Unlocking the potential of single atoms loaded *Geobacter* hybrid catalyst as bifunctional electrocatalyst for water splitting

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Abstract

Single-atom catalysts (SACs) with highly dispersed active metal sites allow maximum atomic utilization. Single-atom metal (SA-M) catalysts have shown good catalytic activity towards oxygen evolution reaction (OER) and hydrogen evolution reaction (HER), which are two crucial reactions of electrochemical water splitting. However, the synthesis of SA-M catalysts is challenging because of high-temperature treatments, leading to a random distribution of atoms and hence, unpredictable structures. Here, we report a reproducible method for the synthesis of SA-Ms on the surface of the electroactive bacterium, *Geobacter sulfurreducens* (GS). Our approach is based on the extracellular electron transfer capability of GS to reduce extracellular metal ions by harvesting the metabolically formed electrons inside the cell. The formation of SA-Ms on the surface of GS was achieved by creating an MN_3 moiety (where $M=Ir, Pt, Co, Ru, Cu$, and Pd , and N = nitrogen), identified by X-ray absorption fine structure analysis. Interestingly, our bio-derived SA-M@GS can be directly used as OER and HER electrocatalysts upon drying without further heat treatments such as pyrolysis or hydrothermal approaches. The SA-Ir@GS electrocatalyst exhibits the best water-splitting performance when compared to other SA-M@GS, showing a low applied potential of 1.65 V to achieve 10 mA cm^{-2} in 1.0 M KOH solution with cycling over 5 h. The as-synthesized SA-Ir@GS electrocatalysts demonstrate exceptionally high current density for both OER (60 mA cm^{-2}) and HER (110 mA cm^{-2}) when compared to the benchmark IrO_2 (18 mA cm^{-2}) and Pt/C (89 mA cm^{-2}) electrocatalysts, at an overpotential of 500 mV for OER and HER, respectively. The high activity of SA-Ir@GS is attributed to the larger adsorption energy of H_2O and moderate adsorption energies of reactants and reaction intermediates, which are revealed by atomic structural and density functional theory studies. Our work provides a novel synthesis method for SA-Ms with good electrocatalytic activity and stability by utilizing the metabolic activity of GS, which is abundant and ubiquitous in nature.

Introduction

Single-atom catalysts (SACs) with atomically dispersed active metal sites possess the advantages of maximum atomic utilization, excellent selectivity, and most exposed active sites, which have recently attracted considerable attention in electrochemical catalysis¹⁻⁵. Furthermore, downsizing the metal nanoparticles to the atomic level can lead to new discrete electronic states, thereby imparting unique performance⁶. Importantly, many SACs have shown outstanding catalytic activity and selectivity in various electrocatalytic reactions, such as oxygen reduction reaction (ORR), hydrogen evolution reaction (HER), oxygen evolution reaction (OER), CO₂ reduction reaction (CO₂RR), and nitrogen reduction reaction (NRR) with high efficiency^{1,3,7-9}. Typically, atomically dispersed metal (M) and nitrogen (N) co-doped carbon (C) nanostructures demonstrated outstanding performance for electrochemical energy conversion applications¹⁰⁻¹². Several single-atom metal (SA-M) catalysts containing MNC (where M=Fe, Mo, Cu, Co, Mn, Ir, and Pt) structure have been demonstrated for OER and HER catalysis. However, most of these electrocatalysts can only have a single function, that is they function as HER or OER^{5,13,14}, which are two important reactions in water splitting, which is a key process in solar fuel production, rechargeable metal–air batteries, and microbial electrosynthesis of chemicals and fuels from CO₂^{15,16}. To date, metal doped carbon nanostructures have demonstrated higher activity towards HER, and a multimetallic catalysts with tailored d-band structure have demonstrated excellent performance for OER¹⁷. Considering practical water splitting applications, both HER and OER must be carried out in the same electrolyte (alkaline solution). Typically, IrO₂ and Pt/C catalysts are utilized for enhancing the kinetics and lowering overpotentials during overall water splitting. However, it is uncommon for a single catalyst to perform efficiently as both OER and HER electrocatalyst. Therefore, the development of a single-atom bifunctional MNC catalysts for both HER and OER is of great importance for promoting water splitting. Currently, pyrolysis of mixtures containing metal, carbon, and nitrogen is the most popular approach for the preparation of MNC type electrocatalysts, but the use of high temperatures will lead to random structures such as presence of nanoparticles along with single atoms and carbonization of polymers covering the catalyst surface¹⁸.

A prerequisite for the successful synthesis of SA-M catalysts is anchoring of SA-Ms on supports, but this remains challenging by the natural tendency of single atoms to aggregate due to

their high surface energies¹⁹. To overcome this, various approaches have emerged, such as reducing the amount of metal in wet chemical synthesis, tailoring support-metal interactions, and creating defects in the supports²⁰. However, the choice of precursors, synthesis conditions, quality control, and process complexity will significantly limit their scalability and real applications²¹. An alternative and more sustainable approach is to harvest the metabolic activity of naturally occurring electroactive bacteria for the synthesis of SA-M catalysts. This is possible because electroactive bacteria utilize a unique respiratory pathway, namely extracellular electron transfer (EET) pathway, which allows them to couple the oxidation of substrates (electron donor) in their cytoplasm with the reduction of insoluble extracellular electron acceptors (e.g., metal ions) for respiration. Few studies demonstrated the synthesis of OER electrocatalysts using the electroactive bacterium *Geobacter sulfurreducens* (GS)^{16,22}. However, synthesis of SA-M electrocatalysts using electroactive bacteria in specific or bacteria in general has not been reported before.

Herein, we report for the first time, the EET capability of GS, as a robust, environmentally friendly approach to generate highly dispersed atomically precise and stable bifunctional SA-M catalysts for both HER and OER in basic conditions. This is possible because GS possesses a vast network of multiheme-containing outer-membrane c-type cytochromes (OM c-Cyts)^{23,24}, which allows them to transfer metabolically generated electrons from the oxidation of acetate (electron donor) to reduce metal ions present outside the outer membrane of the cell (Fig. 1), thus successfully coordinating and stabilizing the SA-M entity. Moreover, GS can act as a support and source for heteroatom doping¹⁶. It should be noted that GS is not capable of synthesizing soluble electron shuttles such as phenazines, flavines, quinones, etc., and the only means of EET is through the expressed membrane-bound c-Cyts^{25,26}. Our detailed study showed that diverse SA-M@GS, where M is Ir, Pt, Co, Ru, Cu, or Pd, adopt an identical MN₃ species with same local atomic coordination implanted on the surface of GS via reduction of metal precursors as revealed by comprehensive analyses of X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS), and imaging by high-angle annular dark-field scanning transmission electron microscope (HAADF-STEM). Bacterial cells served as a support for single atoms and offered more exposed active sites for electrochemical reactions. Our biological approach could produce SA-M catalysts with metal loadings as high as ~1 wt. %. The electrocatalytic efficiencies of various SA-M@GS were evaluated directly for both HER and OER, upon drying without further heat treatments such as pyrolysis or hydrothermal approaches, with

the SA-Ir@GS containing ~1wt. % Ir demonstrated outstanding performance as a bifunctional catalyst. Moreover, the SA-Ir@GS also demonstrated good overall water splitting performance in 1M KOH, operating with reasonable stability as both cathode and anode. Further, the unique structure, composition, low coordination, and exposed active sites of SA-M@GS showed enhanced activity and durability when benchmarked with commercial 10% Pt/C and IrO₂ electrocatalyst under the same testing conditions.

Figure 1. Schematic showing **a**, *G. sulfurreducens* cells with expressed heme-containing c-type cytochromes (c_Cyts) on the outer membrane of the cells, which are responsible for the extracellular electron transfer to the acceptor moieties. **b**, mechanism for the reduction of metal ions (electron acceptors) on the surface of *G. sulfurreducens* cell membrane. The metal cations are reduced into individual metal (M) atoms by metabolically generated electrons from the oxidation of acetate (electron donor) inside the cell that are carried by a series of multiheme-containing outer-membrane c-Cyts (OM c-Cyts) to the surface leading to the reduction of metal ions and formation of isolated single-atom metals (SA-Ms) on the surface of *G. sulfurreducens* cells. M = Ir, Ru, Pt, Co, Cu or Pd.

Results and discussion

Characterization of SA-M@GS

Our approach to achieve SA-M supported on GS proceeds in two steps (Fig. 1). First, we demonstrated that GS could form single-atom Fe by growing an active culture of GS grown using acetate as electron donor and fumarate as electron acceptor (i.e., SA-Fe@GS). The GS cells were killed by ethanol, dried overnight in vacuum dryer, and SA-Fe@GS was then subjected to various characterizations to understand its physiochemical properties. The main source of Fe in SA-Fe@GS was from cytochromes expressed by GS. Scanning electron microscopy (SEM) images of

SA-Fe@GS revealed a crumpled morphology with many grooves on the surface (Fig. 2a), creating a high area surface to facilitate the reactions.

Figure 2. **a**, Scanning electron microscope (SEM) image, inset showing transmission electron microscopy (TEM) image of SA-Fe@GS. **b**, High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) image of SA-Fe@GS. Individual Fe atoms are emphasized by red circles. **c**, Fourier transform spectra of extended X-ray absorption fine structure (FT-EXAFS) at the Fe K-edge of SA-Fe@GS and Fe foil. **d**, HAADF image and scanning transmission electron microscopy-energy dispersive X-ray spectrometer (STEM-EDX) elemental mapping depicting the distribution of C (orange), N (cyan), and Fe (red). **e**, energy dispersive X-ray spectrometer (EDX) spectra showing the elemental composition of the SA-Fe@GS.

The transmission electron microscopy (TEM) image (inset in Fig. 2a) and high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) image (Fig. 2b) demonstrate that there were no observable Fe clusters/nanoparticles on GS surface. Aberration corrected HAADF-STEM images revealed individual Fe atoms highlighted by red circles on the

surface of GS (Fig. 2b). This supports the presence of atomically dispersed Fe species from the expression of heme-containing cytochromes by GS²⁷. We hypothesized that these Fe species will subsequently serve as growth sites for the formation of single atoms of desired chemical identity. Hence, we estimated the content of Fe on the surface of SA-Fe@GS catalyst to be 1.3 wt.%, as confirmed by energy dispersive X-ray (EDX) analysis (Fig. 2e). The Fe content determined herein will serve as a basis for the metal loading to achieve single atom dispersion in the second step of the synthesis process. The X-ray diffraction (XRD) pattern of SA-Fe@GS exhibited only two broad peaks at 9° and 19.4°, and no crystalline peaks corresponding to Fe, Fe₂O₃ and Fe₃O₄ were observed (Supplementary Fig. 1), further confirming the exclusion of clusters/nanoparticles of Fe. Additionally, the STEM-EDX elemental mapping of SA-Fe@GS confirmed the well dispersed Fe on the surface of GS (Fig. 2d). To investigate the local structure of SA-Fe@GS, X-ray absorption fine structure (EXAFS) measurements were performed (Supplementary Fig. 2). The absorption edge of X-ray absorption near-edge structure (XANES) spectroscopy of SA-Fe@GS was situated between those of Fe foil and Fe₂O₃ reference (Supplementary Fig. 2a), suggesting that the single-atom Fe carries a positive charge (+3) (Supplementary Fig. 2b). FT-EXAFS of SA-Fe@GS only presents a distinct peak for Fe–N coordination at 1.46 Å, and absence of Fe–Fe scattering signal at ~2.17 Å, which clearly confirms that Fe atoms are atomically dispersed and coordinated to N (Fig. 2c). Furthermore, EXAFS fitting was performed to determine the structural and coordination information of Fe atoms. The coordination structure around Fe single atoms in SA-Fe@GS was evaluated by fitting the experimental $\chi(r)$ versus r data from 1-2.5 Å assuming Fe–N, Fe–S and Fe–C co-ordination shells. Fe exhibits a first shell Fe–N at 2.01 Å with coordination number 4, while Fe–S interaction is located at 2.21 Å with coordination number 1, and Fe–C at 2.45 Å with coordination number 3 (Supplementary Fig. 3a,c). The R factor of the fitting was 0.003, which suggests it is a good fit.

After confirming the formation of single-atom Fe on GS, different metal cations (Mⁿ⁺, M=Ir, Ru, Pt, Co, Cu or Pd) were introduced separately as electron acceptors into the growth media containing acetate (electron donor) but no fumarate (Fig. 1), forming a series of SA-M@GS. Based on the Fe content of GS cells, we added 1 wt.% of the desired metal precursors assuming Fe species are the only center for the metal reduction and coordination. The addition of 1 wt. % of metal precursors exclusively resulted in the formation of atomic dispersion while higher wt.% of metal

precursors formed clusters/metal nanoparticles on the surface of the GS cells (data not shown). SEM and scanning transmission electron microscopy (STEM) images demonstrate that there are no visible Ir particles in the prepared SA-Ir@GS (Supplementary Fig. 4). The images taken by aberration-corrected HAADF-STEM revealed the presence of high density of individual Ir atoms (Fig. 3a), which were clearly visible from the significant difference in Z contrasts of the image for Ir and GS. The content of Ir was quantified to be 0.18 mg in 20 mg of SA-Ir@GS (i.e., ~1 wt. %) as confirmed by ICP-MS. To confirm that metal cations are reduced into individual metal (M) atoms by metabolically generated electrons from the oxidation of acetate (electron donor) inside the cell that are carried by OM c-Cyts to the surface leading to the reduction of metal cations, we conducted a control experiment by excluding acetate from the media. Ir cations were chosen for this control experiment since it is a heavier atom, thus making it easy to visualize under STEM to see if there are any secondary processes such as adsorption. In the absence of acetate, bright dots of Ir atoms were absent on the GS surface as confirmed by HAADF-STEM images (Supplementary Fig. 5). This observation infers that metabolically generated electrons from the oxidation of acetate were involved in the reduction of Ir⁺³.

The STEM–EDX elemental mapping of SA-Ir@GS revealed uniform distribution of the elements C, N, Fe, and Ir over the entire GS cell (Supplementary Fig. 6). The regional EDX analysis on SA-Ir@GS illustrated that isolated Ir atoms are present on Fe-entity rather than other components of GS (Supplementary Fig. 6), which suggests that coordination and reduction occurred at the iron center.

Figure 3. **a**, High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) image of SA-Ir@GS. Single Ir atoms are highlighted by orange circles. **b**, Fourier transformation spectra of extended X-ray absorption fine structure function (FT-EXAFS) at the Ir L₃-edge of SA-Ir@GS and IrO₂. **c**, The X-ray photoelectron spectroscopy (XPS) patterns of Ir 4f for SA-Ir@GS. **d**, X-ray diffraction (XRD) pattern for SA-Ir@GS. HAADF-STEM images of **e**, SA-Pt@GS, **f**, SA-Co@GS and Co metal, and **g**, SA-Ru@GS. Single Pt, Co, and Ru atoms are highlighted by red, yellow, and green circles, respectively. FT-EXAFS spectra at the **h**, Pt L₃-edge of SA-Pt@GS and Pt foil, **i**, Co K-edge of SA-Co@GS and Co metal, and **j**, Ru K-edge of SA-Ru@GS and Ru metal.

Additional confirmation of atomic dispersion was demonstrated by performing EXAFS (Fig. 3b) and XANES measurements (Supplementary Fig. 7a) for SA-Ir@GS. The oxidation state

of Ir was calculated by the intensity of white line at the Ir L₃-edge in XANES spectra. The Ir XANES results show that the white line intensity of SA-Ir@GS (Supplementary Fig. 7a) is close to that of Ir foil reported in the literature²⁸, indicating that the average oxidation state of Ir is mostly zero. The FT-EXAFS for both SA-Ir@GS and commercial IrO₂ showed a single prominent peak located at 1.8 Å (Ir-N) and 1.5 Å (Ir-O) respectively (Fig. 3b), while Ir foil has been reported to have a major peak at 2.6 Å corresponding to the Ir-Ir bond²⁸. The lack of a peak at 2.6 Å (Ir-Ir bond) in the SA-Ir@GS spectrum supports the atomic dispersion of Ir.

The SA-Ir@GS was further analyzed by X-ray photoelectron spectroscopy (XPS) to obtain information on surface composition and valence state (Fig. 3c). The fitting of the XPS spectra of Ir 4f revealed the existence of reduced Ir (0) along with IrO₂ and IrO₃. The over oxidation of the Ir was observed due to the extended storage of the samples before XPS analysis. The XRD pattern of SA-Ir@GS sample was very similar to that of SA-Fe@GS, displaying only two peaks at 9° and 19.4°, which are typical of amorphous carbon (Fig. 3d). No other crystalline metal phases were observed in the XRD of SA-Ir@GS, further suggesting the lack of Ir nanoclusters/nanoparticles with Ir-Ir stacking on GS surface, consistent with the STEM analysis (Fig. 3a). All the above characterizations lead to an inference that atomically dispersed Ir was successfully synthesized over the surface of GS cells.

X-ray Absorption Spectroscopy (XAS) studies were performed on SA-Ir@GS to probe the local coordination structure around the single metal Ir on the surface of GS (Fig. 4). Subsequently, ab-initio studies were performed to check the stability of the structure. The EXAFS data was fitted to get coordination information and atomic configuration around Ir atom. The coordination structure around Ir single atoms in SA-Ir@GS was evaluated by fitting the experimental $\chi(r)$ versus r data from 1-2.5 Å assuming Ir-N, Ir-Fe and Ir-C co-ordination shells. The Ir single atoms on GS form IrN₃ moiety and are linked to Fe of the heme-containing cytochromes. Ir exhibits a first shell Ir-N at 2.11 Å with coordination number 3, while Ir-Fe interaction is located at 2.31 Å with coordination number 1, and Ir-C at 2.54 Å with coordination number 7 (Fig. 4c). The R factor of the fitting was 0.006, which suggests it is a good fit. The experimental $\chi(r)$ versus r plot of the SA-Ir@GS along with best fit theoretical plot carried out as mentioned above is shown in Fig. 4a.

Figure 4. **a**, X-ray absorption fine structure (EXAFS) fitting of SA-Ir@GS catalyst from 1- 2.5 Å assuming Ir-N, Ir-Fe and Ir-C co-ordination shells. **b**, density functional theory (DFT)-optimized structure of the SA-Ir@GS catalyst with three nitrogen linkage at the Ir center. Atom colors: C (grey), N (royal blue), Fe (purple blue), Ir (cadet blue), H (white), S (yellow). **c**, Table showing the fitted parameters where R is distance between absorber and backscatter atoms, N is coordination number, and σ^2 is Debye-Waller factor.

Most notably, the synthetic approach reported here can be applied to several other metals, including Pt, Co, Cu, Ru, and Pd, which are challenging to obtain via conventional methods such as pyrolysis approach. This was demonstrated by using metal precursors of Pt, Co, Ru, Cu, and Pd to synthesize SA-Pt@GS, SA-Co@GS, SA-Ru@GS, SA-Cu@GS, and SA-Pd@GS, respectively. A noticeable change in the color of the solutions was observed depending on the identity of the metal reduced on the surface of GS (Supplementary Fig. 8). The strong contrast of individual atoms of Pt (~1 wt. %, SA-Pt@GS), Co (~0.4 wt. %, SA-Co@GS), Ru (1 wt. %, SA-Ru@GS), Pd (1 wt. %, SA-Pd@GS), and Cu (1 wt. %, SA-Cu@GS) were finely dispersed on GS surface without visible particles (Fig. 3e-g, Supplementary Fig. 9). We achieved only 0.4 wt.% of Co reduction due to the extremely low reduction potential ($\text{Co}^{2+}(\text{aq}) + 2\text{e}^- \rightarrow \text{Co}(\text{s})$, -0.28 vs. SHE) for the formation of Co metal. FT-EXAFS analysis of all these specimens also revealed the existence of single atoms (Fig. 3h-j), and the adsorption edge of SA-Ms was higher than the related metal foil, suggesting that metal atoms in SA-M@GS was carrying a positive charge (Supplementary Fig. 7).

The XRD spectra of all these samples (Supplementary Fig. 10) were consistent with SA-Ir@GS showing only two peaks at 9° and 19.4°, confirming the lack of metal agglomeration in Pt, Co, Ru, Pd, and Cu containing samples. Similar to SA-Ir@GS, oxidized valence states along with metallic state were also observed in the XPS analysis of SA-Pt@GS, SA-Ru@GS, SA-Cu@GS, and SA-Pd@GS (Supplementary Fig. 11). Based on EXAFS data and DFT stability studies, we derived the optimized geometries of SA-Pt@GS, SA-Co@GS, and SA-Ru@GS (Supplementary Fig. 12) where The SA-M on GS forms MN₃ (where M= Pt, Co, Ru, Cu, and Pd) moiety and are linked to Fe of the heme-containing cytochromes.

Electrocatalytic performance of the SA-M@GS towards OER and HER

After thoroughly characterizing the SA-M@GS, the electrocatalytic OER and HER properties of SA-M@GS were evaluated using a standard three-electrode cell at 30°C in N₂-saturated 1.0 M KOH solution. As a reference point, we also performed HER and OER measurements using commercial 10 % Pt-coated carbon (Pt/C) and IrO₂ under the same conditions. The linear-sweep voltammetry (LSV) curve of SA-Ir@GS recorded at 5 mV s⁻¹ demonstrated an early onset overpotential of 283 mV for generating high HER current density (10 mA cm⁻²), and had a similar LSV curve to commercial Pt/C, which requires 257 mV to deliver 10 mA cm⁻² (Fig. 5a). The overpotentials (η), presented in decreasing order, to reach HER current density of 10 mA cm⁻² were as follows: SA-Ru@GS < SA-Fe@GS < SA-Pt@GS < SA-Co@GS < SA-Ir@GS < 10 % Pt/C, indicating that SA-Ir@GS had the best HER performance compared to the other SA-M@GS. The Tafel slopes (Fig. 5b) resulting from the LSV curves for HER (Fig. 5a), showed that SA-Ir@GS had the lowest Tafel slope (88.4 mV dec⁻¹) when compared to SA-Pt@GS (106 mV dec⁻¹), SA-Ru@GS (114 mV dec⁻¹), SA-Co@GS (126 mV dec⁻¹), and SA-Fe@GS (110 mV dec⁻¹). Low Tafel slopes are an indication of high performance HER electrocatalyst activity. The Tafel slope is often utilized to indicate the electrochemical HER mechanism. The Tafel slope should be 120, 40, or 30 mV dec⁻¹ if Volmer (or discharge), Heyrovsky (or electrochemical desorption), or Tafel (or recombination) reaction is the rate-limiting step of the HER²⁹. The SA-Ir@GS electrocatalyst, in particular demonstrated a lower Tafel slope than 10% Pt/C (128 mV dec⁻¹), indicating favorable HER kinetics under alkaline conditions via a Volmer (40 mV dec⁻¹; H₂O + e⁻ → H* + OH⁻) – Heyrovsky (120 mV dec⁻¹; H₂O + H* + e⁻ → H₂ + OH⁻) pathway.

Similarly, the LSV curves for OER demonstrated superior electrocatalytic activity with SA-Ir@GS (Fig. 5c). The overpotentials, presented in decreasing order, to deliver current density of 10 mA cm^{-2} were as follows: SA-Fe@GS (480 mV) < SA-Pt@GS (470 mV) < SA-Ru@GS (438 mV) < SA-Co@GS (435 mV) < IrO₂ (420 mV) < SA-Ir@GS (418 mV). Also, SA-Ir@GS showed the lowest Tafel slope of 57.2 mV dec^{-1} , compared to SA-Pt@GS (63.5 mV dec^{-1}), SA-Co@GS (63.6 mV dec^{-1}), SA-Ru@GS (64.4 mV dec^{-1}), IrO₂ (75.9 mV dec^{-1}), and SA-Fe@GS ($134.8 \text{ mV dec}^{-1}$) (Fig. 5d), indicating that SA-Ir@GS has excellent OER kinetics possibly due to high exchange current density. The exchange current density is a key parameter of the interfacial reaction kinetics³⁰. The exchange current density for SA-Ir@GS (0.29 mA cm^{-2}) was the highest compared to the other SA-Ms: SA-Pt@GS ($2.4 \times 10^{-4} \text{ mA cm}^{-2}$), SA-Co@GS (0.006 mA cm^{-2}), SA-Ru@GS (0.045 mA cm^{-2}), and SA-Fe@GS ($8 \times 10^{-7} \text{ mA cm}^{-2}$). Notably, SA-Ir@GS demonstrated similar OER kinetics as commercial IrO₂ (0.32 mA cm^{-2}).

Figure 5. **a, c** Linear sweep voltammetry (LSV) of SA-Ir@GS, SA-Ru@GS, SA-Pt@GS, SA-Co@GS, SA-Fe@GS, commercial 10% Pt/C and IrO₂ electrodes towards hydrogen evolution reaction (HER) and oxygen evolution reaction (OER). **b, d**, The corresponding Tafel plots obtained from the LSV curves for HER(a) and OER (c), respectively. **e**, The total overpotential of the appropriate working electrodes obtained at 10 mA cm^{-2} . **f**, The LSV curve of overall water splitting by the SA-Ir@GS electrocatalyst. The HER and OER polarization curves (j - V) were recorded at 5 mV s^{-1} at a rotating speed of 1600 rpm.

SA-Ir@GS had a lower total overpotential (701 mV combining HER and OER) than the other SA-Ms (Fig. 5e), suggesting that it can be applied as an effective bifunctional catalyst for water splitting. Therefore, we loaded SA-Ir@GS catalyst on graphitic sheets (1 cm×1 cm) as anode and cathode in 1.0 M KOH and a current density of 10 mA cm⁻² could be attained with a potential of 1.65 V (Fig. 5f). Further, the SA-Ir@GS catalyst demonstrated stable long-term (5 h) overall water splitting via electrolysis at a constant voltage of 1.65 V (Supplementary Fig. 13). According to Fig. 5e, using 10 % Pt/C for HER and IrO₂ for OER may result in a total overpotential of 677 mV, which is lower than SA-Ir@GS (701 mV). However, this requires the fabrication of two different electrocatalysts and the activity of IrO₂ significantly degraded over time (Fig. 5c).

Theoretical investigations on OER and HER activity

The unique configuration of SA-Ir@GS detected by XANES and XPS would contribute to a discrete electronic structure which is different from bulk metallic Ir and Ir oxides, which inevitably influence the adsorption of reactants and intermediates in OER and HER eventually enhancing their electrocatalytic performance as discussed below. DFT calculations were performed in order to gain more insights on the energetics of OER and HER. The performance of OER and HER was studied in two different model systems. One is isolated Fe(III)-porphyrin molecule, and another is SA-Ms (where M= Pt, Co, Ru, Ir) loaded on Fe(III)-porphyrin molecule. Since HER and OER occur in the presence of the protein environment, the SA-Ms are bound with three ammonia (NH₃) neutral ligands to mimic the protein environment. It can be clearly seen from the optimized geometries that the SA-Ms are adsorbed on the porphyrin ring (Fig. 4 and Supplementary Fig. 3 & 12). As these complexes contain two open-shell metals, we have fully explored the different multiplicities in each case and considered the energetically most stable electronic configurations (details provided in Supplementary Information). The DFT calculations for both OER and HER using the model systems are presented below.

Figure 6. **a**, Proposed oxygen evolution reaction (OER) mechanism with intermediates using the optimized geometry of the isolated Fe(III)-porphyrin molecule (i.e., SA-Fe@GS). Atom colors: C (grey), N (royal blue), Fe (purple blue), O (red), H (white), S (yellow). **b**, Free energy diagram for OER over SA-Fe@GS, SA-Ir@GS, SA-Ru@GS, SA-Co@GS, and SA-Pt@GS at potential $U = 0$ V and $U = 1.23$ V. **c**, Calculated adsorption energies of H_2O on the surface of SA-Ir@GS, SA-Ru@GS, SA-Co@GS, SA-Pt@GS and SA-Fe@GS. **d**, Calculated energy diagram for H_2O dissociation on SA-Ms for the Volmer step in HER. Atomic models with H_2O adsorption, transition state (TS) and final step with OH and H adsorption demonstrated in the insets.

The DFT calculations on SA-M@GS indicated that the catalytic reaction path and activity of OER is strongly dependent on the identity of the metal in SA-M@GS. The OER energy diagram was calculated using DFT at potentials of 0 V and 1.23 V for the various SA-M@GS by following the suggested OER pathways as presented in Fig. 6a,b. The reaction limiting step serves as a descriptor to evaluate the catalytic activity of the catalyst, which can be obtained from the Gibbs free energy of the rate limiting step. At $U = 0$ V, the rate limiting step for SA-Fe@GS was the formation of OH^* with a limiting barrier as large as 2.33 eV. For SA-Ir@GS, SA-Pt@GS, and SA-Co@GS, the rate limiting step was the formation of O^* from OH^* with limiting barriers of 1.88 eV, 2.03 eV, and 2.95 eV, respectively. In the case of SA-Ru@GS, the oxidation of O^* to

OOH* was the rate limiting step. The O₂ desorption from the active sites is the last step in OER, and its energy barrier was largest for SA-Fe@GS (3.55 eV), followed by SA-Co@GS (2.66 eV), SA-Ru@GS (1.63 eV), SA-Pt@GS (1.52 eV), and SA-Ir@GS (1.39 eV).

At U=1.23 eV, the formation of OH* (first step) on SA-Fe@GS was still limiting with a barrier of 1.1 eV, whereas the first step on SA-Ir@GS, SA-Pt@GS, SA-Ru@GS, and SA-Co@GS had a negative Gibbs free energy and occurs spontaneously. Additionally, the energies of the intermediates (O*, OOH*, and O₂*) on SA-Fe@GS were more negative when compared to the other SA-M@GS. This infers that the chemical interaction of SA-Fe@GS with the intermediates was very strong and possess high energy barriers for the evolution of oxygen. Among the SA-M@GS electrocatalysts reported here, DFT calculations showed that SA-Ir@GS has small energy barriers between the reactants and products. These results support its superior OER electrocatalytic activity compared to the other SA-M@GS electrocatalysts (Fig. 5c,d).

As discussed in previous studies, various descriptors of HER activity are proposed to understand the efficiency of electrocatalysts. In the current study, we used the water dissociation process as the descriptor for estimating the efficiency of SA-M@GS electrocatalysts^{3,13}. The water dissociation reaction on metal centers in Fe(III)-porphyrin and SA-Ms (where M= Co, Ru, Ir) loaded on Fe(III)-porphyrin molecule was modeled to estimate the energy barrier for HER. Model predictions revealed that water has the largest adsorption energy on SA-Ir@GS (0.92 eV) and lowest on SA-Fe@GS (0.08 eV) (Fig. 6c). Furthermore, less energy is required to dissociate water molecule when water is adsorbed on SA-Ir@GS (Fig. 6d). The DFT calculations further indicated that the water dissociation process in the case of SA-Ir@GS is exothermic in nature while that of pristine Fe(III)-porphyrin (i.e., SA-Fe@GS) is endothermic. Based on the DFT results, we can conclude that water prefers to dissociate on Ir atoms more than Fe(III) atoms, and SA-Ir@GS is a better HER electrocatalyst than SA-Fe@GS and other SA-M@GS, further supporting its superior HER electrocatalytic activity based on LSV (Fig. 5a,b).

Conclusions

In summary, single atom metal (Ir, Pt, Pd, Ru, Cu, Co) supported GS were obtained by a two-step approach at room temperature. We identified that the SA-Ms (M= Ir, Ru, Pt, Co, Cu and Pd) adopted an MN₃ configuration with M linked to Fe on cytochromes using a combination of

EXAFS, XANES, STEM imaging, and DFT analysis. SA-M@GS demonstrated bifunctional electrocatalytic activities towards HER and OER, with SA-Ir@GS showing superior OER and HER electrocatalytic activity compared to the other SA-M@GS electrocatalysts. SA-Ir@GS demonstrated an overpotentials of 283 mV and 418 mV to achieve current densities of 10 mA cm^{-2} in 1M KOH for HER and OER, respectively. Moreover, SA-Ir@GS was integrated to perform overall water splitting at an applied potential of 1.65 V Vs. RHE to achieve a current density of 10 mA cm^{-2} . The electrocatalytic performance of SA-M@GS may be further improved by increasing the cytochrome expression in GS by altering the growth conditions and thus the density of single atom sites. Our study outlines an efficient biological approach to achieve a MN_3 type SA-M catalyst supported on GS that can be used for various electrocatalytic applications including OER, HER, ORR, CO_2 and N_2 reductions. Also, our work can inspire the use of other efficient electroactive bacteria for synthesizing high-performing and low-cost electrocatalysts for various energy-related applications. Taking into consideration the cost of raw chemicals only, the estimated cost to synthesize 1g of SA-Ir@GS catalyst is 3.81 euros compared to 13.6 euros for 1g of 1 wt.% Ir/C (38330.06; Alfa Aesar) using conventional methods such as impregnation followed by reduction (Supplementary Fig. 14).

Methods

Bacterial strain and culture conditions

Geobacter. sulfurreducens (GS) strain PCA (ATCC 51573) was grown in batch cultures. The growth medium solution contained the following salts (per liter): 1.5 g of NH_4Cl , 0.6 g of Na_2HPO_4 , 0.1 g of KCl , 0.82 g of sodium acetate, 2.5 g of NaHCO_3 , 8.0 g of sodium fumarate, 10 ml of each a vitamin solution and trace element solution. Sodium acetate (10 mM) was provided as the sole carbon and electron source, and fumarate (50 mM) was provided as the electron acceptor. The growth medium was flushed with $\text{N}_2\text{-CO}_2$ (80:20) for at least 40 min to remove oxygen and achieve anaerobic conditions and to maintain the pH at 7. Inoculation was performed in an anaerobic glove box, and the culture bottle was kept in an incubator shaker (130 rpm, 30 °C) for 3 days. Later, the culture suspension was centrifuged at 7000 rpm for 5 min, and the resulting concentrated cell pellet was washed with sterile medium solution (lacking fumarate) three times before being inoculated for the synthesis of single-atom metal (SA-M) catalysts with tunable chemical identity.

Synthesis of SA-M catalysts by GS (SA-M@GS)

For the synthesis of single-atom platinum (Pt), iridium (Ir), ruthenium (Ru), cobalt (Co), palladium (Pd), or copper (Cu) on the outer membrane of GS cells, 16 mg of K_2PtCl_6 , 12 mg of $\text{IrCl}_3 \cdot x\text{H}_2\text{O}$, 16 mg of $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$, 13.2 mg of CoCl_2 , 15 mg of $\text{PdNO}_3 \cdot 2\text{H}_2\text{O}$, or 10 mg of CuCl was added into 500 mL of the above anaerobic growth medium solution (without fumarate) in a rubber septated serum vial. Different amounts of the metal precursors were used to maintain a similar metal loading (3 mg) for the different metals. The concentrated cell pellet was inoculated into the serum vials and incubated anaerobically at 30 °C in the dark for 5 h. The resulting solution was centrifuged at 7000 rpm for 5 minutes, then washed four times with Milli-Q water and 70% ethanol to remove media components and kill GS cells, and then dried in vacuum dryer at room temperature overnight. The dried samples were used for further characterizations.

As for SA-Fe@GS, it was prepared by incubating GS with acetate as electron donor and fumarate as electron acceptor. The Fe is expressed by GS in the form of cytochromes without inclusion of additional iron source in the media. The resulting cell suspension was centrifuged at

7000 rpm for 5 minutes, then washed four times with Milli-Q water and 70% ethanol to remove media components and then dried in vacuum dryer at room temperature overnight.

Material characterizations

X-ray diffraction (XRD) patterns for all the samples were collected using a Bruker D8 Advanced A25 diffractometer (Bruker, UK) with Cu K radiation, which is operated at 40 kV and 40 mA. The data sets were acquired in the step scan mode in the 2θ range of $20\text{--}80^\circ$, using a step interval of 0.05° and a counting time of $10^\circ/\text{min}$.

The morphology of all samples was characterized by transmission electron microscopy (TEM), using a Titan 80-300 ST microscope (Thermo-Fisher Scientific, USA) equipped with a probe-corrector to perform the Cs-corrected scanning transmission electron microscopy (STEM) analysis. It was also equipped with an energy dispersive X-ray (EDX) spectrometer and a post-column energy filter for the determination of the elemental composition of samples. The specimens for STEM analysis were prepared by mixing the powdered samples in a pure ethanol solution. A small amount ($<5\ \mu\text{L}$) of resultant solution from each sample was then placed on Holey-carbon-coated copper grids. These grids were then air-dried at ambient conditions for several hours before performing the analysis. STEM imaging of the samples was completed by operating the microscope at an accelerating voltage of 300 kV, and the images were recorded using a high-angle annular dark-field (HAADF) detector. The scanning electron microscope (SEM) images were taken with a Zeiss Merlin SEM (ZEISS, Germany).

High-resolution X-ray photoelectron spectroscopy (XPS) was used to study the chemical composition and oxidation state of the catalyst surfaces. XPS was carried out using a Kratos Axis Ultra DLD spectrometer (Kratos, UK) equipped with a monochromatic Al $K\alpha$ X-ray source ($h\nu = 1486.6\ \text{eV}$) operating at 150 W, a multichannel plate, and a delay line detector under a vacuum of 1×10^{-9} mbar. The survey and high-resolution spectra were recorded at fixed analyzer pass energies of 160 and 20 eV, respectively, and quantified using empirically derived relative sensitivity factors provided by Kratos analytical. Samples were mounted in floating mode to avoid differential charging. Charge neutralization was required for all samples. Binding energies were referenced to the C 1s peak set at 284.8 eV. The data were analyzed with CasaXPS software (Casa Software Ltd, UK).

X-ray Absorption Spectroscopy (XAS) measurements of SA-M@GS samples have been carried out in fluorescence mode using the scanning X-ray absorption fine structure (EXAFS) beamline (BL-09) at the INDUS-2 Synchrotron Source (2.5 GeV, 200 mA) at the Raja Ramanna Centre for Advanced Technology (RRCAT), Indore, India^{31,32}. The beamline uses a double crystal monochromator (DCM), which works in the photon energy range of 4-25 KeV with a resolution of 10^4 at 10 KeV. A 1.5 m horizontal pre-mirror with meridional cylindrical curvature was used prior to the DCM for collimation of the beam and higher harmonic rejection. The second crystal of the DCM is a sagittal cylinder with radius of curvature in the range 1.28-12.91 meters which provides horizontal focusing to the beam while another Rh/Pt coated bendable post mirror facing down is used for vertical focusing of the beam at the sample position. For measurements in the fluorescence mode, the sample is placed at 45° to the incident X-ray beam and the fluorescence signal (I_f) is detected using a Si drift detector placed at 90° to the incident X-ray beam. An ionization chamber detector is used prior to the sample to measure the incident X-ray flux (I_0) and the absorbance of the sample ($\mu = \frac{I_f}{I_0}$) is obtained as a function of energy by scanning the monochromator over the specified energy range.

The EXAFS spectra were extracted from the absorption spectra as described previously^{33,34}. In order to take care of the EXAFS oscillations in the absorption spectra, the energy dependent absorption coefficient $\mu(E)$ was converted to absorption function $\chi(E)$ defined as follows:

$$\chi(E) = \frac{\mu(E) - \mu_0(E)}{\Delta\mu_0(E_0)} \quad (1)$$

Where E_0 is absorption edge energy, $\mu_0(E_0)$ is the bare atom background and $\Delta\mu_0(E_0)$ is the step in the $\mu(E)$ value at the absorption edge. After converting the energy scale to the photoelectron wave number scale (k) as defined by,

$$k = \sqrt{\frac{2m(E - E_0)}{\hbar^2}} \quad (2)$$

the energy dependent absorption coefficient $\chi(E)$ was converted to the wave number dependent absorption coefficient $\chi(k)$, where m is the electron mass and $\hbar$ is Planck's constant. Finally,

$\chi(k)$ is weighted by k^2 to amplify the oscillation at high k and the functions $\chi(k)$ k^2 are Fourier transformed (FT) in r space to generate the $\chi(r)$ versus r (or FT-EXAFS) spectra in terms of the real distances from the center of the absorbing atom. The k range used for FT is 2-10 Å⁻¹. A set of EXAFS data analysis programs available within the IFEFFIT open source software package³⁵ was used for reduction and fitting of the experimental EXAFS data. This includes data reduction and FT to derive the $\chi(r)$ versus r plots from the absorption spectra, generation of the theoretical EXAFS spectra starting from an assumed crystallographic structure and finally fitting of the experimental $\chi(r)$ versus r data with the theoretical ones using the FEFF 6.0 code.

Electrochemical measurements

The oxygen evolution reaction (OER) and hydrogen evolution reaction (HER) activity of SA-M@GS were tested using a rotating disc electrode (RDE). The working electrode was prepared by the following procedure: the SA-M@GS catalyst (~2 mg) was dispersed in 970 µl of 50% ethanol in water mixture and 30 µl of Nafion (as a binder). The dispersed solution was sonicated for 1 h, and 2 µl of the obtained catalyst ink was drop-coated onto a 3 mm glassy carbon disc electrode (GCE; loading concentration ~ 0.049 mg/cm²) and vacuum dried for 1 h. The electrochemical measurement was carried out using electrochemical working station (BioLogic VMP3, France) in 1 M KOH (Sigma Aldrich, semiconductor grade, pellets, 99.99% trace metals basis) at room temperature using a three-electrodes system, in which Pt mesh and Mercury/Mercury oxide reference electrode (Hg/HgO; 1 M KOH) were used as counter and reference electrodes, respectively. Linear sweep voltammetry (LSV) experiments were performed at a scan rate of 5 mV s⁻¹ while maintaining a constant rotational speed of 1600 rpm under nitrogen environment.

Density functional theory calculations

All geometry optimizations were carried out using density functional theory (DFT) based B3LYP functional with LANL2DZ basis set for Ru, Fe, Co, Ir, and Pt atoms and 6-31G(d) basis set for C, H, O, S, and N atoms in the gas phase. The vibrational frequency analysis was carried out on the optimized geometries at the same level of theory to verify that stationary points are real minima and to obtain thermodynamic energy corrections. All calculations were carried out using Gaussian 16 package (Gaussian, Inc., USA)³⁶.

Oxygen evolution reaction

As suggested by previous theoretical and experimental studies^{18,37}, a four-step mechanism is considered for OER as follows:

1. $\text{H}_2\text{O} + * \leftrightarrow * \text{OH} + \text{H}^+ + \text{e}^-$
2. $* \text{OH} \leftrightarrow * \text{O} + \text{H}^+ + \text{e}^-$
3. $* \text{O} + \text{H}_2\text{O} \leftrightarrow * \text{OOH} + \text{H}^+ + \text{e}^-$
4. $* \text{OOH} \leftrightarrow * + \text{O}_2 + \text{H}^+ + \text{e}^-$

Where * denotes the catalytic active center in Fe-Porphyrin (shown in Fig. 6) or M-Fe-Porphyrin model system where M=Ir, Pt, Co, and Ru. Several intermediates such as *OH, *O, and *OOH are observed in the electrocatalysis.

The free energy change for each step was evaluated for the above-mentioned intermediates using the following equations:

$$\Delta G(*\text{OH}) = \Delta G(*\text{OH}) + 1/2\Delta G(\text{H}_2) - \Delta G(*) - \Delta G(\text{H}_2\text{O}) \quad (3)$$

$$\Delta G(*\text{O}) = \Delta G(*\text{O}) + \Delta G(\text{H}_2) - \Delta G(*) - \Delta G(\text{H}_2\text{O}) \quad (4)$$

$$\Delta G(*\text{OOH}) = \Delta G(*\text{OOH}) + 3/2\Delta G(\text{H}_2) - \Delta G(*) - 2\Delta G(\text{H}_2\text{O}) \quad (5)$$

where, $\Delta G(*)$ is the free energy of the isolated catalytic complex, and $\Delta G(*\text{OH})$, $\Delta G(*\text{O})$, and $\Delta G(*\text{OOH})$ are the energy of adsorption of the *O, *OH, and *OOH on Fe/Ir/Pt/Co/Ru catalytic centers, respectively. The $\Delta G(\text{H}_2)$ and $\Delta G(\text{H}_2\text{O})$ are the free energy of H_2O and H_2 molecules in the gas phase.

The free energy diagrams of OER reaction were calculated according to Nørskov et al.³⁸⁻⁴² computational hydrogen electrode (CHE) model, where the chemical potential of a proton/electron ($\text{H}^+ + \text{e}^-$) is equal to half of the chemical potential of a gaseous H_2 .

The free energy change (ΔG) for each reaction step is given by the following equation:

$$\Delta G_{\text{OER}} = \Delta \Delta G + \Delta G_{\text{U}} + \Delta G(\text{pH}) \quad (6)$$

where $\Delta\Delta G$ is the free energy of reactant and products adsorbed on the catalytic surface. ΔG_U is the effect of an external bias which is shifted by $-eU$. Here, e is the transferred charge and U is the applied bias. ΔG_{pH} is the H^+ free energy correction by the concentration dependence of the entropy:

$$\Delta G_{pH} = -k_B T \ln[H^+] = k_B T \ln 10 \times pH \quad (7)$$

where T and k_B are temperature and Boltzmann constant, respectively.

According to the equation 6, the free energy change of the four reaction steps is given by the expression with the adsorption free energies of OER intermediates as follows:

$$\Delta G_1 = \Delta G_{*OH} - eU + k_B T \ln 10 * pH \quad (8)$$

$$\Delta G_2 = \Delta G_{*O} - \Delta G_{*OH} - eU + k_B T \ln 10 * pH \quad (9)$$

$$\Delta G_3 = \Delta G_{*OOH} - \Delta G_{*O} - eU + k_B T \ln 10 * pH \quad (10)$$

$$\Delta G_4 = 4.92 - \Delta G_{*OOH} - eU + k_B T \ln 10 * pH \quad (11)$$

In the above equations, various conditions such as $pH=14$, $T=303$ K, and $U = 0$ V and 1.23 V are considered. In the final step, we used the term 4.92 eV which is the free energy of the water splitting process as it is difficult to calculate the accurate energy of O_2 molecule using the DFT methods.

Hydrogen evolution reaction

The thermodynamic reaction energies and energy barrier of water O-H bond dissociation were systematically studied to gain insights into HER activity of above-mentioned catalysts. To carry out these reactions, Fe-Porphyrin and M-Fe-Porphyrin ($M = Ir, Pt, Co, \text{ and } Ru$) were considered. All geometries of reactants, products and transition states were fully optimized using B3LYP method. Furthermore, the transition states corresponding to O-H bond dissociation was confirmed by intrinsic reaction coordinates (IRC) analysis at the same level of theory.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Table S1. Inductively coupled plasma mass spectrometry (ICP-MS) analyses of single atom metal catalysts.

Catalyst	Weight of catalyst used for analysis (mg)	Amount of metal added (mg)	Amount of metal in the catalyst ICP-MS (mg)	Amount of residual metal after reduction in media ICP-MS (mg)	Percentage of metal reduction (%)
SA-Ir@GS	20	3	0.178	0.34	89
SA-Pt@GS	20	3	0.182	0.27	91
SA-Ru@GS	20	3	0.196	0.06	98
SA-Pd@GS	20	3	0.2	0.005	100
SA-Cu@GS	20	3	0.198	0.02	99
SA-Co@GS	20	3	0.08	1.74	40

Supplementary Fig. 1. X-ray diffraction (XRD) pattern for SA-Fe@GS derived from *G. sulfurreducens*. No crystalline peaks of Fe were observed, further indicating the absence of Fe clusters/nanoparticles.

Supplementary Fig. 2. **a**, X-ray absorption near-edge structure (XANES) of SA-Fe@GS, Fe_2O_3 , and Fe foil at Fe K-edge. **b**, The plot of the pre-edge peak position vs. oxidation state of Fe for extended X-ray absorption fine structure (EXAFS) of SA-Fe@GS, Fe_2O_3 , and Fe foil at Fe K-edge.

Supporting Fig. 3. **a**, X-ray absorption fine structure (EXAFS) fitting of SA-Fe@GS catalyst from 1- 2.5 Å assuming Fe-N, Fe-S and Fe-C co-ordination shells. **b**, density functional theory (DFT)-optimized structure of the SA-Fe@GS catalyst. Atom colors: C (grey), N (royal blue), Fe (purple blue), H (white), S (yellow). **c**, Table showing the fitted parameters where R is the distance between absorber and backscatter atoms, N is coordination number, and σ^2 is Debye-Waller factor.

Supplementary Fig. 4. **a-b**, High magnification scanning electron microscope (SEM) images showing the crumpled morphology of the SA-Ir@GS cells, indicating the absence of nanoparticle formation. **c-d**, High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images of SA-Ir@GS, indicating the presence of atomically dispersed Ir on the surface of *G. sulfurreducens* (GS) dead cells.

Supplementary Fig. 5. **a-c**, High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images of *G. sulfurreducens* (GS) dead cells fed with Ir^{3+} (electron acceptor) in the absence of acetate (electron donor). Adsorption or formation of Ir atoms on surface of cell was not observed like in Supplementary Fig. 4.

Supplementary Fig. 6. High-angle annular dark-field (HAADF) image and scanning transmission electron microscopy-energy dispersive X-ray spectrometer (STEM-EDX) elemental mapping of SA-Ir@GS. EDX mapping indicating that Ir is present at Fe site, confirming the role of Fe-containing (heme) cytochromes in the reduction of Ir ions to metallic Ir.

Supplementary Fig. 7. X-ray absorption near-edge structure (XANES) spectra of **a**, SA-Ir@GS, **b**, SA-Pt@GS, **c**, SA-Co@GS, **d**, SA-Ru@GS, and **e**, SA-Cu@GS with their corresponding reference materials.

Supplementary Fig. 8. Photo images showing the change in solution color after the reduction of metal cations by *G. sulfurreducens*.

Supplementary Fig. 9. Scanning transmission electron microscopy (STEM) images of **a**, SA-Pd@GS and **b**, SA-Cu@GS confirming the atomic dispersion of single atoms.

Supplementary Fig. 10. X-ray diffraction (XRD) spectra of **a**, SA-Pt@GS, **b**, SA-Ru@GS, **c**, SA-Co@GS, **d**, SA-Cu@GS, and **e**, SA-Pd@GS, indicating the absence of crystalline peaks corresponding to clusters/nanoparticles of the respective metals.

Supplementary Fig. 11. High resolution X-ray photoelectron spectroscopy (XPS) spectra of **a**, Pt in SA-Pt@GS, **b**, Ru in SA-Ru@GS, **c**, Pd in SA-Pd@GS, and **d**, Cu in SA-Cu@GS, showing the metallic states of the respective elements along with their oxide forms.

Supplementary Fig. 12. Optimized geometries of **a**, SA-Ru@GS, **b**, SA-Pt@GS, and **c**, SA-Co@GS complexes as determined using B3LYP functional with LANL2DZ basis set for Ru, Pt and Co atoms and 6-31G(d) basis set for C, H, O, S, and N atoms. Atom colors other than the metals: C (grey), N (royal blue), H (white), and S (yellow).

Supplementary Fig. 13. Stability test for SA-Ir@GS catalyst in 1M KOH at 1.648 V vs. RHE.

Supplementary Fig. 14. Cost estimate of raw chemicals used for the synthesis of 1 g of SA-Ir@GS catalyst. The media contains acetate (0.82 g L^{-1}) as carbon source and electron donor. Fumarate was used as the electron acceptor for the generation of the initial culture of *G. sulfurreducens* to be used for the synthesis of SA-Ms. $\text{IrCl}_3 \cdot x\text{H}_2\text{O}$ was used as the precursor for Ir.