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# Enhanced performances of anaerobic digestion processes treating organic wastes: Role of iron and carbon based nanomaterials

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## ABSTRACT

Issues of climate change, energy demand and antibiotic resistance are calling for advanced methods to convert waste into fuels, value-added materials and safe byproducts. Anaerobic digestion (AD) is a major technology treating organic-rich wastes such as sewage sludge, kitchen waste, and animal manure. AD allows to convert waste into energy such as biogas and electricity, and resources such as fertilizers. Nevertheless, biogas production from organic waste is limited by the low biodegradability of resistant organic matter and the short retention time in AD bioreactors. As a consequence, recent researches have focused on the use of additives to improve the performance of AD. For example, nanomaterials are increasingly used as additives due to their properties in the promotion of direct inter-species electron transfer between microorganisms, and the improvement of the microbial community structure. In this study, we review firstly the contribution of nanomaterials to system stability, digestive performance and risk control of AD processes systematically. Then we sorted the enhanced performances by co-digestion of nano-magnetite with sewage sludge, kitchen waste, livestock and poultry manure thoroughly. Finally we discussed mechanisms on how nanomaterials such as iron- and carbon-based nanomaterials addition could enhance production of volatile fatty acids, and inhibit the production of hydrogen sulfide and the transmission of antibiotic resistance genes simultaneously.

## 1. Introduction

The global energy crisis and climate change are urgently calling for the development of sustainable energy systems since renewable energy sources can prevent resource depletion, ecological degradation, and global warming effectively [1]. The rise of the economy and living standards in many countries is also increasing the production of organic waste. The World bank projected increasing waste generation all over the world. In many cases, the organic waste is landfilled where the energy and nutrients in the organic waste is basically wasted [2]. There are many kinds of solid organic waste, including sewage sludge, kitchen waste, human and animal manure, green plant waste and agricultural waste, mainly containing biodegradable organic components with a water content of 85–90 % [3]. Therefore, organic wastes are a potential source of energy and value-added materials that should be recovered

during waste treatments [4]. For that, many researches are actually focusing on improving anaerobic digestion (AD), a key biological waste treatment technology.

Anaerobic digestion is a complex dynamic system involving microbial, biochemical, and physicochemical processes, with multiple biochemical metabolic pathways [5]. As shown in Fig. 1, AD usually proceeds in four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [6]. In the absence of oxygen, anaerobic bacteria ferment biodegradable substances into methane and convert the organic waste into biomass energy. At the same time, minerals such as ammonium salts, phosphates and sulfides are retained in the digestate, which can be further used to produce soil conditioners [7] and organic fertilizers [8]. During this process, anaerobic microorganisms produce methane by breaking down organic matter. However, the purity (volume content) of methane produced by AD is usually between 50 % and

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70 % [9]. As shown in Fig. 1, other major impurity gases include hydrogen sulfide (H<sub>2</sub>S), gaseous free ammonia (NH<sub>3</sub>), carbon dioxide (CO<sub>2</sub>), and hydrogen (H<sub>2</sub>). These gases are mainly produced by microbial metabolism during AD, where CO<sub>2</sub>, H<sub>2</sub>, NH<sub>3</sub> and H<sub>2</sub>S play different roles. CO<sub>2</sub> and H<sub>2</sub> are common gases produced during AD and play an important role in the system. In addition, H<sub>2</sub> is an important raw material for methane production with CO<sub>2</sub> [10]. H<sub>2</sub>S and NH<sub>3</sub> are mainly produced by sulfate-reducing bacteria and ammonia-oxidizing bacteria. H<sub>2</sub>S can be generated together with methane, but at high concentrations, it can also have a toxic effect. Ammonia nitrogen concentration higher than 1500 mg/L will inhibit AD and affect the metabolism and digestion process of microorganisms. Managing free ammonia content in bulk water is one of the important tasks for AD processes [11].

Researchers are currently attempting to upgrade technologies to achieve better biogas yield and higher methane content, as well as maximize energy recovery and minimize environmental impact, e.g. by adding nanomaterials [12].

Nanomaterials have unique thermal, mechanical, structural, and physicochemical properties such as size, specific surface area, surface structure, solubility, and catalysis, and therefore have a wide range of applications [13]. Nanomaterials applied in AD are mainly because of their properties such as higher specific surface area. Suanon et al. applied nano-zero-valent iron (nZVI) and commercial iron powder to the AD of municipal sludge, and found that the Brunauer-Emmett-Teller surface area of nZVI (23.3 m<sup>2</sup>/g) is about 10 times that of commercial iron powder (2.48 m<sup>2</sup>/g), but the dosage of nZVI is only one-tenth of that of the commercial iron powder [14]. The larger surface area increases the contact between wastes and microorganisms. Various carbon-based nanomaterials are able to enhance the electron exchange between bacteria and methanogens attached to the surface of biochar due to the presence of specific surface functional groups [15].

Nanomaterials have remarkable properties due to their very small

size, diverse morphology, high reactivity, and chemical stability [16]. As a consequence, nanomaterials can interact with the digestion matrix and microorganisms in AD systems to enhance bacterial activity and process performances efficiently [17,18]. In this study, we review the literature on the role of nanomaterials in improving biogas production, focusing on maintaining system stability, suppressing NH<sub>3</sub> and H<sub>2</sub>S production, improving electron transfer within microbial communities, and extracellular polymer production. We also present the use of nanomaterials to inactivate antibiotic resistance genes that occur in wastewater.

### 1.1. Performances and maintainance of the nanomaterials enhanced AD processes

Literature was reviewed to describe the nanomaterials focused on iron-based and carbon-based nanomaterials. To identify papers for the literature review, we reviewed approximately 400 papers on nanomaterials for enhanced AD, and we performed keyword co-occurrence analysis using VOS viewer. The results are shown in Fig. 2. Each keyword is presented in the form of nodes, and the size of the nodes indicates the importance of the keyword. At the same time, the connection between nodes indicates the co-occurrence relationship between these keywords, and the thickness of the connection indicates the strength of the co-occurrence. We count keywords that appear more than 5 times. Of the 2195 keywords, 233 meet the threshold.

Among the keywords that co-occurred more than five times, we found that nZVI, activated carbon, biochar, graphene, magnetite nanoparticles (nFe<sub>3</sub>O<sub>4</sub>), and carbon nanotubes appeared 63, 35, 33, 9, 8, and 6 times, respectively. Results show that most current research is focused on iron-based nanomaterials and carbon-based nanomaterials. This phenomenon may be related to the properties of these two materials. Also, iron- and carbon-based nanomaterials are readily available as raw materials and can be easily recycled for secondary use. This underlined

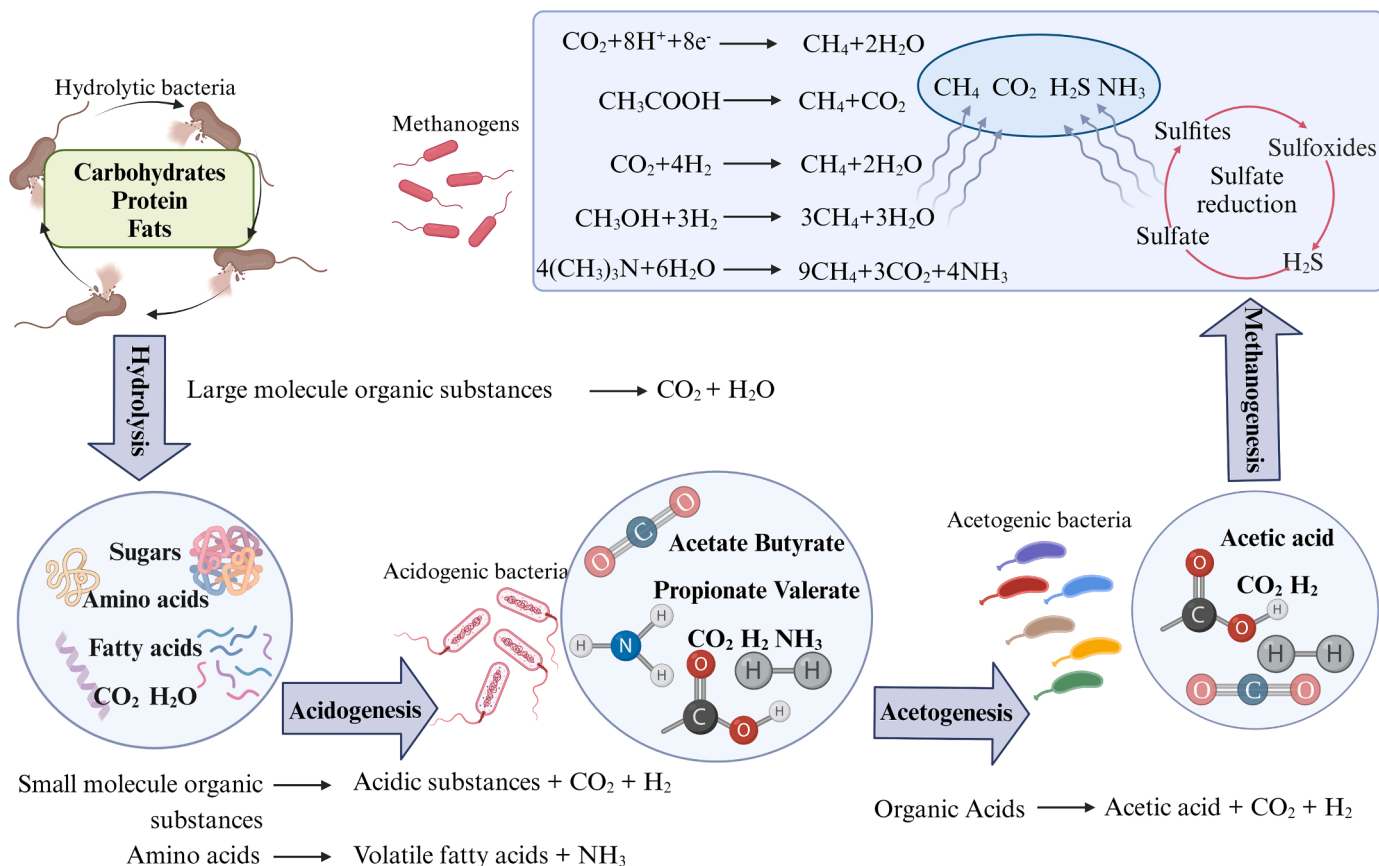


Fig. 1. Diagram of anaerobic digestion and main biogas generation nodes. Created with BioRender.com. Publication license has been obtained.



**Table 1**

Impact of common nanomaterials based on the performance of anaerobic digestions. nZVI: nano zero-valent iron. nFe<sub>3</sub>O<sub>4</sub>: Fe<sub>3</sub>O<sub>4</sub> nanomaterials, rGO-MNPs: reduced graphene oxide materials modified with magnetite nanomaterials.

Type of Nanomaterials	Substrate/ Type of Biochar	Size/ Dosage	Operating Cycle	Digestion Temperature	Biogas Production /Methane Content Increase Rate	Refs.
nZVI	Anaerobic activated sludge	Less than 100 nm 0.25 g/L	14 days	37±3 °C	25.23 % (biogas) 31.38 % (methane)	[41]
nZVI	Activated sludge	20–100 nm 0.50–1.0 g/L	17–80 days	35–37 °C	15.70–30.40 % (biogas) 8.75–40.40 % (methane)	[19] [23] [42] [43]
nZVI	Glucose	100 nm 5.0 g/L	20 days	37 °C	23.90 % (biogas)	[44]
nZVI	Food waste	40–600 nm 5.0 g/L	15–65 days	35–37 °C	8.50–62.58 % (biogas)	[45] [46]
nZVI	Manure	9–80.9 nm 0.20–0.1 g/L	50–69 days	35–37 °C	29.10–45 % (biogas)	[47] [48]
nFe <sub>3</sub> O <sub>4</sub>	Dewatered and secondary sludge	20 nm 1.0 g/L	80 days	35±2 °C	13.44 % (biogas) About 10 % (methane)	[19]
nFe <sub>3</sub> O <sub>4</sub>	Activated sludge	12–30 nm 0.05–0.25 g/L	13–25 days	35–37 °C	60–70 % (biogas) 13.20–50.80 % (methane)	[49] [50] [20]
nFe <sub>3</sub> O <sub>4</sub>	Canola straw and banana plant waste Buffalo dung and banana plant waste	1 µm 2.025 mg /L 1 µm 1.25 mg /L	40 days	37±1 °C	39.40 % (methane) 33.10 % (methane)	[51]
nFe <sub>3</sub> O <sub>4</sub>	Manure/Chicken, cattle dung and poultry litter	4.2–400 nm 0.15–0.20 mg/L	50–80 days	35–37 °C	27.50–66 % (biogas)	[22] [52] [47] [48] [45]
nFe <sub>3</sub> O <sub>4</sub>	Food waste	300–600 nm 5.0 g/L	65 days	37±1 °C	6.00 % (biogas)	
Biochar	Activated sludge/Corn stover, biogas residue, blue algae and coconut shell	0.064–0.12 g/g dry matter	34–35 days	35–55 °C	9.66–39.11 % (biogas) 30.64–46.16 % (methane)	[25] [31] [27]
Biochar	Excess sludge/ Fruitwood	1.0 g/g dry matter	45 days	37 °C	27.80 % (biogas)	[33]
Biochar	Manure/ Residues biochar, wheat straw, Fruitwood and Chicken manure biochar	0.05–0.058 g/g dry matter	60–72 days	35–37 °C	6.97–68.90 % (biogas)	[26] [32] [53]
Biochar	Sorghum/Rice husk	0.5 g/g dry matter	25 days	35 °C	25 % (biogas)	[30]
Biochar	Food waste/Wood chips and biogas residue	0.04–0.08 g/g dry matter	27 days	37–55 °C	10.50–18 % (biogas)	[54] [55]
Biochar	Food waste/ Sludge-derived and wood pellets	0.83–1.06 g/g dry matter	11–46 days	35 °C	214–474 % (biogas)	[56] [57]
nZVI- biochar	Waste activated sludge/Corn stover-derived	59.2 nm 0.21 g/g dry matter	34 days	37±1 °C	29.56 % (methane) 115.39 % (biogas)	[25]
Magnetic biochar	Municipal solid waste/Magnetic rice-straw	25.66 nm 0.5 %, w/w	25 days	35 °C	11.70 % (biogas)	[35]
Magnetic biochar	Artificial dairy wastewater/ Magnetite-contained	Particular amounts	last 8 days	37 °C	38.10 % (biogas)	[36]
Nano-graphene	Anaerobic sludge	0.8–1.2 nm 30 mg/L	55 days	20 ± 2–10 ± 2 °C	14.30 % (biogas)	[39]
rGO-MNPs	Anaerobic sludge	8.3 ± 3.6 nm 50 mg/L	16 days	37 °C	47.00 % (biogas)	[40]

the acetoclastic methanogenesis process is also enhanced. Zhang et al. conducted a series of experiments using nZVI-loaded biochar (nZVI-BC) to enhance AD performances, and found that 0.21 g/g dry matter nZVI-BC can significantly increase the production of total volatile fatty acids (VFAs) and improve VFAs composition, optimize thermodynamic conditions, inhibit propionic acid fermentation, and promote methane production [25]. From the analysis of microbial results, the abundance of *Methanosaeta* dropped from 63.4 % without dry nZVI-BC dosage to 55.93 % of 0.21 g/g dry matter nZVI-BC dosage. The abundance of two hydrogenotrophic methanogens, *Methanobacterium* and *Methanospirillum*, was higher than that of the control group, which implied that the addition of nZVI-BC could change the abundance of systemic archaea and increase the diversity of microbial communities. Therefore, a certain dosage of nZVI-BC can improve the hydrogenotrophic methanogenesis reaction and increase methane production.

Patel et al. used cattle manure as feedstock and dosed biochar at 0–14 mg/L into the reactor; all concentrations of biochar were found to increase the methane content of the AD system [26]. Compared with no added biochar, the methane content was 35.2 % higher in the manure added with 10 mg/L biochar. This is because biochar promotes direct electron or hydrogen transfer between microorganisms, improving the activity of microorganisms.

Liu et al. compared the effects of biochars from biogas residues, coconut shells and corns on the anaerobic co-digestion process [27]. The results showed that the daily mean methane production increased by 46.2 %, 27.7 % and 30.6 % with the addition of the three biochars, respectively, compared with the control group. After the dosage of biochars, the fluorescence intensity of protein and cellulose in the system decreased significantly, indicating that biochar can accelerate the hydrolysis process of protein and ligno-cellular matter and promote AD

performances. The abundance of *Methanobacterium* and *Methanothermobacter*, which are considered to enhance hydrogenotrophic methanogenesis, increased by 2 times and 4 times after the addition of residue biochar respectively [28]. The unique alkaline properties of biochar can enhance the process of hydrogenotrophic methanogenesis. Increased methane production rates are accompanied by slower pH drops because biochar can alleviate the acid inhibition caused by VFAs produced by hydrolytic acidification [29]. Biochar can also increase the conversion rate of microorganisms to VFAs [30].

Jiang et al. found that the addition of blue algae biochar can significantly improve the microbial community structure and greatly increase the abundance of *Methanoscrina* species during the AD of sludge hydrolyzate [31]. Under low inoculum, the rate of VFAs consumption slows down significantly, and the *Methanoscrina* receives additional electrons through cyanobacterial biochar-mediated DIET to enhance AD performances. Pan et al. confirmed that high specific surface area and porous biochar can promote DIET, enabling methanogens such as *Methanosarcina* to establish specialized methanogenic regions [32].

Yan et al. found that when the TS of inoculated sludge was 10 g and the waste apple branch biochar with a sludge concentration of 100 % TS was added, the abundance of methanogens 45 days after AD was 105.4 % higher than before digestion, where the abundance of hydrogenotrophic methanogens such as methane microorganisms is 59.7 % [33,34]. When the added TS of inoculated sludge was 20 g, the concentration of methanogens was found to be approximately 20.6 % higher than the previous group, and the abundance of methane microorganisms increased to 64.1 %. Therefore, biochar can significantly increase the number of Methanomicrobiales and improve hydrogenotrophic methanogenesis.

Qin et al. dosed synthetic magnetic biochar into an AD reactor and found that a large number of microorganisms with hydrolysis, acid-/acetogen production and methanogenic ability were adsorbed on the magnetic biochar, which can significantly shorten the contact distance of microorganisms [35]. The *Moraxellaceae* and *Pseudomonadaceae*, two strains that can participate in DIET, were also detected on the magnetic biochar. M., Wang et al. also confirmed that magnetic biochar with high conductivity can act as an electron shuttle in syntrophic metabolisms in the digestive system, improving the AD performance significantly [36]. Studies have shown that, on the one hand, as the pyrolysis temperature increases, the formation of magnetite increases, so the dissimilatory iron reduction induced by amorphous iron oxide is weakened, and more terminal electrons are used for methane production [36,37]. On the other hand, magnetite can act as an electron conduit to stimulate DIET between syntrophic bacteria and methanogens [38]. Therefore, biochar can significantly increase the number of *Methanomicrobiales* and improve hydrogenotrophic methanogenesis.

Tian et al. applied 30 mg/L nano-graphene to an up-flow anaerobic sludge bed. After 55 days of AD, methane production increased by 14.3 % compared with the control group [39]. Here, the presence of graphene nanomaterials accelerated the cleavage of acetic acid and enhanced the acetoclastic methanogenesis. Covarrubias-García et al. increased methane production by 47.0 % with the addition of reduced graphene oxide materials modified with magnetite nanoparticles (rGO-MNPs) [40]. The application of this composite nanomaterial increased the electron shuttling capacity and the hydrogenotrophic activity of the microorganisms, while iron ions released from the system acted as nutrients for microbial growth. Overall, the addition of nanomaterials induces a major improvement in biogas production and purity, with the best improvement observed for iron-based composite nanomaterials.

In particular, nanomaterials can optimize the two so-called impurity gases,  $H_2$  and  $CO_2$ , and convert them into methane by promoting hydrogenotrophic methanogenesis, and to increase the methane content in the biogas. In short, due to their larger specific surface area and higher reactivity, nanomaterials can participate in reactions as catalysts, microbial supports, electrode catalysts, and mass transfer enhancers, thereby improving reaction rates, product selectivity, and stability. It

can also be concluded from Table 1 that the effect of iron-based and carbon-based nanomaterials on AD is also limited by the effect of the digested substrate. The specific summary is as follows:

Firstly: 0.5–1.0 g/L nZVI, 0.05–0.25 g/L  $nFe_3O_4$ , 0.1 g/g dry matter biogas residue and corn straw biochar are suitable for treating sewage sludge.

Secondly: 5.0 g/L nZVI, 5.0 g/L  $nFe_3O_4$ , and 1.0 g/g dry matter biogas residue and sawdust biochar are suitable for treating kitchen waste.

Finally: 20–100 mg/L nZVI, 15–20 mg/L  $nFe_3O_4$  and 0.05 g/g dry matter fruit wood biochar are suitable for treating livestock and poultry manure.

In addition to the above, the potential of iron-modified biochar nanomaterials for AD is also huge. In the future, research can focus on the mechanism and application of iron-modified biochar in AD.

We performed a simple overall cost analysis of adding nanomaterials to AD. As shown in Table 2, the overall cost is determined by the production cost of the nanomaterial itself and the economic benefits of the methane generated by the nanomaterial. Without considering transportation costs, the price of biochar is approximately 2.43 Yuan/Kg [58]. Since there are few literatures mentioning the manufacturing cost of  $nFe_3O_4$ , we start from the raw materials, especially  $nFe_3O_4$  is mostly synthesized by co-precipitation of ferric chloride and ferrous sulfate. Their costs are 3 Yuan/kg and 0.22 Yuan/kg respectively, so the cost of 1 Kg of  $nFe_3O_4$  is about 7.25 Yuan. In addition, nZVI can be synthesized through the chemical reduction method of ferric chloride and sodium borohydride. However, since the commercial price of sodium borohydride is about 60 times that of ferric chloride, the cost of 1 kg of nZVI is approximately 428.88 yuan. In addition, the price of generated methane is based on the average natural gas price for residential users of 3.91 Yuan/m<sup>3</sup> [59]. Based on the above information, without considering other factors (excipients, energy consumption, etc.) in the production process of nanomaterials, we conducted a rough cost analysis of several typical documents cited in Table 1 based on 1t substrate. If the economic output from additional methane produced is greater than the costs of the nanomaterials, the overall cost effectiveness is displayed as positive, otherwise it is negative. From Table 2, we can see that  $nFe_3O_4$  performs relatively well, probably because its dosage is low but its economic output is high. However, when it comes to kitchen organic waste, it is difficult to achieve good economic results with high dosages. Unless magnetic separation of  $nFe_3O_4$  is used for recycling. In addition, it can be seen from the table that anaerobic co-digestion is also an important means to recover costs [57]. Overall, the application of iron-based and carbon-based nanomaterials can bring cost advantages in terms of increasing gas production rates, reducing gas treatment costs, and improving system stability, making AD a more attractive option for waste disposal and energy recovery methods. However, there are still relatively few applications in actual engineering, and sufficient engineering design and economic evaluation need to be carried out according to specific circumstances to ensure that the advantages of these nanomaterials are maximized.

### 1.1.2. Maintainance of the nanomaterials enhanced AD system

Ammonia nitrogen is an essential nutrient for anaerobic microorganisms during AD processes; at certain concentrations, ammonia nitrogen provides a good buffering effect on the digestion process. However, excessive concentrations of ammonia nitrogen could also inhibit the growth of microorganisms. The two main forms of ammonia nitrogen are ionized ammonium ( $NH_4^+$ ) and gaseous free ammonia ( $NH_3$ ). In general,  $NH_3$  is permeable to bacterial cells, so it is more toxic than  $NH_4^+$  [60]. In the AD process, ammonia is produced mainly from two pathways: ammonia dissociation and anammox reaction. During the AD treatment, organic matter is decomposed by anaerobic bacteria into small molecular compounds, including proteins and amino acids containing amino groups ( $-NH_2$ ). These organic compounds were decomposed under anaerobic conditions, producing substances such as  $NH_3$

**Table 2**

Overall cost analysis of AD with additional nanomaterials.

Type of Nanomaterials	Dosage	Digestive Substrate	Additional Average Methane Production	Economic Output from Additional Methane Produced (¥)	Costs of the Nanomaterials (¥)	Overall Cost Effectiveness
nZVI	1.0 g/L	Sewage sludge	20.58 ml/g VS	1.07	428.88	Negative
nZVI	5.0 g/L	Kitchen waste	36.4 ml/g VS	24.73	2129.4	Negative
nZVI	0.1 g/L	Livestock and poultry manure	78 ml/g VS	201.89	42.888	Positive
nFe <sub>3</sub> O <sub>4</sub>	120 mg/L	Sewage sludge	112 ml/g VS	10.22	0.806	Positive
nFe <sub>3</sub> O <sub>4</sub>	5.0 g/L	kitchen waste	29 ml/g VS	19.70	36.25	Negative
nFe <sub>3</sub> O <sub>4</sub>	20 mg/L	Livestock and poultry manure	53.13 ml/g VS	97.3	0.145	Positive
Biogas residue biochar	0.16 g/g dry matter	Sewage sludge and kitchen waste	136.5 ml/g VS	26.88	19.44	Positive
Corn straw biochar	0.058 g/g dry matter	Sewage sludge	20.84 ml/g VS	4.84	14.094	Negative
Biogas residue biochar	1.0 g/g dry matter	Kitchen waste	32.3 ml/g VS	6.31	14.58	Negative
Sawdust biochar		Kitchen waste and sewage sludge	171.1 ml/g VS	144	60.75	Positive
Fruit wood biochar	0.05 g/g dry matter	Livestock and poultry manure	120 ml/g VS	90.18	33.92	Positive

and some organic acids. Furthermore, the decomposition and transformation of some intermediate products such as amino compounds will generate NH<sub>3</sub>.

Jia et al. added 1000 mg/L nZVI to the AD of sludge and found that the concentration of ammonia nitrogen can be maintained at 600–800 mg/L during the stable period [42]. Compared with the control group, the dosage of 1000 mg/L nZVI reduced the concentration of ammonia nitrogen and created the most favorable conditions for biogas production. Here, nZVI acts as a pH buffer through the dissociation of Fe<sup>0</sup> and Fe<sup>3+</sup>, thus reducing the ammonia nitrogen content and promoting methane production [9]. Amen et al. also found that adding nZVI can remove 18.6 % more total ammonia nitrogen than the control group because nZVI can remove ammonia nitrogen through cation exchange [61]. Zero valent iron was shown to activate the methanogenic activity of *Methanosarcina* in a manner that enhanced electron conversion under ammonia-suppressed conditions. *Methanosarcina* is capable of acetoclastic methanogenesis and hydrogenotrophic methanogenesis, and can directly accept electrons to produce methane through DIET [62]. Zhang et al. found that compared with the control group, under the condition of high ammonia nitrogen concentration, the biogas production after adding nZVI and sulfurized nZVI was still 10 %–60 % higher [63]. The two materials showed the best promoting effect at 1500 and 2500 mg/L ammonia nitrogen concentration, indicating that these two materials can improve the tolerance of the system to ammonia nitrogen and relieve the high inhibitory effect caused by ammonia nitrogen stress. However, when the sulfidated nZVI was added, the abundance of the previously mentioned *Methanosarcina* increased by nearly 3 times, indicating that sulfidated nZVI can enhance methanogenesis through DIET [32].

Sharma et al. proposed that biochar has a good ammonia reduction effect, which is attributed to the adsorption of ammonia ions in the matrix by biochar [64]. W., Yan et al. found that under the conditions of ammonia nitrogen of 1500.0 mg N/L and free ammonia of 49.9 ± 3.9 mg NH<sub>3</sub>-N/L, the time to peak VFAs concentration is prolonged and VFAs accumulation is higher [65]. In all experimental groups, the methane production rate decreased significantly, and the intracellular adenosine triphosphate concentration decreased by 25 to 55 %. This indicates that microbial metabolism is inhibited. Although the AD performance was inhibited, the addition of carbon nanotubes could promote the rapid recovery of methanogenic activity. Iron-modified biochar also exhibited excellent adsorption capacity. Deng et al. successfully increased the ammonia nitrogen adsorption capacity of pure corncob biochar from 5.0 mg/g to 6.4 mg/g using the iron-coated biochar, which ensured the stable operation of the initial stage of AD [66].

In contrast to previous studies, Lü et al. found that biochar mitigated

ammonia inhibition through biochemical rather than physical effects, using biochar of different particle sizes, to alleviate the inhibition of glucose digestion by ammonia nitrogen [67]. Here, biochemical effects promote microbial growth either by facilitating DIET, due to abundant functional groups on the biochar surface, or through the porous structure of biochar. Kizito et al. found that within the concentration range of 250–1000 mg/L ammonia nitrogen in the AD liquid of pig manure, the adsorption rate of wood biochar and rice husk biochar to ammonia nitrogen can reach 80.0 %, and even under higher load (the concentration of ammonia nitrogen reaches 1400 mg/L), the ammonia nitrogen adsorption rate can also reach 50.0 % [68].

Zhang et al. added nZVI-BC to an anaerobic co-digestion of sludge and kitchen waste system [69]. Here, the ability of nZVI-BC to alleviate ammonia inhibition in AD is mainly attributed to the cation exchange properties of carbon materials, hydrogen bonding between organic functional groups and ammonium ions, and the fact that the nZVI improves DIET. Lu et al. found that the application of biochar alone in chicken manure AD did not significantly alleviate the inhibition of high ammonia (5 g/L) [70]. Yet the porous structure of the biochar increased the abundance of *Firmicutes*, an acetate bacterium that plays a major role in acidogenesis and methanogenesis by 28.0–54.0 %. Subsequently, after loading nFe<sub>3</sub>O<sub>4</sub> on biochar, the methane production rate of the system was greatly improved. The presence of nFe<sub>3</sub>O<sub>4</sub> increased the tolerance of microorganisms to high ammonia concentrations and alleviated the inhibitory effect of ammonia on AD performance. Usually, in the early stage of AD, with the hydrolysis of protein, the ammonia nitrogen concentration will go through a stage of rapid increase. But Ning et al. found no significant increase in the ammonia nitrogen concentration after adding phosphoric acid-modified biochar (PBC) and PBC loaded with nZVI to the AD treatment of corn stover [71]. On the one hand, these two modified biochars enrich microorganisms, increase microbial activity and abundance, and convert part of ammonia nitrogen into biological available nitrogen. On the other hand, phosphoric acid modification increases the acidic functional groups on the surface of biochar, which is beneficial to adsorption of ammonia. Su et al. used four different modified biochars (iron, chitosan, iron-chitosan, iron-magnesium-chitosan) to reduce the initial ammonia nitrogen concentration of the kitchen digestive juice from 1600 mg/L to 1000 mg/L [72]. The amino group and hydroxyl group on the surface of chitosan form a stable complex with the added metal ions, which can improve its adsorption performance. In addition, it can also adsorb ammonia nitrogen cations through electrostatic interaction [73].

In general, the removal mechanism of ammonia nitrogen by iron-based and carbon-based nanomaterials in AD mainly covers key processes such as adsorption, chemical reaction, biological reduction, and

ion exchange. However, it can be seen from Table 3 that the dosage of iron-based and carbon-based nanomaterials in AD is not clear. This stems largely from the relatively recent nature of the research field and the complexity of nanomaterials. The application of nanomaterials involves many variables, including the type of nanomaterial, particle size, surface properties, water quality conditions, treatment systems, etc. Under different experimental conditions, the optimal dosage of nanomaterials may be different, and future research needs to deeply explore the long-term impact and possible environmental risks of nanomaterials. Despite the uncertainty of dosage, looking forward to the future, iron-based and carbon-based nanomaterials, as a potential environmental treatment technology, still have great application prospects. With the continuous development and in-depth research of science and technology, we are expected to better understand the mechanism of action of nanomaterials and determine more precise dosage, to achieve a more efficient and sustainable AD process and make positive contributions to the protection of water bodies and the environment.

The level of VFAs is a major indicator of the stability of the digestive system during AD. VFAs are key intermediates in the production of methane, and a certain concentration of VFAs are required for methanogenesis during AD treatment. When the concentration of ammonia nitrogen in wastewater is too high, the growth of acid-producing bacteria will be significantly inhibited, and the production rate of VFAs will decrease, thereby affecting methane production. Therefore, by controlling the rate of VFAs production, the effects of ammonia inhibition can be mitigated. For example, R., Wang et al. added activated carbon and nZVI to an anaerobic reactor, and found that activated carbon enriched the abundance of two acid-producing bacteria, *Trichococcus* and *norank\_f\_Bacteroidetes\_vadinHA17* [44]. This is explained by the porous structure of activated carbon, which enhances enzyme activity during lactate and propionate production. The addition of nZVI increased the activity of pyruvate formate lyase during formic acid production and promoted the conversion of formic acid into acetic acid. Ajayi-Banji found that the addition of nFe<sub>3</sub>O<sub>4</sub> reduced the acetic acid concentration of the system by at least 28 % and promoted the conversion of VFAs [52]. This also inhibited the adverse effects of acidification and free ammonia on the system. As a consequence, the system redox potential and the reproducibility of methanogens increased significantly. Johnravindar et al. added different types of biochar to the kitchen-sludge co-digestion system and found that miscanthus straw biochar had the best performance, which could reduce the accumulated VFAs from the highest 28.6 g/L to 2.9 g/L, which was 2.6 times of the control group [15]. The degradation tendency of rice husk biochar and sewage sludge biochar was also better than that of the control. In addition to the physical properties of biochar itself, the aromatic C—C and C—O bonds on the surface of biochar also matter, because the presence of these functional groups accelerates the electron transfer between bacteria and methanogens, thus promoting VFAs degradation [77].

Ragasri et al. added granular iron hydroxide nanomaterials (GNOF) to an activated sludge AD treatment experiment and found that the mean level of VFAs was higher than that of the control group for all six cycles and that iron promoted sludge hydrolysis [78]. Mostafa and Seongwon obtained more methane production from the AD of oleic acid by adding nFe<sub>3</sub>O<sub>4</sub> and carbon nanotubes, which were 1.5 times more efficient in converting butyric acid to methane [79].

Mostafa and Tolba added nFe<sub>3</sub>O<sub>4</sub>, multi-walled carbon nanotubes, and a composite of these to an activated sludge AD experiment and found an increase of the substrate degradation efficiency by 21.1 %, 18.3 % and 24.4 % respectively [79]. This is explained by the high specific surface area of carbon nanotubes and by providing a source of metal ions. Li et al. also found that the addition of 200 mg/L of magnetic carbon catalyzed the hydrolysis of refractory organic matter, and significantly reduced the accumulation of short-chain fatty acids during AD by increasing DIET, thus avoiding system acidification [80]. Therefore, the addition of these nanomaterials increases the conversion rate of VFAs while increasing the generation of VFAs. On the one hand,

**Table 3**

The role of iron-based and carbon-based nanomaterials in alleviating ammonia-nitrogen inhibition.

Type of Nanomaterials	Substrate	Dosage	Effect	References
nZVI	Excess sludge	1.0 g/L	Maintain ammonia nitrogen concentration at 600–800 mg/L	[42]
nZVI	Activated sludge	1.0 g/L	Total ammonia concentrations were reduced by 18.64 %. Maintain ammonia nitrogen concentration at 545 mg/L	[61]
nZVI	Food waste*	2.0 g/L	Total ammonia concentrations were reduced by 29.7 %	[63]
S-nZVI			Total ammonia concentrations were reduced by 38.8 %	
nZVI	Food waste	4–40 mg/g TS <sub>added</sub>	The addition of nZVI could regulate the concentration of ammonia nitrogen.	[74]
nZVI nFe <sub>3</sub> O <sub>4</sub>	Thermal hydrolysis pretreatment activated sludge	10mmol	Effectively promoting the anti toxic activity of microorganisms during the ammonia inhibition stage	[75]
Pinewood biochar	Activated sludge	4.97 g/g dry matter of sludge	Significantly reduce fluctuations in ammonia nitrogen concentration	[76]
White oak biochar		4.40 g/g dry matter of sludge	during high-temperature AD and decreases the free ammonia by 10.5 %	
Carbon nanotube	Anaerobic sludge	1.0 g/L	Accelerate the recovery of methane production performance	[65]
Iron-coated biochar	Swine manure*	20 g/L	Maintain 94 % of the methanogenesis efficiency under high ammonia nitrogen stress	[66]
Fruitwoods biochar	Glucose	10 g/L	Biochar promotes methanogenesis under high ammonia nitrogen stress	[67]
Wood biochar	Piggery manure	1.0 g/L	The maximum adsorption capacity for ammonia nitrogen is 44.64 mg/g (60 % adsorption rate)	[68]
Rice husk biochar			The maximum adsorption capacity for ammonia nitrogen is 39.8 mg/g (53 % adsorption rate)	
nZVI- biochar (corn straw)	Food waste	3.1 g/g TS	Total ammonia concentrations	[57]

(continued on next page)



**Table 3** (continued)

Type of Nanomaterials	Substrate	Dosage	Effect	References
nFe <sub>3</sub> O <sub>4</sub> biochar (corn straw)	Chicken manure	0.15 g/g dry weight	were reduced by 41.65 % Relieve ammonia inhibition	[70]

\* Ammonia chloride amended.

the increased VFAs can alleviate ammonia inhibition; and on the other hand, the addition of nanomaterials improves the conversion of VFAs so that no acidification of the system occurs.

Nanomaterials can remove free ammonia through adsorption and oxidation on the one hand, reduce the inhibitory effect of ammonia on AD performance, and thus reduce the toxic effect of ammonia on microorganisms. On the other hand, it promotes the metabolic activity of microorganisms in AD, reduces the accumulation of VFAs, and thus alleviates the inhibitory effect of ammonia.

### 1.1.3. Inhibition by hydrogen sulfide in the nanomaterials enhanced AD process

H<sub>2</sub>S is formed by sulfate reduction during the AD process and is a component of the produced biogas. The content of H<sub>2</sub>S in biogas should be as low as possible because H<sub>2</sub>S is toxic to the environment and impairs biogas applications [81]. The production of H<sub>2</sub>S in anaerobic reactors causes problems such as odor in the anaerobic unit area, severe corrosion at the gas-water interface in anaerobic systems, and reduced biogas quality, hence it is necessary to inhibit H<sub>2</sub>S production during the AD process. The basis that nanomaterials can control H<sub>2</sub>S concentration in the AD process is that they can enhance DIET, thereby changing the direction of electron flow and allowing methanogens to obtain more electrons [82]. The special structure and surface properties of nanomaterials can enhance the reactivity of pollutants for rapid conversion, thereby accelerating the oxidative decomposition process of H<sub>2</sub>S by converting it into milder oxides [83,84]. In addition, nanomaterials can also absorb H<sub>2</sub>S in organic wastewater, thereby reducing its concentration and reducing its release to the environment.

Specifically, nanomaterials such as iron-based nanomaterials and iron oxide nanomaterials are widely used in the AD process. These nanomaterials have high activity and surface area in the environment, which can effectively control harmful gases such as H<sub>2</sub>S. In addition, the choice of these nanomaterials is influenced by factors such as their price, stability, and ease of production and handling. Therefore, it is very important to select suitable nanomaterials to control the H<sub>2</sub>S content in AD processes. For instance, the addition of 0.05–0.20 g/g TS of nZVI in the waste activated sludge anaerobic reactor induced a decrease of H<sub>2</sub>S content from 300 mg N/m<sup>3</sup> without nZVI dosage to 0.5–6.1 N/m<sup>3</sup> with nZVI dosage in 20 days [84]. This is explained by the formation of FeS, FeS<sub>2</sub> and S<sup>0</sup> by reduction of H<sub>2</sub>S on the shell surface of nZVI. In an aqueous environment, the surface of nZVI forms an oxide layer consisting of two phases. Among them, the phase near the metal core is Fe (II)/Fe(III) mixed phase, while the phase near the oxide/water interface is mainly composed of Fe(III). Hydroxide exposed to water always exists on the surface of the oxide layer, and these hydroxides can effectively remove H<sub>2</sub>S in sludge. Ferric hydroxide can act as an oxidizing agent to oxidize H<sub>2</sub>S to polysulphides and subsequently pyrite [85]. The research of Farghali et al. also came to a similar conclusion: Nano ferrous oxide removes dissolved sulfide from the system by forming ferrous sulfide precipitation [86]. They added three different concentrations of nano ferrous oxide into an AD reactor respectively, and found that all of them could significantly reduce the H<sub>2</sub>S concentration in the reactor, and the cumulative reduction in the biogas tank was up to 53.52 %. However, compared with nanomaterials in the form of Fe(III), the control effect of nano ferrous oxide on hydrogen sulfide does not appear to be that good.

This is because nano ferrous oxide removes dissolved sulfide by precipitating to form FeS [87]. In contrast, nano-iron in the form of Fe (III) first oxidizes dissolved sulfide to S<sup>0</sup>, and then the reduced Fe(II) continues to react with the remaining dissolved sulfide to form FeS [88]. Therefore, nano-iron oxide is not as effective as nanomaterials in the form of Fe(III). Hassanein et al. applied Fe, Ni, and Co nanomaterials to treat poultry manure by an AD process, and found that the mixed nanomaterials with different concentrations can significantly reduce the generation of H<sub>2</sub>S. While the maximum concentration of H<sub>2</sub>S could be reduced by 100 % [48]. The reduction of H<sub>2</sub>S was related to the increasing concentration of iron, nickel, and cobalt mixture composites, and H<sub>2</sub>S was removed by the precipitation formed by FeS and/or NiS with a strong linear relationship (R<sup>2</sup> was 0.997).

Shen et al. observed H<sub>2</sub>S concentrations below 5 ppb in biogas from a reactor supplemented with biochar [89]. Similarly, the integration of straw biochar columns inside an anaerobic reactor resulted in the rapid removal of 89–98 % of H<sub>2</sub>S, sulfides, and free sulfide [90]. This is likely due to adsorption on the biochar surface due to the presence of COOH, -OH, C=O, and minerals. This is related to the pyrolysis and carbonization process of biochar. As the pyrolysis temperature increases, biochar gradually loses surface functional groups such as OH-, C-bound oxygen and hydrogen atoms and has more COOH, -OH, C=O, and minerals, and these remaining functional groups cause higher H<sub>2</sub>S adsorption. Tsui et al. observed an 80.0 % reduction in H<sub>2</sub>S content after the application of biochar, with approximately 26.6 % of total electrons returning from H<sub>2</sub>S production to methane production [91]. In the absence of sulfide, sulfate-reducing bacteria can use organic matter (such as acetic acid) as an electron acceptor for metabolism. They work by using some special enzymes to degrade organic matter into acetate, releasing electrons and protons at the same time. In the absence of sulfide, the final electron acceptors for sulfate-reducing bacteria are usually carbon dioxide or methyl compounds. In this process, methanogens can use these electrons to reduce carbon dioxide and methyl compounds to methane. Collectively, this syntrophic relationship between sulfate-reducing bacteria and methanogens enables the avoidance of H<sub>2</sub>S formation while utilizing these electrons for methanogenesis.

Iron-modified biochar has also demonstrated good H<sub>2</sub>S adsorption properties. Choudhury et al. found that biochar can effectively remove soluble sulfide in the matrix by adsorption [92]. Compared with no addition of biochar, maple biochar can adsorb 219 mg S<sup>2-</sup>/g biochar of soluble sulfide, while maple biochar loaded with Fe(III) can adsorb soluble sulfide of 269 mg S<sup>2-</sup>/g biochar. Iron and sulfur mainly exist in the form of FeSO<sub>4</sub>·7H<sub>2</sub>O, indicating that the adsorbed sulfide is mainly oxidized to sulfate.

As can be seen from Table 4, from the existing literature, iron-based and carbon-based nanomaterials can eliminate H<sub>2</sub>S in AD. The use of iron-based or carbon-based nanomaterials to remove H<sub>2</sub>S in AD is a research area that has attracted much attention. At present, the types and quantities of nanomaterials added to different treatment systems have not been determined. Future research should focus on different types of iron-based or carbon-based nanomaterials, considering the conditions and requirements of different waste treatment systems, finding the best dosage through experiments and simulations, and exploring the physicochemical properties and activities of different nanomaterials to ensure the efficient use of H<sub>2</sub>S remove.

### 1.2. Applications and mechanisms of nanomaterials enhanced AD processes

Researchers have studied the potential mechanisms underlying the promotion of AD by biochar, and proposed various hypotheses [96]. For instance, the dissolution of nanomaterials provides microbial nutrients that enhance AD [97]. Here we present the main mechanisms that improve microbial community response, interspecies electron transfer, and extracellular polymer changes by nanomaterial addition.

**Table 4**

The potential of iron- and carbon-based nanomaterials for hydrogen sulfide removal during anaerobic digestion.

Type of Nanomaterials	Substrate	Dosage	Effect	References
nZVI	Waste activated sludge	0.20 g/g TS	The removal efficiency of hydrogen sulfide is 99.8 %	[84]
nzvi	sulfate wastewater	5 g/L	Sulfate reducing bacteria utilize ferrous ions to reduce hydrogen sulfide concentration	[93]
Nano ferrous oxide	Cattle manure	1000 mg/L	The removal efficiency of hydrogen sulfide is 53.5 %	[86]
nFe <sub>3</sub> O <sub>4</sub>	Poultry litter	1000 mg/L	The removal efficiency of hydrogen sulfide is 100 %	[48]
nFe <sub>3</sub> O <sub>4</sub>	Swine manure	75 mmol	Reduce approximately 70 % sulfate concentration in the system	[94]
Wood-derived biochar	Chicken manure	5 % of TS	The hydrogen sulfide content has decreased by over 95 %	[95]
Corn stover biochar	Anaerobic sludge	3.64 g/g TS	Hydrogen sulfide removal rate greater than 94.4 %	[89]
Wood-derived biochar	Anaerobic sludge	/	The removal efficiency of gaseous H <sub>2</sub> S is greater than 98 %	[90]
Wood chips biochar	Sewage sludge and leachate	/	Reduced H <sub>2</sub> S content by 80 %	[91]
Corn stover biochar	Dairy Manure	1.82 g/g TS	91.2 % reduction compared to the control	[92]
Maple biochar		1.82 g/g TS	90.0 % reduction compared to the control	
Iron-impregnated corn stover biochar		0.50 g/g TS	100 % reduction compared to the control	
Iron-impregnated maple biochar			97.5 % reduction compared to the control	

### 1.2.1. Microbial community response

Anaerobic methanogenesis results from the synergistic interaction of several microorganisms. The degree of degradation of organic matter depends on the activity of microorganisms. The presence of nanomaterials can optimize the microbial community, in particular by enriching microorganisms possessing the DIET capability, thus promoting anaerobic methanogenesis. Xie et al. analyzed the microbial community changes when nFe<sub>3</sub>O<sub>4</sub> was used in AD of sludge [98]. The results show that nFe<sub>3</sub>O<sub>4</sub> can increase the abundance of *Fibrobacter* from 1.5 to 8.2 %. *Fibrobacter* hydrolyzes cellulose into small molecular carbohydrates. At the same time, the abundance of acetoclastic methanogens (such as *Methanosaeta*) increased 1.78 times. nFe<sub>3</sub>O<sub>4</sub> facilitates AD by reshaping the microbial colony, i.e., enriching the relevant flora of the hydrolysis stage and acetoclastic methanogenesis [93]. Kaur et al. also found that nFe<sub>3</sub>O<sub>4</sub> could enrich more genus *Enterococcus*, *Methanosaeta* and *Methanosarcina* during the AD of *Azolla microphylla* [99]. *Enterococcus* belongs to *Phylum Firmicutes* and can ferment cellobiose into lactic acid and then oxidized into acetate. Most of the genes in the reactor that determine the carbohydrate-active enzymes used by

microorganisms to decompose plant biomass come from *Firmicutes* [100]. The KEGG database also shows that the abundance of genes encoding *acetate kinase* and *phosphate acetyltransferase* used by *Methanosarcina* is much higher than that of the control [101]. Zhou et al. also found that nFe<sub>3</sub>O<sub>4</sub> can promote the activities of key enzymes such as *hydrolase*, *dehydrogenase*, *acetate kinase*, and *coenzyme F<sub>420</sub>* during AD [102]. This further proves the previous conclusion that nFe<sub>3</sub>O<sub>4</sub> can enrich bacterial groups related to hydrolysis and acetoclastic methanogenesis.

Guang Yang et al. found that nZVI could significantly increase the abundance of *Clostridium sensu stricto*, *Proteiniphilum*, *Fonticella* and *Pygmaibacter* in the AD system [103]. These bacteria are all related to the production of VFAs. From the gene analysis of substrate metabolism and VFAs formation in the KEGG database, nZVI promotes the abundance of genes related to hydrolysis, membrane transport and acidogenesis processes, fundamentally increasing the production rate of VFAs. Tao Wang et al. found that adding nZVI, nano-iron oxide (nFe<sub>2</sub>O<sub>3</sub>) could double the gene copy number of bacteria and archaea in the AD system, producing more *α-Proteobacteria*, *β-Proteobacteria*, *Bacteroidetes* and *Methanosaeta* [104]. Qiao Wang et al. also found that *Pseudomonas veronii* appeared in the sludge AD system with nano ferric oxide (nFeO) dosage of 200 mg/L [105]. It not only has strong hydrolysis ability, but also dominates the iron reduction process [106]. And whether it is nFeO, nZVI, or nFe<sub>3</sub>O<sub>4</sub>, it can increase the activity of key hydrolysis enzymes such as Alcohol dehydrogenase 4, D-lactate dehydrogenase, and Pyruvate dehydrogenase E1 component. P. , Wang et al. studied the effect of nZVI, nFe<sub>2</sub>O<sub>3</sub> and nFe<sub>3</sub>O<sub>4</sub> on AD treating food waste [45]. In terms of microorganisms, *Proteiniphilum* and *Fermentimonas* associated with hydrolysis and acetogenesis processes were greatly increased compared with the control, indicating that these three nanomaterials promote the processes of hydrolysis and acetogenesis. In terms of archaea, *Methanosaeta* and *Methanosarcina*, the two kinds of methanogens related to acetoclastic methanogens, also increased significantly, with a total abundance of up to 94.1 %, which indicated that the above three materials were mainly enriched in acetoclastic methanogens rather than hydrogenotrophic methanogens. In addition, the three pilin-encoding genes *mshA*, *mshB*, and *mshC* were only detected in the reactor adding nZVI, nFe<sub>2</sub>O<sub>3</sub> and nFe<sub>3</sub>O<sub>4</sub>, suggesting that these three materials effectively enhanced the DIET potential. Like nFe<sub>3</sub>O<sub>4</sub>, most other iron oxides such as nZVI promote AD by enriching bacteria or archaea in the hydrolysis stage and acetoclastic methanogenesis stage, thereby promoting the production of key enzymes.

Pytlak et al. showed that wood biochar can enrich *Prevotella* in *Bacteroidales* in the sugar beet waste AD system [107]. *Prevotella* can participate in hydrolysis [108] and acetogenesis [109], and the abundance of *Prevotella* was positively correlated with the gene responsible for the synthesis of carbon monoxide dehydrogenase related to the acetate-generating Wood-Ljungdahl pathway. Unlike iron-based nanomaterials enriched in acetoclastic methanogens, the substrate digestion fluid "directly stimulated" by biochar is enriched in hydrogenotrophic methanogens, and their methanogenic capacity is higher, which the authors speculate is related to biochar-mediated DIET [110]. During the AD of cow manure, Su Wang et al. also found that rice straw biochar greatly increased the abundance of *Methanomassiliicoccus* and *Methanoculleus* (hydrogenotrophic methanogens) while reducing the abundance of *Methanosaeta* (acetoclastic methanogens) [111]. Although the system is dominated by acetoclastic methanogens, the KEGG database shows that the relative abundance of genes related to acetoclastic methanogenesis decreased by 67.9 %, while the relative abundance of genes related to hydrogenotrophic methanogenesis increased by 44.6 %, which further proves that biochar can enrich hydrogenotrophic methanogens. G., Wang et al. stated that under high ammonia nitrogen stress in swine manure, sawdust biochar can enrich *Methanosarcina* and *Methanobacterium* and relieve ammonia inhibition [112]. The methanogenesis pathway based on the KEGG database shows that hydrogenotrophic methanogenesis contributes the most to methane

production. *Geobacter* can participate in methanogenesis through the DIET pathway [113]. Cai et al. stated that during the AD of pig manure, high-throughput results and quantitative polymerase chain reaction results showed that biochar can significantly increase the absolute abundance of *Geobacter* [114]. In addition, under the ammonia stress of chicken manure, biochar can specifically enrich *Methanoculleus* and *Methanospirillum*, secreting enzymes related to some hydrogenotrophic methanogenesis, such as the Formylmethanofuran-tetrahydromethanopterin N-formyltransferase, methenyltetrahydromethanopterin cyclohydrolase, and formylmethanofuran dehydrogenase subunit A, thus adapting to the ammonia stress and takes a dominant position in the digestive system [115]. In general, in AD systems, biochar can enrich acetoclastic methanogens, and can also specifically enrich hydrogenotrophic methanogens.

To this end, we summarized the effects of modified biochar based on iron materials on changes in AD microbial communities. Chen et al. found that magnetite-loaded biochar could enrich more *Ruminococcaceae* and a small amount of *Pseudomonas* and *Geobacter*, which are all electroactive bacteria [116]. In addition, the abundance of *Methanotrix*, which is an acetoclastic methanogen but can utilize  $H^+$  and  $e^-$  produced by DIET for methane production, was higher in the reactor with magnetite-loaded biochar dosed [117]. *Methanosarcina* was also enriched, with the highest abundance. As mentioned earlier, *Methanosarcina* is capable of acetoclastic methanogenesis and hydrogenotrophic methanogenesis, and can directly accept electrons to produce methane through DIET [62]. The KEGG database results showed that acetoclastic methanogenic and hydrogenotrophic methanogenic genes increased by 49.9 % and 35.1 % respectively. This illustrates the dual effect of magnetite-loaded biochar on AD. Lim et al. studied the effect of iron-modified biochar (nZVI-BC) on the AD of food waste [118]. Compared with the control group, in the reactors dosed with nZVI, BC and nZVI-BC, the bacteria of *Firmicutes* related to hydrolysis increased. But in terms of the archaea, pure biochar enriched more *Methanoculleus*, nZVI enriched more *Methanosaeta*, while nZVI-BC enriched more *Methanosarcina*.

X., Wang et al. used KEGG database to identify genes for key enzymes associated with different methanogenesis pathways [119]. In nZVI-BC-mediated high-temperature ( $55 \pm 1^\circ\text{C}$ ) AD of food waste, the absolute abundance of genes related to acetoclastic methanogenesis increased by up to 28.56 %, and the absolute abundance of genes related to hydrogenotrophic methanogenesis increased by up to 75.29 %. Similarly, the abundance of enzymes related to methane metabolism such as formylmethanofuran dehydrogenase, formylmethanofuran-tetrahydromethanopterin N-formyltransferase, and methenyltetrahydromethanopterin cyclohydrolase also increased by 47.25 % compared with the control. In addition, the reactor added with nZVI-BC had higher *Thermotogae* [120] and *Methanotrix* [121], which are related to carbohydrate degradation and methanogenesis respectively. Overall, nZVI-BC can increase the abundance of functional genes and enzymes in hydrolysis and methanogenesis stages. Wang and Yu also found that after adding nZVI-BC to the AD of food waste, the abundance of *Methanotrix* increased by 23.09 %, while the proportion of *Methanobacterium* decreased by 21.13 % [122]. The KEGG database results found that the relative abundance of hydrogenotrophic methanogenic pathway decreased by 4.9 %, while the relative abundance of acetoclastic methanogenic pathway increased by 2.8 %. Various evidences show that the addition of nZVI-BC seems to contribute more to the acetoclastic methanogenic pathway, followed by the hydrogenotrophic methanogenic pathway.

Overall, the addition of nanomaterials enriched acetoclastic methanogens in the system and promoted performances of the AD process, but nanomaterial-mediated DIET methanogenesis also made a partial contribution. It provides a large surface area and specific chemical properties to facilitate the attachment and growth of microorganisms. At the same time, it can be used as an electron transfer medium in the microbial community to help the electron transfer between

microorganisms. In addition, the combined effects of adjusting the pH value and microenvironment, and serving as a transport medium for nutrients can optimize the structure and function of the microbial community and improve the efficiency and stability of the AD process.

### 1.2.2. Direct interspecies electron transfer

During the AD process, efficient electron transfer is crucial for the interaction of different microorganisms and directly affects substrate digestion [123]. DIET could be facilitated by conductive nanomaterials, thus increasing the rate of methane production [124]. In the absence of nanomaterials, DIET is done by conductive pili and cytochromes, and is thus limited by the size and abundance of pili and cytochromes (Fig. 3A). Addition of conductive nanomaterials such as carbon and iron nanomaterials accelerates electron transfer because nanomaterials are highly conductive and have a large specific surface area. As shown in Fig. 3B, C, iron-based and carbon-based nanomaterials can form electron channels between microorganisms, forming a similar effect to conductive pili and cytochromes. This results in a faster DIET rate and higher efficiency of anaerobic methanogenesis [125]. F., Liu et al. found that in the presence of granular activated carbon, *G. metallireducens* and *G. sulfurreducens* formed a direct electrical connection (Fig. 3B) [126]. Kato et al. found that  $n\text{Fe}_3\text{O}_4$  can extend the conductive pili of microorganisms and make the microbial population conductive [21]. Rotaru et al. found that the co-culture of *Geobacter metallireducens* and *Methanosarcina barkeri* in granular activated carbon even replaced pili for electron transfer [127].

Nanomaterials also reduce the energy consumption of electrogenic species involved in DIET, while extracellular electron transfer is enhanced in methanogens. Zhu et al. found that microorganisms that have extracellular electron transfer capabilities are enriched in the presence of conductive iron materials [128]. The extracellular proteins of their cells contain amide groups and hydrogen bonds that can transfer electrons to participate in long-range electron transfer (Fig. 3C). Xie et al. demonstrated that magnetite nanomaterials and graphite powder additives enhanced DIET by increasing the abundance of methane-oxidizing bacteria and methanolic bacteria [98]. Li et al. focused on the enhancement of AD performances with nZVI/activated carbon dosage, in terms of enhanced phenol removal, organic matter degradation, and methane production [129]. In the presence of nZVI/activated carbon,  $\text{CO}_2$  is not reduced by  $\text{H}_2$  but receives electrons directly and is reduced to methane in the presence of methanogens [130, 131]. In summary, nanomaterials with good electrical conductivity can improve the DIET efficiency of hydrogen-producing and methanogenesis, while organic matter conversion and methane production can also be improved.

Microbial quorum sensing is an information exchange mechanism between species other than DIET, which achieves information transmission by producing signaling molecules to stimulate the expression of specific genes [132]. Quorum sensing of methanogens is similar to secretion of Gram-negative bacteria, affecting the cell assembly and carbon metabolism flux of methanogens by regulating secreted N-acyl-homoserine lactones (AHLs) [133]. There is evidence that exogenous AHLs promote the production of coenzyme  $F_{420}$ , enhance the methanogenic capacity of anaerobic granular sludge, and regulate the community structure of bacteria and methanogens by adjusting extracellular polymers [134]. Moreover, Pearson correlation shows that functional bacterial flora at each stage of AD are significantly related to certain AHLs signals, such as hydrolytic-fermentative bacteria and  $C_4$ -HSL and  $\text{OXOC}_6$ -HSL,  $C_{14}$ -HSL and aceticlastic methanogenesis and hydrogenotrophic *Methanogenesis* is significantly related [135]. In addition, H., Dang et al. also found that more AHLs were detected in the blackwater AD reactor supplemented with granular activated carbon compared with the control [136]. This is consistent with the methanogenic performance, probably because the abundance of *Methanosaeta* on suspended sludge and granular activated carbon biofilms is much higher than the control. Granular activated carbon may modulate the abundance of *Methanosaeta* through quorum sensing, since microorganisms

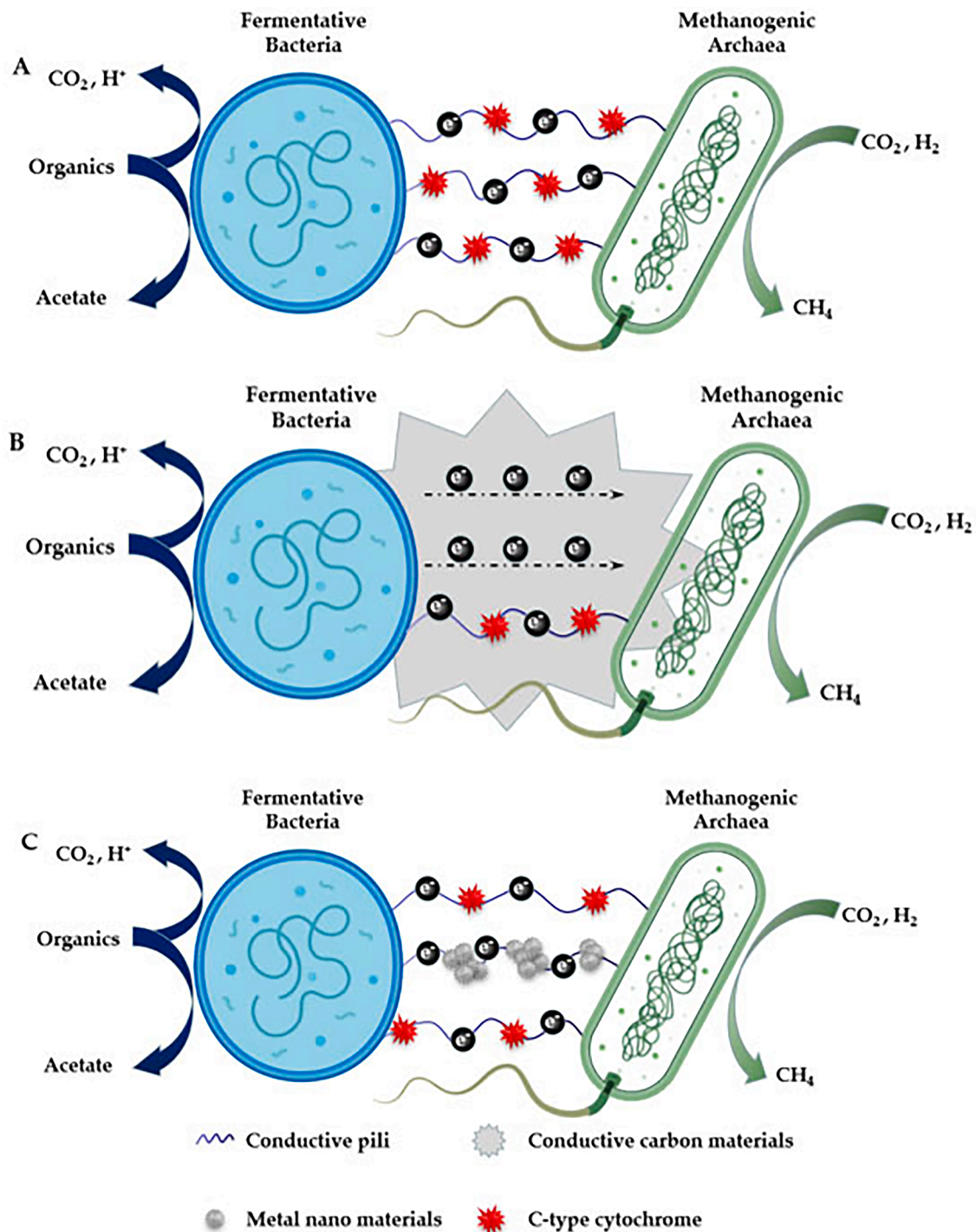
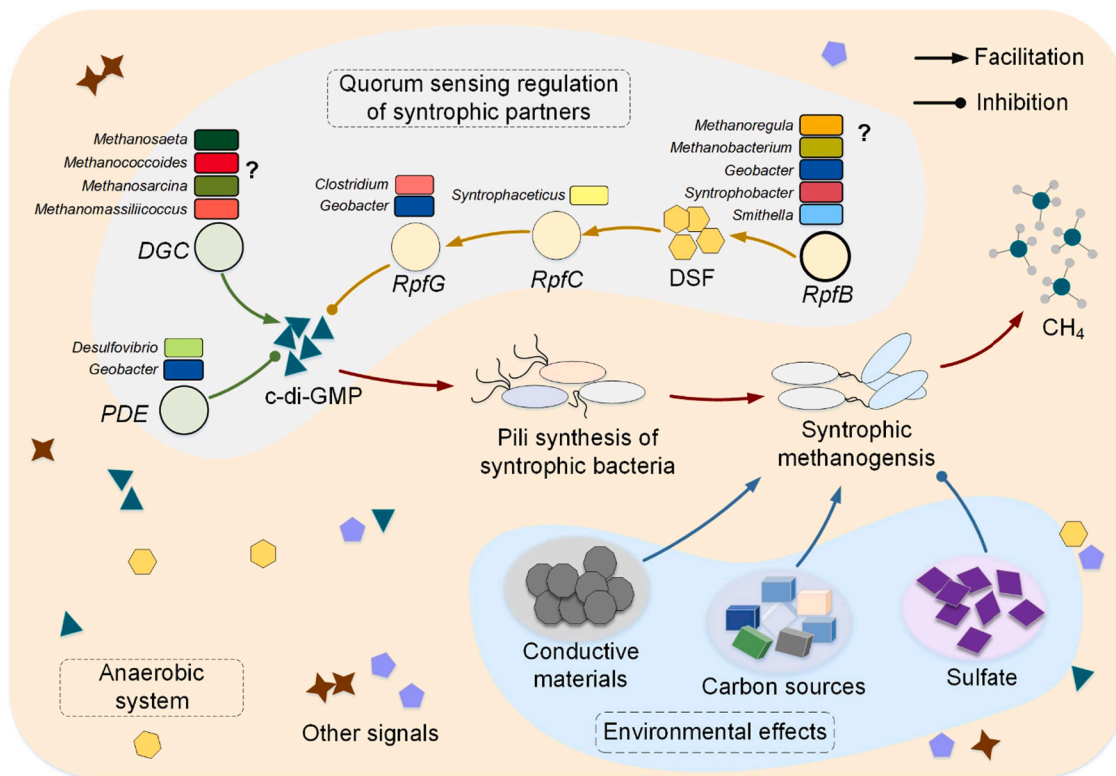


Fig. 3. Mechanisms by which (A) conductive pili and C-type cytochromes promote direct interspecies electron transfer (DIET), (B) conductive carbon materials promote DIET, and (C) conductive iron materials promote DIET.

such as *Pseudomonas*, *Geobacter*, and *Methanosaeta* have been shown to efficiently produce AHLs [133,135,137]. *Pseudomonas*, *Geobacter* was shown to be an electroactive bacterium that promotes DIET of *Methanotherix* (family *Methanosaeta*) through e-pili or c-type cytochromes omcS [138]. The *Pseudomonas*, *Geobacter* and *Methanosaeta* mentioned here have been shown to be effectively enriched by iron-based or carbon-based nanomaterials in the previous section. Therefore, as typical conductive materials, DIET mediated by iron-based and carbon-based nanomaterials can affect the release of signaling molecules in AD systems [139]. In addition to AHLs directly related to methanogens, other signaling substance genes such as autoinducer-2 (AI-2),

diffusible signal factor (DSF), and 3'-5'cyclic diguanosine monophosphate (c-di-GMP) also play an important role in AD of syntrophic microorganisms [140]. For example, the secretion of c-di-GMP can promote the production of type IV pili and promote the formation of biofilms [141]. Fig. 4 introduces the potential role of quorum sensing in syntrophic methane production, which also shows that conductive materials can promote the syntrophic methane production process. Conductive nanomaterials have been proven to replace c-Cyts and conductive pili as electronic connection devices, achieving long-distance electron transmission and accelerating the DIET phenomenon [142]. Therefore, the addition of conductive nanomaterials can have a certain



**Fig. 4.** Potential roles of quorum sensing and direct electron transfer between microbial species in the methanogenesis pathway of syntrophic microorganisms. The entire yellow area represents the AD process. The gray area represents the quorum sensing effect of syntrophic microorganisms, the circles represent different quorum sensing signal molecules, and the rectangles represent different microorganisms related to the methanogenesis process. The blue area represents the influence of other factors such as conductive materials on the methanogenesis process. The two different arrows represent facilitation and inhibition respectively. The figure has been authorized for publication.

impact on the secretion of quorum sensing signal molecules, thereby improving AD, but the specific mechanism requires further research.

Extracellular polymers (EPS) are polymers excreted by microorganisms, accounting for 80 % of the mass of activated sludge, and represent a crucial medium for the exchange of electrons between the inner and outer cell layers [143]. He et al. found a 30 % increase in methane production when exposed to 30 mmol/L nZVI [139]. This suggested that the additional dihydrogen released by nZVI oxidation could stimulate the hydrogenotrophic methanogens. The nZVI particles did not enter the interior of the sludge particles but were adsorbed on the surface of the anaerobic granular sludge in the form of iron oxide aggregates [43]. This protected most microbial cells from contact damage. In addition, the extracellular polymers located on the outer surface of the anaerobic sludge particles can react with nZVI. This accelerates the corrosion of nZVI and reduces the release of dihydrogen during nZVI dissolution. Thus, the toxicity of nZVI to anaerobic microorganisms is further attenuated. Rapid hydrogen production and accumulation following nZVI dissolution can inhibit methanogenesis, while slow dihydrogen release can increase methanogenesis [144,145].

Ye et al. found that the addition of Fe<sub>2</sub>O<sub>3</sub> significantly promoted extracellular polymer production and increased cytochrome concentration, resulting in a 35.52 % increase in methane production [146]. The formation of large and dense bioaggregates and the increase of cytochromes accelerated the direct electron exchange between coprophilous and methanogens [147]. The concentration of humic substances increased significantly after nanomaterials dosage in the study, which reduced the amount of total VFAs, and produced more redox-active mediators that participated in the interspecies electron transfer process through intermediary pathways, thus promoting anaerobic methanogenesis [148]. Therefore, extracellular polymers can store electrochemical energy in sludge and can effectively improve the

DIET process, which ultimately enhances anaerobic methanogenesis.

## 2. Inactivation of antibiotic resistance genes by nanomaterials

The overuse of antibiotics and various antimicrobial drugs in human health and livestock farming etc. is a major health issue since it could accelerate the proliferation of antibiotic-resistant bacteria that diffuse antibiotic-resistance genes in environmental media [149]. The spread of antibiotic resistance genes in environmental media may represent a higher environmental hazard than antibiotics themselves [150,151]. Pathogenic microorganisms can acquire antibiotic resistance genes by vertical or horizontal gene transfer [152,153]. Vertical gene transfer is essentially a self-replication process of resistant microorganisms carrying antibiotic resistance genes. Horizontal gene transfer is mainly driven by mobile genetic elements such as plasmids, transposons, or phages, which are capable of spreading between conspecifics and heterospecifics, and even between resistant and non-resistant microorganisms [154]. Antibiotic resistance genes can remain in various organic wastes such as sewage sludge [155], livestock and poultry manure [156], and kitchen waste [157]. If not disposed of properly, antibiotic resistance genes can quickly spread in large numbers in the human environment and pose a serious risk to human health.

Nanomaterials can reduce gene transfer by immobilizing organisms or inhibiting the growth of drug-resistant microorganisms [158]. For instance, the addition of 160 mg/L nZVI reduced the absolute and relative abundance of antibiotic resistance genes by 5.5–9.4 and 2.0–6.3 logs, respectively, during the AD of cattle manure [159]. Results of redundancy analysis showed that the content of antibiotic resistance genes was directly correlated with the changes in mobile genetic elements. 160 mg/L nZVI reduced the content of four mobile genetic elements, *Int11*, *Int12*, *Tn916/1545* and *ISCR1* by 15.1 %. The gene copy

number of *Tn916/1545* (the highest content) was 8.95 logs lower than that of the control.

P., Wang et al. obtained antibiotic resistance genes removal rates of up to 86.64 % and 31.08 % in high and low-temperature AD reactors for kitchen waste disposal respectively [46]. Results showed that the reduction in the content of antibiotic resistance genes after the addition of nZVI was significantly correlated with the abundance of *Firmicutes*, which dominated the process. The antibiotic resistance gene content decreased as *Firmicutes* abundance decreased from 35.47 to 25.35 % at high temperatures, and from 25.62 to 21.47 % at medium temperatures. *Streptococcus* is the host of a macrolide resistance gene that positively correlates with *int11* [160]. The relative abundance of *Streptococcus*, the dominant microorganism belonging to *phylum Firmicutes* genus in the anaerobic digestive system, decreased from 9.74 to 0.56 % at medium temperature (35 °C), and from 0.19 to 0.17 % at high temperature (55°C) after the addition of nZVI. Lu et al. used Procrustes analysis to find that microbial communities and mobile genetic elements contributed 37.0 % and 58.9 % to the variation of antibiotic resistance genes respectively [161]. Therefore, the addition of nZVI influences the changes in antibiotic resistance genes by inducing changes in microbial communities and mobile genetic elements [141].

Yang et al. found that the relative abundance of most antibiotic resistance genes detected in pig manure AD systems could be reduced by more than 85 % at 5–10 % biochar dosage treatment, and that the removal of *Int11* could be increased by 15 % at 5 % biochar dosage treatment [162]. The addition of biochar had a significant impact on the bacterial community by reducing the relative abundance of antibiotic resistance genes of bacteria. This was related to the porous structure of the biochar, which was able to immobilize microorganisms by adsorption and reduce microbial exchange, thus reducing horizontal gene transfer. In contrast to previous studies, Li et al. applied biochar and nZVI-BC to the AD of sludge and found that both could reduce sludge antibiotic resistance genes, with nZVI-BC addition producing a better effect than biochar dosage alone [163]. Because compared with ordinary biochar, the specific surface area of nZVI-BC is greatly increased, which is beneficial to inducing the oxidative stress response of microorganisms, increasing the antioxidant capacity of microorganisms, and reducing the inheritance of ARGs. At the same time, the higher the antioxidant capacity, the less favorable it is for the occurrence of DNA damage-induced responses, leading to severe damage to the cell membrane and inhibiting the horizontal transfer of ARGs. In addition,  $\text{NH}_4^+\text{-N}$  promoted horizontal gene transfer of antibiotic resistance genes, while biochar and nZVI-BC reduced  $\text{NH}_4^+\text{-N}$  content in the system, alleviating the phenomenon of ammonia stress [164] and effectively removing *Sul1* with antibiotic target replacement as the resistance mechanism.

The study by Yue Lu et al. and Shuo Yang et al. also showed that the removal rate of antibiotic resistance genes was significantly correlated with the methane production of AD, and a higher removal rate of antibiotic resistance genes brought about more methane production [161, 162]. Given the excellent synergistic performance of the composite material with nano-iron and biochar, composite nanomaterials can effectively remove antibiotic resistance genes from the digestive system while enhancing AD performance, which should be a promising development direction for the future.

### 3. Conclusion

This review paper comprehensively summarizes the diverse roles of nanomaterials in the AD process. The findings endorse that nanomaterials enhance AD either by promoting acetoclastic methanogenesis or hydrogenotrophic methanogenesis. From the perspective of various dosages of nanomaterials that promote biogas production or methane content,  $\text{nFe}_3\text{O}_4$  seems to be the best choice. The latter, which utilizes  $\text{H}_2$  and  $\text{CO}_2$  in biogas, has the potential to increase methane yield and presents a promising direction for future development due to its ability to purify biogas in situ. The mechanistic analysis uncovers the

transformative impact of nanomaterials on microbial community structure, modification of extracellular polymeric properties, and reinforcement of interspecies electron transport. Furthermore, the investigation delves into the risk-controlling role of nanomaterials in curtailing the dissemination of antibiotic resistance genes. These discoveries underscore the potential of nanomaterials in optimizing and augmenting the efficiency of AD. Nevertheless, further exploration of the long-term implications of nanomaterials on the environment and ecosystems is imperative to ensure their sustainable application and safety. Studies on the synergistic relationship between composite nanomaterials, such as magnetic iron-carbon composites, and their interaction with microorganisms in the AD system, are necessary to avoid possible hazards in the application of composite nanomaterials. Overall, this study offers valuable insights to advance the application of nanomaterials in the realm of AD.

Currently, iron-based and carbon-based nanomaterials are mainly used in AD. In the future, more types of nanomaterials, such as metal sulfides and metal organic frameworks (MOFs), can be explored and developed to improve catalytic efficiency and adsorption performance. Meanwhile, one can focus on designing nanomaterials with multifunctional properties to meet the needs of different AD. These multifunctional nanomaterials can not only promote biogas production and methane content, but also resist risks posed by various novel pollutants such as antibiotic resistance genes, thereby improving waste treatment efficiency. On the other hand, in future researches, attention should also be paid to the sustainability and environmental friendliness of nanomaterials. This includes research on green synthesis methods, utilization of renewable resources, recycling of waste to reduce adverse impacts on the environment.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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