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# Influence of chlorine discontinuities on the proteobacteria populations in drinking water biofilm

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## Introduction

Water distribution networks seldom function in stationary conditions. According to many parameters (quality of the resource, chlorine rate applied, technical interventions...), networks are constantly subjected to hydraulic, biological as physicochemical discontinuities. This study concerned disinfectant discontinuities (bleach). Few works already published on this topic and limited to discontinuities "all-or-nothing", have thus identified a risk of unsteadiness of the distributed water quality. To our knowledge, no published data exists on discontinuities with a "high and low" sequence of chlorine concentration and none have explored their impact on the bacterial communities.

## Objective

The objective is to appreciate, in drinking water and biofilm, the impact of chlorine discontinuities (changing from 0.1 to 0.4 then 0.1 mg Cl<sub>2</sub>/L of residual chlorine) on:

- the total number of bacteria,
- the viability of bacteria (membrane integrity and culturability),
- the phylogenetic composition of bacteria ( $\alpha$ ,  $\beta$  et  $\gamma$ -proteobacteria).

## Methods

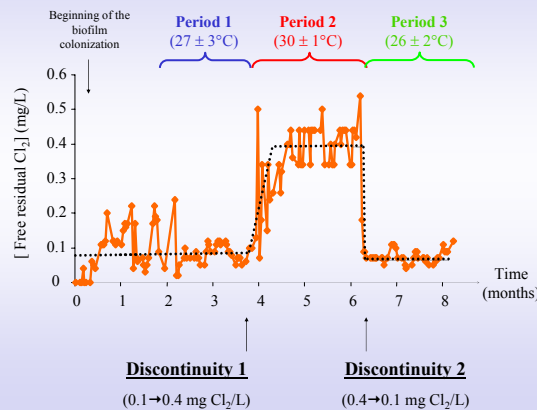
### The experimental distribution system (NanCIE):



Continuous feeding with re-chlorinated drinking water in order to reach 0.1 or 0.4 mg Cl<sub>2</sub>/L in the water at the outlet of the distribution system

Cement-lined cast iron  
Flow rate = 10 L/h    240 L    HRT = 24 h

### Experimental setup:



### Microbiological analysis:

- Regular analysis of water during the three periods
- Punctual analysis of biofilm:
  - before discontinuity 1
  - 24d and 64d after discontinuity 1
  - 18d and 54d after discontinuity 2

### Microbial parameters:

- Total number of bacteria (Syto 9 staining)
- Viable bacteria (with a membrane integrity) (kit BacLight)
- Culturable bacteria (PCA, 22°C, 15days)

### Phylogenetic composition (FISH): (adapted from Batté et al., 2003)

- Eubacteria (probe EUB338)
- $\alpha$ -proteobacteria (probe ALF1b)
- $\beta$ -proteobacteria (probe BET42a)
- $\gamma$ -proteobacteria (probe GAM42a)

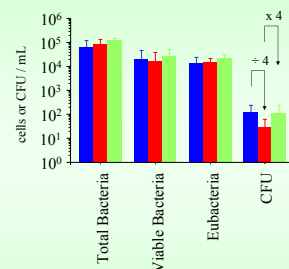
Batté M., Mathieu L., Laurent P. and Prévost M. (2003) Influence of phosphate and disinfection on the composition of biofilms produced from drinking water, as measured by fluorescence *in situ* hybridization. Can. J. Microbiol., 49: 741-753.

## Characteristics of the drinking water at inlet of the distribution system

(average of the 3 periods)

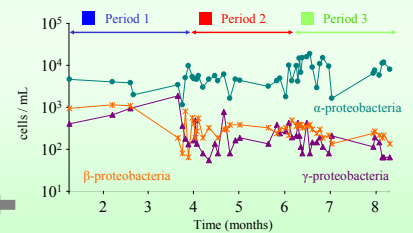
Parameter (mL)	Mean $\pm$ SD	n
Total Bacteria	$1.9 \cdot 10^5 \pm 1.0 \cdot 10^5$	44
Viable Bacteria	$2.2 \cdot 10^4 \pm 2.3 \cdot 10^4$	43
CFU (PCA, 22° C, 15d)	$4.7 \cdot 10^{-1} \pm 8.0 \cdot 10^{-1}$	47
Eubacteria	$3.4 \cdot 10^4 \pm 2.3 \cdot 10^4$	41
$\alpha$ -proteobacteria	$1.4 \cdot 10^4 \pm 9.8 \cdot 10^3$	39
$\beta$ -proteobacteria	$3.9 \cdot 10^2 \pm 6.0 \cdot 10^2$	45
$\gamma$ -proteobacteria	$2.1 \cdot 10^2 \pm 2.6 \cdot 10^2$	45

## Effect of the chlorine discontinuity on the bacterial quality of water



Only the culturability of bacteria is modified during chlorine discontinuities:

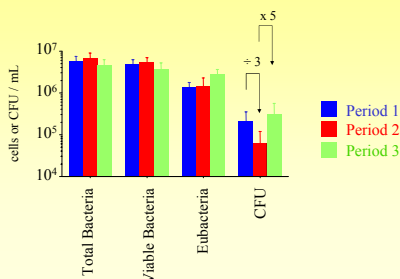
- CFU  $\div$  4 when [Cl<sub>2</sub>] increased from 0.1 to 0.4 mg/L
- CFU  $\times$  4 when [Cl<sub>2</sub>] decreased from 0.4 to 0.1 mg/L



Seasonal variations of the water quality masking the possible effect of chlorine discontinuities.

➔ No significant effect of Cl<sub>2</sub> discontinuities on the phylogeny of bacteria present in drinking water

## Effect of the chlorine discontinuity on the biofilm

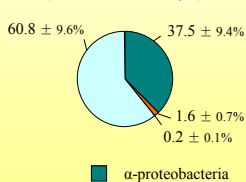


- ➔ The increase of the residual chlorine concentration in distributed drinking water from 0.1 to 0.4 mg Cl<sub>2</sub>/L :
  - alters the culturability of biofilm bacteria (CFU  $\div$  3 after discontinuity), no modification of the total number of bacteria.
  - changes the phylogenetic composition of biofilms:  $\nearrow$  of the  $\beta$ - and  $\gamma$ -proteobacteria and  $\searrow$  of the  $\alpha$ -proteobacteria.

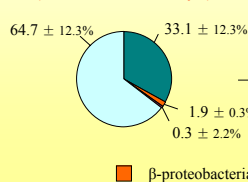
➔ While remaining a minority sub-group, the proportion of  $\gamma$ -proteobacteria, including many bacteria of sanitary interest (coliforms, *Legionella*, *Pseudomonas*, ...), increased by a factor 6

- ➔ This effect is temporary because the signals are reversed when the Cl<sub>2</sub> concentration is again brought back to 0.1 mg Cl<sub>2</sub>/L :
  - the culturable bacteria increased by a factor 5 after discontinuity.
  - the proportions of  $\alpha$ -,  $\beta$ - and  $\gamma$ -proteobacteria find their initial level gradually.

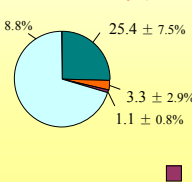
### Period 1: 0.1 mg Cl<sub>2</sub>/L (before discontinuity 1)



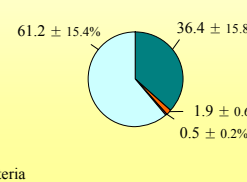
### Period 2: 0.4 mg Cl<sub>2</sub>/L (24d after discontinuity 1)



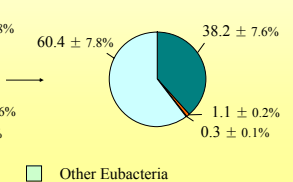
### Period 2: 0.4 mg Cl<sub>2</sub>/L (64d after discontinuity 1)



### Period 3: 0.1 mg Cl<sub>2</sub>/L (18d after discontinuity 2)



### Period 3: 0.1 mg Cl<sub>2</sub>/L (56d after discontinuity 2)



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