Mixture of Sodium Hypochlorite and Hydrogen Peroxide on Adhered Aeromonas hydrophila to Solid Substrate in Water: Impact of Concentration and Assessment of the Synergistic Effect
Chrétien Lontsi Djimeli, Antoine Tamsa Arfao, Olive Noah Ewoti, Mireille Ebiane Nougang, Marlyse Moungang, Geneviève Bricheux, Moïse Nola, Télesphore Sime-Ngando

To cite this version:
Chrétien Lontsi Djimeli, Antoine Tamsa Arfao, Olive Noah Ewoti, Mireille Ebiane Nougang, Marlyse Moungang, et al.. Mixture of Sodium Hypochlorite and Hydrogen Peroxide on Adhered Aeromonas hydrophila to Solid Substrate in Water: Impact of Concentration and Assessment of the Synergistic Effect. International Journal of Bacteriology, Hindawi Publishing Corporation, 2014, 2014, pp.121367. 10.1155/2014/121367 . hal-01983212

HAL Id: hal-01983212
https://hal.archives-ouvertes.fr/hal-01983212
Submitted on 23 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Research Article

Mixture of Sodium Hypochlorite and Hydrogen Peroxide on Adhered Aeromonas hydrophila to Solid Substrate in Water: Impact of Concentration and Assessment of the Synergistic Effect

Chrétien Lontsi Djimeli, 1 Antoine Tamsa Arfao, 1 Olive V. Noah Ewoti, 1 Mireille Ebiane Nougang, 1 Marlyse L. Mounaung, 1 Geneviève Bricheux, 2 Moïse Nola, 1 and Télesphore Sime-Ngando 2

1 University of Yaoundé I, Laboratory of General Biology, Hydrobiology and Environment Research Unit, P.O. Box 812, Yaoundé, Cameroon
2 Laboratoire "Microorganismes: Génome & Environnement", UMR CNRS 6023, Université Blaise Pascal, Complexe Scientifique des Cézeaux, 24 avenue des Landais, BP 80026, 63171 Aubière Cedex, France

Correspondence should be addressed to Moïse Nola; moise.nola@yahoo.com

Received 28 August 2013; Revised 22 December 2013; Accepted 13 January 2014; Published 3 March 2014

Academic Editor: Rodrigo E. Mendes

Copyright © 2014 Chrétien Lontsi Djimeli et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The synergistic effects of the combined treatments of NaOCl and H₂O₂ on the elimination of A. hydrophila adhered to polythene under static and dynamic conditions were evaluated. The concentrations 0.1, 0.2, and 0.3‰ NaOCl and 0.5, 1, and 1.5‰ H₂O₂ were used. The contact periods were 180, 360, 540, and 720 minutes. The abundance of cells adhered reached 2.47 and 2.27 units (log(CFU/cm²)), respectively, under static and dynamic conditions after action of the mixture of disinfectants, whereas it reached 2.41 and 3.39 units (log(CFU/cm²)) after action of NaOCl and H₂O₂ alone, respectively. Increase in the incubation period resulted in a significant decrease in the abundance of cells adhered when the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ was used (P < 0.01). For each cell growth phase, there was a significant difference amongst the mean densities of cells adhered after action of the mixture of disinfectants (P < 0.05). Although the Freundlich isotherm parameters relatively varied from one experimental condition to another, the Kᵥ value registered in the exponential growth phase was relatively higher in static state than in dynamic regime; cells adhered under dynamic condition seem more sensitive to the synergistic action than those adhered under static condition.

1. Introduction

The drinking water distribution network is a source of disquiet regarding the contamination of water during delivery and regrowth of microorganisms that survive after treatment [1]. It is often the scene of many physicochemical and biological reactions resulting from interactions between disinfectants, pipe walls, and the free and fixed biomass [2]. The presence of natural organic matter provides a food source for bacteria that can colonize the inner walls of distribution pipes, forming biofilms that protect and support the growth of microorganisms, some of which are associated to hostile effect on human health [1] and others through their interactions with disinfectants and pipe walls are sometimes the cause of the deterioration of the organoleptic properties of the water supply [2, 3].

In recent years, World Health Organization recognizes A. hydrophila as an opportunistic pathogen, implicated as a pathogenic agent in gastroenteritis, septicemia, cellulitis, colitis, meningitis, and respiratory infections [4–6]. To prevent bacterial regrowth, a residual of a disinfectant is maintained in the water distribution network. Previous work has shown that the bacterium A. hydrophila is widespread in the environment, especially in water intended for human consumption [7, 8]. Its concentration can sometimes reach 10² CFU/mL at the outlet of treatment plants for drinking water. This
concentration may be higher in networks of drinking water distribution due to the growth of *A. hydrophila* on biofilms [7, 9].

The ingestion of water or contaminated food is the common way of progress in the case of *Aeromonas* infection [10]. Numerous studies have been conducted in view of highlighting the inactivation of various waterborne pathogens by various disinfectants, including sodium hypochlorite, hydrogen peroxide, ozone, and chlorine dioxide [11].

The mixture of NaOCl and *H₂O₂* in water resulted in a redox reaction which gave the following equations [12]:

\[
H₂O₂/H₂O: 1.77 v \text{ and } ClO₂^-/ClO^2^- : 0.66 v
\]

\[
\text{ClO}^- + 2\text{HO}^- \rightarrow \text{ClO}_2^- + \text{H}_2\text{O} + 2e^-(1)
\]

\[
\text{H}_2\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O} (2)
\]

(1) and (2): \[
\text{ClO}^- + \text{H}_2\text{O}_2 + 2\text{HO}^- + 2\text{H}^+ \rightarrow \text{ClO}_2^- + 3\text{H}_2\text{O} (3)
\]

\[
\text{ClO}^- + \text{H}_2\text{O}_2 \rightarrow \text{ClO}_2^- + \text{H}_2\text{O} (4)
\]

\[
\text{Na}^+ + \text{ClO}^- + \text{H}_2\text{O}_2 \rightarrow \text{Na}^+ + \text{ClO}_2^- + \text{H}_2\text{O} (5)
\]

\[
\text{NaClO} + \text{H}_2\text{O}_2 \rightarrow \text{NaClO}_2 + \text{H}_2\text{O} (6)
\]

\[
(7)\]

\[
\text{NaClO} + \text{H}_2\text{O}_2 \rightarrow \text{NaCl} + \text{O}_2 + \text{H}_2\text{O} (8)
\]

The reaction between these disinfectants produces singlet oxygen (\(\text{O}_2\)), which is a powerful oxidant that rapidly kills bacterial cells. Singlet oxygen short lifespan (100 nanoseconds in lipid media and 50 nanoseconds in the cytoplasm) can diffuse a short distance and react with certain amino acids leading to structural and functional alteration of the membrane causing liperoxidation [13]. Less data are available on the bacterial behavior or bacterial metabolism when both disinfectants are dissolved in water at the same time. Less information are also available on the cell survival with respect to the both disinfectants concentrations.

Most studies carried out so far provided some information on the doses of disinfectants and adequate contact duration period to effectively control pathogens of public health importance that are commonly used to develop regulations and strategies treatment. Chemical disinfectants cause lethal or nonlethal changes in proteins [14], lipids [15], membrane [16], and DNA [17] of microorganisms. In addition, the mechanisms of disinfection are also highly dependent on the type of microorganism, cell growth stage, and disinfectant [18].

Other studies have considered the impact of disinfectants on *A. hydrophila* adhered to the fragments of polythene immersed in water. It appears that NaOCl is more effective on *A. hydrophila* adhered to polythene than *H₂O₂*. In addition, *A. hydrophila* adhered to polythene under dynamic condition is more sensitive to each of the two disinfectants than that adhered under static condition [18]. However, little data on the combined effect of these disinfectants are available. This study aims to evaluate in microcosm the synergistic effect of NaOCl and *H₂O₂* on *A. hydrophila* cells from different cell growth phases and adhered to fragments of polythene immersed in water.

2. Materials and Methods

2.1. Collection and Identification of *A. hydrophila*. The bacterium *A. hydrophila* was isolated from well water in Yaoundé (Cameroon) using membrane filtration technique, on ampicillin-dextrin agar medium [19, 20]. Cell subculture was performed on standard agar medium (Bio-Rad Laboratories, France). The cells were then identified using standard biochemical methods [21]. These cells are facultative anaerobic, nonsporulated, Gram-negative bacilli, and ferment mannitol, produce indole, and are mobile. They do not possess urease, lysine decarboxylase (LDC), ornithine decarboxylase (ODC), and arginine dihydrolase (ADH). For the preparation of stocks of bacteria, colonies are inoculated into 100 mL of nutrient broth (Oxford) for 24 hours at 37°C. Afterwards, cells were harvested by centrifugation at 8000 rpm for 10 min at 10°C and washed twice with NaCl (8.5 g/L) solution. The pellet was resuspended in NaCl (8.5 g/L) solution and then transferred to 300 μL tubes. The stocks were then frozen stored.

2.2. Assessment of Cell Growth Phase. On the basis of previous studies regarding the different growth phases and biofilm formation, the cell growth phases were assessed at 37°C. The growth of *A. hydrophila* in nonrenewed peptone liquid medium gives 4 growth phases: a lag growth phase from 0 to 2 hours, an exponential growth phase from 2 to 13 hours, a stationary growth phase from 13 to 22 hours, and a decline growth phase which begins as from the 22th hour [18].

2.3. Disinfectants and Adsorbent Substrates Used. The mixture of two disinfectants was used: NaOCl, which belongs to the group of halogen derivatives, and *H₂O₂*, which belongs to the group of oxidants. NaOCl and *H₂O₂* used are, respectively, Colgate-Palmolive (USA) and Gilbert (France) brand. The ease use of these two disinfectants in drinking water treatment justified their choice for this study. The combination concentrations of each disinfectant used ranged from 0.1‰ to 0.3‰ and from 0.5‰ to 1.5‰, for NaOCl and *H₂O₂*, respectively. These concentrations were evaluated by simple method of dilution of crude solution obtained directly from the supplier. The choice of these combination concentrations is justified by their synergistic action. To count the surviving bacteria after disinfection treatment, sterile NaCl solution (8.5 g/L) was used as a diluent.

The substrate used is high dense polythene. It differs from radical low dense polythene and linear low dense polythene by the molecular structure of its sparsely branched chains and its relatively high resistance to shocks, high temperatures, and ultraviolet rays [22, 23]. It is a plastic piping material
obtained directly from the supplier and used in drinking water distribution.

The high dense polythene is obtained by polymerization of the macromolecules of polyolefin family. This polymerization is obtained from gaseous ethylene according to the following equation [24, 25]:

$$n\text{H}_2\text{C}==\text{CH}_2 \xrightarrow{} \left( \begin{array}{c} \text{H} \\ \text{C} \\ \text{H} \\ \text{H} \end{array} \right)^n$$  \tag{9}

The polythene used in this study is commercialized by Goodfellow SARL (France).

2.4. Determination of Activity of Disinfectants Alone or in Combinations. The protocol described by Maris [26] with some modifications was applied. The principle of this protocol consists in preparation of the mixtures of NaOCl (A (B assoc)) and \( \text{H}_2\text{O}_2 \) (B (A assoc)). For it, nine couples of disinfectant concentrations (A (B assoc), B (A assoc)) were studied simultaneously for the preparation of mixtures of disinfectants. The disinfectant concentrations used alone ranged from 0.5‰ to 1.5‰ and from 5‰ to 15‰ for NaOCl (A alone) and \( \text{H}_2\text{O}_2 \) (B alone), respectively. The contaminated substrates are getting in contact with these disinfectant concentrations for 25 to 30 min. The disinfecting effect was stopped by introducing substrates in 10 mL of sterile saline. Antimicrobial activity was assessed after culture of surviving germs and appreciation of the reduction of the bacterial load.

The effect of the association was estimated by calculating the fractional bactericidal concentration (FBC) according to Maris [26]:

$$\text{FBC} = \frac{A \text{ (B assoc)}}{A \text{ (alone)}} + \frac{B \text{ (A assoc)}}{B \text{ (alone)}}$$  \tag{10}

wherein A (B assoc) and B (A assoc) are the respective concentrations of NaOCl and \( \text{H}_2\text{O}_2 \) studied in the mixture. A (alone) and B (alone) are the respective concentrations of the two disinfectants studied alone.

The synergy was then declared for a value of FBC less than or equal to 0.50. The study of this synergy was achieved at each stage of cell growth phase in stationary and dynamic regimes.

2.5. Adhesion Protocol of Cells to Polythene. On the basis of previous studies, parallelepiped shaped fragments of polythene with 13.28 cm² of total surface area suspended with wire of 0.1 mm diameter were immersed in triplicate in the two sets A and B each in four flasks 250 mL Duran A1, A1', and A1'' and B1, B1', and B1'' A2, A2', and A2'' and B2, B2', and B2'', A3, A3', and A3'' and B3, B3', and B3'', and A4, A4', and A4'' and B4, B4', and B4'' each containing 99 mL of NaCl solution (8.5 g/L). Meanwhile, the controls were made and coded A0, A0, 1, A0, 2, A0, 3, and A0, 4 and B0, 1, B0, 2, B0, 3, and B0, 4 [27]. The whole was then autoclaved.

Prior to the experiments, stocks frozen vial containing \( A. \text{ hydrophila} \) cells were thawed at room temperature. Then 100 \( \mu \text{L} \) of the culture was transferred into test tubes containing 10 mL of nutrient broth (Oxford) and incubated at 37°C for 24 hours. Cells from a specific cell growth phase were then harvested by centrifugation at 8000 rpm for 10 min at 10°C and washed twice with sterile NaCl solution (8.5 g/L). The pellets were then resuspended in 50 mL of sterilized NaCl solution (8.5 g/L). After serial dilutions, the initial concentration of bacteria (data at \( t = 0 \)) in each solution was adjusted to 6 \( \times 10^8 \) CFU/mL by reading the optical density at 600 nm using a spectrophotometer (DR 2800) followed by culture on agar [27].

1 mL of the suspension was added to 99 mL of sterilized NaCl solution (8.5 g/L) contained in an Erlenmeyer flask. Triplicate flasks were incubated under dynamic condition for 180, 360, 540, and 720 min at a stirring speed of 60 rev/min, using a stirrer (Rotatest brand). In the same way another triplicate flasks were incubated under static condition for 180, 360, 540, and 720 min. All these incubations were done at laboratory temperature (25 ± 1°C).

2.6. Disinfection Experiments. After each incubation duration, fragments of polythene were drained for 10 seconds in a sterile environment created by the Bunsen burner flame and then introduced into test tubes containing 10 mL of diluted mixture of disinfectant of various concentrations. Fragments removed from flasks A1, A2, A3, A4, B1, B2, B3, and B4 were introduced into mixture disinfected solutions of 0.1% NaOCl and 0.5% \( \text{H}_2\text{O}_2 \). Fragments removed from flasks A1', A2', A3', A4', B1', B2', B3', and B4' were introduced into mixture disinfectant solutions of 0.2% NaOCl and 1% \( \text{H}_2\text{O}_2 \). Similarly, those removed from flasks A1'', A2'', A3'', A4'', B1'', B2'', B3'', and B4'' were introduced into mixture solutions of 0.3% NaOCl and 1.5% \( \text{H}_2\text{O}_2 \). Fragments of polythene flasks from A0, 1, A0, 2, A0, 3, and A0, 4 and B0, 1, B0, 2, B0, 3, and B0, 4 were introduced into 10 mL of sterile NaCl solution (8.5 g/L). The concentration of the disinfectant has not been evaluated after incubation.

After 30 min of incubation at room temperature and under static condition, each fragment was then drained out under sterile condition. Each fragment was then introduced into 10 mL of sterilized NaCl solution (8.5 g/L). The unhooking of adherent cells was performed by vortex agitation at increasing speeds for 30 seconds in three consecutive series of 10 mL sterilized NaCl solution (8.5 g/L). This technique allows for the unhooking of maximum adhered cells [28, 29]. The total volume of the suspension containing the unhooked bacterial cells was 30 mL. The isolation and enumeration of unhooked cells were made by culture on ampicillin dextrin agar, by using spread plat method, followed by incubation on Petri plates at 37°C for 24 hours.

2.7. Data Analysis. The variation of the abundance of adhered \( A. \text{ hydrophila} \) in each experimental condition was illustrated by semilogarithmic diagrams. Standard deviations were not fitted because the curves were too close. Spearman “r” correlation Test was used to assess the degree of correlation between the abundance of adhered cells and other parameters considered. Kruskal-Wallis and Mann-Whitney tests were
used to compare the mean abundance of cells adhered from one experimental condition to another.

The data from absorption experiments were analyzed using the Freundlich isotherm model. This isotherm was chosen because of the number and the relevance of the information it provides on the real adsorption mechanisms on one hand and its remarkable ability to match doses of adsorption on the other hand. The Freundlich isotherm is described by the following equation [30, 31]:

$$C_s = K_f \cdot C^{-1/n}$$

where $C_s$ is the quantity of cells adsorbed in the presence of the mixture of disinfectant solutions, $C$ is the concentration of cells adsorbed in the absence of mixture of disinfectant solutions, $K_f$ is the Freundlich coefficient adsorption which is connected to the adsorption capacity, $1/n$ is coefficient linearity, and $n$ is the intensity of adsorption. Here, $C_s$ is expressed as the number of adherent cells/mixture of disinfectant concentration and $C$ is the number of adherent cells/cm² of polythene. Constructing linear regression log $C_s$ versus log $C$ results in a line of slope $1/n$ which intercepts the $y$-axis at $\log K_f$.

3. Results

3.1. Fractional Bactericidal Concentration (FBC). The FBC values were calculated using the formula indicated above. The different FBC obtained is given in Table 1. To ensure the synergistic action of the two disinfectants, only disinfectant concentrations giving FBC equal to 0.3 were used for the preparation of mixture of disinfectants.

3.2. Abundance of Cells Adhered to Polythene after Action of the Association of Disinfectants in Stationary Regime. The densities of cells adhered ranged from 0.30 to 2.29 units (log (CFU/cm²)) after the action of the mixture of NaOCl and H₂O₂ under static condition. The maximum abundance of cells adhered was recorded in the presence of the mixture of 0.1% NaOCl and 0.5% H₂O₂ and this is after 720 minutes with cells harvested from the lag growth phase. Adhered cells were always partially decimated by the mixture of NaOCl and H₂O₂ (Figure 1).

With cells coming from the lag phase, the abundance of cells adhered under static condition to the control substrate varied throughout from 2.02 to 3.19 units (log (CFU/cm²)) and was always superior to those of fragments tested for disinfection. In addition, they increase with the incubation duration. Maximum cell density was recorded after an adhesion test of 720 minutes. After the action of the mixture of NaOCl and H₂O₂, the densities of cells adhered ranged from 0.30 to 2.29 units (log (CFU/cm²)). The effectiveness of the mixture of NaOCl and H₂O₂ decreased with the length of the adhesion duration test. The maximum cell abundance was recorded in the presence of the mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ after an adhesion test of 720 minutes. The lowest density of adhered cells was observed in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ with cells coming from the adhesion tests of 180 minutes (Figure 1).

The abundance of cells under static condition adhered to the control substrate during the exponential growth phase was lower than that tested for disinfection in the lag growth phase under the same condition. They generally fluctuated between 2.30 and 2.91 units (log (CFU/cm²)). After disinfection test, it was noted that the effectiveness of the mixture of NaOCl and H₂O₂ decreased when the duration of adhesion test increased. Abundance of cells adhered ranged between 0.70 to 1.81 units (log (CFU/cm²)) (Figure 1). The highest cell abundance was recorded in presence of the mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ after an adhesion test of 720 minutes. The lowest density of adhered cells was observed in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ with cells coming from the adhesion tests of 180 minutes (Figure 1).

The stationary growth phase shows the abundance of cells in static regime adhered to the control substrate which varies from 1.92 to 2.49 units (log (CFU/cm²)). They remained higher than those of the fragments tested for disinfection. After disinfection test, abundance of cells adhered ranged between 0.90 and 1.89 units (log (CFU/cm²)). As the duration of adhesion test increased, it was noted that the effectiveness of the mixture of NaOCl and H₂O₂ decreased. The highest density of cells adhered to the polythene was recorded in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 720 minutes incubation duration. The lowest density of adhered cells was observed in the presence of mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 180 minutes incubation duration (Figure 1).

The abundance of cells adhered in static regime to the control substrate during the decline growth phase varied from 1.95 to 2.48 units (log (CFU/cm²)). Adhered cells after the action of NaOCl relatively increased (Figure 1). The maximum density of cells adhered to the polythene was recorded in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 720 minutes incubation duration. The minimum density of adhered cells was observed in the presence of mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 180 minutes incubation duration (Figure 1).

### Table 1: Value of fractional bactericidal concentration (FBC) obtained for each couple of disinfectants concentrations.

| Disinfectants in mixture | Disinfectants alone | FBC |
|--------------------------|---------------------|-----|
| NaOCl (%) | H₂O₂ (%) | NaOCl (%) | H₂O₂ (%) | |
| 0.1 | 0.5 | 0.5 | 5 | 0.3 |
| 0.2 | 1 | 1 | 10 | 0.3 |
| 0.3 | 1.5 | 1.5 | 15 | 0.3 |
| 0.1 | 2 | 0.5 | 5 | 0.6 |
| 0.2 | 3 | 1 | 10 | 0.5 |
| 0.3 | 4 | 1.5 | 15 | 0.46 |
| 0.25 | 5 | 0.5 | 5 | 1.5 |
| 0.5 | 6 | 1 | 10 | 1.1 |
| 0.75 | 8 | 1.5 | 15 | 1.03 |
Figure 1: Temporal evolution of cells adhered under static condition after the action of NaOCl and H$_2$O$_2$ alone and in the mixture of the two disinfectants at different concentrations.
3.3. Abundance of Cells Adhered to Polythene after Action of Association of Disinfectants in Dynamic Regime. The abundance of cells adhered ranged from 0.85 to 2.27 units (log (CFU/cm²)) after the action of the mixture of NaOCl and H₂O₂ under dynamic condition. The maximum abundance of cells adhered was recorded in the presence of mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ and this is after 720 minutes with cells harvested from the lag growth phase.

The density of cells adhered under dynamic condition to the control substrate varied throughout from 2.35 to 3.25 units (log (CFU/cm²)) from the lag phase and was always superior to those fragments tested for disinfection. In addition, they increase with the incubation duration. The maximum cell abundance was recorded in the presence of the mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ after an adhesion test of 720 minutes. The lowest density of adhered cells was observed in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ with cells coming from the adhesion tests of 180 minutes (Figure 2). After action of the mixture of NaOCl and H₂O₂, the densities of cells adhered ranged from 0.85 to 2.27 units (log (CFU/cm²)). The effectiveness of the mixture of NaOCl and H₂O₂ decreased with the length of the adhesion test duration.

Abundance of cells adhered under dynamic condition to control substrate during the exponential growth phase was lower than that tested for disinfection in the lag growth phase under the same condition. They generally fluctuated between 2.47 and 3.19 units (log (CFU/cm²)). After disinfection test, it was noted that the effectiveness of the mixture of NaOCl and H₂O₂ decreased when the duration of adhesion test increased. Abundance of cells adhered ranged between 0.95 and 2.09 units (log (CFU/cm²)) (Figure 2). The maximum cell abundance was recorded in presence of mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ after an adhesion test of 720 minutes. The minimum density of adhered cells was observed in the presence of mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ with cells coming from the adhesion tests of 180 minutes (Figure 2).

The abundance of cells adhered in dynamic regime to the control substrate varied from 2.35 to 2.74 units (log (CFU/cm²)) during the stationary growth phase. It remained higher than those of fragments tested for disinfection. After disinfection test, abundance of cells adhered ranged between 1.30 and 2.13 units (log (CFU/cm²)). As the duration of adhesion test increased, it was noted that the effectiveness of the mixture of NaOCl and H₂O₂ decreased. The maximum density of cells adhered to the polythene was recorded in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 720 minutes incubation duration, whereas the minimum density was observed in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 180 minutes incubation duration (Figure 2).

Density of cells adhered in dynamic condition to the control substrate during the decline growth phase varied from 2.10 to 2.71 units (log (CFU/cm²)). Cells adhered after the action of NaOCl were relatively high (Figure 2). The maximum density of cells adhered to the polythene was recorded in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 720 minutes incubation duration and the minimum in the presence of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ after 180 minutes incubation (Figure 2).

3.4. Freundlich Isotherms of Cells Adsorption. Freundlich isotherms were constructed by considering only the combination concentrations, the number of cells adhered to the substrate, subjected to the test of disinfection, and obtained without exposure to the mixture of disinfectants for each stage of cell growth and each experimental condition. The Freundlich isotherms are shown in Figure 3. It can be noted that, no matter which growth stage cells are, the appearance of the isotherms differs from one incubation condition to another. The linearity coefficient l/n which is related to the adsorption intensity ranged from 0.01 to 0.21 and from 0.02 to 0.15, respectively, under static and dynamic incubation conditions. The adsorption coefficient K_f which is related to the adsorption capacity ranged between 2 and 53 and between 2 and 54 cells adhered, respectively, under static and dynamic incubation conditions. The adsorption coefficient for the lag growth phase ranged between 4 and 53 and between 2 and 54 cells adhered, respectively, under static and dynamic conditions (Table 2). The lowest adsorption coefficient after the mixture of disinfectant treatment was obtained with cell harvested from the lag growth phase (Table 2).

When considering each experimental condition, the adsorption coefficient of cells harvested from the lag phase was relatively higher after the mixture of disinfectant treatment than that of cell harvested from the other cells growth phases (Table 2). It was also noted that for the whole cell growth phases and the whole incubation conditions, the adsorption coefficient values were relatively higher with the mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ concentration than those of the two other mixture of disinfectant concentrations (Table 2).

3.5. Correlation Coefficients between the Abundance of Cells Adhered and Incubation Durations and Concentrations of Disinfectants. Spearman “r” correlation coefficients between the abundances of cells adhered and incubation durations for each concentration of mixture of disinfectant and each experimental condition were assessed and are presented in Table 3. It is noted that the increase in the incubation durations caused a significant decrease in the efficiency of 0.3‰ NaOCl and 0.3‰ H₂O₂ mixture of disinfectant concentration (P < 0.01). This could result in higher abundance of cells adhered as the duration of the cell adhesion process increased.

Spearman “r” correlation coefficients between abundance of cells adhered and concentrations of the mixture disinfectants for each incubation duration and under each experimental condition were also assessed (Table 4). Under static as well as dynamic condition, it was noted that the effectiveness of the mixture of disinfectant concentrations on cells adhered to polythene increased leading to a significant decrease (P < 0.01) in the abundance of bacteria adhered after disinfection treatment.

The degrees of relationship between the mixture of disinfectant concentrations and abundance of cells adhered
Figure 2: Temporal evolution of cells adhered under dynamic condition after the action of NaOCl and H$_2$O$_2$ alone and in the mixture of the two disinfectants at different concentrations.
Figure 3: Freundlich isotherms for cells absorption under static (A1, B1, C1, and D1) and dynamic (A2, B2, C2, and D2) conditions in the presence of the mixture of NaOCl and H$_2$O$_2$ (lag growth phase (A1, A2), exponential growth phase (B1, B2), stationary growth phase (C1, C2), and decline growth phase (D1, D2)).
The aim of this study was to determine the synergistic effect of NaOCl and H$_2$O$_2$ on A. hydrophila adhered to polythene immersed in water under static and dynamic conditions. By contrast, most previous studies have indicated only the effect of NaOCl on one hand and that of H$_2$O$_2$ on the other hand on the adhesion of A. hydrophila to polythene [18, 32, 33]. From the 9 pairs of concentration of disinfectants used for the preparation of mixture of disinfectants, three couples (0.1‰ NaOCl + 0.5‰ H$_2$O$_2$; 0.2‰ NaOCl + 1‰ H$_2$O$_2$; and 0.3‰ NaOCl + 1.5‰ H$_2$O$_2$) were used to evaluate the synergies as they presented an FBC equal to 0.3. A synergy is declared when a value of FBC is less than or equal to 0.50 [26].

The present study showed that the overall abundance of cells adhered to polythene after the action of the mixture of two disinfectants was lower than that obtained after the action of H$_2$O$_2$ alone. Abundance of cells adhered to polythene ranged from 0.30 to 2.29 and 0.85 to 2.27 units (log (CFU/cm$^2$)) after the action of the mixture of NaOCl and H$_2$O$_2$ under static and dynamic conditions, respectively. Previous studies showed that they sometimes reached 2.41 and 3.39 units (log (CFU/cm$^2$)) after the action of NaOCl and H$_2$O$_2$, respectively [18]. These results suggest that the combination of NaOCl and H$_2$O$_2$ leads to a significant synergy in eliminating cells adhered to polythene. This has been also suggested in previous studies [34].

Abundance of cells adhered to polythene after the action of the mixture of NaOCl and H$_2$O$_2$ was relatively higher than those obtained after the action of NaOCl alone.

### 4. Discussion

The overall abundance of cells adhered to polythene after the action of the mixture of NaOCl and H$_2$O$_2$ was lower than that obtained after the action of H$_2$O$_2$ alone. Abundance of cells adhered to polythene ranged from 0.30 to 2.29 and 0.85 to 2.27 units (log (CFU/cm$^2$)) after the action of the mixture of NaOCl and H$_2$O$_2$ under static and dynamic conditions, respectively. Previous studies showed that they sometimes reached 2.41 and 3.39 units (log (CFU/cm$^2$)) after the action of NaOCl and H$_2$O$_2$, respectively [18]. These results suggest that the combination of NaOCl and H$_2$O$_2$ leads to a significant synergy in eliminating cells adhered to polythene. This has been also suggested in previous studies [34].

Abundance of cells adhered to polythene after the action of the mixture of NaOCl and H$_2$O$_2$ was relatively higher than those obtained after the action of NaOCl alone.

### Table 2: Values of adsorption coefficient ($K_J$) (adhered A. hydrophila/mL of mixture of disinfectant) and linearity coefficient ($l/n$) of isotherms under static and dynamic conditions, when using different disinfectants concentrations.

| Disinfectant concentrations and static or dynamic condition | Freundlich isotherm coefficients according to the cell growth phase | Adsorption coefficient (cells adhered/cm$^2$) | Linearity coefficient |
|------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------|----------------------|
| Disinfectant concentrations                               | Condition | Adsorption coefficient (cells adhered/cm$^2$) | Linearity coefficient |
| 0.1‰ NaOCl + 0.5‰ H$_2$O$_2$                               | Static    | 53 | 15 | 18 | 27 | 0.09 | 0.06 | 0.21 | 0.14 |
| Dynamic                                                   | 54        | 41 | 7  | 44 | 0.07 | 0.05 | 0.02 | 0.09 |
| 0.2‰ NaOCl + 1‰ H$_2$O$_2$                                | Static    | 16 | 5  | 2  | 8   | 0.04 | 0.03 | 0.20 | 0.10 |
| Dynamic                                                   | 3         | 20 | 10 | 7  | 0.05 | 0.04 | 0.15 | 0.11 |
| 0.3‰ NaOCl + 1.5‰ H$_2$O$_2$                              | Static    | 4  | 3  | 9  | 5   | 0.01 | 0.01 | 0.19 | 0.06 |
| Dynamic                                                   | 2         | 4  | 5  | 7  | 0.05 | 0.02 | 0.10 | 0.04 |

### Table 3: Spearman $r$ correlation coefficients between the abundances of adhered A. hydrophila and incubation durations for each concentration of mixture of disinfectant and each experimental condition.

| Experimental condition | Mixtures of disinfectant concentrations |
|------------------------|----------------------------------------|
| 0.1‰ NaOCl + 0.5‰ H$_2$O$_2$ | 0.2‰ NaOCl + 1‰ H$_2$O$_2$ | 0.3‰ NaOCl + 0.3‰ H$_2$O$_2$ |
| Static                 | Mixed 0.800                           | Mixed -0.200                    | Mixed -0.400**        |
| Dynamic                | Mixed 0.400                           | Mixed 0.632                     | Mixed -0.949**        |

**$p < 0.01$; ddf = 15.**

### Table 4: Spearman $r$ correlation coefficients between the abundance of adhered A. hydrophila and concentration of mixture of disinfectant for each incubation duration and under each experimental condition.

| Experimental condition | Incubation durations 180 min 360 min 540 min 720 min |
|------------------------|-------------------------------------------------------|
| 0.1‰ NaOCl + 0.5‰ H$_2$O$_2$ | Mixed 1.000** 1.000** 1.000** 1.000** |
| Static                 | Mixed 1.000** 1.000** 1.000** 1.000** |
| Dynamic                | Mixed 1.000** 1.000** 1.000** 1.000** |

**$p < 0.01$; ddf = 15.**

3.6. Comparison of the Mean Abundance of Cells Adhered amongst the Different Stages of Cell Growth. The H test of Kruskal-Wallis was performed in order to compare the mean abundance of cells adhered harvested from different cell growth stages and considering each mixture of disinfectants concentrations. It showed that there is an overall significant difference ($P < 0.05$) between the mean abundance of cells adhered to polythene for each mixture of disinfectant concentration at different cell growth stages. The pair two-by-two comparisons of the mean abundances were then performed using the U test of Mann-Whitney. It was noted that, at each cell growth stage, there was a significant difference ($P < 0.05$) amongst the mean abundance of cells adhered after the action of various mixture of disinfectant concentrations with cells coming from each cell growth phase. With the mixture of 0.1‰ NaOCl and 0.5‰ H$_2$O$_2$ and that of 0.3‰ NaOCl and 1.5‰ H$_2$O$_2$, a nonsignificant difference was observed only with cells harvested from the stationary cell growth phase ($P > 0.05$) (Table 6).

### Table 5: Values of adsorption coefficient ($K_J$) and linearity coefficient ($l/n$) of isotherms under static and dynamic conditions, when using different disinfectants concentrations.

| Disinfectant concentrations and static or dynamic condition | Freundlich isotherm coefficients according to the cell growth phase | Adsorption coefficient (cells adhered/cm$^2$) | Linearity coefficient |
|------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------|----------------------|
| Disinfectant concentrations                               | Condition | Adsorption coefficient (cells adhered/cm$^2$) | Linearity coefficient |
| 0.1‰ NaOCl + 0.5‰ H$_2$O$_2$                               | Static    | 53 | 15 | 18 | 27 | 0.09 | 0.06 | 0.21 | 0.14 |
| Dynamic                                                   | 54        | 41 | 7  | 44 | 0.07 | 0.05 | 0.02 | 0.09 |
| 0.2‰ NaOCl + 1‰ H$_2$O$_2$                                | Static    | 16 | 5  | 2  | 8   | 0.04 | 0.03 | 0.20 | 0.10 |
| Dynamic                                                   | 3         | 20 | 10 | 7  | 0.05 | 0.04 | 0.15 | 0.11 |
| 0.3‰ NaOCl + 1.5‰ H$_2$O$_2$                              | Static    | 4  | 3  | 9  | 5   | 0.01 | 0.01 | 0.19 | 0.06 |
| Dynamic                                                   | 2         | 4  | 5  | 7  | 0.05 | 0.02 | 0.10 | 0.04 |

**$p < 0.01$; ddf = 15.**
The maximum abundance of cells adhered to polythene was recorded under static condition in the presence of the mixture of 0.1‰ NaOCl and 0.5‰ H₂O₂ and this is after 720 minutes with cells obtained in the lag growth phase (Figures 1 and 2). That obtained after the action of NaOCl was recorded during the lag phase under dynamic condition in the presence of 0.5‰ concentrations of NaOCl and this is after an adhesion test of 720 minutes. By cons, the abundance of cells adhered to polythene after the action of the mixture of NaOCl and H₂O₂ was considerably lower than those obtained after the action of H₂O₂.

The maximum abundance of cells adhered after the action of H₂O₂ was recorded during the stationary growth phase under static condition in the presence of 5‰ H₂O₂ concentration after the same period of adhesion test. Due to its highly oxidizing capacity-based production of free radicals that affect the biofilms matrix H₂O₂ was chosen to fight effectively against biofilms formation [35, 36]. In addition, H₂O₂ was chosen as it is highly effective disinfectant in inhibiting biofilms formation at a concentration of 0.05‰. It can also destroy mature biofilms at concentrations between 0.08‰ and 0.2‰ [37]. The reaction between NaOCl and H₂O₂ produces singlet oxygen (¹O₂), which is a powerful oxidant that rapidly kills bacterial cells. In addition, oxygen singlet short lifespan (100 nanoseconds in lipid media and 50 nanoseconds in the cytoplasm) can diffuse a short distance and react with certain amino acids leading to structural and functional alteration of the membrane causing lipoperoxidation [13]. NaOCl and H₂O₂ inhibit the Brownian motion and control the growth of the microbial population [34].

The adhesion of microorganisms to surfaces is the first step in biofilms formation, which is a form of microbial life in aquatic environments [38]. The latter is the source of problems bioburden in various fields such as health, environment, food industry, and water purification [31, 39, 40]. Adhesion is governed by physicochemical interactions of the Van Der Waals and Lewis acid-base types. Fluctuating velocities of adhesion of cells observed during different stages of growth in stationary and dynamic regimes could be explained by changes in the physiology of bacterium at each stage of growth [41, 42]. There are three strategies against biofilms formation: (i) the disinfection time before the biofilms develop, (ii) the disinfection of biofilms using aggressive disinfectants, and (iii) inhibition fixing microbes choosing surface materials that do not promote adherence [43].

By considering separately each condition, it was noted that the increase in incubation durations resulted in a significant decrease (P < 0.01) in the effectiveness of the mixture of 0.3‰ NaOCl and 1.5‰ H₂O₂ (Table 3). This resulted in higher abundance of cells. Indeed, a biofilm can be developed within in a few hours, allowing bacteria therein to become resistant to external agents causing any contamination [44, 45]. In static as well as dynamic condition, increasing the effectiveness of the mixture concentration of NaOCl and H₂O₂ on cells adhered to polythene resulted in a significant decrease in abundance of cells adhered after disinfection test (P < 0.01) (Figures 1 and 2). The treatment of biofilms by combining antimicrobial agents has a synergistic effect on the removal of adherent bacterial cells [34]. Furthermore, this variation of the reaction of cells against the combination of disinfectants may be related to changes in the surface due to a change in their growth phase [46].

It was also noted that for each incubation period and each cell growth phase, a rise in the concentration of disinfectant mixture increases significantly (P < 0.01) the abundance of cells adhered to the substrate (Table 4). Face with antimicrobial agent bacteria develops biofilm formation as a coping
strategy [47, 48]. For each cell growth phase, a significant difference was observed between the mean densities of cells adhered after the action of the different concentrations of the mixture of disinfectants (P < 0.05). The effectiveness of any method of disinfection depends on biotic factors such as the physiological state and the intrinsic microbial resistance to lethal agents [49]. The age of the culture also plays an important role since the adhesion of the bacterium is better during exponential growth phase than stationary growth phase [50].

It is important to remember that bacteria in a biofilm have very different characteristics from their planktonic counterparts including the production of exopolymers [51], a significant increase in antimicrobial resistance and environmental stress [52, 53]. The matrix of exopolymers which presents itself as a mechanical barrier, reducing the penetration of environmental compounds through the biofilms, thus protects bacterial cells embedded in biofilm. This explains the fact that the increase in the concentration of the mixture of disinfectants for each stage of growth leads to a significant increase (P < 0.01) in abundance of cells adhered to the substrates. The adsorption coefficient (Kf) was relatively higher in the static than in the dynamic regime. Thus, the number of cells adhered under static condition were significantly lower than that obtained after the action of H2O2 alone. By contrast, it is significantly lower than that obtained after the action of H2O2 alone. Under static as well as dynamic condition, an increase in the effectiveness of the concentrations of the mixture of NaOCl and H2O2 on cells adhered is noted. For each cell growth phase, the densities of cells adhered differed from a given concentration of a mixture of disinfectants to another. Although the adsorption coefficient (Kf) obtained from the Freundlich isotherm is relatively higher in static state than in dynamic regime, cells adhered to polythene in the presence of the mixture of the two disinfectants under dynamic condition seem more sensitive than under static condition.

5. Conclusion

This study showed that the combination of NaOCl and H2O2 has a synergistic effect on cells adhered to polythene. Abundance of cells adhered to polythene after the action of the mixture of NaOCl and H2O2 is relatively higher than that obtained after the action of NaOCl alone. By cons, it is significantly lower than that obtained after the action of H2O2 alone. Under static as well as dynamic condition, an increase in the effectiveness of the concentrations of the mixture of NaOCl and H2O2 on cells adhered is noted. For each cell growth phase, the densities of cells adhered differed from a given concentration of a mixture of disinfectants to another. Although the adsorption coefficient (Kf) obtained from the Freundlich isotherm is relatively higher in static state than in dynamic regime, cells adhered to polythene in the presence of the mixture of the two disinfectants under dynamic condition seem more sensitive than under static condition.

Conflict of Interests

The authors declare that they have no conflict of interests that could inappropriately influence this work.

References

[1] Comité fédéral-provincial-territorial sur l’eau potable (Canada), “Conseils sur les bactéries pathogènes d’origine hydrique,” 2012, http://www.hc-sc.gc.ca/ewh-semt/alt_formats/pdf/consult/.
[2] P. Mouchet, A. Montiel, and S. Rigal, “Dégradations physico-chimiques de l’eau dans les réseaux de distribution,” TSM. L’Eau, vol. 36, pp. 399–406, 1992.
[3] D. Schoenen, “Role of disinfection in suppressing the spread of pathogens with drinking water: possibilities and limitations,” Water Research, vol. 35, no. 15, pp. 3861–3868, 2002.
[4] K. Krovacek, A. Faris, S. Baloda, T. Lindberg, M. Peterz, and I. Månsson, “Isolation and virulence profiles of Aeromonas spp. from different municipal drinking water supplies in Sweden,” Food Microbiology, vol. 5, no. 3, pp. 215–222, 1992.
[5] A. A. Gabrielson, J. B. P. Landre, and A. K. Lamb, “Incidence of mesophilic Aeromonas within a public drinking water supply in North-East Scotland,” Journal of Applied Microbiology, vol. 84, no. 3, pp. 383–392, 1998.
[6] J. Michael Janda and S. L. Abbott, “Evolving concepts regarding the genus Aeromonas: an expanding panorama of species, disease presentations, and unanswered questions,” Clinical Infectious Diseases, vol. 27, no. 2, pp. 332–344, 1998.
[7] C. Chauret, C. Volk, R. Creason, J. Jarosh, J. Robinson, and C. Warnes, “Detection of Aeromonas hydrophila in a drinking-water distribution system: a field and pilot study,” Canadian Journal of Microbiology, vol. 47, no. 8, pp. 782–786, 2001.
[8] G. E. El-Taweel and A. M. Shaban, “Microbiological quality of drinking water at eight water treatment plants,” International Journal of Environmental Health Research, vol. 11, no. 4, pp. 285–290, 2001.
[9] P. Paymet, E. Franco, and J. Siemiatycki, “Absence of relationship between health effects due to tap water consumption drinking water quality parameters,” Water Science and Technology, vol. 27, no. 3–4, pp. 137–143, 1993.
[10] R. H. W. Schubert, “Aeromonads and their significance as potential pathogens in water,” Journal of Applied Bacteriology, vol. 70, supplement, pp. 131S–135S, 1991.
[11] M. Cho, J. Kim, J. Yoon, and J.-H. Kim, “Mechanisms of Escherichia coli inactivation by several disinfectants,” Water Research, vol. 44, no. 11, pp. 3410–3418, 2010.
[12] S. Rondinini and A. Vertova, “Electroreduction of halogenated organic compounds,” in Electrochemistry For the Environment, pp. 279–306, 2010.
[13] T. Karu, L. Pyatibrat, and G. Kalendo, “Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro,” Journal of Photochemistry and Photobiology B, vol. 27, no. 3, pp. 219–223, 1995.
[14] O. J. Sproul, R. M. Pfister, and C. K. Kim, “The mechanism of ozone inactivation of water borne viruses,” Water Science and Technology, vol. 14, no. 4–5, pp. 303–314, 1982.
[15] P.-C. Maness, S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum, and W. A. Jacoby, “Bactericidal activity of photocatalytic TiO2 reaction: toward an understanding of its killing mechanism,” Applied and Environmental Microbiology, vol. 65, no. 9, pp. 4094–4098, 1999.
[16] S. B. Young and P. Setlow, “Mechanisms of killing of Bacillus subtilis spores by hypochlorite and chlorine dioxide,” Journal of Applied Microbiology, vol. 95, no. 1, pp. 54–67, 2003.
[17] K. Oguma, H. Katayama, H. Mitani, S. Morita, T. Hirata, and S. Ohgaki, “Determination of pyrimidine dimers in Escherichia
coli and Cryptosporidium parvum during UV light inactivation, photoreactivation, and dark repair,” Applied and Environmental Microbiology, vol. 67, no. 10, pp. 4630–4637, 2001.

[18] C. Lontsi Djimel, M. Nola, A. Tamsa Arfo et al., “Effect of disinfectants on adhered Aeromonas hydrophila to polythene immersed in water under static and dynamic conditions,” International Journal of Research in BioSciences, vol. 2, pp. 33–48, 2013.

[19] N. Marchal, J. L. Bourdon, and C. Richard, Culture Media For Isolation and Biochemical Identification of Bacteria, Doin, Paris, France, 1991.

[20] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, USA, 21st edition, 2005.

[21] G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams, Bergey’s Manual of Determinative Bacteriology, Lipponcott Williams and Wilkins, Philadelphia, Pa, USA, 9th edition, 2000.

[22] K. L. Coeyrehourcq, Etude de méthodes rapides d’analyse de la structure moléculaire du polyéthylène [Thèse de Docteurat], Ecole des Mines de Paris Spécialité Science et Génie des Matériaux, 2003.

[23] N. Boutaleb, Etude de la formation de biofilms sur les matériaux couramment utilisés dans les canalisations d’eaux potables [Thèse de Doctorat], Université de Bretagne-sud, 2007.

[24] B. D. Ratner, “Plasmadeposition of organic thin film-control of film chemistry,” Polymer Preprints, vol. 34, pp. 643–644, 1993.

[25] B. D. Ratner, “Surface modification of polymers: chemical, biological and surface analytical challenges,” Biosensors and Bioelectronics, vol. 10, no. 9-10, pp. 797–804, 1995.

[26] P. Maris, “Modes of action of disinfectants,” in Disinfectants: Actions and Applications, H. A. McDaniel, Ed., pp. 47–55, 1995.

[27] O. V. Noah Ewoti, M. Nola, L. M. Moungang, M. E. Nougang, F. Krier, and N. E. Chihib, “Adhesion of Escherichia coli and Pseudomonas aeruginosa on rock surface in aquatic microcosm: assessment of the influence of dissolved magnesium sulfate and monosodium phosphate,” Research Journal of Environmental and Earth Sciences, vol. 3, no. 4, pp. 364–374, 2011.

[28] S. Dukam, P. Pirion, and Y. Levi, “Modélisation du développement des biomasses bactériennes libres et fixées en réseau de distribution d’eau potable,” in Adhésion des Microorganismes aux Surfaces, M. N. Bellon-Fontaine and J. Fourniat, Eds., pp. 149–160, 1995.

[29] O. V. Noah Ewoti, Réétion des bactéries dans le sol et sur des fragments de roches en milieu aquatique : influence du type de cellule et de quelques paramètres chimiques de l’environnement [Thèse], Université de Yaoundé I, 2012.

[30] M. J. Miller, M. M. Critchley, J. Hutson, and H. J. Fallowfield, “The adsorption of cyanobacterial hepatotoxins from water onto soil during batch experiments,” Water Research, vol. 35, no. 6, pp. 1461–1468, 2001.

[31] I.-W. Wang, J. M. Anderson, M. R. Jacobs, and R. E. Marchant, “Adhesion of Staphylococcus epidermidis to biomedical polymers: contributions of surface thermodynamics and hemodynamic shear conditions,” Journal of Biomedical Materials Research, vol. 29, no. 4, pp. 485–493, 1995.

[32] V. Singamaneni, G. Madiraju, and H. Sura, “In vitro effectiveness of different endodontic irrigants on the reduction of Enterococcus faecalis in root canals,” Clinical and Experimental Dentistry, vol. 2, no. 4, pp. 169–172, 2010.

[33] K. Tóth, T. Horemans, D. Vanden Berghe, L. Maes, and P. Cos, “Inhibitory effect of biocides on the viable masses and matrices of Staphylococcus aureus and Pseudomonas aeruginosa biofilms,” Applied and Environmental Microbiology, vol. 76, no. 10, pp. 3135–3142, 2010.

[34] J.-H. Ha, S.-H. Jeong, and S.-D. Ha, “Synergistic effects of combined disinfection using sanitizers and uv to reduce the levels of Staphylococcus aureus in oyster mushrooms,” Journal of Applied Biological Chemistry, vol. 54, no. 3, pp. 447–453, 2011.

[35] C. C. C. R. de Carvalho, “Biofilms: recent developments on an old battle,” Recent patents on biotechnology, vol. 1, no. 1, pp. 49–57, 2007.

[36] C. C. C. R. De Carvalho and M. M. R. Da Fonseca, “Assessment of three-dimensional biofilm structure using an optical microscope,” BioTechniques, vol. 42, no. 5, pp. 616–620, 2007.

[37] M. N. N. Shikongo-Nambabi, B. Kachigunda, and S. N. Venter, “Evaluation of oxidising disinfectants to control Vibrio biofilms in treated seawater used for fish processing,” Water SA, vol. 36, no. 3, pp. 215–220, 2010.

[38] R. M. Donlan, “Biofilms: microbial life on surfaces,” Emerging Infections Diseases, vol. 8, no. 9, pp. 881–890, 2002.

[39] N. Y. Jayasekara, G. M. Heard, J. M. Cox, and G. H. Fleet, “Association of micro-organisms with the inner surfaces of bottles of non-carbonated mineral waters,” Food Microbiology, vol. 16, no. 2, pp. 115–128, 1999.

[40] B. A. Jucker, H. Harms, and A. J. B. Zehnder, “Adhesion of the positively charged bacterium Stenotrophomonas (Xanthomonas) maltophilia 70401 to glass and teflon,” Journal of Bacteriology, vol. 178, no. 18, pp. 5472–5479, 1996.

[41] G. A. O’Toole and R. Kolter, “Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development,” Molecular Microbiology, vol. 30, no. 2, pp. 295–304, 1998.

[42] S. Parot, Electroactifs: formation, caractérisation et mécanismes [Thèse], Institut National polytechnique de Toulouse, 2007.

[43] B. Meyer, “Approches to prevention, removal and killing of biofilms,” International Biodeterioration and Biodegradation, vol. 51, no. 4, pp. 249–253, 2003.

[44] I. B. Beech and C. L. M. Coutinho, “Biofilms on corroding materials,” in Biofilms in Medicine, P. Lens, A. P. Moran, T. Mahony, P. Stoodley, and V. O’Flaherty, Eds., 2003.

[45] I. B. Beech and J. Sunner, “Biocorrosion: towards understanding interactions between biofilms and metals,” Current Opinion in Biotechnology, vol. 15, no. 3, pp. 181–186, 2004.

[46] R. Briandet, Maitrise de l’hygiène des surfaces par la création des biofilms-Aspects physico-chimiques [Thèse de Doctorat], Ecole Nationale Supérieure Agronomique de Rennes, Rennes, France, 1999.

[47] S. Stepanović, I. Ćirkićović, V. Mijač, and M. Švabić-Vlahović, “Influence of the incubation temperature, atmosphere and dynamic conditions on biofilm formation by Salmonella spp,” Food Microbiology, vol. 20, no. 3, pp. 339–343, 2003.

[48] S. Stepanović, I. Ćirkićović, L. Ranin, and M. Švabić-Vlahović, “Biofilm formation by Salmonella spp and Listeria monocytogenes on plastic surface,” Letters in Applied Microbiology, vol. 38, no. 5, pp. 428–432, 2004.

[49] R. Patel, “Biofilms and antimicrobial resistance,” Clinical Orthopaedics and Related Research, no. 437, pp. 41–47, 2005.

[50] P. M. Stanley, “Factors affecting the irreversible attachment of Pseudomonas aeruginosa to stainless steel,” Canadian Journal of Microbiology, vol. 29, no. 11, pp. 1493–1499, 1983.
[51] M. R. Parsek and E. P. Greenberg, “Acyl-homoserine lactone quorum sensing in Gram-negative bacteria: a signaling mechanism involved in associations with higher organisms,” Proceedings of the National Academy of Sciences of the United States of America, vol. 97, no. 16, pp. 8789–8793, 2000.

[52] T.-F. C. Mah and G. A. O’Toole, “Mechanisms of biofilm resistance to antimicrobial agents,” Trends in Microbiology, vol. 9, no. 1, pp. 34–39, 2001.

[53] C. Campanac, L. Pineau, A. Payard, G. Baziard-Mouysset, and C. Roques, “Interactions between biocide cationic agents and bacterial biofilms,” Antimicrobial Agents and Chemotherapy, vol. 46, no. 5, pp. 1469–1474, 2002.

[54] M. Klausen, M. Gjermansen, J.-U. Kreft, and T. Tolker-Nielsen, “Dynamics of development and dispersal in sessile microbrial communities: examples from Pseudomonas aeruginosa and Pseudomonas putida model biofilms,” FEMS Microbiology Letters, vol. 261, no. 1, pp. 1–11, 2006.

[55] D. Büttner and U. Bonas, “Getting across: Bacterial type III effector proteins on their way to the plant cell,” The EMBO Journal, vol. 21, no. 20, pp. 5313–5322, 2002.
