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

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## 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 amoebae 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 immersed 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 ?

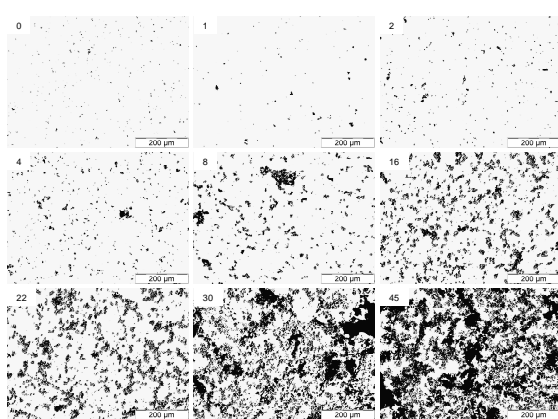


Fig.1. Light microscopy of biofilm forming at 42°C on glass coupons from day 0 to day 45. Bar, 200 µm

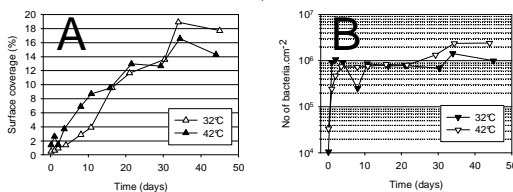


Fig. 2. A Temporal fluctuations in biofilm of surface coverage at 32°C (△) and 42°C (▲)  
Fig. 2. B Temporal fluctuations in biofilm of bacterial concentration at 32°C (▼) and 42°C (▲)

### Influence of the temperature on the amoebic population ?

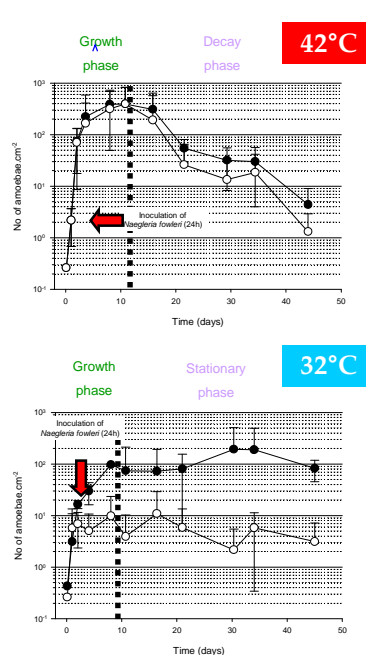


Fig. 3. Temporal fluctuations in biofilm of thermophilic FLA (●) and *Naegleria fowleri* (○) concentrations at 32 and 42°C

### Influence of the bacterial density on the amoebic population ?

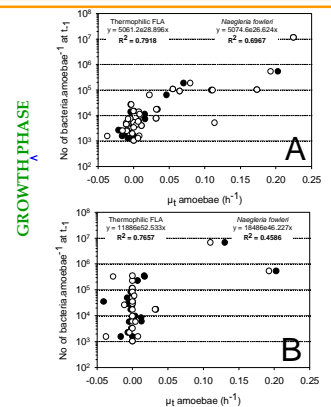


Fig. 4. A and 4. B Thermophilic FLA (●) and *Naegleria fowleri* (○) growth speed in biofilm versus ratio bacteria/amoebae at 42°C (A) and 32°C (B)

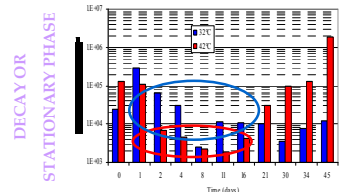


Fig. 5 Temporal fluctuations in biofilm of ratio bacteria/amoebae at 42°C (red) and 32°C (blue)

o The biofilm is characterized by small ovoids microcolonies in size ranged between 4 to 7 µm (fig.1).

o The biofilm growth is regular and gradual (fig.2.A and 2.B).

o According to the parameters considered, the temperature had no significant on the morphological, structural and bacterial development of the biofilm.

o At 42°C *N.fowleri* is the major species of FLA whereas at 32°C others amoebic species are present

o At 42°C *N.fowleri* proliferated whereas at 32°C, it is able to maintained and/or survived

o The amoebic growth rate and extend was higher at 42°C than at 32°C.

o A stationary phase was observed at 32°C whereas a decay phase occurred at 42°C after the growth phase.

o The growth of the amoeba (FLA and *N. fowleri*) could be a function of the the ratio [bacterial density / amoeba].

o 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].