal component analysis (PCA) for investigating the difference in digestibility between sludges was performed by MATLAB R2016b (MathWorks, USA). For the statistical analysis, the Student’s t-test (for two groups of samples) and one-way ANOVA (for multiple groups of samples) were both applied with SPSS Statistics 25 (IBM, USA) to evaluate the significance of differences in BMPs and chemical characteristics between sludges. The significance level of probability (p-value) was 0.05 in this study.

3. Results and discussion

3.1. Sludge characteristics

Particle size distribution of each type of sludge was measured to identify the physical difference between the types of sludge (Fig. 1). It is found that the AGS-RTC sample was dominated by particle sizes larger than 500 μm, i.e. >90% of the total particles, which was clearly different from the other sludge samples. On the contrary, AGS-SD was composed of particle sizes below 500 μm, and therefore showed great similarity with the particle size of WAS. This observed difference in morphology agrees with the results observed by Pronk et al. (2015). In addition, a small fraction of particles in the 500–2000 μm range was observed in PS, likely resulting from settling in the primary sedimentation tank. The distribution of biochemical components of each sludge sample is presented in Table 2. The VS concentrations of all sludge types were adjusted to about 5% by centrifugation, in order to minimize the effect of different substrate concentrations (in VS) on their methane potential (Wang et al., 2015). The used VS concentration is similar to the 3–6 wt% range that is usually fed to full-scale digesters (WWTP Harnaschpolder, The Netherlands). However, the original VS concentrations of sludge extracted from the different flows were different, namely 0.9 ± 0.02, 0.4 ± 0.01, 0.6 ± 0.01 and 3.3 ± 0.03 wt% for AGS-RTC, AGS-SD, WAS and PS, respectively.

Key organic fractions analysed were protein, carbohydrates, lipids and VFAs, since they are considered as the most pertinent indicators in assessing and predicting the anaerobic biodegradability of sewage sludge mixtures (del Rio et al., 2011; Mottet et al., 2010). Results show that proteins and carbohydrates predominated in all types of sludge, representing in total 58–73% of the organic matter (Table 2), which are within the typical range reported for sewage sludge (del Rio et al., 2011; Gonzalez et al., 2018). However, the ratio of proteins and carbohydrates differed. Both AGS-SD and PS showed a much lower protein/carbohydrates ratio than AGS-RTC and WAS. AGS-SD contained a similar carbohydrate content as PS, which was almost double the carbohydrate content in AGS-RTC and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nereda® plant Garmerwolde a</th>
<th>Activated sludge plant Harnaschpolder b</th>
<th>Process parameter</th>
<th>AGS plant AS plant</th>
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<td>Unit</td>
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<td>Effluent mg/L</td>
<td>Load kg/kg TSS/d</td>
<td>Influent mg/L</td>
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<td>TSS</td>
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<td>0.02</td>
<td>58</td>
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<tr>
<td>TP</td>
<td>7.2</td>
<td>0.7</td>
<td>0.003</td>
<td>7.7</td>
</tr>
</tbody>
</table>

a The data was obtained from WWTP Garmerwolde, The Netherlands.

b The data was obtained from WWTP Harnaschpolder, The Netherlands.

c This is an average SRT. The WAGS fraction can have SRT as low as 4 days, whilst the largest granules can be maintained over 150 days due to their settling capabilities (Ali et al., 2019).
WAS. The protein content in AGS-SD was slightly higher than that in PS, but still about 30% lower than that in AGS-RTC and WAS. A large protein fraction in both AGS-RTC and WAS was expected, since these sludges are mainly composed of cells, (exo-?) enzymes and microbial metabolic products (Adav et al., 2008; Gonzalez et al., 2018). Different studies also reported that the proteins/carbohydrates ratio of the extracellular polymeric structures for AGS was usually higher than that for AS (McSwain et al., 2005; Zhu et al., 2018). Different studies also reported that the proteins/carbohydrates ratio of AGS-SD indicates that this sludge fraction is considerably different from the AGS-RTC with big granules. It is known that, in contrast to AS, PS is rich in fibres and contains much less microbial related organics, resulting in the high carbohydrates’ fraction in this type of sludge (Bernat et al., 2017). The high abundance of fibres in AGS-SD has been observed before (Prøn et al., 2015) and agrees with the analyses performed in this study (Fig. 2 c and d).

To verify the fibrous content, composition, cellulose, hemi-cellulose, and lignin contents in all sludge samples were determined (Fig. 3). The used Van Soest method was originally developed for the assessment of fibre-rich materials of plant origin. Therefore, when the Van Soest method is applied for sewage sludge characterization, the resulting fractions are commonly referred to as “like-“ fractions (Mottet et al., 2010; Wu et al., 2015). Fig. 3 shows that the content of lignin-like and hemi-cellulose-like fractions was approximately 9% and 15% of the VS on average, respectively, similar for all sludge samples. The concentration of the cellulose-like fibres in AGS-SD and PS was similar, i.e. 15% and 18% of the total VS on average, respectively. This was almost twice the value in AGS-RTC and WAS, which was 7% and 9% of the total VS on average, respectively. The result was supported by the microscopic examinations presented in Fig. 2, confirming that this organic fraction is one of major components of carbohydrates in AGS-SD.

It needs to be mentioned that the sum of hemicellulose and cellulose in the WAS and AGS-RTC fractions was larger than the total carbohydrates measured (Fig. 3 and Table 2), even though these two fractions are part of the total carbohydrate fraction. This could be caused by the interference of sludge components and the detergents used in the Van Soest method. For example, divalent cations from the sludge matrix could form complexes with the ethylendiaminetetraacetic acid (EDTA) used in the hemicellulose extraction step, resulting in an overestimation of the hemicellulose content. Mottet et al. (2010) indicated that the colorimetric method for direct carbohydrates determination, showed a higher reliability than the Van Soest fractionation. Therefore, the fibre fractionation in this study should only be used as an indicator of the differences between the sludge types, rather than a measure of the actual carbohydrate concentration.

Cellulose embodies between 30 and 50% of the suspended solids in the wastewater of western countries (STOWA, 2010). In conventional sewage treatment, cellulose is partly removed in the primary clarifier, being included in the PS (Champagne and Li, 2009), as is shown in this study as well. Cellulose entering the activated sludge tanks will be (partly) degraded (Ahmed et al., 2019). The Nereda® plant at Garmerwolde receives raw sewage without primary clarification. It is hypothesized that the large suspended solids, like the observed fibres, are not likely to interact with the fully developed compact granules and thus will stay part of the more flocculent fraction that is often observed with the AGS matrix (Prøn et al., 2015). As a result, sludge stratification takes place during settling phase. Together with the selection pressure applied in granular sludge systems to retain large granules, the non-granules fraction, including the mentioned cellulose fibres, can be washed out during the sludge selection discharge.

3.2. Methane production and degradation kinetics of sludges in BMP tests

The different biomass composition, as well as the different degree of stabilization, will likely lead to a different extent and rate of anaerobic digestion. This assumption was verified in a BMP test of which the results are presented in Fig. 4. The BMP of the positive control was 357 mL CH4/g VS on average, which fulfilled the criteria stated by Holliger et al. (2016). Low amounts of total VFAs were detected during the first 6 days of digestion of the four sludge samples, with a maximum concentration of 332, 355, 421 and 402 mg/L at day 3, for AGS-RTC, WAS, AGS-SD and PS, respectively.

The inoculum applied for all batch tests was obtained from the full-scale digester at Harnaschpolder WWTP, treating WAS and PS and was not specifically adapted to the WAGS fractions. del Rio et al. (2014) showed that after long-term acclimation with AGS as sole substrate, the main microbial populations presented were still those commonly found in digesters treating waste municipal sewage sludge. Therefore, the inoculum used was considered similarly effective for all sludge samples tested.

Results clearly show the lowest BMP value for AGS-RTC, namely 194 mL CH4/g VS substrate. This was significantly lower (p-value = 0.01) than the 243 mL CH4/g VS substrate reported for AGS grown in a pilot scale reactor, fed with synthetic domestic wastewater (del Rio et al., 2011). It should be noted that the organic loading rate to the Nereda® in Garmerwolde was higher than that of the activated sludge plant, i.e. 0.20 and 0.16 g COD gTSS/d, respectively, and the COD content of the AGS-RTC was somewhat higher than that of the WAS (Table 2). Nonetheless, the BMP value of AGS-RTC was remarkably lower (p-value = 0.02) than that of WAS. Our results agree with those of Bernat et al. (2017) and suggest an inherently lower anaerobic biodegradability of the aerobic granules compared to activated sludge flocs.

The methane production curves (Fig. 4) could be well fitted with the two-substrate model (Eq. (1), Fig. 4 - dotted line, R² > 0.99 in all studied cases). Results suggest that all biomass samples consisted of a specific fraction that was rapidly biodegradable and one that was more slowly biodegradable. The estimated values of krapid, B0, rapid and kslow, B0, slow for all types of sludge are shown in Table 3.
and demonstrate that AGS-RTC presented a significantly lower $k_{\text{rapid}}$ (p-value $= 0.002$) and $k_{\text{slow}}$ (p-value $= 0.04$) than WAS. This calculation was based on the methane production rate, whereas the VFA production was excluded. Since VFA was detected in the first 6 days, the k-values do not fully represent the hydrolysis rates. However, compared to WAS, the assessed VFA values in AGS-RTC were lower as well, so it can be concluded that the AGS-RTC’s hydrolysis rate was lower than that of WAS. The AGS-RTC used in this study mainly consisted of large granules (Figs. 1 and 2), which limits the surface area to volume ratio. Since the hydrolysis rate is surface proportional (Angelidaki and Sanders, 2004; Sanders et al., 2000), the overall AGS-RTC digestibility might be determined by the morphological structure of the granules.

To clarify the influence of morphology over sludge composition on anaerobic digestion, AGS-RTC and WAS were both mechanically crushed to destroy the physical structure and to achieve a similar particle size distribution (Fig. 3). Results from Fig. 4 and Table 3 show that the BMP of the crushed sludges was similar as the non-crushed ones. The methane production rate, however, showed different behaviour for the two sludge types: all parameters for WAS did not change remarkably (p-value $> 0.05$) by crushing, revealing that the digestion of WAS was not affected by the sludge crushing. Yet AGS-RTC showed a significant increase (p-
value = 0.005) in methane production rate from the rapid convertible fraction ($k_{\text{rapid}}$ and $B_{0, \text{rapid}}$). The values found after crushing of AGS-RTC even approached the values of the rapid digestible fraction of crushed WAS. Kinetics of the slowly biodegradable fraction did not statistically change, although a small shift from $B_{0, \text{slow}}$ to $B_{0, \text{rapid}}$ could be seen (Table 3). This means that destroying the structure of the aerobic granules and increasing its surface area could indeed accelerate the degradation rate of rapidly degradable organics, which was in agreement with the observation found by del Rio et al. (2014) who applied thermal pretreatment to granules. In addition, crushing liberated a fraction of slowly degradable organics to be degraded more rapidly. It should be noted that the total BMP ($B_{0, \text{total}}$) of crushed granules was similar to that of the intact AGS-RTC (Fig. 4 and Table 3), indicating that the sludge structure only limits digestion rate, but not the overall digestibility. The results imply that in practice, sludge pretreatment of the AGS-RTC fraction prior to anaerobic digestion could enhance digester performance.

The BMP of AGS-SD, was just slightly lower than that of PS, and remarkably higher than those of AGS-RTC (p-value = 0.001) and WAS (p-value = 0.005). Also, the methane production rate, i.e. both $k_{\text{rapid}}$ and $k_{\text{slow}}$ of both AGS-SD and PS were distinctly higher (Table 3). Considering the differences in VFA production in combination with the methane production rate, it can be stated that also hydrolysis rates of the AGS-SD and PS are higher than the hydrolysis rates measured for AGS-RTC and WAS. Our present results confirm the high degree of stabilization of AGS-RTC and the low stabilization degree of the more flocculent AGS-SD fraction, supporting the speculations that were reported by Pronk et al. (2015). Apparently, compared to AGS, AGS-SD is characterized by a much lower SRT in the Nereda® tank (Ali et al., 2019), resulting in a much higher BMP.

It should be mentioned that in this study, the tested AGS-SD was taken from a Nereda® reactor with an operational cycle of 6 h in total, which is a common process setting applied in full scale municipal wastewater-fed Nereda® systems (Stubbé, 2016). However, the schedule of the process cycle sometimes changes due to other factors, such as the weather condition (Pronk et al., 2015). It is worth to investigate in future studies if changes of operational parameters of the Nereda® reactor could affect the characteristics and the digestibility of the different WAGS fractions, and its overall digestibility.

### 3.3. Degradation of organic components in sludges during BMP tests

To further elucidate the differences in anaerobic biodegradation of the different sludge types, the main organic fractions in the wet sludge mixtures were analysed at the start and the end of the BMP tests (Table S in Supplementary materials). The converted fractions during the BMP test are shown in Fig. 5. One should take into account that the fractions are presented per 100 g wet sludge mixture, with initial sludge mixture VS concentrations around 2.7 wt% (Table S in Supplementary materials). The COD values used for the calculations were 1.5 g-COD/g proteins, 1.07 g-COD/g carbohydrates, 2.88 g-COD/g lipids, 1.08 g-COD/g acetate and 1.53 g-COD/g propionate (Sum of acetate and propionate represents the total VFAs in this study) (Filipe and Grady, 1998).

### Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>AGS-RTC</th>
<th>AGS-RTC-Crushed</th>
<th>WAS</th>
<th>WAS-Crushed</th>
<th>AGS-SD</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{rapid}}$</td>
<td>1/d</td>
<td>0.41 ± 0.02</td>
<td>0.51 ± 0.01</td>
<td>0.54 ± 0.01</td>
<td>0.54 ± 0.02</td>
<td>0.61 ± 0.01</td>
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</tr>
<tr>
<td>$B_{0, \text{rapid}}$</td>
<td>mL CH4/g VS substrate</td>
<td>140 ± 6</td>
<td>152 ± 5</td>
<td>175 ± 6</td>
<td>179 ± 8</td>
<td>215 ± 4</td>
<td>208 ± 8</td>
</tr>
<tr>
<td>$k_{\text{slow}}$</td>
<td>1/d</td>
<td>0.07 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.01</td>
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<tr>
<td>$B_{0, \text{slow}}$</td>
<td>mL CH4/g VS substrate</td>
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<td>56 ± 3</td>
<td>81 ± 8</td>
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</tr>
<tr>
<td>$B_{0, \text{total}}$</td>
<td>mL CH4/g VS substrate</td>
<td>194 ± 10</td>
<td>198 ± 10</td>
<td>232 ± 11</td>
<td>235 ± 13</td>
<td>296 ± 15</td>
<td>313 ± 11</td>
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</tbody>
</table>
The lower digestibility of aerobic granules should be ascribed to the inherent limited degradation efficiency of carbohydrates and proteins rather than the higher lignin content in AGS (Table 2 and Figs. 3 and 6). The exact reason for the observed difference in biodegradation of carbohydrates and proteins between AGS-RTC and WAS remains unclear. An important fraction of carbohydrates and proteins in AGS-RTC and WAS originates from extra-cellular polymeric substances (EPS) (Chen et al., 2007; Yuan et al., 2014). Recent studies demonstrated that the distinguished sludge morphology between AGS and AS was determined by the chemical and mechanical properties of gel-forming EPS in the sludge of two (Felz et al., 2019; Lin et al., 2013). Possibly, the anaerobic conversion of such polymers in AGS-RTC differs from that in WAS. However, confirmation of this hypothesis needs more study.

4. Conclusions

Based on the results of this research the following conclusions can be drawn:

- The BMP of AGS-RTC was only 80% of that of WAS. Mechanically destroying the compact structure of AGS did not affect its BMP, but accelerated the degradation rate of rapidly biodegradable organics and liberated a fraction of slowly biodegradable ones, resulting in higher methane production rate.
- The BMP of AGS-SD was similar to the BMP of PS, and 1.5 times higher than that of AGS-RTC, mainly due to the slow settleability of highly biodegradable cellulose-like fibres that end up in AGS-SD fraction of WAGS.
- Proteins and carbohydrates in AGS-RTC were more difficult to be degraded than those in WAS, even though the amount of these two fractions was higher in WAS. This difference was hypothesized to be related to the structural differences of EPS between these two biomass morphologies.

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.2020.115617.

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