Received: 19.02.2025 | *Accepted: 27.02.2025*

Veterinary Sciences

doi: 10.31210/spi2025.28.01.41
UDC 615.076.9:636.92
ORIGINAL ARTICLE

Scientific Progress & Innovations
https://journals.pdaa.edu.ua/visnyk

28 (1)
2025

The effect of some materials such as NaCl and acetic acid on bacterial growth

M. A. K. Hameed[1] | H. T. Mahdi[1] | A. D. Salman[2] | A. H. Alsafah[1] | A. A. Fadhal[3]

Article info

Correspondence Author
H. T. Mahdi
E-mail:
hayder.t@uokerbala.edu.iq

[1]College of Veterinary Medicine, University of Kerbala,
56001, Karbala, Iraq

[2]College of medicine, University of Al-Ameed,
56001, Karbala, Iraq

[3]Veterinary Teaching Hospital in Karbala Governorate, Iraqi Ministry of Agriculture,
56001, Karbala, Iraq

Citation: Hameed, M. A. K., Mahdi, H. T., Salman, A. D., Alsafah, A. H., & Fadhal, A. A. (2025). The effect of some materials such as NaCl and acetic acid on bacterial growth. *Scientific Progress & Innovations*, 28 (1), 260–265. doi: 10.31210/spi2025.28.01.41

This observe investigates the anti-bacterial effectiveness in response to salt and acid conditions by exploring both action measures and their impact on bacterial survivability. The osmotic stress created by salt-containing solutions, as well as sodium chloride (NaCl) solutions outside bacterial cells, leads to dehydration of cells through hypertonic environments. External water movement from cells leads to mobile dehydration and plasmolysis and disrupts essential bacterial activities including nutrient transport and enzyme interest as well as DNA replication. Acidic solutions work to decrease outside pH which results in acidifying the bacterial cytoplasm. Healthy bacterial cells experience disruption of membrane pressure through proton loss which triggers damage to metabolic processes before leading to deadly cellular destruction. The research implements microbiological methods including purification steps combined with Gram staining and traditional plate counts to detect bacterial populations before studying salt solution and acid solution inhibitory effects on bacterial growth. Gram staining helps determine whether microorganisms belong to the Gram-high quality or Gram-negative bacterial category which lets researchers comprehend better how different species react to these medications. The standard plate rely method measures bacterial cells before salt and acid treatments to determine how these solutions perform against bacterial growth. Also, the study investigates the persistent effects of salt-acid mixtures, as well as concentration on results, duration of exposure and the influence of bacterial types. Conclusion applies in additional research into the acid and salt solutions antimicrobial system, with probable application in the design of food preservation, disinfection in the medical field and developing antibacterial strategies. This research emphasizes the position of environmental factors in the life cycle of bacteria and points towards the practical way to avoid bacterial growth in different surroundings.

Keywords: Bacterial infection, antimicrobial, zone of inhibition, Gram staining, Salt and Acid Solution.

Вплив NaCl та оцтової кислоти на ріст бактерій

М. А. К. Хамед[1] | Х. Т. Махді[1] | А. Д. Салман[2] | А. Х. Альсафах[1] | А. А. Фадхал[3]

[1]Коледж ветеринарної медицини, Університет Кербела,
Кербела, Ірак

[2]Медичний коледж, Університет Аль-Амед,
Кербела, Ірак

[3]Ветеринарна навчальна лікарня в провінції Кербела, Міністерство сільського господарства Іраку,
Кербела, Ірак

Метою проведеного дослідження було встановлення антибактеріальних і ріст-інгібуючих властивостей гіпертонічного розчину NaCl та розчину оцтової кислоти. Проведеними дослідженнями встановлено, що осмотичний стрес, що створює гіпертонічний розчин (NaCl) на бактеріальні клітини призводить до їх дегідратації. Визначено, що виведення рідини за рахунок дії гіпертонічного розчину NaCl викликає їх плазмоліз, погіршення транспортної функції клітинних мембран і вироблення ферментів такими клітинами, а також порушення реплікації ДНК. Натомість, застосування розчинів кислот, зокрема оцтової кислоти, призводить до зниження pH, як наслідок чого відбувається підвищення рівня кислотності в цитоплазмі таких бактеріальних клітин. В оброблених таким чином культурах бактеріальних клітин спостерігається порушення мембранного тиску та втрата частини протонів. Вищенаведене викликає порушення метаболічних процесів у клітинах та призводить до їх дегенеративних змін та загибелі. У роботі розглянуто послідовність виконання методики мікробіологічних досліджень, що включає етап очищення та фарбування мікроорганізмів за Грамом та етап традиційного їх підрахунку у чашках Петрі для кількісного визначення популяцій мікроорганізмів перед початком вивчення ріст-інгібуючих властивостей гіпертонічного розчину NaCl та оцтової кислоти. Виконання фарбування мікроорганізмів за Грамом дозволяє визначити вплив використаних у досліді сольового розчину та оцтової кислоти на грам-позитивні та грам-негативні бактерії. Отримані результати проведених досліджень дозволяють впроваджувати використання ріст-інгібуючих та антимікробних властивостей кислотних і сольових розчинів у харчовій промисловості (під час консервування харчових продуктів), у медицині (для проведення дезінфекцій) та розробці антибактеріальних препаратів. Таким чином, проведене дослідження додатково розкриває роль факторів довкілля в життєвому циклі бактерій та акцентує увагу на можливі способи практичного застосування ріст-інгібуючих властивостей розчинів солей та кислот.

Ключові слова: бактеріальна інфекція, антимікробний препарат, зона інгібування, фарбування за Грамом, розчин солі та кислоти.

Бібліографічний опис для цитування: *Хамед М. А. К., Махді Х. Т., Салман А. Д., Альсафах А. Х. Фадхал А. А.* Вплив NaCl та оцтової кислоти на ріст бактерій. *Scientific Progress & Innovations*. 2025. № 28 (1). С. 260–265.

Introduction

Bacterial infections endanger public fitness, meals safety, and industrial operations, highlighting the want for effective antimicrobial techniques [1]. Traditional techniques of regulating bacterial boom, including the use of antibiotics, had been challenged via the advent of antibiotic-resistant strains. As a result, there may be a rising hobby in opportunity antimicrobial dealers, consisting of natural materials like salt and acids, that have been used for a while as preservatives and antibacterials [2].

Salt (sodium chloride) and acidic solutions are widely recognized for their ability to inhibit bacterial growth through distinct yet complementary mechanisms [3]. Salt exerts its antibacterial effects primarily by inducing osmotic stress. In hypertonic environments, water is drawn out of bacterial cells, leading to cellular dehydration, shrinkage, and disruption of essential metabolic processes. Additionally, salt can destabilize bacterial membranes, particularly in Gram-negative bacteria, where the lipopolysaccharide layer is vulnerable to osmotic stress. While Gram-positive bacteria possess a thicker peptidoglycan layer that offers some protection, prolonged exposure to high salt concentrations can eventually overcome this defense, leading to cell death or growth inhibition [4, 5].

Acidic solutions, on the other hand, suppress bacterial growth by lowering the pH of the environment, creating conditions that are unfavorable for bacterial survival. Most bacteria thrive in neutral or slightly alkaline pH ranges (6.5–7.5), and exposure to acidic conditions disrupts their metabolic processes. A key mechanism involves the influx of protons (H^+) into bacterial cells, leading to cytoplasmic acidification. This disrupts enzymatic activity, damages cellular proteins and DNA, and impairs the bacteria's ability to maintain homeostasis. Organic acids, such as citric acid and acetic acid, enhance these effects by penetrating bacterial membranes in their undissociated form and releasing protons intracellularly, further exacerbating cellular damage [6, 7].

Despite the widespread use of salt and acids as antimicrobial agents, their mechanisms of action against different bacterial species and their effect are necessary.

The aim of the study

The purpose of this study is to evaluate the bactericidal effects of salt and acid solutions properly on the development of bacteria, focusing on their impact on individual bacterial layers and colonies developed on fixed surfaces.

By clarifying the underlying mechanism and determining their antibacterial effect, this research tries to contribute to the development of effective, non-antibiotic-based strategies to control bacterial infections and preserve food products.

The findings from this study have extensive implications for public health, food security, and industrial applications. For example, salt and acid solutions can be used as a natural protector in the food industry, which reduces the dependence on synthetic additives.

In addition, understanding their mechanisms of action can report the design of new antimicrobial agents targeting bacterial resistance mechanisms. This research also aligns with global efforts to combat antibiotic resistance by detecting an alternative approach to bacterial control.

Materials and methods

For every material used in the study, such as NaCl and acetic acid, the same procedural steps were followed. These steps included culturing, staining, purification, and counting. By maintaining consistency across all materials, the study ensures comparability and reproducibility in the experimental outcomes.

Each of these steps plays a critical role in the methodology. Culturing establishes the baseline conditions for the experiment, while staining helps in visualizing and analyzing the samples. Purification ensures that the materials are free from contaminants, and counting provides quantitative data essential for evaluating the results. Together, these steps form a comprehensive approach to achieving the study's objectives.

Results and discussion

1. Bacterial Strain:

A representative bacterium was used for this study used a pure culture of stress. Stress was selected based on its relevance to ordinary bacterial infections and its sensitivity to the sky and its sensitivity to the sky [8].

- **Purification**: Bacterial tests were cleaned if the plates were cleaned using the stricting method. This technique secured separation of single, viable colonies free of pollution. The purity of culture was confirmed through the village of blur, which also provided information on the Gram classification (gram-positive or gram-negative) of bacteria (***Figure 1***, ***Figure 2***).

Figure 1. The purification streaking method on nutrient agar

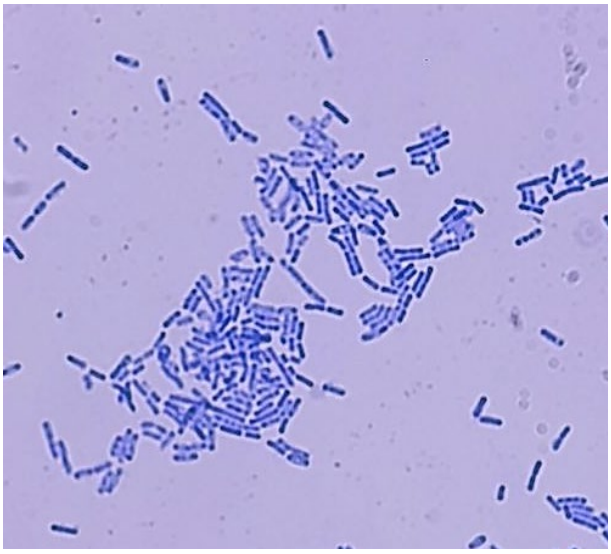

Figure 2. Showed the bacterial population by Gram stain

- **Culture Preparation**: A single colony was inoculated in a sterile nutritional broth and incubated at 37°C for 24 hours to achieve a high density bacterial culture for further use.

2. Standard Plate Count Technique

To determine the bacterial population density and evaluate how well salt and acid solutions inhibited bacterial growth, the conventional plate count approach was utilized. The steps were as follows:

- **Serial Dilution**: ten pipes, each included in 9 ml of phosphate buffer saline (PBS), were made. 1 ml of bacterial broth was added and well mixed into the first tube (pipe 1). Then the pipe was 1 to 1 ml transferred to pipe 2, and this process was repeatedly repeated to the pipe 10. Due to the weakening of this series, the concentration of bacteria in the pipes led to a logical deficiency.
- **Plating**: From Tube 8, 1 mL of the diluted sample was plated onto nutrient agar plates. The plates were then incubated at 37°C for 24 hours to allow bacterial colonies to grow.
- **Colony Counting**: After incubation, the number of visible colonies on the plate was counted. The bacterial population density was calculated using the formula:

Number of bacteria/ml = Number of colonies on the plate × Reciprocal of the dilution factor.

In this technique we used 10 tubes and put 9 ml from pbs and add 1 ml from bacterial broth to tube No 1 and after that give 1 ml from tube No 1 and add to tube No 2 and continue to all tubes and finally we should discarded 1ml and give 1ml from tube No 8 and culturing on nutrient agar after 24 h, 37 c incubation make slide from each tube to see the number of diluted bacterial population and the The result showed that tube No. 8 contains 64*109 bacteria/ml, as appears in ***Figure 3a***, ***b***.

a

b

Figure 3. Showed the serial dilutions (a) and No 8 dilution on nutrient agar (b)

3. Salt Solution Treatment

- **Preparation of Salt Solutions**: Salt solutions with concentrations of 1 % and 0.5 % (w/v) were prepared by dissolving sodium chloride (NaCl) in distilled water [9].

The antibacterial efficacy of the salt solutions was evaluated using the zone of inhibition method. A 1 mL aliquot of the bacterial broth was mixed with 1 % and 0.5 % salt solutions and incubated at 37°C for 24 hours. After incubation, serial dilutions were performed, and the samples were plated onto nutrient agar. The plates were incubated, and the number of colonies was counted to determine the reduction in bacterial density.

The 1 % salt solution demonstrated significant antibacterial activity (***Figure 4***), with fewer than 20 colonies were observed on the nutrient agar plate (***Figure 5***), which indicated a substantial reduction in bacterial viability due to osmotic stress and membrane disruption.

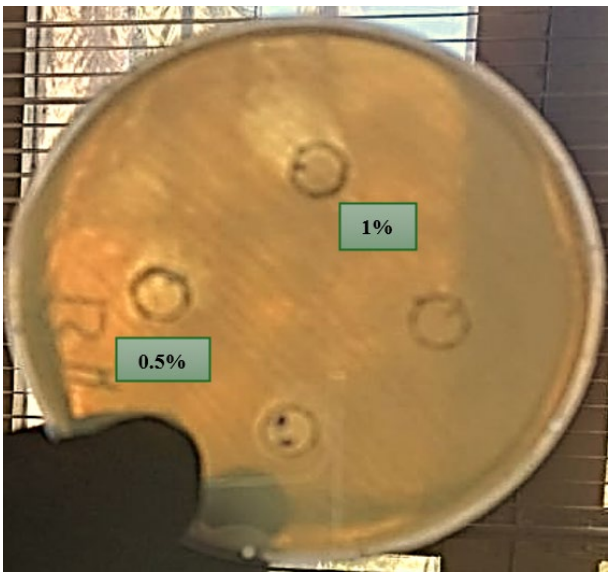

Figure 4. Showed the plate after put salt solution 1 % and 0.5 % in wells on nutrient agar in 37 c and 24 h.

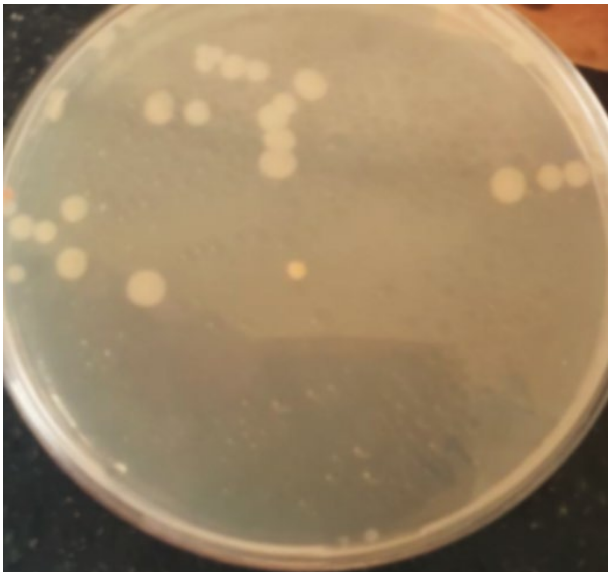

Figure 5. Bacterial colony from No. 8 dilution tube

4. Acid Solution Treatment

- **Preparation of Acid Solutions**: Acidic solutions were prepared by adjusting the pH of distilled water to values below 6.5 using organic acids such as acetic acid. The bacterial broth was exposed to the acidic solutions, and the pH was monitored to ensure consistency. The samples were incubated at 37°C for 24 hours. The acidic environment caused an influx of protons (H^+) into the bacterial cells, leading to cytoplasmic acidification. This disrupted enzymatic activity, damaged cellular proteins and DNA, and impaired the bacteria's ability to maintain homeostasis. Organic acids further enhanced these effects by penetrating the bacterial membrane in their undissociated form and releasing protons intracellularly [10].

The same technical procedures were employed to evaluate the effect of vinegar as an acidic solution (5 % and 2.5 %) on bacterial growth, as illustrated in ***Figure 6***. When a swab was taken from the zone of inhibition of acetic acid at a 5 % concentration of vinegar solution and subjected to serial dilution, the results indicated that the number of bacterial colonies in the No. 8 dilution tube was fewer than 5. This suggests that bacterial growth was completely inhibited, with no visible colonies observed on the nutrient agar, as depicted in ***Figure 7*** [11, 12].

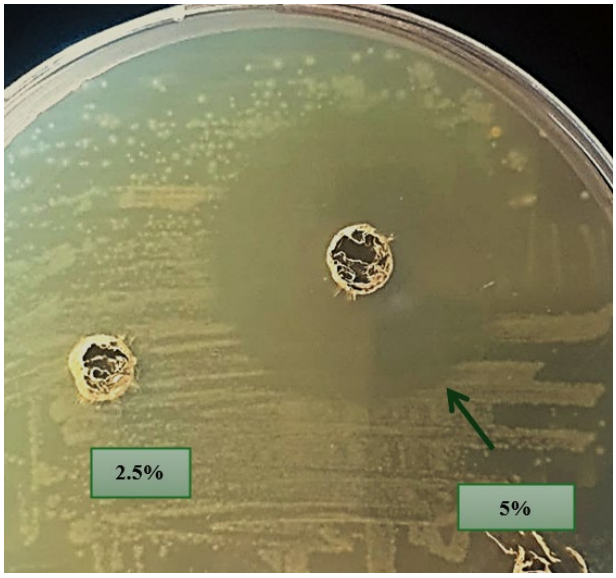

Figure 6. The result after put vinegar solution 5% and 2.5% in wells on nutrient agar in 37c and 24h.

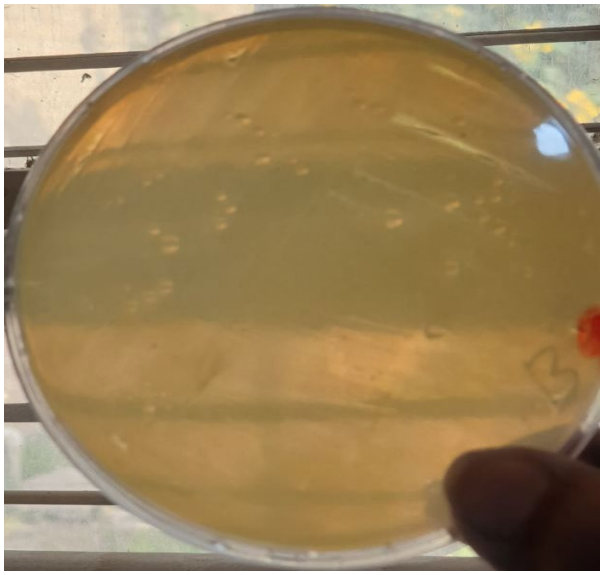

Figure 7. Showed the result of culturing from the No. 8 dilution tube

The findings above confirm that pH significantly impacts the structural integrity of macromolecules that constitute bacterial cells, including lipids, nucleic acids, proteins, and membrane potentials [13]. The acidic environment induces hydrolysis of lipids, triggering chemical reactions that disrupt the cell membrane [14]. In addition, the condition leads to low pH to hydrolysis of RNA and DNA, fractures phosphodister bonds between separate bases and nitrogen bases (Deoxy) RIBOS groups [15]. She provides this resolution disrupts hydrogen bonding, which is necessary for DNA replication and

gene replication and gene replication and Gentral bypass and Gentrial Disriction.

In addition, the low pH environment changes the ionization of functional groups in amino acids, affects hydrogen bonding, and leads to protein renaturation [16]. This results in using a non-functional analogy [17]. Finally, changes in pH proton concentrations affect both inside and outside the bacterial cells. Since cellular respiration depends on a proton concentration gradient, the low pH position prevents the installation of membrane capacity and interferes with cellular functions [18, 19].

The results of the study show that both salt and acid solutions have strong antibacterial properties and effectively inhibit bacterial growth through a number of complementary mechanisms. The results further confirm the value of these solutions in controlling the bacterial population since they align with other studies on the antibacterial effects of osmotic stress and pH alteration.

Salt Solutions and Osmotic Stress

The observed prohibition of the development of bacteria by salt solutions can be attributed to the induction of osmotic stress. In a hypertonic environment, such as that made by high salt concentrations, water is extracted from bacterial cells through osmosis, causing cellular dehydration and shrinkage. This phenomenon disrupts important cellular functions, including enzyme activity, nutrient transport, and metabolic processes, eventually impairing the viability of bacteria [4]. Village-negative bacteria, with their lipopolesacharide-rich outer membranes, are especially susceptible to skystrahery stress, while gram-positive bacteria, despite their coarse peptidoglycan layer, long-term exposure [20] Are not completely resistant to.

Furthermore, by binding free water molecules, salt solution lowers water activity and restricts the amount of water needed for microbial life. This reduction in water activity not only induces osmotic shock but also restricts oxygen solubility, further impairing aerobic bacterial metabolism [5]. The energy expenditure required for bacterial cells to expel sodium ions under high-salt conditions further exacerbates cellular stress, contributing to the observed bacteriostatic and bactericidal effects [6].

Acidic Solutions and pH Modulation

The antibacterial efficacy of acidic solutions is primarily mediated by the disruption of pH homeostasis within bacterial cells. Most bacteria thrive in neutral or slightly alkaline environments (pH 6.5–7.5), and exposure to acidic conditions creates an unfavorable environment for their survival [7]. The influx of protons (H^+) into bacterial cells under acidic conditions leads to cytoplasmic acidification, which disrupts enzymatic activity, damages cellular proteins and DNA, and impairs the bacteria's ability to maintain internal pH balance [4].

Organic acids, such as citric acid and acetic acid, enhance these effects by penetrating bacterial membranes in their undissociated form. Once inside the cell, these acids dissociate, releasing protons and further acidifying the cytoplasm. This dual mechanism of action-direct membrane damage and intracellular acidification-makes organic acids particularly effective against a wide range of bacterial species [5]. The results of this study corroborate previous findings that highlight the synergistic effects of low pH and organic acids in inhibiting bacterial growth [6].

Conclusions

This study confirms that salt and acid solutions effectively inhibit bacterial growth through osmotic stress and pH disruption. Salt solutions dehydrate cells and impair metabolism, while acidic conditions damage cellular functions and DNA. Both methods offer reliable, complementary approaches for bacterial control, supporting their use in antimicrobial applications.

Conflict of interest

The authors state that there is no conflict of interest.

References

1. Ifedinezi, O. V., Nnaji, N. D., Anumudu, C. K., Ekwueme, C. T., Uhegwu, C. C., Ihenetu, F. C., Obioha, P., Simon, B. O., Ezechukwu, P. S., & Onyeaka, H. (2024). Environmental antimicrobial resistance: implications for food safety and public health. *Antibiotics*, 13 (11), 1087. https://doi.org/10.3390/antibiotics13111087
2. Houpikian, P., & Raoult, D. (2002). Traditional and molecular techniques for the study of emerging bacterial diseases: One laboratory's perspective. *Emerging Infectious Diseases*, 8 (2), 122–131. https://doi.org/10.3201/eid0802.010141
3. Li, F., Xiong, X.-S., Yang, Y.-Y., Wang, J.-J., Wang, M.-M., Tang, J.-W., Liu, Q.-H., Wang, L., & Gu, B. (2021). Effects of NaCl concentrations on growth patterns, phenotypes associated with virulence, and energy metabolism in *Escherichia coli* BW25113. *Frontiers in Microbiology*, 12. https://doi.org/10.3389/fmicb.2021.705326
4. Doyle, M. P., & Buchanan, R. L. (Eds.). (2012). *Food Microbiology: Fundamentals and Frontiers. 4th Revised ed.* American Society of Microbiology Press https://doi.org/10.1128/9781555818463
5. Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern Food Microbiology. 7th edition*. Springer US.
6. Ray, B., & Bhunia, A. (2013). Microbiology of Fermented Food Production. (2013). *Fundamental Food Microbiology*, 217–240. https://doi.org/10.1201/b16078-22
7. Toder, K. (2012) *Online Textbook of Bacteriology*. Retrieved from: www.textbookofbacteriology.net
8. Madigan, M. T., Martinko, J. M., Stahl, D. A., & Clark, D. P. (2018). *Brock Biology of Microorganisms* (15th ed.). Pearson. Retrieved from: https://api.pageplace.de/preview/DT0400.9781292235196_A31976983/preview-9781292235196_A31976983.pdf
9. Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2013). *Fundamentals of Analytical Chemistry (9th ed.)*. Brooks/Cole. Retrieved from: https://library.tou.edu.kz/fulltext/buuk/b2539.pdf
10. Davidson, P. M., & Taylor, T. M. (2007). Chemical Preservatives and Natural Antimicrobial Compounds. In M. P. Doyle & L. R. Beuchat (Eds.), *Food Microbiology: Fundamentals and Frontiers, Third Edition*, 713–745. https://doi.org/10.1128/9781555815912.ch33
11. Yue, X., Liu, H., Wei, H., Chang, L., Gong, Z., Zheng, L., & Yin, F. (2023). Reactive and microbial inhibitory mechanisms depicting the panoramic view of pH stress effect on common biological nitrification. *Water Research*, 231, 119660. https://doi.org/10.1016/j.watres.2023.119660
12. Ryssel, H., Kloeters, O., Germann, G., Schäfer, Th., Wiedemann, G., & Oehlbauer, M. (2009). The antimicrobial effect of acetic acid – An alternative to common local antiseptics? *Burns*, 35 (5), 695–700. https://doi.org/10.1016/j.burns.2008.11.009
13. Willdigg, J. R., & Helmann, J. D. (2021). Mini review: Bacterial membrane composition and its modulation in response to stress. *Frontiers in Molecular Biosciences*, 8. https://doi.org/10.3389/fmolb.2021.634438
14. Kocherginsky, N. (2009). Acidic lipids, H+-ATPases, and mechanism of oxidative phosphorylation. Physico-chemical ideas 30 years after P. Mitchell's Nobel Prize award. *Progress in Biophysics and Molecular Biology*, 99 (1), 20–41. https://doi.org/10.1016/j.pbiomolbio.2008.10.013

15. Kornienko, I. V., Aramova, O. Yu., Tishchenko, A. A., Rudoy, D. V., & Chikindas, M. L. (2024). RNA stability: A review of the role of structural features nd environmental conditions. *Molecules*, 29 (24), 5978. https://doi.org/10.3390/molecules29245978
16. Di Russo, N. V., Estrin, D. A., Martí, M. A., & Roitberg, A. E. (2012). pH-dependent conformational changes in proteins and their effect on experimental pKas: The case of Nitrophorin 4. *PLoS Computational Biology*, 8 (11), e1002761. https://doi.org/10.1371/journal.pcbi.1002761
17. Martí, M. A., Estrin, D. A., & Roitberg, A. E. (2009). Molecular basis for the pH dependent structural transition of Nitrophorin 4. *The Journal of Physical Chemistry B*, 113 (7), 2135–2142. https://doi.org/10.1021/jp808055e
18. Lund, P. A., De Biase, D., Liran, O., Scheler, O., Mira, N. P., Cetecioglu, Z., Fernández, E. N., Bover-Cid, S., Hall, R., Sauer, M., & O'Byrne, C. (2020). Understanding how microorganisms respond to acid pH is central to their control and successful exploitation. *Frontiers in Microbiology*, 11. https://doi.org/10.3389/fmicb.2020.556140
19. Ratzke, C., & Gore, J. (2018). Modifying and reacting to the environmental pH can drive bacterial interactions. *PLOS Biology*, 16 (3), e2004248. https://doi.org/10.1371/journal.pbio.2004248
20. Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D.. H., Stahl, D. A., & Brock, T. (2014). *Brock Biology of Microorganisms (14th Edition)*. Hardcover.

ORCID

M. A. K. Hameed https://orcid.org/0000-0001-8076-3371
H. T. Mahdi https://orcid.org/0000-0002-1288-8591
A. D. Salman https://orcid.org/0009-0001-5880-9051
A. H. Alsafah https://orcid.org/0009-0003-8416-4028