Carbon nanotube structured biomimetic catalyst for polysaccharide degradation

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Supplementary Information

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Functionalization of carbon nanotube (CNT): Multi walled carbon nanotube (WMCNT) (40 - 70 nm diameters) was purchased from Wako (Japan). CNT was functionalized by conventional acid treatment. The CNT were mixed with acid mixture (HNO_3:H_2SO_4, 1:3, (v/v)) for 12 h at 140^{o}C under refluxing condition to introduce a carboxyl group on their surface. After acid treatment, functionalized CNT was washed with excess amount of milli-Q water for neutralization.

Fabrication of functionalized CNT matrix: The functionalized CNT matrix was fabricated by casting the functionalized CNT solution (1 mg/mL in ethanol) onto a silane-treated glass substrate surface. After casting, the matrix was stored in an oven at 80 ^{o}C, overnight.

Scanning electron microscope (SEM) observation of CNT matrix: The CNT matrix was imaged using a SEM (FEI Company, DB 235).

Fourier transform infrared spectroscopy (FT-IR) measurement of CNT: FT-IR reflection spectra were obtained using an FT-720 (HORIBA, Japan) with a diamond detector device. The measurements were performed with an average of 49 scans and at a resolution of 4 cm^{-1}. IR spectra were collected from 650 to 4000 cm^{-1}.

Enzymatic measurement of glucose concentration: The glucose concentration as a product from cellobiose via the catalytic reaction of bio-mimetic catalyst was determined by enzymatic methods. We opted for a three enzyme-based assay over Miller's dinitrosalicylic acid-based method because the former is relatively more sensitive, rapid, and consumes less reagents. The glucose in the 24 h reacted solutions was determined by using the following reaction:

$$\alpha\text{-D-glucose} \xrightarrow{\text{mutarotase}} \beta\text{-D-glucose}$$

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} H_2O_2 + \text{Gluconate}$$

$$2H_2O + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Red pigment} + 4H_2O$$

The hydrogen peroxide formed red chromophores through quantitative oxidation

condensation with phenol and 4-aminoantipyrine. The glucose concentration was obtained by measuring the absorbance of the chromophore using a standard glucose.

Neutralization titration of sodium hydroxide with COOH-CNT: The neutralization titration method was used for estimating the number of carboxylic groups on COOH-CNT. The COOH-CNT solution (1 mg/mL in H_2O) was titrated into 10 mL of sodium hydroxide solution (1 mM), incrementally. During titration, the change in pH was detected using a pH meter.

Supplementary Results

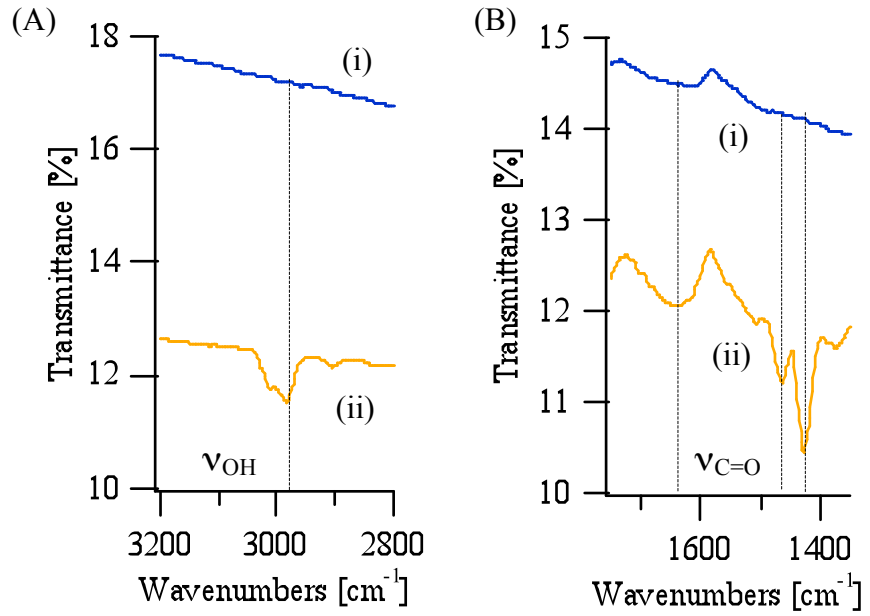

Figure SI 1. FT-IR spectra of CNT and COOH-CNT: (A) The range of OH bending vibration derived from carboxylate, (i) CNT, (ii) COOH-CNT. (B) The range of C=O bending vibration derived from carboxylate, (i) CNT, (ii) COOH-CNT.

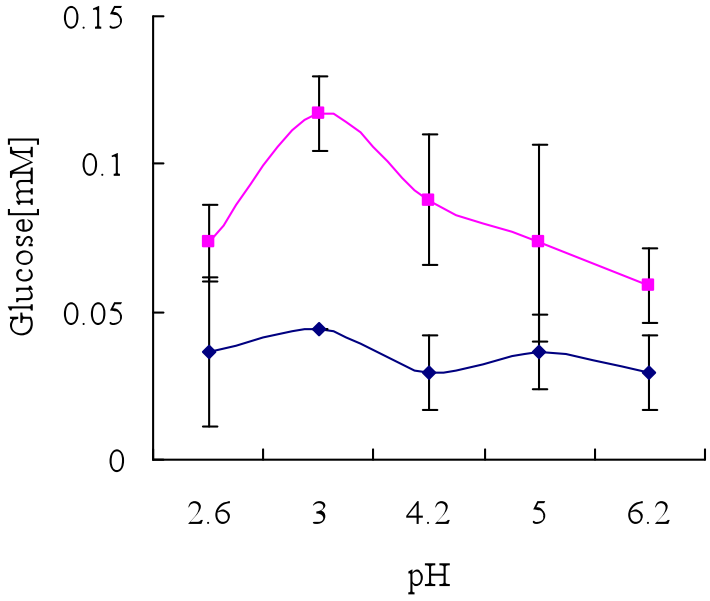

Figure SI 2. Effect of pH on nano-structure based catalyst against degradation of

cellobiose: (i) CNT matrix, (ii) COOH-CNT matrix.

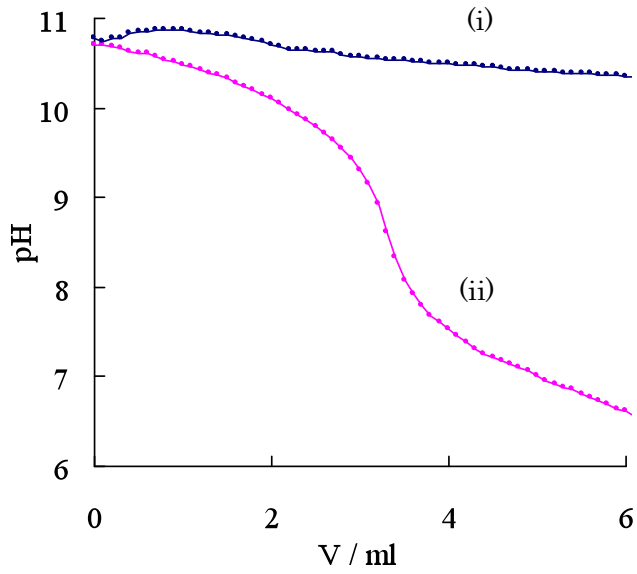

Figure SI 3. Neutralization titration of sodium hydroxide using the COOH-CNT: (i) CNT, (ii) COOH-CNT.

Estimating the number of carboxyl group on COOH-CNT:

During titration, we have the following main reaction:

$CNT\text{-}(COOH)_X + X \cdot NaOH \rightarrow CNT\text{-}(COONa)_X + X \cdot H_2O$

To estimate the number of carboxylic group on COOH-CNT (n[units/CNT]), the following equation was used:

$$X = \frac{n_{NaOH}}{m_{CNT\text{-}(COOH)n} / M_{CNT\text{-}(COOH)n}}$$

n_{NaOH}: 10 [μmol], $m_{CNT\text{-}(COOH)n}$: 3.3 [mg] (the amount of COOH-CNT consumed until equivalence point). $M_{CNT\text{-}(COOH)n}$: 1.15×10^8 [g/mol] (The molecular weight of CNT was calculated on the assumption that one CNT is rod-like structure which has 55 nm in diameter and 1.2 μm in lengths and the length of carbon-carbon bonding is 1.42 angstrom.[1 in SI] As a result, the estimated number of carboxylic group on CNT (n) was 3.48×10^5 [unit/CNT].

Evaluation of the number of active sites per one CNT in a bio-mimetic catalyst:

To estimate the number of the catalytic site per one molecule of catalyst (Y), first we calculated the specific reaction efficiency of cellobiose degradation per one molecule of catalyst (E) using following equation:

$$E\ [\mathrm{nmol/min/molecule}] = \frac{\text{Specific activity [nmol/min/mg]}}{\text{Total number of molecule in one mg}}$$

Supplementary Table 1. Specific activity and total number of molecules of specified catalyst.

Catalyst	Specific activity nmol/min/mg	Molecular weight	Total number of molecule molecule/mg	References
exoglucanase (derived from *Aspergillus sp.*)	440	57500	1.05×10^{16}	13
β-glucosidase (derived from *Aspergillus sp.*)	1020	40000	1.52×10^{16}	13
β-glucosidase (derived from apple seed)	380	120 (kDa)	5.02×10^{15}	14
Bio-mimetic artificial catalyst	3.82	1.15×10^{8}	5.64×10^{12}	This work

Next, we estimated the number of catalytic sites per one CNT in bio-mimetic catalyst, by taking a ratio of the specific reaction efficiency of bio-mimetic catalyst and the natural enzyme (Y). We used the the assumption that each enzyme has one catalytic site and this stite contributes to the cellobiose degradation reaction.

$$Y\ [\mathrm{unit/molecule}] = \frac{E_{\text{Bio-mimetic catalyst}}\ [\mathrm{nmol/min/molecule}]}{E_{\text{Enzyme}}\ [\mathrm{nmol/min/molecule}]}$$

Supplementary references:

(1) Yin. M. T.; Cohen. M. L. *Phys. Rev. B*. **1984**, 29, 12, 6996-6998.

Electronic Supplementary Material (ESI) for Chemical Communications