Enhancement of Anaerobic Digestion with Nanomaterials

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The number of research reporting the addition of nanomaterials to enhance the process of anaerobic digestion has exponentially increased. The benefits of this addition can be observed from different aspects: an increase in biogas production, enrichment of methane in biogas, elimination of foaming problems, a more stable and robust operation, absence of inhibition problems, etc. Several hypotheses have been formulated, with the effect on the redox potential caused by nanoparticles probably being the most accepted, although supplementation with trace materials coming from nanomaterials and the changes in microbial populations have been also highlighted. The types of nanomaterials tested for the improvement of anaerobic digestion is very diverse, although metallic and, especially, iron-based nanoparticles, are the most frequently used.

Keywords: anaerobic digestion ; nanomaterials ; metallic nanoparticles

1. Introduction

Anaerobic digestion (AD) has become a worldwide strategy to obtain renewable energy from organic waste and by-products [1]. The principles of AD are well known, and this technology has been applied to a large number of organic wastes: food waste [2], sewage sludge [3], manure and slurry from farming facilities [4], etc. Briefly, a complex organic waste is composed of polymeric substances, such as proteins, fibers and fats, which are hydrolyzed into simple monomers, converted into volatile fatty acids and, finally, transformed into biogas, a gaseous mixture of methane (50 to 80%) and carbon dioxide (30 to 50%), with methane being produced in the last biological step involved in AD (i.e., methanogenesis) [5].

2. Nanomaterials Used in Anaerobic Digestion

Not intended as a full research, **Table 1** and **Table 2** show several representative examples of recently published research in which nanomaterials improved some aspect of AD, especially an increase in biogas or methane. These tables do not aim to be a complete compilation of works recently published on this topic, as the list would be impossible to present in a single research(a simple search of the Scopus® database for the terms “anaerobic digestion” and “nanomaterials” or “nanoparticles” reports more than 600 research).

Table 1. Examples of studies using inorganic metallic nanoparticles and their effect on anaerobic digestion.

Nanoparticle	Effect Observed	Operation Mode and Particularities	Reference
Cu and Fe oxides	Biogas and methane increase	Batch, iron and copper NPs were synthesized by hydrothermal treatment of corn straw	[6]
Co ferrate	Biogas and methane increase	Batch, NP addition enhanced H_2/CO_2 methanogenesis pathway. Excess NPs revealed negative effects	[7]
Cu and Fe oxides	Biogas and methane increase	Batch, review of microbial mechanisms	[8]
Fe zero-valent	Biogas increase and methane enrichment	Continuous, increase in the biodegradability of fibers due to the presence of NPs	[9]
Zn oxide	Biogas and methane increase	Batch, inhibition observed at high ZnO NPs concentrations	[10]
Fe zero-valent	Methane enrichment	Continuous, increase in the methane content of biogas, under both thermophilic and mesophilic conditions	[11]

Nanoparticle	Effect Observed	Operation Mode and Particularities	Reference
Ti and Fe oxides	**Biogas and methane increase**	**Semicontinuous, NPs and salts boosted methane production for lignocellulosic materials**	[12]
Fe zero-valent	**Methane enrichment**	**Semicontinuous, the increase in the oxidation state of NPs seemed to be related to the loss of effect over time**	[13]
Ti oxide	**Biogas increase and fast hydrolysis and acidogenesis**	**Batch, hydrolysis and acidogenesis rates have been enhanced due to the addition of NPs**	[14]
Fe oxide	**Biogas increase and inhibition of H_2S production**	**Batch, high rate of H_2S production decrease (from 50 to 80%)**	[15]
Fe zero-valent	**Methane increase and better stability**	**Batch, NPs promoted the acidogenesis–acetogenesis without acidification**	[16]

Table 2. Examples of studies using other nanomaterials and their effect on anaerobic digestion.

Nanoparticle	Effect Observed	Operation Mode	Reference
Graphene oxide	**Biogas and methane increase**	**Batch, cumulative methane yield was highly dependent on the dosage**	[17]
Nano-biochar	**Biogas and methane increase**	**Batch, review focused mainly on biochar**	[18]
Graphene	**Biogas and methane increase**	**Batch, low temperature did not affect archaeal community compositions with graphene and methane increase**	[19]
Carbon nanotubes	**Biogas and methane increase; mitigation of ammonia inhibition**	**Batch, carbon nanotubes may mitigate or worsen the ammonia inhibition depending on the total ammonia nitrogen**	[20]
Graphite, graphene and graphene oxide	**Biogas and methane increase**	**Batch, graphene exhibited the best performance by removing some antibiotic resistance genes**	[21]
Graphene	**Biogas and methane increase**	**Batch, direct interspecies electron transfer (DIET) via graphene was established**	[22]

The distribution of the tables was performed according to the materials used: **Table 1** compiles the works that used inorganic metallic NPs, whereas **Table 2** shows the works carried out with other nanomaterials.

As observed in **Table 1** and **Table 2**, as well as in most of the references consulted, several conclusions can be stated regarding the use of different types of nanomaterials in anaerobic digestion:

(a) Among all the nanomaterials used, inorganic metallic NPs were, by far, the most used to enhance the process of anaerobic digestion. In the case of C-based NPs, practically all the works used graphene or graphene oxide;

(b) Among all the inorganic metallic NPs used, those based on iron, zero-valent and oxide forms were the most frequently used, with specific research on this point [23][24];

(c) There existed a significant number of studies related to the use of a combination of metallic NPs, reporting better results than that of a single type of NP (**Table 1**). Obviously, this needs a careful economic assessment, which was not often presented;

(d) A few number of works used metallic NPs covered with a kind of coating, with the main objective being preventing the oxidation of the NPs [25][26];

(e) Another very small number of publications reported a negative effect of NPs on the process of anaerobic digestion [27][28]. Usually, the inhibition provoked by NPs was only observed at high dosages;

(f) There is a concerning lack of information regarding the characteristics of digested materials (both liquid fraction and solid fraction after dehydration). It is clear that some NPs can be negative for a material that is supposed to be applied to soil as an organic amendment.

3. Results According to the Operation Mode

3.1. Batch Mode

As observed in **Table 1** and **Table 2** and reviewing the recent literature, most of the results obtained at this moment of research were still carried out under batch mode conditions. This implies that some results could not be completely reproducible in the semicontinuous or full continuous mode, which are the ways full-scale digesters work. However, some researchers point out that batch experiments, conducted as typical biochemical methane potential (BMP) tests, can be a first approach to the effects of some additives or in anaerobic co-digestion assays [29], especially to obtain kinetic data and methane production. Other research related to the scale-up effects point to several negative effects when comparing batch results and those obtained at higher scales, derived from difficulties in mixing and homogenization, which result in lower methane yields as well as the net electricity produced (20–30% decrease) [30]. Thus, it is evident that batch tests present several limitations. In addition to the above, acclimation to inhibition cannot be determined, a well-known effect observed in the continuous mode for a wide variety of inhibitors (ammonia, volatile fatty acids, long-chain fatty acids, metals, etc.). In addition, it is evident that batch tests can only simulate mixed reactors [31] but not more complex anaerobic reactors [32]. This is the reason why most recent anaerobic digestion studies in which methane yields are used to reproduce full-scale reactors are performed in a continuous mode of operation [11][33][34]. Other possibilities of operation (for instance, semi-batch or fed-batch) are rarely used in pilot of full-scale anaerobic digesters.

3.2. Continuous Operation

For the reasons explained in the previous point, the mini-review focused on recent continuous (often semicontinuous) studies on the effect of nanomaterials in anaerobic digestion. This can be considered an emerging trend in this topic, and it is obviously the previous step to promote full-scale anaerobic digestion operations with NPs. In this case, the number of studies published is small. Moreover, in some cases, the reader must be careful, as some research are not strictly related to nanomaterials as they are defined: sizes between approximately 1 and 100 nanometers [35]. In this case, they must be considered as additives, which is a more conventional topic studied in anaerobic digestion [36].

According to the studies published, the continuous anaerobic digestion process at the pilot scale is often performed under semicontinuous operation, that is, intermittent feeding. Regarding NP feeding, several methods have been reported. For instance, Cerrillo et al. (2021) used a classical semicontinuous method with weekly additions of a zero-valent iron NP pulse in the mesophilic and thermophilic anaerobic digestion of pig slurry [11]. The researchers reported an increase in the content of methane in the biogas in the thermophilic reactor from 64% to a maximum value of 87%. Approximately, the same values were obtained in the mesophilic reactor. The researchers justified this increase by the highest specific methanogenic activity detected with NPs. One important observation is that batch experiments at mesophilic temperature showed an inhibition of methane production at all tested NP dosages (i.e., 42, 84, 168 and 254 mg g^{-1} VSS concentrations), while methane production was boosted with the lowest dosage in thermophilic conditions. This highlights an important fact that has been observed in other works: the results of batch experiments cannot be directly extrapolated to continuous experiments, given the typical phenomenon of the acclimation of anaerobic microorganisms to inhibition conditions [11]. In another work treating wastewater sludge (a mixture of primary and secondary sludge), Barrena et al. (2021) tested the sustained effect of zero-valent iron NPs in the process of anaerobic digestion [13]. The researchers observed some interesting effects. Similar to [11], punctual doses every 5–7 days sustained positive effects with higher methane content. However, NP oxidation was observed by TEM-EELS (transmission electron microscope-electron energy-loss spectroscopy) analysis, which implies the loss of the effect on methane increase over time. The researchers proposed a strategy based on using the magnetic retention of NPs to partially overcome this problem and to reduce the use of NPs, with positive results. When retaining or reusing NPs, it is very important to understand the role of the oxidation state on the enhancement of the anaerobic digestion process. These abovementioned studies [11][13] are of special interest, since they studied the microbial consortium with a marked increase in the relative abundance of members assigned to the *Methanothrix* genus, recognized as an acetoclastic species showing high affinity for acetate, which explains the rise of methane content in the biogas. Other recent works support these findings. For example, Juntupally et al. (2022), when adding iron oxide NPs into the anaerobic digestion of food waste at mesophilic and thermophilic temperatures, observed that the methane content increased from 60% to 74% at 35 °C and 62 to 78% at 55 °C at a dose of 4 g/L of NPs [37]. Again, a syntrophic balance between the bacterial groups (i.e., *Firmicutes, Bacteroidetes, Chloroflexi* and *Thermotogae*) and archaeal groups (i.e., *Methanosarcina, Methanothrix and Methanosaeta*) was observed. Moreover, Dong et al. (2022) also observed that based on a detailed microbial community analysis, biomethanation by using zero-valent iron and zero-valent iron NPs depended on hydrogenotrophic methanogenesis in the anaerobic biomethanation of carbon dioxide [38].

Other works focused on the changes in metabolism when using iron-based NPs. For instance, Zang et al. (2020) attributed the increase in methane production to the consumption of extracellular polymeric substances (EPS) when using iron oxide NPs in the anaerobic digestion of waste sludge that resulted in a considerable decrease in organic matter [39].

Recently, research has been published on the use of NPs to enhance anaerobic digestion. These novel research also observed an increase in methane production, but in addition, they found specific phenomena that are worthy of comment, especially as they were studied in semicontinuous processes, that is, close to realistic AD conditions. One point that was recently observed is the use of genetics. On the one hand, several researchers have used genetic studies (16S rRNA gene sequencing) to confirm that the percentage of hydrogen-utilizing methanogens (*Methanolinea*) was up to 62.6% of total archaeal sequences when using magnetite NPs [40]. One the other hand, other researchers have used genetic techniques to conclude that macrolide, aminoglycoside, and beta-lactam resistance genes are less abundant in the presence of magnetite NPs, which is a new relevant point, as it confirms that the presence of NPs in AD processes is beneficial for the removal of some antibiotic-resistant genes [41]. Another clear field of research is the use of advanced configurations of bioreactors commonly used in AD processes with the addition of NPs. This is the case for UASB (upflow anaerobic sludge blanket) reactors, where zinc oxide nanoparticles immobilized by methylenebisacrylamide were used [42]. In this case, biomass retention capacity was observed to improve carbon dioxide sequestration and to increase methane production using oil palm wastewater. Another later research on granular sludge reported a magnetite nanoparticles-modified *Aspergillus tubingensis* mycelium pellet-based anaerobic granular sludge for AD food waste treatment. In this case, NPs stimulated extracellular polymeric substances (EPS), which protected the microbes from high osmotic pressure, resulting in higher methane yields than activated flocculent sludge [43]. Other research go a step further and report the presence of magnetite NPs in digestate when used as fertilizer for lettuce crops, which also presented a higher presence of NPs in lettuce biomass (21.0–1,920%). This showed that the effects of the NPs remaining in the AD effluent must be considered in future works, an issue that is not treated in the scientific literature [44]. Probably, as this is at an emerging point with new publications appearing each week, new effects of NPs on AD processes will be discovered and become a topic of novel research studies, especially in the use of advanced microbiological techniques and in the development of new AD reactor configurations.

As expected, most of these continuous works were carried out using iron-based NPs, except from a study with silver NPs [45], with the objective to determine the toxicity of this biocide material, which was not observed (even methane production was enhanced), and a study related to the recovery of tellurium NPs by the continuous reduction of tellurite using an UASB reactor [46]. It is evident that the small number of studies related to semicontinuous processes were focused on technical issues, and it is expected that in the short-term future, other aspects will be studied.

3.3. Dosage and Dosing Strategy

Dosage is an important issue in all environmental applications of nanomaterials. In fact, one crucial particularity of these materials is a very high surface/volume ratio, in comparison with non-nanomaterials, which provide enhanced properties in terms of adsorption, catalytic activity, etc. [47]. In consequence, it is expected that the number of nanoparticles to enhance anaerobic digestion is lower than those of other typical additives used in this technology. Thus, it is reported that a significant amount of biochar can retrieve 89% of the ultimate biomethane potential [48], although other researchers point out that the cost of biochar does not compensate for the extra production of methane [49]. A similar situation occurs when using iron for biogas desulfuration, where stoichiometric dosages must be used, although biochar can also have a significant role [50]. In the case of nanoparticles, stoichiometry is not relevant and, consequently, dosages are lower [11][13].

Nevertheless, and considering that the normal mode of operation in full-scale anaerobic digesters is a continuous or semicontinuous substrate feeding, there is some uncertainty on how to feed nanomaterials. In this case, only pilot-scale systems are available in the literature, and the typical strategy is a semicontinuous dosing of NPs, which can be coupled with the substrate addition, although uncoupling between the substrate and NP addition has also been reported (for instance, substrate on a daily basis and NPs every two or three days, or even weekly) [11][13]. It is evident that with the proliferation of continuous studies, the strategy of nanomaterial feeding will play a key role in the enhancement of methane production.

These two critical points are also very important in the life cycle assessment of the overall strategy when using a product such as nanomaterials for the improvement of processes such as anaerobic digestion, which is a technology for obtaining renewable energy. It is clear that a complete sustainability analysis (from environmental and economic perspectives) is necessary. Unfortunately, only very recently have some general reports regarding the sustainable design of engineered nanomaterials and the future prospects of the life cycle assessment of nanomaterials been published [51][52].

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