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### **Broader context**

Electrochemical  $CO_2$  conversion into valuable chemicals is a significant strategy to concurrently curb climate change spurred by the use of fossil fuels and store renewable electricity. Although microbes as bio-catalysts have shown great promise in stability and product selectivity over their non-biological counterparts, their  $CO_2$  conversion rates remain gloomy due to the limited charge transfer speed at the bioinorganic interface. The bio-interfacial charge communication flux is determined by interfaced electroactive microorganism enrichment and charge extraction ability. Particularly, limited progress has been achieved by targeting both factors in a mixed culture due to the complexity of the microbiome and the unclear correlation between the metabolic process and the inorganic catalytic characteristics. In this work, we have demonstrated an atomic-nanoparticle bridge to concurrently improve interfacial microbe enrichment and charge extraction in microbial electrochemical  $CO_2$  fixation. The atomic active sites provide a unique platform to investigate the electro-interaction between the microbes and the electrode while the hierarchical structure of the atomic-nanoparticle bridge affords further convenient modification of the interfacial interaction. This work highlights the significance of interfacial electronic structure engineering in constructing efficient bio-hybrids for various bioelectrochemical systems.

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## Tailoring interfacial microbiome and charge dynamics *via* a rationally designed atomic-nanoparticle bridge for bioelectrochemical CO<sub>2</sub>-fixation<sup>+</sup>

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Bio-electrochemical CO<sub>2</sub> fixation represents a promising strategy for CO<sub>2</sub>-to-chemical conversion, yet it suffers from a low CO2-reducing rate. Limited microorganism attachment and unfavorable charge extraction at the bioinorganic interface are the key determinants that inhibit the reaction kinetics. Herein, we report a judiciously created atomic-nanoparticle bridge composed of cobalt (Co) single atoms covering Co nanoparticles (Co-SA@Co-NP) to concurrently promote the enrichment of the performing microbe and bio-interfacial charge extraction for CO<sub>2</sub> conversion to methane. Finite element analysis (FEA) points to the increased electronegativity and more closely distributed electric intensity of the electrode surface with the introduction of Co nanoparticles underneath, whereby the close-packed biohybrids with enriched performing microbes are developed and assisted by electrostatic forces. The modified surface electronic structure of Co-SA@Co-NP further strengthens the interactions of Co-N<sub>4</sub> and C=O in extracellular humic acid-mediated charge exchange and reduces the activation energy of the intermediator, enabling a high-speed charge transfer channel from the electrode to the microbes. Taken together, an extremely high methane production rate of up to ~2512 mmol m<sup>-2</sup> per day (FE = ~94.1%, V = -1.1 V vs. Aq/AqCl) is delivered with the Co-SA@Co-NP bridgederived biohybrid, which is 70 times that derived with Co-SA only ( $\sim$ 35.47 mmol m<sup>-2</sup> per day). As such, the rationally designed atomic-nanoparticle bridge affords the effective tailoring of microbiome and charge dynamics via interfacial electronic structure engineering, thereby providing a unique platform for developing high-performance bio-electrochemical CO<sub>2</sub>-fixation systems.

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### Introduction

Electrochemical CO<sub>2</sub> conversion to value-added chemical products is an effective approach to storing intermittent renewable electricity.<sup>1-6</sup> However, state-of-the-art electrocatalysts used for CO2 reduction manifest poor performance in terms of product selectivity and stability.<sup>7-10</sup> In this context, microbes that can fix CO2 into valuable chemicals with high product selectivity and long-term stability have been introduced as bio-catalysts into the electrochemical CO<sub>2</sub> fixation system.<sup>11-13</sup> Bioelectrochemical CO2 reduction can be carried out with either type strains or microbiome. Though type strains are much easier for investigating the electron transfer process at the bioinorganic interface,<sup>14-19</sup> their resistance towards the reaction environment and self-regulation are inferior to that of the mixed microbiome.<sup>20</sup> Thus, the superior adaptability makes the mixed microbiome more suitable for practical application. Besides, the multiple electron transfer paths in the mixed microbiome, including mediated electron transfer, interspecies electron transfer, and direct electron transfer, together with various microbial synergies, open up more possibilities for receiving reducing equivalents from the electrode to facilitate CO<sub>2</sub> fixation.<sup>21,22</sup> The CO<sub>2</sub> conversion rates in biohybrid systems with the mixed microbiome, and by extension with type strain, are much lower than in pure electrochemical systems with inorganic catalysts.<sup>21,23-26</sup> The unregulated bioinorganic interface has been determined to be a crucial factor that drags down the CO<sub>2</sub>-reducing rate in bio-electrochemical systems.<sup>27</sup> The influence of the bioinorganic interface is generally based on two aspects, i.e., microorganism enrichment and charge extraction. Abundant microorganism attachment at the surface of the electrode, particularly selective enrichment of the performing microbes that participate in CO<sub>2</sub> conversion, is the precondition for high-performance CO<sub>2</sub> fixation. Previous studies proposed that the interfacial enrichment of microorganisms is highly dependent on the nanostructure of the electrode.28,29 Nevertheless, the inner mechanisms of this dependence are poorly investigated and understood. The other limiting factor, which is the sluggish electron transfer between the interfacing microbiome and electrode, has been drastically discussed in recent years. The unmatched electron flow between the electrode and microbial receivers not only impedes the CO2-reduction rate but also results in various by-products under long-term operation.<sup>15,25,30</sup> Different strategies have been employed to improve the bio-interfacial charge extraction, including gene editing,<sup>31</sup> microbial domestication,<sup>24</sup> and adding electron shuttles,<sup>32</sup> etc. It is worth noting that the specifical interfacial coordination for improving charge extraction remains unrevealed due to complicated interaction sites and unclear electron transfer pathways in mixed microbiome systems.<sup>33,34</sup> The ability to simultaneously enhance interfacial biocompatibility and charge extraction via revealing the correlation between the catalytic characteristics of the electrode and the metabolic process of microbes represents an important endeavor toward high-efficiency bio-electrochemical CO2 fixation. This, however, has yet to be explored.

Herein, we report, for the first time, the rationally designed cobalt (Co) atomic-nanoparticle bridge (Co-SA@Co-NP) crafted in metal-organic framework (MOF)-derived nanosheets to impart concurrent interfacial microbe enrichment and charge transfer enhancement for CO2 conversion to methane in mixed culture. Intriguingly, the biohybrids constructed with the Co-SA@Co-NP bridge-based nanosheets demonstrate dramatically increased methane production rates compared to the pure Co-SA counterpart. The addition of the Co nanoparticles underneath selectively enriches electroactive methanogens (Methanobacterium and Methanoculleus) for CO<sub>2</sub> conversion and enhances the quorum sensing of interfacial electron receiver bacteria (Bacteroidales and Clostridiales) for interspecies electron transfer. Finite element analysis (FEA) reveals that the enrichment and active species in the microbiome community are closely related to the modified surface electronic structure of the Co-SA@Co-NP bridge-based nanosheets. This improved surface electronic structure further affects the electric communication between the microbiome and nanosheet electrode as illustrated by density functional theory (DFT) calculations. The synergistic effects of bio-activity and charge extraction resulting from the Co-SA@Co-NP bridge deliver a dramatically increased methane production rate of ~2512 mmol m<sup>-2</sup> per day (V = -1.1 V vs. Ag/AgCl) with high selectivity (FE =  $\sim$  94.1%). This study demonstrates that interfacial electronic structure engineering may offer great potential for simultaneously tailoring biocompatibility and charge transfer via creating a rich variety of desirable nanostructured catalysts in bioelectrochemical systems.

### Results and discussion

# Crafting the Co-SA@Co-NP bridge in MOF-derived nanosheets for bio-hybrid construction

The large surface area and well-defined porous structure of the electrode are the prerequisites for constructing an efficient biohybrid.<sup>35</sup> In this context, metal-organic frameworks (MOFs)derived nanosheets grown on carbon felt were synthesized, serving as the matrix for Co-SA@Co-NP anchoring and microorganism reproduction. MOFs were selected here due to their effectiveness in building active atomic sites apart from their morphology superiority.<sup>8,36</sup> The construction of bio-hybrids with Co-SA@Co-NP bridge-based nanosheets is exhibited in Fig. 1(A). As depicted, the Co-SA@Co-NP bridge was formed *in situ* on MOFs-derived nanosheets through a simple one-pot synthesis with subsequent pyrolysis. Zinc (Zn) species in the precursors were incorporated to improve the formation of Co single atoms in the nanosheets according to our previous study.<sup>37,38</sup> Notably, the Co-SA@Co-NP bridge can be easily crafted via tailoring the coordination environment through tuning the molar ratios of the Co and Zn precursors (denoted  $Co_x Zn_y$ ). Specifically, a control ( $Co_0 Zn_1$ , *i.e.*, without Co), a Co-SA (Co1Zn8, i.e., Co-SA only), and three Co-SA@Co-NP bridge samples (Co1Zn4, Co1Zn1, and Co1Zn0, respectively) were prepared in this work. Detailed characterizations of these five



**Fig. 1** Biohybrid construction with Co-SA@Co-NP bridge-based nanosheets. (A) Schematic of the nanosheet-microbiome biohybrid construction; SEM images of the biohybrids constructed with (B) Co-SA@Co-NP bridge-based nanosheets and (C) Co-SA-based nanosheets, scale bar 2  $\mu$ m. (D) HADDF-STEM of Co-SA@Co-NP bridge-based nanosheets, scale bar 1 nm; the insert is the HR-TEM image showing the double-layer structure of the Co-SA@Co-NP bridge. (E) X-ray absorption spectra of Co K-edge spectroscopy and (F)  $k^3$ -weighted Fourier transform of EXAFS spectra at the Co K-edge of the Co-SA@Co-NP bridge. (G) EDS mapping and the corresponding SEM image of the biohybrids constructed with the Co-SA@Co-NP bridge-based nanosheets. CLSM images of the biohybrids constructed with (H) Co-SA@Co-NP bridge-based nanosheets and (I) Co-SA-based nanosheets.

prepared samples are shown in Fig. S1–S4 (ESI<sup>†</sup>). The bestperforming Co-SA@Co-NP bridge sample prepared with Co<sub>1</sub>Zn<sub>1</sub> was chosen for comparison with the control and Co-SA samples to reveal the synergistic effect between Co single atoms and Co nanoparticles in biohybrid construction. Thus, all Co-SA@ Co-NP bridges discussed below were prepared with Co<sub>1</sub>Zn<sub>1</sub>. The results obtained with Co-SA@Co-NP bridges prepared using Co<sub>1</sub>Zn<sub>4</sub> and Co<sub>1</sub>Zn<sub>0</sub> can be found in the ESI.<sup>†</sup>

While all the samples maintain the structures of wellassembled nanosheet arrays on carbon felt substrates, the nanosheets with the Co-SA@Co-NP bridge are much rougher compared to that with Co-SA (Fig. S1B and D, ESI<sup>†</sup>). This increased roughness which is supposed to benefit microorganism attachment is due to the emergence of carbon nanotubes on the surface (Fig. S4B, ESI<sup>†</sup>). The continuous addition of Co species leads to excess Zn, the volatilization of which results in the generation of carbon nanotubes during pyrolysis.<sup>39</sup> The presence of Co nanoparticles in the Co-SA@Co-NP bridge-based nanosheets is demonstrated in the HADDF-STEM image (Fig. 1(D)). HR-TEM further depicts that the Co nanoparticles are covered by a MOF-derived carbon layer (Fig. 1(D) inside, and Fig. S5, ESI<sup>†</sup>), where the Co single atoms are supposed to be

built in. This double-layer structure thus imparts an interfacial charge transfer path from the electrode to the microbes, *i.e.*, the Co-SA@Co-NP bridge. Atomically dispersed Co in the Co-SA@Co-NP bridge-based nanosheets was further characterized via K-edge X-ray absorption near edge structure (XANES) and Fourier-transform extended X-ray absorption fine structure (FT EXAFS). The Co K-edge XANES spectra suggest that the nearedge absorption of the Co species in Co-SA@Co-NP bridgebased nanosheets is located between that of the Co foil and phthalocyanine-cobalt (Co-Pc) (Fig. 1(E)), indicating that the valence state of the corresponding Co species is between the 0 and +2. The spectrum of Co-SA-based nanosheets demonstrated a similar structure to that of the Co-SA@Co-NP bridge-based nanosheets, yet the characteristic peak located around 7720 to 7740 eV shifted to Co-Pc (Fig. 1(E)). The XPS spectrum of Co in the Co-SA@Co-NP bridge-based nanosheets is displayed in Fig. S3 (ESI<sup>+</sup>). The two deconvoluted peaks located around 776.8 eV and 781.1 eV were identified as the Co-Co band and the Co-N band, respectively. The FT EXAFS spectra (based on the  $k^3$ -weighted  $\chi(k)$ -function) show scattering paths at  $\approx$  1.4 Å and  $\approx$  2.2 Å for Co-SA@Co-NP bridge-based nanosheets (Fig. 1(F)), which were identified as Co-N<sub>4</sub> and Co-Co according to Co-Pc and Co foil, respectively. Whereas for Co-SA-based nanosheets, only a predominant peak at  $\approx$  1.4 Å was detected. The average coordination number of the Co-N was also quantitatively determined to be 3.6  $\pm$  0.4 by leastsquares EXAFS fitting analysis (Fig. S6, ESI†), further confirming that the atomically dispersed Co was confined in Co-N<sub>4</sub> moieties.

The biocompatibility of the nanosheets was subsequently examined via developing microbial hybrids. The biohybrids were constructed via a stepped start-up process and the detailed process can be found in the ESI.† Though the wellassembled array structure and open porous characteristic of the MOF-derived nanosheets provide abundant surface area for microbe cultivation, only a few microorganisms were found on the control electrode (Fig. S7E, ESI<sup>+</sup>). In comparison, the introduction of Co species into the nanosheets significantly increased microbe attachment (Fig. S7, ESI<sup>†</sup>). The number of attached microbes progressively increased with a higher Co molar ratio, indicating that Co acts as the determining element in the system for microorganism enrichment. Notably, a more closely packed microbial hybrid was developed with Co-SA@Co-NP bridge-based nanosheets compared to that with Co-SAbased nanosheets (Fig. 1(B) and (C)). In addition to the higher loading amount of Co, this improvement can partially result from the rougher surface of Co-SA@Co-NP bridge-based nanosheets. The dense carbon nanotubes located at the surface of Co-SA@Co-NP bridge-based nanosheets (Fig. S1B and S4B, ESI<sup>†</sup>) facilitate the uniform distribution of Co species (Fig. 1(G)), thus providing abundant active sites for microbe attachment. Some nanowires crossing individual microbes were observed on biohybrids developed with Co-SA@Co-NP bridge-based nanosheets (Fig. S7B, ESI<sup>+</sup>), providing a signal for facilitating interspecies charge communication. Quantified biocompatibility evaluation was then carried out via confocal

laser scanning microscopy (CLSM) alive-dead staining assay, in which the green fluorescence represents living cells *via* SYTO9 dye staining (Fig. 1(H), (I) and Fig. S8, ESI†). The living-cells biofilm derived on the Co-SA@Co-NP bridge-based nanosheets ( $\sim$ 130 µm) was much thicker than that on the Co-SA-based nanosheets ( $\sim$ 85 µm). Besides, the viability of the electroactive microbes on Co-SA@Co-NP bridge-based nanosheets was 55.47%, which is higher than that on the Co-SA (51.78%) and the control sample (47.07%), thus confirming the improved biocompatibility of the nanosheets with the Co-SA@Co-NP bridge.

# Microbiome response towards Co-SA@Co-NP bridge-based nanosheets

Note that the CLSM analysis discussed above only exhibited regional microbe attachment; the total biomass concentration on the nanosheet-based electrodes was thus investigated with DNA extraction (Fig. 2(D) and Fig. S9, ESI<sup>+</sup>). Compared to that on the control electrode (5.9 ng  $\mu$ L<sup>-1</sup>), a dramatically increased biomass concentration of 39.6 ng  $\mu L^{-1}$  was observed for the biohybrid constructed with Co-SA-based nanosheets, manifesting a 6.7-fold improvement. Though the construction of biohybrids is often in response to a variety of environmental cues, it is generally accepted that initial microbe adhesion is a key part of the biohybrid development process. This first-step adhesion mainly involves hydrodynamic and electrostatic interactions. Specifically, most microbes have a net negative surface charge and interact preferentially with positively charged surfaces.<sup>40</sup> Co (1.84) has a higher electronegativity than Zn (1.59), representing a greater ability to attract electrons. During the stepped start-up process for constructing biohybrids, the surface of the nanosheet-based electrode is working under a double-layerbased capacitive mode, *i.e.*, the inner surface of the electrode is negatively charged while the outer surface of the electrode is positively charged. Thus, Co with a higher electronegativity in the MOF-derived nanosheets significantly enhances the bioactivity. Similarly, Co nanoparticles in the Co-SA@Co-NP bridgebased nanosheets further modify the overall surface electronegativity, as revealed by FEA simulations (Fig. 2(A), (B), and Fig. S10, ESI<sup>†</sup>). A largely increased surface electric intensity for Co-SA@Co-NP bridge-based nanosheets over that for Co-SAbased counterpart was observed as a direct consequence of enhanced electronegativity (Fig. 2(D)). As a result, the biomass concentration on Co-SA@Co-NP bridge-based nanosheets increased to 72.2 ng  $\mu$ L<sup>-1</sup>, which is 1.82-fold higher than that on Co-SA (Fig. 2(E)). Moreover, it was found that the surface electric intensity progressively increased with the decreased distance between two adjacent nanosheets (Fig. 2(C)). This elucidates the essential reason why building nanostructures is effective in improving biocompatibility. Notably, the addition of Co nanoparticles decelerates the decrease in the surface electric intensity with the increased distance. Thus, a more closely graded surface electric intensity of the nanosheets with Co-SA@Co-NP bridge over that with Co-SA facilitates more uniform dispersion of the microbes. The synergistic effect of increased surface electronegativity and closely graded surface



**Fig. 2** Microbiome response to Co-SA@Co-NP bridge-based nanosheets. (A) SEM image of Co-SA@Co-NP bridge-based nanosheets showing the distance between two adjacent nanosheets used as a parameter in surface electric intensity simulation. (B) Numerical simulation of the surface electric field around Co-SA- and Co-SA@Co-NP bridge-based nanosheets. (C) Surface electric intensity of the nanosheets as a function of the distance between two adjacent nanosheets. (D) Biomass concentration of the biohybrids constructed with different nanosheets. (E) Relative abundance of the bacterial orders and (F) relative abundance of archaea at the genus level for the biohybrids developed with different nanosheets.

electric intensity leads to a densely packed biohybrid on Co-SA@Co-NP bridge-based nanosheets.

Besides the biomass concentration, the microbial community composition in mixed culture is another critical factor in the methanogenesis process. As such, a detailed analysis of the relative abundance of each microorganism was carried out (Fig. 2(E), (F), and Fig. S11, ESI<sup>+</sup>). Generally, microbes contained in the biohybrid can be divided into two categories, *i.e.*, archaea and bacteria. Electroactive methanogens in archaea would directly participate in methanogenesis. While bacteria do not fix CO2 into methane, some conductive species can act as electron acceptors to facilitate methane production via transferring charges to methanogens through interspecies charge exchange. Encouragingly, the performing microbes in archaea and bacteria both increase for the Co-SA@Co-NP bridge-derived hybrid compared to that derived from Co-SA and the control. The relative abundance of electroactive methanogens, i.e., Methanobacterium and Methanoculleus, for the control is 71.8%, and it progressively increases to 90.2% for Co-SA and 95.5% for the Co-SA@Co-NP bridge (Fig. 2(F)), respectively. Particularly, a significantly higher ratio of Methanobacterium, which can conduct direct extracellular electron

transfer as reported,<sup>41,42</sup> was observed for the Co-SA@Co-NP bridge, suggesting that there may be improved direct electron transfer at the bioinorganic interface. Similarly, it was observed that Bacterodiales and Clostridiales in the bacteria are the main types in response to the surface electric intensity change in the electrode, implying that they are the main bacteria for charge communication with the inorganic electrode. The total relative abundance of Bacterodiales and Clostridiales increases from 23.2% for the control to 25.2% for the Co-SA, and further to 56.6% for the Co-SA@Co-NP bridge (Fig. 2(E)). Notably, compared to that in the control- and the Co-SA-derived biohybrids, the electroactive bacteria ratio in the Co-SA@Co-NP bridgedeveloped biohybrid demonstrated a 2-fold increase. This signal implies a largely enhanced interspecies electron transfer between the methanogens and electroactive bacteria, which is supported by the emerging nanowires that cross individual microbes (Fig. S7B, ESI<sup>+</sup>). It is interesting to note that with the continuous increase in the surface electric intensity on the electrode (i.e., from the control to Co-SA, then to the Co-SA@Co-NP bridge), the microbiome response can be divided into two phases. In the first phase, the increase in the microbes is dominated by methanogen reproduction, which can receive

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electrons directly from the electrode *via* either mediated electron transfer or possible direct electron transfer. In the second phase, the microbiome response is dominated by electroactive bacteria multiplying to further facilitate methanogens receiving charges from the electrode *via* boosted interspecies electron transfer. This well illustrates the superior adaptability of the mixed microbiome in maximizing charge communication with the electrode.

# Methane production on the Co-SA@Co-NP bridge-derived biohybrid

CO<sub>2</sub> fixation into methane on the Co-SA@Co-NP bridge-derived biohybrid was carried out in a typical H-type cell (Fig. 3(A)). The biohybrids used in the experiment were obtained with 20 day cultivation under CO<sub>2</sub>/N<sub>2</sub> (20/80, v/v%) mixed gas through stepped start-up potential (Fig. S12, ESI<sup>†</sup>). Before each trial, the chambers in the H-type cell were refilled with new growth medium and the head spaces were re-flushed with 99.999%  $CO_2$  as the only carbon source for methane production.  $CO_2$ fixation was then carried out with the as-prepared biohybrids at various potentials, -0.9 V, -0.95 V, -1 V, and -1.1 V vs. Ag/ AgCl (i.e., -0.287, -0.337, -0.387, -0.487 V vs. SHE). Current densities of the cell with Co-SA@Co-NP bridge-derived biohybrid were tremendously larger than that with control-/Co-SAderived biohybrids during CO<sub>2</sub> fixation (Fig. S13, ESI<sup>+</sup>), indicating the facilitated reactions on the cathode. Specifically, the current density is composed of two parts according to the two competitive reactions on the cathode, namely, CO<sub>2</sub> fixation

and H<sub>2</sub> evolution. More complicatedly, the part for CO<sub>2</sub> fixation consists of methane production *via* methanogens and other product generation happening directly on the inorganic cathode. As such, the performances of the bare control, Co-SA-, and Co-SA@Co-NP bridge-based electrodes (*i.e.*, without microbes on them) in CO<sub>2</sub> conversion were also studied, and it was confirmed that no methane was generated on these catalysts at the studied potentials. There was only a trace amount of CO<sub>2</sub> reduction by-products (*e.g.*, acetic acid, ethanol, *etc.*) generated on these electrodes (Tables S1 and S2, ESI†) due to the catalytic ability of Co single atoms in CO<sub>2</sub> conversion (Fig. S14, ESI†). Therefore, the methane detected in the experiments resulted from the methanogens attached to the hybrids.

The highest production rate and faradaic efficiency (FE) of methane were obtained with the Co-SA@Co-NP bridge-derived biohybrid (Fig. 3(B), (C) and Fig. S15 A, B, ESI<sup>†</sup>) at more negative applied potentials. Moreover, these two parameters for the Co-SA@Co-NP bridge-derived hybrid demonstrate a nearly linear increase with the increased potential, implying a sufficient electron transfer ability at the bio-interface. The methane production rate achieved ~2512 mmol m<sup>-2</sup> per day with high selectivity (FE = ~94.1%) at -1.1 V vs. Ag/AgCl on the Co-SA@Co-NP bridge-derived hybrid, which greatly outperformed that on the control or Co-SA-derived hybrids. Besides, the Co-SA@Co-NP bridge-derived hybrid also depicts highly stable selectivity towards methane under continuous operation for 72 h (Fig. 3(D)). The control-derived hybrid exhibited the lowest methane production rates at various potentials and the



**Fig. 3** Methane production performance of the biohybrid constructed with Co-SA@Co-NP bridge-based nanosheets. (A) Schematic of the bioelectrochemical system for CO<sub>2</sub> fixation into CH<sub>4</sub>; (B) bias-dependent methane production rates; (C) bias-dependent faradaic efficiency of methane; (D) faradaic efficiency of methane production during long-time CO<sub>2</sub> reduction with biohybrid constructed with Co-SA@Co-NP bridge-based nanosheets; (E) methane production rates per unit Co single atoms with biohybrids constructed with Co-SA@Co-NP bridge- and Co-SA-based nanosheets; (F) comparison of production rates in bio-electrochemical systems for CO<sub>2</sub> fixation into methane (the details for the references are shown in Table S3, ESI†).

faradaic efficiency of methane progressively decreased with the increased potential, which is probably due to less microbe enrichment on the electrode and limited interfacial charge transfer speed at high current densities. It is worth noting that for the Co-SA-derived hybrid, a high methane production was obtained at -0.9 V and -0.95 V vs. Ag/AgCl, yet it dramatically decreases with further increased cathode potential; meanwhile, large amounts of H<sub>2</sub> were detected (Fig. S16, ESI<sup>+</sup>). The efficient methane production at low cathode potential on the Co-SA-derived hybrid could be ascribed to the abundant microbe attachment on it, while its suppressed methane generation at high cathode potential could only possibly be derived from the confined charge extraction speed of the microbes. This inefficient charge transfer at the interface greatly enhances H<sub>2</sub> evolution at more negative potentials. Thus, the super methane production performance with Co-SA@Co-NP bridge-derived hybrid is attributed to two aspects, *i.e.*, the abundant performing microbe enrichment and fast interfacial charge extraction. The latter may benefit from the boosted interspecies electron transfer, during which the electroactive Bacterodiales and Clostridiales act as an electron buffer pool for methanogens. Notably, the methane production on the Co-SA@Co-NP bridge-derived hybrid is also much higher than other reported biocathodes at the same applied potential (Fig. 3(F)).42-57 The stability of the as-prepared biohybrids was investigated with a continuous flow of  $CO_2$  gas into the catholyte at a rate of 20 sccm. Notably, the current densities demonstrate nearly no decrease with 12 h operation, depicting the high stability of the electrodes (Fig. S23, ESI<sup>†</sup>).

Since Co nanoparticles are covered by the MOF-derived nanosheets and don't directly come in contact with the

### Electrochemical characteristics of the Co-SA@Co-NP bridgederived biohybrid

To gain more insight into the electron transfer process at the interface, the electrochemical characteristics of the biohybrids were investigated. The linear sweep voltammetry (LSV) and cyclic voltammetry (CV) studies of the bare control-, Co-SA-, and Co-SA@Co-NP bridge-based nanosheet electrodes (*i.e.*, without microbes on them) showed that the Co-SA@Co-NP bridge-based electrode had the highest current density (Fig. 4(A) and (B)), suggesting a much higher conductivity than the control and the Co-SA sample. Similar trends were observed



**Fig. 4** Electrochemical characteristics of the biohybrid constructed with Co-SA@Co-NP bridge-based nanosheets. (A) LSV and (B) CV curves of the bare nanosheets (*i.e.*, without microbes) at a scan rate of 5 mV s<sup>-1</sup>; (C) LSV and (D) CV curves of the nanosheet–microbiome biohybrids at a scan rate of 5 mV s<sup>-1</sup>; (E) EIS and (F) fitted resistance with the equivalent circuit of the nanosheets–microbiome biohybrids.

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for the bio-groups (i.e., control-, Co-SA-, and Co-SA@Co-NP bridge-derived biohybrids), yet with higher current densities compared with the counterparts without microbes (Fig. 4(C)and (D)). The increased current densities demonstrated that the original electrochemical process matched the microbial extracellular electron utilization process, indicating an efficient charge transfer to the electroactive microbes for CO2 conversion.<sup>34</sup> The CV of the groups without microbes revealed a near-rectangular shape, manifesting an electric double-layerbased capacitive response (Fig. 3(B)). In sharp contrast to the bare control and bare Co-SA sample, the bare Co-SA@Co-NP bridge-based electrode demonstrated greatly increased specific capacitance due to the presence of a large amount of Co single atoms and Co nanoparticles that served as active sites and resulted in the enhanced conductivity of the electrode. The attachment of microbes on the electrodes completely changed the CV shapes (Fig. 4(D)), indicating their effectiveness in  $CO_2$ reduction. The Co-SA@Co-NP bridge-derived hybrid displayed stronger catalytic waves than other groups, suggesting more effective electron transfer between active sites and electroactive microbes.

Electrochemical impedance spectroscopy (EIS) was employed to investigate the kinetics of electron-transfer processes on the hybrids. The obtained Nyquist plots are displayed in Fig. 4(E)and modeled with the Randles equivalent circuit (inside Fig. 4(F)). There was no diffusion limitation in the Nyquist plots, that

indicating charge transfer is the dominant factor in CO<sub>2</sub> bioelectric reduction. The high- and low-frequency response arcs of the impedance spectra are ascribed to biofilm resistance  $(R_{bio})$  and extracellular electron transfer resistance ( $R_{ct}$ ), respectively.<sup>34</sup>  $R_{bio}$  is much smaller than  $R_{ct}$  (Fig. 4(F) and Fig. S17F, ESI†), suggesting that extracellular electron transfer is the rate-determining step. The Co-SA@Co-NP bridge-derived hybrid exhibits both decreased  $R_{\rm bio}$  and  $R_{\rm ct}$  compared to that of the control- and Co-SA-derived hybrids. The reduced  $R_{\rm bio}$  originates from the stronger interaction between the electroactive microbes and the active sites on the electrodes, caused by the increased electronegativity of the Co-SA@Co-NP bridge. Notably, the R<sub>ct</sub> of the Co-SA@Co-NP bridgederived hybrid, as the rate-determining step, greatly decreased to 7.2  $\Omega$  from 20.7  $\Omega$  for Co-SA- and 49.8  $\Omega$  for control-derived hybrids, depicting a 7-fold to 4-fold decrease. This implies that the significantly enhanced extracellular electron transfer on the Co-SA@Co-NP bridge-derived hybrid is mainly responsible for its outstanding methane production in CO<sub>2</sub> fixation.

### Extracellular electron transfer on the Co-SA@Co-NP bridgederived biohybrid

It has been demonstrated that microbes generally bond with the electrode through extracellular polymeric substances (EPS).<sup>58,59</sup> Therefore, the electroactive EPS is an ineluctable medium for charge communication between the cells and the electrode, and its abundance can be taken as a rough guide to



**Fig. 5** Mechanisms of the Co-SA@Co-NP bridge-boosted extracellular electron transfer. (A) Excitation–emission matrix spectrum (3D-EEM) of the biohybrid constructed with Co-SA@Co-NP bridge-based nanosheets. (I: tryptophan; II: tyrosine; III: fulvic acid; IV: soluble microbial products; V: humic acid). (B) Fluorescence intensity of electron mediators; (C) CV curves of the model molecule for humic acid with bare nanosheets acting as the working electrodes at the scan rate of 10 mV s<sup>-1</sup>. (D) Charge density difference of the model molecule for humic acid absorbed on Co-SA- and Co-SA@Co-NP bridge-based nanosheets in 3D and vertical 2D views. (E) Free energy diagram for the humic acid hydrogenation reaction (IS: initial state, TS: transition state, FS: final state).

evaluate the extracellular electron transfer at the bio-interface. In this case, the EPS between the microbes and the as-prepared electrodes was extracted with gradient heating, and its abundance was investigated via three-dimensional excitationemission matrix spectroscopy (3D-EEM). The fluorescence distribution in the EEM spectrum is divided into five typical regions (Fig. 5(A) and Fig. S18, ESI<sup>+</sup>), representing different secondary metabolites.<sup>60</sup> Regions I, II, and III are identified as Tryptophan, Tyrosine, and Fulvic acid, respectively, however, they are not conductive and thus make no contribution to interfacial charge exchange. Regions IV (Em/Ex = 400-500 nm/220-325 nm) and V (Em/Ex = 450-500 nm/325-450 nm) were confirmed as the characteristic fluorescence of soluble microbial metabolism and humic acid, respectively, and they are both conductive. It is worth noting that humic acid is the dominant conductive EPS since there are only a few soluble microbial products (Fig. 5(B) and Fig. S18, ESI<sup>+</sup>). Humic acid is well regarded as a carrier of benzoquinonyl compounds for conducting mediated electron transfer. This indicates that the charge exchange at the bioinorganic interface in this work is centered on humic acid-mediated electron transfer, during which humic acid acts as the redox mediator. Specifically, humic acid accomplishes the reduction process by capturing electrons and protons outside the microbes (i.e., at the biointerface) and undergoes the oxidation process inside the microbes to release electrons and protons for CO<sub>2</sub> fixation. Thus, the absolute amount of the humic acid and its redox activity (*i.e.*, the turnover rate of humic acid) are the two critical indexes that reflect the dynamics of mediated electron transfer. The accumulated fluorescence intensity of humic acid for the Co-SA@Co-NP bridge-derived hybrid  $(1.3 \times 10^7)$  is significantly higher than that for Co-SA-  $(7.6 \times 10^6)$  and control-derived  $(5.4 \times 10^6)$  hybrids (Fig. 5(B) and Fig. S19, ESI<sup>†</sup>), demonstrating that there are more redox mediators available on the Co-SA@Co-NP bridge-derived hybrid for conducting mediated electron transfer. The redox activity on the bare Co-SA@Co-NP bridge/Co-SA electrode (i.e., without microbe attachment) was investigated with *p*-benzoquinone as a model compound of humic acid via the CV test (Fig. 5(C) and Fig. S20, ESI<sup>†</sup>). An identical amount of p-benzoquinone was employed in the measurements to rule out the influence of the EPS enrichment difference. Notably, a larger peak current density was obtained with the Co-SA@Co-NP bridge-based electrode at various scan rates compared to that with Co-SA (Fig. S20, ESI<sup>+</sup>), suggesting a much higher turnover rate of the *p*-benzoquinone. The greater abundance and higher turnover rate of humic acid indicated the significantly facilitated mediated electron transfer at the bio-interface with the Co-SA@Co-NP bridge.

It is worth noting that two distinct processes occur concurrently during the reduction of humic acid at the bio-interface, *i.e.*, charge extraction and hydrogenation. Therefore, they were investigated separately to gain more insight into the charge transfer dynamics of the redox mediators. Since charge extraction is closely related to the interfacial interaction between the redox mediators and the electrode, DFT calculations of charge density difference were performed (Fig. 5(D)). Compared to Co-SA-based nanosheets, the introduction of Co nanoparticles into Co-SA@Co-NP bridge-based nanosheets significantly enlarges the redistributed electronic cloud, indicating enhanced electron extraction ability. The gain and loss of electrons suggest bonding between the carbonyl groups (C=O) in \**p*-benzoquinone and Co in  $Co-N_4$  coordination, indicating that Co single atoms are the active sites that come in direct contact with microbes. Yet, a distinct gap between \*p-benzoquinone and Co-SA was observed, which blocks fast charge extraction and thus leads to the accumulation of electrons (Fig. 5(D) and Fig. S21, ESI<sup>+</sup>). Encouragingly, the addition of Co nanoparticles not only efficiently fills up this gap but also significantly enhances electron migration flux. The contribution of Co nanoparticles in this process can be divided into two aspects. Firstly, the introduction of Co nanoparticles improves the interaction between \*p-benzoquinone and Co-N<sub>4</sub> by increasing the localized surface electronegativity around Co-N4 coordination, thereby clearing the gap between them and reducing the energy barrier for charge extraction. Secondly, Co nanoparticles further enlarge the charge communication flux between \*p-benzoquinone and Co-N<sub>4</sub>, probably by collecting electrons from the electrode more efficiently and then passing them to microbes via Co-N<sub>4</sub> sites. Thus, it is the synergistic effect of Co single atoms and Co nanoparticles that strengthens the interfacial charge extraction during mediated electron transfer.

The hydrogenation of *p*-benzoquinone is composed of the adsorption and the reduction of carbonyl groups into hydroxyl groups. This process is thus simulated via DFT calculations. The hydrogenation reaction is dynamically divided into four steps, namely, the initial state 1 (IS1) for humic acid absorption, the transition state (TS) for the hydrogenation of humic acid with the first H, the IS2 for the absorption of the humic acid hydrogenated with one H, and the final state (FS) for the humic acid hydrogenated with two H (Fig. S22, ESI<sup>+</sup>). As shown in Fig. 5(E), Co-SA@Co-NP bridge-based nanosheets display much lower free energy (-3.21 eV) for the adsorption process of p-benzoquinone than the Co-SA-based counterpart (-0.77 eV). Since the hydrogenation steps are all under the zero point, the smaller decrease in free energy during each step suggests less spontaneity for this reaction. For this reason, the limited step was determined to be the hydrogenation of humic acid with the first H. The energy barrier during this ratelimiting step was calculated to be 0.95 eV and 2.07 eV for the Co-SA@Co-NP bridge and Co-SA, respectively, indicating the facilitated hydrogenation of the redox mediators on Co-SA@Co-NP bridge-based nanosheets. This intensive hydrogenation was a response to the enhanced charge extraction at the biointerface, which together increased the turnover rate of the redox mediators and resulted in a strengthened mediated electron transfer.

## Conclusion

In summary, we have developed an understanding of how the judicious design of the surface electronic structure of the

electrode facilitates the concurrent tailoring of microbe enrichment and charge transfer at the bioinorganic interface in CO<sub>2</sub> fixation. The investigation was enabled by crafting an atomicnanoparticle bridge, i.e., the Co-SA@Co-NP bridge. The enhanced electronegativity and more closely distributed electric intensity of the electrode surface rendered by the Co nanoparticles exerted a profound influence on the microbiome response, including increased biomass concentration and the enlarged ratio of electroactive bacteria. The electroactive bacteria act as an electron buffer pool to methanogens via interspecies electron transfer and enable high faradaic efficiency for methane production at more negative potentials. Moreover, the synergetic effect of Co single atoms and Co nanoparticles enhances interfacial charge extraction via strengthening the Co-N<sub>4</sub> and C=O interaction and reduces the energy barrier of the intermediator during hydrogenation, thereby leading to an increased humic acid-mediated electron transfer with a high turnover rate. As a result, an outstanding CO<sub>2</sub> fixation rate with high methane selectivity (~2512 mmol  $m^{-2}$  per day; FE:  $\sim$  94.1%) was achieved under a large flux of reducing equivalent (V = -1.1 V vs. Ag/AgCl). This study offers insight into the tailoring of interfacial microbial interaction via the rational design of surface electronic nanostructures to achieve high-rate and high-efficiency bio-electrochemical CO<sub>2</sub> fixation.

## Conflicts of interest

There are no conflicts to declare.

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### References

- 1 X. Y. Tan and J. Nielsen, *Chem. Soc. Rev.*, 2022, **51**, 4763-4785.
- 2 Q. S. Zhu, C. J. Murphy and L. R. Baker, *J. Am. Chem. Soc.*, 2022, **144**, 2829–2840.
- 3 H. Sheng and C. Liu, Nat. Catal., 2022, 5, 357-358.
- 4 M. Zhang, J. Cheng, X. Xuan, J. Zhou and K. Cen, *Chem. Eng. J.*, 2017, **322**, 22–32.
- 5 D. Pant and S. A. Patil, Joule, 2022, 6, 1399-1401.
- 6 P. Izadi and F. Harnisch, Joule, 2022, 6, 935-940.
- 7 J. Timoshenko, A. Bergmann, C. Rettenmaier, A. Herzog, R. M. Arán-Ais, H. S. Jeon, F. T. Haase, U. Hejral, P. Grosse, S. Kühl, E. M. Davis, J. Tian, O. Magnussen and B. Roldan Cuenya, *Nat. Catal.*, 2022, 5, 259–267.
- 8 A. Wang, J. Li and T. Zhang, Nat. Rev. Chem., 2018, 2, 65-81.
- 9 M. Zhang, X. Xuan, W. Wang, C. Ma and Z. Lin, *Adv. Funct. Mater.*, 2020, **30**, 2005983.
- 10 X. Xuan, M. Wang, M. Zhang, Y. V. Kaneti, X. Xu, X. Sun and Y. Yamauchi, *J. CO2 Util.*, 2022, 57, 101883.

- 11 K. Rabaey and R. A. Rozendal, *Nat. Rev. Microbiol.*, 2010, 8, 706–716.
- 12 X. Xie, M. Ye, P.-C. Hsu, N. Liu, C. S. Criddle and Y. Cui, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 15925–15930.
- 13 Y. Hou, R. Zhang, H. Luo, G. Liu, Y. Kim, S. Yu and J. Zeng, *Process Biochem.*, 2015, **50**, 1103–1109.
- 14 C. Yang, H. Aslan, P. Zhang, S. Zhu, Y. Xiao, L. Chen, N. Khan, T. Boesen, Y. Wang, Y. Liu, L. Wang, Y. Sun, Y. Feng, F. Besenbacher, F. Zhao and M. Yu, *Nat. Commun.*, 2020, **11**, 1379.
- 15 S. Cestellos-Blanco, H. Zhang, J. M. Kim, Y.-X. Shen and P. Yang, *Nat. Catal.*, 2020, **3**, 245–255.
- 16 C. Liu, B. C. Colón, M. Ziesack, P. A. Silver and D. G. Nocera, *Science*, 2016, **352**, 1210–1213.
- 17 K. K. Sakimoto, A. B. Wong and P. Yang, *Science*, 2016, 351, 74–77.
- 18 X. Guan, S. Erşan, X. Hu, T. L. Atallah, Y. Xie, S. Lu, B. Cao, J. Sun, K. Wu, Y. Huang, X. Duan, J. R. Caram, Y. Yu, J. O. Park and C. Liu, *Nat. Catal.*, 2022, 5, 1019–1029.
- 19 F. M. Schwarz, J. Moon, F. Oswald and V. Müller, *Joule*, 2022, 6, 1304–1319.
- 20 E. Blanchet, F. Duquenne, Y. Rafrafi, L. Etcheverry, B. Erable and A. Bergel, *Energy Environ. Sci.*, 2015, **8**, 3731–3744.
- 21 A. Kumar, L. H.-H. Hsu, P. Kavanagh, F. Barrière, P. N. L. Lens, L. Lapinsonnière, J. H. Lienhard V, U. Schröder, X. Jiang and D. Leech, *Nat. Rev. Chem.*, 2017, 1, 1–13.
- 22 C. M. Dykstra and S. G. Pavlostathis, *Environ. Sci. Technol.*, 2017, **51**, 5306–5316.
- 23 Y. Ding, J. R. Bertram, C. Eckert, R. R. Bommareddy,
   R. Patel, A. Conradie, S. Bryan and P. Nagpal, *J. Am. Chem. Soc.*, 2019, 141, 10272–10282.
- 24 B. E. Logan, R. Rossi, A. A. Ragab and P. E. Saikaly, *Nat. Rev. Microbiol.*, 2019, 17, 307–319.
- 25 H. Chen, F. Dong and S. D. Minteer, *Nat. Catal.*, 2020, 3, 225–244.
- 26 P. C. Sahoo, D. Pant, M. Kumar, S. K. Puri and S. S. V. Ramakumar, *Trends Biotechnol.*, 2020, 38, 1245–1261.
- 27 M. Baca, S. Singh, M. Gebinoga, F. Weise, G. Schlingloff and A. Schober, *Adv. Energy Mater.*, 2016, **6**, 1600690.
- 28 E. Ruiz-Hitzky, M. Darder, P. Aranda and K. Ariga, *Adv. Mater.*, 2010, **22**, 323–336.
- 29 T. Zhang, H. Nie, T. S. Bain, H. Lu, M. Cui, O. L. Snoeyenbos-West, A. E. Franks, K. P. Nevin, T. P. Russell and D. R. Lovley, *Energy Environ. Sci.*, 2013, 6, 217–224.
- 30 G. Zhen, X. Lu, G. Kumar, P. Bakonyi, K. Xu and Y. Zhao, Prog. Energy Combust. Sci., 2017, 63, 119–145.
- 31 H. Li, P. H. Opgenorth, D. G. Wernick, S. Rogers, T. Y. Wu, W. Higashide, P. Malati, Y. X. Huo, K. M. Cho and J. C. Liao, *Science*, 2012, 335, 1596.
- 32 R. M. Rodrigues, X. Guan, J. A. Iñiguez, D. A. Estabrook, J. O. Chapman, S. Huang, E. M. Sletten and C. Liu, *Nat. Catal.*, 2019, 2, 407–414.
- 33 X.-W. Liu, W.-W. Li and H.-Q. Yu, *Chem. Soc. Rev.*, 2014, **43**, 7718–7745.
- 34 R. X. Xia, J. Cheng, H. Li, X. Yang, X. Y. Ren, H. Q. Dong, Z. Chen, X. Y. Zhou, R. C. Lin and J. H. Zhou, ACS Sustainable Chem. Eng., 2022, 10, 2890–2902.

- 35 B. Cao, Z. Zhao, L. Peng, H.-Y. Shiu, M. Ding, F. Song, X. Guan, C. K. Lee, J. Huang, D. Zhu, X. Fu, G. C. L. Wong, C. Liu, K. Nealson, P. S. Weiss, X. Duan and Y. Huang, *Science*, 2021, 373, 1336–1340.
- 36 L. Jiao and H.-L. Jiang, Chem, 2019, 5, 786-804.
- 37 W. Xie, Y. Song, S. Li, J. Li, Y. Yang, W. Liu, M. Shao and M. Wei, *Adv. Funct. Mater.*, 2019, **29**, 1906477.
- 38 M. Zhang, Q. Dai, H. Zheng, M. Chen and L. Dai, Adv. Mater., 2018, 30, 1705431.
- 39 Y. Pan, K. Sun, S. Liu, X. Cao, K. Wu, W.-C. Cheong, Z. Chen, Y. Wang, Y. Li, Y. Liu, D. Wang, Q. Peng, C. Chen and Y. Li, *J. Am. Chem. Soc.*, 2018, **140**, 2610–2618.
- 40 H. H. Tuson and D. B. Weibel, Soft Matter, 2013, 9, 4368-4380.
- 41 S. Zheng, F. Liu, B. Wang, Y. Zhang and D. R. Lovley, *Environ. Sci. Technol.*, 2020, 54, 15347–15354.
- 42 J. Li, Z. Li, S. Xiao, Q. Fu, H. Kobayashi, L. Zhang, Q. Liao and X. Zhu, *J. CO2 Util.*, 2020, **35**, 169–175.
- 43 C. Liu, X. Yuan, Y. Gu, H. Chen, D. Sun, P. Li, M. Li, Y. Dang,
  J. A. Smith and D. E. Holmes, *ACS Sustainable Chem. Eng.*,
  2020, 8, 11368–11375.
- 44 D. Liu, T. Zheng, C. Buisman and A. ter Heijne, ACS Sustainable Chem. Eng., 2017, 5, 11346–11353.
- 45 M. F. Alqahtani, K. P. Katuri, S. Bajracharya, Y. Yu, Z. Lai and P. E. Saikaly, *Adv. Funct. Mater.*, 2018, **28**, 1804860.
- 46 H.-Y. Yang, Y.-X. Wang, C.-S. He, Y. Qin, W.-Q. Li, W.-H. Li and Y. Mu, *Appl. Energy*, 2020, **274**, 115292.
- 47 M. C. Van Eerten-Jansen, A. B. Veldhoen, C. M. Plugge,
  A. J. Stams, C. J. Buisman and A. Ter Heijne, *Archaea*, 2013,
  2013, 481784.

- 48 H. Y. Yang, B. L. Bao, J. Liu, Y. Qin, Y. R. Wang, K. Z. Su, J. C. Han and Y. Mu, *Bioelectrochemistry*, 2018, **119**, 180–188.
- 49 H. Xu, K. Wang and D. E. Holmes, *Bioresour. Technol.*, 2014, 173, 392–398.
- 50 G. Zhen, X. Lu, T. Kobayashi, G. Kumar and K. Xu, *Chem. Eng. J.*, 2016, 284, 1146–1155.
- 51 S. Cheng, D. Xing, D. F. Call and B. E. Logan, *Environ. Sci. Technol.*, 2009, 43, 3953–3958.
- 52 Z. Mao, Y. Sun, Y. Zhang, X. Ren, Z. Lin and S. Cheng, *Int. J. Hydrogen Energy*, 2020, 46, 3045–3055.
- 53 M. E. Peña-Méndez, J. Havel and J. Patočka, J. Appl. Biomed., 2005, 3, 13–24.
- 54 Q. Fu, S. Xiao, Z. Li, Y. Li, H. Kobayashi, J. Li, Y. Yang, Q. Liao, X. Zhu, X. He, D. Ye, L. Zhang and M. Zhong, *Nano Energy*, 2018, **53**, 232–239.
- 55 E. M. Nichols, J. J. Gallagher, C. Liu, Y. Su, J. Resasco, Y. Yu, Y. Sun, P. Yang, M. C. Chang and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 11461–11466.
- 56 P. Batlle-Vilanova, S. Puig, R. Gonzalez-Olmos, A. Vilajeliu-Pons, M. D. Balaguer and J. Colprim, *RSC Adv.*, 2015, 5, 52243–52251.
- 57 Q. Li, Q. Fu, H. Kobayashi, Y. He, Z. Li, J. Li, Q. Liao and X. Zhu, Sustainable Energy Fuels, 2020, 4, 2987–2997.
- 58 F. Jia, Q. Yang, X. Liu, X. Li, B. Li, L. Zhang and Y. Peng, *Environ. Sci. Technol.*, 2017, **51**, 3260–3268.
- 59 G. Yang, L. Huang, Z. Yu, X. Liu, S. Chen, J. Zeng, S. Zhou and L. Zhuang, *Water Res.*, 2019, **159**, 294–301.
- 60 J. Cheng, H. Li, L. K. Ding, J. H. Zhou, W. L. Song, Y. Y. Li and R. C. Lin, *Chem. Eng. J.*, 2020, **397**, 125394.