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

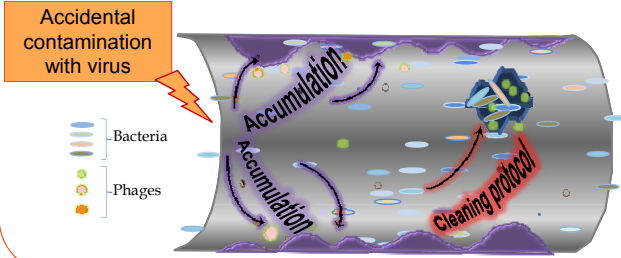


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Context and Objectives



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

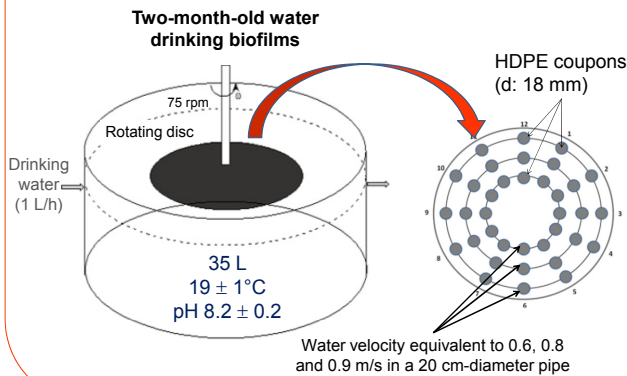
- ⇒ Phages showing differences in their surface properties
- ⇒ Different water velocities in laminar conditions

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

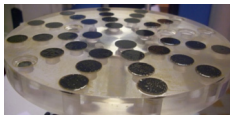
- Specific detection of viral genome by real time RT-PCR (Ogorzaly and Gantzer, 2006)
- Titration by culture method

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

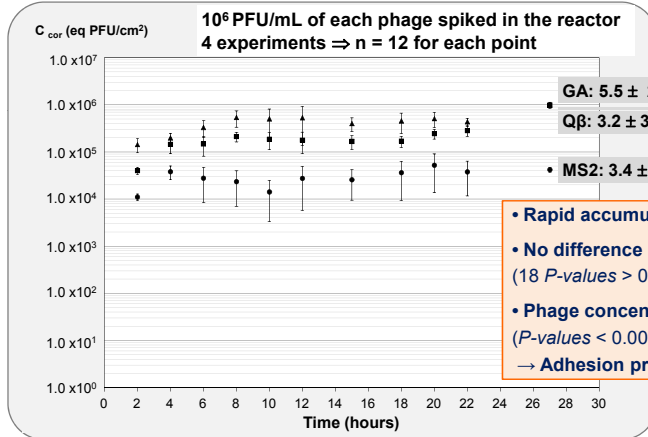
Phages / Virus	MS2	Qβ	GA	Norwalk virus	Hepatitis A virus
Isoelectric point (Michen and Graule, 2010)	2.2 - 3.9	1.9 - 5.3	2.1 - 2.6	5.5 - 6.0	2.8 - 5.8
Negative charge at pH 6.5* (electrophoretic mobility)	MS2 ≈ Qβ ≈ GA				
Hydrophobicity*	MS2 < Qβ < GA				

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

Drinking water biofilm characteristics



1.7x10⁷ cells/cm² (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.

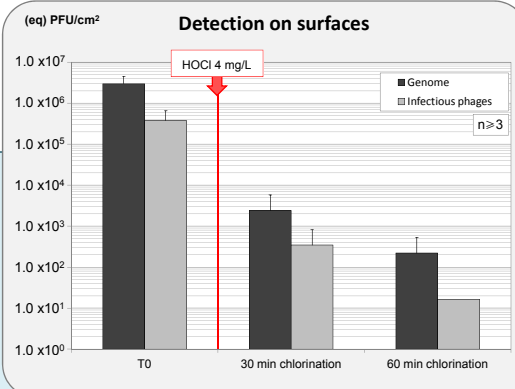
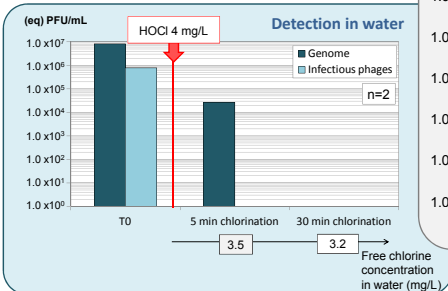
Pelleieux *et al.*, 2012

Protective effect of drinking water biofilm against chlorination

Experimental conditions

Two-month-old drinking water biofilms (pH 8.2 ± 0.2, 19 ± 1°C)
 10⁶ 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 log₁₀ reduction in less than 30 min
 Surfaces: 2.5 log₁₀ reduction in 60 min

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