Supporting Information for

Bactericidal Effect of Zero-Valent Iron Nanoparticles on *Escherichia coli* in Aqueous Solution

Changha Lee,[†] Jee Yeon Kim,[‡] Won Il Lee,[‡] Kara L. Nelson,[†] Jeyong Yoon,[,‡] and David L. Sedlak[*,†]*

†Department of Civil and Environmental Engineering, 657 Davis Hall, University of California, Berkeley, California 94720, and ‡School of Chemical and Biological Engineering, College of Engineering, Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul 151-742, Korea,

Submitted to Environmental Science & Technology

*Address correspondence to either author.

Phone: +82-2-880-8927 (J. Yoon); +1-510-643-0256 (D. L. Sedlak)

Fax.: +82-2-876-8911 (J. Yoon); +1-510-642-7483 (D. L. Sedlak)

E-mail: jeyong@snu.ac.kr (J. Yoon); sedlak@ce.berkeley.edu (D. L. Sedlak)

S1. Detailed procedure for nano-Fe^0 synthesis (modified from the method by Lowry and Johnson, 2004[1])

1. Dissolve 2.0g $FeSO_4 \cdot 7H_2O$ in 200 ml N_2 saturated DI water with gently stirring the solution.
2. Dissolve 0.4 g of $NaBH_4$ in 50 mL DI water and transfer the solution to a separatory funnel.
3. Add the $NaBH_4$ solution to the $FeSO_4$ solution at the rate of 1 ~ 2 drops per second with N_2 gas bubbling.
4. Stir an additional 10 minutes after adding all $NaBH_4$ solution.
5. Transfer the iron suspension into two 50 ml centrifuge tubes by pipetting the suspension while stirring.
6. Centrifuge for 4 minutes at 4000 rpm.
7. Decant supernatant water and refill centrifuge tubes with N_2 saturated 10^{-4} N HCl solution, and resuspend the iron nanoparticles with vigorous shaking the tube vigorously.
8. Repeat the centrifuge and washing processes (steps 6-7) three times; skip the resuspending at the last time.
9. Make 5 ml suspension (stock suspension) by decanting excess water.
10. The nano-Fe^0 concentration of this stock suspension was determined to be 9.0 g/L by weighing the iron particles obtained by drying the stock suspension in a N_2 oven (overnight at 100^{o}C).

S2. Calculation of nano-Fe^0 exposures ($\int$[nano-Fe^0]dt) from the UV/visible spectrophotometric measurements, and comparison with the *E. coli* inactivation curves.

The time-concentration profile of unoxidized nano-Fe^0 ([nano-Fe^0](t)) under air saturation can be obtained from the time-dependent variation in the UV/visible absorption spectrum of nano-Fe^0 suspension (A(t)) using the following equations; UV absorbance at 390 nm was used in the calculation.

$A(t) = A_1 f_1(t) + A_2 f_2(t)$

A(t) = time-dependent UV absorbance of the nano-Fe^0 suspension of specific initial concentration, [nano-Fe^0]$_0$

A_1 = UV absorbance of the unoxidized nano-Fe^0 (the initial value at 0 min under deaerated conditions)

A_2 = UV absorbance of the fully oxidized nano-Fe^0 (the final value at 60 min under air saturated open conditions)

$f_1(t)$ = time-dependent fraction of the unoxidized nano-Fe^0

$f_2(t)$ = time-dependent fraction of the fully oxidized unoxidized nano-Fe^0

$\Rightarrow A(t) = A_1 f_1(t) + A_2(1 - f_1(t)) = (A_1 - A_2) f_1(t) + A_2$

$\Rightarrow f_1(t) = (A_2 - A(t)) / (A_2 - A_1)$

$\Rightarrow$ [nano-Fe^0](t) = [nano-Fe^0]$_0 \times f_1(t)$ = [nano-Fe^0]$_0 \times (A_2 - A(t)) / (A_2 - A_1)$

Figure S2 gives one example of the [nano-Fe^0](t) calculation using the above equation; [nano-Fe^0](t) with 90 mg/L [nano-Fe^0]$_0$ under air-saturated open condition was calculated from A(t) (Figure 1d in the manuscript). Then, the nano-Fe^0 exposures ($\int$[nano-Fe^0]dt, mg·s/L) can be calculated by integrating oxidant concentrations (mg/L) with respect to time (s): $\text{nano-Fe}^0 \text{ exposure} = \int [\text{nano-Fe}^0]\,dt$. Figure S2 also shows the calculated $\int$[nano-Fe^0]dt as a function of time.

The log inactivation of *E. coli* should be proportional to $\int$[nano-Fe^0]dt. The inset of Figure 2S indicates the linear relation between $\int$[nano-Fe^0]dt and *E. coli* inactivation from Figure 2a. Following the same procedure, we calculated the $\int$[nano-Fe^0]dt values (mg·s/L) in various conditions of [nano-Fe^0]$_0$ corresponding to those of Figure 2b in the manuscript, and presented them in Figure 3a.

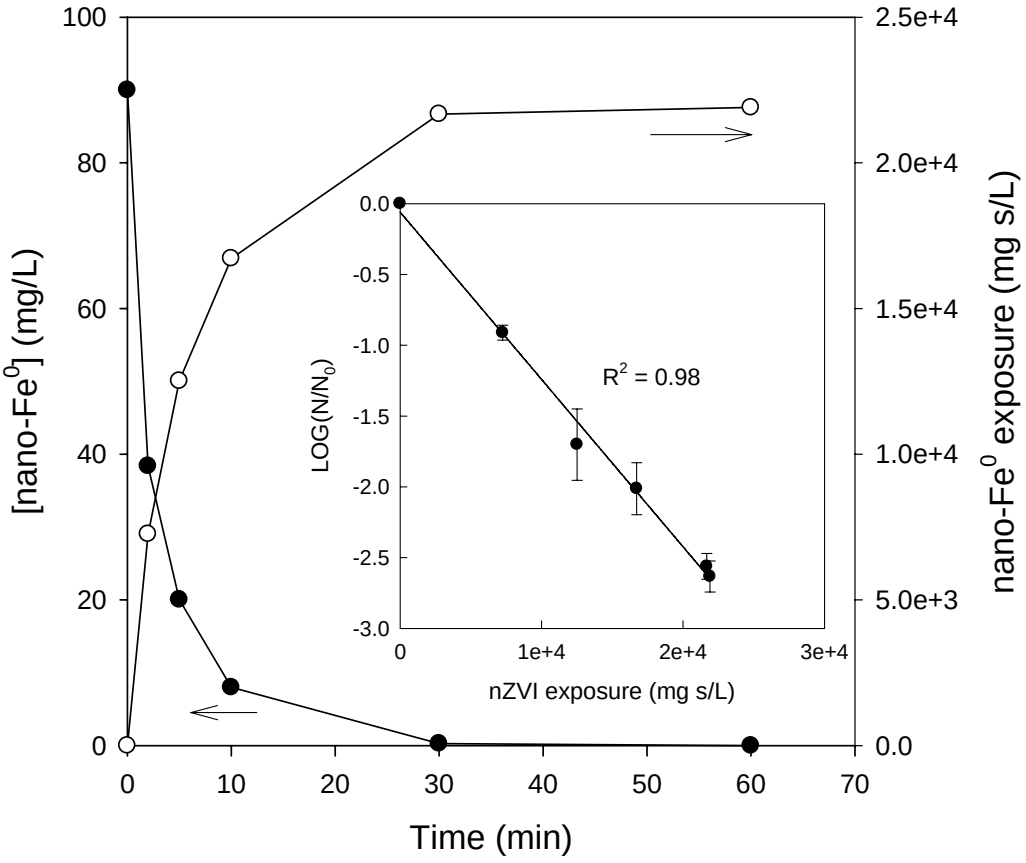


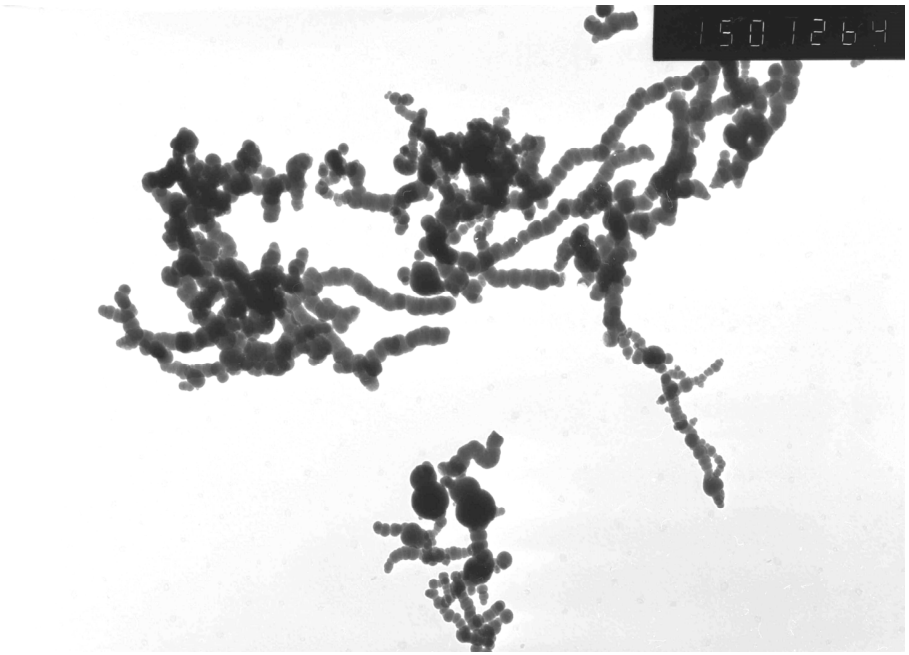
Figure S2. Calculated time profiles of concentration and exposure of nano-Fe^0 under air saturated open condition. Filled circles indicate [nano-Fe^0] (mg/L), and open circles indicate nano-Fe^0 exposure (mg s/L). Inset indicates the linear relation between nano-Fe^0 exposure and *E. coli* inactivation from Figure 2a (pH_0 = 8.0, 2 mM carbonate buffer, [nano-Fe^0]$_0$ = 90 mg/L).

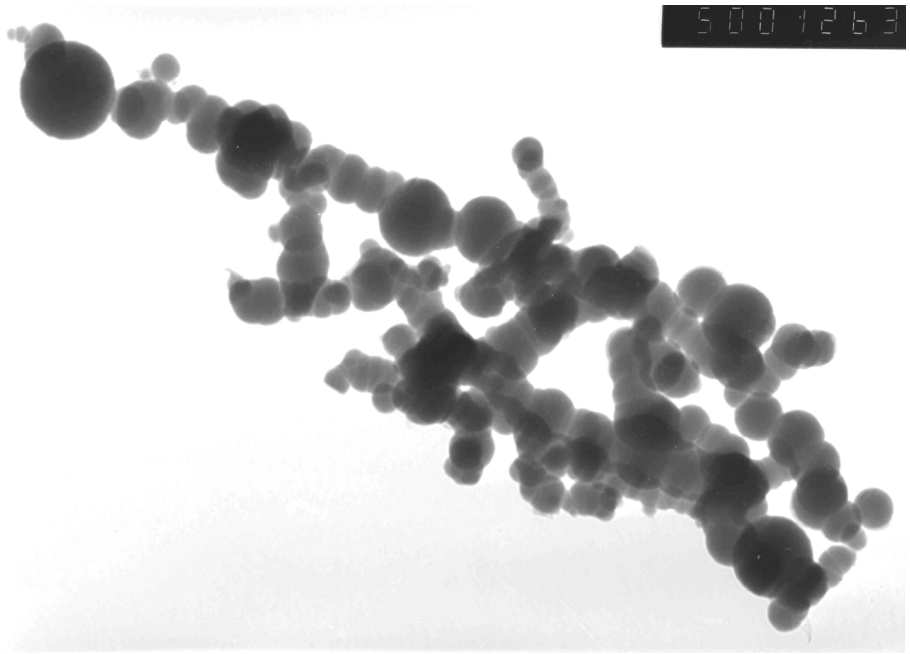
S3. Procedure for nano-Ag^0 synthesis

The synthesis of silver nanoparticles (Nano-Ag^0) via photo-reduction process is well explored[2–6]. Nano-Ag^0 for the experiments 12 & 13 (Table 1 in the manuscript) was simply prepared by photoreduction of silver ion in aqueous solution containing poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers (PEO_{20}-PPO_{70}-PEO_{20}, Pluronic p123, BASF Co.). The aggregates of spherical single nanoparticles ranging over 15 – 20 nm in diameter were observed by TEM analyses[7]. The synthetic procedure is as follows.

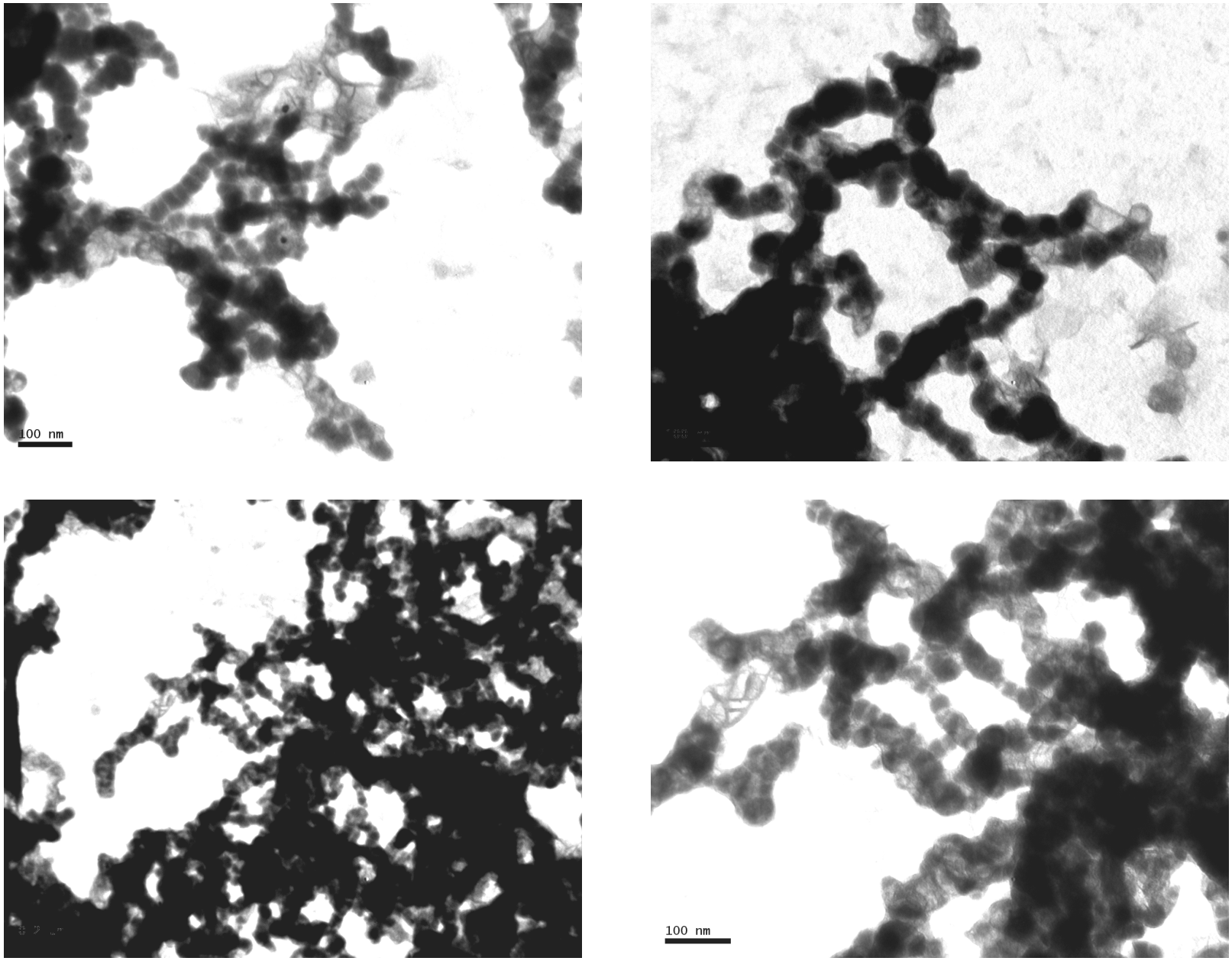
1. Dissolve 1 g Pluronic p123 in 7 ml DI water with gently stirring the solution.
2. Add 17 mg $AgNO_3$ to the Pluronic p123 solution while stirring.
3. Irradiate ultraviolet (UV) light (>350 nm, Black Ray longwave UV lamp B100 AP, UVP Inc.) to the solution for 72 hr.
4. Centrifuge the solution for 10 minutes at 10000 rpm, and dry in vacuum oven at 150^oC for 24 hr

S4. Original TEM images for nano-Fe^0 and *E. coli* cells

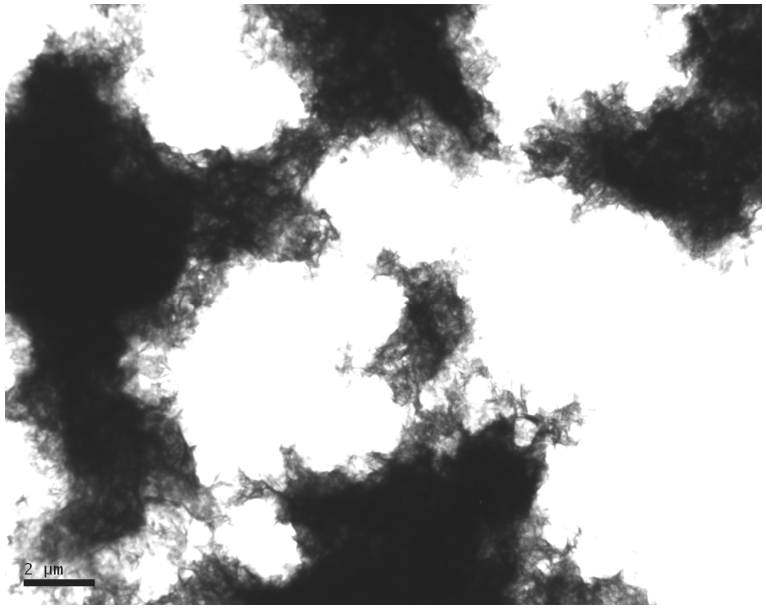


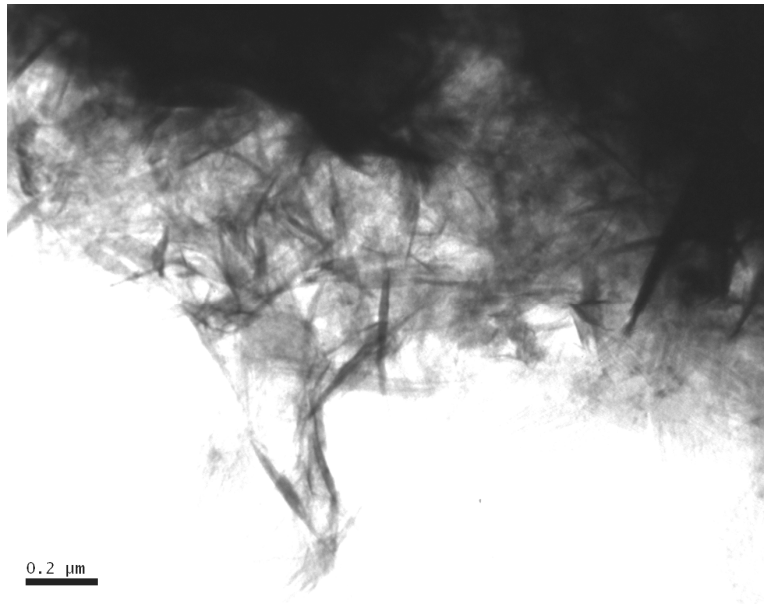


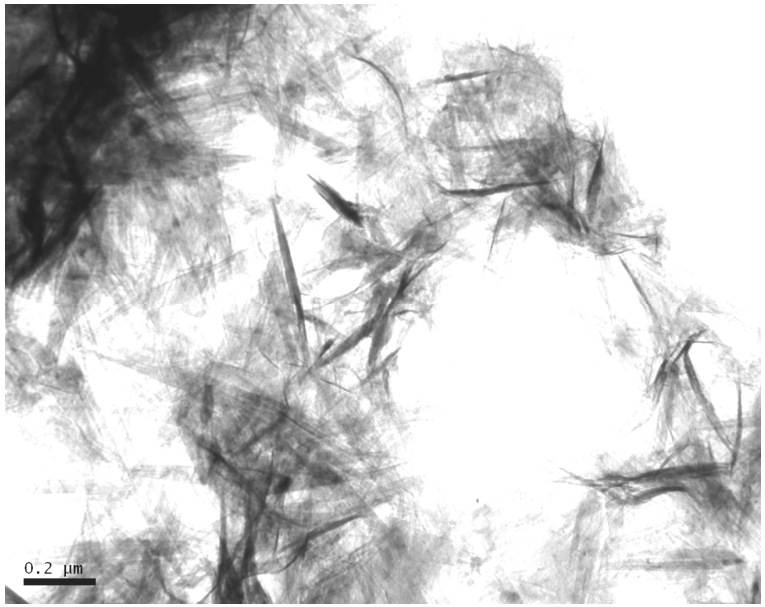
Unoxidized nano-Fe^0

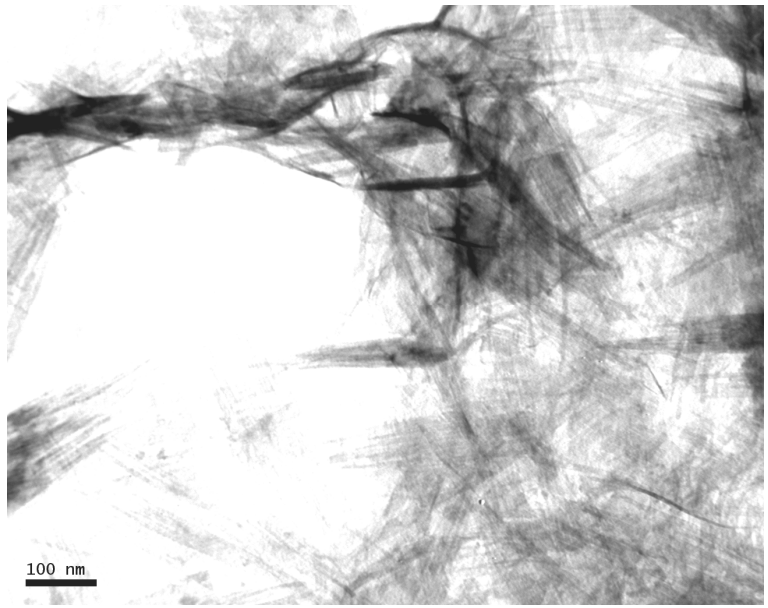


Oxidized nano-Fe^0 (15 sec)

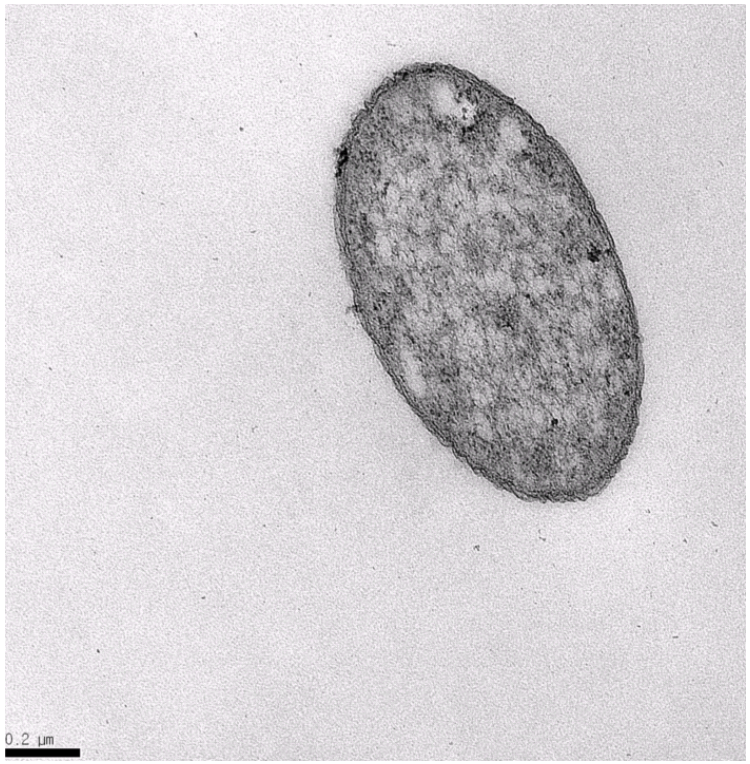


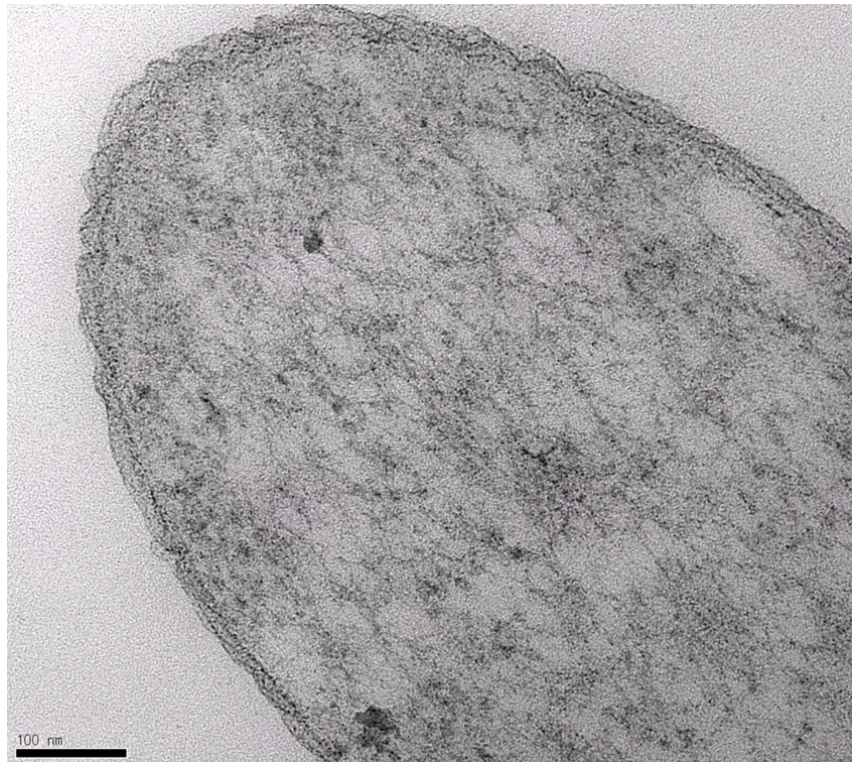






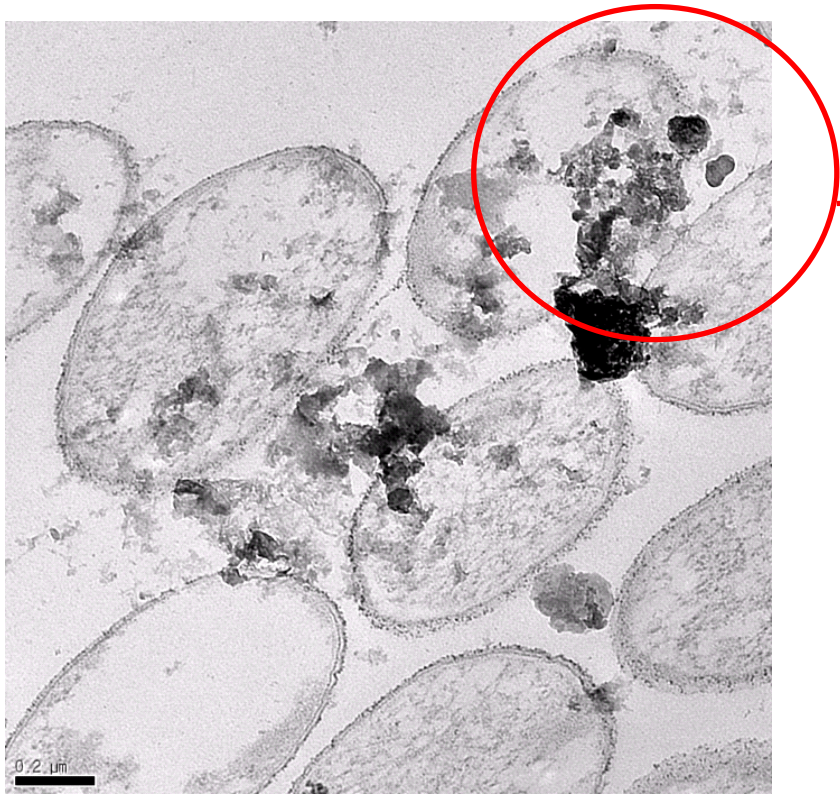
Oxidized nano-Fe^0 (60 min)



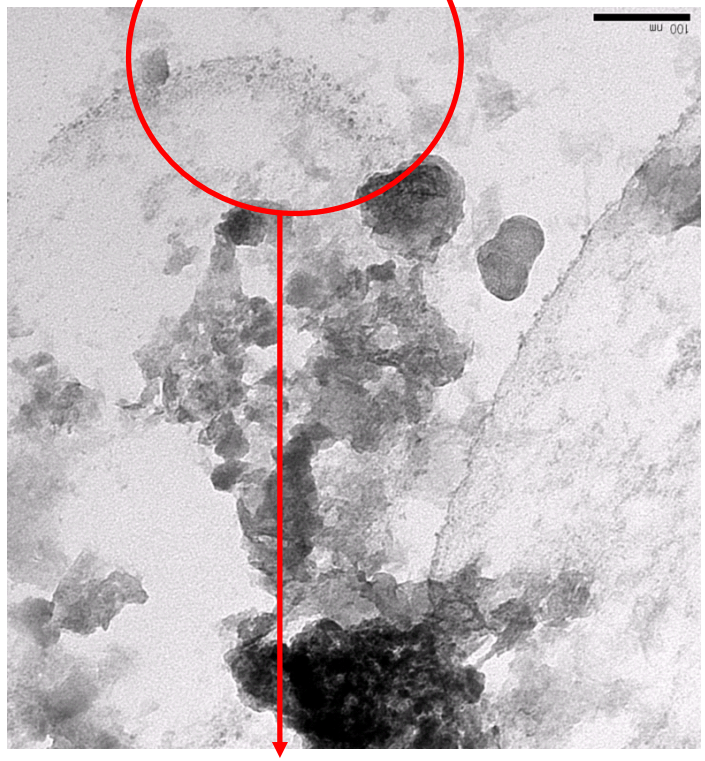


Untreated *E. coli* cells

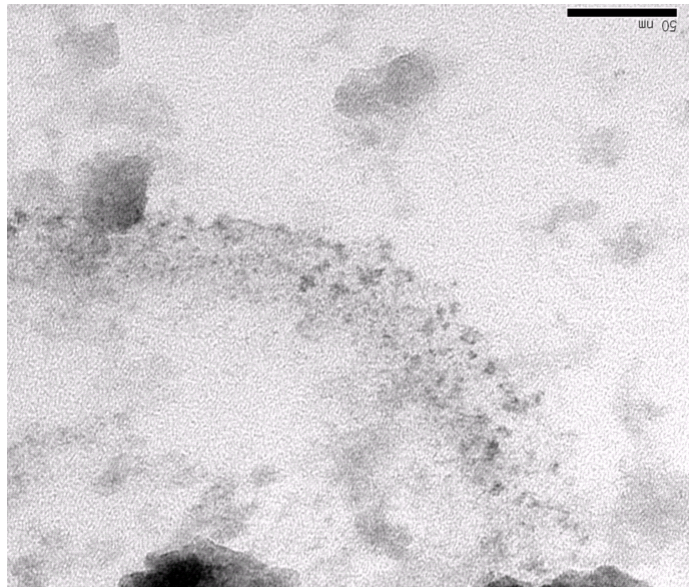
Treated *E. coli* cells with nano-Fe^0



0.2 µm

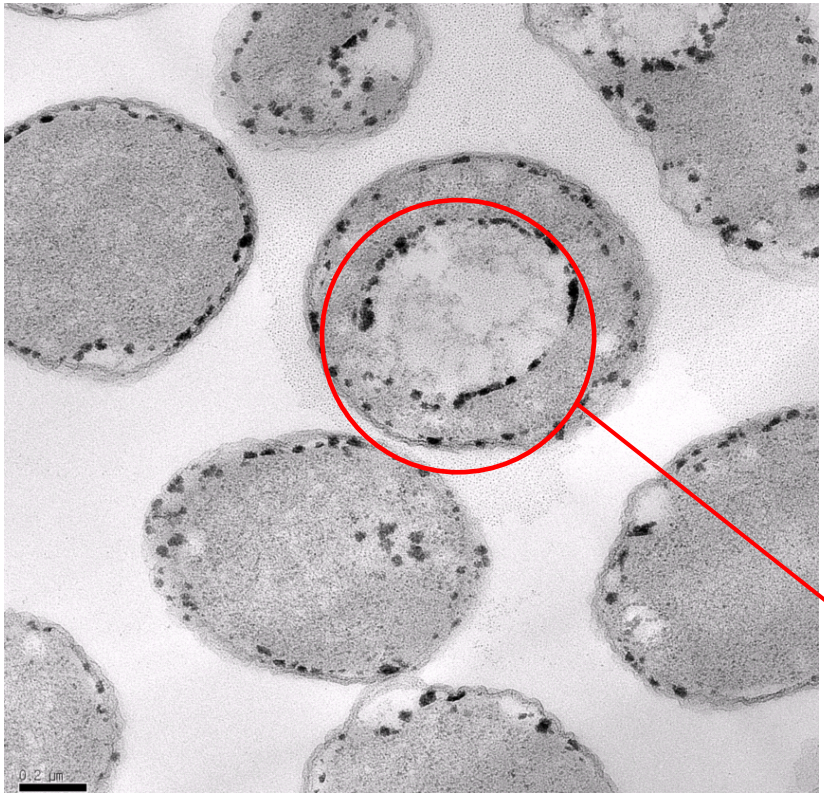


100 nm

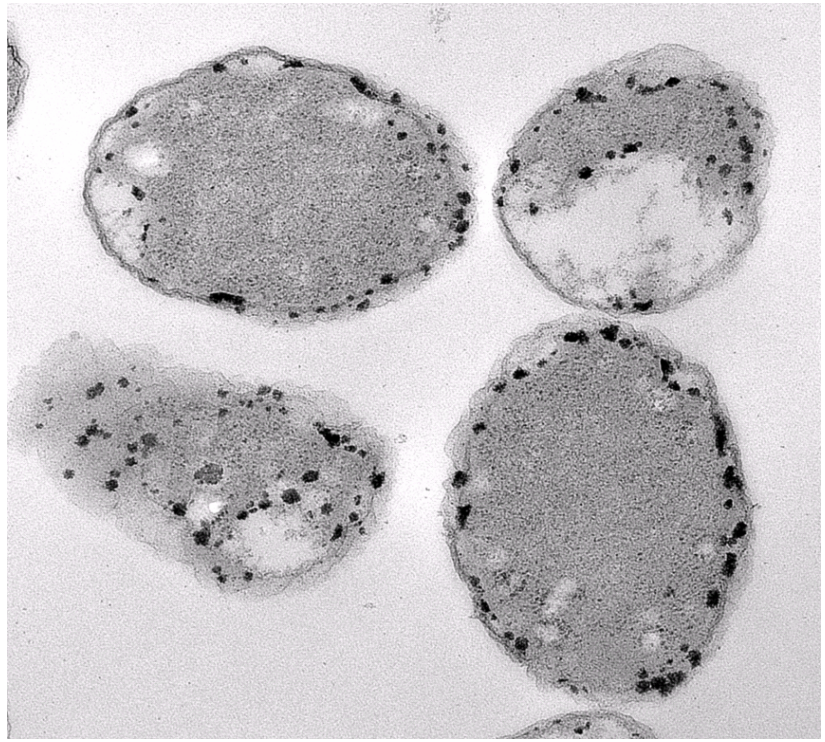


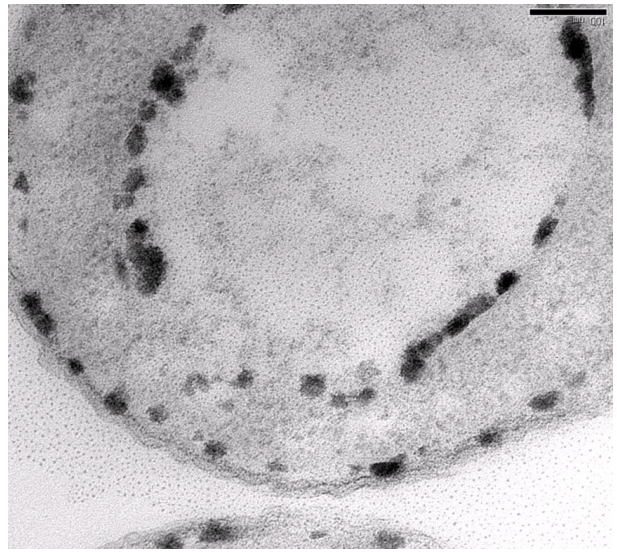
50 nm

Treated *E. coli* cells with Fe(II) ion



0.2 µm





50 nm

REFERENCES

(1) Lowry, G. V.; Johnson, K. M. *Environ. Sci. Technol.* **2004**, *38*, 5208.

(2) Abu Bakar, N. H. H.; Ismail, J.; Abu Bakar, M. *Mater. Chem. Phys.* **2007**, *104*, 276.

(3) Tan, S.; Erol, M.; Attygalle, A.; Du, H.; Sukhishvili, S. *Langmuir* **2007**, *23*, 9836.

(4) Guin, D.; Manorama, S. V.; Latha, J. N. L.; Singh, S. *J. Phys. Chem. C* **2007**, *111*, 13393.

(5) Khanna, P. K.; Singh, N.; Charan, S.; Viswanath, A. K. *Mater. Chem. Phys.* **2005**, *92*, 214.

(6) Zhou, Y.; Yu, S. H.; Wang, C. Y.; Li, X. G.; Zhu, Y. R.; Chen, Z. Y. *Adv. Mater.* **1999**, *11*, 850.

(7) Kim, J. Y.; Kim, S.; Kim, J.; Lee, J.; Yoon, J. *J. Kor. Soc. Environ. Eng.* **2005**, *27*, 771.