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Review

Review on Digestibility of Aerobic Granular Sludge

Mohamed S. Zaghloul, Asmaa M. Halbas, Rania A. Hamza * and Elsayed Elbeshbishy *

Environmental Research Group for Resource Recovery, Department of Civil Engineering, Faculty of Engineering, Architecture and Science, Toronto Metropolitan University, 350 Victoria Street, Toronto, ON M5B 2K3, Canada
* Correspondence: rhamza@torontomu.ca (R.A.H.); elsayed.elbeshbishy@torontomu.ca (E.E.)

Abstract: Full-scale wastewater treatment plants utilizing aerobic granular sludge technology are being built in many countries worldwide. As with all biological wastewater treatment plants, the produced waste biomass must be stabilized to protect the population, wildlife, and the environment. Digestion is usually used to break down the complex organics in the waste sludge; however, the digestibility of aerobic granular sludge still needs to be fully understood compared to the conventional activated sludge. This paper reviews the studies published on the digestibility of waste aerobic granular sludge to date. Studies comparing aerobic granular sludge and activated sludge in terms of composition, properties, and digestibility are highlighted. The impact of biological composition and physical properties on the digestibility of sludge is reviewed in terms of biomethane production and biodegradability. The effect of pre-treatment is also covered. Areas for future research are presented.

Keywords: aerobic granular sludge; wastewater treatment; digestibility; pre-treatment; biomethane production

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1. Introduction

Aerobic granular sludge (AGS) is becoming more popular as an alternative to activated sludge (AS) [1,2]. This is mainly due to the advantages of (AGS) over floccular sludge, such as higher density, microbial diversity leading to simultaneous removal of nutrients and organics, resilience, a 75% smaller footprint, and a 50% reduction in energy demand [3]. The AGS technology is operated in a sequencing batch reactor (SBR) without the need for primary sedimentation tanks. The high concentration of solids in the influent, coupled with the shear force due to the aeration and wastewater up-flow in the SBR, triggers the granulation of the biomass. Controlling the settling time of the SBR cycle allows the reactor to retain the faster-settling granules and wash out the slower-settling biomass flocs, leading to the accumulation of the denser granules inside the reactor [3].

Sludge wasting in biological wastewater treatment is performed primarily to control the solids retention time (SRT) and maintain the biomass concentration in the bioreactor [4]. In the AS process, waste sludge can be taken from the aeration tank's mixed liquor directly or from the return activated sludge (RAS) line, typically the preferred wasting location, as it minimizes the volume of sludge discharged and handled. Most AGS-based bioreactors operate as SBRs, where aeration and settling occur in the same tank, and thus RAS is eliminated. Waste AGS is usually withdrawn between the settling and filling phases to obtain the highest concentrated sludge [5]. Another portion of sludge is removed unintentionally during the decanting phase, which does not fall below the decant port during the settling phase. This process is called the selection pressure of AGS, and it is regarded as one of the main factors that help with granule formation by naturally selecting the faster-settling granules [6]. The other main factor that triggers granule formation is the shear force induced by the air bubbles on the biomass [3,7]. This sludge is usually more floccular and is separated from the effluent using an effluent equalization tank [8]. It has been reported that particle size distribution of the wasted sludge showed that more than 90% of the AGS wasted intentionally (i.e., selective discharge) was larger than 500 µm, while the sludge wasted with the effluent wastewater during decanting had an average particle size smaller than 500 µm [8,9].

The quantity of wasted AGS is determined using Equation (1) [10]. The SRT is a control parameter chosen by the plant designers to maintain the required mixed liquor volatile suspended solids (MLVSS) concentrations, allow for new biological growth, and optimize nutrient removal [11]. The mass of volatile suspended solids (VSS) in the waste sludge is controlled by the operators, where a certain amount of sludge is withdrawn after settling and before filling. The mass of VSS in the effluent is more challenging to measure due to the inconsistency of solids concentration in the effluent stream [8].

$$SRT = \frac{Mass\ of\ MLVSS\ in\ the\ reactor}{Mass\ of\ VSS\ in\ effluent + Mass\ of\ VSS\ in\ Waste\ Sludge} \quad (1)$$

The washed-out sludge leaves the reactor as the effluent begins to be decanted, then once the sludge-liquid interface passes the effluent port, the effluent becomes clear. Theoretically, the only way to accurately sample the VSS in the effluent is by placing the effluent wastewater in a completely stirred reactor and sampling from there, which is not feasible in full-scale applications. Therefore, SRT estimation is often not accurate in AGS bioreactors. In full-scale wastewater treatment plants, however, it was reported that both the conventional AS (i.e., flocculent sludge) and the AGS processes have similar waste sludge production rates. The AGS process at the Nereda® plant in Garmerwolde produced 0.23 kgVSS/kgCOD of waste AGS with an SRT of 28 days, while the AS process at Harnaschpolder produced 0.25 kgVSS/kgCOD at an SRT of 24 days [8]. In Lubawa, Poland, a full-scale AGS facility operated at an SRT of 30 days produced 0.6 kgVSS/kgCOD of biomass [12]. Using synthetic wastewater at a C:N ratio of 100:5, the biomass yield was 0.499 kgVSS/kgCOD [13].

This mini-review article compiled the research published to date on the digestibility of AGS. The literature on the digestibility of AGS in the last ten years is still very limited, and thus, a summary is provided to motivate further research in this area. A recent review article, covering the AGS technology as well as its digestibility has also found that the research is limited [14]. In this article, the biological and chemical characteristics of waste AGS in the reviewed AGS digestibility studies were discussed. The pre-treatment and digestion processes of waste AGS in these studies were summarized. The comparisons made in these studies between the digestion of AGS, waste-activated sludge (WAS), and primary sludge (PS) were highlighted. The effect of the type of AGS and its biological and microbial composition on the pre-treatment and digestibility of waste AGS were also reviewed.

2. Aerobic Granular Sludge Physical Properties

The physical properties of waste AGS can vary according to the type of wastewater treated and the control of the SBR reactor. Wastewater with higher readily biodegradable organics, such as municipal wastewater with high volatile fatty acid (VFA) content, leads to faster granule formation with larger particle sizes and lower density due to the rapid formation of new biomass and larger amounts of slow-settling filamentous growth [15]. Higher ratios of slowly biodegradable to readily biodegradable organics, such as wastewater from the food industry, produce less floccular growth. Denser and faster-settling granules are obtained using the selection pressure by controlling the SBR cycle times, where the settling time is reduced to only allow the denser AGS to remain in the reactor while the floccular sludge is washed out [6].

In a steady-state AGS bioreactor, the washed-out floccular sludge is the amount of biomass growth during each cycle that did not granulate and is usually not used in controlling the reactor SRT [8]. The SRT is controlled by withdrawing settled sludge with fully formed granules. However, washed-out floccular sludge is still collected and requires stabilization similar to the withdrawn waste AGS. The structural morphology and microbial composition differences between the two waste sludge streams of AGS reactors result in differences in their digestibility behavior. Sludge with intact granules contains complex biopolymers that maintain the structural integrity of the granules and have hydrophobic characteristics, which require homogenization and pre-treatment to release the organic contents of the granules. Floccular washed-out sludge, on the other hand, is composed of filamentous bacteria as well as cellulose-like fibers, which are highly biodegradable, which leads to faster digestion and less requirement for pre-treatment [8,16].

The average particle size of aerobic granules in AGS reactors varies according to the type of wastewater treated and the process operation. The aerobic granules, by definition, should have an average particle size of 200 μm [17]. However, a reactor with a granulation percentage of 100% is theoretically not possible. Typically, AGS reactors are hybrid systems of co-existing flocculent and granular biomass [18]. It has been reported that SVI_{30}/SVI_5 can represent the granulation percent, where granular settling would be that of discrete settling rather than flocculent or zone settling [19,20]. During start-up, the AGS reactor is seeded with WAS, which then transforms into aerobic granules. The particle size distribution progression for the first 60 days of operation was plotted to show that the biomass average particle size increases from around 100 μm to above 1700 μm [21]. In comparison to AS, the average particle size at an OLR of 0.93–0.95 gCOD/L.d was 800 μm for AGS and 290 μm for AS, where the AGS was cultivated in the lab, and the AS was collected from a full-scale treatment plant [22]. Table 1 shows the average particle size reported in AGS digestion studies. Other studies have reported that the activated sludge average particle size was 114 μm while AGS was above 1600 μm in both lab and full-scale applications [16,21,23].

Table 1. Average particle size in AGS and AS in various studies.

Sludge Type	Particle Size (mm)	Reference
AGS	1.4–1.7	[23]
AGS	1.3–1.6	[24]
AGS	1	[25]
AGS	1.74	[26]
AGS	0.4–0.8	[22]
AS	0.29	
AGS	1.75	[21]
AGS	1.79	[16]
WAS	0.114	
AGS	0.09–0.35 mm	[27]

The settleability of AGS is typically better than AS due to the larger particle size and faster particle settling velocity. A well-operated granular reactor would have biomass with SVI values below 80 mL/g and an average particle size higher than 200 μm [20,28]. Thermal pre-treatment has been shown to reduce the settleability of AGS due to the transformation of the biomass to a gelatinous consistency with a higher viscosity than the original AGS. It was reported that thermal pre-treatment at temperatures above 115 °C–125 °C drastically increased the granular biomass viscosity, while no change was observed at temperatures less than 100 °C [29,30]. It was shown that this behavior was the opposite of AS under thermal pre-treatment, where the SVI decreased with raising temperature [29,31–33]. The reason for the transformation of AGS to a gel consistency and increased viscosity under thermal pre-treatment is still not understood.

3. Aerobic Granular Sludge Biochemical Properties

The type of influent wastewater affects the fractions of readily to slowly biodegradable organics in the aerobic AGS. Little research is available in the literature on the biodegradability of AGS, but some comparisons were made on the organic composition of AGS cultivated using swine and municipal wastewater [30]. Val del Río et al. [30] observed minimal improvement in biodegradability after the thermal pre-treatment (170 °C–210 °C) of AGS cultivated using municipal wastewater, while AGS cultivated using swine wastewater improved by up to 88%. One of the possible justifications of this finding is that the high content of complex organics in swine AGS was broken down under thermal pre-treatment leading to noticeable digestibility improvement, while municipal AGS already contained a higher fraction of readily biodegradable organics, so thermal treatment had little effect. Table 2 shows the concentrations of chemical oxygen demand (COD), total solids (TS), and volatile solids (VS) in AGS and AS obtained from full-scale and lab-scale reactors reported in different studies. More research is needed to gain further insight into how the wastewater type can influence the composition of complex organics in AGS.

Table 2. Characteristics of AGS and AS from different sources.

Source of Sludge	Type of Wastewater	Sludge Type	COD	TS	VS	Reference
Pilot plant (100 L)	Swine manure	AGS	39.7 g/L	29.6 g/L	27.3 g/L	[30]
	Synthetic wastewater	AGS	85.7 g/L	106 g/L	60.1 g/L	
Pilot plant	Brewery wastewater	AGS	8–31.1 g/L	14–21.1 g/L	7.3–15.9 g/L	[23]
WWTP Calo-Milladoiro, Spain	Municipal wastewater	AS	29 g/L	15.7 g/L	10.9 g/L	
Pilot (100 L)	Swine manure	AGS	7.7–27.5 g/L	9.2–21.1 g/L	8.4–19.2 g/L	[24]
Lab scale SBR	-	AGS	Soluble: 403 (mg/gVSS)	-	-	[34]
Lab scale SBR	-	AS	Soluble: 329 (mg/gVSS)	-	-	
Lab scale SBR (4.5 L)	-	AGS	-	2.28 (%)	1.47 (%)	[25]
WWTP Olsztyn, Poland	Municipal wastewater	AS	-	4.51 (%)	3.46 (%)	
WWTP Olsztyn, Poland	Municipal wastewater	PS	-	1.71 (%)	1.33 (%)	
Lab scale SBR	-	AGS	17.21 g/L	15.3 (gTSS/L)	12.8 (gVSS/L)	[26]
Lab scale SBR (6–8 L)	-	AGS	-	-	-	[22]
Municipal WWTP	Municipal wastewater	AS	-	-	-	
Nereda® plant Garmerwolde	Municipal wastewater	AGS wasted	71.3 (g/L)	6.1 (%)	4.9 (%)	[8]
	Municipal wastewater	AGS Washed out	79.1 (g/L)	6.6 (%)	5.1 (%)	
WWTP Harnaschpolder	Municipal wastewater	WAS	72.4 (g/L)	6.2 (%)	5 (%)	
	Municipal wastewater	PS	77.8 (g/L)	6.4 (%)	5 (%)	
Nereda® plant Garmerwolde	Municipal wastewater	AGS	-	5.41 (%)	4.25 (%)	[16]
WWTP in Harnaschpolder, the Netherlands	Municipal wastewater	WAS	-	5.16 (%)	4.14 (%)	
WWTP in Lubawa (Poland)	Municipal wastewater	AGS	-	1.55 (%)	1.24 (%)	[27]

Additionally, AGS that is wasted after settling was reported to have a different organic composition from washed-out AGS during the decant phase. Guo, van Lier, et al., [8], tested the waste AGS, washed-out AGS, WAS, and PS for three types of fibers: cellulose-like, hemicellulose-like, and lignin-like fibers, as well as proteins, carbohydrates, and lipids. They found that the waste AGS was very similar in composition to the WAS, while the washed-out AGS was similar to the PS. These fibers constitute 30–50% of suspended solids (SS) in wastewater and are typically removed in primary settling in the AS process [35]. However, in the AGS processes, no primary settling is done where raw wastewater enters the AGS reactors directly. Guo, van Lier, et al., [8], found that the fibrous content (as VS) in waste AGS and washed-out AGS was 32.5% and 38%, but the cellulose fraction in the washed-out AGS was double that of the waste AGS. Pronk et al. [9] indicated that fibers do not attach to mature granules but rather remain with the floccular sludge that gets washed out during the AGS process. Guo, van Lier, et al., [8], also indicated that the carbohydrate, lipid, and VFA contents of the washed-out sludge were about double that of the waste AGS, while the proteins were higher in the waste AGS. The difference in organic composition and structural morphology between the two types of sludge directly impacts their biodegradability, where the BMP of washed-out AGS was 1.5 times that of the waste AGS due to the abundance of the biodegradable cellulose-like fibers [8].

The AGS structural integrity relies heavily on a matrix of extracellular polymeric substances (EPS) in which they are entrapped. EPS is a complex mixture of polysaccharides, proteins, nucleic acids, lipids, and humic substances, which provides the cross-linkage between microbial cells in the biofilm matrix in which they are self-immobilized. There is a consensus that EPS synthesis and regulation are linked with microbial interactions, which are further affected by wastewater composition and operating conditions [18,36,37]. EPS production is also highly influenced by environmental stress conditions: shear forces, salinity, and the presence of toxic compounds such as contaminants of emerging concern (CECs) and heavy metals, all of which stimulate EPS production as a defense mechanism. The presence of toxins has been found to increase protein content but to have less effect on polysaccharide content. Although these findings might suggest that the extracellular proteins produced would stimulate the granular formation by promoting nucleation, in fact, excessive EPS could adversely affect granular system performance [38].

The biodegradability of EPS is a key factor in the ease of anaerobic digestion of unhomogenized aerobic granules. Wang et al. [39] indicated that the outer shell of granules that contain aerobic heterotrophic microbes was composed of hydrophobic non-readily biodegradable EPS, while the inner core with anaerobic microbes contained readily biodegradable and soluble EPS. The hydrophobic non-readily biodegradable EPS is responsible for the structural integrity of the granules [40,41]. Homogenization then becomes needed to break the cohesion of the granules to expose the biodegradable organic content, and thermal hydrolysis was used to break down the complex biopolymers into more readily biodegradable compounds. Structural EPS, or only those polymers within the EPS able to form hydrogels, have also been identified as the main component that distinguishes and explains the resistance to degradation of waste AGS compared to AS [8,16]. Table 3 shows the organic biopolymer composition of AGS and AS in different studies.

The selection pressure applied during the operation of the AGS reactors to promote the growth of fast-settling granules also plays a role in the biodegradability of AGS and differentiates it from CAS [8]. The waste AGS extracted to maintain the required SRT is intentionally removed from the settled sludge, which means the granules are more compact and affected by the presence of EPS, while slower-settling sludge is washed out of the reactor. This selective separation leads to the presence of a much higher content of slowly settling readily biodegradable cellulose-like fibers in the washed-out AGS sludge and their absence from the intact granules, further lowering the biodegradability of the waste AGS [9].

Sludge generated from wastewater treatment also contains nutrients and inorganic components. Nutrients are ammonia, nitrogen, and phosphorus, which are used to manufacture plant fertilizer and can cause eutrophication if released untreated into the environment [42]. Inorganic components include several ions, as shown in Table 4. The most commonly present elements, other than carbon, oxygen, nitrogen, and hydrogen, are potassium, calcium, magnesium, and iron.

Table 3. Organic biopolymers composition of AGS and AS from different sources.

Source of Sludge	Type of Wastewater	Sludge Type	Carbohydrates	Proteins (mg/gVS)	Polysaccharides	Lipids (mg/gVS)	VFAs (mg/gVS)	PHA	References
Pilot plant (100 L)	Swine manure	AGS	3.6 g/L	16.6 g/L	-	0.05 g/L	1.4 g/L	0.8 g/L	[30]
	Synthetic wastewater	AGS	6.9 g/L	26.9 g/L	-	0.013 g/L	7.5 g/L	5.5 g/L	
Pilot plant	Brewery wastewater	AGS	-	-	-	-	ND	-	[23]
WWTP Calo-Milladoiro, Spain	Municipal wastewater	AS	-	-	-	-	0.27 (g/L)	-	
Pilot (100 L)	Swine manure	AGS	-	-	-	-	ND–1 g/L	-	[24]
Lab scale SBR	-	AGS	Soluble: 42 (mg/gVSS)	Soluble: 82.7 (mg/gVSS)	-	-	355	-	[34]
Lab scale SBR	-	AS	Soluble: 39.1 (mg/gVSS)	Soluble: 63 (mg/gVSS)	-	-	352	-	
Lab scale SBR (4.5 L)	-	AGS	0.002 (%)	0.926 (%)	-	0.008 (%)	-	-	[25]
WWTP Olsztyn, Poland	Municipal wastewater	AS	0.095 (%)	2.121 (%)	-	0.04 (%)	-	-	
WWTP Olsztyn, Poland	Municipal wastewater	PS	0.162 (%)	0.292 (%)	-	0.01 (%)	-	-	
Lab scale SBR	-	AGS	113.4 (mg/gVSS)	701.6 (mg/gVSS)	-	-	-	-	[26]
Lab scale airlift SBR	-	AGS	-	365 mg/gVSS	135 mg/gVSS	-	-	-	[21]
WWTP in Hong Kong	Municipal wastewater	AS	-	265 mg/gVSS	245 mg/gVSS	-	-	-	
Nereda® plant Garmerwolde	Municipal wastewater	AGS wasted	217 (mg glucose/g sludge)	498	-	37	4.6	-	[8]
	Municipal wastewater	AGS Washed out	429 (mg glucose/g sludge)	301	-	60	9.7	-	
WWTP Harnaschpolder	Municipal wastewater	WAS	190 (mg glucose/g sludge)	389	-	35	5.6	-	
	Municipal wastewater	PS	464 (mg glucose/g sludge)	248	-	73	8.6	-	

Table 4. Inorganic composition of AGS and AS from different sources.

Reference	[34]		[25]			[26]	[21]		[29]	[27]
Sludge Type	AGS	AS	AGS	AS	PS	AGS	AGS	AS	AGS	AGS
NH_4^+	0.7 (mg/gVSS)	1.8 (mg/gVSS)	-	-	-	-	-	-	-	-
TN	39.4 (mg/gVSS)	38.3 (mg/gVSS)	-	-	-	-	-	-	-	-
PO_4^{3-}	9.7 (mg/gVSS)	10.2 (mg/gVSS)	-	-	-	-	-	-	-	-
TP	39.3 (mg/gVSS)	26.7 (mg/gVSS)	-	-	-	31.5 (mg/gVSS)	-	-	9.21–56.51 (g/kgTS)	-
K	18.2 (mg/gVSS)	17 (mg/gVSS)	-	-	-	-	0.34%	0.27%	2.62–3.3 (g/kgTS)	-
Ca	-	-	-	-	-	60.3 (mg/gVSS)	5.34%	0.31%	19.77–188.1 (g/kgTS)	-
Na	-	-	-	-	-	-	5.11%	2.29%	5.42–5.87 (g/kgTS)	-
Mg	10.4 (mg/gVSS)	7.6 (mg/gVSS)	-	-	-	9.1 (mg/gVSS)	0.19%	0.40%	1.9–3.05 (g/kgTS)	-
Mn	-	-	-	-	-	-	-	-	0.025–0.018 (g/kgTS)	-
Al	-	-	-	-	-	2.4 (mg/gVSS)	ND	0.08%	-	-
Zn	-	-	-	-	-	-	0.05%	0.01%	0.009–0.004 (g/kgTS)	295.6 (mg/kgTSS)
Fe	-	-	-	-	-	6.3 (mg/gVSS)	0.02%	0.16%	0.34–0.21 (g/kgTS)	-
Cu	-	-	-	-	-	-	ND	0.01%	0.086–0.058 (g/kgTS)	268 (mg/kgTSS)
Co	-	-	-	-	-	-	-	-	0.009–0.021 (g/kgTS)	-
Ni	-	-	-	-	-	-	-	-	0.009 (g/kgTS)	24 (mg/kgTSS)
Hg	-	-	-	-	-	-	-	-	-	0.0098 (mg/kgTSS)
Cr	-	-	-	-	-	-	-	-	-	39.7 (mg/kgTSS)
Cd	-	-	-	-	-	-	-	-	-	1.6 (mg/kgTSS)
Pb	-	-	-	-	-	-	-	-	-	15.1 (mg/kgTSS)
C	-	-	49.80%	57%	60%	-	38.62%	40.80%	-	-
O	-	-	1.76%	7.56%	9.50%	-	-	-	-	-
N	-	-	7.90%	7%	2.80%	-	9.38%	8.54%	-	-
H	-	-	5%	5.20%	5.60%	-	5.81%	6.41%	-	-
S	-	-	-	-	-	-	0.76%	2.12%	-	-

4. Sludge Preparation Prior to Digestion

Pre-treatment of organic feedstock prior to anaerobic digestion has been proven to improve its biodegradability by breaking down the complex organics in the biological material, which constitute the majority of the WWTP waste sludge components [43–45]. In AGS, the abundance of EPS and other biopolymers, such as alginates, results in more compact granules with hydrophobic properties, which complicates the digestion process [46]. Therefore, pre-treatment may appear necessary to achieve two outcomes: breaking down the compact granular structure and breaking down the complex biopolymers and hydrocarbons. Mechanical or ultrasound homogenization was used to break the structural cohesion of the granules, while thermal pre-treatment was used to break down the complex organics [27,30]. Very scarce research has been done to explore the pre-treatment of AGS for digestion. However, the current literature reported that pre-treatment only improved the methane production rates and had little effect on the final total yield [8]. Zou and Li [34] also reported that thermal pre-treatment improves the recovery of carbon, phosphorus, and hydrogen during anaerobic fermentation.

Mechanical and ultrasound homogenization was used by Cydzik-Kwiatkowska et al. [27] on AGS collected from a full-scale municipal wastewater treatment plant. It was found that the ultrasound was much more effective than mechanical homogenization and resulted in higher methane content in the produced biogas. It was also reported that the digestion of the ultrasound-homogenized AGS was 25% faster than raw AGS. Mechanical crushing was used to homogenize sludge samples in a comparison between AGS and WAS [8]. A household blender was used at 1000 RPM for 5 min. The raw AGS sludge particle size distribution was 70% above 2000 μm, while the raw WAS was 20% between 50 and 100 μm, and 65% between 100 and 500 μm. The homogenized samples were similar in particle size, with around 30% between 0 and 20 μm, 35% between 20 and 50 μm, 20% between 50 and 100 μm, and 15% between 100 and 500 μm. The biogas production analysis also showed a minimal effect of homogenization on the overall yield in AGS, but the rate was faster by about five days to reach the plateau in the accumulated methane production curve. Homogenizing WAS did not have any effect on biogas production. The rate of degradation of readily biodegradable fractions of AGS increased by 24% due to crushing.

The type of wastewater treated was shown to have a significant impact on the effectiveness of thermal pre-treatment. A study by Val del Río et al. [30] compared the digestion of AGS cultivated using municipal and swine wastewater. It was found that the raw AGS cultivated using municipal wastewater was more biodegradable than that cultivated using swine wastewater, with biodegradability of 49% and 33%, respectively. Thermal pre-treatment at 60 °C and 170 °C was found to improve the biodegradability of the swine-based AGS by 20 and 88%, respectively. The more biodegradable municipal AGS was pre-treated at 190 °C and 210 °C with a lower improvement in biodegradability of 14% and 18%, respectively [30].

The compact granular structure was found to have little impact on the final biodegradability and total biogas yield of the AGS, where the overall biodegradability of the AGS was found to be similar to CAS under similar conditions between 30% to 50% [47]. A study used different pre-treatment methods on the same type of AGS and showed that the raw sludge was 44% biodegradable and the anaerobic digestion of the untreated AGS had a 32% solids reduction [24]. Thermal pre-treatment at 133 °C resulted in a 47% improvement in biodegradability. Mixing the untreated AGS with CAS improved the solids reduction during the anaerobic digestion. Val Del Río et al. [24] also compared the biodegradability of CAS with that of AGS and found no major differences in overall biogas yield.

Steam explosion has been found to be an effective pre-treatment method for the anaerobic digestion of AGS cultivated in mineral-rich wastewater [29]. The mineral content inhibits the digestibility of the sludge, leading to lower biogas yields. Steam explosion at 170 °C for 30 min led to a 20% improvement in BMP over CAS under similar conditions [29].

5. Digestion of Aerobic Granular Sludge

The digestibility of aerobic AGS has been studied to investigate its biodegradability and its methane production. It was generally found that the biodegradability of AGS was lower than that of AS due to the granules' morphology and organic biopolymer composition [8,16,34]. Other studies have shown different behavior where both AGS and AS had similar methane production when digested under similar conditions [22,23]. Thermal pre-treatment has been found to improve biomethane production in AGS, where a study compared AGS and AS digestion with and without thermal pre-treatment at 135 °C [24]. Without pre-treatment, the methane production in AS was 20% higher than that of AGS. After pre-treatment, the methane production of AGS was 8% higher than that of AS. It was also found that mixing PS with AGS improved methane production by 16%, but it reduced the methane production of AS by 12% [25].

To the knowledge of the authors, only ten manuscripts were published between 2011 and 2022 that reported the digestibility performance of AGS. Five of these studies used AGS obtained from municipal wastewater treatment, two used AGS obtained from the liquid portion of swine farms wastewater treatment, two used AGS obtained from synthetic wastewater treatment, and one used AGS obtained from brackish wastewater treatment. Table 5 shows the summary of the reported data on the digestion of AGS and the comparison with activated and PS in these studies. It was also noted that all published digestion studies so far were performed as lab-scale experiments with digester volumes between 0.4 and 5 L. Some of these digesters were semi-continuous, while some were just batch experiments. Additionally, only four out of ten studies experimented with the pre-treatment of AGS, and three of them involved thermal pre-treatment. One study only tested ultrasonic homogenization [27]. In the current state of research, the digestion of AGS is still not fully understood. The reported increased viscosity of AGS with heat treatment needs further analysis to identify its reasons and ways to overcome this phenomenon in order to benefit from pre-treatment without increasing the mixing energy demand. Continuous pilot and full-scale digestibility studies should also be performed to validate the experimental results, especially with AGS treatment technology becoming more popular in full-scale applications for wastewater treatment. Further, AGS from different sources, such as food industry wastewater, should be studied to compare their digestibility to municipal AGS.

Table 5. Anaerobic digestion of different AGS and AS substrates.

Type of Sludge	Type of Source Wastewater	Reactor Type	Rector Volume	SRT	OLR	Pre-Treatment	Methane Production (mL/g VS)	Biodegrdability	References
AGS	Swine manure	Batch	0.4 L	-	-	Thermal: 170 °C	337 mL/gVS	62%	[30]
AGS	Synthetic wastewater	Batch	0.4 L	-	-	Thermal 210 °C	404 mL/gVS	58%	
AGS	Brackish wastewater	CSTR	5 L	-	0.4–1.6 (gCOD/L/d)	-	78–136 mL/gCOD	23–42%	[23]
AS		CSTR	5 L	-	1.5 (gCOD/L/d)	-	94 mL/gCOD	27%	
AGS	Swine manure	CSTR–Semi-continuous	5 L	10 days	0.4–1.4 g/L	-	208 mL/gVS	44	[24]
WAS						-	254 mL/gVS	50	
AGS						Thermal: 135 °C	309 mL/gVS	58	
WAS						Thermal: 135 °C	285 mL/gVS	54	
AGS	Synthetic wastewater	Batch	0.6 L	7 days	-	-	0–0.1 mL/gVSS	-	[34]
AS		Batch	0.6 L	7 days	-	-	0–6 mL/gVSS	-	
AGS	Municipal	Batch	-	21 days	2–6 (kgVS/m^{3}d)	-	492.5 m^3/kgVS	-	[25]
AS		-	-	-	2–6 (kgVS/m^{3}d)	-	1178.5 m^3/kgVS	-	
PS:AGS (2:1)		-	-	-	2–6 (kgVS/m^{3}d)	-	574.5 m^3/kgVS	-	
PS: AS (2:1)		-	-	-	2–6 (kgVS/m^{3}d)	-	1035.4 m^3/kgVS	-	
AGS	Municipal	Semi-continuous	0.525 L	20	0.68–0.98 gCOD/Ld	-	285 mL/gVSS	-	[22]
AS		Semi-continuous	0.525 L	22	0.93 gCOD/Ld	-	245 mL/gVSS	-	
AGS	Synthetic	-	-	-	-	-	235 mL/gVS	-	[29]
						Steam Explosion: 170 °C	370–400 mL/gVS	-	
AGS wasted	Municipal	-	-	-	-	-	296.5 mL/gVS	-	[8]
AGS washed-out		-	-	-	-	-	192.9 mL/gVS	-	
WAS		-	-	-	-	-	231.8 mL/gVS	-	
PS		-	-	-	-	-	313.5 mL/gVS	-	
AGS	Municipal	Batch CSTR	2 L	44 days	-	-	197 mL/gVS	-	[16]
WAS		Batch CSTR	2 L	44 days	-	-	242 mL/gVS	-	
AGS	Municipal	Batch	1 L	21 days	-	-	215 (mL/g VS)	-	[27]
						Ultrasound homogenization	300 (mL/g VS)	-	

6. Impact of Microbial Community Structure on AGS Digestibility

Research has shown that the microbial community of AGS was different from that of the flocculent sludge owing to the sludge structure despite being cultivated under the conditions and using the same feed sludge [34], where the microbial community of AGS evolved during the granulation process, and major shifts in the dominated species were observed [48]. The presence of anaerobic and anoxic zones in AGS was reported to promote the survival of anaerobes, which were found to be over 35% more compared to flocculent sludge. These anaerobes facilitated the hydrolysis and acidification during fermentation and thus resulted in more hydrogen production during the early stages of fermentation [34]. The fermentation of nitrifying AGS showed that more soluble chemical oxygen demand (SCOD) and total volatile fatty acids (TVFAs) were released compared to flocculent sludge fermentation, which was attributed to the higher amounts of EPS in AGS and the presence of hydrolytic-acidogenic bacteria [26]. Alkaline fermentation of AGS was reported to favor carbon recovery, whereas acidic fermentation promoted the release of phosphorus in the form of apatite [26].

7. Utilization of Modelling Tools for Prediction of AGS Digestibility

Modeling is a valuable tool that can be used to explain the dynamics of the digestion process of AGS and the factors that influence its rate and methane yields. A partial least square model was used to estimate the anaerobic biodegradability of AGS using the relationship between the initial sludge composition and its BMP [30]. Macroscopic parameters, such as the soluble organic carbon and the COD: TOC ratio, and biochemical parameters, such as the carbohydrate, protein, and lipid concentrations, were used as inputs to the model. The model was used to predict the biodegradability of two AGS samples from different origins (type of influent wastewater) after thermal pre-treatment at various temperatures. A total of 12 samples were modeled, and the predicted biodegradability was within an error of 2–18%. The model was used to quantify the contribution of each of the AGS components, such as the proteins, carbohydrates, lipids, COD/TOC ratio, and soluble organics, to the final biodegradability and add more insight into the behavior of different types of sludge, where the focus was on the particulate and soluble components.

An analysis of the biogas production kinetics using first-order kinetics and non-linear regression analysis was used to compare and understand the dynamics of biodegradation of AGS, WAS, and PS [25]. The models were used to simulate biogas production with an R2 between 88% and 98%. The model was used to estimate the rate, rate constant, and theoretical yield of biogas for each sludge type, as well as mixtures of sludge samples, to simulate co-digestion. Using a set of physicochemical tests, they were able to link the biogas production rates with the sludge characteristics. The impact of lignin content and the TS:VS ratio was found to have a significant impact on the rate and yield of biomethane production. They were also able to identify the benefits of co-digesting AGS with PS rather than WAS.

8. Conclusions and Potential Areas of Research

Studies in the literature have found that the digestibility of AGS is similar to that of AS, making it a viable feedstock for digestion. The AGS morphology and structural polymers slowed down the biomethane production rates, but the overall yield was on par with AS. Thermal pre-treatment was found to improve the methane digestibility of AGS, but it was also found that it can raise the biomass viscosity at temperatures above 125 °C.

The AGS technology is starting to become established as a full-scale wastewater treatment technology, but it is still relatively new compared to the conventional AS technology. Therefore, the available literature on the digestibility of waste AGS is still scarce compared to WAS. There are several gaps in the current literature that allow for future research to be in this area. The first gap is that all the published literature on the digestion of AGS is based on lab-scale digestibility experiments, to the knowledge of the authors, even though full-scale plants already exist in several countries across the world.

In the available literature, only anaerobic digestion was used for AGS. In AS WWTPs, both anaerobic and aerobic digestion provide the same level of stabilization of sludge. Anaerobic digestion has the advantage of the recovery of methane, while aerobic digestion is faster and requires less capital investment than anaerobic digestion. There were no reported studies that investigated the use of aerobic digestion of AGS. It is recommended to compare the aerobic and anaerobic digestion of AGS and study the breakdown of the structural biopolymers.

Additionally, thermal pre-treatment has been used in three out of the four studies that reported using pre-treatment of the AGS. The fourth was ultrasonic homogenization. The impact of pre-treatment on AGS is still not fully understood and requires further analysis. The lack of information in the literature makes it difficult to provide definite conclusions on the efficiency of pre-treatment methods under different conditions.

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