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Effects of hydrodynamics on the behavior of free-living amoeba in freshwater biofilms

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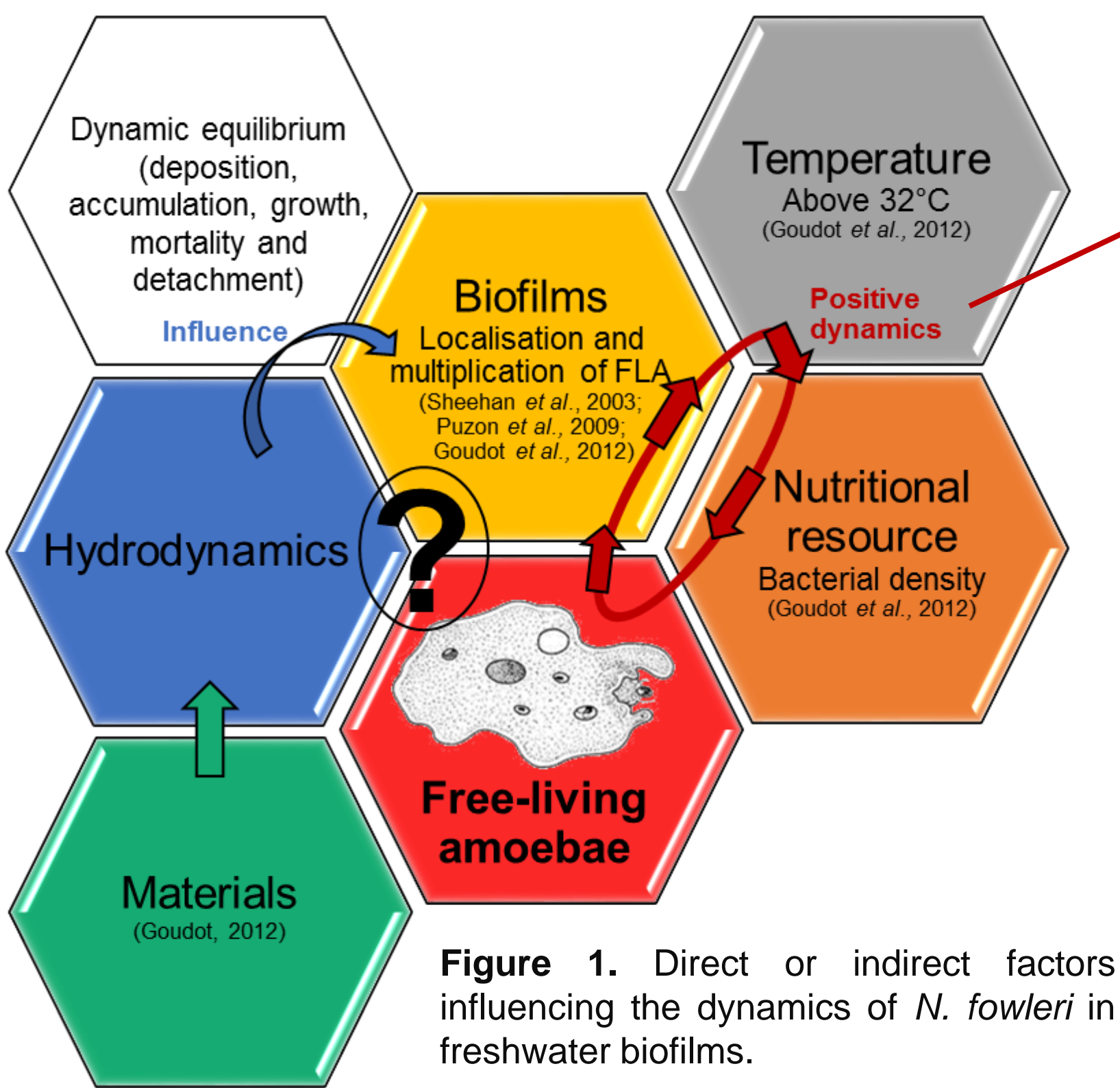
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Naturally present in river water supplied to cooling circuits, free-living amoebae (FLA) can encounter conditions favourable to their multiplication as the water warms up. The temporary presence of the pathogenic *Naegleria fowleri* in industrial cooling circuits represents a potential public health risk in case of dispersion in the river water. To better manage the amoebae problem, it is essential to identify the areas of the cooling system that are especially to their development.



Factors necessary but not sufficient to explain the growth of amoebae *N. fowleri* in biofilms (Figure 1). Biofilms are evolving structures that form as a result of a dynamic equilibrium, which is influenced by the hydrodynamic conditions.

What are the hydrodynamic conditions in cooling circuits? How do they influence the transfer of amoeba to the wall? The dynamics of FLA in biofilms?

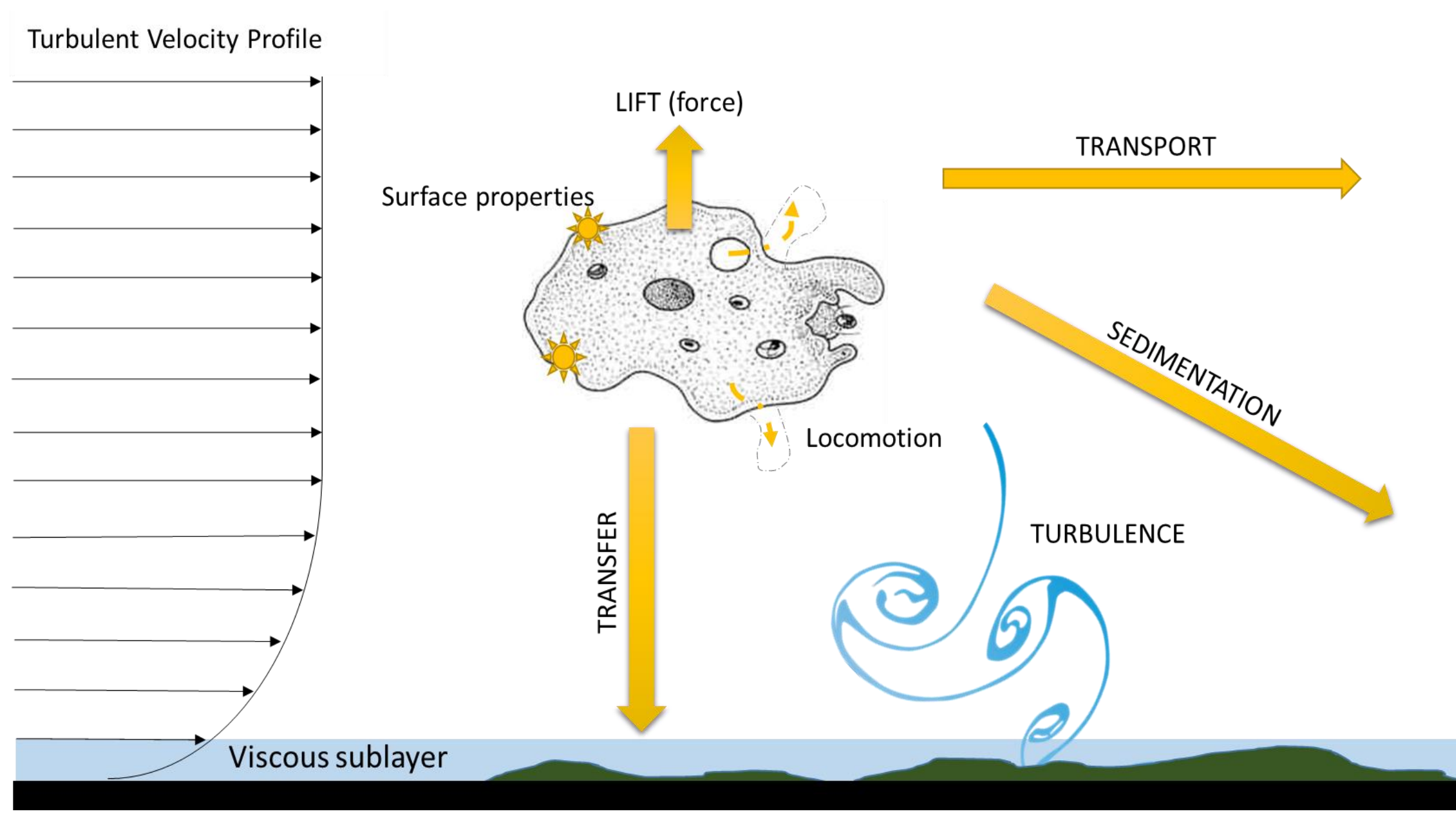
- OBJECTIVES**
- 1 Develop a specific pilot-scale device capable of reproducing a wide range of hydrodynamic conditions found in cooling circuits.
 - 2 Quantify and qualify the establishment, growth and survival of biofilms and of FLA in freshwater biofilms

HYDRODYNAMIC CONDITIONS IN THE COLLING CIRCUITS, TRANSFERT OF AMOEBAE TO THE WALL?

In most of the filled pipes of cooling circuits, the flow is **always turbulent**, characterized by Reynolds number (Re), which is the ratio of inertial forces to viscous forces in the fluid.

The **viscous sublayer** (next to the wall, biofilms) is of very small thickness and characterized by large velocity gradients. Flow in this viscous sublayer is considered to be essentially laminar, with a characterized shear rate (γ).

- $3.80 \times 10^4 < Re < 1.61 \times 10^7$
(at 42°C optimum growth of *Naegleria fowleri*)
- $33,627 < \gamma < 71,793 \text{ s}^{-1}$
(roughnesses of ~ 1 μm for stainless steel and 100 μm for concrete).



Experimental devices have been validated for small particles, such as bacteria. Given the difference between amoebae and bacteria (size and properties), the mass transfer mechanisms are different (Figure 2).

- ❖ Diffusion transfer would be impossible given the size of amoeba (> 20 μm) : **Convection**.
- ❖ In laminar flow conditions, **the lift forces** tend to elevate amoebae away from the wall.
- ❖ **Other mechanisms such as turbulence, sedimentation, locomotion and surface properties**, may explain why FLA may be found in environmental biofilms (Biyela *et al.*, 2012; EDF researches).

Figure 2. Representation of velocity profile, viscous sublayer and different transfer mechanisms (potentially influencing FLA movement towards and adhesion FLA in biofilms), in turbulent pipe flows.

1 REACTOR DESIGN

➔ **Rotating disk biofilm reactor** (Figure 3) (optimized and adapted to Abe *et al.*, 2012; Mathieu *et al.*, 2014; Pelleieux *et al.*, 2012)

- Biofilm that moves relative to the water
→ Rotating disk with removable stainless steel or concrete coupons (7,07 cm²)
- Sedimentation
→ Disc at the bottom of the reactor tank
- Shear rate is a function of the rotational speed of the disk and the radial distance from the axis of rotation (Navier-Stokes equations)

$$\gamma = 0.792 r \frac{w^3}{\nu}$$

(Schlichting, 1979).

rotation
radius

Minimum : 20 rpm $Re = 6.74 \times 10^3$
 $\gamma = 136 \text{ s}^{-1}$

Maximum : 480 rpm $Re = 1.8 \times 10^6$
 $\gamma = 53,382 \text{ s}^{-1}$

- 4 radius positions on the disk= 4 different hydrodynamic conditions for each assay

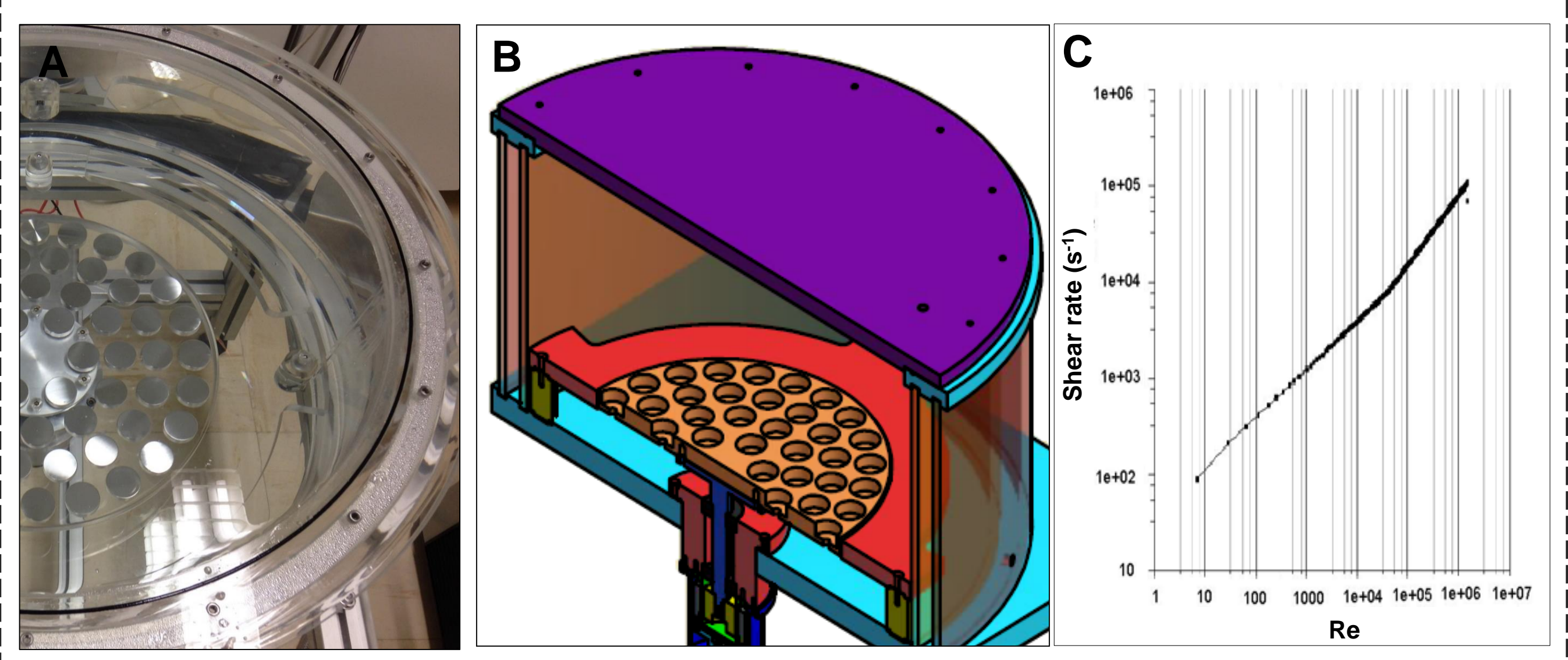


Figure 3. A. (photo) The reactor consist of a 60L, PMMA reaction vessel, 540 mm in diameter and 290 mm high. The main tank is set inside a cylindrical jacket that maintains the temperature at 42°C. B. (design) A fixed ring element (in red), abuts and extends the profile of the rotating disk, assuring both hydrodynamics stability and continuity. The disk is rotated by a magnet drive shaft. C. (FLUENT Simulation) Shear Rate vs Reynolds Number.

2 ESTABLISHMENT OF AMOEBAE

➔ **Assay with rotating disk reactor**

- **350 rpm**
- **Stainless steel**
- **3 hydrodynamics** : 17,727 (RII), 25,483 (RIII) et **33,238 s⁻¹** (RIV)
- Continuously fed with **Loire freshwater at a rate of 1.38 L.h⁻¹**
 - *Hartmannella spp.* were detected at densities 10³ amoebae.L⁻¹ at 30°C and 2x10² at 43°C
 - 8x10⁸ bacteria.L⁻¹ or 10⁹ copies of genomes.L⁻¹ (cell counting and qPCR)
- **Freshwater biofilms** generated were analyzed **quantitatively and chronologically** for presence of FLA and bacteria using microbiological culture and molecular biology methods (Figure 4).

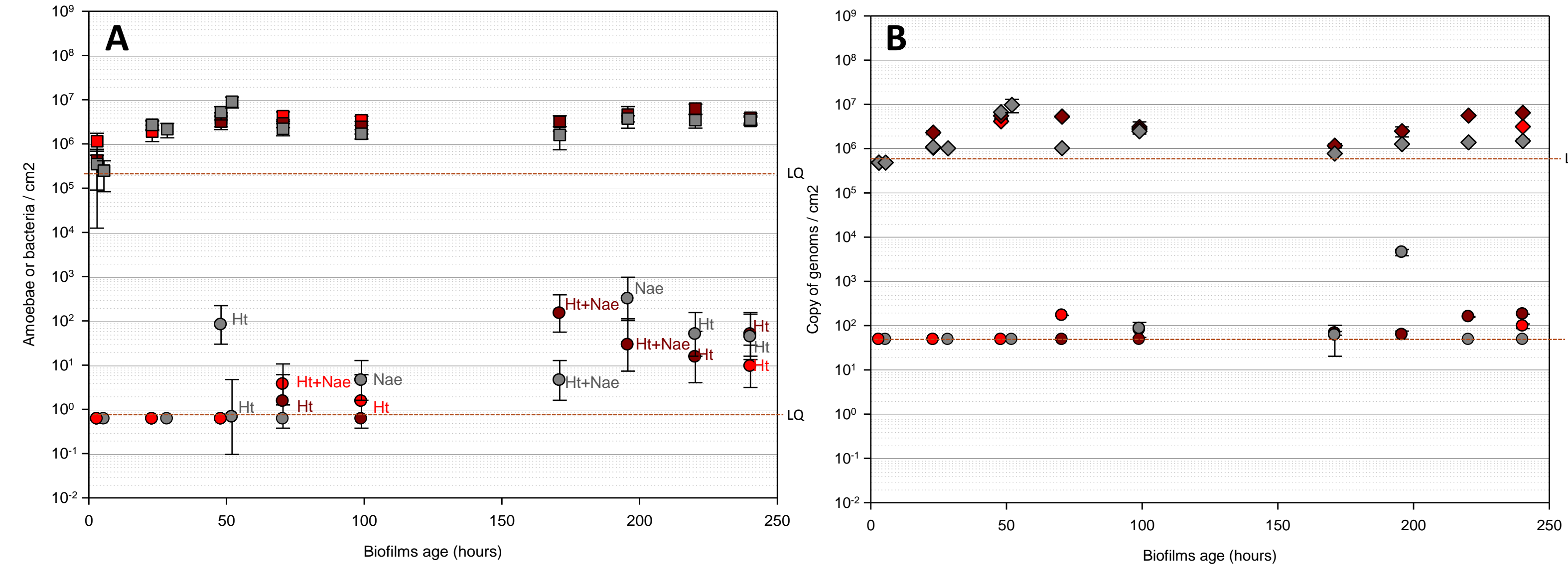


Figure 4. Temporal variations in biofilm for different hydrodynamic conditions RII (■), RIII (■) and RIV (●) in biofilm at 42°C. A. bacterial density (■, square) and thermophilic FLA density (●, circle). Morphological identification amoeba is indicated by an exponent: *Hartmannella spp.* (Ht) and *Naegleria spp.* (Nae). B. bacterial density (◆, diamond) and *Naegleria spp.* density (●, circle) by qPCR. The limit of quantification for each method is indicated by a dotted line (LQ). Samples below the limit does not have a standard deviation.

- **Establishment, growth and survival of indigenous amoebae; *Hartmannella spp.* and *Naegleria spp.* in biofilms at 43°C**
- At stronger hydrodynamics (RIV), for thermophilic FLA in biofilms
 - establishment at 48 hours
 - high concentration 3x10² thermophilic FLA /cm²
- **Correlation between counting and molecular biology methods (bacteria, *Naegleria spp.*)**

The experimental process and technical feasibility have been validated, notably, that FLA colonization of freshwater biofilms is possible at shear rate up to 33,238 s⁻¹.

These results confirm that others mechanisms than convection, have allowed the establishment of FLA in biofilms (turbulence, locomotion or surface properties).

Further assays will follow to enable validate the inter-assay reproducibility and to study the effects of different hydrodynamic conditions on *Naegleria fowleri* behavior in biofilms, as well as the impact of the type of materials (stainless steel or concrete).

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