Effect of Antibiotics in Wastewater on the Activated Sludge Process: The Erythromycin Case

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From antibiotics discharge to water contamination

sludge

WWTP

Urban WWTP

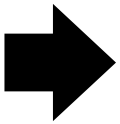
Soil

Aquatic environment

At the WWTP level

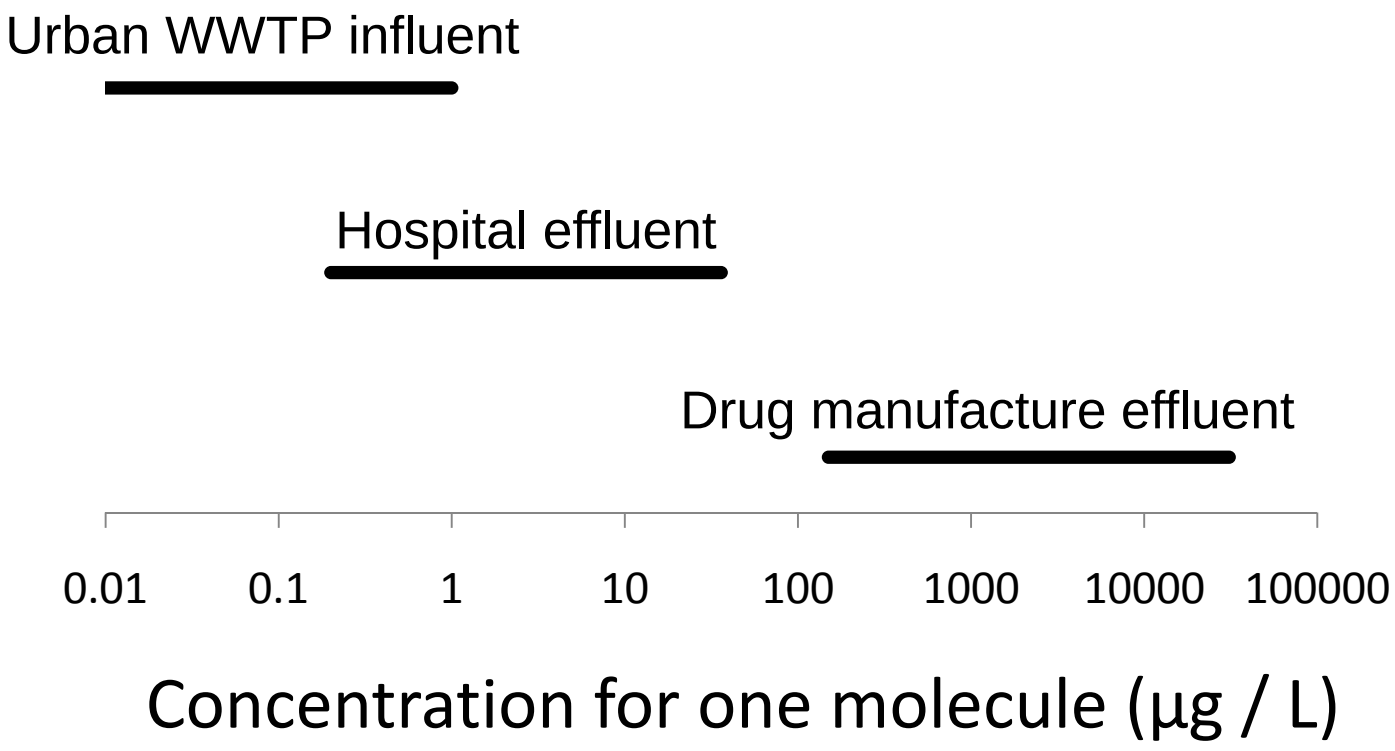
Incomplete antibiotic removal

Concerns about bacterial resistance



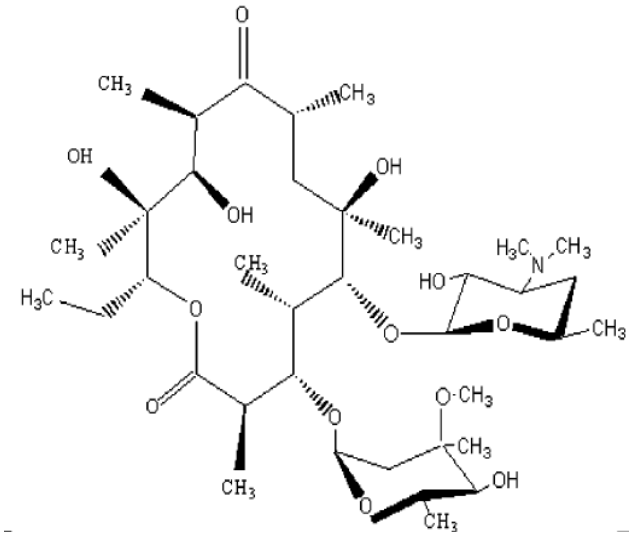
Concerns about toxicity on activated sludge bacteria

Range of antibiotic concentrations in wastewater



Erythromycin in the environment

- One of the most frequently detected antibiotic in untreated drinking water sources (Focazio et al. 2008)
- One of the most harmuful antibiotic for aquatic environment (Lee and al. 2008, Isidori and al.2005,)
- One of the less removed antibiotic by WWTP (Rosal et al. 2010)
- Increase in bacterial resistance (Baquero 2008, Riedel et al. 2007)



Erythromycin

Materials and Methods

1) Comparison of wastewater treatment by activated sludge in control reactors and reactors containing Erythromycin

- Erythromycin concentration (4 µg-20 mg/L)
- Batch experiments lasted for 24h to 90h

Sludge and wastewater collected from two urban WWTPs:

- Nancy WWTP (350,000 person-equivalents; hybrid system of activated sludge and biofilm on sand particles)

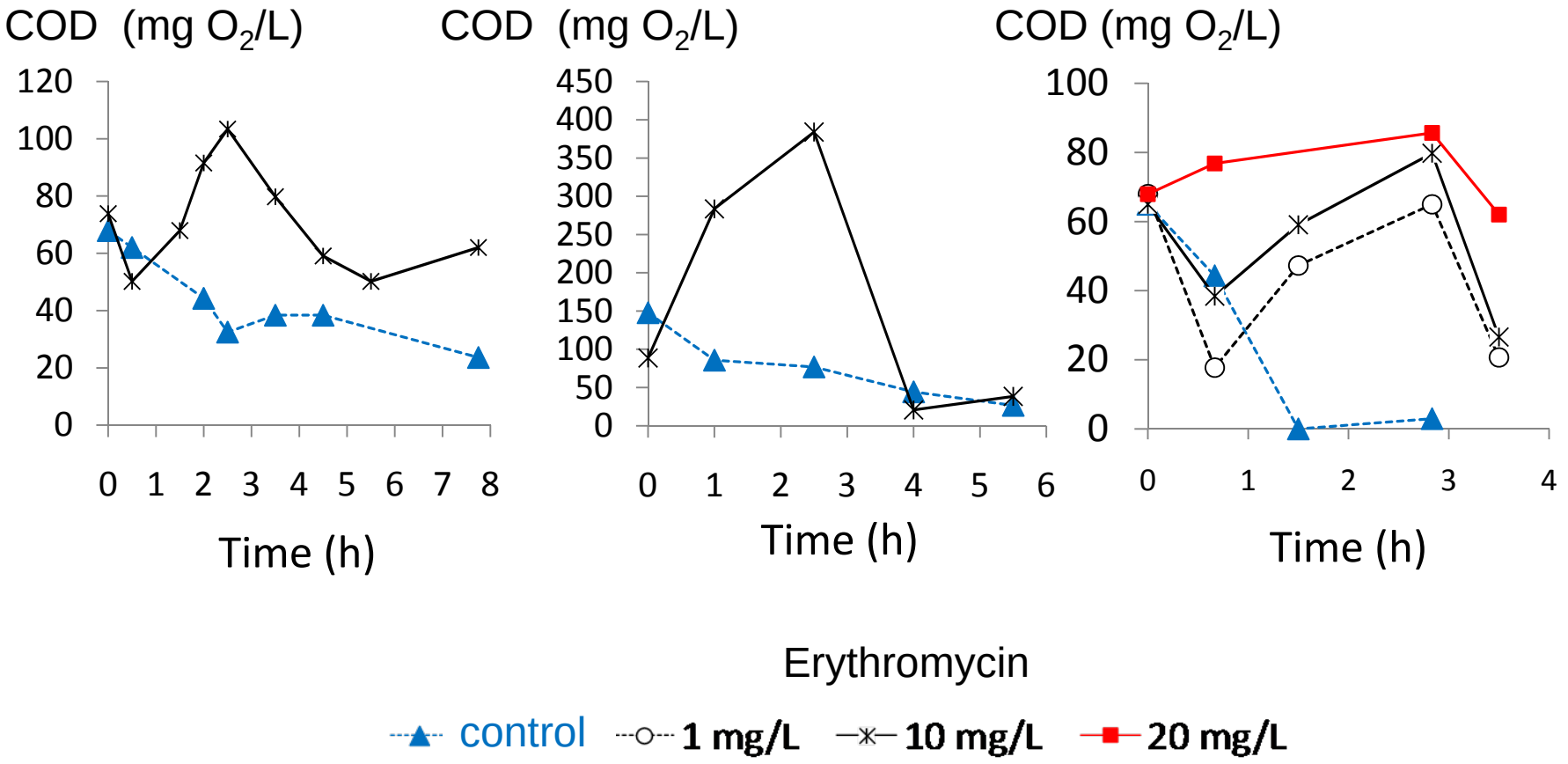
- Epinal WWTP (60,000 person-equivalents; oxidation ditch with no primary settling)

2) Imaging of Erythromycin time-kill activity

- Brightfield microscopy
- CLSMicroscopy, spectral imaging
- CLSMacroscopy
- Epifluorescence combined with Brightfield microscopy

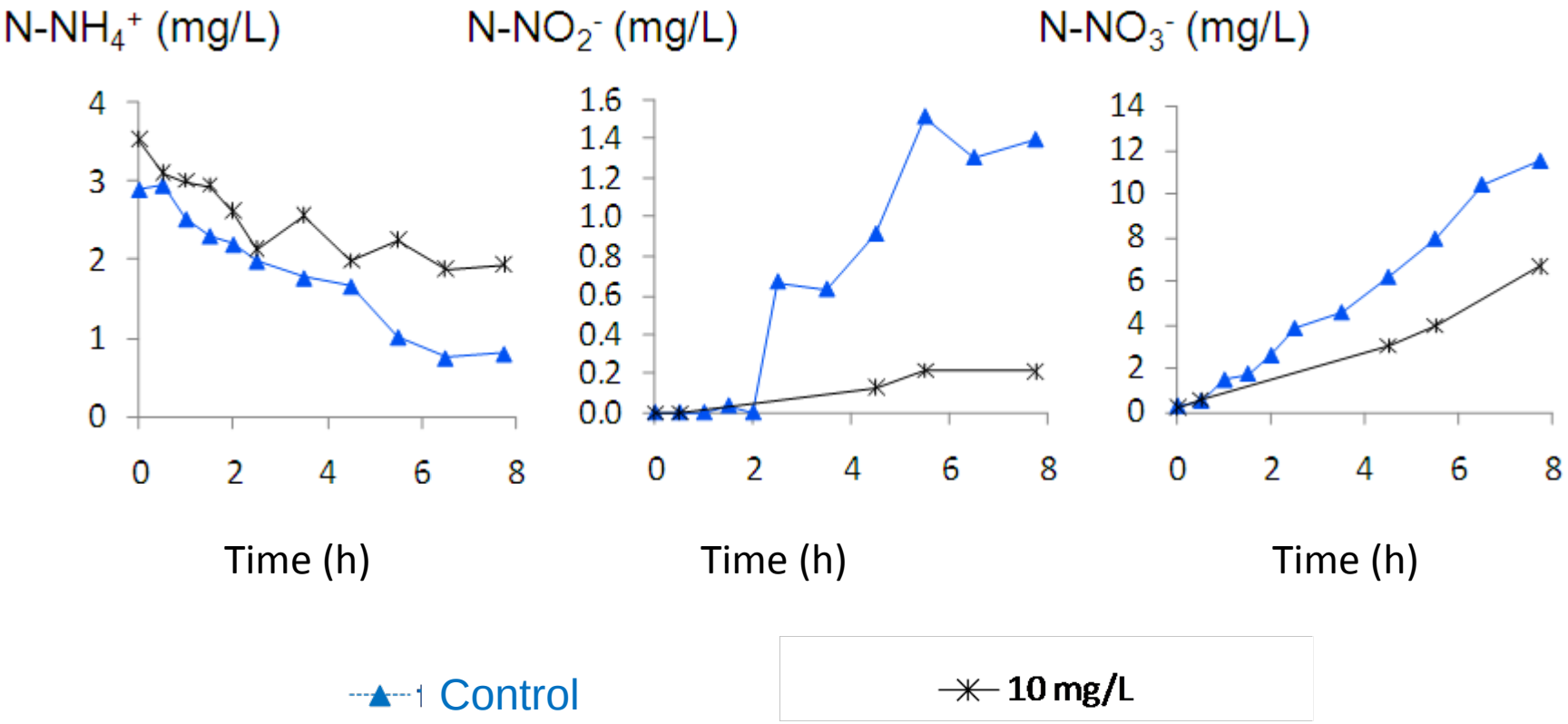
1) Comparison of wastewater treatment by activated sludge in control reactors and reactors containing Erythromycin

Erythromycin inhibited COD removal,
the destroyed sludge increased the pollution level



Nancy Sludge

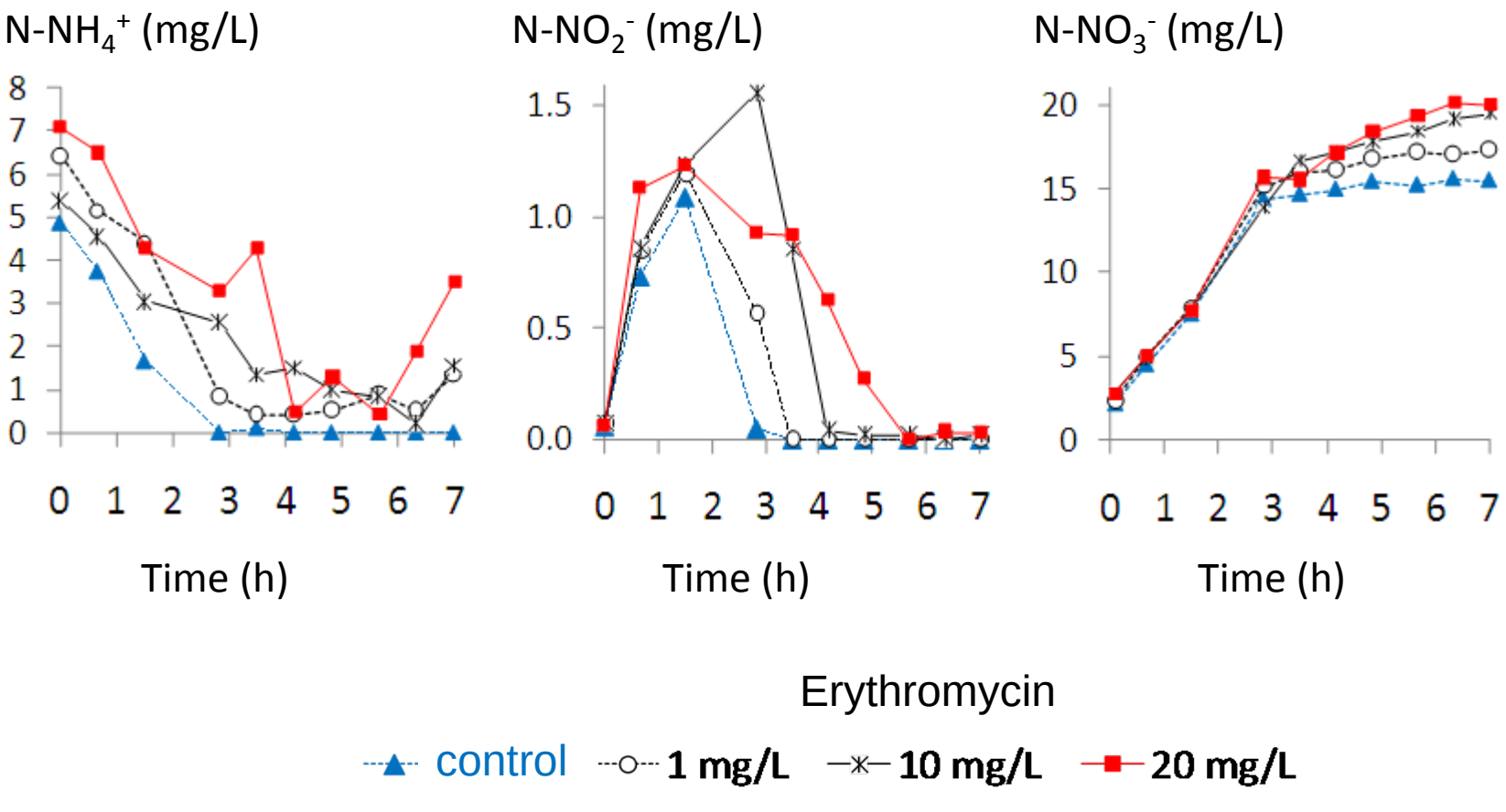
For Nancy WWTP Sludge, nitrifiers were significantly inhibited.



Epinal sludge

Biomass destruction produced organic nitrogen

→ The nitrate production increased in the end of the experiments



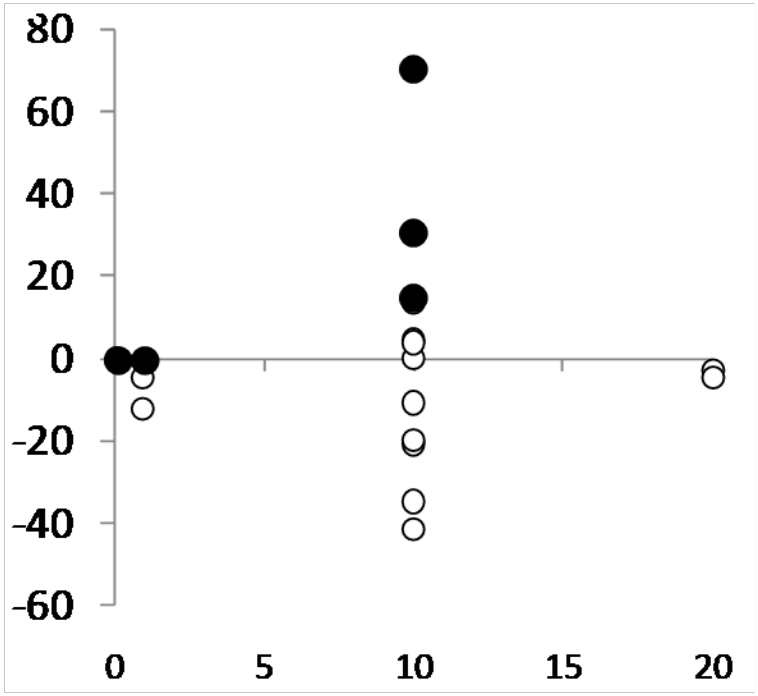
The nitrification inhibition depended on the sludge origin

$$\text{Percent inhibition} = \left(\text{rate}_{\text{control}} - \text{rate}_{\text{erythromycin reactor}}\right) \Big/ \text{rate}_{\text{control}} \times 100.$$

Nitrification inhibition (%)

Nancy Sludge :
Nitrification was inhibited

Epinal Sludge :
Nitrate production increased



Erythromycin concentration (mg/L)

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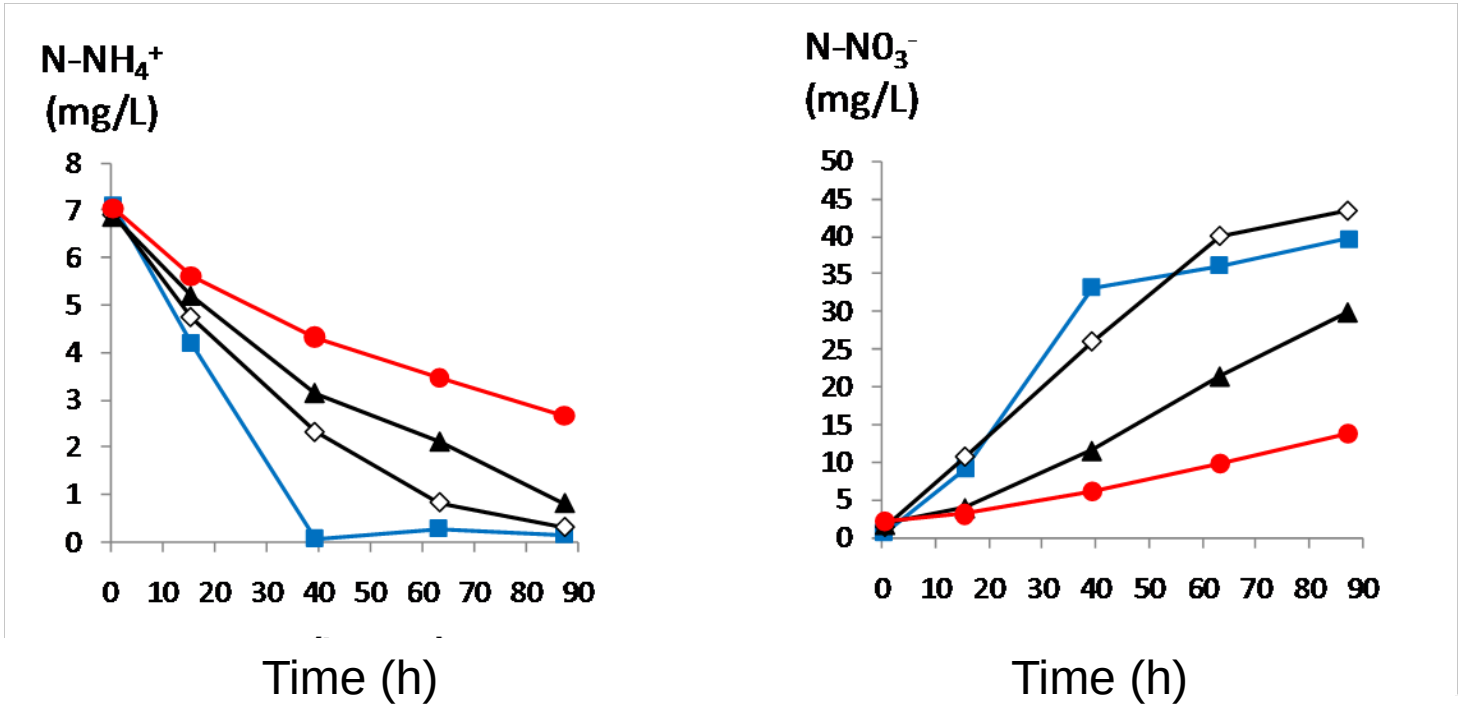
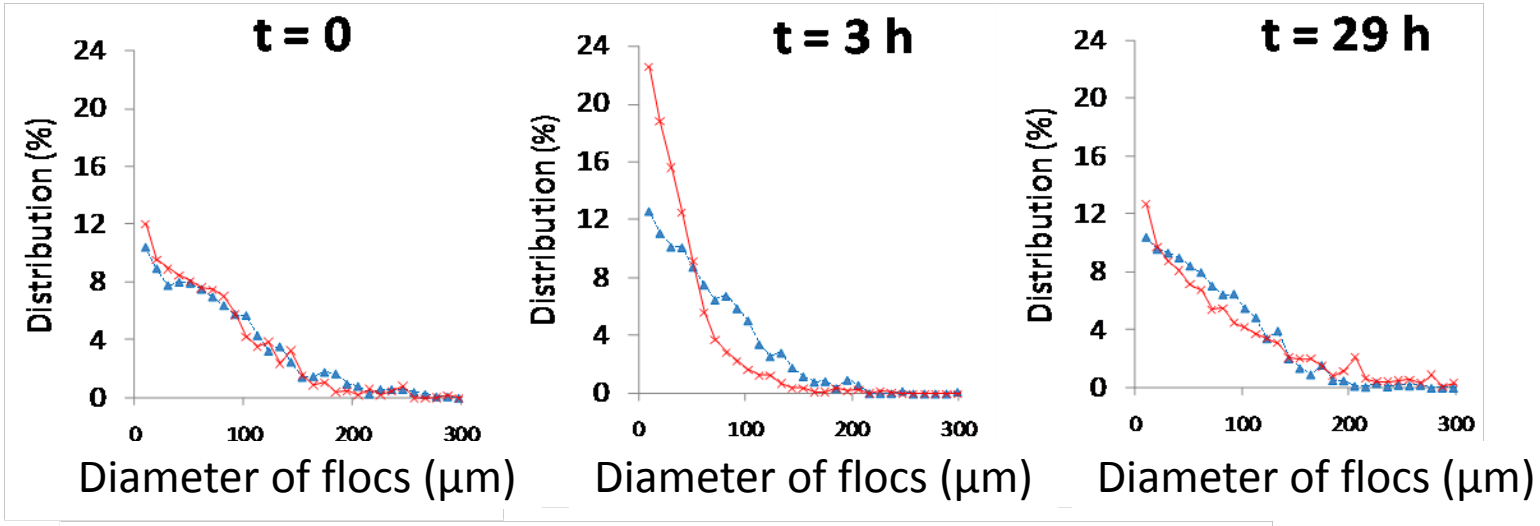
90 h batch experiments:

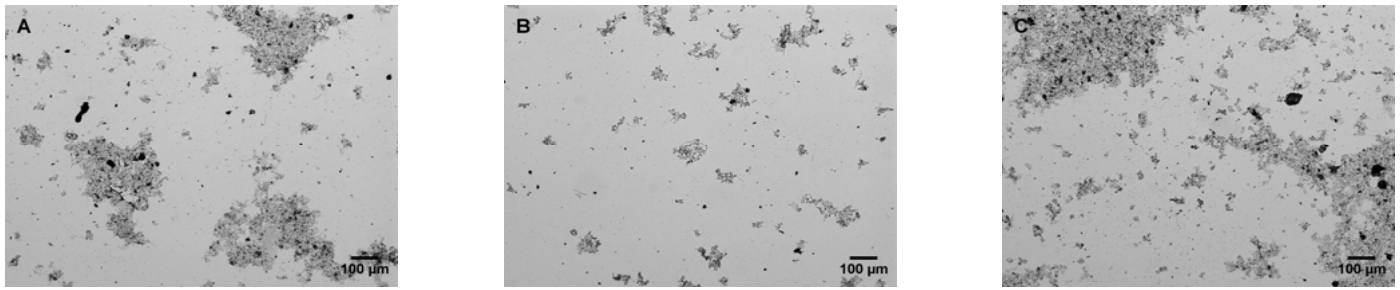
4 µg/L Erythromycin concentration inhibited N-NH_4^+ removal



2) Imaging of Erythromycin time-kill activity

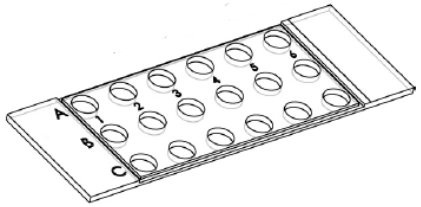
Erythromycin (10 mg/L) caused flocs breakage





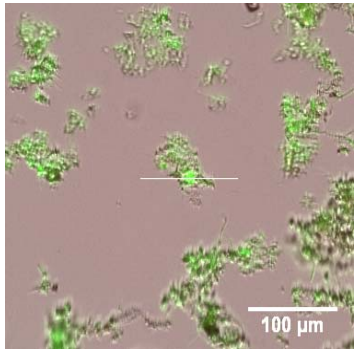
Results observed for both Nancy and Epinal Sludge

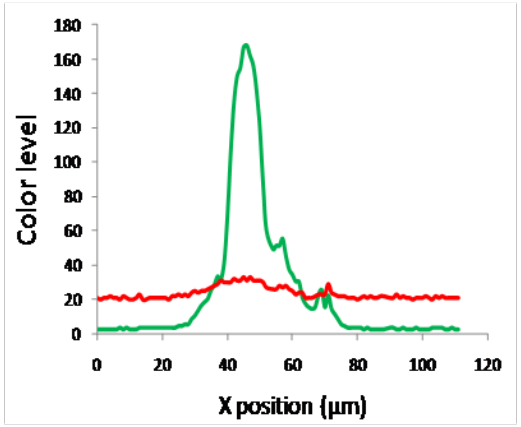
Assessment of antibiotics time-kill activity on activated sludge: Epifluorescence combined with brightfield microscopy



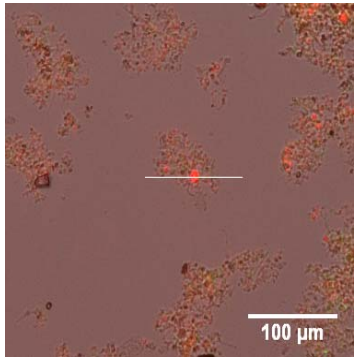
Baclight™ : green living bacteria, red damaged bacteria boues

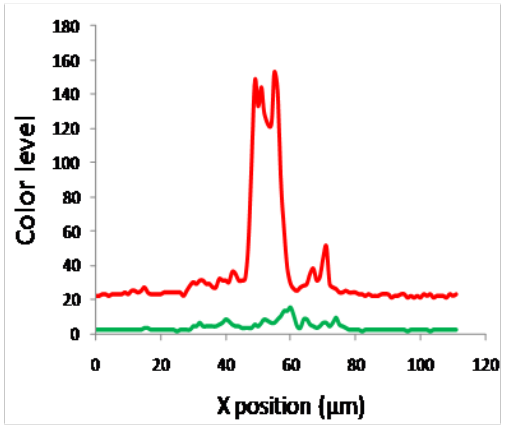
t = 0, 10 mg/L erythromycin

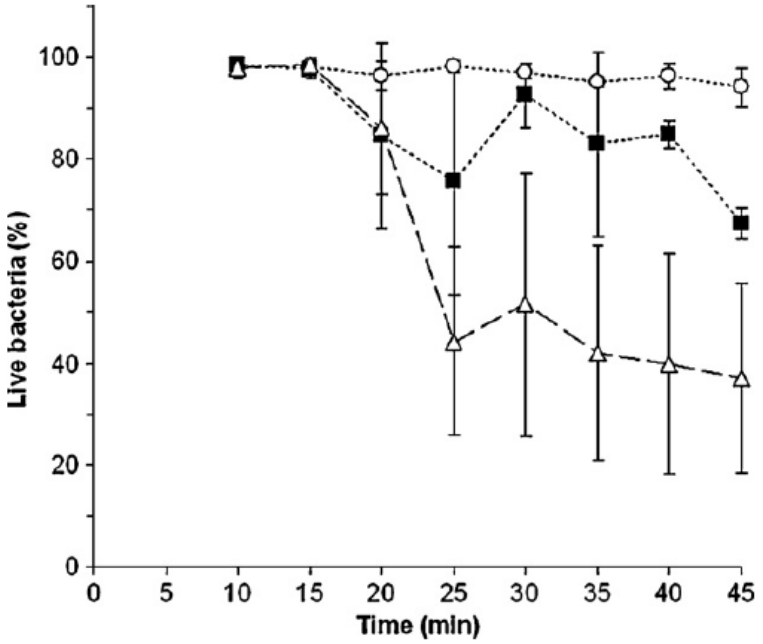




t = 1h10, 10 mg/L erythromycin

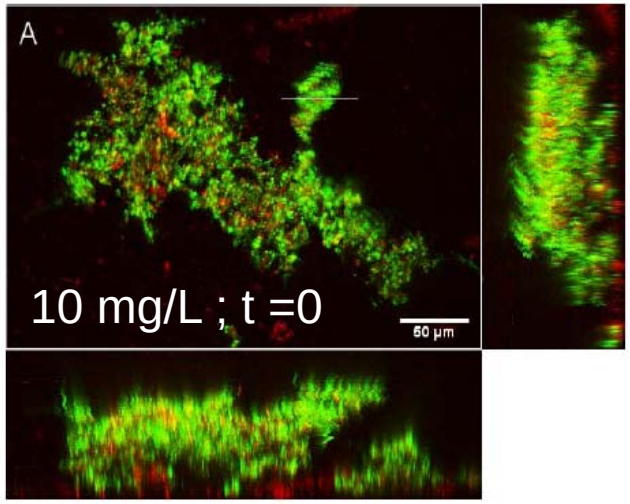


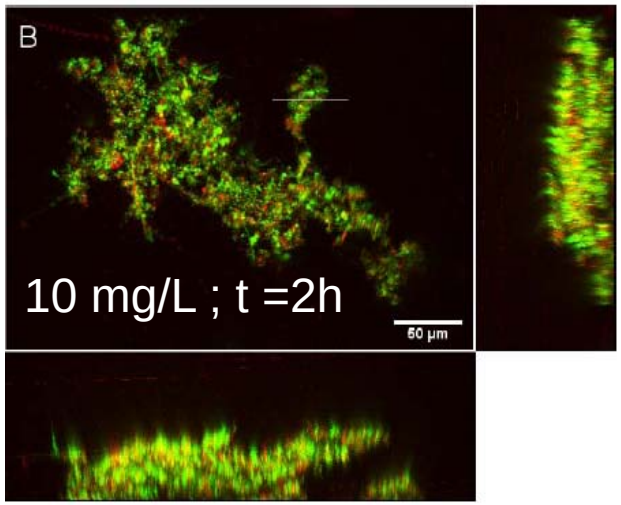


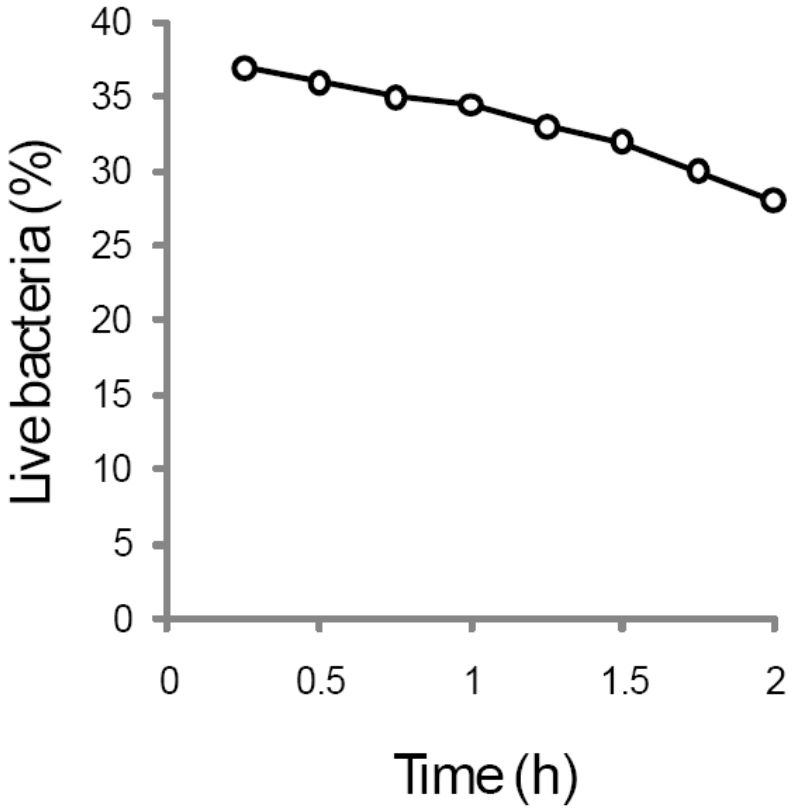


○, control; ■, 0.1 mg/L; Δ, 1 mg/L

Assessment of antibiotics time-kill activity on activated sludge: Timelapse **Confocal Laser Scanning Macroscopy** Imaging

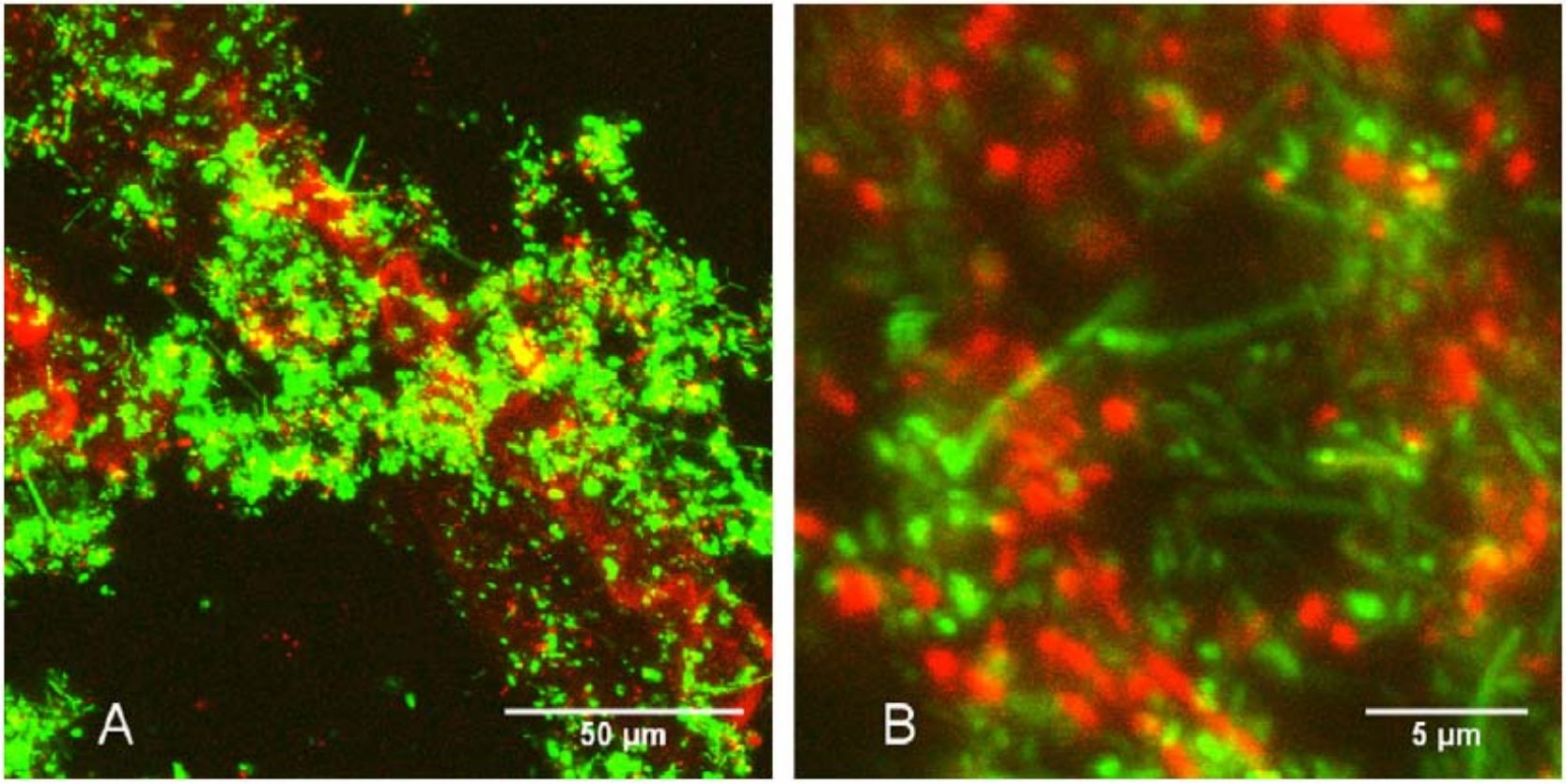






(Nancy Sludge Results)

The higher resolution of the Confocal Laser Scanning Microscopy permitted to clearly observe individual stained cells



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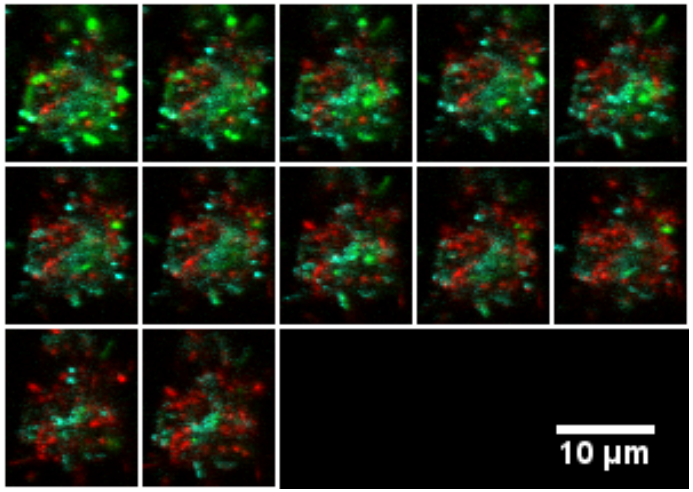
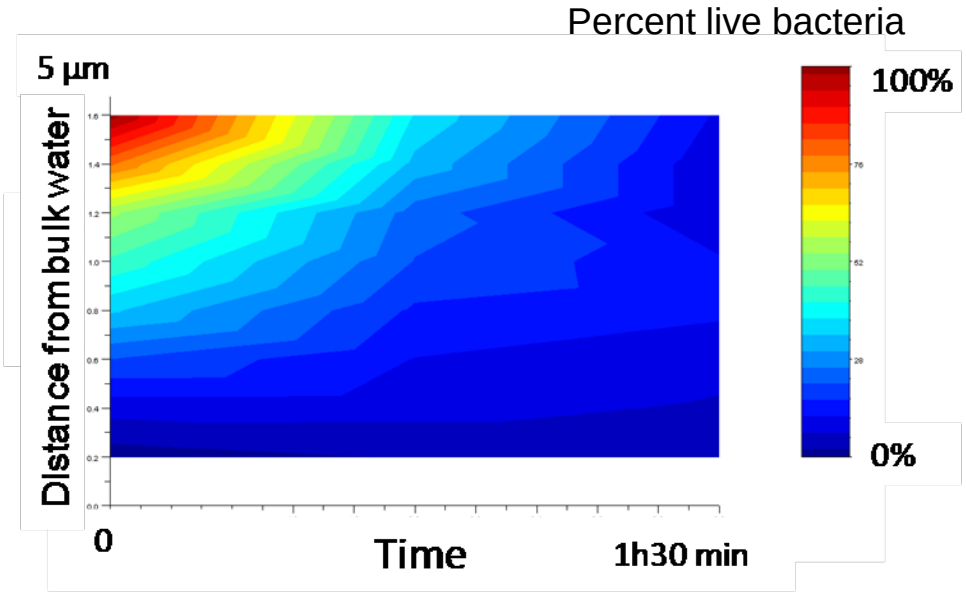
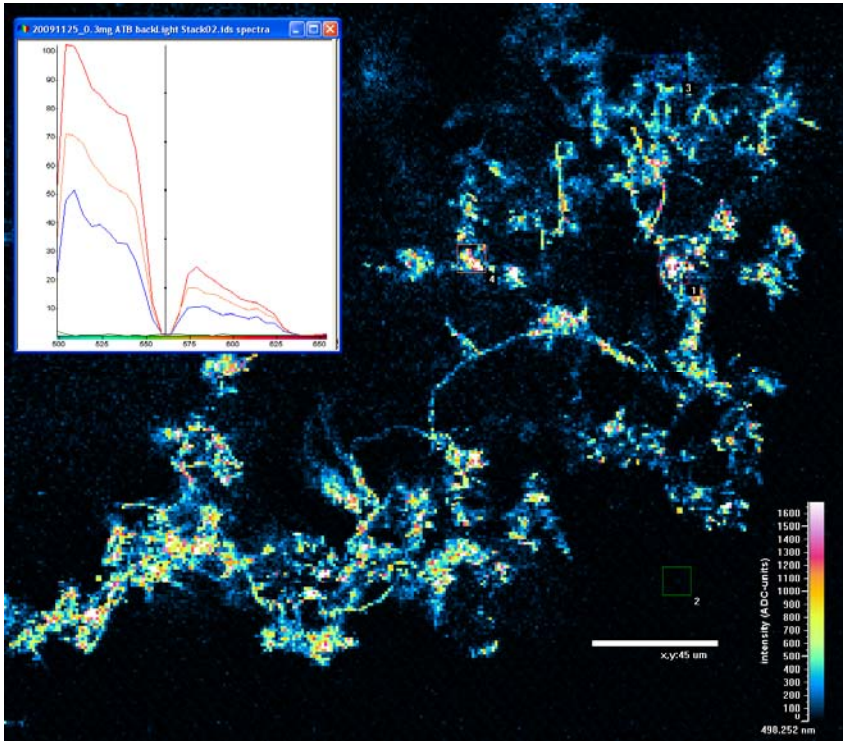
Bacteria at the surface of the flocs were the first to die.





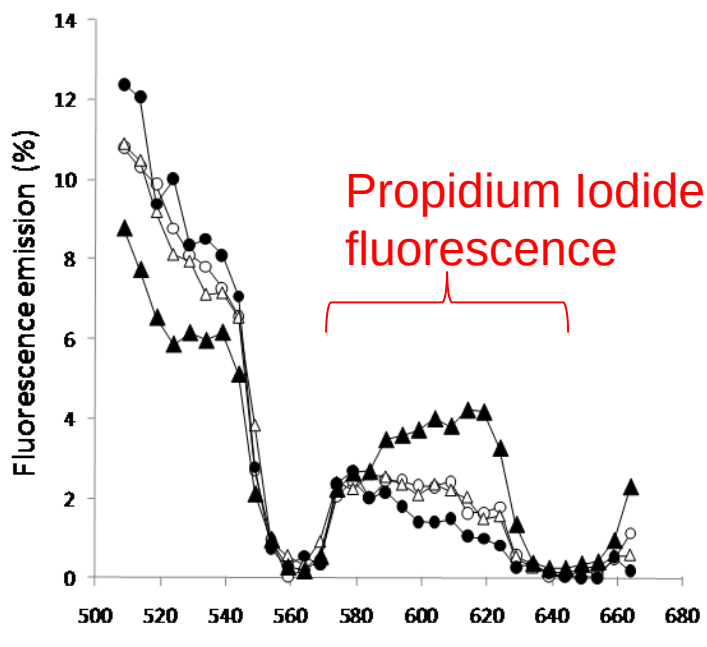
→ importance of antibiotic diffusion through exopolymeric substances and flocs pores network

Assessment of antibiotics time-kill activity on activated sludge:
Confocal Laser Scanning Spectral Imaging: effect of a 0.3 µg/L concentration



Syto 9 fluorescence

Propidium Iodide fluorescence



Control t=0

Control t=1h30

0.3 µg/L erythromycin t=0

0.3 µg/L erythromycin t=1h30

Conclusions

Erythromycin had a toxicity effect on activated sludge even for the lowest concentration tested (0.3 µg/L).

The toxicity effect began after a lag time that depended on the antibiotic concentration.

An inhibition of pollution removal was measured for a 4 µg/L concentration during 90h batch experiments.

Contrary to COD inhibition removal, the effect of erythromycin on nitrification depended on the origin of the sludge.

The antibiotic diffusion into the flocs is expected to control the bacteria death kinetics.

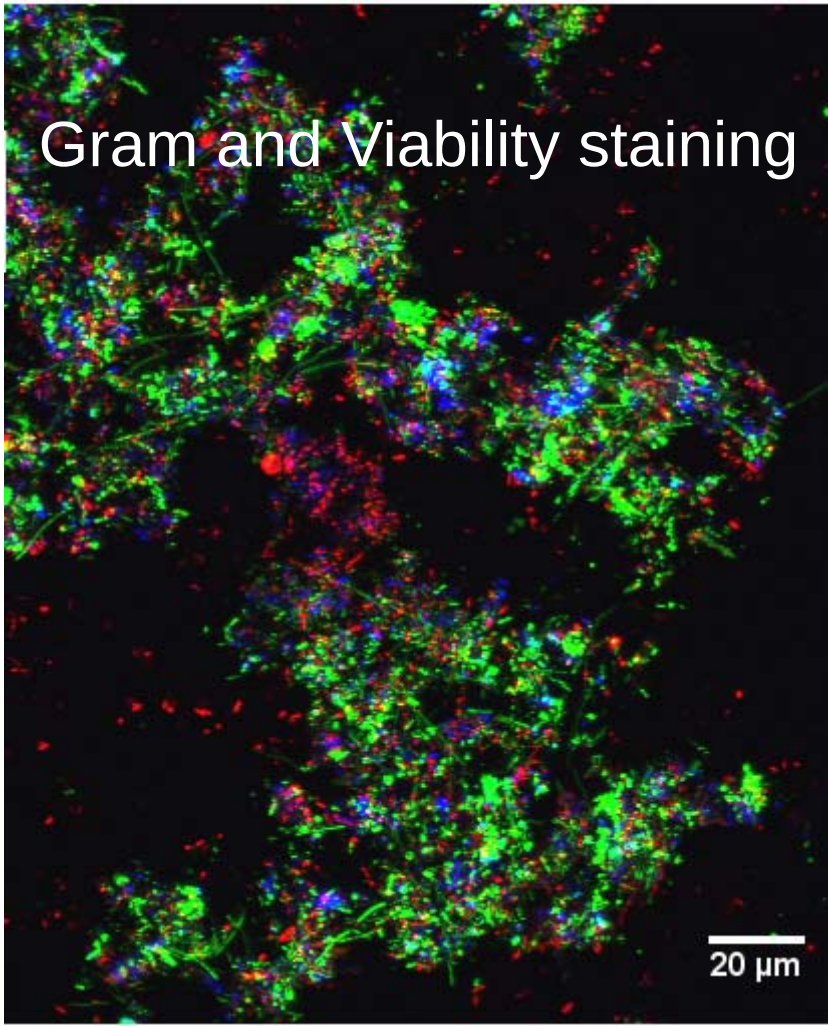
Persectives

Current work focus on

-The characterisation of exopolymeric substances with CLSM and Fourier Transform Infrared Spectroscopy (FTIR)

- The mortality of bacteria as a function of their Gram type

- The relationship between antibiotic biodegradation, toxicity and resistance should be studied



Thank you for your attention