Role of interspecies electron transfer stimulation in enhancing anaerobic digestion under ammonia stress: Mechanisms, advances, and perspectives

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HIGHLIGHTS

- IET stimulation is a promising approach for AD recovery under ammonia stress.
- IET mechanism and the shift of IET pathways with ammonia stress were discussed.
- Findings of ammonia-stressed AD integrated with IET stimulation were summarized.
- Microbial response to IET stimulation in ammonia-stressed AD process was analysed.
- Contribution of IET stimulation to AD under ammonia stress should be emphasized.

GRAPHICAL ABSTRACT
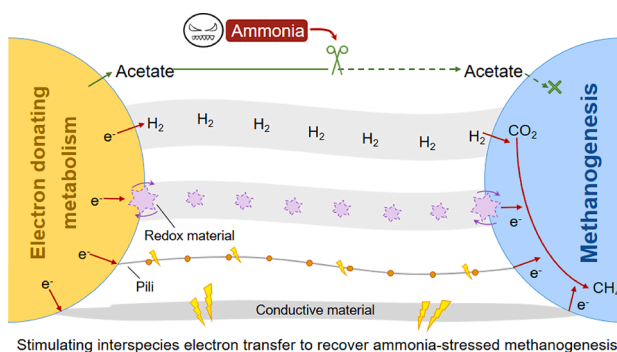
Stimulating interspecies electron transfer to recover ammonia-stressed methanogenesis

ARTICLE INFO

ABSTRACT

Ammonia stress is a commonly encountered issue in anaerobic digestion (AD) process when treating proteinaceous substrates. The enhanced relationship between syntrophic bacteria and methanogens triggered by interspecies electron transfer (IET) stimulation is one of the potential mechanisms for an improved methane yield from the AD plant under ammonia-stressed condition. There is, however, lack of synthesized information on the mechanistic understanding of IET facilitation in the ammonia-stressed AD processes. This review critically discusses recovery of AD system from ammonia-stressed condition, focusing on H_2 transfer, redox compound-mediated IET, and conductive material-induced direct IET. The effects and the associated mechanisms of IET stimulation on mitigating ammonia stress and promoting methanogenesis were elucidated. Finally, prospects and challenges of IET stimulation were critically discussed. This review highlights, for the first time, the critical role of IET stimulation in enhancing AD process under ammonia-stressed condition.

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1. Introduction

Anaerobic digestion (AD) has been widely adopted for treating diverse organic wastes, while generating renewable energy in the form of methane (CH$_4$) (Khanal et al., 2020; Sawatdeenarunat et al., 2015). During AD process, however, about 33 ~ 80% of nitrogenous organic matter, such as urea, proteins, amino acids, and nucleic acids, is hydrolysed and fermented, releasing ammonia nitrogen as an end product (Palakodeti et al., 2021; Yuan and Zhu, 2016). Total ammonia nitrogen (TAN) with concentrations ranging from 1.1 to 11.8 g N/L, including free ammonia nitrogen (NH$_3$-N) and ionic ammonium nitrogen (NH$_4^+$-N), was reported to stress AD process thereby decreasing the specific methanogenic activity (SMA) by half (Chen et al., 2014; Capson-Tojo et al., 2020). Ammonia inhibits the normal metabolic functions of anaerobic microbes by interfering with intracellular pH/proton and K$^+$ balances, intensifying cellular-energy consumption, and suppressing specific enzymatic activities associated with methanogenic metabolism (Wittmann et al., 1995; Kayhanian, 1999; Prochazka et al., 2012). Since methanogens are more sensitive to ammonia than other anaerobic microbes, volatile fatty acids (VFAs) are often accumulated, further exacerbating the deterioration of AD process (Yirong et al., 2017; Peng et al., 2018).

Strategies for ammonia inhibition mitigation via adjustment of operational parameters (e.g., temperature, pH, and carbon-to-nitrogen ratio) (Wang et al., 2014) and conversion or removal of ammonia using physicochemical processes such as dilution (with water, or co-digestion with low-nitrogen containing substrates) (Nielsen and Angel-idaki, 2008), precipitation (Kwon et al., 2018), air stripping (Walker et al., 2011), membrane separation (Lauterbock et al., 2012) and ion exchange (Thornton et al., 2007), have been extensively investigated and reviewed (Yenigun and Demirel, 2013; Rajagopal et al., 2013; Krakat et al., 2017; Jiang et al., 2019; Mutegoa et al., 2020). With the development and advances in molecular techniques in microbial community analysis, particularly 16S rRNA high-throughput sequencing, strategies for alleviating ammonia stress by enhancing microbial functions have emerged. Several studies demonstrated a dramatic shift of methanogenic pathway from acetoclastic methanogenesis (AM) to syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (SAO-HM) under ammonia-stressed conditions (Angenent et al., 2002; Westerholm et al., 2016; Tian et al., 2017). Given this, approaches, such as stepwise ammonia acclimation, SAO-HM consortia bioaugmentation, and in-situ H$_2$ supplementation, were employed to enhance the microbial metabolism and improve the AD performance (Tian et al., 2018; Yang et al., 2019; Wu et al., 2019). The SAO-HM consortia exchange electrons for indirect acetate methanation through H$_2$ as a diffusible electron shuttle (Zabranska and Pokorna, 2018). Thus, acetate methanation is accomplished by SAO-HM, improving CH$_4$ production under ammonia-stressed condition. From the mass flow perspective, SAO-HM promotion approaches aim at stimulating interspecies electron transfer (IET) among syntrophic consortia thereby facilitating the recovery of methanogenesis in the ammonia-stressed AD system.

In addition, other IET mechanisms as alternatives to interspecies H$_2$ transfer have also attracted attention. In the AD process under ammonia-stressed condition, some redox chemicals, such as humic acids and cystine/cysteine, have been reported to accelerate interspecies H$_2$ transfer or potentially mediate electrons as shuttles to promote VFA bioconversion to CH$_4$ (Ho and Ho, 2012; Zhuang et al., 2017). Recently, studies have reported that the amendment of conductive materials in the ammonia-stressed AD systems is an efficient and energy-conserving approach to trigger direct IET between syntrophic microbes and methanogens (Arif et al., 2018; Paritosh et al., 2020; Yin et al., 2020). For example, CH$_4$ production in the ammonia-stressed AD process significantly improved with the amendment of magnetite (Lee et al., 2019), zero-valent iron (ZVI) (Meng et al., 2020), biochar (Pan et al., 2019), and activated carbon (AC) (Johnravindar et al., 2010). However,

studies have barely elucidated the critical roles that IET stimulation plays in recovery of methanogenic activity under ammonia-stressed condition, and therefore further attention is required.

Thus, this review critically examines the recent studies on the effects of IET stimulation on methanogenesis, particularly under ammonia-stressed conditions. This review especially focuses on: (1) IET mechanisms and IET responses to ammonia stress in AD, (2) effects of IET stimulation on CH$_4$ production and CH$_4$ production rate in the ammonia-stressed AD process, and (3) the responses of microbial community to IET stimulation. This review concludes with perspectives and challenges of IET stimulation in the ammonia-stressed AD systems.

2. Electron transfer mechanisms with and without ammonia stress

2.1. Electron transfer pathways in an anaerobic digestion process without ammonia stress

A well-balanced AD process depends on complex interdependent biochemical reactions and the syntrophic metabolism integrated by IET (Nozhevnikova et al., 2020; Ye et al., 2018). The main biochemical reactions integrated with IET in AD process are summarized in Table 1. The bioconversion of polysaccharides, proteins, and lipids into VFAs and then to CH$_4$ as final product coupled with IET is depicted in Fig. 1. H$_2$ transfer, formate transfer, electron shuttle-mediated IET, and direct IET play the crucial roles in syntrophic methanogenesis. H$_2$ and formate transfers driven by hydrogenases and formate dehydrogenases, respectively, are the best-known and the most common pathways (Eqs. 1 ~ 12) (Stams and Plugge, 2009). In some cases, redox compounds, such as quinone/hydroquinone moieties and cystine/cysteine (Lovley, 2017), mediate IET through the transformation of redox states. Redox materials in the oxidized state capture electrons for self-reduction, and then shuttle the electrons for methanogenic metabolism (Eq. 13). In addition to indirect pathways, the direct IET that applies conductive type IV pili and/or external polyheme cytochromes, and exogenous conductive materials was recently identified to accelerate syntrophic methanogenesis (Eq. 14) (Lovley, 2017).

It is noticed that the majority of H$_2$ and formate production reactions in syntrophic process, such as butyrate-, propionate- and acetate-oxidation (Eqs. 4 ~ 5, 8, 10 ~ 11, Table 1), cannot proceed spontaneously under standard conditions due to the high positive Gibbs free energy changes. These indirect syntrophic reactions are available only when H$_2$ and formate are continuously consumed in downstream methanogenic reactions (Eqs. 9, 12, Table 1), thereby creating a thermodynamically favourable condition. In comparison to H$_2$ transfer,

Table 1

Main anaerobic biochemical reactions of electron transfer in AD process (Pan et al., 2021; Leng et al., 2018; Saddy, 2013; Xu et al., 2019).

Bioreactions	$\Delta G^{o'}$ (kJ)
Hydrogen transfer:	
(1) Glucose + 2H$_2$O → 2Acetate + 4**H$_2$** + 2CO$_2$	− 206
(2) Glucose → Butyrate + 2**H$_2$** + 2CO$_2$	− 254
(3) Glucose + 2**H$_2$** → 2Propionate + 2CO$_2$	− 279.4
(4) Butyrate + 2H$_2$O → 2Acetate + 2**H$_2$**	+ 48.4
(5) Propionate + 2H$_2$O → Acetate + CO$_2$ + 3**H$_2$**	+ 76.2
(6) Lactate$^-$ + 2H$_2$O → Acetate + HCO$_3^-$ + 2**H$_2$**	− 4.2
(7) Ethanol + H$_2$O → Acetate + 2**H$_2$**	+ 9.6
(8) Acetate + 2H$_2$O → 2CO$_2$ + 4**H$_2$**	+ 104.6
(9) 4**H$_2$** + CO$_2$ → CH$_4$ + 2H$_2$O	− 135.0
Formate transfer:	
(10) Butyrate$^-$ + 2H$_2$O + 2CO$_2$ → 2Acetate + 2**Formate**$^-$	+ 38.5
(11) Propionate$^-$ + 2H$_2$O + 2CO$_2$ → Acetate + 3**Formate**$^-$ + 2H$^+$	+ 65.3
(12) 4**Formate**$^-$ + 4H$^+$ → CH$_4$ + 3CO$_2$ + 2H$_2$O	− 144.5
Electron shuttle mediated transfer:	
(13) **ES**$_{red}$ + CO$_2$ → CH$_4$ + **ES**$_{ox}$ + H$_2$O	/
Direct electron transfer:	
(14) 8H$^+$ + 8e$^-$ + CO$_2$ → CH$_4$ + 2H$_2$O	/

direct IET is much more energy-favourable due to the breakthrough of thermodynamic barrier. It was previously found that direct IET pathway could conserve 50% of 85 kJ/mol energy of syntrophic propionate oxidation compared with interspecies H_2 transfer (Logan et al., 2002; Wu et al., 2020a). Thus, direct IET pathway is preferred in syntrophic methanogenesis from the energy conservation perspective.

2.2. Response of electron transfer mechanism to ammonia stress

Fig. 2a illustrates the IET response to ammonia stress. Among the IET networks between electron donating bacteria and methanogens, interspecies H_2 transfer may be enhanced. Several studies demonstrated that SAO-HM metabolism dominates over AM metabolism in the ammonia-stressed AD process (Angenent et al., 2002; Hao et al., 2017; Li et al., 2017; Westerholm et al., 2016; Tian et al., 2017; Bonk et al., 2018; Fischer et al., 2019; Yan et al., 2020b; Hao et al., 2021). SAO metabolism occurs through the Wood-Ljungdahl pathway, or the glycine cleavage system pathway coupled with part of the Wood-Ljungdahl pathway (Wei et al., 2020). At high ammonia levels, both the Wood-Ljungdahl pathway and the glycine cleavage system pathway were found to be enhanced in comparison to that at relatively low ammonia levels (Fig. 2b). From the metagenomics perspective, gene counts of acetyl-CoA synthetase (ACSS), CO-methylating acetyl-CoA synthase (ACS), anaerobic carbon monoxide dehydrogenase (CODH), methylenetetrahydrofolate reductase (MTHFR), methylenetetrahydrofolate dehydrogenase (MTHFD), methenyltetrahydrofolate cyclohydrolase (MTHFC), formyltetrahydrofolate synthetase (FTHFS) involved in Wood-Ljungdahl pathway were all upregulated with the increase in ammonia concentration (Ruiz- Sánchez et al., 2018, Yu et al., 2020). From the metaproteomics perspective, protein expressions of amino-methyltransferase (AMT) GcvT, acetate kinase (ACK), glycine dehydrogenase (GDH) subunit 2 and subunit GcvPA involved in glycine cleavage system pathway were upregulated at elevated ammonia level above 4 g N/L (Zhang et al., 2022). Correspondingly,

formylmethanofuran dehydrogenase (FWD), methylenetetrahydromethanopterin reductase (MER), tetrahydromethanopterin S-methyltransferase (MTR) and ferredoxin:CoB-CoM heterodisulfide reductase (HDR) participated in HM pathway, were also promoted (Yu et al., 2020). All these studies supported that CO_2 and H_2 production from acetate for HM, interspecies H_2 transfer as a link, is a favorable pathway in the AD of nitrogenous substrate.

Direct electron flow from electron donors to acceptors could be induced by conductive materials, such as magnetite and ZVI. To date, however, two contradictory findings on the response of direct IET to ammonia stress have been reported. Zhuang et al. (2018) observed that the conductive material-induced direct IET occurs more easily in the AD with a high ammonia spike. The authors reported a 36% higher CH_4 generation rate with magnetite stimulation compared to the control group (without magnetite) at 5.0 g NH_4^+-N/L, while effect was not substantial at 0.5 and 0 g NH_4^+-N/L. In contrast, Yan et al. (2020c) observed the suppression of the direct IET pathway under high ammonia stress at 5.5 g NH_4^+-N/L, despite the presence of conductive material (magnetite and powder AC (PAC)), where abundances of genes involved in the CO_2-reduction methanogenic pathway were downregulated. The completely contradictory findings might be attributed to the difference in specific operational parameters of AD reactors, and the ammonia-tolerance disparity of the microorganisms involved in direct IET. Further studies are needed to better understand the role of conductive materials in stimulating electron transfer to overcome ammonia stress during AD process.

3. Methane production enhancement in the ammonia-stressed anaerobic digestion systems through electron transfer stimulation

3.1. Interspecies H_2 transfer stimulation

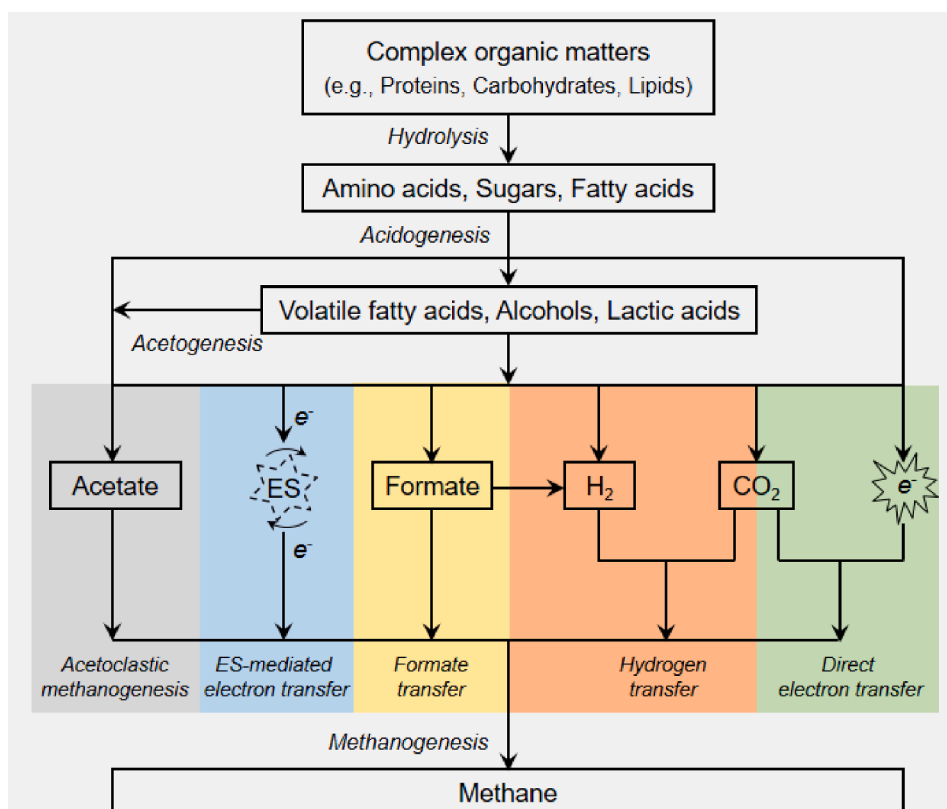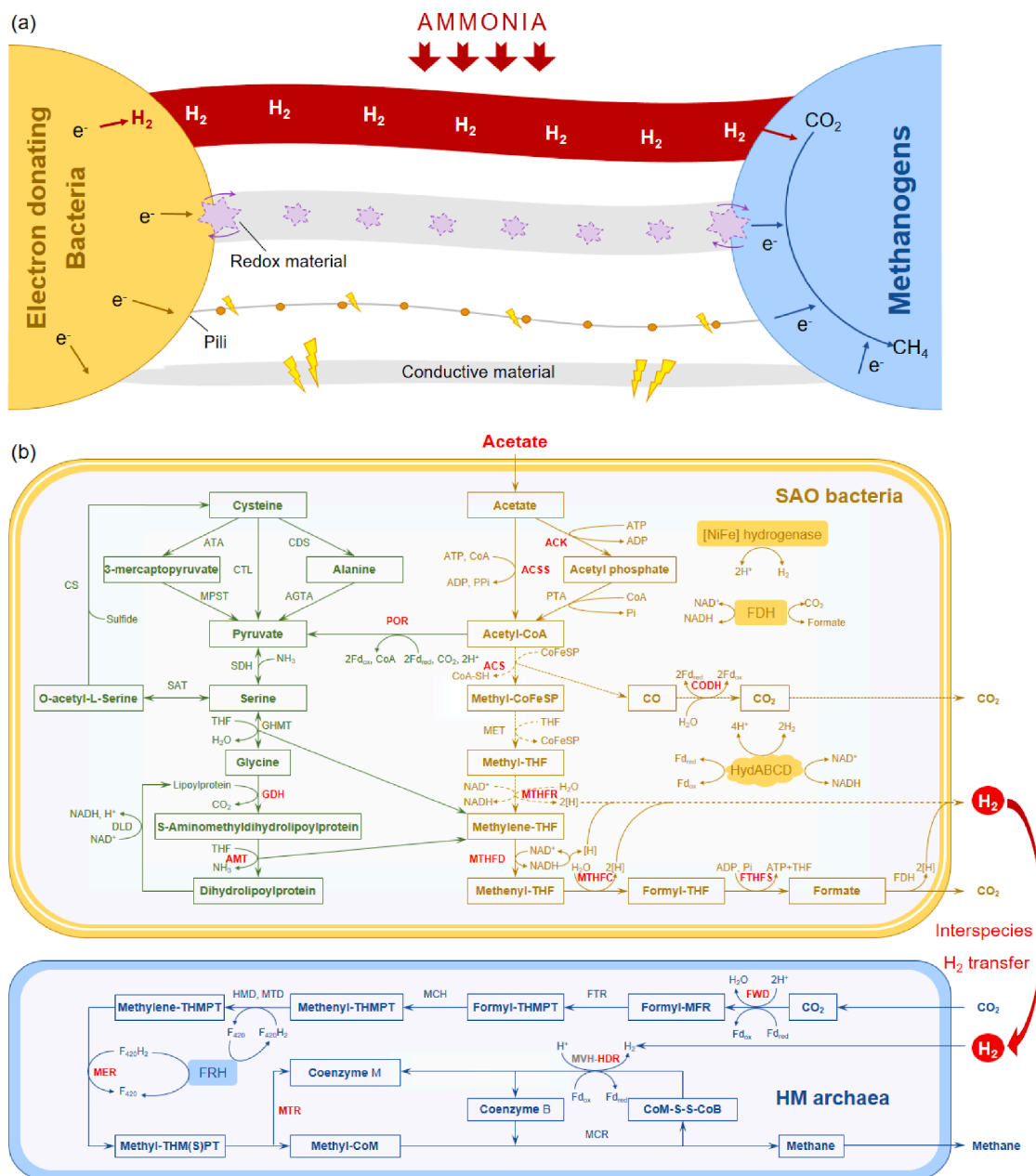Stepwise acclimation to ammonia prior to high ammonia shock load

Fig. 1. Interspecies electron transfer (IET) mechanisms integrated in the conventional AD process (Wang and Lee, 2021; Xiao et al., 2021).

Fig. 2. The response of IET pathway to ammonia stress (a) and the detailed interspecies H_2 transfer mechanism under high ammonia stress (b). In SAO bacteria, the Wood-Ljungdahl pathway and the glycine cleavage system pathway are drawn in green lines and in yellow lines, respectively. The enzymes in red font were reported to be enhanced at high ammonia level. ACK, acetyl-CoA synthetase; PTA, phosphate acetyltransferase; ACSS, acetyl-CoA synthetase; ACS, CO-methylating acetyl-CoA synthase; CODH, anaerobic carbon monoxide dehydrogenase; MET, methylenetetrahydrofolate reductase; MTHFR, methylenetetrahydrofolate reductase; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFC, methenyltetrahydrofolate cyclohydrolase; FTHFS, formyltetrahydrofolate synthetase; FDH, formate dehydrogenase; POR, pyruvate oxidoreductase; ATA, aspartate transaminase; MPST, 3-mercaptopyruvate sulfurtransferase; CTL, cystathionine beta-lyase; CDS, cysteine desulfurase; AGTA, alanine-glyoxylate transaminase; SDH, serine dehydrogenase; GHMT, glycine hydroxymethyltransferase; GDH, glycine dehydrogenase; AMT, aminomethyltransferase; DLD, dihydrolipoyl dehydrogenase; CS, cysteine synthase; FWD, formylmethanofuran dehydrogenase; FTR, formylmethanofuran-tetrahydromethanopterin N-formyltransferase; MCH, methenyltetrahydromethanopterin cyclohydrolase; HMD, methenyltetrahydromethanopterin hydrogenase; MTD, methylenetetrahydromethanopterin dehydrogenase; MER, methylenetetrahydromethanopterin reductase; MTR, tetrahydromethanopterin S-methyltransferase; MCR, methyl-coenzyme M reductase; HDR, heterodisulfide reductase; Hyd, hydrogenase; FRH, coenzyme F420 hydrogenase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

can contribute to the evolution of microbial communities to enhance SAO-HM, which is beneficial to alleviate ammonia inhibition. Studies reported that CH_4 production under ammonia-stressed condition with stepwise acclimation was relatively stable with<10% variation. Specifically, a stepwise increase (i.e., 0.5 g N/L) in TAN concentration from 2.0 to 4.5 N/L did not affect CH_4 production under mesophilic AD condition (Gao et al., 2015). Even at extremely high ammonia concentrations of

9.5 and 10.0 g NH_4^+-N/L, stable CH_4 production (~90% of that without ammonia-stress condition) was achieved (Tian et al., 2018; Yan et al., 2019a). The results of microbial community analysis confirmed that the interspecies H_2 transfer pathway was promoted, contributing to the stable CH_4 production. SAO-HM consortia, for example, classes *Clostridia-Methanobacteriaceae* and species *Clostridium ultunense-Methanoculleus* spp., were stimulated after stepwise acclimation to ammonia

(Gao et al., 2015; Tian et al., 2018). Although the relative abundance of obligate HM consortia like *Methanobrevibacter acididurans* decreased, ammonia tolerant methanogens capable of versatile metabolisms, such as *Methanosarcina soligelidi*, were found to be abundant. The presence of the metabolically versatile genus *Methanosarcina*, which shifts the metabolic pathway from AM to HM in association with SAO bacteria, was considered critical to overcome ammonia inhibition (Yan et al., 2019a).

Microbial acclimation, however, requires relatively long-term consortium adaptation. Thus, such an approach may not be appropriate for the AD system in which ammonia concentration varies frequently. *In-situ* H_2 supplementation is an alternative approach for inducing interspecies H_2 transfer to stimulate SAO-HM metabolism. For example, with external H_2 supplementation under high ammonia stress ($2 \sim 8$ g NH_4^+-N/L), Wu et al. (2019) established microbial community rich in microbes responsible for HM. The use of such enriched microbial community significantly enhanced the CH4 production ($p < 0.05$, compared to the control) during AD of phenol-containing wastewater. However, H_2 supplementation to the ammonia-stressed AD system should be precisely controlled, because a high H_2 partial pressure above ca. 700 mbar would have detrimental effect on methanogenesis due to unfavourable thermodynamic condition (Hao et al., 2017; Cazier et al., 2015).

Compared to direct induction of interspecies H_2 transfer in the ammonia-stressed AD systems, SAO-HM consortia bioaugmentation, i.e., introduction of microorganisms involved in H_2 transfer, can shorten the lag phase of interspecies H_2 transfer stimulation, and thus contribute to a faster relief of ammonia stress (Romero-Guiza et al., 2016). Table 2 details the effects of HM and SAO-HM consortia bioaugmentations on the ammonia-stressed AD that varied in feedstock, temperature, ammonia concentration, and bioaugmentation agent. Hydrogenotrophic methanogen *Methanoculleus bourgensis* MS2, the most frequently applied pure strain, improved CH4 production by $21 \sim 31\%$ in mesophilic AD reactors with $5.0 \sim 13.5$ g NH_4^+-N/L (Fotidis et al., 2014; Tian et al., 2019a; Yan et al., 2020a). In addition, mixture of pure strains of SAO

(*Syntrophaceticus schinkii* and *Tepidanaerobacter acetatoxydans* sp.) and (HM *Methanobacterium bryantii*, *Methanoculleus bourgensis*, *Methanobrevibacter smithii*, versatile *Methanosarcina barkeri* and AM *Methanosaeta harundinacea* sp.) relieved ammonia stress (4 g NH_4^+-N/L), resulting in 4 $\sim 71\%$ higher CH4 production in mesophilic AD system than the control (Yang et al., 2019; Yang et al., 2020). The mitigation should mainly benefit from the increase in the relative abundances of SAO-HM consortia, which could subsequently shorten the inter-microbial distances required for H_2 diffusion, and optimize the thermodynamic condition with efficient H_2 consumption (Fotidis et al., 2017; Tian et al., 2019a; Tian et al., 2019b; Yan et al., 2020a). Notably, the major challenge of SAO-HM consortia bioaugmentation using pure strains is to successfully establish interspecies H_2 transfer pathways between the supplemented strains and the indigenous microorganisms in the reactor. In other words, the failure to establish an efficient interspecies H_2 transfer between SAO bacteria and hydrogenotrophic methanogens, such as *S. schinkii-M. bourgensis* and *T. acetatoxydans-M. bourgensis* as reported by Yang et al. (2019) could have resulted in the poor performance of bioaugmentation strategy.

The mixed culture derived from anaerobic reactors have attracted a significant attention as they could provide closely associated SAO-HM consortia for interspecies H_2 transfer. Generally, mixed strains are obtained from two types of reactors rich in SAO-HM, i.e., H_2/CO_2-fed reactor and high ammonia-adapted reactors. For example, Xu et al. (2019) applied H_2/CO_2-enriched sludge to black water digestion under $0.1 \sim 1.7$ g N/L stress, and found that bioaugmentation resulted in 3 $\sim 16\%$ higher biochemical CH4 potential than control. Fotidis et al. (2017) used the inoculum from an ammonia-adapted A, and then cultured the inoculum with H_2/CO_2 to enrich hydrogenotrophic methanogens *Methanoculleus* spp. and SAO bacteria, such as *Tepidimicrobium* spp., *Aminobacterium* spp., *Petrimonas* spp., and *Defluviitoga* spp. As a bioaugmentation agent, the mixed culture promoted the average CH4 production by >36% under 5 g NH_4^+-N/L. Moreover, the enriched culture was 25% more effective biomethanation inoculum compared to the pure

Table 2
The effects of bioaugmentation using HM and SAO-HM consortia on the ammonia-stressed AD processes.

Reactor type	Feedstock	Temperature	Ammonia concentration	Bioaugmentation agent	Methane recovery	References
CSTR	Animal manure + organic waste	37 °C	5 g NH_4^+-N/L	HM *M. bourgensis* MS2^T	31.3%	Fotidis et al., 2014
CSTR	Cattle manure + microalgae	37 °C	11 g NH_4^+-N/L	HM *M. bourgensis* MS2^T	28%	Tian et al., 2019a
CSTR	OFMSW	37 °C	$9.5 \sim 13.5$ g NH_4^+-N/L	HM *M. bourgensis* MS2	21%	Yan et al., 2020a
CSTR	Glucose	37 °C	4 g NH_4^+-N/L	Eight groups consisting of SAO bacterium (*S. schinkii* and *T. acetatoxydans*) and HM archaea (*M. bryantii*, *M. bourgensis*, *M. smithii* and *M. barkeri*)	30.6 $\sim$ 71.2%	Yang et al., 2019
CSTR	Glucose	37 °C	4 g NH_4^+-N/L	Nine groups consisting of SAO bacterium (*S. schinkii* and *T. acetatoxydans*) and archaea (AM-*M. harundinacea*; HM-*M. smithii* and *M. barkeri*)	4 $\sim$ 49%	Yang et al., 2020
CSTR	Dairy slurry	37 °C	5 g NH_4^+-N/L	The enriched HM cultures (mainly *Methanoculleus* spp.)	>40%	Fotidis et al., 2017
CSTR	Blackwater stock	35 °C	$0.1 \sim 1.7$ g TAN /L	The enriched HM cultures (mainly *Methanobacteriales* and versatile *Methanosarcina*)	3 $\sim$ 16%	Xu et al., 2019
CSTR	Cattle manure	53 °C	5.0 g NH_4^+-N/L	The enriched ammonia-tolerant consortia (mainly AM *M. thermophila*)	after 3 days	Tian et al., 2019b
				The enriched ammonia-tolerant consortia (mainly AM *M. thermophila*) and a strain of HM *M. thermophilus*	17%	
CSTR	Chicken manure	37 °C	$5.0 \sim 8.4$ g NH_4^+-N/L	The enriched propionate-degrading methanogenic culture (mainly *Methanothrix*, *Methanobacterium*, *Levinea*, *Pelotomaculum* and *Syntrophobacter*)	15 $\sim$ 18%	Li et al., 2022
CSTR	Dairy manure	37 °C	4 g NH_4^+-N/L	Consortia acclimated with dairy manure (mainly *Methanosarcina*, *Clostridia*)	26%	Yang et al., 2022
				The enriched ammonia-tolerant consortia (mainly *Methanosarcina*, *Clostridia*)	39%	
	Pig manure			Consortia acclimated with pig manure (mainly *Methanobacterium*, *Clostridia*)	25%	
				The enriched ammonia-tolerant consortia (mainly *Methanosarcina*, *Clostridia*)	49%	

strain of *Methanoculleus bourgensis* MS2[T].

Interestingly, mixed cultures dominated by AM archaea as the bio-augmentation agents, could also stimulate methane production via HM pathway, thus relieving AD system from ammonia stress. For example, Li et al. (2022) applied a propionate-degrading methanogenic culture (AM *Methanothrix* > 70%, and HM *Methanobacterium* < 30%) to the chicken manure-fed AD system under 5.0 ∼ 8.4 g NH_4^+-N/L stress, and observed enhanced CH_4 yield by 15 ∼ 18% compared to the non-bioaugmented condition. With bioaugmentation, syntrophic interactions between propionate-degrading bacteria and H_2 transferring capability of *Methanobacterium* were established, promoting the propionate conversion and CH_4 production under ammonia stress condition. In addition, Yang et al. (2022) employed dairy manure-acclimated consortia (versatile *Methanosarcina* > 40%, AM *Methanosaeta* > 25%, HM *Methanobacterium* > 15%) to bioaugment the AD system under ammonia stress (4 g NH_4^+-N/L), and observed improvement in CH_4 production by 25 ∼ 26% compared to control (without acclimation). Similarly, H_2 transfer in HM pathway was stimulated with bioaugmentation. The common feature of the applied bioaugmentation cultures in these two studies was that HM archaea showed inferiority compared to AM archaea. However, ammonia stressed condition favored the growth of HM archaea over AM archaea. Therefore, HM/SAO-HM dominated mixed culture may not be the only option to stimulate H_2 transfer.

Noticeably, H_2 partial pressure required by SAO bacteria and hydrogenotrophic methanogens is different, which restrains the interspecies H_2 transfer rate, and brings a challenge to interspecies H_2 transfer stimulation to relieve ammonia stress. Briefly, the hydrogenotrophic methanogens need as much H_2 as possible for efficient methanation metabolism. In most cases, the lowest H_2 thresholds required by hydrogenotrophic methanogens should be higher than 0.15 ∼ 8.6 Pa although it varies with species (Feldewert et al., 2020). Such a high H_2 partial pressure might suppress the growth of SAO bacteria based on the Gibbs free energy of the reactions ($\Delta G^{0'} > 0$, Eqs. 4 ∼ 5&8 in Table 1). Future works are suggested to focus on the facilitation of H_2 synthesis capacity in syntrophic consortia and their metabolic tolerance to a high H_2 partial pressure.

3.2. Redox material as an electron shuttle

Indirect IET enhancement via redox material addition is relatively less explored in the ammonia-stressed AD system. So far, humic acid is the only redox compound reported to promote methanogenesis in the ammonia-stressed AD process. Supplementations of 1 ∼ 5 g/L of humic acids contributed to 27 ∼ 29% increments in CH_4 production in the thermophilic (55 °C) livestock wastes-fed AD system at 1.9 ∼ 2.3 g NH_4^+-N/L level (Ho and Ho, 2012). The observed positive effect of humic acid addition was attributed to the increased H_2 utilization thereby creating favourable thermodynamic condition for methanogenesis. Recently, authors' research group found that addition of 100 μM anthraquinone-2,6-disulfonate (AQDS), a representative analogue of humic acid, could improve the cumulative CH_4 production and the maximum CH_4 production rate by 7 and 11%, respectively, and further shorten the methanogenic lag phase by 14% (Xu et al., 2022). AQDS was dynamically stable over the AD process, and was believed to facilitate acetate generation and methanation by acting as an electron shuttle between *Anaerolinea* and *Methanosaeta* (Xu et al., 2022).

The roles of redox materials as electron shuttle on VFA methanation have also been investigated. Since VFA accumulation is a critical issue in the ammonia-stressed AD process, the findings from such studies could be used as references for ammonia stress relief. Zhuang et al. (2017) reported increase in CH_4 production rate by 109, 50, and 31% with the addition of cystine/cysteine at concentrations of 100, 400, and 800 μM, respectively, which was attributed to the cystine/cysteine mediated electron transfer between syntrophic propionate oxidizing bacteria and methanogens. From the aspects of energetics and kinetics, cystine/cysteine are incapable of mediating electron transfer from syntrophic

propionate oxidation (SPO) reaction to CO_2 reduction, since the redox potential of cystine/cysteine (-348 mV) is lower than both of SPO (-290 mV) and CO_2 reduction (-240 mV) under standard conditions. However, at the tested concentrations, the redox potential of cystine/cysteine shifted to a feasible state between −290 and −240 mV (Zhuang et al., 2017). Hence, the stimulation effect of redox materials on syntrophic methanogenic process is concentration dependent. A recent finding by Cai et al. (2021) further corroborated the above statement, in which the authors observed that role of anthraquinone-2-sulfonate as an electron shuttle in syntrophic butyrate oxidation (SBO) for methanogenesis was dependent on its concentration.

Given the current knowledge, however, the results of redox material amendments in AD process are not consistent, especially in terms of CH_4 production and CH_4 production rate (Table 3). A relatively high concentration of redox material might change the redox potential, which is not suitable for methanogenesis, but is beneficial for other biochemical reactions such as quinone respiration (Cervantes et al., 2000). Thus, comprehensive study on the types, dosages, and biochemical relationships of different redox materials is necessary to further elucidate the roles of redox materials as electron shuttle in the ammonia-stressed AD system.

3.3. Direct electron transfer inducement with conductive materials

Direct IET is considered as a more promising approach than interspecies H_2 and formate transfer and redox material-mediated transfer due to the fewer electron transfer steps and favourable thermodynamic characteristics (Wu et al., 2020b). Conductive materials are commonly applied as direct IET inducers for methanogenesis resumption and promotion in AD systems (Wang et al., 2021). To date, metal-based and carbon-based conductive materials, including magnetite, ZVI, biochar, hydrochar, granular activated carbon (GAC), PAC, carbon cloth, carbon felt, carbon fibre, carbon nanotube and graphene, have been tested as direct IET inducers in AD systems (Yin et al., 2020; Wang et al., 2021).

Table 4 summarizes the effects of conductive materials addition on CH_4 production performance, and evidence of direct IET observed in the ammonia-stressed AD processes. Mostly, conductive material amendments are advantageous to methanogenesis. Fig. 3a and 3b illustrate the relationships between conductive material types and CH_4 production, as well as between conductive material types and CH_4 production rate, respectively, in the ammonia-stressed AD processes. Magnetite, ZVI, biochar, GAC, and PAC amendments promoted an average CH_4 production by 67, 42, 37, 71, and 55%, respectively. However, the overall amendment effects were not significantly different in terms of CH_4 production within these conductive material groups ($p > 0.1$). While the amendment effects on CH_4 production rate showed a significant relevance to conductive material types ($p < 0.01$), among which ZVI and PAC were preferred, followed by magnetite and biochar, whereas carbon

Table 3

Effects of redox materials on CH_4 performance at different concentrations.

Applied redox materials	Concentrations (mM)		References
	Promotion	Inhibition	
Cysteine	0.1, 0.4, 0.8	/	Zhuang et al., 2017
Anthraquinone-2,6-disulfonate	0.5, 5	20	Xu et al., 2013
Anthraquinone-2-sulfonate	0.03, 0.05, 0.1	0.15	Cai et al., 2021
9,10-Anthraquinone	/	0.5, 1.0, 5 ppm*	GarciaLopez et al., 1996
Anthraquinone-2,6-disulfonate	/	1, 5	Yang et al., 2012
Humic acids	/	0, 5, 10, 15, 20 (HA:VSS*)	Li et al., 2019

"*" refers to the specific unit of the concentration.

Table 4

Effects of conductive materials on CH$_4$ performances and the potential evidence supporting direct electron transfer in the ammonia-stressed AD processes.

Reactor types	Temperature	Ammonia concentrations	Conductive materials	CH$_4$ performances	Evidences on direct electron transfer	References
Batch	37 °C	5.0 g NH$_4^+$-N/L	Magnetite nanoparticle	36 ~ 58 % increases in rate	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Zhuang et al., 2018
Batch	35 °C	1.6 g NH$_4^+$-N/L	Magnetite	50 % increase in rate	a. Closer biological network (SEM, XRD, FTIR)	Wang et al., 2020
					b. Substitution for electroactive components (3D-EEM and UV/vis) c. Enrichment of electroactive microorganisms (16S rDNA gene sequencing)	
Batch	37 °C	6.5 g TAN/L	Magnetite	15 ~ 58 % increases in yield	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Lee et al., 2019
Batch	35 °C	5.5 g TAN/L	Magnetite	45 ~ 134 % increases in production	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Yan et al., 2020c
					b. Upregulation of CO$_2$ reduction pathway and substitution for electroactive components (TMT quantitative proteomic analysis)	
			ZVI	53 ~ 132 % increases in production	/	
Batch	37 °C	5.0 g TAN/L	ZVI	0.4 ~ 22 % increases in yield	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing, *mcr*A gene quantification)	Meng et al., 2020
Batch	35 °C	7 g TAN/L	Fruitwood biochar	10 ~ 47 % increases in rate	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Lu et al., 2016
Batch	37 °C	5.5 g TAN/g	Wood-pellet biochar	0 ~ 136 % increases in peak daily yield	a. Closer biological network (SEM)	Indren et al., 2020b
					b. Enrichment of electroactive microorganisms (qPCR)	
Batch	37 °C	5.3 ~ 6.5 g TAN/kg	Wood pellet biochar	22 ~ 69 % increase in yield	a. Enrichment of electroactive microorganisms (qPCR)	Indren et al., 2020a
		4.6 g TAN/kg	Wheat straw biochar	Decreased in rate	/	
		4.9 g TAN/kg	Sheep manure biochar	Decreased in rate	/	
Semi-CSTRs	37 °C	1.0 ~ 16.0 g TAN/L	Orchard waste wood biochar	32 ~ 36% increase in production	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Pan et al., 2019
Batch	35 °C	5.5 g TAN/L	PAC	55 ~ 93% increases in production	/	Yan et al., 2020c
Batch	35 °C	1.4 g NH$_4^+$-N/L	GAC	35 ~ 108% increases in yield	a. Closer biological network (SEM)	Johnravindar et al., 2010
Continuous-flow UASB	33 °C	1.2 g NH$_4^+$-N/L	GAC	Successfully start up	a. Enrichment of electroactive microorganisms and upregulation of electroactive components (metagenomic analysis)	Lei et al., 2019
					b. Increase of aggregate conductivity	
Batch	35 °C	1.5 g TAN/L	CNT	18 ~ 71% decreases in rate	/	Yan et al., 2019b
Continuous	20 °C	1.08 g NH$_4^+$-N/L	Carbon fibers	Increase in yield	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Huang et al. 2020
Batch	37 °C	5 ~ 6.5 g TAN/L	ZVI	45.1% decrease in lag phase	a. Enrichment of electroactive microorganisms (16S rRNA gene sequencing)	Liu et al., 2020
			ZVI & AC	70.4% decrease in lag phase		
			ZVI & graphite	64.8% decrease in lag phase		
			Fe & C	63.3% decrease in lag phase		
Continuous	35 °C	4.0 g TAN/L	OLMZ	54 ~ 69% increases in yield	a. Increase of sludge conductivity	Zheng et al., 2020a
					b. Enrichment of electroactive microorganisms (SEM)	

nanotube (CNT) was completely unfavourable to AD with ammonia stress.

ZVI was considered to donate electrons for extra CH$_4$ yields via chemical corrosion and microbial metabolism (Wei et al., 2018). However, since the ZVI corrosion generates only a limited number of electrons, ZVI corrosion alone cannot account for the entire improvement in CH$_4$ production (Meng et al., 2020). Therefore, the improvement in CH$_4$ production with ZVI amendment is most likely attributed to direct IET stimulation in syntrophic methanogenesis. In addition, ZVI was confirmed to act as an intracellular electron shuttle. The conjunction

point of ZVI to the electron transfer system was identified as EtfAB: quinone oxidoreductase (Yan et al., 2020c).

Magnetite and PAC are believed to trigger direct IET among electroactive microbes thereby replacing diffusion-based H$_2$ transfer (Zhuang et al., 2018; Wang et al., 2020; Lee et al., 2019; Yan et al., 2020c). However, it was reported that the genes related to CO$_2$ reduction methanogenic pathway were downregulated in the both magnetite and PAC amendment groups with the elevation of ammonia concentration to 5.5 g N/L, suggesting that direct IET-based methanogenesis was suppressed by these conductive materials under a high ammonia-
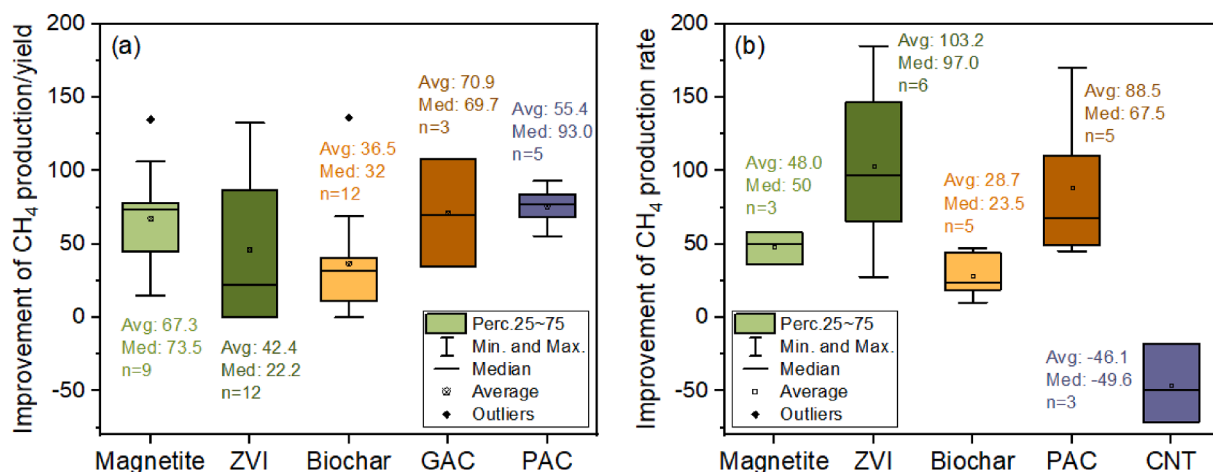
Fig. 3. Overview of the effects of different conductive materials on (a) CH₄ production/yield and (b) CH₄ production rate. n refers to the number of data points for each boxplot. Data with>1.5-fold interquartile ranges above the third quartile or below the first quartile are defined as outliers.

stressed condition (Yan et al., 2020c). Thus, understanding the role of different conductive materials could provide more insight at the intracellular proteomic level.

Biochar and CNT as conductive materials showed relative inferiorities in mitigating ammonia inhibition. The physical properties of conductive materials might influence their ability to resist ammonia inhibition. Indren et al. (2020) attributed the inferior effects of biochar amendment to its microstructure, i.e., smooth surface with fewer pores which may prevent microbial attachment. Yan et al. (2019b) suggested that transmembrane CNT channels might block K^+ transport and reduce the H^+ motive force and adenosine triphosphate (ATP) generation. Moreover, CNTs also involve risks of damaging cell membranes and

inhibiting respiration and enzyme activities (Luongo and Zhang, 2010; Hai et al., 2014; Zhang and Thakor, 2014).

Recently, the modification of conductive materials to resist ammonia inhibition has been explored (Liu et al., 2020; Zheng et al., 2020b). For example, Liu et al. (2020) found that hybrid metal-based and carbon-based materials, i.e., Fe-C, ZVI-AC and ZVI-graphite, could stimulate electroactive microorganisms such as *Methanosarcina* for direct IET acceleration with high ammonia tolerance. The CH₄ production lag phase was reduced by 70, 65, and 63% with the Fe-C, ZVI-AC, and ZVI-graphite amendments, respectively, compared to only 4 ~ 45% reduction with ZVI amendment. In addition, Zheng et al. (2020b) discovered that zeolite modified with oyster shell and lignite could not only trigger
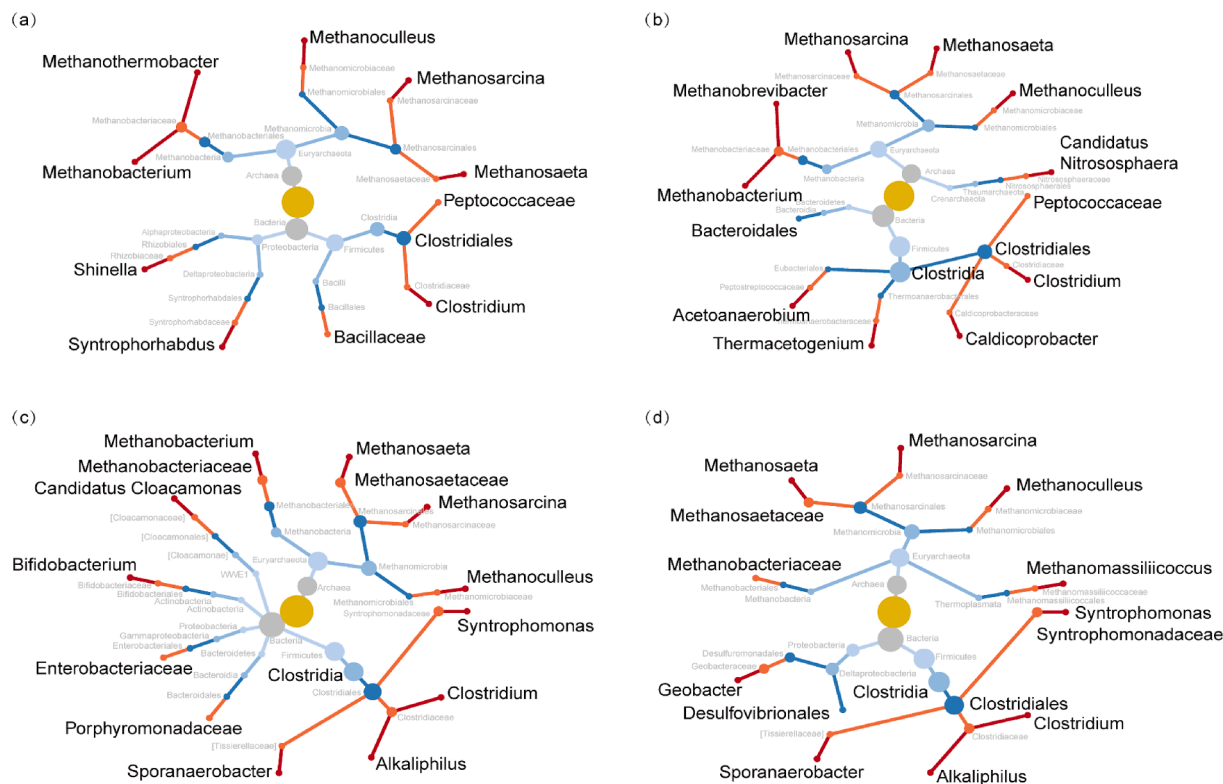

Fig. 4. Phylogenetic trees showing the subordinations and positions of (a) the dominant microbes in the H₂ transfer-stimulated AD system, (b) the enriched microbes with the greatest increase in relative abundance responding to H₂ transfer stimulation strategy, (c) the dominant microbes in the direct IET-stimulated AD system, and (d) the enriched microbes with the greatest increase in relative abundance responding to direct IET stimulation strategy. The taxa in black font were the identified and reported members, while the taxa in grey font were the corresponding parents.

direct IET between electroactive consortia, but also illustrate advances in ammonium adsorption, elements supplementation, and microbial attachment. After its amendment, CH$_4$ yields increased by 54 ~ 69% under 4.0 g N/L stress compared with control. Meanwhile, the modified material after application in ammonia-rich processes could be used as a fertilizer.

Besides the direct IET induction, conductive materials are also reported to maintain pH, provide surface area for microbial immobilization, absorb and adsorb toxicants, and optimize ecological niches for anaerobes (Fagbohungbe et al., 2017; Qiu et al., 2019; Paritosh et al., 2020; Chiappero et al., 2020). However, these mechanisms seemed not crucial. For example, ammonia concentration was not significantly fluctuated before and after conductive material amendment (Mumme et al., 2014; Lu et al., 2016). Further investigations should focus on quantifying the relative contribution of conductive materials to direct IET and the other possible mechanisms.

4. Microbial community and metabolic function

The microbial dynamics revealed more details of the ammonia-stressed AD processes in response to IET stimulation. Fig. 4 shows the phylogenetic trees with the most noteworthy microorganisms after IET stimulation.

As for microbial community with interspecies H$_2$ transfer stimulation, *Shinella*, *Syntrophorhabdus* and *Clostridium* were the dominant bacteria at the genus level (Fig. 4a), most of which are either ammonia-tolerant or are associated with ammonia-utilization (Chen et al., 2016; Zeb et al., 2019). *Methanosaeta*, *Methanosarcina*, *Methanoculleus*, *Methanobacterium*, and *Methanothermobacter* were the most dominant archael genera. Meanwhile, as shown in Fig. 4b, *Clostridium* and *Thermacetogenium* were mostly stimulated in response to ammonia stress. They are both typical SAO consortia associated with hydrogenotrophic methanogens (Pan et al., 2021). *Thermacetogenium phaeum* performs the complete Wood-Ljungdahl pathway for SAO metabolism (Oehler et al., 2012), while *Clostridium ultunense* involves in the glycine cleavage system pathway coupled with part of the Wood-Ljungdahl pathway for SAO metabolism (Wei et al., 2020; Pan et al., 2021). In addition, bacteria capable of H$_2$ production, such as the genera *Caldicoprobacter* and *Acetoanaerobium*, family *Peptococcaceae*, and classes *Bacteroidales* and *Clostridia* (Xu et al., 2020; Bouanane-Darenfed et al., 2011; Bes et al., 2015; Sipma et al., 2007; Iyer et al., 2004), were also enriched. Correspondingly, hydrogenotrophic methanogens, i.e., *Methanobacterium*, *Methanoculleus*, *Methanobrevibacter*, and *Methanosarcina*, were enriched, indicating the augmentation of interspecies H$_2$ transfer to overcome ammonia inhibition.

Following the conductive materials amendments, *Bifidobacterium*, *Alkaliphilus*, *Sporanaerobacter*, *Clostridium*, and *Syntrophomonas* were the dominant bacterial genera, while *Methanoculleus*, *Methanosarcina*, *Methanosaeta*, and *Methanobacterium* were the dominant archaeal genera (Fig. 4c). Meanwhile, bacterial genera such as *Clostridium*, *Syntrophomonas*, *Sporanaerobacter*, *Alkaliphilus*, *Treponema*, and *Geobacter* were observed to be enriched, benefited from their potential electroactivity (Fig. 4d). *Geobacter* in family *Geobacteraceae* can associate with electroactive methanogens via direct IET mechanism (Rotaru et al., 2014a, Rotaru et al., 2014b). Correspondingly, electroactive archaea, such as *Methanosarcina* and *Methanosaeta*, were enriched with significant shifts. The direct IET through pili between *Methanosarcina barkeri* and *Geobacter metallireducens*, as well as between *Methanosaeta harundinacea* and *Geobacter metallireducens*, have been well proven before (Rotaru et al., 2014a, Rotaru et al., 2014b), suggesting the possible direct IET establishment in the ammonia-stressed AD process.

Nevertheless, within a genus of methanogen, interspecies H$_2$ transfer and direct IET may coexist. For example, *Methanosarcina* and *Methanobacterium* can perform CO$_2$ reduction with H$_2$ via interspecies H$_2$ transfer, and with electrons via direct IET (Rotaru et al., 2014a; Yan et al., 2020a). Thus, IET understanding revealed by microbial diversity

are not completely conclusive. Deep insight into function-related information is necessary for the understanding of IET stimulation in the ammonia-inhibited AD systems. To date, only a few studies have investigated fundamental aspects at the levels of gene, enzymatic activity, and protein expression. With bioaugmentation, genes involved in H$_2$-associated CO$_2$ reduction to CH$_4$, i.e., FWD, formylmethanofuran-tetrahydromethanopterin N-formyltransferase (FTR), methenyltetrahydromethanopterin cyclohydrolase (MCH), methylenetetrahydromethanopterin dehydrogenase (MTD), MER, MTR, H$_2$:CoB-CoM heterodisulfide ferredoxin reductase (HDR) and coenzyme-B sulfoethylthiotransferase (MCR), were identified and promoted (Yan et al., 2020a; Yang et al., 2022). With amendment using conductive materials, genes encoding direct IET-related conductive pili, outer membrane c-type cytochromes (OmcS), transmembrane spanning porin-cytochrome complexes, and membrane-bound F$_{420}$ H$_2$ dehydrogenase were stimulated (Lei et al., 2019); fulvic acid-like materials, flavins, and c-type cytochromes in extracellular polymeric substances were promoted (Wang et al., 2020). The enhanced genes, enzymes and proteins supported the possible stimulation of IET mechanism to facilitate VFAs conversion in ammonia-stressed AD process.

5. Prospects and challenges

5.1. Further exploration of electron transfer stimulation mechanism

In general, IET stimulation for efficient syntrophic methanogenesis has great potential to recover CH$_4$ production in the ammonia-stressed AD system. However, evidence of methanogenic improvement with IET stimulation still deserves discussion. Integrated microbiome analyses technologies, i.e., metagenomics, metatranscriptomics, metaproteomics, and metabonomics can provide deeper insights into IET mechanisms. Further studies are expected to reveal more information at the molecular level.

A pure co-culture system is necessary to further understand the occurrence and stimulation of IET in relieving ammonia stress in AD process. In this case, the conversion rate from substrate to CH$_4$ could be a key indicator to demonstrate the IET pathway. For example, pure co-cultures of *Geobacter metallireducens-Methanosarcina barkeri*, as well as *Geobacter metallireducens-Methanosaeta harundinacea*, generated nearly 1.5 mol of CH$_4$ from a mole of ethanol (Rotaru et al., 2014a; Rotaru et al., 2014b). Considering the applied cultures could not utilize H$_2$, the additional 0.5 mol of CH$_4$ should be generated through the direct electron capture for CO$_2$ reduction. In pure co-cultures, noticeably, the target microorganism can be genetically modified to verify the specific metabolism. For example, Zheng et al. (2020b) demonstrated the essential role of PilA in direct IET by employing a mutant strain of *G. metallireducens* in which the gene for PilA was deleted. It was observed that the mutant strain could not form syntrophic associations with *Methanobacterium* strain YSL compared to the wild type.

Additionally, AD coupled with microbial fuel cells (AD-MFCs) may provide a feasible platform to observe electron flow directly, as direct IET through wires from bacteria (at the cathode) to methanogens (at the anode) is the only pathway of syntrophic respiration in such system (Dube and Guiot, 2015).

5.2. Role and contribution of electron transfer in the ammonia-stressed anaerobic digestion

AM was less competitive than IET in ammonia-stressed AD process. Though, with the stimulation of IET, AM showed recovery. For example, Wang et al. (2020) suggested that direct IET and AM were likely simultaneously enhanced with the amendment of magnetite. However, the relative contribution of IET and AM in promoting CH$_4$ production under ammonia-stress condition, has not been reported, which needs further exploration. Within IET network, it is usually acknowledged that direct IET is preferred to interspecies H$_2$ transfer and shuttle-mediated

IET in syntrophic methanogenesis, owing to the convenient transfer steps and its energy-conserving capability (Wu et al., 2020b). Yan et al. (2021) discovered that bioaugmentation of a *Methanothermobacter*-dominated culture, at a dosage of 176 mg volatile suspended solids (VSS)/L and biochar amendments (derived from straw or spruce woodchips, at a dosage of 4 g/L) stimulated the maximum CH$_4$ production rates by 23% and 24 ~ 35%, respectively, in the ammonia-stressed AD reactors compared to the control. It suggested that conductive material amendment to trigger direct IET is more favourable than bioaugmentation to stimulate interspecies H$_2$ transfer for ammonia stress relief. Yet, a comprehensive investigation is required to compare the effects under different types of conductive materials and bio-augmentation microbes, different ammonia stress levels, and different AD operational parameters.

Recent studies showed that combination of different strategies could effectively improve AD performance (Xiao et al., 2019; Yan et al., 2021). For example, Xiao et al. (2019) reported improvement in CH$_4$ production rates by 28 ~ 45% following a combination of bioaugmentation (*Clostridium pasteurianum*) with conductive material amendments (e.g., magnetite and biochar). In the ammonia-stressed process, compared to individual bioaugmentation (*Methanoculleus thermophilus* sp.) and wood biochar amendment, their combination substantially increased CH$_4$ production (Yan et al., 2021). In addition, some redox compounds in soluble microbial products and extracellular polymeric substances, such as humic substances, dipeptides, and flavins, might be involved in electron exchange among insoluble conductive materials and microorganisms, facilitating the IET (Yin et al., 2018). Therefore, the hybrid amendment with redox and conductive materials may have synergistic effects on relieving ammonia stress in AD process.

5.3. Extension of electron transfer stimulation in the ammonia-stressed anaerobic digestion

Innovations in stimulation strategy based on fundamental understanding of IET mechanism are necessary. Regarding direct IET, AD coupled with microbial electrolysis cell (AD-MEC) system provides a platform to stimulate electroactive microbes to trigger direct IET networks (Qin et al., 2021). Recently, light-exposure was proposed as a novel mechanism to trigger direct IET among syntrophic co-operators (Ha et al., 2017; Huang et al., 2019). Whether illumination can enhance direct IET for higher CH$_4$ production to relieve ammonia inhibition is worthy of exploration. In terms of H$_2$ transfer, SAO-HM is not the only metabolic pathway mediated by H$_2$. As summarized in Table 1, interspecies H$_2$ transfer also occurs between SPO reaction (Eq. 5) and HM, as well as SBO reaction (Eq. 4) and HM, among others. Thus, enhancement of other interspecies H$_2$ transfer pathways, such as SPO-HM and SBO-HM, could also recover the ammonia-stressed AD processes.

Meanwhile, process optimization is an important issue to further improve performance of the ammonia-stressed AD system. The wash-out loss of the bioaugmented community or redox/conductive materials from AD system is a major challenge for long-term application of such interventions. Reducing such loss by strengthening their immobilization in AD system could result in sustained stimulatory effects. Some of such immobilization strategies include wrapping additives, applying bio-augmentation with agar gel (Yan et al., 2021), and attaching additives like carbon fibres to AD digestors (Huang et al., 2020), among others.

Economic analysis of IET stimulation strategy is the crucial consideration for its practical application. Most of the applied conductive materials such as magnetite, iron-based oxide, biochar, PAC and GAC, are low in cost, (Nguyen et al., 2021). Compared to other conductive materials, biochar is more economical and can significantly the operating costs, but it cannot be reused/recycled (Qiu et al., 2019). Magnetite biochar as a hybrid material may add extra benefit than biochar in the AD process. On the other hand, CNT is not only relatively high in cost (Nabi et al., 2022), but also ineffective in regulating CH$_4$

production under ammonia stress condition (Yan et al., 2019b); thus CNT seems not favorable based on current knowledge, which needs further investigation. However, most studies aiming at IET stimulation for improving CH$_4$ production under ammonia inhibition were conducted in laboratory-scale bioreactors. More studies based on continuous operation of pilot scale AD systems are necessary for evaluating the long-term effects of such interventions (e.g., bioaugmentation and conductive materials amendments) as well as conducting technoeconomic analysis. Meanwhile, effective separation and recovery methods of amended additives, especially conductive materials, need further scientific exploration for their subsequent utilization.

6. Conclusions

IET stimulation as an approach to enhance CH$_4$ production in the ammonia-stressed AD processes is widely reported. This review comprehensively discussed the IET mechanisms and different strategies to mitigate ammonia stress in AD process. Strategies such as acclimation to ammonia, *in-situ* H$_2$ supplementation, and SAO-HM bioaugmentation can effectively promote interspecies H$_2$ transfer to relieve ammonia stress. To date, redox compound-mediated IET is less explored. The conductive materials amendment-induced direct IET is a promising pathway for recovering CH$_4$ production under ammonia-stressed condition. The role and contribution of IET stimulation to mitigate ammonia stress in AD processes merit further investigation.

CRediT authorship contribution statement

Jun Xu: Conceptualization, Methodology, Visualization, Writing – original draft. **Samir Kumar Khanal:** Conceptualization, Writing – review & editing. **Yurui Kang:** Data curation, Formal analysis. **Jiaxin Zhu:** Data curation, Investigation. **Xia Huang:** Data curation, Investigation. **Yang Zong:** Visualization. **Weihai Pang:** Writing – review & editing. **K.C. Surendra:** Writing – review & editing. **Li Xie:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

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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