Influence of the temperature on the growth dynamic of *Naegleria fowleri* into a complex microbial freshwater biofilm

Goudot Sébastien[1,2*], Herbelin Pascaline[1], Soreau Sylvie[1], Banas Sandrine[2], Mathieu Laurence[2] et Jorand Frédéric[2]

(1) EDF R&D - LNHE Laboratoire National d'Hydraulique et d'Environnement, Chatou, France

(2) LCPME Laboratoire de Chimie Physique et Microbiologie pour l'Environnement, UMR 7564, CNRS - Nancy Université, Jean Barriol Institute, France

(*) Corresponding author : EDF R&D - LNHE, 6 quai Watier 78401 CHATOU Cedex, France, tel : +33130877535, email : **sebastien.goudot@edf.fr**

INTRODUCTION AND OBJECTIVES

The free-living amoebae are ubiquitous organisms that are encountered in natural and artificial environments. The temporary occurrence in industrial cooling circuits of the pathogenic *Naegleria fowleri* is a health topic of interest. The ecology of these thermotolerant amoeba remains poorly documented in biofilms.

This study is intended to **better understand the biotic and abiotic factors influencing the dynamics of *N.fowleri* in freshwater biofilms**. The main factor to investigate **is the temperature. It is well known to be involved in the occurrence of *N.fowleri*** and appears to be a key parameter for its installation, development and survival.

MATERIALS AND METHODS

Two flat-plate open channel reactors made of polyvinylchloride, 41 centimeters wide, 12 centimeters deep and 136 cm long with a working volume of 2.7 liters were fed by freshwater from the Loire River (Loiret, France). A biofilm colonized spontaneously the 70 immerged glass plates served as substrata. **After 24 h, a suspension of the amoeba *Naegleria fowleri* was introduced by a single injection.** Analytical monitoring includes :

- **free living amoeba** (**FLA**) counting (MPN procedure),
- ***Naegleria fowleri*** counting at 43°C (MPN procedure),
- total **bacterial flora** (cell counting using SYBR Green dye)
- **biofilm** surface coverage by transmitted light microscopy and image processing.

Two independent runs were successively conducted within 45 days each, one at **32°C** and the other at **42°C**.

RESULTS

Influence of the temperature on the biofilm growth ?

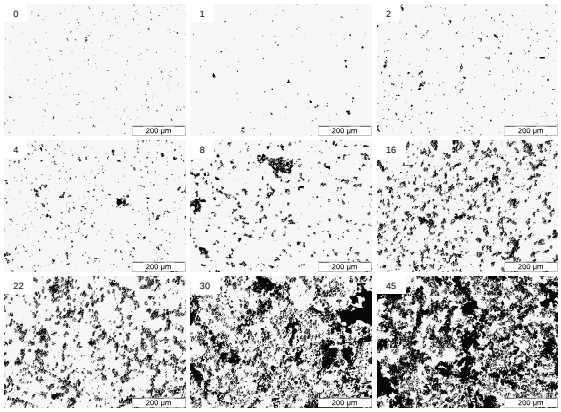


Fig.1. Light microscopy of biofilm forming at 42 °C on glass coupons from day 0 to day 45. Bar, 200 µm

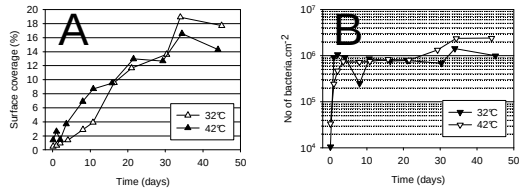


Fig. 2. A Temporal fluctuations in biofilm of surface coverage at 32°C (△) and 42°C (▲)
Fig. 2. B Temporal fluctuations in biofilm of bacterial concentration at 32°C (▽) and 42°C (▼)

- The biofilm is characterized by **small ovoids microcolonies** in size ranged between 4 to 7 µm (fig.1).
- The biofilm growth is **regular and gradual** (fig.2.A and 2.B).
- According to the parameters considered, **the temperature had no significant on the morphological, structural and bacterial development** of the biofilm.

Influence of the temperature on the amoebic population ?

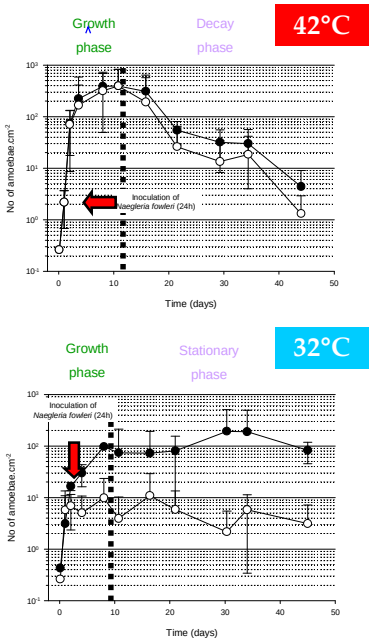


Fig. 3. Temporal fluctuations in biofilm of thermophilic FLA (●) and *Naegleria fowleri* (○) concentrations at 32 and 42 °C

- At 42°C *N.fowleri* is the **major species** of FLA whereas at 32°C **others amoebic species are present**.
- At 42°C *N.fowleri* **proliferated** whereas at 32 ° C, it is able to **maintained and/or survived**.
- The amoebic growth **rate and extend was higher at 42°C than at 32°C.**
- A **stationnary phase was observed at 32°C whereas a decay phase occured at 42°C** after the growth phase.

Influence of the bacterial density on the amoebic population ?

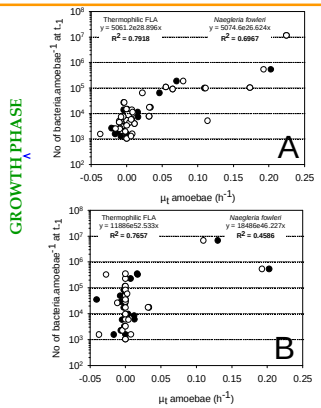


Fig. 4.A and 4.B Thermophilic FLA (●) and *Naegleria fowleri* (○) growth speed in biofilm versus ratio bacteria/amoebae at 42°C (A) and 32 °C (B)

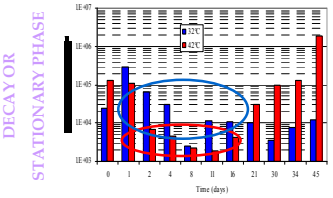


Fig. 5 Temporal fluctuations in biofilm of ratio bacteria/amoebae at 42°C (red) and 32 °C (blue)

- The growth of the amoeba (FLA and N. *fowleri*) could be a function of the the ratio [bacterial density / amoeba].
- One hypothesis to explain the decay phase observed at 42°C **could be a ratio bacteria/amoebae too low** to maintain the amoebic population.

CONCLUSIONS

This study highlighted the **crucial role of temperature on the growth rate of the FLA and *Naegleria fowleri*. Moreover, the focus on *N.fowleri* shows that a temperature of 32°C allows its survival while a temperature of 42°C allows its proliferation.**

Moreover, our works showed that on the one hand **the growth phase of the amoebic dynamics was a function of the ratio [bacterial density / amoebae]** and on the other hand, **the decay and/or stationary phase could be induced by a too low ratio [bacterial density / amoebae].**