



# **Review Aerobic Granular Sludge as a Substrate in Anaerobic Digestion—Current Status and Perspectives**

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**Abstract:** Even though many wastewater treatment systems have been applied so far, there is still a need to develop methods, the implementation of which are technologically and economically justified. The aerobic granular sludge (AGS) method, which has been developed for several years, may represent an alternative to traditional technologies. One of the barriers to AGS deployment is the limited knowledge on the determinants and efficiency of the anaerobic digestion (AD) of AGS, as little research has been devoted to it. Therefore, the aim of the present paper is to summarize the current state of knowledge on the subject, including a review of technological conditions, process performance, and AGS parameters that can impact AD, and currently used pre-treatment methods. The anaerobic stabilization performance of AGS is compared against conventional activated sludge (CAS). The paper also identifies avenues for further research and practical implementations to further optimize the process and to determine whether AD is viable in full-scale plants.

**Keywords:** aerobic granular sludge; anaerobic digestion; wastewater treatment; biogas; methane; pre-treatment

## 1. Introduction

The ever-more stringent quality standards for wastewater treatment effluents call for the development and deployment of efficient, commercially viable, and environmentally friendly wastewater treatment methods [1,2]. These criteria are largely met by established methods for microbial biodegradation and removal of pollutants, whether via conventional activated sludge (CAS) suspended in the wastewater or biofilm deposited on packing elements [3,4]. Nevertheless, in many cases, these technologies can be substituted with the aerobic granular sludge (AGS) method, which has been developed for several years [5,6]. Cases in which the implementation of AGS technology should be considered include the following possibilities: simplifying the technology by resigning from a multi-reactor sewage treatment line [7], changing the qualitative characteristics of the sewage flowing into the treatment plant to which AGS is relatively resistant [8], shortening the work cycle reactors [9], elimination of variable oxygen conditions in order to remove biogenic compounds [10], resignation from sludge separation devices [11], shortening the sedimentation phase [12], resignation from the system of recirculation pumps and agitators [13], reducing energy consumption [14].

AGS sewage treatment systems are now widely accepted as promising and forwardlooking solutions due to their high technical readiness level, optimized processes for cultivating stable granules, as well as established and verified pollutant biodegradation parameters [15]. AGS has a number of clear advantages over CAS. These include, in particular: versatility, well-established treatment performance across different types of effluent, better and faster pollutant removal, improved settleability (and, thus, shorter retention times in AGS separation systems), and reduced bioreactor area/size [16]. This



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). translates to lower investment and operating costs for wastewater treatment, bolstering the commercial competitiveness of AGS technology. Given these benefits, as well as the processing advantages, it is no wonder that AGS has been growing in popularity among researchers and municipal plant operators [9]. The method has been proven to be effective in full-scale systems for municipal/urban wastewater treatment, as well in biodegradation of various industrial pollutants. Large-scale systems have been designed and commissioned all around the world in Nereda<sup>®</sup> technology (Royal HaskoningDHV, Amersfoort, The Netherlands) [17,18].

Recent years have seen a rapid spread of AGS wastewater treatment systems, which help identify and fully explore the strengths of the technology, as well as find solutions to existing technical issues and emergent operational hurdles [19]. By mid-2021, the number of AGS wastewater treatment plants was close to 90 facilities, meaning that it had more than doubled since 2018 [20]. The facilities ranged from 100 to 600,000 m<sup>3</sup> in size and were designed primarily for full biodegradation of organic matter or nitrogen/phosphorus removal from household sewage and from mixed household–industrial sewage [21]. An analysis of data provided by AGS-deploying companies shows there were 13 new full-scale wastewater treatment plants being constructed and commissioned in 2020–2021. A further 11 are being discussed and designed, to be commissioned by 2025 [21], which speaks to the rapidly growing take-up of the technology. Many commissioned AGS installations are actually retrofitted CAS systems, modified to improve pollutant removal and ensure compliance with stringent quality requirements for wastewater-treatment effluent [22]. The number of full-scale AGS installations is provided in Figure 1 [21].



Figure 1. Worldwide number of large-scale AGS wastewater treatment systems from 2005 to 2021.

AGS is an advanced, optimized and well-explored technology incorporated into numerous large-scale installations. Nevertheless, it does have certain well-documented drawbacks that preclude its large-scale deployment and take-up. Researchers and operators have reported issues with granulated biomass instability, especially at longer running times [23,24]. This very often leads to diminished performance of AGS-separation systems, and may also cause the plant effluent to become re-contaminated with the dispersed bacterial suspension. This also leads to higher levels of pollutants detected during the treatment process [25].

Another barrier to the competitiveness of AGS is the limited knowledge on how to manage and, ultimately, neutralize the resultant surplus sludge. One of the most popular ways of militating or fully eliminating the nuisance-inducing sludge is by using anaerobic digestion (AD) [26]. This is a very well explored technology, commonly used as one of the steps in CAS processing to stabilize sludge, remove organic matter, reduce sludge

volume, improve dewaterability, reduce sanitary indicators, limit nuisance smells, improve fertilizing properties and capture methane-rich biogas [27]. Due to the different properties and characteristics of AGS, the processes currently in use need to be tested for suitability and effectiveness in anaerobic treatment of sewage sludge. This means that the underpinnings and technological parameters of the AD process need to be validated and adapted to a substrate with a different chemical composition and properties. Relatively little research to date has focused on analyzing and optimizing anaerobic digestion of AGS, so there is a real need to review the existing findings and find prospective avenues for future research and practical efforts that could further our scientific understanding and practical applicability of the process.

The aim of this review article is to present and summarize the current state of knowledge on technological parameters and performance of anaerobic digestion of AGS. The article also delineates AGS parameters crucial for anaerobic digestion and reviews current methods of improving AGS biodegradability under anaerobic conditions. The literature review served as a basis to analyze the anaerobic stabilization performance of CAS against that of AGS. The paper also identifies directions for further research and practical efforts to optimize the process further and to determine whether AD is viable in full-scale plants.

#### 2. AGS Characteristics and Applications

AGS, as defined by the International Water Association (IWA), is made up of aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settle significantly faster than activated sludge flocs [28]. AGS granules are spherical or elliptical in shape. Their morphology is determined by the technological parameters of the wastewater treatment process (Table 1), including the pollutant load, the age of the sludge, the intensity of aeration and stirring, the type of feedstock, as well as any alternations to the design and operation cycle of the biological reactor [29]. The influence of operating conditions on the AGS characteristics, as well as graphics and photos of granules, have been presented in many scientific publications [30–32].

Operating Conditions	Impacts on Granulation Process	References
Additives Metal Cations	Ca <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>2+/</sup> Fe <sup>3+</sup> intensify granulation by neutralizing negatively charged sludge particles and enhancing adsorption/bridging interactions.	[23,33]
Aerobic starvation	Granulation is initiated by the lack of nutrients, increasing shear force and an increase in the hydrophobicity of the bacteria.	[34]
Coagulant or inert carrier	Effect on the neutralization of negatively charged particles, which promotes aggregation and adsorption of the flocs. The large surface area of coagulants and inert carriers increases the granulation efficiency.	[21,33]
Extracellular polymeric substances (EPS)	EPS aggregates bacterial cells and other solid particles to a granule precursor. The high content of EPS in the system allows the granules to withstand high values of hydraulic and pollutant loading.	[23,35]
Food to microorganism F/M ratio	A high F/M ratio facilitates the formation of large granules. Finding the right F/M ratio is essential for achieving a fast and stable granulation.	[36,37]
Hydraulic retention time (HRT)	Increasing HRT reduces OLR, which limits the granulation efficiency, hinders sedimentation and leads to a decrease in biomass concentration in the technological system, as well as the size and stability of granules.	[36,38]
Hydrodynamic shear force	It regulates the growth of fibres, the porosity and density of the granules as well as the stability of granulation. Higher hydrodynamic shear provides better compaction and density of the granules.	[21,39]
Organic loading rate (OLR)	High OLR allows for quick and efficient granulation, while delayed and difficult granulation formation was observed with low OLR.	[36,40]

Table 1. The influence of operating conditions on AGS characteristics.

Operating Conditions	Impacts on Granulation Process		
Seeding sludge	Type of seed pellets may contain cations and other properties that can help speed up the granulation process. It also acts as a nucleus that promotes the attraction of sludge flocs.	[21,33]	
Settling period	Removes poorly settling, flocculent sludge, enabling the deposition of appropriate granules and the selection of appropriate species of microorganisms.	[21,33]	
Sludge retention time SRT	Prolonged SRT causes deterioration of aerobic granulation, discharge of aging granular sludge and retention of appropriate newly synthesized granules is required for the stability of the aerobic granular sludge process, while shorter SRT results in a reduction of the size of the sludge flocs.	[4,37]	
Temperature	Granulation was successfully carried out in the temperature range of 8–30 °C. It was proven that low temperatures caused an increase in fiber content, causing leaching of bacterial cells and instability of granules.	[4,41]	
Volumetric exchange ratio	High volumetric exchange rates increase the granulation, facilitating the formation and improving the sedimentation properties of the granules.	[36,40]	

Table 1. Cont.

Growing AGS takes time, which can be up to three months in extreme cases, and requires the maintenance of optimal conditions, including suitable hydraulic parameters and organic loads [42]. Granule formation is facilitated by the hydrophobic surfaces of the microorganisms. According to thermodynamics, an increase in bacterial hydrophobicity leads to a reduction in the Gibbs energy of the cell, which causes microbial cells to join together and form aggregates [43]. AGS have been shown to be up to three times more hydrophobic than inoculated CAS. The cell hydrophobicity is an important inducing and maintaining force for cell-to-cell immobilization and cell-to-carrier surface attachment [44,45]. The specific gravity of the oxygen granules is positively related to the hydrophobicity of the cell. High cell hydrophobicity intensifies the intercellular interaction, results in more efficient sedimentation and favors a more stable structure of the AGS community [46]. There are two biological causes that could potentially be responsible for the increase in cell hydrophobicity during bio-granulation [47]: one of them is the community variability in multispecies aggregation of microorganisms, and the other is the change of properties on the cell surface (mainly EPS closely related to the cell surface). It has been shown that the composition of EPS, especially extracellular protein, correlates with the hydrophobicity of cells in the aggregation of microorganisms [48]. The more hydrophobic proteins in AGS are responsible for increasing the adhesion potential, keeping adjacent microbial cells together, accelerating granulation, and maintaining granule stability [49].

The extracellular polymeric substances (EPS) excreted by microorganisms play a major role in AGS formation [48]. EPS are mostly composed of proteins and polysaccharides that form a matrix which immobilizes the activated sludge bacteria within [30]. One of the roles of EPS is that it changes the surface charge of cells, thus reducing their electrostatic repulsive force [50]. Filamentous microorganisms play a huge role in the first phase of AGS formation by serving as a scaffold upon which individual bacterial cells can settle [51]. A diagram showing how a single AGS granule is formed is presented in Figure 2.



PAO-polyphosphate-accumulating organisms

Figure 2. Illustrative chart of AGS granulation process.

The color of the AGS depends on the chemical composition of the wastewater, the process parameters, the reactor design and the taxonomic composition of the granuleforming microorganisms [52]. Granule sizes range between 0.2 and 16.0 mm [53]. Higher pollutant loads in the biomass have been shown to produce larger AGS sizes, whereas smaller granules are formed in reactors with limited availability of organic feedstock over a long period of time [54]. The specific gravity of AGS ranges from 1.004 to 1.100 kg dry matter (DM)/m<sup>3</sup>, which is much more than that of CAS, with water content between 94 and 97%, compared with 99% for CAS [55]. The volume index of AGS is less than 50  $\text{cm}^3/\text{g}$ DM, and can even reach  $20 \text{ cm}^3/\text{g}$  DM in some cases. The surface structures of AGS are peppered with pores, fissures, cracks and channels, allowing feedstocks and metabolites to be carried between the waste and AGS interior [56]. AGS porosity is lower than CAS, and can decrease further as the individual granules become bigger. Pore blockage can hamper feedstock diffusion, which limits microbial activity in the AGS [57]. AGS can be kept in storage for several weeks to several months. The properties of the stored sludge are determined by temperature and the type of medium [58]. However, storage leads to biomass liquefaction and progressive disintegration of AGS [59]. A comparison of AGS and CAS properties is presented in Table 2.

Parameter	Unit	Va	Value	
Tatanicici	Unit	AGS	CAS	Reference
Shape	Shape - Compact and spherical Irregular granular structure s		Irregular and flocculent structure	[39]
Size	μm	>200	50-300	[41]
Settling velocity	m/h	10–130	2–10	[60]
Specific gravity	-	1.010–1.017 1.004–1.100	0.997–1.01 1.002–1.106	[7] [55]
Water content	%	94–97	99	[55]
Sludge Volume Index 5 min 30 min	mL/g	30–60 30–60	- 110–160	[39,61]
Redox microenvironments	-	Aerobic, anoxic, and anaerobic microbial layers	Minimum feasibility for anaerobic zones	[7,62]
EPS synthesis	-	High EPS content in aerobic granules as compared to CAS	Lower EPS content	[7,63]
OLR	-	Capable of withstanding high OLR	Poor removal performance at high OLR	[7,63]
Resistance to shock and fluctuating OLR	-	Able to removePoor removal underpollutants under shockshock or fluctuatingor fluctuating OLROLR		[7]
Tolerance to toxic compounds	-	Higher tolerance to toxic pollutants	Lower tolerance to toxic pollutants	[7,63]

Table 2.	Comparison	of AGS and	CAS characteristics
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The AGS structure can house all kinds of organisms: heterotrophic and autotrophic, aerobic, facultative anaerobic, and obligate anaerobic at the deeper layers of the granule. *Accumulibacter* spp. has been shown to be the primary species at depths up to 200  $\mu$ m, whereas *Competibacter* spp. is the predominant species in the core [64]. In another experiment, the ammonia-oxidizing *Nitrosomonas* spp. was found at a depth of 70–100  $\mu$ m from the edge of the granule, whereas anaerobic bacteria resided at a depth of 800  $\mu$ m and below [65].

The taxonomic structures in the granules change depending on wastewater composition. Granules grown on brewery/malthouse wastewater were colonized mainly by the filamentous *Thiothrix* sp. and *Sphaerotilus natans* [66]. Other researchers have noted that reactors fed with glucose had more filamentous bacteria, whereas acetate grown granules mostly contained bacilliforms [65]. The species composition many be shaped by temperature and dissolved oxygen in the reactor. Song et al. [67] have found *Thermomonas* to be present in the granules at 25 °C, whereas *Curtobacterium ammoniigenes* and *Ottowia* prevailed at 30 °C. *Thiothrix* sp. proliferates in low-oxygen conditions [68], *Microthrix parvicella* can colonize granules at a wide range of oxygen levels [69]. Thanks to the anaerobic and low-oxygen zones, the granules support simultaneous nitrification, denitrification, partial oxygenation of ammoniacal nitrogen into nitrate nitrogen (III), and Anammox processes [70]. Granules are more stable with the presence of slow-growing Anammox bacteria, as well as first- and second-step nitrifying bacteria [71].

AGS has been grown and used to treat dairy [72] and brewery wastewater [73], landfill leachate [74], and municipal sewage [7]. AGS can also be used to treat toxic wastewater. Adapted granules have been demonstrated to effectively degrade phenol at levels of up to 5000 mg/dm<sup>3</sup> [75]. After a long acclimatization period, acetate-grown granules are

able to completely degrade 4-chlorophenol [76]. Phenol derivatives can also be removed when labile carbon is provided as a secondary feedstock [77]. Aerobic granules have been demonstrated to successfully degrade methyl tert-butyl ether with ethanol as a co-feedstock. Ester removal can reach 100%, with the removal rates positively correlating with its levels in the wastewater [78]. Example uses of AGS in the treatment of wastewater of various origins and characteristics are presented in Table 3.

Type of Sewage/Waste	Initial Concentration [mg/dm <sup>3</sup> ]	Reactor Configuration and Operation Conditions	Removal Efficiency [%]	Final Concentration [mg/dm <sup>3</sup> ] *	Reference
Dairy wastewater	COD: 2800; TN: 40; TP: 30	12 L SBR; Cycle period: 8 h	COD: 90; TN: 80; TP: 67	COD: 28; TN: 8; TP: 9.9	[79]
Household wastewater	COD: 506; BOD <sub>5</sub> : 224; TN: 49.4; AN: 39 TP: 6.7;	Two SBR tanks with height of 7.5 m and working volume of 9600 m <sup>3</sup> each; Cycle: 6.5 h in dry season, 3 h in rainy season; VER: 65%	COD: 88; BOD5: 96; TN: 86; AN: 97; TP: 87	COD: 60.7; BOD <sub>5</sub> : 9; TN: 6.9; AN: 1.2; TP: 0.9	[13]
Household sewage (40%) + industrial wastewater (60%)	COD: 1000; AN: 60	SBR; Height: 100 cm and Diameter: 20 cm; Cycle period: 4 h; VER: 50%	COD: 80; TN: 98	COD: 200; AN: 1.2	[80]
Livestock wastewater	COD: 3600; TN: 650; TP: 380	4 L SBR; Cycle period: 4 h; VER: 50%; 27–30 °C	COD: 74; TN: 73; TP: 70	COD: 93.6; TN: 175.5; TP: 114	[81]
Palm oil mill effluents	COD: 69500; AN: 45	3 L SBR; Cycle period: 3 h; VER: 50%	COD: 91.1; AN: 97.6	COD: 69500; AN: 45	[82]
Septic wastewater	COD: 971; AN: 80; TP: 19; SS: 670	Three SBR tanks (7 m height) with maximum capacity of 5000 m $^3$ d $^{-1}$	COD: 94; AN: 99; TP: 83.5; SS: 98	COD: 58.3; AN: 0.8; TP: 3.1; SS: 13.4	[61]
Slaughter house wastewater	COD: $1250 \pm 150$ ; AN: $120 \pm 20$ ; TP: $30 \pm 5$	20 L volume SBR; Cycle period: 6 h; VER: 50%; 18–22 °C	COD: 95.1; AN: 99.3; TP: 83.5	COD: 6.1; AN: 0.8; TP: 5.0	[83]
Rubber industry wastewater	COD: 1850; TN: 248; AN: 49	0.6 L SBR; Cycle period: 3 h; VER: 50%; 27 $\pm$ 1 °C	COD: 96.5; TN: 89.4 AN: 94.7	COD: 64.75; TN: 2.6 AN: 94.7	[84]
Synthetic wastewater with phenol	200 1000	1 L SBR, 12 h cycle, 2 cycles per day, 30 °C, 200 rpm	100 97	- 30	[85]
Synthetic wastewater with p-Nitrophenol (PNP)	200	1 L SBR, 75% VER; 24 h cycle with 23.5 h aeration, 1.6 cm/s SAV, 30 °C	100	-	[86]
Synthetic wastewater with 2,4-Dintrotoluene (2,4-DNT)	10	1 L SBR, 70% VER; 24 h cycle with 23 h aeration, 1.2 cm/s SAV, 30 °C, 100 rpm	90	1	[87]

Table 3. Avenues of AGS use in wastewater treatment.

AN—ammonia nitrogen, BOD<sub>5</sub>—biochemical oxygen demand, COD—chemical oxygen demand, rpm—rotation speed, SAV—superficial air velocity, SBR—sequencing biological reactor, TN—total nitrogen, TP—total phosphorus, VER—volumetric exchange ratio, \* own calculations.

The first pilot AGS-growing plant was constructed in Ede (Netherlands) in 2003 and consisted of two parallel batch reactors with a volume of approx. 1.5 m<sup>3</sup> [88]. Another pilot-scale installation for treating municipal wastewater was established at the Zhuzhuanjing sewage treatment plant in Hefei (China), consisting of two 1 m<sup>3</sup> batch reactors [89]. The first

aerobic-granule sewage treatment plant was commissioned in Gansbaai. It was designed for a 4000 m<sup>3</sup>/d flow capacity and consisted of three parallel batch reactors, 7 m high and 8 m in diameter. A full-scale aerobic-granule system also operates in Epe (Netherlands). The facility is supplied with municipal and food-industry effluent at 8000 m<sup>3</sup>/h [20,39]. Li et al. [22] have described an aerobic-granule municipal wastewater treatment plant in Yanchang. The system is fed with 50,000 m<sup>3</sup>/d wastewater, achieving full biomass granulation after a year's operation. Carbon, ammoniacal nitrogen and total nitrogen removal efficiencies were reported at 85%, ~96% and ~60%, respectively. Selected AGS wastewater treatments systems are presented in Table 4.

Location	Start-Up	Flow Rate [m <sup>3</sup> /d]	Wastewater	Removal Efficiency [%]	Reference
Deodoro, Brazil	2016	Phase I—64,800 Phase II—86,400	Municipal	COD: >90; TN: >60; TP: >50	[39,90]
Epe, Netherlands	2011	8000	Municipal and food-industry	COD: 96.9; BOD <sub>5</sub> : >99.4; TN: >94.7; AN: 99.8; TP: 97.2; total suspended solids (TSS): >98.5	[20,39,91]
Gansbaai, South Africa	2009	4000	Municipal (with a high proportion of industrial slaughterhouse effluent)	COD: 94; TN: 90; TP: >80	[39,90]
Garmerwolde, Netherlands	2013	30,000	Municipal	COD: 89.2; BOD <sub>5</sub> : 96.0; TN: 86.0; TP: 90.3; TSS: 96.4	[92]
Kingaroy, Australia	2016	2625	Municipal	COD: >90; TN: 95; TP: >90	[39,90]
Lubawa, Poland	2017	3200	Mainly municipal, 30–40% dairy effluent	COD: 97.0; BOD <sub>5</sub> : 98.2; TN: 87.0; AN: 99.4 TP: 95.4;	[93]
Ryki, Poland	2015	5320	Municipal	COD: >90; TN: >90; TP: >90	[39,90]
Yancang, China	2008	50,000	Urban (30% domestic sewage and 70% industrial wastewater from printing and dyeing, chemical, textile and beverage)	COD: 85; TN: 59.6; AN: 95.8	[22]

Table 4. Examples of full-scale AGS wastewater treatment systems.

### 3. Basics of Anaerobic Digestion

Anaerobic digestion (AD) of sludge is a complex biochemical process by which organic macromolecules are broken down into simpler compounds, usually carbon dioxide and methane [94,95]. Each step of the digestion is mediated by specialized microorganisms that hydrolyze polymeric substances through enzymatic action. The resultant monomers are further metabolized into alcohols, short-chain fatty acids, H<sub>2</sub> and CO<sub>2</sub> [96,97]. AD has long been used to process sludge, decreasing its total mass and improving dewaterability. It also

helps stabilize sludge by removing easily decomposed organic substances, reducing susceptibility to putrefaction, ensuring partial sanitization and reducing nuisance smells [98]. AD is also used for treating wastewater with high loads of readily biodegradable organic matter [99–101]. Through AD, organic fractions of municipal, industrial and agricultural waste can be harnessed for biotechnological purposes or in dedicated agricultural biogas plants to grow biomass for energy production [102,103].

AD is a four-step processes, with each successive step requiring specific conditions and process parameters [104]. The steps are usually classified as: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The first two are collectively referred to as acidogenic fermentation (after their end product), whereas acetogenesis and methanogenesis are known as methanogenic fermentation, as they lead directly to the production of methane [105]. The conversions of the feedstock during fermentation are presented in flowchart form in Figure 3.



H<sub>2</sub>-hydrogen, CO<sub>2</sub>-carbon dioxide, CH<sub>4</sub>-methane.

Figure 3. AD process flowchart.

AD begins with hydrolysis, which is the degradation of organic polymeric substances (carbohydrates, protein, lipids) into simpler compounds, monomers and dimers [106]. Hydrolysis is mediated by enzymes of specific hydrolyzing bacteria strains, e.g., cellulase, hemi-cellulase, lipase, and other enzymes produced by hydrolytic bacteria [107]. This first step of AD lays the foundation for subsequent biodegradation of organic compounds. The monomers and dimers generated at this stage largely shape the kinetics of the entire process [108]. The hydrolysis step is carried out by a wide spectrum of microbes, including facultative and obligate anaerobes of the genera *Aerobacter, Alcaligenes, Clostridium, Flavobacterium, Lactobacillus, Lactobacterium, Micrococcus, Streptococcus*, and *Pseudomonas* [109].

During the acidogenesis stage, the organic substances produced during hydrolysis are broken down into simpler compounds (volatile fatty acids, alcohols) by facultative bacteria [110]. This part of the process mainly produces organic acids, alcohols and aldehydes, with carbon dioxide and molecular hydrogen as by-products [111]. Acidogenesis

is primarily mediated by the same bacteria responsible for hydrolysis [112]. The next step of AD is acetogenesis by which organic acids are converted into acetic acid, carbon dioxide, and hydrogen [113]. Acetate is generated through one of two pathways. The first oxidizes the long-chain fatty acids formed in the acidogenesis phase, releasing hydrogen, while the second uses hydrogen to reduce carbon dioxide in the presence of homo-acetate bacteria [114]. Acetogenesis is the critical phase for determining biogas generation [113].

Methane production is the final stage of AD, by which methane is generated from acetic acid and hydrogen, whereas the organic acids and other substances formed in the first stage are broken down [115]. Methanogenesis determines the digestion rate, as providing the right conditions for the microorganisms of the last stage is key to the efficiency of the entire process [116]. In addition to methane and carbon dioxide, methanogenesis also produces smaller amounts of hydrogen sulfide, ammonia, and water [117]. An estimated 95% of biodegradable organic matter is metabolized into gaseous products during AD, with the remaining 5% being the bacterial biomass [118]. The methane-producing organisms involved in the process are *Archea*, specifically: *Methanosarcina*, and *Methanosaeta* [119]. The best results in terms of feedstock decomposition are achieved when the conversions of the acidic and fermentation stages occur at similar rates. Anything that slows down the process at the hydrolysis or acidogenesis stages hinders subsequent fermentation steps [120].

AD plant operators benefit from maximizing both methane production and sludge stability [121]. Although biogas production through AD is a well-established process, the majority of commercial AD systems still operate below optimal efficiency [122]. The performance of AD is a function of myriad factors, which must be screened and optimized accordingly [123]. These include the basic design of the process and of the biological reactor itself [124]. Operational guidelines are also important, including the following: sludge pre-treatment, the method and frequency of sludge dosing into the digester, the mixing duration and regime, and maintaining microbial activity in the reactor [125]. Of particular importance is optimization of the digester conditions, such as the temperature, pH, buffering capacity, and fatty acid levels [126,127]. Another crucial aspect to the end-performance of AD are process parameters, including the organic load rate (OLR) and the hydraulic retention time (HRT) [128,129]. Many researchers and operators have suggested that existing sludge AD systems are hamstrung by the lack of reliable sensory equipment for monitoring key parameters and of suitable parallel control systems that could ensure continuous operation at peak efficiency [130].

#### 4. Anaerobic Digestion of AGS

Anaerobic digestion in AGS processing can produce renewable energy and stabilize/upgrade the sludge. Another benefit is that the volume of sludge to be managed and neutralized is significantly reduced [131]. Research has shown that approximately 40 to 60% AGS can be biodegraded via AD, which is comparable to the values for CAS [92]. The anaerobic digestibility of AGS is a function of multiple factors, including the type of wastewater, the size and structure of the aerobic sludge granules, the treatment process, and the AD process parameters (including AGS pre-treatment, if any) [132]. Research work describing and documenting biogas production from AGS has been quite limited, so it is important to summarize existing results and use them to delineate future prospects for this avenue of sludge management.

Despite the growing interest in applying AGS technology for wastewater treatment, full-scale systems remain few and far between compared to CAS [133]. This limits the availability and range of practical, real-world data [134], particularly with regard to anaerobic digestion of AGS. Most studies in the literature focus on laboratory-scale or pilot-scale

efforts, which contribute to the scientific understanding of the issue, but offer little insight into operational difficulties that may emerge in scaled-up systems [131]. This extends both to wastewater treatment applications and to anaerobic digestion of AGS. Bernat et al. [135] found that biogas production potential of AGS was 1.8 times lower than that of CAS. The experiment tested different organic load rates (OLRs). Biogas production from AGS ranged from 0.32 to 0.41 m<sup>3</sup>CH<sub>4</sub>/kgTS, with methane fractions of approx. 56.7–59.5%, depending on the OLR. By comparison, anaerobic digestion of CAS produced 0.536 do 0.781 m<sup>3</sup>CH<sub>4</sub>/kgTS, with the biogas containing between 60.8 and 62.4% methane [135].

The literature classifies AGS into two types, according to the operational profile of the treatment system: excess AGS (AGS-EX) and AGS selection discharge (AGS-SD) [92]. The volume of AGS-EX results from, and is proportional to, the growth of bacterial biomass during waste degradation [21]. AGS-EX is systematically removed from the system to maintain balanced biomass levels in the bioreactors, to control SRT, and to stabilize the ongoing biochemical processes [92]. This is usually a part of processing at flow-through wastewater treatment systems and sequential bioreactors [92]. AGS-SD (AGS selection discharge) is a specific portion of sludge removed every treatment cycle [21]. AGS-SD is more flocculent in structure, with smaller, less compact granules and a lower settling velocity than AGS-EX [21]. Using the selection discharge method, a portion of AGS with lower settleability can be ejected from the system, with the added benefit of avoiding secondary wastewater contamination [136]. This solution is used most often in sequencing biological reactors (SBRs) [136].

The effect of AGS removal method on the AD process and its performance in a wastewater treatment system was evaluated by Guo et al. [92]. The results were compared against those for anaerobic digestion of CAS. The study showed that both AGS-SD and CAS contained high carbohydrate levels of approximately  $429 \pm 21$  and  $464 \pm 15$  mg glucose/gVS, respectively. These carbohydrates were primarily cellulosic fibers. AGS-EX was similar to CAS in terms of protein in the biomass, with levels of  $498 \pm 14$  and  $389 \pm 15$  mg/g sludge VS, respectively. Mesophilically digested AGS-SD was found to have a markedly higher biochemical methane potential ( $296 \pm 15 \text{ mL CH}_4/\text{gVS}$  feedstock) than AGS-EX or CAS. The BMP was almost 1.5 times higher than that of AGS-EX, which is attributed to the slow settleability of the readily-biodegradable cellulose-like fibers that form the majority of AGS-SD sludge. Biogas production from the digested AGS-EX was  $194 \pm 10$  mL CH<sub>4</sub>/gVS, noticeably poorer than yields from anaerobic stabilization of CAS  $(232 \pm 11 \text{ cm}^3 \text{CH}_4/\text{gVS})$ . The study demonstrated that the proteins and carbohydrates in AGS-EX are more resistant to anaerobic biodegradation than CAS, due to the presence of refractory microbial metabolites in the AGS-EX [92]. Furthermore, the chemical and mechanical differences between AGS and CAS in terms of gel-forming extracellular EPS and sludge morphology may explain the variation in anaerobic digestibility of polymers [136]. The study underscores a need to quantify and screen the technological parameters for AGS reactors that impact sludge morphology and the degradability of individual biomass fractions during AD [13]. For example, AGS reactors with lower SRTs are considered to have much higher biochemical methane potential (BMP) [137].

Another experiment managed to produce an average of  $260 \text{ cm}^3/\text{g}$  volatile suspended solids (VSS) methane from AGS grown during municipal wastewater treatment, a significant improvement over yields from CAS ( $240 \text{ cm}^3/\text{g}$ VSS) [132]. The difference in AD performance was the most pronounced for separated pure granules ( $500 \mu$ m and above), as this variant produced 50% more methane than AD of CAS [132]. According to the authors, this improved performance may have been determined by the higher concentrations of extracellular polymeric substances (EPS), as well as the protein and tryptophan levels in the AGS biomass, which were double that of the CAS [132]. Val Del Rio et al. [138] examined the biochemical methane potential of AGS in a pilot-scale SBR fed with the liquid fraction of swine manure. The system yielded 0.35 m<sup>3</sup> biogas/kg VSS input, assumed to contain 60% methane [138].

A study by Palmeiro-Sánchez et al. (2013) evaluated the feasibility of anaerobic biodegradation of AGS under brackish conditions, comparing the results against anaerobic digestion of flocculent activated sludge (FLAS). The results showed similar biodegradability rates across the two substrates—32% and 27%, respectively. These findings clearly indicate that the aggregation status and density of bacterial cells in AGS do not inhibit AD performance [139]. The brackish conditions resulted in elevated sodium and free sulfides in the bioreactor at 2.1–5.2 gNa/dm<sup>3</sup> and 38–93 mg S/dm<sup>3</sup>, respectively, well above the optimal limits for anaerobic digestion. However, the experiment found no inhibition of BMP or biodegradability by this factor. The biogas contained significant H<sub>2</sub>S levels (1.5–3.8%) and would require pre-treatment before any further use, i.e., in energy production [139].

Co-digestion often promotes biogas production through biochemical conversions and improves its qualitative composition. Co-digestion of AGS and CAS leads to higher methane fractions in the biogas and faster production of gaseous metabolites by the microorganisms compared to single substrates [135]. A mass and energy balance model for AGS has shown that cost-effectiveness can be improved by integrating anaerobic digestion with advanced chemical precipitation of phosphorus in the wastewater [140]. One third of the COD input into the wastewater treatments system can be recovered as biogas energy [141]. The commercial attractiveness of the process is further bolstered by the decreased organic load, thus reducing oxygen demand for AGS reactors by up to 45%. Part of the COD load is redirected from the AGS reactor to the digester, lowering COD and total Kjeldahl nitrogen in AGS reactors [141]. The results of studies on AD of AGS are listed in Table 5.

Wastewater	Methane [dm <sup>3</sup> /kg VS]	Biodegradability [%]	Reference
Municipal	272.5–357.2 *	-	[135]
Municipal	AGS-EX: $194 \pm 10$ ; AGS-SD: $198 \pm 10$	COD: >96	[92]
Municipal	260	COD: $51.1 \pm 4.2$ ; VSS: $50.9 \pm 4.2$	[132]
Synthetic	285	COD: $59.6 \pm 4.4$ ; VSS: $59.9 \pm 4.4$	[132]
Liquid fraction of swine manure	210 *	VS: 44; TSS: 32	[138]
Municipal	$197 \pm 11$	VS: $25.4 \pm 1.3$	[136]
Synthetic wastewater	235-310	-	[140]
Urban wastewater	215	-	[131]
Liquid fraction of swine manure	169	BD: 33	[142]

 Table 5. Comparison of experimental results on AD of AGS.

\* own calculations.

#### 5. AGS-Related Determinants of Anaerobic Digestion

An important operational variable in large-scale methane digestion systems is the optimal level and availability of organic feedstock [143]. In fact, the entire AD processing line can be calibrated against this key parameter [144]. Studies to date have shown that specific quantities of AGS biomass generated from waste degradation per COD load removed or BOD<sub>5</sub> are similar to those grown using CAS [145]. This is further supported by observations and analyses of full-scale systems [13]. Therefore, it stands to reason that anaerobic treatment of AGS also faces similar challenges, and anaerobic digestibility of AGS should, thus, be fully and reliably tested. In addition to exploring the primary effects of AGS stabilization, it is also important to identify how the efficiency of anaerobic digestion correlates with the quantity and composition of the resultant biogas [132], as this is crucial to the performance of AD in terms of energy production and cost-efficiency [58].

Other researchers have noted that, although AGS and CAS levels are similar, given the same operating parameters, AGS-based systems require higher sludge age (sludge retention time, SRT), which reduces low biomass growth [146]. Regardless, the rapid proliferation of AGS-based technologies is likely to be followed by a gradual rise in surplus granulated sludge produced, which will not be suitable for disposal into the environment due to its quality [147]. Therefore, there is a real need to develop AGS-handling procedures, including a process guidance protocol to ensure optimal AD performance.

Few studies to date have attempted to determine which parameters of AGS may influence and determine the AD process and its products. It is certain, however, that the morphology, structure, size and chemical composition of AGS can influence the AD process and its efficiency [132]. One of the major determinants of the activity of fermenting microbes and production of gaseous metabolites is the amount of readily-available dissolved organic matter [148]. Efficient hydrolysis and fast dissolution of solid organic matter can change the conversion rate during the first stage of acidic fermentation and directly affect the methanogenesis process and its efficiency [149]. Dissolved CAS provides for efficient hydrolysis, which sets the pace for subsequent anaerobic conversions. This is not the case with AGS, for which increased hydrophobicity, density, granule compactness, EPS levels, and high proportions of filamentous bacteria in the granule structure substantially limit degradability and AD conversion rates [150].

Bernat et al. [135] have demonstrated that anaerobic biodegradation may be hampered by the chemical composition of the granules. Of the filamentous substances present in AGS, approximately 54% are AD-resistant lignocellulosic substances. This means that anaerobic digestion of AGS was less than half as productive as that of CAS (as expressed by methane yields). Specific biogas production fell within the range of 0.3 to 0.4 m<sup>3</sup>/kg AGS dry mass, with methane fractions of approximately 56.7–59.5% [135]. Rollemberg et al. [37] have also noted that high fiber fractions (especially the biodegradation-inhibiting lignin), which are often more than 18% total solids (TS), result in low methane yields in biochemical methane potential (BMP) tests. Though CAS can contain up to 20% fiber, this fiber is usually hemicellulose, which is easier to biodegrade than lignin [135]. Lignin is a highly cross-linked macromolecule composed of three types of aromatic acids, which play a major role in shaping the physical properties of the biomass and protecting it from cellulose-degrading enzymes [151]. The presence of lignin in the complex can harden lignocellulosic materials and bind other sugars into a highly compact, stable complex highly resistant to most enzymes and other substances, such as acids [152]. Lignocellulose can be processed into biogas, but usually requires pre-treatment, as the lignocellulose biodegradation process is severely limited by multiple factors [153,154]. Degradation of lignocellulosic feedstocks can be inhibited by several factors. These include the crystalline structure, low surface availability, the structure of the lignocellulosic matrix, porosity, cell wall thickness, and the variety of biomass molecules [155]. Due to its recalcitrance to enzymes, lignocellulose should be pre-treated prior to waste-to-biogas conversions [156]. This has prompted growing interest over the past several years in exploring methods to decompose lignocellulosic structures and maximize bioprocess performance [157].

Val Del Rio et al. [138] have proven that high levels of functionally essential vesicle protein in AGS can cause ammoniacal nitrogen to accumulate in the bioreactor, resulting in increased toxicity of the medium and reduced metabolic capacity of fermenting bacteria [138]. Nitrogen is an essential nutrient for microbes, usually released as ammonia during hydrolysis and digestion of protein-containing feedstocks [158]. Applying the wrong parameters for sludge AD can lead to elevated nitrogen levels in the digester [159]. Whereas the ammonium ion NH<sup>4+</sup> is synthesized by most bacteria for nitrogen transport [160], non-dissolved NH<sub>3</sub> inhibits microbial metabolism and activity [161]. Ammonia can diffuse freely through the cell membrane, which can lead to changes in the intracellular pH value, higher energy demand, or inhibition of specific enzymatic reactions in the cell [162]. This inhibiting action mostly affects sensitive, methane-producing *Archea* [163]. Comparative investigations on methanogenic media have also shown that high levels of ammonia strongly promote the growth of aceto-clastic methanogens [164].

It has been shown that EPS—an important component of the AGS matrix—can be a readily-digestible and biodegradable source of organic matter for anaerobes [165]. This can

have a significant impact on biogas yields and methane fractions [141]. However, it should be noted that most of the biodegradable fraction of EPS is embedded in the granule interior and core, whereas the surface is mostly composed of structure-forming polymers that are non-biodegradable or hard-to-degrade [166]. The granular interior contains approximately 5 times more EPS than the outer layers [167]. It follows that the efficiency of digestion and EPS degradation is determined by the availability of biodegradable organic structures. The latter can be ensured by increasing the HRT in digesters, using a thermophilic process, or incorporating effective AGS pre-treatment methods into the process [168]. AGS parameters that can potentially affect the AD process and its performance are presented in Table 6.

Factor	Characteristics	Potential Effects on AD	Reference
Structure	Biomass composed of compact and dense granules	Higher biomass retention, resistance to high organic loadings and toxicity, which is beneficial for AD.	[39,166]
Lignocellulosic content	Of the filamentous substances present in AGS, approximately 54% are lignocellulosic substances resilient to AD	Lowers the performance of AGS AD (as expressed by methane yields). The lignocellulosic material usually has to be pre-treated.	[135,156]
Vesicle protein content	Nitrogen is an essential nutrient for microbes, usually released as ammonia during hydrolysis and digestion of protein-containing feedstocks. Selecting the wrong parameters for sludge AD can lead to elevated nitrogen levels in the digester	Can cause ammoniacal nitrogen to accumulate in the bioreactor, resulting in increased toxicity of the medium and reduced metabolic capacity of fermenting bacteria, especially sensitive methane-producing <i>Archea</i> .	[138,159,163]
Fiber content	High fractions of fiber, especially lignin—often more than 18% TS	Lignin inhibits biodegradation and reduces methane yields in BMP tests.	[37,154]
EPS content	A major component of the AGS structure. Most of the biodegradable fraction of EPS is embedded in the granule interior and core, whereas the surface is mostly composed of structure-forming polymers	Can serve as a readily-digestible and biodegradable source of organic matter for anaerobes, thus, significantly improving biogas yields and methane fractions.	[141,165,166]

**Table 6.** Aspects of AGS morphology and composition that can potentially affect the AD process and its performance.

#### 6. Pre-Treatment Influence on AGS Anaerobic Digestion

Feedstock pre-treatment and disintegration facilities are increasingly being incorporated into AD process design to improve performance [169]. This is done to break down the compact and complex structures of the biomass, promote hydrolysis, and dissolve organics, thus enhancing anaerobic biodegradation efficiency [170]. Pre-treatment has also found increasing use in treating sewage sludge [171]. The compactness and hydrophobicity of AGS granules, as well as the prevalence of filamentous bacteria and EPS in its structure, are further indicators that disintegration may be an apt choice for pre-treatment of such sludge [21]. Mechanically destroying the compact structure of AGS-EX has been found to boost biogas production rates by accelerating the breakdown of rapidly biodegradable organics. However, the pre-treatment of AGS-EX produced no significant improvement in the potential energy production, with the biogas yield and composition being comparable to the control [92]. Thus, more research work has to be done to explore the applicability of more advanced pre-treatment techniques. The scheme of the destruction of granule structures during the AGS pre-treatment methods used so far is presented in Figure 4.



Figure 4. Scheme of the AGS pre-treatment of before the AD.

One such technique has combined thermal hydrolysis and anaerobic digestion of AGS sourced from two different wastewater treatment processes [142]. One reactor was fed with swine manure, the other with synthetic municipal wastewater. The results obtained for the untreated AGS showed significant differences in anaerobic digestibility between the two types. The AG sludge grown on swine manure proved 33% biodegradable, whereas the batch grown on municipal wastewater had 49% degradability [142]. The range for CAS is 30% to 50%, depending on source of sludge [172]. Pre-AD heat treatment was found to be advantageous for hard-to-degrade AGS (33% biodegradability). Thermal pre-treatment at 60 °C and thermal hydrolysis at 170 °C improved anaerobic digestibility by 20% and 88%, respectively. However, this pre-processing scheme was found to be of little use for readily-biodegradable AGS (49% biodegradability), as thermal pre-treatment at 190 °C and 210 °C produced only minor improvements in methane yields (by 14% and 18%, respectively) [142].

Another group has examined the structure and morphology of AGS, and similarly concurred that these might be the limiting factors for anaerobic conversion. The researchers conducted an experiment on anaerobic digestion of AGS in three variants: raw AGS, thermally pre-treated AGS, and co-digestion of heat-treated AGS with CAS. The values obtained for anaerobic biodegradability and reduction of solids for raw AGS were 44% and 32%, respectively. Thermal pre-treatment at 133 °C improved digestion performance (solid removal) by approximately 47%. Thermal pre-treated AGS mixed with CAS showed better solid removal performance than the heat-treated AGS alone. The study found that anaerobic stabilization of AGS offers a similar performance to CAS, indicating that the granular structure of the biomass does not seem to limit the anaerobic process [138].

Another study has found that thermal pre-treatment of AGS correlated linearly with production performance and biogas quality in a steam-explosion process. The AD was run at 37 °C under mesophilic conditions [140]. The pre-treated mineralized AGS had a mineral fraction of almost 40%. It was found that AGS with high mineral content is of limited use for anaerobic digestion. Biogas production from non-pre-treated AGS with a 10% mineral fraction can be up to 30% higher than from granules containing 39% minerals [140]. Steam explosion was validated as a very effective way to enhance biogas production from digesting mineral-rich AGS (which tends to have lower BMP). The pre-treated boosted biomethane production at 0.370 dm<sup>3</sup>CH<sub>4</sub>/gVS, compared with the 0.235 dm<sup>3</sup>CH<sub>4</sub>/gVS for CAS, and non-treated mineral-rich (39%) AGS. The low biogas production from non-treated, mineral-rich AGS can be partially alleviated by steam explosion at 170 °C for 30 min. Total methane yields from AGS have been 20% higher than from CAS under similar operating conditions, due to the slow settleability of the readily-biodegradable cellulose-like fibers that end up in the AGS-SD [92].

A study by Cydzik-Kwiatkowska et al. [131] tested the biogas generation potential of AGS sourced from a full-scale municipal wastewater treatment plant. To increase organic uptake by anaerobic bacteria, the AGS was homogenized or pre-treated with ultrasound. Extraction of organic matter from cells was about an order of magnitude higher after ultrasound pre-treatment than after homogenization, with the added benefit of significantly higher production of methane-rich biogas (455 dm<sup>3</sup>/kg VS, approx. 66% CH<sub>4</sub>). The digestion time for the pre-treated AGS was 25% lower than the untreated batch. AGS digestate was rich in Ca (77.0 g/kg TS), Mg (10.9 g/kg TS), N (35.1 g/kg TS) and P (32.4 g/kg TS), with low levels of heavy metals and BMP. The digestate was found to be an environmentally safe, rich source of organic matter and elements essential for soil fertility and stability [131]. Experimental results on pre-treatment performance in improving AD efficiency of AGS are listed in Table 7.

**Table 7.** Comparison of experimental results on pre-treatment performance in improving AD efficiency of AGS.

Wastewater	Pre-Treatment	Methane [dm <sup>3</sup> /kg VS]	Biodegradability [%]	Reference
	Hydrothermal depolymerization at 20 $^\circ \mathrm{C}$	$169\pm7$	BD: $33 \pm 1$	
	Hydrothermal depolymerization at 60 °C	$207\pm10$	BD: $40 \pm 2$	
	Hydrothermal depolymerization at 90 °C	$236\pm 6$	BD: $47 \pm 1$	
Liquid fraction of	Hydrothermal depolymerization at 115 °C	$280\pm12$	BD: $54 \pm 2$	
swine manure	Hydrothermal depolymerization at 140 °C	$308\pm14$	BD: $60 \pm 3$	[142]
	Hydrothermal depolymerization at 170 °C	$337\pm5$	BD: $62 \pm 1$	
	Hydrothermal depolymerization at 190 °C	$311\pm5$	BD: $56 \pm 1$	
	Hydrothermal depolymerization at 210 °C	$314\pm18$	BD: $52 \pm 3$	
	Hydrothermal depolymerization at 20 °C	$243\pm1$	BD: $49 \pm 0$	
Model municipal	Hydrothermal depolymerization at 170 °C	$346\pm7$	BD: 46 ± 1	
wastewater	Hydrothermal depolymerization at 190 °C	$370\pm15$	BD: $56 \pm 2$	[142]
	Hydrothermal depolymerization at 210 °C	$404\pm23$	BD: $58 \pm 3$	
Liquid fraction of swine manure	Heat treatment at 133 °C	-	TSS: 47	[138]
Synthetic wastewater	Steam explosion at 170 $^\circ C$	370–400	-	[140]
Urban wastewater	Ultrasound disintegration	300	_	[131]

It should be emphasized that the amount and scope of research work on the use of pre-treatment to improve the AD efficiency of granulated sludge is still very limited. So far, mainly methods based on hydrothermal depolymerization [173] have been tested. The obtained results confirm the improvement of AGS biogradability and a direct increase in methane production [142]. The experiments conducted so far have been carried out on a laboratory scale, which does not allow for a comprehensive and complete assessment of energy efficiency and economic profitability of the AGS pre-treatment process [131]. Other pre-treatment methods need to be verified, including hydrodynamic cavitation [174], conditioning with solidified carbon dioxide [94], use of chemical [175] and enzymatic techniques [176]. Research on a larger scale in order is also required to obtain operational data, allowing the estimation of the real energy balance of the pre-treatment technology.

#### 7. Conclusions and Future Research

Despite the growing importance of AGS in wastewater treatment, the data on the properties and appropriate handling of AGS has been sorely lacking. Very little progress has been made in verifying how to adapt existing wastewater treatments systems to make the best use of AGS and CAS. This is a key issue for municipal management systems, as sludge generated by AGS and CAS technologies is the largest waste stream generated at wastewater treatment plants.

Anaerobic digestion is a core part of waste management. AD can be used to stabilize sludge, remove organic matter, reduce sludge volume, improve sanitary indicators, limit nuisance smells, improve fertilizing properties and capture methane-rich biogas. This technology is also fully in line with the principles of circular economy, bioeconomy, development of renewables and limiting carbon dioxide emissions. This review of research on anaerobic digestion of AGS shows that it can be considered as valuable a feedstock as CAS. Many have reported no major differences in biogas yields and methane fractions between the two substrates. On the other hand, some authors have found that the structure and morphology of AGS has a negative effect on the process and its products, and, thus, requires pre-treatment or co-digestion with other organic substrates.

It should be noted that research-backed data is severely limited, as is the number of publications to date. Therefore, there is a need to expand the available data and continue the research work to optimize the anaerobic digestion of AGS, identify optimal technological parameters of the process, and explore possible pre-treatments. An important avenue of research that has yet to be pursued is the potential of AGS as a substrate in hydrogen production. The production of hydrogen in dark fermentation is one of the anaerobic digestion technologies of sewage sludge. The feasibility of implementation and the effectiveness of this method has already been repeatedly confirmed for CAS. Satisfactory results related to the stabilization of sewage sludge and the production of hydrogen were obtained. In contrast to CAS, there is a lack of studies allowing assessment of the technological efficiency of hydrogen production using AGS as a source of organic substrate. Hydrogen has been gaining much interest as an energy carrier and as a way to mitigate CO<sub>2</sub> emissions. Hydrogen power targets have been included, for example, in the Hydrogen Strategy for a Climate-Neutral Europe. The strategy cites green hydrogen as one of the key energy carriers that can help reach the goals of the European Green Deal.

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