https://doi.org/10.1016/j.jclepro.2022.131958

Hydrogen peroxide-assisted pasteurization: an alternative for household water disinfection

Kamila Jessie Sammarro Silva, Luan de Souza Leite, Luiz Antonio Daniel, Lyda Patricia Sabogal-Paz*

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, 400 Trabalhador São-carlense Avenue, São Carlos, São Paulo 13566-590, Brazil.

*E-mail of the corresponding author: lysaboga@sc.usp.br

Orcid: Kamila Jessie Sammarro Silva https://orcid.org/0000-0002-6881-4217

Luan de Souza Leite https://orcid.org/0000-0002-2108-2960

Luiz Antonio Daniel https://orcid.org/0000-0002-1765-4209

Lyda Patricia Sabogal-Paz http://orcid.org/0000-0003-2753-3248

Abstract

Decentralized treatments applied at the household level may be alternatives to positively impact global health. Pasteurization is a conventional disinfection method and efforts have been made to increase its efficiency and productivity in point-of-entry (POE) applications. To overcome limitations associated to this practice, we hypothesized that including oxidation prior to pasteurization would improve the treatment. This research aimed to evaluate the performance of hydrogen peroxide-assisted pasteurization and effects of H_2O_2 concentration and temperature organized by a full factorial experimental design. At optimal conditions, >9.3 $\log_{10}$ inactivation of *Escherichia coli* and >5.8 $\log_{10}$ Phi X174 bacteriophage were obtained. Observed values were modelled as empirical equations for *E. coli* and phage inactivation (R^2 = 0.76 and 0.72, respectively). Temperature did not lead to significant differences in H_2O_2 residual, which is favorable for practical implementation. Synergistic effects were found for *E. coli*, and inactivation of Phi X174 tops results

obtained by individual disinfection by pasteurization and H_2O_2 oxidation. Our study also suggests H_2O_2-assisted pasteurization increases oxidation potential, inferred by cell lysis and protein removal. Additionally, analyzing disinfection and H_2O_2 residuals during temperature ramp time endorsed that inactivation might happen at lower temperatures, and stability of H_2O_2 may provide a safer setup when heat sources cannot guarantee pasteurization to occur. Broadly, our research underscores potentials of H_2O_2-assisted pasteurization and, although we recommend further assessments considering pathogens, as well as case studies for specific scenarios, we believe H_2O_2 may improve performance and resilience of classic disinfection by pasteurization as a POE solution.

Keywords: factorial design; point-of-entry disinfection; decentralized water systems; bacteriophage; indicator bacteria

1. Introduction

According to the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), approximately 884 million people worldwide lack basic drinking water settings [1]. That is why Sustainable Development Goal 6 (SDG 6) calls for universal and equitable access to affordable and safely managed drinking water services, an objective to be reached by 2030 [2]. Although, globally, this situation moves towards better conditions, the latest estimate suggest this will not be fully achieved unless progress quadruples [3]. Furthermore, access in specific scenarios is often overlooked, and inequalities remain [2,4], emphasizing the need for new frameworks [5].

Many low-income regions struggle with insufficient water compliance, due to a lack of commitment from authorities involving supply, infrastructure, and a service delivery [6]. This growing gap between demand for safe water and conventional supply has allowed decentralized systems to rise as alternative solutions [7,8]. This approach has been emerging in some urban areas [9], in which, though centralized treatment may be available, measurable levels of pathogens have been found [10]. However, mostly, self-supplied regions, where water quality varies between the source and households [11], are significantly more likely to be contaminated [12], hence these may be very positively impacted by on-site setups for treatment or disinfection.

Decentralized water systems include point-of-use (POU) and point-of-entry (POE) treatments, which are technologies used at the household level, that can make water suitable for potability, particularly in terms of reducing pathogen concentration [13], which is one of the most important issues in drinking water systems [14]. In this regard, pasteurization, i.e., microorganism inactivation by water heating below boiling, has been a classic method for household disinfection due to its simplicity and easy implementation [15]. Nonetheless, it has constraints that research has been dedicating to overcome. Efforts have been made aiming to improve systems design, productivity and the safety threshold for microorganism inactivation [16], considering different heat sources, especially solar

energy [17,18]. However, this too may present limitations, as in low irradiation days [16], which should be compensated for, thus bringing incentives towards the integration of technologies [19] that could guarantee and perhaps increase efficiency.

In order to improve this classic method, here we hypothesized that including an oxidant agent other than chlorine at the point-of-entry could enhance performance or even lead to synergistic effects in conventional pasteurization, therefore reducing dependance on external heat sources, or even lower residence periods. Considering hydrogen peroxide (H_2O_2) has been widely applied in surface [20,21], wastewater [22,23], and drinking water disinfection [24–26], it would be a potential candidate for providing more robustness to household pasteurization. Tough there are reports of H_2O_2 applied in hot water to avoid biofilm formation in hospital settings [27], it does not refer to assisted pasteurization itself, which would be a novel approach, especially considering POE/POU applications. Additionally, the mechanisms involved in microorganism inactivation when H_2O_2 and pasteurization are combined, to our knowledge, have not been reported.

In this light, the aim of this study was to assess the performance of H_2O_2-assisted pasteurization as a potential method for disinfection at the household level, considering fecal contamination. This was carried out in terms of inactivation of *Escherichia coli* (indicator bacterium) and Phi X174 bacteriophage (an enteric virus contamination surrogate). Batch experiments were organized by a full factorial design and observed results were used for suggesting empirical models for each target-organism. Additionally, synergistic effects were evaluated, and inferences of cell lysis were performed by protein quantification and imaging with vital stains.

2. Materials and methods

2.1 Experimental setup

Tests were performed on bench scale, simulating a closed-system environment for pasteurization in glass reagent-bottles wrapped in aluminum foil, to avoid photolysis (30% v v^{-1}, Sigma-Aldrich,

USA). Stock solution was readily tested for molar concentration at acquisition and prior to disinfection assays, so that dosing was consistent through the entire research. The volume of test water used was 300 mL. An inlet was placed on the lid for dosing of chemicals and electrode access. Temperature was maintained by water bath, but combined treatments included a five-minute agitation in contact with H_2O_2 by magnetic stirring prior to heating. Afterwards, sample mixing relied exclusively in convection, as in home-scale pasteurization systems by solar thermal heaters [28]. Assisted-pasteurization was performed for 60 minutes so that tested conditions (further detailed) would fit into Zone C of time-temperature combinations for a desirable inactivation threshold for thermal treatments. This "safety-zone" was recommended by a systematic review and meta-analysis that refined results for microbial inactivation considering data for exposure time and temperature needed to achieve specified $\log_{10}$ reductions [29]. Zone C represents a large variability of conditions, which could be descriptive of a household scenario [29].

All material was previously sterilized. Once each test run was complete, H_2O_2 residuals were measured at 470 nm after subjected to the ferric thiocyanate method, using the Vacu-vials® kit (Chemetrics, USA). Temperature effects on H_2O_2 residuals were investigated by Pearson's linear correlation and of analysis of variance (ANOVA), both at the 95% confidence interval. Residuals were quenched by sodium metabisulfite (Neon, Brazil) at mass ratio of 3:1 [30]. Accordingly, bottles were immediately placed on ice to interrupt temperature effect over microorganisms. Microbiological examinations were carried out without delay, so that any residual activity due to possible slow action of the selected quencher [31] would be avoided. The interval between quenching followed by icing samples to room temperature and microorganism examination would not exceed 10 minutes for *E. coli*. The remaining samples would be placed in the fridge (6 e 10°C) so that phage quantification would be carried out within the next day of each assay. After batch tests, inactivation was calculated according to Eq. (1), where *Y* is the inactivation, *N* is the final number of

microorganisms and N_0 is the inoculum (both in terms of either CFU 100 mL^{-1} or PFU mL^{-1}, for *E. coli* or phage, respectively).

$$Y = -log_{10}(\frac{N}{N_0}) \qquad (1)$$

2.2 Test water

Our intent was to simulate a water source suitable for disinfection, thus followed the recommendations for the validation of household treatment technologies provided by WHO [32,33], without adding solids. The simulated matrix was based on the non-technology-specific general test water (TW), which represents high-quality groundwater (WHO, 2014). Briefly, tannic acid (Sigma-Aldrich, USA) was used as a source of total organic carbon (analyzed by TOC-LCPN, Shimadzu, Japan) and sodium carbonate (Qhemis, Brazil) provided alkalinity input. These parameters were adjusted to 1.19 ± 0.19 mg L^{-1} and 55.8 ± 4.3 mg $CaCO_3$ L^{-1}, respectively. Sulfuric acid (Sigma-Aldrich, USA) was added for pH adjustment to 7.1 ± 0.1. The matrix under test was prepared in ultrapure water. Alkalinity and pH adjustments considered measurements following the Standard Methods prior to microorganism spiking [34]. Interferences of the inoculums in physicochemical quality of the TW were neglected in this research.

2.3 Target organisms

Escherichia coli from a lyophilized commercial strain (ATCC® 11229™) was activated and replicated in tryptone soy broth (Oxoid™, USA), following recommendations from the supplier. TW was inoculated with centrifuged aliquots of the suspensions (1972 ×*g*, 15 min, 4 °C), leading to approximate concentrations that varied between 10^8 and 10^9 CFU 100 mL^{-1}. Detection and quantification of colonies were performed by the membrane filtration technique and *E. coli* was grown in Chromocult® Coliform Agar medium (Merck, USA). Petri dishes were kept at 37 °C, incubated for approximately 24 hours.

Phi X174 bacteriophage (ATCC® 13706-B1™) was used as a virus contamination model and *Escherichia coli* (ATCC® 13706™) as its host. TW was spiked with an approximate order of magnitude that varied between 10^5 and 10^6 PFU mL $^{-1}$ of purified work stocks. Samples tested for phage were filtered in 0.2 µm membranes coupled to sterile syringes. Phi X174 quantification was done by the double-layer agar method [35]. Tryptone soya agar (Oxoid™, USA) was used as culture media, whereas top agar consisted of Tryptone soya agar (Oxoid™, USA) and bacteriological agar (Sigma-Aldrich, USA). Plates were incubated at 37 °C for 18−24 hours. Phage was enumerated in terms of PFU mL^{-1}, according to to Eq. (2).

$$(PFU\ mL^{-1}) = \frac{1000 \times average\ PFUs\ on\ plates}{volume\ (\mu L)\ phage\ or\ sample\ added} \times serial\ dilution\ PFUs\ were\ counted\ at \qquad (2)$$

2.4 Experimental design and response surface analysis

Experiments were organized by a complete factorial design (FFD - two factors and two levels, with central point and two repetitions) in terms of temperature (X_1; °C) and H_2O_2 concentration (X_2; % v v^{-1}). These were treated as continuous variables with coded levels of -1, 0 and 1; corresponding to temperature values of 30, 50 and 70 °C and H_2O_2 concentrations of 0.03, 0.06 and 0.09%. These points were selected considering a conservative approach to boundary conditions, as there is plenty of data on *E. coli* pasteurization at >70 °C [36–38], for instance, and hydrogen peroxide disinfection is often described at much higher concentrations, as in >3% [39–41]. Here we chose to describe a situation in which heat sources would not be available steadily and chemicals should be required at a minimum.

Considering peer research as background [42], a quadratic model was chosen for an attempt to fit results of inactivation of *E. coli* (Y_1) and coliphage (Y_2), as shown in Eq. (3), in order to quantify the effects of each factor on the dependent variables.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \qquad (3)$$

Where β_0 is a constant; β_1, β_2 and β_{12}, are the linear and interaction coefficients, respectively, and β_{11} and β_{22} follow the quadratic terms. The fitted surfaces were obtained in Statistica 13.5 (TIBCO Software Inc.). Statistics consisted of ANOVA and coefficients that were not considered significant ($\alpha = 0.05$) were eliminated, so that model parameters were recalculated by the software. The convenience of the model was evaluated by the coefficients of determinations R^2 and R^2 adj.

Effects of H_2O_2 concentration and temperature levels were assessed by the Pareto chart at a 95% confidence interval. Complementarily, tests considering the individual factors were also carried out, at conditions selected by result-dependent criteria to evaluate occurrence of any synergisms. These are detailed in the discussion section. Additionally, the most suitable combination of independent variables was tested for the disinfectant decay analysis, considering results obtained by the empirical model for each target organism, as well as other criteria: applicability, availability of chemicals and heat source, etc. These are further discussed in the results section.

2.5 Disinfectant decay monitoring

Residual disinfectant was monitored by timed sampling of TW subjected to H_2O_2-assisted pasteurization under conditions selected as adequate, considering criteria detailed in the discussion topic. After each contact time was reached, samples were collected, and residual disinfectant concentration was immediately measured. This monitoring was performed considering time zero as the moment in which samples reached the selected pasteurization temperature. Simultaneously, samples were characterized in terms of pH and ORP (mV), using commercial electrodes (Orion™, USA and Sensorglass™, Brazil, respectively).

This step of the methodology included an extra and result-oriented investigation, as data showed no differences in ORP during the 60-min pasteurization batch. Additionally, an attempt of disinfection kinetics at fixed temperatures was performed, nonetheless absence of microorganisms found after 5, 10 and 15 min of monitoring instigated further inquiry: this analysis was extended to the ramp time,

i.e., the time required for samples to change from initial temperature to target temperature. In the present study, this time had been previously standardized as 10-15 minutes, subjected to equipment limitations. These conditions were replicated for the extra tests seeking to analyze the effect of temperature ramp. Throughout ramp time, samples were monitored for ORP, pH, as well as residual H_2O_2. The latter was measured at the specific times at which samples reached intermediary temperatures, described in the results topic.

2.6 Protein quantification

Seeking to investigate mechanisms of microorganism inactivation, soluble protein content was evaluated for individual conditions and the ideal combinations defined by the analysis of synergistic effect. In this sense, the Bradford reagent (Sigma-Aldrich, USA) was applied for measuring protein (n = 3) at 595 nm. Bovine serum albumin (Sigma-Aldrich, USA) was used as standard.

2.7 Bacteria viability assessment

Inferences on cell lysis were made by investigating cell dye uptake as well as metabolic activity. This was put through by two separate methods: 40,6-diamidino-2-phenylindole (DAPI) staining, as well as a simultaneous vital dye assay, from a commercial kit (ab115347, Abcam®, UK). Samples were concentrated by centrifugation (1972 ×*g*; 10 min; 4°C) to avoid any additional cellular damage during sample processing. Slides were prepared with 10 µL aliquots from a preserved pellet of approximately 5 mL. The two different stains were not applied to the same microscopy wells, so the final micrographs referred to distinct aliquots from the same samples.

Two drops of Fluoroshield™ with DAPI (F6057, Sigma-Aldrich®) were added to each slide well similarly to research that included DAPI to assess viability and cellular morphology integrity [43]. Intracellular DNA was supposed to be observed by DAPI-staining under a maximum excitation of 385 nm and maximum emission of 420 nm.

The live/dead assay was performed according to the manufacturer's protocol for microscopy, considering details described in similar research [44]. Briefly, the concentrated reagent (1000×) was diluted in phosphate buffer saline solution (pH 7.4, PBS tables from Oxoid™, USA). The 10×-solution was overlaid to the suspensions in the same volumes of such, directly in the glass slide. Green fluorescence from the metabolism of esterase substrates were expected from live organisms (visualized under a maximum excitation of 495 nm and 520 nm emission, compatible with FITC). Non-viable bacteria were supposed to be visualized in red because of the incorporation of red dye, impermeable to the membranes. This should increase red fluorescence under 617 nm and 528 nm maximum excitation and emission, allowing observation under FITC as well as the PI-filter (in bright red).

Slide preparation was done in the absence of direct light, in an air flow chamber. No washings of the microscopy glass slides were carried out and wells were sealed with coverslip as soon as they dried. Each slide was stored at 4°C in a Petri dish wrapped in aluminum foil until imaging, which was carried out within the same week as slide preparation. Observations were done in an epifluorescence microscope (BX51, Olympus®) at 1000X magnification with immersion oil. Imaging was obtained by Image-Pro® 6.3.

3. Results and discussion

3.1 Empirical model analysis

Results obtained from FFD experiments led to empirical models for predicting *E. coli* (Y_1) and bacteriophage $\log_{10}$ inactivation (Y_2). Responses were modeled as a function of temperature (X_1) and initial H_2O_2 dose (X_2). Eq. (4) and Eq. (5) represent the respective models for each microorganism, indicating only the individual linear contributions of the independent variables were significant (*p*-value < 0.05) for disinfection. The effects of these statistically significant coefficients are illustrated

by the Pareto charts in Figure 1 and details of ANOVA are available in the supplementary material (tables S.1 and S.2).

$$Y_1 = -1.802 + 0.116X_1 + 34.548X_2 \qquad (4)$$

$$Y_2 = -2.248 + 0.082X_1 + 37.823X_2 \qquad (5)$$

Physically, linear components of the variables presented a positive impact in inactivating both targets. Absolute values of the estimate effect were higher for temperature, as shown by Figure 1, agreeing with the expectations from this study, as pasteurization was the main disinfection method, enhanced by H_2O_2.

Although interaction effects (β_{12}) were not statistically significant within neither empirical model (p > 0.05, thus not represented in Figure 1), adding H_2O_2 prior to pasteurization may still be promising considering scenarios where the heat source is intermittent. In these situations, exposure to the pasteurization temperature could be discontinuous leading to deficiencies in disinfection. In this situation, it would be expected to still present a linear correlation to inactivation of microorganisms, even if only due to hydrogen peroxide. As interaction of the two independent values directly refers to synergistic effects, further discussion (based on observed values) is present in section 3.2.

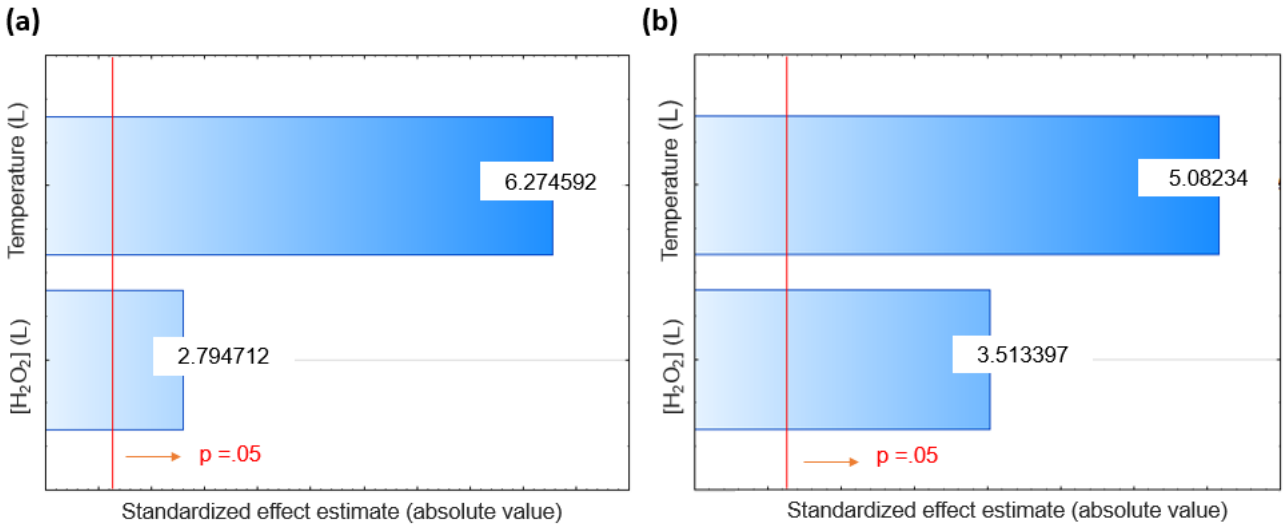

Figure 1 Pareto charts of the significant effects (p-value > 0.05) of temperature and concentration of hydrogen

peroxide on (a) *E. coli* $\log_{10}$ inactivation; (b) Phi X174 $\log_{10}$ inactivation. (L) refers to the linear component of the adjusted model.

Figure 2 displays the fitted surfaces for the inactivation of *E. coli* and phage. R^2 values were 0.76 (R^2 adj = 0.73) and 0.72 (R^2 adj = 0.68) for Y_1 and Y_2, respectively. Neither coefficient of determination met expectations of an overall efficiency of prediction, thus presenting limitations in describing the system. We believe this refers to the limits of quantification in case of absence of microorganisms. However, it is worth pointing out that R^2 and R^2 adj were similar for both empirical equations. Peer research has also worked with this range of R^2 when analyzing effects of different parameters in solar disinfection by multiple regression of full factorial experiments [45].

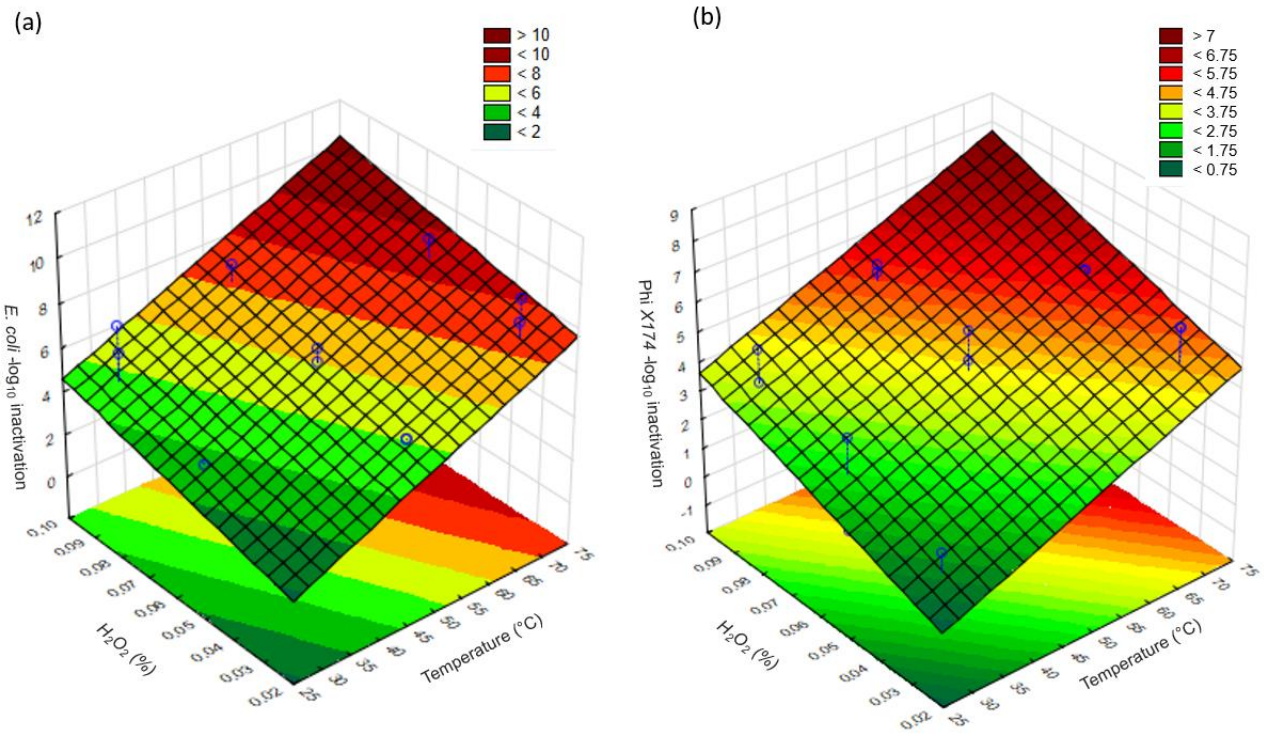

Figure 2 Fitted surfaces and contour plots for the empirical models generated by the FFD. Coefficients not statistically significant (*p*-values > 0.05) were removed prior to surface plotting. Dependent variables: (a) -$\log_{10}$ inactivation (R^2 = 0.76) of *E. coli*; (b) Phi X174 phage (R^2 = 0.72).

In addition, analyzing residues should also be considered when judging model adequacy [46]. These residues refer to the difference between predicted and actual values (table 1). Both models presented

a poorer fit to high levels of inactivation when boundary conditions were considered i.e., high H_2O_2 concentrations and/or high temperatures. Again, that should possibly refer to the limiting effect of the initial microorganism population, i.e., log_{10} inactivation results are equal when there is absence of UFC 100 mL^{-1} or PFU mL^{-1} in treated samples, even if they could be potentially higher. In this sense, the models are not recommended for predictions near extremes, but do provide overall projections of H_2O_2-assisted pasteurization behavior.

Table 1 Actual and predicted values for the inactivation of *Escherichia coli* and Phi X174 phage by hydrogen peroxide-assisted pasteurization

	***E. coli* -log$_{10}$ inactivation**			**Phi X174 -log$_{10}$ inactivation**		
Condition (°C; % H_2O_2)	**Observed**	**Predicted**	**Residues**	**Observed**	**Predicted**	**Residues**
1 (30; 0.03)	1.809	2.725	-0.917	0.716	1.349	-0.633
2 (30; 0.06)	3.534	3.762	-0.227	0.540	2.484	-1.944
3 (30; 0.09)	6.024	4.798	1.226	3.521	3.619	-0.098
4 (50; 0.03)	5.021	5.052	-0.031	1.342	2.991	-1.649
5 (50; 0.06)	5.919	6.089	-0.170	>5.491	4.125	1.366
6 (50; 0.09)	4.393	7.125	-2.732	>5.491	5.260	0.231
7 (70; 0.03)	>7.929	7.379	0.550	>5.803	4.632	1.171
8 (70; 0.06)	>7.929	8.416	-0.486	>5.803	5.767	0.036
9 (70; 0.09)	>7.929	9.452	-1.523	>5.803	6.901	-1.099
10 (30; 0.03)	1.563	2.725	-1.163	1.986	1.349	0.636
11 (30; 0.06)	3.234	3.762	-0.527	3.696	2.484	1.212
12 (30; 0.09)	7.285	4.798	2.487	4.630	3.619	1.011
13 (50; 0.03)	5.029	5.052	-0.023	1.775	2.991	-1.215
14 (50; 0.06)	6.538	6.089	0.450	4.491	4.125	0.366
15 (50; 0.09)	7.874	7.125	0.749	>5.792	5.260	0.532
16 (70; 0.03)	9.006	7.379	1.627	>5.792	4.632	1.160

17 (70; 0.06)	>9.289	8.416	0.873	>5.792	5.767	0.026
18 (70; 0.09)	>9.289	9.452	-0.163	>5.792	6.901	-1.109

From analyzing observed reductions displayed in table 1, the average inactivation obtained equals to 6.089 $\log_{10}$ for bacteria and 4.125 $\log_{10}$ for virus, both temperature- and concentration-independent. This average performance suggests that H_2O_2-assisted pasteurization falls into the 3-star category of protection against bacteria and 2-star against virus, considering criteria set forth to evaluate household treatment options [47,48]. It should be noted that both of the aforementioned categories are comprehensively safe against three of the main classes of waterborne pathogens, particularly considering thermal inactivation [32].

However, this general assessment neglects the poorer inactivation values found for boundary conditions of low temperature and H_2O_2 concentration. Indeed, research on hydrogen peroxide oxidation aiming water treatment often mentions that higher doses and a long contact time are required [49,50], which is why we are focusing on a combined treatment to produce clean water instead of the conventional standalone approaches.

In this sense, it is recommended that any products based on the present treatment should rely on mechanisms that guarantee inactivation thresholds that meet 3-star or 2-star levels of quality. In terms of system design, these could be attained by installing automated dosing devices or thermostatic valves, so that water is only released when a certain temperature is reached. Although this POE adaptation is a topic for further research on practice and field application, there are some references on combined solar plants, for instance, that applied simple thermostatic outlets [51] that could be useful for H_2O_2-assisted pasteurization systems. In addition, shell-and-tube heat exchangers [17], as well as many other improvements that have been discussed on the topic of energy and sanitation [52,53] could be implemented to achieve desired temperature conditions. Such potential indicates that H_2O_2-assisted pasteurization may be an innovative subject for research not only on

disinfection, but also cleaner water production aligned with different SDGs (e.g., affordable and clean energy, etc.).

3.2 Analysis of synergistic effect

Synergic effects were studied by testing temperature and H_2O_2 dosing as single components. Synergism is defined by an enhanced inactivation, which should be higher than the inactivation level obtained by the sum of those achieved when each disinfection mechanism is applied separately [54].

Selected conditions for this assessment were 70°C and H_2O_2 at 0.03 and 0.06%. These were chosen considering the absolute $\log_{10}$ inactivation values obtained for the combined conditions of such concentrations at 70°C, which both led to absence of indicator bacteria and the virus contamination model. Figure 3 displays results for each isolated disinfection method, the sum of their effects, as well as the average observed values (table 1) for the combined treatment, i.e., assisted pasteurization (represented by the baselines).

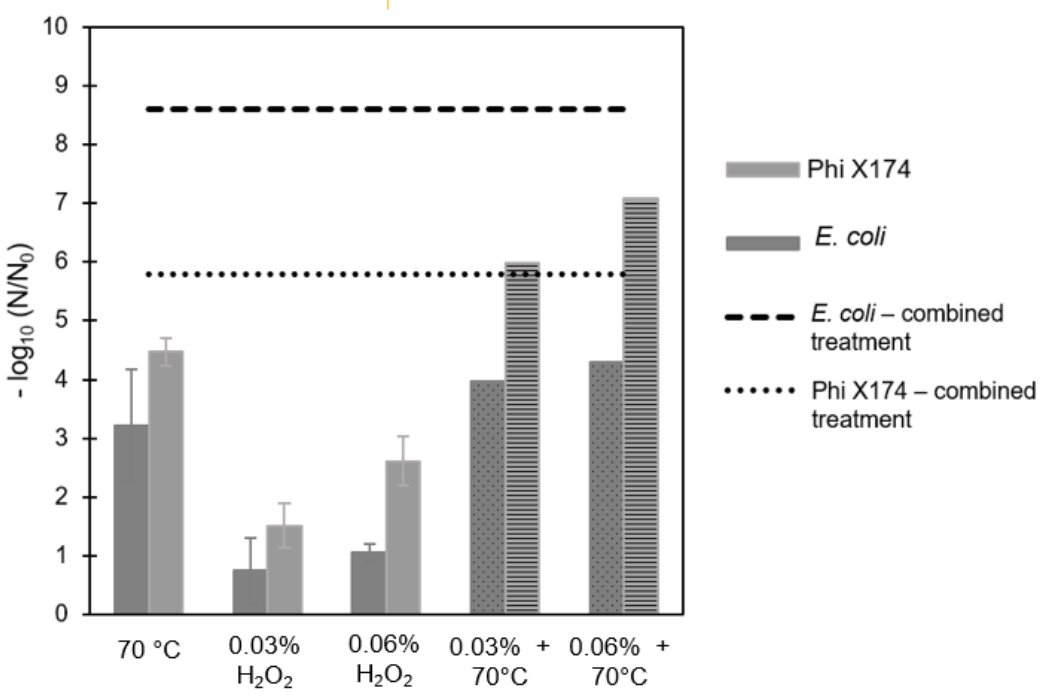

Figure 3 *E. coli* and Phi X174 bacteriophage inactivation by isolated disinfection methods, compared to the sum of standalone components. Textured columns refer to the sum of results obtained by individual

treatments. Baselines indicate the average inactivation obtained by assisted pasteurization (equal at both H_2O_2 doses). Error bars refer to standard deviation.

As standalone pasteurization at 70 °C for 60 min provided a higher absolute value for $\log_{10}$ reduction of both indicator bacteria and phage, comparatively to the oxidation treatments, it is possible to assume it should also play the major role in the combined disinfection. These results align with the inferences from the Pareto chart (figure 1), which suggested that increase in temperature provides more prominent effect in microorganism inactivation than changes in H_2O_2 concentration.

The sum of disinfection mechanisms suggested there might be a synergistic effect in *E. coli* inactivation by assisted pasteurization, as the combined treatments yielded a higher inactivation ($-\log_{10} = 8.609 \pm 0.680$). However, this assumption does not apply to phage. Figure 2 indicates the average inactivation of Phi X174 by the combined treatment ($-\log_{10} = 5.797 \pm 0.005$) surpasses results obtained by pasteurization and both concentrations of H_2O_2 as a sole disinfectant but does not reach the sum of their combined effects, meaning enhancement in performance, but no synergism per se.

These results suggest a satisfactory reduction in oxidant demand while still providing high disinfection efficiency. A recent study that relied on standalone H_2O_2 for water disinfection required a 10-fold higher dose at the same exposure time to obtain an approximate 8-log reduction of *E. coli* [49].

3.3 Temperature effect in hydrogen peroxide residual

Poor correlation was found for temperature and H_2O_2 residuals (Figure 4), but results were not considered significant at a 95% confidence interval. Pearson's coefficients are presented in Table 2, considering residuals grouped by different initial concentrations of H_2O_2. Additionally, these values were analyzed by ANOVA, as data was normally distributed (p-values > 0.05, Shapiro-Wilk test), leading to $p > 0.05$ for all groups. Data shown in Figure 4 refers to significantly similar means for

H_2O_2 residuals in different temperatures, regardless of initial concentrations. This may be beneficial from a practice standpoint, as residuals (to be neutralized) would more likely depend on H_2O_2 concentration, regardless of the temperature that the pasteurization system could provide.

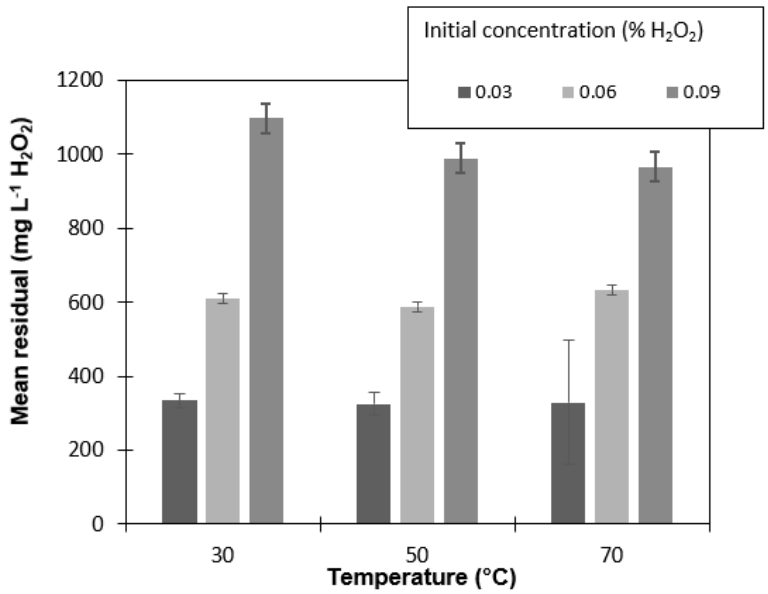

Figure 4 Hydrogen peroxide residuals obtained after assisted pasteurization in different temperatures and initial H_2O_2 concentrations. Error bars refer to standard deviation.

Table 2 Correlation of temperature and hydrogen peroxide residuals after assisted-pasteurization disinfection ($\alpha = 0.05$).

	Initial H_2O_2 concentration (%)		
	0.03	**0.06**	**0.09**
r	-0.140	0.195	-0.424
***p*-value**	0.719	0.614	0.255

3.4 Residual monitoring

Hydrogen peroxide residuals were assessed through time under selected conditions to evaluate the potential of complimentary disinfection. Figure 5 (a) displays the data obtained for residual

concentration during disinfection by 0.06% H_2O_2 at 70 °C for 60 min. Additionally, Figure 5 illustrates the behavior of ORP and pH through time.

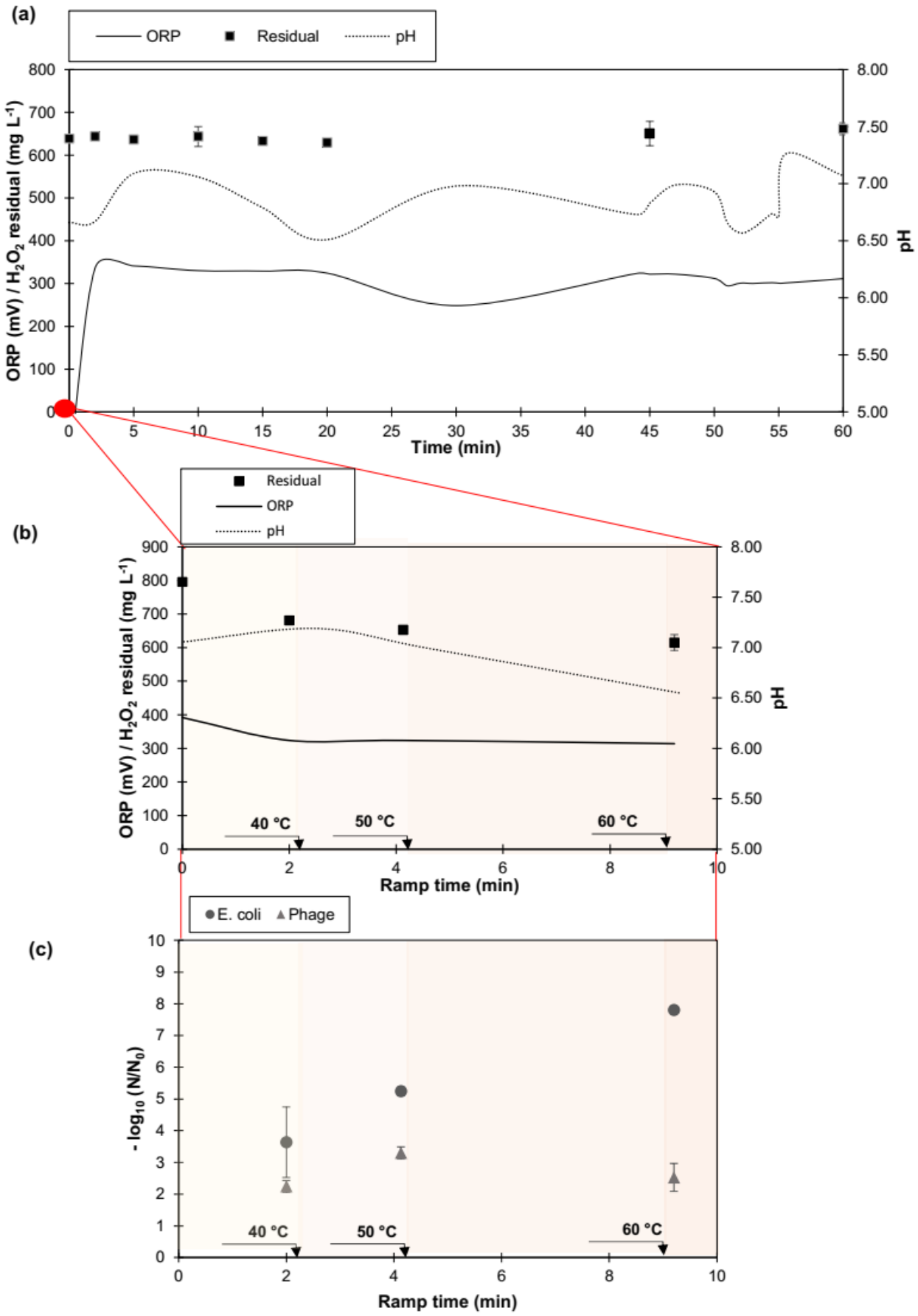
(a)
ORP
Residual
pH
ORP (mV) / H_2O_2 residual (mg L^{-1})
pH
Time (min)
(b)
Residual
ORP
pH
ORP (mV) / H_2O_2 residual (mg L^{-1})
pH
40 °C
50 °C
60 °C
Ramp time (min)
(c)
E. coli
Phage
- $\log_{10}(N/N_0)$
40 °C
50 °C
60 °C
Ramp time (min)

Figure 5 Hydrogen peroxide residuals, ORP and pH during assisted pasteurization at 0.06% initial H_2O_2 concentration (a) through disinfection at 70 °C; (b) through ramp time for reaching 70 º C; (c) *E. coli* and phage inactivation as a function of reached temperature (40, 50 and 60 °C) through ramp time. Error bars refer to standard deviation.

The potential measured using an ORP electrode is affected by all of the redox reactions occurring at the electrode surface, making it difficult to fundamentally relate it to one particular redox reaction [55,56]. However, if the measured potential differs greatly from the theoretical value, it may still provide a useful signal for process control [34]. Nonetheless, Figure 5 (a) did not present any clear shifts in ORP which could possibly correlate to results from residual monitoring. Considering the overall stable pattern found for ORP, pH and H_2O_2 residuals, no inferences were made.

Samples were collected for analyzing the kinetic behavior of microorganism inactivation by assisted pasteurization. At 15-, 10- and 5-min treatment, there was absence of microorganisms, meaning >7.60 and >5.56 absolute $\log_{10}$ inactivation for *E. coli* and phage, respectively. In this light, further investigation was carried out, considering the ramp time, an important feature that is not often specified in pasteurization research seeking disinfection within the sanitation field [57]. This led to results shown in Figures 5 (b) and (c), which ratify observed values from Table 1 for lower temperatures, even though contact time in those conditions was longer.

This general evaluation suggests that assisted pasteurization may be a timely alternative for POU or POE settings, particularly when external heat sources are not stable. Pasteurization research, when focused on industry applications, does not often require ramp time assessment, because of resources availability (e.g., electricity). A study has indicated that high-temperature heating, long- and short-time pasteurization (30 s) were reliable methods for completely inactivating polioviruses in water, milk, and yoghurt [58]. Similarly, microwave heating provided satisfactory levels of bacteria inactivation at 65 °C for 65 to 70 s [59], but this method presents very low ramp time. Applications such as solar pasteurization often deal with longer ramp and contact times. An automated solar

pasteurizer design for water decontamination led to disinfection at 55 °C for 60 min, 60 °C for 45 min, 65 °C for 30 min, 75°C for 15 min, and 85 °C for 15 s [60]. Also, when dealing with natural conditions, as in many reports from literature in pasteurization within the sanitation scene [18,61,62], there is no guarantee of the reached temperature, which is why monitoring is an important aspect. If pasteurization systems do not yield reliable temperatures within the "safety zone" [63], complimentary disinfection methods such as hydrogen peroxide oxidation may play a key role.

Stability in H_2O_2 residual through ramp time implies that most of the demand derived from characteristics of the study water, not the pathogens themselves. In addition, this short period demand corroborates findings from other disinfection studies, as in those that applied oxidants as peracetic acid and chlorine in wastewater and considered a five-minute demand [64]. Here, most H_2O_2 consumption had already occurred at two minutes.

It should be noted, however, that future research on the design of assisted-pasteurization devices or coupled-systems, prior to any implementation in households, should consider residual kinetic decay in time intervals that exceed the treatment assessed in our research (i.e., ramp time + treatment), as well as throughout it. That is because the need for residual neutralization units has to be evaluated, along with toxicity levels that guarantee safety for handling and consuming the treated water effluent.

3.5 Oxidation and cell lysis

Protein removal achieved by 60 min of standalone pasteurization (70 °C), H_2O_2 oxidation (0.06%) and designated optimal conditions of H_2O_2-assisted pasteurization (0.06%; 70ºC) are shown in Table 3. Bacterium organic matter of *E. coli* contains a large proteinaceous fraction (approximately 65% of the dissolved organic carbon) [65], which may cause oxygen demand. From our results, higher removals found for hydrogen peroxide and assisted pasteurization suggest there was oxidation of the samples. Nonetheless, considering the possibility of cell lysis illustrated by the micrographs in

Figure 6, our samples do not refer to a closed system, considering that leaking of intracellular material may increase oxidant demand, and dissolved protein levels might also be affected by denaturation of cell components. Thus, interpretation is limited as we cannot assertively affirm if protein removal refers to dissolved content in the inoculated TW, intracellular protein, or both. Additionally, results from Table 3 were obtained by duplicates, which hinders interpretations based on inferential statistics, probably including experimental error that could be reduced by a larger number of repetitions. If further research focuses on oxidation and cell damage, a more detailed assessment is recommended, also including a mass balance of protein content in microorganisms, suspension media and TW.

Table 3 Protein removals obtained by pasteurization, H_2O_2 oxidation and H_2O_2-assisted pasteurization. Notes: Initial protein content in inoculated test water = 5.72 ± 0.07 mg L^{-1}. Protein removals were calculated in duplicates.

Treatment	Removal (%)
Pasteurization	49.58
H_2O_2	56.30
H_2O_2-assisted pasteurization	57.14

Figure 6 displays illustrative representations of the overall appearance of staining by two different viability assessments. Images above the line refer to a different aliquot from the same sample used for the two micrographs below the line, which is why these first captures do not refer to the same frames as the two below them.

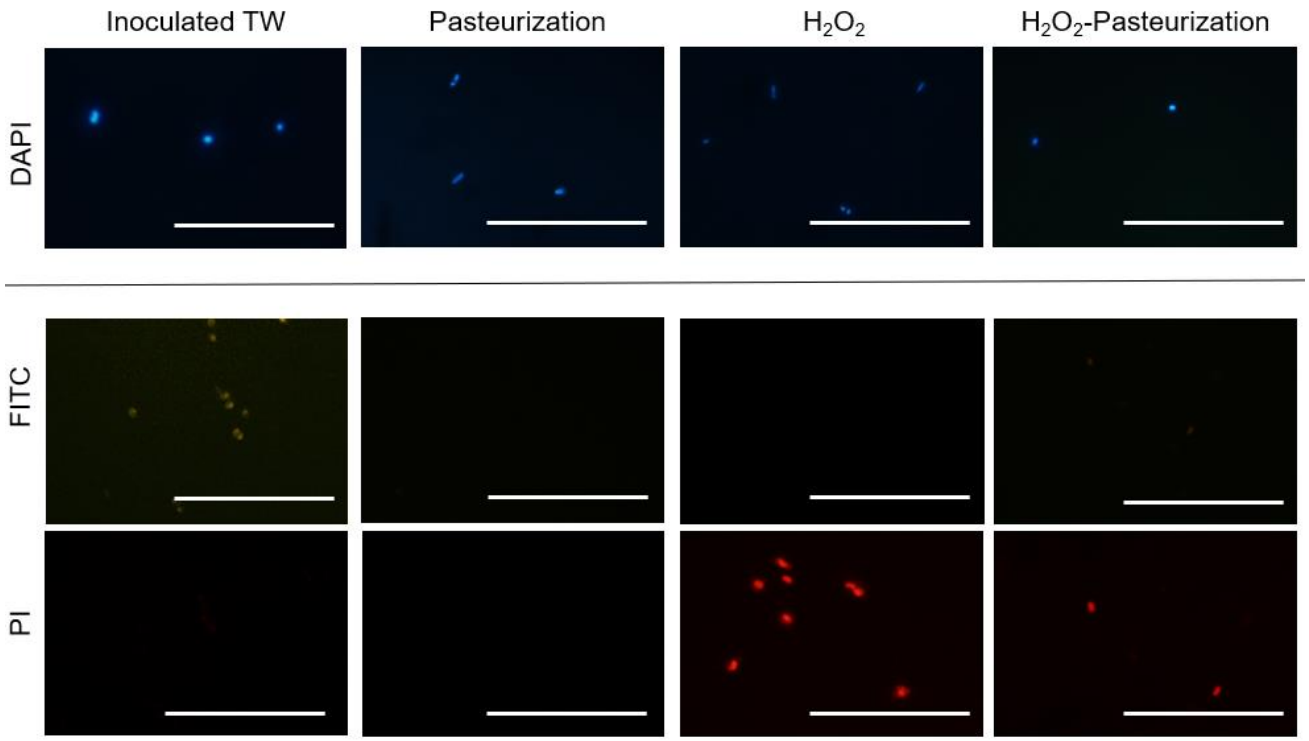

Figure 6 Micrographs of the raw water (positive control) and inactivated *E. coli* stained by different methods. Inactivation treatments are stated in the columns and rows display different microscopy filters. The solid black line horizontally separates micrographs from two different aliquots of the same samples. Representative pictures are shown at 1000× (oil immersion). Notes: TW = Test water; Scale bars = 10 µm.

Observations under FITC did not show high signal for untreated samples, which we believe refers to limitations in the performance of the live/dead kit, whose protocol has not been optimized for the present research. As expected, no cells were visualized under FITC in the microscopy slides of treated samples.

We observed intracellular DAPI signal after pasteurization, which confirms that DNA was retained in the nucleus. This complies with similar research, that tested pasteurization for bacteria inactivation while maintaining cell integrity [43]. No major PI-uptake was noticed in this treatment, endorsing pasteurization under these selected conditions did not lead to considerable cell lysis.

As for oxidation treatments, i.e., H_2O_2 and H_2O_2-assisted pasteurization, although Figure 6 illustratively displays examples of some DAPI-staining, these were very dispersed on the microscopy slides, particularly for the combined disinfection. In this sense, micrographs were shown

representatively, but no major signal was scored under the microscope. The overall aspect of the samples visualized after oxidant treatments had barely shown blue fluorescence and the images shown on Figure 6 were exceptions selected for illustration. We believe leaked DNA could have been stained and this assumption is backed up by intense red staining found under PI-filter.

PI-stained bacteria were easily detected in both hydrogen peroxide inactivation and H_2O_2-assisted pasteurization. This red signal suggests cell lysis in both treatments.

The abovementioned inferences on cell lysis align with protein removal results, as H_2O_2 may have oxidated dissolved protein from inoculated TW, but also led to some membrane damage. We assume that cell lysis would leak DNA from the cells, thus interfering in DAPI signal, as well as enhancing PI uptake, and increasing protein in the samples. Even in this dynamic reactional environment, hydrogen peroxide-assisted pasteurization stood out in oxidation conjectured by decrease in dissolved protein content and cell lysis.

4. Limitations and further research

Here we present an exploratory analysis of H_2O_2-assisted pasteurization at bench scale considering chemical and microbiological aspects in batch experiments. Scaled-up systems and flow-through reactors may present different performances. Such studies are highly encouraged, to not only evaluate and compare efficiencies, but also test different designs for household implementation that can provide safe water and cleaner production in terms of less chemicals and efficient energy use.

Additionally, this work focused on microorganism inactivation of a novel combined treatment, hence TW was intended to be mostly clear of interferents. As for real life situations, seasonal changes in water quality as well as different contamination scenarios may affect oxidation demand and therefore affect outcomes, both in terms of performance, and residual concentration that should be studied for context-specific decay kinetics and possible toxicity. In this sense, we recommend further research with different source waters, so that resilience of H_2O_2-pasteurization settings may be investigated.

Contrarywise, as we only considered non-catalyzed H_2O_2 disinfection, performance could be potentially boosted by the presence of naturally occurring catalysts in source water.

As for the mode of action of assisted pasteurization, although it was speculated in terms of cell lysis, our methods were limited to qualitative viability estimation and protein quantification. Hence, we invite additional investigation including quantitative molecular methods, for instance.

5. Conclusions

The stated purpose of this research was to evaluate the performance of H_2O_2-assisted pasteurization for household water treatment. Boundary conditions for maximum concentration and temperatures led to >9.3 $\log_{10}$ inactivation of *Escherichia coli* and >5.8 $\log_{10}$ Phi X174. Obtained $\log_{10}$ reductions were empirically modeled considering each target-organism. Despite the adherence found for the *E. coli* and phage empirical equations (R^2 = 0.76 and 0.72, respectively), we contend that the FFD overall describes the potential of H_2O_2-assisted pasteurization as a disinfection method within different combined conditions of temperature and H2O2 concentration. It should be noted that temperature did not lead to significant differences in residuals, which is favorable for practical implementation in household settings.

Observed results suggested synergistic effects in inactivation of *E. coli* at selected conditions. Although it does not reach the sum of their combined effects, inactivation of Phi X174 surpasses results obtained by individual disinfection by pasteurization and H_2O_2 oxidation. Besides this increase in disinfectant ability, our results suggest H_2O_2-assisted pasteurization adds an oxidation potential to pasteurization, inferred by cell lysis and protein removal. Additionally, experiments considering ramp time endorsed that inactivation might happen at lower temperatures, and stability of hydrogen peroxide throughout assisted pasteurization may provide a more robust disinfection setup when heat sources are not steady for pasteurization to occur. In short, results indicate

satisfactory performance in producing clean water with the combined treatment, while requiring lower oxidant doses as well as reducing dependance on heat sources.

In general terms of microorganism inactivation, our research underscores potentials of H_2O_2-assisted pasteurization as a combined disinfection method. We recommend further assessments considering pathogens, modeling, as well as case studies for practical applications. We believe H_2O_2 may increase the resilience of classic disinfection by pasteurization and provide a safer alternative to reduce drinking water microbial load.

Declaration of competing interest

Authors declare no known competing interests.

Acknowledgements

This work was supported by The Royal Society (ICA\R1\201373 – International Collaboration Awards 2020) and National Council for Scientific and Technological Development (CNPq-Brazil, process nº 308070/2021-6). The Coordination for the Improvement of Higher Education Personnel (CAPES-Brazil, Financial Code: 001) and the São Paulo Research Foundation (FAPESP – Proc. 2019/05759-1) granted PhD scholarships to K.J.S. Silva and L.S. Leite, respectively. Authors acknowledge Dr. N.M.N. Fava for growing phage stocks.

Appendix A. Supplementary material

Statistical analysis of the empirical model is provided.

References

[1] WHO and UNICEF. Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baseline. World Health Organization, United Nations Children's Fund. World Heal. Organ.. 2017;66.

https://www.unicef.org/publications/files/Progress_on_Drinking_Water_Sanitation_and_Hygiene_2017.pdf.

[2] UNICEF & WHO. Progress on household drinking water, sanitation and hygiene I 2000-2017. UNICEF/WHO 2019;140. https://washdata.org/sites/default/files/documents/reports/2019-07/jmp-2019-wash-households.pdf.

[3] WHO. Progress on Household Drinking Water, Sanitation and Hygiene 2000-2020: Five years into the SDGs. 2021. p. 83.

[4] Price HD, Adams EA, Nkwanda PD, et al. Daily changes in household water access and quality in urban slums undermine global safe water monitoring programmes. Int. J. Hyg. Environ. Health. 2021;231:113632. Available from: https://doi.org/10.1016/j.ijheh.2020.113632.

[5] Brennan M, Rondón-Sulbarán J, Sabogal-Paz LP, et al. Conceptualising global water challenges: A transdisciplinary approach for understanding different discourses in sustainable development. J. Environ. Manage. 2021;298:113361.

[6] Okoro BU, Sharifi S, Jesson M, et al. Characterisation and performance of three Kenaf coagulation products under different operating conditions. Water Res. 2021;188:116517. https://doi.org/10.1016/j.watres.2020.116517.

[7] Hodges BC, Cates EL, Kim JH. Challenges and prospects of advanced oxidation water treatment processes using catalytic nanomaterials. Nat. Nanotechnol. 2018;13:642–650. http://dx.doi.org/10.1038/s41565-018-0216-x.

[8] Zhang H, Guo C, Lv J, et al. Aqueous chlorination of ephedrine: Kinetic, reaction mechanism and toxicity assessment. Sci. Total Environ. 2020;740:140146. https://doi.org/10.1016/j.scitotenv.2020.140146.

[9] Sapkota M, Arora M, Malano H, et al. An overview of hybrid water supply systems in the context of urban water management: Challenges and opportunities. Water (Switzerland). 2015;7:153–174.

[10] Subbaraman R, Shitole S, Shitole T, et al. The social ecology of water in a Mumbai slum: failures in water quality, quantity, and reliability. BMC Public Health 2013;13:173. http://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-13-173.

[11] Sebsibe I, Degaga B, Feye G, et al. Bacteriological and physical quality of fiche drinking water from households and reservoirs, Oromia, Ethiopia. Water Pract. Technol. 2021;00:1–11.

[12] Genter F, Willetts J, Foster T. Faecal contamination of groundwater self-supply in low- and middle- income countries: Systematic review and meta-analysis. Water Res. . 2021;201:117350. Available from: https://doi.org/10.1016/j.watres.2021.117350.

[13] Pooi CK, Ng HY. Review of low-cost point-of-use water treatment systems for developing communities. npj Clean Water. 2018;1. http://dx.doi.org/10.1038/s41545-018-0011-0.

[14] Hafeez A, Shamair Z, Shezad N, et al. Solar powered decentralized water systems: A cleaner solution of the industrial wastewater treatment and clean drinking water supply challenges. J. Clean. Prod. 2021;289.

[15] Nieuwoudt MN, Mathews EH. A mobile solar water heater for rural housing in Southern Africa. Build. Environ. 2005;40:1217–1234.

[16] Carielo G, Calazans G, Lima G, et al. Solar water pasteurizer: Productivity and treatment efficiency in microbial decontamination. Renew. Energy. 2017;105:257–269. http://dx.doi.org/10.1016/j.renene.2016.12.042.

[17] Amsberry A, Tyler C, Steinhauff W, et al. Simple continuous-flow device for combined solar thermal pasteurisation and solar disinfection for water sterilisation. J. Humanit. Eng. 2012;3:1–7.

[18] Reyneke B, Cloete TE, Khan S, et al. Rainwater harvesting solar pasteurization treatment systems for the provision of an alternative water source in peri-urban informal settlements. Environ. Sci. Water Res. Technol. 2018;4:291–302.

[19] Chaúque BJM, Rott MB. Solar disinfection (SODIS) technologies as alternative for large-scale public drinking water supply: Advances and challenges. Chemosphere. 2021;281:130754. https://doi.org/10.1016/j.chemosphere.2021.130754.

[20] Brauge T, Faille C, Leleu G, et al. Treatment with disinfectants may induce an increase in viable but non culturable populations of Listeria monocytogenes in biofilms formed in smoked salmon processing environments. Food Microbiol. 2020;92:103548. https://linkinghub.elsevier.com/retrieve/pii/S0740002020301374.

[21] Hayrapetyan H, Nederhoff L, Vollebregt M, et al. Inactivation kinetics of *Geobacillus stearothermophilus* spores by a peracetic acid or hydrogen peroxide fog in comparison to the liquid form. Int. J. Food Microbiol. 2020;316:108418. https://doi.org/10.1016/j.ijfoodmicro.2019.108418.

[22] Alcalá-Delgado AG, Lugo-Lugo V, Linares-Hernández I, et al. Industrial wastewater treated by galvanic, galvanic Fenton, and hydrogen peroxide systems. J. Water Process Eng. 2018;22:1–12. https://doi.org/10.1016/j.jwpe.2018.01.001.

[23] Yang Y, Cheng D, Li Y, et al. Effects of monochloramine and hydrogen peroxide on the bacterial community shifts in biologically treated wastewater. Chemosphere. 2017;189:399–406. https://doi.org/10.1016/j.chemosphere.2017.09.087.

[24] Liang Z, Keeley A. Comparison of propidium monoazide-quantitative PCR and reverse transcription quantitative PCR for viability detection of fresh *Cryptosporidium* oocysts following disinfection and after long-term storage in water samples. Water Res. 2012;46:5941–5953. http://dx.doi.org/10.1016/j.watres.2012.08.014.

[25] Mohammed AN. Field study on evaluation of the efficacy and usability of two disinfectants for drinking water treatment at small cattle breeders and dairy cattle farms. Environ. Monit. Assess. 2016;188:1–11.

[26] Patil RA, Kausley SB, Balkunde PL, et al. Comparative study of disinfectants for use in low-cost gravity driven household water purifiers. J. Water Health. 2013;11:443–456.

[27] Paduano S, Marchesi I, Casali ME, et al. Characterisation of microbial community associated with different disinfection treatments in hospital hot water networks. Int. J. Environ. Res. Public Health. 2020;17:1–17.

[28] Hoffman LA, Ngo TT. Affordable solar thermal water heating solution for rural Dominican Republic. Renew. Energy . 2018;115:1220–1230. https://doi.org/10.1016/j.renene.2017.09.046.

[29] Espinosa MF, Sancho AN, Mendoza LM, et al. Systematic review and meta-analysis of time-temperature pathogen inactivation. Int. J. Hyg. Environ. Health. 2020;230.

[30] Moore N, Ebrahimi S, Zhu Y, et al. A comparison of sodium sulfite, ammonium chloride, and ascorbic acid for quenching chlorine prior to disinfection byproduct analysis. Water Supply. 2021;1–11.

[31] Wang C, Hofmann M, Safari A, et al. Chlorine is preferred over bisulfite for H2O2 quenching following UV-AOP drinking water treatment. Water Res. 2019;165:115000. https://doi.org/10.1016/j.watres.2019.115000.

[32] WHO – World Health Organization. WHO International Scheme to Evaluate Household Water Treatment Technologies Harmonized Testing Protocol: Technology Non-Specific. 2018;1–5. www.who.int/entity/household_water/scheme/HarmonizedTestProtocol.pdf?ua=1.

[33] WHO – World Health Organization WHO International Scheme to Evaluate Household Water Treatment Technologies Harmonized Testing Protocol: Technology Non-Specific. 2014;22. www.who.int/entity/household_water/scheme/HarmonizedTestProtocol.pdf?ua=1.

[34] APHA, AWWA, WEF. Standard methods for the examination of water and wastewater. 22nd ed. E. W. Rice, Baird RB, Eaton AD, et al., editors. Washington, DC.; 2012.

[35] USEPA - United States Environment Protection Agency. Male-specific (F+) and somatic coliphage in water by two-step enrichment procedure. Washington, DC.: Office of Water, Engineering and Analysis Division; 2001.

[36] Chuah CJ, Mukhaidin N, Choy SH, et al. Prevalence of Cryptosporidium and Giardia in the water resources of the Kuang River catchment, Northern Thailand. Sci. Total Environ. 2016;562:701–713. http://dx.doi.org/10.1016/j.scitotenv.2016.03.247.

[37] Safapour N, Metcalf RH. Enhancement of Solar Water Pasteurization with Reflectors These include : Enhancement of Solar Water Pasteurization with Reflectors. Appl. Environ. Microbiol. 1999;65:859–861.

[38] Sahlström L, Bagge E, Emmoth E, et al. A laboratory study of survival of selected microorganisms after heat treatment of biowaste used in biogas plants. Bioresour. Technol. 2008;99:7859–7865.

[39] Choi JO, Lee YH. Effect of sanitizers and disinfectants in Staphylococcus saprophyticus. Medico-Legal Updat. 2020;20:2064–2068.

[40] Hidber T, Pauli U, Steiner A, et al. In vitro and ex vivo testing of alternative disinfectants to currently used more harmful substances in footbaths against Dichelobacter nodosus. Vaughan L, editor. PLoS One. 2020;15:e0229066. https://dx.plos.org/10.1371/journal.pone.0229066.

[41] Kolar SSN, Manarang JC, Burns AR, et al. Contact lens care solution killing efficacy against Acanthamoeba castellanii by in vitro testing and live-imaging. Contact Lens Anterior Eye. 2015;38:442–450. https://linkinghub.elsevier.com/retrieve/pii/S1367048415300084.

[42] Zang YT, Li BM, Bing S, et al. Modeling disinfection of plastic poultry transport cages inoculated with Salmonella enteritids by slightly acidic electrolyzed water using response surface methodology. Poult. Sci. 2015;94:2059–2065.

[43] Taddese R, Belzer C, Aalvink S, et al. Production of inactivated gram-positive and gram-negative species with preserved cellular morphology and integrity. J. Microbiol. Methods. 2021;184:106208. https://doi.org/10.1016/j.mimet.2021.106208.

[44] Sammarro Silva KJ, Sabogal-Paz LP. Analytical challenges and perspectives of assessing viability of *Giardia muris* cysts and *Cryptosporidium parvum* oocysts by live/dead simultaneous staining. Environ. Technol. 2020;1–10. https://www.tandfonline.com/doi/full/10.1080/09593330.2020.1775712.

[45] Gómez-Couso H, Fontán-Sainz M, McGuigan KG, et al. Effect of the radiation intensity, water turbidity and exposure time on the survival of *Cryptosporidium* during simulated solar disinfection of drinking water. Acta Trop. 2009;112:43–48.

[46] Nair AT, Makwana AR, Ahammed MM. The use of response surface methodology for modelling and analysis of water and wastewater treatment processes: A review. Water Sci. Technol. 2014;69:464–478.

[47] WHO – World Health Organization. Guidelines for drinking-water quality (GDWQ) . 4th ed. 2017. https://www.who.int/publications/i/item/9789241549950.

[48] WHO – World Health Organization. Evaluating household water treatment options: health-based targets and microbiological performance specifications. Geneva; 2011. https://apps.who.int/iris/handle/10665/44693.

[49] Silva KJS, Sabogal-Paz LP. Exploring Potentials and Constraints of H2O2 Water Disinfection for Household Settings. Water, Air, Soil Pollut. 2021;232:483. https://doi.org/10.1007/s11270-021-05434-3.

[50] Wagner EJ, Oplinger RW, Bartley M. Effect of Single or Double Exposures to Hydrogen Peroxide or Iodine on Salmonid Egg Survival and Bacterial Growth. N. Am. J. Aquac. 2012;74:84–91. http://doi.wiley.com/10.1080/15222055.2011.649887.

[51] Monteagudo JM, Duran Segovia A, Israel SM, et al. A novel combined solar pasteurizer/TiO_2 continuous-flow reactor for decontamination and disinfection of drinking water. Chemosphere. 2017;168:1447–1456.

[52] Gautam A, Chamoli S, Kumar A, et al. A review on technical improvements, economic feasibility and world scenario of solar water heating system. Renew. Sustain. Energy Rev. 2017;68:541–562. http://dx.doi.org/10.1016/j.rser.2016.09.104.

[53] Sansaniwal SK. Advances and challenges in solar-powered wastewater treatment technologies for sustainable development: a comprehensive review. Int. J. Ambient Energy. 2019;0:1–34. https://doi.org/01430750.2019.1682038.

[54] Cho M, Kim JH, Yoon J. Investigating synergism during sequential inactivation of *Bacillus subtilis* spores with several disinfectants. Water Res. 2006;40:2911–2920.

[55] Black & Veatch Corporation. White's Handbook of Chlorination and Alternative Disinfectants. 5th ed. Black & Veatch Corporation, editor. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2009. http://doi.wiley.com/10.1002/9780470561331.

[56] Snoeyink LV, Jenkins D. Water Chemistry. Jenkins O, editor. Wiley; 1980.

[57] Lau M, Monis P, Ryan G, et al. Selection of surrogate pathogens and process indicator organisms for pasteurisation of municipal wastewater—A survey of literature data on heat inactivation of pathogens. Process Saf. Environ. Prot. 2020;133:301–314. https://doi.org/10.1016/j.psep.2019.11.011.

[58] Strazynski M, Krämer J, Becker B. Thermal inactivation of poliovirus type 1 in water, milk and yoghurt. Int. J. Food Microbiol. 2002;74:73–78.

[59] Roohi R, Hashemi SMB. Experimental, heat transfer and microbial inactivation modeling of microwave pasteurization of carrot slices as an efficient and clean process. Food Bioprod. Process. 2020;121:113–122. https://linkinghub.elsevier.com/retrieve/pii/S0960308519310193.

[60] Carielo da Silva G, Tiba C, Calazans GMT. Solar pasteurizer for the microbiological decontamination of water. Renew. Energy. 2016;87:711–719. http://dx.doi.org/10.1016/j.renene.2015.11.012.

[61] Bigoni R, Kötzsch S, Sorlini S, et al. Solar water disinfection by a Parabolic Trough Concentrator (PTC): Flow-cytometric analysis of bacterial inactivation. J. Clean. Prod. 2014;67:62–71. http://dx.doi.org/10.1016/j.jclepro.2013.12.014.

[62] Dobrowsky PH, Carstens M, De Villiers J, et al. Efficiency of a closed-coupled solar pasteurization system in treating roof harvested rainwater. Sci. Total Environ. 2015;536:206–214. http://dx.doi.org/10.1016/j.scitotenv.2015.06.126.

[63] Feachem RG, Bradley DJ, Garelick H, et al. Sanitation and disease: health aspects of excreta and wastewater management. Chichester, UK: John Wiley and Sons; 1983.

[64] Freitas B de O, Leite L de S, Daniel LA. Chlorine and peracetic acid in decentralized wastewater treatment: Disinfection, oxidation and odor control. Process Saf. Environ. Prot. 2021;146:620–628. https://doi.org/10.1016/j.psep.2020.11.047.

[65] Leite L de S, Daniel LA, Pivokonsky M, et al. Interference of model wastewater components with flocculation of *Chlorella sorokiniana* induced by calcium phosphate precipitates. Bioresour. Technol. . 2019;286:121352. https://doi.org/10.1016/j.biortech.2019.121352.

Supplementary material

Hydrogen peroxide-assisted pasteurization: an alternative for household water disinfection

Kamila Jessie Sammarro Silva, Luan de Souza Leite, Luiz Antonio Daniel, Lyda Patricia Sabogal-Paz*

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, 400 Trabalhador São-carlense Avenue, São Carlos, São Paulo 13566-590, Brazil.

*E-mail of the corresponding author: lysaboga@sc.usp.br

Table S.1 ANOVA for the fit of the empirical model to *Escherichia coli* inactivation by H_2O_2-assisted pasteurization. Results at 5% significance level for the recalculated model excluding insignificant coefficients. $R^2 = 0.75877$.

Factor	SS	df	MS	*F*-value	*p*-value
Temperature (L)	64.9798	1	64.97983	39.3705	>0.0001
H_2O_2 (L)	12.8909	1	12.89086	7.81042	0.0136
Error	24.7570	15	1.65047		
Total SS	102.6277	17			

Notes: SS = sum of squares; df = degrees of freedom MS = mean square; L = linear

Table S.2 ANOVA for the fit of the empirical model to PhiX 174 bacteriophage inactivation by H_2O_2-assisted pasteurization. Results at 5% significance level for the recalculated model excluding insignificant coefficients. $R^2 = 0.71791$.

Factor	SS	df	MS	*F*-value	*p*-value
Temperature (L)	32.33110	1	32.33110	25.83023	0.0001
H_2O_2 (L)	15.45064	1	15.45064	12.34396	0.0031
Error	18.77515	15	1.25168		
Total SS	66.55690	17			

Notes: SS = sum of squares; df = degrees of freedom MS = mean square; L = linear