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

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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 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 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.

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

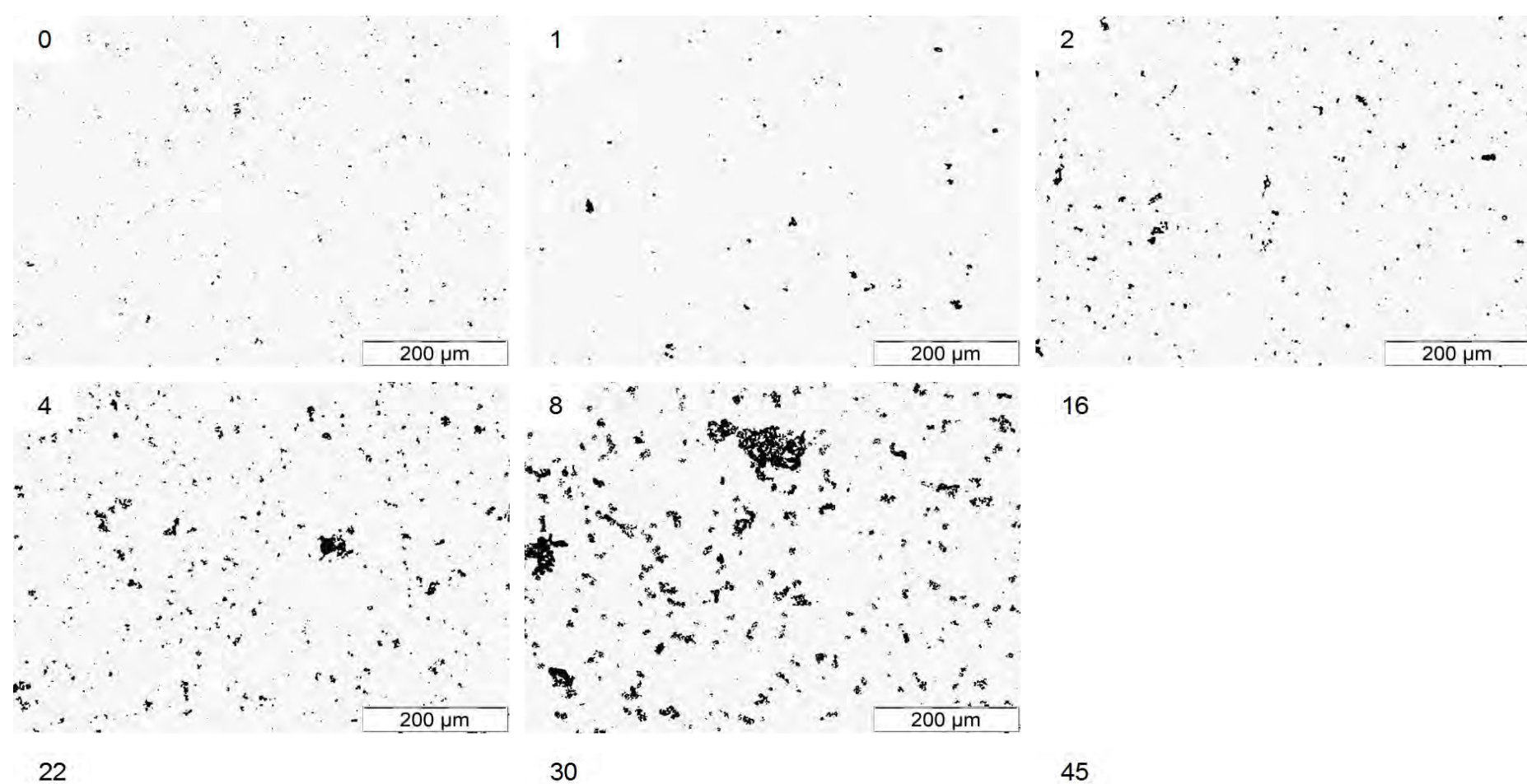
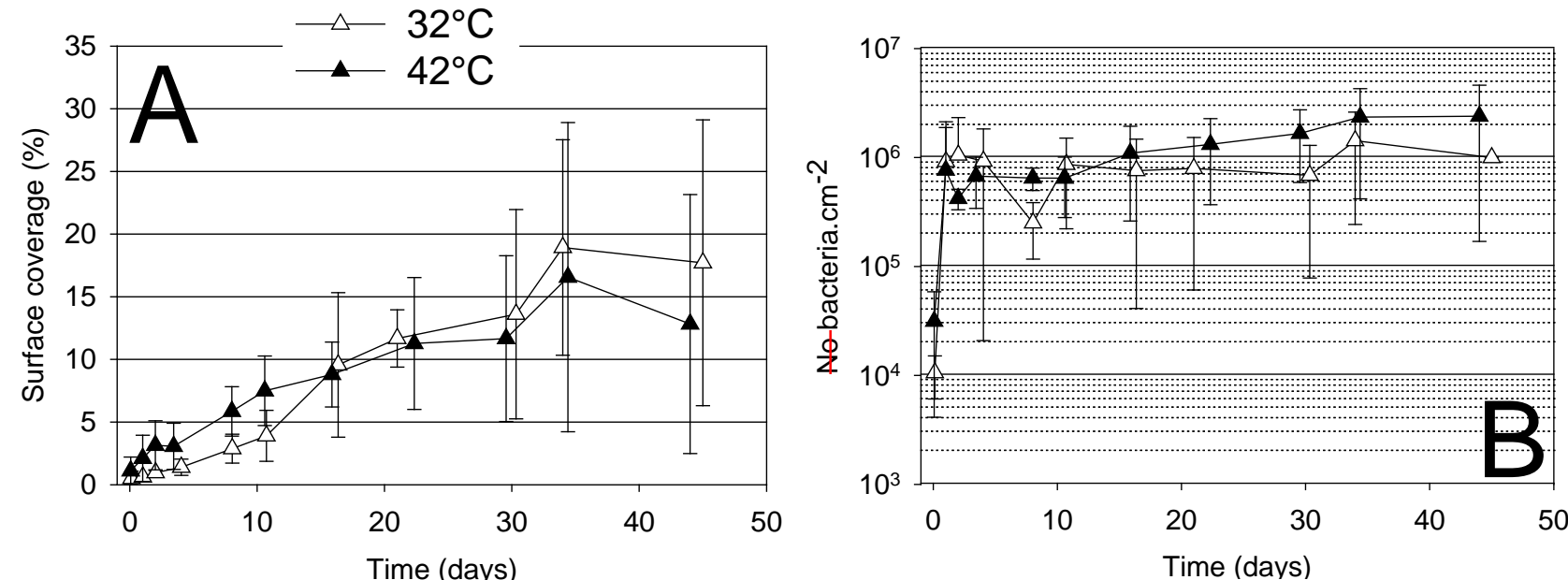
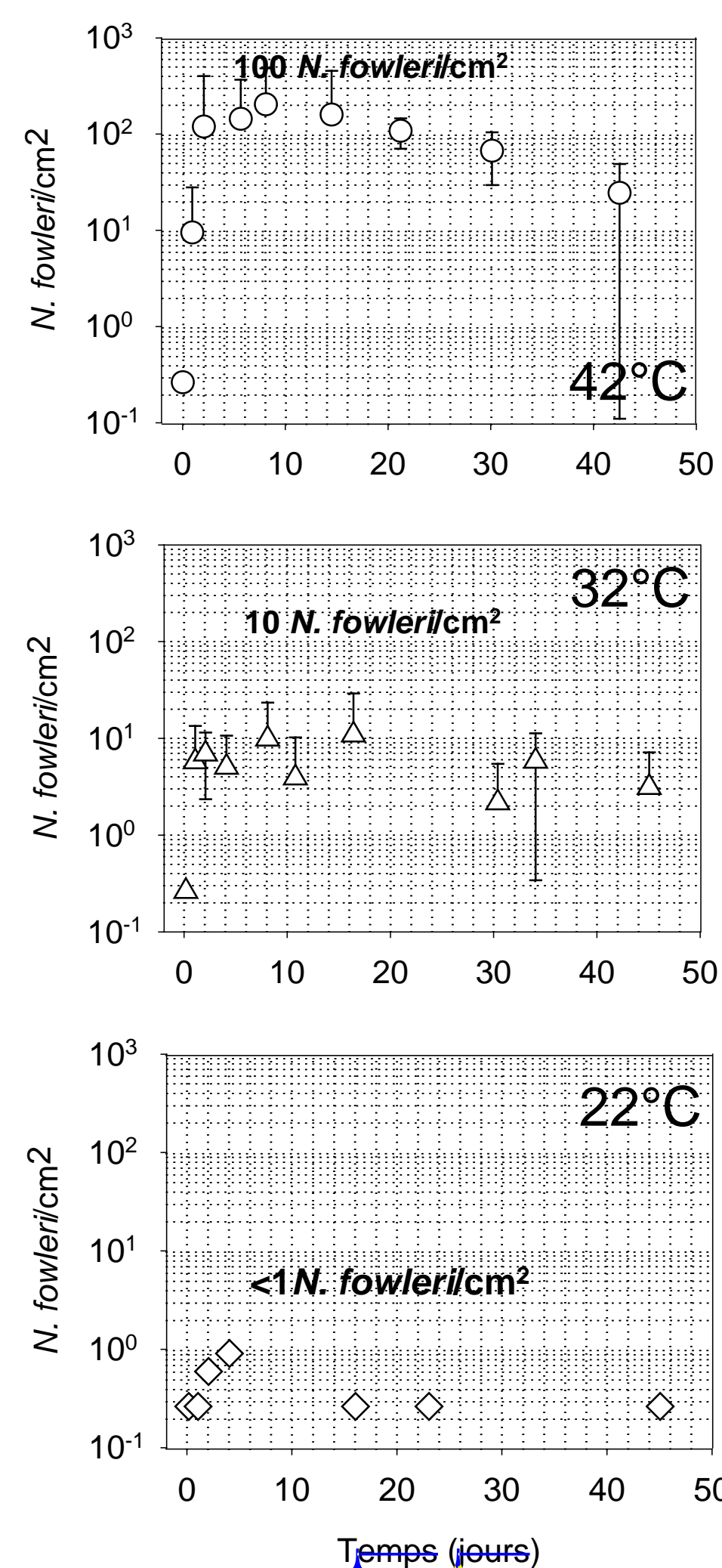


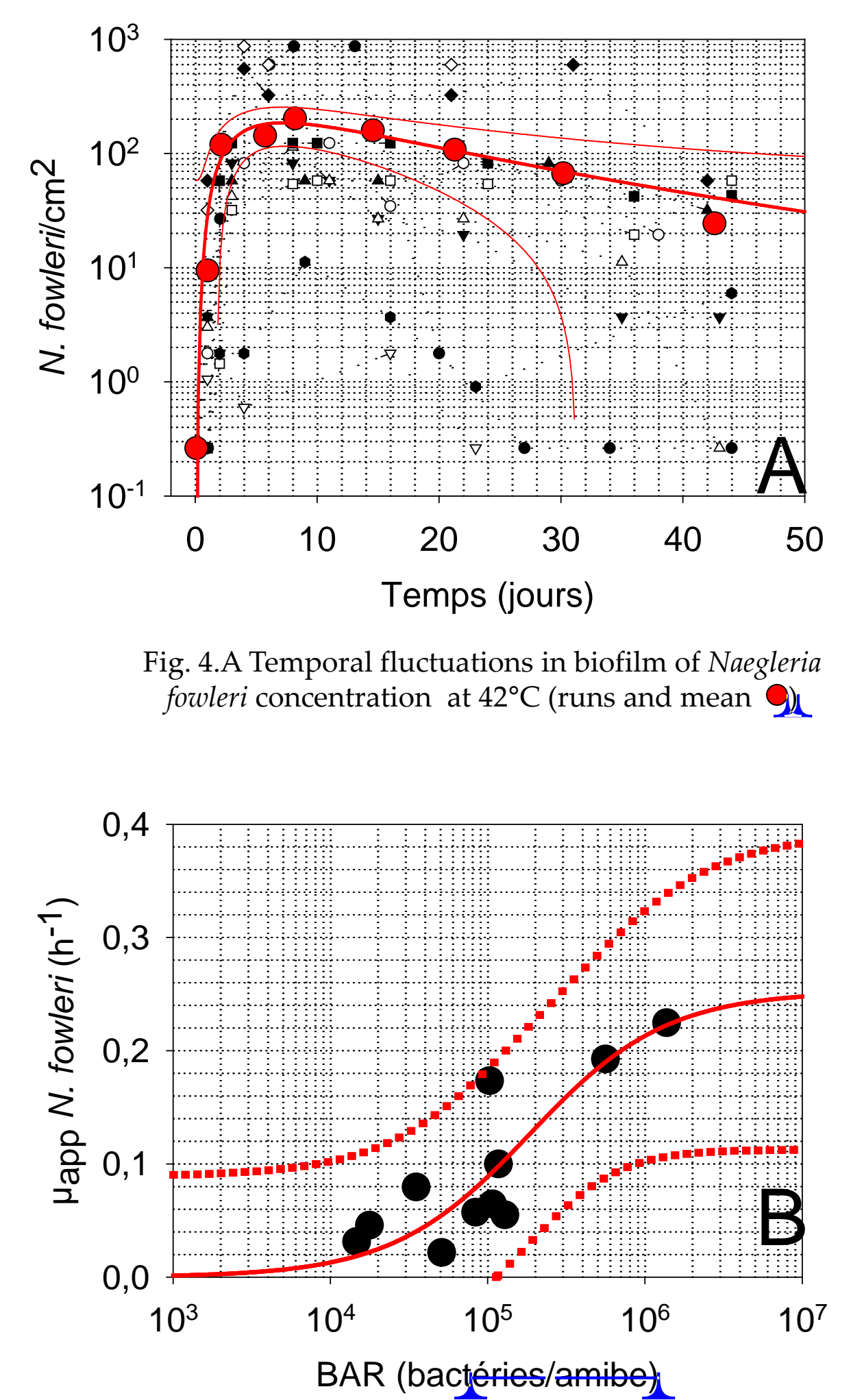
Fig.1. Pictures (light microscopy) of the biofilm at 42 °C on glass coupons from day 0 to day 45.

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

Influence of the temperature on the amoebic population

Fig. 3. Temporal fluctuations in biofilm of *Naegleria fowleri* concentrations at 22, 32 and 42 °C.

Influence of the bacterial density on the amoebic population

Fig. 4.A Temporal fluctuations in biofilm of *Naegleria fowleri* concentration at 42°C (runs and mean)Fig. 4.B Relationship between the specific growth rate (μ) and the amount of substrate (bacteria / amoeba)

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

o At 42°C *N. fowleri* **proliferated** whereas at 32°C, its density was **relatively stable** and at 22°C, *N. fowleri* **disappeared**.

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

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

o The **specific growth** of the *N. fowleri* is a function of the ratio [bacterial density / amoeba].

o μ_{max} is equal to 0.23 h^{-1} , which corresponds to a generation time of 3 **hours**.

o K_s is equal to 1.2×10^5 bacteria/amoeba.

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 amoebic growth is a function of the amount of substrate (ratio bacteria/ amoeba)**.