Continuous and batch reactors to quantify the E. coli response to the exposure to nanoparticles (CAREER, CBET-1350789)

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Introduction

Toxicity assessment are commonly performed in batch conditions. Continuous systems have been extensively used to assess physical and chemical stressor on bacteria phenotype and genotype.

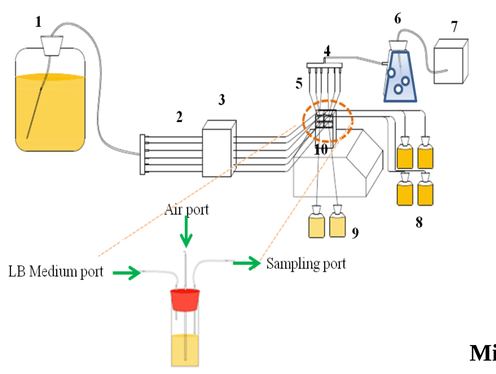
Batch reactors
- Advantages: small volumes, high-through put and simplicity
- Disadvantages: time-dependent

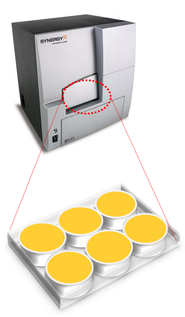
Continuous reactors
- Advantages: steady state, change variables, time independent
- Disadvantages: complexity

Objective

Compare batch and continuous systems to assess the toxicity of casein-coated AgNPs on *Escherichia coli* (*E. coli*)

Setup (I)

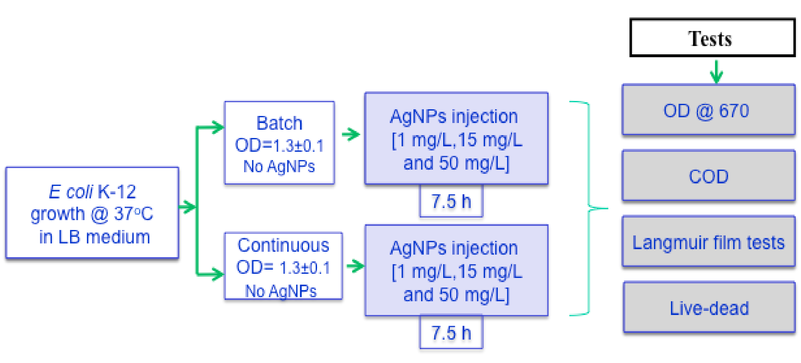




Microplate Reader six wells

Multiplex System
1) sterile media carboy,
2) culture media lines,
3) peristaltic pump,
4) port manifold,
5) airlines,
6) air dehumidifier,
8) collection bottles for samples,
9) collection bottles for controls
10) vessel with ports

Experimental flow chart and analysis tested (I)

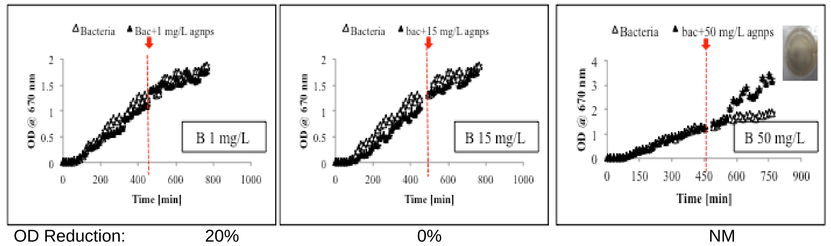


Results (I): Nanoparticle stability

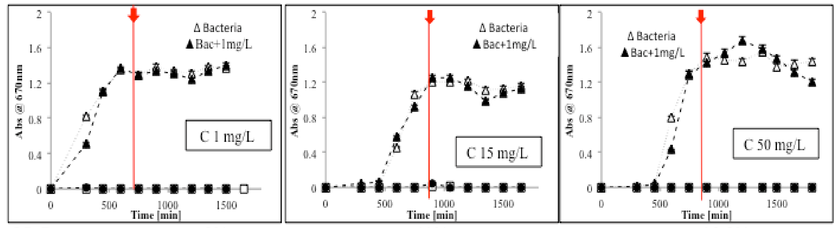
- **Size**: 86 nm to 282 nm after 5 hrs
- **Zeta potential** : -29.7 mV ± 2 mV
- **Ions release**:
  - -Culture media: 4.5% and 8.1% for 15mg/L and 50 mg/L, respectively.
  - -Used media: 0.53% and 0.69% for 15mg/L and 50 mg/L, respectively.

Results (I): Batch vs. Continuous growth

Since biomass concentration in the continuous bioreactor at steady state condition was 1.3 OD_{600}, silver nanoparticles were injected when both, batch and continuous systems reach that concentration value.

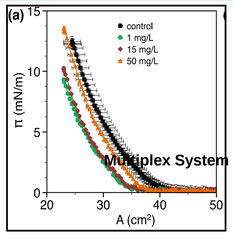


OD Reduction:	20%	0%	NM
COD Reduction:	39%	0%	NM

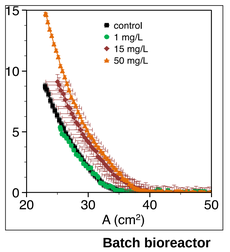


OD Reduction:	0%	11%	16.3%
COD Reduction:	0%	8%	27.6%

Results (I): Langmuir tests



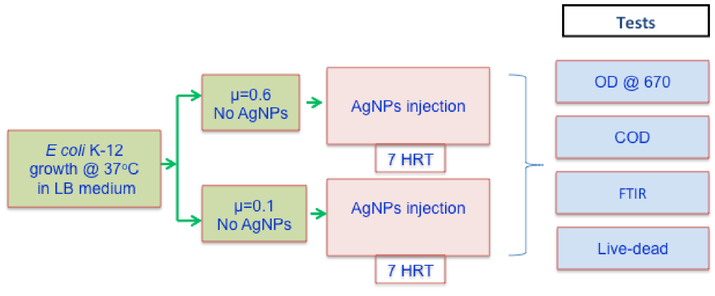
Continuous bioreactor



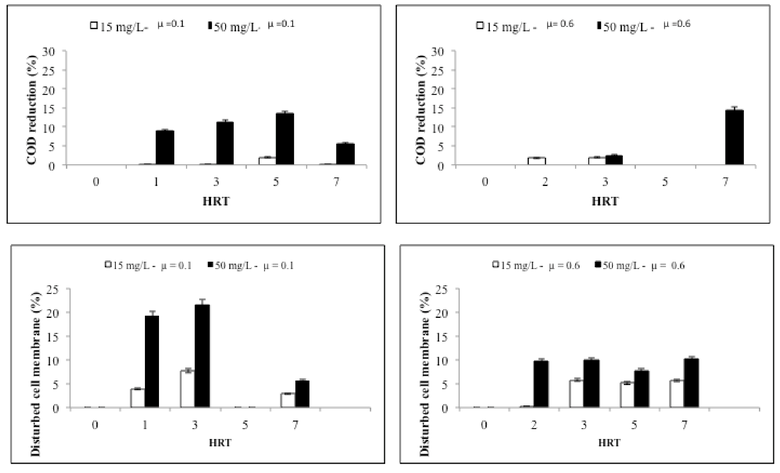
Batch bioreactor

- Batch cultures membrane disruption caused by AgNPs was a destabilizing or fluidizing (disordering) effect. Lipids from extracts where contained more saturated than unsaturated acyl tails. Occupied less area at the air/water interface
- Continuous cultures membrane disruption caused by AgNPs was a *stabilizing or rigidifying (ordering) effect*. Occupied more area at the air/water interface

Experimental flow chart and analysis tested (II)



Results (II):Effect of specific growth rate



Results (II):FTIR results- 50 mg/L of AgNPs (II)

	Vibration mode	Changes detected		
		μ 0.1 h^{-1}	μ 0.6 h^{-1}	
Fatty acid	Asymmetric vibration of (C=O)	Shifting	NF	Changes in the fluidity and the different surface tension between the exposed bacteria and non-exposed one
	Asymmetric vibration of (P=O)	shifting	NF	
	Symmetric vibration of CH_3	NF	Shifting	Changes in the vibration of the carbon atom regarding to hydrogen atoms
Protein	Amide III	Shifting	NF	Damage of conformational/compositional alterations in some of the components of the protein structures that could be intracellular proteins or cell wall peptides (Jiang et al., 2010)

Conclusions

- COD reduction and membrane permeation were detected at high AgNPs concentration used for both growth rates
- Continuous systems can be used to evaluate the inhibitory effect of nanoparticles in continuous culture at different growth rate of bacteria
- The results did not agree with previous studies with regard to the specific growth rate due to the different contact times that were achieved in the continuous at different specific growth rates
- FTIR could provide additional information about the intracellular components affected by the nanoparticles

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