Synthesis and Properties of Manganese Oxide Nanoparticles for Environmental Applications

Alexandra Rutz

Chemistry and Molecular & Cellular Biology, University of Illinois Urbana-Champaign

NNIN REU Site: Nano Research Facility, Washington University in St. Louis, St. Louis, MO

NNIN REU Principal Investigator and Mentor: Young-Shin Jun, Energy, Environmental, and Chemical Engineering, Washington University in St. Louis

Contact: arutz2@illinois.edu, ysjun@seas.wustl.edu

Abstract and Introduction:

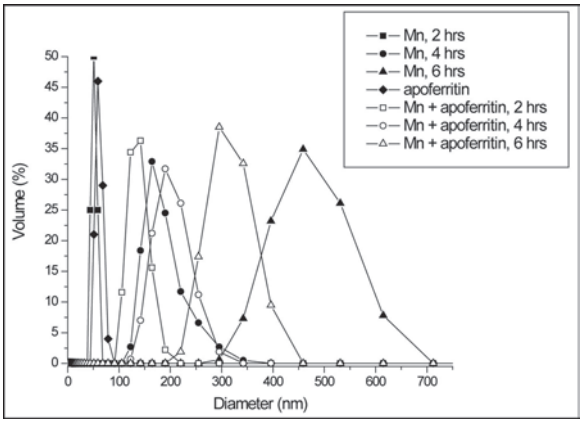
Manganese oxide nanoparticles (MnO_2 NPs) can be utilized for advanced materials in batteries, as well as other applications, such as water treatment and imaging contrast agents [1]. To have more efficient performance, these materials need particles of defined size, while remaining benign to the environment. In this work, we synthesized NPs with a biomineralization approach and investigated antimicrobial effects. MnO_2 NPs were synthesized using the iron storage protein ferritin that provides an 8 nm cavity in which these particles form.

We compared the sizes and morphologies of biomimetically synthesized NPs to those from inorganic synthesis using dynamic light scattering (DLS), transmission electron microscopy (TEM), high resolution TEM (HR-TEM), and atomic force microscopy (AFM). DLS showed sizes of particle aggregates as a function of reaction time. Electron diffraction patterns were collected by HR-TEM to identify the phase of NPs and determine particle size. AFM was used to investigate morphologies and sizes of the particles. To study antimicrobial effects of both synthetic methods, *Shewanella* was studied while in solution with MnO_2 NPs. Our findings from this work will provide fundamental information of the potential toxicities of NPs generated by different pathways.

Materials and Methods:

To synthesize MnO_2 NPs biomimetically, apoferritin and manganese (II) tetrahydrate were added to doubly filtered deionized water. The pH of the reaction volume was brought to 9 with sodium hydroxide, and the reaction was rotated in a test tube for six hours. The inorganic synthesis was prepared in the same manner but without the protein. To investigate particle morphologies, TEM imaging was performed using a FEI Tecnai G^2 Spirit microscope. Samples were prepared by drying the reaction mixture on carbon-coated Formvar-covered copper TEM grids. HR-TEM imaging was performed on JEOL JEM-2100F with imaging using Gatan Orius SD1000B camera to determine particle size and phase by calculating d-spacing. To determine hydrodynamic particle size, DLS data was taken over time during the synthesis incubation using Malvern 1011155 Zetasizer Nanoseries. The particle morphology, before and after trypsin treatment, was examined by AFM with a Veeco Nanoscope microscope.

The protein layer was digested by adding trypsin 1:20 (w:w) to the reaction volume and was incubated for fifteen minutes. To study antimicrobial effects of NPs from different syntheses, manganese oxides at concentrations of 100 μg/mL, 20 μg/mL and 2 μg/mL were incubated in MR-1 media



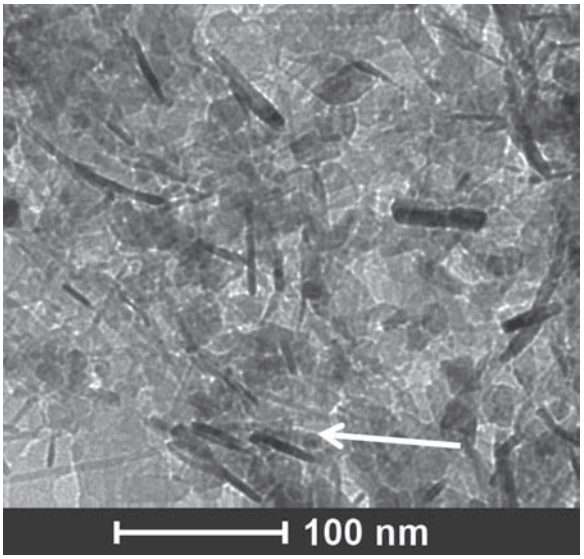


Figure 1, left: DLS data of inorganic synthesis as labeled "Mn" and biomimetic synthesis as labeled "Mn+apoferritin" with the corresponding incubation times. Figure 2, above: TEM image of inorganic synthesis displaying crystalline nanorods of various sizes.

with *Shewanella oneidensis* MR-1 at 32°C. Bacteria was added until the media had an optical density of 0.2 A at 600 nm. Optical density measurements were taken over 24 hours on ThermoScientific Genesys UV spectrophotometer.

Results and Discussion:

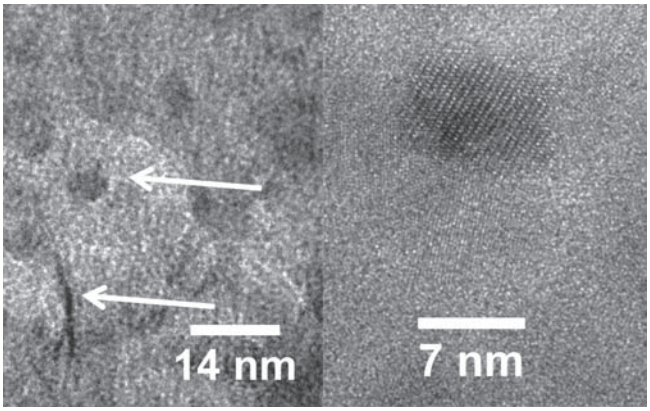
DLS data indicated particles measured were of aggregate size, not individual particle size. The gradual increase of size during the reaction showed aggregation as a function of time. At final incubation time, the biomimetic synthesis yielded smaller particle aggregates than the inorganic synthesis. This trend can be explained by the amount of available aqueous manganese ions in solution for nanoparticle formation.

Crystalline nanorods of various sizes in the inorganic synthesis were observed by TEM while few nanorods and spherical nanoparticles were found in the biomimetic synthesis. Particle size of 7-8 nm was verified by HR-TEM and was consistent with the limits of the protein cavity. The particles showed lattice fringes indicating crystalline material while previous work reported amorphous particles [2]. The d-spacing was calculated to be 3.407 Å corresponding to manganite. Unlike our synthesis, previous work used buffer at pH 9 as the reaction volume. The contribution of the buffer to the crystallinity of MnO_2 needs to be investigated further. Using AFM, we concluded trypsin removed the protein layer. The data of the biomimetic synthesis demonstrated peaks with particle height of 12 nm corresponding to protein and larger heights indicating protein aggregates. Digestion of biomimetic synthesis displayed height peaks at 8 nm indicating manganese oxide nanoparticles without the protein shell.

Antimicrobial studies on *Shewanella* were performed using particles of the biomimetic synthesis with and without the protein layer. The inorganic synthesis was also studied using incubation times of one and six hours. The bacteria exposed to particles of both synthetic methods showed minor inhibition in the lag and log phases of the growth curve, but recovered from the stress in the stable phase. In the log phase at eight hours, the inorganic synthesis showed greater inhibition than the biomimetic synthesis.

Conclusion:

Biomimetic manganese oxides were successfully synthesized from the adopted synthetic method [2]. TEM images indicated the nanoparticles formed within protein shells. We determined the sizes of hydrodynamic and individual nanoparticles by DLS and HR-TEM respectively. The particle morphologies were probed with AFM before and after protein digestion confirming the protein layer was removed from the nanoparticles. Antimicrobial studies of both the biomimetic and inorganic synthesis with respect to *Shewanella* indicated only minor inhibition in the lag and log phases of the growth curve.



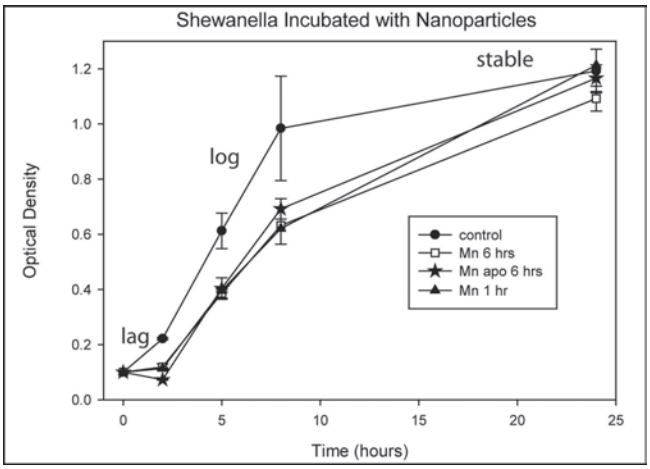


Figure 3, top: HR-TEM images from biomimetic synthesis showing crystalline nanorods and nanoparticles within protein layer.

Figure 4, bottom: Antimicrobial studies of Shewanella measured by optical densities taken over 24 hours.

Our findings report MnO_2 NPs synthesized from a controlled biomimetic method show promise for use in advanced materials that are friendly to the environment.

Acknowledgments:

I would like to thank my Principal Investigator, Dr. Young-Shin Jun, as well as the Jun Group, Kristy Wendt, Amy Sears, Dr. Dong Qin, Dr. Yinjie Tang, and Dr. Bing Wu. I would also like to thank the National Nanotechnology Infrastructure Network Research Experiences for Undergraduates Program and the National Science Foundation for their efforts and support.

References:

[1] Chen, H, et. al; "Facile Synthesis of Monodisperse Manganese Oxide Nanostructures and Their Application in Water Treatment"; J. Phys. Chem., 112, 17540–17545 (2008).

[2] Meldrum, F, et. al; "Synthesis of inorganic nanomaterials in supramolecular protein cages"; Nature, 349, 684-687 (1991).