Impact of Silver Nanoparticles on Wastewater Treatment

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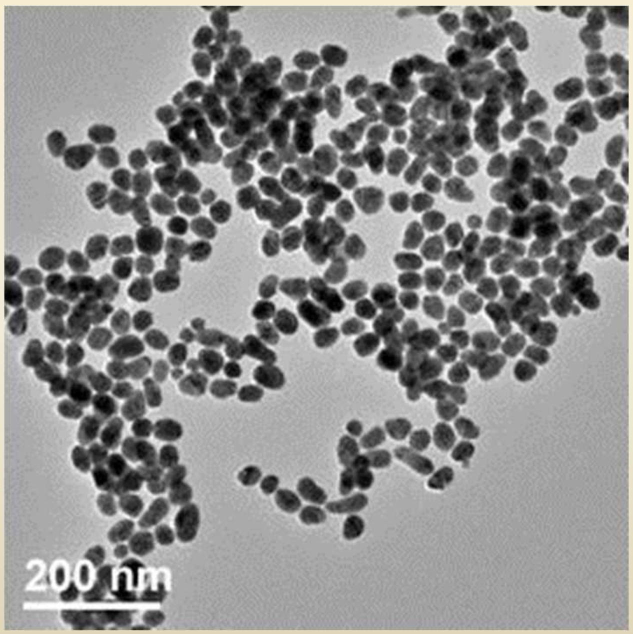
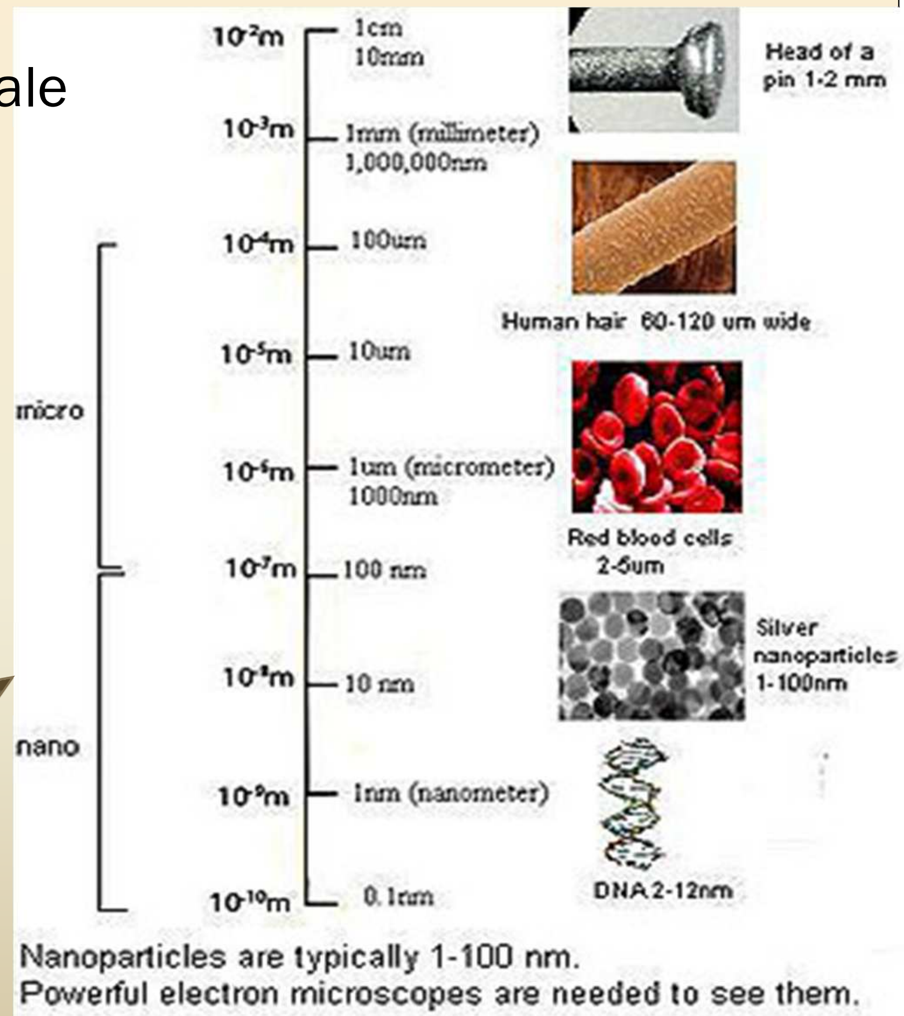
Nanoparticles

Particles with one dimension of nanoscale (typically 1-100 nm)



TEM image of gold nanospheres
Source: bionanotechnology, 2006, Annual meeting, San Francisco, CA



Source: www.beyondpesticides.org/antibacterial/nanosc.

Research Questions

- Environmental Implications
  - How will emerging nanomaterials affect wastewater treatment and anaerobic sludge digestion?
  - How will nanoparticles impact the environment?

- Environmental Applications
  - Can nanomaterials be used for water/wastwater treatment and environmental remediation?
  - How will nano-scale materials be used for water/wastewater treatment while their negative impact is minimized?

Silver Nanoparticles (AgNPs, Nanosilver) as a Emerging Antimicrobial Agent

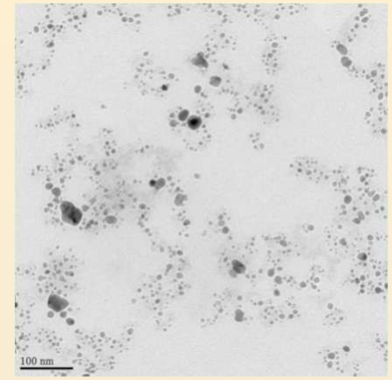
- Silver ions and silver nanoparticles AgNPs (nanosilver): widely used in consumer products.

- Total Ag concentration range from 1.78 to 105 μg/L in wastewater influent (Shafer et al. 1998).
- With a concentration factor of more than 100 in WWTP,the predicted silver concentrations in sludge is between 7 to 39 mg/kg (Blaser et al. 2008).

RESEARCH OBJECTIVES

- Determine the impact of silver nanoparticles on the representative wastewater treatment processes
  - To determine the degree of nanosilver inhibition to bacterial activity and changes of effluent water quality
  - To evaluate the bacterial response to a shock load of silver nanoparticles and the potential change of nitrifying bacterial community structure using molecular tools such as Terminal Restriction Fragment Length Polymorphism (T-RFLP)

MATERIALS & METHODS



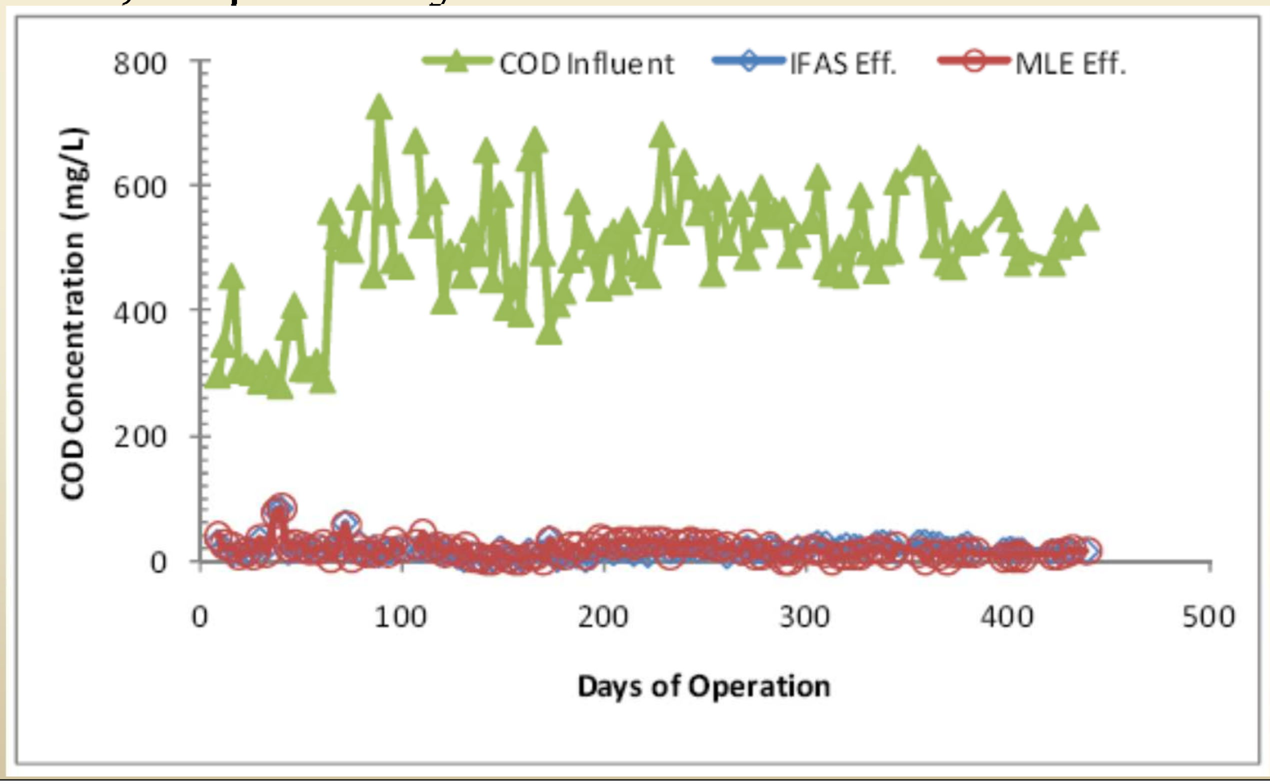
- Nanosilver
  - Made from 0.7 mM $AgNO_3$ by adding equal molar of sodium borohydride ($NaBH_4$) with polyvinyl alcohol (PVA) as a capping agent; Average particle size = 21 – 29 nm (error bar = 100 nm)
- Wastewater Bioreactors
  - One 7L bioreactors using modified Ludzak-Ettinger (MLE) process (alternating anoxic and aerobic)
  - One 7L Integrated Fixed-film activated sludge (IFAS).
  - Two 7L Membrane bioreactor (MBRs) (IFMBR and MLE/MBR) with a 400 cm^2 membrane area hollow fibre membrane module (ZeeWeed from Zenon)
  - Activated sludge from a local wastewater treatment plant was used for seeding.

OPERATING CONDITIONS

- Total reactor volume: 7.2 L for four reactors
- IFMBR (aerobic compartment: 5.0 L, internal clarifier: 2.2 L)
- MLE, IFAS and MBR (anoxic chamber: 1.8L, aerobic chamber:3.6L, internal clarifier:1.8 L)
- Feed flow rate: 7.2 L/day (hydraulic retention time or HRT = 1 d, solids retention time = 20 d)
- Influent COD: 516±80 mg/L
- Influent NH_4-N : 32±2 mg/L (TKN = 50 mg/L)

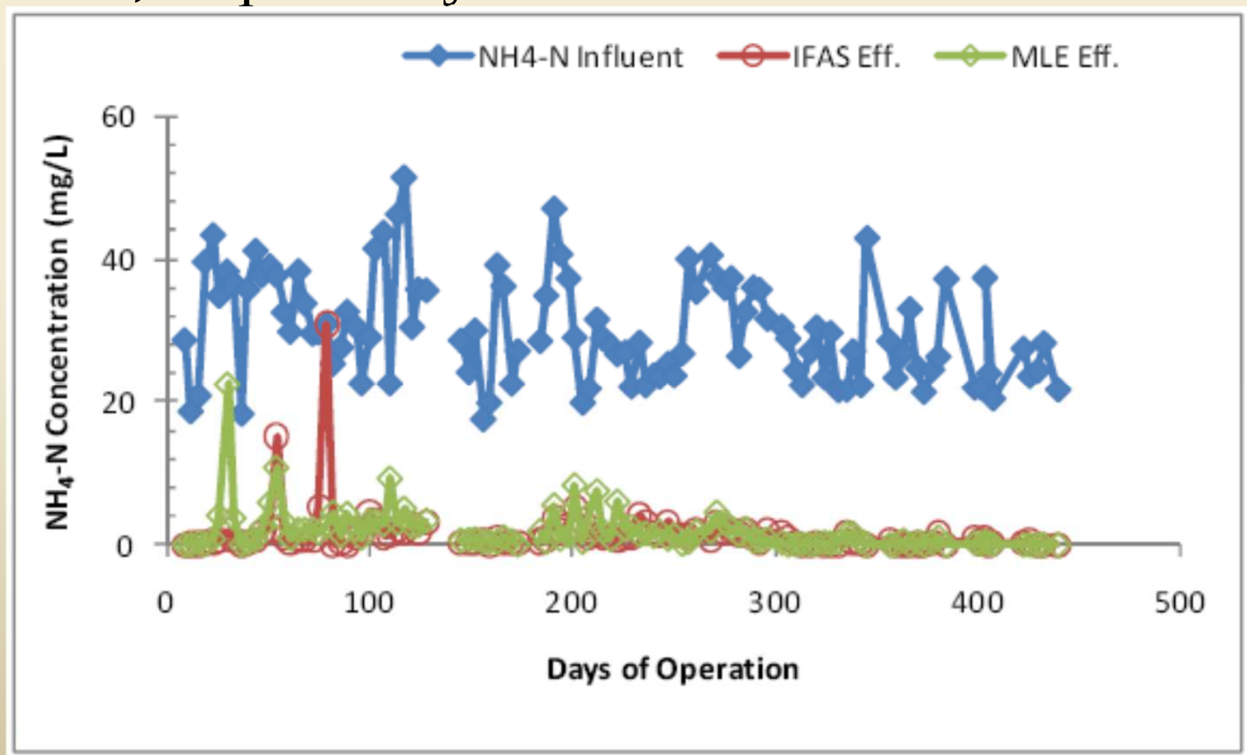
Organic Matter Removal

- Average IFAS and MLE Effluent COD: 16±12 mg/L, 17±13 mg/L, respectively
- COD removal rates in IFAS and MLE were 96.6% and 96.5%, respectively.



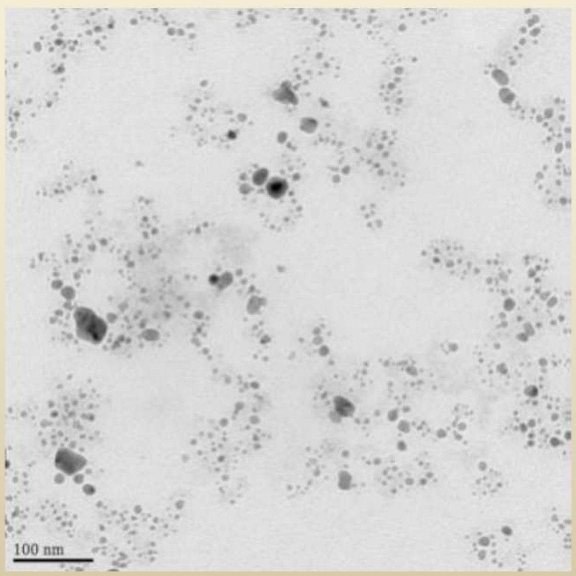
Ammonia-Nitrogen Removal

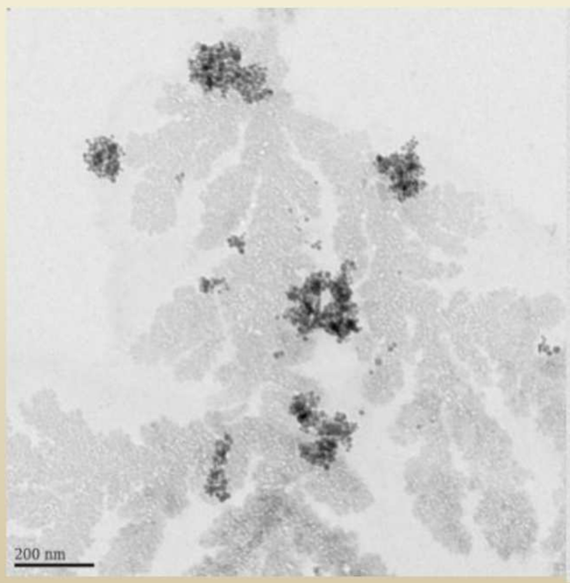
- Concentrations of effluent NH_4^+-N from IFAS and MLE (average) were 1.57 mg/L, 1.86 mg/L, respectively
- NH_4^+-N removal efficiency in IFAS and MLE were 95% and 94%, respectively.

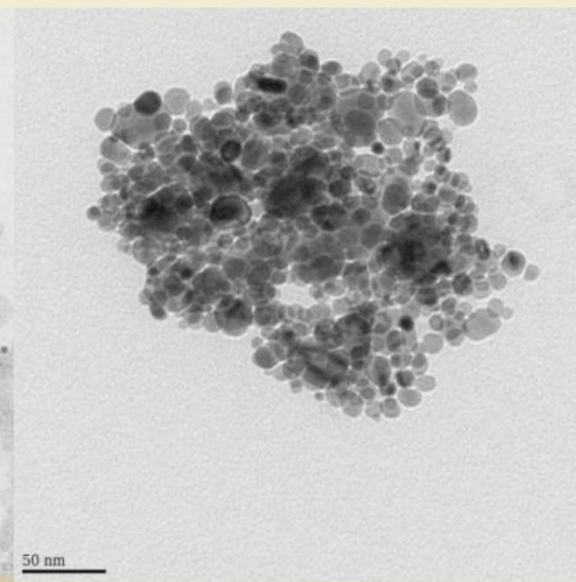


Preliminary Findings

- Silver nanoparticle aggregation in the presence of mixed culture and > 90% of AgNPs was adsorbed to activated sludge biomass
- Continuous and slow release of Ag^{+} from AgNPs resulted in a prolonged period of nitrification inhibition

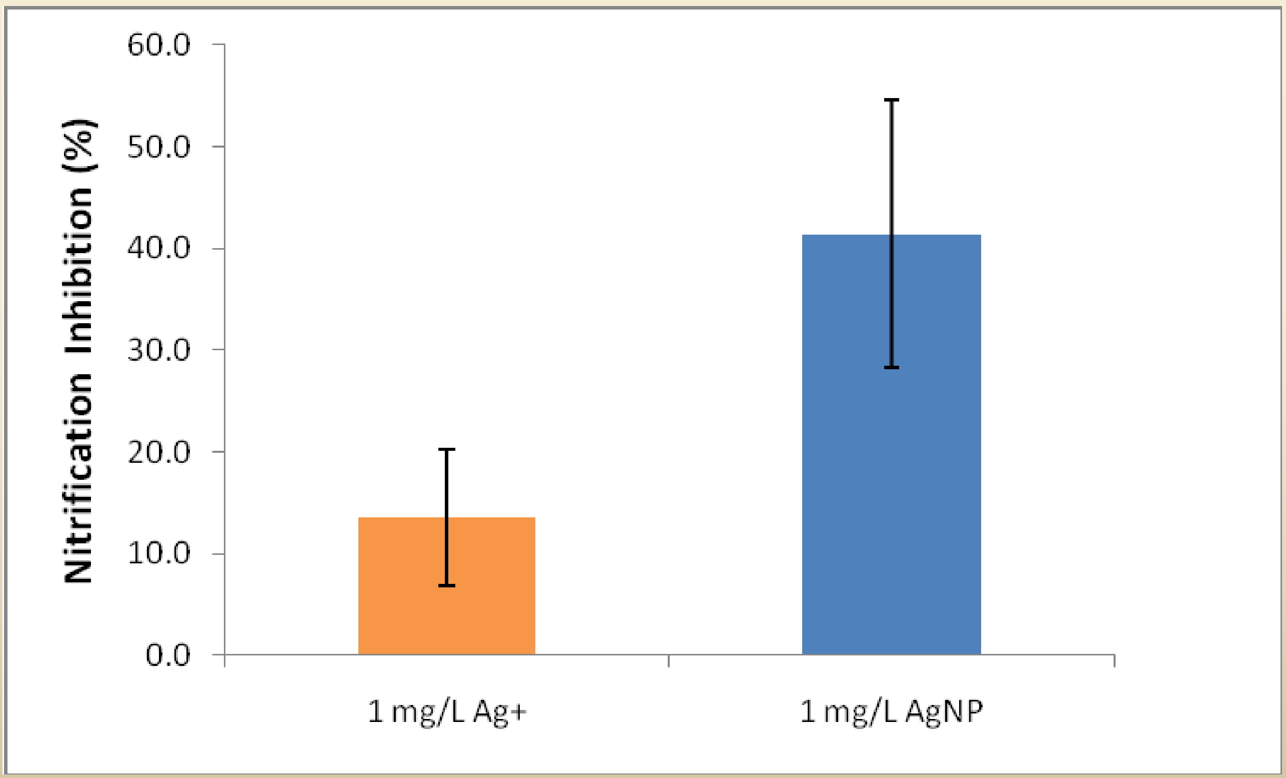




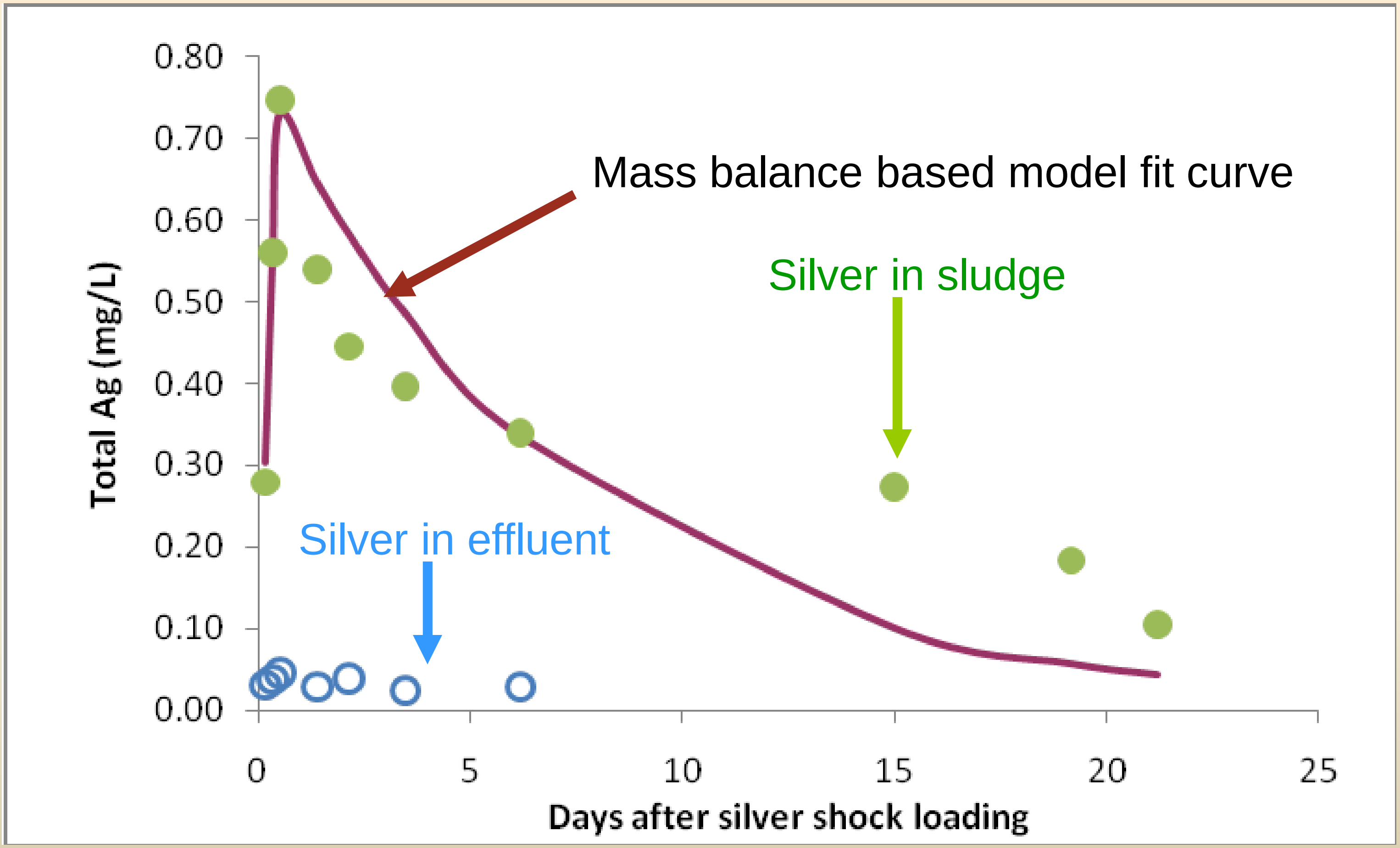


Silver Toxicity to Bacteria

- AgNPs (average size = 21 nm) were more toxic than Ag^+.
- Nitrification inhibition inferred from batch resperiometric assays by AgNPs and Ag^+ ions at 1 mg/L .



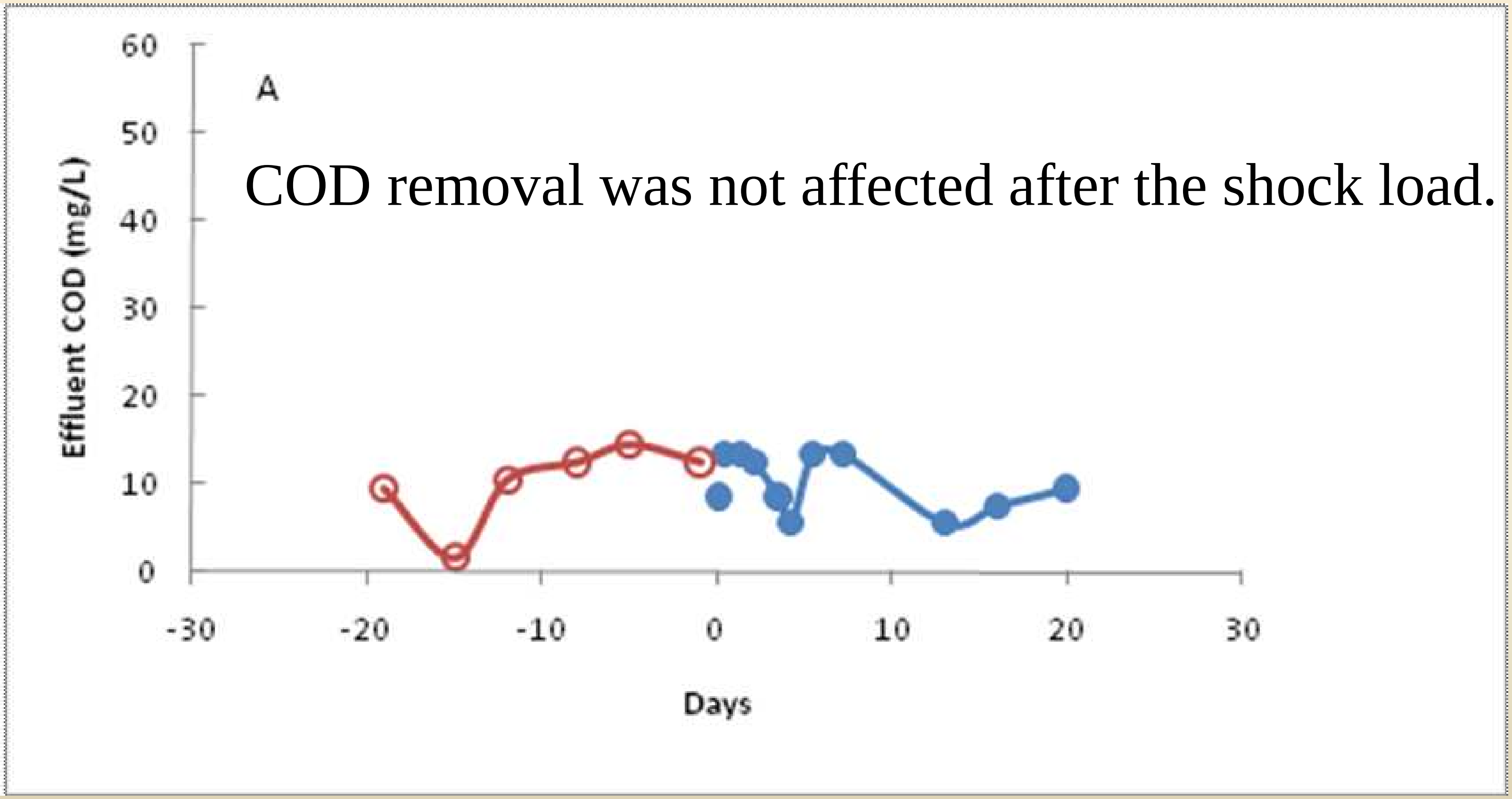
12-h Nanosilver Shock Loading Experiment: Dynamics of Silver in MLE



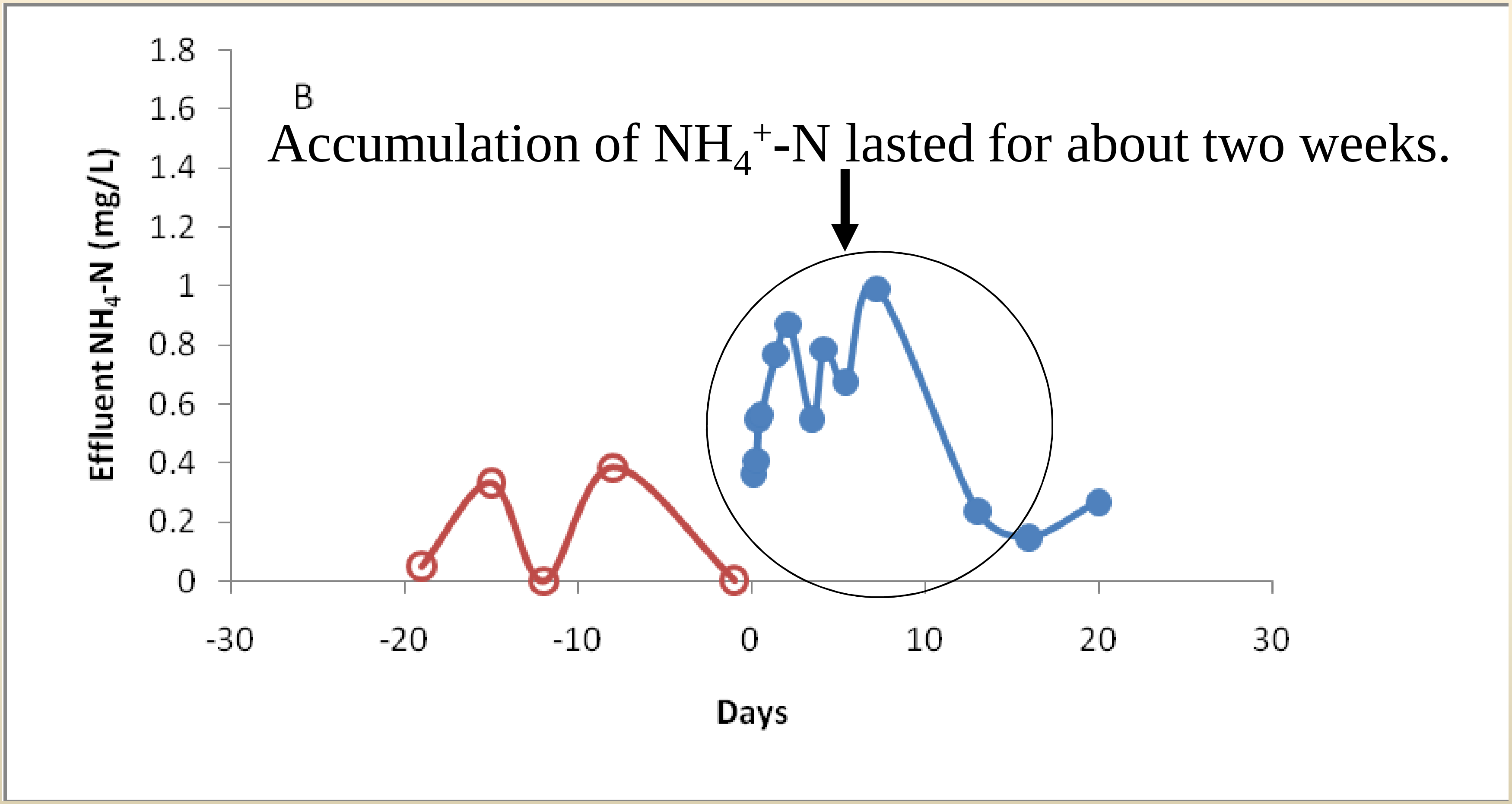
A complete washout of silver in the wastewater system may take more than 25 days

Effluent Water Quality Before and After Nanosilver Shock Load

COD removal was not affected after the shock load.



Effluent Ammonia-N Before and After Nanosilver Shock Load



Effluent Nitrite-N Before and After Nanosilver Shock Load

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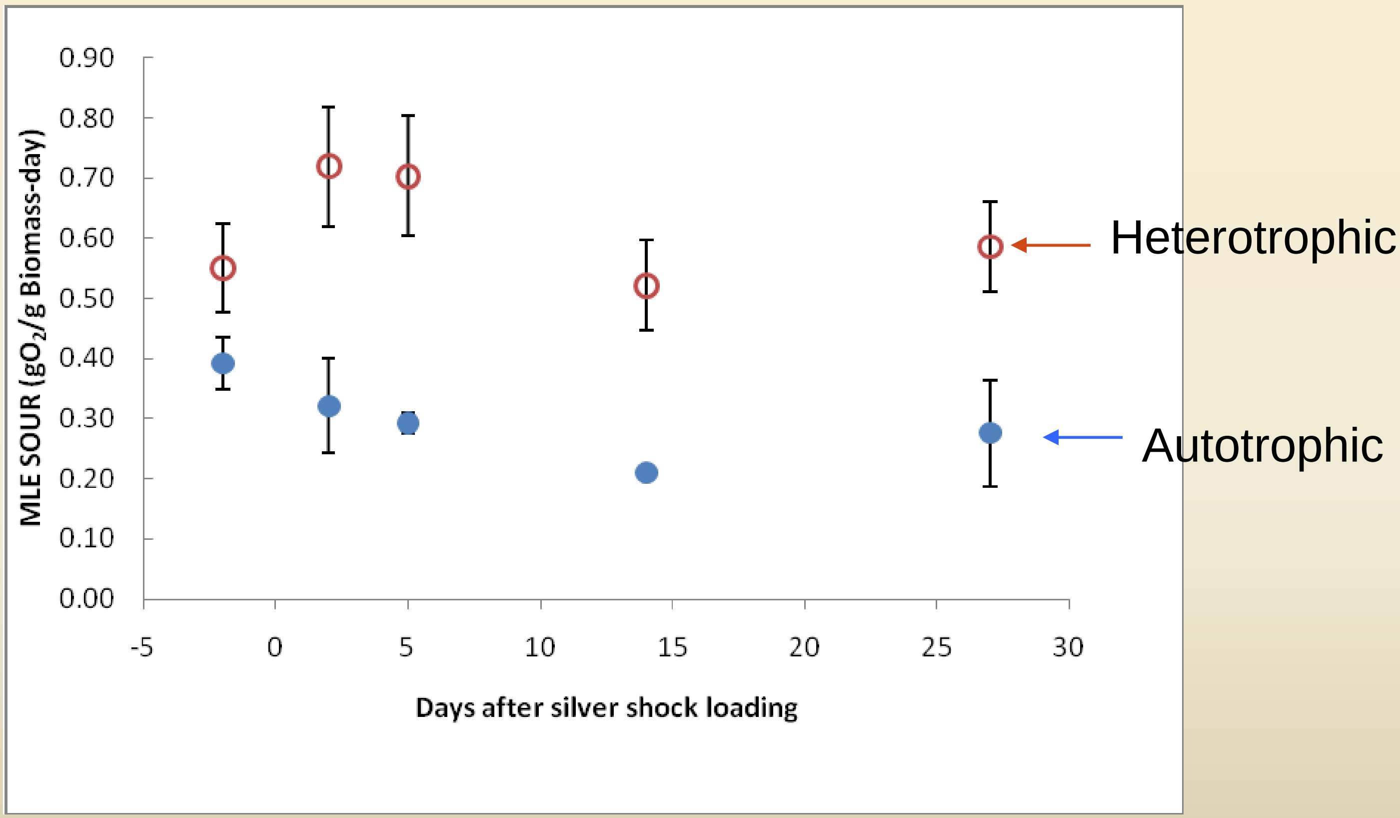
Accumulation of NO_2^--N lasted for about two weeks.

Autotrophic and Heterotrophic Activities in the MLE Before and After Shock Load



Consequence: A prolonged period of nitrification inhibition (>1 month, the highest degree of inhibition = 46.5%)

Change of Bacterial Community Structure in Response to the Shock Load– General Procedure

Sample collection

↓

DNA extraction

↓

Amplification by nested PCR

↓

T-RFLP

↓

Comparison of fragment size

PCR PRIMERS USED

PCR primers

Primer	Sequence (5′–3′)	Specificity
11f	GTTTGATCCTGGCTCAG	Bacteria 16S rRNA gene
1492r	TACCTTGTTACGACT T	Bacteria 16S rRNA gene
Eub338f[a]	ACTCCTACGGGAGGCAGC	Bacteria 16S rRNA gene
Nso1225r	CGCCATTGTATTACGTGTGA	*β-Proteobacteria* AOB 16S rRNA gene
NIT3r	CCTGTGCTCCATGCTCCG	*Nitrobacter* 16S rRNA gene
Ntspa685r	CGGGAATTCCGCGCTC	*Nitrospira* 16S rRNA gene
amoA-1F[b]	GGGGTTTCTACTGGTGGT	AOB *amoA* gene
amoA-2R[a]	CCCCTCKGSAAAGCCTTCTTC	AOB *amoA* gene

Note that K indicates G or T, and S indicates C or G.

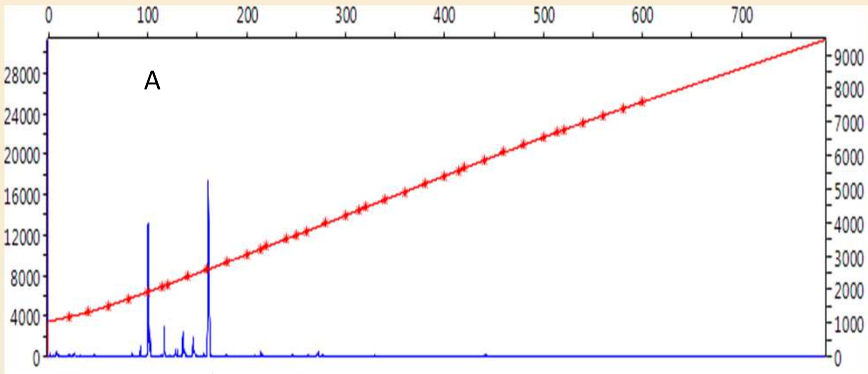
[a] The primer was labeled with the fluorescent dye 6-FAM.
[b] The primer was labeled with the fluorescent dye HEX.

TERMINAL FRAGMENT SIZE

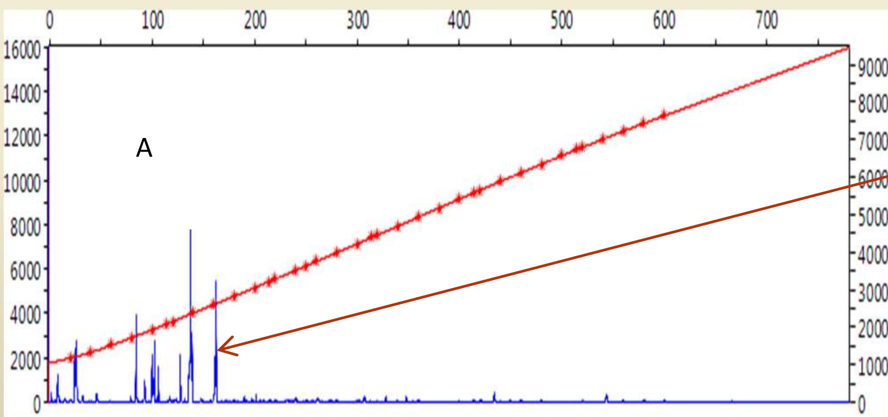
Expected TF sizes and their corresponding AOB and NOB groups based on T-RFLP of 16S rRNA gene

Nitrifier group	TF size (bp)
Group 1 *Nitrosomonas europaea/eutropha* lineage	164–166, 276
Group 2 *Nitrosomonas oligotropha* lineage	276
Group 3 *Nitrosomonas cryotolerans* lineage	276
Group 4 *Nitrosomonas marina* lineage	166
Group 5 *Nitrosomonas communis* lineage	276
Group 6 *Nitrosospira* lineage	105–107
Nitrobacter species	141
Nitrospira species	265–267, 277 (134, 194, 333)

RESULTS OF T-RFLP before and after shock loading ---Ammonia-Oxidizing Bacteria



Nitrosomonas eutropaea (161 bps) (A) was the dominant genus of ammonia-oxidizing bacteria (AOB) before nanosilver shock loading



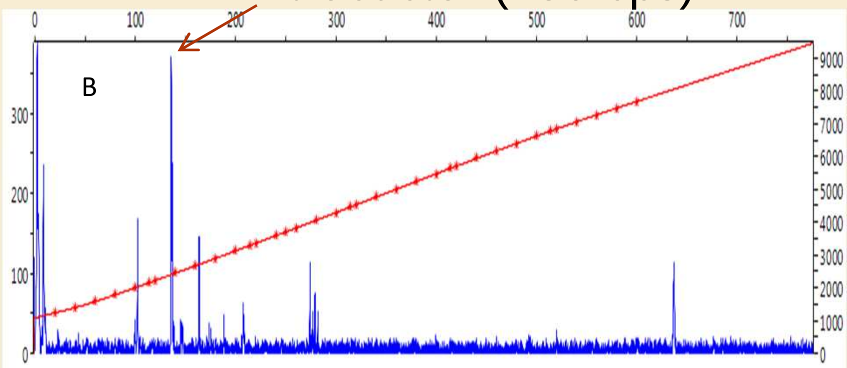
Nitrosomonas eutropaea (161 bps) peak decreased after shock loading

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$

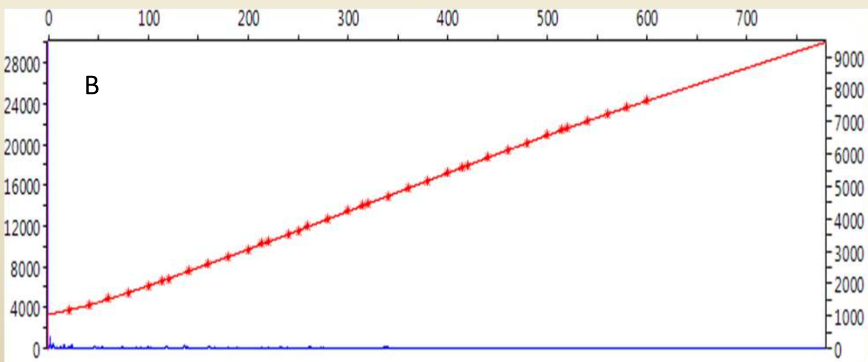
Nitrosomonas eutropaea

RESULTS OF SHOCK LOADING- Nitrite Oxidizing Bacteria

Nitrobacter (136 bps)



Nitrobacter was one of the major nitrite-oxidizing bacteria before shock loading



Nitrobacter was washed out after shock loading

$$NO_2^- + 0.5O_2 \xrightarrow{\textit{Nitrobacter}} NO_3^-$$

SUMMARY

- Batch test results showed AgNPs had higher inhibition on nitrification than Ag^+ ions.

- A 12-h shock load of nanosilver resulted in a peak concentration of 0.75 mg/L Ag in the MLE bioreactor, a prolonged period (> 1 month) of nitrification inhibition and deteriorate effluent water quality.

- The shock-loading event did not affect heterotrophic activity and organic matter removal.

- A shock load of nanosilver changed the nitrifying bacterial community structure, resulting in decreases of *Nitrosomonas* and *Nitrospira* population and wash-out of *Nitrobacter*

Future Work

- Long-term exposure of activated or digested sludge to AgNPs at relevant concentrations
- Impact of AgNPs on membrane based activated sludge systems (membrane bioreactors, MBR)
- Nitrifying bacterial community structure change as a result of nanosilver exposure and a quantitative linkage with nitrification activity
- Methanogenic archaeal community structure change as a result of nanosilver exposure and a quantitative linkage with methane production

Acknowledgements

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