Developing a Yeast Cell Assay for Measuring the Toxicity of Inorganic Oxide Nanoparticles

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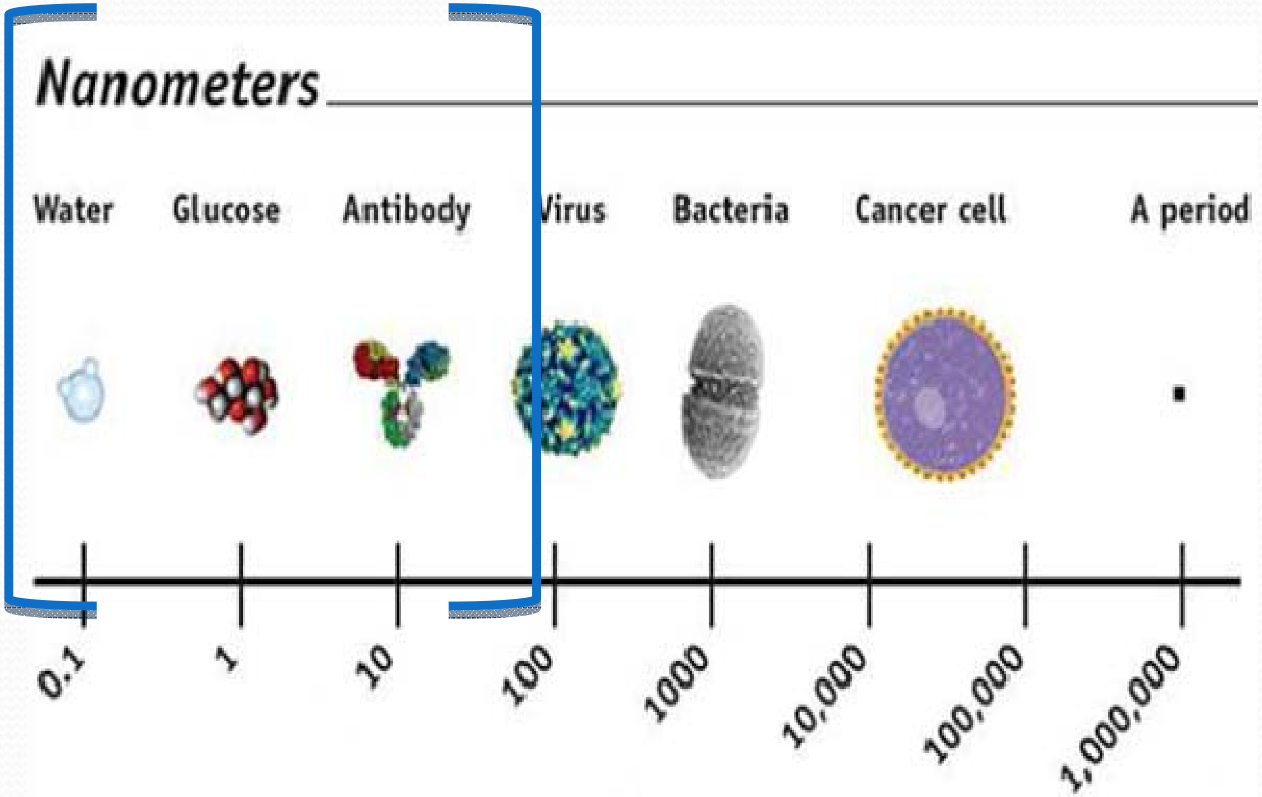
Outline

- *Introduction*
- *Objectives*
- *Materials and Methods*
    - Toxicity Testing using Yeast
    - Characterization of Nanoparticles
- *Results*
- *Conclusions*

Nanotechnology

Understanding and control of matter at dimensions of roughly 100 nm where unique physical properties make novel applications possible



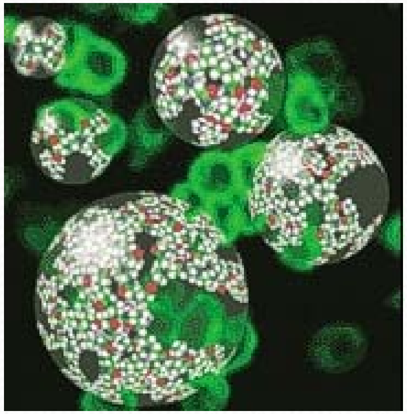
http://www.fda.gov/consumer/updates/nanotech072507.html

Unusual Physicochemical properties

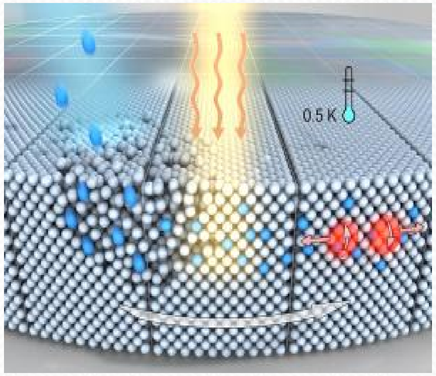
- Small size
- Chemical composition
- Surface structure
- Solubility
- Shape
- Aggregation

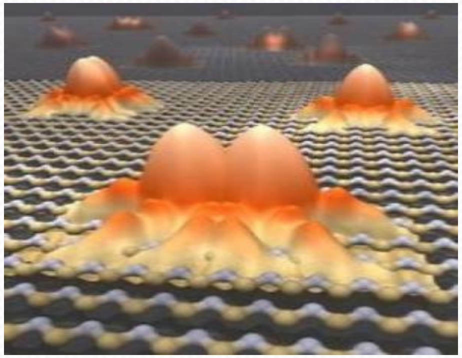
Nanotechnology Applications

Medicine

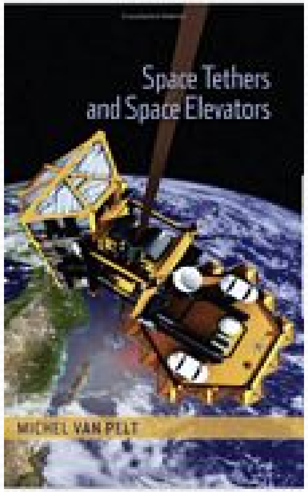


Electronics



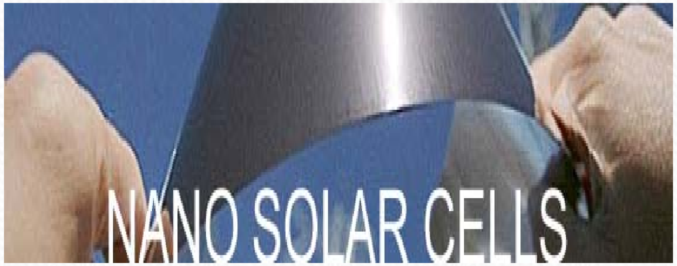


Space



Fuel Cells

Solar Cells



Batteries

Cosmetics

Nanotechnology has an important role to create many new devices and materials with an infinite range of applications

Organization for Economic Cooperation and Development (OECD)

List of Nanoparticles (NPs)

Fullerenes (C60)
Carbon nanotubes
Silver
Iron
Carbon black
✓Titanium dioxide
✓ Aluminum oxide

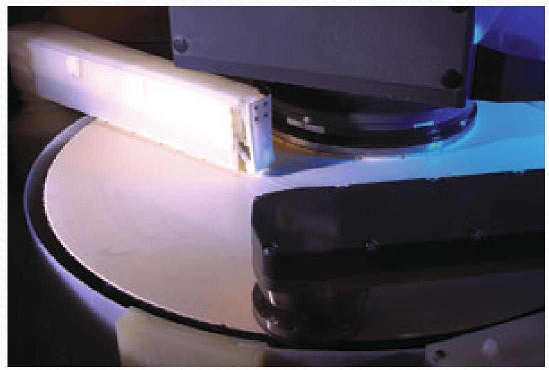
✓Cerium oxide
✓Zinc oxide
✓Silicon dioxide
Polystyrene
Dendrimers
Nanoclays

http://www.nanolawreport.com/2008/07/articles/oecd-to-begin-testing-nanoparticles/

Engineered NPs in Semiconductor Industry

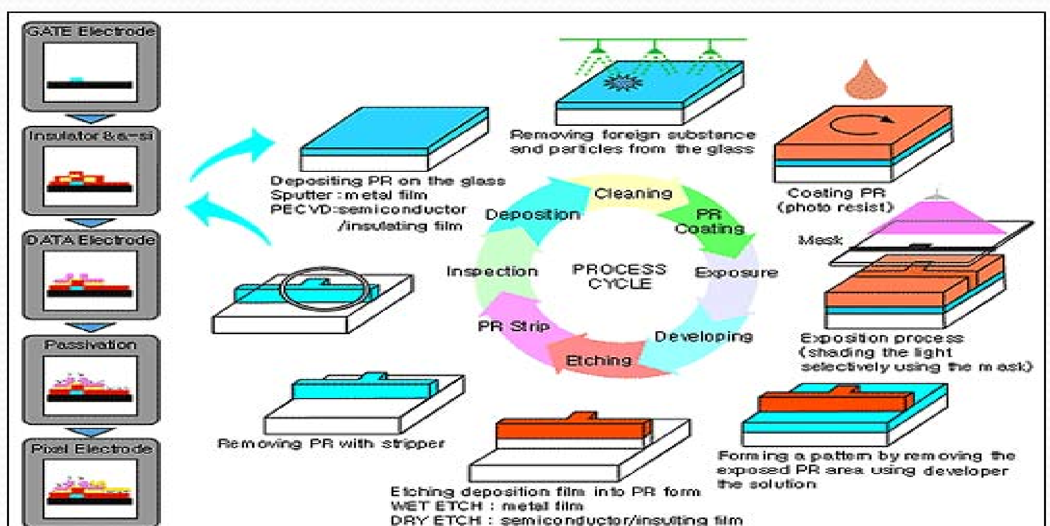
Chemo-Mechanical Planarization (CMP) slurries

- SiO_2
- Al_2O_3
- CeO_2



Immersion Photolithography

- HfO_2



Nanowires

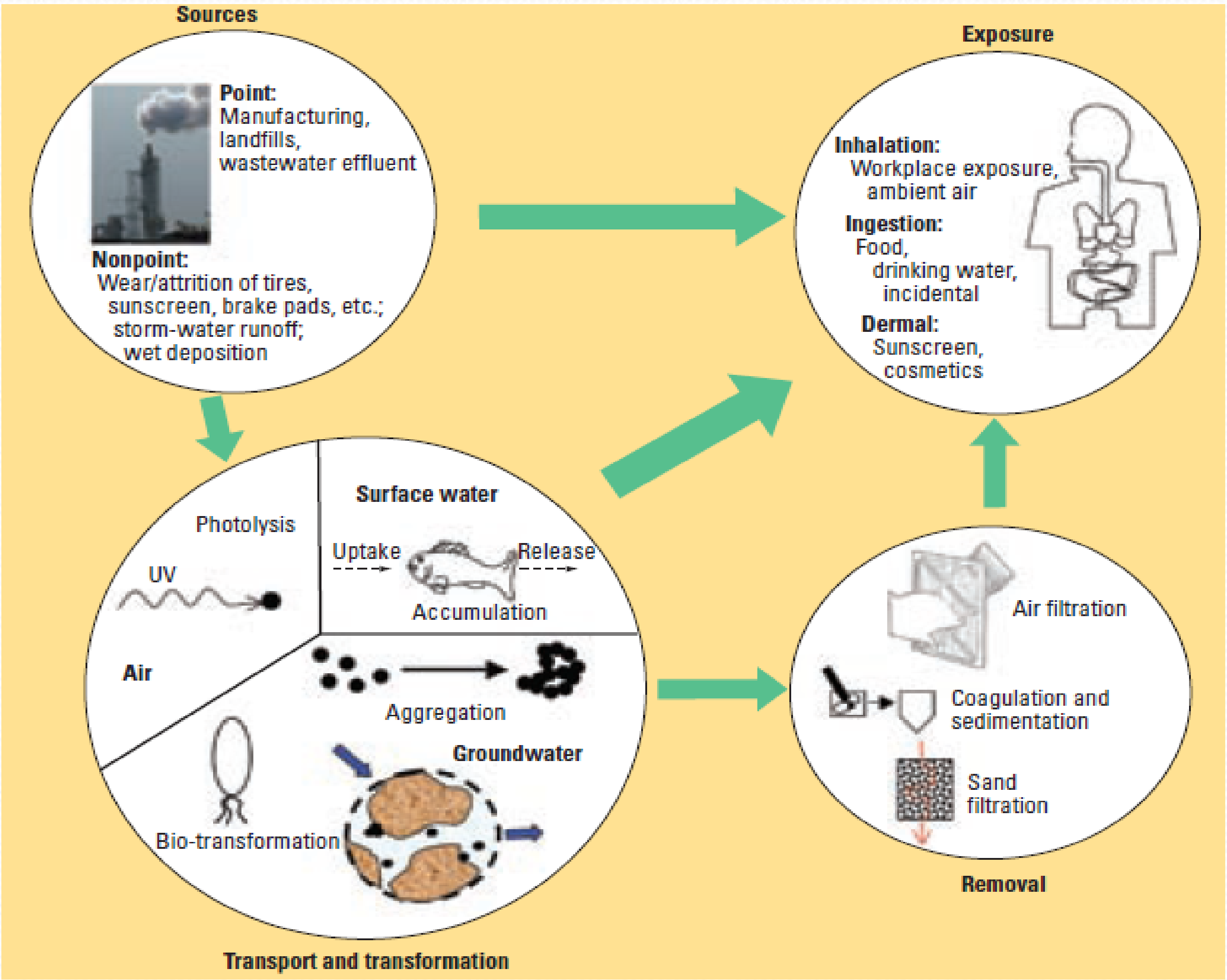
Nanotubes

Quantum dots

Examples of NP Toxicity Studies

NPs	Studies	Reference
SiO_2	In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility.	Brunner et al. 2006
TiO_2	Cytotoxicity of titanium and silicon dioxide nanoparticles	Wagner et al. 2009
ZnO	In vitro cytotoxicity assessment of selected nanoparticles using human skin fibroblasts	Dechsakulthorn et al. 2007
CeO_2	Toxicity of cerium oxide nanoparticles in human lung cancer cells	Weisheng et al. 2006

Release of Nanomaterials to the Environment



(Wiesner *et al.*, 2009)

Methods to Evaluate

Ecotoxicity of Nanoparticles

Ecotoxicity Testing of Nanoparticles

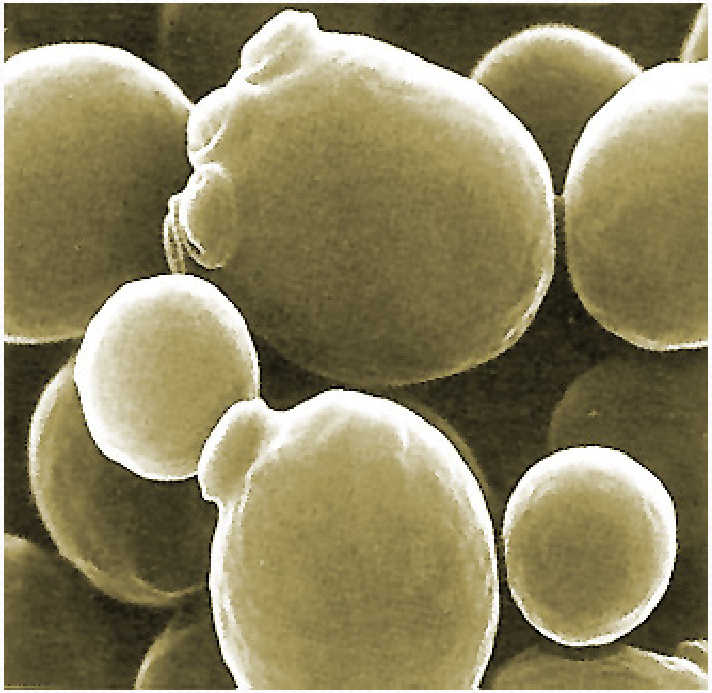
Microorganisms

- Foundation of all known ecosystems
- Basis of food webs and the primary agents for global biogeochemical cycles
- Important components of soil health

Microbial ecotoxicology test are used to study the toxicity of nano-materials and to elucidate cytotoxicity mechanisms that could be extrapolated to eukaryotic cells

YEAST (Saccharomyces cerevisiae)

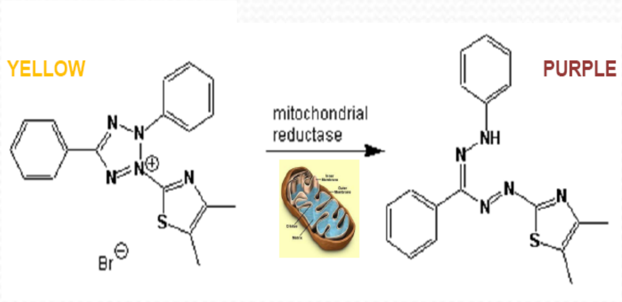
- Unicellular eukaryotic model organisms
- Short generation time
- Used in the toxicological evaluation of chemicals



Concerns About the Evaluation of Nanoparticles Cytotoxicity

- Interferences of NPs on spectrophotometric based techniques

Mitochondrial Toxicity Test (MTT)



- NPs agglomerate in biological medium complicating interpretation of data from toxicity studies

- Poor characterization of NPs

Objectives

- Develop a yeast-based, O_2 -uptake test to evaluate the toxicity of NPs

  - To select non-toxic dispersants to enhance the stability of NPs in biological media using in toxicity testing.

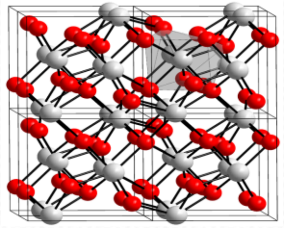
  - To characterize some physicochemical properties of NPs in toxicity assay medium: particle size distribution and NP concentration.

  - Apply the developed method to test the toxicity of NPS utilized in semiconductor manufacturing and in other important industries, in the presence and absence of dispersant.

Materials

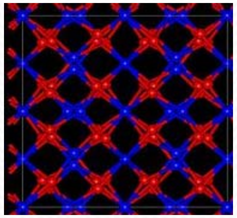
ZrO_2

(20-30 nm)



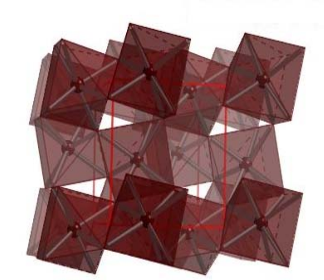
Mn_2O_3

(30-60 nm)



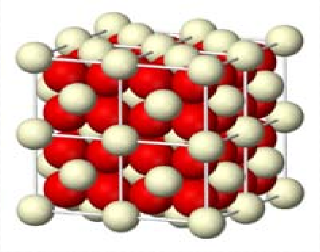
HfO_2

(20 nm)

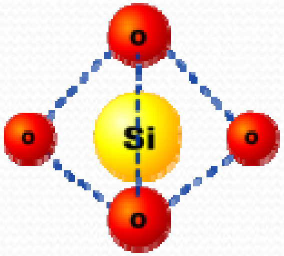


CeO_2

(50 nm)



Nanoparticles (NPs)

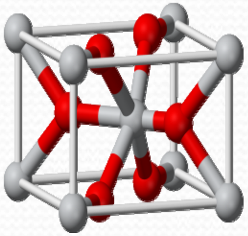


SiO2

(10-20 nm)

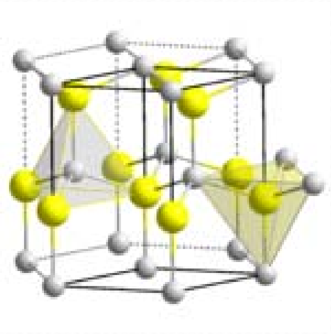
TiO_2

(25 nm)



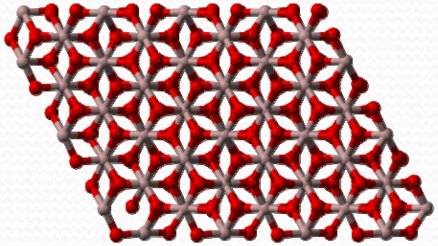
ZnO

(10-30 nm)



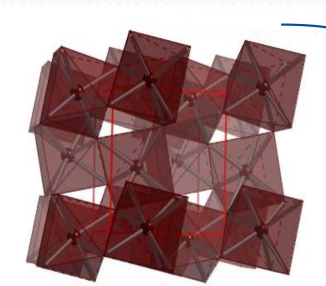
Al_2O_3

(30 nm)



Toxicity Test with Yeast

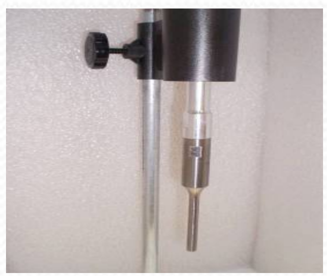
S. cerevisiae (0.1%)
YEPD* medium, pH: 6.5



NPs

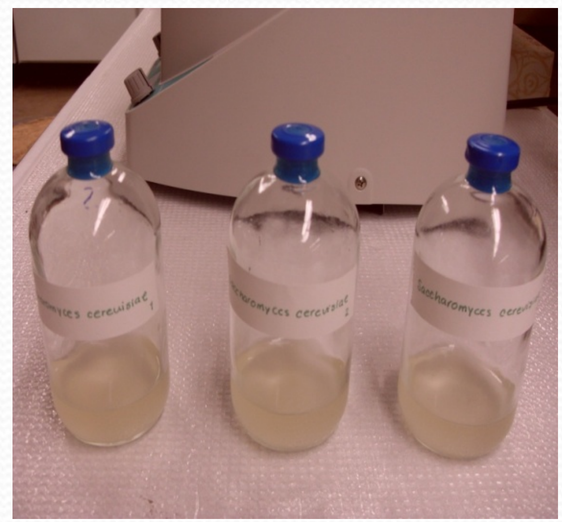
+

Dispersant
(Dispex)
10:1 w/w



Sonicated
5 min.70% amplitude
DEX® 130

20% O_2

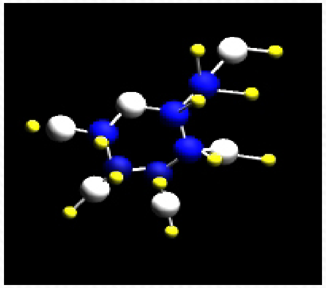


Incubation: 10 h at 30°C and 200 rpm

→ O_2
(GC-TCD)

*YEPD = Yeast Extract Peptone Dextrose

Experiment Design: Estimating O_2 Uptake from Theoretical Oxygen Demand (ThOD)



Glucose

Peptone

Yeast extract

→ *ThOD x (1-cell yield)* →

20% O_2

Head Space → *Volume*

Liquid → *Volume*, *[S]*

Predicted O_2 consumption ~ Amount O_2 in head space

25 mL of YEPD medium, 135 mL of head space and 5 g ThOD/L

Characterization of NPs

10 hrs shaking → 30 min. statics → Samples:

Supernatant
Total suspension

Analytical methods:

- Particle size
- Zeta potential
- Concentration

- Similar conditions to the toxicity test.
- Studies without yeast were carried out.

Particle Size Distribution

Zetasizer®Nano ZS
Malvern Instruments

Dynamic Light Scattering

- Analyzes the velocity distribution of particle movement by measuring dynamic fluctuations of light scattering intensity caused by Brownian motion of the particle.

Zeta potential

- The electrical potential that exists across the interface of all solids and liquids

Zeta Potential [mV]	Stability behavior of the colloid
from 0 to ±5,	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

Concentration of Nanoparticles

<u>ICP- OES</u>

To determine the elemental composition of samples

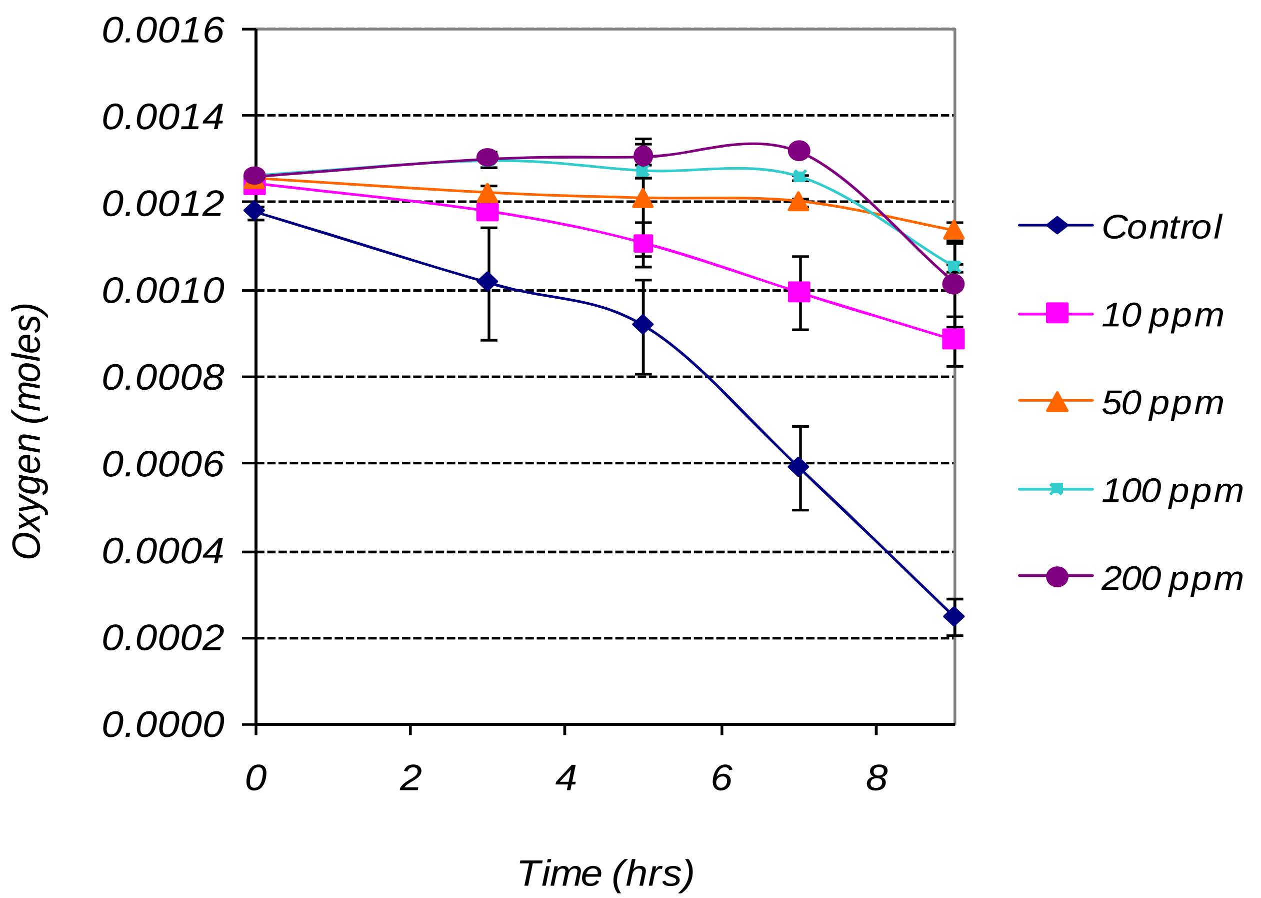
Optima 2100 DV Perkin Elmer ®

<u>Microwave-Assisted Digestions</u>

To reduce interference by organic matter and to convert metals associated with particulates to a form (usually free metal) that can be determined with ICP

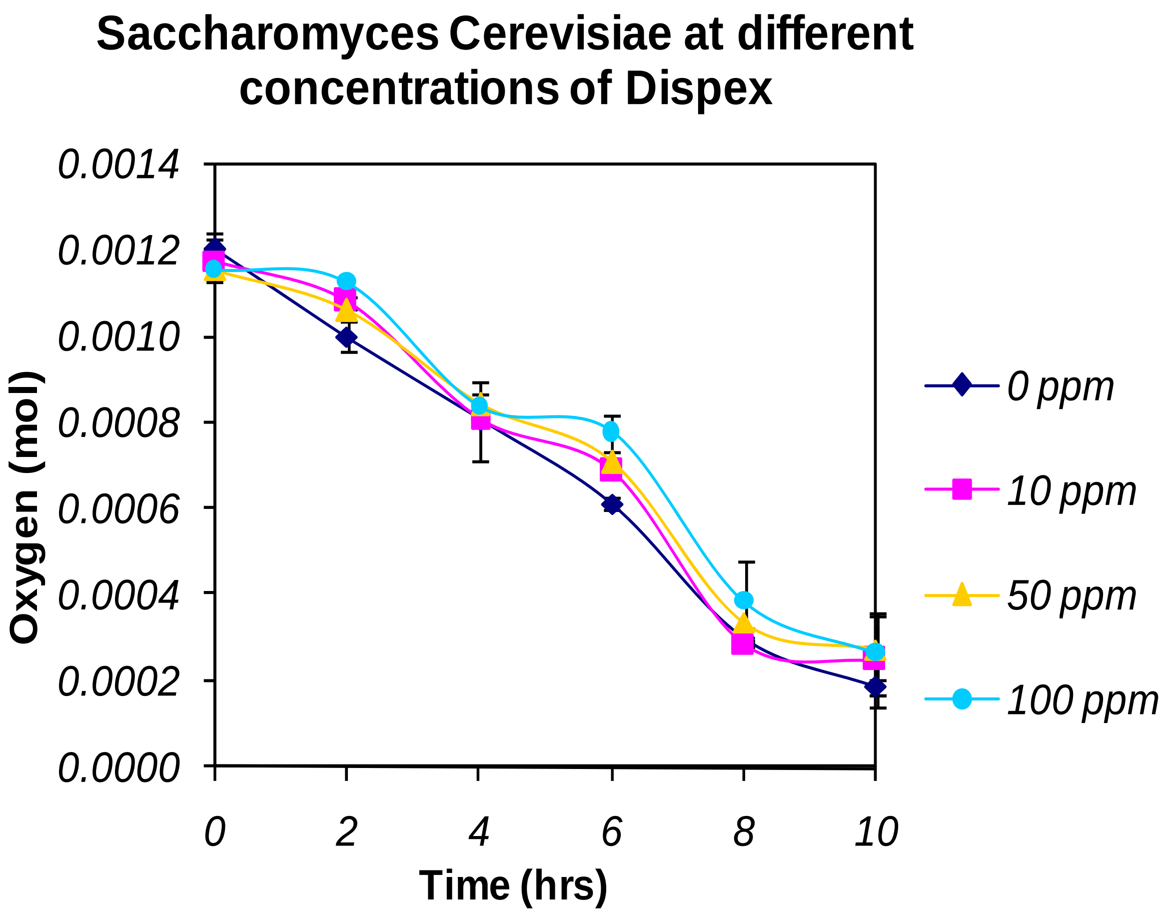
Results…

Toxicity of dispersants Polyethylenimine (**PEI**)



PEI dispersant was toxic to *S. cerevisiae.*

Toxicity of dispersants
Dispex (Ammonium polyacrylate)



Dispex is not toxic to yeast

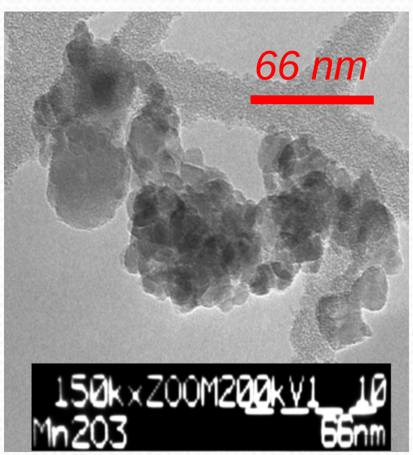
Dispex can be used in toxicity test to disperse NPs.

Dispex:NPs ratio: 1:10 (w/w)

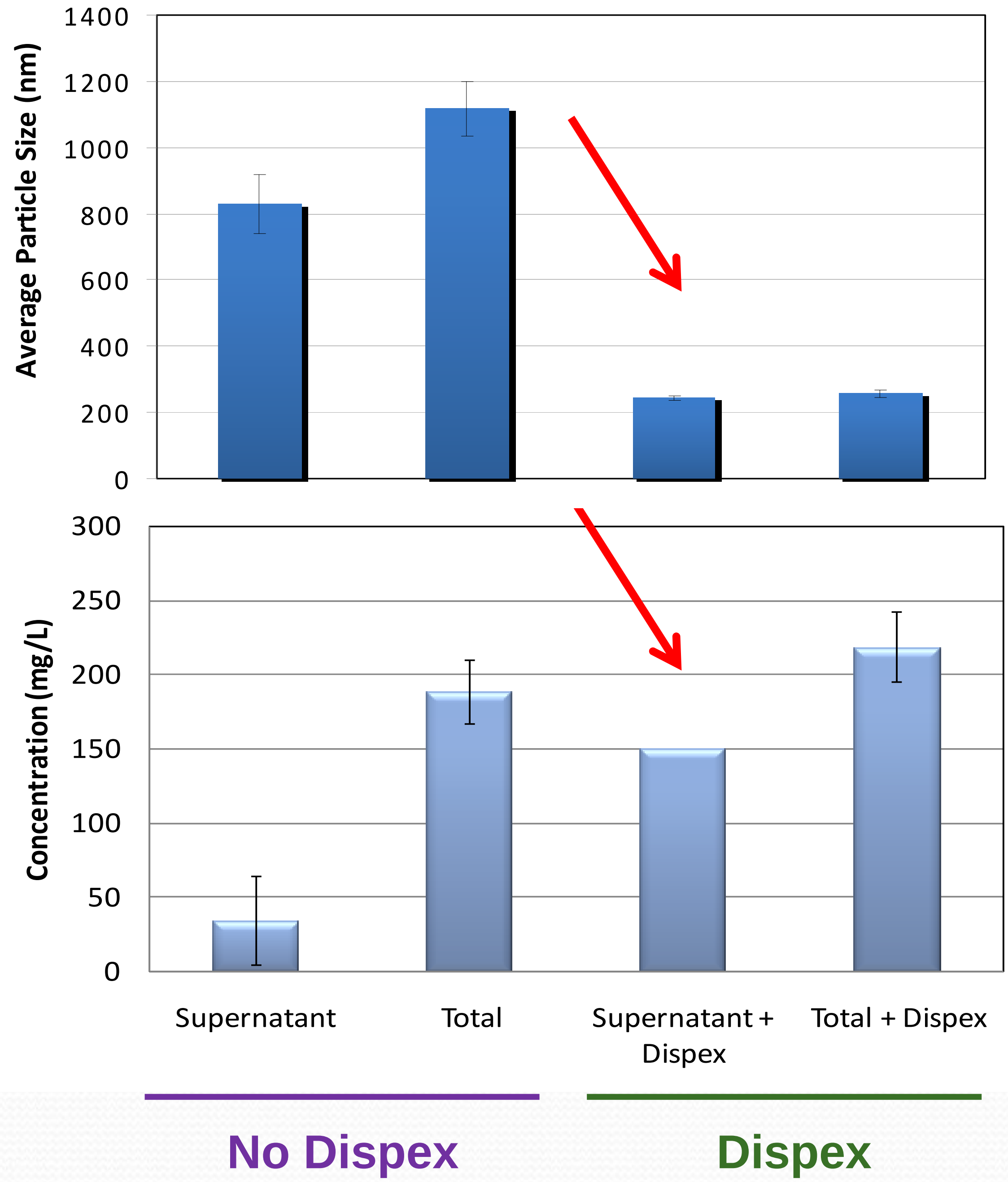
Stability of **Mn_2O_3** NP Dispersions in Demineralized Water

pH= 6

Dispex stabilizes the NP dispersion in water (pH=6)



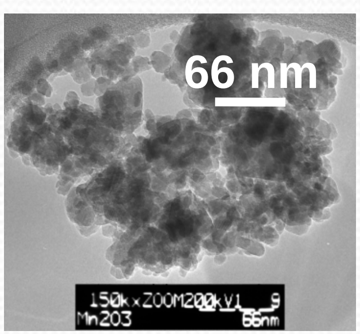
Mn_2O_3 (30-60 nm)



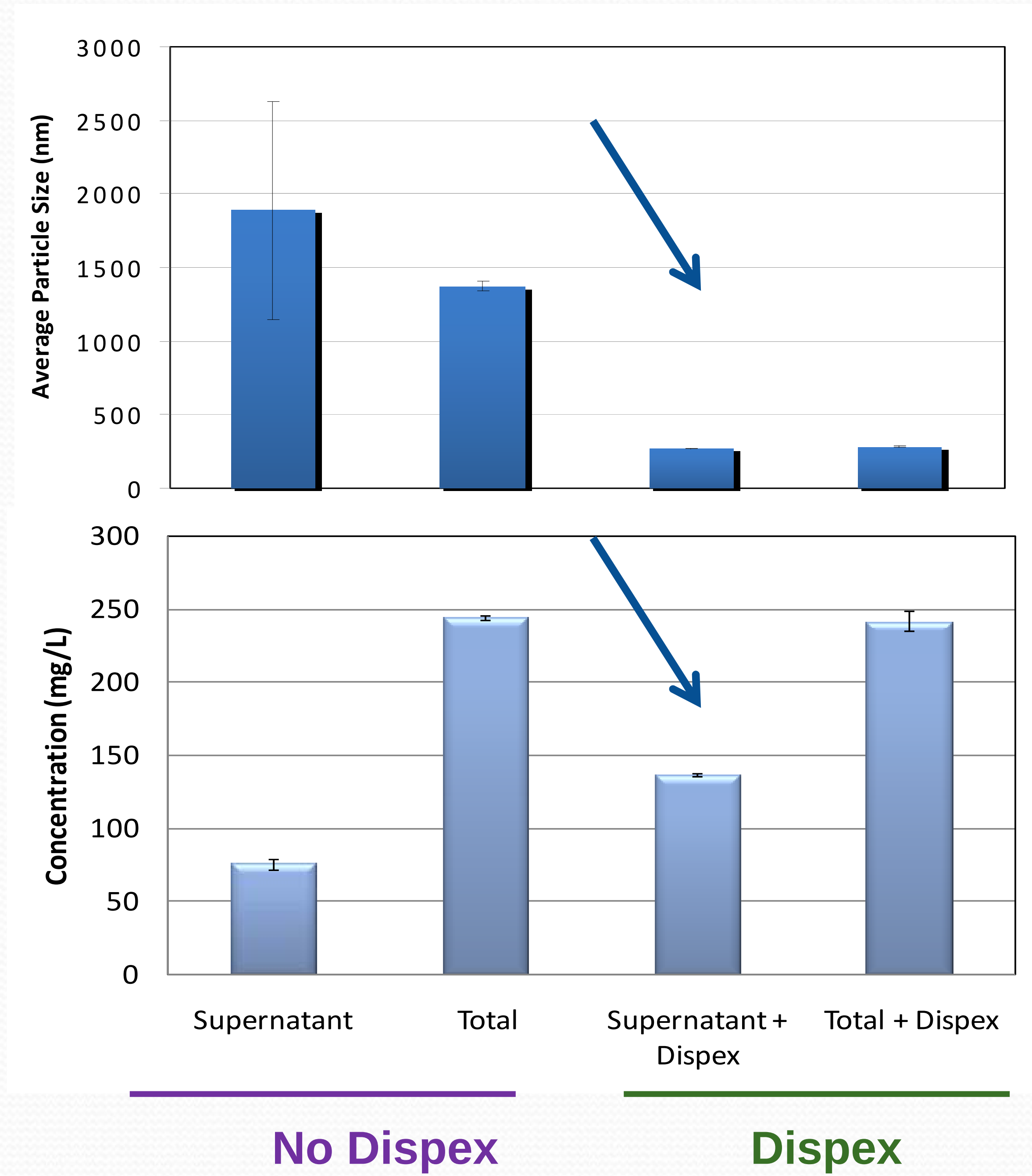
Stability of Mn_2O_3 NPs in Yeast Medium

pH= 6

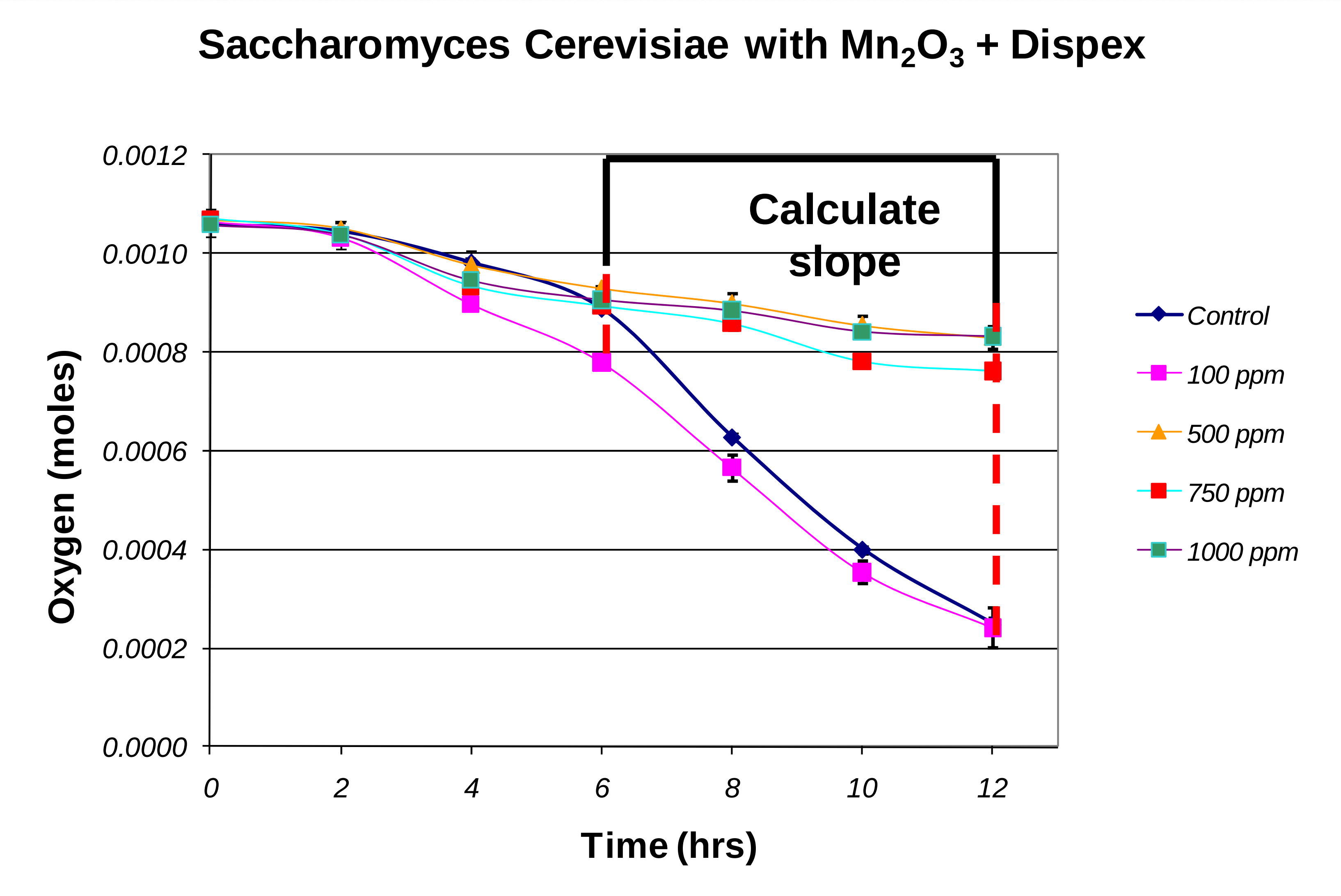
Dispex increased the stability of NP dispersions in biological medium



Mn_2O_3 (30-60 nm)

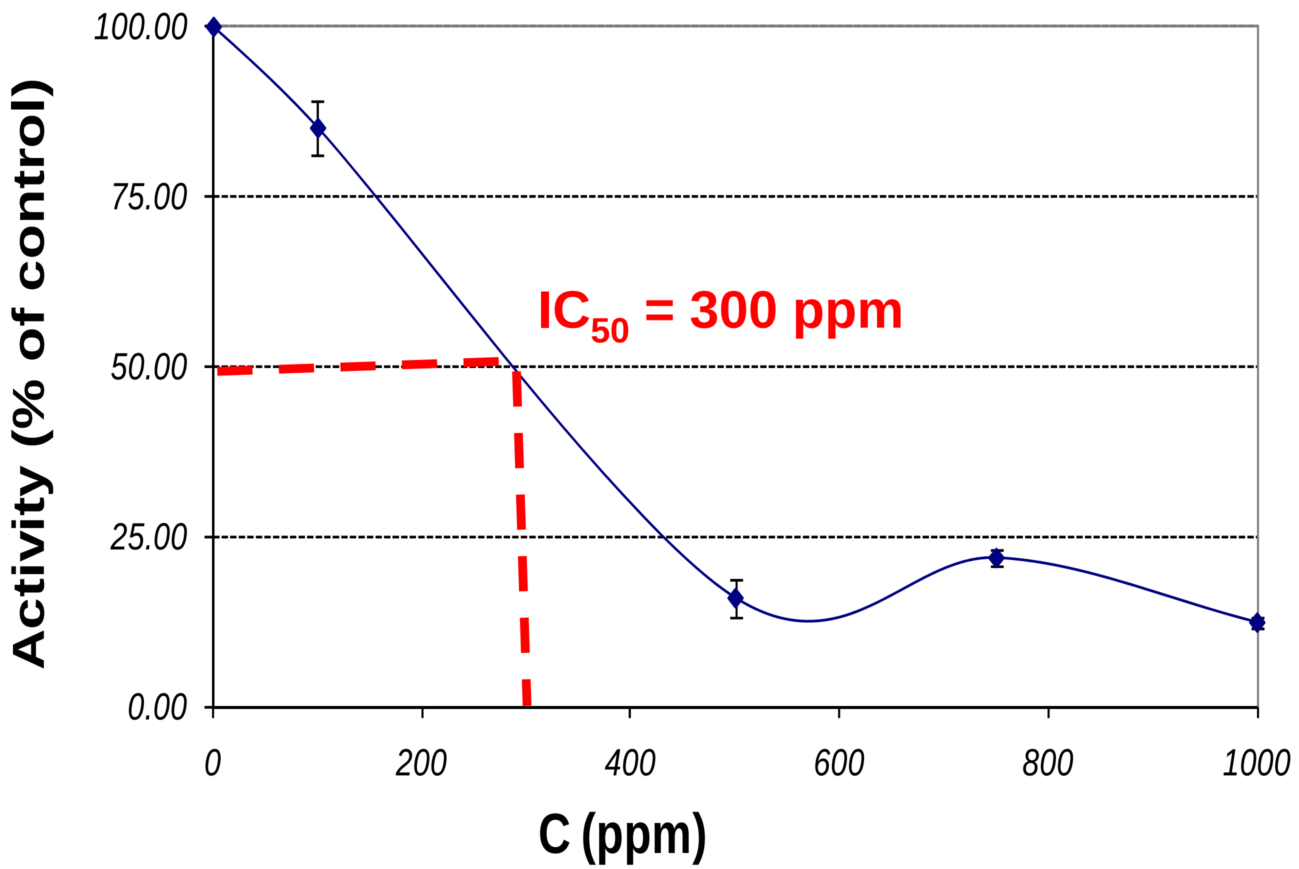


Mn_2O_3 Nanoparticle with Dispex

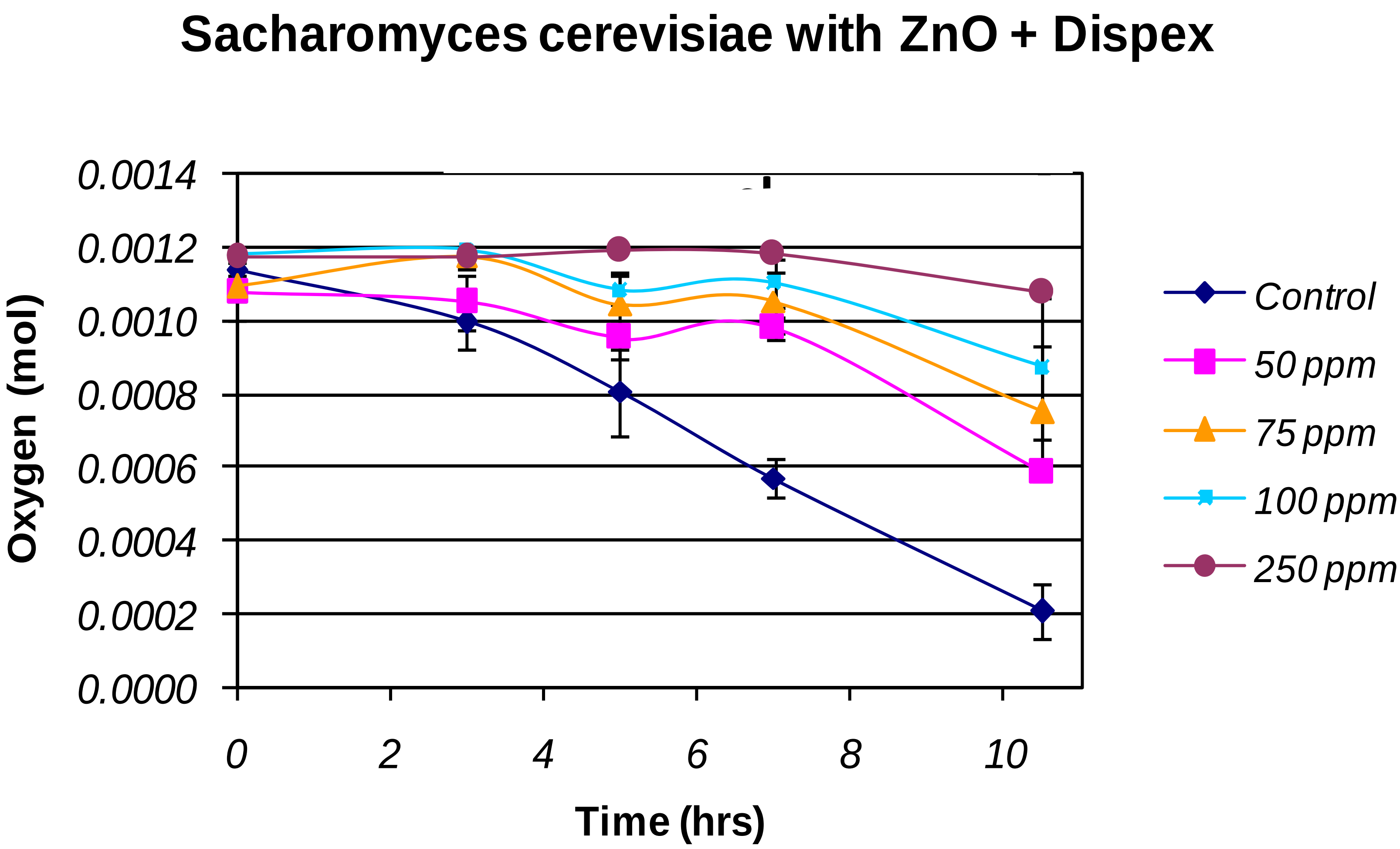


Mn_2O_3 nanoparticle are toxic to yeast at concn. > 500 ppm

Activity of Yeast Respiration Mn_2O_3 Nanoparticles with Dispex

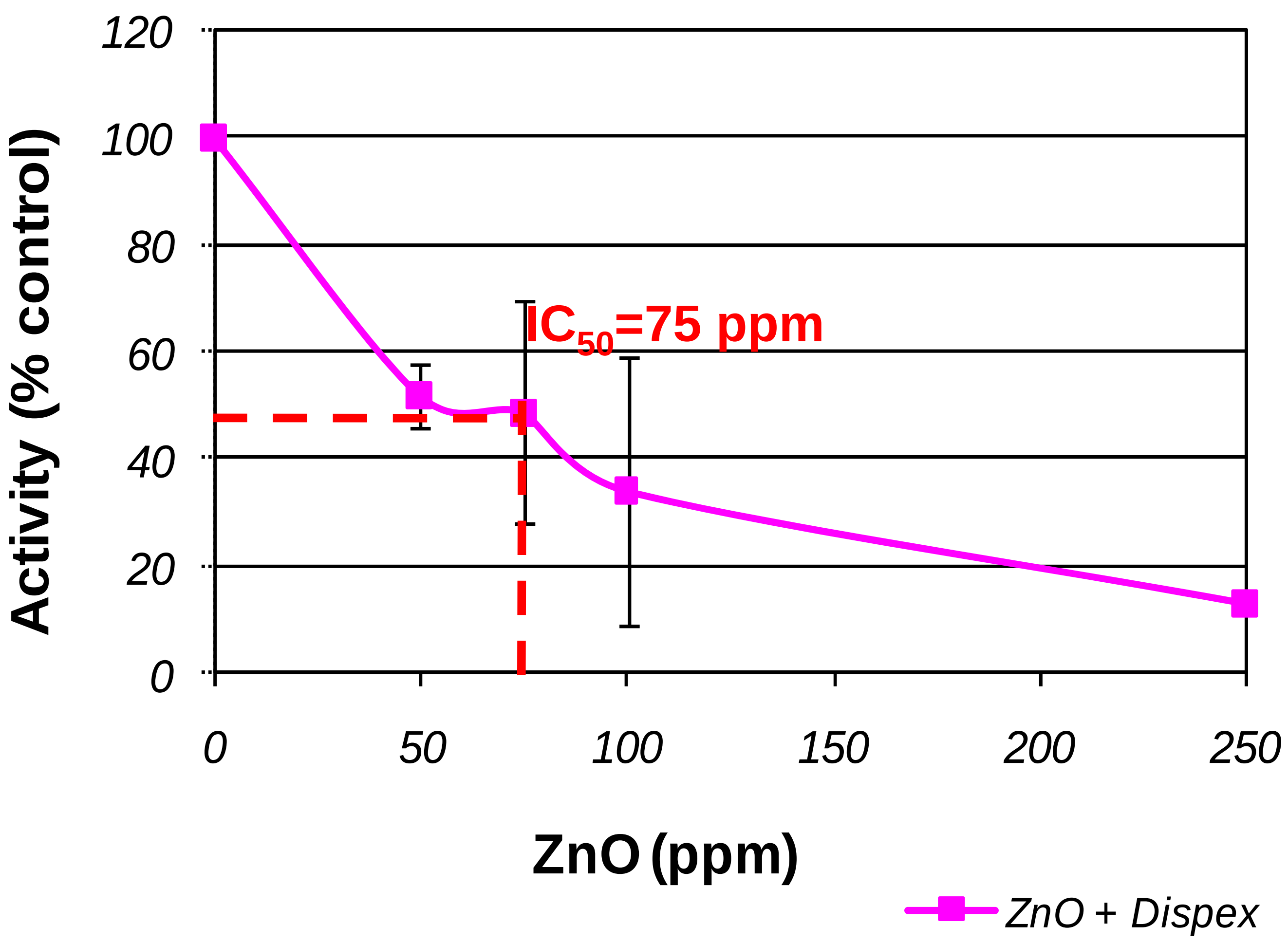


ZnO Nanoparticle with Dispex

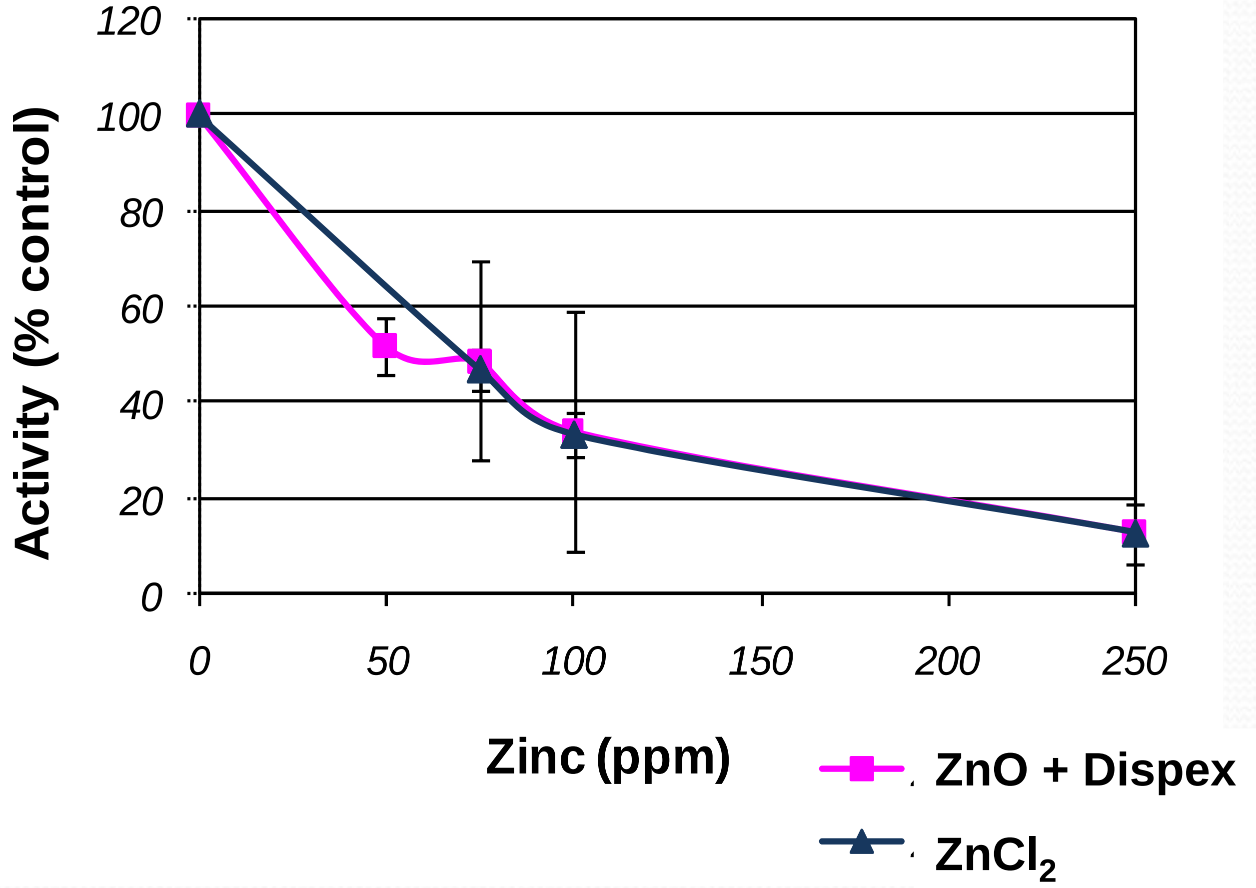


ZnO NPs toxic to yeast at > 50 ppm

Activity of Yeast respiration
ZnO Nanoparticle with Dispex



Possible Mechanism of **ZnO** NP Toxicity



Toxicity observed with ZnO NPs could be associated to the Zn(II) ion

Kasements *et al*. (2009) reported that ZnO toxicity was explained by soluble Zn(II) ion

Nanoparticles Toxicity

NPs	50% inhibition* (ppm)
ZnO	**75**
Mn_2O_3	**300**
CeO_2	**1000**
SiO_2	>1000
HfO_2	>1000
Al_2O_3	>1000
ZrO_2	>1000

***with Dispersant**

Conclusions

- Monitoring of O_2 uptake by yeast cells is a reliable method to study the toxicity of NPs.
- The addition of the dispersant Dispex improved the stability of the NPs in yeast bioassay medium.
- Most NPs were not toxic to yeast at 1,000 mg/L. Only CeO_2, Mn_2O_3 and ZnO displayed toxicity. ZnO was the most toxic compound tested.

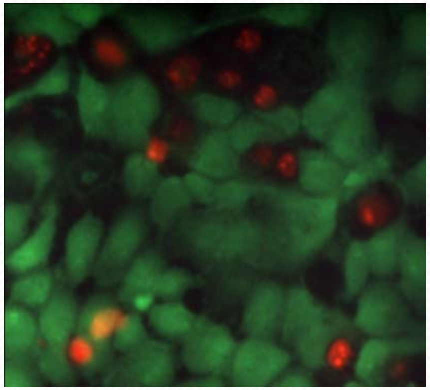
Current and Future Work

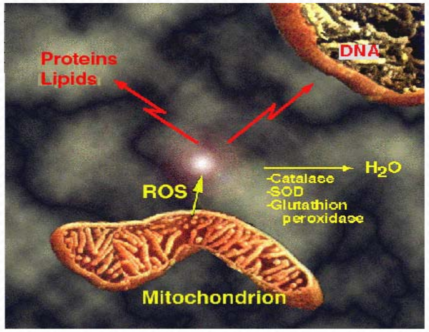
- Complete the characterization of NPs and evaluation of their toxicity to yeast.

- Investigate the mechanisms of toxicity
  - Membrane damage

    (Live/Dead assay, flow cytometry, microscopy)
  - Production of reactive oxygen species (ROS)

    (Commercial kits)





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Thank you!


Questions?