Supplementary Material for:

Biologically Synthesized or Bioinspired Process-Derived Iron Oxides as Catalysts for Living Cationic Polymerization of Vinyl Ether

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Experimental Section

Materials IBVE (TCI; >99.0%), ethyl acetate (Wako; >99.5), and heptane (Nacalai Tesque; >99.0%) were distilled twice over calcium hydride before use. 1,4-Dioxane (Wako; >99.5%) was distilled over calcium hydride and then lithium aluminum hydride. Toluene (Wako; 99.5%) was dried using solvent purification columns (Glass Contour). An adduct of IBVE with HCl [$CH_3CH(O\textit{i}Bu)Cl$; IBVE–HCl] was prepared from the addition reaction of IBVE with HCl.[1] A commercially available α-Fe_2O_3 (Aldrich; 99+%; the crystal structure was determined by powder X-ray diffraction) was used after crushing with a mortar. $\textit{n}Bu_4NCl$ (Fluka; >99.0%) was used without further purification.

The Isolation and Purification of Bacterial Fe_3O_4 Magnetotactic bacteria, *Magnetospirillum magneticum* strain AMB-1 (ATCC700264), were microaerobically grown in an 8-liter fermenter as described previously.[2] After 7 days of cultivation, the cells were harvested by centrifugation and disrupted using a French press (1100 kg/cm^2). The bacterial Fe_3O_4 from the disrupted cells was then collected using a neodymium boron magnet and washed 10 times with HEPES buffer (pH 7.0). The organic components surrounding the bacterial Fe_3O_4 were removed by treating the Fe_3O_4 3 times with 1% (w/v) SDS in a 100 ºC water bath for 30 min.[3] During this process, the sample was treated briefly in an ultrasonic bath. The obtained Fe_3O_4 was then washed with HEPES buffer, ethanol, and toluene. After washing, the Fe_3O_4 was dried in air for 1 day. Before use, the Fe_3O_4 was heated at 100 ºC under reduced pressure for 10 min to remove loosely adsorbed water.

The Synthesis and Characterization of Iron Oxide via a Microbial-Mineralization-Inspired Process An aqueous solution containing 25 mM of disodium dihydrogen ethylenediamine tetraacetate (EDTA, Kanto; 99.5 %) was prepared in a polypropylene vessel using purified water. Then, 25 mM of iron chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, Kanto 99.0 %) was dissolved in 100 mL of the aqueous EDTA solution. The pH of the aqueous solution containing EDTA and $FeCl_2$ was adjusted to 11 using 10 M of NaOH (Junsei 96.0 %) aqueous solution. The precursor solutions were maintained at 90 ˚C for 1 day without stirring. The precipitates were centrifuged and washed with purified water. Then, the resultant powder was dried at 60 ˚C. The crystal phase was identified using powder X-ray diffraction (XRD, Rigaku MiniFlex II, Cu-Kα radiation equipped with a graphite monochromator). Fourier-transform infrared (FT-IR) absorption spectroscopy (Bruker, Alpha-T) was also used to confirm the crystal phase. The FT-IR spectra were obtained using the KBr method. The sample morphologies were observed using a field-emission scanning electron microscope (FESEM, FEI Sirion) and a field-emission transmission electron microscope (FETEM, FEI Tecnai F20 operated at 200 kV). The specific surface area was calculated by the BET method using nitrogen adsorption isotherms obtained at 77 K (Micromeritics, TriStar 3000). Before measuring the specific surface area, the samples were dried under vacuum at 60 ˚C for 5 h.

Thermogravimetric analysis (TG, Seiko TG-DTA 6200) was performed under air atmosphere to estimate the amount of the adsorbed water and hydroxyl groups on the surface of the iron oxides. About 5–10 mg of the specimen powder was put in a platinum pan. Then, the temperature was rose from room temperature to 1000 ºC by ºC the rate of temperature increase set at 5 ºC min^{-1}.

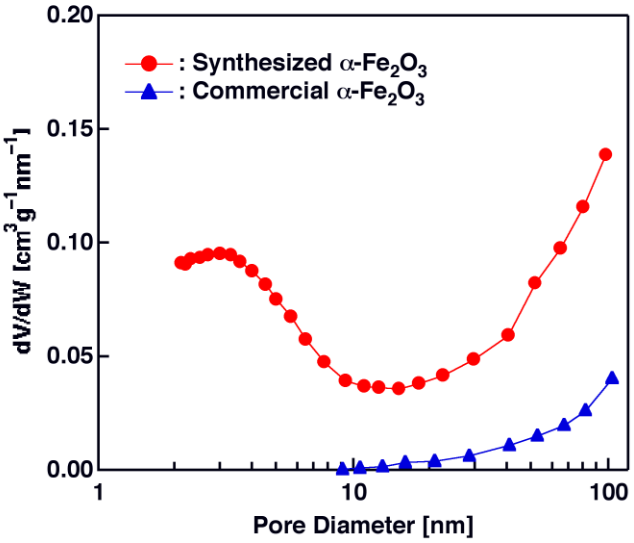
Polymerization Procedures The following is a typical polymerization procedure (the Fe_3O_4 system is used as an example). A glass tube equipped with a three-way stopcock was dried using a heat gun (Ishizaki; PJ-206A; the blower temperature was ~450 ºC) under dry nitrogen for 8 min. Fe_3O_4 (3 mg; 1 × 10^{-2} mmol) was loaded into the tube in air and heated with the heat gun under reduced pressure for 10 min. The heating temperature was controlled by maintaining a suitable distance between the heat gun and the bottom of the tube. The temperature was monitored using a Peltier-type thermometer (Custom; CT-800WP and LK-800WP). After the tube was purged with N_2 and cooled to room temperature, toluene (1.68 mL), heptane (0.12 mL), 1,4-dioxane (0.20 mL; 2.3 mmol), and IBVE (0.25 mL; 1.9 mmol) were successively added into the tube using dry syringes. The polymerization was initiated by the addition of a pre-chilled 40 mM IBVE–HCl solution (0.25 mL; 1.0 × 10^{-2} mmol) in toluene at 0 ºC. During the polymerization, the heterogenous reaction mixture was magnetically stirred using a small, handmade stirring bar constructed from a thin glass tube and a wire. A small amount (~0.05 mL) of the reaction mixture was extracted several times during the reaction to determine the degree of monomer conversion from the residual concentration of IBVE, which was measured using gas chromatography with heptane (5 vol%) as an internal standard. The reaction was finally terminated through the addition of pre-chilled methanol that contained a small amount of an aqueous ammonia solution. The catalyst was removed using a magnet (in reactions using α-Fe_2O_3, the catalyst was separated by centrifugation and decantation). The polymer product was obtained by evaporating the remaining toluene, 1,4-dioxane, and IBVE.

For experiments examining the reuse of the catalyst, the separated iron oxides were successively washed with acetone, ethanol, acetone, and hexane. After washing, the iron oxides were dried in air for a few days. The oxides were heated at a determined temperature under reduced pressure immediately before the 2nd polymerization.

Two different lots of Fe_3O_4 were used for the experiments: the first lot corresponds to entry 1 in Table 1, Figures 2 and S2, and the second lot corresponds to entries 2–5 in Table 1. In addition, two different lots of α-Fe_2O_3 were used: the first lot corresponds to entries 6–8 in Table 1, Figures 3, 4, and S10, and the second lot corresponds to Figures S5 and S11.

Characterization of Polymers The molecular weight distribution (MWD) of the polymers was measured by gel permeation chromatography (GPC) in chloroform at 40 ºC with three polystyrene gel columns [TSK gel G-4000H_{XL}, G-3000H_{XL}, and G-2000H_{XL}; exclusion limit molecular weight = 4×10^5, 6×10^4, and 1×10^4, respectively; bead size = 5 mm; column size = 7.8 mm (internal diameter) × 300 mm; flow rate = 1.0 mL/min] connected to a Tosoh DP-8020 pump, a CO-8020 column oven, a UV-8020 ultraviolet detector, and an RI-8020 refractive-index detector. The number-average molecular weight (M_n) and polydispersity ratio [weight-average molecular weight/number-average molecular weight (M_w/M_n)] were calculated from the chromatographs with respect to 16 polystyrene standards (Tosoh; M_n = 577–1.09×10^6, $M_w/M_n \leq 1.1$). Nuclear magnetic resonance (NMR) spectra were recorded using a JEOL JNM-ECA 500 spectrometer (500.16 MHz for ^{1}H and 125.77 MHz for ^{13}C).

Analysis of Iron Oxides after Heat-Treatment After the thermal treatment, the XRD patterns, FT-IR spectra, and specific surface area were measured using the same methods as those before the thermal treatment.



α-Fe$_2$O$_3$	Specific area (m^{2}g^{-1})
Synthesized	62.3
Commercial	2.0

Fig. S1 BET surface area analysis of microbial-mineralization-inspired process-derived α-Fe_2O_3 and commercially available α-Fe_2O_3.

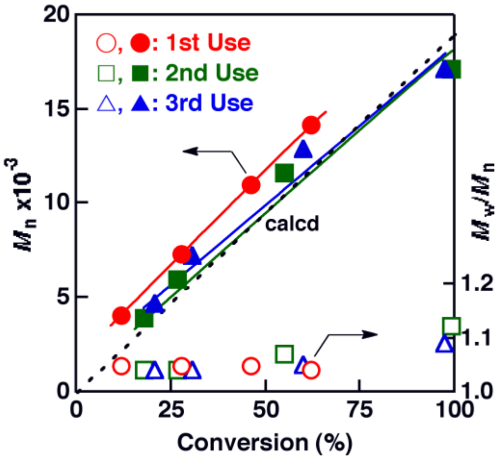


Fig. S2 M_n and M_w/M_n for the polymerization using fresh or reused Fe_3O_4 ($[IBVE]_0$ = 0.76 M, $[IBVE–HCl]_0$ = 4.0 mM, $[Fe_3O_4]$ = 0.7 mol% to IBVE, [1,4-dioxane] = 1.0 M, in toluene at 0 ºC; see Figure 2 for time–conversion curves and MWD curves)

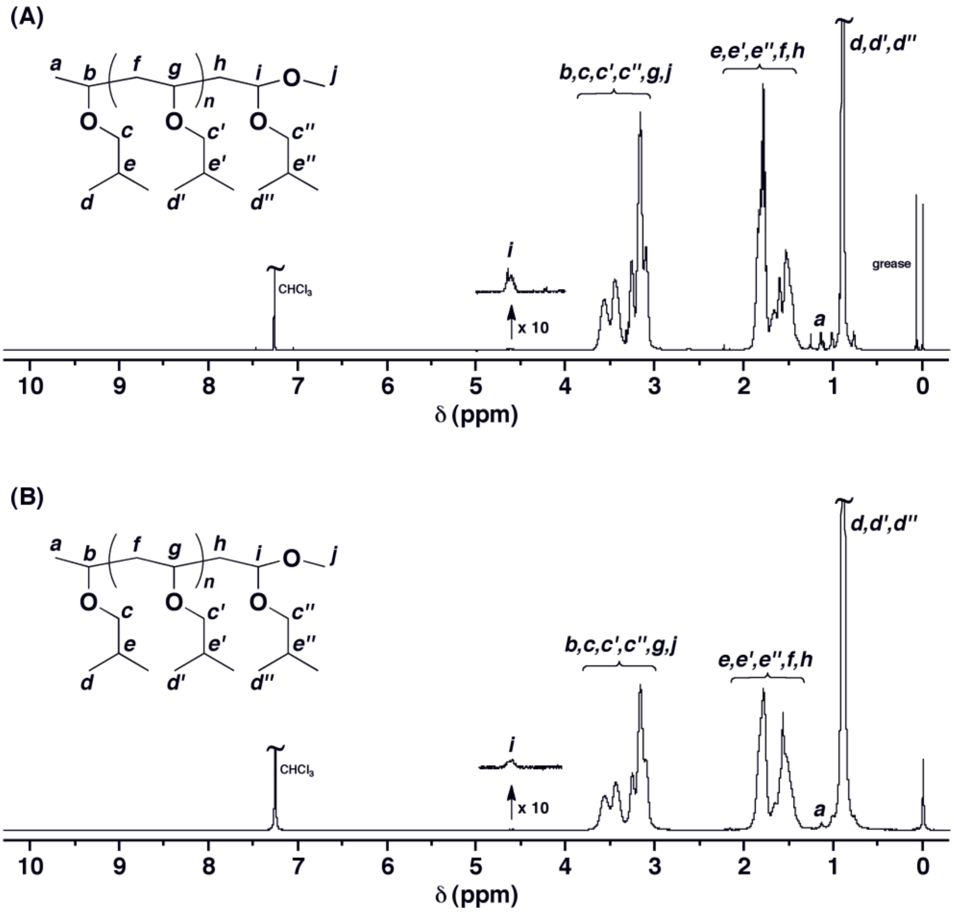


Fig. S3 ^{1}H NMR spectra of poly(IBVE)s obtained with (A) a bacterial Fe_3O_4 and (B) a microbial-mineralization-inspired process-derived α-Fe_2O_3 (in $CDCl_3$ at 30 ºC; analyzed sample: (A) entry 1 and (B) entry 6 in Table 1).

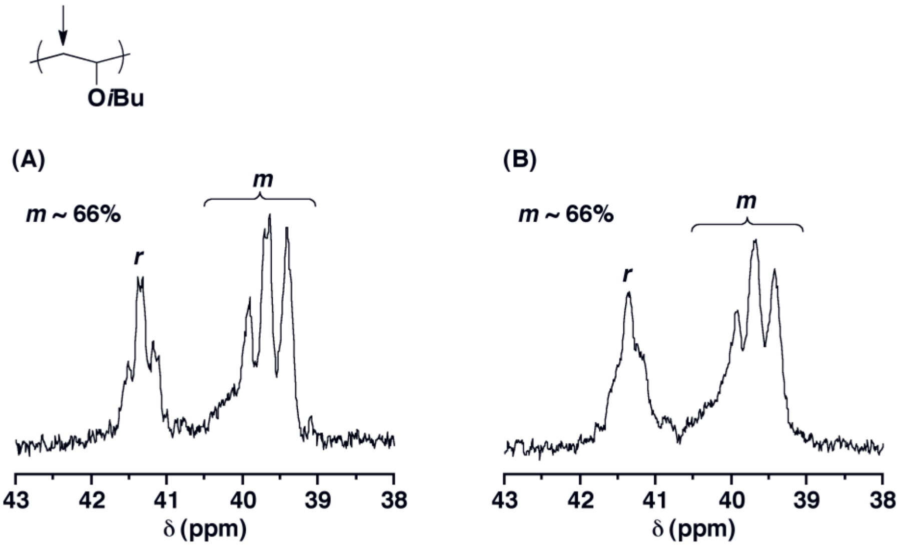


Fig. S4 ^{13}C NMR spectra of poly(IBVE)s obtained with (A) a bacterial Fe_3O_4 and (B) a microbial-mineralization-inspired process-derived α-Fe_2O_3 (the peaks of the main chain methylene carbons; in $CDCl_3$ at 30 ºC; analyzed sample: (A) entry 1 and (B) entry 6 in Table 1).

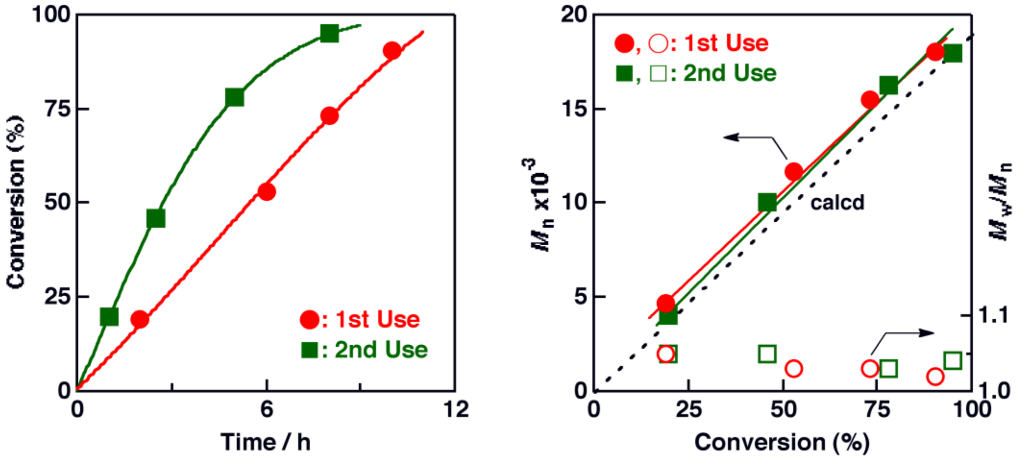


Fig. S5 (A) Time–conversion curves and (B) M_n and M_w/M_n for the polymerization of IBVE using microbial-mineralization-inspired process-derived α-Fe_2O_3 ($[IBVE]_0$ = 0.76 M, $[IBVE–HCl]_0$ = 4.0 mM, [α-Fe_2O_3] = 0.7 mol% to IBVE, [1,4-dioxane] = 1.0 M, in toluene at 0 ºC).

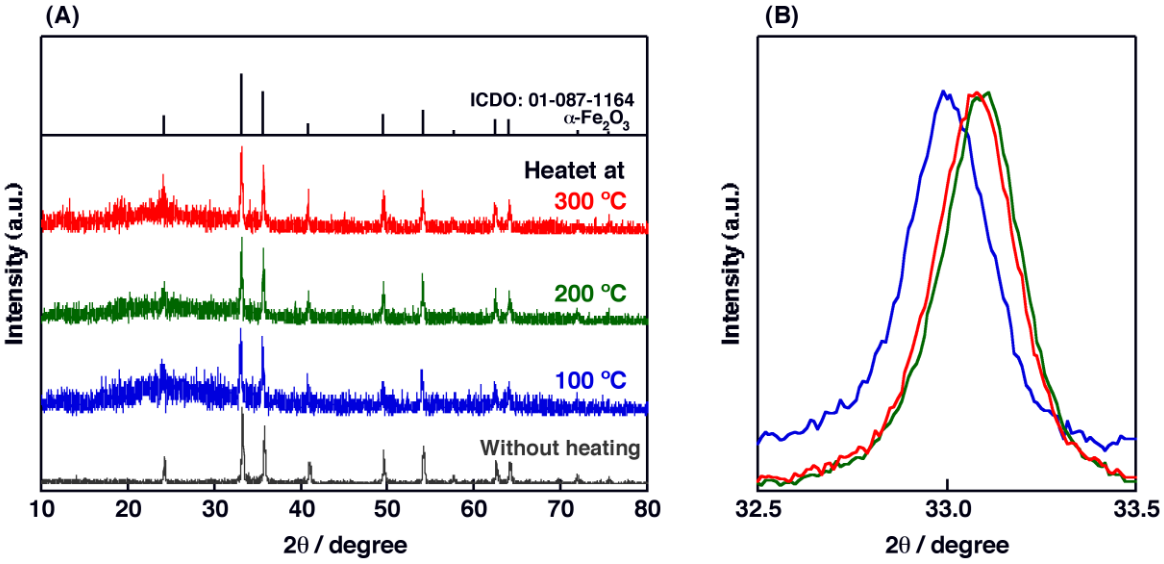
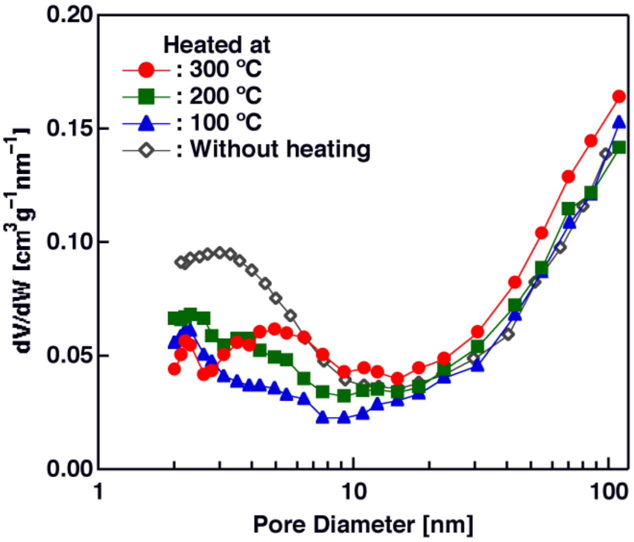


Fig. S6 XRD patterns of microbial-mineralization-inspired process-derived α-Fe_2O_3 heated at 100, 200, or 300 ºC [(A) whole region, (B) enlarged view for comparing half widths].



Heating-temperature	Specific area (m^2g^{-1})
300 °C	56.6
200 °C	63.4
100 °C	61.1
None	62.3

Fig. S7 BET surface area analysis of microbial-mineralization-inspired process-derived α-Fe_2O_3 heated at 100, 200, or 300 °C (N_2 adsorption).

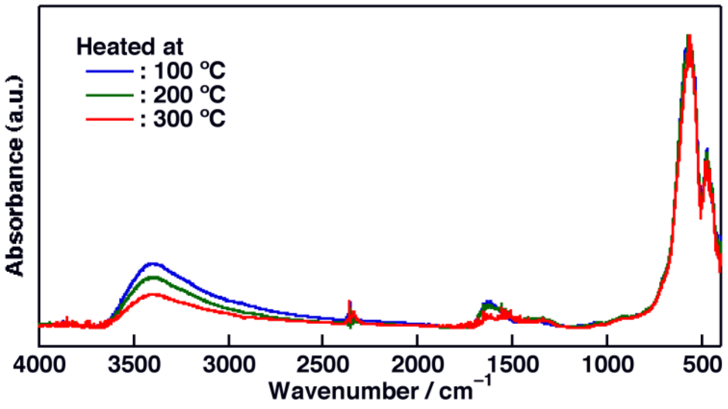


Fig. S8 IR spectra of microbial-mineralization-inspired process-derived α-Fe_2O_3 after heat-treatment (Note: The iron oxides for IR spectrometric analysis were mixed with KBr under air. A part of the OH absorptions may have been attributed to water adsorbed during the preparation step).

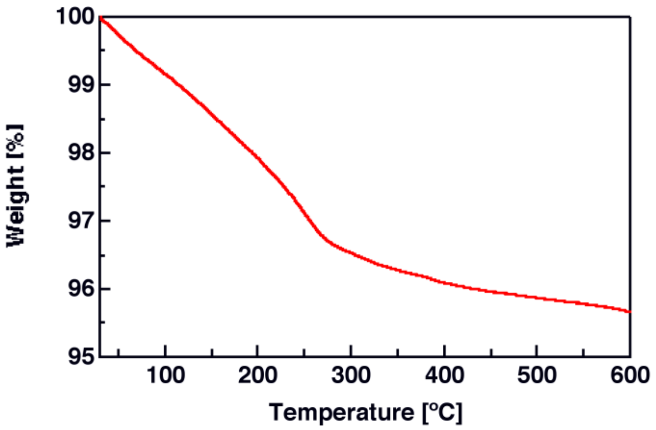


Fig. S9 Thermogravimetric analysis of microbial-mineralization-inspired process-derived α-Fe_2O_3 (the weight loss is probably attributed to the removal of chemically and/or physically adsorbed water molecules).

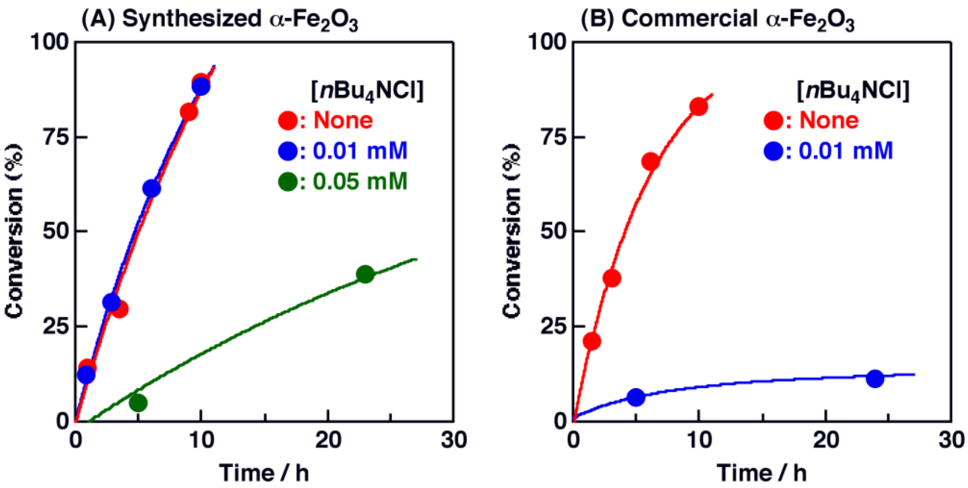


Fig. S10 Time–conversion curves for the polymerization of IBVE using microbial-mineralization-inspired process-derived α-Fe_2O_3 or commercial α-Fe_2O_3 in the presence of a small amount of *n*Bu_4NCl ($[IBVE]_0$ = 0.76 M, $[IBVE–HCl]_0$ = 4.0 mM, [α-Fe_2O_3] = 0.7 mol% to IBVE, [1,4-dioxane] = 1.0 M, $[n\text{Bu}_4\text{NCl}]_0$ = 0, 0.01, 0.05, or 0.1 mM, in toluene at 0 ºC; the iron oxides were heated at ~300 ºC under reduced pressure for 10 min before use).

Note: The chloride anion derived from *n*Bu_4NCl functions as a strong Lewis base and deactivates the active sites on the iron oxides. As shown in Fig. S10, the polymerization using the synthesized α-Fe_2O_3 proceeded in the presence of 0.05 mM of *n*Bu_4NCl but that using the commercially available α-Fe_2O_3 obviously retarded even with 0.01 mM of *n*Bu_4NCl. The results suggest that the amount of the active sites of the synthesized oxide was roughly estimated to be more than five times larger than that of the commercial oxide.

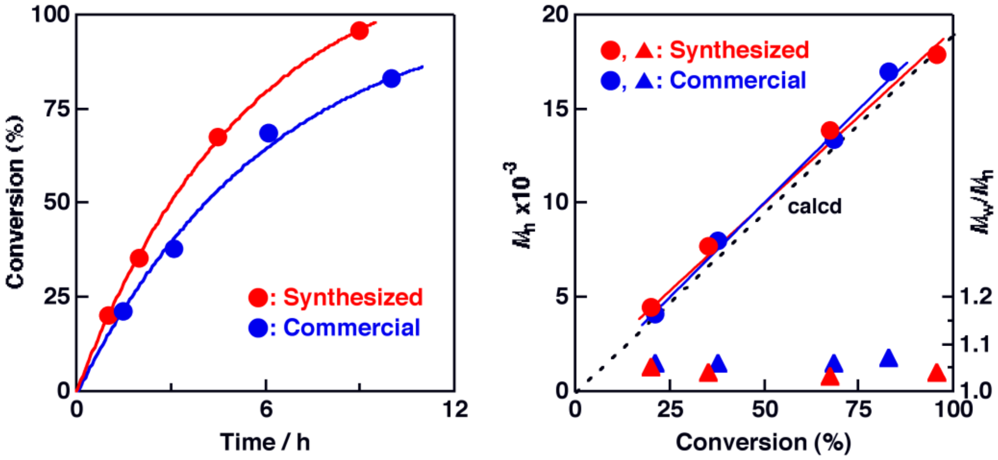


Fig. S11 (A) Time–conversion curves and (B) M_n and M_w/M_n for the polymerization of IBVE using microbial-mineralization-inspired process-derived α-Fe_2O_3 or commercial α-Fe_2O_3 ($[IBVE]_0$ = 0.76 M, $[IBVE–HCl]_0$ = 4.0 mM, [α-Fe_2O_3] = 0.7 mol% to IBVE, [1,4-dioxane] = 1.0 M, in toluene at 0 ºC; the iron oxides were heated at ~300 ºC under reduced pressure for 10 min before use).

References

1. T. Higashimura, M. Kamigaito, M. Kato, T. Hasebe and M. Sawamoto, *Macromolecules*, 1993, **26**, 2670.
2. C. D. Yang, H. Takeyama, T. Tanaka and T. Matsunaga, *Enzyme Microb. Techol.*, 2001, **29**, 223.
3. A. Arakaki, J. Webb and T. Matsunaga, *J. Biol. Chem.*, 2003, **278**, 8745.