

Article

RETRACTED: Exploring the Antimicrobial Efficacy of Low-Cost Commercial Disinfectants Utilized in the Agro-Food Industry Wash Tanks: Towards Enhanced Hygiene Practices

Francisco Chalen-Moreano ¹ , Angélica Saeteros-Hernández ¹ , Paula Abdo-Peralta ² , Catherine Frey ^{2,*} , Lilia Ofir Peralta-Saa ¹, Andrea Damaris Hernández-Allauca ³ , Carlos Rolando Rosero-Erazo ² and Theofilos Toulkeridis ⁴ 

- ¹ Faculty of Public Health, Escuela Superior Politécnica de Chimborazo, Km 1 ½ Panamericana Sur, Riobamba 060155, Ecuador; francisco.chalenm@espoch.edu.ec (F.C.-M.); asaeteros@espoch.edu.ec (A.S.-H.); L.peralta@espoch.edu.ec (L.O.P.-S.)
- ² Independent Researcher, Riobamba 060155, Ecuador; paulitag_abdo@hotmail.com (P.A.-P.); carlos.rosero1733@gmail.com (C.R.R.-E.)
- ³ Faculty of Natural Resources, Escuela Superior Politécnica de Chimborazo, Km 1 ½ Panamericana Sur, Riobamba 060155, Ecuador; andrea.hernandez@espoch.edu.ec
- ⁴ School of Geology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; ttoulkeridis@espe.edu.ec
- * Correspondence: catherine.frey@espoch.edu.ec



Citation: Chalen-Moreano, F.; Saeteros-Hernández, A.; Abdo-Peralta, P.; Frey, C.; Peralta-Saa, L.O.; Hernández-Allauca, A.D.; Rosero-Erazo, C.R.; Toulkeridis, T. RETRACTED: Exploring the Antimicrobial Efficacy of Low-Cost Commercial Disinfectants Utilized in the Agro-Food Industry Wash Tanks: Towards Enhanced Hygiene Practices. *Foods* **2024**, *13*, 1915. <https://doi.org/10.3390/foods13121915>

Academic Editor: Haiying Cui

Received: 1 May 2024

Revised: 7 June 2024

Accepted: 9 June 2024

Published: 18 June 2024

Retracted: 17 July 2025



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The increase in vegetable consumption has underlined the importance of minimizing the risks associated with microbiological contamination of fresh produce. The critical stage of the vegetable washing process has proven to be a key point for cross-contamination and the persistence of pathogens. In this context, the agri-food industry has widely adopted the use of disinfectants to reduce the bacterial load in the wash water. Therefore, we conducted laboratory-scale experiments in order to demonstrate the antimicrobial activity of disinfectants used in the wash tank of agro-food industries. Different wash water matrices of shredded lettuce, shredded cabbage, diced onion, and baby spinach were treated with sodium hypochlorite (NaClO), chlorine dioxide (ClO₂), and peroxyacetic acid (PAA) at recommended concentrations. To simulate the presence of pathogenic bacteria, a cocktail of *E. coli* O157:H7 was inoculated into the process water samples (PWW) to determine whether concentrations of disinfectants inhibit the pathogen or bring it to a viable non-culturable state (VBNC). Hereby, we used quantitative qPCR combined with different photo-reactive dyes such as ethidium monoazide (EMA) and propidium monoazide (PMA). The results indicated that concentrations superior to 20 ppm NaClO inhibit the pathogen *E. coli* O157:H7 artificially inoculated in the process water. Concentrations between 10–20 ppm ClO₂ fail to induce the pathogen to the VBNC state. At concentrations of 80 ppm PAA, levels of culturable bacteria and VBNC of *E. coli* O157:H7 were detected in all PWWs regardless of the matrix. Subsequently, this indicates that the recommended concentrations of ClO₂ and PAA for use in the fresh produce industry wash tank do not inhibit the levels of *E. coli* O157:H7 present in the wash water.

Keywords: antibacterial; disinfectants; food processing industry; vegetables; sodium hypochlorite; chlorine dioxide; peroxy acetic acid; *Escherichia coli*

1. Introduction

The considerable increase in vegetable consumption in recent years is evidence of society's growing sensitivity towards a healthy diet based on the intake of fruit and vegetables, which are considered a fundamental source of nutrients [1–7]. However, factors such as large-scale production, distribution and nature of (raw) consumption contribute to the emergence of illnesses caused by the ingestion of minimally processed fresh produce such as radish seeds, fresh spinach and packaged lettuce that are contaminated with *E. coli*

O157:H7 [8,9]. The Food and Drug Administration (FDA) recommends the implementation of effective control methods during the various stages of production, from storage, washing, drying, processing, packaging and distribution [10–12].

Hereby, the washing stage is considered one of the main risk factors due to potential and very likely “cross-contamination”. If the product originating from the field contaminates the wash water, it would be able to contaminate the following batch of product that has been initially clean. Similarly, if the water in the wash tank is contaminated, it can contaminate the clean product arriving in from the field [11,13,14]. Due to the large amount of products that pass through it, the wash water may be enriched by unusually high concentrations of organic matter, causing changes in its physical-chemical characteristics. A variety of studies have been able to demonstrate high concentrations of organic matter that hinder the inhibition of the microorganisms present in Process WasteWater (PWW), preventing the action of the disinfection treatments used in the agri-food industry [15–25].

As part of this direction of research, the antimicrobial activity of chlorine has been evaluated in numerous studies realized at laboratory scale, where concentrations above 20 ppm and within the range as high as 50 to 100 ppm [13,26] have been used to inhibit the presence of pathogenic bacteria in PWW. However, high concentrations of free chlorine may lead to the appearance of toxic compounds such as trihalomethanes, haloacetic acids and chlorates [27–30]. Nevertheless, several studies also at laboratory scale with low concentrations of free chlorine (2–10 ppm) in water for washing leafy vegetables proved to be effective in avoiding cross-contamination, as long as relevant factors in the process are considered, such as the type of product and the quantity entering the washing tank, which are variables that are decisive when controlling the amount of organic matter present in water [31–33].

Furthermore, some authors have evaluated the efficacy of 25 ppm free chlorine in preventing cross-contamination. In the results obtained by traditional cultivation methods, no cross-contamination was observed, and 25 ppm chlorine was able to inhibit *E. coli* O157:H7 and *Salmonella* pathogens in red chard leaves [33,34]. Additional studies determined the effectiveness of chlorine as a disinfectant in water for washing cut lettuce, demonstrating that when examining the microbiological quality of the water during washing of the product and treatment with disinfectant, the residual concentration of free chlorine at 1 mg/L during washing of lettuce contaminated with *E. coli* O157:H7 (4 log cfu/g) inhibited the pathogen to values below 2.7 and 2.5 log units, respectively [34].

By evaluating the survival of *E. coli* O157:H7 to changes in the concentration of free chlorine according to the amount of organic matter present in the wash water of cut spinach, the results demonstrated that the minimum concentration of 7 ppm free chlorine is effective in inactivating *E. coli* O157:H7 cells in the wash tank under industrial conditions [1]. The overall scientific evidence shows that 10 ppm free chlorine can minimize contamination of the fresh vegetable wash tank [11,35,36]. Disinfection with chlorine dioxide (ClO_2) due to its highly oxidative properties, disinfection mechanism, and the formation of byproducts are different from those of chlorine, as it presents trihalomethane formation and is less sensitive to organic matter. However, after decomposition, it forms inorganic disinfection byproducts such as chlorite, chlorate and perchlorate [28,37,38]. Research on the disinfection of carrots and ready-to-eat salads indicates a low formation of disinfection byproducts below the recommended maximum limit (0.7 mg/L), according to the European Food Safety Authority [30,39].

The antimicrobial capacity of ClO_2 at concentrations of 3–5 ppm in a variety of case studies inhibited *E. coli* pathogens present in the PWW by up to 5 log units with the presence of chlorates below the maximum recommended limit [40]. Despite contradicting results [13,27] which demonstrated that 3 ppm of ClO_2 is just as effective as sodium hypochlorite in inactivating pathogens such as *E. coli*, it proves to be an effective disinfectant to reducing cross-contamination in freshly cut iceberg lettuce, similarly effective as treatments with NaClO without the presence alterations in organoleptic properties and formation of disinfection byproducts such as trihalomethanes. Its mechanism of action acts by altering the permeability of the cell membrane, hindering metabolic activities

such as protein synthesis and preventing the formation of bioparticles, thus making their reactivation difficult. However, its industrial use is limited by its concentration, which could compromise the health of plant personnel due to the complications generated by its instability and reactivity at concentrations greater than 10% [28,41,42].

Disinfection with peroxyacetic acid (PAA) produces fewer harmful byproducts derived from its use since it leaves acetic acid, water, and oxygen as residues [11,27] that are capable of dissolving easily in water and do not present any toxicity indexes [28,29], although it has been indicated that low levels of aldehydes may form in the process [2]. Its limited susceptibility to organic matter due to its slow reaction compared to chlorine means that the doses of disinfectant used to maintain the desired residual in the washing tank are relatively low [11,40].

Further studies describe reductions of 3 log units in *E. coli*, *Salmonella* and *L. monocytogenes* when 80 ppm of PAA, a concentration registered by the United States Environmental Protection Agency (EPA), is applied, with a residence time of 90 s [43]. In the same way, inhibition results of 3 log units were reported in the levels of *Salmonella* and *L. monocytogenes* inoculated in the washing water and treated with PAA concentrations between 30 and 80 ppm, respectively [28,39].

Studies demonstrate that pathogens in the VBNC state, such as those of *E. coli* O157:H7, can be highly virulent when they leave their VBCN state, unlike other research where it is supposed that the VBNC of pathogens such as *Vibrio* and *Legionella pneumophila* remain pathogenic since they continue to express virulence when they are in the VBNC state [44–46]. It must be considered that if VBNC continues to maintain their virulence, their presence constitutes a problem for food safety because bacteria in this state fail to be detected by traditional plate culture methods, which are usually used in agri-food industries in order to control the microbiological quality of water [44,47].

Among the most used techniques to detect VBNC in complex food matrices is the use of photo-reactive dyes combined with molecular techniques based on the quantitative polymerase chain reaction or qPCR. In general, photo-reactive dyes such as monoazide ethidium (EMA) and monoazide propidium (PMA) adhere to cells that have damaged or compromised cell walls, preventing the amplification of dead bacteria present in the sample during the amplification in qPCR [44,48,49]. One of the main drawbacks of using these dyes is the overestimation of the results since, in the samples, there may be dead bacteria with the cell wall not damaged, and that is not inhibited by the dyes in qPCR [49,50].

A recent study [51] demonstrated that the use of qPCR combined with concentrations of 10 mM EMA and 75 mM PMAxx incubated at 40 °C for 40 min and exposed to light for 15 min inhibited the qPCR amplification signal of dead *L. monocytogenes* cells present in the PWW of chopped lettuce. The validation of this method in an industrial environment demonstrated that dead cells and cells in the VBNC state could be significantly distinguished in the PWW treated with chlorine. Therefore, the authors recommended this method as reliable for detecting cells in the VBNC state present in matrices existing in complex water systems in the agri-food industry [27,51].

With this background, the predominant objective of the research has been to demonstrate the most adequate antimicrobial activity of chlorine, chlorine dioxide and peroxyacetic acid used in the washing tank in agri-food industries to avoid cross-contamination and maintain the microbiological quality of the PWW. This will also determine whether disinfectants inhibit bacteria or induce them to a state of viable non-culturable bacteria (VBNC) and also to evaluate whether the VBNC of *E. coli* O157:H7 present in the washing water is able to reverse their state and resurrect. This occurs in the non-culturable viable state of bacteria in the VBNC state, where cells undergo different morphological (size reduction) and metabolic changes, such as modification of their gene expression as well as virulence potential.

2. Materials and Methods

2.1. Preparation of the *E. coli* O157:H7 Cocktail

The *E. coli* O157:H7 strains used in the current study were obtained from the Spanish CECT Type Culture Collection (Valencia, Spain). The stocks of the strains are stored in

glycerol (30%) at 80 °C. 5 µL of each strain were reconstituted in 10 mL of brain heart infusion broth culture medium (BHI, Oxoid, Basingstoke, UK). The tubes were incubated at 37 °C for 24 h until reaching their stationary phase. This process was repeated for two days to produce stability in the bacteria. In order to prepare the strain cocktail, 2 mL of each overnight bacteria was combined to realize a final concentration of 10^8 or 10^9 cfu/mL. On the day of the test, the final concentration of the cocktail was theoretically calculated using optical density with a spectrophotometer at a wavelength of 600 nm (UV1601, Shimadzu Benelux, Tokyo, Japan). Wavelength values between 1.6–2.0 nanometers corresponded to concentrations of 10^8 cfu/mL of bacteria. The actual concentration of the inoculum of each test was confirmed by sowing serial dilutions of the strain cocktail on Cromocult agar (Scharlau, Barcelona, Spain) supplemented with nalidixic acid. The plates were stored at 37 °C for 24 h. This cocktail is a mixture of three *E. coli* O157:H7 strains. The isolates used in the experiments were evaluated, and the presence of the *stx1* and *stx2* genes responsible for producing Shiga toxins was confirmed. This evaluation was carried out by DNA extraction and analysis using qPCR techniques.

2.2. Preparation of Process Water on a Laboratory Scale

Iceberg lettuce (*Lactuca sativa* L.), onion (*Allium cepa*), cabbage (*Brassica oleracea*) and spinach (*Spinacia oleracea*) were purchased in a local supermarket (Murcia, Spain) and transported to the laboratory under re-refrigeration conditions. Lettuce was cut to approximately 3 cm in diameter using a multipurpose vegetable slicer (S547Buffalo, Valencia, Spain). In the same way, with the multipurpose vegetable slicer (Buffalo), the cabbage was grated. The onion was cut into cubes manually using kitchen knives with a blade diameter of approximately 20 cm, while the spinach, due to its size, did not need to be cut.

To mimic the process water of a vegetable washing tank in the agri-food industry on a laboratory scale, 4 kg of each plant material was separated into batches of 1 kg while used and washed in 7 L of water. The plant material was washed for 1 h with 15 min intervals for each 1 kg batch. The concentration of organic matter in each process water obtained was determined by chemical oxygen demand (COD) using the Spectroquant NOVA 60 photometer photometric method (Merck KGsA, Darmstadt, Germany; Table 1). The physicochemical parameters of process wash water (PPW) are described in Table A1.

Table 1. Concentration of organic matter (COD) in the different PPW evaluated.

Process Water Matrix	COD mg/L
Lettuce	3835.0
Cabbage	6412.5
Onion	5972.5
Spinach	1002.5

Chemical Oxygen Demand (COD) was determined photometrically using the Spectroquant NOVA 60 photometric method (Merck KGsA, Darmstadt, Germany). The specific wavelength used for COD measurement is not explicitly detailed in the extracts provided.

In the standard method of measuring COD using a Spectroquant photometer, the wavelength is 600 nm. This is based on the standard practice of measuring the absorbance of the solution resulting from the dichromate oxidation reaction, which is specific for COD quantification.

Experimental procedures and equipment used

Measurement of physical–chemical parameters in which the following were considered:

- Temperature (°C);
- Electrical conductivity (µS/cm);
- Oxidation–reduction potential (ORP) in millivolts (mV);
- Hydrogen potential (pH);

- These parameters were measured using a multi-parameter probe (pH and redox multimeter).

To carry out the experiments, a final COD of 1000 mg/L was required for shredded lettuce, shredded cabbage and diced onion, and 300 mg/L for baby spinach. These selected CODs represent the worst-case scenario for an industrial washing tank because the higher the concentration of accumulated organic matter, the more the disinfectant action may be affected. To obtain this COD, the initial process water was diluted in water at 4 °C temperature. The process water generated from each of the plant matrices was physically and chemically characterized. The parameters determined were temperature (°C), electrical conductivity ($\mu\text{S}/\text{cm}$), oxidation–reduction potential (ORP) expressed in millivolts (mV) and hydrogen potential (pH). All these parameters were measured with a multiparametric probe (multimeter pH and redox device).

2.3. Experimental Design

To evaluate the antimicrobial activity of the disinfectants at the concentrations recommended for the agri-food industry, static batch experiments were performed, 650 mL of each type of process water at the required COD and treated individually with (i) 20 ppm sodium hypochlorite (NaClO ; Industrias Gamer, Murcia, Spain), (ii) 2 ppm chlorine dioxide (ClO_2 ; Chemical Cantabria, Cantabria, Spain) and (iii) 80 ppm Peroxyacetic acid (Peracetic Acid, PAA; $\text{CH}_3\text{CO}_3\text{H}$; Citrocide® Plus T, Citrosol S. A, Valencia, Spain). The water used in the experiments was undisinfected and unsupplemented drinking water, adjusted to simulate high organic matter conditions typical of industrial fresh vegetable washing processes.

In Figure 1. There is a description of the disinfection process; it starts in stage 1 with the preparation of vegetables; four types of vegetables (A, B, C, D) are initially treated in water with additives such as hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaClO). Stage 2 is followed by treatment with disinfectants. Two flasks are shown, one with 20 ppm NaClO and the other with 200 ppm peracetic acid (PAA), both containing thiosulfate. For stage 3, only the bacterial load remained to be established.

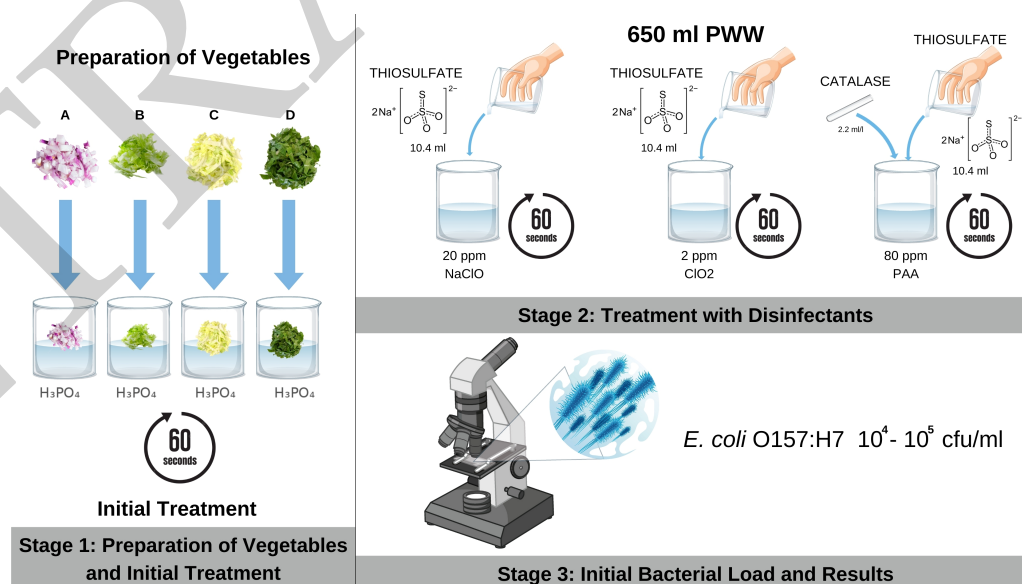


Figure 1. Disinfection process of vegetables to reduce the bacterial load of *E. coli* O157:H7. A-onion, B-lettuce, C-cabbage (*Brassica oleracea*) and D-spinach.

This preparation allowed us to evaluate the effectiveness of the disinfectants under conditions that imitate real industrial operations, representing the worst scenario for a washing tank, where the concentration of accumulated organic matter can affect the action of the disinfectants. One of the main parameters to be controlled in the wash tank was the pH to ensure the efficacy of the disinfectants. In our experiments with sodium hypochlorite

(NaClO), phosphoric acid was used for pH stabilization at 60 s intervals until pH 6–7 was obtained.

Different volumes of disinfectant were added to the process water until the desired concentration was reached to reach the disinfectant residual concentrations indicated above. The residual concentration measurements of the disinfectant were determined by the chlorine (NaClO) and chlorine dioxide (ClO₂) test (Reflectoquant, Darmstadt, Germany); for peroxyacetic acid (PAA), the test (Kemio, Amersham, UK) was used. During the whole process of disinfectant adjustment in the water, the physicochemical water parameters (temperature in °C, electrical conductivity in µS/cm, ORP in mV and pH) were monitored with the multi-parameter probe indicated above.

Once the residual concentrations were reached, the matrices were inoculated with the cocktail of three *E. coli* O157:H7 strains (10⁸ cfu/mL) to obtain a final concentration of 10⁴–10⁵ cfu/mL. To simulate industrial conditions in the wash tank, the contact time between the process water and the disinfectant was 1 min. After this time, the water was collected in sterile bottles with 10.4 mL of sodium thiosulphate pentahydrate (Scharlau, Barcelona, Spain) to neutralize the action of the disinfectant on the NaClO and ClO₂ treated matrices. For PAA neutralization, 10.4 mL/L sodium thiosulphate (Scharlau) plus 2.2 mL/L catalase (Biorad, Hercules, CA, USA) were added.

2.4. Quantification of Culturable *E. coli* O157:H7

The levels of culturable *E. coli* O157:H7 present in the different process water matrices. The 10⁸ cell suspension was diluted to 10⁴ in the process water matrices of lettuce, onion, cabbage and baby spinach. The dilution process involved preparing a cocktail of *E. coli* O157:H7 strains in brain heart broth (BHI) culture medium and combining 2 mL of each strain to achieve a final concentration of 10⁸ or 10⁹ cfu/mL.

Regarding the procedure for mixing disinfectants with cells and food, disinfectants such as chlorine, ClO₂ and PAA were added to the process water matrices after inoculation with *E. coli* O157:H7. For example, in the case of PAA, sodium thiosulfate and catalase were added to the solution. The disinfectants were mixed with the bacterial suspension in the process water matrices, allowing the interaction to occur as part of the disinfection process.

The order of addition and mixing procedures are crucial to ensure the effectiveness of disinfectants against bacterial cells. Proper mixing and contact time between disinfectants and bacterial cells are essential to achieve the desired antimicrobial effect. By following a standardized procedure for mixing disinfectants with cells and food matrices, the study aimed to evaluate the true antimicrobial activity of disinfectants in the context of the agri-food industry after disinfection was quantified by membrane filtration method using a vacuum filtration system (Sartorius-Stedim-Biotech, Madrid, Spain). Different volumes of water (1, 10 and 100 mL) were filtered through nitrocellulose membrane filters of 0.45 µm (pore size) and 47 mm diameter. Filters were aseptically removed and placed on Cromocult[®] agar plates (Merck, Darmstadt, Germany) supplemented with nalidixic acid. All samples were filtered or seeded in duplicate and incubated at 37 °C for 24 h.

2.5. Quantification of Total and Viable *E. coli* O157:H7 Counts (Thidium Monoazide (EMA) and Ropidium Monoazide (PMA) Treatment)

For the determination of viable bacteria present in the process water, the combination of two photo-reactive dyes, thidium monoazide (EMA; Biotium, Hayward, CA, USA) and ropidium monoazide (PMA; Biotium, Hayward, CA, USA), together with quantitative polymerase chain reaction (qPCR), recently published and validated for process water by Truchado [50], for which aliquots (50 mL) of process water were taken from matrices inoculated with *E. coli* O157:H7 and treated with NaClO, ClO₂ and PAA. Centrifuge 10 mL of the samples in duplicate at 4000 rpm at 4 °C for 10 min. We removed supernatants and re-suspended the pellets with 5 µL sterile water. We concentrated the samples by centrifugation at 9000 rpm for 10 min, and discarded the supernatants.

Pellets were treated with a combination of 10 μM EMA and 75 μM PMA (previously dissolved separately in sterile water to obtain a 2 μM stock solution and stored at $-20\text{ }^{\circ}\text{C}$ in the dark until use), and samples were centrifuged at 200 rpm and kept in the dark at $40\text{ }^{\circ}\text{C}$ for 10–60 min. The treated samples were activated by photolysis using PMA-Lite LED blue light (Interchim, Montluçon, France) for 15 min. Treated samples were again centrifuged at 9000 rpm, $4\text{ }^{\circ}\text{C}$, for 10 min. The supernatants were removed, and the pellets treated with the combination of EMA and PMAxx were stored at $-20\text{ }^{\circ}\text{C}$ until DNA extraction.

2.6. DNA Extraction from Process Water

Each sample's genomic DNA was extracted with the Master Pure Complete kit (Epicenter, Madison, WI, USA) following the manufacturer's instructions. DNA quality was determined by absorbance at 260/280 and 260/30 nm using a NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), which quantifies DNA (ng/ μL).

2.7. qPCR Amplification

Real-time qPCR was amplified using an ABI7500 Sequence Detection System thermal cycler (ABI, Applied Biosystems, Madrid, Spain). Primers and probes were commercially synthesized by Applied Biosystem (Madrid, Spain). The final concentrations of primers and probes were those applied by Li and Chen [42]. The amplification and detection of *E. coli* O157:H7 were performed in 96-well plates using the "Kapa Probe Fast Universal qPCR Master Mix" extraction kit (KapaBiosystems, Wilmington, MA, USA).

Each reaction was performed in triplicate in a final volume of 25 μL , for which 5 μL of DNA was taken from the sample with its corresponding dilution, adding (i) 12.5 μL Buffer, (ii) 5.75 μL RNase-free water, (iii) 0.5 μL Forward Primer, (iv) 0.5 μL Reverse Primer, (v) 0.25 μL probe, and (vi) Rox low 0.5 μL . The primers and probe used belong to the sequences (i) Z3276-Forward (F), 5'-GCACTAAAAGCTTGGAGCAGT TC, (ii) Z3276-Reverse (R), 5'AA CAATGGGT CAGCG GTAAGGCTA, and the Z3276-probe, FAM-CGTTGGCGAGGACC. The cycling parameters for qPCR amplification were $95\text{ }^{\circ}\text{C}$ for 10 min for activation of the Taqman probe, followed by 40 cycles of 10 s at $95\text{ }^{\circ}\text{C}$ and $60\text{ }^{\circ}\text{C}$ for 1 min. A negative control was included in each qPCR to rule out any contamination of the samples. These controls are amplified on the same plate as the samples and, therefore, have the same volume.

2.8. VBNC Resuscitation of *E. coli* O157:H7

For resuscitation of viable *E. coli* O157:H7 cells in the NaClO, ClO₂ and PPA-treated process water, 10 mL of each sample was taken in duplicate, previously filtered (0.45 μm filter) to rule out the presence of contaminants. We centrifuged at 4500 rpm for 15 min and removed the swimming envelope. Each pellet was reconstituted in 1 mL of 1% phosphate buffer (PBS), and then each sample was inoculated with two resuscitation media, being (i) IHB (Oxoid) with Tween 80 (1 mL/L, Sigma-Aldrich, Saint Louis, MO, USA) and (ii) IHB (Oxoid) with sodium pyruvate. They were shaken in a vortex and incubated at $37\text{ }^{\circ}\text{C}$ for 18–24 h.

2.9. Statistical Analysis

The number of colonies on each plate was log-transformed and expressed as log ucf/mL. The data represent the mean and standard deviation for each assay. The cfu/mL were transformed to log 10 with Microsoft Excel 2013. Graphs were made with Sigma Plot v 14.0. Analysis of variance (ANOVA) was realized to compare the antimicrobial activity of each disinfectant as a function of the type of process water. Tukey's test was used to determine if there were significant differences between the matrices. When $p < 0.05$, the analysis was considered statistically significant.

3. Results

3.1. Physical–Chemical Parameters of the Process Water

In appendices 1 and 2, the COD values demonstrate the organic matrix levels that simulate the worst-case scenario of microbiological contamination that can occur in the washing tank of an agri-food industry. The pH values for each PWW treated with the different disinfectants stabilized at 5.88 and 6.99, which ranges at which NaClO, ClO₂, and PAA reach their highest effectiveness. Sodium hypochlorite (NaClO) has a significant antimicrobial effect on fresh-cut produce wash water, with operational limits based on microbial inactivation and the presence of disinfection byproducts. [15,26,34] On the other hand, the ORP values for PAA are relatively low in relation to NaClO and ClO₂, which could be due to the fact that its rate of inactivation of microorganisms is lower in relation to the other disinfectants.

3.2. Levels of *E. coli* O157:H7 in the Process Water after Disinfection Treatments

3.2.1. Sodium Hypochlorite

Figure 2 illustrates the cultivable *E. coli* O157:H7 counts obtained using the plate count technique, as well as the levels of viable *E. coli* O157:H7 obtained by using EMA+PMA in combination with qPCR on the different PWW matrices treated with 20 ppm NaClO.

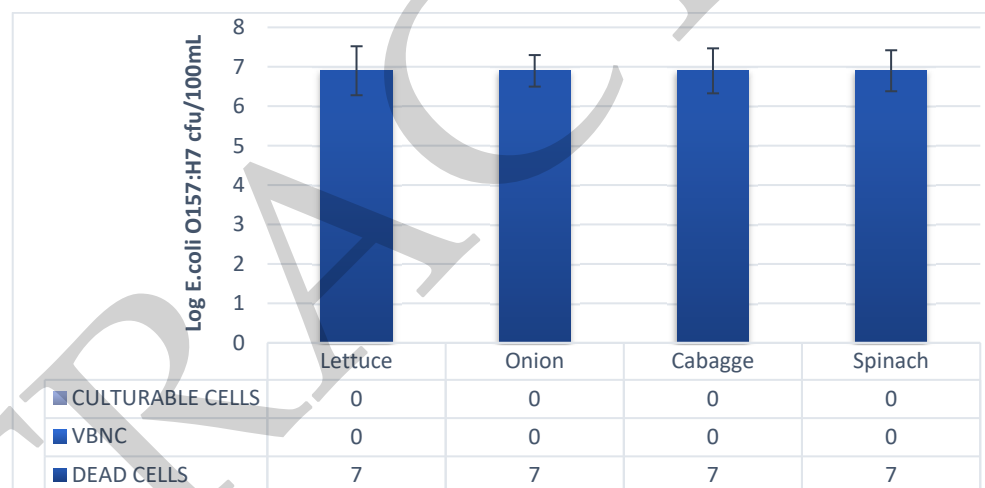


Figure 2. *E. coli* O157:H7 levels (log cfu or cells/100 mL) in the wash water of shredded lettuce, shredded cabbage, diced onion and baby spinach treated with 20 ppm NaClO. Although in the symbols of the legend, VBNC and culturable cells may appear, only dead cells are illustrated and present in this figure.

3.2.2. Chlorine Dioxide

Figure 3 demonstrates the levels of *E. coli* O157:H7 obtained in the different ClO₂-disinfected process water matrices. The residual of 2.2 ppm ClO₂, the initial concentration of culturable *E. coli* O157:H7 in lettuce, shredded cabbage, chopped onion and baby spinach wash water. The levels of culturable bacteria were detected by plate count (culturable), viable cells by EMA+PMAxx-qPCR and dead cells by qPCR. VBNC levels were calculated by comparing viable and culturable bacteria and dead cells between total and dead bacteria. The results obtained indicated that in the case of onion water, the effectiveness of the disinfectant was higher, as in these trials, the highest reduction of culturable bacteria was observed. However, Lin (2017) [52] mentioned that short chlorine treatment reduces antibiotic-resistant *E. coli* counts, but realistic counts of cultivable cells are achieved after extended action [53].

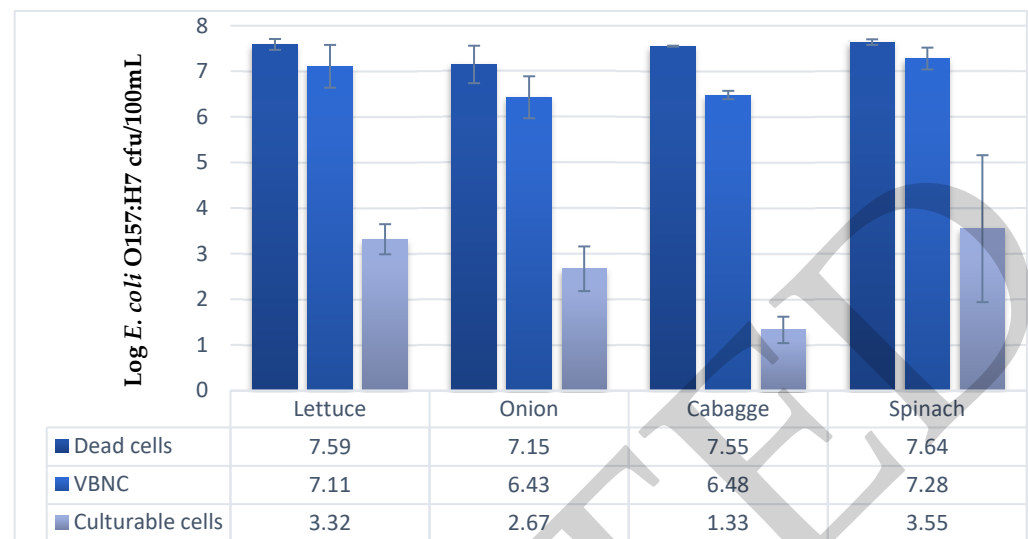


Figure 3. *E. coli* O157:H7 levels (log cfu or cells/100 mL) in the wash water of shredded lettuce, shredded cabbage, diced onion and baby spinach treated with 2 ppm ClO_2 .

3.2.3. Peroxyacetic Acid

When different PWW matrices were treated with 80 ppm PAA, levels of culturable bacteria and VBNC of *E. coli* O157:H7 were detected in all PWWs regardless of the matrix (Figure 4). The results of our study indicate that concentrations of 80 ppm PAA decrease the levels of culturable *E. coli* O157:H7 present in the PWW but do not completely reduce their presence. Higher concentrations or longer contact times are needed for peroxyacetic acid (PAA) to reduce the induction of viable but non-culturable (VBNC) bacteria cells in the wash water. [54] On the other hand, the presence of viable bacteria detected by the combination of EMA+PMA plus qPCR indicates that the use of this disinfectant induces the bacteria present in the wash water to a VBNC state. The combination of EMA and PMA with qPCR is effective in differentiating between dead and VBNC cells in complex water matrices, such as process wash water from the food industry [50]. EMA-qPCR and PMA-qPCR have been shown to be reliable for the rapid identification and quantification of viable cells in various water sources, including opportunistic pathogens [55].

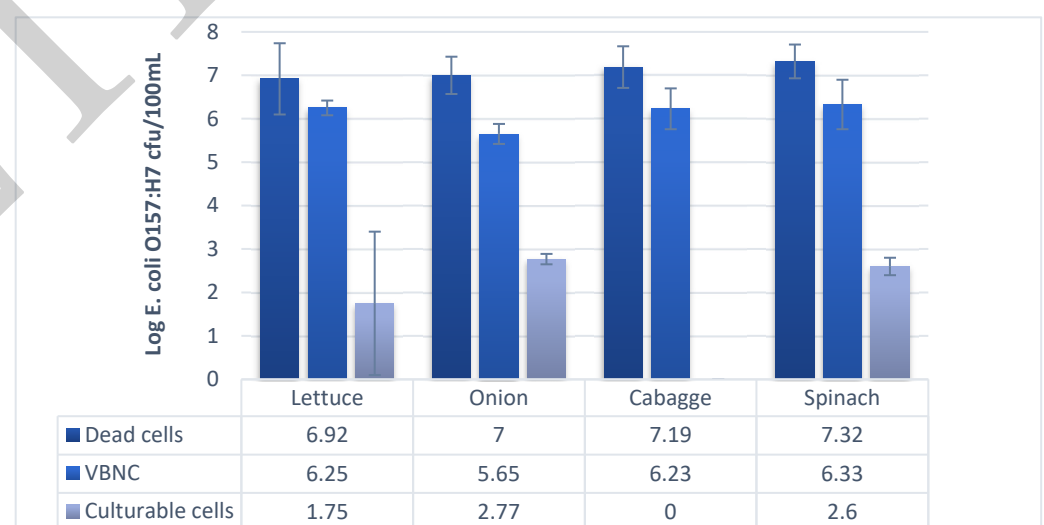


Figure 4. *E. coli* O157:H7 levels (log cfu or cells/100 mL) in the wash water of shredded lettuce, shredded cabbage, diced onion and baby spinach treated with 80 ppm PAA.

3.2.4. Antimicrobial Activity of Disinfection Treatments Recommended for the Agri-Food Industry

The antimicrobial activity was quantified by subtracting the log of total bacteria obtained by qPCR from the log of viable bacteria obtained by EMA+PMA-qPCR. The results obtained are shown in Table 2. Chlorine (20 ppm) was able to inhibit *E. coli* O157:H7 cells inoculated in the different types of process water tested in this work. However, ClO₂ and PAA only reduced the levels of *E. coli* O157:H7 present in the different wash water matrices by −0.36 to −1.36 log units. In general, the results obtained indicated that, with the exception of NaClO, disinfectants such as ClO₂ and PAA at the concentrations recommended for the agri-food industry are not able to inhibit the concentration of the pathogen *E. coli* O157:H7 in the PWW of the different plant matrices studied, and that the use of culture-dependent techniques overestimates the disinfection efficacy of the treatments. Studies demonstrated that at 20 °C, 15 out of 18 food industry disinfectants passed the test against *E. coli* O157:H7, with some exhibiting reduced efficacy at lower temperatures [56]. ClO₂ treatment of food-contact surfaces results in residual lethality to *E. coli* O157:H7 during the drying process [56,57].

Table 2. Concentration of organic matter (COD in mg/L) in the different PWW evaluated.

PWV	Disinfectant (Log Cells/100 mL)								
	NaClO	s	σ	ClO ₂	s	σ	PAA	s	σ
Lettuce	−6.94 ± 0.80	a	0.80	−0.48 ± 0.75	b	0.75	−0.42 ± 0.45	a	0.45
Cabbage	−6.99 ± 0.52	a	0.74	−1.00 ± 0.02	a	1.11	−0.97 ± 0.02	a	0.25
Onion	−6.74 ± 0.74	a	0.52	−0.73 ± 1.11	a	0.02	−1.36 ± 0.25	a	0.02
Spinach	−6.83 ± 0.67	a	0.67	−0.36 ± 0.38	b	0.38	−1.00 ± 0.23	a	0.23

Note: Values are expressed as mean ± S.E. (Standard Error); the letter “s” corresponds to the degree of significance of the treatments; the symbol “σ” corresponds to the standard deviation. the letter “a” and “b” indicate different statistical groups.

In Table 2, there are shown different statistical groups into which the results have been classified. Values with the same letter have no significant differences from each other, while values with different letters do yield significant statistical differences. All values under NaClO have the letter ‘a’, suggesting that there is no significant difference in COD reduction between different vegetables with that disinfectant. On the other hand, values with the letter ‘b’ under ClO₂ and PAA indicate another comparison group. The standard deviation shows the variability in the data, being lower in cabbage with ClO₂ and PAA, suggesting more consistent results with these treatments. The standard deviation data helps to understand the stability and reliability of each disinfection treatment, providing crucial information for the selection of the most appropriate disinfectant. The ANOVA table provides an overview of whether there are significant differences in *E. coli* O157 levels between the different disinfection treatments.

Table 3 reveals an extremely low p-value (0.000046), highlighting significant differences between the treatments. ClO₂ is markedly more effective in reducing *E. coli* O157 compared to NaClO and PAA. The results for NaClO and PAA exhibit greater variability, possibly due to differences in application conditions or concentrations used. In contrast, ClO₂ demonstrates higher effectiveness and greater consistency in disinfectant action. These findings are consistent with previous studies highlighting the efficacy of ClO₂ as a disinfectant. For practical applications, ClO₂ appears to be a more reliable option for the reduction of *E. coli* O157 [56,58,59]. Tukey’s HSD test was used to make paired comparisons between treatments and decide which are significantly different.

Table 3. Differences in the treatments applied.

Source	df	sum_sq	mean_sq	F	PR (>F)
Treatment	2.0	15.5274	7.7637	10.8842	0.000046
Residual	119	84.8828	0.7133		

Note: df—degrees of freedom; mean_sq—mean squared value; sum_sq—sum of squares. The F-value is a number obtained by comparing two variances. In the context of ANOVA, it is used to assess whether the means of three or more groups are different.

Table 4 indicates that ClO₂ versus NaClO has a significant difference with ClO₂ yielding a greater reduction of *E. coli* O157. ClO₂ vs. PAA also indicate a significant difference, with a greater reduction of *E. coli* O157 due to ClO₂. NaClO vs. PAA does not demonstrate a significant difference between treatments.

Table 4. Tukey's HSD test (* Significant difference).

Treatment 1	Treatment 2	Mean Difference	p-Value	Confidence Interval (Lower)	Confidence Interval (Higher)	Reject H ₀
ClO ₂	NaClO	−0.3556	0.0021	−0.5869	−0.1242	*
ClO ₂	PAA	−0.2588	0.0354	−0.5006	−0.0169	*
NaClO	PAA	0.0968	0.6549	−0.1455	0.3391	

The ClO₂ treatment yields the lowest *E. coli* O157 levels among the three treatments and is the most effective. The confidence interval is narrower, suggesting that the data are more consistent and less variable. NaClO had a higher mean *E. coli* O157 than ClO₂ but lower than PAA. The confidence interval is wider than that of ClO₂, indicating greater variability in the results. PAA was therefore the least effective treatment in this analysis. According to [56] the treatments used in their study entitled “Fundamental differences in *E. coli* O157:H7 inactivation mechanisms between chlorine dioxide and sodium hypochlorite” where the data demonstrated that ClO₂ and NaClO damage *E. coli* O157:H7 through measurably different mechanisms and that ClO₂ does not appear to cause substantial oxidative stress to *E. coli* O157:H7 directly, i.e., ClO₂ and NaClO caused cell death using different mechanisms. Non-overlapping confidence intervals indicate statistically significant differences between treatments, which is consistent with the results of ANOVA and Tukey's HSD test.

3.2.5. Resuscitation Capacity of Viable Non-Culturable Bacteria Present in the Process Water

The resuscitation capacity of *E. coli* O157:H7 VBNC detected in the PWV of the different plant matrices treated with the different disinfectants studied was also evaluated (Table 3). The results demonstrated that after disinfection treatment with 20 ppm NaClO, the cells were not able to resuscitate. This finding could indicate that 20 ppm chlorine inhibits the presence of *E. coli* O157:H7 cells inoculated on PWV or that the culture medium and resuscitation conditions used were not sufficient to resuscitate the chlorine-damaged cells. For the resuscitation capacity of *E. coli* O157:H7 VBNCs, the results showed Nd being undetected resuscitation for all types of PWV using the different kinds of disinfectants. *E. coli* O157:H7 cells treated with 20 ppm NaClO were unable to resuscitate, suggesting that this concentration effectively inhibits the presence of VBNC cells or that the resuscitation conditions were insufficient, same as shown in several studies [60,61].

4. Discussion

The chlorine concentration was selected based on the recommendations of recently published studies related to the use of chlorine as a disinfectant in commercial fresh produce processing lines [26,27,31,52]. The corresponding authors recommend free chlorine concentrations to maintain the microbiological quality of the water in the washing tank of

fresh vegetable industries, avoiding the formation of byproducts as a consequence of the degradation of the disinfectant. Our results indicate that regardless of the matrix studied, the levels of *E. coli* O157:H7 present in the PWW were undetectable, regardless of the methodology used. This finding is evidence that concentrations above 20 ppm inhibit the *E. coli* O157:H7 pathogen artificially inoculated into the process water.

The counts of culturable bacteria obtained in our study are similar to those published by other authors who used chlorine residuals of 25 ppm in their trials to inhibit the presence of *E. coli* O157:H7 and *Salmonella* in lettuce and red chard during washing. However, concentrations of 10 ppm free chlorine have been shown to inhibit culturable as well as viable cells of *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* inoculated in lettuce and spinach wash water [51]. In contrast to our results, concentrations between 10–20 ppm chlorine do not induce the pathogen to the VBNC state in the wash tank because they inhibit it. This finding contrasts with the studies conducted on *L. monocytogenes* and *Salmonella*, indicating that these pathogens were induced to the VBNC state after treatment with 12 ppm or 3 ppm chlorine in water, respectively [62].

The viable but non-culturable (VBNC) state of *Escherichia coli* O157:H7, induced by disinfection treatments, represents a significant challenge in the field of food safety. When bacteria enter this state, they stop reproducing and cannot be detected by traditional culture methods, which increases the risk of them going undetected in food quality controls. Although it has been suggested that bacteria in the VBNC state are not capable of causing infections, recent research has indicated that they are able to regain their pathogenic capacity when environmental conditions are conducive. The resuscitation of bacteria in the VBNC state, such as *E. coli* O157:H7, raises serious implications in terms of food safety, as these bacteria could represent a risk to public health if they are not properly detected and controlled. Therefore, it is essential to carry out additional research to better understand the mechanisms that allow bacteria in the VBNC state to regain their infectious capacity. To address this challenge, it is necessary to develop more sensitive and specific detection methods to identify and quantify bacteria in the VBNC state in foods.

Furthermore, it is necessary to investigate how disinfection treatments and other environmental factors can influence the formation and resuscitation of bacteria in the VBNC state, in order to implement effective strategies to prevent risks for consumers. In this context, it is crucial to promote interdisciplinary collaboration between microbiologists, food safety experts, food engineers and other professionals to advance the understanding of the implications of the VBNC status of *E. coli* O157:H7 and its impact on food safety. Only through rigorous research and the development of evidence-based preventive and control strategies can we ensure food safety and protect public health effectively [63–65].

Chlorine dioxide treatments showed that onion process water and applied doses of disinfectant were more efficient due to the presence of antimicrobial compounds from the process matrix. Overall, our findings are in line with previous studies demonstrating reductions of 3 log units of *E. coli* present in lettuce PWW (COD = 1130 mg/L) disinfected with 3 ppm ClO₂ for 1 min. Similar investigations on lettuce PWW (COD = 750 mg/L) inoculated with *E. coli* with 3 ppm ClO₂ confirmed comparable reductions. Furthermore, using the recommended doses of ClO₂ for the disinfection of vegetables in the wash tank avoids the formation of harmful disinfection byproducts such as trihalomethanes, haloacetic acids and chlorates [27,36,42].

Related studies indicate the application of higher concentrations of ClO₂ (5 ppm) reduced *E. coli* bacteria inoculated on PWW by 6 logarithmic units after 1 min of contact [39]. On the other hand, longer contact times (2–3 min) than those used in our trials combined with concentrations of 2–3 ppm ClO₂ also reduced the levels of *E. coli* O157:H7 and culturable *L. monocytogenes* present in the wash water of lettuce, strawberries, and melon by about 5 log units. Until now, there was no scientific evidence from studies assessing the presence of VBNC in PWW after ClO₂ disinfection treatment. However, the presence of VBNC was detected in irrigation water treated with ClO₂ (2 ppm) for irrigation of leafy vegetables [27,65]. The levels of culturable bacteria obtained in the PAA experiments were

similar to those obtained by other authors, such as Banach et al. [66] and López Gálvez et al. [27] observed that concentrations of 80 ppm PAA inhibited the presence of *E. coli* by approximately 4–6 log units inoculated into the PWW (COD, 750 mg/L).

Other investigations also conducted at laboratory scale to determine the efficacy of PAA as a disinfection treatment in the washing stage of fresh vegetables found reductions of 3 log units of *E. coli*, *Salmonella* and *L. monocytogenes* inoculated in the PWW after treatment with 30–80 ppm PAA and a contact time of 90 s. However, it should be noted that the disinfection capacity of PAA depends on the composition of the commercial solution used and the bacteria studied [27]. The different commercial PAA disinfectants contain different concentrations of hydrogen peroxide, which is responsible for the antimicrobial activity [67,68]. As Keskinen et al. mentioned [69] in their study, another factor influencing the effectiveness of the PAA is the presence of bacteria inside the product due to physical processes (cutting), preventing the action of the disinfectant through the formation of biofilms.

A further study found that PAA concentrations of 30 ppm were effective in reducing the presence of culturable *L. monocytogenes* and *E. coli* O157:H7 bacteria inoculated in lettuce and spinach wash water [38]. However, this inhibition in culturable bacteria is in contrast to the results obtained. The results related to viable bacteria published agree with those detected in the experiments performed. They also confirm the ability of PAA to induce food-pathogenic bacteria to the VBNC state. Based on the results obtained, the recommended doses of PAA for disinfecting the wash water of different fresh-cut vegetable matrices (cut lettuce, shredded cabbage, diced onion and baby spinach) were not effective in inactivating the presence of food pathogens. In relation to the antimicrobial activity, it has been evaluated so far using the plate count technique and the recommended doses are based on the results obtained by this methodology [26].

However, recent scientific evidence has indicated that disinfectants may induce VBNC status and do not inhibit the total presence of VBNC in the wash tank, as indicated by plaque count results [64]. To demonstrate the “real” antimicrobial activity of the disinfectants, the concentration of culturable, viable and dead bacteria in the PWW after disinfection processes was quantified. In order to calculate the different physiological states of the pathogen present in the water, the results obtained indicated (i) qPCR (determines both live and dead cells), (ii) EMA+PMA-qPCR (viable bacteria) and plate count (culturable) were used. It should be noted that the results of the resuscitation of chlorine-induced VBNC are controversial. Some research has demonstrated that *E. coli* and *Salmonella Typhimurium* VBNC induced after chlorine treatment could not be resuscitated [70,71].

In the same way, authors such as Zhang et al. 2015 [72] reported that pathogens of *E. coli* O157:H7 in the VBNC state after treatment with sodium hypochlorite could not be resuscitated in TSB (tryptone-soy broth) supplemented with 10% sodium pyruvate. *E. coli* O157:H7 and *Salmonella* cells after treatment with 50 ppm free chlorine were unable to resuscitate in TSB enrichment medium supplemented with 0.3% sodium pyruvate or in synthetic M9 medium supplemented with 0.4% glucose and 0.5 mM of each of 20 amino acids [38]. In contrast to all this work, a study by Chen et al. [54] observed that *E. coli* cells in the VBNC state induced by chlorine treatment can be resuscitated in Luria Bertani (LB) enrichment medium at 37 °C for 24 h.

According to these studies, the resuscitation capacity of VBNC induced by disinfectant treatments is still unclear and not easy to demonstrate. Several authors have described that the resuscitation of VBNC state cells by resuscitation means can be influenced by a wide range of factors [73–76]. In their studies, the authors of [72] noted that resuscitation was achieved, with *E. coli* generating greater resuscitation ability than *P. aeruginosa*. Various factors allowing resuscitation are extracellular resuscitation-promoting proteins and interactions with amoeba [46]. Disinfection-induced VBNC state in *E. coli* can make cells less infectious, but they can regain their pathogenicity after resuscitation [77]. Other factors could include the bacterial species, age of the bacteria, induction conditions to the VBNC state and resuscitation conditions, such as the type of recovery media used, the

supplements added (pyruvate, catalase, amino acids, etc.) and the incubation temperature selected [43,74,78,79].

However, other studies have demonstrated that foodborne pathogenic bacteria-induced after disinfection treatments can be resolved using biological stimuli such as simple animal models and bacteriophages. Highmore et al. [67] observed that chlorine induced the VBNC state of *L. monocytogenes* and *Salmonella enterica*, remaining virulent using the nematode *Caenorhabditis elegans*. Ben Said et al. [80] proposed using a specific lytic phage as a bio-indicator for detecting VBNC in disinfected wastewater. Further studies are therefore needed to elucidate the resuscitation capacity of VBNC foodborne pathogenic bacteria to avoid public health hazards, especially concerning the resuscitation of active but non-culturable bacteria. Although our results are effective, resuscitation was detected according to several studies, thus supposing potential health risks. Thus, it is crucial to use a combined disinfection strategy.

5. Conclusions

The viable but non-culturable (VBNC) state of *E. coli* O157:H7 is a survival mechanism that allows the bacteria to persist in harsh environments. This state poses significant food safety and public health challenges, as VBNC cells can potentially resuscitate under favorable conditions, leading to renewed pathogenicity. This synthesis examines the resuscitation capacity of *E. coli* O157:H7 VBNC cells in different plant matrices treated with various disinfectants, with a focus on the effectiveness of 20 ppm NaClO, ClO₂ and PAA.

At a concentration of 20 ppm, chlorine inhibited the presence of inoculated *E. coli* O157:H7 in the wash water of different plant matrices, such as shredded lettuce, shredded cabbage, shredded onion and baby spinach. However, ClO₂ (2 ppm) and PAA (80 ppm) induced the pathogen present in the different types of PWW to a VBNC state. The presence of viable *E. coli* O157:H7 bacteria detected using EMA+PMAxx-qPCR in the different types of PWW confirms that the culture-dependent techniques overestimate the efficacy of the recommended concentrations of disinfectants for washing fresh-cut vegetables at this stage. The results indicated that cells in the VBNC state can maintain their virulence and gene expression capacity, while others may die due to the experimental conditions. This is because bacteria can respond differently to disinfectant treatments, with some entering the VBNC state while others do not survive.

E. coli O157:H7 VBNC induced by the disinfectants at the recommended doses were not able to resuscitate under the conditions studied. However, further studies are needed to show whether disinfectant-induced VBNC present in the wash tank water can resuscitate or attach to plant tissue and subsequently resuscitate after preservation, so that we can determine whether or not the presence of these bacteria may pose a public health risk. The focus of the study is aimed at simulating industrial conditions where contaminations usually involve multiple strains of bacteria, thus the isolates were not evaluated individually.

Author Contributions: Conceptualization, F.C.-M. and A.S.-H.; methodology, F.C.-M.; software, F.C.-M. and A.D.H.-A.; validation, C.F., C.R.R.-E., L.O.P.-S., A.S.-H. and P.A.-P.; formal analysis, C.F., P.A.-P.; investigation, F.C.-M. and A.S.-H.; resources, A.D.H.-A. and F.C.-M.; data curation, A.D.H.-A.; writing—original draft preparation, F.C.-M.; writing—review and editing, T.T. and C.F.; visualization, C.F.; supervision, C.F. and P.A.-P.; project administration, F.C.-M. funding acquisition, L.O.P.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Physicochemical parameters of process wash water (PWW) inoculated with *E. coli*, chemical oxygen demand (COD), pH, conductivity (EC), oxidation–reduction potential (ORP) and temperature (T^a). Trial 1.

Sanitizer	Wash Water	COD	Sanitizer	pH	EC	ORP	Temperature
	Matrix	(mg L ^{−1})	(mg L ^{−1})		(μS cm ^{−1})	(mV)	(°C)
NaClO							
	Lettuce	1068	21.4	6.50	2590	819	4.1
	Cabbage	1042	19.8	6.84	2310	804	4.1
	Onion	1030	20.2	6.68	2570	845	4.2
	Spinach	332	21.5	6.73	2170	793	4.1
ClO ₂							
	Lettuce	960	1.8	5.88	1866	636	3.9
	Cabbage	1014	2.68	6.99	1523	717	5.0
	Onion	1106	1.78	5.85	1842	685	4.0
	Spinach	278	1.93	6.39	1816	689	4.3
PAA							
	Lettuce	1080	82	6.42	1828	381	4.2
	Cabbage	996	79.7	6.13	1282	409	4.2
	Onion	1066	85.6	6.0	1605	362	4.0
	Spinach	340	79	6.31	1787	362	5.3

Table A2. Physicochemical parameters of process wash water (PWW) inoculated with *E. coli*, chemical oxygen demand (COD), pH, conductivity (EC), oxidation–reduction potential (ORP) and temperature (T^a). Trial 2.

Sanitizer	Wash Water	COD	Sanitizer	pH	EC	ORP	Temperature
	Matrix	(mg L ^{−1})	(mg L ^{−1})		(μS cm ^{−1})	(mV)	(°C)
NaClO							
	Lettuce	1018	19.9	6.62	2500	792	4.1
	Cabbage	1000	19.1	6.73	2613	829	4.2
	Onion	948	18.8	6.81	2795	829	4.3
	Spinach	308	22.9	6.79	1969	786	4.6
ClO ₂							
	Lettuce	1000	2.3	6.99	1611	703	4.2
	Cabbage	944	2.2	6.73	1621	705	4.1
	Onion	968	2.2	6.82	1526	698	4.3
	Spinach	328	2.2	6.49	1930	661	4.3
PAA							
	Lettuce	954	84	6.18	1404	444	5.7
	Cabbage	1180	81.0	6.3	1411	467	6.3
	Onion	1088	85	6.07	1362	450	4.6
	Spinach	362	80	6.34	1514	454	6.8

Table A3. Terminology Clarification.

Acronym	Terminology Clarification
EMA	Ethidium of monoazide
PMA	Propidium of monoazide
qPCR	Quantitative polymerase chain reaction
PAA	Peroxyacetic acid
DQO	Chemical oxygen demand
PWW	Process wash water
VBNC	Viable noncultivable bacteria
FDA	Food and Drug Administration
ClO ₂	Chlorine Dioxide
NaClO	Sodium hypochlorite
pH	Hydrogen potential
ORP	Potential oxidation reduction
ppm	Parts per million
BHI	Brain heart infusion
DNA	Deoxyribonucleic acid
PBS	Phosphate Buffer Saline
TSB	Typtic Soy Broth
μL	Micro liter
μS	Microsiemens
Nm	Nanometer
mV	Milli Volt
μm	Micrometer
mm	Millimeter
°C	Degree Celsius
kg	Kilogram
rpm	Revolutions per minute
log	Logarithm
UV	Ultraviolet radiation
L	Liter
mL	Milli liter
gr	Gram
mg	Milligram

References

- Boeing, H.; Bechthold, A.; Bub, A.; Ellinger, S.; Haller, D.; Kroke, A.; Leschik-Bonnet, E.; Müller, M.J.; Oberritter, H.; Schulze, M.; et al. Critical review: Vegetables and fruit in the prevention of chronic diseases. *Eur. J. Nutr.* **2012**, *51*, 637–663. [\[CrossRef\]](#)
- Fulton, S.L.; McKinley, M.C.; Young, I.S.; Cardwell, C.R.; Woodside, J.V. The Effect of Increasing Fruit and Vegetable Consumption on Overall Diet: A Systematic Review and Meta-analysis. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 802–816. [\[CrossRef\]](#)
- Liu, R.H. Health-promoting components of fruits and vegetables in the diet. *Adv. Nutr.* **2013**, *4*, 384S–392S. [\[CrossRef\]](#)
- Agudo, A.; Cabrera, L.; Amiano, P.; Ardanaz, E.; Barricarte, A.; Berenguer, T.; Chirlaque, M.D.; Miren, D.; Paula, J.; Nerea, L.; et al. Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: Findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). *Am. J. Clin. Nutr.* **2007**, *85*, 1634–1642. [\[CrossRef\]](#) [\[PubMed\]](#)

5. Rissanen, T.H.; Voutilainen, S.; Virtanen, J.K.; Venho, B.; Vanharanta, M.; Mursu, J.; Salonen, J.T. Nutritional Epidemiology Low Intake of Fruits, Berries and Vegetables Is Associated with Excess Mortality in Men: The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. *J. Nutr.* **2003**, *133*, 199–204. [\[CrossRef\]](#)
6. Cao, G.; Booth, S.L.; Sadowski, J.A.; Prior, R.L. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am. J. Clin. Nutr.* **1998**, *68*, 1081–1087. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Ministerio de Agricultura Pesca y Alimentacion. Informe del consumo alimentario en España. 2022. Available online: https://www.mapa.gob.es/es/alimentacion/temas/consumo-tendencias/informe-consumo-2022-baja-res_tcm30-655390.pdf (accessed on 28 April 2024).
8. Olaimat, A.N.; Holley, R.A. Factors influencing the microbial safety of fresh produce: A review. *Food Microbiol.* **2012**, *32*, 1–19. [\[CrossRef\]](#)
9. Sharapov, U.M.; Wendel, A.M.; Davis, J.P.; Keene, W.E.; Farrar, J.; Sodha, S.; Hyytia-Trees, E.; Leeper, M.; Gerner-Smidt, P.; Griffin, P.M.; et al. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of fresh spinach: United States, 2006. *J. Food Prot.* **2016**, *79*, 2024–2030. [\[CrossRef\]](#)
10. U.S. Food and Drug Administration. Guidance for Industry Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. 2008. Available online: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-fruits-and-vegetables> (accessed on 28 April 2024).
11. Gombas, D.; Luo, Y.; Brennan, J.; Shergill, G.; Petran, R.; Walsh, R.; Hau, H.; Khurana, K.; Zomorodi, B.; Rosen, J.; et al. Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables. *J. Food Prot.* **2017**, *80*, 312–330. [\[CrossRef\]](#)
12. Leneveu-Jenvrin, C.; Charles, F.; Barba, F.J.; Remize, F. Role of biological control agents and physical treatments in maintaining the quality of fresh and minimally-processed fruit and vegetables. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2837–2855. [\[CrossRef\]](#)
13. Gil, M.I.; Selma, M.V.; López-Gálvez, F.; Allende, A. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *Int. J. Food Microbiol.* **2009**, *134*, 37–45. [\[CrossRef\]](#)
14. Holvoet, K.; De Keuckelaere, A.; Sampers, I.; Van Haute, S.; Stals, A.; Uyttendaele, M. Quantitative study of cross-contamination with *Escherichia coli*, *E. coli* O157, MS2 phage and murine norovirus in a simulated fresh-cut lettuce wash process. *Food Control* **2009**, *37*, 218–227. [\[CrossRef\]](#)
15. Van Haute, S.; López-Gálvez, F.; Gómez-López, V.; Eriksson, M.; Devlieghere, F.; Allende, A.; Sampers, I. Methodology for modeling the disinfection efficiency of fresh-cut leafy vegetables wash water applied on peracetic acid combined with lactic acid. *Int. J. Food Microbiol.* **2015**, *208*, 102–113. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Gil, M.I.; Selma, M.V.; Suslow, T.; Jaxsens, L.; Uyttendaele, M.; Allende, A. Pre- and Postharvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 453–468. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Mota, J.D.O.; Kooh, P.; Jaffrès, E.; Prévost, H.; Maignien, T.; Arnich, N.; Sanaa, M.; Boué, G.; Federighi, M. First Survey about Current Practices of Environmental Monitoring Programs within French Agri-Food Industries. *Biology* **2022**, *11*, 89. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Cheng, G.; Ning, J.; Ahmed, S.; Huang, J.; Ullah, R.; An, B.; Hao, H.; Dai, M.; Huang, L.; Wang, X.; et al. Selection and dissemination of antimicrobial resistance in Agri-food production. *Antimicrob. Resist. Infect. Control* **2019**, *8*, 158. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Reisman, E. Sanitizing agri-food tech: COVID-19 and the politics of expectation. *J. Peasant. Stud.* **2021**, *48*, 910–933. [\[CrossRef\]](#)
20. Wicaksono, T.; Hossain, M.B.; Illés, C.B. Prioritizing business quality improvement of fresh agri-food SMEs through open innovation to survive the pandemic: A QFD-based model. *J. Open Innov. Technol. Mark. Complex.* **2021**, *7*, 156. [\[CrossRef\]](#)
21. Viles, E.; Santos, J.; Muñoz-Villamizar, A.; Grau, P.; Fernández-Arévalo, T. Lean-green improvement opportunities for sustainable manufacturing using water telemetry in agri-food industry. *Sustainability* **2021**, *13*, 2240. [\[CrossRef\]](#)
22. Bandyopadhyay, S.; Samanta, I. Antimicrobial resistance in agri-food chain and companion animals as a re-emerging menace in post-COVID epoch: Low-and middle-income countries perspective and mitigation strategies. *Front. Vet. Sci.* **2020**, *7*, 620. [\[CrossRef\]](#)
23. Trivedi, R.; Upadhyay, T.K.; Khan, F.; Pandey, P.; Kaushal, R.S.; Sonkar, M.; Kumar, D.; Saeed, M.; Khandaker, M.U.; Emran, T.B.; et al. Innovative strategies to manage polluted aquatic ecosystem and agri-food waste for circular economy. *Environ. Nanotechnol. Monit. Manag.* **2024**, *21*, 100928. [\[CrossRef\]](#)
24. Zhu, X.; Das, R.S.; Bhavya, M.L.; Garcia-Vaquero, M.; Tiwari, B.K. Acoustic cavitation for agri-food applications: Mechanism of action, design of new systems, challenges and strategies for scale-up. *Ultrason. Sonochem.* **2024**, *105*, 106850. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Zhao, G.; Vazquez-Noguerol, M.; Liu, S.; Prado-Prado, J.C. Agri-food supply chain resilience strategies for preparing, responding, recovering, and adapting in relation to unexpected crisis: A cross-country comparative analysis from the COVID-19 pandemic. *J. Bus. Logist.* **2024**, *45*, e12361. [\[CrossRef\]](#)
26. Tudela, J.A.; López-Gálvez, F.; Allende, A.; Gil, M.I. Chlorination management in commercial fresh produce processing lines. *Food Control* **2019**, *106*, 106760. [\[CrossRef\]](#)
27. López-Gálvez, F.; Gil, M.I. La importancia del agua en la industria de alimentos vegetales. *Arbor* **2020**, *196*, e547. [\[CrossRef\]](#)
28. Banach, J.L.; Sampers, I.; Van Haute, S.; van der Fels-Klerx, H.J. Effect of Disinfectants on Preventing the Cross-Contamination of Pathogens in Fresh Produce Washing Water. *Int. J. Environ. Res. Public Health* **2015**, *12*, 8658–8677. [\[CrossRef\]](#) [\[PubMed\]](#)

29. Joshi, K.; Mahendran, R.; Alagusundaram, K.; Norton, T.; Tiwari, B.K. Novel disinfectants for fresh produce. *Trends Food Sci. Technol.* **2013**, *34*, 54–61. [CrossRef]
30. Garrido, Y.; Marín, A.; Tudela, J.A.; Allende, A.; Gil, M.I. Chlorate uptake during washing is influenced by product type and cut piece size, as well as washing time and wash water content. *Postharvest Biol. Technol.* **2019**, *151*, 45–52. [CrossRef]
31. Manzocco, L.; Ignat, A.; Anese, M.; Bot, F.; Calligaris, S.; Valoppi, F.; Nicoli, M.C. Efficient management of the water resource in the fresh-cut industry: Current status and perspectives. *Trends Food Sci. Technol.* **2015**, *46*, 286–294. [CrossRef]
32. Kettlitz, B.; Kemendi, G.; Thorgrímsson, N.; Cattoor, N.; Verzeegnassi, L.; Le Bail-Collet, Y.; Maphosa, F.; Perrichet, A.; Christall, B.; Stadler, R.H. Why chlorate occurs in potable water and processed foods: A critical assessment and challenges faced by the food industry. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2016**, *33*, 968–982. [CrossRef]
33. Tomás-Callejas, A.; López-Gálvez, F.; Sbodio, A.; Artés, F.; Artés-Hernández, F.; Suslow, T.V. Chlorine dioxide and chlorine effectiveness to prevent *Escherichia coli* O157:H7 and Salmonella cross-contamination on fresh-cut Red Chard. *Food Control* **2012**, *23*, 325–332. [CrossRef]
34. Gómez-López, V.M.; Lannoo, A.S.; Gil, M.I.; Allende, A. Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control* **2014**, *42*, 132–138. [CrossRef]
35. Luo, Y.; Zhou, B.; Van Haute, S.; Nou, X.; Zhang, B.; Teng, Z.; Turner, E.R.; Wang, Q.; Millner, P.D. Association between bacterial survival and free chlorine concentration during commercial fresh-cut produce wash operation. *Food Microbiol.* **2018**, *70*, 120–128. [CrossRef] [PubMed]
36. Mahmoud, B.S.M.; Vaidya, N.A.; Corvalan, C.M.; Linton, R.H. Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Poona on whole cantaloupe by chlorine dioxide gas. *Food Microbiol.* **2008**, *25*, 857–865. [CrossRef] [PubMed]
37. Ridenour, G.M.; Ingols, R.S. Bactericidal Properties of Chlorine Dioxide. Available online: <https://www.jstor.org/stable/23349269> (accessed on 5 April 2024).
38. Gu, G.; Bolten, S.; Mowery, J.; Luo, Y.; Gulbranson, C.; Nou, X. Susceptibility of foodborne pathogens to sanitizers in produce rinse water and potential induction of viable but non-culturable state. *Food Control* **2020**, *112*, 107138. [CrossRef]
39. Banach, J.L.; van Overbeek, L.S.; Groot, M.N.N.; van der Zouwen, P.S.; van der Fels-Klerx, H.J. Efficacy of chlorine dioxide on *Escherichia coli* inactivation during pilot-scale fresh-cut lettuce processing. *Int. J. Food Microbiol.* **2018**, *269*, 128–136. [CrossRef] [PubMed]
40. Artés, F.; Gómez, P.; Aguayo, E.; Escalona, V.; Artés-Hernández, F. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biol. Technol.* **2009**, *51*, 287–296. [CrossRef]
41. Parish, M.E.; Beuchat, L.R.; Suslow, T.V.; Harris, L.J.; Garrett, E.H.; Farber, J.N.; Busta, F.F. Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2* (Suppl. S1), 161–173. [CrossRef]
42. van Haute, S.; Sampers, I.; Jacxsens, L.; Uyttendaele, M. Selection criteria for water disinfection techniques in agricultural practices. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1529–1551. [CrossRef]
43. Dong, K.; Pan, H.; Yang, D.; Rao, L.; Zhao, L.; Wang, Y.; Liao, X. Induction, detection, formation, and resuscitation of viable but non-culturable state microorganisms. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 149–183. [CrossRef]
44. Baffone, W.; Citterio, B.; Vittoria, E.; Casaroli, A.; Campana, R.; Falzano, L.; Donelli, G. Retention of virulence in viable but non-culturable halophilic *Vibrio* spp. *Int. J. Food Microbiol.* **2003**, *89*, 31–39. [CrossRef] [PubMed]
45. Zhao, F.; Wang, Y.; An, H.; Hao, Y.; Hu, X.; Liao, X. New Insights into the Formation of Viable but Nonculturable *Escherichia coli* O157:H7 Induced by High-Pressure CO₂. *mBio* **2016**, *7*, e00961-16. [CrossRef] [PubMed]
46. Oliver, J.D. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiol. Rev.* **2010**, *34*, 415–425. [CrossRef] [PubMed]
47. Truchado, P.; Gil, M.I.; Kostic, T.; Allende, A. Optimization and validation of a PMA qPCR method for *Escherichia coli* quantification in primary production. *Food Control* **2016**, *62*, 150–156. [CrossRef]
48. Gensberger, E.T.; Polt, M.; Konrad-Köszler, M.; Kinner, P.; Sessitsch, A.; Kostić, T. Evaluation of quantitative PCR combined with PMA treatment for molecular assessment of microbial water quality. *Water Res.* **2014**, *67*, 367–376. [CrossRef] [PubMed]
49. Nocker, A.; Camper, A.K. Novel approaches toward preferential detection of viable cells using nucleic acid amplification techniques. *FEMS Microbiol. Lett.* **2009**, *291*, 137–142. [CrossRef]
50. Truchado, P.; Gil, M.I.; Larrosa, M.; Allende, A. Detection and Quantification Methods for Viable but Non-culturable (VBNC) Cells in Process Wash Water of Fresh-Cut Produce: Industrial Validation. *Front. Microbiol.* **2020**, *11*, 527470. [CrossRef]
51. Van Haute, S.; Tryland, I.; Escudero, C.; Vanneste, M.; Sampers, I. Chlorine dioxide as water disinfectant during fresh-cut iceberg lettuce washing: Disinfectant demand, disinfection efficiency, and chlorite formation. *LWT* **2017**, *75*, 301–304. [CrossRef]
52. Helbling, D.E.; VanBriesen, J.M. Free Chlorine Demand and Cell Survival of Microbial Suspensions. *Water Res.* **2007**, *41*, 4424–4434. [CrossRef]
53. Lin, H.; Ye, C.; Chen, S.; Zhang, S.; Yu, X. Viable but non-culturable *E. coli* induced by low level chlorination have higher persistence to antibiotics than their culturable counterparts. *Environ. Pollut.* **2017**, *230*, 242–249. [CrossRef]
54. Bommer, A.; Böhrer, O.; Johannsen, E.; Dobrindt, U.; Kuczius, T. Effect of chlorine on cultivability of Shiga toxin producing *Escherichia coli* (STEC) and β -lactamase genes carrying *E. coli* and *Pseudomonas aeruginosa*. *Int. J. Med. Microbiol.* **2018**, *308*, 1105–1112. [CrossRef] [PubMed]

55. Truchado, P.; Gil, M.I.; Allende, A. Peroxyacetic acid and chlorine dioxide unlike chlorine induce viable but non-culturable (VBNC) stage of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in wash water. *Food Microbiol.* **2021**, *100*, 103866. [[CrossRef](#)] [[PubMed](#)]
56. Liu, Y.; Wang, C.; Tyrrell, G.; Hrudefy, S.E.; Li, X.F. Induction of *Escherichia coli* O157:H7 into the viable but non-culturable state by chloraminated water and river water, and subsequent resuscitation. *Environ. Microbiol. Rep.* **2009**, *1*, 155–161. [[CrossRef](#)] [[PubMed](#)]
57. JTaylor, H.; Rogers, S.J.; Holah, J.T. A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa* at 10 and 20 °C. *J. Appl. Microbiol.* **1999**, *87*, 718–725. [[CrossRef](#)] [[PubMed](#)]
58. Bang, J.; Hong, A.; Kim, H.; Beuchat, L.R.; Rhee, M.S.; Kim, Y.; Ryu, J.-H. Inactivation of *Escherichia coli* O157:H7 in biofilm on food-contact surfaces by sequential treatments of aqueous chlorine dioxide and drying. *Int. J. Food Microbiol.* **2014**, *191*, 129–134. [[CrossRef](#)] [[PubMed](#)]
59. Afari, G.K.; Hung, Y.C. Detection and Verification of the Viable but Nonculturable (VBNC) State of *Escherichia coli* O157:H7 and *Listeria monocytogenes* Using Flow Cytometry and Standard Plating. *J. Food Sci.* **2018**, *83*, 1913–1920. [[CrossRef](#)] [[PubMed](#)]
60. Bridges, D.F.; Lacombe, A.; Wu, V.C.H. Integrity of the *Escherichia coli* O157:H7 Cell Wall and Membranes After Chlorine Dioxide Treatment. *Front. Microbiol.* **2020**, *11*, 532441. [[CrossRef](#)] [[PubMed](#)]
61. Visvalingam, J.; Holley, R.A. Evaluation of chlorine dioxide, acidified sodium chlorite and peroxyacetic acid for control of *Escherichia coli* O157:H7 in beef patties from treated beef trim. *Food Res. Int.* **2018**, *103*, 295–300. [[CrossRef](#)]
62. Bridges, D.F.; Lacombe, A.; Wu, V.C.H. Fundamental Differences in Inactivation Mechanisms of *Escherichia coli* O157:H7 Between Chlorine Dioxide and Sodium Hypochlorite. *Front. Microbiol.* **2022**, *13*, 923964. [[CrossRef](#)]
63. Highmore, C.J.; Warner, J.C.; Rothwell, S.D.; Wilks, S.A.; Keevil, C.W. Viable-but-Nonculturable *Listeria monocytogenes* and *Salmonella enterica* Serovar Thompson Induced by Chlorine Stress Remain Infectious. *mBio* **2018**, *9*, e00540-18. [[CrossRef](#)]
64. Cappelletti, J.M.; Besnard, V.; Roche, S.M.; Velge, P.; Federighi, M. Avirulent viable but non culturable cells of *Listeria monocytogenes* need the presence of an embryo to be recovered in egg yolk and regain virulence after recovery. *Vet. Res.* **2007**, *38*, 573–583. [[CrossRef](#)]
65. Dinu, L.D.; Bach, S. Induction of viable but nonculturable *Escherichia coli* O157:H7 in the phyllosphere of lettuce: A food safety risk factor. *Appl. Environ. Microbiol.* **2011**, *77*, 8295–8302. [[CrossRef](#)]
66. Decol, L.T.; López-Gálvez, F.; Truchado, P.; Tondo, E.C.; Gil, M.I.; Allende, A. Suitability of chlorine dioxide as a tertiary treatment for municipal wastewater and use of reclaimed water for overhead irrigation of baby lettuce. *Food Control* **2019**, *96*, 186–193. [[CrossRef](#)]
67. Banach, J.L.; van Bokhorst-van de Veen, H.; van Overbeek, L.S.; van der Zouwen, P.S.; Zwietering, M.H.; van der Fels-Klerx, H.J. Effectiveness of a peracetic acid solution on *Escherichia coli* reduction during fresh-cut lettuce processing at the laboratory and industrial scales. *Int. J. Food Microbiol.* **2020**, *321*, 108537. [[CrossRef](#)] [[PubMed](#)]
68. Hilgren, J.; Swanson, K.M.J.; Diez-Gonzalez, F.; Cords, B. Inactivation of *Bacillus anthracis* Spores by Liquid Biocides in the Presence of Food Residue. *Appl. Environ. Microbiol.* **2007**, *73*, 6370. [[CrossRef](#)]
69. Fatemi, P.; Frank, J.F. Inactivation of *Listeria monocytogenes*/*Pseudomonas* biofilms by peracid sanitizers. *J. Food Prot.* **1999**, *62*, 761–765. [[CrossRef](#)] [[PubMed](#)]
70. Keskinen, L.A.; Todd, E.C.D.; Ryser, E.T. Impact of bacterial stress and biofilm-forming ability on transfer of surface-dried *Listeria monocytogenes* during slicing of delicatessen meats. *Int. J. Food Microbiol.* **2008**, *127*, 298–304. [[CrossRef](#)]
71. Oliver, J.D.; Dagher, M.; Linden, K. Induction of *Escherichia coli* and *Salmonella typhimurium* into the viable but nonculturable state following chlorination of wastewater. *J. Water Health* **2005**, *3*, 249–257. [[CrossRef](#)]
72. Zhang, S.; Ye, C.; Lin, H.; Lv, L.; Yu, X. UV Disinfection Induces a Vbnc State in *Escherichia coli* and *Pseudomonas Aeruginosa*. *Environ. Sci. Technol.* **2015**, *49*, 1721–1728. [[CrossRef](#)]
73. Chen, S.; Li, X.; Wang, Y.; Zeng, J.; Ye, C.; Li, X.; Guo, L.; Zhang, S.; Yu, X. Induction of *Escherichia coli* into a VBNC state through chlorination/chloramination and differences in characteristics of the bacterium between states. *Water Res.* **2018**, *142*, 279–288. [[CrossRef](#)]
74. Pinto, D.; Santos, M.A.; Chambel, L. Thirty years of viable but nonculturable state research: Unsolved molecular mechanisms. *Crit. Rev. Microbiol.* **2015**, *41*, 61–76. [[CrossRef](#)] [[PubMed](#)]
75. Ordax, M.; Biosca, E.G.; Wimalajeewa, S.C.; López, M.M.; Marco-Noales, E. Survival of *Erwinia amylovora* in mature apple fruit calyces through the viable but nonculturable (VBNC) state. *J. Appl. Microbiol.* **2009**, *107*, 106–116. [[CrossRef](#)] [[PubMed](#)]
76. Zhang, S.; Guo, L.; Yang, K.; Zhang, Y.; Ye, C.; Chen, S.; Yu, X.; Huang, W.E.; Cui, L. Induction of *Escherichia coli* into a VBNC State by Continuous-Flow UVC and Subsequent Changes in Metabolic Activity at the Single-Cell Level. *Front. Microbiol.* **2018**, *9*, 390353. [[CrossRef](#)]
77. Zhu, L.; Shuai, X.; Xu, L.; Sun, Y.; Lin, Z.; Zhou, Z.; Meng, L.; Chen, H. Mechanisms Underlying the Effect of Chlorination and UV Disinfection on VBNC State *Escherichia coli* Isolated from Hospital Wastewater. *J. Hazard. Mater.* **2022**, *423*, 127228. [[CrossRef](#)] [[PubMed](#)]
78. Pinto, D.; Almeida, V.; Santos, M.A.; Chambel, L.M.M. Resuscitation of *Escherichia coli* VBNC cells depends on a variety of environmental or chemical stimuli. *J. Appl. Microbiol.* **2011**, *110*, 1601–1611. [[CrossRef](#)] [[PubMed](#)]

79. Senoh, M.; Ghosh-Banerjee, J.; Ramamurthy, T.; Hamabata, T.; Kurakawa, T.; Takeda, M.; Colwell, R.R.; Nair, G.B.; Takeda, Y. Conversion of viable but nonculturable *Vibrio cholerae* to the culturable state by co-culture with eukaryotic cells. *Microbiol. Immunol.* **2010**, *54*, 502–507. [[CrossRef](#)]
80. Said, M.B.; Saad, M.B.; Achouri, F.; Bousselmi, L.; Ghrabi, A. The application of phage reactivation capacity to sens bacterial viability and activity after photocatalytic treatment. *Environ. Technol.* **2021**, *42*, 2836–2844. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.