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**Growth dynamic of *Naegleria fowleri* in a microbial freshwater biofilm**

Goudot Sébastien1,2, Herbelin Pascaline1, Soreau Sylvie1, Banas Sandrine2, Mathieu Laurence2 and Jorand Frédéric2

(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, Institut Jean Barriol, France

(*) Corresponding author : EDF R&D - LNHE, 6 quai Watier 78401 CHATOU Cedex, France, tel : +33130878468, email : pascaline.herbelin@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 cm wide, 12 cm deep and 136 cm long with a working volume of 2.7 L 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.

Several independent runs were successively conducted within 45 days each, respectively at 22°C, 32°C and 42°C.

**RESULTS**

**Influence of the temperature on the biofilm growth**

![Fig.1. Pictures (light microscopy) of the biofilm at 42°C on glass coupons from day 0 to day 45.](image)

- The biofilm is constituted 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 effect on the morphological, structural and bacterial development of the biofilm.

![Fig.2. A Temporal fluctuations of the surface coverage by the biofilm at 32°C (△) and 42°C (▲) (A) B Temporal fluctuations of bacterial density in the biofilm at 32°C (△) and 42°C (▲).](image)

**Influence of the temperature on the amoebic population**

- At 42°C *N. fowleri* proliferated whereas at 32°C, it density was relatively stable and at 22°C, *N. fowleri* disappeared.
- At 42°C *N. fowleri* was the major specie of FLA whereas at 32°C, others amoebic species were present.
- The amoebic growth rate and extend were higher at 42°C than at 32°C.

![Fig.3. Temporal fluctuations in biofilm of *Naegleria fowleri* concentrations at 22, 32 and 42°C.](image)

**Influence of the bacterial density on the amoebic population**

- The specific growth of the *N. fowleri* is a function of the ratio [bacterial density / amoeba].
- μmax is equal to 0.23 h⁻¹, which corresponds to a generation time of 3 hours.
- Ks is equal to 1.2×10⁶ bacteria/amoebae.

![Fig.4.A Temporal fluctuations in biofilm of *Naegleria fowleri* concentration at 42°C (runs and mean).](image)

![Fig.4.B Relationship between the specific growth rate (μ) and the amount of substrate (bacteria / amoeba).](image)

**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 22°C leads to its disappearance, 32°C allows its survival while 42°C allows its proliferation.

Moreover, our works showed that the dynamic of the amoeba growth is a function of the amount of substrate (ratio bacteria / amoebae).