COMPUTATIONAL MODELING AND EXPERIMENTAL ANALYSIS OF BACTERIAL AGGREGATION IN PSEUDOMONAS EXTREMAUSTRALIS 2E-UNGS

E-ICES 20
20° Encuentro del Centro Internacional de Ciencias de la Tierra

Beraha, Natalia[1, 2] - Daniel, María Alejandra[1] - Gotting, Andrea S.[1, 2] - Carusela, María Florencia[1, 2] - Vullo, Diana L.[1, 2]

1) Instituto de Ciencias, Universidad Nacional de General Sarmiento, J.M. Gutiérrez 1150, B1613GSX - Los Polvorines - Buenos Aires - Argentina

2) CONICET, Godoy Cruz 2290, C1425FQB - CABA - Argentina

nberaha@campus.ungs.edu.ar

Introduction

This study explores bacterial aggregation in *Pseudomonas extremaustralis* 2E-UNGS, a bacterium from the polluted Reconquista River. It has properties like biosorption and biotransformation of metals, useful for bioremediation. The study measures bacterial growth and self-aggregation through optical density and microscopy, and develops a numerical model to describe aggregation dynamics based on experimental data.

Objetives

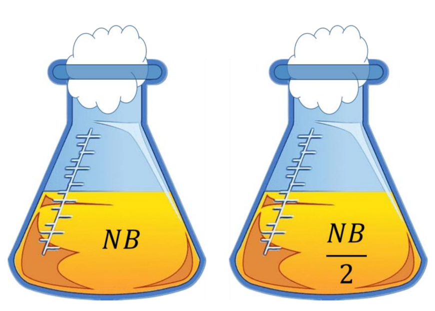
- Study the bacterial aggregation dynamics of *Pseudomonas extremaustralis* 2E-UNGS.
- Develop a deterministic numerical model to describe these dynamics.
- Validate the model using experimental data.

Experimental

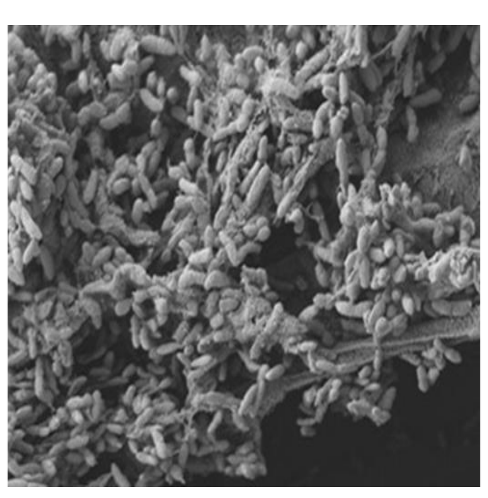
•Microorganism: Pseudomonas extremaustralis 2E-UNGS (GenBank CP091043.1)

• Culture media: Nutrient Broth (g/L: peptone from meat 5.0; meat extract 3.0)

• Incubation: thermostatic bath at 32 ºC under agitation 120 rpm

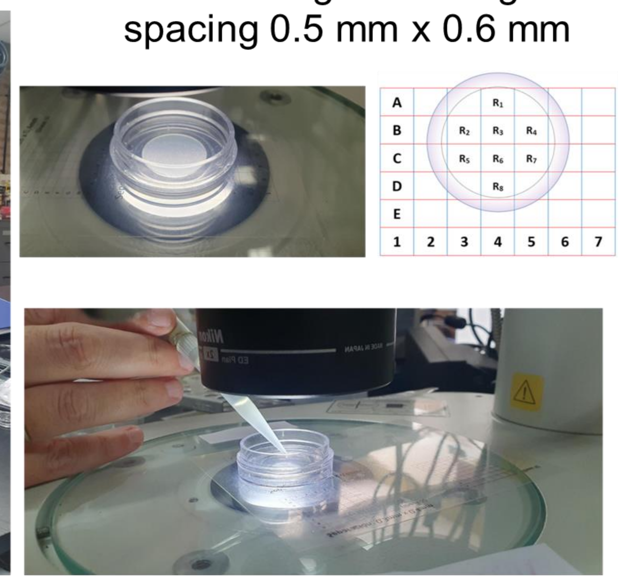


• **2D-Digital images:**
Acquisition of images:



Stereo microscope "NIKON - SMZ 1270"

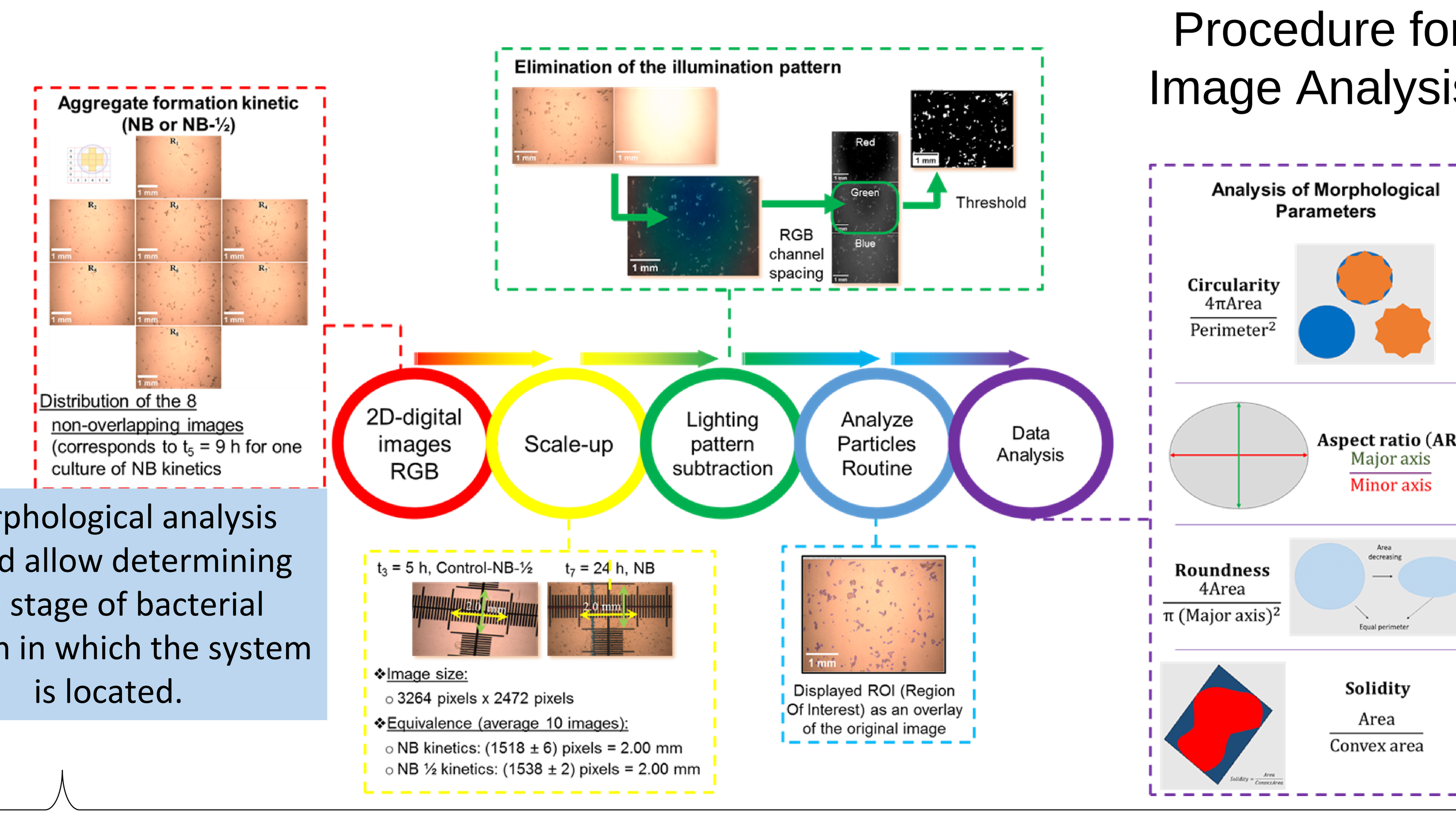
Each petri-dish was divided into in 8 regions with grid spacing 0.5 mm x 0.6 mm



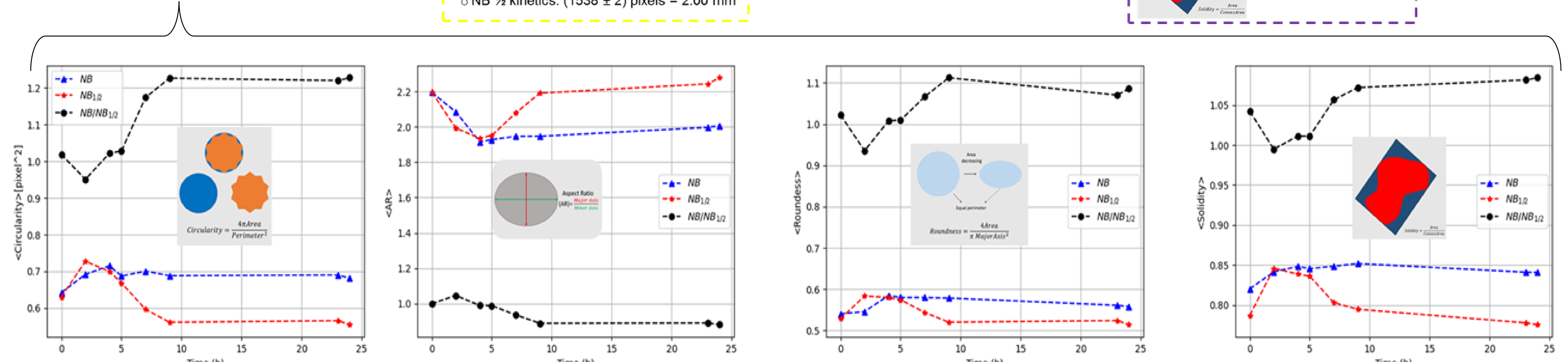
0.300 mL of sampling in petri-dish confocal (in duplicate for each sampling)

Digital Image Analysis: Morphological Study

Procedure for Digital Image Analysis Using FIJI®



Morphological analysis would allow determining the stage of bacterial growth in which the system is located.

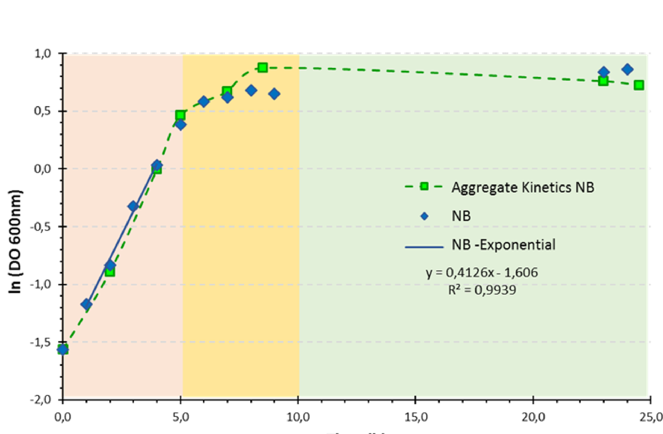


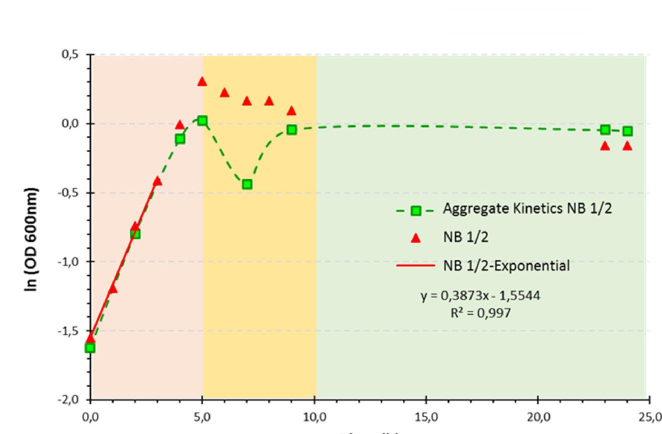
The morphological parameters of the aggregates showed similar behaviors in both media during exponential growth

Upon reaching the stationary phase (time > 10 h), the aggregates adopt a more oblong shape, becoming more elongated and irregular. Consequently, their "solidity" decreases as they fragment due to resource depletion.

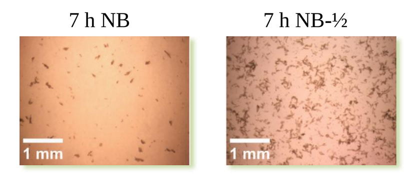
Experimental Data Analysis

Growth curve *of Pseudomonas extremaustralis* 2E-UNGS with NB and NB-1/2





OD_{600nm} becomes a relative parameter of bacterial growth.

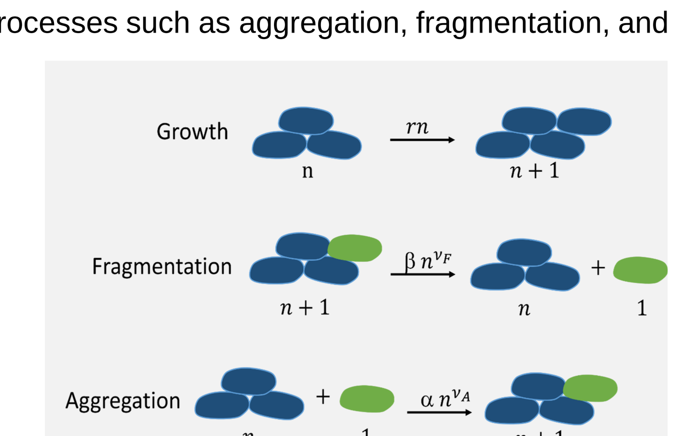


The three stages of bacterial growth can be observed at both nutrient medium concentrations

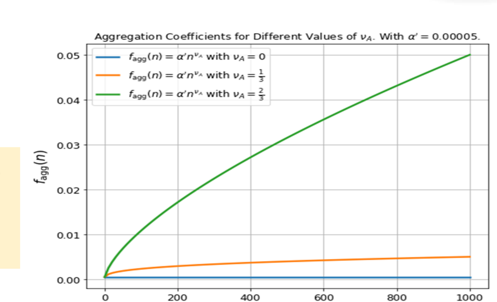
Exponential Phase | Deceleration Phase | Stationary Phase

Modeling Simulation I

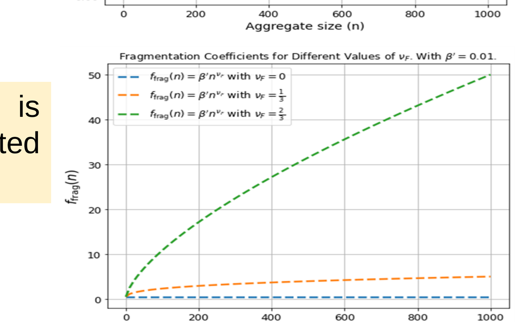
We simulate bacterial aggregate dynamics using a deterministic numerical model implemented in Python, based on a master equation. The model incorporates experimental data as initial conditions and simulates processes such as aggregation, fragmentation, and growth.



The initial conditions are taken from experimental data.



The duplication rate r is experimentally calculated using optical density.



The Adimentional Model

Aggregation term

$$\frac{dc_n}{dt'} = \alpha' (n-1)^{\nu_A} c_{n-1} c_n - \alpha n^{\nu_A} c_n c_1 +$$

Growth term

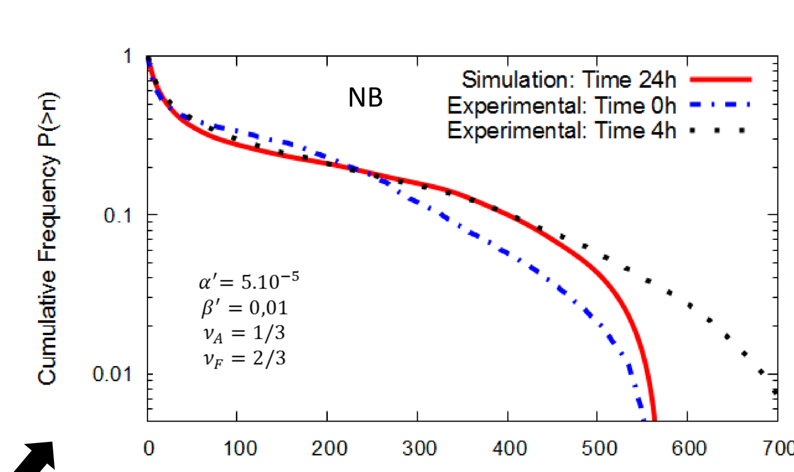
$$+ r' [(n-1) c_{n-1} - n c_n] +$$

$$+ \beta' [(n+1)^{\nu_F} c_{n+1} - n^{\nu_F} c_n + \delta_{n,1} c_1]$$

Fragmentation term

Time: $t' = tr$ Growth rate: $r' = 1$

Aggregation rate: $\alpha' = \frac{\alpha}{r}$ Fragmentation rate: $\beta' = \frac{\beta}{r}$



The numerical simulation fits well with the experimental results!

Modeling Simulation II

Model of discrete bacterial aggregates with simultaneous aggregation and fragmentation of up to three aggregates. Fragmentation uses the Dirichlet distribution to conserve total size. Simulation results are compared with experimental data to assess the observed dynamics.

Generate combinations of aggregates
Calculate all possible combinations of 2 or 3 aggregates that could fuse

↓

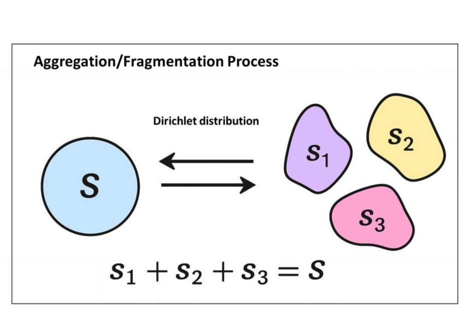
Calculate fragmentation rates
Determine the probability of each aggregate fragmenting

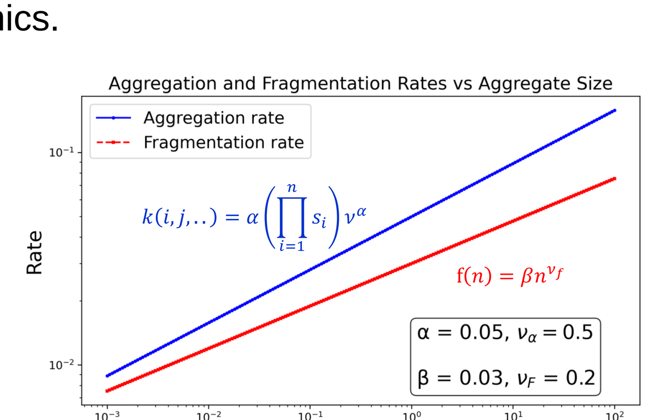
↓

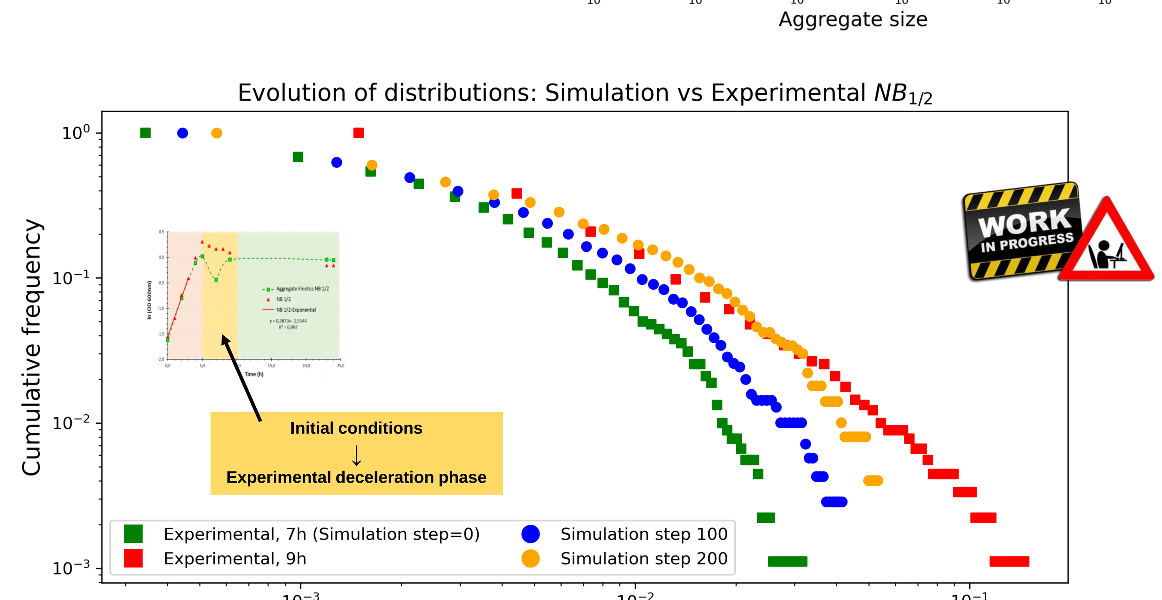
Select event
Choose an event (fusion or fragmentation) probabilistically, proportional to its rate

↓

Update aggregates
Modify the aggregate list based on the selected event, and repeat the cycle







Conclusions

- The morphological analysis of the experimentally recorded images is a good indicator of the stages of bacterial growth.
- A deterministic numerical model was proposed to study the formation of bacterial aggregates, which uses parameters obtained experimentally.
- The discrete multi–interaction model reproduces the experimental size distributions well, capturing both the slope and the characteristic aggregate scale, while only minor deviations remain at the extremes.
- These results contribute to the understanding of the cell aggregation mechanism to optimise the design of bioreactors for the treatment of metal effluents.