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The Effect of Dielectric Barrier Discharge (DBD) Plasma on the Inhibition of Enterococcus Faecalis and Streptococcus Mutans

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Abstract
This study investigates the effect of Dielectric Barrier Discharge (DBD) plasma on the inhibition of bacterial growth. The DBD plasma system operates with a high-voltage power supply at a frequency of 8.4 kHz and an AC voltage of 20 kV. The study utilized two strains of pathogenic gram-positive bacteria, Streptococcus mutans and Enterococcus faecalis. The bacterial species were split into two groups at two distinct dilution levels (108). Following a series of dilution steps ranging from 101 to 109. The bacteria were subjected to DBD plasma treatment for different durations (0.5, 1, 1.5, 2, and 2.5 min). The Dielectric Barrier Discharge (DBD) plasma treatment resulted in a statistically significant increase in bacterial death ($P < 0.05$) compared to the control group. Exposure to DBD plasma effectively resulted in bacterial kill; longer treatment durations yielded greater bacterial inactivation. These results demonstrated the potential of DBD plasma in clinical and environmental applications for bacterial control.

Article Info.

Keywords:
AC (DBD) Plasma, Plasma Bacterial Inactivation, Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), Gram-Positive Bacteria.

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1. Introduction
Nonthermal plasmas have gained considerable attention for their ability to inactivate hazardous microorganisms and pollutants, making them a promising tool in chemical and biological pollution eradication [1-6]. Whether produced under atmospheric pressure or not, these plasmas are considered an advanced method for decontamination at low temperatures [3,4]. Several techniques can produce cold plasmas, including Atmospheric pressure plasma Jet (APPJ), plasma needle, plasma pencil, and dielectric barrier discharge (DBD). Although plasma can kill bacteria and shows potential for industrial use, the methods by which it inactivates microorganisms remain unclear [6]. Many researchers advanced the field, enhancing it and clarifying its core principles [7-22]. Several biomedical applications, such as blood coagulation, wound healing, cancer treatment, and sterilization, have utilized low-temperature atmospheric pressure plasma [23]. Recently, researchers have applied nonthermal plasmas to combat oral diseases due to their potent bactericidal properties [24]. Plasma can generate significant quantities of hydroxyl radicals ($OH^{\cdot}$) and reactive oxygen species (ROS). When nitrogen in the air reacts with water and other gases, reactive nitrogen species (RNS) are formed. These are nitrates (NO_3), nitrites (NO_2), and peroxynitrites ($ONOO^-$) [16-24]. Peroxynitrites occur naturally in the environment [17, 25]. This type of peroxynitrite is particularly effective in eliminating *Enterococcus facials*, *Candida albicans*, *Streptococcus mutans*, and *Escherichia coli* [24-27]. The bacteria mentioned are Pseudomonas aeruginosa [26] and *Lactobacillus casei* [28]. This research aims to design and construct a suitable dielectric barrier discharge system to inhibit two oral pathogenic bacteria: Gram-positive *Streptococcus mutans* and *Enterococcus faecalis*. Different plasma time exposures were employed to assess plasma efficacy in reducing bacterial colonies and understand the underlying mechanisms of bacterial inactivation.

The novelty of this work is its detailed investigation into the time-dependent effects of DBD plasma on the inactivation of gram-positive bacteria, specifically *Streptococcus mutans* and *Enterococcus facials*. What sets this research apart is its focus on understanding the underlying mechanisms of bacteria inactivation through physical and chemical interactions, such as the role of reactive oxygen and nitrogen species and coulomb forces on cell membrane rupture.

2. Experimental Work

2. 1. DBD Plasma System Setup

The AC DBD equipment utilized for this project is depicted in Fig. 1. The electrodes are cylindrical and are made of copper. The cathode electrode has a thickness of 2 cm and a diameter of 3 cm, while the grounded anode is of 4 cm diameter and a 3 cm thickness. Both electrodes are placed in a Teflon container to mitigate the occurrence of electrical sparks at the periphery of the electrodes. The distance between the two electrodes is 6 mm. The surface plasma was formed on the dielectric barrier surface under atmospheric air pressure. The surface plasma discharge was initiated by applying an external AC voltage of 20 kV between the two electrodes.

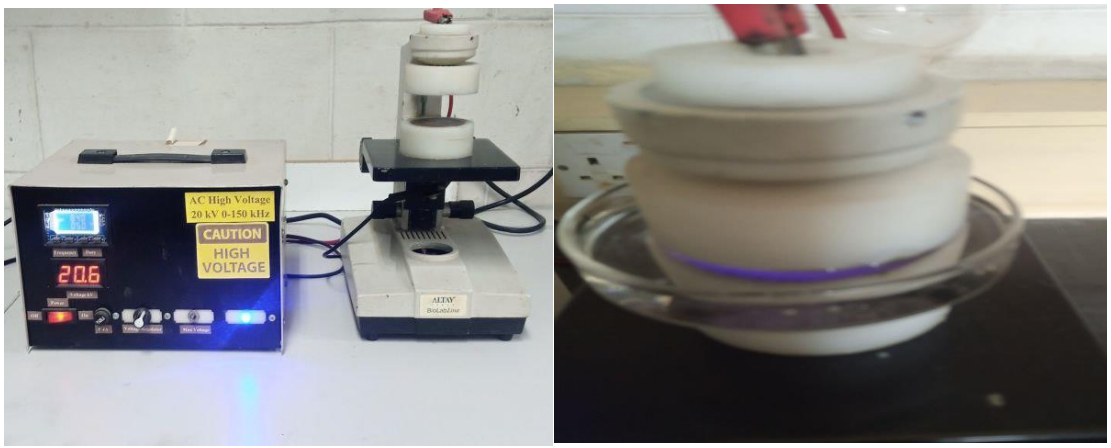

Figure 1: Photograph of the DBD plasma discharge system.

2. 2. Sample Preparation

Two distinct types of microorganisms were utilized in this study: *Streptococcus mutans* and *Enterococcus faecalis;* they are gram-positive bacteria. The microorganisms were acquired from the biology department laboratories at the College of Science, University of Baghdad. Each microbe's pure colony was separated using the Viable Count procedure.

2. 3. Culture Media

Streptococcus mutans and *Enterococcus facials* were cultured in nutrient agar at 37°C for 24 hours [29].

2. 4. Preparation of Serial Dilution

The initial stock culture was subjected to serial dilution in 10 test tubes. Nine millilitres of normal saline were added to each test tube using a measuring cylinder. A 10-fold serial dilution was made by adding 1 ml of the original stock sample to the first tube, which contained 9 ml of normal saline. This tube was labelled as 1/10 dilution. A volume of 1 ml from the first tube was transferred to the second tube, which was labelled as a 1/100 dilution. The method was repeated on each test tube until a dilution ratio of 1/10000 was reached [30].

2. 5. Isolation and Enumeration of Bacteria

For each sample, 0.1ml of a dilution of 10^8 was put onto agar, which was hardened and sterilized plates as shown in Fig.2. The inoculum on each plate was evenly distributed using a sterilized glass rod. Subsequently, the plates were reversed and positioned inside an incubator set at 37℃ for 24 hours. The plate count method was employed to enumerate the total bacteria count. Colonies were observed, numbered, and recorded to get the total number of colonies per milliliter [31].

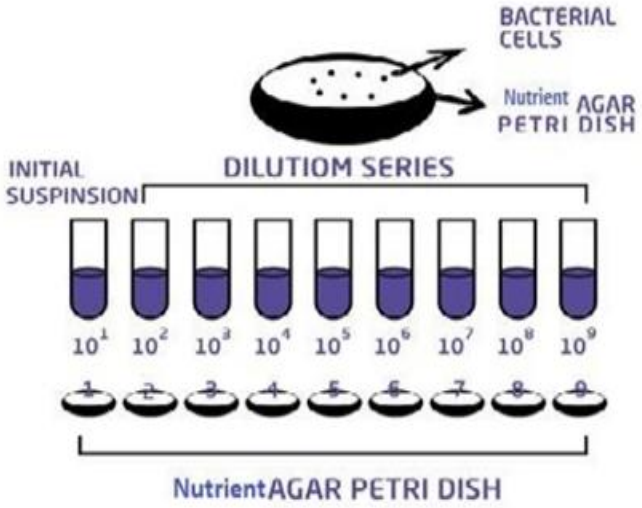

Figure 2: Diagram the serial of dilution.

2. 6. Inhibition of Bacteria by Plasma DBD

A 4 mL solution of *Streptococcus mutans* and *Enterococcus facials* was added to a sterile Petri plate with a diameter of 7.5 cm. Then, 1 mL of normal saline was uniformly poured over the same Petri dish to ensure that the dish's bottom was completely covered with bacteria. The Petri dishes were exposed to plasma under normal atmospheric pressure and at a room temperature of about 25 ℃. We only have one parameter for plasma treatment. The plasma exposure time was changed between 30 and 180 seconds. In this investigation, the discharge was started by supplying a steady AC voltage of 20 kV between the two electrodes at a frequency of 0 to 120 kHz. Following the plasma treatment, the nutrient agar culture medium was promptly placed onto individual Petri dishes and incubated at 37°C for 24 hours. The viability of the colonies was tested using the Viable Plate Count technique.

2. 7. Analytical Statistics

The data was analysed using a one-way analysis of variance (ANOVA) test, and the findings were reported as means ± standard deviation (SD) [32].

3. Results and Discussion

Figs. 3-6 illustrate the relation between the rate of death of both bacteria types and plasma exposure time. The number of colonies for *Enterococcus faecalis* was 15×10^8 before exposure. The number of colonies after 0, 5, 1, 1.5, 2, and 2.5 minutes of plasma exposure was (15, 14, 9, 5, 4, 1) $\times10^8$, respectively. The number of colonies for *Streptococcus mutans* was 20×10^8 before exposure. The number of colonies after 0, 5, 1, 1.5, 2, and 2.5 minutes of plasma exposure was (20, 18, 13, 9, 7, 4) $\times10^8$, respectively. The results shown in Tables 1 and 2 demonstrated that the time factor significantly

affected bacterial inhibition, as evidenced by the bacteria's exposure to plasma at different exposure times. Plasma and reactive species atoms and ions can interact directly with bacteria. The accumulated charges on the cell membrane cause it to burst due to Coulomb forces. Some reactive species generated by plasma can remain active for extended period and may undergo decomposition or secondary reaction in the water layer present on the tooth surface. Bacterial inactivation using plasma jets can occur via two distinct mechanisms: physical and chemical. Physical elements include heat, UV light, and charged particles, whereas chemical agents are the active species. DBD Plasma may cause charged particles to accumulate on the exterior of the cell membrane [30]. These charges combine to produce an electrical force that can break the tensile strength of the cell membrane, causing it to rupture. When bacteria are exposed to plasma indirectly (i.e., without direct contact with the plasma core), the concentration of charged particles decreases due to the rapid recombination of electrons and ions [33]. Exposing bacteria to plasma for long periods increases charge accumulation, leading to faster bacterial killing (15-1 and 20-4) $\times 10^8$. The inhibitory impact of UV photons produced by the DBD plasma on the microorganisms is primarily due to DNA damage. These findings are consistent with previous research [34-35].

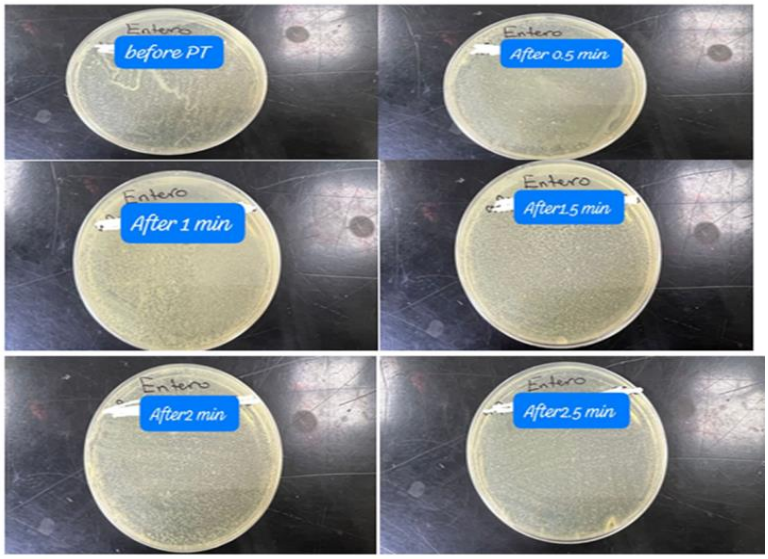

Figure 3: The inactivation of the Enterococcus faecalis at various plasma exposure times.

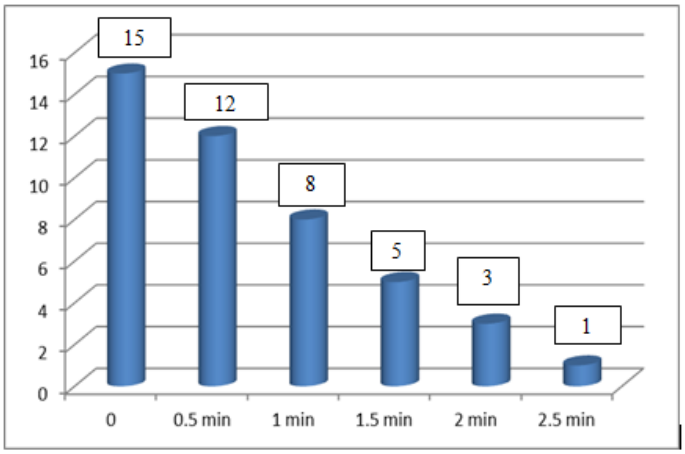

Figure 4: Histogram of the impact of DBD plasma at various plasma exposure times on the Enterococcus faecalis viability in cell Number.

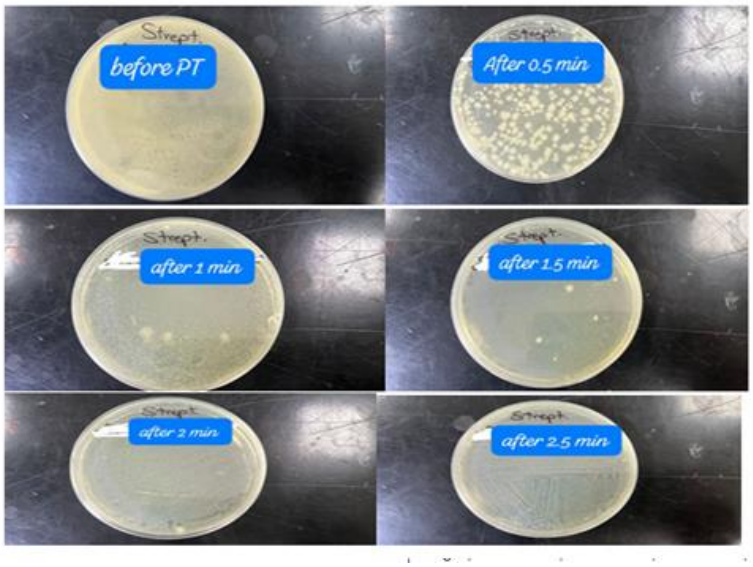

Figure5: The inactivation of the Streptococcus mutans at various times: control, 0.5min, 1min, 1.5min, 2min, 2.5min at 10^8.

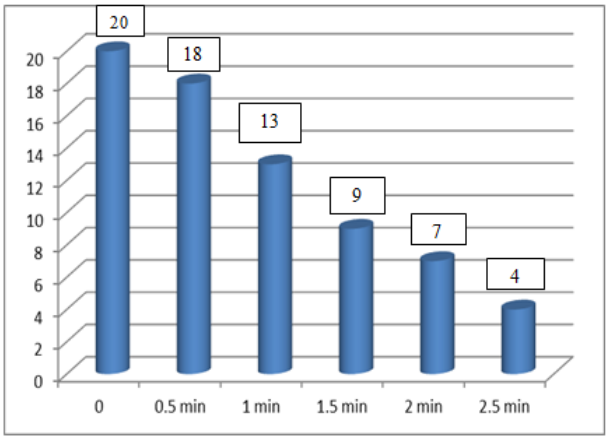

Figure 6: Histogram of the impact of DBD plasma at various plasma exposure times on Streptococcus mutans viability in cell Number.

Table 1: Influence of DBD plasma at various plasma exposure times on Enterococcus faecalis cells number.

Time (min)	**No. of cells x10^8**
Zero(control)	15
0.5min	14
1min	9
1.5min	5
2min	4
2.5min	1

Table 2: Influence DBD plasma at various plasma exposure times of Streptococcus mutans cell number.

Time of plasma	**No. of colonies x10^8**
Zero(control)	20
0.5min	18
1min	13
1.5 min	9
2min	7
2.5min	4

4. Conclusions

This research demonstrates that DBD plasma generated in ambient air under atmospheric pressure is a rapid and effective method for bacterial inactivation. After a 2-minute exposure to the dielectric DBD plasma, 65% of the Streptococcus mutans and 80% of the Enterococcus faecalis bacteria were successfully eliminated. The findings suggest that the heat, electric field, and UV photons produced by DBD plasma contributed to the bacterial inactivation process. These results highlight the potential of DBD plasma as a powerful tool for disinfection and sterilization in different applications.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. S. Moreau, M. Moisan, M. Tabrizian, J. Barbeau, J. Pelletier, A. Ricard, L'H. Yahia, J. Appl. Phys. **88**, 1166 (2000). https://doi.org/10.1063/1.373792.
2. T. A. Hameed and S. J. Kadhem, Iraqi J. Sci. **60**, 2649 (2019). https://doi.org/10.24996/ijs.2019.60.12.14.
3. F. A. Lazem, H. R. Humud, M. J. Alwazzan, Journal of Physics: Conference Series **1963**, 012084 (2021). https://doi.org/10.1088/1742-6596/1963/1/012084.
4. J. G. Birmingham, IEEE Trans. Plasma Sci. **32**, 1526 (2004). https://doi.org/10.12693/APhysPolA.119.803.
5. M. Moisan, J. Barbeau, M.-C. Crevier, J. Pelletier, N. Philip, and B. Saoudi, J. Pure Appl. Chem. **74**, 349 (2002). https://doi.org/10.1351/pac200274030349.
6. X. Guimin, Z. Guanjun, S. Xingmin, M. A. Yue, W. Ning, and L. Yuan, Plasma Sci. Technol. **11**, 83 (2009). https://doi.org/10.1088/1009-0630/11/1/17.
7. H. Qassim, S. J. Kadhem, AIP Conf. Proc. **2922**, 150005 (2024). https://doi.org/10.1063/5.0183148.
8. K. F. Abdullah, S. J. Kadhem, Journal of Optics, **54**, 1100 (2024). https://doi.org/10.1007/s12596-024-01791-4.
9. X. Lu, S. Reuter, M. Laroussi, D. Liu, *Nonequilibrium Atmospheric Pressure Plasma Jets Fundamentals, Diagnostics, and Medical Applications*, CRC Press, (2019). https://doi.org/10.1201/9780429053665.
10. S. J. Kadhem, Indian J. Phys. **98**, 4199 (2024). https://doi.org/10.1007/s12648-024-03155-x.
11. I. K. Abbas, K. A. Adim, Kuwait J. Sci. **50**, 223 (2023). https://doi.org/10.1016/j.kjs.2023.05.008.
12. I. K. Abbas, K, Science & Technology Indonesia, **7**, 508 (2022). https://doi.org/10.26554/sti.2022.7.4.508-513.
13. M. L. Badran, S. J. Kadhem, Iraqi J. Appl. Phys. **20**, 11 (2024).
14. S. N. Rashid, K. A. Aadim, A. S. Jasim, Karbala International Journal of Modern Science **8**, 71 (2022). https://doi.org/10.33640/2405-609X.3210.
15. B. Eliasson, W. Egli, and U. Kogelschatz, Pure Appl. Chem. **66**, 1275 (1994). https://doi.org/10.1351/pac199466061275.
16. N. Gherardi, G. Gouda, E. Gat, A. Ricard, and F. Massines, Plas. Sour. Sci. Technol. **9**, 340 (2000). https://doi.org/10.1088/0963-0252/9/3/312.
17. N. Gherardi and F. Massines, IEEE Trans. plasma Sci. **29**, 536 (2001). https://doi.org/10.1109/27.928953.
18. J. J. Shi, X.-T. Deng, R. Hall, J. D. Punnett, and M. G. Kong, J. Appl. Phys. **94**, 6303 (2003). https://doi.org/10.1063/1.1622110.
19. F. Massines, N. Gherardi, N. Naudé, and P. Ségur, Plasma Phys. Control. Fusion **47**, B577 (2005). https://doi.org/10.1088/0741-3335/47/12B/S42.
20. Murad M. Kadhim, Qusay A. Abbas, Mohammed R. Abdulameer, Iraqi J. Sci. **63**, 2048 (2022). https://doi.org/10.24996/ijs.2022.63.5.20.
21. U. Kogelschatz, IEEE Trans. plasma Sci. **30**, 1400 (2002). https://doi.org/10.1109/TPS.2002.804201.
22. R. Brandenburg, Plasma Sources Science Technology **26**, 53001 (2017). https://doi.org/10.1088/1361-6595/aa6426.
23. S. Kalghatgi, C. M. Kelly, E. Cerchar, B. Torabi, O. Alekseev, A. Fridman, G. Friedman, J. A.-Clifford, PLOS One **6**, e16270 (2011). https://doi.org/10.1371/journal.pone.0016270.
24. I. Koban, M. H. Geisel, B. Holtfreter, L. Jablonowski, N.-O. Hübner, R. Matthes, K. Masur, K.-D. Weltmann, A. Kramer, T. Kocher, Int. Sch. Res. Not. **2013**, 573262 (2013). https://doi.org/10.1155/2013/573262.

25. E. Kvam, B. Davis, F. Mondello, and A. L. Garner, Antimicrobial Agents Chemotherapy **56**, 2028 (2012). https://doi.org/10.1128/aac.05642-11.
26. Y. Zhao, S. Ojha, C. M. Burgess, D. Sun, and B. K. Tiwari, Int. J. Food Sci. Technol. **56**, 721 (2021). https://doi.org/10.1111/ijfs.14708.
27. S. Rupf, A. Lehmann, M. Hannig, B. Schäfer, A. Schubert, U. Feldmann and A. Schindler, J. Med. Microbiol. **59**, 206 (2010). https://doi.org/10.1099/jmm.0.013714-0.
28. Y.-Q. Chen, J.-H. Cheng, and D.-W. Sun, Crit. Rev. Food Sci. Nutr. **60**, 2676 (2020). https://doi.org/10.1080/10408398.2019.1654429.
29. N. A. Abdullah1, and S. J. Kadhem, Journal of Survey in Fisheries Sciences **10**, 1899 (2023).
30. A. N. Alkuraieef, A. M. Alsuhaibani, A. H. Alshawi, N. A. Al faris, D. H. Aljabryn, Food Sci. Technol. 24, e52520 (2022). https://doi.org/10.1590/fst.52520.
31. P. K. Prabhakar, P. P. Srivastav, S. S. Pathak, K. Das, Front. Sustain. Food Syst. **5**, 669473 (2021). https://doi.org/10.3389/fsufs.2021.669473.
32. T. A. Hameed, H. R. Humud, L. F. Ali, Iraqi J. Sci. **64**, 2889 (2023). https://doi.org/10.24996/ijs.2023.64.6.19.
33. Y. Han, J.-H. Cheng, and D.-W. Sun, Crit. Rev. Food Sci. Nutr., **59**, 794 (2019). https://doi.org/10.1080/10408398.2018.1555131.
34. A. Armand, M. Khani, M. Asnaashari, A. AliAhmadi, and B. Shokri, Photodiagnosis and Photodynamic Therapy **26**, 327 (2019). https://doi.org/10.1016/j.pdpdt.2019.04.023.
35. I. K. Abbas, M. U. Hussein, M. H. Hasan, and H. H. Murbat, Iraqi J. Sci. **58**, 1214 (2017). https://doi.org/10.24996/ijs.2017.58.3A.5.

بلازما تفريغ الحاجز العازل (DBD) لتثبيط المكورات المعوية والمكورات العقدية

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الخلاصة

تهدف هذه الدراسة إلى فحص تأثير بلازما تفريغ الحاجز العازل (DBD) على قمع البكتيريا. يعمل نظام بلازما DBD بمصدر طاقة ذات تيار متردد عالي الجهد بتردد 8.4 (كيلو هرتز) وجهد 20 (كيلو فولت). استخدمت الدراسة سلالتين من البكتيريا المسببة للأمراض، وتحديدًا *Streptococcus mutans* و *Enterococcus facials*، وكلاهما إيجابي الجرام. تم تقسيم الأنواع البكتيرية إلى مجموعتين عند مستويين مختلفين من التخفيف بمقدار (10^8). باتباع سلسلة من خطوات التخفيف تتراوح من 10^1 إلى 10^9. كانت البكتيريا موجودة. وقد خضعت لعلاج بلازما DBD لفترات مختلفة (0،5، 1، 1.5، 2 و 2.5) دقيقة. أدى علاج بلازما DBD إلى زيادة كبيرة إحصائيًا في موت البكتيريا ($P < 0.05$) مقارنة بمجموعة التحكم. يعمل التعرض لبلازما DBD بشكل فعال على قمع نمو البكتيريا، حيث تؤدي فترات العلاج الأطول إلى تثبيط أكبر للبكتيريا. تظهر هذه النتائج إمكانات بلازما DBD في التطبيقات السريرية والبيئية للسيطرة على البكتيريا.

الكلمات المفتاحية: تفريغ حاجز عازل التيار المتردد (DBD)، تعطيل البكتيريا، انواع الاوكسجين التفاعلية وانواع النتروجين التفاعلية، بكتيريا موجبة الغرام، تعقيم بالبلازما.