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Research Article

Evaluation of the Desinfectant Activity of Sodium Percarbonate Against *Enterococcus faecalis* Bacteria in Aquatic Microcosm and Role of Some Abiotic Variables

Mouhama Sani Adams Ibn Rabiou[1], Nougang Mireille Ebiane[2], Tamatcho Kweyang Blandine Pulcherie[3], Poutoum Yogne Yves[1], Moungang Luciane Marlyse[1, *], Nola Moïse[1]

[1]Department of Animal Biology and Physiology, University of Yaounde I, Yaounde, Cameroon

[2]Department of Biological Sciences, University of Maroua, Maroua, Cameroon

[3]Department of Microbiology, University of Yaounde I, Yaounde, Cameroon

Abstract

Water pollution can lead to alterations in its physicochemical and microbiological quality (presence of microorganisms). These microorganisms can cause numerous waterborne diseases in humans, hence the need for biological water treatment. Effective water disinfection should indeed take into account biotic and abiotic factors that influence the target microbial species and the disinfection process itself. This study therefore aims to investigate the influence of several abiotic factors on the disinfectant activity of sodium percarbonate in an aquatic microcosm. The study focused on the bacterium *Enterococcus faecalis* because it exhibits antibiotic resistance and is commonly used as an indicator of fecal contamination to assess the hygienic quality of environmental samples. It was isolated using standard techniques from surface waters (Olézoa stream) in the city of Yaounde-Cameroon, using Bile-Esculin-Azide (BEA) agar. The disinfectant used in this study was sodium percarbonate. It was chosen due to its widespread use. Furthermore, it is an environmentally friendly alternative to bleach because it is safe for humans and the environment and also combats bacteria and molds. The biodegradable organic matter used to vary the trophic level of the medium was glucose ($C_6H_{12}O_6$). This was chosen because it is an important nutrient for bacterial metabolism. The chemicals abiotic water parameters considered (pH, electrical conductivity, and dissolved oxygen) were measured using a HANNA pH meter and a HANNA multimeter, respectively. The bacteriological analyses focused on quantitative aspects, and the results were expressed as Colony Forming Units per unit volume of water sample (CFU/21 mL), then converted to CFU/100 mL. The effect of the disinfectant on the microorganism and on some physicochemical parameters was evaluated in the presence and absence of biodegradable organic matter. The incubation periods considered were 2h, 4h and 6h. In the presence of organic matter and disinfectant, *Enterococcus faecalis* densities decreased as incubation time increased. The rates of decline in bacterial density varied depending of the incubation temperature and the concentration of organic matter. Biodegradable organic matter and incubation temperature do not appear to influence disinfection by sodium percarbonate. However, increasing the contact time between bacterial cells and disinfectant in the majority of experimental conditions increases the effectiveness of the disinfectant by decreasing the abundance of cultivable cells. Sodium percarbonate increases the pH and dissolved oxygen levels of the water.

*Correspondence: Moungang Luciane Marlyse (lumou2000@yahoo.fr)

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Keywords

Water, Sodium Percarbonate, Incubation Times, Temperatures, Biodegradable Organic Matter, *Enterococcus faecalis*

1. Introduction

Water is an essential resource for all forms of life on Earth [1]. Poor physicochemical and biological water quality can cause waterborne diseases [2]. Hence the need for biological water treatment.

Several methods aimed at reducing or eliminating microorganisms from water have been developed, including the use of disinfectants. However, despite their effectiveness, these techniques have drawbacks due to the presence of chemical residues and the sometimes high cost of treatment [3]. In addition to the disinfection by products they produce, they have a preferential effect on a specific type of microorganism. Furthermore, if they are not applied under conditions where their lethal activity can be expressed, they promote the selection and even the proliferation of resistant species [4]. Effective water disinfection should indeed take into account biotic and abiotic factors that influence the target microbial species and this process.

While previous studies have established the ability of sodium hypochlorite to reduce microbial load during water treatment and the influence of certain factors on its activity [5, 6], few studies have investigated the combined effects of biodegradable organic matter, temperature, and incubation time on disinfection by sodium percarbonate. This article aims to study the effect of sodium percarbonate on the aquatic bacterium *E. faecalis*. Specifically, it seeks to evaluate the influence of biodegradable organic matter, temperature, and the duration of bacterial-disinfectant contact on the disinfectant activity of sodium percarbonate in an aquatic microcosm and to assess the effect of sodium percarbonate on the temporal evolution of some abiotic water parameters.

2. Materials and Methodes

2.1. Materials

2.1.1. Biological Material

The study focused on the bacterium *E. faecalis* due to its importance in hygiene and public health, as well as its ability to indicate the microbiological quality of drinking water [7, 8]. *E. faecalis* exhibits antibiotic resistance [9], it is commonly used as an indicator of fecal contamination to assess the hygienic quality of environmental samples [10].

2.1.2. Non-biological Material

(i). The Disinfectant

The disinfectant used in this study is sodium percarbonate. It is an environmentally friendly alternative to bleach because it is safe for humans and the environment. It has antibacterial properties and also combats mold [11]. Composed of sodium and hydrogen peroxide, sodium percarbonate decomposes naturally upon contact with water, releasing hydrogen peroxide [12]. Figure 1 shows a photograph of a sachet of the disinfectant used.

Figure 1. *Photograph of a sachet of Sodium Percarbonate.*

(ii). Biodegradable Organic Matter

The biodegradable organic matter used is glucose, with the general formula ($C_6H_{12}O_6$). It was chosen because it is an important nutrient for bacterial metabolism [13]. Three concentration ranges were considered (C1 = 500 mg/L, C2 = 750 mg/L, and C3 = 1000 mg/L). These concentrations were chosen to mimic the concentrations of organic matter found in oligotrophic natural waters [14, 15]. However, the positive control solution does not contain any organic matter. Figure 2 shows a bottle of SIGMA brand glucose.

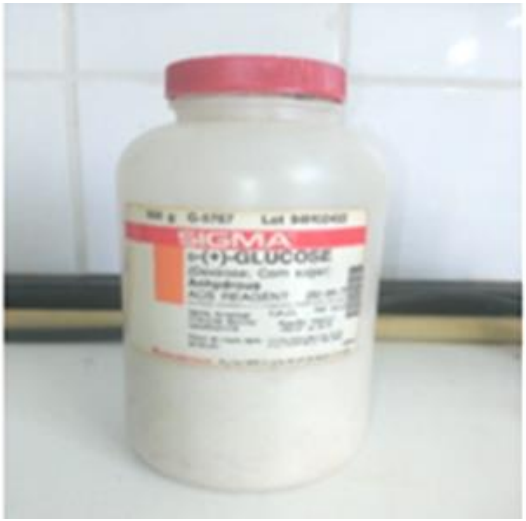

Figure 2. *Photograph of a vial of glucose.*

2.2. Methods

2.2.1. Isolation and Identification of *E. faecalis*

The bacterium *E. faecalis* was isolated using standard technics from surface waters (Olézoa stream) in the city of Yaoundé-Cameroon, using the specific culture medium Bile-Esculin-Azide (BEA). The water sample was collected in sterile glass bottles and transported to the laboratory in a refrigerated container. At the laboratory, 100 µL of the collected sample was inoculated onto BEA agar. After subculture on ordinary agar, wet mount examination, followed by Gram staining of the isolated bacterial cells, was performed. Identification tests were then carried out on the bacterial organism using standard biochemical methods [13, 16, 17]. These tests consist of revealing certain significant and stable characteristics of the metabolism of the bacterial species used.

2.2.2. Preparation of the Bacterial Suspension

A preculture was prepared by bacterial inoculation onto ordinary agar poured at an angle into test tubes, followed by incubation in an incubator for 24 hours at 37 ℃. The resulting pure colonies were collected using a sterile platinum loop and transferred to sterile physiological saline (0.85% NaCl). The homogeneous bacterial suspension obtained after vortexing was prepared at an optical density (OD) of 600 nm between 0.08 and 0.1 (corresponding to the McFarland standard). An OD of (0.08-0.1) corresponds to 10^8 CFU/mL [18]. All of this was carried out under the absolute sterility provided by the blue flame of a Bunsen burner.

2.2.3. Preparation of the Disinfectant Solution

15 g of sodium percarbonate powder were dissolved in one liter of sterile distilled water in a sterile Erlenmeyer flask. The solution was then heated to 60 ℃ to completely dissolve the powder [19]. The resulting solution was left at room temperature for a few minutes (to cool) before disinfection tests were performed [19]. All of this was carried out under the absolute sterility provided by the blue flame of a Bunsen burner.

2.2.4. Disinfection Test

The effect of the disinfectant on the microorganism and on some physicochemical parameters was evaluated in the presence and absence of biodegradable organic matter.

(i). In the Presence of Organic Matter

The aquatic microcosms were represented by 24 Erlenmeyer flasks of 100 mL. 10 mL of the disinfectant solution was introduced into each flask.

The cell densities of the microorganisms in each stock solution were adjusted to an initial concentration of 10^8CFU/mL, corresponding to the McFarland standard, and a volume of 1 mL of the *E. faecalis* suspension was introduced into each flask. The flasks were then divided into three groups (each consisting of 8 flasks), and 10 mL of glucose solution with concentrations C1 (500 g/L), C2 (750 g/L), and C3 (1000 g/L) was introduced into the flasks of groups G1, G2, and G3, respectively. The 8 Erlenmeyers in each group were then separated into two subgroups, one of which was incubated at room temperature and the other at 7 ℃.

(ii). In the Absence of Organic Matter

The aquatic microcosms were represented by eight Erlenmeyer flasks of 100 mL, which served as positive controls. Each flask contained 10 mL of sterile physiological saline (0.85% NaCl) and 10 mL of the disinfectant solution. The cell densities of the microorganisms in each stock solution were adjusted to an initial concentration of 10^8 CFU/mL, corresponding to the McFarland standard. Then, 1 mL of the *E. faecalis* suspension was added to each flask using sterile pipettes. The flasks were then divided into two groups (each consisting of four flasks), one of which was incubated at room temperature and the other at 7 ℃.

Bacteriological analyses and the evaluation of several physicochemical parameters were performed after 2, 4, and 6 hours of incubation.

2.2.5. Preparation of the Control Solution

A negative control group was prepared to evaluate pH, dissolved oxygen, and electrical conductivity in the absence of disinfectant in order to compare the results with those obtained under the action of the disinfectant. For this purpose, 10 mL of sterile physiological saline (0.85% NaCl) was introduced into 24 Erlenmeyer flasks of 100 mL. Then, a volume of the suspension of *E. faecalis* was introduced into each flask using sterile pipettes. Each group was divided into three subgroups G1, G2, and G3. After, 10 mL of glucose solution with concentrations C1, C2, and C3 was introduced into the Erlenmeyer flasks of groups G1, G2, and G3, respectively. The

flasks in each group were divided into two subgroups, one of which was incubated at room temperature and the other at 7 ℃. The evaluation of some physicochemical parameters was carried out after 2h, 4h and 6h of incubation.

2.2.6. Evaluation of the Effect of the Disinfectant on Some Abiotic Parameters of the Environment

The abiotic water parameters considered in this study were pH, electrical conductivity, and dissolved oxygen. These chemical parameters were measured using a HANNA brand pH meter and multimeter, respectively.

2.2.7. Data Analysis

Bacteriological analyses focused on quantitative aspects and were performed on BEA culture medium. The incubation temperature was 37 ℃ for 24 to 48 hours. The results were expressed as Colony Forming Units per unit volume of water sample (CFU/21 mL), then reported as CFU/100 mL. Variations in bacterial abundance as a function of organic matter concentration, temperature, and incubation time, as well as the evaluated physicochemical parameters, were illustrated by histograms using Microsoft Excel 2016.

3. Results

3.1. Isolation and Identification

3.1.1. Macroscopic Examination of Bacterial Colonies

BEA agar was used for the isolation of *E. faecalis*. The *E. faecalis* colonies observed on BEA agar are small (diameter between 0.5 and 0.9 µm), translucent, and surrounded by a black halo. Figure 3 shows a photograph and the cultural characteristics of the bacterial colonies.

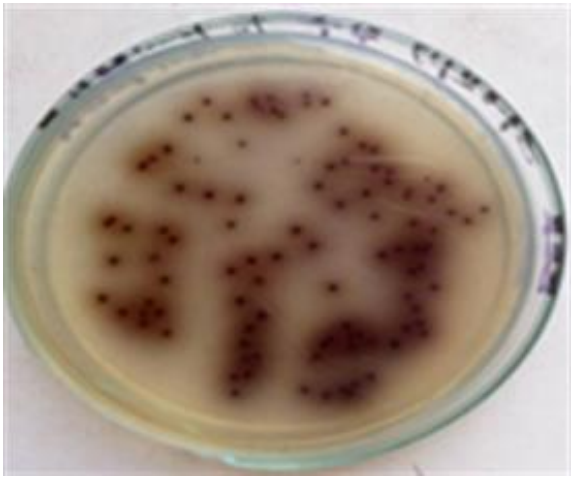

Figure 3. *Photograph of E. faecalis bacterial colonies on BEA medium.*

3.1.2. Microscopic Examination

Microscopic examination of fresh cells and stained smears allowed for the determination of the immobility, shape, flagellation, and Gram staining of the isolated bacteria. This revealed that *E. faecalis* is a non-motile Gram-positive cocci.

3.1.3. Biochemical Tests

The *E. faecalis* colonies were subjected to enzymatic and biochemical reactions. These reactions aimed to detect either an enzyme or a product released after the degradation of a given substrate under experimental conditions.

Taking into account all the results of the identification tests leads to the conclusion that the isolated bacterial species are indeed those sought. Table 1 presents the results of the various biochemical identification tests performed.

Table 1. *Results of biochemical tests.*

Identification tests performed	*E. faecalis*
Mannitol	+
Glucose fermentation	+
Lactose fermentation	+
Oxydase	+
Mobility	-
LDC (Lysine Decarboxylase)	-
ODC (Ornithine Decarboxylase)	-
TDA (Tryptophan-Deaminase)	-
Indole	-
Simmons Citrate	-
ONPG (Ortho-Nitro-Phenyl-β-Galactoside)	+
Urease	-
Gas	-
H_2S	+

+: Positive answer to the test; -: Negative answer to the test

3.2. Effect of Disinfectant on Bacterial Densities in the Presence of Organic Matter

Figure 4 illustrates the variations in bacterial cell density as a function of time, incubation temperature, and glucose (organic matter) concentration. These graphs show a considerable decrease in *E. faecalis* cell density after 2 hours of incubation, regardless of the incubation temperature or organic matter concentration. In the presence of the disinfectant and the various organic matter concentrations, *E. faecalis* cell densi-

ties gradually decreased as the incubation time increased, independent of the incubation temperature.

At room temperature, cell densities decreased to zero (8.68 to 0 log CFU/100 mL) in both the presence and absence of organic matter. The lowest cell density (0 log CFU/100 mL) was recorded in bacterial suspensions both with and without organic matter. Zero values for cell densities were recorded in the majority of cases after 4 hours of incubation (Figure 4A).

At 7 ℃, cell densities ranged from 3.48 to 8.68 log CFU/100 mL in bacterial suspensions lacking organic matter. In the presence of organic matter, they reached 8.68 log CFU/100 mL. The lowest cell abundance was recorded after 6 hours of incubation in bacterial suspensions containing 500 mg/L of organic matter and after 4 hours of incubation in bacterial suspensions containing 750 mg/L of organic matter (Figure 4B).

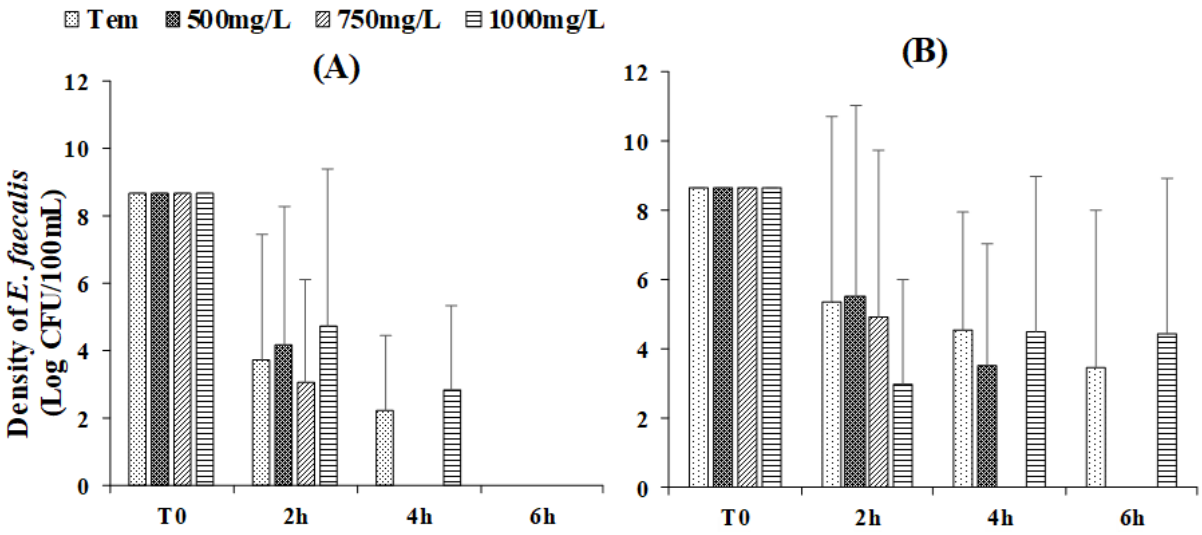

Figure 4. *Variation of E. faecalis densities after 2h, 4h and 6h of contact with sodium percarbonate in the presence of organic matter at room temperature (A) and at 7°C (B).*

3.3. Percentage of Inhibition (PI) of Bacterial Cells

The inhibition percentages were calculated using the formula proposed by [20]. In the presence or absence of biodegradable organic matter, the inhibition percentages (IP) of bacterial cells reached 99% after 2 hours of exposure to sodium percarbonate (Table 2).

Table 2. *Percentage of bacterial cell inhibition.*

Bacterial species	Incubation time	Concentration of biodegradable organic matter (mg/L)							
		Room temperature				7°C			
		Witnesses	500	750	1000	Witnesses	500	750	1000
E. faecalis	2h	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4h	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	6h	99.99	99.99	99.99	99.98	99.95	99.93	99.98	99.99

3.4. Rates of Regression of Cell Densities in Contact with the Disinfectant and in the Presence of Different Concentrations of Organic Matter and the Coefficients of Determination (R^2)

The rates of bacterial density regression varied according to incubation temperature and organic matter concentration. The results obtained are recorded in Table 3.

Table 3. Rates of regression of E. faecalis under the action of the disinfectant.

Bacterial species	Temperature	Disinfection Speeds (CFU/100mL/h) and Coefficient of determination (R^2)			
		Witnesses	C1	C2	C3
E. faecalis	Room temperature	2666,2 (0.77)	7166,2 (0.75)	582.83 (0.75)	25666 (0.76)
	7°C	112167 (0.85)	166083 (0.76)	44083 (0.75)	14417 (0.69)

Overall, *E. faecalis* densities decreased at rates ranging from 582.83 to 166083 CFU/100 mL/h. The lowest rate of decline (582.83 CFU/100 mL/h) was observed at room temperature in solutions containing 750 mg/L (C2) of organic matter. The highest rate of decline (166083 CFU/100 mL/h) was observed at 7°C in solutions containing 500 mg/L (C1) of organic matter.

3.5. Role of Sodium Percarbonate on Some Physicochemical Parameters of Water

pH, dissolved oxygen and electrical conductivity were evaluated as a function of organic matter concentrations, temperature and incubation time in solutions without disinfectant and in those with disinfectant in order to evaluate the influence of the disinfectant on its parameters.

3.5.1. Influence of Sodium Percarbonate on pH Variation

In general, bacterial suspensions containing sodium percarbonate exhibit higher pH values than those without.

Incubated at room temperature, the pH ranged from 7.7 to 8.7 CU in bacterial suspensions without disinfectant and from 11.4 to 11.57 CU in those containing disinfectant. The lowest pH value (7.7 CU) was recorded in bacterial suspensions without disinfectant containing organic matter at concentrations of 500 mg/L (at 0h of incubation) and 1000 mg/L (after 6h of incubation). The highest pH value (11.57 CU) was recorded after 2h of incubation in bacterial suspensions containing both disinfectant and organic matter at concentrations of 500 mg/L and 750 mg/L (Figure 5A).

At 7 ℃, the pH ranged from 7.4 to 8.17 CU in the negative control solutions (not containing the disinfectant) and from 11.4 to 11.62 CU in the solutions containing the disinfectant. The lowest pH value (7.4 CU) was recorded after 2 hours of incubation in the solutions without disinfectant and containing organic matter at a concentration of 500 mg/L. The highest pH value was recorded after 2 hours of incubation in the bacterial suspensions containing sodium percarbonate and organic matter at a concentration of 500 mg/L (Figure 5B).

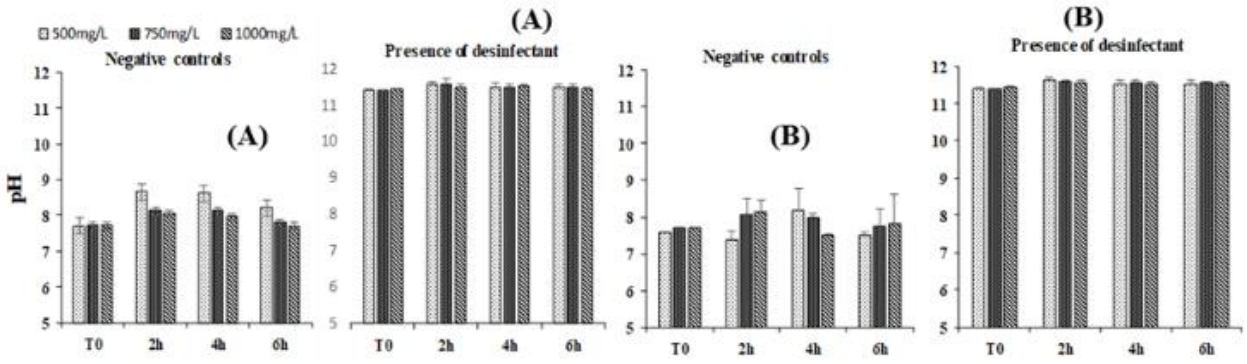

Figure 5. Temporal variation of the pH of cell suspensions in the presence and absence of disinfectant at room temperature (A) and at 7°C (B).

3.5.2. Influence of Sodium Percarbonate on the Variation of Dissolved Oxygen Content

In both the presence and absence of disinfectant, dissolved oxygen gradually decreased as incubation time increased.

When incubated at room temperature, dissolved oxygen levels after 6 hours ranged from 2 to 9.3 mg/L in bacterial suspensions not treated with disinfectant and from 17.3 to 28.6 mg/L in those treated with disinfectant. The lowest dissolved oxygen value (2 mg/L) was recorded after 6 hours of incubation in solutions without disinfectant and containing organic

matter at a concentration of 1000 mg/L. The highest dissolved oxygen value (28.6 mg/L) was recorded at the initial time in bacterial suspensions containing both disinfectant and organic matter at concentrations of 500 mg/L and 750 mg/L (Figure 6A).

At 7 ℃, dissolved oxygen ranged from 1.65 to 9.25 mg/L in solutions without disinfectant and from 27.52 to 30.92 mg/L in those containing disinfectant. The lowest dissolved oxygen value (1.65 mg/L) was recorded after 6 hours of incubation in bacterial suspensions without disinfectant and containing organic matter at a concentration of 1000 mg/L. The highest dissolved oxygen value (30.92 mg/L) was recorded after 2 hours of incubation in solutions containing both disinfectant and organic matter at a concentration of 1000 mg/L (Figure 6B).

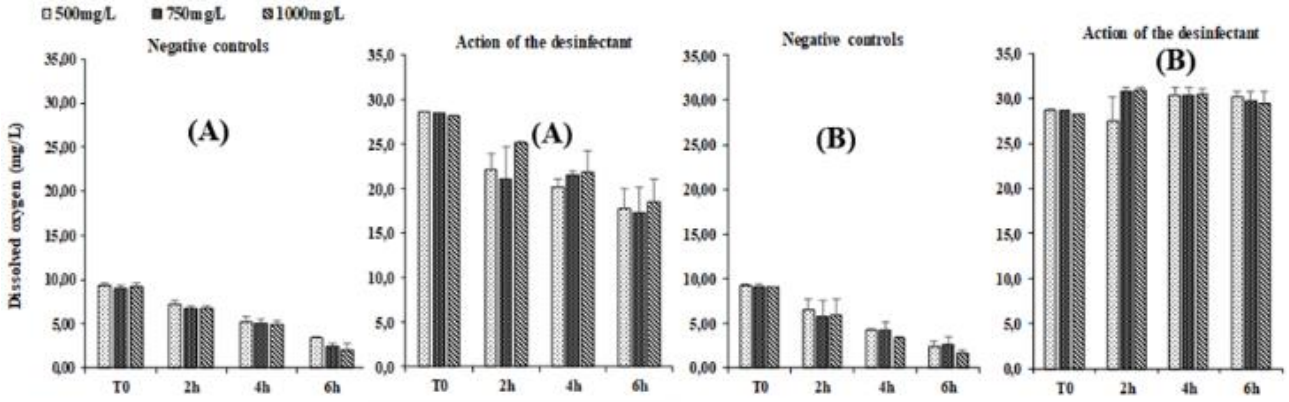

Figure 6. *Temporal variation of dissolved oxygen in cell suspensions in the presence and absence of disinfectant at room temperature (A) and at 7°C (B).*

3.5.3. Influence of Sodium Percarbonate on the Variation of Electrical Conductivity

In bacterial suspensions incubated at room temperature, electrical conductivity ranged from 7 to 7.70 µS/cm in solutions without disinfectant and from 4.8 to 8.1 µS/cm in those with disinfectant. The lowest electrical conductivity value (4.8 µS/cm) was recorded after 2 hours of incubation in solutions without disinfectant and containing organic matter at a concentration of 500 mg/L. The highest electrical conductivity value (8.1 µS/cm) was recorded after 6 hours in solutions containing both the disinfectant and organic matter at a concentration of 750 mg/L (Figure 7A).

At 7 ℃, electrical conductivity fluctuated between 4.8 and 8.03 µS/cm in solutions without disinfectant and between 5.13 and 7.68 µS/cm in those containing disinfectant. The lowest electrical conductivity value (4.8 µS/cm) was recorded at the initial time in solutions without disinfectant and containing organic matter at a concentration of 500 mg/L. The highest electrical conductivity value (8.03 µS/cm) was recorded after 4 h of incubation in solutions without disinfectant and containing organic matter at a concentration of 1000 mg/L (Figure 7B).

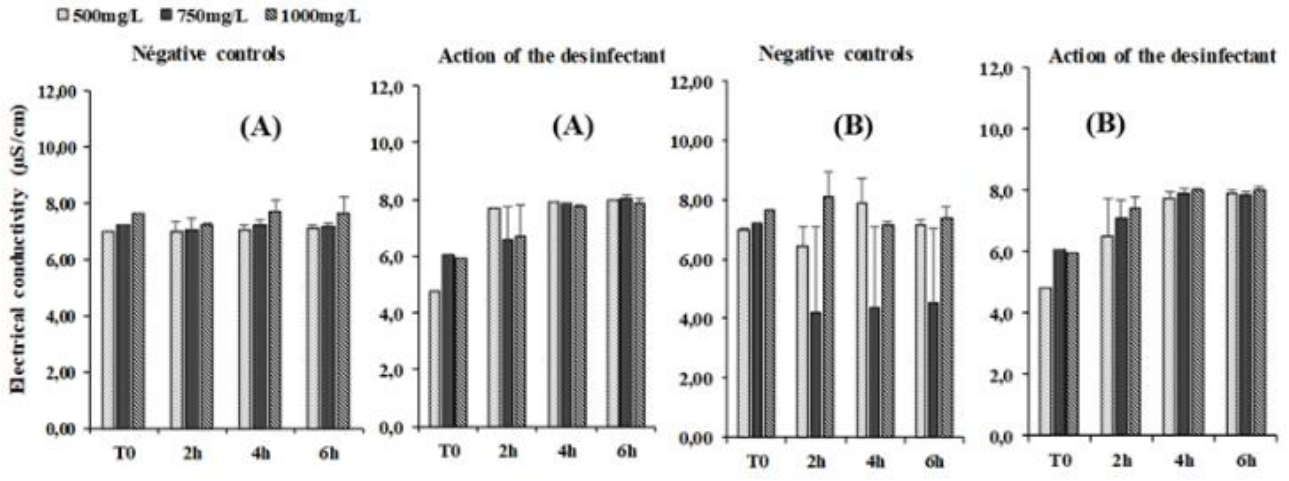

Figure 7. *Temporal variation of the electrical conductivity of cell suspensions in the presence and absence of disinfectant at room temperature (A) and at 7°C (B).*

3.6. Correlations Between the Evaluated Parameters

3.6.1. Correlation Between Average Bacterial Densities and Organic Matter Concentrations

Overall, no significant correlation (P>0.05) was observed between bacterial density and biodegradable organic matter concentration at any temperature or incubation time. The decrease in bacterial cell density with increasing incubation time in the disinfectant was not related to variations in organic matter concentration.

3.6.2. Correlation Between Average Bacterial Densities and Incubation Times

Table 4 Shows a highly significant negative correlation between mean bacterial densities and incubation times (P<0.01). An increase in incubation time would lead to a decrease in bacterial densities in the presence of the disinfectant.

Table 4. *Spearman correlation coefficient "r" between mean bacterial densities and incubation times.*

	Enterococcus faecalis
Incubation times	-0.839**

** P<0.01 ddl = 31

3.6.3. Correlation Between Average Bacterial Densities and Incubation Temperatures

Spearman's rank correlation coefficient (r) revealed no significant association (P>0.05) between mean bacterial densities and incubation temperatures. In the presence of the disinfectant, the variation in bacterial densities was not related to the variation in incubation temperature.

3.7. Comparisons Between the Analyzed Variables

3.7.1. Comparison Between the Average Densities of Bacterial Cells Evaluated at Different Incubation Temperatures

The Kruskal-Wallis H-test revealed no significant difference between the mean cell densities of *E. faecalis* incubated at room temperature and those incubated at 7 ℃. Therefore, the cell densities assessed at room temperature are not different from those assessed at 7 ℃. Thus, the incubation temperature does not appear to influence the disinfection of water by sodium percarbonate.

3.7.2. Comparison Between the Average Densities of Bacterial Cells Evaluated at Different Concentrations of Organic Matter

The Kruskal-Wallis "H" comparison test revealed no significant difference between the mean *E. faecalis* densities assessed in solutions devoid of organic matter and those assessed in solutions containing organic matter. Biodegradable organic matter does not appear to have a significant influence on water disinfected by sodium percarbonate.

3.7.3. Comparison Between the Average Densities of Bacterial Cells Evaluated at Different Incubation Times

The Kruskal-Wallis H-test revealed a significant difference between the mean *E. faecalis* densities assessed as a function of incubation time. Thus, the number of bacteria eliminated by sodium percarbonate differs according to the incubation time. This number appears to decrease as the incubation time increases. Table 5 presents the results obtained.

Table 5. *Comparison between the average densities of bacterial cells evaluated as a function of incubation time.*

	Enterococcus faecalis
Incubation time	P=0.0001

Significant difference at p< 0.05 ddl = 31

3.7.4. Comparison Between the Averages of the Physicochemical Parameters

The Kruskal-Wallis H-test revealed a significant difference between dissolved oxygen levels and electrical conductivity measured at different incubation times. Sodium percarbonate appears to affect these parameters differently over time. No significant difference was observed between the pH levels measured at different incubation times. This same test revealed no difference between dissolved oxygen, pH, and electrical conductivity measured at different incubation temperatures and organic matter concentrations. Table 6 presents the results.

Table 6. Comparison of the mean physicochemical parameters measured at different organic matter concentrations, temperatures, and contact times.

Physicochemical parameters	Dissolved oxygen	pH	Electrical conductivity
Incubation temperature	P=0.072	P=0.498	P=0.592
Contact time	P=0.021	P=0.444	P=0.001
Organic matter concentration	P=0.995	P=0.838	P=0.921

Significant difference at p< 0.05 ddl = 31

3.7.5. Comparison Between the Average Physicochemical Parameters Evaluated in the Control Solutions and Disinfectant Solutions

The Mann-Whitney U test revealed a significant difference between the dissolved oxygen levels and pH measured in the control solutions and those measured in the solutions containing the disinfectant. Sodium percarbonate appears to increase the dissolved oxygen level and pH of the water. Table 7 presents the results obtained.

Table 7. Comparison between the average physicochemical parameters evaluated in the control solutions and those evaluated in the solutions containing the disinfectant.

	Desinfectant solution		
	Dissolved oxygen	pH	Electrical conductivity
Witnesses	0.0001	0.0001	0.0001

Significant difference at p< 0.05 ddl = 31

4. Discussion

During the study, *E. faecalis* densities varied according to organic matter concentrations, contact time, and incubation temperature. According to [21], the distribution of enteric bacteria in aquatic environments is related to the physicochemical and meteorological parameters of the environment.

The densities of these bacterial cells decreased considerably after 2 hours (more than 99% of these bacteria were destroyed). No bacterial cells were observed after 6 hours in the solution of percarbonate. The Kruskal-Wallis H-test showed that the number of bacteria eliminated by sodium percarbonate differs according to the incubation time, and high efficiency was noted after 2 hours of incubation. Similarly, highly significant negative correlations ($P < 0.01$) between mean bacterial densities and incubation times were observed. This shows that the decrease in *E. faecalis* cell densities is highly significantly linked to an increase in incubation time. These results corroborate those of [5], who noted that increasing the duration of the disinfection process, in most cases, leads to a significant decrease in cell abundance in each solution treated with the disinfectant.

The rates of bacterial density regression varied according to the incubation temperature and the concentrations of organic matter. This could be explained by the variation in the physiological state of the microorganisms at each incubation temperature. Indeed, bacteria can adopt several strategies to survive in a hostile environment. Some differentiate into metabolically inactive resistance forms. Others, on the other hand, develop regulatory systems to control the stress experienced by adapting their survival to maximize energy and nutrient conservation [22].

Spearman's rank correlation coefficient (r) revealed no significant association ($P > 0.05$) between the mean bacterial densities and incubation temperatures. Similarly, the Kruskal-Wallis H-test showed no significant difference between the mean bacterial densities assessed at room temperature and those assessed at 7 ℃. This could be explained by the fact that the enzymatic components of *E. faecalis* are readily synthesized under mesophilic conditions [23]. It could also be explained, according to [23], by the fact that the effect of temperature on bacterial survival depends on other factors such as nutrients. When these nutrients are present in relatively high concentrations, cell survival and growth occur if temperatures are close to the optimum. These results are contrary to those

of [24] which state that the vital mechanisms (growth, nutrition, and metabolism) of all microorganisms are affected by temperature.

Furthermore, no significant correlation was observed between the average bacterial densities and the different concentrations of biodegradable organic matter. In addition, the Kruskal-Wallis H-test revealed no significant difference between these densities and the organic matter concentrations. The results obtained show that during water disinfection with sodium percarbonate, bacterial densities did not vary according to the concentrations of biodegradable organic matter. Thus, organic matter does not appear to influence water disinfection by sodium percarbonate. According to [11], once dissolved in water, sodium percarbonate decomposes into its two constituents: sodium carbonate and hydrogen peroxide. According to [25], hydrogen peroxide destroys the biodegradable organic matter present in the water. The bacteria can no longer use this organic matter as a carbon source. Therefore, this organic matter will no longer influence culturable cell densities. These results contradict those obtained by [6], who showed that an increase in the concentration of biodegradable organic matter during water disinfection with sodium hypochlorite leads to an increase in culturable cell densities of *Staphylococcus aureus*.

Similarly, the Mann-Whitney U test revealed a significant difference between the dissolved oxygen levels and pH measured in the control solutions and those measured in the solutions containing the disinfectant. These parameters were higher in the disinfectant solutions. This difference is likely related to the disinfectant's effect on these parameters. Thus, sodium percarbonate appears to increase the pH of the water and improve oxygenation. Indeed, according to [26], sodium carbonate resulting from the dissolution of sodium percarbonate in water is a weak base that increases the water's alkalinity. [27] points out in this regard that in aqueous solutions, sodium percarbonate decomposes into active oxygen and active carbonate; it is therefore known as a bifunctional ingredient because it offers both the benefits of hydrogen peroxide and the alkalinity of sodium carbonate.

Dissolved oxygen levels varied overall depending on the incubation time. According to [28], dissolved oxygen participates in the majority of chemical and biological processes in aquatic environments.

5. Conclusion

This study aimed to evaluate, in an aquatic microcosm, the influence of biodegradable organic matter, temperature, and incubation time on the effect of sodium percarbonate on the bacterium *E. faecalis*. The results obtained reveal that incubation temperature and the concentration of biodegradable organic matter do not influence the biological treatment of water by sodium percarbonate. However, incubation time does influence the effect of sodium percarbonate on *E. faecalis*. Similarly, highly significant negative correlations were observed between cell density and incubation time. Thus, increasing the residence time of *E. faecalis* in the disinfectant generally increased the disinfectant's effectiveness. Sodium percarbonate also increased the pH and dissolved oxygen levels in the water.

Abbreviations

BEA	Bile-Esculin-Azide
NaCl	Sodium Chloride
OD	Optical Density
LDC	Lysine Decarboxylase
ODC	Ornithine Decarboxylase
TDA	Tryptophan-Deaminase
ONPG	Ortho-Nitro-Phenyl-β-Galactoside
IP	Inhibition Percentage

Author Contributions

Mouhama Sani Adams Ibn Rabiou: Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing

Nougang Mireille Ebiane: Formal Analysis

Tamatcho Kweyang Blandine Pulcherie: Resources

Poutoum Yogne Yves: Data curation

Moungang Luciane Marlyse: Conceptualization, Methodology, Validation, Visualization, Supervision, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing

Nola Moïse: Supervision, Investigation

Conflicts of Interest

The authors declare no conflicts of interest.

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