Research Article

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Anaerobic biodegradation and dewaterability of aerobic granular sludge

Lydia Jahn,* Ernis Saracevic, Karl Svardal and Jörg Krampe

Abstract

BACKGROUND: Although a growing number of full-scale wastewater treatment plants have already been constructed and operated with aerobic granular sludge (AGS), only limited information is available about further post-treatment, in particular about sludge stabilization and dewaterability. The aim of the present study was to investigate the biodegradation and methane yield of AGS by the use of anaerobic laboratory-scale reactors operated under mesophilic conditions and hydraulic retention times of 25 and 40 days.

RESULTS: The methane yield of AGS was *ca* 260 mL $gVSS^{-1}$ (volatile suspended solids) and thus slightly increased compared to that of suspended activated sludge (SAS; 240 mL $gVSS^{-1}$). A clear difference between the methane yield was found for separated pure granules (500 μm), which was *ca* 50% higher compared to that for SAS. VSS removal of AGS during anaerobic degradation was *ca* 52%. Dewaterability of AGS after anaerobic digestion was slightly lower compared to SAS. Extracellular polymeric substance (EPS) extraction and fluorescence analysis showed tryptophan contents which were almost twice as high compared to the EPS extracted from SAS.

CONCLUSIONS: Overall, the anaerobic digestion of AGS was found to be a suitable stabilization strategy with the benefit of recovering energy in the form of methane. Further tests are needed to validate the decreased dewatering behaviour with full-scale applications. The presented approach for tryptophan measurement allows the transfer of qualitative results from a fluorescence analysis into quantitative values and could be further adapted for identifying relevant EPS constituents.

Keywords: anaerobic digestion; biogas; SBR; sewage; AGS; biodegradation

INTRODUCTION

Aerobic granular sludge (AGS) is an upcoming wastewater treatment technology, which is increasingly applied in full-scale wastewater treatment plants (WWTPs). The compact and dense sludge structure offers excellent settling properties and the possibility for enhancing the activated sludge concentration and thus the biological treatment capacity. Aerobic granules are characterized by the formation of zones with different substrate and dissolved oxygen levels, which allow biological phosphate and simultaneous nitrogen removal. A rising number of full-scale sequencing batch reactors (SBRs) are operated using the commercial Nereda process, which is reported to be more cost-efficient compared to the conventional wastewater treatment technology[1] with an overall lower energy consumption.[2] Although numerous studies have focused on the process stability and treatment performance of AGS cultivated under laboratory-scale as well as under full-scale conditions,[3–7] information about the impacts on advanced sludge stabilization is limited.

During biological wastewater treatment, biomass growth leads to a continuous surplus sludge generation. For suspended activated sludge (SAS), the surplus sludge production depends on the sludge retention time (SRT) and is normally between 0.5 and 1.0 gTSS $gBOD_5^{-1}$. Muda *et al.*[8] reported a biomass yield for aerobic granules in the range 0.2 to 0.4 gTSS $gCOD^{-1}$, which is lower compared to that for suspended sludge. Ni and Yu[9] explained the lower sludge production of AGS by the fact that heterotrophs grow partially on internal stored polyhydroxybutyrate which leads to an overall lower grow rate. One further reason for the lower surplus sludge generation is an overall longer SRT for the granules. However, the generated surplus sludge has to be removed from the biological system constantly. Almost all larger WWTPs apply an aerobic (external or simultaneous) or anaerobic sludge stabilization, which primarily serves to decrease the organic compounds of the biomass. Sludge stabilization with an enhanced degradation of organic compounds improves the dewaterability of the sewage sludge and finally reduces the polymer demand and disposal costs.[10] Most large WWTPs apply an anaerobic stabilization process using digesters, where the organic sludge compounds are converted into carbon dioxide (CO_2) and energy-rich methane (CH_4). The benefits of this anaerobic stabilization strategy compared to aerobic stabilization are an overall

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reduced energy demand and a smaller required aerobic tank volume. A subsequent water separation step by centrifuges, screw presses or chamber filter presses is necessary to reduce the volume of the digested sludge in order to ensure low disposal costs.

For economic reasons, the biodegradation and dewaterability of AGS should be considered in detail, especially since the structure and composition of the aerobic granules are quite different compared to SAS. Moreover, characterization of AGS has found increased content of extracellular polymeric substance (EPS).[11–13] EPS is a gel-like hydrated secretion which is produced by microorganisms themselves[13] and consists mainly of proteins, polysaccharides, glycoproteins, nucleic acids and phospholipids.[14] The function of the EPS to protect the biomass against external environmental influences and to enhance the stability of microbial aggregates can adversely affect the degradability and dewaterability. Wang *et al.*[16,17] investigated the biodegradability of EPS extracted from AGS and found that the EPS from the outer layer was not biodegradable in contrast to EPS found in the inner layer. The biodegradability is thereby linked to the composition of the EPS. Especially, the presence of multivalent ions and crosslinks was found to reduce the biodegradability. Leenen[17] showed that Ba-Ca-EPS is not biodegradable, whereas Sawabe *et al.*[18] reported a simple degradability of EPS with Na^+ compounds.

Further studies focused on the correlations between the EPS content and the dewaterability of activated sludge. For example, Jin *et al.*[19] quantified the filterability of activated sludge samples by a capillary suction time test and compared the results with the amount of extracted EPS. Those authors found that a high amount of total extracted EPS was associated with low capillary suction time, indicating a better filterability. Similar results were published by Mikkelsen and Keiding:[20] while the EPS improved the floc stability and filterability, the cake solid content decreased with higher EPS content. Moreover, Kopp and Dichtl[21] reported that the EPS content affects the polymer demand during dewatering. Skinner *et al.*[22] investigated the dewaterability of digested sludge samples from different WWTPs and identified a strong correlation between the volatile suspended solids (VSS) and the final cake solid content. Cetin and Erdincler[23] found higher cake solid concentrations with lower EPS protein. Regarding that fact, EPS proteins seem to be an important parameter for the dewatering behaviour and water holding capacity.

Although a number of studies have investigated the dewaterability of digested SAS, limited comparative information exists for aerobic granules. Lehmann and Kaper[24] reported a lower total suspended solids (TSS) concentration after thickening an AGS compared to a conventional sludge using the same polymer dosage. Here again, the EPS proteins were named as a potential reason due to their increased water binding capacity. In contrast to these results, the polymer demand to thicken the AGS of another WWTP operating the Nereda process was only half that for a conventional activated sludge.[24] These contradictory results confirm that more investigations are needed to predict the dewaterability of AGS. Some further studies reported the methane production and biodegradation of AGS. For example, Val del Rio *et al.*[25] investigated the anaerobic digestibility of AGS samples, which were cultivated using pig manure and synthetic wastewater. Those authors found a specific methane production for the granules grown on synthetic wastewater with 243 mL $gVSS^{-1}$ and 170 mL $gCOD^{-1}$ as well as a VSS reduction of 49%. Thermal pre-treatment was found to slightly enhance the biodegradation. Hogendoorn[26] investigated the VSS removal of AGS sludge from a Nereda plant and reported a VSS degradation of *ca* 42.5% with hydraulic retention times of 12 and 20 days. Palmeiro-Sánchez *et al.*[27] compared the anaerobic biodegradation of AGS and flocculent sludge under saline conditions and found a higher degradation of AGS with 32% compared to the flocculent sludge with 27%. The overall reduced biodegradation of that study was explained by an increased salt concentration in the treated wastewater.

Since only a few publications are so far available concerning the anaerobic stabilization of AGS, the purpose of the study reported here was to characterize the biodegradation and dewatering behaviour of AGS compared to SAS. Laboratory-scale tests were applied to investigate VSS and COD (chemical oxygen demand) removal as well as the specific methane production. Moreover, the digested sludge samples were centrifuged and compared in terms of their water separation. The outcomes of the study were used to quantify the AGS post-treatment in terms of anaerobic biodegradation and dewatering efficiency compared to conventional SAS. EPS was extracted and subsequently analysed via fluorescence spectroscopy to quantify the tryptophan content. The decision to investigate the tryptophan content was driven by the fact that tryptophan is a typical EPS protein, which can be easily detected using fluorescence spectroscopy.

MATERIALS AND METHODS

SBRs (AGS)

Laboratory-scale SBRs with a reactor volume of 6–8 L were operated over a period of 36 months to investigate various operational strategies. Cycle times were in the range 3–6 h with an anaerobic plug-flow feed of 60–90 min. Settling times were varied between 1 and 10 min. The biomass was mainly fed with sewage from a municipal WWTP. However, there were some tests with synthetic wastewater containing $C_{12}H_{22}O_{11}$, $C_6H_8O_7$, CH_4N_2O and K_2HPO_4 in a similar composition to that of municipal sewage. After a start-up period of *ca* 28 days, the granules were removed from the SBR to control the SRT and afterwards used for anaerobic tests. Further information about the SBR operation can be found in Jahn *et al.*[28]

Anaerobic digestion

Five identical flasks with a volume of 525 mL were operated to characterize the anaerobic digestion of AGS under semi-continuous feeding conditions. The feed was carried out on 5 of 7 days, where 25 mL of input sludge was added per day. Due to the low feeding quantity, the digested sludge was removed only once a week (125 mL) and analysed for the parameters COD, TSS and VSS. Hydraulic retention time in the digesters was on average 25 days. Since the degradation also depends on the reactor operation, the temperature was always adjusted to 37 °C using a thermostatic bath. The produced biogas was collected in gas-tight cylinders with gas capture reset once a week. Methane content in the biogas was measured with a gas analyser (GFM series). COD balances were calculated to control the analysed parameters and the measured methane gas.

Table 1 summarizes the mean composition of the input sludges and the experimental settings of the anaerobic reactors. During the reference run, the anaerobic reactor was fed with thickened SAS from a municipal WWTP. During runs I to IV, the anaerobic reactors received thickened AGS from the laboratory-scale SBR. The dosed granules during the first and second runs were cultivated

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Table 1. Composition of feed sludge and settings of anaerobic reactors

Run	Feed (origin)	VSS/TSS	COD/VSS	OLR (gCOD L^{-1} day^{-1})	Wastewater (SBR)	SRT (feed) (days)
Ref.	SAS (WWTP)	0.73	1.48	0.93	Municipal	15
I	AGS (SBR)	0.78	1.44	0.68	Municipal	25
II	AGS (SBR)	0.83	1.48	0.96	Municipal	25
III	AGS (SBR)	0.90	1.36	0.89	Synthetic	25
IV	AGS (SBR)	0.90	1.37	0.76	Municipal	>40

Table 2. Particle size distribution and volumetric equivalence diameter[a] of AGS and SAS

Run	Input sludge	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	Diameter[a] (μm)	Granulation grade (%)
Ref.	SAS (WWTP)	83	235	571	287	–
I	AGS (SBR)	143	548	1255	630	83.5
III	AGS (SBR)	453	854	1451	899	95.7
IV	AGS (SBR)	166	366	693	400	84.7

with the same municipal sewage as the reference activated sludge. However, the third run was carried out with granules, which grew on synthetic wastewater. Since the biodegradation correlates with the SRT, the anaerobic tests were performed for granules operated under different SRTs. AGS during the fourth run came from a reactor operated with a SRT above 40 days, while the granules for runs I to III were cultivated under a SRT of 25 days.

All feed sludges (SAS and AGS) were thickened via centrifuge to *ca* 4.0 to 5.0% TSS before feeding. During the experiments the organic loading rates (OLR) of the anaerobic reactors were in the range 0.7–0.9 gCOD L^{-1} day^{-1}. AGS samples offered an overall increased VSS/TSS ratio, which can be explained by a lack of precipitation agents added to the reactor. Biological phosphate removal was the highest during runs I and II. During these tests, slightly lower VSS/TSS ratios were observed compared to runs III and IV. The increased inorganic compounds were probably related to mineralic precipitations inside the granules. Earlier studies assume that increased phosphorus removal can cause apatite precipitation inside the granules, especially under anaerobic conditions and phosphate release.[29] In contrast, a reduced biological phosphate removal and a lower VSS/TSS ratio were found during run III and the use of a synthetic medium.

In the frame of the tests, the particle size distributions of the feed sludges were measured with a Malvern Matersizer 2000 (Table 2). Aerobic granules normally coexist within a mixture of sludge flocs; thus the applied measurement analyses all particle sizes including flocs and granules. The smallest particle sizes were found for the SAS with 50% being smaller than 235 μm. During the second run, *ca* 50% of the particles reached a size above 550 μm. The largest particles were found for the AGS investigated during the third run, for which about 90% of the particles were larger than 453 μm. This observation can probably be attributed to the synthetic feed and a more compact and regular growth of the granules. The smallest diameters of the aerobic granules were observed during the fourth run, for which 50% of the particles were smaller than 166 μm. The particles size distribution provides an indication of the degree of granulation of the sludge. Pronk *et al.*[1] reported for a full-scale WWTP with AGS that *ca* 80% of the granules were larger than 0.2 mm. Our measurements confirm similar granulation grades, since the fraction of flocs (<200 μm) was only between 4.3 and 16.5%. The highest granulation grade of 95.7% was found for the granules cultivated under synthetic wastewater.

Table 3. Composition of feed sludge and settings of anaerobic reactors

Run	Feed (origin)	VSS/TSS	COD/VSS	SV_{10}/SV_{30}
I	Sludge flocs (<500 μm)	0.87	1.11	1.39
II	Granules(>500 μm)	0.86	1.24	1.17
III	AGS (mixed)	0.86	1.27	1.10
IV	AGS (mixed)	0.86	1.32	1.13

Anaerobic batch tests

Anaerobic batch tests were performed to differentiate the methane yield of the AGS as well as from the separated sludge fractions (large granules and sludge flocs). This method is characterised by a one-time feed, while the gas production is subsequently measured over a period of 21 days. Glass flasks with a volume of 525 mL were placed in a temperature-controlled (37 °C) water bath. Gas was collected in graduated tubes. Overall, there were two measurements with AGS as a mixture of sludge flocs and granules as well as two tests with the sieved fractions. For this purpose, AGS from a SBR was separated by a sieve with a mesh size of 500 μm and then divided into flocculent and granulated fractions. For the dosage of the input sludge, a recommended ratio of maximum 50% to the VSS load of the seed sludge was taken into account (VDI 4630, 2016).[55] Table 3 presents the mean composition of the samples investigated with the batch tests as well as the SV_{10}/SV_{30} ratio before thickening.

Dewaterability tests

The quantification of the full-scale dewatering results with laboratory-scale tests is very challenging, since several parameters, like the polymer dosage and the operation of the dewatering aggregate, affect the results significantly. Moreover, there are many dewatering aggregates used at WWTPs, like screw press, decanter or chamber filter press, which achieve different TSS in the dewatered sludge. Since the quantification of TSS is challenging for the different aggregates, the study focused on given qualitative information on the dewaterability of AGS compared to conventional SAS. The applied method used in this study was based on von der Emde and Sadzik,[30] whereby those author centrifuged primary and surplus sludge under a constant centrifugation

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time and *z* values between 20 and 2000 g. The procedure allows calculating approximately the solid content of the dewatered sludge under full-scale conditions. To comply with the method described of von der Emde and Sadzik,[30] a laboratory centrifuge (Sigma 3K30) was used to investigate the sludge cake solids of the digested probes. A sample volume of 30 mL was centrifuged for 10 min at 20 000×*g* (14 000 rpm), whereby a higher *z* value was chosen to get an almost complete separation of the loosely bound water. After centrifugation, the centrate was removed from the centrifuge tube and analysed for NH_4-N and PO_4-P. TSS and VSS contents in the remaining residue were determined by drying the probe at 105 and 550 °C.

EPS extraction

Different kinds of EPS are described in literature as soluble EPS, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS).[31] EPS outside cells can be subdivided into bound EPS (sheaths, capsules, polymers, gels) and soluble EPS (soluble macromolecules, colloids, slimes).[15,33] The structure of the bound EPS can be illustrated as a two-layer model.[14] The inner layer consists of TB-EPS, which is in contact with the cell surface. The outer area comprises a loosely bound dispersible EPS layer without clear delimitation. The proportion of LB-EPS in microbial aggregates is reported to be smaller than that of TB-EPS.[34,35] Different methods are applied for the extraction of the different EPS types. A detailed list of such extraction methods can be found in More *et al.*[35]

For this study, a method published by Adav *et al.*[36] and Liu and Fang[37] was used to extract the bound EPS from SAS and AGS samples. Sludge samples were washed three times with distilled water before extraction. A triple determination was prepared for each probe with a sample volume of 10 mL. For a sufficient breakup of the granule structure, the samples were pre-treated in an ultrasonic bath for 30 min. Subsequently, 0.06 mL of formamide was dosed to stabilize the cell walls and to avoid cell lysis and contamination through inner cell content. During a reaction time of 1 h, the samples were stored at 4 °C. After the reaction time, 4 mL of NaOH (1 N) was dosed to increase the pH and to subsequently dissociate acidic groups in EPS.[10] The samples were stored again for 3 h at 4 °C. Afterwards, the samples were centrifuged for 10 min at 10 000×*g*. The supernatant was separated by a 0.2 µm filter, whereby the filtrate contained the dissolved components of the bound EPS. Beside the extraction of the bound EPS, the soluble EPS was separated from the biomass by filtration through a 1 µm glass microfibre filter.

Further EPS analysis is based on three-dimensional fluorescence spectroscopy. The recording of the fluorescence spectrum makes it possible to obtain qualitative information on the distribution of different substance groups. This technique was also used in earlier studies to determine characteristic EPS compounds.[10] Especially, aromatic proteins can be detected through fluorescence analysis. Zeng *et al.*[38] reported by means of measured fluorescence intensities that tryptophan-like substances are the major group of fluorescence substances in EPS. Since tryptophan is a characteristic protein of EPS, the general idea was that higher tryptophan concentrations correlate with increased EPS proteins. Therefore, the extracted EPS was analysed using an Aqualog fluorescence absorption spectrometer (Horiba), which simultaneously records the absorption and fluorescence spectra as excitation (Ex) emission (Em) matrix (EEM). Figure 1 shows the assignment of areas in an EEM for detectable substance groups. Tryptophan-like substances are visible at 280/350–360 nm (Ex/Em), while tyrosine-like substances appear in a region of 270–280/305–310 nm (Ex/Em).[39]

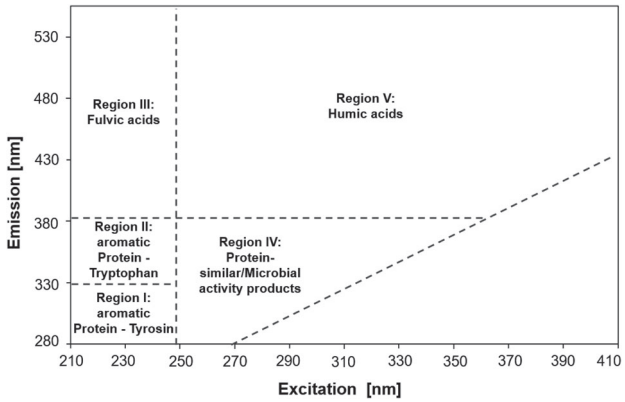

Figure 1. Assignment of regions of different substance groups in EEM.[41,42]

The investigation of this study focused on the tryptophan content in the extracted bound EPS of AGS and SAS samples. For the reason that the EEM gives only qualitative values in the form of intensity peaks, the samples were spiked with known tryptophan concentrations and afterwards measured using the fluorescence spectrometer. This approach allows the transfer of qualitative results into quantitative values.

RESULTS AND DISCUSSION

EPS protein

This section summarizes the results of the EPS examination. For the reason that the EEM gives only qualitative values in the form of peaks, the samples were enhanced with known tryptophan concentrations to determine the increase in the peak intensity. In a first step, tryptophan was spiked to deionat in different concentrations. The resulting fluorescence peaks in the region of tryptophan (Ex/Em: 273/347 nm) were correlated to the added tryptophan concentrations, which resulted in a linear correlation as shown in Fig. 2 (left, black dots). This validation indicates that it is possible to recalculate from peak intensity to substance concentration by having the same background matrix.

The tryptophan concentration in the extracted EPS was analysed for all probes with the described spiking method. Figure 2 shows two examples of fluorescence peaks, which resulted from added tryptophan concentration (+0.5, +1.0, +1.5, +2.0 mg L^{-1}). The examples relate to the extracted EPS from a SAS (left) and AGS (right). The peaks in the EEM appeared in the characteristic area of tryptophan with 273/347 nm (Ex/Em). A clear link was found between the measured EEM peaks and the increased tryptophan concentrations, while the double determination confirms a coefficient of determination of *ca* 0.99. Since bacteria produce the EPS, the concentrations were related to the VSS content to obtain comparable results. The calculated tryptophan concentrations for these samples were 1.2 mg $gVSS^{-1}$ (Fig. 2, left: SAS) and 2.5 mg $gVSS^{-1}$ (Fig. 2, right: AGS).

Figure 3 shows the analysed tryptophan concentrations of the soluble and bound EPS. AGS samples were taken from a laboratory-scale SBR operated for aerobic granulation; the SAS samples were collected from a pilot plant operated in continuous flow with sludge flocs. The left-hand panel of Fig. 3 shows the tryptophan concentrations of the soluble EPS of samples were collected over a two-month period. The results show that the tryptophan concentrations were always higher in the soluble EPS extracted from the granular sludge probes compared to the

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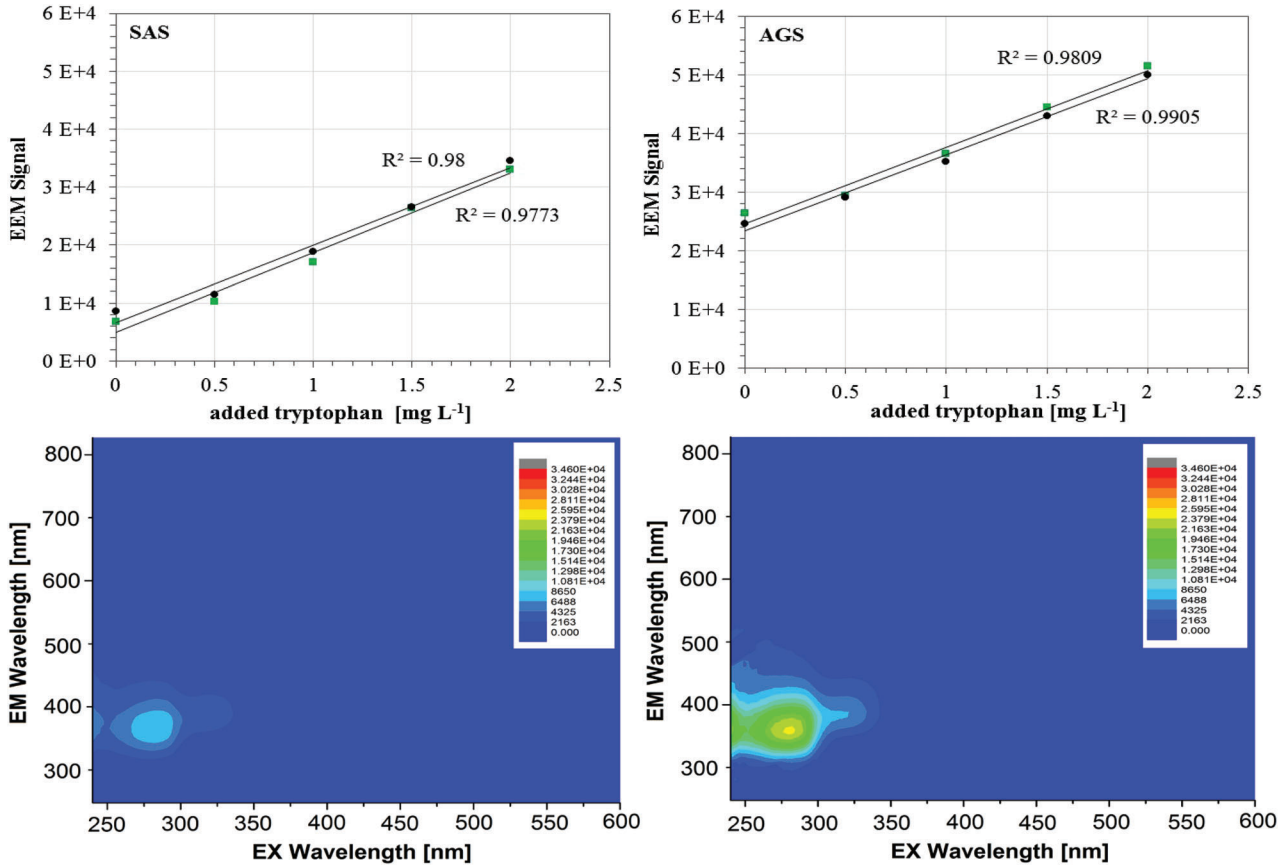

Figure 2. EEM signal (273/347 nm) with added tryptophan concentrations for EPS extracted from SAS and AGS (top) and EEM of the untreated samples (bottom).

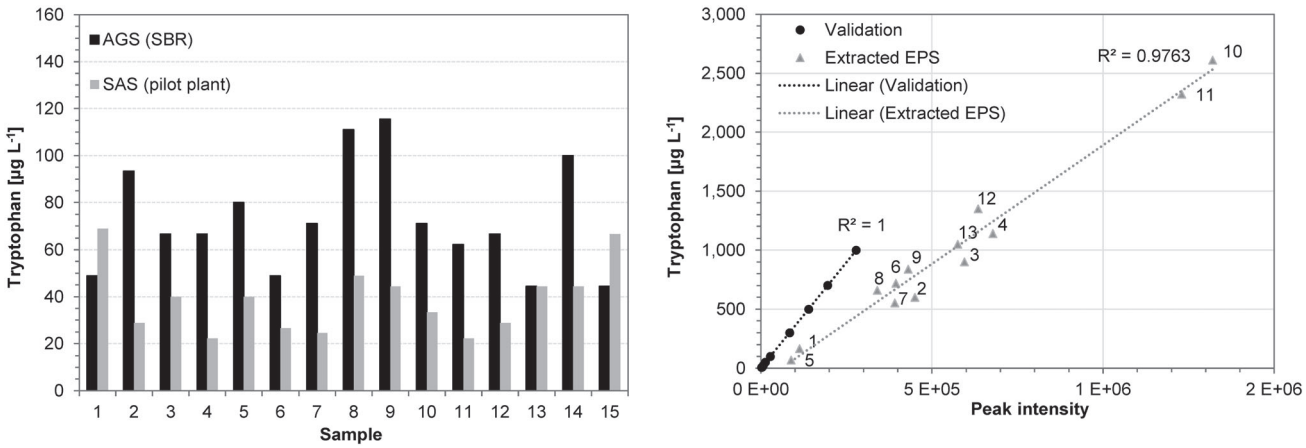

Figure 3. Tryptophan of soluble EPS (left); tryptophan of extracted bound EPS, validation (right).

EPS of the SAS. In a further step, the tryptophan concentrations were related again to the organic solid contents. The results for the EPS from the AGS samples were found in a range between 30 and 75 µgTry gVSS^{-1} (mean 55 µgTry gVSS^{-1}). This result was approximately three times higher than for the EPS extracted from the SAS reaching on average 17 µgTry gVSS^{-1}.

Figure 3 (right) summarizes the results for the bound EPS extracted from AGS and suspended sludge. The results confirm a significantly increased tryptophan concentration in the AGS compared to SAS (data points 1, 5). Although all measurements result in a linear correlation, the fluorescent peaks were overall lower than for the validation with deionized water. This observation is probably due to a different background matrix (salts, ions) which is known to slightly affect the fluorescence signal.

The tryptophan concentration of the soluble EPS was *ca* 10% of that measured for the bound EPS. Guo *et al.*[31] investigated the fraction of soluble EPS, LB-EPS and TB-EPS and found a ratio of 0.1:0.3:1. This ratio would explain the overall lower tryptophan concentration in the soluble EPS. All measurements reveal tryptophan concentrations in the extracted bound EPS from the SAS in a range of 1.2 to 1.9 mg gVSS^{-1}, while the tryptophan concentration in the bound EPS from the AGS ranged between 2.5

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Table 4. COD and VSS removal and specific methane yield during runs I to IV

Parameter	Run	Ref.	Run I	Run II	Run III	Run IV
	SRT (days)	15	25	25	25	>40
	Evaluation time (days)	36	28	36	14	25
VSS removal	(%)	45.9 ± 2.5	51.1 ± 4.2	52.6 ± 4.5	59.9 ± 4.4	37.2 ± 2.4
COD removal	(%)	47.5 ± 2.5	50.9 ± 4.2	52.4 ± 4.5	59.6 ± 4.4	35.2 ± 2.4
Methane yield	(mL $gVSS_{IN}^{-1}$)	245 ± 13	263 ± 21	271 ± 23	285 ± 21	168 ± 12
Methane yield	(mL $gCOD_{IN}^{-1}$)	166 ± 9	178 ± 15	183 ± 16	209 ± 15	123 ± 8

and 4.6 mg $gVSS^{-1}$. The total protein contents of the extracted EPS were measured by a modified TKN analysis according to DIN EN ISO 11732.[54] Proteins in the EPS extracted from AGS ranged between 211 and 338 mg $gVSS^{-1}$, wheras proteins in suspended sludge EPS were *ca* 150 mg $gVSS^{-1}$. The amount of tryptophan was 1.2 ± 0.12% of the measured protein content. The reported amount of proteins in EPS varies widely; while Dai *et al.*[42] reported 3.5 mg g^{-1} in a municipal raw sludge, Zeng *et al.*[38] measured proteins in a range between 35 and 68 mg $gVSS^{-1}$ and Adav *et al.*[10] found concentrations even up to 540 mg $gVSS^{-1}$ in AGS. Our outcomes are thus in a similar range compared to the literature values.

Anaerobic digestion

Table 4 summarizes the VSS and COD removal as well as the specific methane production of the anaerobic tests with AGS and SAS. COD balances were satisfactory for all reactors with variances below 10%. The pH values of the reactors were within an optimal range of 6.7 to 7.5. For the reference test with SAS, the VSS and COD removal was calculated at 46 and 47%, which are slightly above the reported literature values of 30–40%.[44,45] Biogas yield for waste activated sludge depends on the sludge age and is typically between 250 and 350 mL $gVSS^{-1}$.[45] AGS used for the first and second run was fed with the same municipal wastewater as the reference sludge, which allows a direct comparison of the results. In the digested sludge, the granules were no longer visible, which confirms that the compact structure was completely degraded. Methane production of the AGS was about 20 mL $gVSS^{-1}$ higher than for the reference run and thus in a range comparable to that for the suspended sludge samples. A slightly increased COD and VSS removal of *ca* 51% (runs II and III) was calculated for the AGS, which is in line with findings of Palmeiro-Sánchez *et al.*,[27] who also reported a slightly increased biodegradation of AGS. Since the formation of aerobic granules is associated with the implementation of an anaerobic feeding phase at the beginning of the SBR cycle, glycogen- and phosphate-accumulating organisms are enriched inside the biomass. The metabolism of these organisms is coupled to an increased formation of internal stored substrates (polyhydroxyalkanoates) and volatile fatty acids formation. Val del Rio *et al.*[25] state that the degradation of polyhydroxyalkanoates and volatile fatty acids can contribute to the biodegradability and methane production. A further reason for the higher specific gas production of AGS can be seen in the increased EPS. The applied extraction method and fluorescence measurements revealed clear increased tryptophan and protein concentrations in the EPS of the AGS. An increased protein content in the EPS of AGS was also reported by Zhang *et al.*[46] EPS in microbial aggregates has numerous sites for the adsorption of metals as well as organic substances such as aromatics, aliphatics and carbohydrates.[47] Due to the high number of carboxyl and hydroxyl groups, the EPS has a very high binding capacity.[47] A high absorption rate of EPS was also reported by Wei *et al.*[48] It can be assumed that the higher specific methane production was caused by an increased uptake of easily degradable organic substances into the EPS.

A clear increased VSS and COD removal of about 60% was achieved within the third run, where the granules were cultivated using synthetic wastewater. The increased specific methane production is probably attributed to the sewage composition with an overall increased easily available carbon source. In contrast, a clear decreased removal was found for the fourth run. Here, VSS and COD removal reached only 35 and 37%. Moreover, the methane production was decreased to about 170 mL $gVSS^{-1}$. This observation can be explained by a longer SRT in the SBR. Mennerich *et al.*[45] investigated the biogas production of activated sludge samples from WWTPs with different SRTs and showed a clearly declining gas production with longer SRT. Since the SRT of the reference sludge was only 15 days, it can be assumed that a higher SRT would result in a slightly lower methane yield and a greater difference between the methane potential calculated for SAS and AGS. According to a regression calculated by Mennerich *et al.*,[45] a lowering of methane yield by *ca* 10% would result from changing the SRT in the biological stage from 15 to 25 days.

Anaerobic batch test

Figure 4 shows the cumulative curves of gas production for the batch tests recorded for a period of 18 to 21 days. Since AGS always contains a fraction of flocs and granules, two batch tests were performed for AGS as complete mixture of flocs and granules. The samples were removed from a SBR operated under similar conditions (SRT of *ca* 25 days, same feed). The gas development appeared similar for both tests with a specific methane production of 250 and 260 mL $gVSS^{-1}$. These results are in line with the findings of the continuous anaerobic tests. In a further test, the

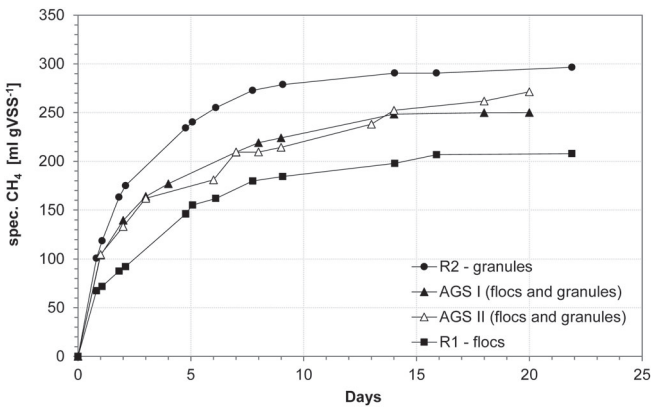

Figure 4. Sum curves of gas production for batch tests.

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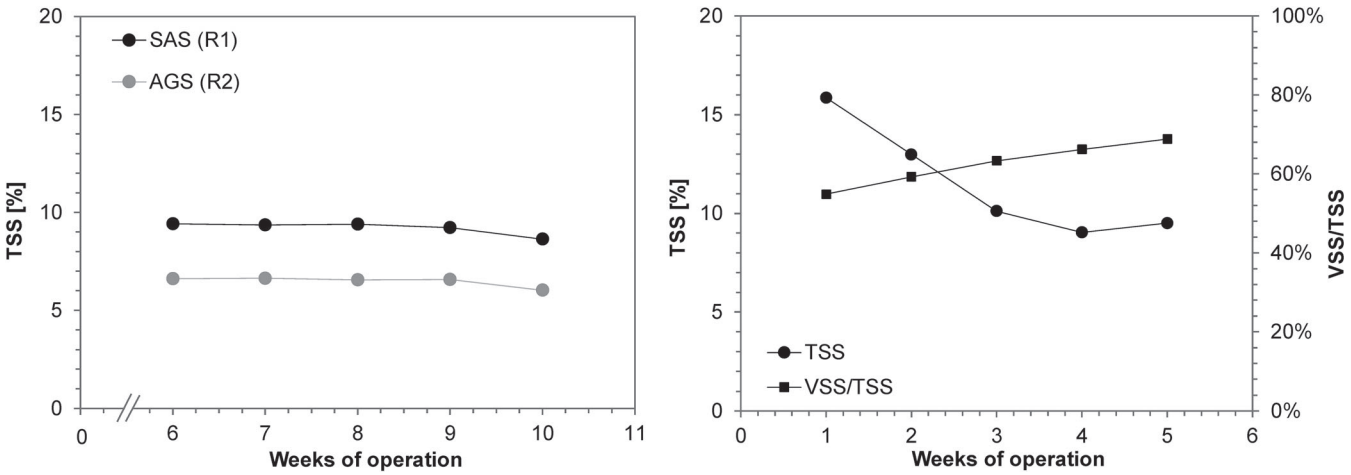

Figure 5. TSS in residue (left: run I, after start-up; right: during start-up of run IV).

granulated sludge was separated into a flocculent fraction (R1) and granules with sizes above 500 µm (R2). The specific methane production of the pure granules was 294 mL $gVSS^{-1}$ and thus clearly increased compared to the batch test with the flocculent fraction (206 mL $gVSS^{-1}$). Although there was no EPS extracted from these samples, the separated granules were covered by a shiny gel-like matrix which was characteristic of EPS.

Dewaterability

Figure 5 shows the results of the dewatering tests as TSS in the digested granules after centrifugation. A clear decreased dewatering behaviour was found during the start-up of phase IV with TSS decreasing from 16 to 9% within 35 days (Fig. 5, right). In the same time, a clear increased VSS fraction was observed for the digested sludge samples. Similar results of the dewatering behaviour were found for the digested reference sludge and the granules from the first run. The biomass from both runs was fed with the same municipal wastewater, which allowed a direct comparison of the dewatering results. The digested and dewatered SAS comprised a TSS of 9.6%, while the TSS for the AGS was only about 6.6%. During these tests, the organic fraction of the digested granules was significantly increased with 69.1% compared to the digested reference sludge (61.1%).

From the dewatering results, it can be concluded that the increased organic fraction caused a lower water separation. This is line with the results of Thomé-Kozmiensky,[49] who reported a lower dewaterability of sewage sludge with a higher ignition loss. Organic compounds have a higher water binding capacity, which hinders an advanced water separation. Moreover, the lower dewatering behaviour was probably a result of the increased PO_4-P concentrations in the AGS. Hougthon and Stephenson[50] reported that increased phosphate concentrations can negatively affect the dewatering results. Increased concentration of orthophosphate (PO_4-P) enhances the water binding capacity and finally the demand for polymer agents.[51] A steady increase from 13 to 186 mgPO_4-P L^{-1} in the centrate was found during the start-up of run IV (AGS). Furthermore, the centrate of the digested AGS of the first run was on average 396 mgPO_4-P L^{-1} and 1645 mgNH_4-N L^{-1}, while the concentrations in the centrate of the flocculent sludge were significantly lower at 143 mgPO_4-P L^{-1} and 1400 mgNH_4-N L^{-1}. Biological phosphate removal during the treatment process with AGS is responsible for increased phosphate uptake under aerobic conditions, while the stored phosphate is released during anaerobic conditions and the degradation of organic compounds. Additionally, the increased EPS can be named as a further potential reason for a decreased dewaterability. However, the EPS left after the anaerobic treatment was not analysed in this study. Further tests are recommended for quantifying the degradation of EPS under anaerobic conditions.

The outcomes indicate that there are various parameters having an impact on the dewaterability of digested AGS. However, two prospects can be named in order to improve the dewaterability of digested sludge samples. For example, it can convenient to apply an increased hydraulic retention time in the digester in order to decrease the organic solids and EPS through further degradation. However, an extended hydraulic retention time for the digester operation can only be applied when there is enough digester volume available. Another possibility for improving the dewatering behaviour could be selective PO_4-P removal. Kopp[52] and Bergmans *et al.*[53] observed an improved sludge dewaterability after a target struvite formation. The conclusion from these studies was that a better mechanical dewatering can be expected with less dissolved PO_4-P in the sewage sludge. Further tests for the quantification of the dewaterability are recommended at this point with respect to the EPS and PO_4-P content of AGS.

CONCLUSIONS

Anaerobic degradation and methane production of AGS and SAS were investigated in this study using laboratory-scale reactors. The specific methane production of AGS cultivated with municipal wastewater was found to be *ca* 260 mL $gVSS^{-1}$ and was thus slightly increased compared to that of conventional activated sludge. However, it could be confirmed that SRT above 40 days led to a clearly decreased gas yield (−35%). A higher specific methane production was found for granules separated from a mixed granular sludge, whereby the higher methane yield was probably linked to an increased EPS content. Proteins and tryptophan were clearly increased in the extracted EPS of the granules. The use of fluorescence to analyse EPS allowed the transfer of qualitative results into quantitative data. This approach can be further adapted to investigate special substance groups in the extracted EPS. The dewatering test showed a slightly decreased dewatering behaviour of the digested granules, which was probably caused by an increased organic fraction due to an absence of chemical precipitation and

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an increased amount of PO_4-P. The dewatering results require further validation with full-scale aggregates under real operational conditions.

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