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Sandra Pelleïeux, Laurence Mathieu, C. Gantzer, Isabelle Bertrand. Accumulation of phages in drinking water biofilms: a way to elude chlorine?. *Biofilm* 5, 2012, Paris, France. hal-03749875

**HAL Id: hal-03749875**

**<https://hal.univ-lorraine.fr/hal-03749875v1>**

Submitted on 11 Aug 2022

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# Accumulation of phages in drinking water biofilms: a way to elude chlorine?

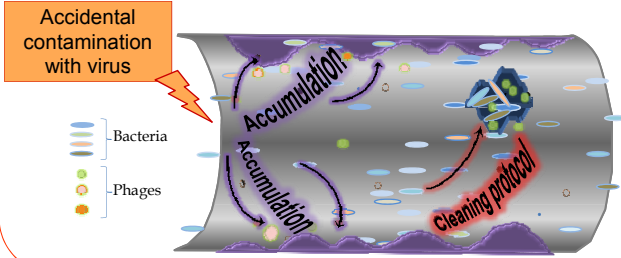


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## Context and Objectives



## Accumulation of F-specific RNA bacteriophages MS2, GA and Qβ on surfaces colonised with drinking water biofilms

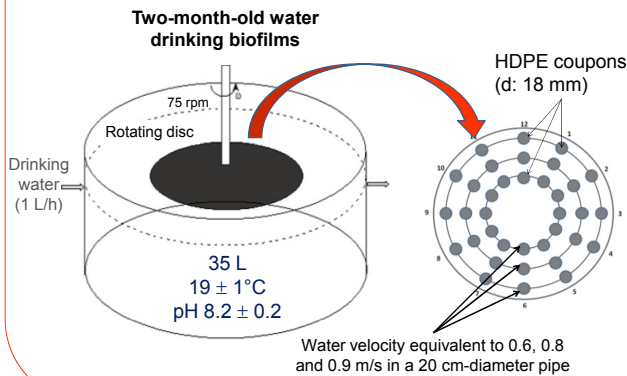
- ⇒ Phages showing differences in their surface properties
- ⇒ Different water velocities in laminar conditions

## Efficiency of a cleaning protocol based on high level chlorination

- ⇒ On phages in suspension in water
- ⇒ On phages accumulated in the biofilm

## Materials & Methods

### Rotating disc reactor



### F-specific RNA bacteriophages MS2, GA and Qβ



- **Structural similarities with enteric pathogenic viruses**  
Size: 20-30 nm  
Non-enveloped capsid and RNA genome

### Detection methods

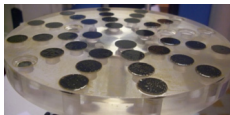
- Specific detection of viral genome by real time RT-PCR (Ogorzaly and Gantzer, 2006)
- Titration by culture method

### Surface properties recently studied (\*Langlet et al., 2008)

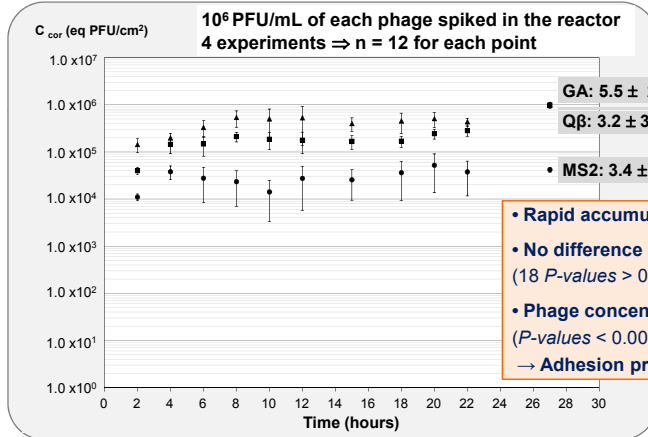
Phages / Virus	MS2	Qβ	GA	Norwalk virus	Hepatitis A virus
<b>Isoelectric point (Michen and Graule, 2010)</b>	2.2 - 3.9	1.9 - 5.3	2.1 - 2.6	5.5 - 6.0	2.8 - 5.8
<b>Negative charge at pH 6.5* (electrophoretic mobility)</b>	MS2 ≈ Qβ ≈ GA				
<b>Hydrophobicity*</b>	MS2 < Qβ < GA				

## Accumulation of MS2, GA and Qβ phages on HDPE coupons colonised with drinking water biofilm

### Drinking water biofilm characteristics



1.7x10<sup>7</sup> cells/cm<sup>2</sup> (Live/Dead BacLight kit)  
whatever the wall shear rate  
≈ 0.1% cultivable (15 d)  
> 95% showing intact membranes



- **Rapid accumulation of phages on biofilm within the first 3 hours.**
- **No difference in the phage concentration between 0.6 and 0.9 m/s (18 P-values > 0.05; α = 0.05, Mann-Whitney)**
- **Phage concentration on surfaces: MS2 < Qβ < GA (P-values < 0.0001, Mann Whitney)**  
→ Adhesion profile of phages may be related to their hydrophobicity.

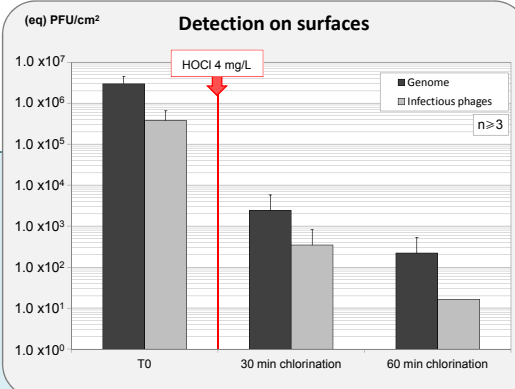
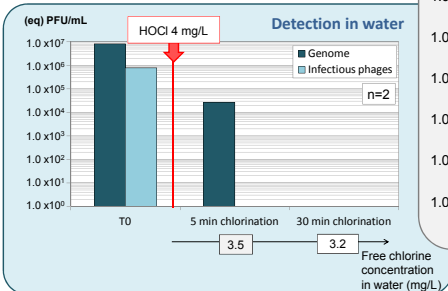
Pelleieux et al., 2012

## Protective effect of drinking water biofilm against chlorination

### Experimental conditions

Two-month-old drinking water biofilms (pH 8.2 ± 0.2, 19 ± 1°C)  
10<sup>6</sup> PFU/mL of each phage spiked in the reactor

- **Adsorption step:** 3 hours (75 rpm) (T0)
- **Chlorination step:** 4 mg/L of sodium hypochlorite (HOCl)



- **Rapid inactivation of phages in water by chlorine**
- **Protective effect of the biofilm against chlorination (4 mg/L) for adsorbed phages**  
Water: 5 log<sub>10</sub> reduction in less than 30 min  
Surfaces: 2.5 log<sub>10</sub> reduction in 60 min

**Acknowledgements:** These results were obtained within the scope of the research program "Biofilm VIII" funded by the Agence de l'Eau Seine-Normandie (AESN), VEOUA Environment Recherche & Innovation (VERI) and the Syndicat des Eaux d'Ile-de-France (SEDFI), and coordinated by Université de Lorraine.

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