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Effects of hydrodynamic conditions on the bacterial and amoebic biomass in freshwater biofilms

A Perrin, P Herbelin, Frédéric Jorand, Salaheddine Skali-Lami, Laurence Mathieu

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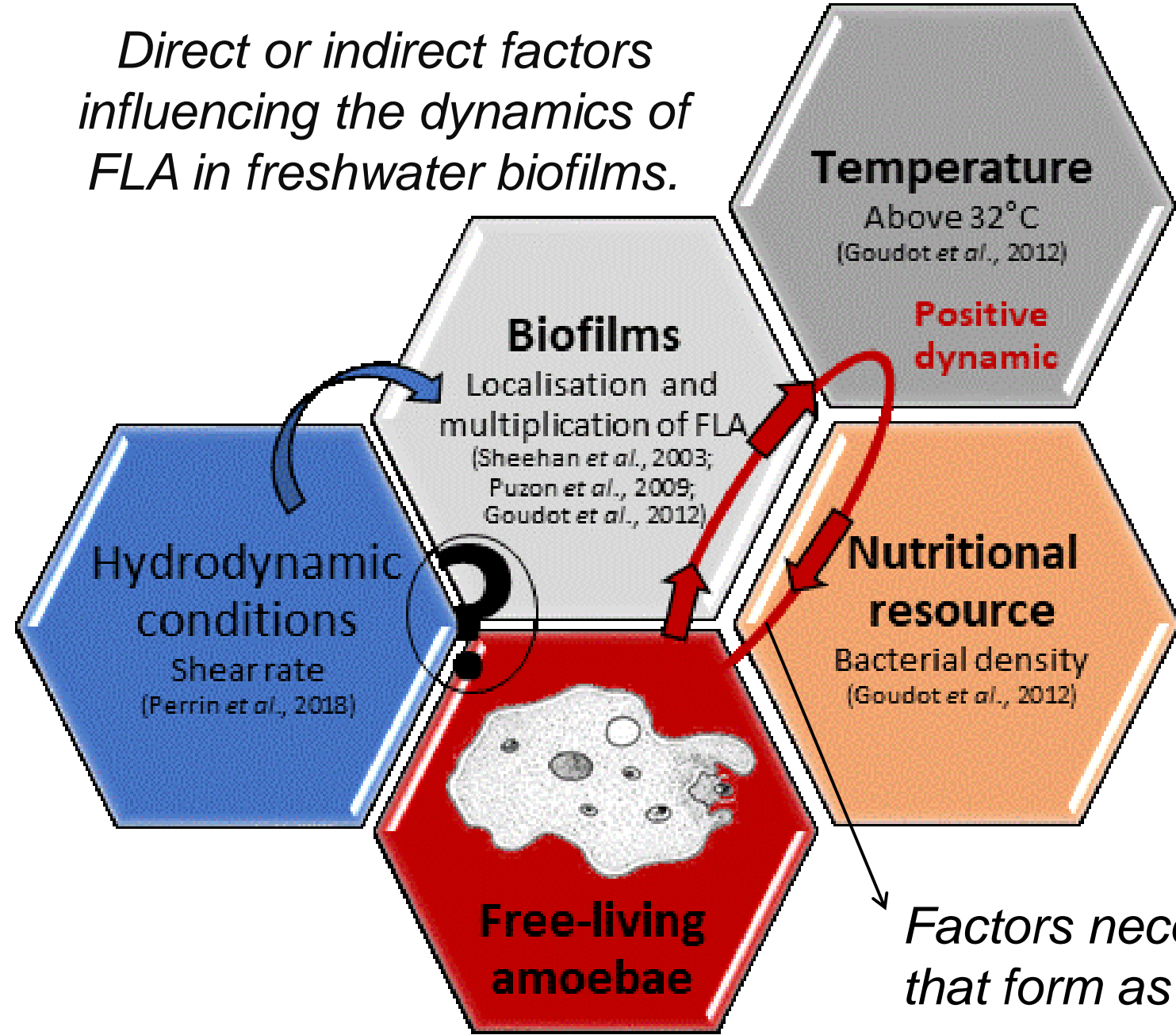
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Naturally present in river water supplied cooling circuits, bacteria and free-living amoebae (FLA) can encounter conditions favourable to their multiplication in freshwater biofilms, especially since the water warms up. The presence of freshwater biofilms onto surface of industrial cooling circuits can reduce the thermal performance and represents a potential public health risk. Several factors play a role on the behavior of freshwater biofilm. Among those factors and to better manage those industrial problems, it is essential to understand how hydrodynamic conditions influence the dynamics of microorganisms into biofilms and identify the areas of the cooling system where it could occur.



Factors necessary but not sufficient to explain the growth of free-living amoebae in biofilms (Goudot, 2012). Biofilms are evolving structures that form as a result of a dynamic equilibrium between nutrients, bacteria and FLA, which are influenced by hydrodynamic conditions.

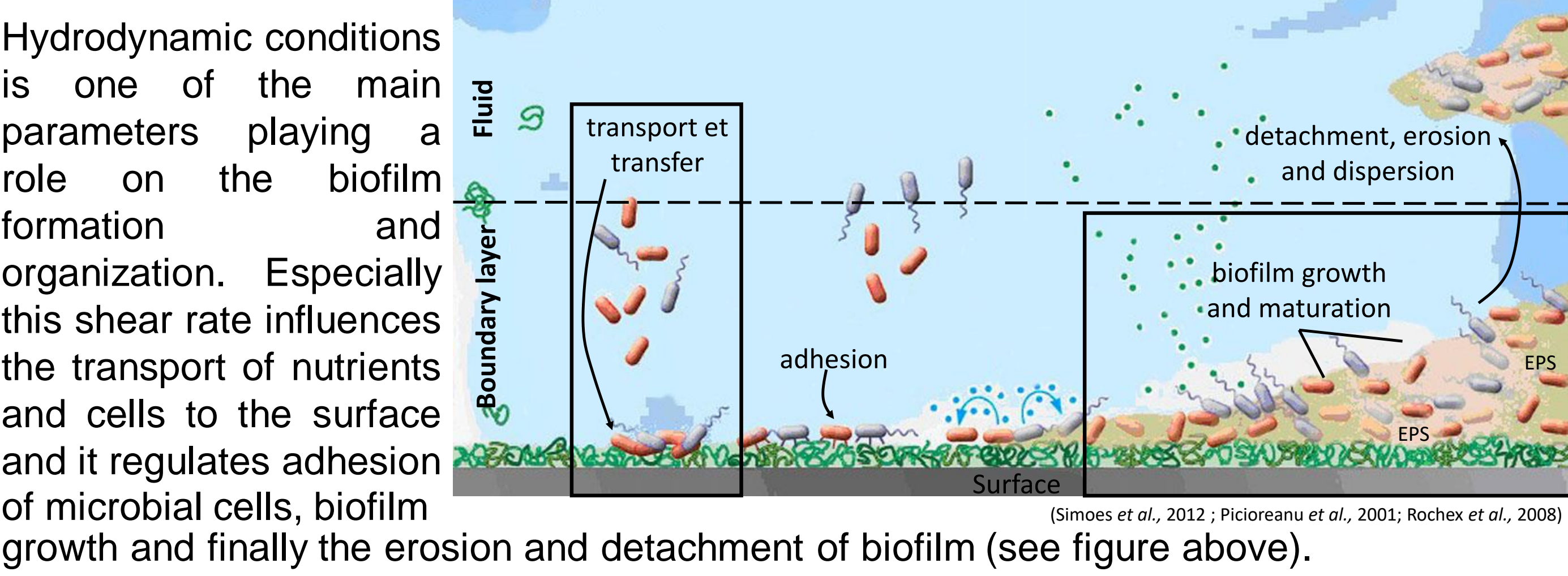
What are the hydrodynamic conditions in cooling circuits ? How do they influence the dynamics of bacteria and free-living amoeba into biofilms on surface ?

OBJECTIVES

- Develop a specific pilot-scale device able of reproducing a wide range of hydrodynamic conditions of cooling circuits
- Quantify and qualify the establishment, growth and survival of bacteria and FLA in freshwater biofilms

1 HYDRODYNAMIC CONDITIONS IN COOLING CIRCUITS

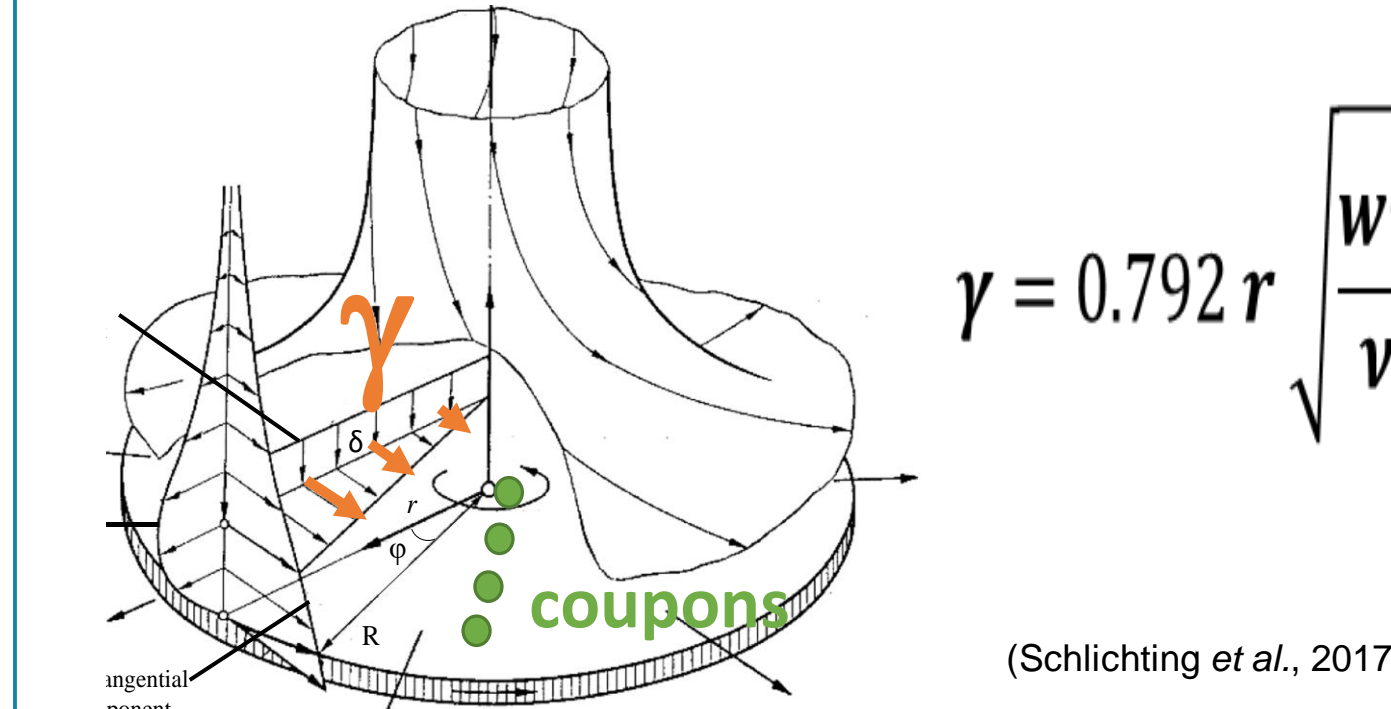
In most of the pipes of cooling circuits, the flow is turbulent with a Reynolds number (Re), ratio of inertial forces to viscous forces in the fluid, ranging from $4 \cdot 10^4$ to $2 \cdot 10^7$. The viscous sublayer (next to the surface), with a very small thickness, is characterized by large velocity gradients. Flow inside this viscous sublayer is considered to be essentially laminar, with a characterized shear rate (γ).



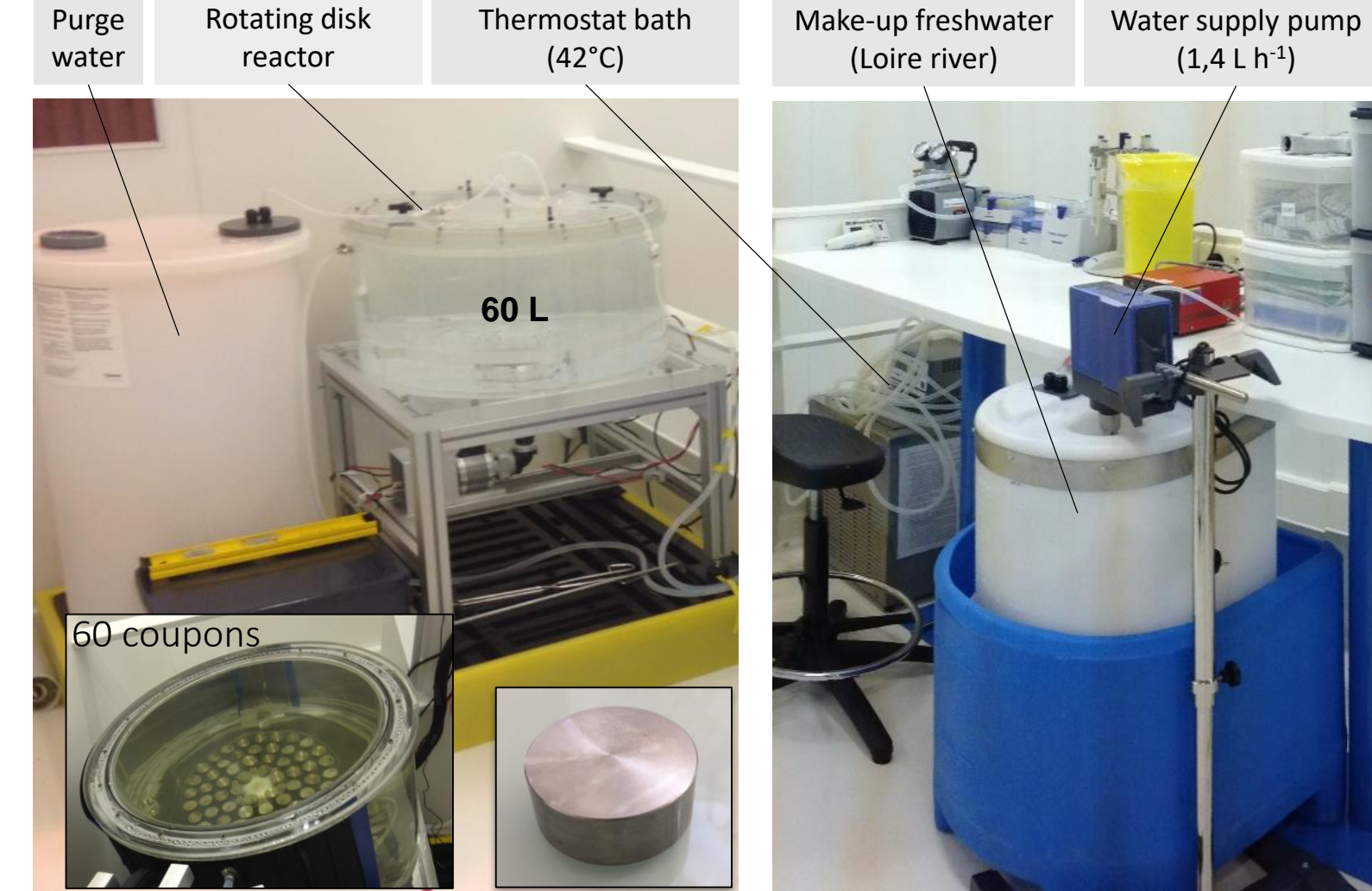
The shear rate in cooling circuits ranges from $34,000 \text{ s}^{-1}$ to $72,000 \text{ s}^{-1}$ (Perrin, 2019).

2 ROTATING DISK BIOFILM REACTOR

Shear rate (γ) is a function of the rotational speed (ω) of the disk and the radial distance (r) from the axis of rotation.



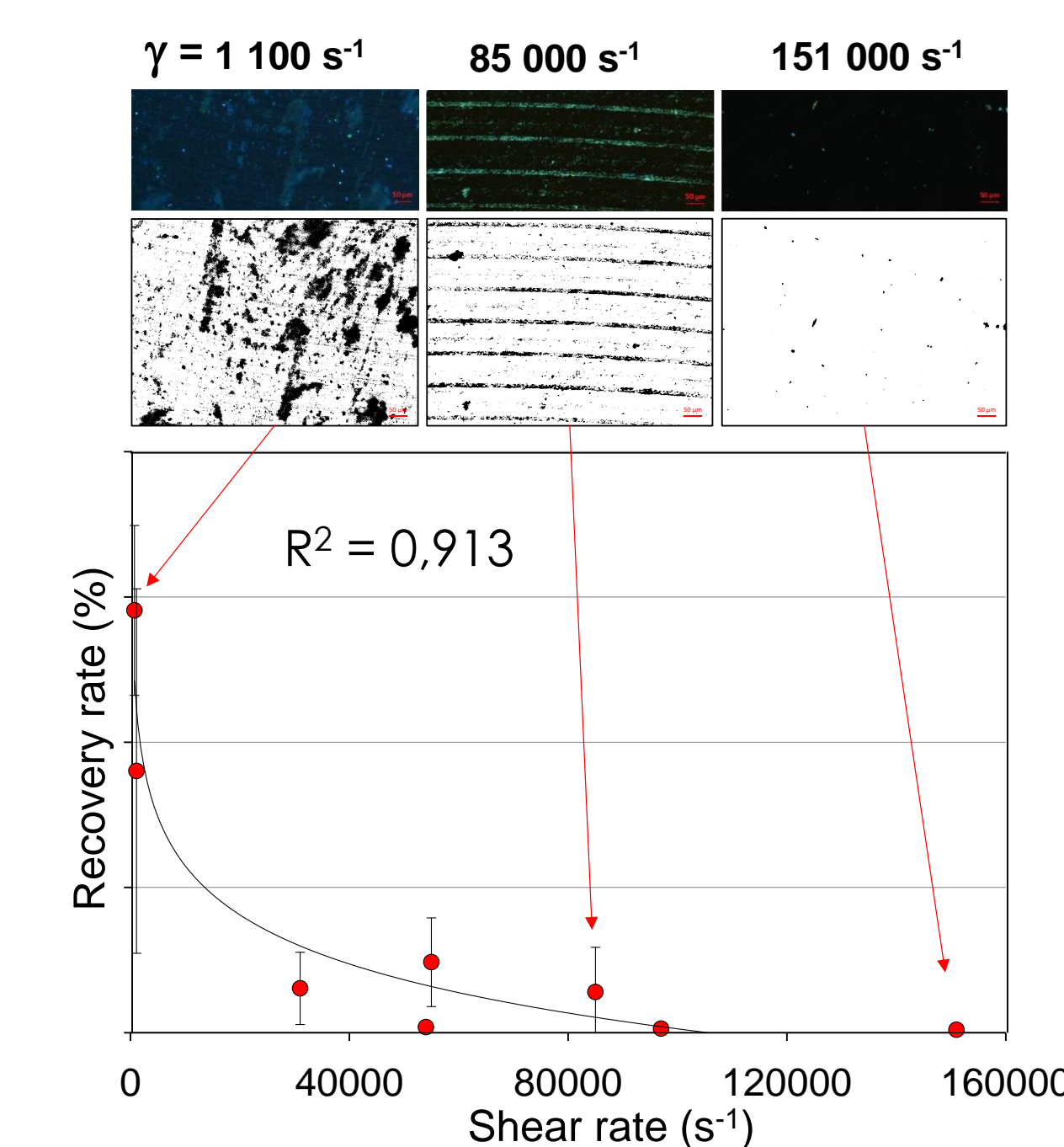
| Rotating disk reactor | 30 rpm | 350 rpm | 480 rpm |
|---|-----------------------|-------------------|-------------------|
| Nombre de Reynolds Re (sans dimension) | RI 1.0×10^4 | 1.2×10^5 | 1.6×10^5 |
| | RIV 1.1×10^5 | 1.3×10^6 | 1.6×10^6 |
| Taux de cisaillement $\gamma \text{ (s}^{-1}\text{)}$ | 250 | 13 000 | 22 000 |
| | 1 000 | 85 000 | 151 000 |



The reactor consists of a 60L, PMMA reaction vessel, and contains sixty stainless steel coupons. It is supplied with freshwater. The main tank is maintained at warm temperatures (42 degrees Celsius). Four radius positions on the disk corresponds to four different hydrodynamic conditions.

3 BACTERIA : RECOVERY RATE AND DENSITY

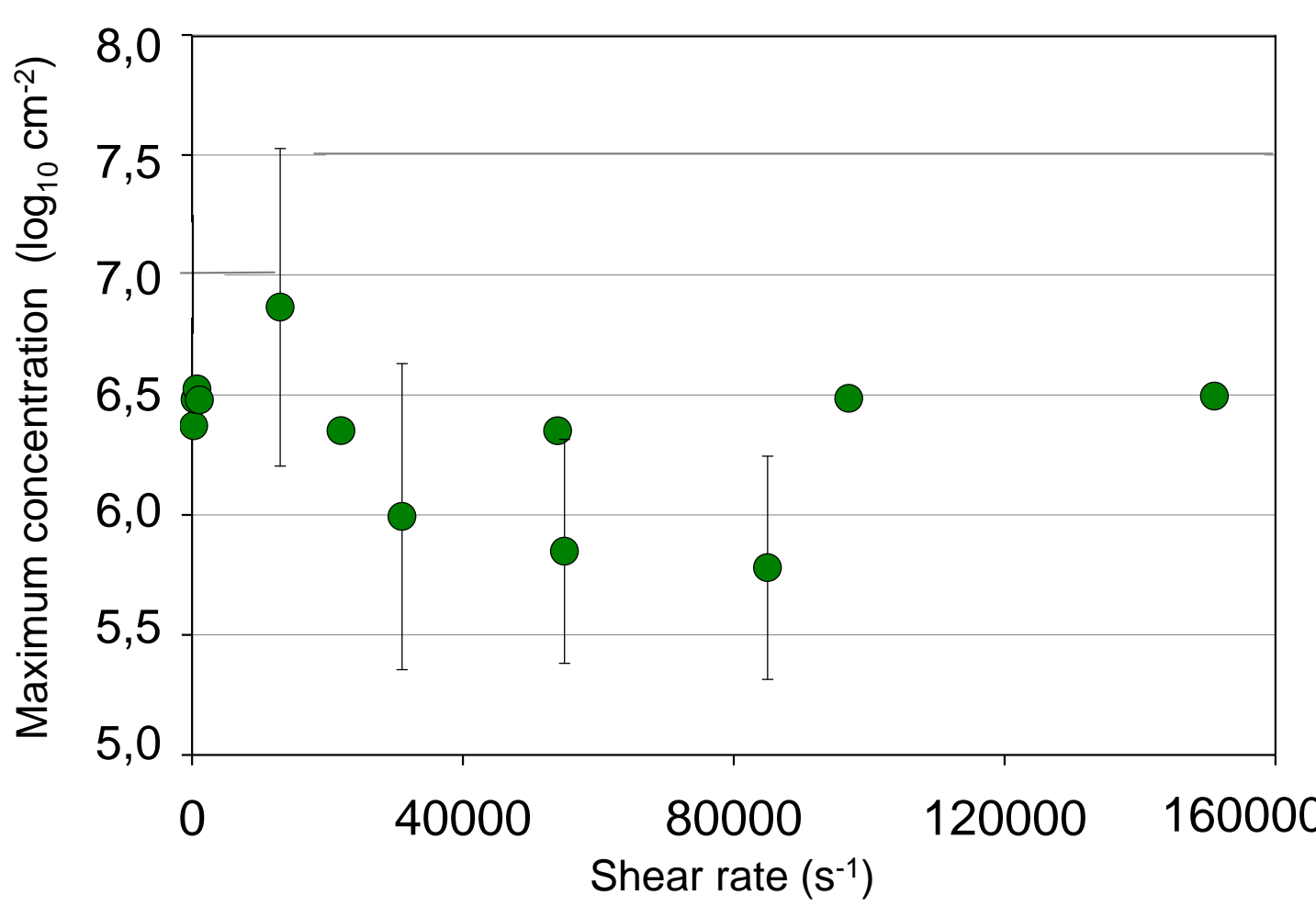
Recovery rate and spatial organization were determined by cell nuclear DAPI staining and epifluorescence microscopic observation (Olympus BX50) (100-500x magnification). Ten microscopic views were captured on each coupon (Nikon DS-Fi2), which were analysed with the software MATLAB® in order to produce the recovery rate according to the script of Lewandowski and Beyenal (2013).



- Cell recovery rate on the coupons decreases as shear rate increases. Only one layer appears on the surface.
- The spatial organization differs according to shear rates. Microbial clusters on surface under low shear rates are larger and in greater numbers than at high shear rates.

Each collected coupon was gently washed by immersion in phosphate buffer saline to remove deposits loosely attached to the substrata. Then deposits of biofilm were dispersed by sonication (15 min, ultrasonic bath) (Goudot et al., 2012).

Bacterial cells nucleic acid were stained with SYBR® Green I. Cells were recovered by filtration through a $0.2 \mu\text{m}$ pore-size polycarbonate membrane and counted with an epifluorescence microscopy (Olympus BX50) (Noble et Fuhrman, 1998 ; Patel et al., 2007). Quantitative PCR with 16S rRNA-gene-targeted specific primers (Eub338F et Eub518R) have been also done to quantify bacterial cells (Ferrand et al., 2014) (data not shown, similar results as microscopy).

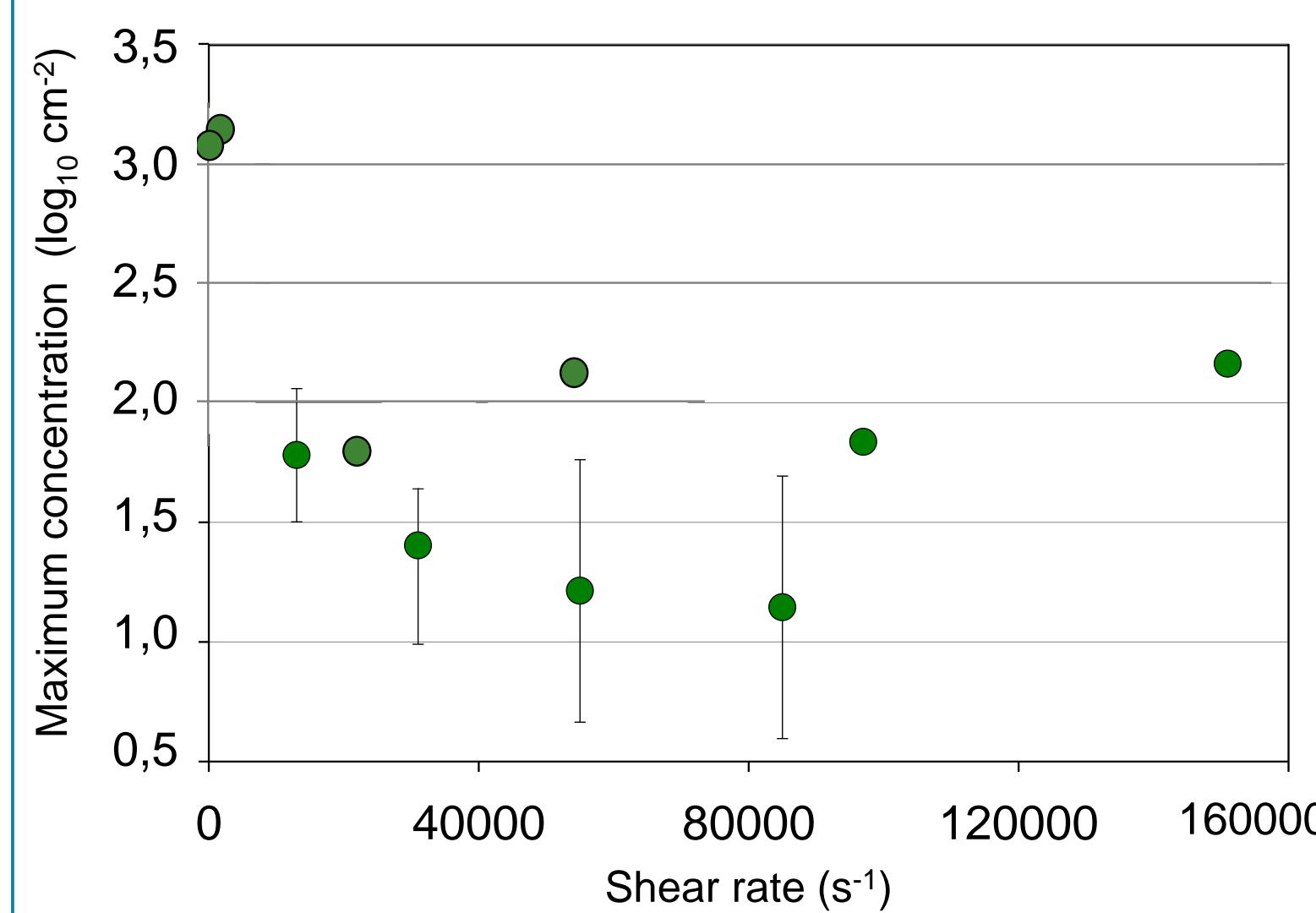


- Rapid colonization and stabilization in 7 days at about $10^6 \text{ cells cm}^{-2}$.
- Bacterial densities do not differ statistically according the wide range of shear rates comprised between $1,000$ to $151,000 \text{ s}^{-1}$.

4 FREE-LIVING AMOEBAE : DENSITY AND DIVERSITY

Thermophilic free-living amoebae were cultured on non-nutrient agar plates overlaid with *E. coli*. Amoebic migration fronts were observed on an inverted microscope (80-160x magnification). Amoebic clades were determined according to morphologic criteria (size and shape of cyst and trophozoite stages).

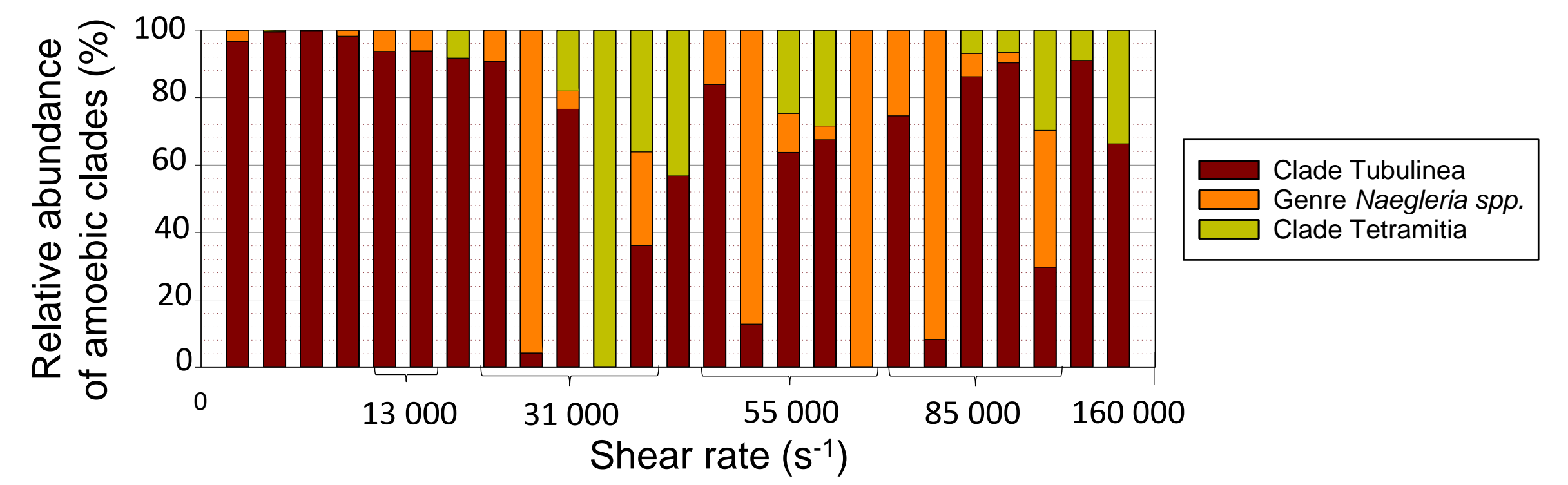
DENSITY



- Establishment of viable eukaryotic microorganisms within biofilms under high shear rates, in opposition to theoretical concepts.
- Rapid colonization after 1-2 days
- Amoebic concentration ranging from 10^1 to $10^3 \text{ cells cm}^{-2}$ and values are a hydrodynamic-independent phenomena. Densities do not differ statistically.
- However, amoebic density would trend to increase as shear rate decreases (tests required to confirm). The hypothesis are :
 - A low shear rate would be favourable to amoebic-cell sedimentation.
 - A high shear rate could be responsible for erosion of biofilm and cell detachment would occur.
 - Amoebic density might depend on the bacteria density.

DIVERSITY

- Amoebic diversity is hydrodynamic-independent.
- One of the two clades, *Tubulinea* clade, has been mostly identified on stainless steel. Belonging to the *Tetramitia* clade, *Naegleria* genus is also frequently observed in balance with *Tubulinea*.



DISCUSSION AND PROSPECTS

The designed experimental process has enabled to highlight that FLA colonization of freshwater biofilms is possible at shear rate up to $151,000 \text{ s}^{-1}$, even if convection mechanism doesn't technically provide it. These results confirm that other mechanisms, such as turbulence, locomotion or surface properties have allowed the establishment of FLA in biofilms.

Finally, hydrodynamic conditions have shaped the spatial organization of microbial cells on the surface : higher shear stresses have reduced the recovery rates and number of clusters. However no significantly difference in the bacterial and amoebic densities has appeared between the hydrodynamics conditions despite of the wide range of shear rates (high shear rates tending to reduce FLA concentration into biofilm needs to be confirmed).

How the recovery rate and the number of clusters could be affected by the shear stresses without modify microbial charge ? Given the relevance of hydraulics on the biofilm growth, a study of the biofilm cohesive strength and the density, porosity and thickness of biofilms will be helpful to better understand the structure of freshwater biofilms into cooling circuits, considering different materials (concrete, PVC) and microbial factors (microbial diversity and amoebic morphology).

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