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Review

Bioelectrochemical systems for enhanced nitrogen removal with minimal greenhouse gas emission from carbon-deficient wastewater: A review

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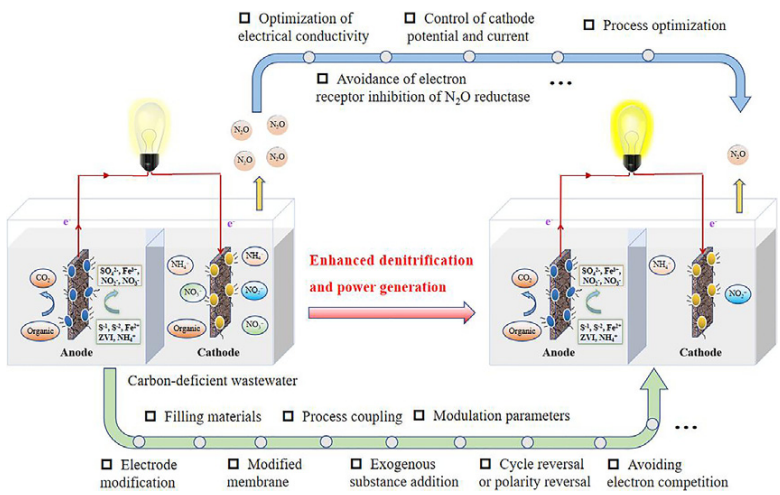
- Classical treatments do not remove N entirely from carbon-deficient wastewater.
- Coupling BES with emerged methods improves nitrogen removal and produces electricity.
- BES also allows one to reduce N_2O emissions through multifactorial modulation.
- Novel insights and future directions to upscale BES technologies are provided.



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A B S T R A C T

Nitrogen pollution and the rising amount of wastewater generation are calling for advanced wastewater treatments, which is particularly necessary for carbon-deficient wastewater that contains multi-species inorganic nitrogen, since conventional heterotrophic denitrification processes cannot remove nitrogen completely when carbon sources are insufficient. For that, bioelectrochemical systems (BES) have been recently developed because they can simultaneously produce electricity and remove resistant nitrogen from the carbon-deficient wastewater. However, the simultaneous removal of multi-species inorganic nitrogen cannot be achieved by electroautotrophic denitrification using BES alone. Moreover, the efficiency of nitrogen removal and power generation has been thwarted by the low energy output, high internal resistance of the device, and electron competition in non-denitrification pathways. This review article discusses the latest developments for nitrogen removal through BES-enhanced denitrification and elucidates multiple coupled BES-based denitrification pathways to remove multi-species inorganic nitrogen simultaneously. Focus points of the research area include coupling BES technologies with emerged methods, electron transfer enhancement, and avoiding electron competition that improves performance with less cost. The prospect of reducing emissions of greenhouse gases is also critically reviewed, in the hope of reducing potential intermediate products of denitrification, such as nitrous oxide (a potent greenhouse gas), through multi-factor regulation. We imply that BES is a good choice for future scale-up applications of MFC coupled with MEC to treat carbon-deficient wastewater. Overall, this review will provide useful information for the development of advanced technologies to treat carbon-deficient wastewater with less emission of greenhouse gases.

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Abbreviations

BES	Bioelectrochemical systems;
NH_4^+	ammonia nitrogen
NO_3^-	nitrate nitrogen
NO_2^-	nitrite nitrogen
BOD	biochemical oxygen demand
TN	total nitrogen
MFC	Microbial fuel cell
MEC	Microbial electrolytic cell
N_2	nitrogen gas
SND	simultaneous nitrification-denitrification
Anammox	anaerobic ammonia oxidation
N_2O	nitrous oxide
SHE	standard potential with standard hydrogen electrode
SDAD	Sulfur-driven autotrophic denitrification
NZVI	nano-zero valent iron
FeS_2	pyrite
H_2	Hydrogen gas
AOB	ammonia-oxidizing bacteria
NOB	nitrite-oxidizing bacteria
DEA	Diethylamine-functionalized polymers
PRBES	Periodic reversal bioelectrocoagulation system
NLCE	nematic liquid crystal electrode
PyAno	pyrite as an anodic
BER	biofilm-electrode reactor
Pt	platinum
Gf	graphite felt
GAC	granular activated carbon
3D-BES	three-dimensional electrode BES
HAlk	protonated alkalinity
Nar	NO_3^- reductase
Nir	NO_2^- reductase
Nor	nitric oxide reductase
Nos	nitrous oxide reductase
FNA	free NO_2^- acid

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1. Introduction

The rising stringent laws of environmental protection call for improved treatment efficiency of wastewater (Hu et al., 2020; Porse et al., 2020). In particular, advanced methods are required to remove nitrogen-containing pollutants completely (Chu et al., 2022). Carbon-deficient wastewater refers to the lack of biodegradable organic matter due to the lack of carbon source itself or excessive consumption of carbon source due to unreasonable wastewater treatment process design. Biochemical oxygen demand (BOD_5)/total nitrogen (TN) ratio of the typical carbon-deficient wastewater is generally below 2 and thus is difficult to carry out heterotrophic denitrification. Carbon-deficient wastewater includes a wide range of municipal and industrial sources and secondary effluents from biochemical treatment of domestic, industrial, and agricultural wastewater. Carbon-deficient wastewater also includes tail-water with limited carbon source such as toilet recycling flushing water, secondary effluents from wastewater treatment plants, and industrial and agricultural wastewater tail-water from the fertilizer, coking, breeding, livestock and poultry industries (Cao et al., 2019; Chen et al., 2022). The annual production of carbon-deficient wastewater is estimated to reach 60 billion tons by 2030 in China for municipal treatment plants (Chang and Wang, 2015). Therefore, the development of effective technologies for enhanced denitrification of carbon-deficient wastewater is of rising interest.

Enhanced nitrogen removal from carbon-deficient wastewater is a challenge, due to the lack of biodegradable organic matter in the wastewater and its co-exiting with multi-species of nitrogen pollutants such as ammonia nitrogen $(NH_4)^+$, nitrate nitrogen (NO_3^-) and nitrite nitrogen (NO_2^-) (Yu et al., 2019). For efficient heterotrophic denitrification, the BOD_5/TN ratio should be higher than 4, whereas the typical BOD_5/TN ratios is generally lower than 2 in the carbon-deficient wastewater. Consequently, methanol and sodium acetate are often added to provide sufficient electron donors for heterotrophic denitrification. But this strategy increases the operating cost and causes greenhouse gas (CO_2 equivalents) emissions (Jin, 2017). Similarly, complementary methods such as reverse osmosis, electrodialysis, ion exchange, and chemical ammonia oxidation do not meet carbon neutrality concepts due to their high energy consumption and expensive operating costs (Singh et al., 2022). Therefore, sustainable technologies are needed to recover resources such as electricity and remove nitrogen from carbon-deficient wastewater to reduce greenhouse gases emissions. For ameliorating nitrogen removal in carbon-deficient wastewater, bioelectrochemical systems (BES) such as microbial fuel cells (MFC) and microbial electrolytic cells (MEC) are currently under intense research (Mier et al., 2021; Qin et al., 2022; Zhang et al., 2022).

BES technologies use microorganisms attached on both the cathode and the anode as enzymatic catalysts. The electrons generated on anode reach the cathode through an external circuit, and they are utilized by the cathode microorganisms for reduction of oxidized nitrogen (NO_3^-/NO_2^-) to nitrogen gas (N_2) (Su and Chen, 2022). BES allows partial or complete autotrophic denitrification of carbon-deficient wastewater, with either reduced or zero extra dosage of chemicals acting as electron donors.

So far, most studies concerning BES-enhanced nitrogen removal from wastewater are conducted mainly under single-contaminant conditions, such as autotrophic denitrification of a single nitrogen-compound in water. Whereas multiple nitrogen compounds coexist in most actual effluents (Ahmed et al., 2012), it is necessary to seek a coupled BES-based denitrification pathway. Performance enhancement with BES technologies have been confirmed in processes such as anodic ammonia oxidation (Oksuz and Beyenal, 2021), simultaneous nitrification-denitrification (SND) (Wang et al., 2020a), short-range SND (Dong et al., 2020), anaerobic ammonia oxidation (Anammox) (Zhang et al., 2020), and their combinations. However, the efficiency of nitrogen removal and electricity production has been thwarted by the following challenges: the low voltage output of a single MFC, high internal resistance, and electron competition with non-denitrification pathways (Cai et al., 2020). These issues can be ameliorated by coupling BES and emerging methods (e.g., electrical stimulation, photocatalysis) or by the multi-factor modulation. At the same time, BES coupling strategies are effective in reducing the emission of nitrous oxide (N_2O), a notorious gas with strong greenhouse potential (Nguyen and Babel, 2022). Of course, whether other greenhouse gases are reduced through BES coupling is also of interest.

Therefore, BES is a promising technology with advantages of simultaneous removal of multi-species inorganic nitrogen, reduced extra dosage of chemicals, high nitrogen removal rate and reduction of greenhouse gas emissions. BES-based methods for wastewater treatments have been wildly applied in nitrogen removal and had been recently reviewed in terms of energy consumption analysis and reactor configurations (Cecconet et al., 2018; Su and Chen, 2022). However, there have been no specific reviews on BES for enhanced denitrification of the carbon-deficient wastewater. Advances in BES-based denitrification need to be comprehensively reviewed. Here, we review BES-enhanced denitrification technologies for multi-species inorganic nitrogen carbon-deficient wastewater, and summarize the coupled denitrification pathways based on BES, focusing on BES coupled with emerged methods, electrode material modification, cathode electron competition factors, and electron transfer rates. Under the context of counting greenhouse gas emissions during wastewater treatment, N_2O control emissions from wastewater treatment plants had been reported in detail (Thakur and Medhi, 2019). However, there are few reviews of BES-based N_2O control emissions through multifactor regulation, and most of the current studies have been conducted in small-scale BESs. Thus, key principles on how to up-scale the BES enhanced technologies are another purpose of the review.

2. Bioelectrochemical autotrophic denitrification of multiple nitrogen compounds

The overall principle of denitrification in BES involves biodegradation of organics coupled with electrons generation at the anode, and the electrons are transferred to the cathode for NO_3^- reduction (Fig. 1). Electrons can also be produced by other electron donors. Therefore, organic and/or inorganic wastewater such as the brewery wastewater, starch processing

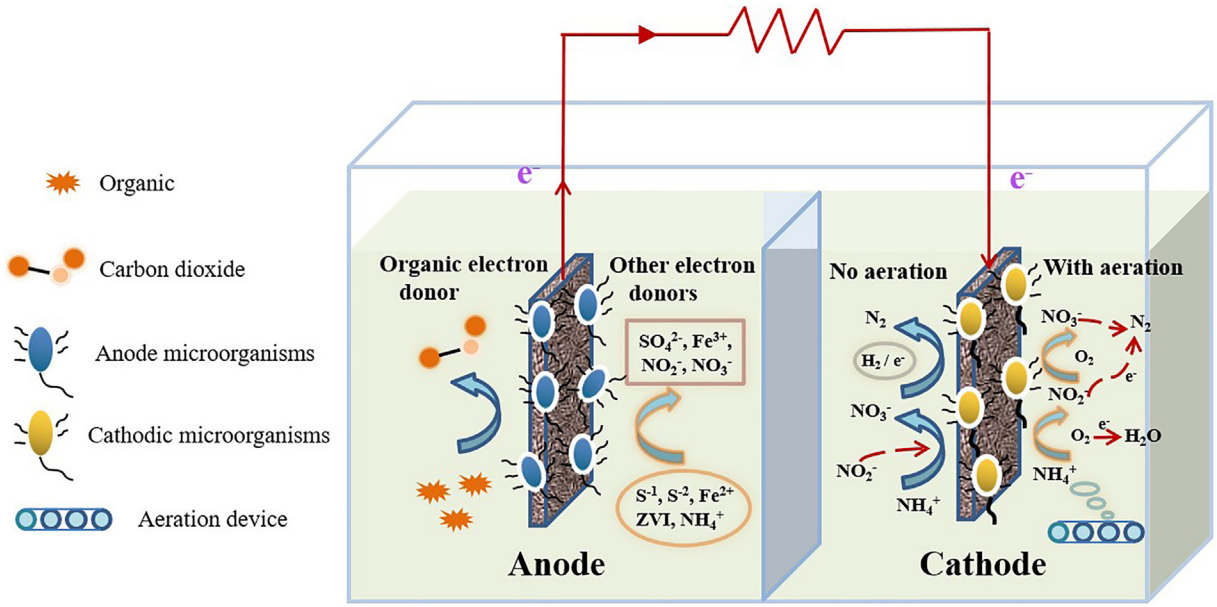

Fig. 1. Overview of electron donors and terminal electron acceptors for achieving denitration in a two-chamber bioelectrochemical systems (BES) configuration. The anode chamber is divided into conventional organics electron donors and other electron donors. The cathode chamber can undergo electro/hydrogen autotrophic denitrification and anaerobic ammonia oxidation (Anammox) in the absence of aeration. Simultaneous nitrification-denitrification (SND), short-range SND, and oxygen competition for electrons can occur under aeration.

plant wastewater, pharmaceutical wastewater, and tannery wastewater can be dosed to the anode of the reactor and used as electron donors.

2.1. Supply path of anode electrons

2.1.1. Electron supply based on organic matter

Organic matter-driven electroautotrophic denitrification uses organic matters as the electron donor (Eq. (1), Clauwaert et al., 2007). Those electrons are then utilized by the electroautotrophic denitrifying bacteria at the cathode to reduce electron acceptors such as NO_2^- and NO_3^- to N_2, and to generate electricity simultaneously. The denitrification process can be divided into four reduction steps, where the complete reduction of 1 mol NO_2^- and 1 mol NO_3^- to N_2 requires the consumption of 3 mol and 5 mol of electrons, respectively (Eqs. (2)–(5)). Therefore, the reduction of nitrogen compounds such as NO_2^- and NO_3^- depends on the electron flow from the biocathode (Puig et al., 2011).

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad E^{o\prime} = +0.433 \text{ V vs SHE} * \tag{1}$$

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \quad E^{o\prime} = +0.433 \text{ V vs SHE} \tag{2}$$

$$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O \quad E^{o\prime} = +0.350 \text{ V vs SHE} \tag{3}$$

$$NO + e^- + H^+ \rightarrow 1/2N_2O + 1/2H_2O \quad E^{o\prime} = +1.175 \text{ V vs SHE} \tag{4}$$

$$1/2N_2O + e^- + H^+ \rightarrow 1/2N_2 + 1/2H_2O \quad E^{o\prime} = +1.355 \text{ V vs SHE} \tag{5}$$

* SHE: standard potential with standard hydrogen electrode.

2.1.2. Sulfide-based electron supply

Sulfur-driven autotrophic denitrification (SDAD) uses sulfur and sulfur compounds such as sulfide and thiosulfate as electron donors and reducing sludge production, without dosing organic carbon chemicals (Wang et al., 2020a). The anode of MECs is controlled at the positive potential to produce electrons by sulfide oxidation (Eq. (6), Kato et al., 2012).

$$S^{2-} - 2e^- \rightarrow S \tag{6}$$

The oxidation of sulfide to sulfur depends on the oxidation-reduction potential, while increasing the anodic potential promotes further oxidation of sulfur (Eq. (7), Rabaey et al., 2006).

$$S^{2-} - 8e^- + 4H_2O \rightarrow SO_4^{2-} + 8H^+ \tag{7}$$

Sulfur can be recycled as a resource, while sulfate is an undesirable contaminant. Wang et al. (2018a) found that sulfur production decreased and sulfide removal increased with increasing anode potential. The optimal anode potential for sulfur recovery and sulfide removal ranges from −188 mV to −146 mV. Therefore, the anode potential should be controlled for simultaneous sulfur recovery and denitrification.

2.1.3. Iron-based electron supply

Iron can also act as electron donor under anaerobic conditions. For example, Li et al. (2019) designed an iron fuel cell bioreactor with iron as the abiotic anode and the electron donor. The generated electrons convert a portion of NO_3^- to NO_2^- at the cathode, enhancing the heterotrophic denitrification efficiency of the carbon-deficient wastewater. It avoids the reaction between nano-zero valent iron (NZVI) and NO_3^- to generate NH_4^+ at the cathode, which reduces the inhibitory effect on microorganisms. To reduce the operation cost, Ge et al. (2020) used pyrite (FeS_2), which is prevalent in artificial wetlands, to provide a dual electron donor of iron and sulfur to enhance nitrogen removal from the carbon-deficient wastewater while achieving electricity production (Eqs. (8)–(9)).

$$Fe^{2+} - e^- \rightarrow Fe^{3+} \tag{8}$$

$$S^{-1} + 4H_2O \rightarrow SO_4^{2-} + 8H^+ + 7e^- \tag{9}$$

2.1.4. NH_4^+-based electronic supply

When a current is applied between the cathode and anode of the MEC, the bacteria on the anode can oxidize NH_4^+ to NO_2^- (Koffi and Okabe, 2021). In addition, NH_4^+ can be anaerobically oxidized to NO_3^- if the MEC anode acts as the electron acceptor (Qu et al., 2014). Therefore, NH_4^+ can be used as an anode fuel in MEC. However, the simultaneous oxidation of organic carbon and ammonia at the bioanode is more complex

and flexible than the anodic ammonia oxidation alone (Zheng et al., 2020). This co-oxidation process releases electrons. Some electrons flow to the anode microorganisms to trigger metabolic activities, or reach the cathode to react with dioxygen. Therefore, it is crucial to direct the electrons generated by the anode to the denitrification pathway.

2.1.5. Hydrogen-based electron supply

Hydrogen autotrophic denitrification has received attention in recent years as a clean and economical process (Eqs. (10), (11)). Hydrogen gas (H_2) is one of the most favorable thermodynamic electron donors for denitrification (Di Capua et al., 2019). For instance, the additional voltage in MECs is small, of 0.2–0.8 V, to drive H_2 evolution at the cathode, which is much lower than the voltage required for water electrolysis (1.8–-3.5 V) (Katuri et al., 2019). In addition, the H_2 production rate of MEC is proportional to the applied voltage over a range of values, making the process controllable (Call and Logan, 2008).

$$H_2O + e^- \rightarrow H_2 + OH^- \tag{10}$$

$$NO_3^- + 2.5H_2 + H^+ \rightarrow 0.5N_2 + 3H_2O \tag{11}$$

2.2. Autotrophic simultaneous nitrification and denitrification

The autotrophic nitrification and denitrification (SND) coupled pathway enables simultaneous removal of several NH_4^+-containing compounds, where nitrification consists of a two-step process in which the ammonia-oxidizing bacteria (AOB) oxidizes NH_4^+ to NO_2^- under aerobic conditions, and the nitrite-oxidizing bacteria (NOB) oxidizes NO_2^- to NO_3^- (Eqs. (12), (13)). The SND biofilm presents a structure in which the electroautotrophic denitrifying bacteria is attached to the electrode surface and nitrifying bacteria surround them to occupy the outer part of the biofilm (Lai et al., 2020).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \tag{12}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \tag{13}$$

SND has two main advantages: (1) The products of the nitrification reaction, NO_2^- and NO_3^-, are the direct substrates for the denitrification reaction, which accelerates the nitrification reaction. (2) The nitrification reaction can consume the alkalinity released by denitrification, which makes the pH relatively stable in the system. Dissolved oxygen is a critical factor in achieving cathodic SND. At dissolved oxygen levels below 2 mg/L, NO_3^- and NO_2^- are used as electron acceptors. In contrast, at dissolved oxygen levels above 2 mg/L, dioxygen is the primary cathodic electron acceptor (Wang et al., 2020c). The cathode is the main factor limiting the high and stable power generation of MFC (Tran et al., 2022). In particular, dioxygen competes with NO_3^- for electrons in the cathode chamber, affecting the cathode denitrification rate.

Initially, MFCs were combined with an external aerobic nitrification reactor to convert NH_4^+ to NO_3^- externally, which was then recycled to the MFC cathode chamber for denitrification (Virdis et al., 2008). However, the system design with an external nitrification bioreactor is difficult to apply in the practical engineering. The designed tubular or three-chamber MFC (dual cathode) implements nitrification in the external cathode, an aerobic cathode, and bioelectrochemical denitrification in the internal cathode, an anoxic cathode (Zhang and He, 2012). However, separation with an anion/cation exchange membrane between the anode and the dual cathode increases operating costs. For this reason, single-chamber MFCs have been investigated, using a bottom anode and a top rotating cathode, where the SND reaction occurs at the cathode. Here, the three phases of air, solution, and biofilm interact to complete external nitrification and internal denitrification for carbon-deficient wastewater denitrification. To further improve coulombic efficiency, voltage output and power density, the addition of sponges can reduce the charge transfer resistance and solution resistance of the cathode (Deng et al., 2018).

2.3. Autotrophic short-range simultaneous nitrification and denitrification

In recent years, short-range SND in BES with low NOB activity, and thus low NO_3^- production, has been widely studied because this minimizes aeration costs with minimum C/N requirements (Nguyen and Babel, 2022). Theoretically, this pathway saves 25 % of the required aeration and 40 % of the carbon source compared to using NO_3^- as the electron acceptor, since the C/N ratio required for the reduction of NO_2^- is 1.72 versus 2.86 required for the reduction of NO_3^-. In addition, NO_2^- can be reduced twice faster than NO_3^-, and sludge production is reduced by 40 % in the short-range SND process. Therefore, the short-range SND is more advantageous in treating carbon-deficient wastewater.

Dissolved oxygen is also a critical factor. On the one hand, organic matter in the wastewater will be degraded under aerobic condition. Oxygen can be transferred from the cathode to the anode, reducing the coulomb efficiency (Bavasso et al., 2018). On the other hand, high dissolved oxygen in the cathode chamber can inhibit denitrification. Ammonia oxidation at low dissolved oxygen, around 0.5 mg/L, resulted in NO_2^- accumulation. Whereas at dissolved oxygen levels higher than 5.0 mg/L, NH_4^+ is directly oxidized to NO_3^- without NO_2^- accumulation (Li et al., 2016). Since very low dissolved oxygen (below 1 mg/L) can adversely affect the power generation of BES and high dissolved oxygen can lead to higher aeration costs, the dissolved oxygen concentration should be optimized.

The C/N ratio significantly affects the denitrification efficiency. Relatively high C/N ratios above 2.6 did not always produce high nitrogen removal loading rates, probably due to the competition between heterotrophic and autotrophic organisms for carbon sources. Considering nitrogen removal and SND efficiency, a C/N of 2.0 is considered to be an optimal operating condition (Yang et al., 2021). Therefore, the activity balance of heterotrophic and autotrophic organisms enables short-range SND more suitable for denitrification of the carbon-deficient wastewater (Jiaqi et al., 2020).

2.4. Anammox-denitrification/ammonia oxidation-anammox coupled pathway

Anammox is a process of anaerobic oxidation of NH_4^+ by a specific microbiota using NO_2^- as the electron acceptor to produce N_2. As a consequence, anammox could be widely used in the world of wastewater treatment when the influent composition is suitable (Yangin-Gomec et al., 2017). The advantages of the anammox process save cost for aeration and external carbon source dosage. When 1 mol NH_4^+ is oxidized, 1.32 mol NO_2^- is consumed, but 0.26 mol NO_3^- by-products are produced (Eq. (14), Chen et al., 2021).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \tag{14}$$

Therefore, the theoretical maximum TN removal in anammox is only 89 % (Liang et al., 2015). A BES-denitrification unit could be integrated into the anammox system. 96.3 % of TN removal was achieved using anammox sludge as an anode microbial catalyst to drive the electrocatalytic reduction of NO_3^- in the SnCu-Pd/CFC catalytic cathode, with maximum voltage and power density of 39.8 mV and 21.20 ± 0.05 mW/m^3, respectively (Zhang et al., 2020). The aeration system used in the ammonia oxidation-BES still consumed a lot of energy. Replacing the aeration system with biological oxygen production technologies would reduce the carbon emissions of ammonia oxidation-BES. For instance, the integration of ammonia oxidation and microalgae is promising. Indeed, the dioxygen produced by microalgae during the day would provide sufficient dissolved oxygen for partial conversion of NH_4^+ to NO_2^-, and at night the microalgae would consume dissolved oxygen to form an anoxic state, which would be more favorable for anammox to remove the remaining ammonia (Ghimire et al., 2021).

3. Challenges and strategies for BES-based treatment with scale-up applications

3.1. Challenges of BES treatment for carbon-deficient wastewater with scale-up applications

Surprisingly, after more than two decades of development, the bottlenecks of unstable performances and low power production of carbon-deficient wastewater treatments with BES remain unsolved, especially in scale-up applications (scaling BES from the lab to the practical engineering applications). Power density and reactor configuration have been reported as major influencing factors (Selvasembian et al., 2022). Therefore, given the current situation of low energy output, it is wiser to use the weak current generated by BES to enhance denitrification, instead of recycling current as electric energy (Liu et al., 2021). Other influencing factors and optimization strategies are necessary to be explored for future practical applications.

3.2. Strategies to enhance BES synchronous denitrification and power production

Several strategies could enhance nitrogen removal and electricity production simultaneously (Fig. 2). The first strategy is to couple with emerging methods such as electrical stimulation and photocatalysis, to enhance autotrophic denitrification. There are also strategies such as modifying the electrode materials, filler materials, modified membrane materials, and parameter optimization to achieve a controllable design with a high electron transfer rate. These strategies improve nitrogen removal and electricity production efficiencies from carbon-deficient wastewater. It can be expected that the scaled-up application of BES enhanced denitrification of carbon-deficient wastewater can be achieved through the synergistic optimization of multiple strategies.

Over the last decade, progresses have been made in simultaneous BES-enhanced denitrification and power production. The upper part of the arrow in the figure depicts the development of electrode materials, while the lower part of the arrow represents other strategies, including specific strain screening, filler materials, and cyclic polarization (Jin et al., 2018; Lu et al., 2011; Srinivasan et al., 2019; Wang et al., 2021a; Xu et al., 2018; Yan et al., 2012, 2021; Zhu et al., 2015).

3.2.1. Coupling of multitype BES-based processes to improve the lack of electron donors

Due to the lack of organic electron donors in carbon-deficient wastewater, the power generation performance and denitrification efficiency of BES are limited. In addition to the previously described addition of inorganic electron donors, electrical stimulation techniques can also achieve enhanced denitrification in carbon-deficient wastewater. For instance, Zhang et al. (2014) fed electrical energy generated by MFC to a biofilm-electrode reactor (BER) and observed an increase in denitrifying bacteria abundance and enhanced nitrogen removal. To reduce the operating cost and to investigate the effect of weak electricity on enhanced denitrification, Liu et al. (2021) developed a MFC-resistive electrically stimulated denitrification process. Results showed an increase in aromatic amino acids and enhanced electron transfer under 0.2 V microbial weak electricity stimulation, which increased the denitrification rate to 98.1 %. In another study, a MFC was coupled with a MEC, and the MEC was stimulated in situ using ultra-low microbial weak electricity of 0.1 V generated by MFC (Qin et al. 2022). Findings show the formation of aromatic proteins and the increase of tightly bound extracellular polymers involved in electron transfer, and the TN removal efficiency increased from 88.7 % to 91.3 %, thus revealing the activation of autotrophic microorganisms and the inhibition of some heterotrophic microorganisms.

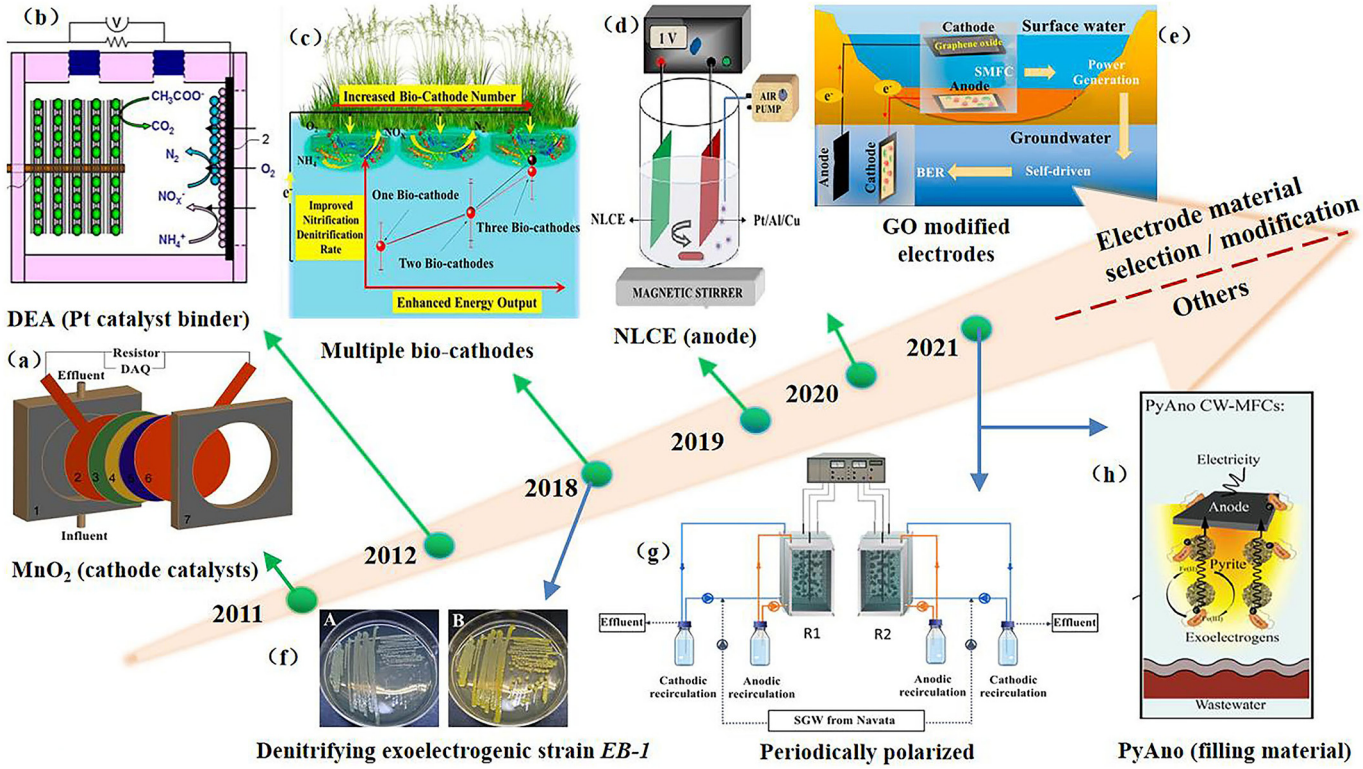

Fig. 2. (a) β-MnO_2 as alternative cathode catalysts in air-cathode microbial fuel cells (MFCs). (b) DEA was used as the 0.5 mg-Pt/cm^2 catalyst layer on the cathode. DEA polymer solution was composed of a tetrahydrofuran solvent. (c) Three bio-cathodes constructed wetland-MFC system. (d) Conductive NLCE as the anode, obtained from an inutile desktop monitor. (e) GO modified electrodes, and GO was prepared using the modified Hummers method from flake graphite (Sigma-Aldrich). (f) Exogenous denitrifying biofilms were enriched from the anodes of MFCs fed with mineral salt medium containing acetic acid, and *EB-1* was then isolated. (g) Under a 30 s open-circuit/30 s polarization mode. (h) Using pyrite as the anodic filling material and quartz sand as the cathodic filling material. Where, BES: bioelectrochemical systems; DEA: Diethylamine-functionalized polymers; NLCE: nematic liquid crystal electrode; GO: Graphene oxide; PyAno: pyrite as an anodic.

The coupling of photocatalysis with BES enhanced the redox potential of BES and improved the ammonia oxidation performance of the anode chamber. Ultimately, the power yield of BES in carbon-deficient wastewater, as well as the nitrification and denitrification rates were improved (Xu et al., 2015). Visible light-algae-BES phase coupling is possible for the mariculture wastewater with carbon-deficient, low discharge limits, and high salinity (Sun et al., 2020). Here, at the anode, the algae assists the electricity-producing bacteria to generate electricity and improves the pollutant removal. At the cathode, the algae absorb nitrogen and phosphorus from the wastewater firstly and then forms a "bioaerator" that release dioxygen uniformly through photosynthesis under visible light. This synergistic effect of the catalytic cathode, algae, and cathode denitrifying bacteria in SND enhanced denitrification. However, these emerging coupled processes are currently experimental in synthetic wastewater. Their feasibility in real wastewater treatment should be further investigated.

3.2.2. Modification and optimization of electrode materials

Electrode materials play an important role in BES, and largely determine the ability of BES to act as a "highway" of electron transfer. Table 1 presents recent progress on the material selection and modification of BES applied, to improve the performance of simultaneous nitrogen removal and energy recovery from the carbon-deficient wastewater.

3.2.2.1. Non-biological electrodes. It is crucial to select a highly conductive electrode for electron transfer in order to minimize the ohmic voltage loss in the BES. For most metals, the resistivity of electron transfer at 20 °C is about 10^{-7}–10^{-8} Ω·m, while the resistivity of carbon to π electrons is about 3.5×10^{-5} Ω·m (Serway and Beichner, 1988). Thus, abiotic electrodes such as platinum (Pt) anodes have excellent organic removal properties, while stainless steel and Pt perform better as cathode materials because of their strength, manageability, and better electrokinetic properties, which facilitate biofilm-driven reduction. With Pt catalyst loaded on the cathode, the system voltage was significantly increased from 95 mV to 450 mV and the power density was 117.7 mW/m^2, which was 16 times higher than that without Pd catalyst in the system (Fang et al., 2011). Although the NO_3^- removal rate is increased by 3.5 times, it is costly due to the presence of Pt. Biocathode MFC has the lowest efficiency due to its higher internal resistance value of 533.9 Ω (Raychaudhuri et al., 2022). From the application point of view, precious metals are more expensive than carbon materials. Moreover, precious metals are more susceptible to corrosion and

Table 1
Enhanced denitrification of carbon-deficient wastewater and electricity production by electrode material modified bioelectrochemical systems.

Wastewater type	Removed substrate	C/N	Modified electrode	Electrode material	Treatment efficiency	Power or cell voltage	Coulombic efficiency	Reference
Domestic wastewater	NO_3^-	0	Cathode	Pb	NO_3-N^-: 2 mg/ L day^{-1}	117.7 mW/m^2	66 %	(Fang et al., 2011)
Artificial wastewater	NO_3^-	0.18	Anode, cathode	Graphitized carbon nanofibers	NO_3^-: 89.3 %	1.26 mW/m^2	NA	(Alharbi, 2013)
Artificial wastewater	NO_3^-, NH_4^+	2.16	Cathode	Pb	NO_3^-: 88.93 g m^{-3} NCC[a] day^{-1}	593 mV	23.54 %	(Al-Mamun and Baawain, 2015)
Constructed wetland wastewater	NH_4^+	4.38	Cathode	Graphite felt[b]	NH_4^+: 163.55 ± 11.88 mg/(m^2·d)	26.16 mW/m^2	NA	(Xu et al., 2018)
Artificial wastewater	NO_3^-	0	Cathode	MWCNT[d]/carbon felt	NO_3^-: 83.58 %	NA	NA	(Safari et al., 2014)
	NO_3^-	0	Cathode	MWCNT/carbon cloth	NO_3^-: 79.66 %	NA	NA	
	NO_3^-	0	Cathode	MWCNT/graphite	NO_3^-: 63.98 %	NA	NA	
Groundwater	NO_3^-	3	Anode	Fe	NO_3^-: 63.72 ± 1.18 %	NA	NA	(Li et al., 2019)
Supernatant liquid taken from the suspended sludge reactor	NO_3^-	0	Cathode	HCl pretreatment graphite	NO_3^-: 19.96 mg/ L d^{-1}	NA	NA	(Chen et al., 2017)
	NO_3^-	0	Cathode	H_2O_2 pretreatment graphite	NO_3^-: 14.82 mg/ L d^{-1}	NA	NA	
	NO_3^-	0	Cathode	HNO_3 pretreatment graphite	NO_3^-: 13.93 mg/ L d^{-1}	NA	NA	
	NO_3^-	0	Cathode	HNO_3 + heat pretreatment graphite	NO_3^-: 27.36 mg/ L d^{-1}	NA	NA	
Artificial wastewater	NO_3^-	2	Anode	C—C	NO_3^-: 79.1 %	NA	NA	(Zhu et al., 2015)
	NO_3^-	2	Anode	Fe—C	NO_3^-: 90.3 %	NA	NA	
Groundwater	NO_3^-	1.25	Cathode	Graphene oxide modified	NO_3^-: 99.9 %	900 mV	NA	(Yang et al., 2021)
Artificial wastewater	NO_3^-	3	Anode	Fe—C	NO_3^-: 50 %	NA	NA	(Qian et al., 2018)
Artificial wastewater	NH_4^+	3.51	Cathode	Carbon brush + proton exchange membrane	NH_4^+: 51.7 ± 0.7 %	31.91 mW·h	NA	(Huang et al., 2021)
	NH_4^+	3.51	Cathode	Graphite felt+ proton exchange membrane	NH_4^+: 68.0 ± 1.6 %	46.11 mW·h	NA	
	NH_4^+	3.51	Cathode	Carbon brush + cation exchange membrane	NH_4^+: 47.7 ± 0.1 %	21.49 mW·h	NA	
Artificial wastewater	NH_4^+	7.8	Cathode	Nafion binder	NH_4^+: 90.7 %	945 ± 42 mW/m^2	26 %	(Yan et al., 2012)
	NH_4^+	7.8	Cathode	Diethylamine-functionalized polymer[c]	NH_4^+: 96.8 %	900 ± 25 mW/m^2	25 %	
Landfill leachate	NH_4^+	2.73	Anode, cathode	Anode: carbon nanotubes/carbon nanofibers; Cathode: TiO_2/carbon nanofibers	NH_4^+: 66.6 % TN: 67.9 %	216 ± 13 mW/m^2	NA	(Cai et al., 2020)
	NH_4^+	2.73	Anode, cathode	Anode: carbon nanotubes/carbon nanofibers; Cathode: carbon nanotubes/carbon nanofibers;	NH_4^+: 78.2 % TN: 77.8 %	301 ± 13 mW/m^2	NA	
Artificial wastewater	NH_4^+	2	Anode	Polypyrrole/microbial cellulose	NH_4^+: 97.42 %; TN: 62.47 %	NA	NA	(Hossini et al., 2015)

[a] Net cathodic compartment;
[b] Multiple bio-cathodes.
[c] Pt catalyst binder.
[d] MWCNT: multi-wall carbon nanotube.

interference with the denitrification process. Therefore, reducing the internal resistance of bioelectrodes through modification and other methods is necessary.

3.2.2.2. Bioelectrodes. An important factor determining the efficiency of BES is the performance of bioelectrodes, with the interaction between electrodes and microorganisms to catalyze electrochemical reactions (Wang et al., 2021b). These microorganisms are usually attached to the electrode surface and exchange electrons with the solid electrode to facilitate microbial metabolism. Carbon-based materials, such as graphite felt (Gf), carbon felt, and carbon cloth are well suited for biofilm formation due to their stability, high specific surface area, roughness, and electrochemical properties (Zhang et al., 2021b). Nonetheless, the low electrochemical activity of Gf electrodes due to poor kinetics and high internal resistance properties limits their wide application (Safari et al., 2014). Therefore, modification of such electrode materials to improve their electrochemical performance has received much attention.

Several studies have shown that carbon nanomaterials can facilitate electron transfer during the electroreduction and electrooxidation of electroactive substances, such as NADH and hydrogen peroxide (Atta et al., 2019). Carbon nanomaterials are carbon materials with at least one dimension less than 100 nm in the dispersed phase scale. By dispersing carbon nanomaterials on the electrode surface to form a randomly dispersed high surface area array, the surface area of carbon electrodes can be expanded. In addition, their porous structure provides better wetting properties for electrodes. The MFC system modified with carbon nanomaterials achieved more than 95 % NO_3^- removal (Alharbi, 2013). The β-MnO_2 in manganese oxides exhibits better catalytic activity of the process (Huang et al., 2019). Carbon nanotube-loaded β-MnO_2 MFC showed a high power density of 97.8 mW/m^2 with an internal resistance as low as 165 Ω (Lu et al., 2011). In addition, brittle carbon materials that increase the ohmic loss of the electrode, carbon or graphite electrode materials can be supported by stainless steel mesh, a conductive metal collector (Song et al., 2020). Coating graphite electrodes with electron mediators, polyaniline, and quinone groups can likewise improve their performance (Deng et al., 2017).

However, slow biofilm build-up rates and system instability often limit pollutant removal and power output. Ammonia oxidation pretreatment, sulfuric acid, hydrochloric acid, hydrogen peroxide, or nitric acid oxidation pretreatment of bioelectrodes can accelerate electrode biofilm formation. This is achieved by generating oxygen-containing functional groups, mainly in the form of carboxyl groups, thus facilitating the growth of metabolically active bacteria and enhancing the electron transfer between bacteria and the electrode surface (Bi et al., 2022). Similarly, pretreatments such as ultrasound and autoclave disinfection can also modify the electrode to improve BES electricity production and the removal of contaminants simultaneously (Sivasankar et al., 2019). Chen et al. (2017) found that the cathode surface oxygen content of a BER after coupled heat treatment with HNO_3 was 11.54 %, which was 1.98 %, 4.10 % and 7.70 % higher respectively after HNO_3, H_2O_2, and HCl treatment, promoting the biofilm growth and reduction rate of the cathode-driven autotrophic denitrification. The presence of functional groups in anodes, which consists of conductive linear liquid crystal electrodes such as C≡N and C═O, is the key factor in the rapid formation of stable biofilms, effectively ameliorating the issues of long BES initiation times and frequent biofilm shedding or erosion (Srinivasan et al., 2019).

Optimizing the surface area of electrodes and the number of cathodes is another option, if the electrodes are optimized from the perspective of convenience to enhance the performance of BES in terms of electricity production and denitrification. The TN removal rate increases proportionally to the electrode surface area (Oksuz and Beyenal, 2021). Xu et al. (2018) found that increasing the number of biocathodes from 1 to 3 resulted in a significant reduction in energy loss on both the anode and cathode (from 97.85 mV to 46.09 mV for the anode and from 221.5 mV to 45.89 mV for the cathode respectively). The maximum power density of the system increased significantly from 12.56 mW/m^2 to 26.16 mW/m^2. Thus, the enhanced power production performance contributed to higher nitrification rates (from 98.59 ± 4.53 mg/(m^2·d) to 179.11 ± 7.65 mg/(m^2·d)) and denitrification rates (from 89.64 ± 4.57 mg/(m^2·d) to 163.55 ± 11.88 mg/(m^2·d)). The relationship between the number of biocathodes, power production, and denitrification efficiency in the carbon-deficient wastewater has not been completely understood. Further studies are needed to explore the applicability of multiple biocathodes for various scenarios.

3.2.3. Filling materials

A key obstacle in the practical application of BES is the poor electron transfer between the microorganisms and the anode (Yang et al., 2021). In addition to modifying the electrodes to improve their electrochemical performance, filling inexpensive conductive particles between BES electrodes is an economical and convenient option (Table 2). Activated carbon, metals such as zinc, iron, manganese and graphite are commonly used as conductive particles in three-dimensional electrode systems (Zhang et al., 2021c). These particles can form microelectrodes in an electric field, and their high specific surface area and high electrical conductivity help accelerate mass transfer and enhance electron transfer.

Biochar, which has an excellent biocompatibility, is now widely studied and applied by scholars. Dong et al. (2020) selected three biochar-modified electrodes and found that BES filled with the MgO-modified biochar achieved the highest power density of 2.40–4.45 W/m^3, compared to 1.31 W/m^3 for the BES without biochar filling. The anode chamber and the cathode chamber were filled with granular activated carbon (GAC) and MnO_2/TiO_2/g-C_3N_4@GAC respectively to construct a dual-chamber three-dimensional electrode BES (3D-BES, Zhang and Liu, 2020). Under the electric field, the filled particles were polarized to form a large number of charged microelectrodes, which significantly improved the current efficiency of the BES (Feng et al., 2018).

Power density and coulombic efficiency in bigger scales (L-level) were lower by 2–3 orders of magnitude compared to the mL-level MFCs (Wang et al., 2017). This is because the removal of organic matter by the electron-producing bacteria at the anode alone is inefficient. To extend the range of electroactivity in the anode region, inexpensive natural conductive pyrite (PyAno) can be used as a filling material (Yan et al., 2021). As a conductive mineral, pyrite is capable of long-range bio-current transmission in various environments. Materials such as gravel and sand are also used to fill the biocathodes, enhancing nitrogen removal from groundwater (Cecconet et al., 2019).

3.2.4. Improving the wettability of membrane materials

Exchange membranes are essential components of BES, and the structure and composition of the membrane should accommodate the passage of both cations and anions. To ensure the diffusion of protons from the anode chamber to the cathode chamber. The pH gradient on both sides of the membrane would increase the ohmic voltage loss, which tends to reduce the stability and nitrogen removal of BES (Daud et al., 2018). Therefore, the exchange membrane should have good ionic conductivity, stability, and durability.

Microfiltration membranes in BES outperform ultrafiltration membranes due to their larger pore size that induces less internal resistance (Sun et al., 2009). Nonetheless, when dioxygen penetrates into the anode chamber of an anaerobic chamber, the anode coulombic efficiency and bioelectrocatalytic activity decrease (Pant et al., 2010). Therefore, balancing dioxygen permeation and proton transfer in the BES dual chamber is critical, especially in processes requiring cathodic aeration such as SND. Changing only the pore size of the exchange membrane does not solve this issue. Improving the wetting properties of the exchange membrane improves the proton transfer efficiency and reduces dioxygen permeation (Zhou et al., 2021). For example, expanded polytetrafluoroethylene prepared with ferric hydroxide colloidal solution, $Fe(OH)_3$, acrylic acid, and $C_3H_4O_2$ is hydrophilic, which could be used to separate BES. This membrane improved the overall electrical conductivity and power generation capacity of BES while reducing dioxygen permeability (Yao et al., 2015).

Table 2
Bioelectrochemical systems with filling material for wastewater denitrification and electricity production.

Wastewater type	Removed substrate	C/N	Filling material	Treatment efficiency	Power density	Voltage	Coulombic efficiency	Reference
Groundwater	NO_3^-	0	Sand medium	NO_3^-: 86 ± 4 %	NA	NA	NA	(Cecconet et al., 2019)
	NO_3^-	0	Gravel medium	NO_3^-: 95 ± 2 %	NA	NA	NA	
Constructed wetland wastewater	NH_4^+, NO_3^- [a]	5.4	Quartz sand	NH_4^+: 20.1 % ± 2.7 % TN: 30.7 % ± 3.4 %	14.4 mW/m^2	270.2 mV ± 71.3 mV	NA	(Yan et al., 2021)
	NH_4^+, NO_3^- [a]	8.5	Pyrite	NH_4^+: 25.7 % ± 2.1 % TN: 30.2 % ± 3.4 %	27.8 mW/m^2	309.1 mV ± 130.0 mV	NA	
	NH_4^+, NO_3^- [a]	3.1	Quartz sand + pyrite	NH_4^+: 32.5 % ± 2.7 % TN: 36.7 % ± 3.6 %	22.0 mW/m^2	416.0 mV ± 65.4 mV	NA	
Domestic wastewater	NH_4^+	50	Biochar	NH_4^+: 91.7 ± 4 % TN: 80.1 ± 2.8 %	2.40 ± 0.28 W/m^3	262 mV	3.11 ± 0.19 %	(Dong et al., 2020)
	NH_4^+	50	Biochar and zeolite mixture	NH_4^+: 92.7 ± 3.1 % TN: 80.5 ± 2.6 %	3.13 ± 0.27 W/m^3	332 mV	3.51 ± 0.27 %	
	NH_4^+	50	MgO-modified biochar	NH_4^+: 93.6 ± 3.2 % TN: 84.8 ± 2 %	4.45 ± 0.21 W/m^3	400 mV	6.01 ± 0.25 %	

[a] Multi-species inorganic nitrogen wastewater.

However, these membranes are relatively expensive to prepare and need to be replaced regularly, making them difficult for practical applications. It would be more practical to use membrane-free BES or find an inexpensive alternative such as a ceramic membrane (Guo et al., 2022).

3.2.5. Addition of exogenous substances

Adding exogenous substances is also a feasible strategy to intensify the denitrification of carbon-deficient wastewater, as it can act as an electron donor and enhance electron transfer; while adding hydroxyl-methylfurfural or NZVI in BES increases dihydrogen production and improves the denitrification efficiency of H_2 production (Malik et al., 2021). The addition of NZVI causes changes in microbial populations, mainly depending on its dosage amount. The addition of montmorillonite-loaded sodium alginate immobilized NZVI in a denitrification zone of the vertical flow CW and thus increased the NO_3^- denitrification efficiency, with increased biodiversity and abundance level in the system (Zhao et al., 2018).

Trace amounts of quinolone or other compounds acting as electron shuttle to BES increase biomass and biogenic power production significantly (Shehab et al., 2017). However, dissolved electron shuttles are usually toxic and easily washed out from the effluents. Alternatively, humin is an insoluble electron shuttle working within a broad pH scope, which is physicochemically stable and widely available in the environment. Electrochemical denitrification performance of *Pseudomonas stutzeri JCM20778* was enhanced after the addition of humin to BES (Xiao et al., 2016). Therefore, there is a great potential for future BES applications to improve the electron transfer between electrodes and bacteria for enhanced nitrogen removal. However, the immobilization method and dosage should be optimized to avoid possible reduction of the charge transfer rate of the biocathode.

3.2.6. Role of buffers and their by-products

Many factors affect the performance of BES denitrification, including pH, temperature, electrode spacing, and external resistance, which were covered by a previous review paper (Sun et al., 2020). Therefore, we focus on buffers and their by-products that assist in the regulation of pH control, and on the effects of ionic strength, electrode potential, and substrate concentration. To maintain neutral conditions, an alkaline buffer is usually added to the solution. For that, carbonates are better than phosphate buffers because carbonates act as a source of inorganic carbon and have a high diffusion coefficient in water, allowing rapid transport of protonated alkalinity (HAlk) in biofilms (Marcus et al., 2011). Eqs. (15)–(18) show that the anode solution tends to be more acidic after reaction. The CO_2 produced can be circulated within the cathode and dissolved into water to form H_2CO_3, which then reacts with OH^- step by step to eventually form CO_3^{2-} (Eqs. (19)–(20)). Thus, carbonates and bicarbonates can be reused for pH control, and the CO_2 produced can increase the conductivity of the solution and enhance electron transfer (Buvik et al., 2021). Since CO_2 produced could be utilized by BES in-situ, the emission of the greenhouse gas from the wastewater treatment process is controlled.

$$Alk^- + H^+ \leftrightarrow HAlk \tag{15}$$

$$CO_3^{2-} + H^+ \leftrightarrow HCO_3^- \tag{16}$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \tag{17}$$

$$H_2CO_3 \leftrightarrow CO_2 + H_2O \tag{18}$$

$$H_2CO_3 + OH^- \rightarrow H_2O + HCO_3^- \tag{19}$$

$$HCO_3^- + OH^- \rightarrow H_2O + CO_3^{2-} \tag{20}$$

Low anodic NO_2^- concentrations (below 60 mg/L) could act as a co-substrate to promote BES electricity production in the absence of organic matter (Wang et al., 2020b). When the NO_2^- concentration is too high, the biotoxicity of NO_2^- inhibits the growth of biofilm and suppresses the production of cytochrome C and extracellular polymeric substances (EPS), which is an important component of biofilm and promotes the adhesion and growth of microbial cells on the electrode surface. In addition, redox molecules in EPS, such as outer membrane cytochromes, contribute to electron transfer. Thus, high NO_2^- concentrations increase the resistance to charge transfer in the anode biofilm, which in turn inhibits the activity of electrogenic bacteria. The presence of ammonium ions in the anode chamber promotes electron transfer from the anode solution, so that the NO_3^- removal rate from 3.07 ± 0.26 g/(m^3·d) to 7.89 ± 0.78 g/(m^3·d) and current density from 28.2 ± 19.4 mA/m^2 to 176.1 ± 31.7 mA/m^2, could be much higher compared to that in the NH_4^+-free system (Kim et al., 2014). Jin et al. (2018) inoculated *Geobacter metallireducens* in the anodic denitrification region, and the current output of MFC was strongly inhibited at NO_3^- concentrations higher than 56 mg N L^{-1}. Therefore, the NH_4^+ and NO_3^- concentration in carbon-deficient wastewater controls the BES power production.

It is unclear to what extent the energy used for microbial growth and maintenance influences the overpotential at the biocatalytic electrode. Yet, capacities of electroactive microorganisms anodic oxidation and cathodic reduction are closely related to electrode potential. For example, acetate-oxidizing microorganisms showed enhanced biomass activity after 30 days at an anodic potential of −0.2 V vs SHE compared to 0 V or 0.2 V (Aelterman et al., 2008). Altering the electrode potential results in the inability of some species to respire under such redox conditions, while other electroactive species are retained at a competitive advantage. When the applied cathodic potential is lower than the theoretical reduction potential of the target pollutant, the pollutant can be directly reduced. However, too high a potential (−1.8 V) or higher current intensity (600 mA) can

disrupt microbial metabolism and even cause microbial death (Zhu et al., 2021a).

3.2.7. Cycle reversal and/or polarity reversal

Increased microbial activity and promoted nitrogen denitrification could be achieved by applying an electric field or changing the electrical properties of the species (Qian et al., 2018, 2017). Periodic reversal bioelectrocoagulation system (PRBES) differs from BES mainly in that the former use active metals such as iron and inert materials as electrodes, which release cations that form flocs with the activated sludge in the system (Qian et al., 2017). Activated sludge could be further stimulated by electrochemistry to increase the biomass weight and biological activity of the nitrification system. Due to the periodic reversal of the DC power supply, this system can alleviate the dissolution of the anode active metal and reduce the anode passivation, which is one of the periodic reversal advantages. Qian et al. (2018) embedded Fe-C electrodes in PRBES and found that iron ion leaching increased denitrifying bacteria abundance, led to enhanced nitrogen removal.

BES under cyclic polarization such as 30 s open - 30 s polarization, electroactive heterotrophs such as *Geobacter* sp. can store excess electrons in their redox proteins (E.g. polyhemoglobin c-type cytochromes), then release these electrons as transient currents in addition to steady-state currents when repolarization is activated. Thus, optimized cyclic polarization enhances the charge storage capacity and conductivity of these anodically electroactive biofilms (Wang et al., 2021a). Nonetheless, NO_2^- accumulation can limit the overall denitrification process and is a challenge for BES-enhanced denitrification. Furthermore, Liang et al. (2019) created a bi-directional electron transfer cathodic biofilm dominated by *Geobacter* sp. through anodic polarity reversal, where electrochemically active bacteria such as *Geobacter* sp. act as an "electron bridge" at the anode, thus providing an additional channel for denitrifying bacteria to obtain electrons from the cathode, with a denitrification rate of 98 %. Overall, cycle inversion or polar inversion is feasible for enhanced denitrification and electricity production in carbon-deficient wastewater, but polar inversion needs to be supplemented with organic matter immediately after operation. Future studies should explore how to avoid or reduce the addition of organic matter to obtain bi-directional electron transfer cathode biofilm as well.

3.2.8. Electron competition

For both anodic and cathodic reactions in BES, electron competition or incomplete denitrification processes significantly reduces the charge transfer efficiency, and the coulombic efficiency decreases correspondingly (Parameswaran et al., 2009). Electron competition mainly occurs during BES-SND, or short-range SND, due to the high concentration of dissolved oxygen and penetration into the cathode. This phenomenon leads to a decrease in the coulombic efficiency and denitrification efficiency of BES.

At the anode, coulombic efficiency reduction could be explained by two mechanisms: (1) Under absolute anaerobic conditions, the methanogenic bacteria compete with the electrogenic bacteria for organic carbon sources and cause a reduction in hydrogen production by MEC. Therefore, dissolved oxygen in the anode chamber (slightly greater than 0.2 mg/L) might be beneficial to reduce the production of CH_4 (a strong greenhouse gas). (2) Cathodic dissolved oxygen infiltration into the anode leads to organic matter oxidation by the aerobic microorganisms at the anode, which weakens the anaerobic environment of electrophilic bacteria. At the cathode, the main electron transfer pathways include: (1) Transfer of electrons to dioxygen and NO_3^- along the external circuit. (2) Transfer of electrons to dioxygen and NO_3^- by organic matter. (3) Transfer of electrons to dioxygen by ammonia. Since the redox potential of O_2/H_2O, of $+0.82$ V, is higher than that of NO_3^-/N_2, of $+0.75$ V, dioxygen and NO_3^- would compete for electrons in the complicated processes. In addition, high dissolved oxygen inhibits the activity of denitrifying bacteria and leads to compromised denitrification. As the autotrophic denitrification rate increased, denitrifying bacteria tends to fully utilize the cathode electrons. And the competitiveness of autotrophic denitrification in denitrification gradually increases with the nitrification rate (Ling et al., 2020).

Therefore, dissolved oxygen is a key factor in SND systems. Lacking of dissolved oxygen inhibits nitrification. On the one hand, the lack of dissolved oxygen inhibits nitrification. On the other hand, abundant dissolved oxygen will cross the outer layer of the biofilm to reach the inner layer, disrupting the micro-anoxic environment in the biofilm and thus inhibiting denitrification. Li et al. (2016) found that NOB growth remained limited and NO_2^- accumulated to achieve short-range denitrification when dissolved oxygen was 3.5 mg/L. High dissolved oxygen is favorable for BES power generation but causes an increase in aeration energy consumption. Therefore, the effect of dissolved oxygen concentration on power generation, denitrification efficiency, and operational cost on BES enhanced denitrification should be considered.

3.2.9. Other strategies

Although the redox enzymes of some exoelectric species are located on the biofilm's outer surface, only the bacteria's outer layer is electrochemically active. Some *Geobacter* and *Shewanella* species help microbial delivery to electrodes by producing nanowires or conductive hairs (Aiyer, 2020). In addition, some microorganisms can synthesize redox mediators through primary and secondary metabolites to achieve high-speed electron transfer. For example, phenazines produced by *Pseudomonas aeruginosa* are used for electron transfer between bacteria and anodes (Rhodes et al., 2021). Jin (2018) isolated a strain of *Mycobacterium* sp. *EB-1* and found it could generate electrical energy through direct electron transfer, which exhibited stable denitrification and power generation properties and utilized limited carbon sources more efficiently in the carbon-deficient wastewater. Therefore, microbial interactions can be used to optimize the bacterial-electrode electron transfer and ultimately improve the overall performance of BES.

Thick biofilm deposition impedes electron flow (Mohan et al., 2014). Therefore, controlling biofilm thickness is important to achieve high power output. In addition, heavy fouling on the cathode reduces the BES power generation by up to 85 % (Kook et al., 2016). Biofilm thickness can be controlled by optimizing parameters such as current density. Last but not least, the resultant ohmic voltage loss consists of two types of charge transfer in BES: Ion transfer through the electrolyte and electron transfer through the electrode (Clauwaert et al., 2008). The ohmic voltage loss increases with the reactor size. Stacking multiple suitably sized 3D-BES may be a more feasible strategy for BES scale-up. Liang et al. (2018) realized the amplification application of real municipal wastewater in BES by assembling three 3D-BES modules (1000 L).

4. Challenges and strategies reducing N_2O production in BES

To control N_2O production in BES facilitated processes, researchers have developed strategies such as optimizing conductivity, controlling cathode potential and current, process optimization, and avoiding electron acceptor inhibition of N_2O reductase (Fig. 3).

4.1. Challenges of N_2O reduction based on the principles of BES

Issues of climate change, waste production, and energy shortage are calling for wastewater treatment processes that maximize resource and energy recovery. For that, BES is an emerging technology that has the potential to recover potential energy from wastewater and for future application in wastewater treatment plants. BES application to improve wastewater denitrification performance has been extensively studied recently (Liang et al., 2019; Liu et al., 2020; Zhu et al., 2021b). However, incomplete denitrification in this denitrification process produces intermediate products such as N_2O. A slight increase in N_2O accumulation over a long atmospheric life cycle would have seriously negative environmental impacts (Otte et al., 2019). Furthermore, in BES enhanced denitrification processes, the accumulation of intermediate products decreases the cathode coulombic efficiency. Therefore, an in-depth understanding of the principles of N_2O production in BES is important to control greenhouse gas emissions.

NO_3^- reductase (Nar), NO_2^- reductase (Nir), nitric oxide reductase (Nor), and N_2O reductase (Nos) are four key enzymes in the process of

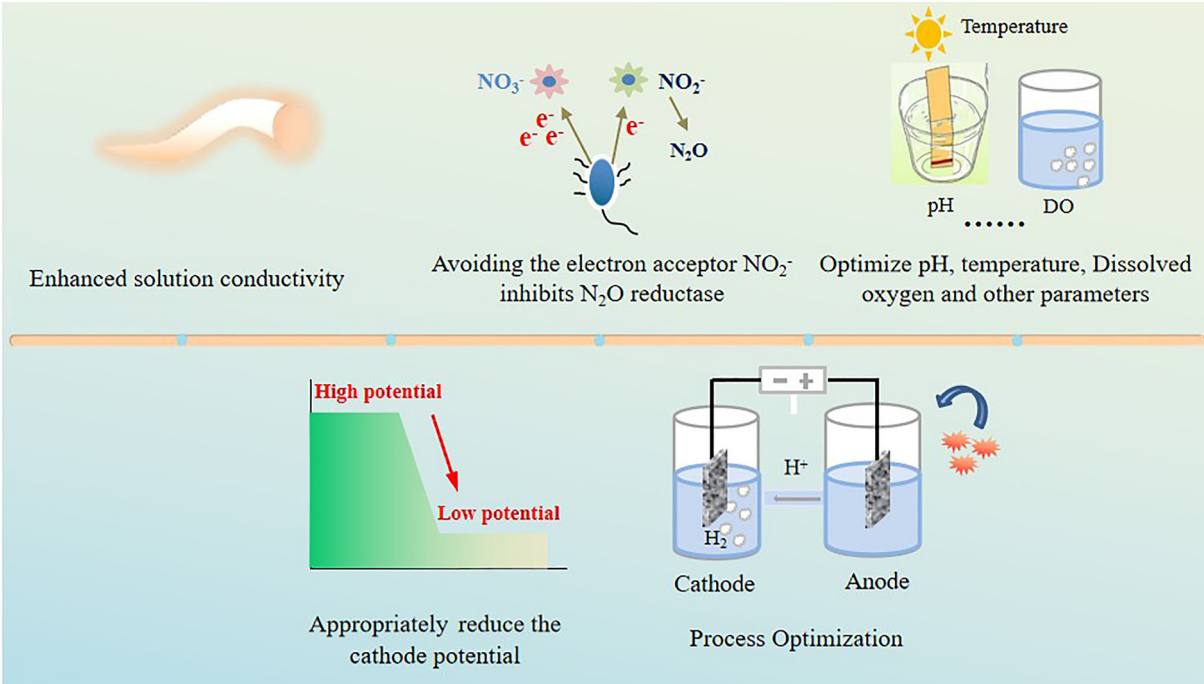

Fig. 3. Strategies for BES N_2O emission reduction. N_2O production can be reduced by enhancing solution conductivity, reducing electrode potential, process optimization, avoidance of electron receptor inhibition of N_2O reductase, and optimizing parameters such as temperature and pH.

complete denitrification (Zhang et al., 2021a). These enzymes are controlled by genes *narG*, *nirK*, *nirS*, and *nosZ* and determine the rate of NO_3^- reduction. The reasons for the variation in N_2O production potential are manifold and include differences in the main physicochemical conditions, such as temperature, dissolved oxygen, conductivity, pH, cathodic potential, the nature of the electron acceptors, and denitrification composition of the bacterial flora. Influenced by these factors, N_2O reductase is often inhibited in BES, thereby reducing the proportion of electrons allocated to N_2O reductase. When the electron flux to N_2O reduction is lower than that to NO_2^- reduction, it leads to the accumulation of the intermediate N_2O (Pan et al., 2013), and decreases the total rate of biological denitrification. Therefore, improving N_2O reductase activity in BES is a challenge that should be overcome in future studies. We present a detailed review of the numerous factors that influence N_2O reductase activity in BES and provide references on how to modulate these factors. Achieving minimum greenhouse gas emissions and power production stability while contributing to the complete conversion of N to nitrogen in carbon-deficient wastewater.

4.2. Strategies to reduce N_2O production in BES

4.2.1. Optimization of electrical conductivity

The BES cathodic reduction reaction is influenced by conductivity, and studies showed that a low conductivity below 1600 $\mu S \cdot cm^{-1}$ limited the denitrification efficiency and power generation capacity of BES, resulting in the incomplete reduction of NO_3^- and accumulation of denitrification intermediates such as N_2O (Puig et al., 2012). Carbon-deficient wastewater such as urban sewage plant tailwater is characterized by low conductivity below 1000 $\mu S \cdot cm^{-1}$, which leads to irreversible energy losses (over potential) (Usikalu et al., 2021). The accumulation of N_2O is facilitated by the limitation of electron and proton transport. At high conductivity, electrons generated at the anode are transferred to the oxidized nitrogen compound near the cathode, which establishes the proton motive force for the production of ATP, and generally yields higher power density and current than the low conductivity wastewater.

The most direct and faster way to increase the conductivity of carbon-deficient wastewater is to dose sodium chloride or sodium bicarbonate solution, which acts as electrolytes (Puig et al., 2012). However, this method may cause secondary contamination. Alternatively, studies have shown that a larger electrode size increases the conductivity of BES (Gadegaonkar et al., 2020). Therefore, the applied liquid electrolyte and dosage should be selected rationally according to the actual situation. Considering the environmental impact, other strategies such as passing CO_2 or controlling its cathode potential by the MEC that uses the applied power supply to reduce N_2O production could also be used.

4.2.2. Control of cathode potential and current

An appropriate cathodic potential or current is important in order to achieve BES enhanced denitrification. The levels of NO_3^- reduction end-products, such as NO_2^-, N_2O, and N_2, vary with cathodic electrode potential, and the accumulation of N_2O at the cathode is the leading cause of coulombic efficiency reduction. It was shown that NO_3^- removal increased from 26.8 % to 75.6 % accompanied by higher coulombic efficiency of 72 % when the applied cathode potential was continuously reduced from +250 mV to −500 mV. Low cathode potential significantly reduced the proportion of electrons transferred and released in the form of N_2O under certain current conditions. However, Xiao et al. (2013) found that low potentials may alter the metabolic pathway of glucose to organic acids. Applying a cathodic potential of −0.2 V enhanced the activity of NO reductase and N_2O reductase in the biofilm. When −0.4 V cathodic potential was applied, the lower N_2O concentration (0–10 μm) in the biofilm could be caused by the decrease in electron supply. Therefore, both too low and too high cathodic potentials can negatively affect the complete denitrification of the BES cathode, and the above study tabulated that a cathodic potential around −400 mV is more appropriate.

The structure and gene expression of the active biofilm community may be significantly changed at different current densities. Virdis et al. (2009) found that at a current of 15 mA, the rate of NO_3^- reduction was comparable to the rate of N_2O accumulation. When decreasing to a current of 5 mA, the rate of NO_3^- reduction was higher than the rate of N_2O accumulation. This suggests that N_2O is reduced simultaneously with NO_3^- reduction, confirming the reduced inhibition of *nosZ* at low currents.

4.2.3. Process optimization

The treatment of nitrogenous wastewater in BES is often inhibited by anode passivation and low activity of functional denitrification genes enzymes, resulting in N_2O accumulation. Using ZVI as an electron donor can drive autotrophic denitrification, and as the active center of oxidase, iron is an important element of the electron transport chain. Iron ions contribute to the enhancement of biomass and denitrifying bacteria, and increased abundances of functional marker genes such as *Nirs* and *Nosz* leads to less accumulation of N_2O (Qian et al., 2018). In addition, coupled electric field based on iron ions could further enhance the nitrogen transfer rate and the activities of four enzymes, i.e. Nar, Nir, Nor, and Nos, thereby enhancing the denitrification process. Qian et al. (2018) found that the periodic reversal bioelectric coalescence system constructed by iron-carbon electrodes reduced N_2O by 65.48 % compared to the conventional SBR operating under a low temperature of 10 °C. However, enzyme activity is strongly influenced by temperature factors, and it is worth further exploring on N_2O reduction mechanisms under low temperature conditions.

4.2.4. Avoidance of electron receptor inhibition of N_2O reductase

According to chemical kinetics, NO_2^- forms free NO_2^- acid (FNA) spontaneously according to pH of the aqueous medium (Al-Mamun and Baawain, 2015). N_2O reductase was inhibited at an FNA concentration of 0.0014 mg HNO_2^- L^{-1} (Al-Mamun and Baawain, 2015). Since the NO_3^- reduction potential E0′ of +0.433 V is higher than that of NO_2^- as +0.350 V, the cathodic denitrification of NO_3^- under autotrophic conditions performs better. The energy available for growth in the cathode chamber using NO_3^- as electron acceptor was 11 % higher than NO_2^- according to thermodynamic calculation, which increases the maximum coulombic efficiency while contributing to the reduction of N_2O accumulation.

The influent load of NO_3^- affects denitrifying microbial activity. Al-Mamun and Baawain (2015) found that when the influent load exceeded 0.1 kg NO_3^- m^{-3} NCC d^{-1}, the pH of the cathode solution decreased with increasing cathode NO_3^- loading rate, and the formation of FNA increased exponentially, thus inhibiting the complete denitrification process of microorganisms on the cathode surface. In summary, the activity of Nos is not only related to the accumulated FNA concentration but also to the pH of the medium.

4.2.5. Other strategies

In BES, pH affects not only the activity of denitrification-related enzymes but also the species of nitrogen in water. Under low pH conditions, N_2O reductase is inhibited by generating free nitrite, leading to N_2O accumulation during denitrification (Wang et al., 2018b). In an open circuit, N_2O concentration in the biofilm is mainly influenced by pH, with higher activity of N_2O reductase than NO reductase in the pH range of 6.8–7.5 or higher than 8.0. In the pH range of 7.5–8.0, or less than 6.8, the NO reductase activity is higher than the N_2O reductase (Xiao et al., 2013).

Temperature affects N_2O production mainly through chemical equilibrium, microbial activity, and enzymatic activity (Reino et al., 2017). Temperature changes lead to an imbalance in the oxidation reaction of NH_4^+ and NO_2^-. In addition, temperature affects the growth rate of AOB and NOB in BES. The optimal temperature for NOB survival is 25–30 °C, which is sensitive to high temperatures (Bo et al., 2007). In contrast, AOB can survive at a relatively high temperature (45 °C). Therefore, the growth rate of the AOB is faster than the NOB at high temperatures, which may lead to the accumulation of NO_2^-, while the inhibitory effect of 0.0007 mg/L of FNA leads to the accumulation of N_2O (Zhou et al., 2008). Therefore, the temperature should be kept in the range of 20–25 °C to achieve minimum greenhouse gas and stability of electricity production.

Too low or too high dissolved oxygen will lead to the accumulation of N_2O. In enhanced denitrification of carbon-deficient wastewater treated by BES, 25–39 % of the TN is transformed to N_2O due to low dissolved oxygen concentration in the nitrification phase, relatively deficient carbon source in the denitrification phase, and high NO_2^- accumulation (Virdis et al., 2011). In recent years, AOB has been considered to be the main cause of N_2O production (Blum et al., 2018). However, under hyperoxic conditions, the N_2O reductase is inhibited, leading to the accumulation of the intermediate denitrifying compound N_2O (Al-Mamun and Baawain, 2015). In most cases, reactors with intermittent aeration mode would emit less N_2O.

In addition to biotic pathways, abiotic pathways are also present in BES in some cases. For example, chemical reactions between redox-active metals (Fe and Mn), organic matter (humic and xanthic acids), and the nitrogen cycle may produce N_2O (Zhu-Barker et al., 2015). For example, NH_2OH can be oxidized by Fe(III) or Mn(IV) to further generate N_2O (Chen et al., 2020). Although abiotic pathways mainly involve chemical reactions, they may be enhanced in wastewater containing heavy metals (Chen et al., 2020), such as secondary effluents of waste leachate, domestic sewage, and other carbon-deficient wastewater.

5. Conclusion and future prospects

Principles, development, and low carbon emission strategies of the BES-based enhanced autotrophic denitrification technologies for carbon-deficient wastewater treatment are critically reviewed. The main conclusions are as follows.

(1) Most studies focus mainly on the enhanced autotrophic denitrification of BES with monomorphic nitrogen water quality and mostly using artificial water, but multi-species inorganic nitrogen often co-exist in the actual carbon-deficient wastewater. BES-based autotrophic denitrification coupled with other denitrification pathways is being studied toward the simultaneous removal of multi-species inorganic nitrogen in real wastewater.
(2) Here are several strategies to enhance nitrogen removal and electricity production in BES. The first strategy is to couple with emerged treatment methods to enhance the autotrophic removal of nitrogen. There are also strategies such as modifying the electrode materials, filling materials, modified membrane materials, cycle inversion or polarization inversion, and avoidance of electron competition to achieve a controllable design with a high electron transfer rate.
(3) Influenced by environmental factors, N_2O reductase is often inhibited in BES, resulting in a decrease in the total rate of biological denitrification. Therefore, improving N_2O reductase activity in BES is a challenge that should be overcome in future studies. The literature has reported that N_2O emissions in BES may be reduced by BES coupling strategies, optimization of conductivity, control of cathode potential and current, avoidance of inhibition of N_2O reductase by electron acceptors, and parameter optimization.

Our proposals for future researches and considerations are as follows:

(1) Multi-species inorganic nitrogen often co-exist in the actual carbon-deficient wastewater. For the practical application of BES, future research should focus on understanding the competing mechanisms of nitrogen removal from different species in BES, coupling of new biological denitrification technologies and BES, directionally coupling denitrification pathways according to the actual nitrogen pollution species in wastewater, achieving optimal distribution of nitrogen substrate and electron supply between different denitrification paths, holding a great promise to achieve high-efficient, stable and cost-effective treatment of various nitrogen co-existent contaminants.
(2) Due to the low energy output and high internal resistance of the device and electrodes, the rate and degree of denitrification are affected by electron competition in non-denitrification pathways. Therefore, future technological breakthroughs and scaled-up applications of BES require the cooperation of traditional environmental experts, biological experts, and energy technicians. The effects of interactions such as selection and modification of electrode materials, filling materials, membrane materials, the addition of exogenous substances, polarization reversal, and proper optimization of parameters such as pH, temperature, and electrode potential on enhanced nitrogen removal should be

appropriately evaluated in laboratory-scale studies to answer these questions. Large-scale studies are urgently needed to assess field-scale issues such as lifetime and management. This will provide guidance for engineering applications.

(3) Screening microorganism strains and inoculating it in BES has superiority in simultaneous denitrification and electricity production. This helps produce less sludge volume and reduces subsequent power consumption for residual sludge treatment, which is in line with the carbon neutrality context.

(4) BES is a good choice for future scale-up applications of MFC coupled with MEC for treating carbon-deficient wastewater. However, two key principles need to be noted, which are priority regulation and end control. Firstly, a directional coupling weak current regulation pathway is designed, and the MEC denitrification microbial activity is stimulated weakly by MFC to achieve the optimal allocation of nitrogen substrates and electron supply among different denitrification pathways. Then, the MFC is directed to produce CO_2 through the MEC as an inorganic carbon source by controlling the dissolved oxygen concentration and increasing the solution conductivity. The excess microbial weak power generated during this process is stored in an energy storage battery, which is used for aeration and reduces carbon emissions from daily electricity consumption. The pollutants are turned into "green power". Of course, this requires the efforts of energy technicians to improve the efficiency of battery energy storage continuously.

(5) N_2O, a greenhouse gas with high warming potential, is not aspired to be produced at BES and its production can be reduced through proper process design optimization and operation. Although initial strategies have been developed, they need to be validated through full-scale applications to achieve scalability and commercialization of sustainable BES, which will help the realization of carbon neutrality and finally bring about significant cost benefits and eco-friendly opportunities in the 21st century.

CRediT authorship contribution statement

- Hongbo Liu: Conceptualization, funding acquisition, project administration, supervision, original draft
- Song Qin: Methodology, data curation, original draft
- Anzhe Li: Methodology, data curation
- Jian Wen: Methodology, data curation
- Eric Lichtfouse: Review & editing
- Heping Zhao: Review & editing
- Xianzhong Zhang: data curation

Data availability

Data will be made available on request.

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.

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