World Journal of
Experimental Biosciences

Volume 7, Number 1: 1 -4
ISSN: 2313-3937
2019

Review article

The low concentrations of hydrogen peroxide reduce biofilm formation

Ghada Mohammed Saleh[1*]

ABSTRACT

Biofilm formation considers a serious problem that faces the physician in treating infectious diseases associated with medical devices. The present study aims to highlight the effect of hydrogen peroxide (H_2O_2) on biofilm formation by bacteria and yeasts. The current study confirms the anti-biofilm effect of H_2O_2 on the biofilm formed by bacteria and yeast. The effects of H_2O_2 on biofilm formation were against the ability of a microorganism to attach to the surface by the effect on either the appendages or the mechanisms of attachment, also it was found that the effect on biofilm can be explained by the effect on the microorganism growth. The current study highlighted the future prospect of using H_2O_2 by increasing the effectiveness of common antibiotics against high-resistance bacteria by increasing the ways of entering antibiotics by forming pores in the biofilm mass and the microorganism cells.

Keywords: Biofilm, Extracellular Polymeric Substances (EPS), H_2O_2, Reactive Oxygen Species (ROS).

Citation: Saleh GM. (2019) The low concentrations of hydrogen peroxide reduce biofilm formation. *World J Exp Biosci* **7**:1-4.

Received January 24, 2019; Accepted May 5, 2019; Published june 16, 2019.

1. INTRODUCTION

Hydrogen peroxide is a chemical compound with the formula H_2O_2. It is a clear, colorless liquid that is slightly more viscous than water. It is used as an oxidizer, bleaching agent, and antiseptic, usually as a dilute solution (3%–6% by weight) in water for consumer use, and in higher concentrations for industrial use [1]. It has been found to reduce biofilm formation. Hydrogen peroxide has antimicrobial properties and can effectively kill bacteria and other microorganisms. When applied at appropriate concentrations, hydrogen peroxide can disrupt the formation of biofilms by interfering with bacterial attachment and inhibiting the growth of biofilm-forming microorganisms. Biofilms are communities of microorganisms that attach to surfaces and form a protective matrix, making them highly resistant to antimicrobial treatments. The effect of the H_2O_2 on the biofilm formation are vary depending on the environmental condition and the species of microorganisms that produce the biofilm [2].

There are a lot of applications of H_2O_2 besides the industrial application there are a lot of clinical and Lab applications such as using in low concentration as a substrate in the very important test of catalase test that use in the identification of different pathogenic and environmental bacterial isolates [3].

2. GENARL PROPRTIES OF H_2O_2

Hydrogen peroxide (H_2O_2) is a chemical compound composed of two hydrogen atoms bonded to two oxygen atoms. It is widely used for various purposes due to its oxidizing and disinfecting properties. The molecular weight of Hydrogen peroxide is 34.0147 grams per mole. It is a clear, colorless liquid at room temperature [1]. It has a slightly bitter taste and a characteristic odor and is miscible with water in all proportions. Hydrogen peroxide is a powerful oxidizer. It readily donates oxygen atoms that can react with other substances, causing oxidation reactions.

* Correspondence: **Ghada Mohammed Saleh**. E. mail: ghada90m@gmail.com
Department of Biology, College Science, University of Baghdad, Baghdad, Iraq.
Full list of author information is available at the end of the article.

This property makes it useful in various applications. Hydrogen peroxide is relatively unstable and can decompose over time, especially when exposed to heat, light, or certain metals. To maintain its effectiveness, it is often stored in opaque containers and kept away from direct sunlight or heat sources [4]. Concentrated hydrogen peroxide can be hazardous and potentially corrosive. It should be handled with care, and protective measures such as gloves and goggles should be used when working with higher concentrations. Diluted solutions are generally safer for household and personal use.

3. THE APPLICATION OF H_2O_2

There are many general applications of H_2O_2 including as a cleaning agent, disinfectant, and bleaching agent. One of the important characteristics of hydrogen peroxide is its antimicrobial properties which can kill a wide range of microorganisms, including bacteria, viruses, and fungi. It disrupts the cell walls and membranes of microorganisms, leading to their destruction. It is commonly used as an antiseptic to clean wounds and prevent infection. It can be used to disinfect surfaces, tools, and equipment in medical settings. It also uses in hair bleaching products and teeth whitening products. However, it should be used with caution and in appropriate concentrations to avoid adverse effects [5].

4. BACTERIA BIOFILM

Biofilm formation is a complex process in which bacteria adhere to surfaces and form a structured community embedded within a protective matrix. The formation of biofilm required several steps i.g. 1. Attachment: In the initial stage, bacteria attach to a surface. This attachment can occur through various mechanisms, including physical adhesion, production of adhesive molecules, and interactions with the surface. 2. Colonization: Once attached, bacteria start to multiply and form microcolonies on the surface. They begin to secrete extracellular polymeric substances (EPS), which form the matrix of the biofilm. EPS consists of polysaccharides, proteins, DNA, and other molecules that help stabilize the biofilm structure.3. Growth and Maturation: The microcolonies continue to grow and merge, forming a more complex and mature biofilm structure. Bacteria within the biofilm exhibit altered gene expression patterns, leading to the development of specialized phenotypes that promote biofilm survival and persistence.4. Maintenance and Dispersal: The mature biofilm reach a steady state, with bacteria continuously dividing, shedding, and dispersing. Dispersal allows bacteria to colonize new surfaces and form biofilms elsewhere. Dispersal can be triggered by environmental cues or stress signals, leading to the release of individual bacteria or small aggregates [6].

Biofilms provide several advantages to bacteria, including increased resistance to antimicrobial agents, protection from host immune responses, and enhanced survival in various environments. The EPS matrix acts as a barrier, limiting the penetration of antimicrobial agents and hindering the effectiveness of immune cells. Biofilms are commonly associated with various medical and industrial issues, such as chronic infections, device-associated infections, and biofouling of surfaces and equipment [7,8]. Understanding the mechanisms involved in biofilm formation is crucial for developing strategies to prevent or disrupt biofilm growth. This includes targeting bacterial attachment, EPS production, and the signaling pathways involved in biofilm development and that will be key to treating the complicated infections caused by the biofilm of different pathogenic bacteria [9].

5. H2O2 REDUCES BACTERIAL BIOFILM

It has been found to reduce biofilm formation. Biofilms are communities of microorganisms that attach to surfaces and form a protective matrix, making them highly resistant to antimicrobial treatments. Hydrogen peroxide has antimicrobial properties and can effectively kill bacteria and other microorganisms [10]. It's important to note that the effectiveness of hydrogen peroxide in reducing biofilm formation can vary depending on the specific microorganisms involved, the concentration and exposure time of hydrogen peroxide, and the environmental conditions. Additionally, it's crucial to use hydrogen peroxide at appropriate concentrations to avoid potential cytotoxic effects on human tissues. When applied at appropriate concentrations, hydrogen peroxide can disrupt the formation of biofilms by interfering with bacterial attachment and inhibiting the growth of biofilm-forming microorganisms [11].

5.1. Disruption of bacterial attachment

Hydrogen peroxide can interfere with the ability of bacteria to attach to surfaces. It can disrupt the initial steps of biofilm formation by preventing bacterial cells from adhering and colonizing the surface, thus impeding the formation of a biofilm. The disruption of bacterial attachment, which is an important initial step in biofilm formation. H_2O_2 can interfere with bacterial attachment by damaging pili and adhesins, which are surface structures in bacteria that facilitate attachment to surfaces [12]. Hydrogen peroxide can cause oxidative damage to these structures, compromising the ability of bacteria to adhere to surfaces effectively. This damage can occur through the generation of reactive oxygen species (ROS) by H_2O_2. Exposure to H_2O_2 can induce oxidative stress in bacterial cells [13]. The oxidative stress disrupts cellular functions and may alter the expression of genes involved in attachment mechanisms, inhibiting the bacteria's ability to attach to surfaces. It also disruption of quorum sensing which is a cell-to-cell communication system used by bacteria to coordinate biofilm formation. Hydrogen peroxide has been shown to interfere with quorum sensing, thereby inhibiting the production of signaling molecules necessary for bacterial attachment and biofilm development [14]. By disrupting bacterial attachment, hydrogen peroxide reduces the initial step of biofilm formation. This disruption can prevent bacteria from establishing a foothold on surfaces, inhibiting the subsequent growth and maturation of the biofilm. It's important to note that the effectiveness of H_2O_2 in disrupting bacterial attachment may depend on factors such as the concentration and exposure time of hydrogen peroxide, the bacterial species involved, and the specific conditions in which biofilm formation occurs. Additionally, the concentration of hydrogen peroxide must be carefully regulated to avoid cytotoxic effects on human cells or tissues [15].

5.2. Oxidative stress

Hydrogen peroxide generates reactive oxygen species (ROS) when it comes into contact with cells. These ROS can cause oxidative damage to bacterial cells, including DNA, proteins, and lipids. The oxidative stress induced by hydrogen peroxide can inhibit the growth and survival of bacteria, thereby reducing biofilm formation [16].

5.3. Biofilm matrix degradation

Hydrogen peroxide can degrade the extracellular matrix that holds the biofilm together. The matrix primarily consists of polysaccharides, proteins, and DNA, and hydrogen peroxide

can break down these components, destabilizing the biofilm structure and making it more susceptible to removal [16].

6. H2O2 REDUCES YEAST BIOFILM

Hydrogen peroxide has been found to reduce the biofilm produced by yeast. Biofilms formed by yeast, such as Candida species, can contribute to various infections, including oral thrush and vaginal yeast infections. Reducing the yeast biofilm can be done in several ways such as 1. Oxidative stress: Hydrogen peroxide generates reactive oxygen species (ROS) when it comes into contact with cells. These ROS can cause oxidative stress in yeast cells, leading to damage to cellular components and impaired biofilm formation. The oxidative stress disrupts the metabolic processes and signaling pathways involved in biofilm development, thereby reducing the ability of yeast to form a robust biofilm [17]. 2. Disruption of extracellular matrix: The extracellular matrix in yeast biofilms consists of polysaccharides, proteins, and other molecules that provide structural support and protection. Hydrogen peroxide can degrade and disrupt the extracellular matrix, weakening the biofilm structure. This disruption hinders the ability of yeast cells to adhere to surfaces and form a cohesive biofilm [18]. 3. Inhibition of yeast growth: Hydrogen peroxide can inhibit the growth and proliferation of yeast cells. By inducing oxidative stress and damaging cellular components, hydrogen peroxide reduces the viability and growth of yeast within the biofilm [19]. This inhibition of yeast growth limits the formation and expansion of the biofilm. By inducing oxidative stress, disrupting the extracellular matrix, and inhibiting yeast growth, hydrogen peroxide can effectively reduce the biofilm produced by yeast [17]. However, it's important to note that the effectiveness of hydrogen peroxide may vary depending on the specific yeast strain, the concentration of hydrogen peroxide, and other environmental factors. Additionally, the concentration of hydrogen peroxide should be carefully controlled to avoid cytotoxic effects on surrounding tissues or cells [20].

7. Future prospects

Many studies have shown that hydrogen peroxide can affect biofilm formation and also can lead to biofilm removal by preventing biofilm maturation. One of the most important effects of hydrogen peroxide is that affects the biofilm as well as the bacterial cell body or yeast cell through holes in the biofilm or the wall or cell membrane of the microorganism and this can be employed by increasing the effect of antibiotics on the biofilm. Recent studies have used hydrogen peroxide to increase the effectiveness of antibiotics [10, 21]. This trend is considered one of the new hopes that scientists rely on to counter the significant increase in bacterial resistance to antibiotics, which has greatly affected public health.

8. Conclusion:

The present study showed the negative role of H2O2 in reducing the microorganism (bacteria and yeast) biofilm formation and that can be done by the effect on the appendages or mechanisms that use by microorganisms to attach to different surfaces. Moreover, H_2O_2 may affect microorganism growth (microorganism biomass) by interfering with the growth process. That all explains the anti-biofilm effect of H_2O_2 that is produced by either bacteria or yeast.

Funding information

This work received no specific grant from any funding agency.

Conflict of interest

The authors declare that they have no conflict of interests.

9. REFERENCES

[1] **Tredwin CJ, Naik S, Lewis NJ, Scully C.** (2006) Hydrogen peroxide tooth-whitening (bleaching) products: review of adverse effects and safety issues. *Br Dent J* **200**:371-6. doi: 10.1038/sj.bdj.4813423. PMID: 16607324.

[2] **Watson F, Keevil CW, Wilks SA, Chewins J.** (2018) Modelling vaporised hydrogen peroxide efficacy against mono-species biofilms. *Sci Rep* **8**:12257. doi: 10.1038/s41598-018-30706-0. PMID: 30115938; PMCID: PMC6095907.

[3] **Saginur R, Clecner B, Portnoy J, Mendelson J.** (1982) Superoxol(catalase)test for identification of Neisseria gonorrhoeae. *J Clin Microbiol* **15**: 475-7. doi: 10.1128/jcm.15.3.475-477.1982. PMID: 6804487; PMCID: PMC272120.

[4] **Wang GS, Liao CH, Chen HW, Yang HC.** (2006) Characteristics of natural organic matter degradation in water by UV/H2O2 treatment. *Environ Technol* **27**:277-87. doi: 10.1080/09593332708618638. PMID: 16548208.

[5] **Li Y.** (1996) Biological properties of peroxide-containing tooth whiteners. *Food Chem Toxicol* **34**:887-904. doi: 10.1016/s0278-6915(96)00044-0. PMID: 8972882.

[6] **Quirynen M, Bollen CM.** (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *J Clin Periodontol* **22**:1-14. doi: 10.1111/j.1600-051x.1995.tb01765.x. PMID: 7706534.

[7] **Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O.** (2010) Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* **35**:322-32. doi: 10.1016/j.ijantimicag.2009.12.011. Epub 2010 Feb 10. PMID: 20149602.

[8] **Del Pozo JL.** (2018) Biofilm-related disease. *Expert Rev Anti Infect Ther* **16**:51-65. doi: 10.1080/14787210.2018.1417036. Epub 2017 Dec 19. PMID: 29235402.

[9] **Petruzzi B, Inzana TJ.** (2016) Exopolysaccharide Production and Biofilm Formation by Histophilus somni. *Curr Top Microbiol Immunol* **396**:149-60. doi: 10.1007/82_2015_5013. PMID: 26853691.

[10] **Shirato M, Nakamura K, Kanno T, Lingström P, Niwano Y, Örtengren U.** (2017) Time-kill kinetic analysis of antimicrobial chemotherapy based on hydrogen peroxide photolysis against Streptococcus mutans biofilm. *J Photochem Photobiol B* **173**:434-440. doi: 10.1016/j.jphotobiol.2017.06.023. Epub 2017 Jun 21. PMID: 28666212.

[11] **Lee HJ, Kim HE, Lee C.** (2017) Combination of cupric ion with hydroxylamine and hydrogen peroxide for the control of bacterial biofilms on RO membranes. *Water Res* **110**:83-90. doi: 10.1016/j.watres.2016.12.014. Epub 2016 Dec 11. PMID: 27998786.

[12] **Huang CJ, Wang ZC, Huang HY, Huang HD, Peng HL.** (2013) YjcC, a c-di-GMP phosphodiesterase protein, regulates the oxidative stress response and virulence of *Klebsiella pneumoniae* CG43. *PLoS One* **8**:e66740. doi: 10.1371/journal.pone.0066740. PMID: 23935824; PMCID: PMC3720812.

[13] **Imlay JA.** (2002) How oxygen damages microbes: oxygen tolerance and obligate anaerobiosis. *Adv Microb Physiol* **46**:111-53. doi: 10.1016/s0065-2911(02)46003-1. PMID: 12073652.

[14] **Chen M, Liu H, Yan F.** (2019) Oscillatory dynamics mechanism induced by protein synthesis time delay in quorum-sensing system. *Phys Rev E* **99**:062405. doi: 10.1103/PhysRevE.99.062405. PMID: 31330665.

[15] **Duan D, Scoffield JA, Zhou X. Wu H.** (2016) Fine-tuned production of hydrogen peroxide promotes biofilm formation of *Streptococcus parasanguinis* by a pathogenic cohabitant Aggregatibacter actinomycetemcomitans. *Environ Microbiol* **18**:4023-4036. doi: 10.1111/1462-2920.13425. Epub 2016 Jul 22. PMID: 27348605; PMCID: PMC5118171.

[16] **Lee K, Jeong JE, Kim IH, Kim KS, Ju BG.** (2015) Cyclo(phenylalanine-proline) induces DNA damage in mammalian cells via reactive oxygen species. *J Cell Mol Med* **19**:2851-64. doi: 10.1111/jcmm.12678. Epub 2015 Sep 28. PMID: 26416514; PMCID: PMC4687708.

[17] **Lu H, Xiong J, Shang Q, Jiang Y, Cao Y.** (2016) Roles of RPS41 in Biofilm Formation, Virulence, and Hydrogen Peroxide Sensitivity in *Candida albicans*. *Curr Microbiol* **72**:783-7. doi: 10.1007/s00284-016-1019-7. Epub 2016 Mar 7. PMID: 26952720.

[18] **Lefevre S, Sliwa D, Auchère F, Brossas C, Ruckenstuhl C, et al.** (2012) The yeast metacaspase is implicated in oxidative stress response in

frataxin-deficient cells. *FEBS Lett* **586**:143-8. doi: 10.1016/j.febslet.2011.12.002. Epub 2011 Dec 7. PMID: 22155640.

[19] **Marrero EJ, Silva FA, Rosario I, Déniz S, Real F, et al.** (2017) Assessment of in vitro inhibitory activity of hydrogen peroxide on the growth of *Malassezia pachydermatis* and to compare its efficacy with commercial ear cleaners. *Mycoses* **60**:645-650. doi: 10.1111/myc.12637. Epub 2017 May 30. PMID: 28557001.

[20] **Kean R, McKloud E, Townsend EM, Sherry L, Delaney C, et al**. (2018) The comparative efficacy of antiseptics against *Candida auris* biofilms. *Int J Antimicrob Agents* **52**:673-677. doi: 10.1016/j.ijantimicag.2018.05.007. Epub 2018 Oct 8. PMID: 29775686.

[21] **Sgibnev A, Kremleva E.** (2017) Influence of Hydrogen Peroxide, Lactic Acid, and Surfactants from Vaginal Lactobacilli on the Antibiotic Sensitivity of Opportunistic Bacteria. *Probiotics Antimicrob Proteins* **9**:131-141. doi: 10.1007/s12602-016-9238-6. PMID: 27832440.

Author affiliation

1. *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*