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Enzymeless multi-sugar fuel cells with high power output based on 3D graphene-Co$_3$O$_4$ hybrid electrodes†

Yun Chen, Kenath Priyanka Prasad, Xuewan Wang, Hongchang Pang, Ruyu Yan, Aung Than, Mary B. Chan-Park, Peng Chen*

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Biofuel cells (BFCs), which use enzymes as the catalysts to harvest energy from green and sustainable fuels abundantly producible from biological systems, are promising next-generation energy devices. But the poor stability and high specificity to only one fuel type of these bio-catalysts largely limit the practice use of current BFCs. In this contribution, we demonstrate a unique fuel cell which, equipped with two identical enzyme-free electrodes based on Co$_3$O$_4$ coated 3D graphene, is able to efficiently harvest electricity from various sweet biofuels (glucose, sucrose, or lactose). Taking advantages of the dual catalytic ability of nanostructured Co$_3$O$_4$ for both glucose oxidation and oxygen reduction as well as the exceptional electrical and structural properties of 3D graphene, our glucose-powered fuel cell, with good long-term stability, offers high open circuit voltage (~1.1 V) and power density output (2.38 ± 0.17 mW cm$^{-2}$).

1. Introduction
The continuously accelerated consumption of the traditional non-renewable energy makes it ever urgent for researchers to find replaceable new power sources allowing conversion of chemical energy into electrical energy. Biofuel cells (BFCs) are promising next-generation energy devices which are able to use enzymes or microorganisms as catalysts to harvest energy from green and sustainable fuels producible from biological systems in gentle and environmental-friendly conditions.1-5

Most enzymatic BFCs use glucose as the biofuel due to its vast abundance, readily availability, and green chemistry. And as glucose is a universal energy currency in living beings, glucose based BFC is a potential power source for bionic implants.3, 6-8 Current glucose-based BFCs, however, rely on glucose specific enzymes, namely, glucose oxidase (GOD) and glucose dehydrogenase (GDH), to oxidize glucose molecules at the anode. To complete the circuit, usually oxygen (O$_2$) is reduced at the cathode by another enzyme, laccase or bilirubin oxidase (BOD). However, the poor stability of the bioenzymes largely limits the practical use of BFCs.1, 2 and the high specificity of these enzymes excludes the utilization of other biofuels (e.g., other sugars) also available in the biomasses or biofluids. Furthermore, non-ideal coupling between enzymes and electrodes compromises the BFC performance. Redox mediators are often required in a BFC to facilitate electron transfer from the embedded catalytic center of the enzyme to the electrode.1-3, 9 These mediators are sometimes toxic and unstable,1, 2 and indirect electron transfer through mediators sacrifices open circuit voltage (OCV),3 thus the power output. Therefore, it is imperative to overcome these current problems of enzymatic BFCs.

Nanostructured materials (e.g., nanoparticles of precious metals or metal oxides) which exhibit exceptional catalytic properties provide novel, stable, and efficient alternatives to bioenzymes.10-12 Among them, cobalt oxides (Co$_3$O$_4$) have attracted widespread attention for various applications due to its extraordinary electrochemical and catalytic properties.13-15 Recently, the excellent catalytic ability of Co$_3$O$_4$ nanostructures towards glucose oxidation has been demonstrated and exploited for enzyme-free glucose detection.16-18 This implies the possibility of using nanostructured Co$_3$O$_4$ as a low-cost and stable substitution to glucose enzymes. However, just like other metal oxides, Co$_3$O$_4$ is poorly conductive. Therefore, it needs to be functionalized onto or hybridized with conductive materials in order to construct an active and conductive electrode. Owing to its extraordinarily high conductivity, specific surface area and chemical inertness, graphene (one-atom-thick crystalline carbon sheet) is a new and attractive electrode material to provide support for catalysts and pathways for electron transfer/conduction.19-22 But compositing other nanomaterials with small individual graphene sheets often suffer from the problems, such as, aggregation, reduced conductivity, compromised surface area, and poor infiltration. Recently, 3D architectures of 2D graphene have been demonstrated, and used as novel 3D electrodes for various applications taking advantages of its high conductivity, large surface area, and macroporous structure.23-28

In this study, Co$_3$O$_4$ hybridized 3D graphene electrodes are fabricated as both the anode for glucose oxidation and the cathode for oxygen reduction (Scheme 1). Such enzyme and mediator-free fuel cell (FC) offers high open circuit voltage (~1.1
Volts, close to the theoretical limit), high power output (2.38 ±
0.17 mW cm⁻²), and good long-term stability (only ~27% run-
down after 30 days). Moreover, we demonstrate that this FC is
able to use other sugar fuels as well (sucrose and lactose).

2. Experimental

2.1 Fabrication of 3D graphene and 3D graphene-Co₃O₄ hybrid electrodes

As reported previously, 3D graphene was synthesized by
chemical vapor deposition (CVD) under atmospheric pressure
using nickel foam (Alumont Advanced Technology Materials,
China) as the substrate and ethanol as the carbon source. Nickel foam was then etched away in 3M HCl leaving 3D graphme foam free-standing. Subsequently, with Co(NO₃)₂·6H₂O solution (0.1 M) as the electrolyte, Co(OH)₂ nanostructures were electrochemically deposited onto bare 3D graphene by applying a constant potential of -1.0 V (vs. Ag/AgCl reference electrode) for 800 s. After thorough rinsing with DI-water, the 3D graphene-Co₃O₄ hybrid was annealed at 400 °C for 4 h to transform Co(OH)₂ to Co₃O₄. Finally, the obtained 3D graphene-Co₃O₄ foam (~0.7 x 0.8 cm, 0.5 mm thick) was mounted onto a glass slide, and an electrical lead was made by silver paint and copper wire insulated with silicone rubber.

2.2 Chemicals and solutions

All chemicals were purchased from Sigma-Aldrich. The sugar stock solutions (1 M) were prepared at least 24 h before use. pH 5.0 buffer solution (0.2 M) was made of sodium acetate and acetic acid; pH 7.0 buffer (0.2 M) was made of NaH₂PO₄ and Na₂HPO₄; pH 11.0 solution (0.2 M) was freshly prepared with NaOH and NaCl.

2.3 Material characterizations and electrochemical measurements

Field emission scanning electron microscope (FESEM, JMS-6700F), confocal Raman system (WITec CRM200 using 488 nm laser), and X-ray diffraction (XRD) system (Bruker D8 Avance diffractometer using Cu Kα radiation) were used to characterize our samples. Contact angles were examined with a FTA200 Dynamic Contact Angle Analyzer. Electrochemical measurements were performed using an electrochemical workstation (CHI 660D). Cyclic voltammetric (CV) measurements were conducted with a three-electrode configuration including a Pt wire as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode, and a 3D graphene-Co₃O₄ hybrid electrode as the working electrode. The open circuit potential of the electrode was determined with a two-electrode configuration (SCE as the reference).

2.4 Fuel cell design and test

The FC house-made with acrylic glass was divided into the anodic and cathodic compartments by a proton exchange film (perfluorosulfonic acid/PTFE copolymer membrane, DuPontTM Nafion®, 25.4 μm thick). The anodic chamber was filled with nitrogen-saturated buffer solution containing different kinds of fuels (sugars) at defined concentrations while the cathodic chamber was filled with oxygen-saturated buffer solution. The open circuit voltage (Eocv) was continuously monitored by CHI-660D electrochemical station after assembling of the FC. Once Eocv reached the steady-state, FC was loaded with various external resistance (100 Ω ~ 100 kΩ) and the corresponding output voltages were recorded with a precision digital multimeter. These results were used to determine the polarization curves and the power density curves.

3. Results and discussion

3.1 Material Characterizations

The structure and surface morphology of the bare 3D graphene and 3D graphene-Co₃O₄ hybrid were investigated by FESEM. As shown in Fig. 1A, 3D graphene exhibits a monolithic macroporous structure (pore size of ~200 μm). After electrochemical deposition, a continuous and regular layer of flower-like Co₃O₄ nanostructure forms around the 3D graphene scaffold (Fig. 1B). In the Raman spectrum depicted in Fig. 1C, the absence of D band (at ~1350 cm⁻¹) and the ratio between G (1584 cm⁻¹) and 2D (2710 cm⁻¹) peaks suggest that the 3D graphene is defect-free and mainly few-layered. In comparison, the Raman spectrum of 3D graphene-Co₃O₄ hybrid manifests additional peaks at 487, 532, 621, and 694 cm⁻¹, corresponding to E₁g, F₂g, E₂g, and A₁g modes of crystalline Co₃O₄, respectively. The XRD pattern of 3D graphene gives two characteristic diffraction peaks at 26.5° and 54.6° (20) due to the (002) and (004) reflections of graphitic carbon, respectively (Fig. 1D). In addition to these graphitic peaks, the 3D graphene-Co₃O₄ hybrid shows ten diffraction peaks resulted from the (111), (220), (311), (222), (400), (422), (511), (440), (531) and (620) planes of crystalline Co₃O₄. Both Raman and XRD characterizations indicate the success deposition of Co₃O₄ with high crystallinity on defect-free graphene. Furthermore, the contact angle measurement suggests that the Co₃O₄ coating renders the highly hydrophobic bare 3D graphene hydrophilic (Fig. 1E). This ensures that the electrolyte can easily and fully penetrate into the 3D electrode (optical image shown in Fig. 1F).

As shown in Fig. S1 in Electronic Supplementary Information (ESI), the morphology of deposited Co₃O₄ microflowers evolves with the electrochemical deposition duration, it reaches almost full blossom when the deposition duration is increased to 800 s. In all the following experiments, we use electrodes with 800s electrochemical deposition of Co₃O₄ (same as shown in Fig. 1B).
inset) because the open circuit potential of such electrodes reaches its maximum while the electrodes using less deposition time delivers lower open circuit potential.

3.2 The anodic properties

Serving as the anode, the open circuit potential ($E_{ocp}^{an}$) of 3D hybrid electrode is measured at acidic (pH 5.0), neutral (pH 7.0), and basic (pH 11.0) conditions (Fig. 2A). As expected, without glucose, the steady-steady $E_{ocp}^{an}$ is essentially zero (0.048 ± 0.021 V, n = 3). Interestingly, in the presence of glucose (200 mM), $E_{ocp}^{an}$ is the maximum at the acidic condition (-0.591 ± 0.012 V, n = 3), in comparison to $E_{ocp}^{an}$ at the neutral (-0.538 ± 0.009 V, n = 3) and at basic conditions (-0.261 ± 0.025 V, n = 3). $E_{ocp}^{an}$ at the acidic condition essentially reaches the theoretical thermodynamic equilibrium potential of the gluconolactone/glucose couple at pH 5.0 ($\varphi' = -0.57$ V vs. SCE), suggesting that the nanostructured Co$_3$O$_4$ acts as a perfect catalyst to oxidize glucose ($\text{glucose} \rightarrow \text{gluconolactone} + 2\text{H}^+ + 2e^-$). To the best of our knowledge, the previously reported anodes based on glucose-specific enzymes (GOD or GDH) are not able to reach this ideal $E_{ocp}^{an}$. Not to mention that, enzymes are not stable and expensive.

To understand the reason for optimal operation in the acidic condition, cyclic voltammogram (CV) of the electrode was conducted. The CV obtained at pH 5.0 without glucose (Fig. 2B) shows two oxidation peaks. Co$_3$O$_4$ (sometimes written as CoO$_2$CoO$_2$) is a mixed valence compound containing both Co$^{II}$ and Co$^{III}$ oxidation states. The lower oxidation peak (at ~0.31 V) is probably ascribed to the oxidation of Co$^{II}$ to Co$^{III}$ in the following reaction:

$$2\text{Co}_3\text{O}_4 + \text{H}_2\text{O} \leftrightarrow 3\text{Co}_2\text{O}_3 + 2\text{H}^+ + 2e^-$$  \hspace{1cm} (1)

while the higher oxidation peak (at ~0.56 V) is likely due to the oxidation of Co$^{III}$ to Co$^{IV}$ as formulated below:

$$\text{Co}_3\text{O}_4 + \text{H}_2\text{O} \leftrightarrow 2\text{CoO}_2 + 2\text{H}^+ + 2e^-$$  \hspace{1cm} (2)

It is known that both Co$^{II}$ and Co$^{IV}$ have strong oxidative ability while converting to Co$^{III}$ and Co$^{IV}$, respectively. In acidic conditions, the theoretical equilibrium potentials of Co$^{III}$ to Co$^{II}$ conversion ($\varphi' = 0.48$ V vs. SCE) and Co$^{IV}$ to Co$^{III}$ conversion ($\varphi' = 0.909$ V) are high, suggesting their strong ability to oxidize glucose. In the presence of glucose, Co$^{IV}$ and Co$^{III}$ would be more readily reduced to Co$^{II}$ whereby promoting reaction (1) and (2). As a consequence, both oxidation peaks increase while the reduction peak (at ~0.27 V) disappears with addition of 1 mM glucose (Fig. 2B). However, it should be pointed out that only Co$^{III}$ exists in the native state of Co$_3$O$_4$, and is responsible for the open circuit potential.

In basic conditions, Co$_3$O$_4$ electrocatalyzes glucose in a different way as previously reported. The two electro-oxidation reactions (at the two oxidation peaks) are given as follows:

$$\text{Co}_3\text{O}_4 + \text{OH}^- + \text{H}_2\text{O} \leftrightarrow 3\text{CoOOH} + e^-$$  \hspace{1cm} (3)

$$\text{CoOOH} + \text{OH}^- \leftrightarrow \text{CoO}_2 + \text{H}_2\text{O} + e^-$$  \hspace{1cm} (4)

The thermodynamic equilibrium potentials of Co$^{III}$ to Co$^{II}$ conversion ($\varphi' = 0.140$ V) and Co$^{IV}$ to Co$^{III}$ conversion ($\varphi' = 0.480$ V) are significantly lower as compared with those in acidic conditions, suggesting that the oxidative ability of high valence Co is compromised by high pH. In support of this, it is observed that glucose induced increase of the oxidation current at the lower potential peak (Co$^{III}$ as the catalyst) vanishes while the increase at the higher potential (Co$^{IV}$ as the catalyst) is much less as compared with that in the acidic condition (231 ± 10 $\mu$A cm$^{-2}$, n = 3 vs. 469 ± 37 $\mu$A cm$^{-2}$, n = 3) (Fig. 2D). Not surprisingly, the CV obtained in neutral condition demonstrates the insignificant oxidative ability of Co$^{III}$ and moderate oxidative ability of Co$^{IV}$ (Fig. 2C).
electrolyte with glucose (iv). The dashed-line indicates the theoretical limit. (B-D) The CVs of the hybrid electrode without (black) or with (red) glucose (1 mM), in pH 5.0 (B), pH 7.0 (C), or pH 11.0 electrolyte (D). The dashed-arrows indicate the changes of oxidation peaks; and all solutions were saturated with nitrogen.

The native Co₃O₄ contains both Co³⁺ and Co⁵⁺. We further demonstrate that the CoOOH (containing only Co³⁺) coated 3D graphene was able to produce a significant $E_{ocp}^{anop}$ of -0.587 ± 0.049 V (n = 3) resulting from glucose oxidation whereas Co(OH)$_2$ (Co⁵⁺ only) coated 3D graphene electrode could not give an appreciable $E_{ocp}^{anop}$ (Fig. S2 and S3 in the ESI). This experiment suggests that it is the strong oxidative ability of Co⁵⁺ (not Co³⁺) in Co₃O₄ (particularly in acidic condition) responsible for the high anodic $E_{ocp}^{anop}$.

3.3 The cathodic properties

Co³⁺, which has been shown to possess reducing ability (towards, e.g., oxygen and H$_2$O$_2$) due to Co⁵⁺ to Co³⁺ conversion, equally co-exists in Co₃O₄, implying that the same 3D graphene-Co₃O₄ electrode may also be able to serve as the FC cathode. The open circuit potentials ($E_{ocp}^{anop}$) of the 3D graphene-Co₃O₄ cathode, which is result from the reaction: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$, were measured at acidic (pH 5.0), neutral (pH 7.0) and basic conditions (pH 11.0), with saturated oxygen. As shown in Fig. 3A, like the anodic potential $E_{anop}^{catalyst}$, $E_{ocp}^{anop}$ is also most significant in the acidic (0.520 ± 0.010 V, n = 3), in contrast to $E_{ocp}^{anop}$ at neutral (0.312 V ± 0.014 V, n = 3) and basic (0.056 ± 0.009 V, n = 3) electrolyte. And this value is comparable to the theoretical equilibrium potential ($\phi'$ = +0.637 V vs. SCE) of the O$_2$/H$_2$O couple at pH 5.0.

The optimal performance in the acidic condition is not unexpected because the oxidative capability of O$_2$ decreases with the increasing pH because of the decreasing $\phi'$ (0.637 V at pH 5.0, 0.519 V at pH 7.0, and 0.164 V at pH 11.0). To further investigate this phenomenon, the CVs were obtained at the different pH levels.

In the acidic conditions, the reduction peak in the CV (Fig. 3B) is attributed to the following reaction:

$$\text{Co}_3\text{O}_4 + 2\text{H}^+ + 2e^- \leftrightarrow 3\text{CoO} + \text{H}_2\text{O} \quad (5)$$

Obviously, this reaction is favoured at a low pH. $\phi'$ of this reaction at pH 5.0 (0.239 V vs. SCE) is significantly lower than that of oxygen reduction (0.637 V), indicating the good catalytic ability of Co³⁺ to Co³⁺ conversion to oxygen reduction. In agreement with this view, the introduction of oxygen largely increases the reduction current (Fig. 3B). In basic conditions, the reduction peak in the CV from the reaction below:

$$\text{Co}_3\text{O}_4 + 4\text{H}_2\text{O} + 2e^- \leftrightarrow 3\text{Co(OH)}_2 + 2\text{OH}^- \quad (6)$$

The equilibrium potential $\phi'$ of this reaction at pH 11.0 (0.810 V vs. SCE) which is higher than $\phi'$ of oxygen reduction (0.164 V), indicating that in a basic condition Co⁵⁺ loses its ability to reduce oxygen. Consequently, the introduction of oxygen fails to enhance the reduction current in the CV as shown in Fig. 3D. Consistently, the reducing ability of Co⁵⁺ is moderate in the neutral condition as compared to that in the acidic condition (125 ± 9 μA cm$^{-2}$ vs. 162 ± 10 μA cm$^{-2}$ as the oxygen-enhanced reduction current, n = 3).

We further show that the Co(OH)$_2$ coated 3D graphene electrode offered a large $E_{anop}$ of 0.543 ± 0.020 V (n=3) whereas CoOOH coated 3D graphene electrode only produced a trivial $E_{anop}$, indicating the reducing ability of Co³⁺ (but not Co⁵⁺) to oxygen (Fig. S3 in the ESI). Together with the results demonstrated in the previous section, it can be concluded that the dual catalytic ability of Co₃O₄ (thus its ability to serve as both BFC anode and cathode) is due to the strong oxidative ability of Co⁵⁺ to glucose and the excellent reducing ability of Co³⁺ to oxygen.

![Fig. 3](image_url) (A) The open circuit potential of the 3D graphene-Co₃O₄ hybrid cathode in pH 5.0 (i), pH 7.0 (ii), or pH 11.0 (iii) electrolyte solution saturated with oxygen. The dashed-line indicates the theoretical limit. (B-D) The CVs of the hybrid electrode, with saturation of nitrogen (black) or saturation of oxygen (red), in pH 5.0 (B), pH 7.0 (C), or pH 11.0 (D) electrolyte solution. The dashed-arrows indicate the changes of reduction peaks.

3.4 The performance of the sugar-powered fuel cell

The fuel cell (FC) equipped with both 3D graphene-Co₃O₄ anode and cathode works as illustrated in Scheme 1. At the anode, Co³⁺ sites in the Co₃O₄ nanostructures efficiently catalyze glucose oxidation via its conversion to Co⁵⁺; the thereby obtained electrons are rapidly conducted away by the 3D-multiplexed conductive highways of the graphene foam to the cathode leading to restoration of the converted Co⁵⁺ back to Co³⁺ state; at the cathode, the Co⁵⁺ sites of Co₃O₄ layer reduce oxygen via its conversion to the higher valence states (Co⁷⁺ or Co⁵⁺) which soon return to the divalent state while receiving electrons from the anode; Co³⁺ sites at the cathode may promote chemisorption of O$_2$ molecules through their cationic d-orbitals; to complete the circuit, the current in the solution is carried by the proton flux from the anode to the cathode. The generated electricity will last as long as the fuel molecules (glucose and oxygen) are not depleted.
glucose as the fuel while wasting other possible fuels in the biomass. Uniquely, we find that our enzyme-free FCs are able to harvest energy from other sugar molecules (specifically, disaccharide sucrose and β-lactose) in addition to glucose (monosaccharide) (Fig. 4C). Also as shown in Fig. 4C, the maximum power output \(P_{\text{max}}\) is fuel-concentration dependent (highest \(P_{\text{max}}\) reached at ~200 mM for glucose, ~50 mM for sucrose, and ~100 mM for β-lactose). Decrease of \(P_{\text{max}}\) at a too-high sugar concentration is presumably, at least in part, due to increase of internal resistance and decrease of diffusion process (because of increase of viscosity). Moreover, we show that the device is able to harvest electricity from the mixture of three sugar species (50 mM glucose, 25 mM sucrose, and 25 mM β-lactose) and deliver an output higher than that from any individual species alone at the same concentration (100 mM) (Fig. 4). As a demonstration of its practical use, we show that two FCs in series can light up a yellow LED (turn-on voltage ~1.8 V).

Because BFCs are promising powering units for implanted devices due to the abundant availability of biofuels in the living systems, we examined the cytotoxicity of the 3D graphene-Co\(_3\)O\(_4\) electrode. As shown (Fig. S4 in ESI), the hybrid electrode didn’t impose appreciable cytototoxicity to the tested cell model (PC12 cells – a cell line derived from rat adrenal medulla).

Fuel cells using noble metal nanoparticles as the catalysts for glucose oxidation have been demonstrated in many studies. But in addition to the problems that noble metal nanoparticles are expensive and prone to poisoning by the reaction intermediates, these FCs have to operate in highly basic conditions (thus often called as glucose alkaline fuel cells) and sometimes high temperature.

In constrast, our FC using inexpensive Co\(_3\)O\(_4\) as the catalyst can operate in mild and physiological conditions and is a superior alternative to enzymatic biofuel cells. At pH 7.0, our glucose-powered fuel cell is able to deliver a power output density of 1.55 ± 0.11 mW cm\(^{-2}\) \((n = 3)\) which outperforms that of the previously reported BFCs and is much higher than that of all glucose alkaline fuel cells whose outputs drastically drop to a few μW cm\(^{-2}\) at neutral pH.

### 4. Conclusion

We herein demonstrate a unique fuel cell which, equipped with two identical enzyme-free electrodes based on Co\(_3\)O\(_4\) coated 3D graphene, is able to efficiently harvest electricity from various sweet fuels (glucose, sucrose, or lactose). This development takes advantage of the fact that Co\(_3\)O\(_4\) is a mixed valence compound, in which, the higher valence state (Co\(^{IV}\)) can act as an ideal oxidation catalyst towards glucose while the lower valence state (Co\(^{II}\)) can serve as an excellent reduction catalyst towards oxygen. Using glucose as the fuel, our FC offers the highest open circuit potential and power density output ever reported. Such high performance is owing to not only the exceptional dual catalytic abilities of nanostructured Co\(_3\)O\(_4\) but also the extraordinary properties of 3D graphene. The graphene foam provides a 3D scaffold with large surface area to support abundant Co\(_3\)O\(_4\) catalysts, highly-conductive 3D network for rapid charge transport, and macroporous structure to ensure...
 unhindered diffusion of ions and substances. Finally, without the use of bioenzymes, our sugar-powered FC is of low-cost and high stability.

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