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INACTIVATION KINETICS OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* BY A HYDROGEN PEROXIDE BASED DISINFECTANT

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Abstract

Pathogenic bacteria in food industries may have several origins, including raw materials, workers, equipment and containers. If these bacteria are not destroyed during processing, they can grow during production, thus reducing the quality and safety of food. Hydrogen peroxide possesses many advantages comparing to other disinfectants. One of them is that it does not produce toxic residues. The aim of this study was to evaluate the efficiency of a hydrogen peroxide based disinfectant against *Escherichia coli* and *Staphylococcus aureus*, to reveal the nature of the inactivation kinetics and to determine the decimal reduction time (D-value) for mentioned bacteria.

Quantitative suspension test method was designed for the evaluation of bactericidal activity of mentioned disinfecting agent. The requirement of this standard is a minimum 5 log10 reduction within 5 min. The contact times were 0, 1, 2, 3, 4, 5 minutes. The D-value experiments were done by inoculating 8 mL of disinfecting solution with 1 mL of a bacterial suspension. At regular intervals (every 1min.), 1 mL aliquots of this mixture were transferred to 8 mL of growth media containing a neutralizing agent, and incubated at 37 ^{0}C. Modeling of survival curves was carried out by using the first order log-linear regression model $\log[Nt/No] = -t/D$. The D-value was determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the survivor curves ($\log^{10}$ CFU/mL versus time of exposure to the disinfectant solution).

After 5 min. of contact time hydrogen peroxide possessed better inhibition activity (more than 6 $\log^{10}$ reduction) against *S. aureus* comparing with inhibition activity against *E. coli* (more than 5 $\log^{10}$ reduction) ($P < 0.05$). Linear death kinetics was observed during 5 min. contact frame. D-values for 2% solution of hydrogen peroxide based disinfectant at 20 ^{0}C were *E. coli* (D = 0.89 min.) and *S. aureus* (D = 0.81 min.).

The results of this study have shown that log-linear inactivation models could be applied to suspension test for modeling of efficiency of disinfecting agents during particular contact time frame.

***Key words**: Inactivation kinetics, Modelling, D-value, Hydrogen peroxide, Disinfectant, Suspension test, Foodborne pathogens.*

1. Introduction

Food borne pathogens cause millions of illnesses and thousands of deaths every year worldwide [9]. The fecal coliforms, e.g., *Escherichia coli*, are used as an indicator of the sanitary conditions. The presence of the marker groups such as coliforms and *E. coli* in the processed products demonstrates possible process related to the contamination and indicates poor manufacturing practices and inadequate factory hygiene standards.

Staphylococcus aureus is a major human pathogen capable of causing a wide range of infections. The intoxication cases caused by methicillin-resistant *S. aureus* remains a problem and of particular concern [2].

For proper control of food borne pathogenic bacteria in food processing facilities one of the important tasks is to study the effect of the extrinsic parameters

of the processing and storage (temperature, freezing, irradiation, cleaning & disinfection dehydration, packaging, humidity) on survival and growth of bacteria. Sanitation is an important practice for reducing the occurrence of foodborne outbreaks [3].

Hydrogen peroxide is widely used as a sanitizer, particularly in applications where its decomposition into non-toxic by-products is important. The bactericidal efficacy of hydrogen peroxide has been demonstrated in both water and food systems [6], with Gram negative organisms having the most susceptibility (Davidson and Branen, [4]). Their main advantages are broad-spectrum activity, which includes efficacy against bacterial endospores, their lack of environmental toxicity following their complete degradation, and the fact that, with imaginative formulation, their surface corrosiveness and smell (for peracetic acid-based products) have been greatly reduced [7].

The aim of this study was to (i) evaluate the efficiency of a hydrogen peroxide based disinfectant (Bioxil) against *Escherichia coli* and *Staphylococcus aureus* and (ii) to determine the decimal reduction times (D-value) for mentioned bacteria. Action of the preparation Bioxil is based on activation of hydrogen peroxide by a composite synergistic composition [5].

2. Materials and Methods

2.1 Microorganisms and bacterial working cultures

Staphylococcus aureus (DSM 799) and *Escherichia coli* (DSM 301) strains were obtained from Leibniz-Institute DSMZ culture collection and were stored under -20 ^{0}C before future use. Subcultures on Tryptic Soy Agar (TSA) and Nutrient agar plates were prepared from the stock cultures and incubated for 18 - 24 h at 37 ^{0}C. Bacterial test suspensions were adjusted approximately to 10^6 cfu/mL and 10^7 - 10^8 cfu/mL for *E.coli* and *S. aureus* respectively. Determination of viable concentrations bacteria in suspension were performed in accordance with bacterial growth curve analyzing method.

2.2 Bactericidal activity

This quantitative suspension test method was designed for the evaluation of bactericidal activity of hydrogen peroxide based disinfectant used in food areas. The requirement of this standard is a minimum reduction by a factor of 10^5 within 5 min. The efficiency of the sanitizer were also assessed after a: 1, 2, 3 and 4 minutes of contact time, to better understand the reduction kinetics during 5 minutes period. One milliliter of sterile distilled water was added to 1 mL of bacterial test suspension. After 2 minutes, 8 mL of tested sanitizer was added to the mixture and shaken. After above mentioned time periods, 1 mL of the test mixture was transferred into a tube containing 8 mL of neutralizer and 1 mL of sterile distilled water, and mixed. After a neutralization time of 5 minutes, solutions were serially diluted and viable counts were performed on TSA plates. Plates were incubated for 24 h at 37^{0}C, counted, and then re-incubated for a further 24 h to detect slow-growing colonies.

2.3 Statistical analysis

Student's t-test were performed with the Excel (Excel, Microsoft Corporation, Redmond, WA, USA) software. P values of 0.05 were considered significant. The decimal reduction time (D-value), the interval of time required to reduce one decimal logarithm of the initial bacterial population, at a specified disinfectant concentration (at constant temperature of 25 ^{0}C), was determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the survivor curves (log^{10} CFU/mL versus time of exposure to the chemical solution, at constant temperature) [8].

3. Results and Discussion

Surviving populations of each bacterium treated with hydrogen peroxide-based sanitizer (containing 0.5 - 2% H_2O_2) are shown in Figure 1. Initial population of the microorganisms was approximately 6 log CFU/mL

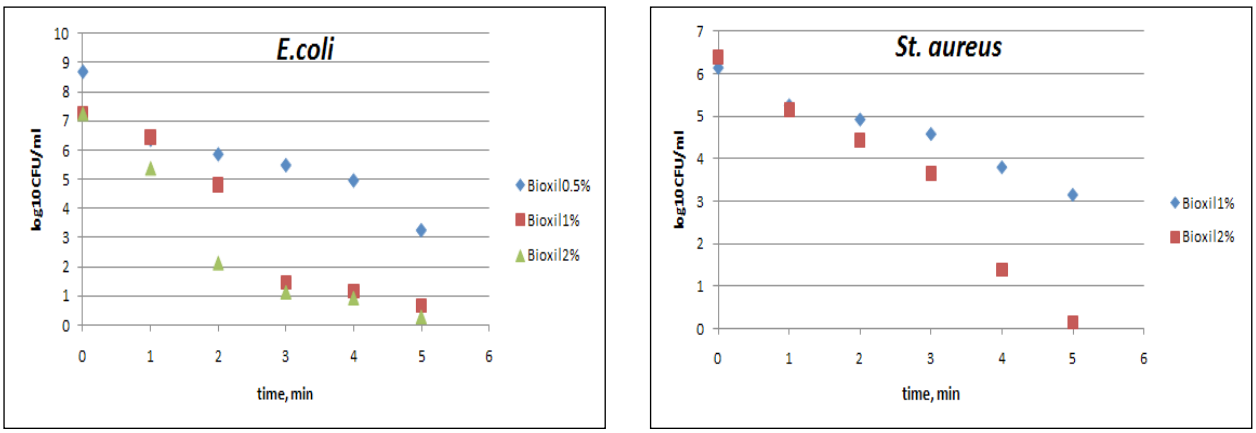


Figure 1. Survivor curves showing the reduction of *E. coli* and *S. aureus* exposed to different concentrations of Bioxil

and 7 log CFU/mL for *E. coli* and *S. aureus,* respectively. After 5 min. of contact with 1% and 2% solutions of mentioned sanitizer the number of *E. coli* decreased up to 0.3 log CFU/mL and 0.65 log CFU/mL, respectively.

Figure 2 shows the antibacterial activity of different concentrations of hydrogen peroxide based sanitizer against *E. coli* and *S. aureus* during 5 min. contact time period.

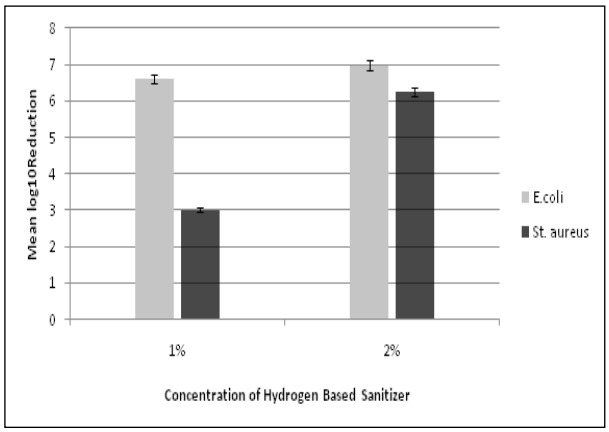


Figure 2. Log[10] reduction of *E. coli* and *S. aureus* after 5 min. contact time with 1% and 2% of hydrogen peroxide based biocide solutions

2% solution of hydrogen peroxide based sanitizer was significantly effective ($P < 0.05$) against *S. aureus* comparing to 1% solution of the same biocide. *S. aureus* was significantly ($P < 0.05$) resistant to 1% solution of the biocide in comparison with *E. coli*. There was no significant difference ($P \geq 0.05$) between resistance of *E. coli* and *S. aureus* in case of 2% solution of hydrogen based biocide.

For a better understanding of a disinfectant's effect during 5 minutes contact time with each bacterial strain the reduction after every minute has been studied and the decimal reduction times (D-values) have been calculated for each bacterial strain at constant temperature (25 ^{0}C) using different concentrations of biocide.

The use of the test microbial suspension is to monitor the disinfection procedure and its performance is dependent on both the initial test microbial suspension population (N_0) and the D-value [10]. The D-values were determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the survivor curves (log10 CFU/mL as versus time of exposure as shown in Figure 3).

In case of 1% and 2% H_2O_2 based biocide solution (Bioxil) the decimal reduction values of *S. aureus* were 1.77 and 0.88 minutes respectively. Amer *et al.*, [1] obtained similar results. From tested 3 bacterial species the most resistant vegetative cell to 2% hydrogen peroxide was *S. aureus* (D = 8.7 min). Thus, the same concentration of modified hydrogen peroxide solution possesses better efficiency against *S. aureus* in comparison with conventional hydrogen peroxide solution.

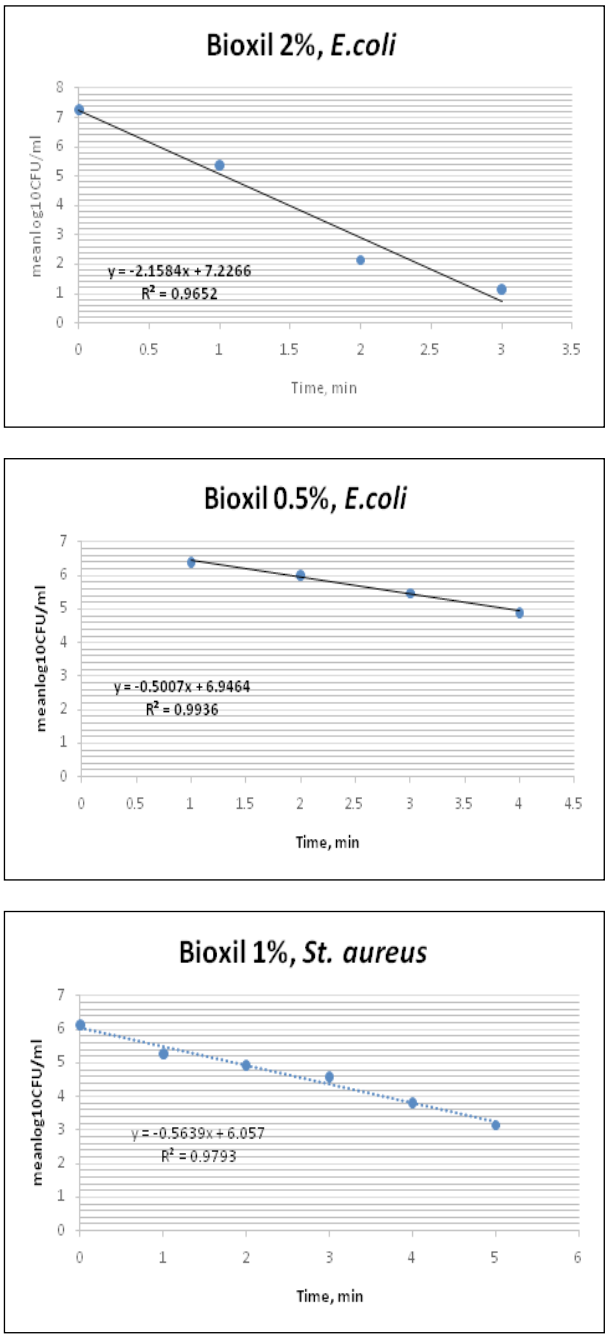
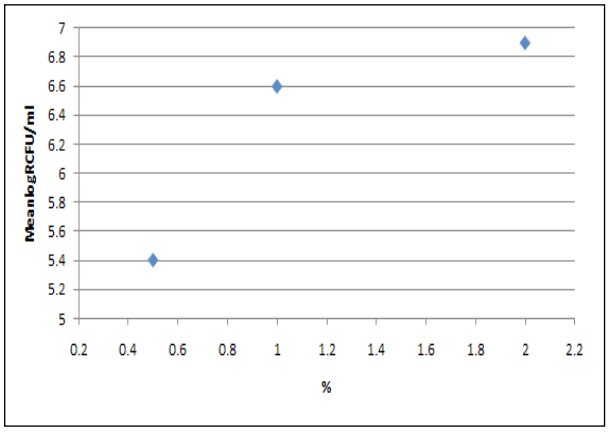


Figure 3. Linear portions of the survivor curves showing the reduction of *E.coli* and *S. aureus* exposed to different concentrations of Bioxil

E. coli possessed more sensitivity against 1% and 2% solutions of modified hydrogen peroxide based solution, with 0.67 and 0.47 min D-values respectively. 0.5% solution of the Bioxil had less efficiency against *E. coli* (D = 1.1). Linear death kinetics was observed during 5 minutes contact time of 1% solution of Bioxil with *S.*

aureus. High correlation was noticed between different concentrations of Bioxil, decimal redaction times and log reductions (logCFU/mL) of *E. coli* as shown in Figure 4.



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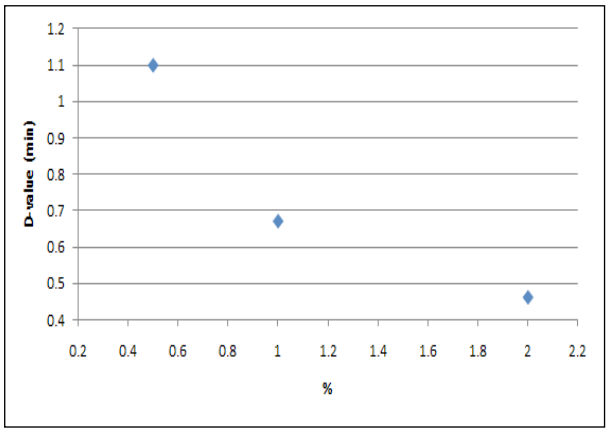

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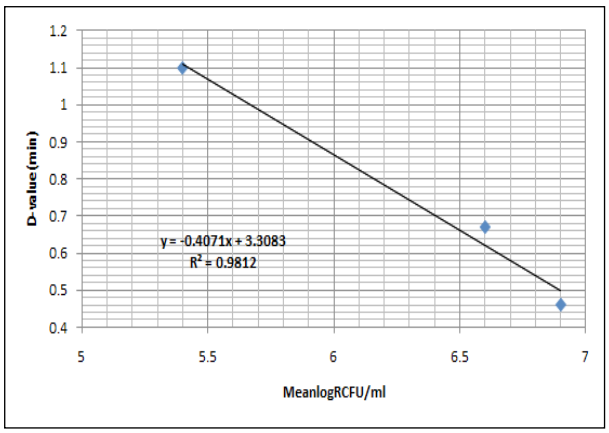

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Figure 4. Correlation between a) concentration of Bioxil and log-reduction, b) concentration of Bioxil and D-value and c) D-value and log-reduction of *E. coli*

All tested concentrations of hydrogen peroxide based disinfectant possessed sufficient antibacterial activity against *E. coli* 5 min. time period. Low concentrations of the mentioned disinfectant did not pass 5 log reduction factor in case of *S. aureus*, however the decimal reduction time was much lower in comparison with conventional hydrogen peroxide sanitizers.

4. Conclusions

- This study shows that high concentrations of the studied hydrogen peroxide based disinfectant can be used as effective sanitizer against *E. coli* and *S. aureus*.

- High correlation between D-values and log-reduction numbers of *E. coli d*etermination of determination of D-values can be used for testing the efficiency of chemical disinfectants by suspension test method.

5. References

[1] Amer A., Mady S., Yusef H., Sabry S. (2013). *Determination of decimal reduction time (D-value) of chemical agents used in hospitals for killing airborne isolated bacteria*. African Journal of Microbiology Research, 7, pp. 3321-3330.

[2] Ammerlaan H., Kluytmans J., Wertheim H., Nouwen J., Bonten M. (2009). *Eradication of methicillin-resistant Staphylococcus aureus carriage: A systematic review*. Clin. Infect. Dis., 48, pp. 922-930.

[3] Choi N., Baek S., Yoon J., Choi M., Kang D., Lee, S. (2012). *Efficacy of aerosolized hydrogen peroxide-based sanitizer on the reduction of pathogenic bacteria on a stainless steel surface*. Journal of Food Control, 27, pp. 57-63.

[4] Davidson M. P., Branen L. A. (1993). *Antimicrobials in Foods* (2nd Ed.). Marcel Dekker Inc., New York, USA.

[5] Grigoryan K., Badalyan G., Martiryan M., Al-Fageeh B. M., Agabekov V., Tavadyan L., Nersesyan A. L. (2014). *Antifungal activity of hydrogen peroxide based disinfectant bioxil*. J. Microbiol. Biotech. Food Sci., 3, (6), pp. 519-521.

[6] Liao C., Sapers M. G. (2000). *Attachment and growth of Salmonella chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments*. J. Food Prot., 63, (7), pp. 876-883.

[7] Linley E., Denyer S., McDonnell G., Simons C., Maillard J. (2012). *Use of hydrogen peroxide as a biocide: new consideration of its mechanisms of biocidal action*. Journal of Antimicrobial Chemotherapy, 67, pp. 1589-1596.

[8] Mazzola P. G., Penna V., Martins M. (2003). *Determination of decimal reduction time (D-value) of chemical agents used in hospitals for disinfection purposes*. BMC Infec. Dis., 3, pp. 24-34.

[9] Oh S. W., Dancer G. I., Kang D. H. (2005). *Efficacy of aerosolized peroxyacetic acid as a sanitizer of lettuce leaves*. Journal of Food Protection, 68, pp. 1743-1747.

[10] Penna V., Mazzola P. G., Martins M. (2001). *The efficacy of chemical agent in cleaning and disinfection programs*. BMC Infect. Dis., 1, pp. 1-16.