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KAMILA JESSIE SAMMARRO SILVA

Hydrogen peroxide in household water treatment and disinfection technologies

CORRECTED VERSION

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Hydrogen peroxide in household water treatment and disinfection technologies

PhD thesis presented to the Graduate Program in Hydraulic Engineering and Sanitation at São Carlos School of Engineering, University of São Paulo, to obtain the degree of Doctor of Science.

Supervisor: Lyda Patricia Sabogal-Paz

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I dedicate this thesis to my grandmother Carmen, *in memoriam*, and to all of those who have also experienced loss while this work was being carried out and these lines were being written. Let us not reduce pain and injustice to meaningless numbers.

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“No matter the self-conceited importance of our labors we are all compost for worlds we cannot yet imagine.”

— David Whyte

ABSTRACT

SILVA, K. J. S. **Hydrogen peroxide in household water treatment and disinfection technologies.** 2022. Doctoral thesis, São Carlos School of Engineering, University of São Paulo, São Carlos, 2022.

This thesis was divided into chapters aiming to approach hydrogen peroxide application in household water treatment (HWT) and disinfection technologies by both literature analysis and experimental research, according to aims and hypotheses presented in Chapter 1. Chapter 2 consisted of a review on H_2O_2 as a standalone disinfectant in the last decade and indicated it has not been much explored in sanitation, less even in HWT. Results from content analysis revealed a knowledge gap for this disinfectant at the household level, as well as practical knowledge research gap due to lack of real-life applications and inconsistencies in operational conditions among the analyzed papers published in the last 10 years. Such opportunities for research were explored in the following chapters. Potentials and constraints of liquid H_2O_2 individual use in domestic settings were discussed in Chapter 3, which presented a preliminary assessment of hydrogen peroxide compared to chlorine, a classic disinfectant in water treatment plants and at the point of use. Chlorine disinfection efficiency based exclusively on *Escherichia coli* inactivation was insufficient at the tested conditions and H_2O_2 was more efficient than chlorine in inactivating Phi X174 bacteriophage. This chapter also indicated that photometric assays may be misleading to evaluate organic matter oxidation by H_2O_2. Chapter 4 presented effects of the water matrix when H_2O_2 was applied as a preoxidant, for conditioning natural source waters to a (non-specified) main HWT to follow. Hence, lower concentrations and exposure times were explored (if compared to Chapter 3). Results for H_2O_2 preoxidation indicated a reduction in virus and *E. coli* contamination levels in river water, implying that this pretreatment may improve microbiological quality of such matrix prior to other treatments, particularly considering the presence of natural catalysts that might have enhanced oxidation performance for clarification and disinfection. H_2O_2 preoxidation of groundwater for reducing microbiological load was not encouraged at the tested doses, but further research on H_2O_2 may help improving the lifespan of the main HWT. A combined treatment was proposed and tested in Chapter 5, and it was based on pasteurization, a well-known intervention for water decontamination in households, assisted by H_2O_2, leading to satisfactory removals of *E. coli* and at a wide range of conditions for temperature and hydrogen peroxide dose at a fixed contact time. Empirical models were proposed for inactivation of both target organisms, and synergistic effects were obtained for *E. coli* inactivation. In Chapter 5, H_2O_2 has shown to be a possibility

for increasing robustness of pasteurization setups for HWT. Overall, this thesis elucidated some of the possibilities and drawbacks of the application of hydrogen peroxide in households and provided background and insight for future work on its implementation as a point-of-use or point-of-entry disinfectant, as well as for design of water treatment systems that include this oxidant at the household level.

Keywords: Point-of-use. Oxidation. Drinking water. Microorganism inactivation. SDG 6.

RESUMO

SILVA, K. J. S. **Uso de peróxido de hidrogênio em tecnologias domiciliares de tratamento de água e desinfecção.** 2022. Tese (Doutorado) – Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos, 2022.

Esta tese foi dividida em capítulos visando abordar a aplicação do peróxido de hidrogênio no tratamento de água e tecnologias de desinfecção em nível doméstico (HWT) por meio de análise de literatura e pesquisa experimental, conforme hipóteses e objetivos apresentados no Capítulo 1. O Capítulo 2 consistiu em uma revisão sobre H_2O_2 como desinfetante individual e indicou que não tem sido muito explorado em saneamento, e ainda menos como HWT. Resultados da análise revelaram uma lacuna de conhecimento sobre esse desinfetante em nível residencial, bem como uma lacuna de conhecimento prático devido à falta de aplicações em situações reais e inconsistências nas condições operacionais exploradas nas publicações dos últimos dez anos. Essas oportunidades foram exploradas nos capítulos seguintes. Potenciais e limitações do uso individual de H_2O_2 líquido em ambientes domésticos foram discutidos no Capítulo 3, que apresentou uma avaliação preliminar do H_2O_2 comparado ao cloro, desinfetante clássico em estações de tratamento de água e no ponto de uso. A eficiência da desinfecção com cloro baseada exclusivamente na inativação de *Escherichia coli* foi insuficiente nas condições testadas e H_2O_2 foi mais eficiente que o cloro na inativação do bacteriófago Phi X174. Este capítulo também indicou que ensaios fotométricos podem ser enganosos para avaliar a oxidação da matéria orgânica por H_2O_2. O Capítulo 4, por sua vez, apresentou os efeitos da matriz quando o H_2O_2 foi aplicado como um pré-oxidante, para condicionar as águas de fonte natural a uma HWT principal a seguir (não especificada). Assim, foram explorados concentrações e tempos de exposição mais baixos (se comparados ao Capítulo 3). Os resultados para a pré-oxidação usando H_2O_2 indicaram uma redução nos níveis de contaminação por vírus e *E. coli* em água proveniente de rio, o que implica que este pré-tratamento pode melhorar a qualidade microbiológica dessa matriz antes de outros tratamentos, principalmente considerando a presença de catalisadores naturais que podem ter melhorado o desempenho da oxidação para clarificação e desinfecção. A pré-oxidação da água subterrânea com H_2O_2 para reduzir a carga microbiológica não foi recomendada nas doses testadas, mas incentivam-se pesquisas adicionais sobre H_2O_2 para aumentar a vida útil da HWT principal. Um tratamento combinado foi proposto e testado no Capítulo 5, baseado na pasteurização, intervenção bem conhecida para descontaminação de água em residências, assistida por H_2O_2, levando a remoções satisfatórias de *E. coli* e fagos uma ampla gama de condições de temperatura e dose de H_2O_2 em um tempo

de contato fixo. Modelos empíricos foram propostos para a inativação de ambos os organismos-alvo, e efeitos sinérgicos foram obtidos para a inativação de *E. coli*. No Capítulo 5, o H_2O_2 mostrou ser uma possibilidade para aumentar a robustez das configurações de pasteurização como tratamento de água domiciliar. No geral, esta tese elucidou algumas das possibilidades e desvantagens da aplicação do H_2O_2 em residências e forneceu subsídios e *insights* para trabalhos futuros sobre sua implementação como desinfetante de ponto de uso ou ponto de entrada, bem como para o projeto de sistemas de tratamento de água que incluem este oxidante em nível doméstico.

Palavras-chave: Ponto de uso. Oxidação. Água para consumo. Inativação de microrganismos. ODS 6.

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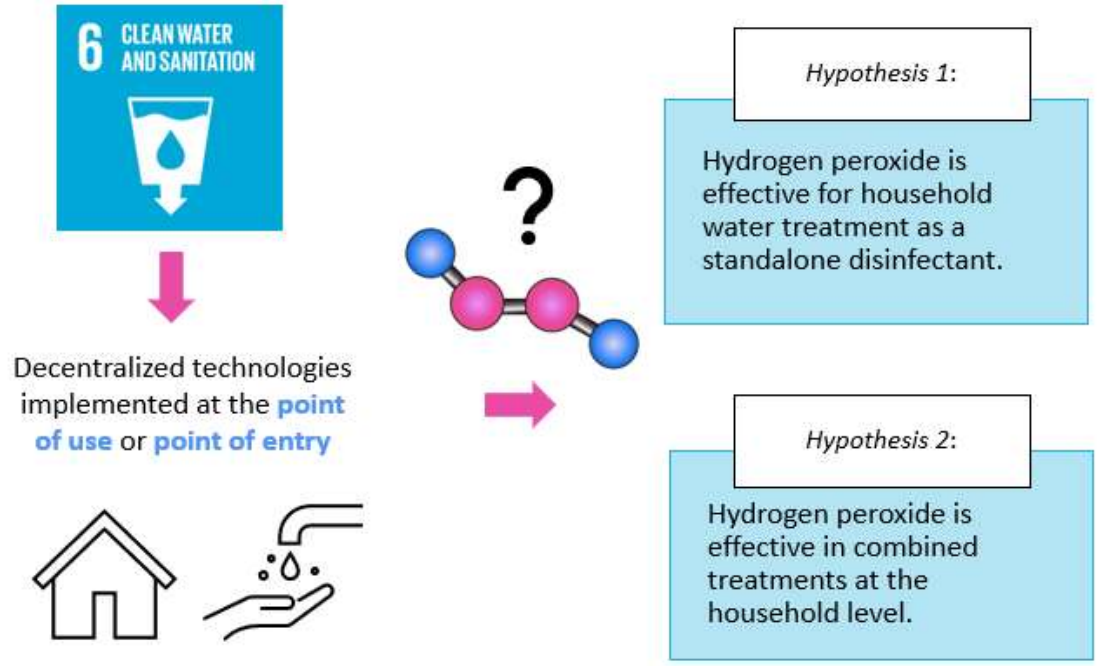

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1.1 General introduction and background

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 (WHO; UNICEF, 2017). 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 (UNICEF; WHO, 2019).

Although, globally, this situation moves towards better conditions, the latest estimate suggests this will not be fully achieved unless progress quadruples (WHO, 2021). Furthermore, access in specific scenarios is often overlooked, and inequalities remain (UNICEF; WHO, 2019; PRICE et al., 2021), emphasizing the need for new frameworks (BRENNAN et al., 2021).

Many low-income regions struggle with insufficient water compliance, due to a lack of commitment from authorities involving supply, infrastructure, and service delivery (OKORO et al., 2021). While policymakers are in search of long-term solutions to water insecurity, a recent meta-ethnographic synthesis has identified that some of the coping strategies could be as simple as providing purification of water prior to consumption (ACHORE et al. 2020). In fact, a large fraction of the global population relies on small supply systems (DEBIASI; BENETTI, 2019), in the form of wells, boreholes or harvested rainwater usually owned and maintained by individual families (FOSTER et al., 2021).

This growing gap between demand for safe water and conventional supply has allowed decentralized systems to rise as alternative solutions (HODGES; CATES; KIM, 2018; ZHANG et al., 2020). This approach has been emerging in some urban areas (SAPKOTA et al., 2015), in which, though centralized treatment may be available, measurable levels of pathogens have been found (SUBBARAMAN et al., 2013). However, mostly, self-supplied regions, where water quality varies between the source and households (SEBSIBE et al., 2021), are significantly more likely to be contaminated (GENTER; WILLETTS; FOSTER, 2021), hence these may be very positively impacted by on-site setups for treatment or disinfection.

In this sense, plain decontamination solutions would be effective and desirable interventions (PATIL et al. 2020) for providing safe drinking water at households. When locally applied, these are known as household water treatment (HWT) systems and could be employed as point-of-use (POU) or point-of-entry (POE) technologies, which can play a strategic role to help meeting households' immediate water needs (POOI; NG 2018) and, as a result, help overcoming inequalities.

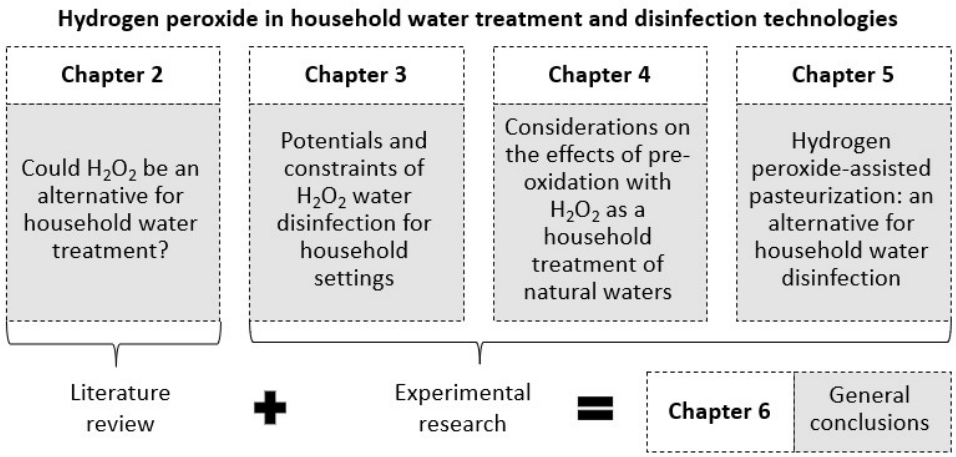
There are different approaches for HWT, which vary from portable devices (PATIL et al., 2020; MONTENEGRO-AYO et al., 2020) to in-home installed systems, e.g., photovoltaic

powered ultraviolet and visible light-emitting diodes (LUI et al., 2014), household slow sand filters (FREITAS et al., 2022), etc. Other examples of decentralized treatment schemes rely on even simpler interventions, such as chlorination, which has been used in disinfection since the early 1900s (USEPA 1999).

As much as conventional treatments, HWT technologies also face emerging challenges. Chlorination, for instance, which is a widely spread POU method (MITRO et al., 2019; CLAYTON; THORN; REYNOLDS, 2021), is associated to the formation of toxic disinfection by-products (DBPs) (HU et al., 2018; LEITE et al., 2022). Hydrogen peroxide, comparatively, is considered as a cleaner substance, as it is usually decomposed into oxygen and water molecules, avoiding the DBP formation upon successful disinfection (FARINELLI et al., 2021; HERRAIZ-CARBONÉ et al., 2021). In fact, H_2O_2 is an alternative oxidant for controlling the generation of by-products themselves (POLENENI, 2020), rising as a promising candidate for HWT applications (SILVA et al., 2021). In addition, H_2O_2 has been employed in addressing other challenges in disinfection, as in inactivation of antibiotic resistant (AR) microorganisms (CADNUM et al., 2015; MCKEW et al., 2021), as well as pathogenic protozoa, known to be resistant to conventional disinfection (LIANG; KEELEY 2012; QUILEZ et al. 2005).

All of these factors point to H_2O_2 potential for POU/POE water treatment technologies. However, to our knowledge, it has not been much explored as such. Based on this, this thesis presents an exploratory analysis divided into chapters, and carried out by both a literature review perspective, and experimental research on liquid hydrogen peroxide as a disinfectant aimed at being applied at the household level, for either standalone or combined treatments, as detailed further. Figure 1-1 displays an overview of the organization of this thesis, considering the chapters that will follow.

Figure 1-1 - Organization of the thesis



Source: the author.

1.2 Hypotheses and objectives

This thesis considers two main hypotheses:

Hypothesis 1: Hydrogen peroxide presents efficacy for household water treatment as a standalone disinfectant. Premise for hypothesis 1: there is plenty of information on the efficacy of H_2O_2 disinfection in different areas of research.

Hypothesis 2: Hydrogen peroxide presents efficacy in combined treatments at the household level. Premises for hypothesis 2: H_2O_2 has been used in preoxidation, conditioning water for the main treatment. Additionally, as hydrogen peroxide is popular in combined treatments (e.g. photocatalysis, Fenton, etc), in which synergistic effects have been described, classic techniques for water treatment at the household level (e.g, pasteurization) could also benefit from it.

In order to test the two aforementioned hypotheses, this work had the primary objective of exploring hydrogen peroxide in household water treatment and disinfection technologies. This main goal was divided into objectives specifically related to each chapter previously listed in Figure 1-1. These specific aims are:

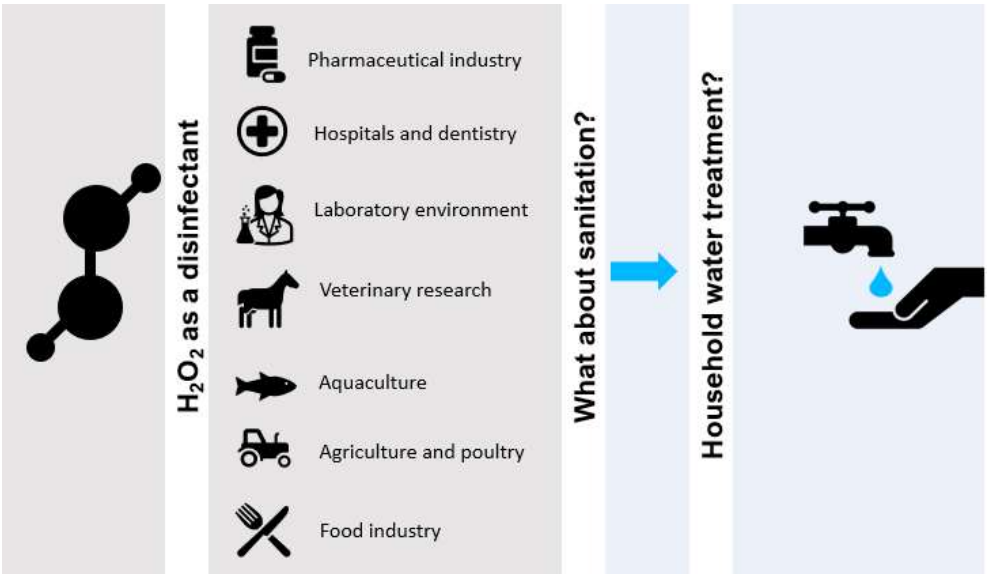
- Chapter 2 (which relates to hypotheses 1 and 2): Describe the main applications reported in literature for hydrogen peroxide disinfection; Identify research gaps, challenges, and perspectives for implementing H_2O_2 at the household level based on published data.
- Chapter 3 (which relates to hypothesis 1): Experimentally explore potentials and constraints of H_2O_2 as a point-of-use or point-of-entry disinfectant using general test water; Describe oxidation effects of the individual use of hydrogen peroxide on the referred matrix.
- Chapter 4 (which relates to hypotheses 1 and 2): Evaluate effects of the water quality on the performance of H_2O_2 for preoxidation as a pretreatment for household water technologies.
- Chapter 5 (which relates to hypothesis 2): Assess the performance of H_2O_2-assisted pasteurization as a potential HWT; Describe the expected efficiency of this combined treatment by empirical models of microorganism inactivation; Estimate synergistic effects; Provide inferences on the treatment mode of action based on cell lysis and protein quantification.

Aims from each chapter were responded within their partial conclusions section. Chapter 6 provides a general perspective of the findings in this thesis, linking them to the primary goal and the two established hypotheses.

2- Chapter 2

Literature review

A 10-year critical review on hydrogen peroxide as a disinfectant: could it be an alternative for household water treatment?



Source: the author.

Highlights:

- A decade of H_2O_2 was analyzed showing only 1% of research dedicated to sanitation.
- Retrieved records do not include data on H_2O_2 as an HWT.
- Operational conditions found for liquid H_2O_2 use often favor catalytic treatments.
- Context-specific studies are recommended to evaluate HWT feasibility.

2.1 Introduction

Although recent research (described in Chapter 3) has explored some advantages and constraints of H_2O_2 as a potential HWT by conducting a laboratory scale experiment (SILVA; SABOGAL-PAZ, 2021), to our knowledge, literature lacks current and systematically organized information in that regard.

Therefore, this chapter presents a critical review aimed to provide an overview of the applications of hydrogen peroxide in the last decade and use this data to shed light onto the hypothesis of H_2O_2 as an alternative for water disinfection at the household level, that is, a strategy to tackle inequalities in access to safe water.

2.2 Methods

The main research question here was: "could hydrogen peroxide be used as a water disinfectant at the household level?" In order to answer it with a broad notion, a literature review on H_2O_2 disinfection was performed, so that trends and gaps could be identified through qualitative synthesis and a critical discussion.

2.2.1 *Research strategy and data curation*

The research strategy was an adaptation of the PRISMA model (Preferred Reporting Items for Systematic Reviews and Meta-analyses) (LIBERATI et al., 2009). Articles were identified from the Scopus database, restricting documents from 2011 to 2021 using "hydrogen peroxide disinfection" as keywords (with Boolean descriptors: "hydrogen AND peroxide AND disinfection").

From the total retrieved results, papers that utilized plasma treatment, foam, and cleaning wipes were removed in screening at the title and abstract levels. Combined and catalytic treatments were also dismissed, as well as electrogeneration, because these involve more parameters than individual applications do, thus exceeding the scope of our present discussion. Studies on decontamination of medical, as well as personal protection equipment (PPE) were not considered, as most of these publications were context-oriented within specific healthcare applications or emergencies (such as the COVID-19 pandemic). Review articles were also excluded.

Independent extraction of eligible articles was carried out using predefined data filters including purpose/context (e.g., room decontamination, agriculture, aquaculture, sanitation, etc.), matrix (surface, water, wastewater, etc.), target organism, method of application, main parameters, and relevant notes. At this level of screening, air disinfection was removed from

eligible papers, as well as decontamination of tissue and vaccine industry applications, which were only identified after data extraction.

The final qualitative synthesis included studies narrowed to the sanitation field. Even so, obtained information from the remaining eligible articles was still integrated as scope for discussion in this critical review, as well as general data visualization.

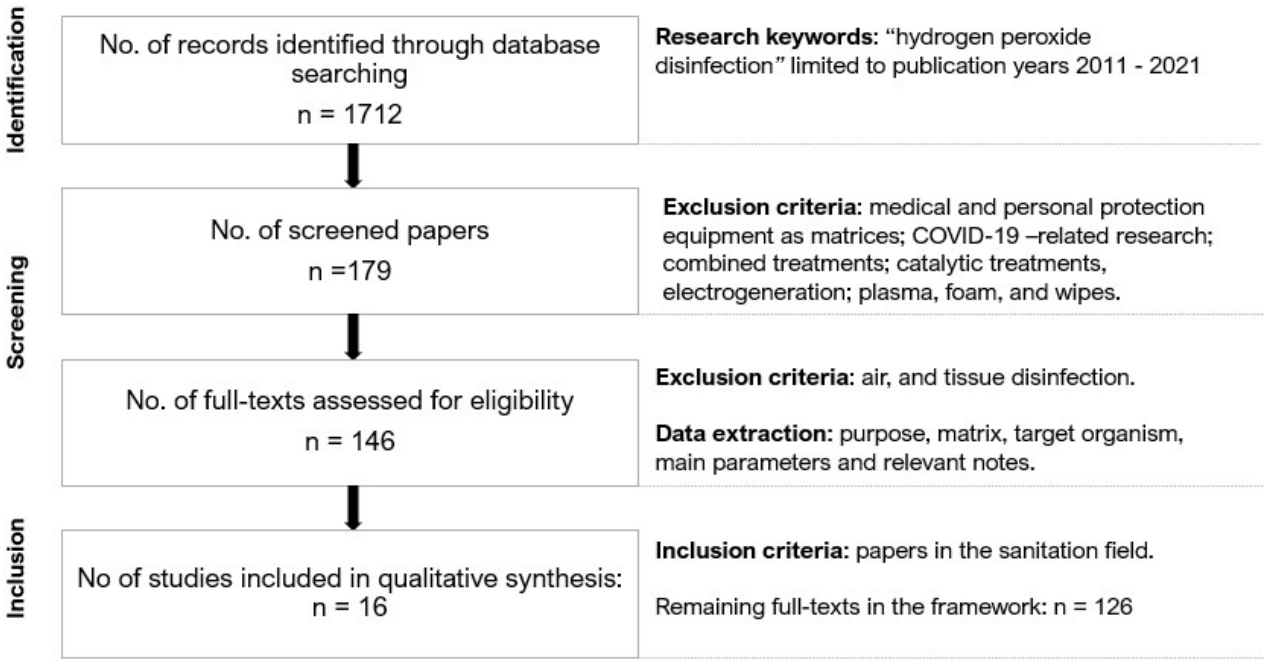
2.2.2 Data visualization

Filtered information from selected articles was organized into networks built on Cytoscape (SHANNON et al., 2003) for a broader visualization. All of the additional references from extracted data, as well as detailed information are listed in table A1, available in Appendix 1.

2.3 Results and discussion

A flowchart of the number of records from each phase of the research strategy is shown in Figure 2-1. From those, only 1% of publications were from the sanitation field. Although this result may be influenced by a supposed increase in pandemic-related titles (retrieved and excluded in the identification phase) that proportionally reduce other areas of study, it still indicates a lack of research in H_2O_2 standalone disinfection of water and wastewater.

Figure 2-1 Flow of information through different phases of the systematic review of H_2O_2 disinfection



Source: the author.

2.3.1 Overview of hydrogen peroxide disinfection

Despite this review found it to be unpopular as a standalone disinfectant in sanitation, due to scarce literature when compared to total retrieved documents, hydrogen peroxide is a widely known disinfectant and biocide. The modes of action related to H_2O_2 inactivation action rely on intra and extracellular effects, as well as inhibition of peroxide activity and internal Fenton process (MAILLARD, 2002).

Figure 2-2 and Figure 2-3 display networks built out of data extracted from the 142 selected papers (n = 16 in sanitation; n = 126 from other applications, details in Appendix 1). Density of connections (lines) indicate the frequency in which such relationships are present in retrieved documents.

Figure 2-2 illustrates different scenarios where H_2O_2 has been applied and the methods by which it was applicated. By observing the network, it is possible to identify that the main method of H_2O_2 application was found to be through liquid and vapor (i.e., fog), but it has also been used as liquid applied as spray, and aerosol (i.e., dry mist). In sanitation, hydrogen peroxide has been reported in uses only as a liquid, mainly pure but also with peroxygen-based disinfectant formulas.

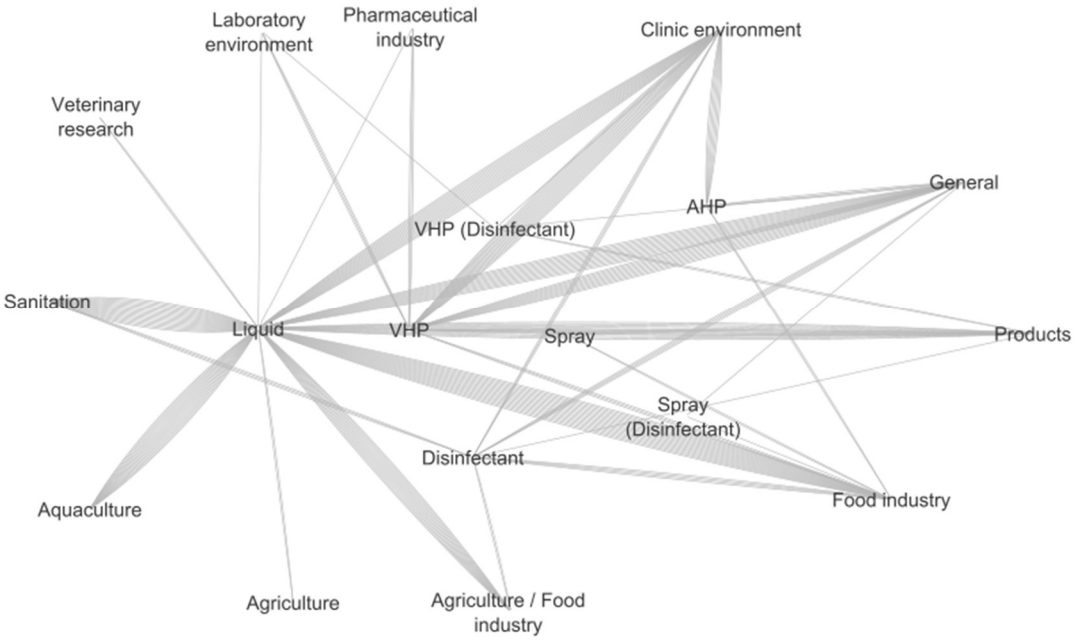
It should be noted that depending on the application form, different operational conditions apply. Vaporized hydrogen peroxide (VHP) systems often generate vapor by adding > 30% H_2O_2 solutions to a vaporizer to be heated at 130 ºC and then produce vapor that is aimed to condense onto surfaces (OTTER et al. 2010; HOLMDAHL et al. 2011). Aerosol systems (AHP) rely on pressure to produce aerosols with a particular particle size and often include lower H_2O_2 concentrations and mixtures of silver cations, for instance (HOLMDAHL et al., 2011). This variety in application form indicate a certain versatility of hydrogen peroxide as a disinfectant but must be carefully considered when determining working conditions for different field uses.

Figure 2-3 shows a network that illustrates the decontamination matrices found in H_2O_2 disinfection research, as well as the main target-organism groups. Most research is focused on surface decontamination, but there are liquid matrices relevant to sanitation as in water and wastewater. Details of disinfection settings are present in Appendix 1.

Overall, a wide range of target-organisms was found for H_2O_2 disinfection, but the main targets were bacteria, regardless of the matrix. In clinic environments, particularly, these even include antibiotic resistant (AR) bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* (VRE) (CADNUM et al., 2015; AMAEZE et al., 2020). Other groups of microorganisms have also been explored, as viruses

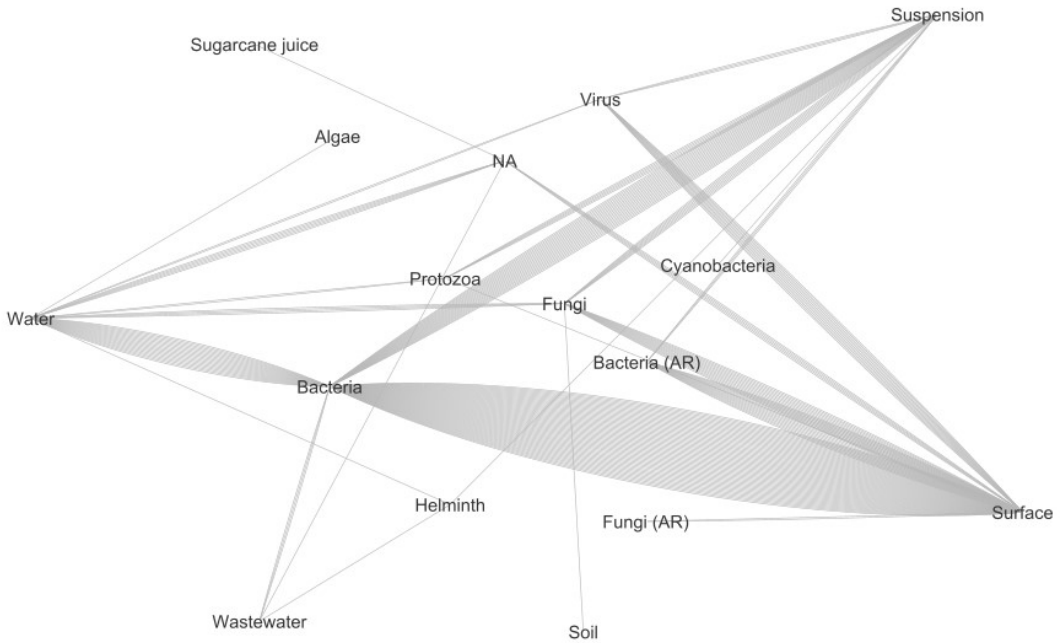
and fungi. The latter should be highlighted, as there is research on H_2O_2 applied against emerging threats to public health like the fungus *Candida auris* (CADNUM et al., 2015; COBRADO et al., 2021; MCKEW et al., 2021). Details of targeted microorganism groups and their references are available in Appendix 1.

Figure 2-2 - Network of the main areas of hydrogen disinfection research and forms of application (2011 – 2021)



Notes: AHP = aerosolized hydrogen peroxide; General = decontamination of room or in-house environments. Disinfectant = peroxygen-based products that may contain a small percentage of other substances (e.g., alcohol, peracetic acid, silver nitrate, quaternary ammonium, etc.). VHP = Vaporized hydrogen peroxide
Source: the author.

Figure 2-3 - Network of retrieved information of matrices and target-organisms in hydrogen disinfection research (2011 – 2021)



Notes: AR = antibiotic / antifungal resistant. NA = not available.
Source: the author.

2.3.2 H_2O_2 in sanitation research

In sanitation, the main applications observed were related to microorganism inactivation per se, as laid out in Table 2-1. Target-organisms were often from bacteria groups (especially fecal contamination indicators, e.g., *Escherichia coli*), but there were also studies contemplating protozoan (oo)cysts and helminth eggs. *Giardia* spp. and *Cryptosporidium* spp. are particularly relevant parasites for studies on technologies to be applied at the household level, because their infective forms are resistant, associated to worldwide diseases outbreaks (EFSTRATIOU et al., 2017), and have been recently reported in water sources in rural regions, including both surface and groundwater (CHUAH et al., 2016; CHIQUE et al., 2020; KIFLEYOHANNES; ROBERTSON, 2020). Helminth eggs are not only appropriate targets due to their resistance to disinfection, but also because they are considered social indicators of a country (GUADAGNINI et al., 2013), thus directly relevant to future studies on HWTs aiming to reduce inequalities. Less attention was directed to cyanobacteria, viruses, and fungi, but they were still present, and point to pertinent targets for further and directed research.

HWT research has shown that added H_2O_2 may be promising with solar light and Fenton processes, producing fast killing effects in resilient microbial contaminants like fungi spores (SICHEL et al., 2009), and virus (ORTEGA-GÓMEZ et al., 2015). These and similar studies were not included in this review because they refer to combined treatments, but definitely showcase potentials of hydrogen peroxide in household applications.

But as for standalone H_2O_2 in households, a knowledge gap (JACOBS, 2011) was found. From retrieved documents in the sanitation context, only one study aimed at POU water treatment, which refers to Chapter 3 of this thesis, hence not detailed at this point in order to avoid redundancies. This document was recovered from the data base, because it was published by Silva and Sabogal-Paz (2021), who explored liquid H_2O_2 as a potential HWT, benchmarking it against chlorine for the inactivation of indicator bacteria and a virus contamination model, as described in Table 2-1. This research, however, highlighted the need for site-specific information, including a broader assessment that includes different microorganism groups. This point has also been raised in a commentary (MRAZ et al., 2021) that illustrated that decisions regarding water and sanitation should not only rely on indicators, but also include enteric pathogens. That was demonstrated considering calculated probabilities of infection risk, which are significantly higher when inactivation information for pathogens is included. In order to illustrate a water treatment setting, Mraz et al. (2021) considered chlorination of surface water.

This could be an analogous situation for H_2O_2 as an HWT, thus inviting further research to describe whether interventions are realistic for each contamination scenario.

Table 2-1 - Summary of aims and targets of research on H_2O_2 disinfection in sanitation (2011 – 2021)

Main purpose	**Target**	**Relevance**	**Microorganism group**	**Reference**
Validate viability assessment protocol	*Cryptosporidium parvum*	Resistant pathogen	Protozoa	(LIANG; KEELEY 2012)
Inactivation	*Ascaris suum*	Resistant pathogen	Helminth	(MORALES et al., 2013)
Inactivation	TC, *Escherichia coli*; *Ascaris* spp.	Resistant pathogen	Bacteria; helminth	(GUADAGNINI et al., 2013)
Inactivation	*E. coli*	Indicator	Bacteria	(PATIL et al. 2013)
Kinetics and effects of pH	TC, *E. coli*	Indicator	Bacteria	(VARGAS et al., 2013)
Inactivation	*Giardia duodenalis*	Resistant pathogen	Protozoa	(GUIMARÃES et al., 2015)
Inactivation	TC, *E. coli, Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp.	Field study	Bacteria	(MOHAMMED, 2016)
Monitor shifts in microbial communities	General bacteria profiling	Complex matrix	Bacteria	(YANG et al., 2017)
Inactivation	Algae; *E. coli*	Complex matrix	Algae; bacteria	(FARINELLI et al., 2021)
Inactivation	*Hymenolepis nana*	Resistant pathogen	Helminth	(LANDRY et al., 2021)
Inactivation	*E. coli*; Phi X174	Indicator	Bacteria; virus	(SILVA; SABOGAL-PAZ, 2021 - Chapter 3)
Inactivation; toxin removal[1]	*Microcystis aeruginosa*	Complex matrix	Cyanobacteria	(FAN et al., 2014)
Removal of organic matter[1]	N/A	Complex matrix	N/A	(ALCALÁ-DELGADO et al., 2018)
Dechlorination[1]	N/A	Quenching agent	N/A	(QIAN et al., 2015)
Inactivation [2]	*Legionella pneumophila*	Biofilm	Bacteria	(FARHAT et al., 2011)
Inactivation [2]	*Verticillium dahliae*	Field study	Fungi	(SANTOS-RUFO; RODRÍGUEZ-JURADO 2016)

Notes: [1]Oxidation experiments. [2]Study applied a peroxygen-based disinfectant. TC = total coliforms. N/A = does not apply.

2.3.3 Operational conditions in sanitation studies

In order to shed light onto conditions in which non-catalyzed oxidation with H_2O_2 may be applied for water treatment, details of peroxidation within the scope of sanitation in the last

decade are present in Table 2-2. Practical knowledge gaps (JACOBS, 2011) were found particularly in working conditions and technology implementation.

Table 2-2 - Operational details of disinfection experiments using H_2O_2 in sanitation (2011 – 2021)

Scale	Matrix		Operational parameters	Quencher	Reference
Bench (batch)	Suspension		28.64 mg L^{-1} for 58 min	Sodium thiosulfate	(MORALES et al., 2013)
			15, 60, and 6000 mg L^{-1} for 5.5 s, 60 min and 30 min, respectively	NA	(GUIMARÃES et al., 2015)
	W	Artificially contaminated surface and disinfected water	0.10%, 0.60%, 1%, 3%, 6%, 10%, 20% and 30% for 1 h. Kinetic tests: 0.1%, 0.6% and 3% for 36 h, sampled at various time points (1, 2, 4, 6, 8, 12, 16, 24, 30 and 36 h)	None[1]	(LIANG; KEELEY 2012)
		Artificially contaminated groundwater	10, 100, 1,000 and 10000 mg L^{-1} inactivation for 10, 30, 60, and 120 min	None[2]	(PATIL et al. 2013)
		Drinking water for cattle	25, 35, and 40 mg L^{-1} from 12 to 24 h	NA	(MOHAMMED, 2016)
		Groundwater contaminated with receiving leachate	0 to 15 mM for 2 h	NA	(FARINELLI et al., 2021)
		Microcosm containing helminth eggs recovered from wastewater and fecal sludge	0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 cl L^{-1} for 24 h	None[3]	(LANDRY et al., 2021)
		Artificially contaminated test water	0.01, 0.03, 0.05, 0.1, 0.3 and 3% for 30 min; 3% for 60 min	Sodium metabisulfite	(SILVA; SABOGAL-PAZ 2021 - Chapter 3)
	WW	Treated sewage; artificially contaminated synthetic WW	0 to 300 mg L^{-1} for 10 min. Kinetic tests: 25, 50, 75 and 100 mg L^{-1}. Aliquots sampled at various time points until 60 min	NA	(VARGAS et al., 2013)
		Artificially contaminated treated sewage	Initial doses: 0.0, 10.2, 30.6, and 51 mg L^{-1}. Exposure time: 2 days	Sodium thiosulfate	(FAN et al., 2014)
		Treated sewage	7 mg L^{-1} for 10 and 60 min	None[1]	(YANG et al., 2017)
		Industrial	7840 mg L^{-1} was dosed at 1, 5, 10, and 15 min during a treatment time of 120 min. pH: 2.8.	NA	(ALCALÁ-DELGADO et al., 2018)
		Treated sewage	30 mg L^{-1}. NA exposure time.	NA	(GUADAGNINI et al., 2013)

Bench; pilot (batch)	W	Suspension; Artificially contaminated surface water for irrigation	In-vitro experiments: 0.2, 0.8, 3.2, 12.8, and 51.2 mL L^{-1} OX-VIRIN®; 5.2, 15.5, 46.4, 139.2, and 417.5 µL L^{-1} OX-AGUA AL 25®. Exposure times: 1 min, 5, 15, and 30 days. Natural conditions: 0.8, and 3.2 mL L^{-1} OX-VIRIN®; 46.4 mL L^{-1} OX- AGUA AL 25®. Exposure times: 0, 7, 14, and 18 days after infestation.	Sodium thiosulfate	(SANTOS-RUFO; RODRÍGUEZ-JURADO 2016)
Pilot (flow-through)	W	Hot water flowing through biofilm	1,000 mg L^{-1} at a 20 mL min^{-1} flow for 3–6 hours	None[4]	(FARHAT et al., 2011)

Notes: [1]Washing with PBS followed by centrifugation. [2]Considers complete dissolution of hydrogen peroxide residuals.[3]Washing with distilled water followed by centrifugation. [4]The total volume of treated water was renewed until residuals could not be detected. NA = Not available information. W = water. WW = wastewater. Peroxygen-based commercial disinfectants: OX-VIRIN® = 25% H_2O_2 plus 5% peracetic acid and 8% acetic acid; OX-AGUA AL 25® = 5% H_2O_2 plus 25% alkyl dimethyl benzyl ammonium chloride.

Results suggest there is some bias regarding the idea of hydrogen peroxide to be inefficient in the sanitation field because only few studies investigate disinfection with different methods by employing equivalent biocidal efficiency levels. Yang et al. (2017) has done so to compare the effects of monochloramine and hydrogen peroxide on the biological community of treated wastewater and found that minimum inhibitory concentration of the former (0.7 mg L^{-1}) was 10 times lower than H_2O_2 (7 mg L^{-1}), using *Pseudomonas aeruginosa* as a contamination model. Authors still raise the discussion that lab-cultured *P. aeruginosa* may respond differently from a strain native to wastewater, as well as from other organisms present in environmental matrices (LINLEY et al., 2012; YANG et al., 2017).

Additionally, several works on combined and catalytic treatment, for instance, apply H_2O_2 alone as a control, hence its low doses may reflect on ineffective results. A study that compared hydrogen peroxide to a Fenton-type nanocatalyst (MORALES et al., 2013), for example, selected a dose of 28.64 mg $L^{-1}H_2O_2$ based on the optimal Fe:H_2O_2 ratio (DI PALMA et al. 2003). Similarly, another work incorporated in Table 2-2 applied the 30 mg L^{-1}dose for a hydrogen peroxide oxidation, when it was, in fact, a control experiment to describe enhanced performance of H_2O_2/UV on disinfecting wastewater. Similarly, a control study in contrast to galvanic Fenton (GF) treatment investigated sole hydrogen peroxide by applying a 7840 mg L^{-1} H_2O_2 dose on industrial wastewater at pH 2.8, also following the Fe:H_2O_2, in this case, optimal for GF (ALCALÁ-DELGADO et al., 2018), also highlighting the variety in working conditions for hydrogen peroxide as a disinfectant. Although these papers prove a point in terms of possible synergism, their conclusions should not be escalated to hydrogen peroxide efficiency itself,

which has been known as satisfactory, as long as adequate operational conditions apply. These have been found to vary a lot according to specific challenges such as matrix or target-organism.

Here, the author recommends that if H_2O_2 is investigated for HWT uses, benchmarking other treatments should consider equivalent working conditions in terms of biocidal efficiency, particularly because the mode of action of each technology is not the same. Catalytic treatments rely on the formation of superoxide and hydroxyl radicals, which are highly reactive, thus, easily, and rapidly able to oxidize a wider range of molecules and recalcitrant pollutants. That said, catalytic processes are attractive for removing toxins, for instance, (MANSOURI et al., 2019), as well as resistant pathogens (ABELEDO-LAMEIRO et al., 2017), as described by peer literature. Nevertheless, such challenging purposes may not necessarily be the goal of liquid H_2O_2 as a HWT (e.g., in replacement of in-house chlorination), which should often target fecal bacteria and similar threats considering the water source, ideally with high quality. Additionally, HWTs are aimed to be low-cost and user-friendly, which are not necessarily the case of, for example, Fenton processes, that require a narrow acid pH range, and their iron lost to acidic sludge may be a hazardous waste (GARRIDO-RAMÍREZ et al., 2010).

It should be noted, however, that depending on the source water, it is possible that non-catalyzed hydrogen peroxide disinfection benefits from the presence of metallic ions present in the matrix, as previously reported for treated sewage (VARGAS et al., 2013). Contrariwise, the presence of carbonates and bicarbonates, which is frequent in groundwater, could hamper oxidation, as described by a research on H_2O_2 as a POU disinfectant that used water from a local well, considering this it is a common supply source in low-income regions (PATIL et al. 2013). This illustrates the importance of properly characterizing the water source and context when designing a HWT (SILVA et al., 2021), whether it relies on non-catalyzed H_2O_2 or not. Few studies on kinetics of peroxidation aimed at disinfection are reported in literature, as previously stated by peers (VARGAS et al., 2013), and confirmed by this review.

The same applies to exposure time, which varies depending on the treatment's purpose (e.g., shock disinfection, conventional disinfection, challenging matrices, etc.). From this literature analysis, and as displayed by Table 2-2, exposure times varied from seconds to days, not necessarily presenting an equivalent change in the order of magnitude of the H_2O_2 concentration under test. This makes sense when considering short and long-term effects, but not necessarily indicate efficiency or feasibility of a project, which should be discussed in future work for HWT. Moreover, few of the selected papers evaluate disinfectant demand prior to selecting contact time. This gap emphasizes the importance of the investigation of inactivation kinetics and residual disinfectant decay to assist the proposal of proper H_2O_2-based

technologies, considering local particularities. A study on cell viability of cyanobacteria and toxin removal by different oxidants performed a disinfectant demand experimental screening and determined chlorine acts in a matter of 30 min to get effective results, whereas ozone takes 5 min, potassium permanganate requires 180 min, and hydrogen peroxide could demand almost 2 days (FAN et al., 2014). This type of information would allow properly assessing costs and boost the design of household device and their efficiency, as well as proportionally compare performance to other technologies currently available.

Target-organisms also play an important role when determining operational conditions. In food industry, surface disinfection should consider the combination of contact time and concentration that considers the most resistant contaminant, in agreement with a "worst case scenario approach" (VISCONTI et al., 2021). This notion may also apply to household water treatment, which endorses the need for kinetic experiments, as well as an investigation of a diverse range of microorganisms, including resistant pathogens prior to any intervention, particularly when working with complex contaminated matrices, which may require larger biocidal concentrations to target persistent/surviving microorganisms (FARINELLI et al., 2021).

It should also be pointed out, that there is a lack of standardization in units of measure regarding H_2O_2 dosing, which we decided to present *verbatim* in Table 2-2. Even within the sanitation field, some papers report mg/L, while others treat it as cL L^{-1}, mmol L^{-1} and % (v v^{-1} or w v^{-1}). The latter is the most common approach found when screening eligible papers for this research (considering various decontamination scenarios). Although units can be easily converted, this variety may cause misinterpretations at first glance. Here, we recommend the use of % (v v^{-1} or w v^{-1}) in future research regarding non-catalyzed H_2O_2 in HWT, as it could simplify the understanding of dilutions from the users' perspective, especially because commercial hydrogen peroxide is often available as such.

2.3.4 Quenching

Residual H_2O_2 activity will determine the need for quenching. For drinking water purposes, regulation sources do not include standards for residual concentration, supposedly because H_2O_2 is not a conventional disinfectant in water treatment utilities (i.e., it has not been mentioned in classic guidance manuals such as USEPA (1999)). Such documents provide technical data and engineering information aimed at full-scale drinking water treatment plants, hence not applying to HWT systems conception, to which quenching may still be a concern.

As for food decontamination, comparatively, H_2O_2 appears in the tolerance exemptions list from USEPA (2002) on all commodities at the rate of ≤1% hydrogen peroxide

per application on growing and postharvest crops. The Food and Agriculture Organization of the United Nations (FAO) along with the World Health Organization (WHO) mentions that H_2O_2 excess is destroyed after its application for bactericidal effect in dairy products and foodstuffs. Toxicological considerations, thus, apply only to possible interference in nutritional value of treated products or the formation of toxic substances, but not to residual hydrogen peroxide (FAO; WHO, 1974). Treated with antimicrobial washing solutions, small residues on food at the time of consumption would not pose a safety concern (FAO; WHO, 2004).

Though not present in reports by international entities of the water sector, some eco-toxicity data is provided by scientific literature on sanitation. A study on GF treatment (ALCALÁ-DELGADO et al., 2018) has found that a 40 mg L^{-1} H_2O_2 residual does not affect *Lactuca sativa* germination. However, hydrogen peroxide standalone disinfection, which led to a 1570 mg L^{-1} residual, strongly inhibited the germination of lettuce seeds. Studies that evaluated kinetics of H_2O_2 decay in treated effluent (FAN et al., 2014) found that it remained relatively stable after a 6-day period (a final residual of 45.7 mg L^{-1}, which is higher than the initial dose of many treatments, as described in Table 2-2). Such scenarios indicate that residual hydrogen peroxide must be accounted in HWT conceptualization and design, particularly considering water quality, oxidant demand and working concentrations of disinfectant, as its residual may possibly not be so small compared to antimicrobial solutions applied in food decontamination, for example.

Table 2-2 includes a list of quenching agents applied for neutralizing hydrogen peroxide in sanitation and illustrates how it has been explored in peer scientific work. Research on neutralization of H_2O_2 following a UV-based advanced oxidation process found that chlorine is preferred over bisulfite for neutralization of the natural water matrix under test, both reacting at a 1:1 stoichiometric ratio (WANG et al., 2019). As for individual use of H_2O_2 in water treatment, such detailed investigation of chemical quenching is lacking. Likewise, there are limited reports in full-scale applications, that could be analogous for HWT. From our perspective, and considering gathered data, quenching should be considered as an operational parameter in HWTs, i.e., it is of major importance to determine whether the neutralizing agent is necessary, its dosing ratio and application form, so that system design is proper conceptualized and there are no risks in consumption, handling, and disposal.

2.3.5 Implementation challenges

Scaling may be one of the main future challenges in implementation, even if it is at the household level, particularly because peroxidation is not a conventional method recognized by the water sector. Table 2-2 indicates that sanitation research using H_2O_2 have mostly relied on

bench-scale studies. An assessment on chlorine as a HWT solution found that efficacy under laboratory controlled conditions was significantly better than POU chlorination, when both were evaluated on their log reductions and their ability to meet microbiological safety standards (LEVY et al., 2014). Likewise, if H_2O_2 is to be a candidate for HWT, it is highly recommended that context-specific conditions are considered (SILVA; SABOGAL-PAZ 2021), as previously mentioned.

Cultural particularities should be also considered at the development of the implementation strategies. This is a key gap found in this review, as there were no retrieved reports on standalone H_2O_2-based interventions at households. The author believes that challenges may be similar to chlorination in regard to community acceptance and follow-up. Hence, benchmarking strategies is encouraged, aiming to potentialize facilitators and avoid barriers, some of which have been reported for chlorine use (MITRO et al., 2019).

As for engineering aspects, authors do not consider hydrogen peroxide local storage to be a hazard (DOMÈNECH et al. 2001), but corrosive properties should be taken into account. Resistance to corrosion has been explored in research on plumbing materials commonly used in hospital settings (GIOVANARDI et al., 2020) and the effect of various disinfectants have also been studied on experimental coupons (MARCHESI et al., 2016). This should also be considered for HWT applications, aiming at longer device lifespan and a design that is safe to users.

An alternative to cope with these issues, both from the public acceptance and supply infrastructure perspectives, is the implementation of H_2O_2 disinfection at community collection points or as a POE solution. This could reduce the dependance on behavior change by relying on in-line devices without requiring major infrastructure (POWERS et al., 2021) and effort from the users. This brings opportunity for the conceptualization of automated and-or in-line hydrogen peroxide dosing mechanisms.

2.4 Concluding remarks

Some of the limitations of the work present in this chapter relate to the methodological choices of the research question, search string, filters, and the selected database. In addition, there is intrinsic interpretation bias in any content analysis. The multi-method approach (network visualization and content analysis) was an attempt to mitigate this constraint.

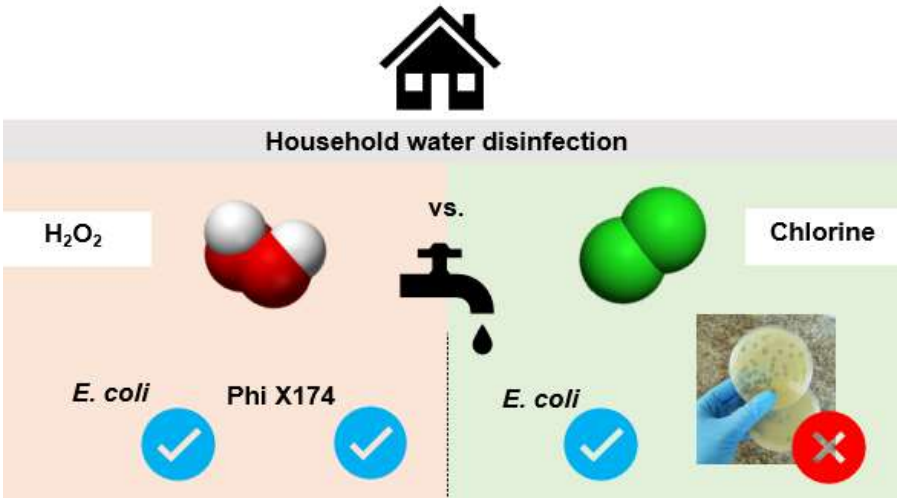
From gathered literature data, H_2O_2, has not been much explored in sanitation in the last decade and has not been much investigated as a POU/POE technology, even though

research in different areas point it as a promising approach. This brings up a knowledge gap, despite the attention that hydrogen peroxide disinfection has attracted in other disciplines.

This review showed that it is difficult to find consistency in dosing and exposure time due to scarce specific literature and because several studies on hydrogen peroxide as a disinfectant for water or wastewater treatment actually do so as a control for combined treatments. Additionally, matrix-specific kinetic experiments are lacking in the sanitation sector, as well as detailed information on residual neutralization, which impedes immediate application of this disinfection solution, especially at the household level, where there is a practical knowledge gap. Hence, unexplored dimensions on working conditions of H_2O_2 as a standalone method invite exploratory research that tackle different disinfection challenges, so that this alternative could be evaluated specifically for implementation as a HWT technology.

3- Chapter 3

Potentials and constraints of H_2O_2 water disinfection for household settings



Source: the author.

Highlights:

- Hydrogen peroxide was more efficient than chlorine in inactivating Phi X174.
- The virus model led to a higher disinfectant demand for both chlorine and H_2O_2.
- Photometric assays are misleading to evaluate organic matter oxidation by H_2O_2.

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3.1 Introduction

One of the main methods for point-of-use disinfection for drinking water is chlorination followed by safe storage. Chlorine has historically supplied microbiologically safe drinking water in collective water systems and, likewise, chlorine has also been introduced as a low-cost HWT in rural and marginalized communities (NIELSEN et al. 2022).

However, chlorine in the presence of natural organic matter (NOM) is associated to the formation of disinfection by-products (DBPs) (HU et al., 2018; MAZHAR et al., 2020). Thus, investigating alternative disinfectant products that could be potentially applied at the household level would avoid such concern, whereas leading to satisfactory pathogen inactivation.

In this sense, hydrogen peroxide (H_2O_2) is a potential candidate, considering that it has been widely employed in a variety of research fields, as explored in Chapter 2. Although there are reports of its application (both standalone and combined use) in disinfection of water sources (GUIMARÃES et al., 2014; KAREL, 2018) recreative water (ROSENDE et al., 2020) and wastewater (KOIVUNEN; HEINONEN-TANSKI, 2005; GUADAGNINI et al., 2013; FORMISANO et al., 2016), Chapter 2 demonstrated that research has not focused on individual use of liquid H_2O_2 at the household level for either POU/POE applications, nor humanitarian emergency water supply.

As much of the effective application of chlorine can be limited by uncertainties regarding the determination of initial dose (WU; DOREA, 2021), such difficulty also applies to hydrogen peroxide disinfection, which lacks straight-forward information for household-scale treatments. In order to shed light onto the possible application of H_2O_2 as a POU sole disinfectant for drinking water, it is important to initially evaluate its performance in laboratory-controlled settings, contemplating different microbial contamination scenes.

It should be noted that, from a research standpoint, probabilities of infection risk statistically increase when survival information for different microorganisms are used comparatively to indicator species data (MRAZ et al., 2021). In other words, relying on indicator bacteria alone for assessing treatment efficiency may underestimate the health risk to consumers (MRAZ et al., 2021), which encouraged us to explore other contaminants along with *Escherichia coli*.

Recent studies have underscored effluents as sources of viral contamination (YANG et al., 2021) and numerous reports have dedicated to the detection of viruses in surface water (HATA et al., 2014; GUO et al., 2018), freshwater (MASACHESSI et al., 2020), groundwater (EMELKO; SCHMIDT; BORCHARDT, 2019; JI et al., 2020) and even drinking water

(WANG et al., 2020). However, most household purification systems (and that includes chlorination) are characterized by their efficiency in removing bacteria, but not viruses in general (LUGO; LUGO; PUENTE, 2021). Timely, bacteriophages that infect coliform bacteria have been considered as possible surrogates for enteric viruses in surface and groundwater, as well as disinfected samples (SAVICHTCHEVA; OKABE, 2006; LAU et al., 2020). Hence, simulating contamination with bacteriophages as enteric viruses' models should be a suitable complementary analysis to standard indicator organisms, particularly because coliform bacteria and *E. coli* are not necessarily representative markers for viral contamination (PANG et al., 2021).

Therefore, the aim of this chapter was to assess the performance of hydrogen peroxide as a standalone disinfectant for potential point-of-use applications, considering a water source with low levels of natural organic matter, thus simulating a matrix compatible with direct disinfection, but high microbial load as if there was microbiological contamination. This was achieved by a comparison to conventional chlorine disinfection, considering a microbiological contamination simulated by seeded *Escherichia coli* as an indicator from the bacterial group, and Phi X174 bacteriophage as a virus model. This part of the thesis was also aimed at making some preliminary considerations on H_2O_2 effects on organic matter, in order to elucidate challenges and perspectives from the oxidation standpoint.

3.2 Materials and methods

3.2.1 Experimental procedure

Disinfection tests were carried out in reagent glass bottles previously disinfected. These were wrapped in aluminum foil, in order to avoid photo-degradation of hydrogen peroxide. Reactional conditions were provided by slow magnetic stirring. Raw and treated samples were characterized in terms of pH, temperature, and conductivity, as well as chemical parameters that required analytical methods further detailed.

Specific volumes of disinfectant stock solutions (sodium hypochlorite 10-15 % and hydrogen peroxide 30 %, both purchased from Sigma-Aldrich, USA) were added into 500 mL of artificially contaminated test water to achieve the desired initial doses, listed in Table 3-1. The selected concentrations for chlorine disinfection referred to preliminary demand tests carried out using seeded test water. In short, the 1.5 mg L^{-1} dose was motivated considering that typical chlorine doses in final treated water range from 0.2–2.0 mg L^{-1} of free chlorine (BRANDT et al., 2017; GOVERNMENT OF SUDAN, 2017). The demand assay indicated 0.2

mg L^{-1} free chlorine even at an initial concentration as low as 0.5 mg L^{-1} (Appendix 2). This concentration was therefore reproduced here, though at a shorter contact time (15 min), so that a critical scenario could be explored.

As for the chosen doses for hydrogen peroxide, this research considered information from literature, mainly on inactivating microorganisms' suspensions, which often require higher concentrations and exposure times. Thus, we started from 3 % H_2O_2 (KOLAR et al., 2015; SCANO et al., 2019; CHOI; LEE, 2020; TUVO et al., 2020), then tested lower doses laid out in Table 3-1

Table 3-1, which were explored stepwise, based in the obtained results. Hydrogen peroxide concentrations are present in % (v v^{-1}) for practical convenience, considering common ground in their commercial applications. However, concentrations in mg L^{-1} were checked prior to every test, considering stock solutions, initial dose, and residuals, so that coherence was obtained throughout this assessment.

Table 3-1 - Experimental conditions tested for *Escherichia coli* inactivation in test water

Disinfectant	**Exposure time**	**Dose**
Chlorine	30 min	1.50 mg L^{-1}
	15 min	0.50 mg L^{-1}
Hydrogen peroxide	60 min	3.00%
	30 min	3.00%
		0.30%
		0.10%
		0.05%
		0.03%
		0.01%

Note: Hydrogen peroxide concentrations in mg L^{-1} were confirmed prior to each assay. The same applies to chlorine, obtained by sodium hypochlorite, diluted into working solutions also tested for active disinfectant in terms of mg L^{-1} Cl_2.

After the contact time was completed, the residual concentration of the disinfectant under test was assessed according to analytical methods commercially available. Physicochemical characterization was performed, and disinfectant residuals were quenched by sodium metabisulfite (Neon, Brazil), as recommended by contemporary literature (MOORE et al., 2021). Microbiological examinations were carried out immediately afterwards, so that any residual activity regarding slow action of the quencher (WANG et al., 2019) would be avoided. Inactivation was calculated according to Equation 3-1.

$$Y = -log_{10}(\frac{N}{N_0}) \quad \text{Equation 3-1}$$

Experiments described in Table 3-1 were brought about considering *E. coli* as a target organism. After data analysis, Phi X174 inactivation was evaluated for the chlorine treatment that led to the highest log_{10}-inactivation of *E. coli*. As for experiments targeting the

bacteriophage, efficacy criteria considered no *E. coli* CFU mL^{-1} found in prior tests, as well as statistically similarity of means compared to chlorine treatment.

Controlled samples were kept for: test water without inoculum nor disinfectant (negative control), seeded test water without disinfectant (positive control), test water without inoculum but subjected to treatment. The latter was a reference for microbiological demand, when comparing residuals to the treated samples, whereas the positive control indicated the microbial input.

3.2.2 Test water

Study water was prepared based on the recommendation of the World Health Organization for the validation of household treatment technologies (WHO, 2018). An adaptation of general test water (presented here as TW), which is not technology-specific and represents high-quality groundwater or rainwater (WHO, 2014), was produced in order to simulate a matrix suitable for disinfection. In short, total organic carbon (TOC) from TW derived from tannic acid (Sigma-Aldrich, USA) and sodium carbonate (Qhemis, Brazil) provided alkalinity input. pH was adjusted with sulfuric acid (Sigma-Aldrich, USA). Test water characterization, prior to microorganism inoculum, consisted of TOC (TOC-LCPN, Shimadzu, Japan), alkalinity and pH (APHA; AWWA; WEF, 2012). UV absorbance at 254 nm and 274 nm wavelengths were also measured, as described in the analytical methods section.

3.2.3 Target organisms and microbiological analyses

In order to allow evaluating disinfection efficiency, although a high-quality water was tested, microbial load was added to the TW. This scenario could simulate on-site contamination, and the order of magnitude of the inoculums was based on the WHO International Scheme to Evaluate Household Water Treatment Technologies (WHO, 2018).

A lyophilized *Escherichia coli* strain (ATCC® 11229™) was activated, replicated, and cultivated in nutrient medium. Aliquots leading to an approximate concentration of 10^7 to 10^8 CFU 100 mL^{-1} were spiked into test water for artificial contamination. After treatments were performed, detection was carried out by the membrane filtration technique and *E. coli* colonies were grown in Chromocult® Coliform Agar medium (Merck, USA). Petri dishes were kept at 37 °C for 18−24 hours of incubation, and counts were performed in terms of CFU 100 mL^{-1}.

This study has used bacteriophage Phi X174 (ATCC® 13706-B1™) as a virus model and *Escherichia coli* (ATCC® 13706™) as its host. Seeding of test water was done with an approximate order of magnitude of 10^6 to 10^8 PFU mL^{-1}. Phi X174 was counted by the double-layer agar method (Kim et al. 2017; USEPA 2001). Tryptone soya agar (Oxoid™, USA) was

used as culture media and Tryptone soya agar (Oxoid™, USA) and bacteriological agar (Sigma-Aldrich, USA) consisted of the top agar. Considering these were non-selective media, samples were filtered in 0.2 μm membranes coupled to sterile syringes. Filtered samples were added to top agar together with the same volume of host *E. coli* suspensions and then overlayed onto the culture media. Plates were incubated at 37 °C for 18−24 hours and enumerated in terms of PFU mL^{-1}, according to Equation 3-2.

$$(\frac{PFU}{mL}) = \frac{1000 \times average\ PFUs\ on\ plates}{volume\ of\ sample\ added\ (\mu L)} \times serial\ dilution\ PFUs\ were\ counted\ at \quad \text{Equation 3-2}$$

3.2.4 Analytical methods

Free chlorine concentrations, as well as residual hydrogen peroxide were measured by colorimetric assays using a DR 3900 spectrophotometer (Hach, USA). The former was carried out by the USEPA DPD (N,N-diethyl-p-phenylenediamine) method using powder pillows (Hach, USA) of immediate reaction analyzed at λ = 530 nm. The latter was performed by the ferric thiocyanate method, using the Vacu-vials® kit (Chemetrics, USA) analyzed at 470 nm wavelength.

Total organic carbon was not measured in artificially contaminated test water, nor treated samples. Instead, spectrophotometric methods were used to assess organic matter after experiments were performed, using one-centimeter quartz cuvettes (Nanocolor UV/vis II, Macherey-Nagel, Germany). Absorbance was measured at 254 nm, representing dissolved organic carbon. The relationship between UV absorbance and tannic acid concentration was established by Equation 3-3 (r^2 = 0.9984, detection limit of 0.09 mg L^{-1} and limit of quantification of 0.30 mg L^{-1}). Thus, the 274 nm wavelength was additionally measured, in order to indirectly monitor organic matter derived from the tannic acid, main source of organic carbon from the test water. Details are provided in Appendix 2, including peaks at 274 nm obtained by spectrum scanning and relationships to tannic acid concentrations and TOC.

$$\text{Abs 274 nm} = 0.0423 \times \text{tannic acid concentration (mg } L^{-1}) + 0.0026 \quad \text{Equation 3-3}$$

Any hydrogen peroxide interferences in photometric assays were accounted for using blank standardized curves, considering found residuals. These are provided in the supplementary material.

3.2.5 Data analysis

Descriptive and inferential statistics was performed using PAST 3.2 software (HAMMER; HARPER; RYAN, 2001). Probability distribution of the samples was verified by Shapiro-Wilk normality test under a 95% confidence interval. Normally distributed data was tested by one-way ANOVA and the *post hoc* Tukey's test. For two-sample tests, Student's *t* test was used.

3.3 Results and discussion

3.3.1 Matrix characterization

Table 3-2 displays the physicochemical characteristics of the test water as a function of the seeded microorganisms used in this study. Therefore, test water used in this research was trusted as similar to matrices considered compatible to disinfection (apart from the microbial load, intended to be high) (WHO, 2014). That is because these matrices present low concentrations of organic carbon, thus not requiring separation treatments. Disinfection, instead of the removal of microorganisms, results in their inactivation.

Table 3-2 - Physicochemical characterization of general test water (TW) and effects of microbial load

Parameter	**Unit**	**TW**	**TW + *E. coli***	**TW + Phi X174**
Temperature	°C	25.0 ± 1.0	23.2 ± 1.0	21.2 ± 0.4
pH	-	7.07 ± 0.05	7.09 ± 0.16	6.62 ± 0.00
Conductivity	$\mu S\ cm^{-2}$	232.1 ± 17.8	215.2 ± 18.3	305.2 ± 3.9
TOC	$mg\ L^{-1}$	1.186 ± 0.191	NM	NM
Abs 274 nm	-	0.106 ± 0.013	0.097 ± 0.003	0.082 ± 0.001
Abs 254 nm	-	0.064 ± 0.006	0.063 ± 0.006	0.055 ± 0.003
Alkalinity	$mg\ L^{-1}\ CaCo_3$	55.81 ± 4.33	NM	NM

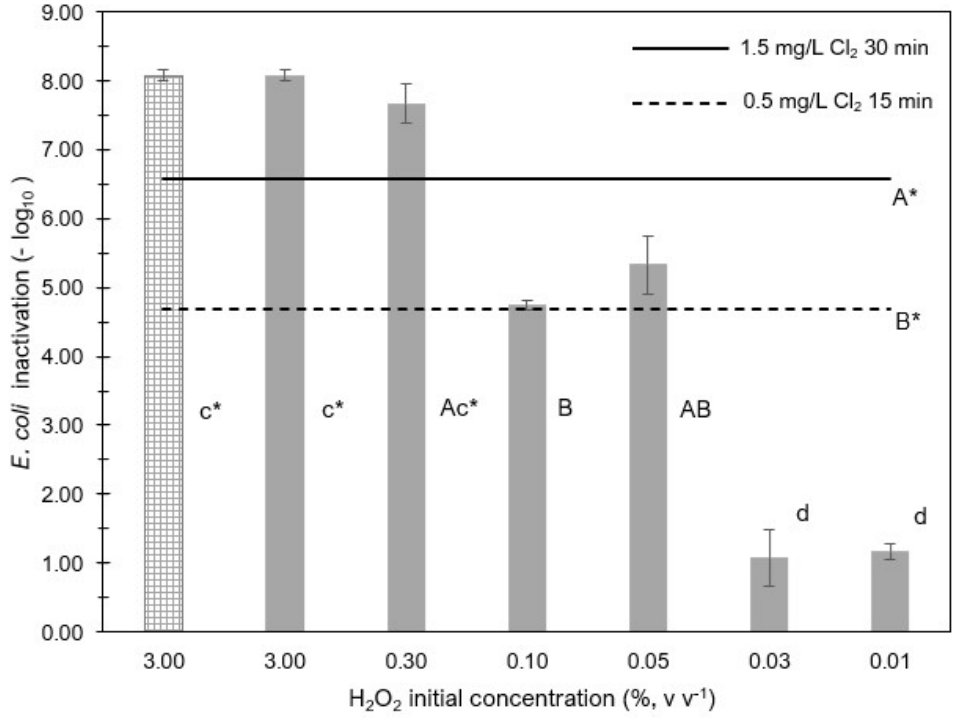
Notes: NM = not measured. TOC = total organic carbon. All the displayed values consist of average from the replicates and respective standard deviation. All repetitions referred to genuine replicates (different samples). Replicates for GTW characterization: n = 7, except for TOC and alkalinity, which n = 3. Samples inoculated with *E. coli*: n = 3. Samples inoculated with Phi X174: n = 2.

3.3.2 Disinfection

Inactivation of indicator bacteria obtained for different treatments (Table 3-1) is exhibited in Figure 3-1. Baselines indicate the log_{10}-reductions obtained by chlorine disinfection at different concentrations and exposure times. The 0.5 $mg\ L^{-1}$ Cl_2 concentration was intentionally low, in order to simulate free residual concentrations within storage tanks. During 15 min exposure time, this dose provided a 4.69 ± 0.54 log_{10}-inactivation of *E. coli*. Although recommended in the literature as an adequate residual for water in pipelines, it is most

likely not sufficient for storing water at home (LANTAGNE; CLASEN, 2009) or providing treatment per se. As for 1.5 mg L^{-1} Cl_2 in contact with contaminated water for 30 min, no colony forming units were found, providing a >6.58 log_{10} of inactivation. These are promising results, as they are refer to lower chlorine concentrations, as in some recommendations of dosing at 5 mg L^{-1}, which is likely to exceed the taste acceptability threshold (LANTAGNE; CLASEN, 2009).

Figure 3-1 - Mean log_{10}-reductions of *E. coli* as a function of disinfectant dose after 60-min exposure for grid-patterned columns and 30-min for solid-filled ones



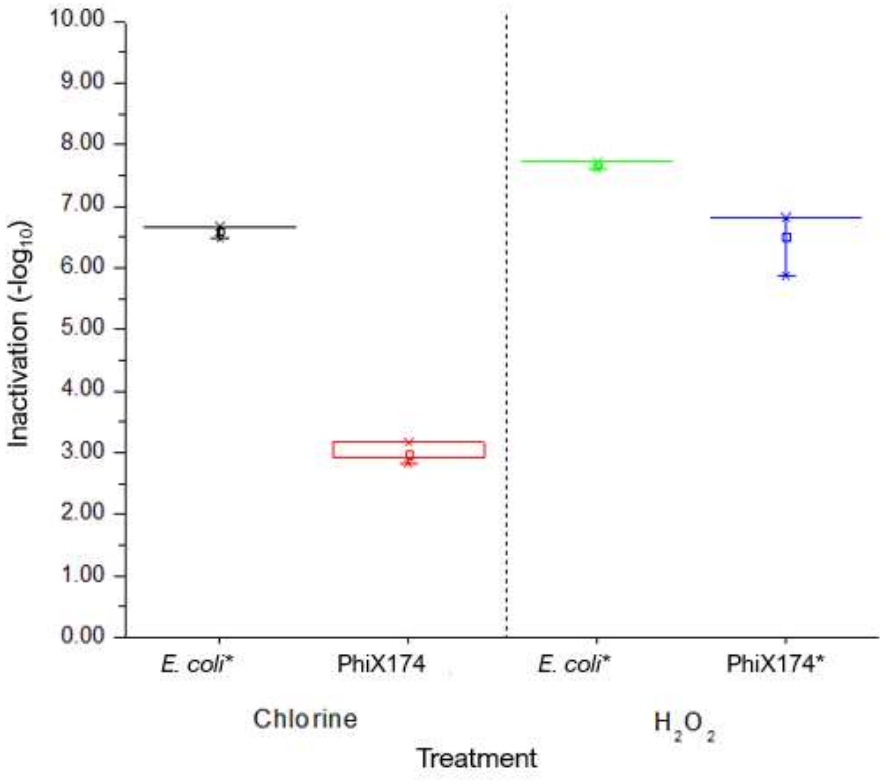
Notes: Baselines refer to log_{10}-reduction by chlorine disinfection. Letters denote statistically significant differences (Tukey's pairwise; $\alpha = 0.05$). Error bars indicate standard deviation (n = 3). Asterisks indicate conditions in which *E. coli* (CFU $100mL^{-1}$) was not detected in one or more replicates of treated samples.
Source: the author, also published in Silva and Sabogal-Paz (2021).

Results obtained from hydrogen peroxide disinfection displayed in Figure 3-1 support that, as a standalone disinfectant, H_2O_2 requires high doses and a long exposure time (WAGNER; OPLINGER; BARTLEY, 2012). An assessment of disinfection performance in pool water artificially contaminated with *E. coli* and *Pseudomonas aeruginosa* concluded that hydrogen peroxide was not effective as a biocide at 1.2 mg L^{-1} (ROSENDE et al., 2020), which is a compatible disinfectant concentration to reports of pools in use, but much lower than other H_2O_2 applications. Taking other studies into account, the 3% (v v^{-1}) concentration provided limited effect in shock disinfection followed by 1 hour flushing of dental settings (TUVO et al., 2020), suggesting exposure time is also an important parameter. Decontamination of footbath

for ovine footrot, targeting the bacteria *Dichelobacter nodosus* led to a 7.2 log_{10}-reduction, but dosing was as high as 5% (v v^{-1}) (HIDBER et al., 2020). In the present chapter, results showed limited *E. coli* inactivation at lower doses (0.03 and 0.01%), but 0.05% and higher concentrations of H_2O_2 for 30 min led to statistically similar or greater log_{10}-removals to chlorine treatments.

As *E. coli* is considered a suitable model organism for disinfection studies, particularly when fecal contamination of drinking water is assessed (WHO, 2011a), the highest values obtained for its inactivation were picked for the following test runs. These were carried out targeting Phi X174 and Figure 3-2 illustrates bacteriophage inactivation in a boxplot graph. For this assay, the selected chlorine concentration vs time (CT) values were 1.5 mg L^{-1} for 30 minutes, while 0.3% for 30 min was the chosen CT for hydrogen peroxide. The latter referred to a more conservative approach, as its choice was based on similarity to chlorine disinfection ($\alpha = 0.05$) and lower standard deviation (SD = 0.29) compared to the log_{10}-inactivation obtained by 0.05% H_2O_2 for 30 min (SD = 0.42). Note that CT values refer to dosed disinfectant.

Figure 3-2 - Boxplot of log_{10}-reductions obtained for *E. coli* and Phi X174 for different disinfectants during 30 min contact time



Note: Dashed line separates results obtained for chlorine at 1.5 mg L^{-1} Cl_2 and H_2O_2 0.3%. Asterisks denote treatments in which there was absence of microorganisms in treated samples.
Source: the author, also published in Silva and Sabogal-Paz (2021).

Comparison between chlorine and H_2O_2 treatments in test water contaminated with Phi X174 lead to a statistically significant difference in mean inactivation ($p < 0.001$; *t*-Student's test for chlorine against viral average log_{10}-inactivation as a given mean). Hydrogen peroxide was considered a better disinfectant alternative when virus are targets, achieving $>6.505 \pm 0.450$ log_{10}-inactivation, whilst chlorine led to 2.914 ± 0.147.

Analyzing the performance on different target organisms (Figure 3-2), chlorine reached a higher log-inactivation for *E. coli* compared to virus ($p < 0.001$; *t*-Student's test for two samples). This result endorses the fact that studies relying on indicator bacteria alone may overestimate treatment efficiency (MRAZ et al., 2021), which poses a risk to its prompt application in POU settings without considering different pathogen groups. That is because chlorine disinfection under the concentration versus time evaluated in this research was not deemed safe in scenarios of virus contamination, even if the literature has considered this concentration of free chlorine "good" for virus inactivation, in a scale from "excellent" to "poor"(GRAY, 2013). Disinfection treatments that lead to a minimum 4-log_{10} virus reduction are considered justifiable for matrices as in groundwater in absence of more detailed information in virus occurrence, enumeration, and dose-response (EMELKO; SCHMIDT; BORCHARDT, 2019). This threshold was not achieved by chlorine at the CT under study.

Although, apparently, the same outcome (*E. coli* log_{10}-inactivation > Phi X174's) was found for H_2O_2 disinfection ($p = 0.0014$; Student's *t* test for chlorine against viral average log_{10}-inactivation as a given mean), in this comparison, no PFU mL^{-1} were detected in treated samples. The log_{10}-inactivation obtained for virus (>6.505), lower than the one reached for *E. coli* (>7.678), may be explained by variations in the order of magnitude of the inoculum. Hence, hydrogen peroxide disinfection was considered efficient within the scope of the present work. However, further research comprising other groups of microorganisms e. g. protozoa and helminths is recommended.

3.3.3 Oxidation

Table 3-3 exhibits the physicochemical characterization of disinfected samples (targeting *E. coli*), as a function of contact time and concentration of both chlorine and hydrogen peroxide. Similarly, Table 3-4 displays these characteristics for TW spiked with Phi X174.

Chlorine treatments displayed in Table 3-3 imply an oxidation of natural organic matter (NOM, simulated by tannic acid and represented by absorbance at 274 nm), as well as organic carbon in general, represented by the absorbance at 254 nm wavelength. This can be inferred by comparing such properties with the raw water (TW spiked with *E. coli*, Table 3-2).

Assessing oxidation efficiency by chlorine, when water was contaminated with bacteriophage (Table 3-4), however, did not meet expectations. Although there was a slight removal of abs 274 nm, suggesting oxidation of NOM, absorbance at 254 nm increased. That said, evaluation of H_2O_2 oxidation performance was not considered fully reliable in this chapter.

Table 3-3 - Physicochemical characterization of treated samples and residual disinfectant concentration for treatments targeting *E. coli*

Parameter	Chlorine (mg L^{-1})		Hydrogen peroxide (%)						
	30 min	15 min	60 min	30 min					
	1.5	0.5	3.00	3.00	0.30	0.10	0.05	0.03	0.01
Temperature (°C)	23.9	25.1	22.1	22.1	22.5	22.8	22.5	23	22.5
pH	7.52	7.73	5.59	5.13	7.24	7.21	7.07	7.04	7.07
Conductivity (µS cm^{-2})	308.4	308.4	253.1	231.2	221.2	221.2	225.6	225.4	223.5
Abs 274 nm	0.018	0.020	NA	NA	NA	0.120	0.118	0.100	0.111
Abs 254 nm	0.030	0.010	NA	2.203	0.414	0.163	0.021	NA	NA
Mean residual (mg L^{-1}) ± SD	0.54 ± 0.02	0.25 ± 0.03	31,955± 2,363	35,931± 1,373	3,811 ± 2.18	1,059 ± 50.45	627.07 ± 0.94	364.94 ± 34.73	114.63 ± 0.08

Notes: NA = not available. SD = standard deviation. UV absorbance data for H_2O_2 treatments was corrected according to a second-order polynomial equations, adjusted to different hydrogen peroxide concentrations. Residual values were used as input, but if abs interference was superior to the obtained values or > 3.5, data was not considered and displayed as "NA". Residual concentrations of disinfectants were measured in duplicates and values of 3 % initial dose required 1000-fold dilutions prior to residual measurements.

Table 3-4 - Physicochemical characterization of treated samples and residual disinfectant concentration for treatments targeting Phi X174 bacteriophage

Parameter	Chlorine	H_2O_2
	30 min	30 min
	1.5 mg L^{-1}	0.3%
Temperature (°C)	21.9	21.6
pH	6.72	6.62
Conductivity (µS cm^{-2})	309.4	309.1
Abs 274 nm	0.077	0.083
Abs 254 nm	0.088	0.092
Mean residual (mg L^{-1}) ± SD	0.04 ± 0.00	3763.28 ± 0.00

Notes: SD = standard deviation. UV absorbance data for H_2O_2 treatments was corrected according to a second-order polynomial equations, adjusted to different hydrogen peroxide concentrations. Residual concentrations of disinfectants were measured in duplicates.

Table 3-3 and Table 3-4 display the high residuals found, which may have hindered photometric assays, even though blank curves were prepared (Appendix 2), and values displayed within these tables were corrected accordingly. This remaining interference was also endorsed by the increase in UV absorbance at 274 nm, which was supposed to have been associated exclusively to NOM (simulated by tannic acid), whereas 254 nm should had

represented a broader perspective. Therefore, within the scope of our study, interpretations regarding release of intracellular organic matter and oxidation of NOM were not made for hydrogen peroxide treatments.

This issue has been reported for chemical oxygen demand (WU; ENGLEHARDT, 2012), but here we expand it to other photometric assays. It is suggested that any UV absorbance analyses are carried out after residual removal, so photolysis of hydrogen peroxide is avoided during measurements. If quenching with catalase enzyme is performed (FLORES et al., 2012; ARVIN; PEDERSEN, 2015), it is important to notice if there is any increase in the organic load of the samples. Further research is recommended, including total organic carbon as a parameter, not only to avoid H_2O_2 interference, but especially because chlorine-based oxidation of NOM-enriched water may lead to the formation of disinfection byproducts (GOSLAN et al., 2009).

3.3.4 General limitations and further research

Considering variations in water quality, disinfectant decay studies should be performed prior to any implementation. It is recommended that these are carried out within different contamination scenarios (as in various organic loads, turbidities, and target microorganisms), in order to provide notions on required dose, as well as to assess the need of residual H_2O_2 neutralizing. Tests on natural matrices are also encouraged.

Similar research has considered hydrogen peroxide a promising alternative to chlorine-based disinfection, but also raised a concern towards performance in different community settings, as well as corrosion effects in pipelines (MARCHESI et al., 2016). In this sense, though we present an overall assessment the performance of liquid H_2O_2 as a POU/POE disinfectant, case studies would allow exploring context-specific potentials and challenges for different source waters and household settings.

3.4 Conclusions

Results from this chapter reiterated that relying on indicator bacteria alone may be misleading or underestimate microbiological risk of treated water. This was inferred because inactivation obtained by chlorine and hydrogen peroxide were considered statistically similar targeting *Escherichia coli*, though the disinfectants efficacy were dramatically different when Phi X174 bacteriophage was a target. In this scenario, hydrogen peroxide was more effective than chlorine, as the former led to an approximate >6.5 $\log_{10}$-inactivation and the latter reached around 3.0 under the most ideal tested conditions.

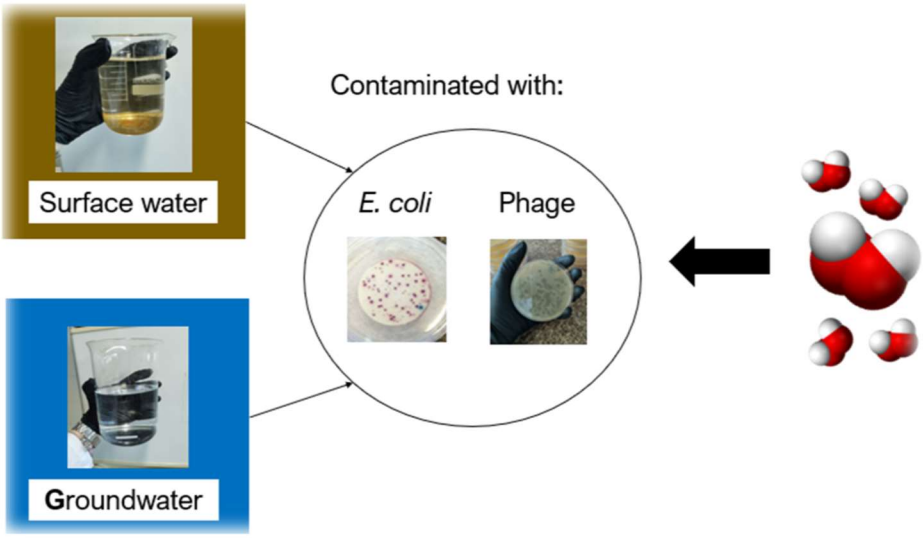
Although a comparison of *E. coli* and Phi X174 was presented, a broader assessment of the H_2O_2 disinfection effectiveness should be performed. It is recommended that disinfection efficiency evaluation is extended to different groups of pathogens, as well as different strains within each group prior to implementing hydrogen peroxide as a POU intervention. Residual decay assays, as well as prediction models considering different contamination scenarios and hydrogen peroxide concentrations are also advised for future studies.

Similarly, oxidation of natural organic matter should be studied considering total organic carbon as a parameter. That is because UV absorbance data (at 254 nm and 274 nm wavelengths) was not considered consistent as an inference of organic load, even though effects from residuals were accounted for.

From a batch experiment carried out in bench scale, this chapter suggests hydrogen peroxide may be promising as a point-of-use disinfectant aiming to achieve SDG6, but further evaluations are required prior to any interventions. Additionally, though this chapter presented a general perspective of some advantages and constraints, investigation within specific household settings is recommended.

4- Chapter 4

Considerations on the effects of pre-oxidation with H_2O_2 as a household treatment of natural waters



Source: the author.

Highlights:

- H_2O_2 preoxidation reduces virus and *E. coli* contamination levels in surface water.
- 5-min oxidation with H_2O_2 led to >3.0 $\log_{10}$ inactivation of *E. coli* from surface water.
- H_2O_2 preoxidation may improve microbiological quality of surface water prior to other treatments.
- H_2O_2 preoxidation of groundwater for reducing microbiological load is not encouraged at the tested doses.
- Natural catalysts from surface water may have enhanced H_2O_2 preoxidation performance.

A modified version from this chapter was published in:

SILVA, K.J.S., LEITE, L.S., FAVA, N.M.N., DANIEL, L.A., SABOGAL-PAZ, L.P. Effects of hydrogen peroxide preoxidation on clarification and reduction of the microbial load of groundwater and surface water sources for household treatment. **Water Supply**, v. 23, n. 3, p. 1–11, 2021. Available at: < https://doi.org/10.2166/ws.2021.421>.

4.1 Introduction

Household water treatment (HWT) systems e.g., solar disinfection (SODIS), filtration, and others, are limited by the quality of the source water (ROSE, 2005; GAO et al., 2011), particularly when it contains high levels of natural organic matter (NOM) associated to turbidity and color. NOM removal is therefore essential, as it conveys color and taste to the water, makes it unattractive to consumers, provides substrate for bacterial regrowth in the distribution system and storage, and potentially imparts adsorbed organic and inorganic contaminants, as well as microorganisms (EXALL; VANLOON 2000).

As for HWT performance, such unfavorable conditions may also cause rapid membrane fouling or clogging of filter media, increasing maintenance frequency, and reducing water production (POOI; NG 2018), as well as increasing the risk of microorganisms to permeate through (GWENZI et al., 2015). In addition, NOM raises chemical demand and costs in traditional treatments (XIE et al., 2016), hence similar impairments apply to HWT technologies.

Pretreatment processes in drinking water production typically rely on screening, preconditioning, and/or other site-specific processes aiming to improve and adapt water quality in such a way that the main technology has its life extended (PANGULURI et al., 2014). Oxidation of organic and inorganic molecules is a common approach for clarification (as well as disinfection), hence chlorination has been a popular method for achieving this goal (BLACK & VEATCH CORPORATION, 2009). Nonetheless, the use of chlorine products in the presence of NOM is associated to the formation of carcinogenic disinfection by-products (DBPs) (HU et al., 2018). An effective approach for containing DBP formation is removing precursors by alternative treatments such as preoxidation with alternative oxidants (SHARMA et al., 2005; LIN et al., 2012). Besides chlorine, permanganate and ozone are the main oxidants for preoxidation of feed water (ZHANG et al., 2013; LU et al., 2015).

Besides the wide applicability described in Chapter 2, hydrogen peroxide is not often contemplated in preoxidation (XIE et al., 2016). This encourages exploring its potential, particularly considering it may be an alternative for conditioning source waters to household treatment systems that require turbidity and color to not exceed a certain range, as well as to assess removing DBP precursors. Additionally, information on its effectiveness in reducing fecal contaminants in natural waters is lacking in literature. This should be timely within the

context of household treatments, whose goal is mostly based on improving water quality from a public health perspective regarding waterborne diseases (EHDAIE et al., 2020).

In this scene, the aim of this chapter was to overall evaluate the effects of H_2O_2 preoxidation of two natural water matrices (surface water and groundwater) in bench-scale batch tests. Pretreatment performance was assessed in terms of physicochemical parameters and microbial load, considering indicator bacteria (*Escherichia coli*) and an enteric virus contamination model (Phi X174). Insights on how water quality influences preoxidation raised a discussion toward potentials of H_2O_2 in HWT.

4.2 Methods

4.2.1 Experimental design

In this chapter, effects of hydrogen peroxide preoxidation of different water sources, artificially contaminated with a high microbial load were investigated. The microorganisms under analysis were an enteric virus contamination model (Phi X174) and an indicator bacterium (*Escherichia coli*). These were selected, as well as the order of magnitude of the inoculums, based on the international scheme to evaluate HWT technologies by the World Health Organization (WHO, 2018).

The first experiment consisted of assessing H_2O_2 initial demand by the water sources, by measuring hydrogen peroxide residuals and pH after two minutes of reaction with 500 mL samples. Another batch of experiments followed, in which a preoxidation setup was simulated within the same conditions, but extending the contact time to five minutes, so that disinfection potential could be evaluated. In these preoxidation tests, physicochemical parameters were then measured, as well as microorganism inactivation. Here, a short exposure time was chosen, assuming a conservative approach, i.e. worst scenario for a household setting, considering preoxidation experiments might range from five up to 100 minutes (LV et al. 2019; LIU et al. 2020), depending on the matrix, goal, and available conditions.

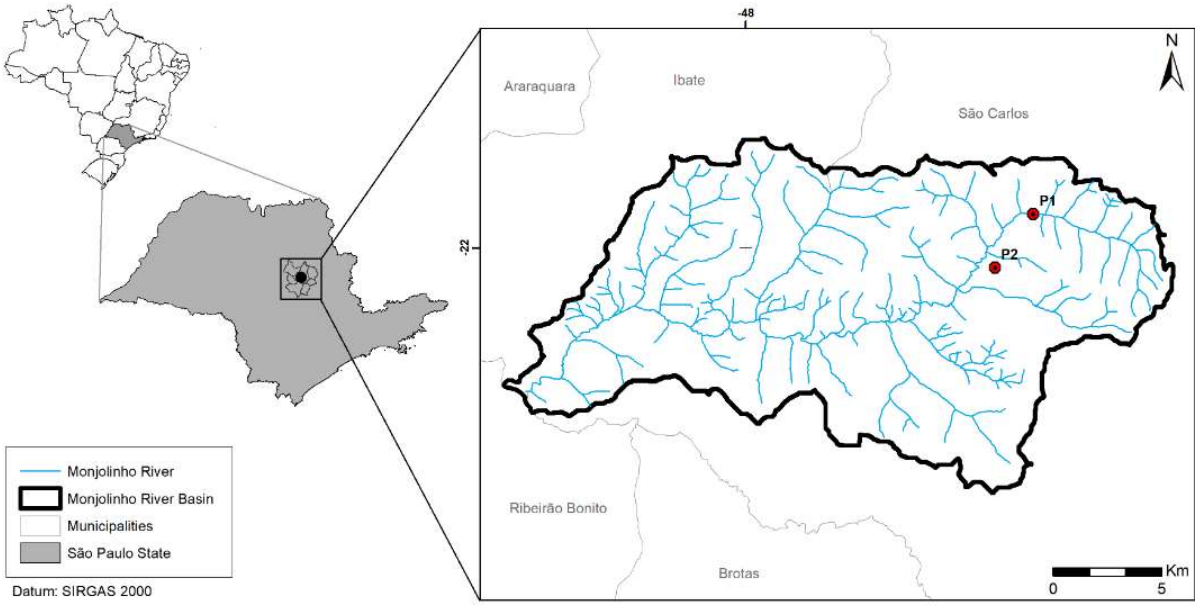
Experiments were performed in previously sterilized reagent bottles, wrapped in aluminum foil to prevent photolysis. Magnetic stirring provided the mixture environment. Hydrogen peroxide (30 % v v^{-1}) was purchased from Sigma-Aldrich®, USA.

4.2.2 Test matrices

This study considered two natural matrices, into which indicator bacteria and an enteric virus contamination model were spiked. Samples were characterized before and after the inoculum. Surface water samples were collected from Monjolinho River, a water source

located in the municipality of São Carlos (São Paulo State, Brazil). Groundwater samples were obtained from a well located in the same municipality, accessed from São Carlos School of Engineering (SCSE, USP, Brazil). Collection sites are displayed in Figure 4-1.

Figure 4-1 - Location of the test waters' collection sites



Notes: P1: Monjolinho River (superficial water source); P2: well from São Carlos School of Engineering.
Source: elaborated by Larissa Lopes Lima, as published in Silva et al., (2021).

4.2.3 Physicochemical tests and analytical methods

Both test waters were characterized according to Standard Methods (APHA *et al*. 2012) prior to inoculum and after microorganisms were spiked into them. Zeta potential measurements were performed using Zetasizer Nano-ZS (Malvern, UK) at 25 °C. Iron was quantified by USEPA FerroVer® Method using the Iron Reagent Powder Pillows (Hach, USA) analyzed at 510 nm wavelength in a DR 5000 spectrophotometer (Hach, USA).

Residual hydrogen peroxide was measured by the ferric thiocyanate method, and the presence of free chlorine in both raw waters was assessed by the USEPA DPD (N,N-diethyl-p-phenylenediamine) method, both described in section 3.2.4 Analytical methods). Quenching was also performed according to the referred section,

4.2.4 Target organisms and microbiological analyses

In order to represent fecal contamination, an *Escherichia coli* strain (ATCC® 11229™) was inoculated to the samples as indicator bacterium. Additionally, Phi X174 (ATCC® 13706-B1™) bacteriophage was used as viral indicator of water quality and *Escherichia coli* (ATCC® 13706™) as its host. Inoculum and quantification were performed according to section 3.2.3 Target organisms and microbiological analyses). However, in this chapter, the order of

magnitude of the bacteria inoculum (ATCC® 11229™) was approximately 10^8 UFC 100 mL^{-1}. As for phage (ATCC® 13706-B1™), natural waters were spiked at approximately10^5 PFU mL^{-1}, but there was some die-off of working stocks, as explained in the discussion section of this chapter.

4.2.5 Data analysis

PAST 3.2 software (HAMMER et al. 2001) was used for descriptive and inferential statistics. Pearson's correlation was applied for evaluating the association between physicochemical variables and H_2O_2 concentrations. As for microbiological assessment results, Shapiro-Wilk normality test under a 95% confidence interval determined the probability distribution of the samples, so that normally distributed results were analyzed by one-way ANOVA and the *post hoc* Tukey's test.

4.3 Results and discussion

4.3.1 Physicochemical characterization and oxidant demand

The general characterization of the water sources is shown in Table 4-1. It indicates microorganism spiking did not cause any major differences in physicochemical characteristics of the test waters. Differences in water quality obtained for the two sources also draw attention to the importance of such characterization. That is because, even though an HWT may be considered efficient under certain conditions, its ability to improve water safety within a village setting may vary as a function of source water characteristics c

In addition to Table 4-1, it should be noted that testing for free chlorine carried out for all matrices (both raw and seeded with microorganisms) led to concentrations lower than 0.1 mg L^{-1} Cl_2. Therefore, any chlorine effects on microorganisms, as well as possible interferences in analytical methods, were considered negligible.

Table 4-1 - Characteristics of the test waters before and after inoculum with *E. coli* and Phi X174 phage

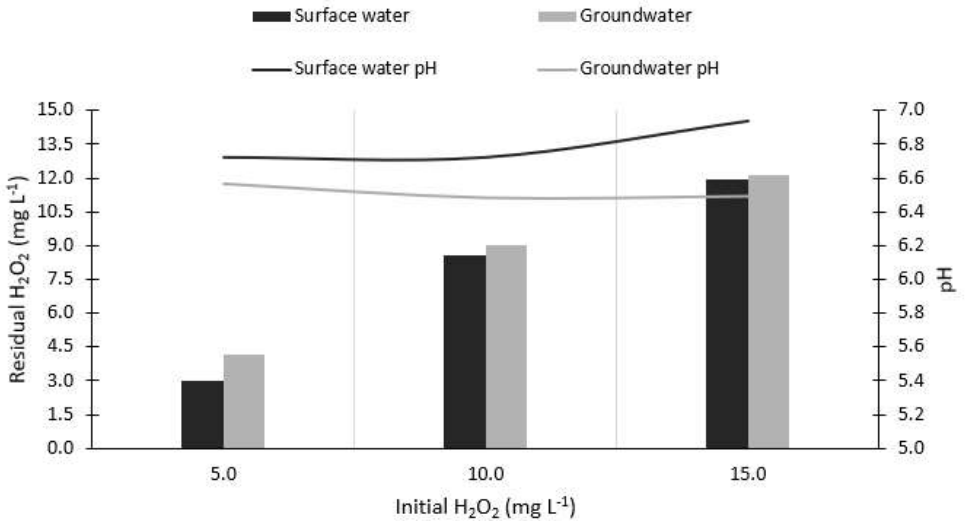
Parameter	Unit	Surface water		Groundwater	
		Raw water	Seeded water	Raw water	Seeded water
pH	-	6.38	6.63	6.21	6.47
Turbidity	NTU	19.00	17.60	0.17	1.16
Apparent color	HU	118.0	113.0	0.9	4.1
Abs 254 nm	-	0.317	0.317	0.004	0.041
Total alkalinity	mg $CaCO_3$ L^{-1}	22.22	NM	28.05	NM
Conductivity	µS cm^{-1}	55.77	187.40	53.37	76.82
Iron	mg Fe L^{-1}	1.44	NM	<0.01	NM
Zeta potential	mV	-16.4	-19.7	-12.9	-14.2
Escherichia coli	CFU 100 mL^{-1}	2.9 x 10^3	6.7 x 10^9	ND	2.5 x 10^8
Total coliforms	CFU 100 mL^{-1}	1.5 x 10^4	6.7 x 10^9	ND	2.5 x 10^8

Phage	PFU mL^{-1}	NM	1.2×10^5	NM	5.9×10^4

Notes: ND refers to not detected and NM refers to not measured.

Figure 4-2 shows the residuals found after two minutes of the reaction of hydrogen peroxide to the different matrices, as an inference of initial demand. The oxidant demand represents the consumed disinfectant after it immediately reacted to the sample, considering the presence of competing species, prior to actively reacting towards the inactivation of microorganisms (FREITAS et al., 2021). It is known that initial demand is directly associated to the water quality (AMERIAN et al., 2019), but no major differences were found when comparing neither H_2O_2 residuals nor shifts in pH of (both seeded) surface water and groundwater, as displayed by Figure 4-2. That should be explained by the fact that both source waters under test present low levels of organic matter and strong competitors as in sulfide compounds (WANG et al., 2017), when compared to more contaminated matrices also often designated to oxidation treatments, e. g. domestic sewage (MEDEIROS; DANIEL, 2017; FREITAS; LEITE; DANIEL, 2021) or agro-industrial wastewater (SARTORI et al., 2015; MANDRO et al. 2017). This may come as an advantage from the preoxidation standpoint, as lower doses would be required to directly target microorganisms or provide water clarification, considering the dosed oxidant is supposed to be readily available.

Figure 4-2 - Residual concentrations of hydrogen peroxide found for surface water and groundwater after two minutes of exposure



Notes: Primary y axis refers to columns the secondary ordinate refers to lines.
Source: the author, as published in Silva et al., (2021).

4.3.2 Water clarification

The relative removals obtained for the major physicochemical quality parameters are shown in Table 4-2. Strong correlations were found for the applied dose and clarification of

surface water (r = 0.93 for turbidity removal; r = 0.93 for color removal) and oxidation of organic matter measured by absorbance at 254 nm wavelength (r = 0.95).

As for groundwater, a Pearson correlation of 0.59 was observed for turbidity reduction. This could be explained by the good quality of the raw water itself, which led to clarification up to almost 100% at the lowest H_2O_2 concentration. Final turbidity obtained for all of the tested doses was <0.3 NTU for groundwater. Removals of color and abs 254 nm led to r = 0.92 and 0.94, respectively.

Table 4-2 - Hydrogen peroxide residuals and effects in physicochemical characteristics of both seeded surface water and groundwater after 5 min, as a function of applied dose.

Parameter		Surface water			Groundwater		
		5 mg L^{-1}	10 mg L^{-1}	15 mg L^{-1}	5 mg L^{-1}	10 mg L^{-1}	15 mg L^{-1}
Final pH	-	6.46	6.76	6.74	6.80	6.90	6.84
Final zeta potential	mV	-15.1	-21.3	NA	-12.6	-18.4	-16.7
Turbidity removal	%	28.8	59.0	64.5	79.3	80.8	80.2
Color removal	%	8.0	29.2	50.4	44.4	60.5	63.0
Abs 254 nm reduction	%	1.7	35.4	44.8	14.6	26.8	24.4
H_2O_2 residual	mg L^{-1}	2.76	5.15	5.71	4.17	8.51	8.65

Notes: NA refers to data that is not available.

A lower, yet satisfactory, reduction in absorbance at 254 nm was found when compared to turbidity and color removals of both matrices. Differences in absorbance at 254 nm may have occurred by oxidation of carbon, without, however, effectively reducing dissolved organic carbon. It is recommended that total organic carbon is tested coupled to absorbance in the UV spectrum so that alterations in organic matter could be assessed more precisely. Additionally, it should be noted that groundwater presented a low 254 nm absorbance prior to treatment (Table 4-2). As for surface water, the obtained performance was considered adequate for further treatments. Some HWT systems such as household slow sand filters rely on physical barriers as in non-woven synthetic fabric to adequate physicochemical parameters to their limitations (FARIA MACIEL; SABOGAL-PAZ, 2018). This pretreatment has led to relative removals of approximately 46 % turbidity and 21 % apparent color (FREITAS et al., 2021; TERIN et al., 2021), falling into similar efficiencies of H_2O_2 preoxidation found in our study.

Additionally, results obtained for zeta potential did not show any trend in groundwater samples after preoxidation. As for surface water, although there is unavailable data for the highest hydrogen peroxide tested concentration and a lower value was found at 10 mg L^{-1} H_2O_2, the decrease in absolute zeta potential obtained at 5 mg L^{-1} is suggestive of a reduction in negative charge density of organic matter, a behavior reported in preoxidation literature (LIU et al., 2020). This encourages further research, because, additionally, in the presence of metals

such as iron or manganese, preoxidation may cause a rupture in complexes of the metallic ions, resulting in an in-situ production of coagulant (XIE et al., 2016).

This endorses that characterization of source water quality is essential for selecting site-specific HWTs, which might be potentially improved by pretreatments such as preoxidation. Within the scope of this research, river water presented iron, as displayed by table 1. Fe(III) positive charge might contribute to increasing zeta potential, by reducing electrostatic repulsive interaction through electrostatic neutralization (HE et al., 2015). Considering similar water quality, particularly if a coagulation treatment was planned as the main HWT (CRUMP et al., 2004), optimization of such mechanism is highly recommended in order to take advantage of natural water conditions. Accordingly, other HWTs based on activated carbon, sand, or membrane filtration, for instance, could also be favored. That is because surface charge properties influence adsorption (HIJNEN et al., 2007) and there is data on attenuation of membrane fouling and decreasing formation potential of DBPs after preoxidation correlating to the reduction of negativity of zeta potential (HE et al., 2021; KHAN et al., 2020).

4.3.3 Microorganism inactivation

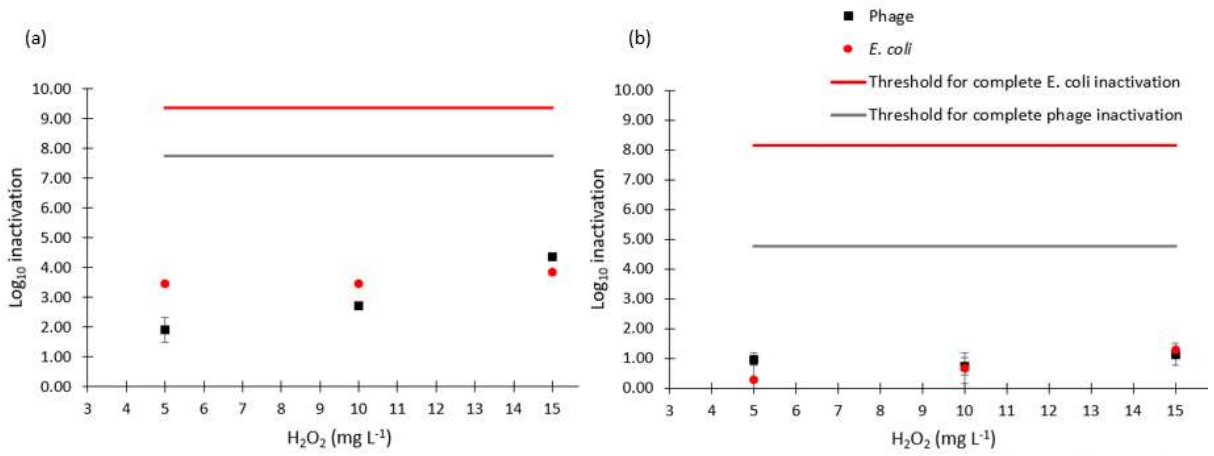
Figure 4-3 displays the results obtained for Phi X174 phage and *E. coli* in surface water and groundwater. Baselines indicate the desired level for complete inactivation, considering controlled samples with microorganism spiking, but no oxidation treatment. It is worth noting that although experimental procedures were repeated rigorously, there was some die-off of both E. *coli* and phage spiked into the samples, which is seen by comparing Table 4-1 to Figure 4-3. In addition, different microorganism resistance was not assessed due to the variation in order of magnitude between inoculums. The same applies to the effects of dosing in different matrices. Therefore, this chapter investigated the inactivation of microorganisms, individually, within each matrix.

Considering surface water (Figure 4-3, a), the 15 mg L^{-1} hydrogen peroxide dose provided 4.35±0.04 log_{10} inactivation of phage and an average of 1.90 ± 0.30 log_{10} at 5 mg L^{-1}. Targeting *E. coli*, the highest reduction amongst the concentrations under study was also obtained at 15 mg L^{-1} H_2O_2 (3.84 ± 0.08 log_{10}), and the lowest, likewise, referred to the 5 mg L^{-1} dose (3.45 ± 0.07 log_{10}). These results suggest preoxidation applications in surface water with similar characteristics to the present one may be useful to reduce disinfectant demand in further steps of treatment, as even low concentrations of hydrogen peroxide led to reduction in microbial load of the matrix. This inference is endorsed by one-way ANOVA, which recommends rejecting the null hypothesis of similar means for the log_{10} inactivation of phage (p = 0.0007), as well as *E. coli* (p = 0.0019) at the 95% confidence interval. Tukey's *post hoc*

test results are shown in Table 4-3, indicating that the 15 mg L^{-1} H_2O_2 concentration provided statistically significant results against the other tested doses for log_{10} reductions considering both target-organisms.

As for groundwater (Figure 4-3, b), only 15 mg L^{-1} H_2O_2 reached >1.0 log_{10} reduction (1.14 ± 0.38 for phage and 1.27 ± 0.04 for *E. coli*), which is still far from a desired dejection in microbial load. Inferential statistics imply similar means for data on both phage (p = 0.3464) and *E. coli* (p = 0.1483) inactivation in groundwater at the different H_2O_2 doses under test. Such low effects on microbial concentration do not encourage hydrogen peroxide preoxidation of this matrix.

Figure 4-3 - Mean log_{10}-reductions of *E. coli* and Phi X174 as a function of H_2O_2 concentration during 5-min preoxidation in (a) surface water, and (b) groundwater



Notes: Error bars refer to standard deviation (n=3) and baselines indicate the log_{10} levels that would refer to complete inactivation of each inoculum.
Source: the author, as published in Silva et al., (2021).

Table 4-3 - p-values of Tukey's pairwise test (α = 0.05) for log_{10} microorganism inactivation of surface water

H_2O_2 concentrations compared	**Phage**	***E. coli***
5 mg L^{-1} vs. 10 mg L^{-1}	0.0514	0.9706
5 mg L^{-1} vs. 15 mg L^{-1}	**0.0005**	**0.0033**
10 mg L^{-1} vs 15 mg L^{-1}	**0.0071**	**0.0375**

Notes: Results in bold refer to significant differences in means.

A straightforward treatment approach is thus recommended to water sources with quality such as the seeded groundwater from our study. Although the lack of oxidation competitors (Figure 4-2) suggests oxidative radicals would be more available for microorganism inactivation of this matrix, results obtained have shown otherwise. Additionally, pretreatments would be unnecessary as low NOM levels were found in groundwater (Table 4-1), hence preoxidation would be an avoidable extra step.

Comparing the inactivation levels obtained in the two source waters, Figure 4-3 clearly illustrates that preoxidation provided a better performance for inactivating spiked microorganisms from river water. It should be noted that natural water sources may contain catalytic species. Iron, copper and zinc, for instance, provide good catalytic activities (KITANOSONO et al., 2018). By analyzing Table 4-1, an iron concentration of 1.4 mg Fe L^{-1} was found in the surface water sample. Considering the presence of the catalyst, we believe that a non-intentional Fenton process may have acted during peroxidation, improving disinfection performance in river water, even though pH and stoichiometric conditions were not ideal. In this process, hydroxyl radicals (·OH), which present powerful oxidation ability, are produced from the reaction between aqueous ferrous ions and H_2O_2 (POLO-LÓPEZ et al., 2019), according to:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

In order to obtain good disinfection rates, higher amounts of iron are usually required (POLO-LÓPEZ et al., 2012). However, humic substances may either consume or catalyze the formation of hydroxyl radicals, depending on their concentration and molecular form (VIONE et al., 2004) Here, we also raise the hypothesis that they may have acted as catalysts to the Fenton process, which is favorable for practical reasons. Considering that reagents are one of the most impairing costs for (POLO-LÓPEZ et al., 2019), the presence of natural catalysts in source waters may be advantageous. We highlight this potential, especially if H_2O_2 preoxidation is intended prior to solar disinfection (SODIS) treatments (VILLAR-NAVARRO et al., 2019; JIN et al., 2020), for instance, either providing or improving a photocatalysis setting.

In short, results strongly suggest the influence of natural catalysts in river water, which improved the inactivation performance of H_2O_2 on the target-organisms by giving means to the formation of hydroxyl radicals. It should be noted, additionally, groundwater presented a little higher total alkalinity compared to the surface water source, which may have prevented the formation of hydroxyl radicals (BURNS et al., 2012), along with the lack of natural catalysts.

Although H_2O_2 is a thermodynamically powerful oxidant, its reaction rates are typically slow compared to those of free radicals (BURNS et al., 2012). It is generally believed that microbial inactivation by hydrogen peroxide does not directly result from oxidative properties of its molecular state, but the consequence of the activity of other strongly oxidant chemical species derived from it (LABAS et al., 2008). In this sense, implementing a preoxidation stage should consider advantages and constraints related to water quality and the main HWT, in order to obtain the best from H_2O_2 potentials within specific settings.

4.4 Conclusions

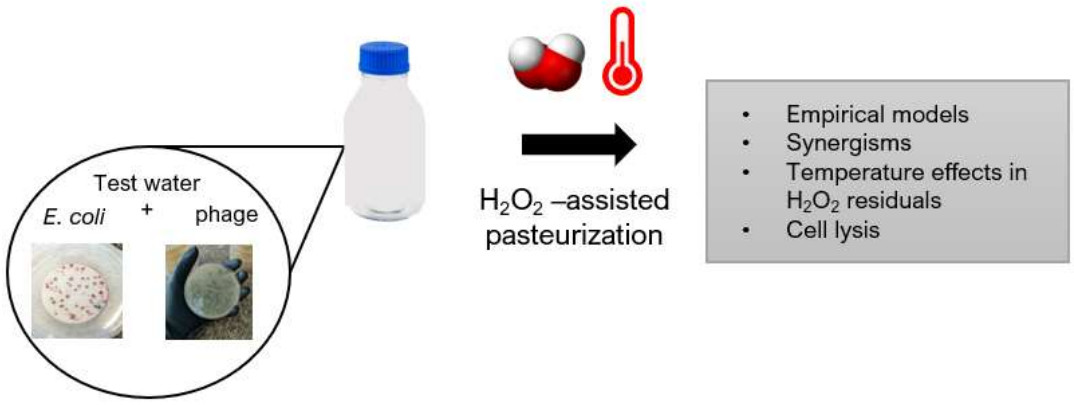
Hydrogen peroxide was considered efficient in improving physicochemical characteristics of both surface and groundwater. As for surface water, particularly, turbidity and color removals may considerably increase the life of the following HWT.

Reduction in microbial load was surprisingly low for seeded groundwater, which suggests this matrix is suitable for more straightforward treatments as in household disinfection itself. It should be noted that, in our research, a contamination scenario was simulated with microorganism spiking. As for surface water, H_2O_2 preoxidation reduced virus and *E. coli* contamination levels at >4.0 and >3.0 $\log_{10}$, respectively, at the 15 mg L^{-1} dose. This indicates H_2O_2 preoxidation may improve microbiological quality of highly contaminated surface water, making it less demanding from the main treatment. Here, the author hypothesizes that iron content of the natural surface water may have provided catalytic activity to the preoxidation, but more repetitions of this assessment are invited.

Our results highlight the importance of evaluating water quality, which can be either impairing or favorable to a HWT implementation. Although design for practical applications of H_2O_2 preoxidation was not within the scope of this study, further research is encouraged for assessing its performance and cost-effectiveness in different conditions, water sources, and coupled to specific HWTs.

5- Chapter 5

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



Source: the author.

Highlights

- H_2O_2-assisted pasteurization led to >9.3 $\log_{10}$ removal of *E. coli* and >5.8 phage.
- Synergistic effects were obtained for *E. coli* inactivation.
- Quadratic empirical models for *E. coli* and phage inactivation were proposed.
- No correlation was found for H_2O_2 residuals and water temperature.
- H_2O_2 may increase robustness of pasteurization setups for POE applications.

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5.1. Introduction

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 (NIEUWOUDT; MATHEWS, 2005). 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 (CARIELO et al., 2017), considering different heat sources, especially solar energy (AMSBERRY et al., 2012; REYNEKE et al., 2018). However, this too may present limitations, as in low irradiation days (CARIELO et al., 2017), which should be compensated for, thus bringing incentives towards the integration of technologies (CHAÚQUE; ROTT, 2021) that could guarantee and perhaps increase efficiency.

In order to improve this technique, this chapter lays a hypothesis 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 (BRAUGE et al., 2020; HAYRAPETYAN et al., 2020), wastewater (YANG et al., 2017; ALCALÁ-DELGADO et al., 2018), and drinking water disinfection (LIANG; KEELEY, 2012; PATIL et al., 2013; MOHAMMED, 2016), 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 (PADUANO et al., 2020), 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 chapter 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.

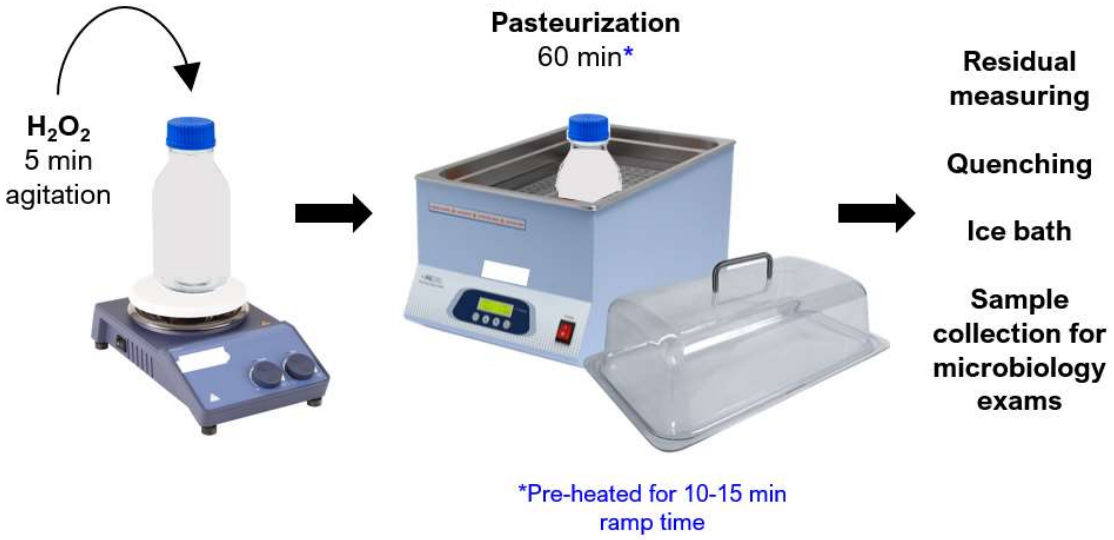
5.2. Materials and methods

5.2.1 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 (HOFFMAN; NGO, 2018). 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 (ESPINOSA et al., 2020). Zone C represents a large variability of conditions, which could be descriptive of a household scenario (ESPINOSA et al., 2020).

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 (MOORE et al., 2021). 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 (WANG et al., 2019) 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 Equation 3-1. Figure 5-1 displays a simplified scheme of the setup for the assisted pasteurization experiments.

Figure 5-1 - Scheme of the experimental setup for hydrogen peroxide assisted pasteurization



Source: the author.

5.2.2 Test water

Considering the aims of this chapter, it was necessary to simulate a water source suitable for disinfection, thus followed the recommendations for the validation of household treatment technologies provided by WHO (WHO, 2014, 2018), without adding solids, as described in section 3.2.2 Test water. Interferences of the inoculums in physicochemical quality of the TW were neglected in this chapter.

5.2.3 Target organisms

TW was inoculated with centrifuged aliquots of *Escherichia coli* (ATCC® 11229™) suspensions (1972 ×*g*, 15 min, 4 °C), leading to approximate concentrations that varied between 10^8 and 10^9 CFU 100 mL^{-1}. 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. Phage was enumerated in terms of PFU mL^{-1}, according to Equation 3-2. Details of microorganism quantification and working stocks preparation are present in section 3.2.3 Target organisms and microbiological analyses.

5.2.4 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 (SAFAPOUR; METCALF, 1999; SAHLSTRÖM et al., 2008; CHUAH et al., 2016), for instance, and hydrogen peroxide disinfection is often described at much higher concentrations, as in >3% (KOLAR et al., 2015; CHOI; LEE, 2020; HIDBER et al., 2020). A situation in which heat sources would not be available steadily and chemicals should be required at a minimum was described.

Considering peer research as background (ZANG et al., 2015), 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 Equation 5-1, in order to quantify the effects of each factor on the dependent variables.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad \text{Equation 5-1}$$

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 of this chapter. 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 of this chapter.

5.2.5 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 chapter, 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.

5.2.6 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. The Bradford reagent (Sigma-Aldrich, USA) was applied for measuring protein (n = 3) at 595 nm (DR 5000 spectrophotometer, Hach, USA). Bovine serum albumin (Sigma-Aldrich, USA) was used as standard.

5.2.7 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 (TADDESE et al., 2021). 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 (SAMMARRO SILVA; SABOGAL-PAZ, 2020). 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.

5.3. Results and discussion

5.3.1 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). Equation 5-2 and Equation 5-3 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 5-2 and details of ANOVA are available in Appendix 3.

$$Y_1 = -1.802 + 0.116X_1 + 34.548X_2 \quad \text{Equation 5-2}$$

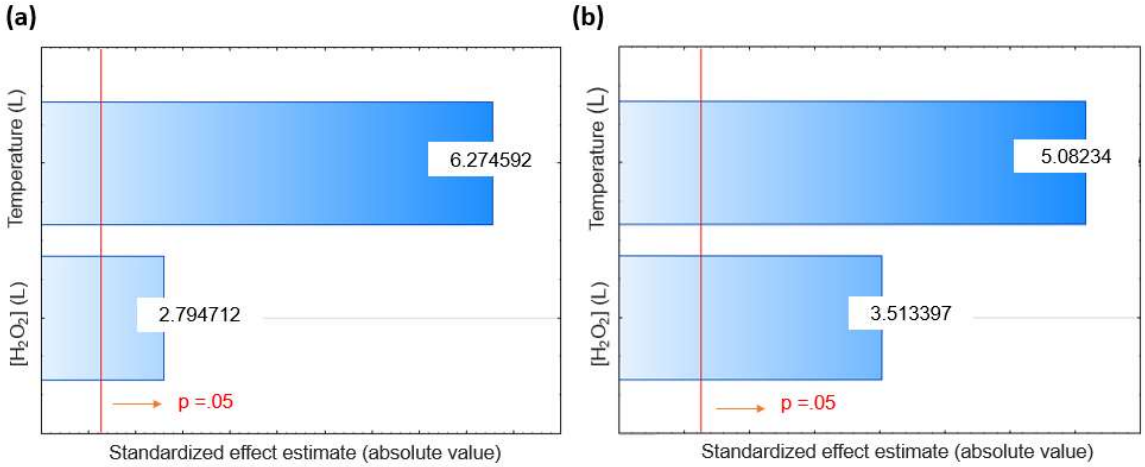
$$Y_2 = -2.248 + 0.082X_1 + 37.823X_2 \quad \text{Equation 5-3}$$

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 5-2, agreeing with the expectations from this chapter, 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 5-2), 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. Hence, 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 5-2 - 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



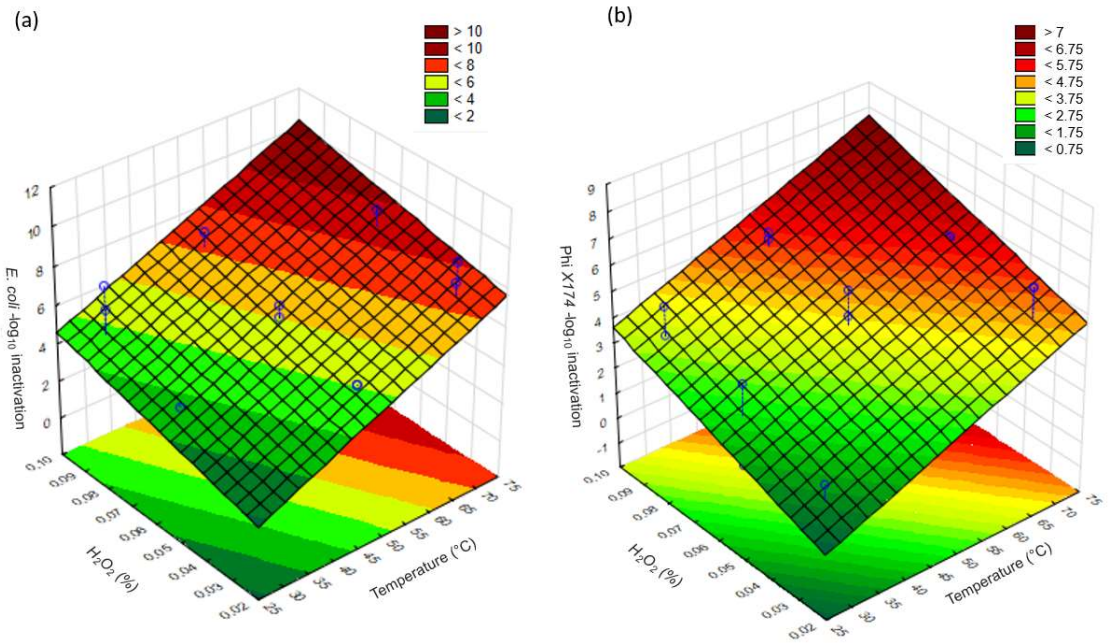
Source: the author (SAMMARRO SILVA et al., 2022).

Figure 5-3 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. The author believes 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 (GÓMEZ-COUSO et al., 2009).

In addition, analyzing residues should also be considered when judging model adequacy (NAIR; MAKWANA; AHAMMED, 2014). These residues refer to the difference between predicted and actual values (Table 5-1). 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.

Figure 5-3 - Fitted surfaces and contour plots for the empirical models generated by the FFD



Notes: 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 ($R^2 = 0.72$).
Source: the author (SAMMARRO SILVA et al., 2022).

Table 5-1 - Actual and predicted values for the inactivation of *E. 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 5-1, 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 (WHO, 2011b, 2017). 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 (WHO, 2018).

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 (WAGNER; OPLINGER; BARTLEY, 2012; SILVA; SABOGAL-PAZ, 2021), 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 (MONTEAGUDO et al., 2017) that could be useful for H_2O_2-assisted pasteurization systems. In addition, shell-and-tube heat exchangers (AMSBERRY et al., 2012), as well as many other improvements that have been discussed on the topic of energy and sanitation (GAUTAM et al., 2017; SANSANIWAL, 2019) 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.).

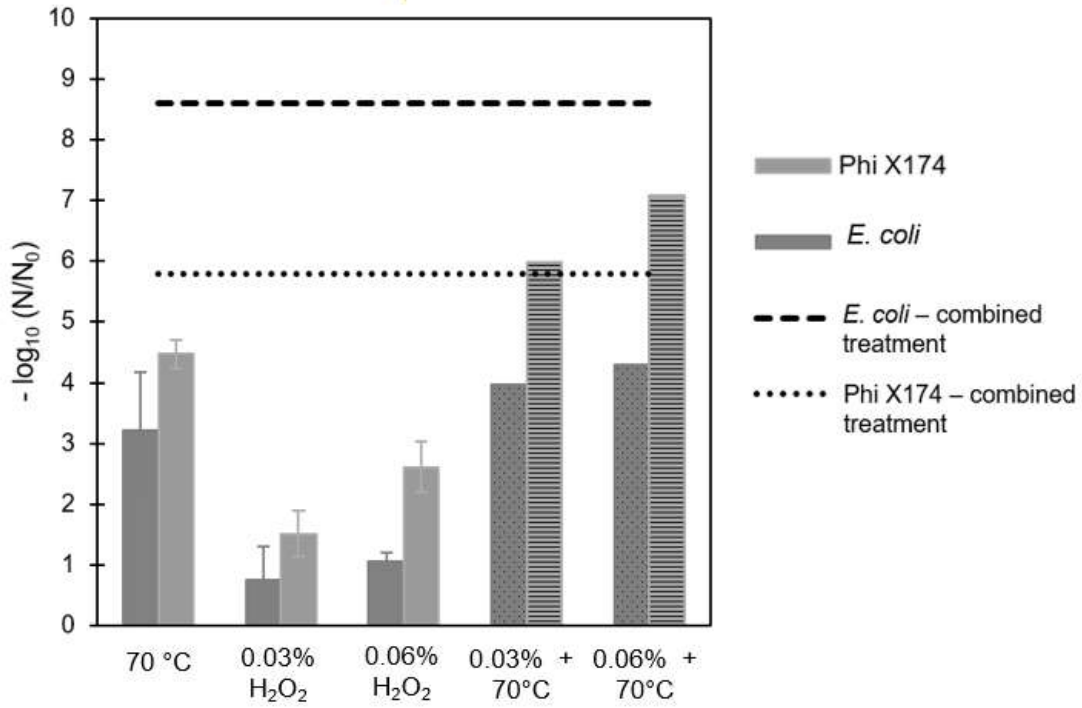
5.3.2 Analysis of synergistic effect

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 (CHO; KIM; YOON, 2006).

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 5-4 displays results for each isolated disinfection method, the sum of their effects, as well as the average observed values (Table 5-1) for the combined treatment, i.e., assisted pasteurization (represented by the baselines).

Figure 5-4 - *E. coli* and Phi X174 bacteriophage inactivation by isolated disinfection methods, compared to the sum of standalone components



Notes: 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.
Source: the author (SAMMARRO SILVA et al., 2022).

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 5-2), 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 5-3 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.

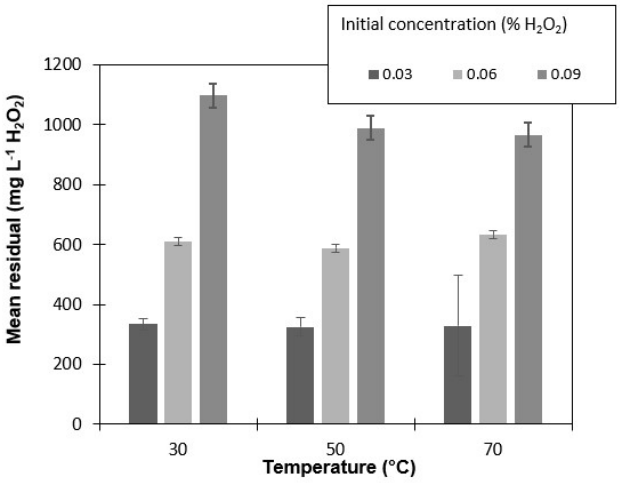
These results suggest a satisfactory reduction in oxidant demand while still providing high disinfection efficiency. A recent study that relied on standalone H_2O_2 for water disinfection required a 10-fold higher dose at the same exposure time to obtain an approximate 8-log reduction of *E. coli* (SILVA; SABOGAL-PAZ, 2021).

5.3.3 Temperature effect in hydrogen peroxide residual

Poor correlation was found for temperature and H_2O_2 residuals (Figure 5-5), but results were not considered significant at a 95% confidence interval. Pearson's coefficients are presented in Table 5-2, 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 5-5 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 5-5 Hydrogen peroxide residuals obtained after assisted pasteurization in different temperatures and initial H_2O_2 concentrations



Notes: Error bars refer to standard deviation.
Source: the author (SAMMARRO SILVA et al., 2022).

Table 5-2 - 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

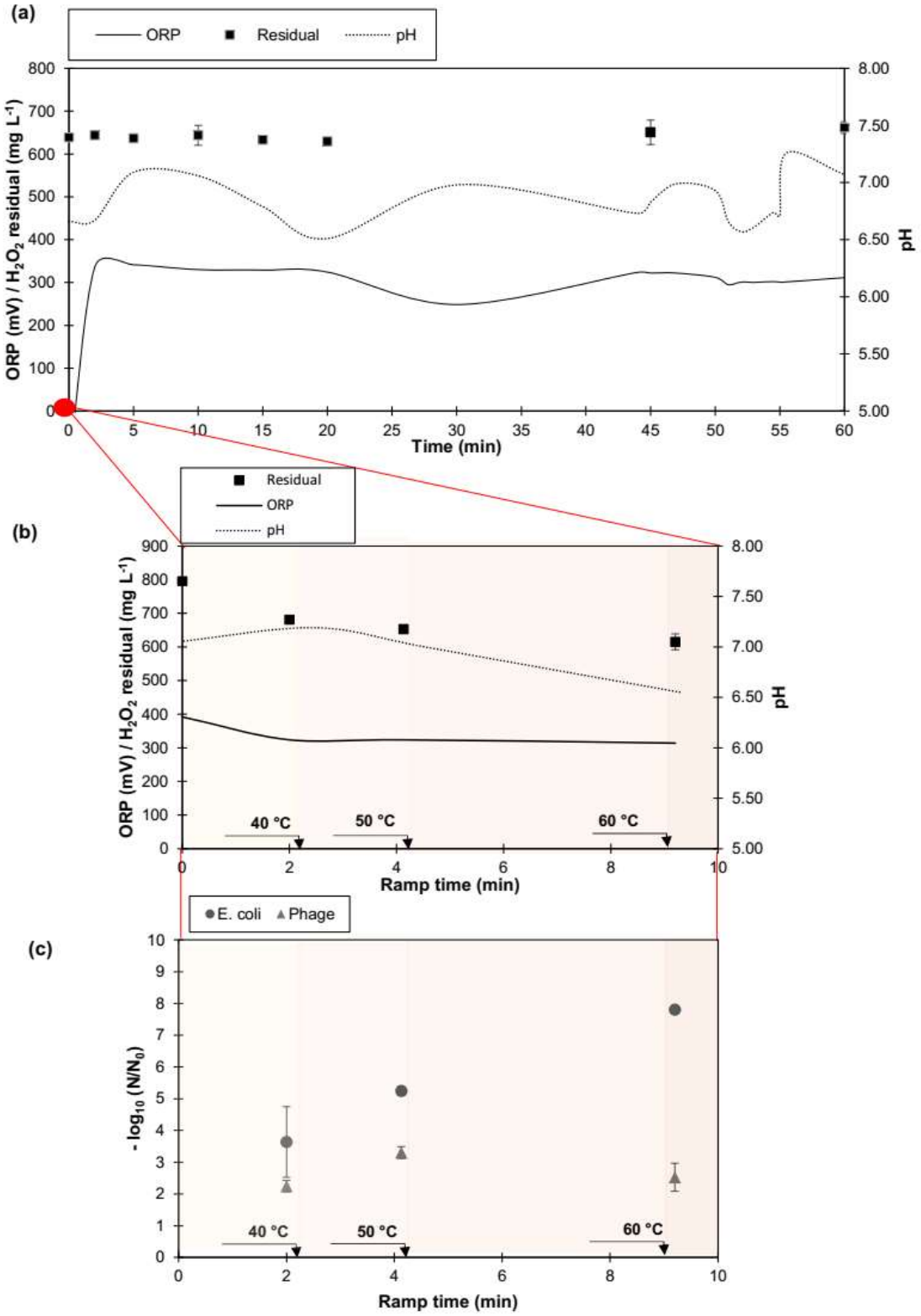
5.3.4 Residual monitoring

Hydrogen peroxide residuals were assessed through time under selected conditions to evaluate the potential of complimentary disinfection. Figure 5-6 (a) displays the data obtained for residual concentration during disinfection by 0.06% H_2O_2 at 70 °C for 60 min. Additionally, Figure 5-6 illustrates the behavior of ORP and pH through time.

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 (SNOEYINK; JENKINS, 1980; BLACK & VEATCH CORPORATION, 2009). However, if the measured potential differs greatly from the theoretical value, it may still provide a useful signal for process control (APHA; AWWA; WEF, 2012). Nonetheless, Figure 5-6 (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 (LAU et al., 2020). This led to results shown in Figure 5-6 (b) and (c), which ratify observed values from Table 5-1 for lower temperatures, even though contact time in those conditions was longer.

Figure 5-6 – H_2O_2 residuals, ORP and pH during assisted pasteurization at 0.06% initial [H_2O_2] (a) 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



Notes: Error bars refer to standard deviation.
Source: the author (SAMMARRO SILVA et al., 2022).

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 (STRAZYNSKI; KRÄMER; BECKER, 2002). Similarly, microwave heating provided satisfactory levels of bacteria inactivation at 65 °C for 65 to 70 s (ROOHI; HASHEMI, 2020), 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 (CARIELO DA SILVA; TIBA; CALAZANS, 2016). Also, when dealing with natural conditions, as in many reports from literature in pasteurization within the sanitation scene (BIGONI et al., 2014; DOBROWSKY et al., 2015; REYNEKE et al., 2018), 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” (FEACHEM et al., 1983), 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 (FREITAS; LEITE; DANIEL, 2021). Here, most H_2O_2 consumption had already occurred at two minutes, aligning to results obtained for hydrogen peroxide demand in Chapter 4.

Future research on the design of assisted-pasteurization devices or coupled-systems, prior to any implementation in households, should however 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.

5.3.5 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 5-3. Bacterium organic matter of *E. coli* contains a large

proteinaceous fraction (approximately 65% of the dissolved organic carbon) (LEITE et al., 2019), 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 5-7, 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 5-3 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 5-3 - Protein removals obtained by pasteurization, H_2O_2 oxidation and H_2O_2-assisted pasteurization

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

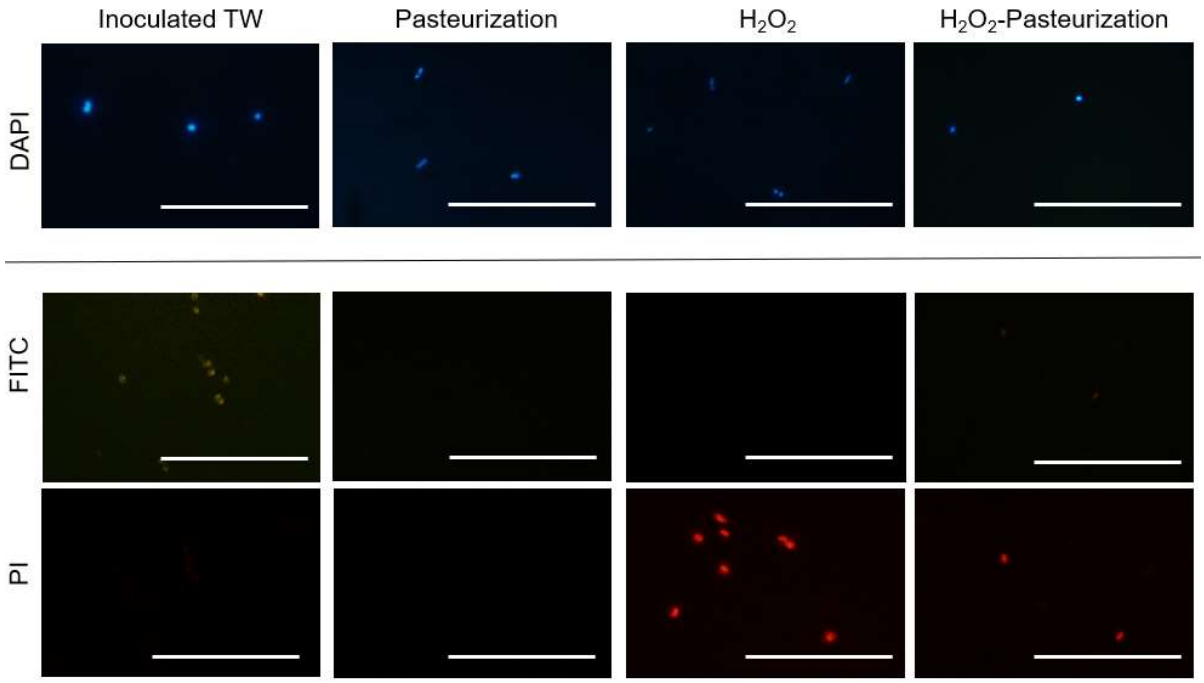
Notes: Initial protein content in inoculated test water = 5.72 ± 0.07 mg L^{-1}. Protein removals were calculated in duplicates, which is why the standard deviation is not presented.

Figure 5-7 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.

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.

Intracellular DAPI signal was observed after pasteurization, which confirms that DNA was retained in the cell. This complies with similar research, that tested pasteurization for bacteria inactivation while maintaining cell integrity (TADDESE et al., 2021). No major PI-uptake was noticed in this treatment, endorsing pasteurization under these selected conditions did not lead to considerable cell lysis.

Figure 5-7 - Micrographs of the raw water (positive control) and inactivated *E. coli* stained by different methods



Notes: 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.
Source: the author (SAMMARRO SILVA et al., 2022).

As for oxidation treatments, i.e., H_2O_2 and assisted pasteurization, although Figure *5-7* 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 in Figure 5-7 were exceptions selected for illustration. The author believes that 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. The author also assumes 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.

5.4. Limitations and further research

This chapter presented 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 lead to 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. A preliminary design for H_2O_2-assisted solar pasteurization has been conceptualized during the elaboration of this thesis and is detailed in Appendix 3.

Additionally, this step of the 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, further research with different source waters is recommended, so that resilience of H_2O_2-pasteurization settings may be investigated. Contrariwise, 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 suggested in Chapter 4.

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, additional investigation including quantitative molecular methods, for instance, is invited.

5.5. Conclusions

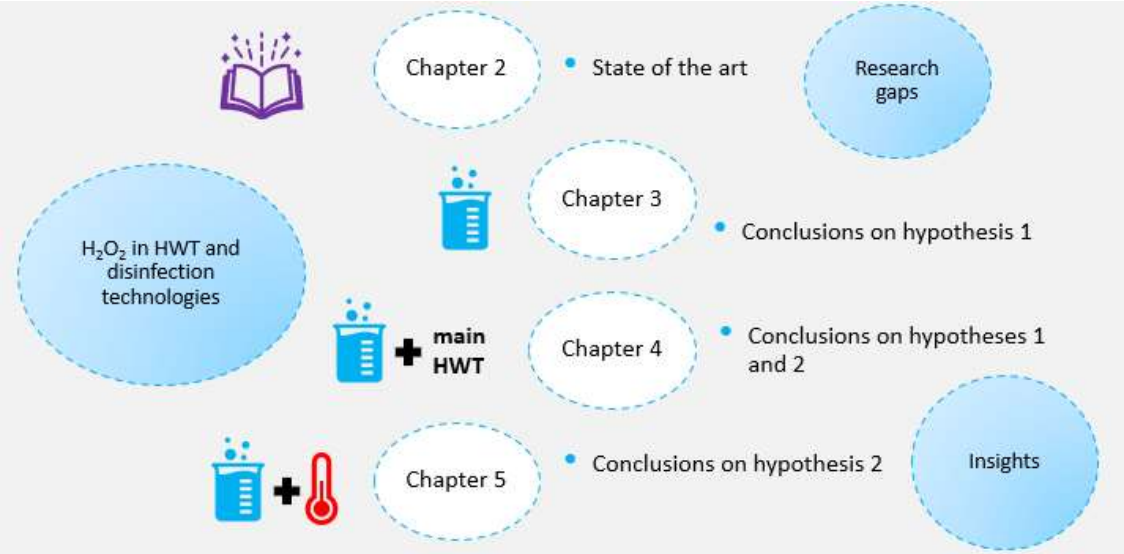
The stated purpose of this chapter was to evaluate the performance of H_2O_2-assisted pasteurization for household water treatment. Boundary conditions for maximum concentration and temperatures led to >9.3 $\log_{10}$ inactivation of *Escherichia coli* and >5.8 $\log_{10}$ Phi X174. Obtained $\log_{10}$ 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), the author contends that the FFD overall describes the potential of H_2O_2-assisted pasteurization as a disinfection method within different combined conditions of temperature and H_2O_2 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, this chapter underscores potentials of H_2O_2-assisted pasteurization as a combined disinfection method. Further assessments considering pathogens, modeling, as well as case studies for practical applications are recommended, but results endorse that H_2O_2 may increase the resilience of classic disinfection by pasteurization and provide a safer alternative to reduce drinking water microbial load.

6- Chapter 6

General conclusions



Source: the author.

6.1 Remarks on the hypotheses

The chapters included in this doctoral thesis discussed whether hydrogen peroxide would be effective in household water treatment for standalone disinfection (Hypothesis 1) and combined applications (Hypothesis 2). Specific objectives of each step of the research were included in their respective chapters. Regarding the hypotheses, it should be noted that:

- The systematic review in Chapter 2 indicated that H_2O_2 is not popular in sanitation, even though it is a widespread disinfectant, considered efficient in different areas of research (a premise to Hypothesis 1). In this sense, secondary information found in peer literature was insufficient to respond to any of the two hypotheses, but rather encourage experimental study on H_2O_2 application at the household level. Thus, Chapter 2 elucidated a knowledge gap, as well as an implementation gap in research regarding the primary objective of this thesis.
- Chapter 3 consisted of a preliminary assessment, so that challenges and potentials could be identified at bench scale when working with hydrogen peroxide aiming at its use as an HWT. Conclusions from this chapter suggested that Hypothesis 1 should be accepted, as H_2O_2 was considered efficient at some of the experimental conditions, benchmarked against chlorine (a classic disinfectant, even at the point of use). It should also be noted, that besides the information obtained in this step of the thesis, limitations found in this preliminary experimental study (e.g., those regarding residuals and analysis of oxidation efficiency) endorse the research gaps initially pointed. Overall, Chapter 3 invites additional research on hydrogen peroxide as a standalone disinfectant targeting different contamination scenarios.
- Chapter 4 presented a different perspective, in which water quality varies. A potential to standalone disinfection was tested by measuring differences in microbial load when subjecting two different natural waters to preoxidation using H_2O_2 (which is carried out in lower concentrations than those tested in Chapter 3). Clarification efficiency and results for the inactivation of phage and bacteria, especially in river water, implied that Hypothesis 1 should be partially accepted. That is because Chapter 4 details water quality of both matrices under test, suggesting that enhanced effects may have taken course due to the presence of natural catalysts, also partially suggesting acceptance of Hypothesis 2, so there is evidence of catalytic effects. The idea in this chapter was to present hydrogen peroxide as a technique to condition water to a main HWT. Although oxidation reactions were quenched for analyzing treatment efficiency, additional research on combined treatments were also proposed, so that Hypothesis 2 would be further tested in different household water treatment frameworks.
- In Chapter 5, a combined treatment was investigated, in which hydrogen peroxide was applied prior to pasteurization, which is a common approach to obtain safe drinking water at

the point of use. Specific conclusions from this chapter indicated efficiency in H_2O_2-assisted pasteurization in inactivating phage and *E. coli*, which implies that Hypothesis 2 is true for the tested conditions of the proposed HWT. As this chapter proposed a novel topic, it raised research gaps of its own, so that future work on Hypothesis 2 is fomented.

6.2 Overall comments and future work

Broadly, this thesis restates tackling inequalities in access to safe water is a challenge. In this sense, interventions based on decentralized water treatment would play a valuable role for addressing such matter.

Here, hydrogen peroxide application in household water treatment and disinfection was explored by different approaches. Each chapter's methodology, however, was limited to a certain scope, which included its own objectives, considering specific target-organisms, water matrices and operational conditions. These were selected considering not only scientific relevance, but also budget, the schedule available for this study, human and laboratory resources, and COVID-19 restrictions at both national and state[1] levels.

When summing up conclusions from the individual chapters in this thesis, it was noteworthy that there are knowledge and practical knowledge research gaps to be filled. Considering points in common from both literature and experimental data collected in this

[1] State of São Paulo decrees related to the quarantine: N° 64881 (03/22/2020) - Decree quarantine throughout the state of São Paulo due to the COVID-19 pandemic; Nº 69420 (04/06/2020) - Extends the statewide quarantine for another 15 days, for the period from April 8 to 22, 2020; Nº 64946 (04/17/2020) - Extends the quarantine measure dealt with in Decree Nº. 64,881 of March 22, 2020. Nº 64949 (05/08/2020) - Extends the quarantine until May 31 to the entire state, a measure established by Decree Nº. 64.881, of March 22, 2020; Nº 64987 (05/19/2020). Suspends the working hours of state public offices headquartered in the municipality of São Paulo on May 22, 2020 and takes related measures; Nº 64994 (05/28/2020) - Extends the quarantine valid for the entire state of São Paulo until June 15 and institutes the São Paulo Plan; Nº 65014 (06/10/2020) - Extends the quarantine measure dealt with in Decree Nº. 64,881, of March 22, 2020, until June 28. Nº 65032 (06/27/2020) - Extends the quarantine measure dealt with in Decree Nº. 64,881, of March 22, 2020, until July 14; Nº 65056 (07/10/2020) - Extends the quarantine measure referred to in Decree Nº. 64,881, of March 22, 2020, until July 30, 2020; Nº 65088 (07/24/2020) - Extends the quarantine measure referred to in Decree Nº. 64,881, of March 22, 2020, until August 10, 2020; Nº 65114 (08/07/2020) - Extends the quarantine measure referred to in Decree Nº. 64,881 of March 22, 2020 until August 23; Nº 65143 (08/21/2020) - Extends the quarantine measure until September 6, which is dealt with in Decree Nº. 64881, of March 22, 2020; Nº 65184 (09/18/2020) - Extends the quarantine measure until October 9, which is dealt with in Decree Nº. 64.881, of March 22, 2020. Nº 65237 (10/09/2020) - Extends the quarantine measure until November 16, which is dealt with in Decree Nº. 64,881, of March 22, 2020; Nº 65295 (11/16/2020) - Extends the quarantine measure until December 16, which is dealt with in Decree Nº. 64.881 of March 22, 2020; Nº 65320 (11/30/2020) Extends the quarantine measure until January 4, 2021, mentioned in Decree No. 64.881, of March 22, 2020; Nº 65437 (12/30/2020) - Extends the quarantine measure until February 7, 2021 mentioned in Decree Nº. 64881, of March 22, 2020; Nº 65545 (03/03/2021) - Extends the quarantine measure until April 9, 2021; Nº 65635 (04/16/2021) - Extends the quarantine measure referred to in Decree Nº. 64,881, of March 22, 2020, institutes transitional measures, of an exceptional nature, aimed at dealing with the COVID-19 pandemic, and takes related measures.
Laboratory work has been resumed from November 2020 until state lockdown was reestablished. Activities began again in May 2021.

work, the following topics could lead to future study in individual and combined use of H_2O_2 at the household level:

- Testing effects on natural water matrices and specific contaminants of local relevance to household settings.
- Designing point-of-use and point-of-entry technologies based on hydrogen peroxide.
- Addressing residuals as a main research topic, considering decay, modeling, and prediction, as well as quenching and toxicity.
- Implementation campaigns and behavior change studies once the methodology development reaches a safe status for community interventions.

Overall, this thesis proposed different approaches to hydrogen peroxide, which is a widely known disinfectant, but had not yet been explored as a point-of-use or point-of-entry technology. Hence, this study displayed some of the potentials and limitations for H_2O_2 application in households, aiming to tackle the remaining inequalities in access to safe water, but does not suffice within its own scope, instigating further investigations and bringing up challenges and insights for future work.

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Appendix 1

This appendix supports Chapter 2.

A1.1 Extracted content from the systematic review

Table A1.1 shows categorized information extracted from retrieved records of the systematic review, which were used as input for building visualization networks in Cytoscape.

Table A1.1. Details of retrieved records on hydrogen peroxide disinfection (2011-2021)

Process	Context	Matrix	Goal	Microorganism group	Reference
Liquid	Veterinary research	Suspension	Disinfect.	Bacteria	(GUTIÉRREZ-MARTÍN et al., 2011)
AHP	Clinic environment	Surface (carrier disks)	Disinfect.	Bacteria (AR)	(PISKIN et al., 2011)
Liquid	Products	Surface (lens cases)	Disinfect.	Bacteria	(WU et al., 2011)
AHP; VHP	Clinic environment	Surface (room)	Disinfect.	Bacteria	(HOLMDAHL et al., 2011)
Liquid	Products	Suspension	Disinfect.	Protozoa	(KOBAYASHI et al., 2011)
Liquid	Food industry	Surface (carrier disks)	Disinfect.	Bacteria	(RUSHDY; OTHMAN 2011)
Disinfectant	Sanitation	Water (hot water)	Disinfect.	Bacteria	(FARHAT et al., 2011)
VHP (Disinfectant)	General	Surface (room; air-conditioning ducts)	Disinfect.	Bacteria (AR)	(TANEJA et al., 2011)
VHP	Pharmaceutical	Surface (carrier disks)	Disinfect.	Virus	(BERRIE et al., 2011)
Spray (Disinfectant)	General	Surface (different carrier materials)	Disinfect.	Bacteria	(WOOD et al., 2011)
Liquid	Agriculture / Food industry	Surface (wheat seeds/sprouts)	Disinfect.	Bacteria; fungi	(TORNUK et al., 2011)
Liquid	Agriculture / Food industry	Surface (fresh-cut apple)	Disinfect.	Bacteria	(ABADIAS et al., 2011)
Liquid	Food industry	Suspension and surface (biofilm)	Disinfect.	Bacteria	(YUN et al., 2012)
Liquid	General	Suspension	Disinfect.	Fungi	(VÝROSTKOVÁ et al., 2012)
Liquid	Sanitation	Water (surface water and disinfected water)	Disinfect.	Protozoa	(LIANG; KEELEY 2012)
VHP	Clinic environment	Surface (carrier disks)	Disinfect. (impact of suspending media)	Bacteria (AR)	(OTTER et al. 2012)
VHP	Clinic environment	Surface (hospital settings)	Disinfect.	Bacteria	(CHMIELARCZYK et al., 2012)
VHP	Clinic environment	Surface (carrier disks; hospital settings)	Disinfect.	Bacteria	(HAVILL et al. 2012)
AHP; VHP	Clinic environment	Surface (carrier disks)	Disinfect.	Bacteria (AR)	(FU et al. 2012)
VHP	General	Surface (carrier disks)	Disinfect.	Virus	(TULADHAR et al., 2012)
VHP	General	Surface (carrier disks)	Disinfect.	Virus	(BENTLEY et al., 2012)

Process	Context	Matrix	Goal	Microorganism group	Reference
VHP	Clinic environment	Surface (carrier disks; hospital settings)	Disinfect.	Bacteria	(BARBUT et al. 2012)
Liquid	Aquaculture	Water	Oxid.	NA	(PEDERSEN; PEDERSEN 2012)
Liquid	Aquaculture	Water (egg collection from aquaculture)	Disinfect.	NA	(WAGNER et al. 2012)
Liquid	Products	Suspension	Disinfect.	Protozoa	(BOOST et al., 2012)
Disinfectant	Food industry	Surface (artifcially contaminated chicken breasts)	Disinfect.	Bacteria	(LU; WU 2012)
Liquid	Sanitation	Suspension	Disinfect.	Helminth	(MORALES et al., 2013)
Liquid	Sanitation	WW	Disinfect.	Bacteria; helminth	(GUADAGNINI et al., 2013)
Liquid	General	Suspension	Disinfect. (avoid germination)	Bacteria	(SETLOW et al., 2013)
Liquid	Sanitation	Water (artificially contaminated groundwater)	Disinfect.	Bacteria	(PATIL et al., 2013)
Liquid	Sanitation	WW (artificially contaminated synthetic WW and treated sewage)	Disinfect.	Bacteria	(VARGAS et al., 2013)
Liquid	Agriculture / Food industry	Suspension (buffer and potato extracts)	Disinfect.	Bacteria	(CZAJKOWSKIET et al. 2013)
VHP	Food industry	Surface (filtration membrane)	Disinfect.	Bacteria	(MALIK et al., 2013)
Liquid	Products	Surface (lens cases)	Disinfect.	Protozoa	(PADZIK et al., 2014)
VHP	Laboratory environment	Surface (carriers; different points in a room)	Disinfect.	Bacteria	(KASPARI et al., 2014)
Liquid	Agriculture / Food industry	Surface (Fresh-cut cabbage)	Disinfect.	Bacteria	(LEE et al. 2014)
Liquid	Sanitation	Suspension	Disinfect.; toxin removal	Cyanobacteria	(FAN et al., 2014)
Liquid	Food industry	Suspension and surface (biofilm)	Disinfect.	Bacteria	(JAHID; HA 2014)
Disinfectant	Clinic environment	Water (dental units settings)	Disinfect.	Bacteria	(DALLOLIO et al., 2014)
Liquid	Clinic environment	Surface (artificially contaminated curtain fabric)	Disinfect.	Bacteria (AR)	(SOOD et al., 2014)
VHP	General	Surface (carrier disks)	Disinfect.	Virus	(GOYAL et al., 2014)
VHP	General	Surface (container simulating confined space)	Disinfect.	Bacteria	(LIANG et al. 2014)
VHP	Clinic environment	Surface (hospital settings)	Disinfect.	Bacteria	(BEST et al., 2014)
Disinfectant	General	Suspension; surface (biofilm)	Disinfect.	Bacteria (AR)	(PERUMAL et al., 2014)
Disinfectant	Agriculture / Food industry	Suspension	Disinfect.	Bacteria	(OOSTERIK et al., 2014)
AHP; VHP	Clinic environment	Surface (room)	Disinfect.	Bacteria	(BARBUT, 2015)
Liquid	Sanitation	Suspension	Disinfect.	Protozoa	(GUIMARÃES et al., 2015)
Liquid	Food industry	Sugarcane juice	Oxid.	NA	(SARTORI et al. 2015)
Liquid	Aquaculture	Suspension	Disinfect.	Bacteria	(CHANG et al., 2015)

Process	Context	Matrix	Goal	Microorganism group	Reference
Liquid	Products	Suspension	Disinfect.	Protozoa	(KOLAR et al., 2015)
Liquid	Agriculture	Soil	Disinfect. (preventing development)	Fungi	(GARCIA-BARREDA et al. 2015)
Liquid	Agriculture / Food industry	Water (wash water from a full-scale leafy vegetables washing process)	Disinfect.	Bacteria	(VAN HAUTE et al., 2015)
VHP	General	Surface (carriers; hard to reach areas in a room)	Disinfect.	Bacteria (AR)	(LEMMEN et al., 2015)
VHP	Clinic environment	Surface (artificially contaminated curtain fabric)	Disinfect.	Bacteria (AR)	(CADNUM et al., 2015)
Liquid	Sanitation	Surface (biofilm from sand filters)	Oxid.	NA	(GUO et al., 2015)
Liquid	Agriculture / Food industry	Surface (biofilm immersed into suspension)	Disinfect.	Bacteria	(HOWARD et al., 2015)
Liquid	Sanitation	Water (drinking water)	Dechlorination	NA	(QIAN et al., 2015)
Liquid	Products	Surface (lens cases)	Disinfect. (preventing microorganism development)	Fungi	(MELA et al., 2015)
Liquid	Aquaculture	Water (egg collection from aquaculture)	Disinfect.	Bacteria	(EL-DAKOUR et al. 2015)
Liquid	General	Surface (biofilm on glass and wood)	Disinfect.	Bacteria	(MUAZU et al., 2015)
AHP	General	Surface (cover glasses; carrier disks)	Disinfect.	Virus	(ZONTA et al., 2016)
VHP	General	Surface (carrier disks)	Disinfect.	Bacteria (AR)	(MURDOCH et al., 2016)
Liquid	Clinic environment	Water (dental units settings)	Disinfect.	NA	(PAWAR, 2016)
VHP	Clinic environment	S (carrier disks; hospital settings)	Disinfect.	Bacteria (AR)	(ALI et al., 2016)
Liquid	Clinic environment	Water (hot water; hospital settings)	Disinfect.	Bacteria	(MARCHESI et al., 2016)
Liquid	Sanitation	Water (Drinking water for cattle)	Disinfect.	Bacteria	(MOHAMMED, 2016)
Disinfectant	Sanitation	Water (Suspension and surface water for irrigation)	Disinfect.	Fungi	(SANTOS-RUFO; RODRÍGUEZ-JURADO 2016)
VHP	Clinic environment	Surface (hospital settings)	Disinfect.	Bacteria	(YUI et al., 2017)
Liquid; Disinfectant	Food industry	Suspension	Disinfect.	Bacteria	(IÑIGUEZ-MORENO et al., 2017)
VHP	General	Suspension (saturated paper bedding pieces)	Disinfect.	Bacteria	(BENGA et al., 2017)

Process	Context	Matrix	Goal	Microorganism group	Reference
Liquid; Disinfectant	Agriculture / Food industry	Suspension (PVC coupons)	Disinfect.	Bacteria	(MAHARJAN et al., 2017)
VHP	General	Surface (carrier disks; hard to reach areas in a room)	Disinfect.	Virus	(MONTAZERI et al., 2017)
Liquid	Clinic environment	Water (hospital settings)	Disinfect.	Bacteria	(CASINI et al., 2017)
Liquid	Sanitation	WW	Disinfect.	Bacteria	(YANG et al., 2017)
Liquid	Sanitation	WW (Industrial)	Oxid.	NA	(ALCALÁ-DELGADO et al., 2018)
VHP	Products	Surface (historical objects)	Disinfect.	Bacteria; fungi	(WAWRZYK et al., 2018)
Liquid	General	Surface (artificially contaminated vs non-spiked toilet bowls after flushing)	Disinfect.	Virus	(SASSI et al., 2018)
VHP	Clinic environment	Surface (hospital settings)	Disinfect.	Bacteria	(CHIGUER et al., 2019)
Liquid	Aquaculture	Water (egg collection from aquaculture)	Disinfect.	Bacteria	(PATRICK et al., 2019)
Liquid	General	Suspension	Disinfect. (gene expression alterations)	Bacteria	(LIGOWSKA-MARZĘTA et al., 2019)
Liquid	Laboratory environment	Surface (biofilm on titanium disks)	Disinfect.	Bacteria	(HOMAYOUNI et al., 2019)
Liquid; Disinfectant	General	Surface (biofilm)	Disinfect.	Bacteria	(CHOWDHURY et al., 2019)
Liquid	Food industry	Surface (hatching eggs)	Disinfect.	Bacteria; fungi	(MELO et al., 2019)
Liquid	General	Suspension (paper disks)	Disinfect.	Bacteria	(MONTAGNA et al., 2019)
Disinfectant	Food industry	Suspension	Disinfect.	Bacteria	(SKOWRON et al., 2019)
Liquid	Clinic environment	Suspension	Disinfect.	Bacteria	(SANDLE, 2019)
Liquid	Food industry	Surface (room)	Disinfect.	Bacteria	(MØRETRØ et al., 2019)
Liquid	Agriculture / Food industry	Surface (plastic and wood)	Disinfect.	Fungi	(BERNAT et al., 2019)
Liquid	Veterinary research	Suspension	Disinfect.	Bacteria	(SCANO et al., 2019)
Disinfectant	Food industry	Surface (biofilms formed upon smoked salmon processing environment)	Disinfect.	Bacteria	(BRAUGE et al., 2020)
VHP	Products	Surface (historical objects)	Disinfect.	Bacteria; fungi	(WAWRZYK et al. 2020)
Spray	Food industry	Surface (hatching eggs)	Disinfect.	Bacteria; fungi	(WLAZLO et al., 2020)
VHP	General	Surface (different carrier materials)	Disinfect.	Bacteria	(ESCHLBECK et al. 2020)

Process	Context	Matrix	Goal	Microorganism group	Reference
Liquid	General	Water (Synthetic water containing known concentrations of endotoxins)	Oxid.	NA	(HUMUDAT et al. 2020)
Liquid	Clinic environment	Water (dental units settings)	Disinfect.	Bacteria; protozoa	(TUVO et al., 2020)
Liquid; VHP	Food industry	Suspension; surface (carrier disks)	Disinfect.	Bacteria	(HAYRAPETYAN et al., 2020)
Liquid	Clinic environment	Water (hot water)	Disinfect.	Bacteria	(PADUANO et al., 2020)
VHP; VHP (Disinfectant)	Laboratory environment	Suspension; surface	Disinfect.	Virus	(KINDERMANN et al., 2020)
Liquid	Clinic environment	Surface (room)	Disinfect.	Bacteria	(OON et al., 2020)
Liquid	Products	Suspension (sport mouthgard suspended in artificially contaminated saliva solution)	Disinfect.	Bacteria; Fungi	(D'ERCOLE et al., 2020)
Liquid	Food industry	Suspension (biofilm-derived cells of Salmonella Enteritidis)	Disinfect.	Bacteria	(ROMEU et al., 2020)
VHP	Laboratory environment	Surface (carrier disks)	Disinfect.	Bacteria	(POTTAGE et al., 2020)
Disinfectant	Products	Surface (lens cases with or without contact with solution)	Disinfect.	Bacteria	(YAMASAKI et al., 2020)
Liquid	General (pools)	Water (artificially contaminated pool water)	Disinfect.	Bacteria	(ROSENDE et al., 2020)
Liquid	Food industry	Surface (hatching eggs)	Disinfect.	NA	(TEBRÜN et al., 2020)
Liquid	Agriculture / Food industry	Water (footbath for ovine footrot)	Disinfect.	Bacteria	(HIDBER et al., 2020)
Liquid	Clinic environment	Surface (dental units settings)	Disinfect.	Bacteria	(CHOI; LEE, 2020)
VHP (Disinfectant)	Clinic environment	Suspension	Disinfect.	Bacteria (AR)	(AMAEZE et al., 2020)
Liquid	Agriculture / Food industry	Suspension	Disinfect.	Bacteria	(ZOU et al., 2020)
Liquid	Agriculture / Food industry	Water (wash water for artificially contaminated strawberry processing)	Disinfect.	Bacteria; virus	(ORTIZ-SOLÀ et al., 2020)
Spray (Disinfectant)	Food industry	Surface (artificially contaminated eggshell samples)	Disinfect.	Bacteria (AR)	(MOTOLA et al., 2020)
Disinfectant	Clinic environment	Surface (carrier disks)	Disinfect.	Fungi (AR)	(SEXTON et al., 2020)
VHP (Disinfectant)	Products	Suspension; surface (sterile polyethylene flat-top caps)	Disinfect.	Bacteria (AR)	(SOOHOO et al., 2020)
VHP	Clinic environment	Surface (dental units settings)	Disinfect.	Bacteria; fungi	(WAWRZYK et al., 2020b)

Process	Context	Matrix	Goal	Microorganism group	Reference
Liquid	Clinic environment	Suspension	Disinfect.	Virus	(EGAWA et al., 2021)
Liquid	Products	Surface (artificially contaminated toothbrushes)	Disinfect.	Bacteria	(CAYO-ROJAS et al., 2021)
AHP; Liquid	Food industry	Surface (carrier disks); Suspension	Disinfect.	Fungi	(KURE et al. 2020)
Disinfectant	Food industry	Suspension	Disinfect.	Protozoa	(OMRAN et al., 2021)
VHP	Pharmaceutical	Surface (artificially contaminated stainless-steel surfaces)	Disinfect.	Virus	(AJORIO et al. 2021a)
Liquid	Aquaculture	Water (aquaculture fresh and salt microcosms); suspensions	Disinfect.	Fungi	(YAZDI; SOTO 2021)
Liquid	Aquaculture	Water (recirculation water in aquaculture)	Disinfect.; oxigenation	Bacteria; fungi	(BÖGNER et al., 2021)
Disinfectant	General	Suspension	Disinfect.	Virus	(LEE et al. 2021)
VHP	General	Surface (carrier disks)	Disinfect.	Bacteria	(CHEN et al., 2021)
Liquid	Products	Surface (polymethylmethacrylate)	Disinfect.	NA	(MOHAMMED; MAHMOOD 2021)
Liquid	Aquaculture	Suspension; surface (biofilm)	Disinfect.	Bacteria	(ACOSTA et al., 2021)
AHP	General	Surface (dried ceramic tiles)	Disinfect.	Bacteria	(KNOBLING et al., 2021)
Liquid	Sanitation	Water (groundwater contaminated with receiving leachate)	Disinfect.	Algae; bacteria	(FARINELLI et al., 2021)
VHP	General	Surface (common indoor materials)	Disinfect. (residual removal)	NA	(POPPENDIECK et al.2021)
AHP	Clinic environment	Surface (hospital settings)	Disinfect.	Bacteria; fungi	(RAMIREZ et al., 2021)
Liquid	Aquaculture	Water (aquaculture tanks)	Disinfect. (reduction of fish mortality)	Fungi	(DICOCCO et al., 2021)
Liquid	Clinic environment	Surface (PVC, stainless-steel, linoleum, napa leather, and formica coupons)	Disinfect.	Bacteria (AR); fungi (AR)	(COBRADO et al., 2021)
Liquid	Products	Surface (artificially contaminated dental impressions)	Disinfect.	Fungi	(ASLANIMEHR et al., 2021)
AHP	Clinic environment	Surface (hospital settings)	Disinfect.	Bacteria (AR)	(MCKEW et al., 2021)
Liquid; VHP	Pharmaceutical	Surface (artificially contaminated stainless-steel surfaces)	Disinfect.	Virus	(AJORIO et al. 2021b)
Liquid	Aquaculture	Water (recovered fertilized fish eggs; seawater)	Disinfect.	Bacteria	(MAAPEA et al. 2021)
Liquid	Aquaculture	Suspension (fertilized contaminated fish eggs in solution)	Disinfect.	Bacteria; fungi	(LAHNSTEINER, 2021)

Process	Context	Matrix	Goal	Microorganism group	Reference
AHP	Public transportation	Surface (spore discs placed in public buses)	Disinfect.	Bacteria	(ARUNWUTTIPONG et al., 2021)
Liquid	Agriculture	Surface (cannabis seeds immersed in solution)	Disinfect.	NA	(PEPE et al., 2021)
Liquid	Sanitation	Water (microcosm containing helminth eggs recovered from WW and faecal sludge)	Disinfect	Helminth	(LANDRY *et al.*, 2021)
Liquid	Clinic environment	Surface (artificially contaminated bone discs)	Disinfect.	Bacteria	(DANTAS et al. 2021)
Liquid	Food industry	Suspension	Disinfect.	Fungi	(VISCONTI et al., 2021)
Disinfectant	Clinic environment	Surface (carrier disk)	Disinfect.	Bacteria (AR)	(CADNUM et al., 2021)
Liquid	Sanitation	Water (artificially contaminated test water)	Disinfect.	Bacteria; virus	(SILVA; SABOGAL-PAZ 2021)

Notes: AR = antibiotic / antifungal resistant; General = decontamination (room or in-house environments, unless stated). disinf. = disinfection; NA = not available; oxid. = oxidation; WW = wastewater.
Carrier disks are made of stainless-steel, unless stated.
Disinfectants refer to peroxygen-based products that may contain a small percentage of other substances (e.g. alcohol, peracetic acid, silver nitrate, quaternary ammonium, etc.).

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Appendix 2

This appendix supports Chapter 3 and part of it has been published as supplementary material to the following article:

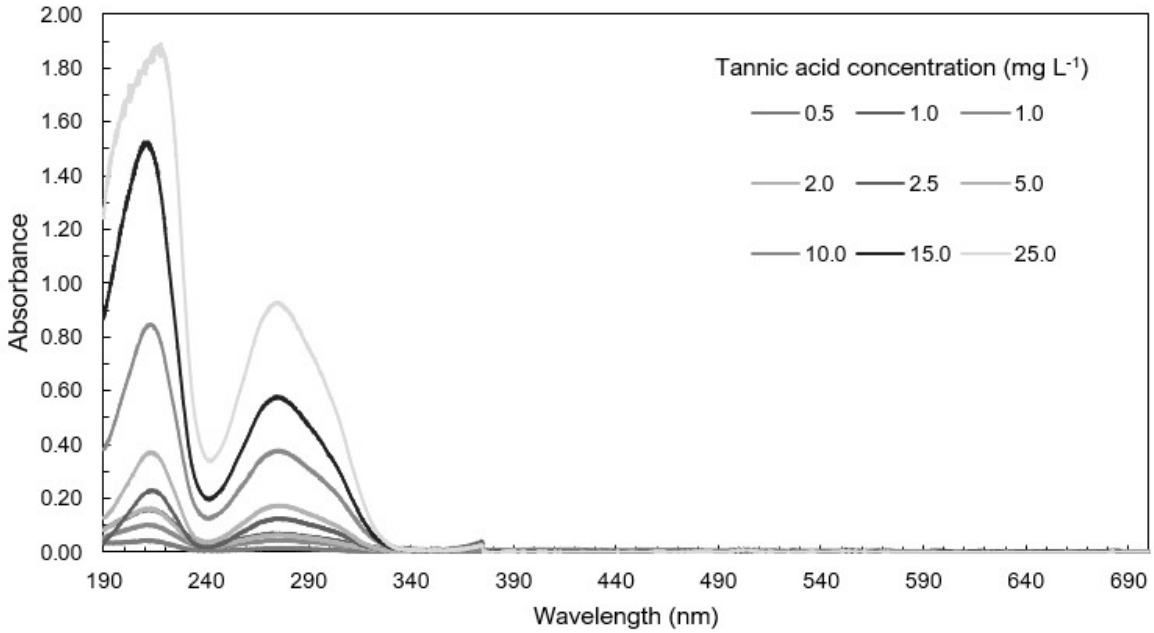
SILVA, K,J,S,, SABOGAL-PAZ, L.P, Exploring Potentials and Constraints of H_2O_2 Water Disinfection for Household Settings, *Water Air, and Soil Pollution* **232,** 483 (2021), https://doi,org/10,1007/s11270-021-05434-3

A2.1. Test water

The study water was prepared aiming to adjust the TOC so that it contained around 1.0 mg L^{-1} without adding color to the matrix. For this, different doses of tannic acid were added. Simultaneously, the electromagnetic spectrum was scanned (190 to 700 nm) for different concentrations of tannic acid (figure A2-1). Through the analysis of the peaks in the scan, it was identified that the absorbance at 274 nm is representative, according to the correlation indicated in figure A2-1.

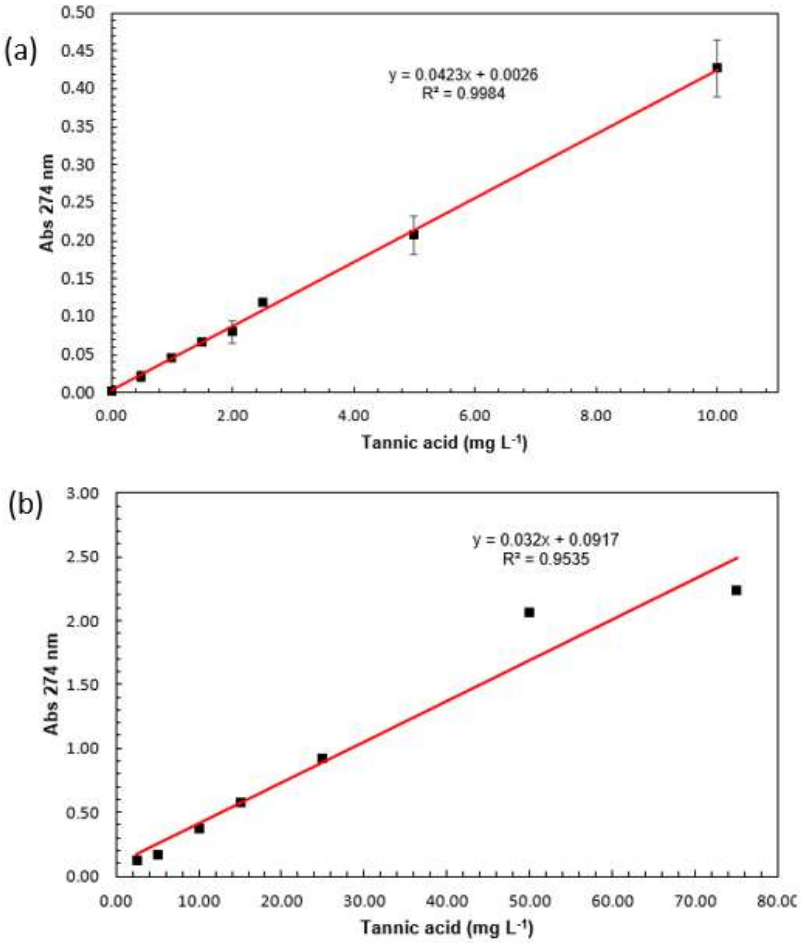
Based on the results obtained for abs 254 nm, an interval was inferred in which the quantification of total organic carbon would be evaluated. The relationship between TOC and tannic acid concentration as a representative of NOM is shown in figure A2-3.

Figure A2-1 - Spectrum scanning between 190 to 700 nm considering tannic acid concentrations



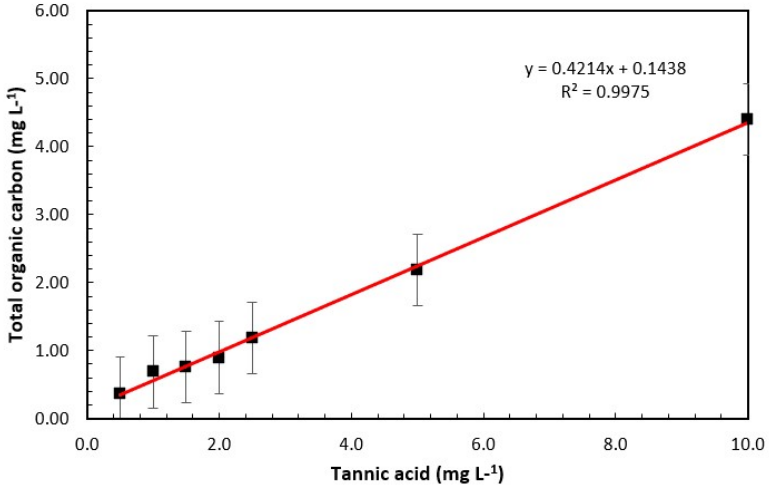
Source: the author, also published in Silva and Sabogal-Paz (2021).

Figure A2-2 - Relationship between absorbance at 274 nm for low (a) and (b) high tannic acid concentrations



Notes: Error bars refer to standard deviation calculated for n = 3 in low concentrations. Repetitions were not performed for high concentrations of tannic acid.
Source: the author, also published in Silva and Sabogal-Paz (2021).

Figure A2-3 - Total organic carbon as a function of tannic acid concentration



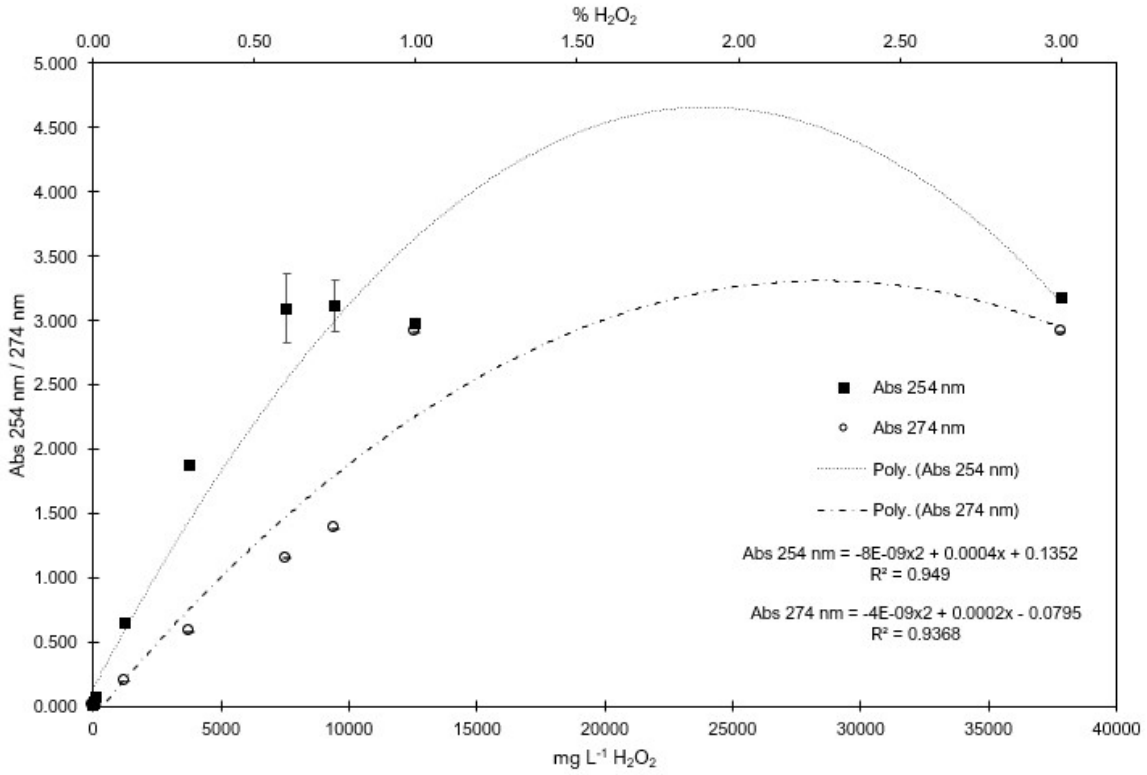
Notes: Error bars refer to standard deviation calculated for n = 3.
Source: the author, also published in Silva and Sabogal-Paz (2021).

A.2.2. Hydrogen peroxide interference in photometric assays

"Blank" curves were prepared in order to describe the interference of only hydrogen peroxide on the absorbance at 254 nm and 274 nm measured by the spectrophotometer, as a function of H_2O_2 concentration.

Obtained results are shown in Figure A2-4, as well as the polynomial curves and respective R^2 for each wavelength (given by Microsoft Office® Excel).

Figure A2-4 - Hydrogen peroxide contributions for absorbance at 254 and 274 nm



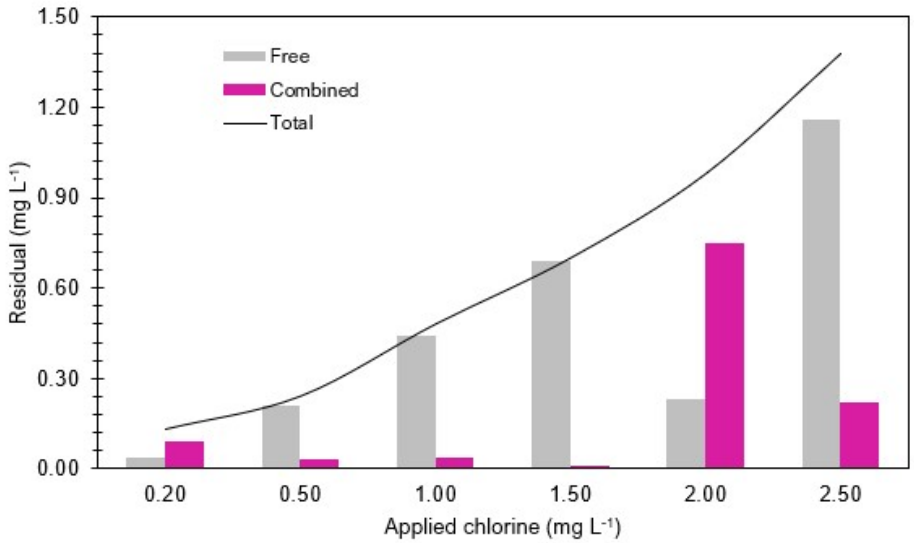
Notes: Error bars refer to standard deviation calculated for n = 3.
Source: the author, also published in Silva and Sabogal-Paz (2021).

A2.3. Chlorine demand

A test for the determination of residual chlorine was performed (without genuine replicates) aiming at finding a preliminary notion to assist in the selection of disinfectant doses. In this test, there was inoculum of Phi X174, as well as the suspension of *Escherichia coli*, which could be responsible for increasing chlorine demand and simulating contamination.

The obtained results for free, combined, and total chlorine are shown in figure A2-5.

Figure A2-5 - Chlorine residuals obtained in artificially contaminated test water after an exposure time of 30 min. Samples were mixed at 700 s^{-1} for ~7 s at kept at 30 s^{-1} velocity gradient during contact time



Source: the author, also published in Silva and Sabogal-Paz (2021).

Appendix 3

This appendix supports Chapter 5 and part of it (section A3.1) has been published as supplementary material to the following article:

SAMMARRO SILVA SILVA, K.J.S., LEITE, L.S., DANIEL, L.A., SABOGAL-PAZ, L.P. Hydrogen peroxide-assisted pasteurization: an alternative for household water disinfection. **Journal of Cleaner Production** (2022). Available at: <https://doi.org/10.1016/j.jclepro.2022.131958>

A3.1 Statistical analyses for the empirical models

Tables A3.1 and A3.2 display the output for ANOVA of the empirical models considering the two target organisms under test for assessing H_2O_2-assisted pasteurization.

Table A3.1 - ANOVA for the fit of the empirical model to *E. coli* inactivation by H_2O_2-assisted pasteurization

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: Results at 5% significance level for the recalculated model excluding insignificant coefficients. R^2 = 0.75877. SS = sum of squares; df = degrees of freedom MS = mean square; L = linear

Table A3.2 ANOVA for the fit of the empirical model to PhiX 174 bacteriophage inactivation by H_2O_2-assisted pasteurization

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: Results at 5% significance level for the recalculated model excluding insignificant coefficients. R^2 = 0.71791SS = sum of squares; df = degrees of freedom MS = mean square; L = linear

A3.2 A design proposal for hydrogen peroxide-assisted solar pasteurization

This section summarizes a product of the co-orientation of Nicholas Picin Casagrande for his undergraduate thesis in Civil Engineering at the University of São Paulo and it is present as a component of this doctoral thesis for credit and participation disclosure:

CASAGRANDE, N. P. **A proposal for rural residential water treatment system by solar pasteurization assisted by oxidation** (Original title in Portuguese: "Proposta de sistema residencial rural de tratamento de água por pasteurização solar assistida por oxidação") Undergraduate thesis (not published), São Carlos School of Engineering, University of São Paulo, São Carlos, 2020

Aims and methods

The project aimed to present a layout of an H_2O_2-assisted pasteurization solar system, based on rural settings in Brazil, providing an integrated concept that addresses Sustainable Development Goal 6 (SDG 6 – safe water and sanitation for all) and SDG 7 (access to affordable, reliable, sustainable and modern energy for all) (UNICEF; WHO, 2019) in household water treatment.

The conceptualization of the system considered a standard residence aligned with the first range of the "Minha Casa Minha Vida Program" (PMCMV), in order to guarantee the scope of application. The household of reference has a structural system in concrete walls, capable of safely supporting the treated water reservoir further indicated.

As for water consumption, four full-time residents were adopted at the household, who must have their demands for drinking water supplied by the system. Daily needs were defined as 480 L d^{-1}, based on literature information for water consumption in family households (TSUTIYA, 2006).

It was assumed that it was a self-supplied residence, sourced by groundwater with a minimum flow rate to feed the system's demand. The water quality was also assumed to be compatible with the proposed technology, obtained from a tubular well (200 mm diameter; ≤ 50 m deep). This water source was selected for the project due to its representativity in sourcing at a national level (CPRM, 2020).

Concept

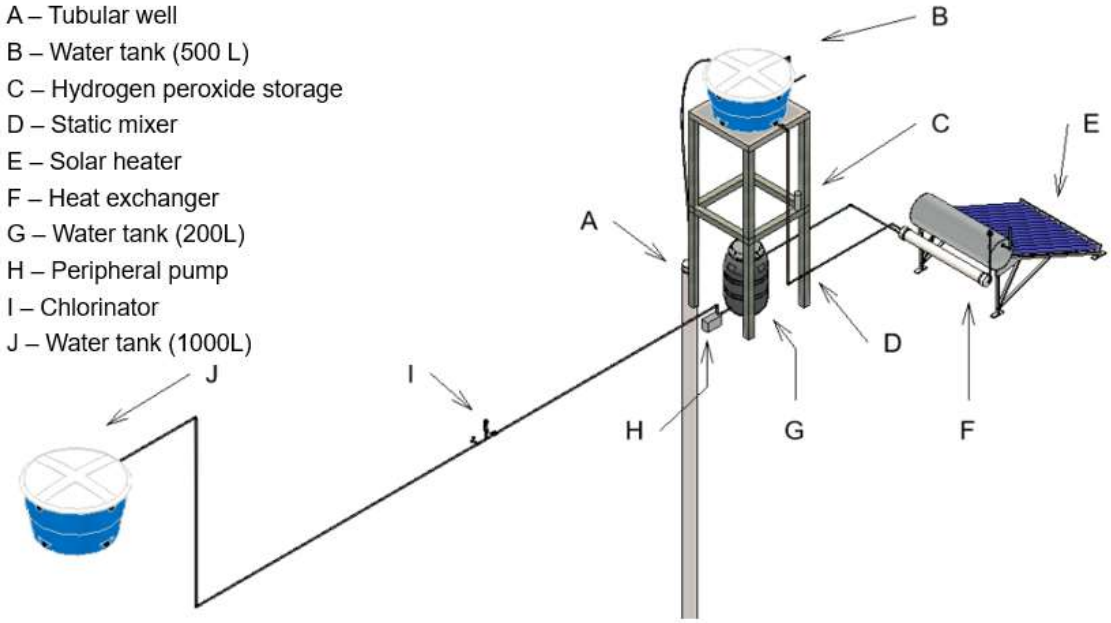
Figure A3.1 displays a schematic diagram of the hydrogen peroxide-assisted solar pasteurization system, its components, and associated stages. Each step is explained as follows:

- Stage A: Water collection. The vibrating pump is submerged in the well and its activation is controlled by a level float located in the raw water reservoir (B).

- Step B: Raw water storage. In the indicated 500-L tank, the water is accumulated for providing adequate pressure for the system. The water output is controlled by a hydraulic float valve located in tank (G).
- Step C: Hydrogen peroxide dosing. H_2O_2 is stored in the indicated container, from which the metering pump will direct it to point (D). The pump activation is done by the level sensor installed in tank (G), which is supposed to contain a 200 L volume capacity.
- Step D: Mixing. The static mixer (commercially available) provides agitation, dispersing H_2O_2 into the water.
- Step E: Solar pasteurization. Water will flow through the collector tubes, heating up and circulating through the heater tank by convection. For this step, a commercially available solar heater was chosen, consisting of a vacuum storage tank with no electricity backup resistor connected to borosilicate glass tubes. The capacity of the selected solar pasteurizer is 150 L.
- Step F: Heat exchange. Leaving the solar heater, the water flows through a CPVC pipe to the heat exchanger, which will be filled with raw water at its natural temperature. This water will receive part of the heat from the pasteurized water. Note that there is no mixing between raw and treated water, only contact between the hot water pipe and the raw water.
- Stage G: Intermediate storage for flow control. Treated water is accumulated in tank (G). It has a float valve that will closes the water inlet when the reservoir level is reached, interrupting the flow of the entire system. The level sensor that controls Step C will be aligned with the level valve, activating the metering pump only when there is water flowing through the system.
- Stage H: Supply of treated water. The peripheral pump is responsible for the water flow from tank (G) to the upper reservoir (J) located in the residence.
- Step I: Chlorination. The user must daily add the recommended amount of chlorine to guarantee safe storage. This is the only user interaction with the system.
- Stage J: Storage. Home storage of treated water and distribution in the house's internal network.

Details for the system’s hydraulic and electrical conceptualization (using photovoltaic panels) are available in the original manuscript by Nicholas Picin Casagrande (2020). Additionally, the project contains budgeting and a study on solar irradiation for a hypothetical residence situated in the municipality of São Carlos (São Paulo State, Brazil), so that the H_2O_2-SOPAS system is adequately positioned in the household.

Figure A3.1 – Scheme of the H_2O_2-SOPAS residential system and its components (no scale)



Source: Nicholas Picin Casagrande (2020).

Additional references

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