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Effects of heavy metals and antibiotics on performances and mechanisms of anaerobic digestion

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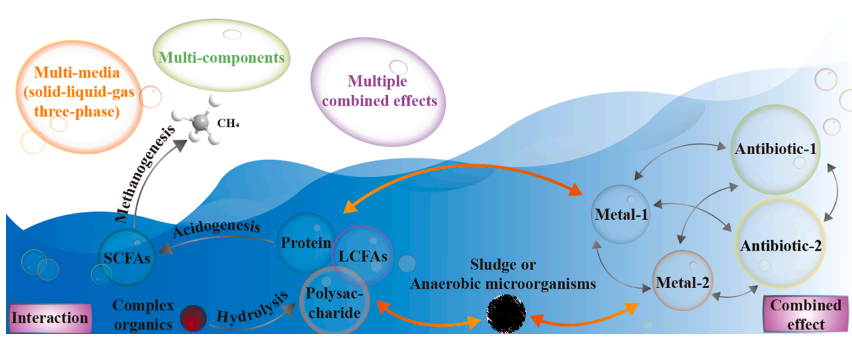
HIGHLIGHTS

- The effects of different kinds of metals or antibiotics on AD performance are summarized and compared.
- The joint action and interaction mechanisms of metals or antibiotics or both with anaerobic microorganisms are reviewed.
- The key active species and pathways for electronic or signaling transmissions are identified and commented.
- Control strategies for enhanced performance and metal recovery in AD are proposed.
- Future research needs for AD are suggested.

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic digestion (AD) is an efficacious technology to recover energy from organic wastes/wastewater, while the efficiency of AD could be limited by metals and antibiotics in substrates. It is of great significance to deeply understand the interaction mechanisms of metals and antibiotics with anaerobic microorganisms, as well as the combined effects of metals and antibiotics, which will help us break the inherent dysfunction of AD system and promote the efficient operation of AD. Therefore, this paper reviews the effects of metals, antibiotics and their combinations on AD performance, as well as the combined effects and interactional mechanisms of metals and antibiotics with anaerobic microorganisms. In addition, control strategies and future research needs are proposed. This review provides valuable information for the enhancement strategies and engineering applications of AD for organic wastes/wastewater containing metals and antibiotics.

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1. Introduction

Anaerobic digestion (AD) is an efficacious bioenergy strategy for the treatment of organic wastes (e.g. animal manure, straw and food waste) and highly concentrated organic wastewater (e.g. swine wastewater) (Chan et al., 2019a; Ghofrani-Isfahani et al., 2020; Liu et al., 2021a; Qi et al., 2021; Tan et al., 2021; Wang et al., 2019). Compared with aerobic treatment, AD not only has the characteristics of low nutrient requirements, high organic loading rate (OLR) and low operating cost, but also has the advantages of energy saving and productivity, and 50 % to 70 % of methane can be recovered from the biogas it produces (Park et al., 2018). Methane is a critical renewable energy source (Shen et al., 2016). The complete combustion of methane per unit mass (1 g) can release 55.7 kJ of heat (Shen et al., 2016). Compared with other hydrocarbons, methane releases more heat and produces less carbon dioxide (Shen et al., 2016). At the same time, the generation and use of methane has promoted the global carbon circulation and energy utilization.

AD consists of a series of microbial processes, the essence of which is to complete the three stages of hydrolysis, acidogenesis (acetogenesis) and methanogenesis through the syntrophic collaboration between non-menthanogens and methanogens (Pavlostathis and Giraldo-Gomez, 1991). In this syntrophic metabolism between syntrophic bacteria and methanogens, the slowest kinetics becomes the rate-limiting step of the entire AD process. (1) When the substrate is complex organic matter or carbohydrate, hydrolysis is the rate-limiting step (Ai et al., 2018; Pavlostathis and Giraldo-Gomez, 1991; Yu et al., 2017). (2) When the substrate is biodegradable, the rate-limiting steps are the utilization of long chain fatty acids (LCFAs) and short chain fatty acids (SCFAs) and methane production (Chan et al., 2019a; Novak and Carlson, 1970), and the increase of saturated fatty acids chain length leads to the decrease of AD rate (Novak and Carlson, 1970). The main challenge of AD is to overcome the inherent obstinacy of mass transfer between complex organic substances/LCFAs and anaerobic microorganisms.

Because there are great differences in environmental sensitivity, nutrient requirements and growth kinetics between non-methanogens and methanogens, maintaining a good syntrophic collaboration relationship between non-methanogens and methanogens is the key to stabilize AD system (Park et al., 2018; Pavlostathis and Giraldo-Gomez, 1991). Antibiotics/heavy metals in industrial wastewater and animal manure/wastewater will break the original AD balance, lead to the change of anaerobic microbial population or change the normal physiological function of anaerobic microorganisms, which shows the following trends: (1) low concentration promotes the utilization of organic acids/methane production and establishes a new equilibrium at the same time, and (2) high concentration inhibits (Banks et al., 2012; Chan et al., 2019a; Li et al., 2021; Liu et al., 2021a; Qi et al., 2021; Wang et al., 2018b; Yenigün et al., 1996; Zhao et al., 2019; Zhu et al., 2021). High concentrations of antibiotics/heavy metals further hinder the performance of AD on the basis of the original mass transfer barrier. Moreover, it is often complicated to recover the activity of anaerobic microorganisms from AD system inhibited by antibiotics/heavy metals (Liu et al., 2021a; Mushtaq et al., 2022; Qi et al., 2021; Zhou et al., 2021).

The reinforcing effect of "fighting poison with poison" can be realized by the combined effects between metals and metals, antibiotics and antibiotics, metals and antibiotics. Studies have shown that the presence of certain metals/antibiotics can subside the toxicity of another metal/antibiotic and even enhance the efficiency of AD (Aydin et al., 2015; Zhang et al., 2019b). In addition, certain metals/metal nanoparticles (M−NPs) can regulate the interspecies electron transfer pathway and take direct interspecies electron transfer (DIET) as the dominant role, thus achieving the purpose of improving AD performance (Gorby et al., 2006; John et al., 2017; Lovley and Holmes, 2022; Ye et al., 2018a). DIET can not only effectively promote the hydrolysis process of complex organic matter, but also accelerate the utilization of LCFAs/SCFAs and methanogenesis (Chan et al., 2019a; Qi et al., 2021; Wang et al., 2018a; Wang et al., 2019). In addition, the AD reactor dominated by DIET can eliminate the adverse impacts of antibiotics and high concentration of metals and improve the performance of AD.

This paper mainly reviews the effects of metals, antibiotics and their combinations on AD, and the main objectives are: (1) to understand how metals, antibiotics, and their combinations promote or inhibit the performance of AD reactors; (2) to reveal the combined effects and interaction mechanisms of metals, antibiotics, and their combinations with anaerobic microorganisms; (3) to identify the key active substances and electronic/signaling transmission pathways; and (4) to propose some control strategies and the future research prospects from the micro point of view.

2. Parameters affecting the stability of anaerobic digestion systems

The stability of AD system is mainly judged by LCFAs, SCFAs, pH value, total ammonia nitrogen (TAN) and hydrogen partial pressure (Chan et al., 2019a; Park et al., 2018; Qi et al., 2021; Rasit et al., 2015; Zhou et al., 2018). It is reported that the AD system collapses when the hydrogen partial pressure is higher than 10^{-4} atm (Park et al., 2018). The pH value suitable for AD is 6–8 (Clark and Speece, 1971). The concentration of SCFAs should be less than 1000–1500 mg/L (Buyukkamaci and Filibeli, 2004). Hill et al. (1987) proposed that when the ratio of propionic acid to acetic acid was greater than 1.4 or the concentration of acetic acid was greater than 800 mg/L, the AD system was about to collapse. Different LCFAs inhibited AD at different thresholds, such as 30–300 mg/L for oleic acid, 100–300 mg/L for stearic acid and 30 mg/L for linoleic acid (Fernández et al., 2005). When the concentration of TAN was 1500–7000 mg/L, it might cause instability of AD system (Rajagopal et al., 2013). The variability of inhibition thresholds of these parameters may be due to the influence of substrate type, inoculum characteristics, temperature, redox potential, hydraulic retention time (HRT), operation mode and exogenous substances (Fernández et al., 2005; Rajagopal et al., 2013; Zhou et al., 2018). Moreover, in some cases, the reported inhibition thresholds are much higher than the above-mentioned inhibition concentration ranges due to the adaptation of anaerobic microorganisms to highly toxic environments (Fernández et al., 2005; Rajagopal et al., 2013).

LCFAs are the main inhibitors of AD (Rasit et al., 2015). It is reported that lipid-rich substrates (e.g. animal manure, food waste, municipal sewage, food processing wastewater and slaughterhouse wastewater) will be hydrolyzed into more glycerol and LCFAs (Chan et al., 2019a; Rasit et al., 2015). Because of the slow β-oxidation process of LCFAs, LCFAs cannot be rapidly converted into SCFAs, and the degradation rate of saturated LCFAs (e.g. palmitic) is 5 times slower than that of unsaturated LCFAs, so LCFAs is easy to accumulate in AD reactor, which seriously affects the stability of AD system (Chan et al., 2019a; Pavlostathis and Giraldo-Gomez, 1991). In addition, the characteristics of substrates may also affect the removal of LCFAs (e.g. palmitic) (Fernández et al., 2005). It is reported that the main reason for palmitic accumulation may also be attributed to its high melting point (61–62.5 ℃) (Chan et al., 2019a). In the thermophilic AD system, the heterogeneity of solid palmitic and anaerobic sludge may limit mass transfer (Chan et al., 2019a). The mechanism of LCFAs inhibiting AD is that LCFAs can produce toxicant effects on the adsorbed cell wall of microorganisms (Rasit et al., 2015). In addition, heavy metal cations can improve the performance of AD by reducing the toxicity of LCFAs or removing LCFAs (Table 1) (Chan et al., 2019a; Chan et al., 2019b).

pH value, HRT, OLR, sulfate and heavy metals will affect the removal and production pathways of SCFAs (Table 1) (Chan et al., 2019a; Chan et al., 2019b). It is reported that SCFAs production is highest when pH value is between 5.0 and 6.0, while SCFAs production is inhibited when pH value is less than 4.0 (Wang et al., 2014). Propionic acid is easily produced in extremely acidic environment (pH ≈ 4.0) (Wang et al.,

Table 1
Conditions that affect the production/utilization of LCFAs/SCFAs of AD.

Type	Dose	Substrate	Inoculum	OLR	HRT	pH	Effect on LCFAs	Effect on SCFAs	Reference
$ZnSO_4$ or $ZnCl_2$ [a]	5–100 mg/L	Co-digestion[c]	Seeded sludge[e]	3.8 g COD/L/day	10 days	7.7 ± 0.2	Boosted its utilization	Boosted its utilization[i,j]	(Chan et al., 2019a)
ZnO NPs[b]	0.5–5.5 g VS/L	Petroleum wastewater	Seeded sludge[f]	15.9 g COD/L	–	7.6	Boosted its production and utilization	Boosted its production and utilization[i]	(Ahmad, 2020)
CuO[b]	120 mg/L	Cattle manure	Seeded sludge[e]	–	–	6.9 ± 0.3	–	Inhibited its utilization	(Luna-delRisco et al., 2011)
ZnO NPs[b]	0.32–34.5 mg Zn/L	Mixture of SCFAs	Seeded sludge[g]	Increase stepwise from 1 to 3 g COD/L/day	Reduced stepwise from 36 to 12 h	–	–	Inhibited its utilization	(Otero-González et al., 2014)
ZnO NPs[b]	100 mg/g TSS 200 mg/g TSS	Synthetic wastewater[d]	Seeded sludge[f]	2000 mg COD/L	–	6.9 ± 0.1	–	Inhibited its utilization[ijk] Inhibited its production and utilization[ijk]	(Mu et al., 2012)
$CuSO_4$ or $CuCl_2$ [a]	1–10 mg/L	Co-digestion[c]	Seeded sludge[e]	3.8 g COD/L/day	10 days	7	Boosted its utilization	Boosted its utilization[ijk]	(Chan et al., 2019b)
$CuSO_4$ [b]	1–10 mg/L	Glucose	Seeded sludge[h]	20000 mg COD/L	4 days	5.7	–	Inhibited its production[ijk]	(Yenigün et al., 1996)
$ZnSO_4$ [b]	5–40 mg/L							Inhibited its production[ijk]	

[a] Pilot scale.
[b] Lab scale.
[c] Mixture of food waste and domestic wastewater.
[d] Contained glucose and sodium acetate.
[e] From a local wastewater treatment plant.
[f] From long-term operated reactors.
[g] From a full-scale reactor treating brewery wastewater.
[h] From the acidification tank of a full scale anaerobic treatment plant.
[i] Acetic acid.
[j] Propionic acid.
[k] Butyric acid.

2014; Zhou et al., 2018). When the pH value is 4.0–7.0, the production of butyric acid shows a downward trend, while acetic acid is opposite, but when the pH value is 4.0–6.0, butyric acid still accounts for the largest proportion, followed by acetic acid (Zhou et al., 2018). When pH value is 6.5–7.0, the content of butyric acid and acetic acid is basically the same (Zhou et al., 2018). Qiao et al. (2016) confirmed that when HRT was greatly shortened or OLR was excessively increased, pH value would decrease and SCFAs would accumulate significantly, among which propionic acid accounted for the largest proportion, and the AD system inhibited by propionic acid was difficult to recover for a long time. The consumption of acetic acid and butyric acid can be considered as the signal of the recovery of propionic acid oxidation bacterial activity (Qiao et al., 2016). Sulfate-reducing bacteria (SRB) can take exogenous sulfate as electron acceptor, promote partial oxidation of LCFAs to acetic acid or acetic acid and propionic acid, and accelerate the utilization of propionic acid, thus restoring AD performance (Qiao et al., 2016; Rabus et al., 2006).

3. Effects of heavy metals and antibiotics on performance of anaerobic digestion systems

3.1. Heavy metals

Metals are crucial to the rate and stability of AD. These trace elements could be introduced in the chemical speciation of salts, elementary substances, oxides, or smaller nanoparticles (NPs) (Chan et al., 2019a; Ma et al., 2019; Qi et al., 2021; Ye et al., 2021). However, high concentrations of metals can also cause toxicity and interfere with the normal metabolism of anaerobic microorganisms (Zhang et al., 2019b).

3.1.1. Hormesis effects of heavy metals on performance of hydrolysis and acidogenesis

3.1.1.1. Zinc. Many studies have confirmed that zinc is essential for hydrolysis and acidogenesis process of AD (Table 2).

The promotion or inhibition of ZnO NPs in the hydrolysis stage should be discussed in stages. The 5–100 mg/g TS ZnO NPs significantly affected the dissolution and hydrolysis of organic compounds in the process of AD of cow manure (Qi et al., 2021). Soluble chemical oxygen demand (SCOD) increased at the low concentration of 5 mg/g TS ZnO NPs (Qi et al., 2021). Meanwhile, 5–100 mg/g TS ZnO NPs resulted in significant accumulation of two degradation products (i.e., soluble protein (SP) and soluble polysaccharide (SC)), as well as NH_4^+-N (Qi et al., 2021). It was confirmed that ZnO NPs could significantly boost the dissolution and hydrolysis of complex organic compounds, especially at low concentrations, and inhibited the hydrolysis of SP and SC (Qi et al., 2021). Similarly, Zhang et al. (2019b) showed that ZnO NPs of 5–100 mg/g-TSS inhibited protein degradation, and speculated that this inhibition might be attributed to the increase of Zn^{2+} concentration. Zhao et al. (2019) reported that with the extension of reaction time, the inhibition of 30 mg/g ZnO NPs on protein degradation showed a trend of first strengthening and then weakening. The highest inhibition rate was 28.3 percent at 14 h, but reduced to 7.7 percent at 36 h, and no significant inhibition was observed after 84 h (Zhao et al., 2019).

The inhibition of ZnO NPs on hydrolysis and acidogenesis process can be receded by introducing other metals. It was reported by (Zhang et al., 2019b) that adding ZnO NPs alone could inhibit the hydrolysis and acidogenesis process, while adding TiO_2 NPs alone had no effect on the hydrolysis and acidogenesis process. However, adding TiO_2 NPs and ZnO NPs mixture promoted the hydrolysis and acidogenesis process (Zhang et al., 2019b). The presence of TiO_2 NPs could attenuate the toxicity of ZnO NPs and boost the degradation of proteins and volatile fatty acids (VFAs), because the combination of TiO_2 NPs and ZnO NPs

Table 2
Conditions related to the promotion/inhibition of zinc on hydrolysis, acidogenesis and methanogenesis process of AD.

Type	Dose	Particle size	Reactor type	Substrate	Inoculum	Temp. (℃)	Effect on hydrolysis	Effect on acidogenesis	Effect on CH_4 production	Reference
Salt	50 mg/L	–	Batch	Pig manure	Seeded sludge[d]	35	–	–	Decreased by 26.34 %	(Yang et al., 2020)
ZnO NPs	5–100 mg/g TS	80–100 nm	Batch	Cattle manure	–	55	Promoted/ Inhibited[f]	Inhibited	Decreased by 84.55 % to 93.72 %	(Qi et al., 2021)
$ZnSO_4$ or $ZnCl_2$	5–100 mg/L	–	Semi-continuous	Co-digestion[b]	Seeded sludge[e]	35 ± 1	–	Promoted	Promoted by 26 % to 78 %	(Chan et al., 2019a)
ZnO NPs	0.5–5.5 g VS/L	0.35 nm	Batch	Petroleum wastewater	Seeded sludge[d]	37	Promoted	Promoted	Promoted	(Ahmad, 2020)
ZnO NPs	120 mg/L	50–70 nm	Batch	Cattle manure	Seeded sludge[e]	36	–	–	Decreased by 43 %	(Luna-delRisco et al., 2011)
ZnO		1 µm							Decreased by 18 %	
ZnO NPs	5–100 mg/g TSS	200 nm	Batch	Waste active sludge	Seeded sludge[e]	37 ± 1	Inhibited	Inhibited	–	(Zhang et al., 2019b)
ZnO NPs	100 mg/g TSS	140 ± 20 nm	Batch	Synthetic wastewater[c]	Seeded sludge[d]	35 ± 1	–	No significant impact	Decreased by 56.5 %	(Mu et al., 2012)
	200 mg/g TSS							Inhibited	Decreased by 74.9 %	
Immobilized ZnO NPs[a]	150 g/L	3.93 mm	Continuous	Palm oil mill effluent	–	37	Promoted	Promoted	Promoted	(Ahmad and Reddy, 2020)
ZnO NPs	0.4 mg/L	531 nm	Continuous	Synthetic wastewater[c]	Seeded sludge[e]	35	Inhibited	Inhibited	Decreased to 0	(Chen et al., 2019)
ZnO NPs	1 mmol/L	30 ± 10 nm	Batch	Soluble starch and mixed protein	Seeded sludge[d]	37	–	Promoted	Decreased by 50.7 %	(Zhu et al., 2020)

[a] ZnO NPs immobilized by methylenebisacrylamide.
[b] Mixture of food waste and domestic wastewater.
[c] Contained glucose and sodium acetate.
[d] From long-term operated reactors.
[e] From a local wastewater treatment plant.
[f] It boosted the dissolution of organic matter, but inhibited the degradation of SP and SC.

could improve the activity of key enzymes (i.e., protease and acetate kinase), cell viability and related bacterial abundance (i.e., *Proteobacteria, Firmicutes, Bacteroidetes, Chloroflexi, Actinobacteria* and *Acidobacteria*) in AD system, and decreased the concentration of Zn^{2+} in the AD system (Eqs. (1) and (2)) (Zhang et al., 2019b). Nevertheless, there are still a lack of research on the combined effects of different metals on AD system. In future studies, we need to pay attention to the combined effects and mechanisms between different kinds of metals and anaerobic microorganisms, which can help us understand and control the role of metals in AD.

$$ZnO(s) + 2H^{+}(aq) \rightleftarrows Zn^{2+}(aq) + H_2O(l) \quad (1)$$

$$Zn^{2+}(aq) + \equiv TiOH(s) \rightleftarrows \equiv TiOZn^{2+}(s) + H^{+}(aq) \quad (2)$$

However, current studies differ on the threshold value at which zinc can promote or inhibit hydrolysis or acidogenesis process. Some researches have pointed out that Zn^{2+} at 50 and 70 mg/L could effectively promote palmitic acid removal (Chan et al., 2019a). Other researches have indicated that 20–100 mg/L $ZnSO_4$ or $ZnCl_2$ has few effect on the hydrolysis and acidogenesis in the anaerobic co-digestion process of domestic wastewater and food waste (Chan et al., 2019a). However, at low concentrations (5–10 mg/L), Zn^{2+} slightly promoted the hydrolysis and acidogenesis process of dairy wastewater, and at high concentrations (10–400 mg/L), Zn^{2+} inhibited the production of VFAs, and the inhibition effect was positively correlated with Zn^{2+} concentration, and moreover, both low and high concentrations of Zn^{2+} inhibited the production of acetic acid (Yu and Fang, 2001). Yenigün et al. (1996) found that 5–40 mg/L Zn^{2+} weakened the production of propionic acid, butyric acid and acetic acid in an AD reactor with glucose as substrate. When Zn^{2+} concentration was higher than 20 mg/L, propionic acid production stopped completely (Yenigün et al., 1996). Therefore, we can speculate that the appropriate concentration of zinc can facilitate hydrolysis or acidogenesis processes. However, it is also dependent on the properties of the substrate and operating conditions, including temperature, substrate, flora or seeded sludge, food to microorganism ratio, HRT, etc.

At present, most studies focus on the effect of metals on each stage of AD system, but ignore the interaction mechanisms between the intermediates produced in each stage of AD and metals. It is of great significance for us to understand and control the role of metals in AD, maintain the stability of AD system, and speed up the rate-limiting step of AD.

3.1.1.2. Copper. Wang et al. (2018b) proved that the impact of Cu^{2+} (in the speciation of $CuSO_4$) on AD of swine manure mainly occurred at the stage of hydrolysis and acidogenesis. They found that the presence of Cu^{2+} at a concentration of 5000 mg/kg prominently facilitated the hydrolysis of proteins and carbohydrates, and the hydrolysis time was prolonged by Cu^{2+} (Wang et al., 2018b). At the same time, the accumulation of VFAs was also increased in the Cu^{2+} added reactor, which proved that the acidification process of AD could also be conspicuously boosted by the introduction of Cu^{2+} (Wang et al., 2018b). Rai et al. (2019) cloned a high-temperature resistant laccase with Cu as the active center from *Geobacillus* sp. *strain WSUCFI*. This enzyme could increase the hydrolysis of lignocellulosic biomass by 2.02–132 times (Rai et al., 2019). Wyman et al. (2018) used $CuSO_4$ as an enzyme inducer based on lignocellulose. The results showed that laccase enzyme activity increased by 16 % (Wyman et al., 2018). At present, there are few reports on AD hydrolysis or acidogenesis of Cu, and further research is needed.

3.1.1.3. Other metals. With 2.0–20 g/L waste iron powder, the hydrolysis rate of dairy manure was expedited by two times and the lag time was shortened by half, and the hydrolysis rate was independent of the amount of waste iron powder added (Andriamanohiarisoamanana et al., 2018). 80 and 160 mg/L nZVI (nano-zero valent iron) stimulated the enrichment of bacteria related to hydrolysis and acidogenesis during AD of cattle manure (Ma et al., 2019). Adding 0.01 mg/L Mo, 0.1 mg/L Mn and 1 mg/L Se in the chemical speciation of salt could prominently expedite the removal rates of VS and TS in the AD system of rice straw with lignocellulose as the principal ingredient (Cai et al., 2018). A previous study showed that Ba supplementation enhanced hydrolase activity (Muñoz et al., 2016). Whereas, Wyman et al. (2019) reported that Ba at 2, 20, 200 mg/L had little or no effect on the hydrolysis of cellulose and complex organic substrates, while the degradation of cellulose was completely inhibited at up to 2000 mg/L Ba, which was ascribed to the change of the chemical balance of the AD system when Ba was added. However, there is a lack of further experimental evidence to elucidate the mechanism of Ba indirectly inhibiting the hydrolysis of AD.

Studies showed that the chemical speciation of metals would affect the performance of AD. Wang et al. (2018a) reported that when the carbon source of AD was simple glucose, Fe^{3+} or ZVI with a concentration of 20 mg/L could effectively boost the transformation of LCFAs into SCFAs. However, compared with ZVI, Fe^{3+} could remove COD faster (Wang et al., 2018a). Besides, Fe^{3+} could also boost sludge granulation, while ZVI could reduce hydrogen partial pressure and redox potential, and convert the Gibbs free energy required to convert propionic acid to acetic acid into a negative value, thereby boosting the formation of acetic acid and improving the stability and redox ability of AD system (Wang et al., 2018a). Therefore, Fe^{3+} may be mainly responsible for the early stage of acidogenesis, while Fe^{2+} may be more helpful to convert SCFAs into acetic acid.

3.1.2. Hormesis effects of heavy metals on performance of methanogenesis

Appropriate concentrations of Fe, Co, Ni, Cu, Zn, Mo, Se and Mn could effectively promote methane production during AD (Cai et al., 2018; Park et al., 2018; Wang et al., 2018a; Wang et al., 2019). Low concentrations of LCFAs (oleate and stearate) have been reported to inhibit all stages of the AD process in cow manure and this inhibition is irreversible (Angelidaki and Ahring, 1992). Nevertheless, zinc is essential for methanogenesis (Table 2). 20–100 mg/L Zn^{2+} could effectively reduced the accumulation of LCFAs and SCFAs and promoted the conversion of SCFAs into methane, and there was a positive correlation between methane yield and Zn^{2+} concentration (Chan et al., 2019a). Ye et al. (2021) reported that ZVI can produce H_2 (Eq. (3)) after corrosion, and H_2 must participate in the process of CO_2 conversion to CH_4 (Eq. (4)). Therefore, ZVI boosts the conversion of CO_2 to CH_4 and promotes the formation of CH_4.

$$Fe^0 + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^-, \quad \Delta G^{0'} = -5.02 kJ/mol \tag{3}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O, \quad \Delta G^{0'} = -131\, kJ/mol \tag{4}$$

However, high concentrations of the metals inhibited methane yield in the AD process (Otero-González et al., 2014; Qi et al., 2021). Metal particle size may also affect metal toxicity to methanogens (Luna-del-Risco et al., 2011).

Many researches have reported that the results of batch and long-term continuous feeding experiments in AD reactors in the presence of metals are quite different (Otero-González et al., 2014; Wang et al., 2019). Unlike other toxic pollutants, metals cannot be degraded by microorganisms and long-term exposure to them can lead to bio-accumulative toxicity (Otero-González et al., 2014). Moreover, anaerobic microorganisms could tolerate low concentrations of metals in a certain time range (Otero-González et al., 2014). Therefore, in future studies, we need to explore the effect of metals on anaerobic microorganisms, especially methanogens, through long-term continuous feeding reactors. Meanwhile, we also need to attach importance to the study of pilot-scale and full-scale reactor to obtain more reliable and useful results.

These differences indicate that the hormesis effects of metals on the hydrolysis, acidogenesis and methanogenesis of AD are controlled by the chemical composition of substrates, operating conditions, types of metals, total concentration of metals, particle size effect and chemical speciation of metals.

3.1.3. Hormesis effects of heavy metals on performance of anaerobic digestion systems

3.1.3.1. Organic loading rate. In the actual production process, the OLR of wastewater will fluctuate greatly (Wang et al., 2021). Besides, in order to maximize cost savings, engineers will use the maximum OLR parameters and the lowest HRT as possible while expecting the highest biogas yield. However, a momentary change in OLR can turn the AD system into an overload state (Wang et al., 2021). This kind of organic load shock is easy to cause the problem of poor interspecific synergistic effect in AD system, and then result in the cumulation of LCFAs and SCFAs, leading to the permanent inhibition of AD system: (1) irreversibly inhibits the activity of methanogens; (2) VFAs lead to disintegration and loss of granular sludge (Angelidaki and Ahring, 1992; Wang et al., 2021). As we have detailed in sections 2.1.1. and 2.1.2., appropriate amounts of metals can avoid or even eliminate this inhibitory effect while achieving higher biogas production. However, high concentrations of metals will have the opposite effect.

3.1.3.2. Total ammonia nitrogen. It has been shown that appropriate amounts of metal elements can avoid ammonia inhibition in AD system. Banks et al. (2012) demonstrated that metal trace elements (Co, Se, W, Mo and Ni) could reduce the accumulated TAN in mesophilic AD system with ammonia concentrations of 5000–6100 mg/L. In addition, AD system without trace element supplementation cannot remain stable at 2.0 kg vS m^{-3} day^{-1} OLR (Banks et al., 2012). However, additional supplementation of Se and Co could increase OLR up to a maximum of 5.0 kg VS m^{-3} day^{-1} (Banks et al., 2012). Moreover, Se and Co may help to enhance the biological function of hydrogenotrophic methanogens under high ammonia concentration (Banks et al., 2012). Bardi and Aminirad. (2020) reported that at high OLR level, the introduction of co-trace elements (Fe, Zn, Ni, Mo, Cu and Co) not only prevented the inhibition of ammonia, but also promoted the degradation of propionic acid, which enhanced the stability of AD system and significantly increased biogas production. However, trace elements were limited in maintaining the stability of the AD system: (1) when OLR increased to a certain extent, trace elements could no longer maintain the stability of the system, and the AD system collapsed (Bardi and Aminirad, 2020); (2) excessive metals could accelerate the accumulation of TAN in AD system (Qi et al., 2021).

3.1.3.3. Hydrogen partial pressure. The essence of AD is the process of interspecific electron transfer (IET) through the syntrophic collaboration between non-methanogens and methanogens. IET determines whether hydrolysis, acidogenesis and methanogenesis of organic matter can proceed, and the efficiency of the entire AD process relies on the rate and pathway of IET.

H_2 is essential for methanogenesis because it acts as an electron donor during AD and is involved in the earliest and most studied interspecies hydrogen transfer (IHT) process (Stams and Plugge, 2009). However, IHT plays a dominant role in traditional AD reactors, and H_2 partial pressure determines whether the AD process can proceed normally (Stams and Plugge, 2009). Nevertheless, hydrogenotrophic methanogens are very low in abundance in traditional digesters (Stams and Plugge, 2009). Only when there are enough hydrogenotrophic methanogens in the reaction system to carry out hydrogenotrophic metabolism with other microorganisms, so as to continuously consume H_2 and maintain the H_2 partial pressure at a low level (H_2 less than 10^{-4}atm), can the metabolism of anaerobic microorganisms proceed normally (Park et al., 2018). Otherwise, the balance of the AD system will be broken, resulting in the accumulation of a large number of VFAs in the AD reactor, especially propionic acid and butyric acid (Liu et al., 2021b). Finally, the failure of AD process is caused (Liu et al., 2021b).

Moderate metals supplementation may change the IET pathway, avoid the disadvantages of IHT and promote the enrichment of hydrogenotrophic methanogens, which maintains the balance of AD system and improve economic efficiency. Some studies have shown that the addition of nickel foam under high H_2 partial pressure and the supplementation of Fe_3O_4 under high VFAs could enrich hydrogenotrophic methanogens (Li et al., 2021; Zhu et al., 2021). They prevented the collapse of the AD system caused by the cumulation of propionic and butyric acid, and may promote the metabolism of propionic and butyric acid through DIET pathway to boost biogas yield (Li et al., 2021; Zhu et al., 2021). However, the role of metals in the IHT pathway has been neglected in current reports. There is still a lack of in-depth analysis of metabolic pathways of syntrophic bacteria and methanogens at molecular and electronic levels.

3.2. Antibiotics

Many studies have reported the effects of tetracyclines, sulfonamides and macrolides antibiotics on various stages of AD (Aydin et al., 2015; Liu et al., 2021a; Mushtaq et al., 2022). Most of the researches showed that different antibiotics had different inhibitory effects on hydrolysis, acidogenesis and methanogenesis of AD (Gaballah et al., 2021; Liu et al., 2021a). These studies occurred at the mg/L level, while the actual antibiotics concentration in animal wastewater is at the μg/L level (Wei et al., 2011; Zhou et al., 2021).

At present, only a few researches have indicated the impacts of low concentrations of antibiotics on AD of animal wastewater. The results indicated that the concentration of antibiotics directly affected the performance of AD, which showed the trend of promoting or not affecting at low concentration and inhibiting at high concentration (Liu et al., 2021a; Mushtaq et al., 2022). For example, Mushtaq et al. (2022) found that oxytetracycline at low concentration (0.12–1.2 mg/L) had no effect on biogas production by AD of cattle manure, but oxytetracycline at concentration $\geq$ 3 mg/L could inhibit biogas yield. Liu et al. (2021a) operated simulated swine wastewater containing 2 and 4 mg/L tetracycline in a semi-continuous AD reactor for 54 days. The results showed that 2 and 4 mg/L tetracycline could slightly boost the hydrolysis and methane production of organic matter (Liu et al., 2021a). However, when the concentration of tetracycline increased to 8 mg/L, the results of 48-day semi-continuous experiments showed that 8 mg/L tetracycline significantly inhibited the hydrolysis and methane production of organic matter (Liu et al., 2021a). It has been reported that antibiotics are mainly removed by biodegradation and adsorption during AD (Gaballah et al., 2021). The removal rate of various veterinary antibiotics in animal manure ranges from 17.8 % to 100 %, with an average of 73 % (Gaballah et al., 2021). However, most reports lack researches on the pathways of antibiotics removal (Gaballah et al., 2021).

The effects of high concentrations of antibiotics on AD help us to discover the mechanisms of toxic action. However, the effect of low concentrations of antibiotics on the long-term continuous feed reaction system can give us a more reliable and useful understanding of the role of antibiotics in AD in practical situations.

In addition, different classes of antibiotics have a combined effect during AD, which is different from the effect of single antibiotics on AD performance. Aydin et al. (2015) studied the effects of sulfamethoxazole-tetracycline (ST) and erythromycin-tetracycline-sulfamethoxazole (ETS) on methane yield. The results proved that erythromycin showed antagonistic effect on ST, and ST combination showed stronger inhibitory effect on methanogenesis than ETS (Aydin et al., 2015). At present, many reports focus on the effects of single antibiotics on AD. In actual wastewater, however, antibiotics are usually present as mixtures. Therefore, we need to pay attention to the combined effects of multiple antibiotics, including additive effect, synergistic effect, potentiation, antagonistic effect.

3.3. Combined effects of heavy metals and antibiotics

At present, there are few researches on the impact of the combined effects of metals and antibiotics on AD. Wang et al. (2018b) pointed out that in an AD system based on swine manure, the separate addition of chlortetracycline (CTC) (0.5 g/kg dry weight, dw) and Cu^{2+} (5 g/kg dw) promoted hydrolysis, acidogenesis and methanogenesis, but slowed down the reaction rate. However, the simultaneous addition of CTC (0.5 g/kg dw) and Cu^{2+} (5 g/kg dw) not only reduced the reaction rate, but also severely inhibited the hydrolysis and acidogenesis processes, causing a conspicuous reduction in the total production of biogas (Wang et al., 2017; Wang et al., 2018b). Tong et al. (2015) studied the combined effect of tetracycline (TC) and Cu^{2+} to luminescent bacteria (*Vibrio fischeri*). The research indicated that the toxicity of Cu^{2+}-TC complex was lower than that of Cu^{2+} or TC alone, and the Cu^{2+}-TC complex showed strong antagonistic effect (Tong et al., 2015). Zhao et al. (2019) found that supplementing ZnO NPs and sulfamethazine (SM2) respectively had weak or no inhibitory effect on protein and carbohydrate degradation. However, this inhibitory effect was enhanced in AD system where ZnO NPs and SM2 existed at the same time (Zhao et al., 2019). Moreover, the simultaneous addition of ZnO NPs and SM2 had a significant synergistic inhibition on methane production in a short time, however, this inhibition decreased with the prolongation of time (Zhao et al., 2019). Generally speaking, different kinds of antibiotics of the same class will have different combined effects with the same metal. In practice, many antibiotics and metals exist simultaneously in AD system. At present, researchers focus on single factor research, while ignoring the combined effect of multiple poisons.

4. Metabolic mechanisms and regulation of syntrophic bacteria and methanogens

4.1. Heavy metals

4.1.1. Regulation of interspecies electron transfer

The IHT pathway of traditional anaerobic digester requires the participation of multiple enzymes and consumes a lot of energy (Stams and Plugge, 2009). More importantly, this process is prone to the accumulation of toxic intermediates such as propionic acid due to high organic load, resulting in the failure of AD process (Stams and Plugge, 2009).

The emergence of a new syntrophic mode between syntrophic bacteria and methanogens based on DIET undoubtedly opens up a new situation for IET, which makes the electron transfer process between syntrophic bacteria and methanogens faster and has higher environmental resistance, and solves the problems of poor interspecific synergy and easy collapse of IHT mechanisms in traditional AD reactors (Gorby et al., 2006; John et al., 2017; Lovley and Holmes, 2022).

Numerous studies have shown that metals can regulate the IET pathway in AD system, and DIET is the dominant position, not IHT. Therefore, metals can efficiently promote the hydrolysis, acidogenesis and methanogenesis of AD.

At present, the generally accepted mechanisms of DIET process in which metals act as electron donors/acceptors can be summarized as follows (Fig. 1): (1) electrons are transported to the outer surface of cells through porin-cytochrome complexs, Multi- haem c-type cytochrome (c-Cyts) and electron shuttles (e.g. flavins, quinones and phenazines) (Lovley and Holmes, 2022); (2) electron shuttles act as transport media to transfer electrons between cells and extracellular electron acceptors or other cells, or through direct contact with electron acceptors by c-Cyts, conductive pili (e-pili), bacterial nanowires, intercellular nanotubes and metal-containing non-biological conductive materials (CMs) (Gorby et al., 2006; John et al., 2017; Lovley and Holmes, 2022); (3) metals can further improve the efficiency of DIET by promoting the

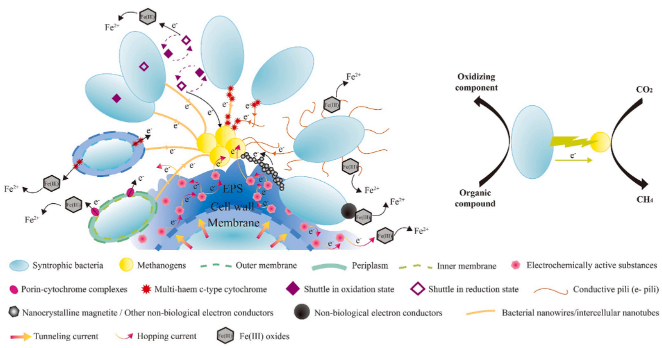

Fig. 1. Mechanisms of DIET process of metals as electron donors/acceptors.

secretion of c-Cyts or other electrochemically active substances by anaerobic microorganisms (Ye et al., 2018a); (4) extracellular polymeric substances (EPS) promotes DIET process by storing electrochemical active substances (e.g. flavins and c-Cyts) (Xiao et al., 2017).

Among them, the research on the interaction between bacterial nanowires and metals was reported by (Gorby et al., 2006). There exist few studies to fill the knowledge gap. The discovery of nanowires is helpful for us to understand the mechanisms of metal oxides and long-distance electron transfer. However, the research on electron transfer between metals and nanowires is relatively lacking at present. Up to now, no one has clarified the complete composition of nanowires, the mechanisms of electron acceptance/giving by nanowires and conductive metal solids, and the interaction mechanisms between anaerobic microorganisms/electrochemically active substances and nanowires.

Dubey and Ben-Yehuda. (2011) discovered the bacterial communication mediated by nanotubes for the first time, which occurs between the same/different kinds of bacteria and promotes the exchange of cytoplasmic contents. In the report of (John et al., 2017), an intercellular nanotube similar to bacterial nanowires but different from bacterial nanowires was first proved in AD microbial community. The length of nanotubes and nanowires is more than 5 μm, but the diameter of nanotubes is 0.13–0.47 μm, which is much higher than that of nanowires (50 to > 150 nm) (Gorby et al., 2006; John et al., 2017). They found that the generation of nanotubes is due to the stress of microorganisms under environmental pressure, such as Fe_2O_3 NPs and insoluble microcrystalline cellulose, which leads to microbial stress and induces the establishment of nanotubes, and speculated that the microbial communication mode established by nanotubes may be ubiquitous in AD (Gorby et al., 2006; John et al., 2017).

EPS plays an important role in AD of DIET system. It has been reported that EPS can store electrochemically active substances, such as flavin and c-Cyts, which is helpful for microorganisms to carry out DIET (Xiao et al., 2017). Because every microorganism is coated with EPS and the thickness of EPS layer can be as high as 1 μm, we can't ignore the role of EPS in AD electron transfer system (Dohnalkova et al., 2011; Xiao et al., 2017). However, in the process of AD water treatment and organic solid wastes treatment, there are almost no studies on the interaction between EPS and metals. In addition, the effect of EPS was not considered when explaining the role of c-Cyts and CMs in DIET.

In the existing reports, researchers have found that EPS affects the transformation of metals in AD system. Because of the strong absorption ability of EPS, EPS can bind, accumulate and isolate metals in the environment, and affect the redox reaction of metals (Dohnalkova et al., 2011). It has been shown that c-Cyts and EPS participate in the formation of M–NPs and store M–NPs in EPS (Kang et al., 2014; Marshall et al., 2006). Among them, Kang et al. (2014) reported that the reduction of Ag^{+} to Ag NPs was ascribed to the hemiacetal groups of sugars in EPS. Therefore, in the presence of metals, EPS may be an important part of anaerobic microorganism participating in electron transfer. Xiao et al. (2017) proposed that electron "hopping" is the most possible mechanism for electrons to pass through EPS. However, it is necessary to systematically and deeply study the interaction mechanisms between metals, anaerobic microorganisms, electrochemically active substances and EPS.

Metals also affect EPS. Metal cations are helpful to bridge EPS and neutralize the negative charge on the surface of sludge (Li et al., 2012). Some metals (e.g. Fe and Mn) can also promote the secretion of EPS in sludge, including tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS) (Ye et al., 2018a). It has been reported that both EPS and metal cations contribute to the formation of microbial aggregates because EPS has functional groups (e.g. hydroxyl, phosphoric and carboxyl groups) (Li et al., 2012; Ye et al., 2018a). EPS helps the microorganism to attach to the electron acceptors/donors, facilitates the long-distance IET between the syntrophic bacteria and methanogens, and saves energy for reaching the acceptors/donors (Xiao et al., 2017). Therefore, metal cations may reduce the distance of IET between methanogens and syntrophic bacteria through the augment of EPS and the formation of microbial aggregates, thus achieving the effect of high efficiency, rapid and energy saving.

Among them, Fe is the most studied metal. It has been reported that Fe can increase the percentage of biological aggregates larger than 100.0 μm (Ye et al., 2018a). Besides, Fe can also change the structure of EPS (Ye et al., 2018a). Ye et al. (2018a) found that proteins, lipids and b-d-pyranose polysaccharides in biological aggregates were concentrated in the outer layer of granules in the experimental reactor with exogenous Fe addition, instead of the interwoven network constructed by uniform distribution in the control reactor. Electron donating/accepting capacities analysis of EPS showed that Fe significantly improved the redox properties of EPS and promoted the electronic exchange ability of EPS (Ye et al., 2018a). The analysis of electrochemical active substances in EPS showed that Fe could conspicuously boosted the production of c-Cyts and humus. Among them, quinone in humus also contributes to electron exchange (Ye et al., 2018a).

At present, the researches on DIET mechanisms of metal-enhanced organic hydrolysis, acidogenesis and methanogenesis mainly focus on the electrochemical test to prove that metal can effectively enhance the redox reaction in AD process and reduce the electron transfer resistance, and they proved that metals enriched microorganisms with DIET activity through 16S rRNA high-throughput sequencing, thus reaching the conclusion that metals promoted the DIET between syntrophic bacteria and methanogens (Ye et al., 2018a; Ye et al., 2018b; Yun et al., 2021). However, these conclusions are somewhat arbitrary. After all, there is no research to confirm the exact mechanisms of DIET, and the essence of anaerobic microbial metabolism is that microorganisms exchange information and carry out basic life activities through electronic transport.

4.1.2. *Toxicity of heavy metals to anaerobic microorganisms*

The toxicity of M–NPs to anaerobic microorganisms has been widely reported. However, the toxicity mechanisms of M–NPs to anaerobic microorganisms are still controversial. In terms of ZnO NPs, many studies have reported that the principal reason for the toxicity of ZnO NPs to anaerobic microorganisms is the release of Zn^{2+} by ZnO NPs (Zhang et al., 2019b). However, some studies have confirmed that a small amount of Zn^{2+} dissolved by ZnO NPs has no toxic effect on *E. coli*, but can be used as a nutrient to promote the survival of *E. coli* (Brayner et al., 2006; Wang et al., 2016). The NPs of ZnO themselves are the main reason of sterilization (Brayner et al., 2006; Wang et al., 2016). In fact, M–NPs themselves and metal ions/metal ions released by M–NPs can poison anaerobic microorganisms. However, the mechanisms of cytotoxicity induced by M–NPs and metal ions have not been systematically and deeply studied.

M–NPs and metal ions can poison cells in many ways including (1) reducing the number of bacteria and archaea and reducing the diversity of microbial community (Zhang et al., 2019b); (2) increasing the level of extracellular lactate dehydrogenase (LDH), thus destroying the integrity of cell membrane and causing damage to cell membrane (Zhang et al., 2019b); (3) inducing reactive oxygen species (ROS) production and impairing cell function (Zhang et al., 2019b); (4) interrupting transmembrane electron transfer, pitting cell wall, oxidizing cell components and reducing the activities of various key enzymes (Li et al., 2008; Zhang et al., 2019b); (5) inhibiting biological activity (i.e., quorum sensing signal molecules and ATP) and inhibiting the expression of EPS and bacterial kinetic related genes, thus causing toxic effects on anaerobic microorganisms (Cheng et al., 2019); (6) affecting DNA replication, damaging DNA and inhibiting DNA repair (Li et al., 2008); (7) M−NPs with small size (about 5 nm) will lead to the change of local electronic structure on the surface of NPs, which will produce electronic effect and increase the reactivity of the surface of M−NPs (Morones et al., 2005); (8) metal ions and small-sized M−NPs can interact with bacteria directly, attach to the surface of membrane and enter bacteria, which affects the permeability of membrane and interferes with the respiration of bacteria (Morones et al., 2005). In addition, different kinds of M−NPs/metal ions can also produce combined effects to affect cytotoxicity (Zhang et al., 2019b).

Advanced technologies (e.g. molecular biology and machine learning) provide technical support for us to explore and predict the toxicity of M−NPs/metal ions to anaerobic microorganisms. In the future research, we need to explore the combined effects mechanisms of metals at the molecular level. Strive to find new toxicological pathways on the basis of traditional toxicological mechanisms. This provides theoretical and technical support for the design of efficient and economical AD reactors in the future.

4.1.3. Mitigation of stress of heavy metals on anaerobic microorganisms

EPS not only plays an active role in AD of DIET, but also acts as a protective barrier to prevent anaerobic microorganisms from being damaged in the presence of high concentrations of metals (Dohnalkova et al., 2011). By transforming metals into M−NPs and sealing them in EPS, the M−NPs can be isolated from cells and prevented from entering cells directly (Gorby et al., 2006; Kang et al., 2014; Wang et al., 2016). Wang et al. (2016) confirmed that the sealing of ZnO NPs by EPS was realized by forming NP-EPS complexes. The carboxylate-like structures and major protein-like structures (amide II) and/or glycoprotein in EPS were participated in the complexation of ZnO NPs (Wang et al., 2016). Every microorganism is wrapped in EPS (Xiao et al., 2017). Therefore, EPS acts as an important part to the defense system of metals invading microbial cells.

Plenty of studies have proved that metals can enhance the protective effect of EPS by motivating the secretion of EPS by microorganisms (Kang et al., 2014; Ye et al., 2018a).

EPS can also reduce the ROS level of AD system, thus stabilizing the performance of AD (Zakaria and Dhar, 2021). However, the interaction mechanisms between EPS and ROS needs further study.

The formation of intercellular nanotubes mentioned in 3.1.1. is also a microbial communication mode spontaneously established by anaerobic microorganisms in response to the toxicity of Fe_2O_3 NPs to cells, so as to resist the influence of adverse environment (John et al., 2017). However, the mechanisms of nanotube communication between anaerobic microorganisms under metal stress are still unclear.

4.2. Antibiotics

4.2.1. Stress on bacteria

According to the different types of mechanisms of antibiotics on bacteria, they are divided into different categories (Tenover, 2006). Tetracycline antibiotics inhibit the synthesis of bacterial protein by acting on 16S r-RNA conserved sequence of 30S ribosomal subunit, thus preventing the combination of *t*-RNA to site A (Kapoor et al., 2017). Macrolide antibiotics can inhibit the early stage of bacterial protein synthesis, which is due to the targeted binding of macrolide antibiotics to 50S ribosomal subunit (Kapoor et al., 2017; Tenover, 2006). Different kinds of sulfonamides can inhibit different steps of folic acid metabolism (Kapoor et al., 2017). Sulfonamides inhibit DNA synthesis by inhibiting bacterial metabolic pathway (Tenover, 2006).

It has also been reported that tetracyclines and macrolides can promote the release of LDH, which leads to cell membrane damage. Liu et al. (2021a) pointed out that low levels of tetracycline (2 mg/L) hardly effected LDH release, however, high levels of tetracycline (8 mg/L) led to a sharp increase in LDH release. Liu et al. (2018) found that high concentration of azithromycin (69 mg/L) induced LDH release from anaerobic sludge, and the release amount of flocculent sludge was more than that of granular sludge. This indicates that azithromycin can destroy the integrity of cell membrane, and the resistance of granular sludge is stronger than that of flocculent sludge.

4.2.2. Stress on archaea

The effects of the same class of antibiotics on methanogens are quite different. (1) Tetracycline antibiotics: He et al. (2020) reported that oxytetracycline (100–200 mg/L) enriched methanogens with more affinity for hydrogen and acetic acid, which were *Methanobacterium* and *Methanosaeta*. However, Sanz et al. (1996) found that chlortetracycline (152 mg/L) can completely inhibit acetoclastic methanogens. (2) Macrolide antibiotics: Ni et al. (2020) pointed out that roxithromycin (0.01–1 mg/L) inhibited the function of acetoclastic methanogens. However, another study found that tylosin (25–250 mg/L) had no influence on acetoclastic methanogens (Sanz et al., 1996). The effects of antibiotics on methanogens are different, and the influence rule of a certain class of antibiotics on methanogens has not been found, and there is no report on the response of methyltrophic methanogenic archaea to antibiotics. In addition, there is no report on the response of methyltrophic methanogens to antibiotics.

4.3. Combination of heavy metals and antibiotics

The mechanisms of combined effects between metals and antibiotics are related to the types of metals/antibiotics. In the AD system where SM2 and ZnO NPs exist simultaneously, SM2 will cause (1) ZnO NPs to release more Zn^{2+} in a short time; (2) an increase in the concentration of Zn^{2+} and SM2 complexes (Zhao et al., 2019). Therefore, the existence of SM2 and ZnO NPs at the same time can enhance the toxicity of AD compared with the existence of SM2 or ZnO NPs alone (Zhao et al., 2019). However, another study reported that Cu^{2+}-TC complex was formed due to the strong complexation between Cu^{2+} and TC (Tong et al., 2015). The toxicity of Cu^{2+}-TC complex was significantly lower than that of the system in which Cu^{2+} or TC acted alone, showing an antagonistic effect (Tong et al., 2015). Wang et al. (2017) pointed out that the combination of CTC and Cu^{2+} can enrich *Treponema* in a short time compared with the AD system with CTC or Cu^{2+} alone, which means that CTC and Cu^{2+} can terminate AD in advance.

5. Effective control strategies

5.1. Addition of nanoparticles coated with a variety of metals

NPs have preeminent physical and chemical properties, such as high specific surface area, high reactivity and high specificity, which exhibit extraordinary properties that bulk materials and micron-sized materials usually do not show (Hassanein et al., 2021; Wu et al., 2018). Many researches have shown that when M−NPs (1–100 nm) are added into AD system, M−NPs can serve as electron channels between syntrophic bacteria and methanogens, and enhance DIET process (Hassanein et al., 2021; Qi et al., 2021; Zhou et al., 2021). Ghofrani-Isfahani et al. (2020) indicated that Fe_2O_3-TiO_2 NPs and NiO-TiO_2 NPs accelerated the hydrolysis and acidogenesis stages of lignocellulose AD process compared with metal salts (i.e., $FeCl_3$ and $NiCl_2$). In the rate-limiting step of lignocellulose AD, NPs showed better performance than metal ions. In addition, metal ions can also be reduced to M−NPs by EPS as electron acceptors during AD (Gorby et al., 2006; Kang et al., 2014). It can be speculated that part of the positive effects of metal ions on AD are realized through NPs. It has been proved that coating metal oxides on inert carrier nanomaterials (e.g. TiO_2, Al_2O_3, SiO_2) can increase the proportion of metal atoms exposed to NPs, and improve the reactivity of metals, thus increasing the bioavailability of metal elements (Ghofrani-Isfahani et al., 2020). At the same time, we can't ignore the toxic effect of the direct interaction between small-sized NPs and cells (Morones et al., 2005).

In Part 2.1., we have described that different metals have different positive/negative effects on the hydrolysis, acidogenesis and methanogenesis stages of AD under different substrates and operating conditions, and the mechanical mixing of different metals has a combined effect on anaerobic digestion. In Part 2.3., we have also explained the combined effects of metals and antibiotics. Many studies have confirmed that the comprehensive effects of mechanical mixing of different metals/metals and antibiotics on AD are not simple superposition. For example, the adverse effects of ZnO NPs on AD can be removed by TiO_2 NPs, however, TiO_2 NPs alone have no effect on AD (Zhang et al., 2019b).

In practice, multiple metals, antibiotics or metals and antibiotics exist simultaneously in AD system, so we need to focus on the combined effects of multiple metals, antibiotics or metals and antibiotics in AD process. This is helpful for us to understand and control the AD process, and make it develop in the direction of high efficiency and stability, so as to achieve the purpose of saving costs and increasing economic benefits. For example, the introduction of other metals can eliminate the toxicity of some metals/antibiotics and even enhance the performance of AD.

In view of the unique functional characteristics of NPs on anaerobic microorganisms and the combined effects of various metals, antibiotics or metals and antibiotics, we can combine them to develop attenuated and efficient composite M−NPs according to the metals/antibiotics characteristics of different AD substrates, so as to adapt to the future trend of circular economy.

5.2. Addition of composite of metal nanoparticles and carbon-based conductive materials

A large number of researches have indicated that carbon-based CMs (e.g. granular activated carbon (GAC), carbon fiber cloth and biochar) could conspicuously boosted the performance of AD, mainly by facilitating methanogenesis (Liu et al., 2021b; Park et al., 2018; Zhang et al., 2019a; Zhou et al., 2021). However, GAC, as an enhancer of DIET, still has many shortcomings. For example, EPS hinders the direct contact between microbial cell surface and GAC, which makes it impossible for cells and GAC to improve the ability of electron transfer through tight aggregation, resulting in GAC not completely overcoming the distance barrier between cells and electron acceptors (Yang et al., 2020). Therefore, the electron capture process between microbial cell surface and GAC may be an important factor for DIET limitation (Yang et al., 2020). However, M−NPs/metal salt particles can be used as electron donors to boost the electron capture process between metals and microorganisms (Yang et al., 2020). But M−NPs/metal ions are easy to flow out in AD reactor, and M−NPs are easy to interact to form aggregates, thus reducing the bioavailability of metal elements on the surface of NPs (Ghofrani-Isfahani et al., 2020; Hassanein et al., 2021; Yang et al., 2020). GAC with high specific surface area and high stability provides a recoverable physical carrier for the uniform distribution of M−NPs (Park et al., 2018). Experiments have proved that the composites of GAC and M−NPs could overcome the shortcomings of single GAC/M−NPs, enhanced the performance of AD system, and showed the advantages that simple mechanical mixing did not (Song et al., 2019; Yang et al., 2020).

Besides GAC, carbon fiber cloth and biochar also have excellent physical and chemical properties (Park et al., 2018). However, there are few researches on the AD performances and mechanisms of composites of carbon-based CMs and M−NPs. We need to focus on the interaction between carbon-based CMs and M−NPs, so that we can control the properties of composites at molecular and electronic levels, and make them more efficient and conducive to industrial application. Moreover, the composites of carbon-based CMs and M−NPs need to further understand the mechanisms of IET at the molecular level by using metagenomics and metabolomics techniques.

5.3. Recycling of metal resources via sulfate-reducing bacteria

Thermodynamically, SRB is more favorably converted to hydrogen, acetate and propionate by sulfate reduction than by methanogens (Eqs. (5)-(11)) (Kumar et al., 2021; Qiao et al., 2016; Ye et al., 2021). In anaerobic environment, SRB can transform metals into metal sulfide precipitates at very low metal concentrations, thus realizing the dual advantages of metal removal and metal recovery in anaerobic reactors (Eqs. (12) and (13)) (Kumar et al., 2021). Because of its characteristics of no secondary pollution, low cost, green and low carbon, different bioreactor systems have been used to recover metals at present (Kumar et al., 2021). Among them, the metal sulfide recovered in nano-form has a wide application prospect in many fields (Kumar et al., 2021). Nevertheless, few researches have applied metal sulfide NPs to improve AD performance.

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O, \quad \Delta G^{0'} = -136\,kJ/mol \tag{5}$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-, \quad \Delta G^{0'} = -31\ kJ/mol \tag{6}$$

$$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + 3H_2 + HCO_3^- + H^+, \quad \Delta G^{0'} = +76.1\,kJ/mol \tag{7}$$

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+, \quad \Delta G^{0'} = +48.1\ kJ/mol \tag{8}$$

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O, \quad \Delta G^{0'} = -152\ kJ/mol \tag{9}$$

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-, \quad \Delta G^{0'} = -48\,kJ/mol \tag{10}$$

$$4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 3HS^- + 4CH_3COO^- + 4HCO_3^- + H^+, \quad \Delta G^{0'} = -150\,kJ/mol \tag{11}$$

$$SO_4{}^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3{}^- \tag{12}$$

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+ \tag{13}$$

It has been reported that the formation of metal sulfide effectively reduces the toxicity of ZnO NPs and CuO NPs to methanogens (Gonzalez-Estrella et al., 2015). Metal sulfide can also enhance the degradation kinetics of LCFAs and oleate (Yekta et al., 2017). In addition, the formation of metal sulfide effectively alleviated the propagation of antibiotic resistance genes of CuO NPs (Huang et al., 2019). Zhang et al. (2019a) pointed out that adhering FeS to the surface of nZVI can reduce the passivation and toxicity of nZVI, promote the interaction between nZVI and microorganisms, and improve the electron transfer process of nZVI.

Therefore, metal sulfide/metal sulfide NPs can significantly promote the performance of AD, and the recovery and utilization of metal sulfide/metal sulfide NPs can adapt to the trend of circular economy in the future. In the future, we need to pay attention to the strategy of applying metal sulfide/metal sulfide NPs to AD. In addition, it is indispensable to systematically and deeply study the interaction between anaerobic microorganisms and metal sulfide/metal sulfide NPs, and the interaction mechanism between metal sulfide/metal sulfide NPs and pollutants (e.g. antibiotics) in the presence of other pollutants.

6. Prospects for future research

(1) In recent reports, more emphasis has been placed on the effect of total metal concentration on AD, which is crucial to evaluate the bioavailability and ecotoxicity of metals during AD. Nevertheless, the chemical speciation of a metal usually determines the toxicity and bioavailability of the metal (Wu et al., 2017b). However, at present, there are still some controversial areas, such as changes in chemical speciation, bioavailability and toxicity of metals after AD (Chen et al., 2015; Wu et al., 2017a; Zheng et al., 2020). In future studies, we should clarify the interaction between anaerobic microorganisms and metal chemical speciation, as well as the reason and process of metal chemical speciation change. However, there are few studies in this area, and further exploration and proof should be carried out, which will be more helpful for us to predict and avoid the negative effects of metals on the AD process, and to control the role of metals in the AD process.

(2) The hormesis of metals on AD system is affected by metal particle sizes. However, few studies have paid attention to the effects of metal particle sizes. For a given metal, the micron and nano forms of metal can produce varying degrees of hormesis on the performance of AD. Moreover, smaller M−NPs can not only cause local electronic structure changes on the surface of M−NPs, but also interact directly with bacteria, even enter the interior of bacteria, interfering with the normal life activities of bacteria. Therefore, it is of great significance to study the effects of different metal particle sizes on AD performance for efficient and economical practical large-scale application.

(3) Current studies have attributed the ability of metals to promote AD to the DIET process. However, the DIET mechanism has not been fully confirmed, and the conclusions of these studies are somewhat arbitrary. In the future research, we need to identify more common key active substances which are decisive to biochemical reactions in AD system, thus systematically and deeply understanding the electronic/signaling transmission system, which is the basis of realizing efficient resource transformation. In addition, we need to use molecular biology techniques (e.g. metabolomics and metagenomics), molecular cytogenetics techniques (e.g. fluorescent in situ hybridization), transmission electron microscope, information technology and other cutting-edge advanced means to deeply explore the interaction mechanisms and change laws between metals and anaerobic microorganisms at molecular, electronic and proton levels, and calculate the electron flux, which makes it possible to confirm the electronic/signaling transmission pathways. At the same time, we need to pay attention to the role of EPS.

(4) It is an urgent problem for us to synthesize efficient and recyclable CMs at the lowest economic cost, and regulate the IET pathway directionally, so as to minimize the oxidation reaction of AD system, make more carbon convert to methane directionally and reduce the CO_2 content in biogas. Promising control strategies are NPs coated with a variety of metals, composite of M−NPs and carbon-based CMs and metal sulfide/metal sulfide NPs recovered by SRB.

(5) AD is a complex system with multi-media (solid–liquid-gas three-phase) and multi-components, and there are multiple interactions. It is of great significance for us to clarify the mechanisms and laws of metals-metals, antibiotics-antibiotics, metals-antibiotics combined effects, which is of great significance for us to control the process of AD in actual conditions. However, at present, researchers focus on the influence of single factor.

(6) The amount of intermediate products (e.g. LCFAs, SCFAs) accumulated during AD determines whether the AD system can operate stably. Therefore, it is necessary to reveal the interaction mechanisms between intermediate products and metals/antibiotics, which is very important to maintain the stability of AD system and accelerate the rate-limiting steps of AD.

(7) The results of batch and long-term continuous feeding experiments are quite different. Therefore, we must pay attention to the research of long-term continuous feeding experiment in pilot-scale and full-scale reactor in order to obtain more reliable and useful results.

(8) High concentrations of antibiotics are more helpful for us to understand the mechanisms of toxic action. However, in practice, the highest concentration of antibiotics in animal wastewater is only at the level of μg/L. Therefore, we must simulate the effect of low concentration antibiotics on AD of animal wastewater in order to obtain more useful results. In addition, the mechanisms and laws of antibiotics action on archaea have not been clarified.

7. Conclusions

Metal ions/M−NPs have been shown to facilitate/inhibit various stages of AD. The key to enhancing AD is to determine the effects of metal species, particle size, concentration and chemical speciation. In most cases, antibiotics inhibit AD to varying degrees. However, these reports are carried out under high concentrations and short-term feeding conditions, and cannot be used to evaluate the effect of antibiotics on AD in actual cases. Because multiple metals, antibiotics, or metals and antibiotics often coexist in the environment, it is of great significance for us to explore their combined effects for breaking the inherent dysfunction of AD.

CRediT authorship contribution statement

Zhiwei Huang: Conceptualization, Methodology, Visualization, Writing – original draft. **Qiuya Niu:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Wenkai Nie:** Methodology, Writing – review & editing. **Xiang Li:** Methodology, Writing – review & editing. **Chunping Yang:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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

No data was used for the research described in the article.

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References

Ahmad, A., 2020. Bioprocess evaluation of petroleum wastewater treatment with zinc oxide nanoparticle for the production of methane gas: Process assessment and modelling. Appl. Biochem. Biotechnol. 190 (3), 851–866.
Ahmad, A., Reddy, S.S., 2020. Performance evaluation of upflow anaerobic sludge blanket reactor using immobilized ZnO nanoparticle enhanced continuous biogas production. Energy Environ. 31 (2), 330–347.

Ai, S., Liu, H., Wu, M., Zeng, G., Yang, C., 2018. Roles of acid-producing bacteria in anaerobic digestion of waste activated sludge. Front. Env. Sci. Eng. 12 (6), 1–11.
Andriamanohiarisoamanana, F.J., Shirai, T., Yamashiro, T., Yasui, S., Iwasaki, M., Ihara, I., Nishida, T., Tangtaweewipat, S., Umetsu, K., 2018. Valorizing waste iron powder in biogas production: Hydrogen sulfide control and process performances. J. Environ. Manage. 208, 134–141.
Angelidaki, I., Ahring, B., 1992. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. Appl. Microbiol. Biotechnol. 37 (6), 808–812.
Aydin, S., Ince, B., Cetecioglu, Z., Arikan, O., Ozbayram, E.G., Shahi, A., Ince, O., 2015. Combined effect of erythromycin, tetracycline and sulfamethoxazole on performance of anaerobic sequencing batch reactors. Bioresour. Technol. 186, 207–214.
Banks, C.J., Zhang, Y., Jiang, Y., Heaven, S., 2012. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. Bioresour. Technol. 104, 127–135.
Bardi, M.J., Aminirad, H., 2020. Synergistic effects of co-trace elements on anaerobic co-digestion of food waste and sewage sludge at high organic load. Environ. Sci. Pollut. Res. 27 (15), 18129–18144.
Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M.F., Fiévet, F., 2006. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. 6 (4), 866–870.
Buyukkamaci, N., Filibeli, A., 2004. Volatile fatty acid formation in an anaerobic hybrid reactor. Process Biochem. 39 (11), 1491–1494.
Cai, Y., Zheng, Z., Zhao, Y., Zhang, Y., Guo, S., Cui, Z., Wang, X., 2018. Effects of molybdenum, selenium and manganese supplementation on the performance of anaerobic digestion and the characteristics of bacterial community in acidogenic stage. Bioresour. Technol. 266, 166–175.
Chan, P.C., de Toledo, R.A., Iu, H.I., Shim, H., 2019a. Effect of zinc supplementation on biogas production and short/long chain fatty acids accumulation during anaerobic co-digestion of food waste and domestic wastewater. Waste Biomass Valorization. 10 (12), 3885–3895.
Chan, P.C., Lu, Q., de Toledo, R.A., Gu, J.-D., Shim, H., 2019b. Improved anaerobic co-digestion of food waste and domestic wastewater by copper supplementation – Microbial community change and enhanced effluent quality. Sci. Total Environ. 670, 337–344.
Chen, L., Hu, Q., Zhang, X., Cai, Z., Wang, Y., 2019. Effects of ZnO nanoparticles on the performance of anaerobic membrane bioreactor: An attention to the characteristics of supernatant, effluent and biomass community. Environ. Pollut. 248, 743–755.
Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D., Zhang, J., 2015. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnol. Adv. 33 (6), 745–755.
Cheng, H., Guan, Q., Villalobos, L.F., Peinemann, K.-V., Pain, A., Hong, P.-Y., 2019. Understanding the antifouling mechanisms related to copper oxide and zinc oxide nanoparticles in anaerobic membrane bioreactors. Environ. Sci. Nano. 6 (11), 3467–3479.
Clark, R., Speece, R., 1971. The pH tolerance of anaerobic digestion. Adv. Water Pollut. Res. 1, 1–13.
Dohnalkova, A.C., Marshall, M.J., Arey, B.W., Williams, K.H., Buck, E.C., Fredrickson, J. K., 2011. Imaging hydrated microbial extracellular polymers: comparative analysis by electron microscopy. Appl. Environ. Microbiol. 77 (4), 1254–1262.
Dubey, G.P., Ben-Yehuda, S., 2011. Intercellular nanotubes mediate bacterial communication. Cell. 144 (4), 590–600.
Fernández, A., Sánchez, A., Font, X., 2005. Anaerobic co-digestion of a simulated organic fraction of municipal solid wastes and fats of animal and vegetable origin. Biochem. Eng. J. 26 (1), 22–28.
Gaballah, M.S., Guo, J., Sun, H., Aboagye, D., Sobhi, M., Muhmood, A., Dong, R., 2021. A review targeting veterinary antibiotics removal from livestock manure management systems and future outlook. Bioresour. Technol. 333, 125069.
Ghofrani-Isfahani, P., Baniamerian, H., Tsapekos, P., Alvarado-Morales, M., Kasama, T., Shahrokhi, M., Vossoughi, M., Angelidaki, I., 2020. Effect of metal oxide based TiO_2 nanoparticles on anaerobic digestion process of lignocellulosic substrate. Energy. 191, 116580.
Gonzalez-Estrella, J., Puyol, D., Sierra-Alvarez, R., Field, J.A., 2015. Role of biogenic sulfide in attenuating zinc oxide and copper nanoparticle toxicity to acetoclastic methanogenesis. J. Hazard. Mater. 283, 755–763.
Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S., 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc. Natl Acad. Sci. 103 (30), 11358–11363.
Hassanein, A., Naresh Kumar, A., Lansing, S., 2021. Impact of electro-conductive nanoparticles additives on anaerobic digestion performance - A review. Bioresour. Technol. 342, 126023.
He, Y., Tian, Z., Yi, Q., Zhang, Y., Yang, M., 2020. Impact of oxytetracycline on anaerobic wastewater treatment and mitigation using enhanced hydrolysis pretreatment. Water Res. 187, 116408.
Hill, D., Cobb, S., Bolte, J., 1987. Using volatile fatty acid relationships to predict anaerobic digester failure. Trans. ASAE. 30 (2), 496–0501.
Huang, H., Zheng, X., Yang, S., Chen, Y., 2019. More than sulfidation: Roles of biogenic sulfide in attenuating the impacts of CuO nanoparticle on antibiotic resistance genes during sludge anaerobic digestion. Water Res. 158, 1–10.
John, M., Trzcinski, A.P., Zhou, Y., Ng, W.J., 2017. Microbial stress mediated intercellular nanotubes in an anaerobic microbial consortium digesting cellulose. Sci. Rep. 7 (1), 1–14.
Kang, F., Alvarez, P.J., Zhu, D., 2014. Microbial extracellular polymeric substances reduce Ag^{+} to silver nanoparticles and antagonize bactericidal activity. Environ. Sci. Technol. 48 (1), 316–322.
Kapoor, G., Saigal, S., Elongavan, A., 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. J. Anaesthesiol. Clin. Pharmacol. 33 (3), 300–305.
Kumar, M., Nandi, M., Pakshirajan, K., 2021. Recent advances in heavy metal recovery from wastewater by biogenic sulfide precipitation. J. Environ. Manage. 278, 111555.
Li, Q., Mahendra, S., Lyon, D.Y., Brunet, L., Liga, M.V., Li, D., Alvarez, P.J.J., 2008. Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. Water Res. 42 (18), 4591–4602.
Li, X., Mo, H., Zhou, C., Ci, Y., Wang, J., Zang, L., 2021. Nickel foam promotes syntrophic metabolism of propionate and butyrate in anaerobic digestion. ACS Omega. 6 (32), 21033–21042.
Li, H., Wen, Y., Cao, A., Huang, J., Zhou, Q., Somasundaran, P., 2012. The influence of additives (Ca^{2+}, Al^{3+}, and Fe^{3+}) on the interaction energy and loosely bound extracellular polymeric substances (EPS) of activated sludge and their flocculation mechanisms. Bioresour. Technol. 114, 188–194.
Liu, P., Chen, J., Shao, L., Tan, J., Chen, D., 2018. Responses of flocculent and granular sludge in anaerobic sequencing batch reactors (ASBRs) to azithromycin wastewater and its impact on microbial communities. J. Chem. Technol. Biotechnol. 93 (8), 2341–2350.
Liu, Y., Li, X., Tan, Z., Yang, C., 2021a. Inhibition of tetracycline on anaerobic digestion of swine wastewater. Bioresour. Technol. 334, 125253.
Liu, Y., Li, X., Wu, S., Tan, Z., Yang, C., 2021b. Enhancing anaerobic digestion process with addition of conductive materials. Chemosphere. 278, 130449.
Lovley, D.R., Holmes, D.E., 2022. Electromicrobiology: the ecophysiology of phylogenetically diverse electroactive microorganisms. Na. Rev. Microbiol. 20 (1), 5–19.
Luna-delRisco, M., Orupõld, K., Dubourguier, H.-C., 2011. Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. J. Hazard. Mater. 189 (1), 603–608.
Ma, J., Gu, J., Wang, X., Peng, H., Wang, Q., Zhang, R., Hu, T., Bao, J., 2019. Effects of nano-zerovalent iron on antibiotic resistance genes during the anaerobic digestion of cattle manure. Bioresour. Technol. 289, 121688.
Marshall, M.J., Beliaev, A.S., Dohnalkova, A.C., Kennedy, D.W., Shi, L., Wang, Z., Boyanov, M.I., Lai, B., Kemner, K.M., McLean, J.S., 2006. c-Type cytochrome-dependent formation of U (IV) nanoparticles by *Shewanella oneidensis*. PLoS. Biol. 4 (8), e268.
Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology. 16, 2346–2353.
Mu, H., Zheng, X., Chen, Y., Chen, H., Liu, K., 2012. Response of anaerobic granular sludge to a shock load of zinc oxide nanoparticles during biological wastewater treatment. Environ. Sci. Technol. 46 (11), 5997–6003.
Muñoz, C., Fermoso, F.G., Rivas, M., González Grau, J.M., 2016. Hydrolytic enzyme activity enhanced by barium supplementation. AIMS Microbiol. 2 (4), 402–411.
Mushtaq, M., Zeshan, Zeeshan, M., Nawaz, I., Hassan, M., 2022. Effect of low levels of oxytetracycline on anaerobic digestion of cattle manure. Bioresour. Technol. 349, 126894.
Ni, B.-J., Zeng, S., Wei, W., Dai, X., Sun, J., 2020. Impact of roxithromycin on waste activated sludge anaerobic digestion: Methane production, carbon transformation and antibiotic resistance genes. Sci. Total Environ. 703, 134899.
Novak, J.T., Carlson, D.A., 1970. The kinetics of anaerobic long chain fatty acid degradation. J. Water Pollut. Control Fed. 42, 1932–1943.
Otero-González, L., Field, J.A., Sierra-Alvarez, R., 2014. Inhibition of anaerobic wastewater treatment after long-term exposure to low levels of CuO nanoparticles. Water Res. 58, 160–168.
Park, J.-H., Kang, H.-J., Park, K.-H., Park, H.-D., 2018. Direct interspecies electron transfer via conductive materials: A perspective for anaerobic digestion applications. Bioresour. Technol. 254, 300–311.
Pavlostathis, S.G., Giraldo-Gomez, E., 1991. Kinetics of anaerobic treatment. Water Sci. Technol. 24 (8), 35–59.
Qi, L., Liu, X., Miao, Y., Chatzisymeon, E., Yang, P., Lu, H., Pang, L., 2021. Response of cattle manure anaerobic digestion to zinc oxide nanoparticles: Methane production, microbial community, and functions. J. Environ. Chem. Eng. 9 (6), 106704.
Qiao, W., Takayanagi, K., Li, Q., Shofie, M., Gao, F., Dong, R., Li, Y.-Y., 2016. Thermodynamically enhancing propionic acid degradation by using sulfate as an external electron acceptor in a thermophilic anaerobic membrane reactor. Water Res. 106, 320–329.
Rabus, R., Hansen, T.A., Widdel, F., 2006. Dissimilatory sulfate-and sulfur-reducing prokaryotes. The prokaryotes. 2, 659–768.
Rai, R., Bibra, M., Chadha, B.S., Sani, R.K., 2019. Enhanced hydrolysis of lignocellulosic biomass with doping of a highly thermostable recombinant laccase. Int. J. Biol. Macromol. 137, 232–237.
Rajagopal, R., Massé, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632–641.
Rasit, N., Idris, A., Harun, R., Ghani, W.A.K., 2015. Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: An overview. Renew. Sust. Energ. Rev. 45, 351–358.
Sanz, J., Rodriguez, N., Amils, Á., 1996. The action of antibiotics on the anaerobic digestion process. Appl. Microbiol. Biotechnol. 46 (5), 587–592.
Shen, L., Zhao, Q., Wu, X., Li, X., Li, Q., Wang, Y., 2016. Interspecies electron transfer in syntrophic methanogenic consortia: From cultures to bioreactors. Renew. Sust. Energ. Rev. 54, 1358–1367.
Song, X., Liu, J., Jiang, Q., Zhang, P., Shao, Y., He, W., Feng, Y., 2019. Enhanced electron transfer and methane production from low-strength wastewater using a new granular activated carbon modified with nano-Fe_3O_4. Chem. Eng. J. 374, 1344–1352.

Stams, A.J., Plugge, C.M., 2009. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat. Rev. Microbiol. 7 (8), 568–577.

Tan, Z., Li, X., Yang, C., Liu, H., Cheng, J.J., 2021. Inhibition and disinhibition of 5-hydroxymethylfurfural in anaerobic fermentation: A review. Chem. Eng. J. 424, 130560.

Tenover, F.C., 2006. Mechanisms of antimicrobial resistance in bacteria. Am. J. Med. 119 (6), S3–S10.

Tong, F., Zhao, Y., Gu, X., Gu, C., Lee, C.C., 2015. Joint toxicity of tetracycline with copper (II) and cadmium (II) to *Vibrio fischeri*: effect of complexation reaction. Ecotoxicology. 24 (2), 346–355.

Wang, R., Chen, M., Feng, F., Zhang, J., Sui, Q., Tong, J., Wei, Y., Wei, D., 2017. Effects of chlortetracycline and copper on tetracyclines and copper resistance genes and microbial community during swine manure anaerobic digestion. Bioresour. Technol. 238, 57–69.

Wang, J., Fang, H., Jia, H., Yang, G., Gao, F., Liu, W., 2018a. Effect of zero-valent iron and trivalent iron on UASB rapid start-up. Environ. Sci. Pollut. Res. 25 (1), 749–757.

Wang, Q., Kang, F., Gao, Y., Mao, X., Hu, X., 2016. Sequestration of nanoparticles by an EPS matrix reduces the particle-specific bactericidal activity. Sci Rep. 6 (1), 1–10.

Wang, C., Liu, Y., Wang, C., Xing, B., Zhu, S., Huang, J., Xu, X., Zhu, L., 2021. Biochar facilitates rapid restoration of methanogenesis by enhancing direct interspecies electron transfer after high organic loading shock. Bioresour. Technol. 320, 124360.

Wang, J., Westerholm, M., Qiao, W., Mahdy, A., Wandera, S.M., Yin, D., Bi, S., Fan, R., Dong, R., 2019. Enhancing anaerobic digestion of dairy and swine wastewater by adding trace elements: evaluation in batch and continuous experiments. Water Sci. Technol. 80 (9), 1662–1672.

Wang, K., Yin, J., Shen, D., Li, N., 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH. Bioresour. Technol. 161, 395–401.

Wang, R., Zhang, J., Liu, J., Yu, D., Zhong, H., Wang, Y., Chen, M., Tong, J., Wei, Y., 2018b. Effects of chlortetracycline, Cu and their combination on the performance and microbial community dynamics in swine manure anaerobic digestion. J. Environ. Sci. 67, 206–215.

Wei, R., Ge, F., Huang, S., Chen, M., Wang, R., 2011. Occurrence of veterinary antibiotics in animal wastewater and surface water around farms in Jiangsu Province. China. Chemosphere. 82 (10), 1408–1414.

Wu, S., He, H., Inthapanya, X., Yang, C., Lu, L., Zeng, G., Han, Z., 2017a. Role of biochar on composting of organic wastes and remediation of contaminated soils—a review. Environ. Sci. Pollut. Res. 24 (20), 16560–16577.

Wu, X., He, H., Yang, W.L., Yu, J., Yang, C., 2018. Efficient removal of atrazine from aqueous solutions using magnetic Saccharomyces cerevisiae bionanomaterial. Appl. Microbiol. Biotechnol. 102 (17), 7597–7610.

Wu, S., Shen, Z., Yang, C., Zhou, Y., Li, X., Zeng, G., Ai, S., He, H., 2017b. Effects of C/N ratio and bulking agent on speciation of Zn and Cu and enzymatic activity during pig manure composting. Int. Biodeterior. Biodegrad. 119, 429–436.

Wyman, V., Henríquez, J., Palma, C., Carvajal, A., 2018. Lignocellulosic waste valorisation strategy through enzyme and biogas production. Bioresour. Technol. 247, 402–411.

Wyman, V., Serrano, A., Borja, R., Jiménez, A., Carvajal, A., Lenz, M., Bartacek, J., Fermoso, F.G., 2019. Effects of barium on the pathways of anaerobic digestion. J. Environ. Manage. 232, 397–403.

Xiao, Y., Zhang, E., Zhang, J., Dai, Y., Yang, Z., Christensen, H.E., Ulstrup, J., Zhao, F., 2017. Extracellular polymeric substances are transient media for microbial extracellular electron transfer. Sci. Adv. 3 (7), e1700623.

Yang, B., Xu, H., Liu, Y., Li, F., Song, X., Wang, Z., Sand, W., 2020. Role of GAC-MnO_2 catalyst for triggering the extracellular electron transfer and boosting CH_4 production in syntrophic methanogenesis. Chem. Eng. J. 383, 123211.

Ye, J., Hu, A., Ren, G., Chen, M., Tang, J., Zhang, P., Zhou, S., He, Z., 2018a. Enhancing sludge methanogenesis with improved redox activity of extracellular polymeric substances by hematite in red mud. Water Res. 134, 54–62.

Ye, J., Hu, A., Ren, G., Zhou, T., Zhang, G., Zhou, S., 2018b. Red mud enhances methanogenesis with the simultaneous improvement of hydrolysis-acidification and electrical conductivity. Bioresour. Technol. 247, 131–137.

Ye, W., Lu, J., Ye, J., Zhou, Y., 2021. The effects and mechanisms of zero-valent iron on anaerobic digestion of solid waste: A mini-review. J. Clean Prod. 278, 123567.

Yekta, S.S., Ziels, R.M., Björn, A., Skyllberg, U., Ejlertsson, J., Karlsson, A., Svedlund, M., Willén, M., Svensson, B.H., 2017. Importance of sulfide interaction with iron as regulator of the microbial community in biogas reactors and its effect on methanogenesis, volatile fatty acids turnover, and syntrophic long-chain fatty acids degradation. J. Biosci. Bioeng. 123 (5), 597–605.

Yenigün, O., Kizilgün, F., Yilmazer, G., 1996. Inhibition effects of zinc and copper on volatile fatty acid production during anaerobic digestion. Environ. Technol. 17 (11), 1269–1274.

Yu, T., Deng, Y., Liu, H., Yang, C., Wu, B., Zeng, G., Lu, L., Nishimura, F., 2017. Effect of alkaline microwaving pretreatment on anaerobic digestion and biogas production of swine manure. Sci Rep. 7 (1), 1–8.

Yu, H.Q., Fang, H., 2001. Inhibition on acidogenesis of dairy wastewater by zinc and copper. Environ. Technol. 22 (12), 1459–1465.

Yun, S., Xing, T., Han, F., Shi, J., Wang, Z., Fan, Q., Xu, H., 2021. Enhanced direct interspecies electron transfer with transition metal oxide accelerants in anaerobic digestion. Bioresour. Technol. 320, 124294.

Zakaria, B.S., Dhar, B.R., 2021. Characterization and significance of extracellular polymeric substances, reactive oxygen species, and extracellular electron transfer in methanogenic biocathode. Sci. Rep. 11 (1), 1–13.

Zhang, D., Shen, J., Shi, H., Su, G., Jiang, X., Li, J., Liu, X., Mu, Y., Wang, L., 2019a. Substantially enhanced anaerobic reduction of nitrobenzene by biochar stabilized sulfide-modified nanoscale zero-valent iron: Process and mechanisms. Environ. Int. 131, 105020.

Zhang, L., Zhang, Z., He, X., Zheng, L., Cheng, S., Li, Z., 2019b. Diminished inhibitory impact of ZnO nanoparticles on anaerobic fermentation by the presence of TiO_2 nanoparticles: Phenomenon and mechanism. Sci. Total Environ. 647, 313–322.

Zhao, L., Ji, Y., Sun, P., Deng, J., Wang, H., Yang, Y., 2019. Effects of individual and combined zinc oxide nanoparticle, norfloxacin, and sulfamethazine contamination on sludge anaerobic digestion. Bioresour. Technol. 273, 454–461.

Zheng, X., Liu, Y., Huang, J., Du, Z., Zhouyang, S., Wang, Y., Zheng, Y., Li, Q., Shen, X., 2020. The influence of variables on the bioavailability of heavy metals during the anaerobic digestion of swine manure. Ecotox. Environ. Safe. 195, 110457.

Zhou, Q., Li, X., Wu, S., Zhong, Y., Yang, C., 2021. Enhanced strategies for antibiotic removal from swine wastewater in anaerobic digestion. Trends Biotechnol. 39 (1), 8–11.

Zhou, M., Yan, B., Wong, J.W.C., Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. Bioresour. Technol. 248, 68–78.

Zhu, R., He, L., Li, Q., Huang, T., Gao, M., Jiang, Q., Liu, J., Cai, A., Shi, D., Gu, L., He, Q., 2021. Mechanism study of improving anaerobic co-digestion performance of waste activated sludge and food waste by Fe_3O_4. J. Environ. Manage. 300, 113745.

Zhu, K., Zhang, L., Mu, L., Ma, J., Wang, X., Li, C., Cui, Y., Li, A., 2020. Antagonistic effect of zinc oxide nanoparticle and surfactant on anaerobic digestion: Focusing on the microbial community changes and interactive mechanism. Bioresour. Technol. 297, 122382.