ACS APPLIED MATERIALS & INTERFACES

Letter

www.acsami.org

Cellulosic Carbon Fibers with Branching Carbon Nanotubes for Enhanced Electrochemical Activities for Bioprocessing Applications

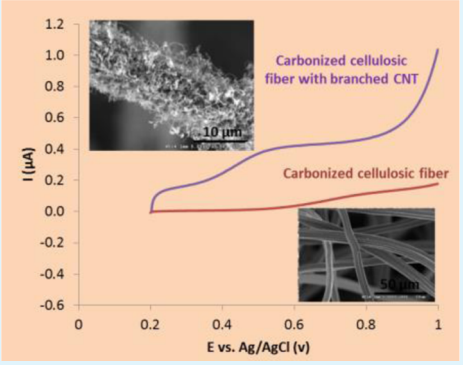
Xueyan Zhao,[†,‡] Xin Lu,[†,‡] William Tai Yin Tze,[†] Jungbae Kim,[*,§] and Ping Wang[*,†,‡]

[†]Department of Bioproducts and Biosystems Engineering and [‡]Biotechnology Institute, University of Minnesota, Saint Paul, Minnesota 55108, United States

[§]Department of Chemical and Biological Engineering, Korea University, Seoul, Korea

Supporting Information

ABSTRACT: Renewable biobased carbon fibers are promising materials for large-scale electrochemical applications including chemical processing, energy storage, and biofuel cells. Their performance is, however, often limited by low activity. Herein we report that branching carbon nanotubes can enhance the activity of carbonized cellulosic fibers, such that the oxidation potential of NAD(H) was reduced to 0.55 V from 0.9 V when applied for bioprocessing. Coordinating with enzyme catalysts, such hierarchical carbon materials effectively facilitated the biotransformation of glycerol, with the total turnover number of NAD(H) over 3500 within 5 h of reaction.



KEYWORDS: *carbon nanotubes, cofactor regeneration, dihydroxyacetone, glycerol biotransformation, porous carbon electrode, cellulosic fibers*

INTRODUCTION

Hierarchical nanostructured carbon materials promise fine-tuned physicochemical properties and high specific surface areas and have been greatly attractive for high-performance microdevices such as microchips and biosensors.[1] Cellulosic fibers are considered to be some of the most abundant renewable resources for carbon materials, ideal especially for large-scale applications such as energy storage and chemical processing. However, carbonized cellulosic fibers generally lack the desired features of nanomaterials, offering low specific surface areas and weak electrochemical activities. Previous studies have shown the possibility of surface fabrication by deposition of carbon nanotubes (CNTs) on carbon materials for improved electrochemical performance for biosensor and biofuel cell applications.[2,3] Because the deposited CNTs lay recumbently on the surface or are embedded inside polymeric coatings with a large portion of the surface areas covered by neighboring materials, the potentials of CNTs may not get fully capitalized.

EXPERIMENTAL SECTION

Materials. All solvents and reagents were purchased from Sigma-Aldrich and used without further purification.

Fabrication. In the current work, we explore the fabrication of cellulose carbon fibers with branched CNTs, generating a hierarchical structure with fully exposed CNTs. Carbonization can be achieved via pyrolysis, through which the pyranose units of cellulose in the fibers are dehydrated to carbonaceous polymer intermediates, which can be transformed into a polyaromatic structure[4] that provides electrical conductivity characteristics. To introduce branching CNTs to the surface of cellulose carbon fibers, a carbonization process was developed in the current work by generating metal nanoparticles on the surface of the fibers to catalyze the growth of CNTs. This was achieved by spreading a metal-containing salt solution on the surface of cellulose fibers before carbonization. Specifically, cellulose fibers (medical-grade cotton wool) were pretreated by dipping into a well-suspended dimethylformamide solution containing 3.3% $Fe(C_5H_7O_2)_3$ at room temperature for 2 h. The pretreated sample was dried at room temperature before being carbonized in a high-temperature tubular furnace (Sentro Tech, model STT-1200-3.5-12, Cleveland, OH). The sample was heated at 250 °C in argon for 3 h, and the Fe^{3+} ions loaded on the cellulose fibers were then reduced to iron nanoparticles by introducing hydrogen at 500 °C. The cellulose fibers were then carbonized at 850 °C for 30 min, and growth of the CNTs was achieved at 700 °C by introducing hexane vapor as a carbon source for 3−20 min (depending on the desired length of the CNTs). Iron nanoparticles formed on the surface of carbon cotton fiber catalyzed the growth of CNTs, generating the desired branching structure.

Received: July 19, 2013
Accepted: September 10, 2013
Published: September 10, 2013

   dx.doi.org/10.1021/am402916g | *ACS Appl. Mater. Interfaces* 2013, 5, 8853−8856

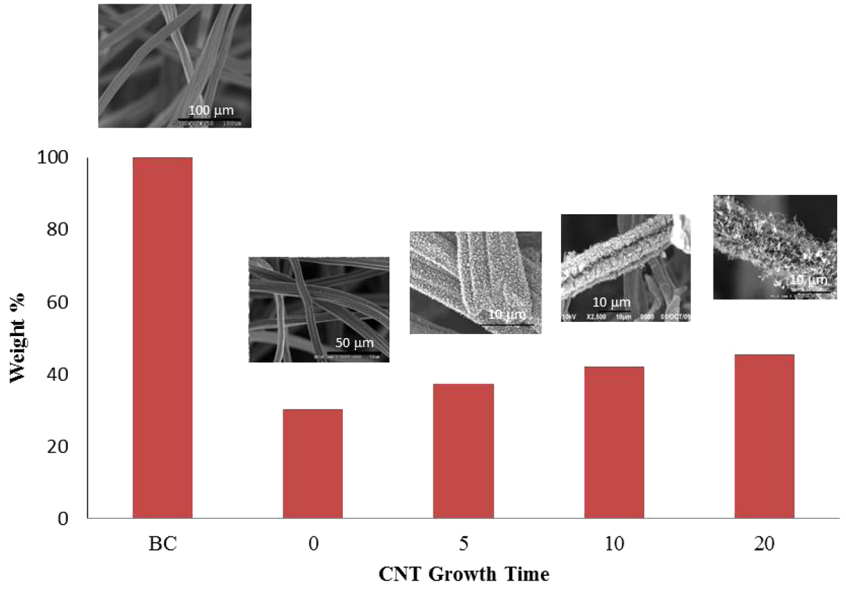


Figure 1. Carbonization of cellulosic fibers and CNT growth. Images from left to right: cotton cellulose fibers before carbonization (BC); SEM image of carbonized cellulose fibers (weight loss ~ 70%, time 0); carbonized cellulose fibers with branched CNTs grown on the surface (growth time 5, 10, and 20 min). The weight gain after carbonization shows the progress of CNT growth.

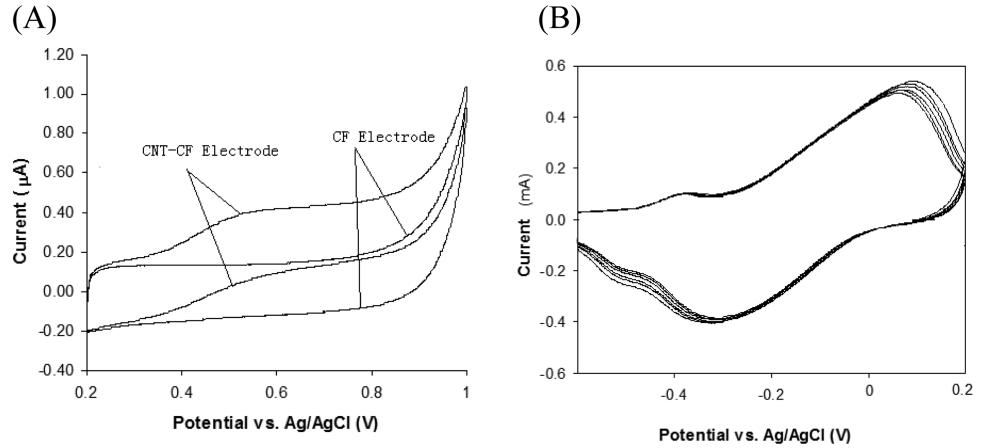


Figure 2. Cyclic voltammetry of NADH with a carbonized cellulosic electrode. (A) CNT-carbon fiber hierarchical (CNT-CF) electrode, with the oxidation peak of NADH reduced to 0.55 V from 0.9 V as a result of CNT branching. (B) CNT-CF electrode with a coated Meldola Blue mediator, realizing NADH oxdidation at 0.1 V. The electrolyte used was a 0.5 mM NADH solution (pH 7.0) in a 50 mM phosphate buffer. Scan rate 100 mV/s.

RESULTS AND DISCUSSION

The original cellulose fibers have an average diameter of ~20 μm, which was reduced to 15 μm after carbonization (Figure 1). The carbonized fibers prior to CNT growth retained 30% of the original weight. The hexane exposure time controlled the length of CNT. The CNT-branched carbon fibers exhibited a weight gain that corresponded well to an increase of the CNT length, linearly with growth time until it reached ~45% of the original cotton fiber weight (Figure 1). Brunauer–Emmett–Teller surface area measurement revealed that blank carbon fibers have surface areas below the measurement limit (<10 m^2/g), while that for CNT-branched fibers could be as high as 36 m^2/g.

In addition to surface area improvement, CNT also enhanced the electrochemical activity of the carbon fibers. Cyclic voltammetry tests were conducted in a three-electrode electrochemical cell with an Ag/AgCl reference electrode and a platinum counter electrode. A biochemical redox species, NADH, was selected for the test for its importance in both bioanalytical and bioprocessing applications. The CNT-branched carbon fiber realized oxidation of NADH at 0.55 V, whereas 0.9 V was needed for fibers without CNT (Figure 2). This result is comparable to the previously published oxidation potential (0.4 V) of a multiwalled CNT fiber microelectrode.[5] In addition, the specific current of NADH oxidation on CNT-grown carbon fibers was over 10 times higher than that without CNTs, a result of the increased surface area. We further fabricated the hierarchical carbon electrode with poly-(methylene green) through an electrodeposition procedure. Cyclic voltammetry tests showed that oxidation of NADH could be realized at 0.1 V (vs Ag/AgCl; Figure 2B).

The cellulose-derived hierarchical carbon electrode was then examined for enzymatic glycerol biotransformation to demonstrate its potential in bioprocessing applications. Glycerol is the byproduct of the production of biodiesel and surfactants, and it is becoming an overwhelming stream of biowaste because of

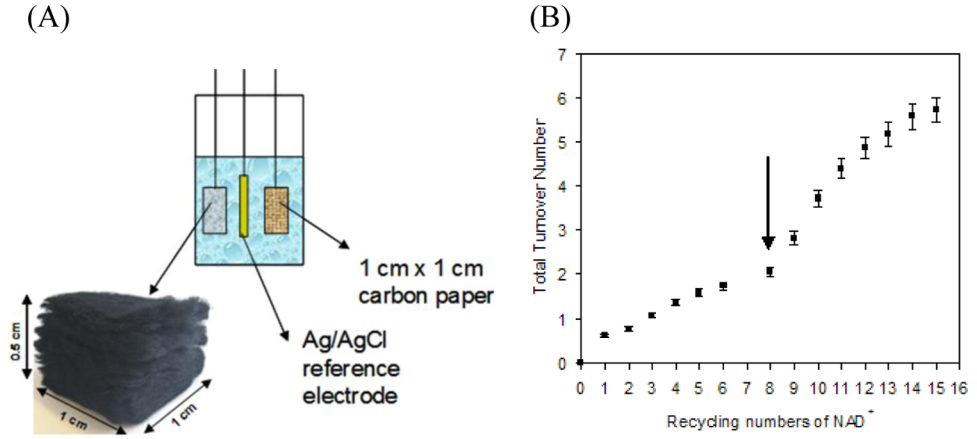


Figure 3. Cellulose carbon electrode coupled with DHA synthesis with in situ coenzyme regeneration. (A) Electrochemical cell with a 3D carbon electrode. (B) TTNs of NADH enabled with electrochemical regeneration of NAD^+ coupled with glycerol oxidation for the synthesis of DHA. The arrow indicates the point of glycerol amendment after consumption of the original feed. TTN was measured as 2512 and 3547 for reaction times of 2 and 5 h, respectively.

the rapidly growing demands for biodiesels. Glycerol dehydrogenase (GDH) in the presence of NAD^+ is capable of catalyzing the oxidation of glycerol into dihydroxyacetone (DHA), a valuable diol for personal care products and chemical synthesis. The reduced form of the cofactor, NADH, needs to be oxidized to NAD^+ to enable continued reaction without amendment. In this study, a his-tag recombinant GDH (rGDH), overexpressed in *Escherichia coli* BL21 (DE3), was applied for enzymatic glycerol oxidation. A 3D hierarchical carbon electrode with dimensions of 1 × 1 × 0.5 cm was prepared (Figure 3) from cellulose fibers and tested for the electrochemical regeneration of NAD^+ from NADH to facilitate the production of DHA from glycerol. The applied regeneration potential was 0.6 V (vs Ag/AgCl).

rGDH was immobilized on ProBond resin particles to catalyze the reaction. In a batch operation mode, the glycerol oxidation reaction produced DHA (product) and NADH; the reaction solution containing NADH was applied to the electrode to allow cofactor regeneration, regeneration of NAD^+, and release of protons. The released protons were converted into water by another enzyme, laccase. DHA generated from the reaction was monitored using high-performance liquid chromatography to calculate the total turnover number (TTN) of NAD^+. The results of a typical batch operation with reaction−regeneration cycles are shown in Figure 3 with the amount of DHA accumulated as a function of NAD^+ regeneration cycles. The TTN of NAD^+ (defined as the ratio of DHA produced versus the total amount of NAD^+ added) reached a plateau (~2) at cycle 8 because of depletion of glycerol in the reaction solution. When fresh glycerol was added, the yield and concentration of DHA continued to increase.

To allow a continuous operation mode, the reaction system was retained in the reactor using an ultrafiltration cell equipped with a membrane with a pore size of 1.2 μm, while glycerol was continuously fed to the reactor. The particles with immobilized enzymes and coenzyme were suspended in the reaction solution by stirring at 200 rpm via magnetic stirring. With continuous feeding of glycerol, the TTNs of NAD^+ calculated based on the initial amount of NAD^+ applied were 2512 at 2 h and 3547 at 5 h, indicating a much more intensified reaction system than the packed-bed reactor mode.

CONCLUSION

In conclusion, we have demonstrated that CNT branching could effectively increase the specific surface areas of carbon fibers and reduce its overpotential for biochemical redox reactions. Such hierarchical carbon materials afforded much intensified productivity for bioprocessing applications. Coenzyme-enabled biotransformations promise valuable biosyntheses, and there has been rapidly growing interest in cell-free synthesis using such multienzyme systems to realize complicated reaction pathways with enhanced reaction kinetics and simplified product purification.[6,7] However, such a biosynthesis is often limited by the high cost of coenzymes. Coenzymes can be regenerated via either chemical or electrochemical methods,[8] while the latter is preferred for large-scale applications because it offers fast kinetics and neat reactions. Traditional electrochemical coenzyme regeneration has been achieved with glassy carbon or metal electrodes with low energy efficiency. It has been reported, for example, that direct electrochemical oxidation of NADH at pH 7.0 occurred at 1.1 V on the carbon electrode[9] and 1.3 V on the platinum electrode.[10] With much improved electrochemical activity, we expect that the renewable hierarchical carbon electrodes in combination with enzyme reaction systems can be efficiently employed for a variety of coenzyme-dependent enzymatic syntheses.

ASSOCIATED CONTENT

Supporting Information

Enzyme (rGDH) structure and purity and activity data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: jbkim3@korea.ac.kr.
*E-mail: ping@umn.edu. Tel: +1-612-624-4792.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are thankful for support from a Biocatalysis Initiative grant from University of Minnesota and the

"International Collaborative R&D Program" of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Trade, Industry & Energy (Grant 20118510020020).

REFERENCES

(1) Mazurenko, I.; Etienne, M.; Tananaiko, O.; Urbanova, V.; Zaitsev, V.; Walcarius, A. *Carbon* **2013**, *53*, 302−312.
(2) Wang, M.; Zhao, F.; Liu, Y.; Dong, S. *Biosens. Bioelectron.* **2005**, *21*, 159−166.
(3) Swamy, B.; Venton, B. *Analyst* **2007**, *132*, 876−884.
(4) Plaisantin, H.; Pailler, R.; Guette, A.; Daudé, G.; Pétraud, M.; Barbe, B. *Compos. Sci. Technol.* **2001**, *61*, 2063−2068.
(5) Wang, J.; Deo, R.; Paulin, P.; Mangey, M. *J. Am. Chem. Soc.* **2003**, *125*, 14706−14707.
(6) Hodgman, C.; Jewett, M. *Metab. Eng.* **2012**, *14*, 261−269.
(7) Zhang, Y.; Huang, W. *Trends Biotechnol.* **2012**, *30*, 301−306.
(8) Liu, W.; Wang, P. *Biotechnol. Adv.* **2007**, *25*, 369−384.
(9) Moiroux, J.; Elving, P. *Anal. Chem.* **1978**, *50*, 1056−1062.
(10) Haegfeldt, H. *J. Electroanal. Chem.* **1980**, *110*, 295−302.