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Sustainable carbon management in aerobic granular sludge for municipal wastewater treatment

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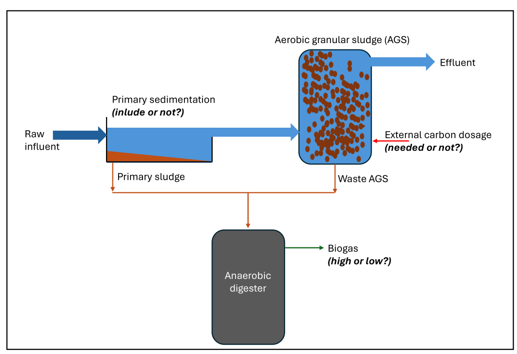
HIGHLIGHTS

- A pilot-scale AGS reactor was operated with and without primary sedimentation.
- Primary sedimentation resulted in 25% higher overall biogas production.
- No primary sedimentation enabled efficient denitrification without external carbon.
- Price of biogas and biomethane potential of sludge affect sustainability assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

Aerobic granular sludge (AGS) operated with pre-settled wastewater enables separation of organics with high biomethane potential. However, organic compounds are also needed to support denitrification, and external carbon may be needed to achieve low effluent nitrogen concentrations. This study evaluated the impact of primary sedimentation on carbon management in AGS processes. A pilot-scale reactor reached effluent nitrate concentrations of 2–3 mg NO_3-N/L when fed with pre-settled wastewater with the addition of 0.8 $\pm$ 0.2 g COD/g N as methanol in the post-denitrification phase, or when fed with raw wastewater without an external carbon source. The biogas potential of the whole process was 25 % higher with primary sedimentation. A sustainability assessment showed that the benefits of increased biogas production with primary sedimentation could outweigh the drawbacks associated with the use of methanol as external carbon source both in terms of economy and CO_2 emissions, but methane price and biogas yield affect the assessment.

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1. Introduction

As the world strives towards a more holistic approach to sustainability (Richardson et al., 2023), wastewater treatment plants (WWTPs) are expected to not only meet more stringent effluent demands, but to also consider factors such as climate impact, energy and chemical usage (Capodaglio & Olsson, 2020). The aerobic granular sludge (AGS) process is a wastewater treatment technology that was first described in the 1990s (Beun et al., 1999; Morgenroth et al., 1997). The sludge is grown under environmental conditions such as high substrate concentrations, high shear stress, low growth rates, and short biomass settling times that favour the formation of compact granular structures with high settling velocity (Bengtsson et al., 2019; Beun et al., 1999), which also have the ability to perform simultaneous nitrification and denitrification as well as biological phosphorus removal within different parts of the same granule (Beun et al., 2001; Lochmatter & Holliger, 2014). This results in a process with lower costs as well as lower energy-, land-, and chemical use compared to conventional activated sludge (CAS) (Bengtsson et al., 2019; Ekholm et al., 2023; Pronk et al., 2017). Because of these advantages, AGS has been shown to be an attractive alternative to the widely implemented CAS process.

The excess sludge generated by biological wastewater treatment processes such as AGS and CAS is typically treated using anaerobic digestion, which produces biogas. Production of biogas is an opportunity for WWTPs to cover costs and reduce waste sludge volume (Kehrein et al., 2020). The produced biogas also serves as a climate-friendly substitute to fossil fuels. While the number of full-scale AGS installations is increasing, with approximately 100 Nereda®Technology plants installed across the world, the research on biogas production from AGS plants is still limited. Some previous studies have indicated that waste sludge from AGS systems have lower biogas potential than waste activated sludge (WAS) from CAS systems (Bernat et al., 2017; Val Del Río et al., 2014), while others show contrary results (Guo et al., 2020). Design and operational factors of the AGS system likely affect the biogas potential of the excess sludge.

Primary sedimentation is commonly applied at full-scale WWTPs, and the produced primary sludge is digested along with the waste sludge from the biological treatment. The digestibility and biogas output from primary sludge is known to be high in comparison to excess sludge from AGS and CAS (Abdelrahman et al., 2023; Guo et al., 2020). Designing an AGS process without primary sedimentation will reduce the footprint of the WWTP and likely improve the biogas potential of the excess sludge, as has previously been shown for a high-loaded activated sludge system (Jimenez et al., 2015). However, the overall biogas output from the WWTP may decrease.

At WWTPs with strict effluent limits, another important aspect of carbon management arises. The organic carbon-to-nitrogen (C/N) ratio must be high enough to reach low concentrations of nitrogen in the effluent. This can typically be achieved when the AGS or CAS system is fed with raw influent, i.e. influent that has not undergone primary sedimentation. The presence of primary sedimentation prior to an AGS process has been shown to negatively affect nutrient removal because of the low C/N ratio (Ekholm et al., 2022; Kosar et al., 2022). In CAS systems, addition of external carbon source, such as methanol or ethanol, for post-denitrification is common practice when space is limited and low effluent nitrogen is required, but so far this is not practiced in full-scale AGS installations. Thus, when constructing an AGS plant, initial design choices such as whether to include primary sedimentation will affect both the potential for biogas production from the excess sludge and the need for external carbon source to support nitrogen removal. It is unclear if the benefits of including primary sedimentation with the likely potential for higher total biogas production would outweigh the drawbacks of requiring external carbon for denitrification. Further research on carbon management in AGS systems is needed to design processes that are as sustainable as possible. There is also a need for research on systems operated with real municipal wastewater as most of the scientific literature on the AGS process is based on studies with synthetic wastewater (e.g. Kosar et al., 2022).

The goal of this study was to examine trade-offs in carbon management in AGS systems by evaluating results from a large pilot reactor operated for 1.5 years treating municipal wastewater. The biogas potential of excess sludge and the need for external carbon source to support denitrification were investigated. The experimental results were used to assess differences in costs and carbon dioxide emissions when operating an AGS process with or without primary sedimentation.

2. Materials and methods

2.1. Rya wastewater treatment plant

The pilot plant was installed at the Rya WWTP in Gothenburg, Sweden, which treats municipal wastewater from about 800 000 population equivalents. The process consists of 20 mm coarse bar screens, sand and grit removal, 2 mm fine bar screens followed by primary sedimentation tanks. A high loaded activated sludge process reduces organic matter, nitrate and phosphate (by chemical precipitation with iron sulfate), followed by nitrification in trickling filters and moving bed biofilm reactors (MBBR), post-denitrification in MBBR, and finally micro filtration. Excess sludge from the high-loaded AS system is co-digested with primary sludge in two anaerobic digesters operated in series at approximately 35 °C.

2.2. Pilot-plant

The AGS pilot plant was a Nereda® installation consisting of an influent buffer and a 1.5 m^3 reactor (diameter 0.58 m, height 5.7 m). The pilot plant was run with a simultaneous feeding and decanting phase, an anoxic pre-denitrification phase followed by an aerobic phase, an anoxic post-denitrification phase, and lastly a sedimentation phase. The total cycle time varied from 3 to 5 h depending on rain or dry weather conditions. Waste aerobic granular sludge was taken out of the reactor after the feeding phase.

The pilot test was divided into three periods: Pre-settled 1, Raw, and Pre-settled 2. During pre-settled 1 period, the reactor was fed with pre-settled wastewater for 149 days before switching to raw wastewater for 54 days, followed by a second period with pre-settled wastewater that lasted 54 days. In the last period, methanol was dosed directly after the aeration phase to support denitrification in the post-denitrification phase. An initial dose corresponding to 0.2 g COD/g N was added to the reactor. After 4 days, the dose was increased to 0.7–1.0 g COD/g N. After operating the pilot with this dose for 46 days, the dose was decreased to 0.4 g COD/g N. The methanol was diluted with water to a concentration of 10 %.

2.3. Biochemical methane potential

Biochemical methane potential (BMP) was tested in anaerobic batch assays using AMPTS II equipment (Bioprocess Control). BMP tests were carried out on AGS sampled at the end of the pre-settled 1 period (this set of BMP tests are referred to as Setup I) and the raw period (Setup II). Waste activated sludge (WAS) and primary sludge (PS) from the full-scale Rya WWTP were also tested. As a positive control, microcrystalline cellulose (Sigma-Aldrich) was used. All the substrates were tested in at least two replicates (see supplementary material).

Two types of excess sludge were extracted from the pilot reactor. Mixed AGS was sampled during aeration to ensure the sludge in the reactor was fully mixed. Suspended solids concentration at the time of sampling of mixed AGS was 5.0 g/L during pre-settled 1 and 6.0 g/L during the raw period. This sludge contains both granules and flocs. The second sludge type, waste AGS, was sampled from the sludge buffer tank where the sludge discharge was collected. This sludge contains only flocs and no visible granules. The sludge buffer tank was continuously stirred to avoid sedimentation. Sludge samples that were sampled when operating the AGS pilot with raw wastewater are labelled *Mixed AGS raw* and *Waste AGS raw* and samples collected during the period with pre-settled wastewater as feed are labelled *Mixed AGS PSED* and *Waste AGS PSED*.

PS and WAS were sampled from the full-scale processes at Rya WWTP. Sludge inoculum was extracted from the existing sludge buffer tank following the full-scale anaerobic digesters at Rya WWTP. Process conditions of the AGS pilot plant and the full-scale WWTP during the time of sampling are shown in the supplementary material. The AGS pilot was operated at a higher sludge age (14–37 d) than the full-scale plant (6.5 d), as Rya WWTP is a high loaded activated sludge for BOD removal and denitrification. The waste AGS had a much lower suspended solids concentration (0.75–2.6 g/L) than the WAS (17 g/L), especially when the AGS was fed with pre-settled wastewater.

The volume of each BMP test bottle was 500 ml, and the total volume of substrate and inoculum was 400 ml, leaving 100 ml headspace. The inoculum-to-substrate ratio was approximately 2 (dry weight). The WAS and AGS samples were thickened by first allowing the samples to settle whereafter the supernatant was decanted, and the sludge pellet was thickened further using a Hermle Z510 centrifuge at a velocity of 3700 rpm for 10 min. A portion of the supernatant was again decanted to reach the desired range of 20–60 g VS/L to follow standardized recommendations provided by Holliger et al. (2016). To mimic the conditions of the full-scale digesters at Rya WWTP, the bottles were kept at 35 °C in a water bath throughout the BMP tests. BMP was calculated using Equation (1).

$$BMP = \frac{V_S - V_B\left(\frac{m_{IS}}{m_{IB}}\right)}{m_{vs,sS}} \tag{1}$$

where V_S is the accumulated volume of methane produced from the reactor with sample (i.e. inoculum and substrate), V_B is the accumulated volume of methane produced by the blanks (i.e. test bottles with inoculum but without substrate), m_{IS} is the total amount of inoculum in the sample, m_{IB} is the total amount of inoculum in the blank, and $m_{vs,sS}$ is the amount of organic substrate contained in the sample bottle.

Degree of digestion was calculated according to Equation (2) (Schnürer & Jarvis, 2009).

$$Degreeofdigestion = \left(\frac{TS_{IN} \times VS_{IN} - TS_{EF} \times VS_{EF}}{TS_{IN} \times VS_{IN}}\right) \tag{2}$$

where TS_{IN} and TS_{EF} are the total solids content (percent dry solids) at the start and the end of the BMP test, and VS_{IN} and VS_{EF} are the fractions of volatile solids as percentage of TS.

2.4. *Analytical methods*

Total chemical oxygen demand (COD) of the influent and effluent wastewater were analyzed using the sealed tube method, ISO 15705:2002. Total solids (TS) and volatile solids (VS) were analyzed with standard method (APHA, 2023). NH_4-N and NO_3-N in the effluent of the pilot was analyzed according to ISO 15923-1:2013 Annex B and ISO 15923-1:2013 Annex C, respectively.

Total carbohydrate contents in the substrates and inoculum were estimated as glucose-equivalent concentration using a phenol–sulfuric acid assay (DuBois et al., 1956) using glucose as standard (D-(+)-glucose, Sigma-Aldrich). Protein was determined by a modified protein assay kit with BSA as standard (Thermo Scientific). Total COD was determined by Hach (DR890). Prior to analysis of carbohydrates, protein and COD_{TOTAL} the sludge samples were sonicated for 1 min at 50 W placed on ice to get homogenous samples and diluted with MilliQ water as required for the chemical analyses. The concentrations of carbohydrate and protein were calculated as COD using the values 1.07 g COD/g carbohydrate and 1.5 g COD/g protein (Guo et al., 2020).

Statistical analysis of differences in BMP between was carried out using one-way analysis of variances (ANOVA) and Tukey's honestly significant difference (HSD) test as implemented in Scipy (Virtanen et al., 2020).

2.5. *Sustainability assessment*

A sustainability assessment was conducted in which the climate impact and cost of running AGS reactor at Rya WWTP with either raw influent or with pre-settled influent including addition of methanol, i.e. identical to the "pre-settled 2" period, were compared. The functional unit for the sustainability assessment was person equivalent (pe), i.e. a biochemical oxygen demand (BOD) load equivalent of 70 g BOD_7/person/d.

Climate impact was calculated based on global warming potential with a 100-year time horizon. For the climate impact calculation, it was assumed that the methanol used was produced from natural gas with a climate impact of 0.6 kg CO_2e/kg methanol from production (Ecoinvent, 2021) and 1.4 kg CO_2e/kg methanol from respiration, based on the reaction in Equation (3).

$$6NO_3^- + 5CH_3OH + 6\ H^+ \rightarrow 3\ N_2 + 5\ CO_2 + 13\ H_2O \tag{3}$$

The produced biogas was assumed to replace natural gas with a climate impact of 2.5 kg CO_2e per Nm^3 CH_4 (Gode et al., 2012). For the economic calculation, a biogas price of 0.9 euro per Nm^3 CH_4 and a methanol price of 0.54 euro/kg was assumed in accordance with average methanol prices on the European market in 2022 (Methanex, 2024). Moreover, it was assumed that 10 % of the biogas was consumed in the upgrading process. Sensitivity analyses were performed for cases with a lower gas price (0.2 euro per Nm^3 CH_4) as well as for a case with biogas potential for AGS fed with raw wastewater according to Guo et al. (2020).

3. Results and discussion

3.1. *Chemical oxygen demand in the influent and external carbon requirements*

Influent COD and C/N ratio were highest when raw influent was fed to the AGS and lowest in the first period of operation with pre-settled influent (Table 1). Incoming COD concentrations varied between the first and second pre-settled periods due to differences in infiltration and inflow into the sewer system, but the overall C/N ratio was very similar for the two periods. Considering that the COD removal efficiency was 80–88 %, the C/N ratio in terms of biodegraded COD was 12–20 % lower than the values provided in Table 1. Methanol was added with the target to achieve an effluent nitrate concentration of less than 2 mg NO_3-N/L. On average this required 0.8 g COD/g N entering the reactor, which corresponds to approximately 14 % of the total COD/N load. It may be possible to optimize the methanol dosing further in a full-scale application. At the end of the aeration phase when methanol was dosed, there was still some oxygen left in the reactor. This may have contributed to aerobic degradation of methanol rather than the intended anoxic denitrification. In full scale, it would also be possible to control the dosing in

Table 1
Influent- and effluent characteristics during the pilot test periods (average ± standard deviation). The average and standard deviations were calculated based on 25 measurements for the pre-settled 1 period, 6 for the raw period, and 5 for the pre-settled 2 period.

Parameter	Pre-settled 1	Raw	Pre-settled 2
COD influent (mg/L)	138 ± 75	279 ± 145	203 ± 36
NH_4 influent (mgN/L)	20 ± 8	18 ± 8	32 ± 5
C/N (gCOD/gN)	5.9 ± 1.1	12.4 ± 2.0	5.8 ± 0.9
COD reduction (%)	80 ± 14	88 ± 4	86 ± 2
Methanol dose (gCOD/gN)	–	–	0.8 ± 0.2
NO_3^- effluent (mgN/L)	5.8 ± 1.9	3.0 ± 0.9	2.0 ± 0.8
NH_4^+ effluent (mgN/L)	0.62 ± 0.16	0.43 ± 0.21	0.24 ± 0.14

relation to actual nitrate concentrations in the reactor at the end of the aeration phase. Thus, methanol dosing could be adjusted to the targeted effluent nitrate concentration. Although the average methanol dose was 0.8 g COD/g N in the pilot reactor, the dose was decreased to 0.4 g COD/g N at the end of the experimental run, suggesting it is possible to reach low effluent nitrate concentration with lower methanol dose.

3.2. Sludge composition

The composition of the substrates and inoculum is presented in Table 2. Mixed AGS PSED contained less carbohydrate, protein and COD_{TOTAL} than Mixed AGS raw. This is expected due to the approximately 30 % higher load of organics when raw wastewater was fed into the reactor (see supplementary material). Waste AGS had a slightly different composition than Mixed AGS, containing more protein, carbohydrate and COD_{TOTAL} when primary sedimentation was applied. Waste AGS contains primarily flocculated sludge, which is younger than mature granules and has a similar chemical composition to activated sludge. Previous research has shown that AGS contains more protein compared to activated sludge and that larger size fractions of the granules contain relatively more protein (Feng et al., 2024). However, when fed with raw wastewater, waste AGS contained more carbohydrate but less protein than with primary sedimentation. Carbohydrates in wastewater are derived from fecal and food residues and cellulose fibers, and the attachment of particulate organic matter to the biomass is higher for flocs than for AGS (Layer et al., 2020), which may explain the higher concentration of carbohydrates in the waste AGS, which mainly contains flocs. When comparing WAS and waste AGS with primary sedimentation, lower concentrations of carbohydrate and protein were observed for WAS. The distribution of extracellular polymeric substances (EPS), an important binding component of sludge, differs between aerobic granular sludge and activated sludge with the former containing more protein (Zhu et al., 2015). When comparing WAS and waste AGS with primary sedimentation, lower concentrations of carbohydrate and protein were observed for WAS. The PS contained substantially lower concentration of protein, but higher concentration of carbohydrate compared to both AGS and WAS.

In the literature, variable compositions of AGS, activated sludge and PS have been reported. In a detailed study comparing the composition of AGS, AS and PS, higher concentrations of carbohydrate and protein in AGS compared to activated sludge were observed (Guo et al., 2020). In accordance with our study, they also found that PS contained higher concentrations of carbohydrates compared to activated sludge and AGS but less proteins. A fraction of the carbohydrates in the sludge is made up of cellulose and hemicellulose, which largely end up in the primary sludge. Lipids is the fraction that gives most methane during digestion, and it has previously been found that PS contains relatively high concentrations compared to AGS and activated sludge (Bernat et al., 2017; Guo et al., 2020).

3.3. Biochemical methane potential

Results from the BMP tests are shown in Fig. 1. There was a statistically significant difference in BMP between the tested substrates ($p < 0.001$, ANOVA). As expected, the control with cellulose was the most readily biodegradable substrate, followed by PS. The inoculum used in Setup II had a higher biodegradability than the one used in Setup I, indicating a variability in the inoculum over time (Table 3), but the difference was not statistically significant ($p = 0.41$, Tukey's HSD). Microcrystalline cellulose, which was used as a model substrate to test the quality of the digestion setup and the inoculum, had a BMP of 307–322 mL CH_4/g VS in the first setup and 338–367 ml CH_4/g VS in Setup II. The theoretical BMP of cellulose is 350 ± 29 ml CH_4/g VS (Raposo et al., 2011). The degree of digestion for the cellulose samples slightly exceeds 100 % (Table 3). A probable cause is related to the accuracy of the TS and VS analyses.

The WAS had a BMP of 284–289 mL CH_4/g VS, which is somewhat higher than some previous studies that found BMP of 232 ± 11 mL CH_4/g VS (Guo et al., 2020) and 239 mL CH_4/g VS (Liu et al., 2019). This is likely due to the short sludge age at the Rya WWTP, around 6 days, which leaves a lot of organic carbon available for energy recovery (Ge et al., 2017). It has previously been concluded that with extended sludge ages, the biodegradability of protein, polysaccharides, and lipids in sludge decline (Chen et al., 2020).

When the AGS pilot was fed pre-settled wastewater, the mixed AGS sludge had a higher BMP (181–235 mL CH_4/g VS) than the waste AGS sludge (176–197 mL CH_4/g VS) (Table 3), but the difference was not statistically significant ($p = 0.51$, Tukey's HSD). Previously, waste AGS was shown to have higher BMP than mixed AGS (Guo et al. 2020) due to higher content of highly biodegradable cellulose-like fibers, similar to PS. The differences in the results may be due to differences in the composition of the wastewater entering the AGS reactor. Initially, the BMP was lower for mixed AGS compared to waste AGS but increased over time to eventually reach a higher value (Fig. 1). Guo et al. (2020) found that by mechanical disruption of the compact AGS structure accelerated the degradation rate by releasing rapidly biodegradable organics and releasing slowly biodegradable organics, ultimately resulting in a higher methane production rate. Although waste AGS contained higher concentrations of protein and carbohydrates compared to WAS, BMP was lower. Similar results were observed by Guo et al. (2015), and it was suggested to be due to structural differences of EPS between the two biomasses.

When the AGS pilot was fed with raw wastewater, the waste AGS had a BMP of 209–246 mL CH_4/g VS, which was slightly higher than the mixed AGS with 195–226 ml CH_4/g VS, but the difference was not statistically significant ($p = 0.97$, Tukey's HSD). PS had the highest BMP value of all tested substrates with 360–373 mL CH_4/g VS ($p < 0.01$, Tukey's HSD). This is expected, as the PS is formed of readily degradable colloidal organic matter whereas, e.g., WAS consists of microorganisms with difficult-to-degrade cell walls (Bernat et al., 2017). Waste AGS contained a higher concentration of carbohydrates and similar COD_{TOTAL} to PS, suggesting that more of the bound material from the raw

Table 2
Composition of the sludge samples tested for biomethane potential (BMP).

Parameter	Mixed AGS PSED	Mixed AGS raw	Waste AGSPSED	Waste AGS raw	WAS	PS	Inoculum*
Carbohydrate (mg/gVS)	15.8 ± 1.0	27.5 ± 1.6	23.6 ± 1.0	44.2 ± 2.6	19.4 ±1.2	59.1 ± 3.5	29.7 ± 1.8
Carbohydrate (mgCOD/gVS)	16.9 ± 1.0	29.4 ± 1.8	25.2 ± 1.5	47.3 ± 2.8	20.7 ± 1.2	63.2 ± 3.8	31.8 ± 1.9
Protein (mg/gVS)	360 ± 14	501 ± 20	738 ± 27	473 ± 19	662 ± 27	262 ± 11	666 ± 27
Protein (mgCOD/gVS)	541 ± 22	751 ± 30	1107 ± 44	710 ± 29	993 ± 40	392 ± 16	999 ± 23
Protein/carbohydrate	23	18	31	11	34	4	22
COD_{TOTAL} (mg/gVS)	790 ± 76	1752 ± 90	1365 ± 133	2330 ± 133	1415 ± 94	2150 ± 204	2765 ± 84

*Anaerobic digester sludge used as inoculum in the biomethane potential tests in Setup II (see supplementary material).

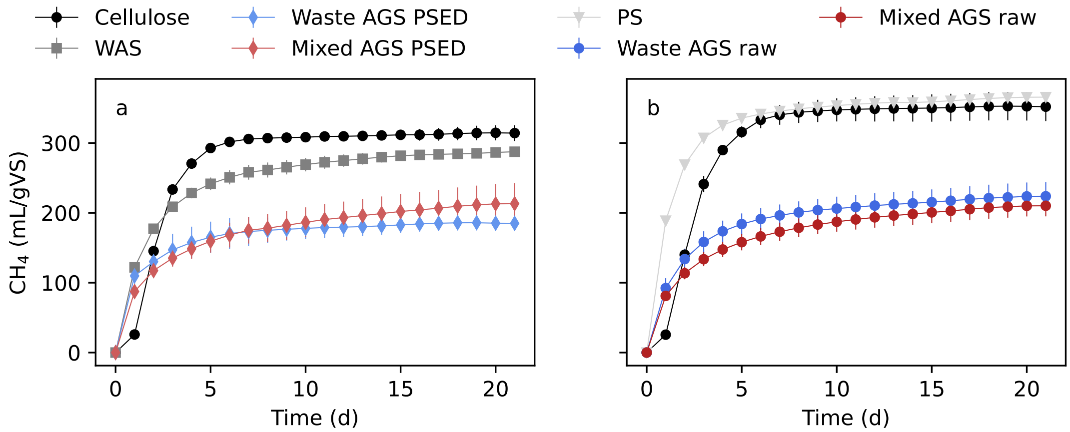

Fig. 1. Biochemical methane potential of the different types of sludge tested in setup I (a) and II (b). Markers and error bars show means and standard deviations. The number of replicates was 2–3 (see supplementary material).

Table 3
Characteristics of substrates, inoculum to substrate ratio and results from BMP tests. For the BMP, the average and standard deviation are shown based on the number of replicates specified in the supplementary material.

Substrate	TS (%)	VS (% of TS)	Inoculum/ substrate ratio	Degree of digestion (%)	BMP (ml CH_4/g VS)
Inoculum I	3.2	58	–	18	89 ± 1
Cellulose I	2.3	103	1.7	102	314 ± 11
WAS	4.1	69	1.5	68	287 ± 2
Mixed AGS PSED	3.2	76	1.7	48	213 ± 29
Waste AGS PSED	3.0	74	2.2	52	185 ± 10
Inoculum II	3.1	57	–	21	67 ± 5
Cellulose II	2.5	96	1.8	103	352 ± 20
PS	3.6	82	1.5	90	365 ± 7
Mixed AGS raw	4.1	81	1.3	57	208 ± 15
Waste AGS raw	3.4	77	1.7	61	223 ± 19

wastewater ends up in the flocculent fraction in waste AGS. The BMP for PS was higher than the BMP obtained in Guo et al. (2020), which was 313 ± 11 mL CH_4/g VS. The most notable difference from previous studies is that the BMP of waste AGS was found to be comparatively low. It was 185 ± 10 and 223 ± 19 ml CH_4/g VS in this study when the reactors were fed with pre-settled and raw wastewater, respectively. Other studies have reported values of 225 ml CH_4/g VS (Cydzik-Kwiatkowska et al., 2022) and 279–348 ml CH_4/g VS (Bernat et al., 2017; Guo et al., 2020) for AGS fed with raw wastewater.

3.4. Sustainability implications

Production of biogas generates economic revenue for WWTPs and reduces climate impact by replacing natural gas consumption in society (Bakkaloglu & Hawkes, 2024; Campello et al., 2021). On the other hand, the addition of methanol as an external carbon source for denitrification is an economic cost and is associated with a climate impact. The sustainability of five scenarios for implementing AGS for wastewater treatment was calculated based on the BMP results as well as the sludge production and need for methanol addition observed in the pilot plant. Scenario 1 was an AGS reactor operated with pre-settled influent and methanol added for post-denitrification. The sludge production was 39 g/(pe·d) total solids of PS and 33 g/(pe·d) of waste AGS. Scenario 2 was an AGS reactor operated with raw influent and no addition of methanol. The waste AGS was 100 g/(pe·d) total solids. In scenario 1, the higher biogas production with primary sedimentation more than made up for the cost and climate impact of the methanol (Fig. 2). When the price of gas is relatively low as demonstrated in scenario 3 and 4, the case without primary sedimentation is more cost effective, but the case with primary sedimentation is still better in terms of climate impact. When the gas yield for WAGS is substantially higher than in this pilot trial, e.g. as in Guo et al. (2020) (scenario 5), the case without primary sediments is better both in terms of cost and climate impact. This suggests that the optimal carbon management strategy can be different for different AGS systems, and that it is important to assess biogas potential on a case-by-

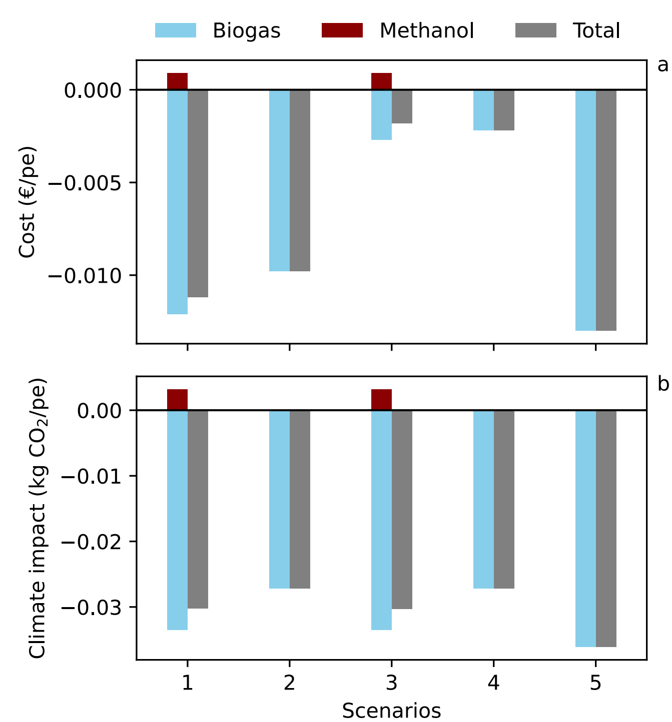

Fig. 2. Costs (a) and climate impact (b) of five scenarios representing possible implementations of AGS systems. Scenario 1 includes primary sedimentation, addition of methanol, biogas production of 15 L/pe and a biogas price of 0.9 €/Nm^3; and scenario 2 includes raw wastewater as influent to the AGS, no methanol addition, and a biogas production of 12 L/pe and a price of 0.9 €/Nm^3. Scenarios 3 and 4 are the same as 1 and 2, respectively, but with a biogas price of 0.2 €/Nm^3. Scenario 5 is the same as 2, but with a biogas production of 16 L/pe. Negative costs in panel *a* represents revenue obtained from selling biogas. Negative values in panel *b* represents avoided climate impact from biogas substituting natural gas.

case basis. Furthermore, there may be an optimum where part of the load is bypassed the primary sedimentation to provide just enough carbon for denitrification. The biogas production will then be maximized based on available carbon in the influent wastewater. It should also be noted that the available carbon in the raw wastewater varies over time due to infiltration and inflow into combined sewer systems. Thus, the optimum bypassed load may vary over time. Another possibility to limit the climate impact is to produce carbon sources in the form of volatile fatty acids from primary sludge. However, fermented primary sludge typically contains relatively high concentrations of NH_4 (Ossiansson et al., 2023), which is not suitable for post-denitrification, but it could be added to the influent. It may also be possible to improve carbon management by chemically-enhanced primary sedimentation, which increases the amount of PS that could be used for biogas production (Song et al., 2024).

4. Conclusions

A pilot-scale AGS reactor treating municipal wastewater achieved an effluent concentration of 3.0 ± 0.9 mgNO_3^--N/L with raw wastewater. With pre-settled wastewater, the nitrate concentration was 5.8 ± 1.9 mgNO_3^--N /L and 0.8 ± 0.2 gCOD/gN methanol was needed to reach 2.0 ± 0.8 mgNO_3^--N/L.

Waste AGS had 20 % higher biomethane potential when the reactor received raw wastewater than when it was fed pre-settled wastewater, but the process was estimated to produce 25 % more biogas when primary sedimentation was included. The sustainability analysis suggested that in terms of economic value and CO_2 emissions, the increased biogas production obtained with primary sedimentation in the process design could outweigh drawbacks associated with usage of methanol as external carbon source for denitrification. Inclusion of primary sedimentation is, thus, an option worth consideration in the design of AGS plants, especially if primary sedimentation basins are already available, such as when retrofitting an existing CAS plant.

CRediT authorship contribution statement

Therese Areskoug: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Johanna Arita Mendoza:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Oskar Modin:** Writing – review & editing, Visualization. **Dag Lorick:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Susanne Tumlin:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Britt-Marie Wilén:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis.

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.biortech.2025.132624.

Data availability

Data will be made available on request.

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