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**Application of nano-structured materials in anaerobic digestion: Current status
and perspectives**

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Abstract

Nanotechnology is gaining more attention in biotechnological applications as a research area with a huge potential. Nanoparticles (NPs) can influence the rate of anaerobic digestion (AD) as the nano-sized structures, with specific physicochemical properties, interact with substrate and microorganisms. The present work has classified the various types of additives used to improve the AD processes. Nanomaterials as new additives in AD process are classified into four categories: Zero-valent metallic NPs, Metal oxide NPs, Carbon based nanomaterials, and Multi- compound NPs. In the following, application of nanomaterials in AD process is reviewed and negative and positive effects of these materials on the AD process and subsequently biogas production rate are discussed. This study confirms that design and development of new nano-sized compounds can improve the performances of the AD processes.

Keywords

Anaerobic digestion; Nanomaterials; Additives; Biogas production; Trace metals

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1. Introduction

Anaerobic digestion (AD) of organic waste streams has been a fast-growing green technology along with the increased environmental awareness to overcome energy and environmental challenges (Zidanšek et al., 2009). Clean fuel (biogas) is produced by the AD of different groups of organic wastes, i.e. animal manures, agriculture waste, forestry waste, sludge, food processing waste, and organic household waste (Angelidaki et al., 2003; Pieta et al., 2018). Four major steps are involved in complex AD process, i.e. hydrolysis, acidogenesis (primary fermentation), acetogenesis (secondary fermentation), and methanogenesis (Deublein and Steinhauser, 2011). Performance and efficiency of the AD process is highly dependent on various microorganisms involved in the digestion steps and also the type of substrate (Siddique and Wahid, 2018). Biogas production rate could be enhanced by accelerating the slowest step known as the rate limiting step. The rate limiting step depends on the substrate type and its concentration, reactor temperature, configuration of the reactor and the organic loading rates. However, there is no fixed rate limiting step in all AD processes (Ganzoury and Allam, 2015). Many researchers reported that hydrolysis or solubilisation can be considered as the rate-limiting step in the AD of complex organic substrate, i.e. lignocellulosic substrate (Fernandes et al., 2009; Lynd et al., 2002; Rafique et al., 2010). On the other hand, in case of easily biodegradable substrates methanogenesis can be considered as the rate limiting step, since the methanogenic archaea have relatively slower growth rate compared microorganisms performing previous steps of the AD process (Lu et al., 2008). It is necessary to increase the overall reaction rate by accelerating the rate limiting steps.

In order to enhance the biogas production and assuring stability of the AD process,

different strategies have been implemented. These methods include co-digestion with dissimilar substrates (to overcome obstacles as non-optimal C:N ratio or limited nutrients availability) (Luo and Angelidaki, 2013; Siddique and Wahid, 2018), pre-treatments of recalcitrant substrate (to release the bio-degradable organic matter for microbial attack) (Dahunsi et al., 2017; Tsapekos et al., 2018b), modifying reactor configuration (Li et al., 2017), adjusting operation conditions (Leitão et al., 2006), and addition of organic/inorganic additives (to stimulate microbial activity) (Nzila, 2017; M S Romero-Güiza et al., 2016). Additives used to enhance the biogas production in AD processes can be classified into organic and inorganic additives. Organic additives include green biomass, microbial cultures, and enzymes (Angelidaki et al., 2018; M. S. Romero-Güiza et al., 2016). Inorganic additives can be sub-divided into macro-nutrients, micro-nutrients, and carbonous materials (Dang et al., 2016). The macro-nutrients, i.e. phosphorus (P), nitrogen (N), sulfur (S) are added to feedstock of AD process in the form of salts to improve the system buffer capacity. Micro-nutrients, i.e. nickel (Ni), cobalt (Co), iron (Fe), molybdenum (Mo), etc., are crucial constituents of co-factors and enzymes in numerous biological reactions involved in AD and their addition to anaerobic bioreactors has been proven to stimulate the performance of biogas production reactors (Demirel and Scherer, 2011; Gustavsson et al., 2013). These trace elements can be added to AD process in the form of salts, pure metal/metal oxides (bulk material), and recently in the form of nano-structured materials. Significant research efforts have been focused on adding carbonous materials, as conductive non-biological materials, to anaerobic digesters. Results from these studies revealed that carbonous materials (such as granular activated carbon, biochar, graphite, carbon-based nano materials) can act as an electron conduit for direct interspecies electron transfer

(DIET) between syntrophic microorganisms and enhance the biomethane yield and rate (Barua and Dhar, 2017; Martins et al., 2018; Salvador et al., 2017). The additives used in AD processes can be summarized as shown in Fig. 1. Researchers have used the inorganic additives in the form of nano-structured materials to study the catalytic effect of nanomaterials on the AD process. Furthermore, nanomaterials have been used in anaerobic treatment processes to enhance the microbial degradation of recalcitrant pollutants, such as halogenated pollutants, phenolic compounds, aromatic compounds, etc. (Cervantes et al., 2016; Lei Li et al., 2016; Pat-Espadas et al., 2016).

Figure 1. Overall classification of additives used in AD processes (*CNT: carbon nanotube)

Nano-sized materials (1-100 nm) as additive could have negative or positive effect on AD processes. Due to the unique thermal, mechanical, structural properties and high specific area of nano-scale materials, nanotechnology has become a growing technology in environmental, medical, and chemical industries (Ünşar and Perendeci, 2018). These unique properties (that are attributed to very small size) of nanomaterials motivate researchers to investigate the effects of these materials on diverse biological

113 applications such as AD process to develop the green energy production.

114 This review presents an overview of the recent attempts in the application of
115 nanomaterials in the AD due to their capacity to improve the performance of this process.

117 **2. Application of nanomaterials in anaerobic digestion**

118 Nanomaterials, with any external dimension in the size range from approximately
119 1-100 nm, have extensively been used in many applications including energy,
120 agriculture, coating, catalysts, automotive, sensors, food products, and environmental
121 processes and researches are continued to find new applications (Keller et al., 2013;
122 Zhou et al., 2011). Nanomaterials have excellent physico-chemical properties (i.e. size,
123 specific surface area, surface structure, solubility, catalytic properties) which make them
124 to be useful in wide applications (Hussein, 2015). As particle size decreases, the
125 surface-to-volume ratio increases. The properties of nanoscale material can be totally
126 different from their corresponding properties in either bulk form or as part of larger
127 scale entities (Krol et al., 2012). Nanomaterials possess promoted mobility in
128 environmental media , so they have high potential to be as a new functional material and
129 device with unique activity for biological systems (Abdelsalam et al., 2016; Lu et al.,
130 2016). Nanomaterials have an important property in interaction with biological systems
131 since they are able to penetrate cell membranes, thus facilitating the interaction with the
132 immune system (Huangfu et al., 2018; Luna-delRisco et al., 2011).The positive effects
133 of nanomaterials are achieved by specific characteristics as high surface area, high
134 reactivity, high specificity (Abdelsalam et al., 2016). It has been proved that
135 supplementation of micro-nutrients in the form of nanoparticles improved the AD
136 performance (Casals et al., 2014). The slow dissolve of NPs facilitates the

bioavailability in the AD media and enhances the enzymatic activity (E. Abdelsalam et al., 2017). On the other hand, enzymatic adsorption onto NPs will cause conformational transitions, accompanied by the inhibition or enhancement of enzyme activity depending on concentration (Chen et al., 2017). All nanomaterials used in AD processes can be categorized in four groups, i.e. zero valent metallic NPs, metal oxide NPs, carbonous nanomaterials, and multi- compound NPs (Fig. 2). A gap of knowledge in the design and application of multi-compound NPs with predetermined properties exists. In the following, the applications of various types of NPs are reviewed based on the mentioned classification.

Figure 2. Classification of nanomaterials and amount (%) of their usage in AD processes

2.1. Zero valent metallic nanoparticles

Nanoscale zero valent metals (NZVMs), i.e. Fe, Ni, Cu, Co, Ag, Au, etc., with significant strong chemical reducibility, high efficiency, and large specific surface have the most promising applications in environmental processes compared to zero valent metals (ZVMs) (Lingyun Li et al., 2016). In fact, the percentage of atoms located at the surface of particle increases with the decrease in particle size regarding the mass of

particle, which can raise the tendency to adsorb, interact, and react with other atoms, molecules, and complexes (Crane and Scott, 2012). Table 1 summarizes the efforts of zero valent NPs reported in the literature. As mentioned before, the AD is carried out by different groups of microorganisms. The flow of electrons within these communities governs the occurrence of reactions and their rates (Cheng and Call, 2016). Interaction between acetogenesis and methanogenesis, mediated by interspecies electron transfer between syntrophic bacteria and methanogenic archaea plays a vital role in improving the AD efficiency (Stams and Plugge, 2009). Mediated interspecies electron transfer (MIET) based on the exchange of metabolites (i.e. H₂, formate) between two microorganisms is one of the earliest discovered mechanisms of external electron transfer (Storck et al., 2016). In MIET, the electron-donating microorganism reduces protons to hydrogen and the electron-accepting microorganism oxidizes the hydrogen with the reduction of an electron acceptor (Sieber et al., 2012). MIET is feasible only at low enough concentrations of H₂/formate metabolites due to the thermodynamic constraints (Cruz Viggi et al., 2014). Intrinsically, MIET via diffusive carriers (i.e. H₂ and formate) is a slow mechanism due to slow diffusion rate of metabolites in the reaction media, (Kato et al., 2012). Recently, researchers showed that DIET via electrically conductive materials, i.e. nano zero valent iron (NZVI) and carbonous materials, energetically have more advantage over MIET, because DIET does not require the multiple enzymatic steps to produce hydrogen as an electron carrier (Zhao et al., 2015). Addition of electrically conductive materials, to anaerobic digesters is an engineered approach to stimulate DIET and subsequently promote biogas production.

Table 1. Reported applications of the nano zero valence metal NPs in anaerobic digestion processes

NPs	Mean Size, nm	Concentration of NPs	Substrate type	Temperature (°C)	Effect on AD process	Ref.
Ni	17 nm	2 mg/L	Manure slurry	37 °C, batch	Decrease lag phase, 101% increase in biogas production, 74% increase in methane production	(Abdelsalam et al., 2016; E. Abdelsalam et al., 2017)
	100 nm	5-10 mg/KgVS	Sewage sludge	37 °C, batch and CSTR	increased methane yield up to 10%	(Tsapekos et al., 2018a)
Cu	40-60 nm	10-1500 mg/L	Granular sludge	30 °C, batch	Highly inhibitory to acetoclastic and hydrogenotrophic methanogens with IC ₅₀ values of 62 and 68 mg/L	(Gonzalez-Estrella et al., 2013)
Co	28 nm	1 mg/L	Manure slurry	37 °C, batch	Decrease lag phase, 64% increase in biogas production, 86% increase in methane production	(Abdelsalam et al., 2016; E. Abdelsalam et al., 2017)
NZVI	128 nm	10 mg/g TSS	Waste activated sludge	35 °C, batch	Increase 120% of methane production	(Wang et al., 2016)
	9 nm	5, 10, 20 mg/L	Raw manure	37 °C, batch	45% increase in biogas production,	(E Abdelsalam et al., 2017)

					59% increase in methane production	
	160 nm	100 mg/L	Sewage sludge	37 °C, batch	25.2 % increase of methane production, 10 % increase in COD removal	(Suanon et al., 2017)
	20 nm	10 mg/L	Sewage sludge	37 °C, batch	30.4% increase in biogas production, 40.4% increase in methane production	(Su et al., 2013)
	45 nm	1000 mg/L	Domestic sludge	37 °C, batch	105.46% increase in biogas production	(Amen et al., 2017)
	55 nm	56 , 560, and 1680 mg/L	Digested sludge	37 °C, batch	20% decrease in methane production	(Yang et al., 2013)
	46-60 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)
Ag	170 nm	500 mg/g TSS	Waste activated sludge	35 °C, batch	Decrease 27% of methane production	(Casals et al., 2014)
	30 nm	130 mg/L	Cellulose	37°C, 55°C, batch	Toxicity effect, decrease 33-50% biogas production	(García et al., 2012)
	10-15 nm* cationic	0.5, 1, 5 and 100 mg/L	Biosolid from wastewater	37 °C, batch	Up to complete inhibition of the anaerobic biogas	(Gitipour et al., 2016)

	silver NPs		treatment plant		production	
	20-40 nm	5-1000 mg/gTS	municipal waste activated sludge	37 °C, batch	12.1% decrease in the methane production at the highest dosage	(Ünşar et al., 2016)
	<100 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez- Estrella et al., 2013)
Au	20 nm	75 mg/L	Cellulose	37°C, 55°C, batch	Zero or slight toxicity effect on ordinary heterotrophic organisms, ammonia- oxidizing bacteria, and anaerobic bacteria	(García et al., 2012)

NZVI has been widely investigated as a nanoscale zero valent metallic additive in the field of biogas production. Suanon et al. (2017) showed that the application of 100 mg/L NZVI (160 nm) in batch bio-digesters enhanced the methane production from sewage sludge up to 25.2% . The results were in accordance with previous findings reported in elsewhere (Su et al., 2013; Xu et al., 2004). Su et al. (2013) reported the promotion of biogas and methane production up to 30.4% and 40.4%, respectively, by addition of 0.1 wt% NZVI (10 mg/L) to the AD process of sewage sludge during 17 days of batch experiment.

The effects of 9 nm NZVI NPs on the methanogenic archaea during the AD of raw

manure (feces and urine) in a batch mode were also investigated by Abdelsalam et al. (2017). They reported that addition of 20 mg/L of NZVI NPs to bio-digester under mesophilic temperature stimulates the methanogenic archaea and increases its activity and subsequently increasing biogas and methane production up to 45 and 59%, respectively.

On the contrary, Yang et al. (2013) showed that NZVI NPs (55 nm) with a concentration of 1 mM (56 mg/L) inhibited methanogenesis more than 20% during the AD of sludge. They reported that the inhibition was due to the disruption of cell integrity in exposure to NZVI NPs. In addition, rapid H₂ production was observed in the presence of NZVI NPs under anaerobic conditions ($\text{Fe} + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^-$) as reported by Liu et al. (2006). The produced H₂ takes part in bacterially controlled hydrogenotrophic process (i.e., $2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}$) in AD. Addition of 30 mM (1680 mg/L) of NZVI to bio-digester during AD process caused a significant increase of soluble COD (due to microorganism cell disruption) and volatile fatty acids in the bioreactor medium, resulting in 69% decrease of methane production.

Abelsalam et al. (2016) also declared that introducing 1 mg/L Co NPs (28 nm), 2 mg/L Ni NPs (17 nm) or 20 mg/L NZVI (9 nm) to the AD process of raw manure increase the biogas yield by 50%, 70%, and 80% compared to controlled condition, respectively. The yield of methane increased significantly by 67%, 117%, and 100% more than the methane yield of the controlled condition, respectively. Ni, Co, and NZVI NPs applied in this study improved various biological processes involved in biogas and methane production. This observation is in accordance with the results obtained by Gustavsson et al. (2013) who stated that Ni and Co concentrations in digester substrates, organic matters, promote biogas production by increasing methane production and

making the AD process stable.

Wang et al. (2016) investigated the effects of NZVI (128 nm) and zero valent Ag (170 nm) NPs on methane production during the AD of waste activated sludge under mesophilic conditions. Based on their results, introducing 10 mg/g total suspended solid (TSS) NZVI to the digester increased the methane production more than 120%, while introducing 500 mg/g TSS zero valent Ag NPs produced 26.48% lower amounts of methane. The results indicated that the number of microorganisms and activity of key enzymes, such as protease, acetate kinase and coenzyme F₄₂₀, are boosted by adding low concentration of NZVI NPs, but high concentration of zero valent Ag NPs inhibits the microbial growth and activity. Microbial analysis showed that the population of active bacteria and archaea (i.e. α -proteobacteria, β -Proteobacteria, bacteroidetes and methanosaeta) is increased by introducing 10 mg/g of TSS NZVI.

Abdelsalam et al. (2017) studied the effect of Ni and Co NPs (0.5, 1, and 2 mg/L) on the AD of manure at mesophilic conditions. They showed that the addition of the examined NPs led to reduction of lag phase and increase of biogas and methane production rates. The results revealed that Ni and Co NPs have strong biostimulating effects on the methanogenic activity. The cumulative amount of biogas and methane production were directly proportional to the concentration of Ni NPs up to 2 mg/L. the results confirmed that the addition of 0.5, 1 and 2 mg/L Ni NPs and 1 mg/L NiCl₂ to the substrate increased the biogas production by 46, 72, 74 and 44%, respectively. These results are in agreement with those obtained by Demirel and Scherer (2011) who stated that the stimulatory concentrations of Ni for batch cultures of methanogens were in the range of 0.012 to 5 mg/L. Introducing 0.5 mg/L Co, 1 mg/L of Co NPs, and 1 mg/L of CoCl₂ increased the biogas production up to 36, 64, and 32% higher than the volume of

biogas produced under controlled condition, respectively. Enhancement of biogas and methane production using 1 mg/L Co NPs was in accordance with the results obtained by Demirel and Scherer (2011) and Feng et al. (2010) who reported that Co is an important metal for methanogenesis as it acts as metallic enzyme activator. On the other hand, the addition of Co NPs with concentration more than 1 mg/L, i.e. 2 mg/L, showed a toxicity effect on the methanogenic archaea which decreased 5% of biogas production and 12.7% of methane, compared to the controlled assays, during 50 days of AD process. The undesired effect of 2 mg/L Co NPs on biogas and methane production are in agreement with those reported by Facchin et al. (2013) declaring that the supplementation of excess metals could have a negative effect on acetoclastic methanogenesis or on other metabolic pathways of an AD process.

Tsapekos et al. (2018a) investigated the effect of Ni NPs (<100 nm) on anaerobic digestion of sewage sludge in batch and continuous mode at thermophilic condition. Results showed that 5 mgNi-NPs/kgVS in the presence of nitrilotriacetic acid (NTA) as chelating agent enhanced the methane yield up to 10% compared to the control sample in batch mode. The Ni NPs also boosted the methane yield of continuous digester up to 8%.

García et al. (2012) investigated the effects of Ag (30 nm) and Au (20 nm) NPs on activity of key microorganisms such as ordinary heterotrophic organisms, ammonia oxidizing bacteria, and other anaerobic bacteria presented in municipal wastewater. Based on their results, Ag NPs had a toxicity effect and inhibited the biogas production by 33-50%, whereas Au NPs showed zero or slight inhibition for all kind of tested substrates.

Gitipour et al. (2016) stabilized Ag NPs with different capping agents at

concentrations of 0.5, 1, 5, and 100 mg/L and used them for AD of biosolids from a WWTP. Based on their results, negatively charged citrate-coated NPs (citrate-Ag NPs) and minimally charged polyvinylpyrrolidone coated Ag NPs (PVP-Ag NPs) showed no toxicity and inhibitory effects on biogas production at any concentration. In the case of citrate-Ag NPs, the lack of toxicity might be due to aggregation and subsequent settling which can lower the bioavailability of NPs for the microbiome. The minimum surface charge on PVP-Ag NPs may have resulted in lower attraction between the NPs and the negatively charged bacterial membrane, exhibiting low toxicity. Relatively high concentration (i.e. 100 mg/L) of positively charged branched polyethyleneimine coated Ag NPs (BPEI-Ag NPs) demonstrates elevated toxicity, similar in magnitude to that of Ag⁺ ion, in comparison to PVP-Ag NPs and the anionic citrate-Ag NPs.

Ünşar et al. (2016) also investigated short-term, i.e. 72h, and long-term, i.e. 48 days, inhibition effects of 20-40 nm Ag NPs on the AD of waste activated sludge (WAS). They observed that Ag did not cause significant impacts on AD process during short-term exposure of NPs. In the long-term test, the introduction of Ag NPs at the highest dosage (i.e. 500 mg/gTS) caused 12.1% decrease in the methane production. Slow and long term silver release from Ag NPs dissolution cause inhibition of methane production after. Release of silver ions after long-term AD creates imposes antibacterial effects by making defects in cell membranes or causing enzyme inactivation or protein dephosphorylation (Prabhu and Poulouse, 2012).

Inhibitory effects of Ag, Cu and Fe NPs on acetoclastic and hydrogenotrophic methanogenic activities of anaerobic granular sludge were investigated by Gonzalez-Estrella et al. (2013). Their results revealed that 1500 mg/L of Cu NPs (40-60 nm) was highly inhibitory to both acetoclastic and hydrogenotrophic methanogens due to the

release of toxic Cu^{2+} ions caused by corrosion and dissolution of the NPs. In contrary, methanogens were not inhibited when exposed to high concentrations (1500 mg/L) of Ag and Fe NPs. This is in agreement with the results of other researches (García et al., 2012; Yang et al., 2012) reported that high concentrations of Ag NPs did not cause methanogenic inhibition. Although Ag is toxic to a number of microorganisms, relatively high concentrations of Ag NPs (40-43 mg/L) did not cause methanogenic inhibition. Oxysulfidation and sulfidation of Ag NPs in anaerobic media caused precipitation of Ag NPs in the form of AgS, a non-toxic form of Ag (Choi et al., 2009). Therefore, the sulfide concentration in the various studies should be expected to have a considerable effect on the inhibitory potential of the Ag NPs.

Cervantes et al. (2016) studied the catalytic effect of bimetallic alloys of Pd-Cu NPs on microbial reduction of p-nitrophenol (PNP) to p-aminophenol (PAP). Results showed that addition of 20 mg/L of Pd-Cu NPs to methanogenic sludge incubations caused increasing threefold reduction rate of PNP with negligible accumulation of toxic intermediates compared with sludge incubations lacking metallic NPs. Metallic NPs facilitate transfer of reducing equivalents derived from lactate/ethanol fermentation towards the nitro group in PNP.

Pat-Espadas (2016) investigated the effect of Pd NPs on biotransformation of two recalcitrant halogenated pollutants, 3-chloro-nitrobenzene (3-CNB) and iopromide (IOP), in anaerobic granular sludge media in batch and continuous upflow anaerobic sludge blanket (UASB) reactors. Pd-immobilized sludge increased removal efficiency of IOP and 3-CNB up to 80% and 100%, respectively, compared with the control (not exposed to Pd) indicating Pd NPs has an important catalytic effect during the biotransformation of IOP. Pd- enriched sludge act as solid-phase redox mediators in

the biotransformation of recalcitrant pollutants. In the case of these halogenated compounds, that are highly resistant to oxidation, Pd NPs can help to achieve dehalogenation since this NPs is able to convert hydrogen gas to adsorbed atomic hydrogen which is a powerful reducing agent that reacts with oxidized functional groups under methanogenic conditions.

2.2. Metal oxide nanoparticles

Metal oxide NPs play a very important role in many areas of chemistry, physics and materials science (Rodríguez and Fernández-García, 2007). Metal oxide NPs can exhibit unique physical and chemical properties due to their small sizes and high density of corner or edge surface sites (Fernández-García and Rodriguez, 2011). There are numerous types of metal oxide NPs such as ZnO, CuO, TiO₂, MgO, NiO, Fe₂O₃ nanoparticles etc. (Noguera, 1996). In the developing field of nanotechnology, it is tried to benefit from metal oxide NPs in process of biogas production. From this point of view, researchers have evaluated the impacts of metal oxide NPs on the efficiency of AD processes (table 2).

Table 2. Metal oxide NPs added to AD process

NPs	Mean Size, nm	Concentration of NPs	Substrate type	Temperature (°C)	Effect on AD process	Ref.
Mn ₂ O ₃	-	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)

Fe ₃ O ₄	7 nm	100 mg/L	crystalline cellulose	37 °C, batch	180% increase in biogas production, 8% increase in methane production	(Casals et al., 2014)
	7 nm	5, 10, 20 mg/L	Raw manure	37 °C, batch	66% increase in biogas production, 96% increase in methane production	(Abdelsalam et al., 2017)
	15-22 nm	50-125 mg/L	Municipal solid waste	37 °C, batch	Up to 117% increase in methane production	(Ali et al., 2017)
Fe ₂ O ₃	128 nm	100 mg/g TSS	Waste activated sludge	35 °C, batch	Increase 117% of methane production	(Casals et al., 2014)
	-	750 mg/L	Granular sludge	36 °C, batch	Increase 38% of methane production	(Ambuchi et al., 2016)
	40 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)
Al ₂ O ₃	<50 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)
MgO	154 nm	500 mg/g TSS	Waste activated sludge	35 °C, batch	Decrease 99% of methane production	(Casals et al., 2014)
CuO	30 nm	7.5- 480 mg/L	Cattle manure	36 °C, batch	Inhibition of biogas production up to 96%	(Luna-delRisco et al., 2011)
	30-50	11, 110, 330, 550, and 1100 mg/L	municipal waste activated sludge	37 °C, batch	Inhibition of biogas production up to 84%	(Ünşar et al., 2016)

	40 nm	10-1500 mg/L	Granular sludge	30 °C, batch	Inhibited acetoclastic methanogens with IC ₅₀ value of 223 mg/L	(Gonzalez-Estrella et al., 2013)
CeO ₂	12 nm	640 mg/L	Cellulose	37 °C, 55°C, batch	Toxicity effect, decrease nearly 100% biogas production	(García et al., 2012)
	-	100, 500, 1000 mg/L	Sludge	30 °C, batch	Up to 35 % decrease in biogas production	(Nguyen et al., 2015)
	15- 30 nm	11, 110, 330, 550, and 1100 mg/L	municipal waste activated sludge	37 °C, batch	caused 9.2% increase in the methane production at the highest dosage	(Ünşar et al., 2016)
	50 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)
ZnO	140 nm	10, 300, 1500 mg/L	Waste activated sludge	35 °C, batch	Inhibition of methane production up to 75.1 %	(Mu and Chen, 2011)
	145 nm	5, 50, 100, 250 and 500 mg/L	Waste activated sludge	37 °C, batch	VFA accumulation in the AD process, 25% reduction on biogas and 50% reduction on methane production.	(Zhang et al., 2017)
	120-140 nm	42, 210, 1050 mg/L	Mixed primary and excess	35 °C, batch	Decreased the abundance of methanogenic	(Zheng et al., 2015)

			sludge		archaea, inhibition of methane production	
	-	100, 500, 1000 mg/L	Sludge	30 °C, batch	Up to 65.3 % decrease in biogas production	(Nguyen et al., 2015)
	50-70 nm	7.5- 480 mg/L	Cattle manure	36 °C, batch	Inhibition of biogas production up to 74%	(Luna-delRisco et al., 2011)
	10-30 nm	10-1500 mg/L	Granular sludge	30 °C, batch	highly inhibitory to acetoclastic and hydrogenotrophic methanogens with IC ₅₀ values of 87 and 250 mg/L	(Gonzalez-Estrella et al., 2013)
SiO ₂	10-20 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)
TiO ₂	7.5 nm	840 mg/L	Cellulose	37°C, 55°C, batch	No effect	(García et al., 2012)
	150-170	42, 210, 1050 mg/L	Mixed primary and excess sludge	35 °C, batch	No measurable impact on methane production	(Zheng et al., 2015)
	25 nm	1500 mg/L	Granular sludge	30 °C, batch	No toxic effects on the methanogenic activity	(Gonzalez-Estrella et al., 2013)

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330 Wang et al. (2016) investigated the impacts of metal oxide NPs (i.e. Fe₂O₃, MgO)

331 on the AD of WAS in dosages up to 500 mg/ g TSS. The authors reported that the

332 supplementation of 100 mg/g TSS Fe₂O₃ NPs increased 117% the methane production

rate, while 500 mg/g TSS MgO NPs produced 98.92% lower amounts of methane. These results indicated that number of microorganisms and enzymatic activity is increased by introducing low concentrations of Fe₂O₃ NPs, but high concentrations of MgO NPs had an inhibitory effect. Measuring lactate dehydrogenase (LDH) concentration, as a cell-membrane damage indicator, showed that LDH concentration in case of adding 500 mg/g TSS MgO NPs was significantly higher than that in the reactor containing 100 mg/g TSS Fe₂O₃ NPs. This shows that high concentrations of MgO NPs probably damage cell membranes and release more LDH contents. Lower concentration of MgO NPs (≤ 100 mg/ g TSS) did not cause any beneficial or inhibitory effect on biogas production rate. Abdelsalam et al. (2017) studied the effects of Fe₃O₄ magnetic NPs on the methanogens during the AD of raw manure. Their research revealed that 20 mg/L Fe₃O₄ NPs at 37 °C stimulated the activity of methanogenic populations and subsequently, led to higher methane production rate. In addition, Fe₃O₄ magnetic NPs provided a uniform distribution of Fe ions in the media, which maintained a sustainable supply of Fe ions in the digester.

Ali et al. also studied the effects of Fe₃O₄ NPs (15-22 nm) on the AD of municipal solid waste under mesophilic conditions (Ali et al., 2017). Results showed that Fe₃O₄ NPs caused the enhancement of methane production rate by stimulating the enzymatic activities of methanogens up to 117%. In addition, the presence of NPs ensured a uniform distribution of iron ions in the solution, which maintained a sustainable supply of Fe ions in the reactor. The addition of 50 and 75 mg/L of NPs were more effective in improving the methane production rate. In contrast, higher concentrations (i.e. 100 and 125 mg/L) reduced the methane production. Ali et al. concluded that the NPs guaranteed a uniform distribution of iron ions in the media, which maintained a

sustainable supply in the aqueous media. The findings are in agreement with the results obtained by Abelsalam et al. (2017) who stated that iron and iron oxide NPs can improve the performance of bioreactors due to the graduate release of $\text{Fe}^{+2}/\text{Fe}^{+3}$ ions. Regarding the metabolism of microorganisms, iron element can enhance activities of methanogenic archaea as reported by Qiang et al. (2012). Also, Su et al. (2013) and Liu et al. (2015) concluded that the addition of iron-based NPs could provide resistance against the formation of hydrogen sulfide (H_2S) and increased stability by pH buffering. Furthermore, the use of Fe_3O_4 NPs has been associated with facilitation of DIET in syntrophic methanogenesis. Such an effect is highly increased when Fe_3O_4 is utilized in nanoscale range, because of numerous active sites that nano-scale materials provide (Ali et al., 2017).

Casals et al. (2014) controlled the dosage of $\text{Fe}^{3+}/\text{Fe}^{2+}$ ions during an AD process by size and surface-state engineering of Fe_3O_4 NPs. Results showed that the Fe_3O_4 NPs (100 ppm of 7 nm) dispersed in an anaerobic waste treatment reactor and gradually dissolved in form of $\text{Fe}^{3+}/\text{Fe}^{2+}$ ions, acting as effective elements. This strategy keeps the concentration of $\text{Fe}^{3+}/\text{Fe}^{2+}$ ions in optimal range during the AD process and boost the bacterial activity over an extended time leading to 180% higher biogas production rate during 50 days of process. Nguyen et al. (2015) studied the effect of CeO_2 and ZnO NPs on the AD of sludge in an UASB. Results showed that adding NPs at low concentration (i.e. 10 mg/L, of CeO_2) boosted the performance of biogas reactor about 11% as compared with the controlled experiment. In contrast, the bacterial toxicity assays showed that higher concentrations of NPs are toxic to microbiome, reducing the biogas production rate. Adding CeO_2 NPs, above 100 mg/L, resulted in decrease of biogas production rate. The reductions of the total biogas production volume due to adding

CeO₂ NPs at 100, 500, and 1000 mg/L were 32.2%, 32.3%, and 35.1%, respectively. Also, the authors reported that inhibition of biogas production with ZnO NPs is dosage dependent. At a low concentration of ZnO NPs, 10 mg/L, the reduction in biogas volume was 8.4% and the biogas inhibition reached to 65.3% in the presence of 1000 mg/L of ZnO NPs.

García et al. (2012) studied the effect of TiO₂, and CeO₂ NPs on the activity of microorganisms presented in WWTP. They reported that 640 mg/L CeO₂ inhibited the biogas production up to 100%, whereas TiO₂ NPs showed zero or slight inhibition for all tested biomasses at mesophilic temperature. Introducing TiO₂ NPs had a slightly positive effect (10% increases) on the biogas production in thermophilic conditions. Likewise, CeO₂ NPs caused an intense inhibition (90%) at thermophilic conditions. In another work, Zheng et al. (2015) stated that TiO₂ NPs had no significant impact on hydrolysis, acidification, and methane production during the AD of sludge, even at high concentrations (i.e. 150 mg/g TSS), both in short- and long-term exposure.

Ambuchi et al. (2016) investigated the role of iron oxide NPs (Fe₂O₃) in the enhancement of the AD performance of granular activated sludge. The results showed that Fe₂O₃ NPs at a concentration of 750 mg/L caused faster utilization of substrate and biogas production rates than the controlled condition. In their study, microbial community analysis revealed the role of *Anaerolineaceae* and *Longilinea* bacteria, predominant bacterial genera in the biodegradation process, could have highly been boosted by NPs.

The influence of ZnO NPs on the methane production rate during the AD of waste activated sludge was investigated by Mu et al. (2011). Their results indicated that 1 mg/g-TSS of ZnO NPs had no positive or negative effect on the methane production

rate, while 30 and 150 mg/g-TSS of ZnO NPs caused 18.3% and 75.1% inhibition, respectively. The toxic effect of ZnO NPs on the methane production rate was mainly attributed to the release of Zn^{2+} ion, which inhibited the activities of protease, acetate kinase, and coenzyme F_{420} , leading to inhibition of hydrolysis and methanation steps (Zheng et al., 2015).

Zhang et al. (2017) declared that addition of ZnO nanomaterials during the AD of activated sludge increased the accumulation of VFAs. The promoted VFAs accumulation in the presence of ZnO nanomaterials was due to the slow production and slower consumption rates of VFAs during the AD process. The decrease of production rate of VFAs was mainly contributed to the inhibition of protein hydrolysis by Zn^{2+} ions. Decrease in biogas and methane production was in agreement with the results obtained by Mu and Chen (2011) that proved the slow consumption of VFAs in the presence of ZnO nanomaterials.

Luna-del Risco et al. (2011) investigated the effects of various concentrations of bulk and nano-sized CuO and ZnO powder on the biogas production rate during the AD of cattle manure at 36 °C. Their results showed that bulk and nano-size powder at concentrations higher than 120 and 15 mg/L for CuO and 240 and 120 mg/L for ZnO could remarkably inhibit the biogas production rate. Similar to the work of Mu et al. (2011), the inhibition of biogas and methane production in the presence of these NPs was attributed to the bioavailable portion of the existed metal . The results showed that the most toxic compounds to anaerobic microorganisms were CuO NPs (30 nm), ZnO NPs (50-70 nm), bulk ZnO and bulk CuO, respectively. It is noteworthy that the biogas production rate increased in the last days of experiment, indicating that the anaerobic bacteria were adapted to the NPs and were able to tolerate in such an environment.

Ünşar et al. (2016) investigated inhibition impacts of CuO and CeO₂ NPs on the AD of WAS in case of short-term and long-term exposure. CuO NPs had the most toxic effect on microorganisms during the AD process. Increase of CuO NPs concentration, from 11 to 1100 mg/L, increased the inhibition levels from 5.8 to 84.0%. Based on their results, CeO₂ NPs did not cause intense effects on the AD process compared to CuO NPs. Long-term exposure of 1100 mg/L of CeO₂ NPs caused 9.2% increase in the methane production rate. Furthermore, the hydrolysis rate constant (k_H) of WAS in all tests of CeO₂ NPs was 0.0277 d⁻¹, while the k_H of WAS in the presence of high concentration of CuO NPs was 0.0016 d⁻¹. The toxic effect of CuO NPs could be attributed to their heavy metal characteristics.

Gonzalez-Estrella et al. (2013) investigated the inhibitory effect of inorganic oxide NPs (i.e. Al₂O₃, CeO₂, CuO, ZnO, Fe₂O₃, Mn₂O₃, SiO₂, TiO₂) on acetoclastic and hydrogenotrophic methanogenic activities in the AD of granular sludge. The results revealed that CuO and ZnO NPs are highly inhibitory to activity of acetoclastic and hydrogenotrophic methanogens. 62 and 68 mg/L of CuO NPs and 87 and 250 mg/L of ZnO NPs caused 50% inhibition towards acetoclastic and hydrogenotrophic methanogens, respectively. The inhibition is mainly due to the release of toxic Cu²⁺ and Zn²⁺ ions caused by corrosion and dissolution of the NPs. In contrary, methanogens were not inhibited when exposed to high concentrations (1500 mg/L) of Al₂O₃, CeO₂, Mn₂O₃, Fe₂O₃, SiO₂, and TiO₂ NPs, suggesting that anaerobic treatment processes could tolerate high concentrations of these types of NPs.

The number of reports concerning the toxicity of NPs indicate that addition of natural organic matter (NOM) to the different NPs resulted in lower toxicity to the majority of the organisms (Grillo et al., 2015). NOM could form complexes with

released metal ions and lowering their bioavailability in the media, hence lowering the toxicity (Gao et al., 2012). In the other scenario the surface charge of NPs could be changed due to bonding with the NOM, changing the interaction with other compounds (Romanello and Cortalezzi, 2013). Chen et al. (2011) reported using 10 mg/L of humic acids (HA), as a model for NOM, in contaminated water with hazardous oxidized pollutants significantly decreased toxicity of NZVI towards gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis*. The TEM images showed that the surface of NZVI and microorganisms covered by NOM. This decreased the zeta potential of NZVI from -30 to -45 mV and caused electrosteric hindrance to minimize direct contact with microorganisms, which decreased cell membrane destruction. Alvarez and Cervantes (2012) investigated the effect of bare and coated γ -Al₂O₃ NPs with HA on specific methanogenic activity in an anaerobic granular sludge consortium. Their results revealed that HA-coated γ -Al₂O₃ NPs mitigated the toxicological effects of γ -Al₂O₃ NPs on methanogenic microorganisms. The HA-coated NPs could enhance Al³⁺ sorption, thus decreasing the toxicological effects of this ion.

2.3. Carbon based nanomaterials

The carbon based nanostructures can be found at different types such as graphene, diamond, nanotube and nanofibers. They have high surface area for the deposition of conducting polymer and metal/ metal oxide NPs, which facilitate the efficient ion diffusion. Furthermore, they have small dimensions, high chemical stability, high thermal conductivity and low resistivity (Bose et al., 2012). Table 3 summarizes the researches regarding the applications of carbon based nanostructures in the AD process.

Table 3. Summary of the reported applications of carbonous nanomaterials in AD processes

NPs	Mean Size, nm	Concentration of NPs	Substrate type	Temperature (°C)	Effect on AD process	Ref.
MWCNT ^a	-	1500 mg/L	Granular sludge	36 °C, batch	Increase 43% of methane production	(Ambuchi et al., 2016)
SWCNT ^b	1-2 nm diameter, 5-20 nm length	1000 mg/L	Sucrose	35 °C, batch	No impact on the maximum methane yield, increasing methane production rate by 100%	(Li et al., 2015)
	1-2 nm diameter, 5-30 µm length	1000 mg/L	Glucose	55 °C, batch	CH ₄ production rate increased by 92%	(Yan et al., 2017)
Graphene	4-20 nm	0.5- 2 g/L	Ethanol	35 °C, batch	Increase 25% in methane yield and 19.5% in biogas production rate	(Lin et al., 2017)
	-	30-120 mg/L	Glucose	35 °C, batch	Up to 51.4% increase in methane production rate	(Tian et al., 2017)
Fullerene (C ₆₀)	-	Up to 50000 ng/Kg of biomass	Waste water sludge	Ambient temperature, batch	No effect	(Nyberg et al., 2008)

^a Multi wall carbon nanotube, ^b Single wall carbon nanotube

The effect of graphene as a highly conductive carbon based nanomaterial on the efficiency of AD process of ethanol, as a key intermediate product, was studied by Lin

et al. (2017). They observed that, the addition of 1.0 g/L graphene resulted in an enhancement of 25% in methane yield and 19.5% in biogas production rate. Interspecies electron transfer between bacteria and archaea plays a vital role in promoting the efficiency of AD processes. Microbial analysis revealed that electrogenic bacteria of *Geobacter* and *Pseudomonas* along with archaea *Methanobacterium* and *Methanospirillum* might participate in DIET. Graphene-based DIET can sustain higher electron transfer flux than conventional hydrogen transfer because of high electrical conductivity of graphene along with its high surface area. The lag phase time and peak time decreased in the presence of graphene. Furthermore, the degradation rate of ethanol was simultaneously enhanced by adding graphene, which is possibly due to the high electrical conductivity and specific surface area of graphene. On the other hand, methane yield slightly decreased with increasing the graphene concentration to 2.0 g/L. This was probably due to the microbial inhibition effect caused by high concentration of graphene, indicating that cytotoxicity could become an undesired phenomenon when applying carbonous nanomaterials in AD. Cytotoxicity of carbon nano structures (i.e. graphene and CNT) to microorganisms has been presented using different microbial strains such as *Escherichia coli* (Liu et al., 2011).

Tian et al. (2017) studied short-term and long-term effects of graphene on AD processes using pure glucose as substrate at 35°C. Results showed that short-term exposure of graphene with concentration of 30 and 120 mg/L had significantly positive effects on methane production rate, which increased by 17.0% and 51.4%, respectively. However, long-term exposure to graphene at 120 mg/L showed slight inhibition of methanogen at ambient temperature (10-20 °C). High concentration of graphene causes microbial inhibition, indicating that cytotoxicity could become an adverse effect when

using nanomaterials in AD processes. They concluded that acetate-consuming methanogenesis was enhanced by adding graphene to the AD process at mesophilic conditions. Furthermore, *Geobacter* enrichment was observed with graphene, suggesting that the DIET might be promoted. This conclusion is due to the fact that DIET between *Geobacter* species and *Methanosaeta* or *Methanosarcina* species is recognized as an effective form of syntrophy in methanogenic communities (Bueno-López et al., 2018; Dubé and Guiot, 2015). Graphene act as electric conduits between *Methanosaeta* and *Geobacter* promoting DIET process. Then, *Methanosaeta* consume electrons produced from the oxidation of propionate or butyrate to acetate (Zhao et al., 2016).

Li et al. (2015) reported that the electrical conductivity of sludge was enhanced in the presence of single wall carbon nanotubes (SWCNT) at mesophilic temperature, which might promote DIET among fermentative bacteria and methanogens in the AD process. Thus, it is hypothesized that materials with higher conductivity could play a more significant role in promoting the DIET. They showed that introducing 1000 mg/L of SWCNT to the sludge for 180 h had no effect on cumulative methane production rate in comparison to the controlled experiment, while SWCNT dosing caused faster substrate utilization and methane production rate.

In another research, Yan et al. (2017) studied the effects of single wall carbon nanotubes SWCNT, as a conductive material, on the AD of glucose. They also found that conductive materials such as SWCNT could promote the start-up of thermophilic process. Their results showed that the methane production rate in conductive materials supplemented reactors was almost 100% higher than the controlled reactors. The promoted biogas production rate was also related to the establishment of DIET.

Ambuchi et al. (2016) investigated the role of multi wall carbon nanotubes (MWCNTs) in enhancing the AD performance of granular activated sludge. They showed that MWCNTs at the concentration of 1500 mg/L facilitated the utilization of substrate and biogas production rate. This finding suggests that MWCNTs could be a good enhancer of direct interspecies electron transfer leading to increased biogas production rate.

Nyberg et al. (2008) studied the effect of fullerene (C₆₀) on the AD of wastewater sludge. Their findings suggest that C₆₀ fullerenes have no significant effect on the anaerobic community during few months of exposure and biogas production rate is affected by introducing C₆₀ nanomaterial.

Furthermore, researchers indicated that carbon based nanomaterials such as (reduced) graphene oxide and CNT can facilitate the reduction of recalcitrant pollutants, such as azo dyes (Colunga et al., 2015; Lu et al., 2014; Pereira et al., 2014), nitroaromatic compounds (Lei Li et al., 2016; Wang et al., 2014) and halogenated pollutants (Chen et al., 2014; Toral-Sánchez et al., 2018, 2017) under methanogenic condition. It has been reported that, these materials could mediate the redox conversion of recalcitrant pollutants in biological treatment systems by enhancing electron transfer processes (Cervantes et al., 2000; Pereira et al., 2014).

2.4. Multi-compound nanoparticles

The relatively high density minerals in the form of NPs may cause non-uniform dispersion and agglomeration of NPs in the AD media (Khalil et al., 2017), which could decrease the availability of the elements for microorganisms and weaken the physico-chemical interaction between NPs and microorganisms (Nel et al., 2009). A novel

approach is to encapsulate or bind multiple compounds in a combined NPs and take advantage of their unique characteristics (Da Silva et al., 2017). Supporting/coating the NPs onto carrier additives to: 1) overcome the inhibitory effects of agglomeration of NPs and 2) strength the contacts between microorganisms and NPs, could be served as an efficient strategy to increase reactivity and performance of NPs (Elreedy et al., 2017). Distributing metal/metal oxide NPs over the support materials (i.e. TiO₂, Al₂O₃, SiO₂, zeolite, carbon based materials, etc.) increase the fraction of surface- exposed atoms of NPs, and consequently, increases the reactivity due to their desired dispersion on the surface of the support (Astruc et al., 2005). As an example, Khalil et al. (2017) concluded that the ratio 2:1 of NZVI to activated carbon as a support was the optimum ratio that caused the maximum nitrate and phosphate removal from aqueous solutions. In another research, Fateminia and Falamaki (2013) studied the effect of freshly NZVI-Cu bimetallic supported on zeolite. The results proved a great advantage of nitrate anions removal from groundwater under un-buffered conditions. The efforts about using multi-compound NPs in biogas production processes are summarized in Table 4.

Table 4. Summary of reported multi- compound NPs as additive in AD process

NPs	Mean Size, nm		Concentration of NPs	Substrate type	Temperature (°C)	Effect on AD process	Ref.
Zeolite	7.13 µm		4 g/L	Domestic sludge	37 °C, batch	24.86% increase in biogas production	(Amen et al., 2017)
NZVI and zeolite mixture	-		500 mg NZVI+ 4 g/L zeolite	Domestic sludge	37 °C, batch	130.87% increase in biogas production	(Amen et al., 2017)
NZVI coated zeolite	45 nm NZVI NPs coated on 7.13 µm zeolite particles		500, 1000 mg/L regard to NZVI	Domestic sludge	37 °C, batch	149.95% , 286.75% increase in biogas production	(Amen et al., 2017)
Iron oxide-zeolite system	20 nm iron oxide NPs coated on 0.5-1 mm zeolite particles		Provide (20 mM) 1120 mg/L Fe element	Cow manure+ rice straw	35 °C, batch	372.85% increase in cumulative methane yield	(Lu et al., 2017)
M/SiO ₂	M=	-	10 ⁻⁵ mol/L	Acetate	55 °C, batch		(Al-Ahmad et al., 2014)
	Ni					70% increase in methane production	
	Co					48% increase in methane production	
	Fe					7% increase in methane production	
	Pt					6% increase in methane production	

NiO/TiO ₂	~ 10 nm	0.252 mg/gTS	Wheat straw	55 °C, batch	up to 13% increased soluble COD and up to 67% increase of VFAs concentration, increasing methane production rate	(Isfahani et al., 2018)
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579 Amen et al. (2017) studied the effects of various additives, i.e. NZVI, zeolite, NZVI
580 and zeolite mixture, and NZVI coated zeolite (ICZ) particles, on the AD of domestic
581 sludge at mesophilic conditions. Experimental monitoring showed that addition of ICZ
582 initially induced a lag phase before generation of significant biogas volume as a result
583 of ammonia accumulation at the beginning of digestion period. This is due to the fact
584 that high concentration of ammonia (>400 mg/L) inhibits specific enzyme reactions.
585 However, in case of using ICZ, after the lag phase the cumulative production increased
586 more than that of the other examined additives, i.e. NZVI, zeolite, NZVI and zeolite
587 mixture. In addition, higher concentration of Fe NPs on the surface of zeolite particles
588 led to an increase in the biogas generation. The high biogas production rate is probably
589 due to the action of ICZ particles (1000 mg/L) that dominate the non-uniform dispersion
590 in the digester media, increase the bioavailability and electron transfer interaction
591 between the external additives and microbiome.

592 Lu et al. (2016) synthesized iron oxide NPs supported on natural zeolite and
593 investigated their effects on the AD of cow manure and rice straw at mesophilic
594 temperature. In the presence of iron oxide-zeolite system, the cumulative methane
595 production rate was 74.11% higher than that of the mixture of iron oxide. In addition,
596 when zeolite was used as additive, the cumulative production rate was 373% higher

than that of the controlled condition. A rational behind the positive effects can be due to the enhanced activity of methanogens immobilized on the surface of the iron oxide-zeolite system. Zeolite is a non-cytotoxic mineral having a number of channels and pores on its crystalline structure boosting the immobilization of microorganisms (Borja et al., 2007). The coenzyme F₄₂₀ assay proved a significantly promoted methanogen activity by the addition of the iron oxide-zeolite system. This is due to the fact that the iron oxide-zeolites nanoparticle is an efficient carrier, i.e. immobilization support, for methanogens. More microorganisms immobilized on the newly designed NPs because of their larger specific surface area. On the other hand, the iron oxide minerals on the surface of the zeolite accelerated the activity of the methanogen microorganisms by accelerating the electron transfer in the methanogenic process and subsequently increasing the concentration of F₄₂₀ coenzyme. This novel form of NP facilitates the bioavailability and electron catalysis of iron oxides due to the close distance and convenient contact with the immobilized microorganisms. In addition, the outstanding ion exchangeable ability of iron oxide-zeolite system ensured a more stable pH due to the great buffering capacity of the system.

Al-Ahmad et al. (2014) investigated the effect of metal NPs (i.e. Fe, Pd, Pt, Ni, Co, Ag, Cu) encapsulated in porous SiO₂ matrix on the AD of acetate and glucose at 55 °C and concentration of 10⁻⁵ mol/L. The results showed that, methane production was catalyzed by Ni, Co, Fe and Pt NPs encapsulated in porous SiO₂ by transferring more efficiently electrons to acceptors and enhancing the activity of microorganisms. Addition of these compounds to the media during the AD of acetate increased the methane production rate 70, 48, 7 and 6%, respectively. The authors also investigated the effect of the other concentrations of Ni/SiO₂ NPs (i.e. 10⁻⁴, and 10⁻⁶ mol/L) and

confirmed that the higher concentrations (10^{-4} mol/L) improved the methane production rate. Furthermore, they concluded that introducing 10^{-5} mol/L of Cu/SiO₂ NPs have neither a positive nor negative impact on the methane production rate, while Pd/SiO₂ and Ag/SiO₂ inhibited methane production rate.

In another research, Beckers et al. (2013) investigated the enhancement effect of 2-3 nm metallic (Pd, Ag and Cu) or metallic oxide (Fe_xO_y) NPs, encapsulated in porous SiO₂, on hydrogen production from glucose by a *Clostridium butyricum* strain. Using very low concentrations (10^{-6} mol/L) of Fe₂O₃/SiO₂ NPs in a batch mode increased 38% of hydrogen production and 58% of maximum H₂ production rate in comparison with those obtained without NPs or with silica particles only. These results suggest an improvement of the electron transfer through combinations between enzymes and inorganic materials. An enhancement of the H₂ production rate confirmed interactions of microorganisms with NPs. The addition of the NPs would improve the bacterial hydrogen production rate through catalysis involving extracellular mediated-molecules. Furthermore, the encapsulation of the NPs in the silica matrix decreases the risk of agglomeration of the active sites. The NPs didn't show an inhibiting effect because the and/or without SiO₂). In fact, it is recognized that Ag and Cu behave as antimicrobial elements since they destroy the membrane cells by contact between the microorganism and the NPs (Bagchi et al., 2012; Sotiriou and Pratsinis, 2010). Iron oxide NPs encapsulated in SiO₂ caused the highest H₂ production rate. A rational to explain this result can derive from the interaction between NPs and bacteria that resulted in a faster hydrogen production rate. Furthermore, since the SiO₂ (without NPs) did not show any significant effect, it can be concluded that only the central active site (i.e. the metal or metal oxide NPs) could play a role in the improvement of the hydrogen production.

Considering the low concentration of iron oxide NPs, it is suggested that the enhancement of hydrogen production rate with $\text{Fe}_2\text{O}_3/\text{SiO}_2$ catalysts is related to a catalytic activity working in parallel with the enzymes involved in electron transfer as the hydrogenases, *c*-cytochromes or/and with extracellular electron mediators. Indeed, the immobilized iron oxide active sites could be used by the bacteria for oxidation/reduction chemical reaction (considering the redox couple $\text{Fe}^{2+}/\text{Fe}^{3+}$) to help the bacteria transferring faster its electrons without consuming or metabolising the iron when it is added to the medium in a dissolved form. However, considering the small size of the iron oxide NPs (around 3 nm), the surface effect is greatly enhanced and may improve the ability of the NPs to react with the electrons transported by mediators and transfer them efficiently to electron acceptors or eventually protons.

Isfahani et al. (2018) investigated the effect of 5.0 wt% NiO-coated TiO_2 NPs on the AD of wheat straw at thermophilic condition. Results showed that addition of different dosages of NiO- TiO_2 NPs to batch assays, i.e. 0.252 mg of NiO- TiO_2 /gTS, resulted in up to 13% increased soluble chemical oxygen demand (COD) and up to 67% increase of volatile fatty acids (VFAs) concentration in comparison with control experiments. Methane production rate was increased while no significant increase of final methane production was observed in batch experiments.

3. Summary and perspectives

Supplementation of inorganic additives in form of nanomaterials caused enhancement in the AD performance because it offers unique physio-chemical properties, i.e. high surface area, increased number of active sites, high reactivity, high specificity, self-assembly, promoted mobility in the AD media, and dispersibility.

Furthermore, the stimulating effects of nanomaterials can be related to the cellular uptake of nanomaterials inside the microorganisms and integrating with the metabolic intermediates and key enzyme activity involved in AD processes. All the nanomaterials used in AD processes were categorized into four groups, namely zero valent metallic NPs, metal oxide NPs, carbonous nanomaterials, and multi- compound NPs. Results clearly indicate that using nanomaterials can be a useful strategy to improve the performance of the AD process. Using multi-functional nanomaterials, that are capable of accomplishing multiple objectives, is a promising method to improve the AD process. The objectives included facilitating direct interspecies electron transfer (DIET), preventing sulfur/ammonia inhibition, controlled nutrient and trace elements supplementation or providing an active substrate for enzyme immobilization.

Fe, Ni, Co, Mo, Se, Cu, Zn are constituent of enzymes/microorganisms and are known to be fundamental trace elements for numerous AD reactions. Depending on type and composition of feedstock, the need for these trace elements is variable. It is crucial to ensure that the proportion of trace elements in AD media is optimal. Deposition of trace elements in form of metal/metal oxide NPs on different supports with optimal proportion is an idea to take advantages of support and deposited materials at a same time, as well as avoiding uneven dispersion of trace elements. Designing multi-functional nanomaterials using above mentioned trace elements (metal/metal oxide) could increase the bioavailability of the trace elements and strengthen the interaction between NPs of trace elements and functional microorganisms (which are required for AD). Actually deposition of metal/metal oxide NPs over the support materials, i.e. carbonous nano structures, TiO_2 , Al_2O_3 , SiO_2 , increases the fraction of surface- exposed atoms of NPs, and consequently increases the reactivity of these metals due to desire

dispersion of metal/metal oxides on surface of support. Supports should be non-hazardous, cheap and show high surface area, chemical stability as well as capability for dispersing metal/metal oxide NPs highly over the surface. Synthesizing a recyclable support, such as magnetic carriers or supports in form of pellet, could be an ideal choice that make possibility to collect NPs, regenerate them and use continuously in the process.

The perspective could be design, development and fabrication of multi-functional nanomaterial to overcome the bottleneck steps in AD processes using different feedstock. In the future, the following stages should be considered to achieve a novel multi-functional nanomaterial: (1) Deep understanding of the effects of different nanomaterials on each step of the AD process by using model substrates. (2) Fabrication of multi-functional nanomaterials, i.e. deposition of several metal/metal-oxide NPs on supporting materials (3) Tuning the activity of multi-functional nanomaterial via optimization of composition ratios. (4) Assessment of fabricated nanomaterial through application in the presence of different types of substrates

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