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Review

Enhancing Methane Yield in Anaerobic Co-Digestion of Primary Sewage Sludge: A Comprehensive Review on Potential Additives and Strategies

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Abstract: Traditionally, anaerobic digestion has been applied to mixed sludge, combining primary sludge (PS) with secondary sludge. However, recent research has unveiled the advantages of dedicated PS digestion due to its higher energy content. Anaerobic digestion (AD) of primary sewage sludge can offer a sustainable solution for managing sewage sludge while generating renewable energy. The present study provides a comprehensive examination of the current state of knowledge regarding the anaerobic digestion of PS. Co-digestion of PS with organic substrates, including food waste and agro-industrial residues, emerges as a promising approach to boost biogas production. Additionally, the utilization of additives such as glucose and clay minerals has shown potential in improving methane yield. Critical factors affecting AD, such as pretreatment methods, carbon-to-nitrogen (C/N) ratio, temperature, pH, volatile fatty acids (VFAs) levels, organic loading rates (OLR), inoculum-to-substrate ratio (ISR), and the role of additives, have been meticulously studied. Finally, this review consolidates existing knowledge to advance our understanding of primary sewage sludge anaerobic digestion, fostering more efficient and sustainable practices in sludge management and renewable energy generation.

Keywords: anaerobic digestion; primary sludge; co-digestion; additives; biogas production; methane yield; renewable energy; sustainability

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Citation: Sakaveli, F.; Petala, M.; Tsiridis, V.; Darakas, E. Enhancing Methane Yield in Anaerobic Co-Digestion of Primary Sewage Sludge: A Comprehensive Review on Potential Additives and Strategies. *Waste* **2024**, *2*, 29–57. https://doi.org/10.3390/waste2010002

Academic Editor: Catherine N. Mulligan

Received: 30 October 2023
Revised: 30 November 2023
Accepted: 11 January 2024
Published: 16 January 2024

1. Introduction

The European Commission since March of 2020 has agreed on Europe's new agenda for sustainable growth. One of the primary policy areas of the new circular economy action plan aims to ensure the release of less waste through the improvement in their management, stimulation of innovation in recycling, and the limitation of landfilling [1]. One key process for developing the objectives of waste and recycling management in the context of a circular economy and the EU's Green Deal is the increase in biomethane production. According to the European Biogas Association, the transition of Europe to clean energy systems will be implemented through applications that engage biogas, biomethane, and other renewable gases based on circular economy fundamentals. It is also estimated that "renewable gases will enable carbon-neutral (or even carbon-negative) Europe with zero pollution by 2050" [2].

Anaerobic digestion is a well-known and established practice in municipal wastewater treatment plants (WWTP), which target energy recovery through biogas production, sludge volume reduction, and sludge stabilization through the inactivation of pathogens. Sludge is then converted into a biologically stable material so that it can be safely disposed of or used in applications such as agriculture, composting, etc. In addition, as landfilling is prohibited, the large quantities of sludge that are generated during its treatment process must be accordingly handled.

Normally, anaerobic digestion is applied to the sludge stream that is composed of the primary sludge (PS) collected at the bottom of primary clarifier and the excess of secondary sludge that is removed from the bottom of the secondary sedimentation tank [3,4]. However, recent studies [5,6] have shown that the solo processing of primary sludge may be an advantageous process, as primary sludge has higher energy content than secondary sludge, and the treatment of the primary biosolids as a separate waste stream may reduce the total cost of the management of biosolids, which can reach 50% of the total operational costs of municipal wastewater treatment plants. Resource recovery from primary sludge is economically viable due to its high level of the available biodegradable substrate (e.g., fatty acids and lipids), whereas secondary sludge contains organics of less energy content since it has already undergone significant decomposition [7]; thus, due to the nature of primary sludge, the hydrolysis step during the anaerobic digestion process is accelerated compared to the rate of hydrolysis observed during the anaerobic digestion of secondary sludge.

Although sewage sludges are a readily biodegradable substrate, they have a high moisture content, averaging approximately 98% [8], which leads to the underutilization of anaerobic digesters in wastewater treatment plants due to the low organic loading rates [9]. Such digesters' features could be optimized by the co-digestion of primary sludge with other organic fractions or by using additives that have been confirmed to improve hydrolysis during the anaerobic digestion process. Practices like that will allow extra loading to be applied to the digesters with inputs of high-energy substrates and lower moisture contents, providing an additional biogas production. The most commonly used additive for the co-digestion of primary sewage sludge seems to be the organic fraction of food wastes, as many studies have investigated this method in batch assays, while there are few that have employed it in semi-continuous or continuous systems. Digesting primary sludge along with the organic fraction of food waste seems to be an effective way to maximize the capacity of the digester and increase methane production. This is consistent with circular economy measures in terms of Europe's transition to clean energy systems. Other organic-rich wastes such as agro-industrial wastes and crude glycerol have been used as co-substrates. In addition, substances such as biochar or activated carbon have been shown to promote volatile fatty acid (VFA) consumption. Furthermore, elements such as iron, nickel, cobalt, etc., have been found to enhance the metabolic activity of methanogens, thereby increasing methane yield [10,11].

Despite the extreme interest in anaerobic digestion and anaerobic co-digestion, and the interest in the enhancement of biogas production from the enhanced anaerobic digestion of primary sewage sludge, the effect of co-substrates and operating conditions on biogas production from the co-digestion of primary sludge has not been critically evaluated. This review aims to present a comprehensive and critical review on the effects of factors like pretreatment practices, C/N ratio, temperature, VFAs, organic loading rate (OLR), and additives in an effort to extend the scientific knowledge of anaerobic digestion of primary sludge for the advanced production of biogas.

2. Methodology

For the purposes of this work, the examined literature was retrieved by querying the database of Scopus and Google Scholar, using selection criteria including keywords such as "anaerobic digestion, anaerobic digestion of primary sludge, enhanced anaerobic digestion of primary sludge, co-digestion of primary sludge, etc." (Figure 1). Recent peer-reviewed papers were included in this work. Books and older studies were also included, when deemed necessary, to complete the research. The discussed studies were selected after screening the abstracts and then fully analyzing the text.

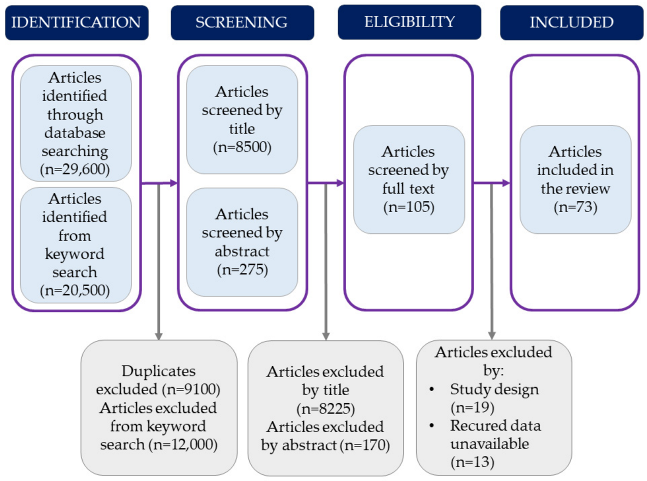

Figure 1. Flow diagram of literature selection process.

As mentioned, this work aims to try to extend the scientific knowledge of anaerobic digestion of primary sludge for the advanced production of biogas. To achieve that, the enhancement of anaerobic digestion of primary sludge with the use of different additives or different treatment processes is presented according to the following structure:

- Anaerobic co-digestion of primary sludge and biowastes;
- Minerals and nanometal particles in the anaerobic digestion of primary sludge;
- The co-digestion of primary sludge with wastewater and water treatment residues;
- Treatment processes and enhancement of primary sludge anaerobic digestion;
- Microalgae and primary sludge.

Data in the literature are often not straightforward to compare. In principle, independent experimental variables are many and expressed differently according to the experimental protocols. For example, inoculum-to-substrate ratio (ISR), although it principally refers to the VS ratio between the substrate and inoculum, it can be expressed in different ways, e.g., ISR can be either expressed as g of volatile solids (VS) of the inoculum to g of VS of the substrate or as mass or volume ratios, posing difficulties in comparing results across studies. In this review, we aimed to standardize the ISR within each table using the available data to ensure uniformity in units and to produce comparable results. This approach under a unifying basis was used to appraise the impact and contribution of each specific additive to the anaerobic digestion of PS.

3. Anaerobic Co-Digestion of Primary Sludge and Biowastes

Primary sludge is a valuable substrate for anaerobic digestion as it contains a higher percentage of fatty acids and lipids compared to secondary sludge [12,13], although its carbon-to-nitrogen ratio is relatively low [14] due to its inherent deficiency of carbon [15]. This limiting factor of C/N ratio can be overwhelmed by the co-digestion of primary sludge with organic fractions such as agricultural byproducts and municipal solid wastes. The operating principle of this practice is based on the fact that organic fractions such as agricultural byproducts contain a high percentage of carbon and a low percentage of nitrogen, so the co-digestion of primary sludge with different organic fractions, such as animal manure, agricultural residues, organic fractions of municipal waste, or vegetable residues, may improve the balance of nutrients, provide buffering capacity, adjust the C/N ratio, reduce the concentration of ammonia, and hence its inhibitory effects, and overall promote the process of methanogenesis [16,17].

3.1. Food Waste (FW), Fruit and Vegetable Waste (FVW), and the Organic Fraction of Municipal Solid Waste (OFMSW)

Table 1 summarizes the findings of studies that investigated the impact of the co-digestion of primary sludge with food waste (FW), fruit and vegetable Waste (FVW), and the organic fraction of municipal solid waste (OFMSW) on biogas production efficiency. Obulisamy's et al. [9] studied the anaerobic co-digestion of FW–primary sludge mixture under mesophilic and thermophilic conditions. Prior to digestion, primary sludge was further thickened using chemical agents (flocculants). The best performance as regards methane production was obtained at mesophilic conditions, while it was favored at decreased food-to-waste concentrations, i.e., decreased FW/CEPT ratio. For example, a decrease from 1:1 to 1:2 in the FW/CEPT ratio resulted in about a 40% increase in methane production per VS unit mass. Specifically, for the 1:1 FW/CEPT ratio, methane production averaged around 100 mL CH_4/g VS, while for the 1:2 ratio, it increased to approximately 140 mL CH_4/g VS. This was probably attributed to better hydrolysis of organics and enhanced efficiency of acetogenesis process. A further increase in the overall efficiency was observed in a later study of Chakraborty et al. [18] who added lime for improving the alkalinity of tested substrates and tested even lower FW-to-CEPT ratios. Kang and Liu [19] agreed well with the studies of Obulisamy et al. [9] and Chakraborty et al. [18], verifying that an increase in CEPT fraction in the substrate favors the production of biomethane and limits problems associated with increased acidogenesis inside the anaerobic digestors. Interestingly, the amount of cumulative methane produced by the 1:4 FW/CEPT mixture, which was about 2750 mL CH_4, at the end of a 20-day anaerobic digestion procedure was almost two-fold higher than the amount produced by the 3:2 FW/CEPT mixture. On the other hand, Xie et al. [20] found that anaerobic digestion of primary sludge with FW was highly efficient for methane production (799 mL CH_4/g VS) at a ratio of 1:1 for FW to PS, while Rakić et al. [21] found the highest biogas production equal to 619 mL/g VS at a FW/PS ratio equal to 3:1. Alternatively, Xie et al. [20] used paper pulp reject to improve the C/N ratio of PS; however, methane production capacity was inferior when compared to food waste/PS substrate ratio. The crucial role of alkalinity-related problems during the co-digestion of primary sludge with food waste was moreover addressed by Gomez-Lahoz et al. [22], who revealed that the addition of $NaHCO_3$ in the mixture of FVW and PS at a ratio of 1:1 significantly improved the efficiency of methane production. Lately, Elsayed et al. [23] observed the highest methane yield (141 mL CH_4/g VS) at a FVW-to-PS ratio of 1:1 on a VS basis, while a further increase in the FVW fraction did not improve biogas production. On the contrary, Gómez et al. [15] studied the digestion of primary sludge and the co-digestion of primary sludge with fruit and vegetable wastes in ratio of around 1:3.5 and found that the addition of fruit and vegetable wastes enhanced the organic loading in the digesters and produced higher amounts of biogas when compared to single digestion of primary sludge, overlooking inhibition problems related to acidogenesis and alkalinity. Comparable results were reported by Habagil et al. [24] when a mixture of municipal organic solid wastes and primary sludge was subjected to anaerobic digestion. In this study, 404 mL CH_4/g VS/d were produced using as a substrate mixture at ratio of 4:1 FW/PS on a VS basis. Meanwhile, when Ahmed et al. [25] studied the anaerobic digestion of the organic fraction of the municipal solid wastes and primary sludge at a 1:1 ratio, their findings revealed a biogas production rate of 107 mL/g VS. In general, the term FW includes a multitude of wastes that originate from diverse sectors and activities, such as household or manufacturing, wholesale/retail, and food sale. Therefore, FW as a substrate in anaerobic digestion may be highly diverse in terms of its content of carbohydrates, fats, and proteins. Food wastes containing components such as meat, bones, cheese, and eggs are rich in proteins and fats, whereas bread, potatoes, rice, and flour are rich in carbohydrates, while legumes and fresh vegetables such as spinach present a more even composition of carbohydrate and protein content [26]. The origin of FW is critical for anaerobic digestion, considering that methane yield depends on the carbon source of the substrate; for example, lipids have a higher methane potential and can achieve 0.70

to 1.01 L CH_4/g VS, although they require a digestion time up to 50–65 days compared to proteins, whose maximal methane yield ranges between 0.42 and 0.85 L CH_4/g VS after digestion for about 15–25 days [27,28]. Based on these data, it might have been expected that an increase in FW contribution to the PS substrate would enhance the methane production yield. Yet, during the first step of hydrolysis, FW induces extended acidogenesis via lactic acid and VFA production, which inhibit methanogenesis through different pathways. For this reason, the addition of lime or the increase in PS to the substrate favors the buffering capacity of the system and the overall co-digestion of FW and PS. In addition, as suggested by Chakraborty et al. [18], the activity of various enzymes, such as the a-amylase and b-galactosidase, is limited at lower concentrations of FW in the substrate, thus enhancing the production of bioenergy. Moreover, the addition of PS advances the production of methane with less H_2S content, independently of the substrate-to-inoculum ratio [29].

The formation of VFAs significantly contributes to their accumulation in digesters that may negatively impact their operation and methane productivity. Although butyric and acetic acid favor methanogenesis through the activity of specific bacteria, propionic acid deteriorates methane productivity due to slow degradation kinetics [11]. Progressive VFA accumulation may alter the VFA distribution (acetate-to-butyrate and acetate-to-propionate ratios) and even the microbial population dynamics. An increase in PS concentration can improve the distribution of VFAs and acetoclastic microbes. To this aim, PS advances the presence of nutrients, such as iron, that are valuable elements for methanogens' viability and proliferation and related metalloenzymes that contribute to methane production. Fe contributes to the precipitation and thus the inactivation of sulfur, while it promotes the activity of specific metalloenzymes known to enhance methane production, i.e., carbon monoxide dehydrogenase (CODH) for the degradation of acetic acid and F420 co-enzyme that is involved in methanogenic reactions.

3.2. Agro-Industrial Wastes

Agro-industrial wastes are more homogeneous than food wastes, but they present certain constraints. The quality of such wastes depends on the type of crops found in a region, while their production occurs in specific periods. This phenomenon impacts associated logistics and storage capacities of raw materials in the anaerobic digestion unit. Though anaerobic digestion of agro-industrial wastes is quite familiar in the literature, only the last year's co-digestion of such wastes with primary sludge is conducted. According to the best of the authors′ knowledge, research studies have mainly focused on the co-digestion of agro-industrial wastes with sewage sludge and have thoroughly investigated the terms to facilitate the biological conversion of that biomass into bioenergy. Anaerobic digestion of primary sludge with wastes from agricultural and livestock activities has been mainly studied regarding corn stover biochar, wheat straw, buckwheat husk, fallen leaves, grass, leaves, cow manure, and brewery sludge. Table 2 summarizes the outcomes of these studies in a comprehensive yet conclusive way to understand what kind of substrates are the most promising ones.

Table 1. Co-digestion of primary sewage sludge with food waste, fruit and vegetable waste, and organic fraction of municipal solid wastes.

	PS Substrate [1]	Co-Substrate [2]	Inoculum [3]	ISR * (*w*/*w*)	OLR + initial g VS/L/d	Tested Concentrations	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
1.	CEPT	FW	ADS + UASB	1:5	-	FW/CEPT 1:1, 1:2, 3:1, 1:2, 1:3 *v*/*v*	B	L	M TH	-	[9]
2.	CEPT	FW	ADS + UASB	1:5	-	FW/CEPT 1:7, 1:5, 1:3 *w*/*w*	B	L	M	+++	[18]
3.	CEPT	FW	ADS	1:1	-	CEPT/FW 1:4, 2:3, 3:2, 4:1 *w*/*w*	B	L	M	++	[19]
4.	PS	FW	ADS	1.5:1	-	FW/PS 1:1 *w*/*w* VS	B	L	M	+++	[20]
5.	PS	FW	ADS	2:1	-	FW/PS 3:1, 1:1, 1:3	B	L	M	++	[21]
6.	PS	FVW	ADS	-	2.5	PS/FVW 1:4.5 *w*/*w* TS^-	C	L	M	+	[15]
7.	PS	FVW	-	-	-	PS/FVW 0–100% *w*/*w* $NaHCO_3$ 4–16 g/kg $NaHCO_3$+ $Ca(OH)_2$ 6 + 4 g/kg	B	L	M	+++	[22]
8.	PS	FVW	CM AS ES	2:1	-	PS/FVW—(CM) 2.3:1, 1:1, 1:2.3, 2:4 *w*/*w* VS PS/FVW—(CM, AS, ES) 1:1 *w*/*w* VS	B	L	M	+ +++	[23]
9.	PS	OFMSW	ADS	-	1.0	OFMSW/PS 1:1, 3:1, 4:1 *w*/*w* VS	SC	L	M	-	[24]
10.	PS	OFMSW	UASB	1:1	-	OFMSW/PS 1:1 *v*/*v*	B	L	M		[25]

[1] PS substrate, CEPT: chemically pretreated primary sludge; PS: primary sludge. [2] Co-substrate, FW: food waste; FVW: fruit and vegetable waste; OFMSW: organic fraction of municipal solid waste. [3] Inoculum, ADS: anaerobic digested sludge; UASB: up-flow anaerobic sludge blanket digestion; CM: cow manure; AS: activated sludge; ES: excess sludge. [4] Mode, B: batch; C: continuous; SC: semi-continuous. [5] Scale, L: lab scale. [6] T: temperature; M: mesophilic; TH: thermophilic. [7] Efficiency, +: 0–40%; ++: 41–80%; +++: >81% enhancement of biomethane production compared to single digestion of primary sludge. * Inoculum-to-substrate ratio: ISR. + Organic loading rate: OLR. Total solids: TS.

Elsayed et al. [30] studied the co-digestion of primary sludge with either wheat straw (WS) or buckwheat husk (BH) along with wheat straw (WS). The mix of primary sludge with WS at a ratio close to 1:2 on a VS basis with an organic loading of 7.5 g VS/L produced around 345 mL CH_4/g VS, while the combined use of BH and WS resulted in a higher methane yield of 481 mL CH_4/g VS at a ratio of PS/mix of WS and BH equal to 1:1 on a VS basis, with a C/N equal to 10.07 and an organic loading of 7.50 g VS/L. The obtained efficiency was three times higher than that of single PS digestion. Subsequently, Elsayed et al. [31] investigated the co-digestion of primary sludge with fallen leaves (FL) and grass (GR), focusing on the impact of the C/N ratio on methane production. The experimental results showed that a C/N ratio of 13, corresponding to a ratio of PS/mix of FL and GR almost equal to 1:2 on a VS basis, showed the highest methane yield, 352 mL CH_4/g VS, two times higher than that of primary sludge, and the shortest lag phase (about 14 d) among the C/N ratios tested. In the same context, Elsayed et al. [32] examined the impact of sugarcane leaves (SL) and Corchorus stalks (CS) on the digestion of PS. The highest methane production, almost three times higher than that of single PS, was obtained during the co-digestion of PS with the mix of SL and CS at a ratio of 2:1 (PS/mix) on a VS basis, indicating that in this case, more PS biomass was required to achieve high methane production yields. Similar results were found in a later study by Elsayed et al. [33] who investigated the co-digestion of primary sludge with sugar beet pulp (SBP). Among the examined ratios, the highest methane production was achieved at a ratio of PS/SBP equal to 7:3 *w*/*w* VS. At this ratio, methane production reached 307 mL CH_4/g VS, nearly doubling the methane yield achieved during the exclusive digestion of primary sludge. Overall, the efficiency of each substrate was related to the type of waste/additive that prescribes the characteristics of organic matter, as well as the mixing ratio of PS/additives and the overall organic loading rate in the digester.

The most essential factor to take into consideration when designing such units is the carbon source characteristics. Organic material in agro-wastes contains to a great extent lignocellulose, e.g., hemicellulose, cellulose, and lignin. Cellulose is the most abundant organic compound on earth, and its chemical structure consists of linear chains of glucose units linked by β-1,4-glycosidic bonds [34]. Hemicellulose is known as the second most abundant carbohydrate material, and in contrast to cellulose, which is a polymer of only glucose, hemicellulose is a polymer of different monosaccharides (e.g., glucose, mannose, xylose, arabinose, and fructose), and it is generally easier to degrade enzymatically than cellulose [35,36]. Finally, lignin is a polyphenolic structural constituent of wood and other native plant materials and its high crystallinity makes it difficult to be degraded, as it is composed of aromatic alcohols and their ramifications (e.g., syringyl alcohol, guaiacyl alcohol, and p-coumaryl alcohol) [37]. Even though cellulose and hemicellulose appear to be favorable substrates for degradation, it is well known that during the anaerobic digestion of lignocellulosic biomass, cellulose and hemicellulose are often surrounded by lignin, resulting in a stable polymer that is quite resistant to degradation during digestion. One promising approach for improving lignocellulose biomass hydrolysis appears to be that of organic acid pretreatment. In fact, Dharmalingam et al. [38] observed that when treating a mixed lignocellulose biomass with citric acid, biogas production was increased by fivefold over the untreated biomass. However, the resistance of lignocellulose biomass to degradation does not apply in the same way for all crops which are expected to present differences in cellulose, hemicellulose, and lignin content. From the lignocellulosic substrates used for the co-digestion of primary sludge, buckwheat husk is generally characterized by a high concentration of cellulose, 40–52%, and lower concentrations of hemicellulose and lignin, 17–32 and 27–29%, respectively [39,40]. Probably, its higher concentration of cellulose enhanced the hydrolysis rate and resulted in improved methane production when it was used at an optimal ratio of PS/mix of WS and BH equal to 1:1 on a VS basis (Figure 2). On the other hand, the addition of fallen leaves and grass into primary sludge resulted in lower methane production efficiency, although the concentration of lignin in this substrate was expected to be rather low [41,42]. The obtained results could be possibly attributed to other inhibitory phenomena, potentially involving the release of inhibitory compounds, such as vanillin, syringaldehyde, humic acids, etc., resulting from the degradation of the lignocellulose biomass [43]. These compounds can have an inhibitory effect on the anaerobic digestion process, leading to a reduction in methane yield [43]. A suitable measure to confront such limitations would be to increase the amount of primary sludge in the slurry to be digested. In this context, Elsayed et al. [32] investigated the co-digestion of primary sludge with the mix of sugarcane leaves and Corchorus stalks at a ratio of 2:1 on a VS basis (C/N = 18). Yet, the methane production was not further increased, indicating the impact of other operational conditions, such as the organic loading rate, the overall C/N ratio, and the type of inoculum, as shown in Table 2, that may affect the microbial communities inside the digester.

Anaerobic co-digestion of livestock residues, for example, manure, together with other organic wastes or energy crops is common practice [44] and has been applied at an industrial scale for quite some years. Co-digestion is more favorable against the single digestion of manure due to its recalcitrant biodegradability potential and inhibitory behavior from high ammonia content. Several studies report that the co-digestion of manure with C-rich wastes improves the C/N ratio and thus the digestibility and methane production capacity of manure [45,46]. To this regard, the co-digestion of manure with primary sludge may present some potential, though it has not been extensively studied. Nansubuga et al. [47] examined the co-digestion of primary sludge with cow manure (CM) or brewery sludge (BS) at ratios of 1:1 and 3:1, as well as the co-digestion of PS with a mix of CM and BS at a ratio of 2:1:1, respectively. Livestock addition to PS did not contribute to biogas production, regardless of the examined mixing ratio. On the contrary, BS addition enhanced considerably the biogas production in all tested conditions, i.e., the biogas production was five times higher than that of single PS digestion at a mixing ratio of 1:1. Interestingly, the biogas produced by the mix of PS with CM and BS at a ratio of PS/CM/BS = 2:1:1 was three times higher than that of single PS. Such biogas yield was comparable to that of PS and BS at a ratio of 3:1, suggesting that livestock was difficult to treat anaerobically, presumably because of poor hydrolysis and decomposition, and thus did not contribute to biogas production. Moreover, Shilton et al. [48] managed to boost up the production of biogas during anaerobic digestion of PS with whey by adding moderate quantities of manure, whereas at the same time, they achieved better control of the alkalinity of the system. The increased process efficiency was obtained at an operational ratio of PS/WH/CM equal to 1:0.8:0.2 on a mass basis.

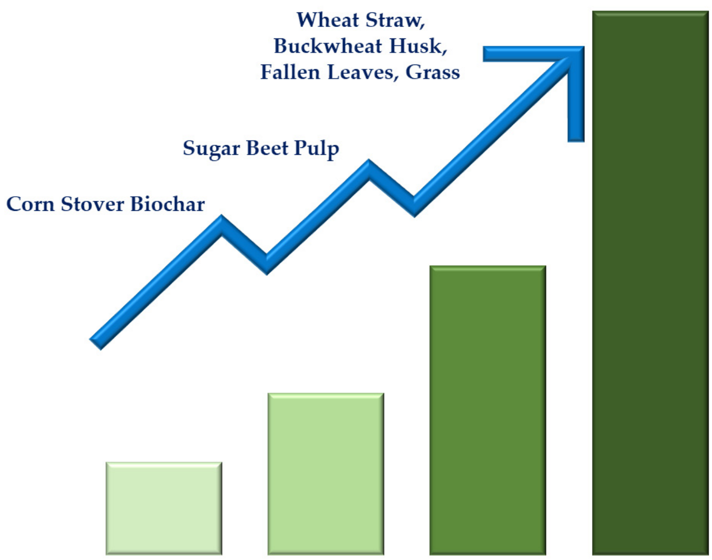

Figure 2. Performance of anaerobic co-digestion of PS with agro-industrial wastes under comparable experimental conditions in terms of inoculum origin, ISR, and OLR.

Table 2. Co-digestion of primary sewage sludge and agricultural wastes.

	PS Substrate [1]	Co-Substrate [2]	Inoculum [3]	ISR *	OLR + initial	Tested Concentrations	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
1.	PS	CSB	ADS	2:1 *w*/*w* VS	-	1.82, 2.55, 3.06 g/g TS⁻	B	L	TH	+	[6]
						1.82 g/g TS + 0.12 g/g TS/d	C				
2.	PS	WS BH	CM	2:1 *w*/*w* VS	3.0, 6.0, 7.5, 8.0, 10.0, 12.0 g VS/L	PS/WS 1:2 *w*/*w* VS	B	L	M	+++	[30]
					-	PS/WS BH C/N = 10.07, 13.06, 15.01, 20.03, 25.25				+++	
3.	PS	FL GR	ADS + WAS	2:1 *w*/*w* VS	-	PS FL GR C/N = 10, 13, 16, 20, 23	B	L	M	+++	[31]
					0.5 g VS/L/d	PS FL GR C/N = 13	SC				
4.	PS	SBP	ADS	2:1 *w*/*w* VS	-	PS/SBP 7:3, 1:1, 3:7 *w*/*w* VS	B	L	M	++	[33]
5.	PS	SL CS	CM RC	2:1 *w*/*w* VS	-	PS SL CS C/N = 18, 21, 25, 30, 35	B	L	M	+++	[32]
					-	PS SL CS—CM, C/N = 18 PS SL CS—RC, C/N = 20.70	B				
					0.5 g VS/L/d	PS SL CS C/N = 18	SC				
6.	PS	CM BS	UASB	-	0.71 g COD/L/d	PS/CM 3:1, 1:1 *w*/*w*	C	L	M	+	[47]
						PS/BW 3:1, 1:1 *w*/*w*				+++	
						PS/CM:BW 2:1:1 *w*/*w*				+++	

Table 2. *Cont.*

	PS Substrate [1]	Co-Substrate [2]	Inoculum [3]	ISR *	OLR + initial	Tested Concentrations	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
7.	PS	WH CM	ADS	-		PS/WH 4:1 *w/w* PS/WH 1:1 *w/w* + Ca(OH) PS/WH/CM (final) 1:0.8:0.2 *w/w*	SC	L	M	+	[48]

[1] PS substrate, PS: primary sludge. [2] Co-substrate, CSB: corn stover biochar; WS: wheat straw; BH: buckwheat husk; FL: fallen leaves; GR: grass; SBP: sugar beet pulp; SL: sugarcane leaves; CS: Corchorus stalks; CM: cow manure; BS: brewery sludge; WH: whey. [3] Inoculum, ADS: anaerobic digested sludge; CM: cow manure; WAS: waste-activated sludge; RC: rumen content of cattle; UASB: up-flow anaerobic sludge blanket digestion. [4] Mode, B: batch; C: continuous; SC: semi-continuous. [5] Scale, L: lab scale, [6] T: temperature; M: mesophilic; TH: thermophilic. [7] Efficiency, +: 0–40%; ++: 41–80%; +++: >81% enhancement of biomethane production compared to single digestion of primary sludge. * Inoculum-to-substrate ratio: ISR. + Organic loading rate: OLR. Total solids: TS.

4. Minerals and Nanometal Particles in the Anaerobic Digestion of Primary Sludge

Other major groups of additives that have been utilized in the anaerobic digestion of organic wastes are minerals and nanometal particles. Minerals with adsorption capacity such as clay minerals and magnetite, due to their surface properties, porosity, and chemical composition, can regulate ammonia toxicity, promote microbial attachment, growth, and enrichment, sequent community formation, and increase hydrolysis [49–51]. In parallel, nanometal particles are suitable for anaerobic digestion processes due to their high activity, large specific surface area, and excellent specificity and dispersibility [52]. Examples of nanometal particles that have been studied as additives in anaerobic digestion of organic wastes are nanometal monomers such as zero-valent iron (ZVI), nanometal ions such as cobalt and nickel, and nanometal oxides such as ZnO and TiO_2. It is widespread knowledge that several metals, such as zinc, iron, nickel, and cobalt, are indispensable for the biodegradation of organic matter in the anaerobic digestion process, as they are directly involved in the enzymatic activity of anaerobic bacteria [10]. According to the above, it is apparent that research on the effect of such additives in the anaerobic digestion of primary sludge is of major importance. Even though the results for such additives' performance may not be directly comparable, as research outcomes of the respective studies are likely to be influenced by the additive type, nonetheless, in the present work, some trends have been identified and summarized in Table 3. Figure 3 illustrates major findings of PS digestion in the presence of such compounds after normalization of biogas production efficiencies (on VS basis) and a comparison of these results with biogas production efficiency rates of single PS.

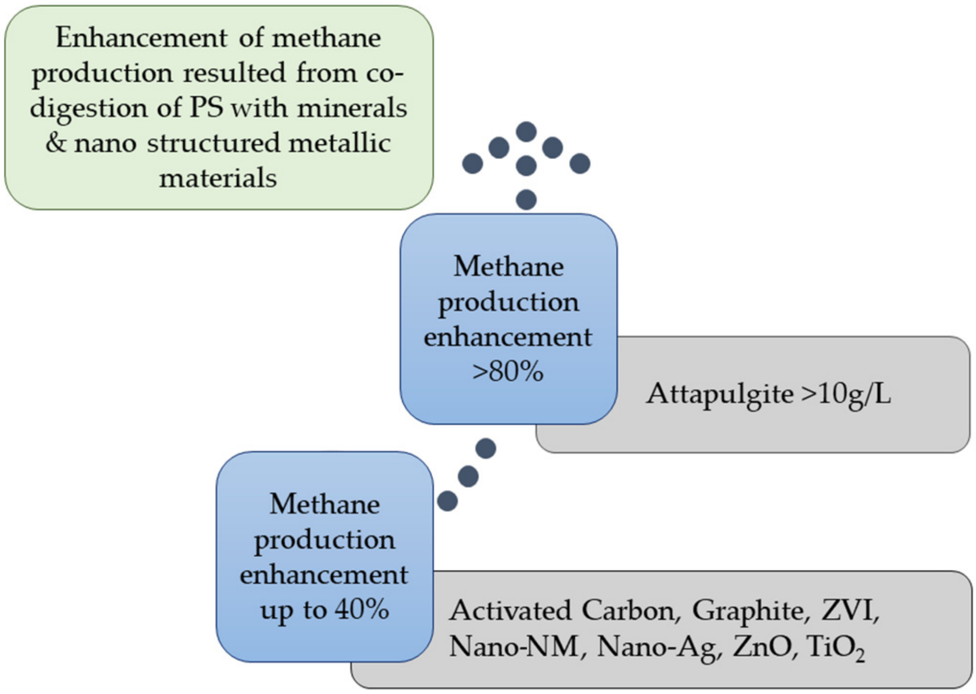

Figure 3. Comparative graph of biogas production enhancement using various minerals and nanoparticles during the anaerobic digestion of primary sludge (ZVI: Zero Valent Iron and NM: Nano-Magnetite).

Within this context, the performance of PS's anaerobic digestion with the addition of a clay mineral, attapulgite, was probed thoroughly by Sakaveli et al. [5]. Specifically, different testing conditions, such as the digestion of untreated PS (as received from the WWTP) or conditioned PS, with or without the addition of attapulgite, were evaluated in two ratios of inoculum to substrate equal to 1:1 and 2:1 on a VS basis. According to their results, the optimum inoculum-to-substrate ratio was that of 1:1 on a VS basis. At this ratio, the methane yield of the single digestion of untreated PS was almost 1.2 times higher than that at the ratio of 2:1. At this optimum ratio, the highest methane production occurred when conditioned PS was co-digested with attapulgite at a dose of 15 g/L. However, this effect was attributed to the addition of polyelectrolytes which led to an increase in organic matter in the digestive system. The optimal efficiency in methane production, without the additional increase in organic matter, was recorded when attapulgite was co-digested with untreated primary sludge at the same dose. In this case, the methane production was almost 1.5 times higher than the single digestion of untreated PS at the same ratio (1:1). Furthermore, their kinetics analysis revealed that the addition of attapulgite increased the hydrolysis rate (k = 0.16 d^{-1}) compared to that of PS (k = 0.11 d^{-1}). Their overall results suggested that single digestion of primary sludge with the addition of attapulgite may accomplish higher methane production capacities at lower digestors' volume, increasing their overall efficiency and productivity, due to the underlying ability of attapulgite to enhance methane production. Specifically, in a later study by Sakaveli et al. [4], which was conducted in an effort to standardize the process and the results, it was revealed that during the anaerobic digestion of primary sludge with attapulgite at a dose of to 20 g/L, the relative abundance of Methanofastidiosaceae (e.g., Candidatus Methanofastidiosum), which produce methane via the reduction of methylatedthiol compounds [53], was increased, while that of Methanocorpusculaceae (e.g., Methanocorpusculum), which use H_2/CO_2 and formate as methane substrates [54], was decreased. The above suggest that the present of attapulgite in the digestion mixture can shift the methanogenesis to a more methyl-thiol dominated hydrogenotrophic methanogenesis. Additionally, the produced digestate exhibited a positive dewatering influence, while the dried biosolids, when applied to soil, amended its properties and enhanced nutrient bioavailability, without showing any negative effects on plant growth.

The use of nanoparticles (NPs) in commercial products and industrial applications has increased significantly in recent years. Therefore, their transfer to the environment, as well as their impact on wastewater treatment processes, is a cause for concern. In this regard, several researchers have tried to approach the effects of NPs when they are present in anaerobic digestion. Sakarya et al. [55] specifically evaluated the effect of nano-TiO_2, nano-ZnO, and nano-Ag on methanogenesis. For all tested NPs, both tested doses (1 and 10 mg/g TS) showed neither significant VS degradation nor a significant effect on methane production compared to the control groups. In addition, the VFA concentration was very low in each test, indicating that there were no stress conditions in the system. These results are most likely due to the relatively low exposure of methanogens to NPs, as major amounts of NPs were adsorbed onto the sludge or settled [55–58].

The influence of other additives such as $NiCl_2/CoCl_2$, nano magnetite (NM) [59], graphite powder (GP), and activated carbon (AC) in the anaerobic digestion of primary sludge were evaluated by Xie et al. [60]. These additives serve distinct functions in the anaerobic digester. Co and Ni are associated with the growth of methanogenic bacteria during enzyme synthesis [61]. Magnetite has been found to accelerate propionate oxidation as an electron acceptor [59]. Graphite powder was reported to enhance the direct interspecies electron transfer as an alternative to interspecies hydrogen/formate transfer during anaerobic digestion [62], while activated carbon has widely been used in anaerobic digestion due to its conductive properties [63]. According to Xie et al.'s [60] results, the addition of $NiCl_2/CoCl_2$ did not enhance methane production; on the contrary, the application of the highest dose of $NiCl_2/CoCl_2$ inhibited methane production by 24%, which is probably due to the accumulation of heavy metals which resulted in a toxic effect on enzyme functions. Respectively, NM also failed to significantly enhance methane production. On the contrary, GP and AC led to a relative increase in methane production. The application of AC enhanced hydrolysis (the hydrolysis rate for all tested AC concentrations was approximately 0.70 d^{-1}, whereas that of PS stood at around 0.50 d^{-1})and hydrogenotrophic methanogenesis, while electron transport played a key role in enhancing the anaerobic digestion of primary sludge when GP was used as an additive, and this was evident from the increase in Methanosaeta and Methanolinea which use directly conductive materials instead of conventional hydrogenotrophic methanogenesis. These results were in line with the study by Guo et al. [64] that used as substrate the joint sludge stream from primary and secondary sedimentation.

Another inorganic additive that, in recent decades, has gained interest as an additive in anaerobic digestion and has been also used in the anaerobic digestion of primary sewage sludge is zero-valent iron (ZVI). ZVI which is a non-toxic and cost-effective metal monomer [65]. It has been reported that with the addition of ZVI into an anaerobic digester, more organics can directly be hydrolyzed and fermented into acetate due to its ability to serve as an electron donor in microbial metabolism [66]. Wei et al. [67] evaluated the effect of ZVI on the anaerobic digestion of PS, with a series of doses equal to 1, 4, and 20 g/L. Their results showed the ability of ZVI to increase the hydrolysis rate of primary sludge and to make the biodegradable substances degrade rapidly. Indeed, based on their conducted kinetic analysis, the hydrolysis rate of PS stood at 0.29 d^{-1}, while for the doses of 1, 4, and 20 g/L, the hydrolysis rate corresponded to 0.33, 0.40, and 0.39 d^{-1}, respectively. Remarkably, the dose of 4 g/L, which exhibited the highest hydrolysis rate, also yielded the highest methane production. Specifically, the dose of 4 g/L enhanced the methane production by 27%, compared to the single digestion of PS.

While the mentioned research discusses the potential benefits and limitations of these diverse additives in the anaerobic digestion of primary sludge, a lack of standardization and comparability between most of the studies is confirmed. Hence, it is challenging to draw definitive conclusions about the effectiveness of these additives across different experimental setups. In conclusion, further studies with standardized methodologies and comprehensive assessments of their long-term impacts are needed to provide more robust and widely applicable conclusions in this field.

Table 3. Minerals and nanometal particles in the anaerobic digestion of primary sewage sludge.

	PS Substrate [1]	Co-Substrate [2]	Inoculum [3]	ISR *	Tested Concentrations	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
1.	PS CEPT	AT	ADS	1:1 *w*/*w* VS 2:1 *w*/*w* VS	AT 15 g/L	B	L	M	+++	[5]
2.	PS	AT	ADS	1:1 *w*/*w* VS	AT 10, 20, 40 g/L	B	L	M	+++	[4]
3.	PS	Nano-TiO_2	ADS	1:2 *w*/*w* VS	Nano-TiO_2 1, 10 mg/g TS^-	B	L	M	+	[55]
		Nano-Ag			Nano-Ag 1, 10 mg/g TS					
		Nano-ZnO			Nano-ZnO 1, 10 mg/g TS					
4.	PS	NM $NiCl_2$/$CoCl_2$ GP AC	ADS	1.5 *w*/*w* VS	NM 50, 100, 200 mg/L $NiCl_2$/$CoCl_2$ 10:10, 100:100 mg/L GP 250, 500, 1000 mg/L AC 10,000, 15,000, 20,000 mg/L	B	L	M	+	[60]
5.	PS	ZVI	ADS	2:1 *w*/*w* VS	1, 4, 20 g/L	B	L	M	+	[67]

[1] PS substrate, PS: primary sludge; CEPT: conditioned PS. [2] Co-substrate, AT: attapulgite; NM: nano magnetite; GP: graphite powder; AC: activated carbon; ZVI: zero-valent iron. [3] Inoculum, ADS: anaerobic digested sludge. [4] Mode, B: batch. [5] Scale, L: lab scale. [6] T: temperature; M: mesophilic. [7] Efficiency, +: 0–40%; +++: >81% enhancement of biomethane production compared to single digestion of primary sludge. * Inoculum-to-substrate ratio: ISR. Total solids: TS.

5. Co-Digestion of Primary Sludge with Wastewater and Water Treatment Residues

Numerous practices in both wastewater and water treatment geared toward streamlining operations lead to the generation of byproducts or residues that necessitate appropriate handling. When utilized efficiently, these substances can possess beneficial properties to enhance anaerobic digestion and subsequent processing. Table 4 encompasses the assorted studies that have examined the application of such substances in enhancing the anaerobic digestion of primary sludge.

Sewage sludge represents the primary residual product in wastewater treatment plants. Methods such as sewage sludge incineration are employed to generate energy while simultaneously reducing the volume of sewage sludge considerably. Nevertheless, sewage sludge incineration yields two distinct types of residues: bottom ash and fly ash. Bottom ash (BA) is a non-hazardous waste with low heavy metal content, typically characterized by a high calcium oxide and silica content [68]. Resnet studies has shown that the utilization of bottom ash in anaerobic digestion can enhance methane production [69]. The beneficial impact of bottom ash on anaerobic digestion performance primarily results from its CaO content, which contributes alkalinity to the system and the presence of metal ions. During the anaerobic digestion process, metal ions have the potential to leach out from the ash and become available for use by anaerobic bacteria, and it is unlikely that these metal ions would reach inhibitory levels when bottom ash is used in reasonable doses [68,70,71]. Wei et al. [72] investigated the influence of co-digesting primary sludge with bottom ash derived from the incineration of wastewater-activated sludge, and their findings indicated an acceleration in the hydrolysis and acidogenesis processes. The highest methane production was detected at a dose of 0.9 g/g TS (hydrolysis rate k = 0.43 d^{-1}), and it was 18% higher compared to the control (digestion of primary sewage sludge without the addition of bottom ash) (hydrolysis rate k = 0.29 d^{-1}). In contrast, when a higher dose, 1.2 g/g TS, was applied, no enhancement of methane production was observed, possibly due to the toxic effect of metals on methanogens, which are characterized as more sensitive to the presence of metals, against hydrolytic and acidogenic bacteria. However, it is noteworthy that for all of the tested doses of bottom ash, 0.6, 0.9, and 1.2 g/g TS, the dewaterability in the digestate was significantly improved. This was attributed to the presence of Ca^{2+} in the bottom ash which can be used as a flocculation agent to enhance the dewatering capacity of sewage sludge [72].

Another practice that aims to manage the process and disposal cost of sludge is hydrothermal carbonization (HTC). Hydrothermal carbonization is a procedure that results in two kinds of byproducts· hydrochar and a liquid fraction (orocess water, PW) [73]. The explosion of these byproducts in an anaerobic digestion process may be an effective and economical choice, as hydrochar is a product with a high heating value, and PW contains at least 15% of the sludge′s initial carbon content and 30% of its total COD [73–78]. HTC can treat dewatered waste-activated sludge without prior drying, and the resulting PW of such a practice comprises high organic matter and nitrogen content and contains heterocyclic organic compounds (pyrroles and pyridines), phenols, ketones, aldehydes, and alcohols [74,75,79]. In this context, Villamil et al. [80] studied the anaerobic digestion of primary sludge with PW of dewatered waste-activated sludge at ratios of PW/PS of 10:90 and 5:95 on a COD basis for different organic loading rates (OLRs) equal to 1.5 and 2.5 g COD/L/d at mesophilic and thermophilic temperature regimes. Mesophilic digestion obtained better results, as during the thermophilic anaerobic digestion, inhibition effects were revealed. At mesophilic conditions and for an OLR of 1.5 g COD/L/d, the optimum compositions of PW/PS were found to be 10:90, with a methane yield 15% higher than that of the control PS digestion. In contrast, at the higher OLR, the highest methane yield CH_4/g COD was recorded during the digestion of the control PS, which was 1.4 times greater than the methane yield obtained from the control at the lowest OLR.

During drinking water treatment, the addition of coagulants or flocculants, which aim to remove turbidity, color, pathogens, and organic matter, results in substantial amounts of drinking water treatment sludge (DWTS). This sludge is rich in aluminum or iron, as the most used chemicals in water treatment include aluminum-based coagulants such as aluminum sulfate ($Al_2(SO_4)_3$) and iron-based coagulants such as ferric chloride ($FeCl_3$) [81]. Even though DWTS is characterized by low organic matter, which may not contribute significantly to the anaerobic digestion of sewage sludges, its high concentrations of Al and Fe may be beneficial for the downstream processes of biosolids generated in wastewater treatment plants. As both Al and Fe in low concentrations are considered as beneficial nutrients in agriculture [82], and their presence in the produced biosolids can stimulate soil fertility. Xie et al. [83] evaluated the impact of two types of drinking water treatment sludges treated with Fe (Fe-DWTS) or Al (Al-DWTS) on the anaerobic digestion of primary sludge. According to their results, in both cases, methane yield was inhibited, with Al-DWTS showing a higher inhibition of up to 45–55% compared to the control (inoculum with primary sludge). As iron is known to promote methane production, the restricted methane production can be attributed to the presence of humic substances that can suppress microbial growth [84], while the inhibition effects of Al-DWTS were likely due to the combined effect of humic substances, aluminum, and sulfate. As sulfate promotes the activities of sulfate-reducing bacteria, methane production could be inhibited due to the competition of sulfate-reducing bacteria with methanogens and fermentative bacteria for common substrates [83,85]. However, it was observed that the addition of DWTSs affected the structure of the microbial community. The abundance of hydrogenotrophic methanogens and acetoclastic methanogens were increased in the presence of Al-DWTS and Fe-DWTS, respectively. Moreover, a positive downstream impact was achieved during the anaerobic digestion of primary sewage sludge with DWTS, since both Fe-DWTS and Al-DWTS significantly reduced H_2S concentration in biogas and phosphate concentration in the digestate. Phosphate removal was primarily due to the precipitation of phosphate with the iron or aluminum ions provided by Fe-DWTS and Al-DWTS, respectively. Furthermore, the addition of Fe-DWTS and Al-DWTS improved the dewaterability of the digestates, as the presence of iron or aluminum salts can remove extracellular polymeric substances and improve sludge dewaterability [83].

Table 4. Co-digestion of primary sewage sludge with water and wastewater treatment residues.

	PS Substrate [1]	Co-Substrate [2]	Inoculum [3]	ISR *	OLR $^{+}$ g COD/L/d	Tested Concentrations	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
1.	PS	BA	ADS	2:1 *w*/*w* VS	-	0.60, 0.90, 1.20 *w*/*w* TS^{-}	B	L	TH	+	[72]
2.	PS	PW	ADS	-	1.5, 2.5	PW/PS 5:95, 10:90 *w*/*w* COD	SC	L	M	+	[80]
					2.5	PW/PS 5:95 *w*/*w* COD			T	-	
3.	PS	Fe-DWTS	ADS	1.5:1	-	Fe-DWTS 10, 20, 30, 40 % *v*/*v*	B	L	M	-	[83]
		Al-DWTS				Al-DWTS 10, 20, 30, 40 % *v*/*v*				-	

[1] PS substrate, PS: primary sludge. [2] Co-substrate, BA: bottom ash; PW: process water from hydrothermally treated WAS; Fe-DWTS and Al-DWTS: sludge from a drinking water treatment plant. [3] Inoculum, ADS: anaerobic digested sludge. [4] Mode, B: batch; SC: semi-continuous. [5] Scale, L: lab scale. [6] T: temperature; M: mesophilic; TH: thermophilic. [7] Efficiency, +: 0–40% enhancement of biomethane production compared to single digestion of primary sludge. * Inoculum-to-substrate ratio: ISR. $^{+}$ Organic loading rate: OLR. Total solids: TS.

6. Treatment Processes and Enhancement of Primary Sludge Anaerobic Digestion

During AD, several factors such as poor hydrolysis rate can lead to the requirement of extended retention times and the use of larger bioreactors, as well as causing lower methane production. To overcome such problems, several pretreatment techniques such as chemical, mechanical, physical, thermal, biological, and biochemical [86] can be applied to improve the anaerobic digestion of sewage sludge. Table 5 summarizes various pretreatment techniques that have been used in the anaerobic digestion of primary sludge. Subsequently, the effects of the various treatment processes that have been applied to enhance the anaerobic digestion of primary sludge are discussed in terms of improved biogas production.

In the quest to overcome poor hydrolysis during the anaerobic digestion of primary sewage sludge, one promising approach is its enzymatic pretreatment. This method harnesses the enzymatic breakdown of organic compounds and cell walls, thus rendering them more accessible during anaerobic digestion. A recent study by Bahreini et al. [87] highlighted the impact of cellulase on the enhancement of primary sludge fermentation. Their findings indicated that the addition of cellulase enhanced the VFA yield of fermentation from 78–192 to 87–202 mg COD/g VS compared to the untreated primary sludge. In this regard, Tongco et al. [88] conducted a comprehensive investigation into enzymatic hydrolysis of primary sludge. Specifically, they explored different protease (P) and lipase (LP) ratios and found that the highest degradation rate (33%) for volatile suspended solids (VSS) when primary sludge was treated was achieved at a ratio of P/LP = 1:3. Subsequently, biochemical methane potential (BMP) tests of the hydrolyzed substrate unveiled the significant potential of enzymatic pretreatment, with a 90% boost in methane production and a noteworthy 10% increase in methane yield compared to untreated primary sludge. An effective thermal pretreatment technique for enhancing the digestion process of primary sludge is hydrothermal treatment (HTT) due to its ability to enhance biodegradability and energy/nutrient recovery from organic wastes. The advantage of this method lies in its simplicity and avoidance of using chemicals. In a recent study conducted by Yuan et al. [89], primary sludge treated within the temperature range of 130–210 °C for 30 min showed promising results. At 150 °C, the highest methane yield was achieved, boasting a 31% increase compared to untreated primary sludge. Additionally, conditions at 170 °C demonstrated a substantial 27% surge in methane production, signifying its potential for boosting biogas generation.

The enhancement of biogas production can be achieved by altering primary sludge characteristics, or by improving its performance in anaerobic digestion, by utilizing other organic or inorganic media. As chemical pretreatment of primary sludge is a popular practice in wastewater treatment facilities and organic matters such as cellulose are key components in municipal sewage, Zhuang et al. [90] conducted a comprehensive investigation into the transformation of cellulose carbon and cellulolysis metabolism for methane production during the anaerobic digestion of CEPT sludge. The research outcomes shed valuable light on the biological aspects crucial for ensuring the stability of anaerobic digesters when dealing with cellulose-rich substrates like food waste, lignocellulosic biomass, and non-recyclable paper. Specifically, the research revealed the significance of cellulose and protein degradation at lower organic loading operations and emphasized lipid degradation at higher loading conditions.

Another wildly applied method which alters the characteristics of primary sludge is chemical flocculation. An alternative process to the chemical pretreatment of primary sludge is the "advanced primary separation" [91,92]. This process involves the recirculation of secondary sludge into the primary settling tank, resulting in the formation of larger flocs with increased settling velocities, indicating a greater potential for biogas production during anaerobic digestion. This phenomenon is attributed to the presence of extracellular polymeric substances (EPS) in the secondary sludge stream [93–95]. As proposed by Araneda et al. [95], remarkably, the application of this technique could result in a substantial 50% increase in biogas production. Furthermore, this approach holds promise not only for managing primary sludge but also for wastewater treatment, as it effectively reduces the energy demands associated with aeration during secondary treatment processes.

Furthermore, the pretreatment of primary sludge with free nitrous acid (FNA) is another pretreatment method that aroused the interest of researchers. While FNA pretreatment has shown benefits in waste-activated sludge digestion, Zhang et al. [96], conducted a study to investigate the effects of FNA pretreatment specifically on primary sludge methane production. Their approach involved subjecting primary sludge to different doses of HNO_2-N and conducting BMP tests. Additionally, the investigators separated the supernatant from the solid phase of FNA-pretreated primary sludge in parallel tests to examine the impact on these fractions individually. The outcomes of these experiments consistently showed reduced methane production in all tested conditions. This reduction was attributed to differences in the macromolecular components and floc structures between primary sludge and waste-activated sludge. Primary sludge contains higher levels of fatty acids, whereas FNA and its derivatives primarily target proteins and amino sugars, which are more abundant in waste-activated sludge. As a result, the study suggests that FNA pretreatment technology should be exclusively applied to waste-activated sludge to maximize methane production during the co-digestion of these two sludge streams.

In addition to the aforementioned pretreatment methods, Li et al. [97] introduced a unique procedure in their study involving the extraction of 5% of sludge from a semi-continuous digester 4 hours before the midpoint of a 24-hour digestion cycle. The extracted sludge underwent disintegration with 0.1 mol/L NaOH, neutralization, and subsequent reintroduction into the digestion system. The outcomes of this alkaline post-treatment revealed a notable increase in the content of soluble organic substances, particularly VFA and polysaccharides, within the extracted sludge. As a result of this innovative approach, biogas production witnessed a substantial 33% boost, and the degradation rate of sludge organic matter increased from around 40% to 45% when compared to the control group. Nevertheless, it is worth noting that when the proportion of recycled sludge was further increased to 10% or 15%, there was a limited increase in biogas production due to unjustifiable inactivation of anaerobic bacteria within the digester.

Recently, the hydrolysis and acidification of PS was promoted significantly by the mechanical cutting pretreatment (MCP) method [98]. The maximum cumulative biogas production in the pretreatment group was about six times that in the control group. Sludge disintegration by mechanical pretreatment resulted in higher bacteria populations, especially Firmicutes, which were almost double that in the control group. However, MCP had little effect on archaea in the anaerobic digestion of PS, and the populations of methanogens in the control and pretreatment reactors demonstrated comparable results.

The reviewed studies, each with its advantages and considerations, emphasize the possibilities of improving the anaerobic digestion of primary sewage sludge by employing different treatment methods. The overall conclusion is that a customized approach tailored to the specific sludge composition and co-digestion elements is essential in order to attain the highest possible methane production.

Table 5. Treatment processes to enhance anaerobic digestion of primary sludge.

	Substrate [1]	Treatment [2]	Inoculum [3]	ISR *	Tested Conditions	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
1.	CEPT	-	ADS	-	OLR 2, 1.5, 2.25, and 3 g VSS/L/d	SC	L	M	-	[90]
2.	PS SS	Advanced primary separation	ADS-LAB	-	SS: 0.5 and 1.5 g/L	-	L	-	-	[95]
3.	PS	FNA (HNO_2-N)	ADS	1.5–2 *w*/*w* VS for sludge, *w*/*w* TCOD for supernatant	0.77, 1.54, 2.31, 3.08, and 3.85 mg HNO_2-N/L	B	L	M	-	[96]
4.	PS	Enzymatic treatment (P, LP)	ADS-LAB	1:1 g VSS/g VS	P/LP 3:1, 1:1, 1:3, 0:1 *w*/*w*	B	L	M	+	[88]
5.	PS	NaOH	EBS	4:1	NaOH 0.1 mol/L to 5, 10, 15% recycled sludge	SC	L	M	+	[97]
6.	PS	MCP	ADS	0.3:1 *v*/*v*	2, 4, 6, 8, and 10 min of MCP	B	L	M	+++	[98]
7.	PS	HTT	ADS	0.3:1 *w*/*w* VS	130, 150, 170, 190, and 210 °C for 30 min	B	L	M	+	[89]

[1] Substrate, CEPT: chemically pretreated primary sludge; PS: primary sludge; SS: secondary sludge. [2] Treatment, FNA: free nitrous acid; P: protease; LP: lipase; MCP: mechanical cutting pretreatment; HTT: hydrothermal treatment. [3] Inoculum, ADS: anaerobic digested sludge; ADS-LAB: anaerobic digested sludge from lab-scale reactor; EBS: excess biofilm sludge. [4] Mode, SC: semi-continuous; B: batch. [5] Scale, L: lab scale. [6] T: temperature; M: mesophilic. [7] Efficiency, +: 0–40%; +++: >81% enhancement of biomethane production compared to single digestion of primary sludge. P: protease; LP: lipase. * Inoculum-to-substrate ratio: ISR.

7. Co-Digestion of Primary Sludge with Crude Glycerol or Microalgae Biomass

7.1. Co-Digestion of Primary Sludge with Crude Glycerol

Crude glycerol is a major byproduct of biodiesel production which raises environmental concerns due to its increasing production [99], which is estimated to reach 4.04 million tons by the end of 2025 [100]. While an appealing management method seems to be its co-digestion with primary sewage sludge, its high biodegradability can notably increase the organic loading in anaerobic digesters, resulting in the inhibition of methane production. To prevent this, it is commonly suggested to maintain crude glycerol levels below 3% *v/v* [101]. One of the studies that have analyzed the co-digestion of primary sludge with crude glycerol is that of Alves et al. [102] (Table 6). Alves et al. [102] noticed a considerable increase in methane production, almost up to 167%, when 3% *v/v* crude glycerol was introduced into the digester. This was ascribed to the improvement in the C/N ratio, e.g., doses of 1 and 3% *v/v* crude glycerol optimized the C/N ratio of primary sludge from 9.2 to 14.1 and 17.3, respectively. Interestingly, in the case of a 3% *v/v* crude glycerol dose, methane production did not follow the normal pattern of methane production, where methane is produced in significant amounts during the early days of digestion. Instead, a hysteresis was observed, probably due to stimulant activity of acidogenic microorganisms that were required to cope with the increased loading rates and the delayed response of methanogenic archaea which require more time to grow to consume the excess VFA, H_2, and CO_2. Consequently, the CO_2 production contributed to increased biogas rates, yet the CH_4 content in biogas was lower compared to CH_4 content in the biogas produced by PS. To further investigate the perspectives of co-digestion, Alves et al. [103] tested the digestion of crude glycerol with a mix of PS and food waste (at a ratio of 1:1). Although the C/N ratio was further increased, compared to the previous study, the results showed that the key factor for the quantity and the quality characteristics of produced biogas was the concentration of crude glycerol. These studies are in line with studies where other substrates are used and denote limitations in the addition of crude glycerol during anaerobic digestion. In this regard, Nartker et al. [104] suggested that a stepwise supply of crude glycerol in the digester increases both biogas production and the resilience of the system at increased crude glycerol rates. In particular, a systematic increase in glycerol dose from 1 g to 10 g in the digestion liquor resulted in increased biogas production from around 350 to 920 mL/g VS d, while the maximum gain in biogas, the difference between the primary and the co-digested primary sludge, was observed in the 4–9 g of glycerol loading range. These results confirmed that high glycerol loadings can be achieved if proper loading rates are employed to allow the bacterial community to be properly regulated. Such results are promising and should be further investigated. Furthermore, Li and Shimizu [105] applied re-inoculation with fresh inoculant as a countermeasure to VFA inhibition during anaerobic co-digestion of crude glycerol and FW, but this technique proved to exhibit only short-term beneficial results. On the other hand, the addition of biochar was promising in regulating the alkalinity of liquor and facilitating the conversion of VFAs and the activation of methanogenesis. All in all, hindrances related to process adjustments during long-term AD operation, logistics, feedstock sufficiency, and possible impacts on downstream product quality limit the usage of crude glycerol in AD systems that treat PS.

7.2. Co-Digestion of Primary Sludge with Microalgae Biomass

Oswald et al. [106] presented an early study on the use of microalgae to treat municipal wastewater. Today, there are several examples of microalgal-based wastewater treatment systems [107] where microalgae are used to remove nitrogen, phosphorus, and other pollutants from wastewater. After the biological treatment step, microalgae biomass (MB) should be handled properly, e.g., by harvesting and digestion, prior to its final disposal. The digestion of microalgae may be assisted by mixing MB with other waste streams. Studies report that microalgae may cause inhibitory effects on anaerobic digestion due to their high N content and low C/N ratio that can lead to high ammonia levels [108,109]. To overcome this issue, it has been proposed to co-digest MB with primary sludge, as through this process, the C/N ratio is optimized and bioenergy production is increased significantly [27]. The optimization of the C/N ratio during the co-digestion of microalgae with primary sludge is due to the fact that primary sludge yields a more readily biodegradable carbon-rich substrate and has a lower protein content [110], and so, primary sludge may increase C/N levels and enhance biogas production. Moreover, the co-digestion of primary sludge and microalgae may balance the moisture content and optimize the organic loading rate during the anaerobic digestion process. Solé-Bundó et al. [27] analyzed the anaerobic co-digestion of microalgae and primary sludge (1:3 on a VS basis) in continuous reactors for 20 days. The results showed a lower risk of ammonia toxicity and doubling of biogas production during the co-digestion of microalgae and primary sludge compared to production observed when MB was digested alone. This may be due to the fact primary sludge has a higher amount of lipids (45%) and a lower amount of proteins (29%), and it is more biodegradable compared to microalgae that consist of a higher proportion of proteins (58%). Recently, a promising pilot plant study by Mora-Sánchez et al. [111] was implemented. The experiments were conducted to investigate the efficiency of an anaerobic membrane bioreactor that co-digested microalgae and primary sludge. The system operated steadily for 576 days and yielded efficient performance as regards biological degradation and the filtration process, and 215 mL CH_4/g COD was produced on average at 35 °C [111].

Table 6. Co-digestion of primary sewage sludge and glycerol.

	PS Substrate [1]	Co-Substrate [2]	Inoculum [3]	ISR *	OLR + $_{initial}$	Tested Concentrations	Mode [4]	Scale [5]	T [6]	Efficiency [7]	Reference
1.	PS	GL	ADS	2:1 *w/w* VS	-	GL 1, 3% *v/v*	B	L	M	+++	[102]
2.	PS	GL FW	ADS	2:1 *w/w* VS	-	GL 1, 3% *v/v*	B	L	M	+++	[103]
3.	PS	GL	ADS	-	0.98 kg VS/m^3/d	GL 0.8–8% *w/w*	C	L	M	+++	[104]

[1] PS substrate, PS: primary sludge. [2] Co-substrate, GL: crude glycerol; FW: food waste. [3] Inoculum, ADS: anaerobic digested sludge. [4] Mode, B: batch; C: continuous. [5] Scale, L: lab scale. [6] T: temperature; M: mesophilic. [7] Efficiency, +++: >81% enhancement of biomethane production compared to single digestion of primary sludge. * Inoculum-to-substrate ratio: ISR. + Organic loading rate: OLR.

8. Conclusions

Large amounts of sludge are produced during the wastewater treatment process, making its treatment and disposal of major concern. In this regard, the last year's primary sludge is considered an energy source rather than waste, as its energy capacity is proportional to the amount of biodegradable organic compounds in its mass. Biogas, which is produced during the anaerobic digestion of primary sludge, can be used to produce electrical and thermal energy. The present work summarizes the recent research on anaerobic digestion of primary sludge in the context of biogas improvement with various additives (Figure 4).

This literature review revealed that although primary sludge has a high energy content, the anaerobic digestion process may face difficulties due to its low C/N ratio. However, the digestion of primary sludge in conjunction with other organic-rich materials, such as food waste, appears to be one possible way to address this problem, as modifying the C/N ratio when modifying the raw material ratios can significantly increase methane production. However, conflicted results can be met during the co-digestion of primary sludge with livestock residues, where the digestion process can greatly be influenced by the carbon source, as lignin can be quite resistant to degradation. Another organic-rich material which is highly biodegradable and can lead to increased methane production when the loading rates are controlled to avoid inhibition of the methanogenic microorganisms is crude glycerol.

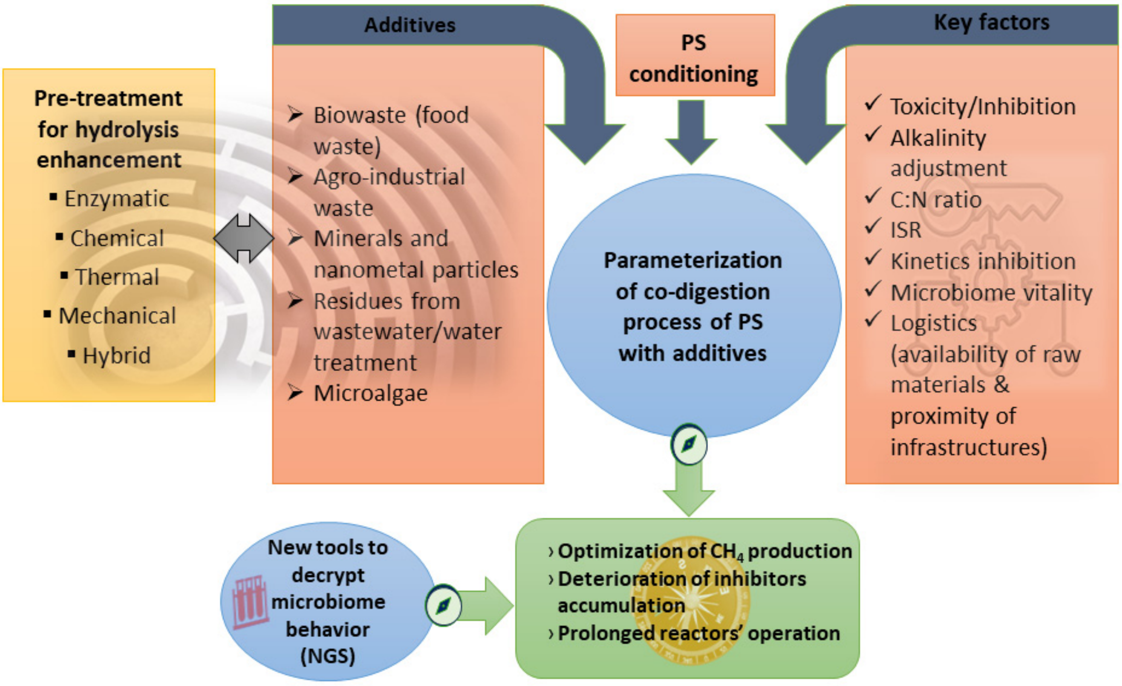

Figure 4. Mapping of anaerobic co-digestion of primary sludge with additives of different origin.

In addition to organic additives, also minerals and nanometal particles seem to be able to optimize various aspects, such as ammonia toxicity, the influence of microbial attachment and community composition, and hydrolysis enhancement, of primary sludge anaerobic digestion. Notably, increased methane production has been demonstrated by the use of attapulgite and ZVI in the proper dosages. However, further studies using standardized methodologies and thorough assessments are required to fully realize the advantages of these additives in order to provide more reliable and broadly applicable conclusions in this area.

Overall, the results of this study suggest that the anaerobic co-digestion of primary sludge with various organic substrates or minerals and nanometal particles has significant potential in enhancing methane production in WWTP and mitigating toxicity during digestion. However, the efficiency of these strategies depends on a thorough understanding of primary sludge and additive characteristics, mixing ratios, and efficient operational parameters. Equally important is the control of organic loading rates to avoid inhibitory effects such as the accumulation of volatile fatty acids. The co-digestion of primary sludge with food waste under controlled feedstock inflow and a relatively low substrate-to-inoculum ratio is promising to achieve high biogas flowrates and is in line with the sustainable development goals (SDGs). Finally, it is deemed necessary for more research to be carried out to develop standardized procedures and integrated assessments that will lead to the optimization and widespread adoption of such practices.

Author Contributions: Conceptualization, F.S. and M.P.; data curation, F.S.; formal analysis, M.P. and F.S.; investigation, F.S.; methodology, F.S., M.P. and V.T.; resources, M.P.; supervision, M.P. and E.D.; validation, F.S. and V.T.; visualization, F.S.; writing—original draft, F.S. and M.P.; writing—review and editing, M.P., F.S. and V.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AC	activated carbon
AD	anaerobic digestion
ADS	anaerobic digested sludge
ADS-LAB	anaerobic digested sludge from lab-scale reactor
Al-DWTS	drinking water treatment sludge treated with Al
AS	activated sludge
HTT	hydrothermal treatment
ZVI	zero-valent iron
AT	attapulgite
B	batch
BA	bottom ash
BH	buckwheat husk
BMP	biochemical methane potential
BA	bottom ash
BS	brewery sludge
C	continuous
C/N	carbon-to-nitrogen ratio
CEPT	chemically pretreated primary sludge
CM	cow manure
CS	Corchorus stalks
CSB	corn stover biochar
DWTS	drinking water treatment sludge
EBS	excess biofilm sludge
EPS	extracellular polymeric substances
ES	excess sludge
Fe-DWTS	drinking water treatment sludge treated with Fe
FL	fallen leaves
FNA	free nitrous acid
FVW	fruit and vegetable waste
FW	food waste
GL	crude glycerol
GP	graphite powder
GR	grass
HTC	hydrothermal carbonization
ISR	inoculum-to-substrate ratio
k	hydrolysis rate constant
L	lab scale
LP	lipase
M	mesophilic
MB	microalgae biomass
MCP	mechanical cutting pretreatment
NM	nano magnetite
NPs	nanoparticles
OFMSW	organic fraction of municipal solid waste
OLR	organic loading rates
P	protease
PS	primary sludge
PW	process water from hydrothermally treated waste-activated sludge
RC	rumen content of cattle
SBP	sugar beet pulp
SC	semi-continuous
SL	sugarcane leaves
T	temperature
TH	thermophilic

UASB	up-flow anaerobic sludge blanket digestion
VFAs	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids
WAS	Waste-activated sludge
WH	whey
WS	wheat straw
λ	lag phase

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