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Review

Effect of Emerging Micropollutants on the Anaerobic Digestion of Sewage Sludge

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Abstract: The recovery of valuable resources from wastewater treatment plants (WWTPs) has received a great deal of attention as part of the concept of a circular economy. Anaerobic digestion for stabilizing sewage sludge in WWTPs, which produces biogas and stabilized biosolids, is a mature technology used worldwide. However, despite the necessity of achieving safe and reliable organic recycling, studies on the effect of some emerging micropollutants on this process are rare. This knowledge gap is of growing relevance because of the increasing use of some endocrine-disrupting compounds (EDCs), microplastics (MPs), and engineered nanoparticles (NPs) in industry and human life. These compounds are ubiquitous in wastewater streams and, therefore, may have serious effects on the course of the anaerobic digestion of sewage sludge, raising concerns about their effects on the environment. This article provides a comprehensive overview of the mechanisms by which selected EDCs, MPs, and NPs affect the valorization of sewage sludge, with a focus on the production of CH_4, H_2, and volatile fatty acids. This study takes into consideration the performance during all stages of anaerobic digestion, the shifts in microbial abundance and diversity, and the activity of key enzymes during the treatment process.

Keywords: sewage sludge; anaerobic digestion; endocrine disrupting compounds; microplastics; engineered nanoparticles; pharmaceuticals

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1. Introduction

The increasing concern for the environment and the development of analytical techniques have led to investigations into the occurrence of pollutants in the environment, such as endocrine-disrupting compounds (EDCs), that are associated with some disorders of the reproductive system of exposed organisms. EDCs include pharmaceuticals, hormones, bisphenol A (BPA), microplastics (MPs), engineered nanoparticles (NPs), etc. Due to the common and increasing use of these compounds in industry and personal care products, and the mechanisms by which they are transported in the human body or in the environment, they enter wastewater treatment plants (WWTPs), leading to a potential environmental hazard [1]. Pharmaceuticals in particular are the most abundant emerging pollutants in wastewater because they are intensively used in human and veterinary medicine, as well as to promote the growth of farmed fish and livestock, and they are easily metabolized into polar and soluble forms in living organisms [2]. Depending on their hydrophobicity, these pollutants demonstrate medium to strong sorption onto solids. For example, about 40% of BPA and more than 50% of pharmaceuticals can adsorb to the solid fraction of sewage and are thus present in waste-activated sludge (WAS) [3–5].

The growing energy demand has resulted in an urgent search for alternative and clean energy sources. A promising technology for CH_4 recovery from organic sources is anaerobic digestion. This technology is used worldwide for the utilization of sewage sludge, which contributes to global carbon neutrality objectives via the recovery of two valuable resources, biogas and stabilized biosolids. Accordingly, anaerobic digestion has received

great attention in the quest to achieve a circular economy. In addition to the production of an energy source [6], the widely recognized advantages of this process include low sludge generation, low investment and operating costs, low energy consumption, and simple design and operation. However, the literature indicates that the effectiveness of microbiological conversions during anaerobic digestion can be disrupted by the presence of emerging pollutants or their by-products [7]. Another important aspect is that micropollutants and the products of their metabolism can be retained in the digestate, which affects the safe disposal of WAS.

AD comprises various steps, namely hydrolysis, acetogenesis, acidogenesis, and methanogenesis. In the hydrolysis phase, complex organic substances that cannot be directly utilized by bacteria are broken down into soluble monomers by extracellular hydrolytic enzymes. Hydrolysis is carried out by bacteria from the group of relative anaerobes belonging, e.g., to the genera *Streptococcus* and *Enterobacterium* [8]. During acidogenesis, acid-producing fermenting bacteria convert soluble monomers into end products and volatile fatty acids (VFAs) that can be recovered as a bioproduct. These are facultative anaerobes that utilize the oxygen accidentally introduced into the process and thus create favorable conditions for the development of obligate anaerobes such as *Pseudomonas* sp., *Bacillus* sp., or *Clostridium* sp. In the acetogenesis phase, bacterial activity leads to the formation of acetic acid and hydrogen. In the final methanogenic phase, the acidified products are converted into methane by various mesophilic bacterial species [9]. Anaerobic methanogens are very sensitive to changes in the operating conditions in the reactor [10], and their metabolism is particularly susceptible to disturbances caused by the presence of micropollutants. In general, the fermentation of the sludges reduces the concentration of emerging pollutants in the digestate. However, many studies have reported that BPA did not biodegrade during anaerobic mesophilic or thermophilic digestion [11–13]. Regarding pharmaceuticals, under anaerobic conditions, they are degraded from a medium to high extent [14]. On the other hand, MP degradation in anaerobic conditions is mainly related to the type of polymer.

Because CH_4 production from sewage sludge belongs to the most vulnerable treatment processes, and since the anaerobic microorganisms are sensitive when exposed to certain conditions, such as the presence of toxic pollutants [15], special attention should be paid to the effect of emerging pollutants on the performance of anaerobic digestion. Even though emerging pollutants are frequently detected in WAS and anaerobic treatment predominates in WWTPs, the interference of emerging pollutants with anaerobic digestion is overlooked. The fact that the influence of emerging micropollutants on the anaerobic digestion of sewage sludge in WWTPs has not been systematically reported may result from the fact that simple and rapid analytical methods for the accurate quantification of these pollutants in complex matrices such as sewage sludges were not yet developed [16]. In addition, the lack of systematic reports on the effect of emerging pollutants on anaerobic digestion results from the huge diversity of these compounds, whose structure and physicochemical properties strongly affect their behavior under environmental conditions [17]. Furthermore, their effect on the anaerobic process is often dose-dependent [18,19]. In addition, some compounds, like NPs, affect anaerobic digestion positively or negatively, depending on their composition [20,21].

The objective of this manuscript is to review the effects of the presence of selected EDCs, MPs, and engineered NPs on CH_4, H_2, and VFA production during anaerobic digestion. The ways in which these compounds influence the utilization of substrates for CH_4 conversion, the performance of all stages of anaerobic digestion, the shifting of microbial abundance and diversity during the treatment, and the potential impact of selected pollutants on key enzyme activities are discussed.

2. The Effect of BPA on Anaerobic Digestion

2.1. The Presence of BPA in the Environment and Its Degradation

BPA is an alkylphenol composed of acetone and phenol. It is a plastic additive that is most commonly used to produce different industrial and personal care products, e.g., polycarbonate, polysulphone, epoxy, polyacrylate, polyetherimide resins, thermal paper, beverage containers, dental sealant, and so on [1]. This persistent organic pollutant is considered an environmental endocrine disruptor. It is one of the most common man-made pollutants with an adverse effect on humans and the environment, and it has been defined as an exogenous chemical by the US Environmental Protection Agency (EPA), listed on the Candidate List of Substances of Very High Concern (SVHC), and recommended for inclusion in the REACH authorization list by the European Chemicals Agency (ECHA) [22]. BPA has been detected in surface waters (up to 56 μg/L), sediments (up to 20 mg/kg dry weight (d.w.)], tap water (14 ng/L), effluents from WWTPs (370 μg/L), sewage sludge and biosolids (from 10 to >100,000 μg/kg d.w.), and waste landfill leachates (17 mg/L) [23,24]. It has been reported to be one of the five most commonly occurring organic micropollutants in wastewater in the United Kingdom [25]. As wastewater is considered the main source of BPA in the environment, different methods for its removal from wastewater have been reported: adsorption [1], ozonation [26], Fenton and photo-Fenton oxidation [27,28], electrolysis [29], biodegradation [3,30,31], membrane techniques [32], and membrane bioreactors [33].

Microbial degradation is the most efficient process for reducing the persistence of BPA [31,34]. However, due to its hydrophobicity, about 40% of BPA can adsorb to primary sludge and adapted and non-adapted activated sludge [3,4]. A concentration of BPA in sludge of 25.6 mg/kg d.w. has been reported [13]. If sludge containing BPA is used as fertilizer, soil pollution, and health problems may occur, as BPA can be absorbed by plants [35] and then enter the bodies of animals and humans as food. Therefore, the treatment of sludge before its application to the soil is a widespread practice. However, many studies have reported that BPA did not biodegrade during anaerobic mesophilic or thermophilic digestion [11–13]. Moreover, in some studies, BPA concentration increased in the mesophilic digesters because of the degradation of polycarbonate polymers, which released the BPA monomer [36]. After anaerobic digestion, sewage sludge may contain up to 36.7 mg BPA/kg d.w. [37]. As can be seen, even after anaerobic digestion, BPA will remain in the final sludge applied to agricultural fields. As an endocrine-disrupting compound, which can profoundly affect organisms at low concentrations, the tolerable daily intake of BPA was identified as 50 μg/(kg body weight·day) by the EPA and was lowered to 4 μg/(kg body weight·day) by the European Food Safety Authority (EFSA) [38].

2.2. The Effect of BPA on Methane Production from Sewage Sludge

Because sewage sludge valorization by biogas production is widely applied in the management of WWTPs, some studies have focused on the effect of BPA on biogas production. Digester sewage sludges and co-digester sewage sludges (with silage, farm manure, livestock and farming waste, food waste, and the organic fraction of municipal solid waste) have been investigated under mesophilic methanogenic conditions [16]. These sludges contained endogenous BPA at levels of up to 10,973 μg/kg d.w. (digested sludges) and up to 9069 μg/kg d.w. (co-digested sludges). The addition of BPA (228 μg/kg) did not affect biogas production and the efficiency of BPA removal reached 50%. It was concluded that BPA removal was triggered by the high endogenous BPA concentrations, which allowed for the acclimation of the microbial community, thus improving BPA degradation. This explanation seems to be correct because, in other studies, even a small amount of BPA released from polyvinyl chloride (PVC) MPs in sewage sludge (1.8–3.6 μg/L) decreased CH_4 production by approximately 24% [39]. BPA leaching caused the solubilization of extracellular polymeric substances (EPS) and the rupture of microbial cells, which released lipids and nucleic acids, thus increasing concentrations of soluble COD. Simultaneously, the abundance of some hydrolytic bacteria decreased, and acidogenesis was negatively affected, which reduced the production of VFAs, decreasing the abundance of fermentative bacteria.

In anaerobic granular sludge spiked with 40 mg BPA/L, there were numerous indicators of the toxic effects of BPA [40]. Firstly, COD removal was substantially reduced to less than 65%, which was 30% lower than COD removal in the control variant (without BPA spiking), due to the inhibition of the hydrolysis–acidification process. Secondly, sludge stabilization was adversely affected. In the structure of EPS, the tryptophan-like proteins that protect microbes against toxicity disappeared when BPA was added, whereas they were present in the absence of BPA. BPA also affected the activity of protease, acetate kinase, and coenzyme F_{420}. The concentration of coenzyme F_{420}, which is unique to methanogens and related to CH_4 production, decreased from 0.0045 to 0.0017 μmol/L in the presence of BPA. BPA also improved the protease activity of anaerobic granular sludge, thus increasing protein hydrolysis. An increase in the activity of acetate kinase, which controls the transformation of acetyl-CoA into acetic acid, indicated that BPA stimulated the production of large concentrations of acetic acid, which inhibited methanogens' growth. This conclusion was supported by the increase in the relative abundance of Bacteroidetes (from about 13% to over 22%), which play a significant role in the acetogenic phase of anaerobic digestion. In addition, BPA was strongly toxic to *Hydrogenophaga* sp., which can utilize various organic compounds; its abundance decreased sharply (from 15.58% to 0.12%) after BPA spiking. The inhibition of sludge hydrolysis by the exposure of waste sludge to BPA has been attributed to the denaturation of α-amylase [41], which is an enzyme widely distributed in waste sludge that plays a significant role in hydrolysis. BPA changed the secondary structure of α-amylase after interacting with the enzyme via hydrophobic and hydrogen bonding.

The effect of BPA on methane fermentation was related to the production of reactive oxygen species (ROS). BPA leached from polycarbonate MPs (1.26 ± 0.18 mg/L) decreased the production of ROS, thereby increasing enzyme activity, biomass viability, and the abundance of *Methanosarcina* sp. and *Methanobacterium* sp., and thus improving CH_4 production by up to 24.7% [42]. On the other hand, when a larger amount of BPA leached (4.02 ± 0.15 mg/L), the production of ROS was stimulated, resulting in decreased biomass viability and even apoptosis, thus decreasing CH_4 production by 8.1%.

The effect of BPA on CH_4 production has been investigated in bioelectrochemical systems, which include microbial electrolysis cells and microbial fuel cells; these systems can both generate CH_4 and increase the anaerobic degradation of resistant compounds. In a bioanode single-chamber microbial electrolysis cell, organic compounds are degraded to electrons, CO_2, and H^+ by exoelectrogenic microorganisms on the anode. The electrons are transferred to the anode by exoelectrogens. Then, electrons migrate to the cathode, where they are combined with protons to form H_2. Additionally, CO_2 and H_2 combine to form CH_4 and water. Under optimum conditions (applied voltage 0.8 V, BPA concentration 10 mg/L, hydraulic retention time (HRT) 24 h, C/N ratio 50), 95.4% BPA removal, 94.9% COD removal, a rate of CH_4 production of about 120 mL/(L·day), and a CH_4 content of over 90% were obtained [29]. These values were higher than those of the control system (a system containing electrodes without any energy input), which were 54.2%, 61.3%, 83.1 ± 2.2 mL/(L·day), and 71.1%, respectively. The removal of COD and BPA, and the CH_4 production rate and content, were increased as the C/N ratio was increased from 20 to 50. Increasing the BPA concentration from 10 to 80 mg/L decreased the CH_4 production rate from about 118 to about 97 mL/(L·day) and the CH_4 content from 93.1 to 79.8%. These decreases were explained by the fact that a substantial fraction of the electrons was consumed to reduce BPA, decreasing the amount of H^+ evolved into H_2, which would be consumed by hydrogenotrophs.

2.3. The Effect of BPA on VFA Production from Sewage Sludge

Anaerobic digestion cannot only degrade organic substances and transform them into CH_4 but can also transform them into VFAs. VFAs can be used as raw materials for generating higher-value compounds, including polyhydroxyalkanoates (PHAs), CH_4, or alcohols [43–45], and as carbon sources for nutrient removal in WWTPs [46,47]. A BPA concentration of 0–200 mg/kg d.w. increased VFA accumulation; the largest increase in VFA production (2095 mg COD/L) was observed at 50 mg BPA/kg d.w. and was

1.3 times higher than the production in the control [4]. This effect was mainly due to acetic acid production, which accounted for 70% of the VFAs (3.6 times more than in the control). Large amounts of acetic acid were produced as a result of 20 and 40% increases in the activities of acetate kinase and phosphotransacetylase in the presence of BPA. The VFA content increase was attributed to the increase in protein content in the total EPS caused by BPA. Although BPA did not affect the solubilization of WAS, it increased sludge hydrolysis 1.1-fold by increasing the activities of protease and α-glucosidase. The improved bioconversion of proteins, carbohydrates, lipids, and other compounds in the presence of BPA was indicated by significantly higher relative abundances of genes coding enzymes involved in microbial metabolism. Due to the higher protein content in EPS, anaerobe cells were better protected, and more substrate was available for VFA production. The increase in acetic acid concentrations was not attributed to its production from CO_2 and H_2 by homoacetogenic bacteria because BPA did not affect the consumption of H_2. The increase in VFA production could have resulted from the changes in the abundance of microorganisms. For example, with BPA, the abundance of Proteobacteria and Firmicutes, which can utilize VFAs, decreased from 37.2 to 30.2% and from 14.0 to 11.7%, respectively. Conversely, the abundance of *Actinobacteria* sp. and *Clostridium* sp., which can produce VFAs, increased from 6.2 to 27.0%, and from 1.4 to 1.7%, respectively. When BPA was present, the relative abundance of genes encoding enzymes involved in VFA production increased. For example, the abundance of *gltB* (involved in glutamate synthesis) increased 1.7 times; that of *asdA* (involved in transferring nitrogenous groups to L-alanine) increased 1.2 times; *aspC* and *yhdR* (encoding aspartate aminotransferase and participating in oxaloacetate synthesis) increased 1.5 and 1.8 times, respectively; AGXT (involved in pyruvate synthesis) increased 1.6 times; *dsdA* (involved in hydrolysis of D-serine to pyruvate) increased 1.2 times; *fabG* (encoding of acyl reductase and important in fatty acid synthesis) increased 1.3 times; *fas* (encoding fatty acid synthetase) increased over 4 times; and *desA1* (related to acyl reductase, which participates in the VFA formation) increased 6 times. In addition, the quorum sensing system was improved after BPA addition. The presence of BPA increased the relative abundance of *rpfC*, *crp*, and *rpfG* genes, which are responsible for the synthesis of EPS and the formation of biofilm, thus protecting cells from toxic environmental conditions.

2.4. The Effect of BPA on Hydrogen Production from Sewage Sludge

The production of H_2 from biomass and organic waste is considered a potential alternative energy source and H_2 is regarded as a clean and CO_2-free fuel with a high energy density. Digestion for H_2 production offers numerous advantages, such as low sludge production and energy requirements [48]. H_2 is considered a promising energy source; therefore, the effective transformation of biomass and organic waste into H_2 is urgently sought. However, the major disadvantage of anaerobic digestion to produce H_2 is its high sensitivity to toxins [15]. The inhibitory effect of BPA on H_2 production from the organic fraction of municipal solid waste was studied with different BPA concentrations from 0.5 to 25 mg/L [49]. The reduction in H_2 yield by 9.2–75.3% in the presence of BPA was due to the shift in the metabolic pathway from butyrate to propionate production. In the control, the cumulative H_2 production was 227.9 ± 10.5 mL which decreased to 58.9 ± 10.4 mL in the experimental batches supplemented with BPA. The decline in the H_2 yield was 37.1%. Also, the inhibitors decreased the efficiency of COD removal.

3. The Effect of Pharmaceuticals on Anaerobic Digestion

3.1. The Presence of Pharmaceuticals in the Environment and Its Degradation

Pharmaceuticals, as parent compounds or their metabolites, are largely excreted via urine. In municipal wastewater, the pharmaceuticals with levels over 100 μg/L include antibiotics (ciprofloxacin, trimethoprim, tetracycline), anti-inflammatories (ibuprofen, diclofenac, naproxen), β-blockers (metoprolol), neuroleptics (carbamazepine), antidiabetics (metformin, hydrochlorothiazide), analgesics (paracetamol), and biocides (triclosan) [50]. The concentrations of pharmaceuticals in municipal sewage sludge are lower; they barely

reach 100 μg/L. The total content of pharmaceuticals in sewage sludge included diuretics (19%), lipid-modifying agents (16–21%), diclofenac, hydrochlorothiazide, clarithromycin, furosemide, atorvastatin, and carbamazepine [51]. The mean concentrations of selected pharmaceuticals in sewage sludge applied to agricultural land were 125 μg/kg d.w. (diclofenac), 20 μg/kg d.w. (estrone), 20 μg/kg d.w. (17β-estradiol) [52], 300 μg/kg d.w. (ibuprofen), 45 μg/kg d.w. (trimethoprim), 100 μg/kg d.w. (erythromycin), 4 μg/kg d.w. (sulfamethoxazole), 150 μg/kg d.w. (carbamazepine), and 10,000 μg/kg d.w. (triclosan) [53]. However, these concentrations vary depending on the region, sampling period, and active substance. Once released into the environment, pharmaceuticals accumulate in soils and leach into groundwater, thus negatively affecting the environment and human health [50,54].

In general, pharmaceuticals can be eliminated mainly by electrooxidation, ozonation, thermal hydrolysis, ultrasonic treatment, sorption to solids, bioaugmentation, and biotransformation [17,51], and the efficiency of elimination depends on the physicochemical features of pharmaceuticals, operating conditions, microbial diversity, and enzymatic activity in biological systems. Some studies have reported that anaerobic digestion can efficiently remove most of the pharmaceuticals (>70%), even those present in concentrations of, e.g., 7.027 mg/L (acetaminophen), 6.304 mg/L (metformin) or 111.4 μg/L (naproxen) [54]. On the other hand, moderate removal (31 and 45% after mesophilic and thermophilic digestion, respectively) was reported, whereas some pharmaceuticals (atenolol, valsartan, and hydrochlorothiazide) were almost completely degraded [55]. According to Gonzalez-Gil [14], sulfamethoxazole, for example, was completely removed, whereas most pharmaceuticals were removed to a moderate extent (35–70%) during methanogenesis. At the organic loading rate (OLR) typical of sewage sludge digesters (1–2 g COD/(L·d)), biodegradation was not supported by cometabolism. However, other compounds, such as β-blockers and anti-inflammatories, are persistent in anaerobic digestion [56].

3.2. The Effect of Pharmaceuticals on Anaerobic Digestion

Most studies report that the presence of pharmaceuticals inhibits anaerobic digestion. One of the reasons for this is the difficulty in keeping the syntrophic interactions between bacteria and methanogenic microorganisms [57]. However, the different physical and chemical properties of pharmaceuticals mean that their effect on fermentation varies. For example, triclocarban promoted the release of organic substances (dissolved organic matter, proteins, and polysaccharides) from the WAS, which favored acidogenesis and acetogenesis, but inhibited methanogenesis, thus leading to VFA accumulation and lowered CH_4 production [58]. A change in the ratio between methanogens, due to an increase in the abundance of acetoclastic methanogens and a decrease in the abundance of hydrogenotrophic methanogens, may have been the reason for the inhibition of methanogenesis. In comparison, the neuroleptic fluoxetine (FLX) inhibited the activity of the key enzymes involved in hydrolysis, acidogenesis, and methanogenesis, leading to a decrease in CH_4 production. This effect was dose-dependent. At a low dose (0.1 mg FLX/kg d.w.), no significant effect on CH_4 production was observed. At 2.0 mg FLX/kg d.w., the cumulative methane CH_4 was about 90 mL/g VSS (volatile suspended solids), which was about 60% of the control (without FLX) [19]. Similarly, the instability of the anaerobic process leading to the accumulation of VFAs was observed during the digestion (38 °C, OLR 1.3 g VS/(L·d), HRT 43 d) of synthetic sewage sludge spiked with environmentally relevant pharmaceuticals (clotrimazole, clarithromycin, fluoxetine, erythromycin, ibuprofen, and a few others) [59]. The VFA accumulation that led to a decrease in biogas production was attributed to changes in the microbial community, mainly the increase in the abundance of Firmicutes (>70%) and the decrease in the abundance of Bacteroidetes and Euryarchaeota (<5%). As another example, carbamazepine limits the anaerobic digestion of wastewater from lactic acid synthesis, as demonstrated by a decrease in CH_4 yield from 275.12 to 182.04 mL/g when the carbamazepine dosage was increased from 0 to 0.14 mM [60]. Carbamazepine promoted sludge solubilization, resulting in the release of polysaccharides

and proteins; however, hydrolysis, which is commonly considered a rate-limiting step, was inhibited. Acidification was enhanced, leading to an accumulation of VFAs due to the increasing abundance of *Chloroflexi* sp. (from 21.5 to 26.7%). This caused disturbances in acetogenesis and methanogenesis, resulting in a significant decrease in CH_4 yield. The changes in the methanogen distribution in the microbial community may have been the reason for this; the acetoclastic methanogen *Methanosaeta* sp. decreased from about 60 to 45%, while the hydrogenotrophs *Methanobacterium* sp. and *Methanomassiliicoccus* sp. increased from 28% and 0.5% to 37.9% and 4.1%, respectively. The negative effect of carbamazepine on anaerobic digestion decreased in the following order: acetogenesis > methanogenesis > hydrolysis > acidogenesis > solubilization.

The effects of pharmaceuticals on the efficiency of anaerobic digestion for CH_4 production were discussed in terms of the physical properties of pharmaceuticals and their effects on the most sensitive microbial groups involved in the process—acetogens and acetoclastic methanogens. Three pharmaceuticals commonly found in sewage sludge, namely ofloxacin, propranolol hydrochloride, and diclofenac sodium, were investigated [61]. The reduction in CH_4 production in the presence of these pharmaceuticals was associated with their hydrophobic nature. In general, the more hydrophobic compound, the more detrimental its effect on methanogenesis. Therefore, propranolol hydrochloride was the most inhibitory pharmaceutical, while ofloxacin was the least inhibitory to both microbial groups. Due to its chemical nature, the effect of propranolol hydrochloride is similar to that of a surfactant. It changes the bilayer organization of biological membranes, affecting their permeability, protein structure, etc., and leads to membrane destruction at higher concentrations. Increasing the concentrations of propranolol hydrochloride and diclofenac sodium resulted in an increasing inhibition effect, whereas, in the case of ofloxacin, the inhibition did not depend on the concentration. It was concluded that typical concentrations of these pharmaceuticals in sewage sludge should not inhibit anaerobic digestion.

The effect of pharmaceuticals on biogas production during anaerobic digestion depends on the degree of sludge adaptation. In the study of anaerobic digestion with the spiking of sludge with two concentrations (10 and 500 μg/L) of antibiotics (amoxicillin and ciprofloxacin), analgesics (ibuprofen, diclofenac, and tramadol), β-blocker (atenolol), psychoactive compounds (carbamazepine, caffeine, and cotinine), and a mixture of them stimulated anaerobic digestion in unadapted sludge [62]. For example, 500 μg ibuprofen/L increased biogas production by 61%. In the adapted sludge, pharmaceuticals decreased biogas production. For example, 500 μg ciprofloxacin/L reduced biogas production by 52%.

Although a strong bactericidal effect of some pharmaceuticals on the microbial structure of the anaerobic digestion environment and biogas production has been observed, this effect was observed mainly in concentrations that are higher than the concentrations typically found in sewage sludge. Therefore, in some cases, under normal operating conditions, the negligible inhibition of anaerobic digestion was considered [50]. Although the recovery of CH_4 is most valuable in sludge management in terms of so-called clean production, it is recommended VFAs be recovered from sludges with a high dosage of pharmaceuticals to limit their inhibitory effect on CH_4 production [60]. Furthermore, to reduce the toxic effect of inhibitors on CH_4 production, the dilution of inhibitory substances was recommended by, for example, the co-digestion of sewage sludge with food waste, which is pharmaceuticals-free and rich in biodegradable substances [63].

4. The Effect of Engineered NPs on Anaerobic Digestion

4.1. NPs in Wastewater and Sewage Sludge

Due to their unique magnetic, electrical, and optical properties, NPs are widely used in the electronic industry, and in medicine, pharmacy, and cosmetics [64]. NPs are leached from products to ultimately enter landfills and WWTPs to finally end up in wastewater and sewage sludge [65]. Their specific physicochemical properties include chemical stability, extremely small size (1–100 nm), diverse morphology, and high reactivity [66]. For

these reasons, NPs entering wastewater and WAS can influence biological treatment both positively [20] and negatively [21].

4.2. The Effect of NPs on Anaerobic Digestion

Regarding the positive effect of NPs on anaerobic digestion, most metallic NPs with a trace element base (e.g., iron, nickel, cobalt), within an optimal concentration, improved H_2 and CH_4 production in terms of gas production and effluent quality [67]. Since enzyme synthesis during the growth of methanogens depends on Fe, Co, and Ni, NPs stimulate the activity of anaerobic microorganisms by providing important nutrients and supporting the synthesis of enzymes and co-enzymes. CH_4 production was more sensitive to the addition of NPs than H_2 production. NPs without a trace element base provided active sites for microorganisms and absorbed inhibitory substances. In addition, the improvement in biogas generation was attributed to electron transfer between species using electrically conductive materials such as NPs, which is one of the mechanisms responsible for the increase in CH_4 yield [68].

In the study with the addition of silver NPs and ionic silver, the CH_4 content was not significantly changed compared to the control, but the addition of NPs positively affected the kinetic parameters of anaerobic digestion [69]. As a result of the five-fold increase in the abundance of *Methanosarcina* sp., the maximum CH_4 production rate was significantly higher, and a shorter lag phase was observed.

The effect of NPs on anaerobic digestion is dose-dependent. For example, when investigating the addition of iron (Fe) and magnetic iron oxide (Fe_3O_4) at concentrations of 5, 10, and 20 mg/L, the highest biogas and CH_4 production was obtained for 20 mg Fe/L and 20 mg Fe_3O_4/L [18]. At these concentrations, the biogas volume increased 1.45- and 1.66-fold compared to the control with the addition of salt ($FeCl_3$), and the CH_4 volume increased 1.59- and 1.96-fold. At 20 mg Fe_3O_4/L, the specific biogas and CH_4 production were the highest (584 mL biogas/g VS and 351.8 mL CH_4/g VS), which was 1.6 and 2.0 times higher than the control, respectively. In another study, the effect of TiO_2, NiO-TiO_2, and Fe_2O_3-TiO_2 on anaerobic digestion was investigated using simple substrates such as glucose, cellulose, acetic acid, and a H_2–CO_2 mixture, as well as complex substrates such as municipal biopulp [70]. The hydrolysis rate increased with a higher dose of both metals (Fe and Ni) coated with TiO_2. For example, the hydrolysis rate of cellulose substrate increased up to 58% at a concentration of 23.5 mg NiO–TiO_2/L, whereas a higher dose inhibited the hydrolytic activity. On the other hand, a low dose of NiO–TiO_2 improved CH_4 production by up to 24%, which was due to the acceleration of the enzymatic activity of acetoclastic methanogens.

The opposite effect of adding NPs on anaerobic digestion was found in the study of pilot anaerobic digesters with WAS exposed to a mixture of Ag_2O, TiO_2, and ZnO at concentrations of 250, 2000, and 2800 mg/kg d.w., respectively [66]. The NP dosage increased the accumulation of VFAs by 1.2-fold and decreased the number and diversity of methanogens by 1.4-fold and 1.8-fold, respectively, compared to the control. The increased abundance of *Methanosarcina acetivorans* (6-fold) and *Methanosarcina barkeri* (11-fold) indicated that these microorganisms are NP-tolerant.

When investigating the effect of ZnO on methane fermentation, 1 mg ZnO/g d.w. (total suspended solids) did not alter CH_4 production, but 30 and 150 mg ZnO/g d.w. led to an inhibition of 18.3 and 75.1%, respectively [21]. Higher concentrations of ZnO reduced the activities of protease and coenzyme F_{420}, and the abundance of methanogenic Archaea. The release of Zn^{2+} from the NP, which may inhibit hydrolysis and the methanation stages of fermentation, was considered to be responsible for the decrease in CH_4 production.

When investigating the addition of NPs such as carbon NPs, Al_2O_3, ZnO, and CuO, it was found that ZnO and CuO not only significantly diminished the microbial diversity and altered the structure of the microbial community but also increased the risk of antibiotic resistance [71].

4.3. The Effect of NPs on Wastewater Treatment

Another aspect of the application of NPs that should also be noted is that the physical and chemical characteristics of NPs, such as metal oxides, give them adsorption properties, which is related to their photocatalytic capacity, which can be beneficially applied in the treatment of organics-contaminated water. For example, the use of synthesized NPs between two or more metal oxides (e.g., TiO_2 and ZnO, TiO_2 and SiO_2, TiO_2 and WO_3, CeO_2 and SiO_2, ZnO and CuO, ZnO and Al_2O_3 and TiO_2), between metal oxides and polymers (TiO_2-SiO_2 and polypyrrole, SnO_2 and polypyrrole, ZnO and polypyrrole), or between metal oxides and carbon materials (TiO_2 and graphene, Cu_2O-SnO_2 and graphene) has been suggested as an efficient solution for improving water treatments, including the degradation of dyes, insecticides, pesticides, or antibiotics [72]. These hybrid NPs absorb visible light and are considered cost-effective solutions for pollutant removal, which positively affects the performance of water treatment plants. However, the separation of NPs from purified water and their reuse remain limiting factors.

5. The Effect of MPs on Anaerobic Digestion

5.1. MPs in Sewage Sludge

WWTPs are regarded as significant point sources of MPs to the environment [73]. It has been previously proved that WWTPs can retain over 90% of the MPs present in wastewater. A review of the data in the literature indicates that, for WWTPs, the MP concentrations were from 61 to 5600 μg/L and from 0.5 to 170.0 μg/L in influent and effluent, respectively, representing a removal efficiency of 93.8–99.8%. The number of MP particles per L of raw wastewater varies from 1.01 to 31,400 [74]. An analysis of urban and urban/industrial WWTPs in the Agadir metropolis indicated the most abundant polymers were polyester, polyethylene (PE), PP, and polystyrene (PS) in the shape of fibers, with the predominance of the 100–500 μm fraction [75]. Preliminary and primary treatments are mostly responsible for MP removal; the efficiency of secondary and tertiary treatments is determined by the choice of technology. The results of Carr et al. [76], from their study conducted in tertiary WWTPs and secondary WWTPs, indicate that tertiary effluent is an insignificant source of MPs and that MPs are mostly removed via skimming and settling processes and are thus retained in the sewage sludge.

Some reports inform us that MPs do not negatively affect the performance of biological rectors and communities of bacteria crucial for wastewater purification such as nitrifiers and denitrifiers [77,78]. Others indicate that specific processes, e.g., denitrification, can be affected by the presence of MPs. Su et al. [79] observed that low concentrations of polypropylene (PP) MPs slowed down the NO_2^-–N reduction, while the concentration of PP MPs of 60 mg/L caused the significant accumulation of NO_2^-–N in the effluent. Simultaneously, the release of greenhouse gas N_2O increased with increasing PP concentrations in wastewater. There is no doubt, however, that the high abundance of MPs strongly affects the sludge disposal and quantity and quality of bioproducts recovered from sludge.

Based on sixty-five studies on MP contamination in sewage sludge and biosolids spanning twenty-five countries, it was concluded that MP concentrations varied considerably with a median MP concentration of 22.41 particles/g d.w. [80]. Chaudhary et al. [81] reported the occurrence of MP in three Indian sewage sludge in the ranges of 29.2–60.8 $\times$ 10^3 particles/kg d.w.

Sewage sludge from WWTPs, including digestate from anaerobic digestion, is often used as a fertilizer for agricultural soils. A large number of MPs were estimated to enter fields via biosolids application. An analysis of data collected for sixteen countries indicated that the amount of deposited MP varied from 8.2 $\times$ 10^{10} to 1.29 $\times$ 10^{15} particles/year, although there was no difference in MP amounts between fields with a history of biosolids application and control fields [82]. Large amounts of MPs in sewage sludge can be hazardous to crop production when such sewage sludge is applied [81]. The most desirable solution is to remove MPs before entering the biological treatment line of WWTPs, where they accumulate in the sewage sludge [83].

EPS production, whose content in biomass plays an important role during anaerobic digestion, strictly depends on MP presence in sludge. PE MP concentrations in wastewater (1, 10, 50 mg/L) stimulated EPS and alginate production in granules. The alginate content increased from about 240 mg/g MLSS in the control to about 440 mg/g MLSS (mixed liquor-suspended solids) at the highest PE load in wastewater [78]. The structure of EPS changes during anaerobic digestion from tightly bound to sludge particles to slime fraction. A comparison of the EPS fractions in the raw sludge and digestate indicated that, during anaerobic digestion, tyrosine-like compounds were converted to tryptophan-like compounds, and the content of humic-like substances in EPS increased [84]. Ma et al. [85] observed that EPS increased CH_4 production (25.0–36.5%) compared to the control, probably due to a shift in the digester microbiome. EPS presence enriched functional microorganisms, including Actinobacteria, Firmicutes, Synergistetes, and Chloroflexi, which are important for hydrolysis and acidification. EPS also increased the abundance of cytochrome c, which accelerated the direct interspecies electron transfer between syntrophic and methanogenic bacteria (*Methanosaeta* sp.). A comparison of the biogas production of sludge and sludge deprived of EPS indicated that, after removing EPSs from sludge, cumulative biogas production and yield were raised by 9.4 and 28.8%, respectively [86].

An additional threat to the efficiency of sludge fermentation is the fact that, after biological treatment, MP is fragmented, its surface is rougher and more porous [87], and the number of functional groups such as C–O and O–H is increased in comparison with pristine MPs, which results in a considerable enhancement in adsorption properties. It was observed that the adsorption capacity for Cd of MPs from the biological treatment line of WWTPs was one order of magnitude higher (up to about 2.5 mg/g MP) than that of pristine MPs [88]. As a result, MPs may serve as vectors for such metal pollutants as Cd, Pb, and Co, which are known inhibitors of methanogenesis.

Some effects of MPs on fermentation can be related to the degradation of plastics in anaerobic conditions and thus a release of organics that can be a substrate for methanogenesis. Studies on the anaerobic biodegradation of natural polyesters, such as poly(β-hydroxybutyrate-co-11.6%-β-hydroxyvalerate) (PHBV), poly(β-hydroxybutyrate) (PHB), and the synthetic polyester poly(ε-caprolactone) (PCL), indicated that PHB and PHBV films were efficiently degraded within 6–10 weeks, as concluded by weight loss and biogas formation, and that PCL was also degraded but at a slower rate than the two natural polyesters [89]. To improve the plastic biodegradability, conventional plastics can be spiked with various additives or bio-based plastics or natural fiber composites can be used. Gómez and Michel [90] reported that digestion for 50 days caused the 20–25% degradation of the bio-based materials, while, in the case of the additive-containing plastics, less than 2% of their mass was transformed to CH_4 and CO_2. Regarding bio-based plastics and natural fibers, only a PHA-based plastic was biodegraded within the time typically used for solid waste utilization. These results indicate that MPs present in WAS will affect anaerobic digestion.

5.2. The Effect of MPs and Its Additives on Anaerobic Digestion

One of the most important factors affecting anaerobic digestion is the size and concentration of MPs. Exposure to 80 nm and 5 μm PS MPs at concentrations of 0.2 g/L or less did not affect the cumulative CH_4 production. The inhibitory effects of nanoplastics on fermentation were more significant than those of MPs. Additionally, 80 nm and 5 μm PS MPs in concentrations above 0.25 g/L decreased CH_4 production by 19.3 and 17.9%, respectively. The negative effect of nanoplastics and MPs was mainly due to the inhibition of the acidification and methanation stages of anaerobic digestion [91]. An analysis of the effect of PS nanoplastics and MPs on the core microbiome and functional genes during WAS digestion revealed that nanoplastics suppressed acidogenesis by inhibiting acetate kinase activity and subsequently diminished CH_4 production [92]. MPs of 1 and 10 μm did not affect CH_4 production, while nanoplastics of 50 nm decreased CH_4 yield to 15.5%. The authors suggest that this decrease was due to the predomination of the genera *Candidatus* Methanofastidiosum, *Sulfurovum*, and *Methanobacterium* in the biomass. During the co-digestion of WAS and food waste, the

addition of polyethylene terephthalate (PET) MPs with particle sizes of 30 and 250 µm in amounts of 2.70 mg/g d.w. decreased CH_4 production by 21.63% (30 µm) and 15.87% (250 µm) compared to the control [93]. Short exposure to PE MPs at lower concentrations (i.e., 10, 30, and 60 particles/g d.w.) had no significant effect on CH_4 production, but concentrations of PE MPs of 100 and 200 particles/g d.w. reduced CH_4 production by 12.4–27.5% and lowered the CH_4 production potential and the efficiency of hydrolysis. The negative effect of PE MPs was probably due to the generation of ROS rather than the leaching of acetyl tri-n-butyl citrate from MPs. The generation of ROS reduced cell viability, which inhibited sludge hydrolysis, acidification, and methanogenesis [94]. For polycarbonate (PC) MPs, a dose-dependent effect on the anaerobic digestion of WAS was observed. PC MPs in concentrations from 10 to 60 particles/g d.w. increased methanation up to about 25% (at 30 particles/g d.w.), while 200 particles/g d.w. of PC MPs diminished CH_4 production by about 8% [42]. In the studies on anaerobic digestion in reactors with granular sludge, the addition of PET MPs at a low dose of 15 particles/L did not improve performance. The addition of 75–300 MP particles/L decreased COD removal efficiency and CH_4 yield by 17.4–30.4% and 17.2–28.4%, respectively. At the same time, an increase in the accumulation of VFAs of about 119–228% was observed. PET MPs at higher concentrations suppressed the formation of EPS and reduced the abundance of important acidogens (e.g., *Levilinea* sp.) and methanogens (e.g., *Methanosaeta* sp.) [95]. Dose-dependent effects on CH_4 production were also observed for PS MPs. The addition of 20–40 particles PS MP/g d.w. improved the CH_4 yield by 3.38–8.22%, while the addition of 80–160 particles/g d.w. resulted in a decrease of 4.78–11.04% [96].

Plastic additives (PAs) occur in the environment as a result of their release from plastics/MPs. PAs include plasticizers or lubricants (e.g., BPA) and non-intentionally added substances such as dodecanoic acid, α-terpineol, oleic acid, benzophenone, isodecyl diphenyl phosphate, or butylated hydroxytoluene [97,98]. PAs are usually not, or only weakly, chemically bound to polymers and are therefore easily released during the aging of MP [99]. Phthalates (PAEs) are commonly used as plasticizers in PVC, personal care products or food packaging, and are the most abundant in sludge. They are followed by phenolic stabilizers such as bisphenol analogs, synthetic phenolic antioxidants, and nonylphenols [74]. Microorganisms and their enzymes are the most promising solution for PA removal [100], although the effect of PAs on their metabolism may vary.

There is a growing number of studies on the potential impact of PAs released from MPs on anaerobic digestion showing that PAs can affect every phase of the process. During hydrolysis in sewage sludge reactors, organic material is degraded by extracellular hydrolases into simple soluble monomers or dimers. The effect of PAs on hydrolysis depends strongly on their dose and the operating conditions in the reactors. For example, BPA released from PVC at concentrations of 1.8 to 3.6 µg BPA/L inhibited sludge hydrolysis at a pH from 7.0 to 7.2 and consequently reduced both acidification and CH_4 production [39]. The presence of PVC MPs and their PAs led to a shift of the microbiome in the anaerobic digestion reactor and to the growth of microorganisms that did not support hydrolysis, acidification, and, finally, methanation. BPA leached from 200 particles/g d.w. of PC MPs (4.02 ± 0.15 mg/L) stimulated the production of ROS, resulting in reduced biomass viability and even apoptosis [42]. On the other hand, BPA in the range of 0–200 mg/kg d.w. had a positive effect on VFA production. Acetic acid yield increased from 563 in the control reactor to 1010 mg COD/L at 50 mg BPA/kg d.w. BPA leached from 30 particles/g d.w. of PC MPs (1.26 ± 0.18 mg/L) decreased intracellular ROS production, resulting in increased enzyme activity and biomass viability, and increased the abundance of *Methanobacterium* sp. and *Methanosarcina* sp. that increased CH_4 production [42]. A positive effect on anaerobic digestion was also reported for caprolactam (CPL). Chen et al. [101] observed that the leaching of CPL from polyamide 6 (PA6) MPs significantly increased CH_4 production during the fermentation of WAS. The presence of 10 particles of PA6/g d.w. increased CH_4 generation from about 124 to 173 L CH_4/kg d.w. The presence of PA6 promoted the destruction of volatile solids and increased CH_4 production potential, while, via increasing key enzyme activities, CPL increased acidification and methanogenesis.

The soluble polymers or monomers produced during hydrolysis are utilized by acidogenic bacteria and converted into H_2 and VFAs such as acetic, propionic, or butyric acid. The VFA production was increased by 4.9% in the presence of 10 particles PVC MP/g d.w., which corresponded to about 0.5 µg/L of leached BPA. However, it decreased from 6.9 to 16.8% when the dosage of PVC MPs was increased from 20 to 60 particles/g d.w. (related to 1.8–3.6 µg/L BPA). The reasons for the reduced production of VFAs were the decrease in the abundance of acidogens such as *Proteiniborus* sp. or *Garciella* sp. and the inhibition of acetate kinase [39]. The presence of 20–200 mg nonylphenol/kg d.w. during alkaline sludge digestion (pH 10) increased acetic acid production but did not affect the production of other VFAs [102]. The authors observed that the presence of nonylphenol increased the cell viability, growth rate, and acetate kinase activity in acetogen *Proteiniphilum acetatigenes*. The enhancement of nonylphenol biodegradation and VFA accumulation during WAS digestion can be achieved by pH control (pH 10) and the addition of sodium lauryl sulfate. Under these conditions, the biodegradation of nonylphenol increased by about 30% and the concentration of VFAs doubled as a result of the increase in the abundance of nonylphenol-degrading microorganisms and acidifying bacteria in the microbiome [103]. In the study on the effect of PET MP on the anaerobic co-digestion of WAS and food waste, PET MPs negatively affected the process through the leaching of toxic dibutyl phthalate (DBP) and diisobutyl phthalate (DIBP). DBP and DIBP reduced the abundance of the most important hydrolyzing bacteria (*Bacteroides vadin* HA17) and acidifying bacteria (*Clostridium* sp. and *Sphaerochaeta* sp.), reduced CH_4 production, and strongly affected daily CH_4 production in the presence of small-size MPs [93]. DBP leaching has also been cited as a major factor in the toxicity of PET MPs in anaerobic digesters containing granular sludge. The presence of DBP induced the formation of ROS and led to increased cell mortality and the release of lactate dehydrogenase [95].

During methanogenesis, the final step of anaerobic digestion, hydrogenotrophic and acetotrophic methanogens utilize the acidification products to produce CH_4. BPA (3.6 µg/L) leached from PVC MPs at a concentration of 60 particles/g d.w. inhibited CH_4 production by altering the microbiome structure. In the microbiome, the abundance of *Methanosaeta* sp. was significantly reduced by about 16.5%. As a result, the cumulative CH_4 production in BPA-contaminated sewage sludge was reduced by 21.5% compared to the control. Diethylhexyl phthalate (DEHP) is a plasticizer commonly used in industry. DEHP mainly affected sludge solubilization during anaerobic digestion and changed the content of soluble proteins and polysaccharides in the system. However, it also increased the CH_4 yield in the initial phase of fermentation due to an increased number of methanogens [104]. CPL released from 10 particles PA6/g d.w increased the activities of key enzymes involved in methanogenesis, resulting in almost 40% higher effective CH_4 production than in the control system [101].

The summary of the MP effect on AD is presented in Figure 1.

Some studies suggest that granular activated carbon (GAC) can be used to reduce the negative effects of nanoplastics/MPs during WAS digestion. In fermenters containing 150 µg/L PS nanoplastics, CH_4 yield decreased by 32.3% due to increased ROS levels. The addition of 5 and 15 g of activated carbon per liter of digester volume improved CH_4 production, probably due to the enrichment of microbes performing a direct interspecies transfer of electrons and the adsorption of PS nanoplastics by GAC [105].

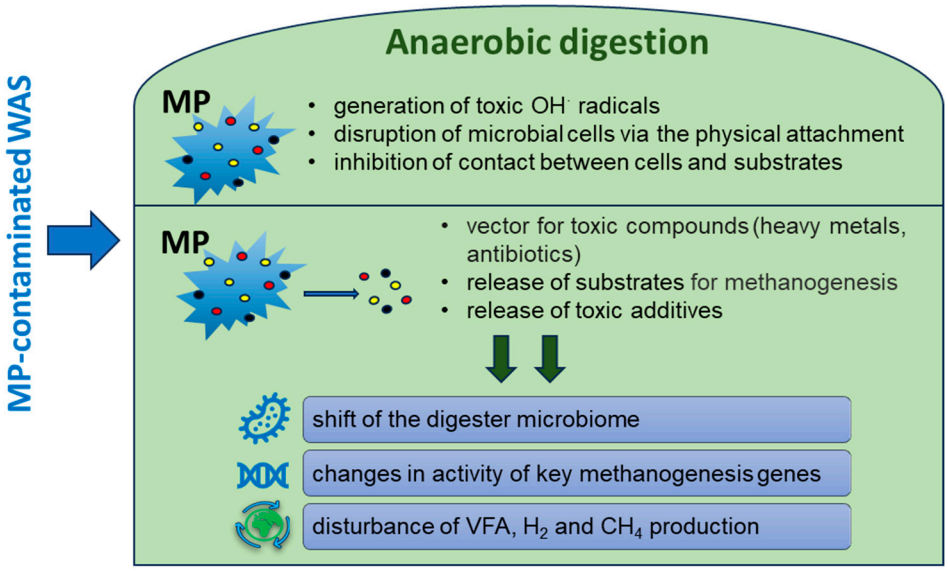

Figure 1. The mechanisms of the effects of MP on anaerobic digestion.

5.3. Mechanisms of MP Inhibition

Many researchers are focusing on the mechanisms of MP inhibition in anaerobic digestion in pure culture studies. The inhibition of methane fermentation by Pd-doped polystyrene nanoplastics (Pd-PS) was investigated during the short-term exposition of *Methanosarcina acetivorans* C2A. The Pd-PS limited methanogenesis; CH_4 production was reduced by over 14% at a concentration of 2.36×10^{10} Pd-PS particles/mL. Pd-PS nanoplastics interacted with proteins in EPS and inhibited the transcription of the genes, *mtaA* and *mcrA*, which encode the enzymes involved in CH_4 production [106]. A similar negative effect of nanoplastics on the growth and metabolism of carbohydrate-fermenting *Acetobacteroides hydrogenigenes* was also reported [107]. In studies on mesophilic digestion, the presence of 0.2 g nanoplastics/L in the reactor reduced the CH_4 yield and maximum daily CH_4 yield by 14.4 and 40.7%, respectively, compared to the control system. The presence of nanoplastics prolonged the start-up phase of the mixed anaerobic digestion system and altered the microbial structure by reducing the relative abundance of the taxa Cloacamonaceae, Porphyromonadaceae, Anaerolinaceae, and Gracilibacteraceae [107]. The presence of PS nanoplastics during anaerobic digestion decreased the abundance of genes coding enzymes responsible for carbon decomposition (e.g., *lig*, *xylA*) and genes related to the phosphorus cycle (e.g., *phnK*). The absolute abundance of *mcrA*, which encodes methyl-coenzyme M reductase, was also more than 50% lower in the biomass exposed to nanoplastics than in the control sample [92]. PS MPs, during anaerobic digestion, promoted sludge solubilization and hydrolysis but inhibited acidogenesis. The activities of key functional enzymes were stimulated at low PS MP concentrations, while they were almost completely inhibited by the cumulating oxidative stress induced by high concentrations of PS MPs. Low levels of PS MPs enhanced methanogenesis by acetoclastic and hydrogenotrophic microorganisms, while high levels of PS MPs promoted methanogenesis via the hydrogenotrophic pathway. PS MPs, after the digestion process, had greater toxicity than pristine PS due to its increased ability to adsorb pollutants [96].

5.4. H_2 Production

Kaykhaii et al. [108] reported that both the size and the number of MPs in the biomass significantly affect H_2 formation during fermentation. The authors observed that nanoplastics at the studied concentrations always suppressed H_2 production, while MPs can increase or decrease it. For example, the presence of 60 PET MP particles/L reduced H_2 production by 30%, while 200 PET MP particles/L provided 63.6% more H_2. High pH during WAS fermentation inhibits homoacetogenesis and methanogenesis and thus ensures effective

H_2 production. Wei et al. [109] reported that, under such conditions, H_2 production from WAS decreased; the yield of H_2 at a concentration of 60 particles PET/g d.w. was about 30% lower than in the control system. Despite the lower H_2 consumption under alkaline conditions (pH 10), PET MPs reduced hydrolysis, acidogenesis, and acetogenesis in alkaline anaerobic digestion, decreasing H_2 production. The presence of PET MPs in the fermenters reduced the number of microorganisms conducting hydrolysis and acidification. PET MPs leached the toxic DBP and stimulated the formation of ROS, leading to the death of cells and reduced production of H_2.

In biological processes, MP particles are incorporated into the biomass, changing its properties and thus influencing the efficiency of H_2 production. The study on the acute exposure–response relationship between several coexisting MPs and the anaerobic granules showed that the MPs covered the surface of the granule. The shock loading of MPs in the wastewater (up to 80 mg/L) caused the granules to become loose and even collapse as EPS production decreased (by over 16%). A leachate of 80 mg MP/L caused oxidative stress that increased cell death by up to 14.7% and decreased H_2 production by 18.7% [110].

MPs distinctively affect H_2-producing and CH_4-producing sludge systems. In the presence of PS MPs (50 μm) and nanoplastics (50 nm), the plastics inhibited gas accumulation; an exception to this was the increased H_2 production in the presence of PS MPs, which was due to enhanced sludge solubilization [111]. PS MPs showed no notable effects on the hydrolytic microorganisms but reduced the abundance of microorganisms associated with CH_4 production. The strong inhibition of H_2 production by PS nanoplastics was caused by the strong inhibition of hydrolysis, despite the enhanced solubilization. Different levels of inhibition of acidification and methanation jointly contributed to reduced CH4 accumulation in the presence of PS particles in the sludge.

The literature presents some solutions that appear to mitigate the negative effects of MPs on H_2 production. For example, Zhang et al. [112] studied the different stress responses of H_2-producing granular sludge (HPG) on PE, PET, and PVC MPs. In the presence of PE MPs, PET MPs, and PVC MPs, H_2 production decreased to about 82, 72, and 67% of the control values, respectively, due to the different toxicities of the leachates and oxidative stress caused by different MPs. The presence of MPs induced granule disintegration and deteriorated EPS production, but the addition of biochar mitigated these negative effects. The effective mitigation was the result of the strong adsorption of MPs on the biochar surface, which reduces the contact between microorganisms and MPs. The superior mitigation of HPG contaminated by PVC MPs resulted from the stronger affinity of biochar to PVC MPs and the effective reduction in toxicity of PVC leachate. The biological processes of sludge treatment, such as anaerobic digestion, can also be affected by the fact that the negative effects of the presence of MPs are enhanced by additional micropollutants such as antibiotics. For example, the co-occurrence of triclosan and PS, PVC, and PA in biological reactors led to an increase in EPS production, a reduction in microbial diversity, and a shift in microbial communities in the biomass [113].

5.5. The Effect of MPs on Sludge Disposal

The presence of MPs in the anaerobic digesters not only affects the efficiency of CH_4 production but also the quality of the digestate and thus sludge disposal. MPs reduce the flocculation capacity and dewaterability of sludge [79]. A study by Xu et al. [114] showed that MP particle size is more important for sludge dewatering capacity than the polymer type. MPs of about 4 mm reduced sludge dewaterability by about 30–48%, mostly as a result of the physical crushing of MPs on sludge flocs. Nanoplastics (213 nm), at a dose of 100 mg/L, also decreased sludge dewatering, but the mechanism was different. It was concluded that nanoplastics diminished sludge activity and the abundance of EPS-producers, which changed the spatial distribution and composition of EPS and worsened sludge dewatering. The presence of MPs during methane fermentation causes the digestate in the reactor with MPs to contain a higher concentration of organics and nutrients, pointing to the inhibition of CH_4 formation potential with incomplete digestion in the presence of

MPs [115]. This conclusion is supported by other studies. In a continuous test lasting over 130 days, the addition of 200 particles of PE MPs/g d.w. to WAS reduced the destruction of volatile solids by up to 27% at the end of the test and led to a 9.1% increase in the amount of digested sludge to be disposed of [94].

To summarize the effects of the above micropollutants on biogas production from sewage sludge, Table 1 shows some examples for each group of compounds which are the focus of this study.

Table 1. Summary of the effects of micropollutants on biogas production.

Micropollutant	Dose of Micropollutant	Effect of Micropollutant on Biogas Production	References
BPA	228 µg/kg	No effect	[16]
	3.6 µg/L	Decreasing CH_4 production by 24%	[39]
	1.26 mg/L	Increasing CH_4 production by 24.7%	[42]
	4.02 mg/L	Decreasing CH_4 production by 8.1%	[42]
	10–80 mg/L	Decreasing CH_4 production by 18%	[29]
	0.5–25 mg/L	Decreasing H_2 production by 9.2–75.3%	[49]
Pharmaceuticals	0.1 mg fluoxetine/kg d.w.	No effect	[19]
	2.0 mg fluoxetine/kg d.w.	Decreasing CH_4 production by 40%	[19]
	0–0.14 mM carbamazepine	Decreasing CH_4 production by 33%	[60]
	500 µg ibuprofen/L	Increasing biogas production by 61%	[62]
	500 µg ciprofloxacin/L	Decreasing biogas production by 52%	[62]
Engineered NPs	40 mg Ag-NPs/L	Increasing CH_4 production by 15%	[69]
	5–20 mg Fe-NPs/L	Increasing biogas production by 45% and methane production by 59%	[18]
	1 mg ZnO/g d.w.	No effect	[21]
	30 mg ZnO/g d.w.	Decreasing CH_4 production by 18.3%	[21]
	150 mg ZnO/g d.w.	Decreasing CH_4 production by 75.1%	[21]
MPs	0.2 g/L	No effect	[91]
	above 0.25 g/L	Decreasing CH_4 production by19.3 and 17.9%, depending on the particle size	[91]
	2.70 mg/g d.w.	Decreasing CH_4 production by 21.63 and 15.87%, depending on the particle size	[93]
	10, 30, and 60 particles/g d.w.	No effect	[94]
	100 and 200 particles/g d.w.	Decreasing CH_4 production by 12.4–27.5%	[94]
	10 to 60 particles/g d.w.	Increasing CH_4 production by up to 25%	[42]
	200 particles/g d.w.	Decreasing CH_4 production by 8%	[42]
	75–300 particles/L	Decreasing CH_4 production by 17.2–28.4%	[95]
	20–40 particles/g d.w.	Increasing CH_4 production by 3.38–8.22%	[96]
	80–160 particles/g d.w.	Decreasing CH_4 production by 4.78–11.04%	[96]
	150 µg/L	Decreasing CH_4 production by 32.3%	[105]

6. Conclusions

This review has highlighted the dose-dependent relationships between selected EDCs, pharmaceuticals, engineered NPs, and MPs and the processes of organic transformation under anaerobic conditions and has provided new insights into the effects of these pollutants on sewage sludge digestion. Importantly, it has shown that most studies have only investigated the effects of individual pollutants on anaerobic digestion, which can be very different from their effects in actual wastewater containing mixtures of these compounds. In addition, the comparison of different studies does not allow any general assumptions to be made about the effects of the reported compounds on sewage sludge digestion. Rather, the operating parameters, environmental conditions, and microbial communities can alter the effects that these compounds have on the digestion process. Therefore, to minimize the health and environmental risks connected with the reuse of digestate and make a significant contribution to the achievement of a circular economy, further research is needed on both

the fate of these emerging contaminants during anaerobic digestion and their potential effect on this process.

7. Future Directions

In the context of methane fermentation, micropollutants include a wide range of chemical compounds that can potentially interfere with microbial processes and affect the overall efficiency of the system. These substances often enter the fermentation process through the contamination of feedstock or as by-products of various industrial activities. Dealing with micropollutants requires not only the identification and quantification of these compounds but also the implementation of targeted strategies for their removal. Future directions in dealing with micropollutants in methane fermentation involve a multidimensional approach aimed at improving the efficiency and sustainability of the process. Researchers are developing advanced analytical techniques to detect and quantify trace pollutants and thus gain a more comprehensive understanding of their fate and impact on methane fermentation. An increasing emphasis should be placed on the development of innovative micropollutant removal technologies, such as bioaugmentation with specialized microbial strains that can degrade these contaminants. The integration of advanced treatment processes, such as activated carbon adsorption and membrane filtration, allows the selective removal of micropollutants from reject water from anaerobic digesters. In addition, the operating parameters, such as temperature, pH, and HRT, should be optimized to create conditions conducive to both efficient CH_4 production and micropollutant reduction. The presence of micropollutants in digestate poses a significant risk because, if contaminated digestate is applied to agricultural soils, there is a possibility that these micropollutants will enter crops and, subsequently, the food chain. Micropollutants can remain in the soil, seep into groundwater, or be transported off-site by runoff, which can have far-reaching ecological consequences. Therefore, comprehensive monitoring, treatment, and regulatory measures are essential to mitigate the potential risks associated with the agricultural use of micropollutant-contaminated digestates and to ensure the sustainable and safe implementation of anaerobic digestion in agricultural practice. Overall, a holistic and synergistic approach combining technological advances, process optimization, and microbial engineering paves the way for the more sustainable and environmentally friendly management of micropollutants in biogas plants.

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