How shape and binding to wool fibers influences silver nanoparticle toxicity towards *Nitrosomonas europaea* and *Pseudomonas putida*

Tyler S. Radniecki and Alyssa Deline
School of Chemical, Biological and Environmental Engineering
Oregon State University

Steven Oldenburg
nanoComposix, Inc.
San Diego, CA
The Rise of Ag-NP

Major Nanoparticles

Number of Products

- Silver
- Carbon
- Titanium
- Gold

March 2006
March 2011
Release of Ag-NP to WWTP

Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics

TROY M. BENN* AND PAUL WESTERHOFF
Civil and Environmental Engineering, Arizona State University, Box 5306, Tempe, Arizona 85287-5306

The Behavior of Silver Nanotextiles during Washing

L. GERANIO, † M. HEUBERGER, † AND B. NOWACK*, †
Technology and Society Laboratory, and Laboratory of Advanced Fibers, Empa - Swiss Federal Laboratories for Materials Testing and Research, CH 9014 St. Gallen, Switzerland
Biological Nitrogen Cycle

Highly Sensitive to Disruption!
The Influence of Shape

nanoComposix, Inc.
http://www.nanocomposix.com
The Influence of Shape

Capping Agents
Polyvinylpyrrolidone (PVP)
The Influence of Shape

Diameter

Thickness = 10 nm

nanoComposix, Inc.
www.nanocomposix.com
**Nitrosomonas europaea**

- The model Ammonia Oxidizing Bacteria
- Oxidizes $\text{NH}_3 \rightarrow \text{NO}_2^-$
- Gram Negative
- Chemolithoautotroph
- 8-12 h Doubling Time

Radniecki, et. al., ES&T, 2008
Experimental Set-up

- 2.5 mM (NH$_4$)$_2$SO$_4$
- 30 mM HEPES
- pH 7.8
- 6 mg protein/L
- 250 rpm
- 30°C in the dark
- 3 h duration
- Measure NO$_2^-$
Experimental Set-Up

Activity = Slope

mmol NO$_2^-$ /mg protein

Control

Activity = Slope

Minutes
Experimental Set-Up

mmol NO$_2^-$ /mg protein

Control

Treatment

Minutes
Experimental Set-Up

\[
\%\text{ Nitrification Activity} = \frac{\text{Treatment Slope}}{\text{Control Slope}} \times 100\%
\]

mmol NO$_2^-$ /mg protein

Minutes
AgNP Toxicity

Ag-NP Dissolution to Ag$^+$
Quantifying AgNP Dissolution

Ultrafiltration-ICP method

AgNP in Test Media
(No cells)

Pass through a 3kDa filter
(∼ 0.2 nm)

[Ag] measured in filtrate
(ICP)
Quantifying AgNP Dissolution

Absorbance

Wavelength (nm)
Influence of Shape on Toxicity

% Nitrification Activity

- 170 nm Ag plate
- 110 nm AgNPs
- 30 nm AgNPs
- 10 nm AgNPs

0.86 ppm
Influence of shape on toxicity

Nitrification Activity %

- 170 nm Ag plate
- 110 nm AgNPs
- 30 nm AgNPs
- 10 nm AgNPs

0.86 ppm
Influence of Shape on Toxicity

% Nitrification Activity

170 nm Ag plate
110 nm AgNPs
30 nm AgNPs
10 nm AgNPs

0.86 ppm
Influence of Shape on Toxicity

% Nitrification Activity

ppm silver

50 nm AgNPs
Influence of Shape on Toxicity

% Nitrification Activity

ppm silver

- 50 nm AgNPs
- 50 nm Ag nanoplates
Influence of Shape on Dissolution

Silver (ppm)

Minutes

50 nm AgNPs

60 nm Ag nanoplates
Influence of Shape on Dissolution

Silver (ppm)

50 nm AgNPs

50 nm Ag nanoplates

Minutes

0 0.25 0.5 0.75

0 45 90 135 180
Influence of Shape on Dissolution

\[
\text{Ag}^+ + 2 \text{NH}_3 \rightarrow \text{Ag(NH}_3\text{)}_2^+ 
\]
Influence of Shape on Dissolution

Influence of Shape on Dissolution
Influence of Attachment to Wool

% Nitrification Activity

100

75

50

25

0

10 nm AgNPs

170 nm Ag plate

35 nm Ag plate

3 mg Ag/in² cloth
Influence of Attachment to Wool

% Nitrification Activity

3 mg Ag/in² cloth

10 nm AgNPs

170 nm Ag plate

35 nm Ag plate
Pseudomonas putida Toxicity Tests

Pseudomonas putida

Wool with nanoAg
Pseudomonas putida Toxicity Tests
Pseudomonas putida Toxicity Tests
**Pseudomonas putida toxicity tests**

CFU under nanoAg cloth

3 mg Ag/in² cloth

- Control Cloth
- 10 nm AgNPs
- 35 nm Ag plates
- 170 nm Ag plates
Pseudomonas putida Toxicity Tests

CFU outside of nanoAg cloth

3 mg Ag/in$^2$ cloth

- Control Cloth
- 10 nm AgNPs
- 35 nm Ag plates
- 170 nm Ag plates
N. europaea and BSA Protection

% Nitrification Activity vs. BSA (ppm)

- 50 nm AgNPs
- 50 nm Ag microparticles
N. europaea and BSA Protection

% Nitrification Activity

50 nm AgNPs
50 nm Ag nanoplates

BSA (ppm)
N. europaea and Alginate Protection

% Nitrification Activity

Algininate (ppm)

50 nm AgNPs

50 nm Ag nanoparticles
N. europaea and Alginate Protection

% Nitrification Activity

50 nm AgNPs

50 nm Ag nanoplates

Alginate (ppm)
Conclusions

• AgNP shape greatly influences toxicity

• In the presence of NH$_3$, Ag nanoplates are more toxic than AgNPs due to increased dissolution

• In the presence of proteins and LPS, AgNPs are more toxic than Ag nanoplates due to decreased interactions with the biological macromolecules

• Proper test media matters!
Acknowledgements

San Diego State University

Dept. of Civil, Construction & Environmental Engineering

Mrs. Alyssa Deline – Undergraduate Researcher*
  *Current PhD student at Oregon State University

Mr. Matthew Tallone – Undergraduate Researcher

Mr. Issa El Haddad – Undergraduate Researcher

Mr. Tanner Houston – Undergraduate Researcher

nanoComposix, Inc.

Steven Oldenburg, CEO
Acknowledgements

Funding

National Science Foundation (CBET – Environmental Health and Safety of Nanotechnology - # 1067572)

United State Department of Agriculture (Training Grant # 2011-38422-31204)

nanoComposix, Inc.
Acknowledgements