

Electronic Supplementary Information

Electroenzymatic tandem catalysis for the conversion of nitrate to ammonia

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EXPERIMENTAL

Enzyme preparation and reagents

The culture of *Caldariomyces fumago* and the extraction and purification of chloroperoxidase (CPO) were carried out according to the method reported by Morris and Hager. CPO activity was 5900 U·mL⁻¹ according to a standard monochlorodimetholone (MCD) assay. The R_z (purity) of CPO was 1.15 ($R_z = A_{398}/A_{278} = 1.40$ is regarded as pure enzyme).

The chemical reagents used in the experiment were sodium nitrite (NaNO₂), sodium nitrate (NaNO₃), sodium hydroxide (NaOH), hydrochloric acid (HCl), dipotassium hydrogen phosphate (K₂HPO₄), potassium dihydrogen phosphate (KH₂PO₄), ethanol (C₂H₅OH) and acetone purchased from Sinophasic Chemical Reagents Co., LTD. ¹⁵N-(NH₄)₂SO₄, ¹⁵N-NaNO₃ were from Sigma-Aldrich Co., LTD. Silver foil (99.9985%, thickness 0.5mm) was from Thermo Fisher Scientific Co., LTD. All the above chemicals were analytically pure.

Synthesis of CPO-IL_{EMB}/3D-Ag biocomposite

Synthesis of 3D-Ag: The purchased silver foil (99.9985%, 0.5mm thick) was cut into 1×1 square pieces, polished with 3000-mesh sandpaper, and washed with ethanol and acetone sequentially for 15 minutes, and then the cleaned silver foil was fixed on the electrode clamp. A 50 Hz square wave pulse centered at 1.85 V (vs. RHE) was applied to the silver foil using 0.2 M NaOH as the electrolyte, followed by continuous oxidation using a three-electrode system. The surface was completely oxidized by repeating the cycle 3000 times. The surface is quickly reduced using a reduction voltage of -0.8V (vs. RHE). Rinse with ultrapure water/ethanol and vacuum dry and prepare.

Synthesis of CPO-IL_{EMB}: Pre-prepared CPO solution (200 U mL⁻¹) was added to 1-ethyl-3-methylimidazolium bromide (IL_{EMB}) and complexed for 25 min. The remaining unsuccessfully bound IL_{EMB} and CPO were removed by centrifugal washing twice to obtain CPO-IL_{EMB}.

Synthesis of CPO-IL_{EMB}/3D-Ag biocomposite: The prepared 3D-Ag was put into 1 mL of CPO-IL_{EMB} and shaken at a constant temperature for 2 hours, so that the enzyme was evenly immobilized on the surface and in the voids of 3D-Ag. The quality of CPO-IL_{EMB} fixation on the 3D-Ag surface was determined by measuring the change in the characteristic peak height of CPO at 398 nm before and after fixation using a ultraviolet-visible (UV-vis) spectrophotometer. Then dried at room temperature to obtain CPO-IL_{EMB}/3D-Ag biocomposite. Calculation formula for CPO-IL_{EMB} fixation quality:

$$\text{Fixation quality} = \frac{\Delta A_{398} \times V \times M}{\varepsilon \times l} mg \quad \textcircled{1}$$

where ΔA_{398} is the change in height of the characteristic peak before and after fixation, V represents the solution volume of 1 mL, M represents the relative molecular mass of CPO of 42000, ε is the molar absorption coefficient of CPO of 91200 L, mol⁻¹, cm⁻¹, and l is the width of the quartz cuvette (0.5 cm).

Instruments used for analysis

The crystal structure and elemental composition of the material were analyzed by X-ray Powder diffractometer (MiNiFlex 600) and X-ray photoelectron spectrometer (Axis UltraDL). The surface structure and morphology of the material were analyzed by Field emission transmission electron microscope (Tecnai G2 F20), Scanning electron microscope (SU8220) and Environmental scanning electron microscope (Quanta 200) T. The color reaction of nitrate, nitrite and ammonia was tested using ultraviolet-visible (UV-vis) spectroscopy (Cary 60). The zeta (ζ) potential of 3D-Ag was carried out by Malvern Zetasizer (Nano ZS90). All electrochemical tests were carried out on CHI Electrochemistry 660e workstation.

Test method for products of NO₃⁻RR

Determination of NH₄⁺-N: The NH₄⁺ contained in the product was determined using the hypochlorite-phenol method. Firstly, the dried (NH₄)₂SO₄ solution was used to prepare different concentrations of standard NH₄⁺-N solution. The peak height at 655 nm was measured by UV-vis spectrophotometer and the standard curve was drawn. The specific steps of the test reaction product were to take 500 μL of the electrolyte, add ultrapure water and dilute it to 5 mL, then add 200 μL of phenol alcohol reagent, 200 μL of sodium ferrocyanide and 500 μL of alkaline and sodium hypochlorite mixed oxidant in turn. After standing for 3 hours, the concentration was determined according to the UV-visible peak curve combined with the standard fitting curve. The faradaic efficiency and yields of NH₃ was calculated as follows:

$$FE = \frac{8 \times F \times c_{\text{NH}_3} \times V}{17 \times Q}$$

$$\text{Yield} = \frac{c_{\text{NH}_3} \times V}{m \times t}$$

Determination of NO₂⁻-N: The NO₂⁻ concentration in the product was determined using the ethylenediamine hydrochloride method. Firstly, the dried NaNO₂ solution was used to prepare different concentrations of standard NO₂⁻-N solution. The peak height at 542 nm was measured by UV-vis

spectrophotometer and the standard curve was drawn. After removing 500 μL of the electrolyte, 4.5 mL of ultrapure water was added, 500 μL of naphthalene ethylenediamine tetraacetic acid solution was added for standing reaction 10-15 minutes, and then 500 μL of p-aminobenzosulfonamide solution was added. After standing for 15 minutes, the concentration was determined according to the UV-visible peak curve combined with the standard fitting curve.

Determination of NO_3^- -N: The NO_3^- concentration in the product was determined using the aminosulfonic acid-UV spectrophotometry. Firstly, the dried NaNO_3 solution was used to prepare different concentrations of standard NO_3^- -N solution. The peak height at 210 nm was measured by UV-vis spectrophotometer and the standard curve was drawn. After removal of 500 μL of electrolyte, 4.5 mL of ultrapure water was added and diluted to 5mL. Then, 1 mL of hydrochloric acid (1 M) and 10 μL of aminosulfonic acid solution (0.8%wt) were successively added to the electrolyte, the concentration was determined according to the UV-visible peak curve combined with the standard fitting curve. Calculation formula for NO_3^- conversion rate:

$$\text{Conversion rate} = \frac{\Delta C}{C_0}$$

Isotope labeling experiment: To determine the source of NH_4^+ -N in the products, isotopic ^{15}N labeling experiments were performed, using 99% isotopic ^{15}N nitrate as standard sample and detecting the reaction products by ^1H NMR spectroscopy. Since the increase of NH_4^+ concentration in the electrolyte will lead to the rise of pH, making the conversion of NH_4^+ to NH_3 , which is not conducive to the NMR test, the PBS electrolyte was adjusted to pH = 3 for the test. When ^{15}N labeled nitrate was used as the substrate, the product NH_4^+ corresponded to the special double peaks of ^{15}N . When normal ^{14}N nitrate was used as the substrate, the product NH_4^+ corresponded to the three peaks of ^{14}N , which indicated that the NH_4^+ in the product was completely derived from the nitrate added in the reaction.

Figures

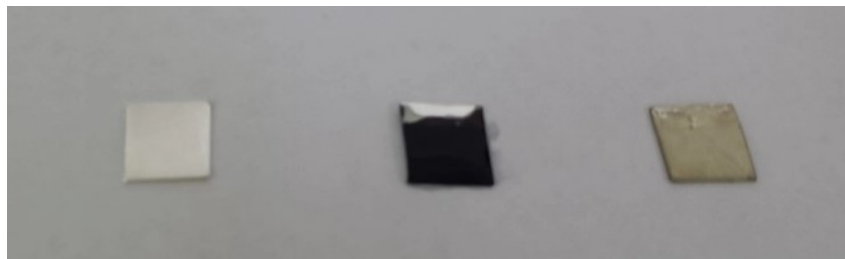


Fig. S1. Actual photo of 3D-Ag preparation process.

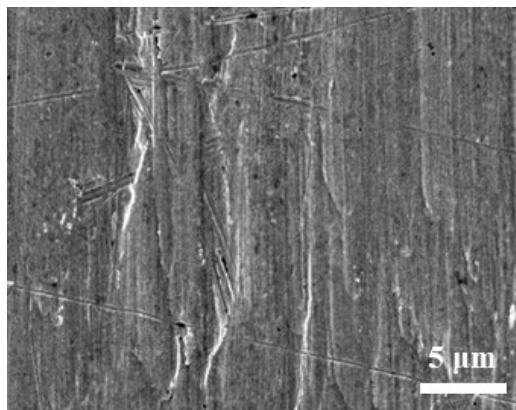


Fig. S2. SEM image of Ag foil.

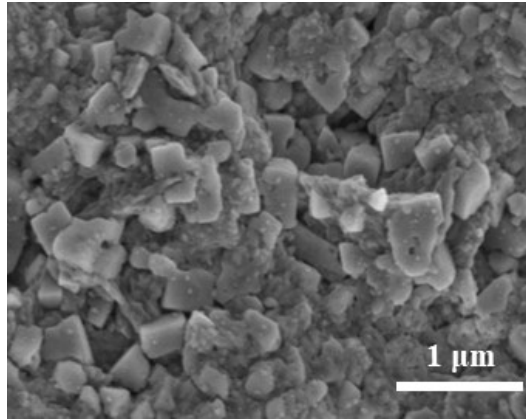


Fig. S3. SEM image of Ag₂O₂.

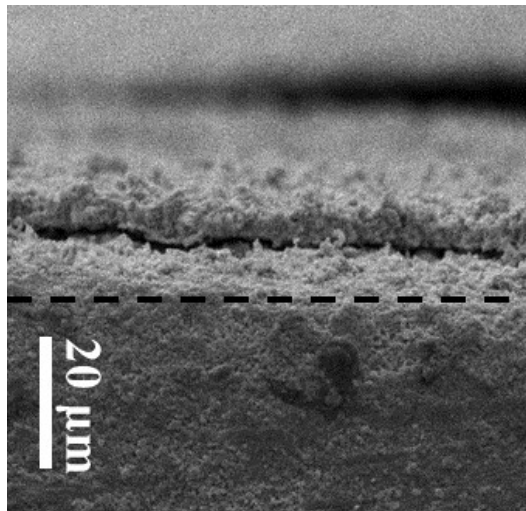


Fig. S4. Cross section SEM image of Ag₂O₂.

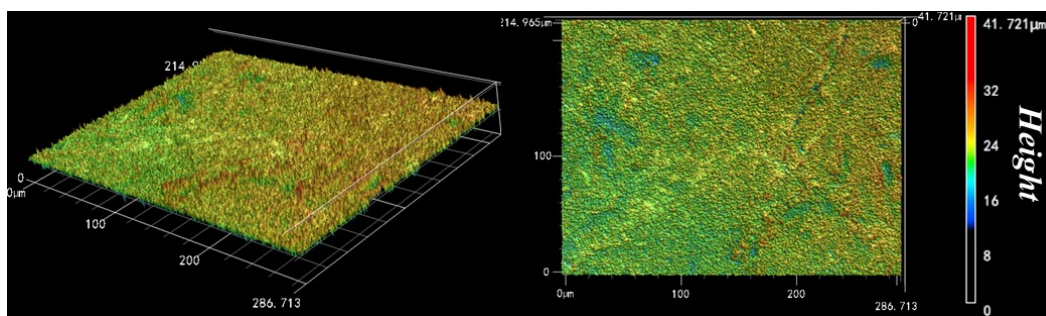


Fig. S5. CLSM pattern of 3D-Ag.

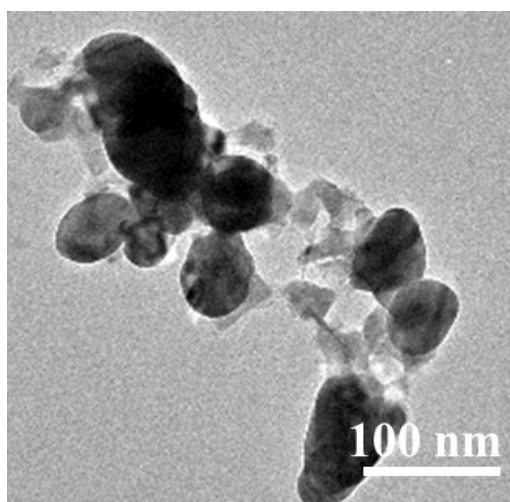


Fig. S6. TEM image of Ag nanoparticles at 3D-Ag.

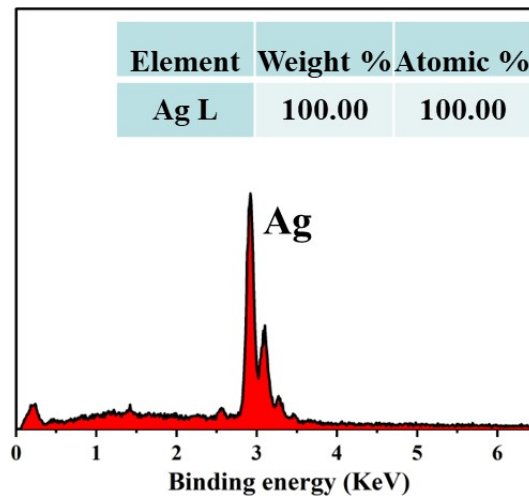


Fig. S7. EDX pattern of 3D-Ag.

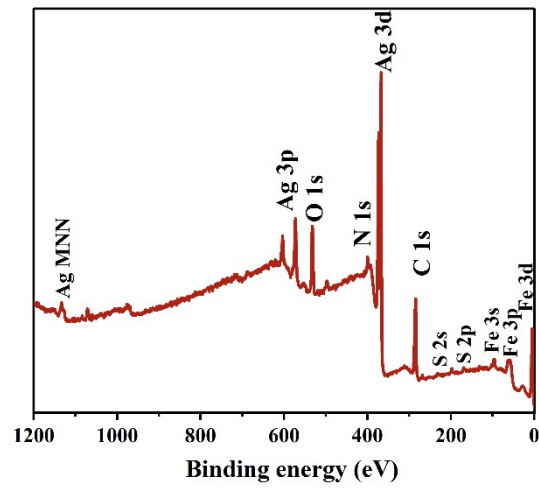


Fig. S8. XPS survey spectrum of CPO-IL_{EMB}/3D-Ag.

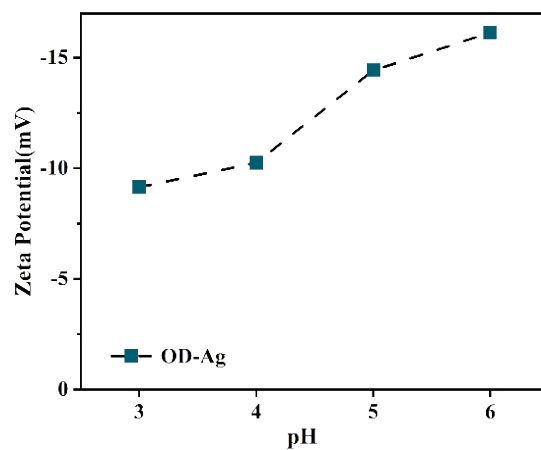


Fig. S9. Zeta potential of 3D-Ag at different pH values.

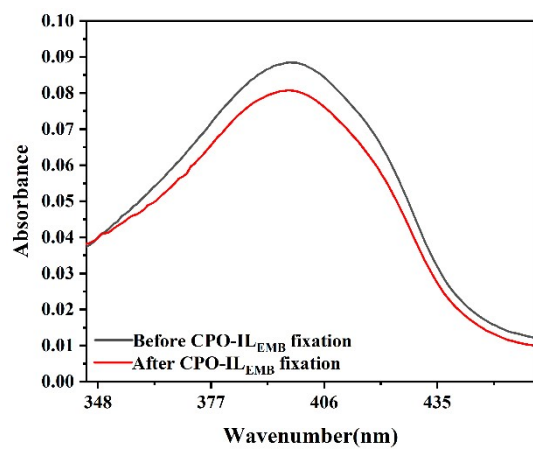


Fig. S10. UV-vis spectra of the change in characteristic peak heights of CPO at 398 nm before and after immobilization.

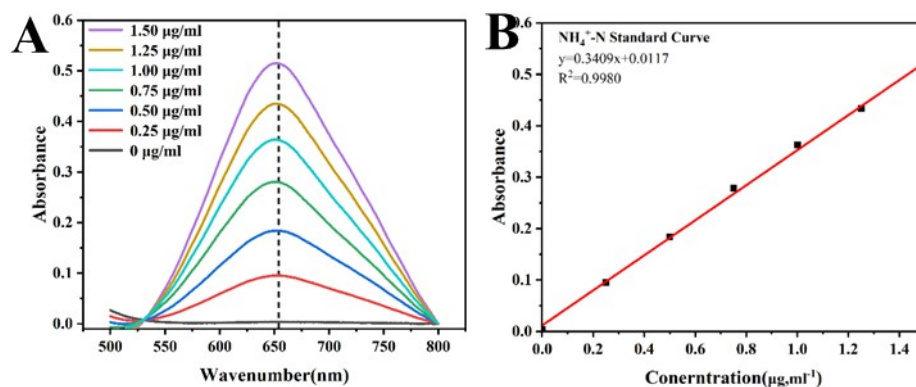


Fig. S11. (A) UV-vis curves of phenate assays after in darkness for 3 h at room temperature. (B) Calibration curve used for estimation of NH₃ by NH₄⁺ ion of different concentrations.

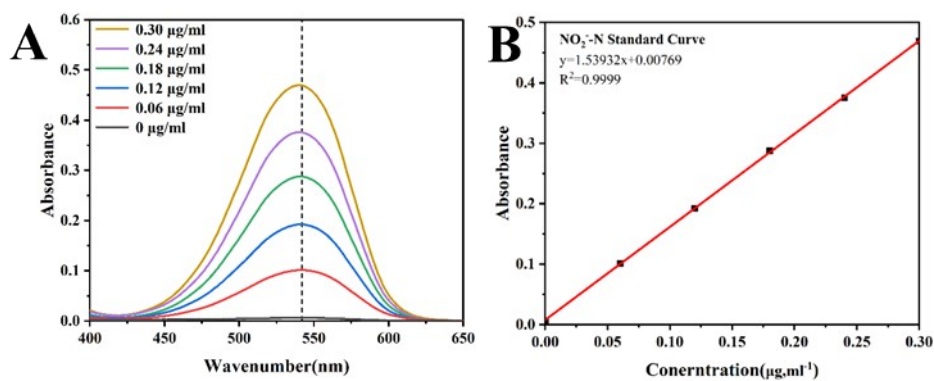


Fig. S12. NO₂⁻-N (A) UV-vis curves of NO₂⁻ ions after 20 min at room temperature. (B) calibration curve used for estimation of NO₂⁻ by NO₂⁻ ion of different concentrations.

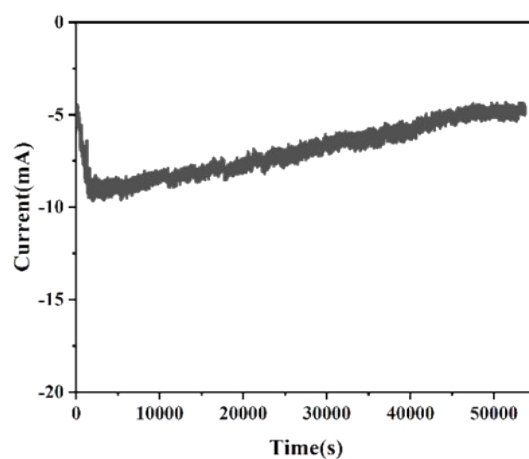


Fig. S13. 15 h chronoamperometry test curve.

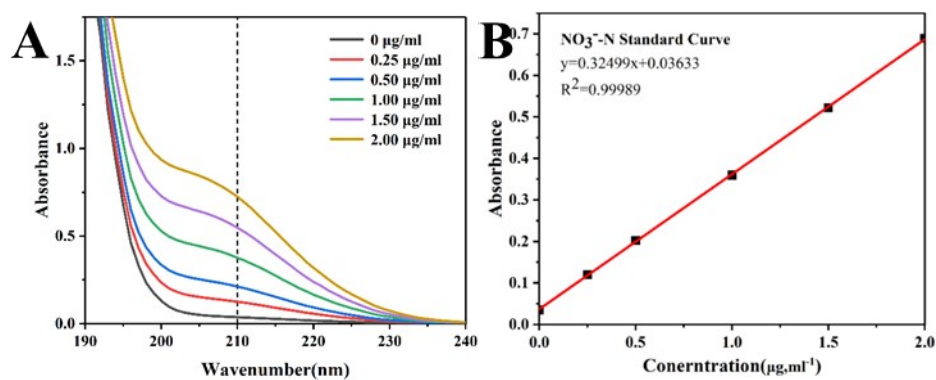


Fig. S14. NO_3^- -N (A) UV-vis curves of NO_3^- ions after 10 min at room temperature. (B) Calibration curve used for estimation of NO_3^- by NO_3^- ion of different concentrations.

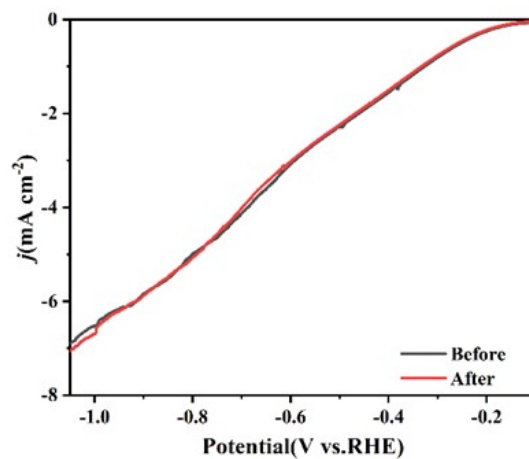


Fig. S15. LSV curves of CPO-IL_{EMB}/3D-Ag before and after 15 h chronoamperometry test in phosphate buffer solution (pH = 5.0) at 10 mV s⁻¹.

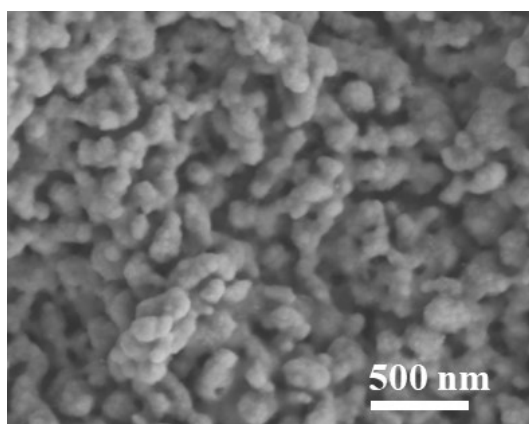


Fig. S16. SEM image of 3D-Ag after 15 h reaction.

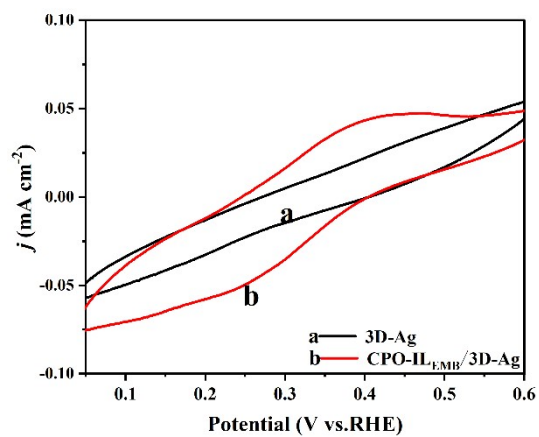


Fig. S17. CV curves of CPO-IL_{EMB}/3D-Ag in phosphate buffer solution (pH = 5.0) at 10 mV s⁻¹.

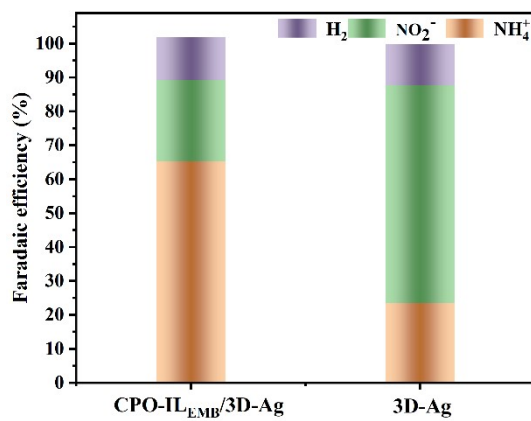


Fig. S18. Faraday efficiency comparison of 3D-Ag vs. 3D-Ag/CPO-IL_{EMB}

Table S1. Comparison of Faraday efficiency, NH_4^+ yield, onset potentials of NO_3^- RR at different electrocatalysts.

| Material | Reaction potential (vs. RHE) | FE | NH_4^+ yield | Onset potential | Cite |
|-----------------------------------|------------------------------|---------|-------------------------------------------------------|-----------------|-----------|
| $\text{Fe}_3\text{O}_4/\text{TP}$ | -0.9 V | 79.4 % | 5.985 $\text{mg h}^{-1} \text{cm}^{-2}$ | -0.45 V | 21 a) |
| TiO_2/TP | -0.9 V | 78.2 % | 4.096 $\text{mg h}^{-1} \text{cm}^{-2}$ | -0.3 V | 21 a) |
| 1:2 MnPc:RGO | -1.5 V | 98.3 % | 20.316 $\text{mg h}^{-1} \text{mg}_{\text{cat}}^{-1}$ | -0.75 V | 21 b) |
| CoPc-RGO 1D-2D HT | -0.2 V | 43.69 % | 0.143 $\text{mg h}^{-1} \text{mg}_{\text{cat}}^{-1}$ | -0.2 V | 21 c) |
| $\text{Co}_3\text{O}_4/\text{CC}$ | -0.9 V | 69.7 % | 2.181 $\text{mg h}^{-1} \text{cm}^{-2}$ | -0.3 V | 8 |
| Bare CC | -0.9 V | 30 % | 0.202 $\text{mg h}^{-1} \text{cm}^{-2}$ | -0.22 V | 8 |
| CPO-IL _{EMB} /3D-Ag | -0.76 V | 65.3 % | 22.27 $\text{mg h}^{-1} \text{mg}_{\text{cpo}}^{-1}$ | -0.24 V | This work |