UNIVERSITÀ DEGLI STUDI
DI MILANO

REPORT

Evaluation of the minimal inhibitory concentration (MIC) on *Escherichia coli* biofilm

September 18th, 2017

Performed activities

1. Inhibition of planktonic growth. With this assay, it was evaluated the minimum concentration of Easychlor able to inhibit the planktonic growth of *E. coli*. Three different inoculating bacterial concentrations were considered, i.e. 10^6 cell/ml, 10^5 cell/ml and 10^4 cell/ml.

2. Inhibition of cell adhesion to surfaces. With this assay, it was evaluated the minimum concentration of Easychlor able to inhibit the surface adhesion and biofilm formation. Three different inoculating bacterial concentrations were considered, i.e. 10^6 cell/ml, 10^5 cell/ml and 10^4 cell/ml.

3. Inhibition of pre-grown biofilm. With this assay it was evaluated the minimum concentration of Easychlor able to inhibit the growth of *E. coli* cells pre-grown as biofilm on a surface.

4. Break up of pre-grown biofilm following a short contact with the products. With this assay, it was evaluated the minimum concentration of Easychlor able to detach pre-formed bacterial biofilm from surfaces in a short contact period.

Experimental details

- The experimental trials were performed using the biofilm-producing bacterium *Escherichia coli* DSM30083, pathogenic to chickens (https://www.dsmz.de/catalogues/details/culture/dsm-30083)

- Tested concentrations:

0, 10, 50, 100, 150, 200, 250, 500, 750, 1000, 2000, 2500 ppm of free chlorine. The products were diluted in sterile distilled water (or LB medium) according to the following conversions:

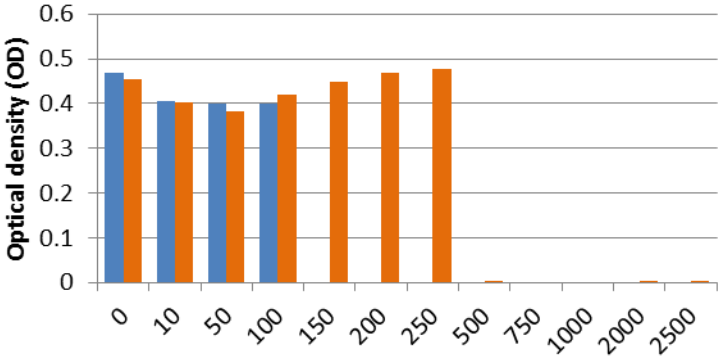
- 1 Easychlor tab dissolved in 1000 L of water corresponds to 5.59 ppm free chlorine;
- 14.5 vol Ipochlorite correspond to 145,000 ppm free chlorine

- Experiments were performed with four replicates

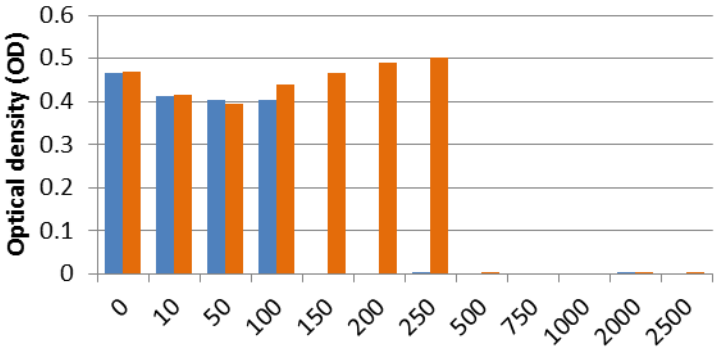
1 - Inhibition of planktonic growth

Biocidal effect of Easychlor and Ipochlorite on the bacterial cells

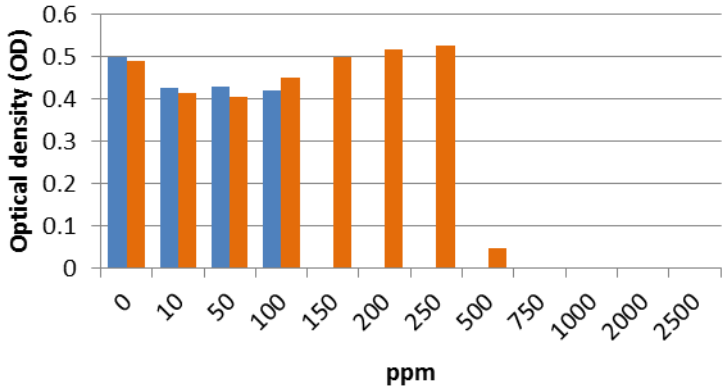
10^4 cell/ml



10^5 cell/ml



10^6 cell/ml



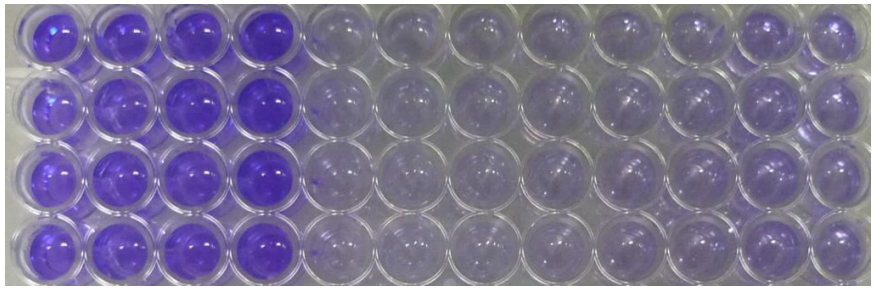
Easychlor

Ipochlorite

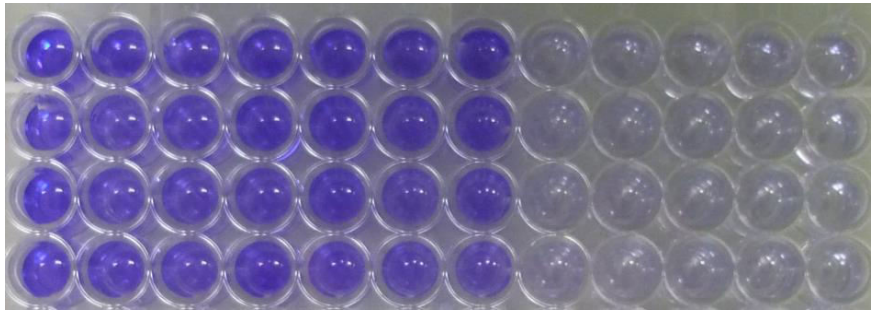
2 - Inhibition of cell adhesion to surfaces

[molecule]

Easychlor

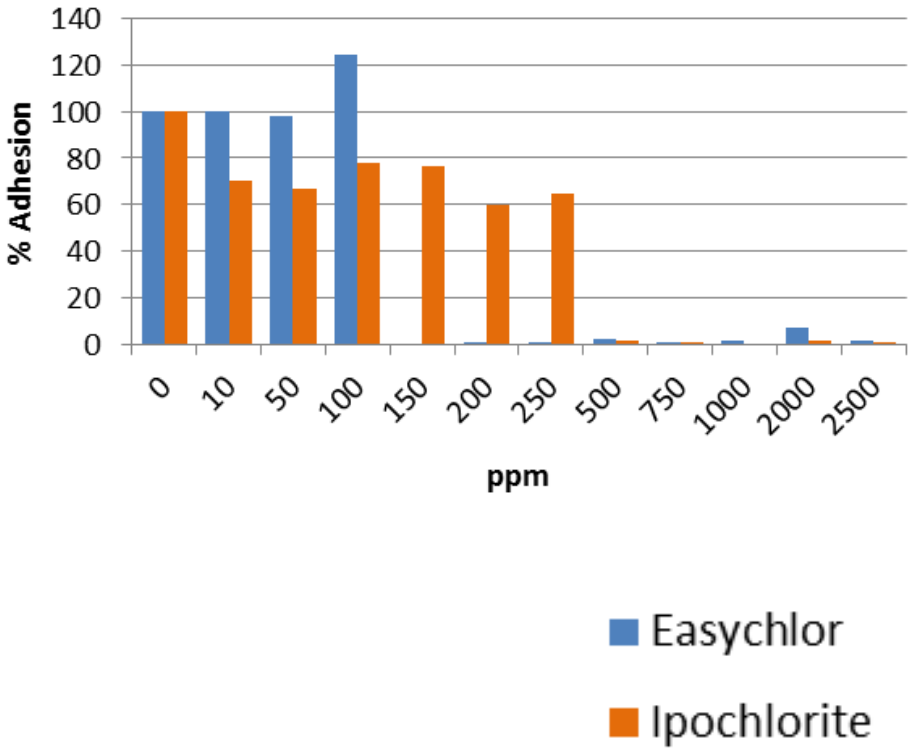


Ipochlorite



E. coli inoculum: 10^4 cell/ml

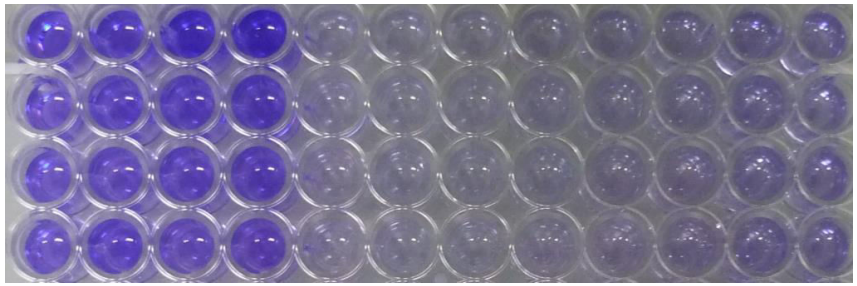
Anti-adhesion effect of the molecules on the surfaces



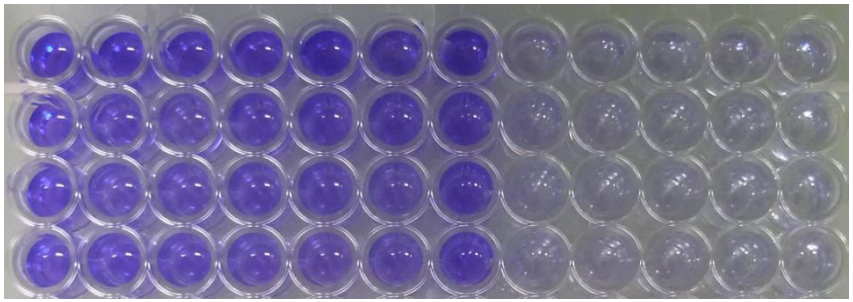
2 - Inhibition of cell adhesion to surfaces

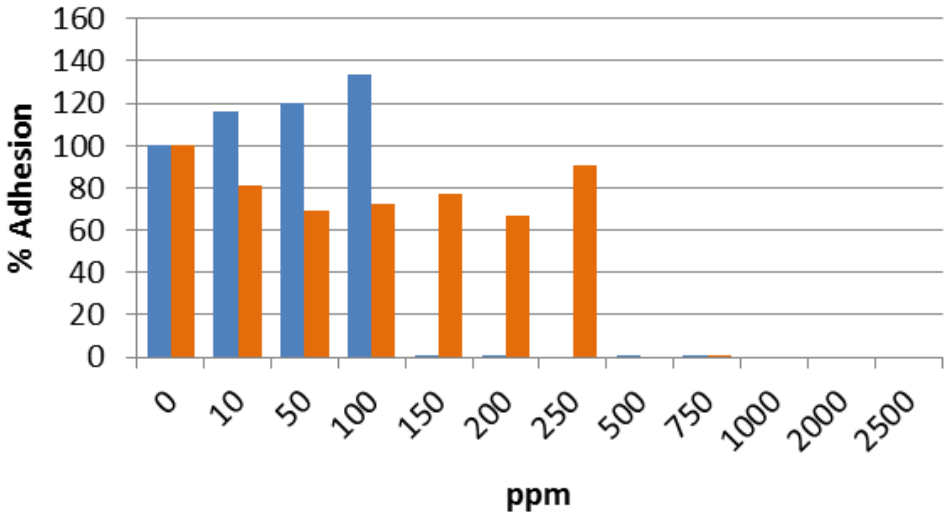
[molecule]

Easychlor



Ipochlorite



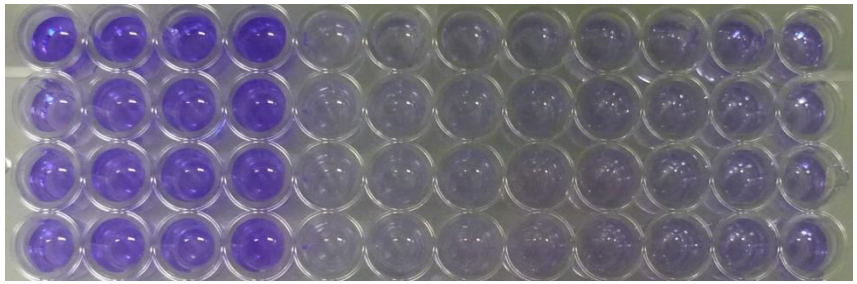


E. coli inoculum: 10^5 cell/ml

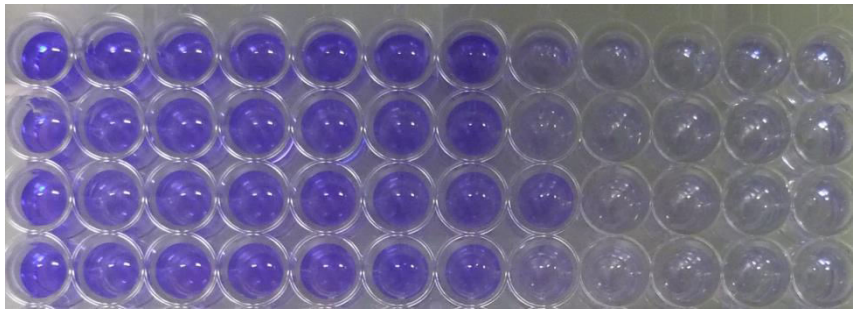
2 - Inhibition of cell adhesion to surfaces

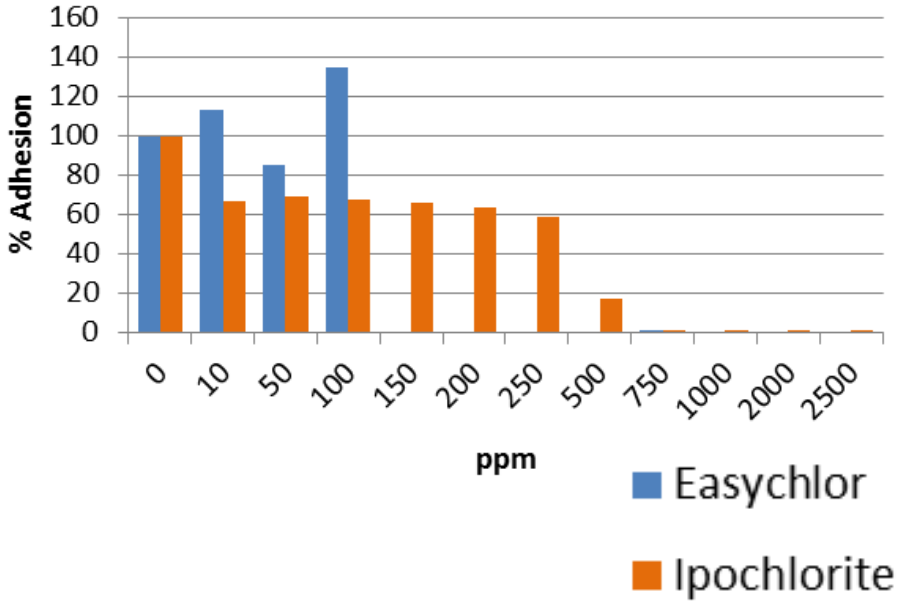
[molecule]

Easychlor



Ipochlorite





E. coli inoculum: 10^6 cell/ml

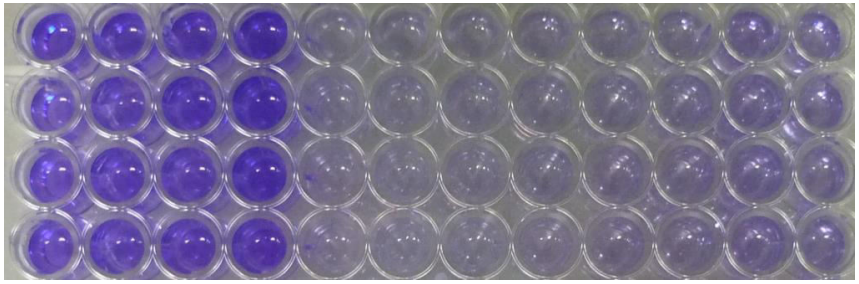
2- Inhibition of cell adhesion to surfaces

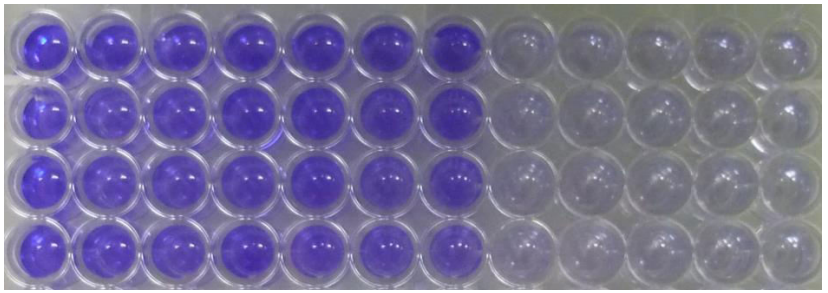
[molecule]

Easychlor

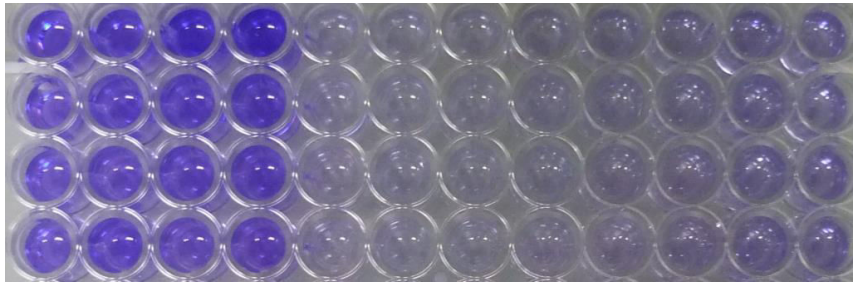
Ipochlorite

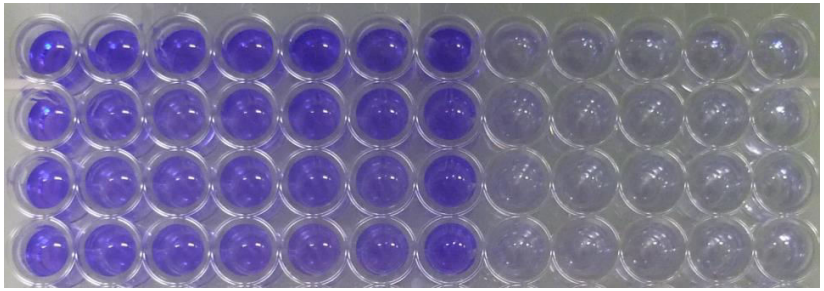
10^4



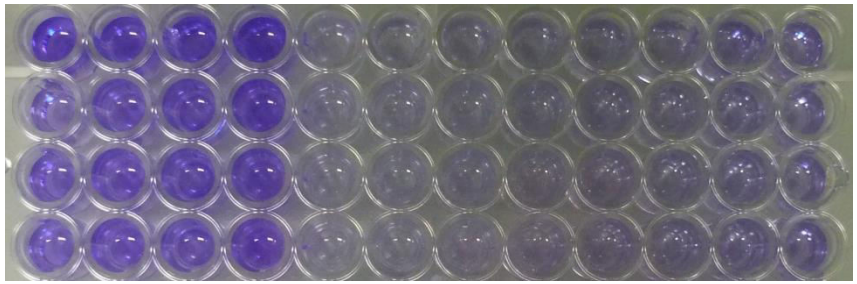


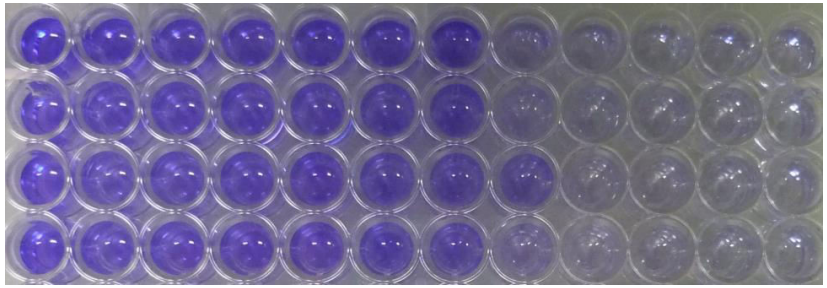
10^5





10^6

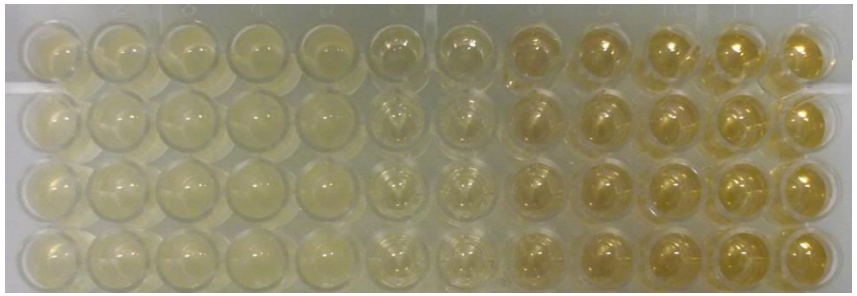




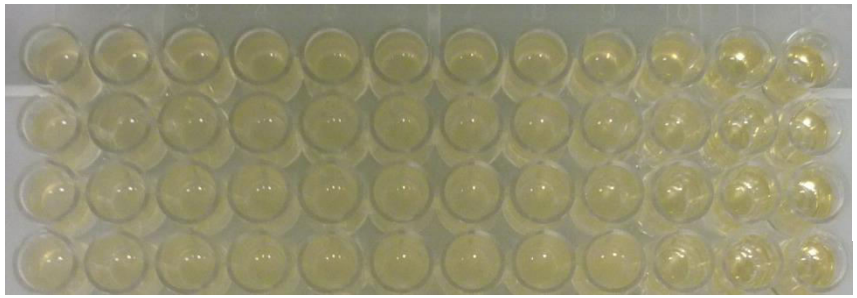
3 - Inhibition of pre-grown biofilm

[molecule]

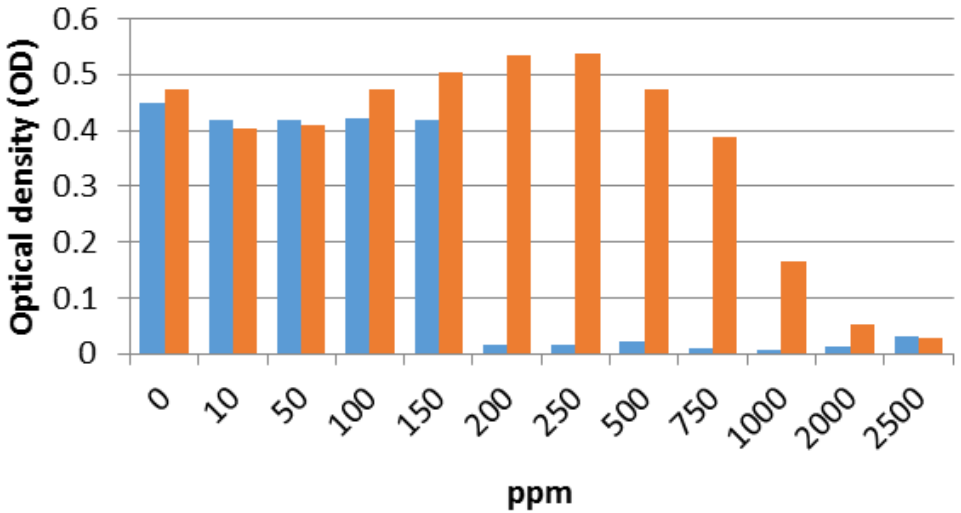
Easychlor



Ipochlorite



We observed the cell growth inhibition

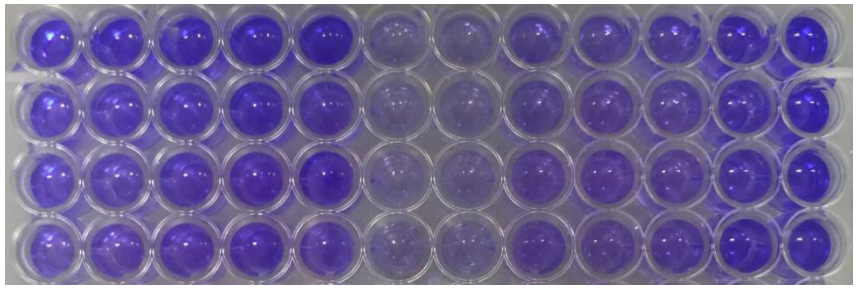


Ipochlorite: more gradual decrease of cell turbidity with high concentrations

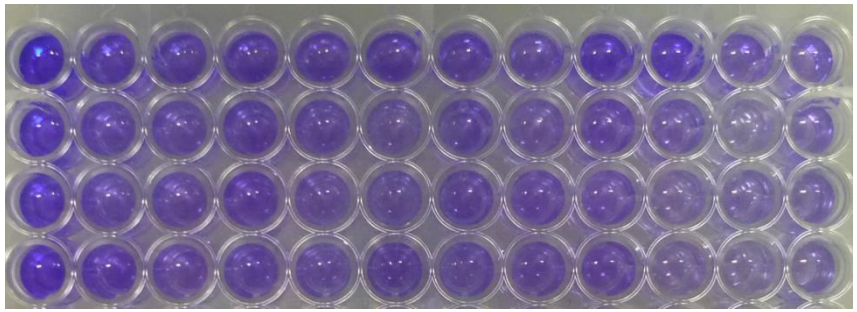
3 - Inhibition of pre-grown biofilm

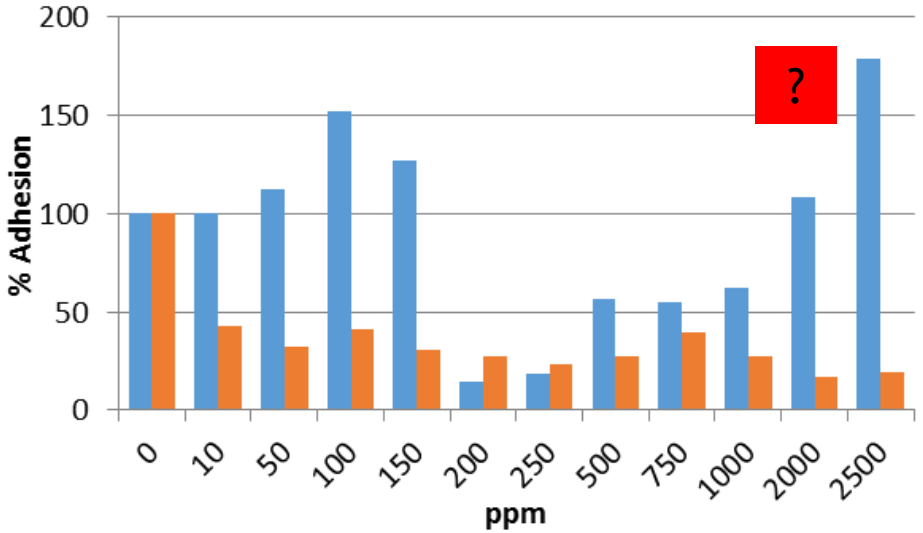
[molecule]

Easychlor



Ipochlorite



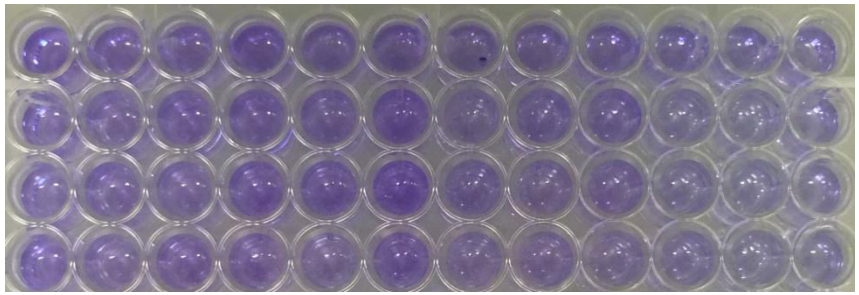


Biocidal effect on the pre-grown biofilm

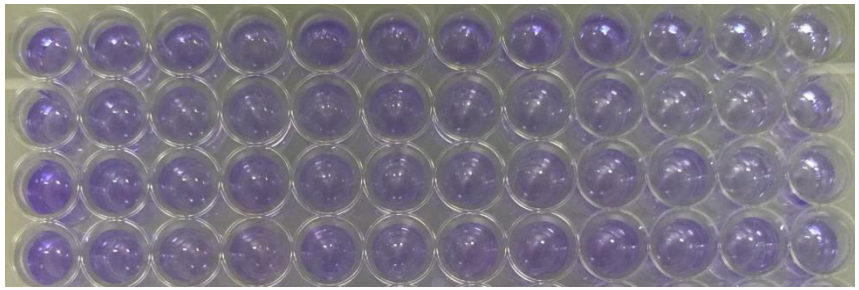
4 - Break up of pre-grown biofilm - short contact

[molecule]

Easychlor

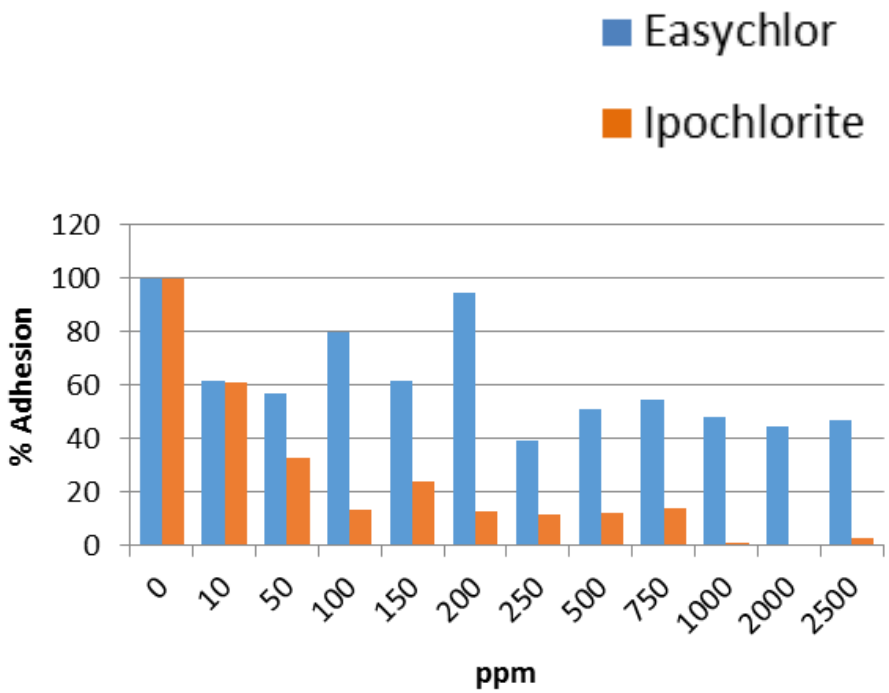


Ipochlorite



Disruptive effect of the molecules on the biofilm

The products were administered at different concentrations in distilled water on a 24 hours pre-grown biofilm. After 2.5-hours contact, the residual biofilm was quantified.



Conclusions

1. Inhibition of planktonic growth.
Easychlor inhibits *E. coli* growth at 100 ppm, while Ipochlorite is active at 250 (500) ppm. Ipochlorite is more sensitive to the inoculum concentration than Easychlor.

2. Inhibition of cell adhesion to surfaces.
Easychlor inhibits *E. coli* adhesion at 100 ppm, while Ipochlorite is active at 250 (500) ppm. Ipochlorite is more sensitive to the inoculum concentration than Easychlor.

3. Inhibition of pre-grown biofilm.
Easychlor: not clear situation.
Ipochlorite: about 50% inhibition of pre-grown biofilm with concentrations >10 ppm.

4. Break up of pre-grown biofilm following a short contact with the products.
Following 2.5 hours-contact, Easychlor and Ipochlorite show reductions of the pre-grown biofilms with concentrations >10 ppm (not for all the cases). Ipochlorite is more active to remove the pre-grown biofilm than Easychlor.