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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

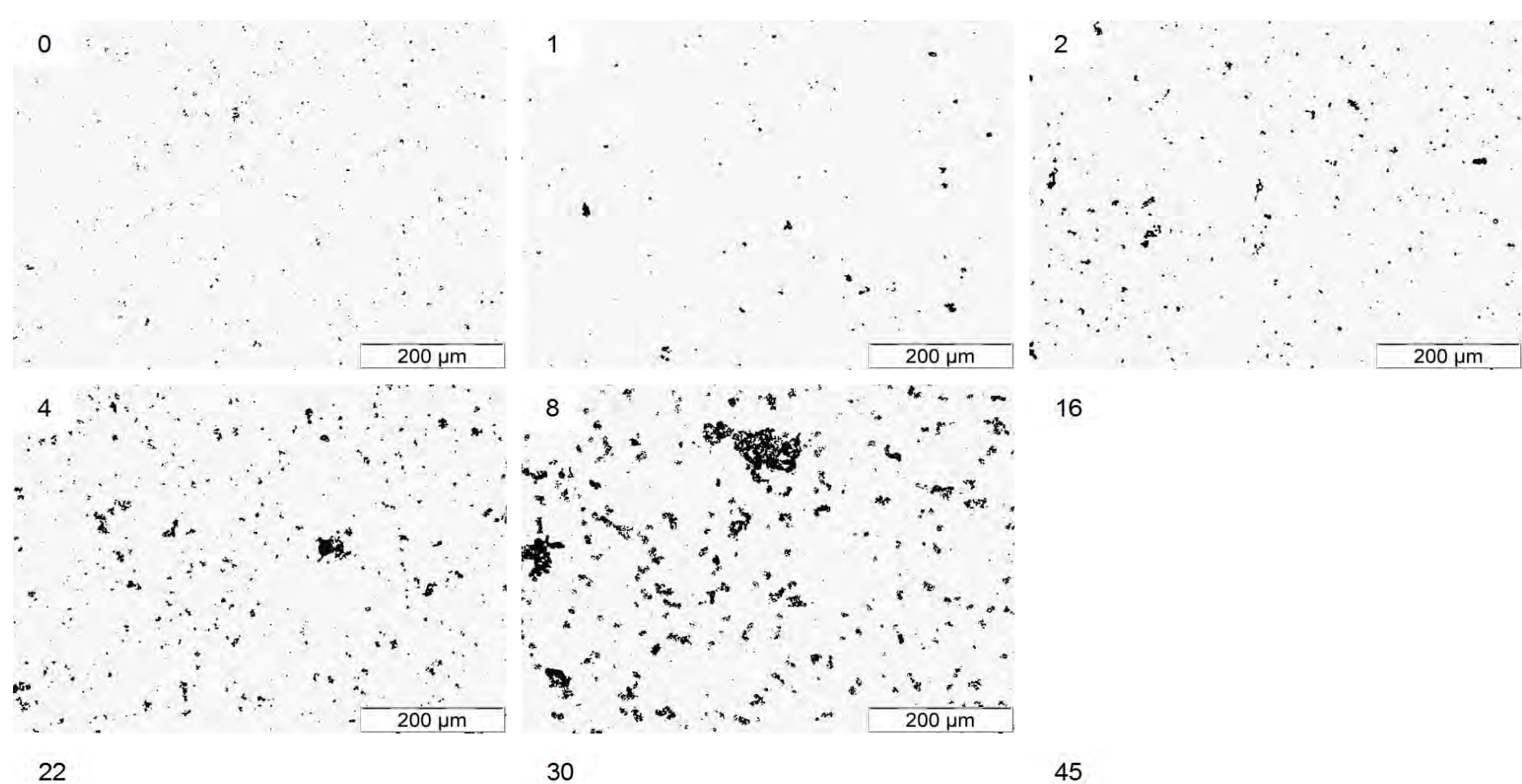


Fig.1. Pictures (light microscopy) of the biofilm at 42 °C on glass coupons from day 0 to day 45.

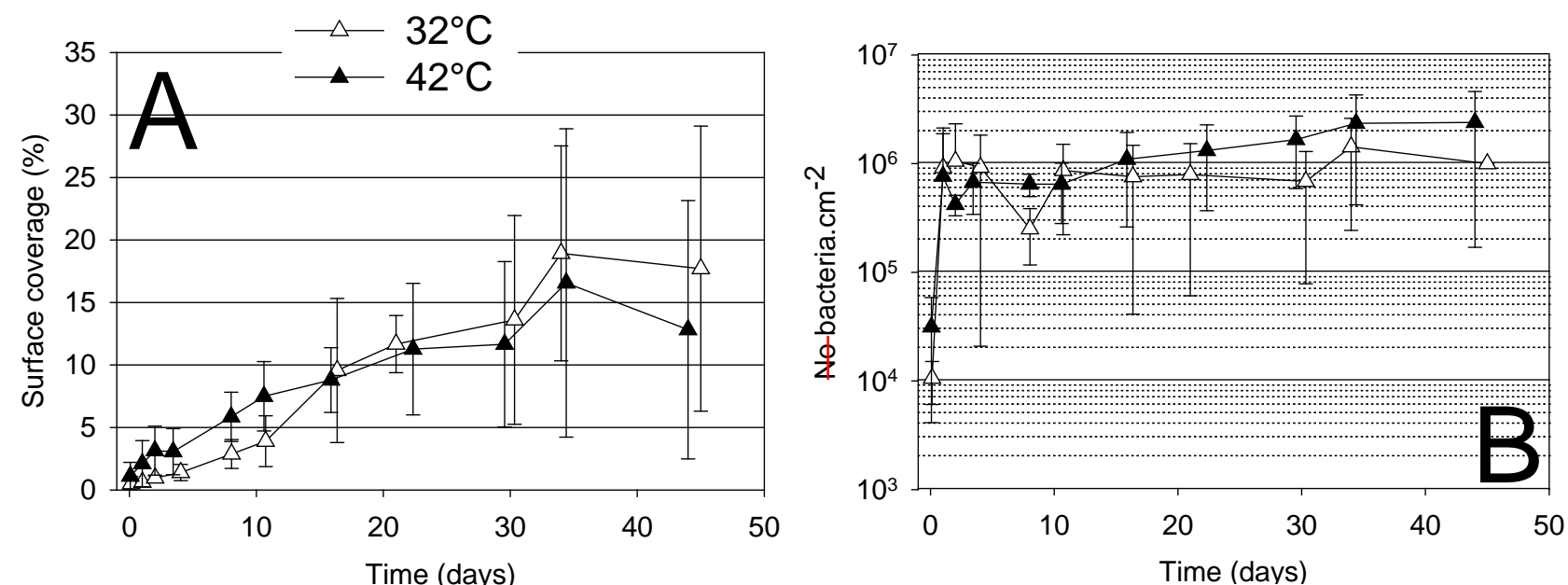


Fig. 2. A Temporal fluctuations of the surface coverage by the biofilm at 32°C (△) and 42°C (▲)  
Fig. 2. B Temporal fluctuations of bacterial density in the biofilm at 32°C (△) and 42°C (▲)

### Influence of the temperature on the amoebic population

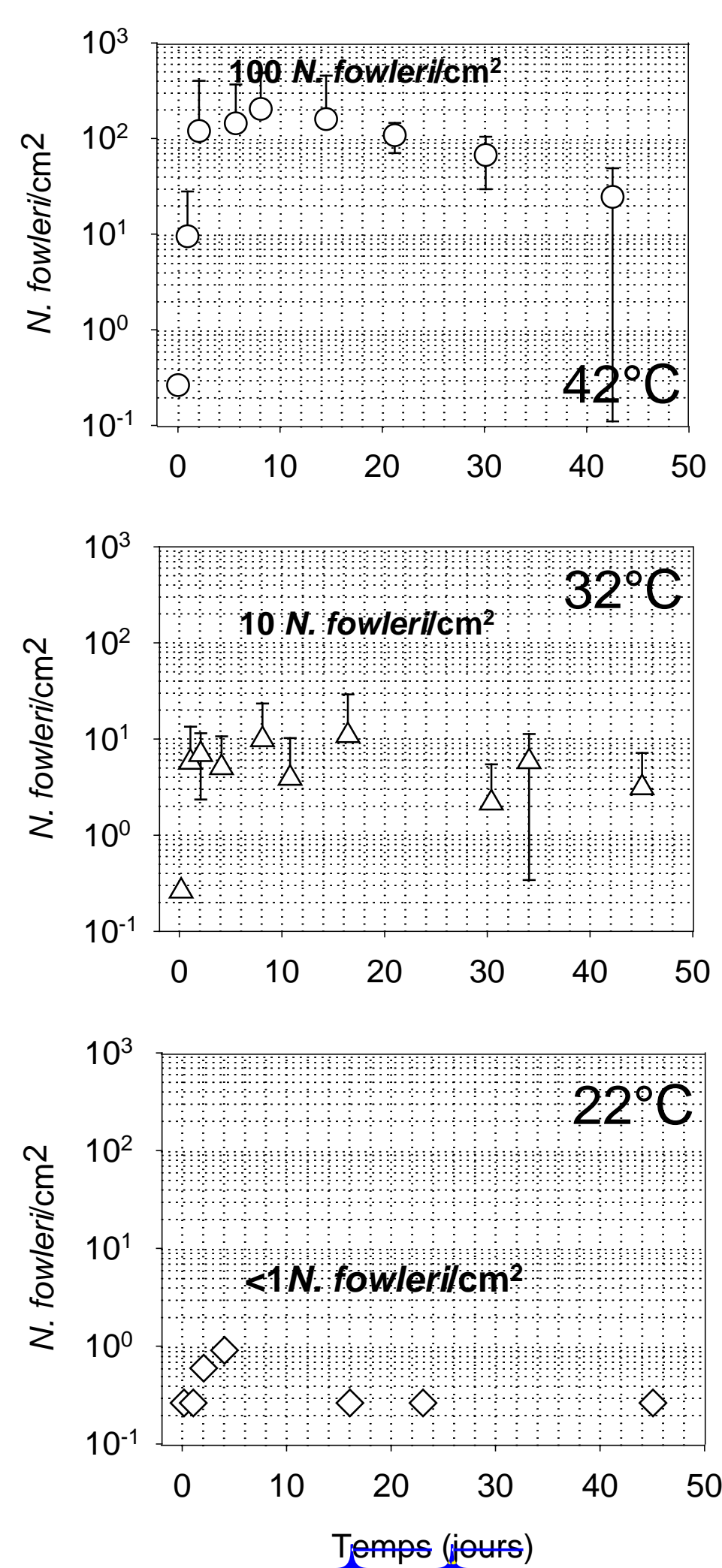


Fig. 3. Temporal fluctuations in biofilm of *Naegleria fowleri* concentrations at 22, 32 and 42 °C.

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

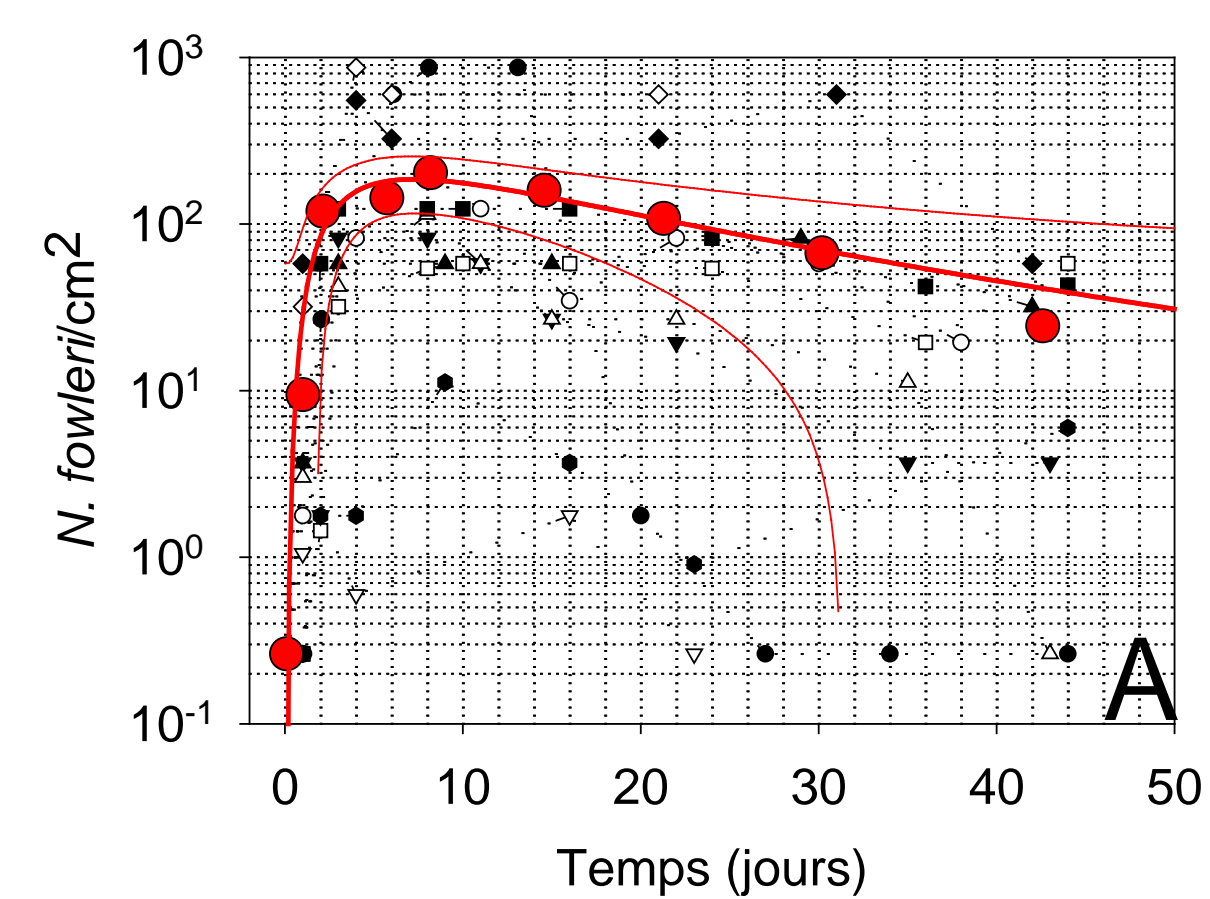


Fig. 4.A Temporal fluctuations in biofilm of *Naegleria fowleri* concentration at 42°C (runs and mean)

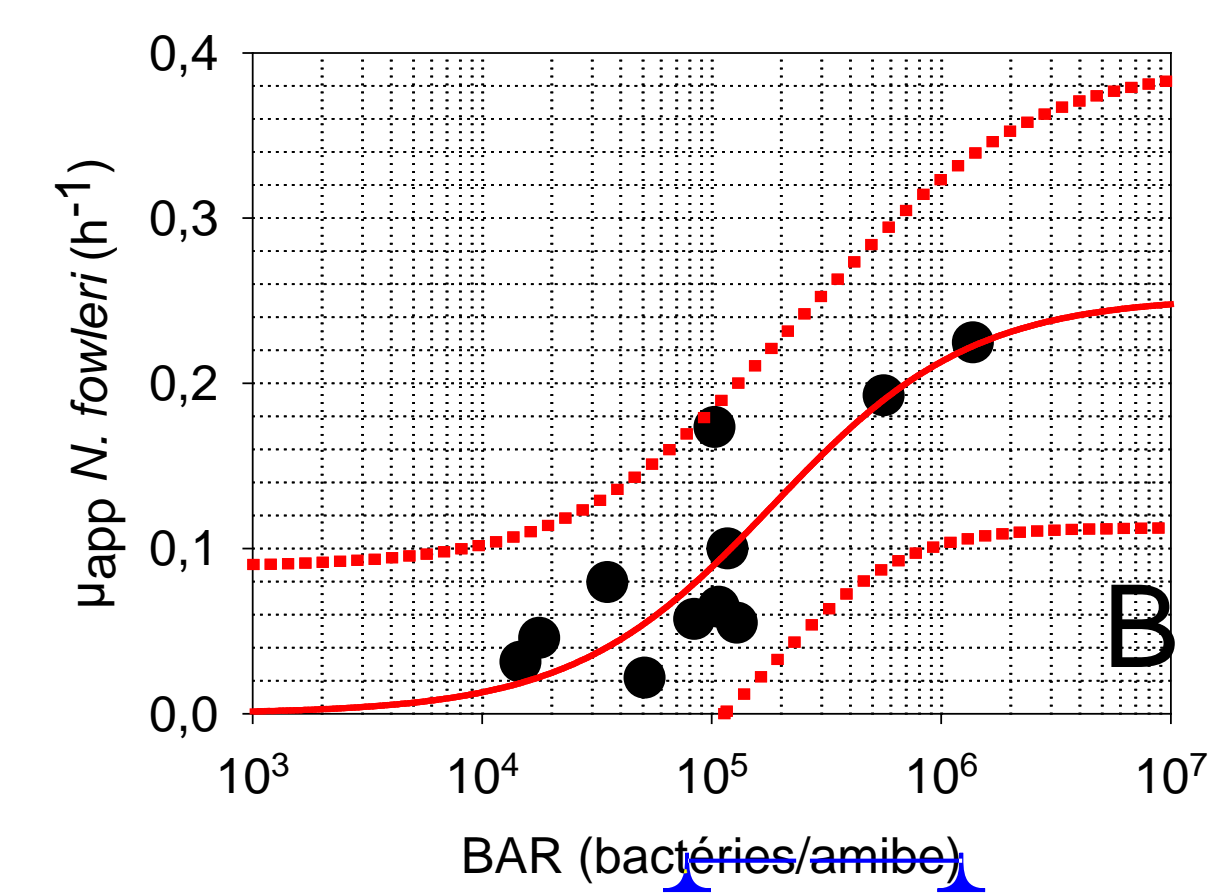


Fig. 4.B Relationship between the specific growth rate ( $\mu$ ) and the amount of substrate (bacteria / amoeba)

o The biofilm is constituted by **small ovoids microcolonies** in size ranged between 4 to 7  $\mu\text{m}$  (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  $\mu_{\text{max}}$  is equal to 0.23  $\text{h}^{-1}$ , which corresponds to a generation time of 3 hours.

o  $K_s$  is equal to  $1.2 \times 10^5$  bacteria/amoebae.

## 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/ amoebae)**.