Impact of Hydrogen Peroxide Treatment on Environmental *Escherichia coli* Strains

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Abstract The impact of three hydrogen peroxide (H₂O₂) concentrations (250, 300 and 350 mg.L⁻¹) at 30, 60, 90 and 120 min time intervals was determined on environmental and ATCC reference *E. coli* strains (n=11). Variation between strains was evident and treatment resulted in significantly different log reductions after the 120 min contact time. The environmental strains were generally more resistant than the reference strains. A H₂O₂ resistant environmental strain (M53) and a potential pathogenic strain (W1371) were used to determine bactericidal effect at higher (and a lower) H₂O₂ concentrations of 50, 350, 700 and 1 000 mg.L⁻¹ on the microbial inactivation. Bacterial inactivation increased as concentration increased, with 50 mg.L⁻¹ resulting in low microbial inactivation and 1 000 mg.L⁻¹ resulting in an effective (>4 log) reduction. A significant difference in microbial reduction was not observed at H₂O₂ concentrations between 350 and 700 mg.L⁻¹. The potential influence of the COD (chemical oxygen demand) of river water on the H₂O₂ treatment was also determined. It was observed that the water COD, at the levels investigated, might influence H₂O₂ efficacy treatment over shorter treatment times (30 min), but not over longer periods (90-120 min). Different levels of catalase activity were also measured for the test strains. A trend was observed between H₂O₂ resistance and an increased amount of HPⅡ catalase activity. However, it was also observed that *E. coli* can also employ other protection mechanisms, as two of the most resistant environmental *E. coli* strains only indicated average catalase activity.

Keywords: H₂O₂, biocide, irrigation, water treatment, *E. coli*, resistance, catalase

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1. Introduction

Water scarcity is a global problem, and a reality in South Africa [1,2]. In many developing countries, water scarcity can be linked to poverty due to the decrease in food production [3]. It is therefore of the utmost importance that the South African agricultural industry keep up with growing food demands in spite of a limited water supply.

Due to a variety of reasons the quality of irrigation water sources is deteriorating [4]. This a major cause for concern since foodborne disease outbreaks have been linked to irrigating fresh produce with faecally contaminated water [5]. On-farm treatment options of water are therefore needed in order to decrease the high microbial loads prior to irrigation.

The disinfectant properties of H₂O₂ have been known for many years. It acts as a disinfectant due to the formation of highly reactive hydroxyl and superoxide radicals with strong oxidising properties [6]. This is seen as a significant advantage for fresh produce farmers when considering water treatment options. Factors such as concentration, contact time and organic matter content can all influence the efficacy of H₂O₂ as a treatment option [7,8]. Presence of catalases might furthermore contribute to microbial tolerance at lower concentrations [9]. Generally there is limited literature available on H₂O₂ concentrations used for irrigation water treatment. Studies on wastewater have, however, shown that a 250 mg.L⁻¹ H₂O₂ treatment resulted in a 2.2 log reduction after 120 min [10], and that the organic matter content in water can also decrease the efficiency of H₂O₂ treatment [8].

H₂O₂ has been used for microbial inactivation of a variety of viruses and other pathogenic microorganisms, as well as for both gram positive and gram negative vegetative bacteria [11]. According to the WHO (World Health Organization) [12] faecal coliforms must not exceed 1000 faecal coliforms per 100 mL water intended for irrigation of fresh produce to ensure food safety. Therefore, in this study the effect that H₂O₂ can have on *E. coli* strains was studied.

It is furthermore evident that variation between bacterial strains exists with regards to survivability after exposure to different treatment options. A study done by
Cherchi and Gu [13] on chlorine disinfection indicated that E. coli O157:H7 was more sensitive to chlorine disinfection compared to E. coli K12. Wojciecka et al. [14] has also reported that E. coli O157:H7 were less sensitive than reference strains (after a free chlorine and monochloramine treatment). However, other heterotrophic environmental strains were also included in the study, and the results indicated that they were easily inactivated compared to reference strains [14]. Mazzola et al. [15], on the other hand, reported that environmental strains isolated from a water purification system showed higher resistance against chemical disinfectants compared to standard reference strains. Environmental strains might not always have the same inactivation kinetics as standard reference strains. It is, however, important to investigate strain-strain variation and determine the most resistant strains. These strains should then be used during optimization studies of water treatment options in order to determine the most effective treatment parameters. Therefore, in this study the effect that H2O2 can have on different environmental and reference E. coli strains was studied.

2. Materials and Methods

2.1. Escherichia coli Cultures

In this study, 12 E. coli strains (three ATCC strains and nine environmental strains) were used (Table 1). The environmental E. coli strains had been isolated previously from river water and produce (Department of Food Science, Stellenbosch University) and stored in 40% (v.v-1) glycerol (Fluka Analytical, Germany) at -80°C. Strains were resuscitated in Nutrient Broth (NB) (Biolab, Merck), after which single colonies was obtained by streaking on Levine Eosin Methylene-Blue Lactose Sucrose Agar (L-EMB) (Oxoid, South Africa. The Escherichia coli colonies had a metallic green sheen on L-EMB agar [16]. Each strain’s identification was confirmed using API 20E (BioMérieux, South Africa) as described previously [17].

| Strain   | Source | Resistance |
|----------|--------|------------|
| M29      | River  | C, TM      |
| MJ58     | Parsley| None       |
| MJ56     | Parsley| None       |
| M53      | River  | T, TM, AMP, S |
| E12.1    | River  | None       |
| E22.1    | River  | None       |
| E11.1    | River  | None       |
| F11.2    | River  | T          |
| ATCC 11772| ATCC  | None       |
| ATCC 25922| ATCC  | None       |
| ATCC 35218| ATCC  | AMP, C, S  |
| W1371 (EPEC) | River | TM, AMP, S |

C: chloramphenicol, T: tetracycline, TM: trimethoprim, AMP: ampicillin, S: streptomycin, ATCC: American Type Culture Collection.

2.2. E. coli Inoculum Preparation

Physiological Saline Solution (PSS) (0.85% m.v-1 NaCl) was used as the test medium for all the laboratory studies discussed in this report. Before each treatment an inoculum was prepared for each strain by diluting an overnight culture (incubated in NB at 35°C) in SSS to achieve a turbidity equal to 0.5 McFarland standard (BioMérieux, South Africa).

2.3. E. coli Enumeration

A dilution series was prepared both before (control), and after all specific treatments and time intervals. Enumeration of E. coli were done on Violet Red Bile Agar (VRBA) (Biolab, Merck, South Africa). Plates were poured in duplicate, inverted and incubated at 35°C for 24h.

2.4. H2O2 Treatments

H2O2 30% (V.v-1) (Merck, South Africa) was used to prepare the H2O2 concentrations (50, 250, 300, 350, 700 and 1000 mg.L-1) that were used in this study. The H2O2 concentrations were confirmed by a Spectroquant® Hydrogen Peroxide Cell Test (2.0 – 20.0 mg.L-1) (Merck, South Africa).

2.5. Study Design

2.5.1. Study 1: E. coli Resistance to H2O2 Treatment/Effect of H2O2 Concentration and Contact Time on Selected E. coli Strains

The impact of three H2O2 concentrations (250, 300 and 350 mg.L-1) were determined on all the E. coli strains at five intervals (0 (initial counts), 30, 60, 90 & 120 min) in triplicate in PSS. One of the most resistant environmental strains was then selected and exposed to a wider range of H2O2 concentrations (50, 350, 700 & 1000 mg.L-1) at five time intervals (0 (initial counts), 30, 60, 90 & 120 min) in PSS. An environmental E. coli strain identified as carrying enteropathogenic (EPEC) gene sequences, W1371, was also exposed to the wider range of H2O2 concentrations (50, 350, 700 & 1000 mg.L-1).

2.5.2. Study 2: Influence of COD (Chemical Oxygen Demand)

The impact that COD content (taken as an indicator of organic matter content) could have on H2O2 treatment was also determined. The most resistant environmental strain from Study 1 was inoculated into PSS, as well as into autoclaved river water from two different sources (River 1 and River 2), and exposed to 350 mg.L-1 H2O2 at five time intervals (0 (initial counts), 30, 60, 90 & 120 min).

2.5.3. Study 3: Catalase Activity of E. coli Strains

The catalase activity of all 12 E. coli strains was measured to determine if higher catalase activity corresponded with increased H2O2 resistance. Two different methods were used in this study. The first method (method 1) [18], only gave an indication of total catalase activity and was based on interpreting the visual reduction of 30 % (v.v-1) H2O2 by each culture on a glass slide. Results were recorded based on the robustness of the bubbling: ‘-‘ (no bubble formation); ‘+‘ (little bubble formation); ‘++‘ (average bubble formation) and ‘++++‘ (immediate, strong bubble formation).
The second method (method 2) [19], was used to quantify HPI (hydroperoxidase I), HPII (hydroperoxidase II) as well as total catalase activity. The method involved the transfer of cell suspension (0.01 g of wet weight and 100 μL of PSS) to a test tube, after which 100 μL Triton X-100 was added, followed by the addition of 100 μL H₂O₂ solution (30% v/v). The height of the foam in the test tube was measured after the foam height had remained constant for 15 min. This value was then used as an indication of total catalase activity. In order to distinguish between the two types of catalase, HPI and HPII, a second aliquot in PSS was also prepared. This aliquot underwent a heat treatment of 55 °C for 15 min. The heat treatment was done before adding the cell suspension to the test tube with Triton X-100 and H₂O₂. The heat-stable catalase (HPII activity) was determined by measuring the height of foam that had been stable for 15 min of the heat treated aliquots. The heat–labile (HPI activity) catalase activity was then determined by subtracting the heat-stable catalase activity from the total catalase activity.

2.6 Statistical Analysis

Main effects ANOVA using Statistica 12.5 (Statsoft Inc.) was used, with E. coli strain and time as the two effects. The main purpose of doing the ANOVA was to determine significant differences between means. A mixed model repeated measures ANOVA was used to analyze the E. coli counts and a two-way ANOVA was used to analyze the log reductions achieved in study 1. In study 2, a mixed model repeated measures ANOVA was used to analyze the E. coli counts in the PSS, River 1 and River 2 samples.

Post hoc tests were conducted using the Fisher least significant difference (LSD) testing. A 5% significance level (p < 0.05) was used as guideline for determining significant results. If no E. coli growth was observed it was recorded as 30 cfu.mL⁻¹ (1.48 log cfu.mL⁻¹) (as the lower limit) for all the statistical analyses done in studies 1 and 2.

3. Results

3.1. Study 1: E. coli Resistance to H₂O₂ Treatment/ Effect of H₂O₂ Concentration and Contact Time on Selected E. coli Strains

In order to determine the bactericidal effect that the three H₂O₂ concentrations (250, 300 and 350 mg.L⁻¹) had at the individual time intervals, the colony counts of all the E. coli strains, were pooled and are presented in Figure 1. The highest H₂O₂ concentration (350 mg.L⁻¹) was the most effective and resulted in a higher log reduction over 120 min (Figure 1). When comparing the 250 and 300 mg.L⁻¹ treatments, 300 mg.L⁻¹ resulted in a slightly higher log reduction (Figure 1). This indicated a trend that the higher H₂O₂ concentration would result in a greater log reduction. However, although a trend was observed when comparing results at the different H₂O₂ concentrations, a significant difference could not be proven (p < 0.05) between the 250, 300 and 350 mg.L⁻¹ H₂O₂ treatments. The average colony counts obtained for each strain at all three H₂O₂ concentrations, were, therefore, pooled before log reductions of the individual strains were compared.

Figure 1. Effect of H₂O₂ concentration and contact time (0, 30, 60, 90 and 120) on the 11 E. coli strains used in study B, the E. coli strains were pooled in order to observe only the effect of H₂O₂ concentration and contact times on the E. coli strains. Error bars represent error at a 95% confidence interval.

The log reductions achieved for the individual E. coli strains exposed to H₂O₂ for the 120 min contact time varied from 2.13 – 5.48 (Figure 2). It was observed that ATCC 11775 was the most sensitive to the H₂O₂ treatment (5.48 log reduction). ATCC 25922 showed a 5.14 log reduction after 120 min. A significant difference was not seen between the log reductions achieved for ATCC 11775 and ATCC 25922 at 120 min (p = 0.19). These strains were considered the most sensitive of all the strains tested. ATCC 35218 was the most resistant ATCC strain to H₂O₂ treatment and only a 2.14 log reduction was achieved after 120 min (Figure 2).

The environmental Escherichia coli strains MJ58 and M53 showed the same log reduction (2.17 log reduction...
after 120 min), while M29 resulted in a 2.27 log reduction after 120 min (Figure 2). A significant difference was not seen between M53, MJ58 and M29 (p > 0.05) after 120 min, and these strains were considered the most resistant of the environmental E. coli strains evaluated in this study. Environmental strains F11.2, E22.1 and E11.1 indicated 2.82, 3.08 and 2.9 log reductions, respectively, after 120 min.

A significant difference was not observed between F11.2, E22.1 and E11.1 (p > 0.05), although these strains did however, differ significantly from, M53, MJ58 and M29 (p < 0.05) (Figure 2). A 3.52 log reduction was achieved after the 120 min contact time for E12.1. Consequently, E. coli E12.1 was considered one of the more sensitive environmental strains to the H₂O₂ treatment. E12.1 differed significantly from M29, M53, MJ58, F11.2 and E11.1, however a significant difference was not seen between E12.1 and E22.1 (p = 0.08) (Figure 2). MJ56 was the most sensitive environmental strain to the H₂O₂ treatment, resulting in a 3.92 log reduction after 120 min. MJ56 differed significantly (p < 0.05) from all the other environmental E. coli strains used in the study, except for E. coli E12.1 (p = 0.11). Growth was observed for all the environmental E. coli strains after 120 min, however for ATCC 11775 no growth was present after 120 min and therefore a statistical lower limit of 30 cfu.mL⁻¹ (1.48 log cfu.mL⁻¹) was used for the data analysis.

Figure 2. Log reductions achieved for the 11 E. coli strains after the 120 min contact time (T₀ – T₁₂₀) (Pooled H₂O₂ concentrations). Error bars represent error at a 95% confidence interval.

Figure 3. Effect of four H₂O₂ (50, 350, 700 and 1000 mg.L⁻¹) concentrations on M53 and W1371. No growth was recorded as 30 cfu.mL⁻¹ (1.48 log cfu.mL⁻¹). Error bars represent error at a 95% confidence interval.
M53 was selected as one of the most resistant environmental *E. coli* strains to H$_2$O$_2$ treatment and exposed to four H$_2$O$_2$ concentrations (50, 350, 700 and 1 000 mg.L$^{-1}$), to determine the effect that both low and high H$_2$O$_2$ concentrations would have. When 50 mg.L$^{-1}$ H$_2$O$_2$ was used, a significant difference was not seen between the counts obtained at 0, 30, 60, 90 and 120 min (Figure 3) (p > 0.05), with an overall log reduction of 0.22. At 350 mg.L$^{-1}$, the overall log reduction increased to 2.06 (Figure 4). When the H$_2$O$_2$ concentration was doubled to 700 mg.L$^{-1}$ the log reduction increased slightly from 2.06 to 2.27, and no significant difference was observed between the log reductions achieved with the 350 and 700 mg.L$^{-1}$ H$_2$O$_2$ treatments (p = 0.24) (Figure 4). At 1 000 mg.L$^{-1}$ growth was observed at 90 min (Figure 3), where a 4.3 log reduction was achieved. However, no growth was present after the 120 min contact time. For W1371, as for M53, no significant difference was seen between the log reductions at 350 and 700 mg.L$^{-1}$ (p = 0.05) (Figure 4). At 1 000 mg.L$^{-1}$ growth was observed at 90 min, where a 4.7 log reduction was achieved. However, no growth was present after the 120 min contact time (Figure 3). To facilitate statistical analyses no growth was recorded as 30 cfu.mL$^{-1}$ (1.48 log cfu.mL$^{-1}$).

![Figure 4. Log reductions achieved after 120 min for M53 and W1371 at 50, 350, 700 and 1 000 mg.L$^{-1}$. Error bars represent error at a 95% confidence interval](image)

![Figure 5. Effect of 350 mg.L$^{-1}$ H$_2$O$_2$ on M53 in PSS, River 1 and River 2 water samples. Error bars represent error at a 95% confidence interval](image)
3.2. Study 2: Effect of Organic Matter (COD) on the Efficacy of the \( \text{H}_2\text{O}_2 \) Treatment

River water was sampled from two rivers, and autoclaved before the samples were inoculated with \( \text{E. coli} \) M53. The COD values for River 1 and River 2 were 18 and 55 mg.L\(^{-1} \), respectively. A comparison was made between the log reductions achieved after the \( \text{H}_2\text{O}_2 \) treatment (350 mg.L\(^{-1} \)) in PSS (which served as a control), River 1 and River 2 at time intervals at 0, 30, 60, 90 and 120 min (Figure 5).

After 30 min, a significant difference was seen between the log reductions achieved for strain M53 in the PSS and River 1 (\( p = 0.01 \)), as well as the PSS and River 2 (\( p = 0.02 \)), with numbers decreasing quicker in the PSS sample. After 120 min, 2.12, 2.18 and 2.28 log reductions were achieved in the PSS, River 1 and River 2 samples, respectively. These did not differ significantly (PSS and River 1, \( p = 0.90 \); PSS and River 2, \( p = 0.70 \); River 1 and River 2, \( p = 0.80 \)).

3.3. Study 3: Catalase Activity of \( \text{E. coli} \) Strains

The total catalase activity was determined for all 12 \( \text{E. coli} \) strains using method 1 [19]. MJ58, M53, M29, as well as E22.1, was observed to have the highest catalase activity (+++) according to the amount of bubbles formed. F11.2, E12.1, E11.1, MJ56, W1371 and ATCC 35218 showed a slightly lower catalase activity (++). The ATCC strains 25922 and 11775 had the lowest catalase activity (+) in comparison to all the other \( \text{E. coli} \) strains tested.

In order to quantify the catalase activity, all the strains were evaluated using method 2 [20]. The amount of total catalase activity varied between 120.2 - 557 units (U per 1 mg wet weight) for all strains tested (Figure 6).

![Figure 6. Catalase activity of all 12 \( \text{E. coli} \) strains in the study. Error bars represent error at a 95% confidence interval](image)

**E. coli strains**

M53, M29, MJ58 and E22.1 had 557, 548.3, 513.7 and 505.1 U total catalase, respectively, and had the highest amount of total catalase activity. These results corresponded with the observations of method 1. These four strains also had the highest amount of HPII activity (Figure 6). The HPI activity varied between 0 – 224 U catalase for all strains tested (Figure 6). It was observed that \( \text{H}_2\text{O}_2 \) resistant strain (W1371) indicated no HPI activity and ATCC 25922, a sensitive strain to the \( \text{H}_2\text{O}_2 \) treatment, had 224 U HPI catalase activity

4. Discussion

Although a trend was observed in Study 1, no significant differences could be proven between the bactericidal activity of the 250, 300 and 350 mg.L\(^{-1} \) \( \text{H}_2\text{O}_2 \) treatments. Similar results were seen in a study done by Vargas et al. [8] where little statistical significant variation was found between the following \( \text{H}_2\text{O}_2 \) concentrations: 100, 150, 200, 250 and 300 mg.L\(^{-1} \). ATCC 35218 was the most resistant ATCC strain to \( \text{H}_2\text{O}_2 \) treatment. ATCC 35218 is also resistant to three antibiotics including ampicillin, chloramphenicol and streptomycin (Table 1). Log reductions obtained for ATCC 35218 differed significantly from both those obtained for ATCC 11775 and ATCC 25922. Thus, significant variations exist between the \( \text{H}_2\text{O}_2 \) resistance of the three ATCC \( \text{E. coli} \) strains tested.

Variation in strain resistance to the \( \text{H}_2\text{O}_2 \) treatment was also clearly evident for the eight environmental strains tested at the 250, 300 and 350 mg.L\(^{-1} \) \( \text{H}_2\text{O}_2 \) treatments. The log reductions of the three most resistant
environmental strains (M53, M29 and MJ58) did not differ significantly from the reduction observed for ATCC 35218. Interestingly, M53 and M29 also exhibited multiple antibiotic resistances (Table 1), as was observed for ATCC 35218. The other five environmental strains all showed varying resistance to H₂O₂ treatments, but were all significantly more resistant than the two ATCC strains (25922 and 11775) (Figure 2). This highlights the fact that careful consideration should be given to the choice of strain when testing the efficacy of water treatment strategies. Choosing a sensitive strain, like ATCC 25922, might result in the development of strategies which is not properly optimized for the reduction of environmentally adapted strains.

Studies by Britz et al. [20,21] indicated high faecal contamination of certain South African rivers and recommended a 3 – 4 log reduction to achieve irrigation water of a safe quality. The log reductions obtained for the four most resistant strains (Figure 2) were all in the range between 2.14-2.27. Ortega de Velásquez et al. [10] reported similar reductions in a study done using H₂O₂ to treat wastewater. They found that faecal coliforms decreased by 2.2 log reduction after 120 min, in the presence of 250 mg.L⁻¹ H₂O₂. However it must be noted that the treatment was done in wastewater, and therefore high levels organic matter present in the water could have further reduced the efficiency of the H₂O₂ treatment [7,10]. The impact of higher concentrations of H₂O₂ on the log reduction of resistant (M53) and pathogenic strains (W1371) were, therefore, also evaluated.

A study done by Linley et al. [6] hypothesized that at lower H₂O₂ concentrations, bacterial inactivation is a result of DNA damage, and is closely related to the Fe²⁺ ions associated with the DNA molecule. They argued that DNA-associated iron may be the limiting factor when H₂O₂ reacts with DNA, thus indicating that increasing the H₂O₂ concentration may not result in an increased rate of DNA damage. It was hypothesized that the biocidal effect of H₂O₂ at higher concentrations is a result of oxidation of proteins and lipids [6]. A low and high H₂O₂ concentration was thus included to determine if it would result in at least a three log reduction.

Results indicated that survival decreased as the concentration increased (Figure 3 & Figure 4). The fact that no significant differences were observed between the log reductions of both strains at 350 and 700 mg.L⁻¹ H₂O₂ treatments suggests that there might be a certain resistance threshold in resistant E. coli strains which enables the strains to withstand higher H₂O₂ concentrations up to a certain level. The same “threshold” can be observed for the 1000 mg.L⁻¹ H₂O₂ treatment up to 60 min (Figure 3). After 60 min a rapid decrease in numbers were observed for both strains. This is an important factor to consider, as the concentration selected to treat irrigation water has cost implications. It would not be worth doubling the concentration of H₂O₂, and in turn the chemical cost, if similar log reductions could be achieved at both concentrations. Less contaminated water might only need a 2 log reduction in E. coli levels to reach the recommended irrigation water limit. For more contaminated sources, implementing a 1 000 mg.L⁻¹ treatment for at least 90 min could result in a sufficient log reduction, unless water quality parameters interfere with H₂O₂ efficacy.

High organic matter may limit the efficiency of H₂O₂ [7]. Study 1 was done in PSS, but since H₂O₂ will oxidize all the organic matter present, log reductions achieved in river water might be negatively impacted if the water has a high COD. It was therefore expected in Study 2 that the H₂O₂ treatment of samples from River 1 and River 2 would be less effective that the control sample in PSS. This was true for the first 30 min, where the levels of M53 decreased quicker in PSS than in the river water samples (Figure 5). After the 120 min treatment it was however clear that the PSS control was not more efficient that the River water samples. It was therefore concluded that, a low COD value (18 – 55 mg.L⁻¹) did not appear to influence the efficiency of the H₂O₂ treatment over the 120 min exposure time.

H₂O₂ can be catalyzed into water and oxygen by the catalase enzymes hydroperoxidase I (HPI) and hydroperoxidase II (HP II), therefore reducing the toxicity of H₂O₂. [22]. KatG genes are responsible for encoding HPI and KatE genes are responsible for encoding HP II [19,22,23,24]. These two catalase enzymes have different functions. HPI is induced by low H₂O₂ concentrations, whereas HP II is induced during the stationary phase or other stresses [25,26]. Thus, catalase is an enzyme that can protect the cell against sub-lethal concentrations of H₂O₂. Catalase levels may be increased due to phenotypic adaptation to protect the cell against oxidative stress. Consequently, active resistance against H₂O₂ may develop [27]. The aim of Study 3 was to determine if the E. coli strains which showed resistance to the H₂O₂ treatments in Study 1, also had high catalase activities. This would in turn indicate the importance of catalase as a mechanism by which E. coli strains can protect themselves against H₂O₂ treatment.

The results from Study 3 indicated that no correlation existed between H₂O₂ resistance and HPI catalase activity. In fact the two strains with the highest HPI catalase activity (ATCC strains 25922 and 11775), were the most sensitive strains to the H₂O₂ treatment (Figure 6). In contrast, it was observed that HPII could be an important enzyme to protect strains against H₂O₂ treatment. The H₂O₂ resistant strains M53, M29, MJ58 also had the highest HPII catalase activity, while the most sensitive strains (ATCC strains 25922 and 11775) showed no HPII catalase activity.

It was however concluded that HPII catalase activity could not be the only mechanism that determines resistance against H₂O₂ treatment. Two of the most resistant strains to H₂O₂ treatment (ATCC 35218 and W1371) only indicated average HPII catalase activity. These strains may have other properties that enable them to withstand high H₂O₂ concentrations. Both these strains, for instance, display multiple antibiotic resistances (Table 1). The mechanisms that enable them to resist certain antibiotics might also aid in protecting the strains against high H₂O₂ concentrations. Loui et al. [28] determined the resistance of E. coli to H₂O₂ in the presence of chloramphenicol, which inhibits protein synthesis by inhibiting peptide bond formation. Their results indicated that chloramphenicol enhanced the effect of H₂O₂, as greater bacterial inactivation was seen with the use of H₂O₂ and chloramphenicol than either one of the substances alone. It was concluded that the synergetic effect of using H₂O₂ and chloramphenicol indicated that
protein synthesis is important for E. coli resistance to H$_2$O$_2$. In this study both M29 and ATCC 35218, showed antibiotic resistance against Chloramphenicol, and both these strains were considered highly resistant to the H$_2$O$_2$ treatment. It could be that, as a result of their unique genetic make-up, the same physiological properties that protect protein synthesis in these strains from Chloramphenicol, will also protect these strains from H$_2$O$_2$ treatment. Thus, the resistance of the E. coli strains may not be due to catalase activity alone, especially in the strains indicating H$_2$O$_2$ resistance, yet displaying only average catalase activity. Non-specific efflux pumps is another strategy that bacteria can employ to protect them against non-specific biocides (such as H$_2$O$_2$) as well as against target-specific antibiotics [29]. High efflux pump activity might explain why certain multi-drug resistant strains in this study with only average catalase activity, (W1371 and ATCC 35218), were highly resistant to H$_2$O$_2$ treatment. This will, however, need to be investigated further.

5. Conclusion

Overall, a trend was seen between the H$_2$O$_2$ concentrations tested, with the highest H$_2$O$_2$ concentration resulting in the highest log reduction. Strain-to-strain variation was observed for all the strains tested indicating that environmental strains are usually better adapted to withstand the H$_2$O$_2$ treatment that ATCC strains. It was concluded that resistant environmental E. coli strains are capable of surviving high H$_2$O$_2$ concentrations (1 000 mg.L$^{-1}$), for long time periods (up to 90 min) provided high microbial loads are present. Careful consideration should therefore be given to the choice of a test strain for treatment method optimization studies. Exposure time during H$_2$O$_2$ treatment appear to be a critical parameter for two reasons: Firstly, when considering the “threshold” tolerance that certain H$_2$O$_2$ resistant strains exhibited in this study at concentrations of 1000 mg.L$^{-1}$ H$_2$O$_2$ and lower; and secondly, considering the influence of water COD levels (18 – 55 mg.L$^{-1}$) on H$_2$O$_2$ efficacy at shorter treatment times. Finally, although catalase activity (more specifically HPII activity) appears to be an important microbial defense mechanism against H$_2$O$_2$ treatment, it is evident that E. coli can also employ other protection strategies.

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References

[1] Department of Water Affairs & Forestry, South Africa. National Water Resource Strategy – first edition. The Government Printer, Pretoria, 2004.
[2] Norton-Brandllo, D., Scherenberg, S.M. and Van Lier, J.B., “Reclamation of used urban waters for irrigation purposes – A review of treatment technologies,” Journal of Environmental Management, 122, 85-98, 2013.
[3] FAO (Food and Agriculture Organization). Coping with water scarcity challenge of the twenty-first-century- UN Water. 2007. [Internet document]. Available: http://www.fao.org/in/water/docs/escarety.pdf. [Accessed Aug. 31, 2013].
[4] Jabadenyini, O.A. and Buya, E.M., “Irrigation water and microbiological safety of fresh produce; South Africa as a case study: A review,” African Journal of Agricultural Research, 7 (35). 4848-4857. 2012.
[5] Heaton, J.C. and Jones, K., “Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review,” Journal of Applied Microbiology, 104. 613-626. 2008.
[6] Linley, E., Denyer, S.P., McDonnell, G., Simons, C. and Maillard, J-Y, “Use of hydrogen peroxide as a biocide: new consideration of its mechanism of biocidal action,” Journal of Antimicrobial Chemotherapy, 67 (7). 1589-1596. 2012.
[7] Newman, S.E, “Disinfecting Irrigation Water for Disease Management.” in 20th Annual Conference on Pest Management on Ornamentals. San Jose, California, Colorado State University. 2004.
[8] Vargas, G.D.L.P., Moreira, R.F.P.M. and José, D.S.H.I., “Treated domestic sewage: Kinetics of Escherichia coli and total coliform inactivation by oxidation with hydrogen peroxide,” Quim Nova, 36 (2). 252-256. 2013.
[9] McDonnell, G. and Russell, A.D, “Antiseptics and disinfectants: activity, action and resistance,” Clinical Microbiology Reviews, 12. 148-179. 1999.
[10] Orta de Velásquez, M.T., Yáñez-noguez, I., Jiménez-cisneros, B. and Luna Pabello, V.M., “Adding silver and copper to hydrogen peroxide and peracetic acid in the disinfection of an advanced primary treatment effluent,” Environmental Technology, 29. 1209-1217. 2008.
[11] Labas, M.D., Zalazar, C.S., Brandi, R.J. and Cassano, A.E, “Reaction kinetics of bacteria disinfection employing hydrogen peroxide”, Biochemical Engineering Journal, 38. 78-87. 2008.
[12] WHO (World Health Organization), “Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture,” WHO, Technical Report Series no. 778, Geneva, Switzerland. 1989.
[13] Cherchi, C. and Gu, A.Z, “Effect of bacterial growth stage on resistance to chlorine disinfection,” Water Science & Technology, 64 (1). 7-13. 2011.
[14] Wojciecka, L., Hofmann, R., Baxter, C., Andrews, R.C., Liére, J., Miller, T., Chauret, C. and Baribeau, H., “Inactivation of environmental and reference strains of heterotrophic bacteria and Escherichia coli O157:H7 by free chlorine and monochloramine,” Journal of Water Supply: Research and Technology-AQUA, 56 (2). 137-149. 2007.
[15] Mazzola, P.G, Martins, A.M.S and Penna, T. C.V, “Chemical resistance of gram-negative bacteria to different sanitizers in a water purification system,” BMC Infections Disease, 6. 131-142. 2006.
[16] Merck, Microbiology Manual, 12th edition. Merck, Darmstadt, Germany. 2005.
[17] Lamprecht, C., Romanis, M., Huisamen, N., Carinus, A., Schoeman, N., Sigge, G.O. and Britz, T.J, “Escherichia coli with virulence factors and multidrug resistance in the Plankenburg River,” South African Journal of Science, 110(9/10), Art.#2013-0347. October 2014. [Online]. Available: http://www.scielo.org.za/scielo.php?pid=S0038-23532014001000013&script=sci_arttext. [Accessed May 30, 2015].
[18] Chen, C.-H., Hofmann, C.S., Cottrell, B.J., Strobaugh, T.P., Paoli, C.G., Nguyen, L.-H., Yan, X. and Uhlich, G.A, “Phenotypic and genotypic characterization of biofilm forming capabilities in non-O157 Shiga toxin producing Escherichia coli strain,” PLoS ONE, 8(12), e84863, December 2013. [Online]. Available:
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0084863. [Accessed May 30, 2015].

[19] Iwase, T., Tajima, A., Sugimoto, S., Okuda, K., Hironaka, I., Kamata, Y., Takada, K. and Mizuno, Y., “A simple assay for measuring catalase activity: A visual approach,” Scientific Reports, 3. 3081; October 2013. [Online]. Available: http://www.nature.com/srep/2013/131030/srep03081/full/srep03081.html?message-global=remove. [Accessed May 30, 2015].

[20] Britz, T.J., Sigge, G.O., Buys, E.M., Schmidt, S., Potgieter, N. and Taylor, M.B., “Baseline study on extent (types and quantities) of contamination found in irrigation water at selected sites (K5/1773)” In: Quantitative investigation into the link between irrigation water quality and food safety (Volume 2). WRC Report No. K5/1773/4, Pretoria, 2012.

[21] Britz T.J., Sigge, G.O., Huisamen, N., Kikine, T., Ackermann, A., Lötter, M., Lamprecht, C. and Kidd, M., “Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenburg and Eerste Rivers, Western Cape, South Africa,” Water SA, 39 (4). 457-465. 2013.

[22] Loewen, P.C., Switala, J. and Triggs-Raine, B.L., “Catalase HPI and HPII in Escherichia coli are induced independently,” Archives of Biochemistry and Biophysics, 243 (1). 144-149. (1985).

[23] Heimberger, A. and Eisenstark, A., “Compartmentalization of catalases in Escherichia coli,” Biochemical and Biophysical Research Communications, 154 (1). 392-397. 1988.

[24] Storz, G. and Imlay, J., “Oxidative stress,” Current Opinion Microbiology, 2. 188-194. 1999.

[25] Mulvey, M.R., Switala, J., Borys, A. and Loewen, P.C., “Regulations of transcription of KatE and KatF in Escherichia coli,” Journal of Bacteriology, 172 (12), 6713-6720. 1990.

[26] Visick, J.E. and Clarke, S., “RpoS- and OxyR-Independent induction of HPI catalase at stationary phase in Escherichia coli and identification of rpoS mutations in common laboratory strains,” Journal of Bacteriology, 179 (13). 4158-4163. 1997.

[27] Morató, J., Mir, J., Codony, F., Mas, J. and Ribas, F., “Microbial response to disinfectants” in The Handbook of Water and Wastewater Microbiology (edited by D. Mara & N. Horan), Academic Press, London, 2003, 657-670

[28] Loui, C., Chang, A.C. and Lu, S., “Role of the ArcAB two-component system in the resistance of Escherichia coli to reactive oxygen stress,” BMC Microbiology, 9, 183-196.

[29] Ortega Morente, E., Fernández-Fuentes, M.A., Burgos, M.J.G., Abríouel, H., Publido, R.P. and Gálvez, A., “Biocide resistance in bacteria,” International Journal of Food Microbiology, 162 (1). 13-25. 2013.