



Supporting Information

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Designed Fabrication of Magnetically Switchable Bioelectrocatalytic System Using Crosslinked Enzyme Aggregates Shipped in Magnetic Mesocellular Carbon Foam**

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1. Experimental Section

Materials: Glucose oxidase (GOx), D-(+)-glucose, and glutaraldehyde (GA, 25 %) were purchased from Sigma. Tetraethylorthosilicate (98 %), divinylbenzene (98 %), 1, 3, 5-trimethylbenzene, and ferrocene methanol (FcMeOH) were from Aldrich. Pluronic P123 (EO₂₀PO₇₀EO₂₀, M_{av} = 5800) was obtained from BASF. All chemicals of analytical grade were used as received without further purification.

Synthesis of Mag-MCF-C: MCF silica template was first prepared following the reported procedure.^[S1] Briefly, 4 g of P123 was completely dissolved in a solution composed of 130 mL of deionized water and 21 mL of hydrochloric acid (36 wt. %), and temperature of the solution was raised to 313 K. Then, 4.3 mL of trimethylbenzene (TMB) was added into the P123 polymer solution. Following addition of 9.2 mL of TEOS, the resulting solution was aged at 313 K for 20 h and another 24 h at 373 K. The resulting white precipitate was filtered, dried at room temperature and finally calcined at 550 °C in air to obtain MCF silica template. For the synthesis of Mag-MCF-

C, 1 g of dried MCF silica was wetted with polymer precursor solution composed of 1 mL divinylbenzene and free radical initiator, 2,2'-azobisisobutyronitrile (AIBN) with a molar ratio of 15 : 1. Polymerization was performed by heating at 85 °C for 12 h under argon atmosphere. 0.71 g of Fe(NO)₃·9H₂O dissolved in ethanol was incorporated into the pores of MCF/poly(DVB) composite through the impregnation method. The composite was heated at 800 °C for 1 h under nitrogen atmosphere (heating rate = 2 °C min⁻¹). Dissolution of the MCF template using 1 M NaOH (ethanol:water = 1:1 v/v) at 100 °C yielded magnetically separable mesocellular carbon foam, designated as Mag-MCF-C.

Characterization of Mag-MCF-C: Argon (or N₂) adsorption and desorption isotherms were measured at 77 K using a Micromeritics ASAP 2000 Gas Adsorption Analyzer after Mag-MCF-C was degassed at 10 μtorr for 5 h at 423 K. The pore size distribution was determined from analysis of the adsorption branch of the argon (or N₂) isotherm using the BJH (Barrett–Joyner–Halenda) method. Transmission electron micrographs (TEM) were obtained by using an electron microscope (JEOL JEM-2010). Scanning electron microscopic images were obtained on a JSM-840A microscope. The XRD pattern was analyzed with a diffractometer (Rigaku D/Max-3C) equipped with a rotating anode and a Cu Kα radiation source ($\lambda = 0.154056$ nm).

The magnetic properties of Mag-MCF-C were investigated by measuring the temperature-dependent magnetization after zero-field cooling (ZFC) and field cooling (FC) procedures in an applied magnetic field of 100 Oe between 2 K and 350 K. We used a commercial superconducting quantum interference device (SQUID) (Quantum Design, MPMS5XL).

Immobilization of GOx in Mag-MCF-C: Mag-MCF-C (10 mg) was washed successively with distilled water, 0.2 M sodium acetate buffer (pH 5.1), and 0.1 M sodium phosphate buffer (pH 7.4), followed by separation with a magnet. The resulting Mag-MCF-C was mixed with 1.5 mL of 4 mg mL⁻¹ GOx solution in acetate buffer. The reaction mixture was incubated at 25 °C with shaking (250 rpm) for 20 min. In an effort to increase the retention of enzymes within pores, the Mag-MCF-C/GOx was treated with GA for crosslinking. The Mag-MCF-C/GOx was resuspended in GA solution (0.1 % in 0.1 M sodium phosphate buffer, pH 8.0) and incubated for 30 min at room temperature with shaking. After washing with phosphate buffer and 0.1 M Tris·HCl (pH 8.0), remaining aldehyde groups were blocked by incubation in Tris·HCl for 30 min at room temperature with shaking. The resulting Mag-MCF-C/CLEA-GOx was washed three times with phosphate buffer and stored at 4 °C until use. The GOx concentration in buffer solution was determined by Bradford method (Bio-Rad) and used for the calculation of enzyme loading within Mag-MCF-C.

Assay of GOx activity: GOx assay kit from Molecular Probes was employed for the determination of GOx activity following the manufacturer's instruction. Briefly, sample solutions containing GOx were diluted to proper range in 50 mM sodium phosphate buffer (pH 7.4). Standards and sample solutions of GOx (50 µL each) were loaded into wells of 96-well microplate, followed by addition of 50 µL of assay solution (100 µM Amplex Red, 0.2 U/mL horseradish peroxidase, and 100 mM glucose). The reaction product (resorufin) was quantified by measuring the absorbance at 570 nm using microplate reader (Bio-Rad). The absorbance increased linearly for 30 min, and the enzymatic activity was determined from slope of the absorbance change and calibration curve prepared using

GOx standards of known activities. The activity of immobilized GOx in Mag-MCF-C was also determined following the same procedure after appropriate dilution. In this dilution range, suspended particles had a negligible effect on the absorbance measurement. The assay mixture was continuously shaken during the reaction to facilitate mass transport.

Retention of immobilized GOx within Mag-MCF-C: 10 mg of Mag-MCF-C/GOx was suspended in 10 mL of 0.1 M sodium phosphate buffer (pH 7.4). The retention of GOx was tested by repeating the following steps: shaking for 5 min at 250 rpm, separation by a magnet, and the buffer exchange. At intervals, the activity of immobilized GOx in Mag-MCF-C and replaced buffer was checked as described above. For the evaluation of long-term stability, 10 mg of Mag-MCF-C/GOx was suspended in 10 mL of phosphate buffer and incubated at room temperature under continuous shaking (at 250 rpm). At intervals, aliquots (100 μ L each) were sampled, and the activity of immobilized GOx was assayed after buffer exchange with fresh one to exclude the contribution from leached enzymes.

Construction of magnetically switchable glucose biosensor: Gold substrate prepared by resistive evaporation of 150 nm of Au (99.999 %) onto titanium-primed (40 nm Ti) Si[100] wafer was used as a working electrode after cleaning with piranha solution (1:4 v/v mixture of 30 % H₂O₂ and conc. H₂SO₄) for 5 min. The working electrode was attached to the bottom of electrochemical cell (4 mL), exposing the surface area of ~ 0.1 cm². 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1 mM FcMeOH as a redox electron mediator was used as a base electrolyte after deoxygenation by nitrogen bubbling for 30 min. Mag-MCF-C/CLEA-GOx (5 mg) was suspended in the electrolyte

followed by electrochemical measurements using voltammetric analyzer (BAS CV-50W). A standard three-electrode configuration with a platinum wire counter electrode and an Ag/AgCl (3M NaCl, BAS) reference electrode was used. Magnetic on-off switching of enzyme electrode was conducted by alternate positioning of the magnet below and above the electrochemical cell. When placed below the electrochemical cell, the magnet attracted Mag-MCF-C/CLEA-GOx to the electrode surface, which generated the anodic current by the ferrocene-mediated electron transport from FAD redox center of GOx to the electrode (switch on). Meanwhile, positioning of the magnet above the electrochemical cell removed the Mag-MCF-C/CLEA-GOx from the electrode, resulting in a switched off state.

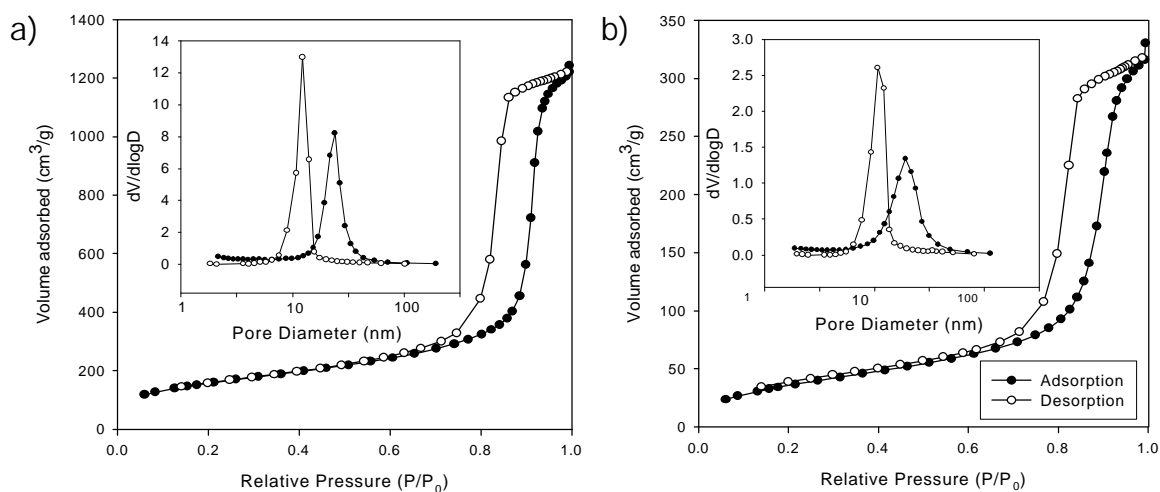


Figure S1 N₂ isotherm and pore size distributions of (a) MCF silica template (b) MCF silica/poly(DVB) composite.

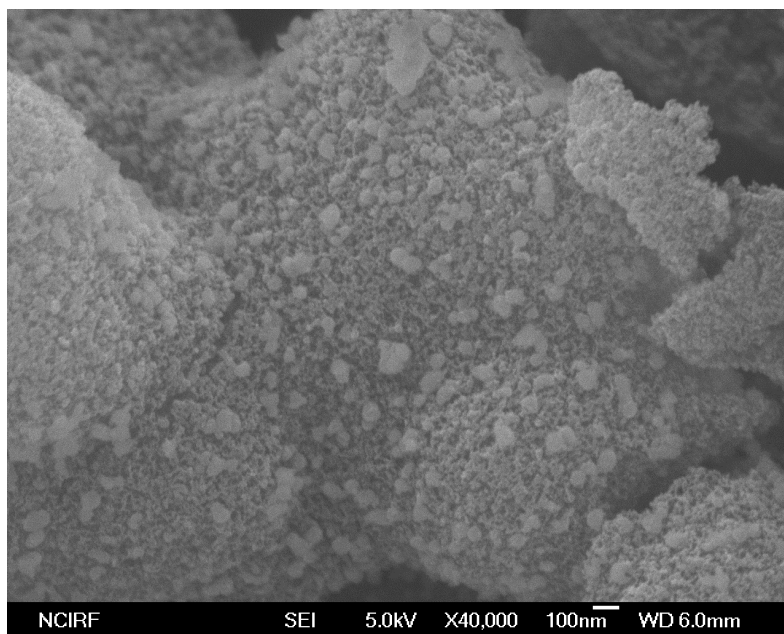


Figure S2. SEM image showing the formation of magnetic nanoparticles on the exterior surface of Mag-MCF-C.

2. Formation Route for MNPs during Carbonization

To investigate the formation route for α -Fe MNPs during carbonization, iron nitrate-impregnated MCF/poly(DVB) composite was carbonized at various temperatures under N_2 atmosphere (see Figure S3) There appeared only magnetite (Fe_3O_4) phase after heat treatment at 400 °C. On the contrary, $Fe(NO_3)_3 \cdot 9H_2O$ impregnated in three-dimensionally interconnected mesoporous silica (MCM-48) was converted to hematite (α - Fe_2O_3) phase after heat treatment under N_2 atmosphere at 400 °C.^[S2] Bulk $Fe(NO_3)_3 \cdot 9H_2O$ salt was also converted α - Fe_2O_3 under the same condition. As the heating temperature increased to 550 °C, XRD peaks became intense and narrow, which indicates that magnetite nanoparticles became bigger. No change in the magnetite phase was observed. Further increase in temperature to 700 °C yielded sharp α -Fe peaks and decreased intensity of magnetite phase. It was reported that magnetite phase converts to α -Fe via reduction process during carbonization.^[S3] We observed that magnetite phase completely disappeared in the XRD pattern after carbonization at 800 °C (see Figure S4)

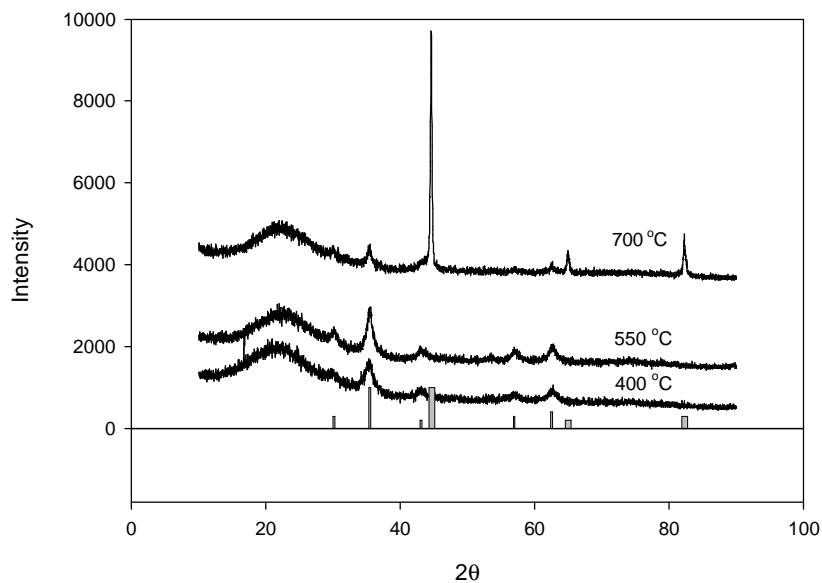


Figure S3. XRD patterns of composites obtained by heat treatment of MCF/poly(DVB)/iron nitrate composite at various temperatures. Thick and thin bar indicate α -Fe peak and magnetite (Fe_3O_4) peak from JCDPS (Joint Committee for Powder Diffraction Standard cards), respectively.

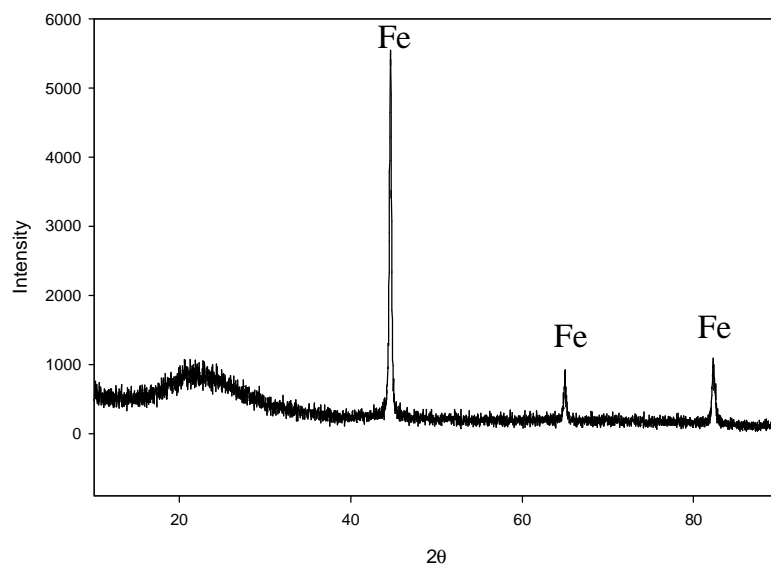


Figure S4. XRD pattern of Mag-MCF-C/silica composite.

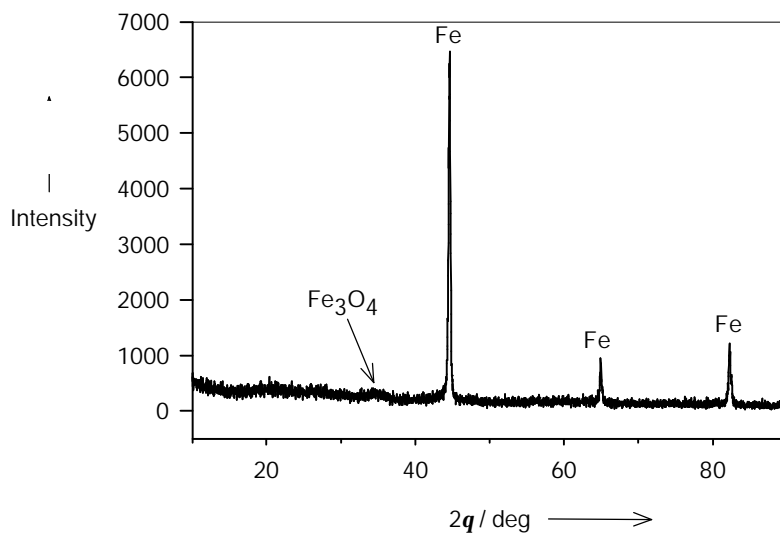


Figure S5. XRD pattern of Mag-MCF-C.

The peak for Fe_3O_4 identified in XRD pattern of Mag-MCF-C (see Figure S4) indicates that $\alpha\text{-Fe}$ is partially converted to magnetite (instead of non-magnetic $\alpha\text{-Fe}_2\text{O}_3$) during hot NaOH etching process. It was reported that magnetite is usually formed under basic condition.^[S4] As small Fe_3O_4 peak implies, the oxidation seems not to be significant. This result indicates that Fe_3O_4 passivation layer is formed on the surface of $\alpha\text{-Fe}$, which prevented excess oxidation of $\alpha\text{-Fe}$ under etching conditions (at 100 °C under air).

2. Characterization of Core-shell Morphology of MNPs Using Convergent Beam Electron Diffraction (CBED) and Energy Dispersive X-ray Spectroscopy (EDX).

Core-shell structure of MNP was characterized by combination of CBED and EDX. The beam size was 25 nm. When the convergent beam was focused on the shell part of MNPs, the molar ratio of oxygen to iron was 1.10. While the convergent beam was moved to core part, the molar ratio of oxygen to iron was decreased to 0.22. This result clearly shows that the iron core was surrounded with iron oxide shell (see Figure S5).

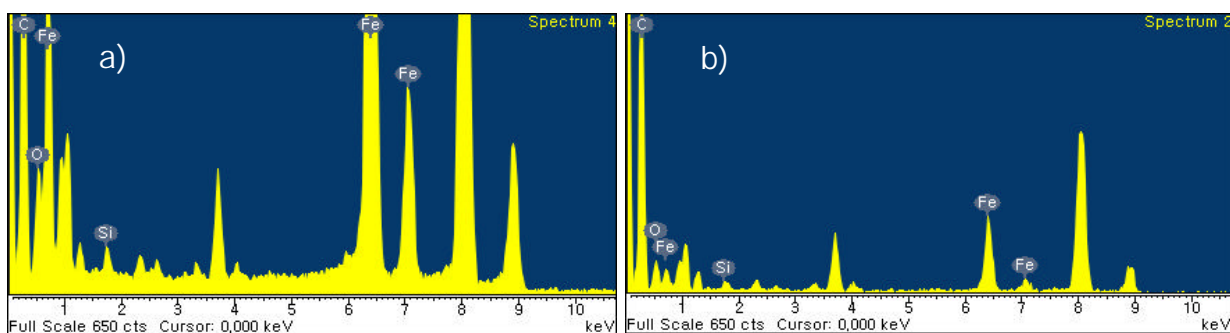


Figure S6. EDX data of (a) core part and (b) shell part.

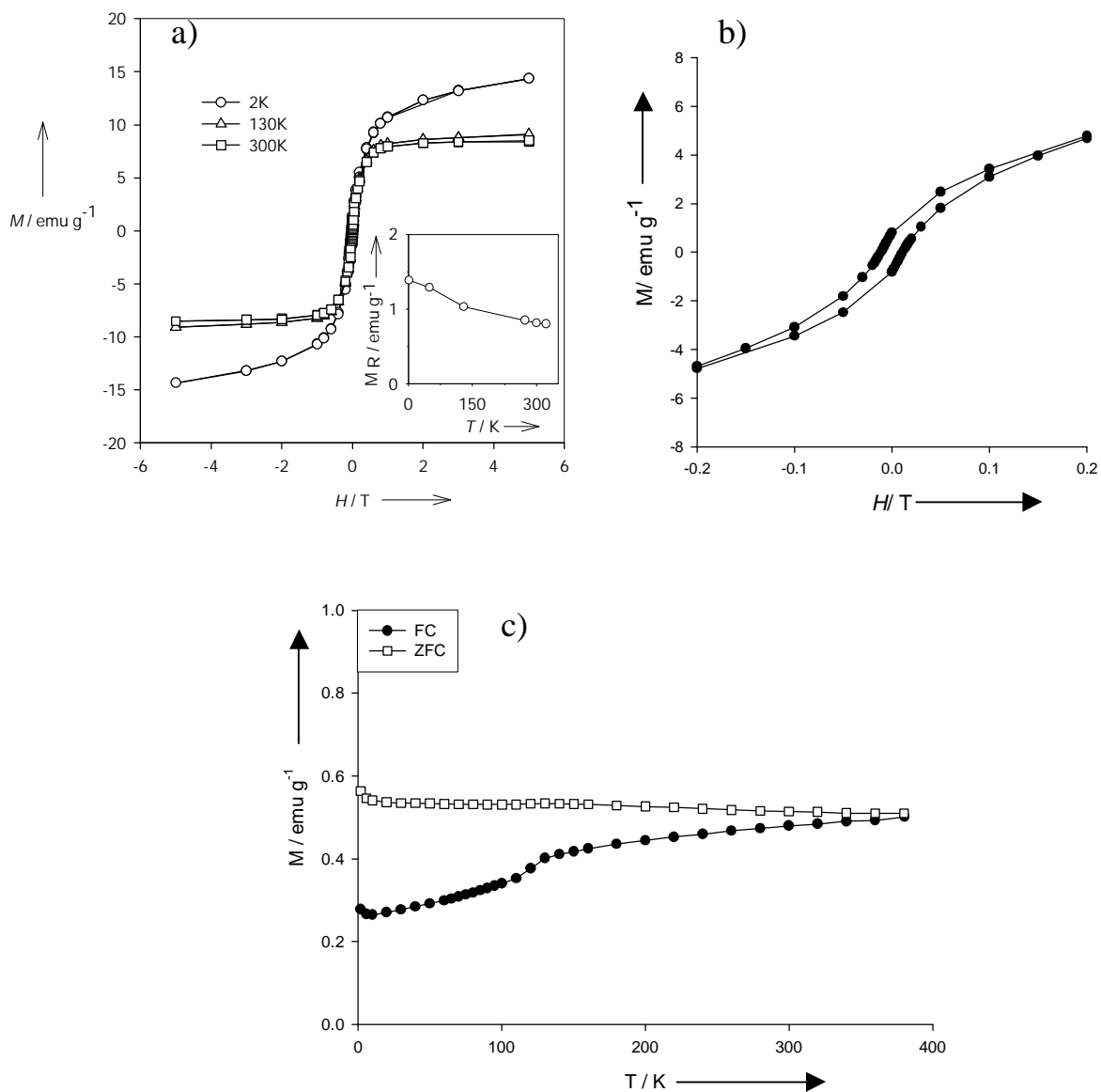


Figure S7. (a) Magnetization vs. applied magnetic field of Mag-MCF-C. (inset) Temperature dependent remanent magnetization. (b) enlarged magnetic data obtained at 300 K to show the hysteresis. (c) Zero-field cooled and field-cooled magnetization data measured in an applied field of 100 Oe. This result clearly shows that the magnetic nanoparticles in Mag-MCF-C is ferromagnetic at room temperature.

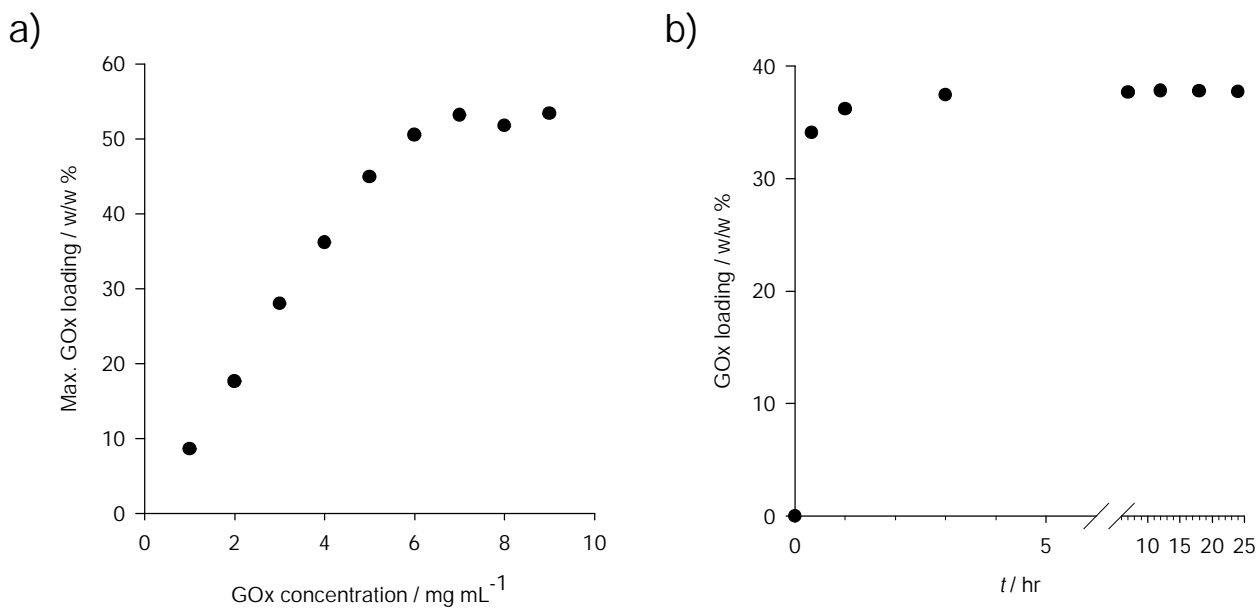


Figure S8. (a) Adsorption isotherm of GOx into Mag-MCF-C. The Mag-MCF-C (10 mg) was incubated in GOx solution of specified concentration under shaking (at 250 rpm) at 25 °C for 48 hr to get the maximal enzyme loading. (b) Time-course of GOx adsorption into Mag-MCF-C. The Mag-MCF-C (10 mg) was incubated in 4 mg/mL GOx solution under shaking (at 250 rpm) at 25 °C.

Data points represent the average of duplicate experiments.

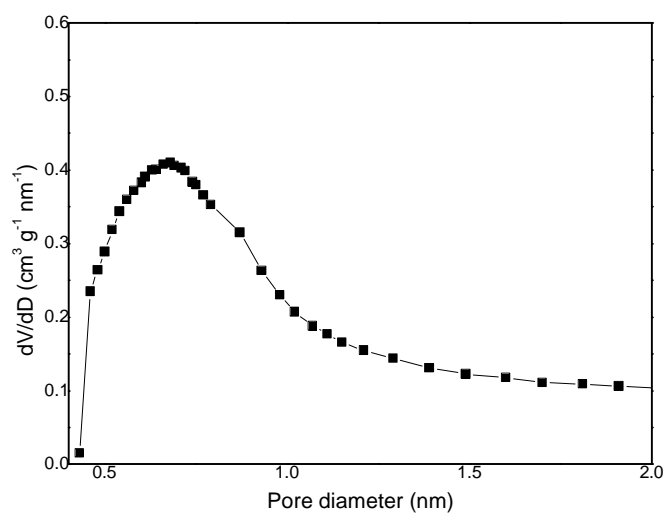


Figure S9. The distribution of micropore sizes in Mag-MCF-C. Micropore size was centered at 0.65 nm and micropore volume is $0.321 \text{ cm}^3 \text{ g}^{-1}$. The micropore below 2 nm was calculated from the analysis of the adsorption branch of Ar isotherms using the Horvath-Kawazoe formalism.

S10. Concentration of GOx in Mag-MCF-C

The concentration of GOx in Mag-MCF-C can be estimated from the GOx loading and the volume of pores with sizes larger than 7.7 nm ($0.78 \text{ cm}^3/\text{g}$ -calculated from cumulative pore volume data).

Since 300 mg of GOx is immobilized in 1 g Mag-MCF-C having 0.78 mL total pore volume, the concentration of GOx in the pores of Mag-MCF-C is calculated to be 384 mg/mL.

4. References

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