# Rapid assessment of chlorination on drinking water biofilm by measuring fluorescence of SYBR® Green II stained surfaces



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#### **Objective**

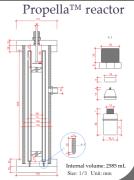
One of the objectives of the European project SecurEau is to assess rapidly the effectiveness of chlorine  $(Cl_2)$  used for microbial decontamination of the drinking water pipe walls after a deliberate contamination (Task 6.7).

A new method of disinfection control based on the rapid detection (within 30 min) of bacterial damages with fluorochromes was developed and applied to surfaces (biofilm). The major hypothesis is that attached bacteria exposed to  $\text{Cl}_2$  should be difficult to stain with fluorochrome like SYBR® Green II (Sybr II). Such a low fluorescence signal should be fast measurable with a fluorescence reader and related to the effective disinfection of the surfaces.

# Fluorescence reader

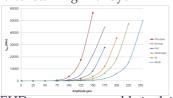
Propella<sup>TM</sup> coupons





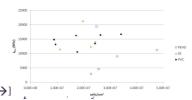
#### Fluorescence reading to assess biofilms

**Background of fluorescence** of bare materials **PVC**, **PEHD and SS** before and after staining with Sybr II



→PEHD coupons are usable to detect fluorescence intensity of biofilms.

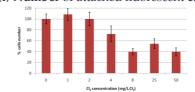
The fluorescence intensity of unchlorinated coupons with biofilms is not predictive of cell number.



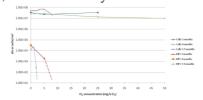
measurements (before *versus* after chlorination) can be achieved with fluorescent reader.

### Effects of chlorination on drinking water biofilms

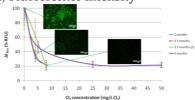
Effect of Cl<sub>2</sub> in static conditions on:
(i) Number of attached fluorescent cell



(ii) Cell culturability



(iii) Fluorescence intensity



 $\rightarrow$  A threshold concentration of around 10 mg/L Cl<sub>2</sub> for 1 h is needed to disinfect pipes wall.

## Chlorination of biofilms contaminated with spores

**Effect of Cl<sub>2</sub>** in dynamic conditions (Propella reactor continuously supplied with 10 mg/L Cl<sub>2</sub>)

(i) Stripped coupons after 6 days of chlorination

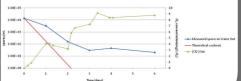
d0 0



(ii) Biofilm **clumps in bulk water** on day 4 of chlorination



(iii) Release/desorption of viable spores (*Bacillus subtilis*) from drinking water biofilms in bulk water



#### Conclusion

Treatment of drinking water biofilm with 10~mg/L free  $\text{Cl}_2$  results in (i) a 70~% decrease of attached fluorescent cells, (ii) a 100% decrease of bacteria culturability and (iii) a 80~% decrease of fluorescence intensity of biofilms stained by Sybr II.

Thus, variation in fluorescent signal may constitute a reliable and rapid indicator about the effectiveness of water mains wall disinfection.

