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Identification of Reactive Species Produced by Surfaces Dielectric Barrier Discharge Nonthermal Plasma with Gas Sources Variation (Air, N_2, O_2) to Kill Bacteria

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Abstract: Non-thermal plasma is one of the new techniques that is being focused on in the medical world, one of which is used for sterilization because non-thermal plasma is known to have a bactericidal effect. Reactive species produced by non-thermal plasma are antimicrobial. One factor affecting plasma species' reactive composition is the gas source used. Therefore, this study aimed to determine the effect of reactive species produced from non-thermal plasma generation of SDBD using different gas sources, namely free air, oxygen, and nitrogen, in treating Escherichia Coli bacteria. The gas flow used is 1 liter/minute. Reactive species produced by plasma were characterized using Optical Emission Spectrometry (OES). SDBD Non-thermal plasma treatment for Escherichia Coli bacteria samples was repeated three times for each treatment, and then the Anova test was performed. The results of this study indicate that bacterial death at the decontamination level depends on the composition of the gas used during treatment. Plasma using an O_2 gas source has a more effective inactivation ability, namely 6 x 10^7 CFU/ml, compared to the control, which is 409 x 10^7 CFU/ml. At the same time, the treatment results with free air and nitrogen gas sources were 6.33 x 10^7 CFU/ml and 41.67 x 10^7 CFU/ml. These results indicate that the composition of ROS and RNS influences bacterial inactivation, where ROS is more effective in inactivating bacteria than RNS.

Keywords: Nonthermal plasma; SDBD; ROS; RNS; E. coli; air, oxygen, nitrogen.

Introduction

Plasma is the fourth ground state of matter, from solids, liquids, and gases. Plasma is a partially ionized gas produced at ambient temperature and pressure. Depending on the experimental conditions, a non-equilibrium state is achieved, which keeps the plasma close to room temperature with the production of a reactive mixture containing electrons, positive and negative ions, groups of reactive species such as reactive oxygen species (ROS), or reactive nitrogen species (RNS), reactive species neutral in the ground state and excited (metastable and radiation state) and highly energetic photons (Dezest et al., 2017; Xu et al., 2015; Zhou et al., 2015).

Nonthermal plasma has the potential to kill microorganisms (Hati et al., 2012; Patinglag et al., 2021). Nonthermal plasma technology kills microorganisms by utilizing ionized gas in an electric discharge between two electrodes (Lopes et al., 2013). In recent decades, the use of plasma technology has been widely used in various medical fields such as cancer therapy (Ahn et al., 2014), accelerating wound healing (Dubey et al., 2022), and tissue sterilization (Amalda et al., 2020; Martines et al., 2009). Dielectric barrier discharge (DBD) and plasma jet are two methods that are often used to produce nonthermal plasma. From the generated plasma, various types of reactive oxygen species and nitrogen reactive

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species (ROS and RNS) are formed, including hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), ozone (O_3), atomic oxygen (O), superoxide anions (O_2^-), nitric oxide (NO), and peroxynitrite anion ($ONOO^-$), which are considered plasma components that have a role in inactivating microorganisms (Lotfy, 2020; Xu et al., 2015)(Nupangtha et al., 2021).

Reactive species produced by nonthermal plasma are considered effective in killing many microorganisms (Hati et al., 2012)(Lotfy et al., 2022). However, which reactive species are the most effective or influential in inactivating microorganisms is not known. For this reason, it is necessary to identify the most effective reactive species in inactivating microorganisms. One of the reactive species compositions is influenced by the gas source used (Zhou et al., 2015). Therefore, this study aimed to identify the most effective reactive species between ROS and RNS in inactivating bacteria using three different gas sources, namely free air, oxygen, and nitrogen by using surface dielectric barrier discharge (SDBD) non-thermal plasma. SDBD is a new technique for producing plasma (Nupangtha et al., 2021). Some of the advantages of SDBD are that it uses small power, reduces the burning of cells, and covers a wide sterilization area. In this study, variations of gas sources were used to determine the effect of the concentration of the gas source on the reactive species produced in killing bacteria. So that by doing this research, it is possible to obtain a new sterilization method that is effective, inexpensive, and efficient compared to conventional methods in general, as well as several non-thermal plasma sterilization techniques that have been carried out. In this study, E. coli was used as a sample. The inactivation of E. coli cells was evaluated by counting colony forming units (CFU) in petri dishes.

Method

Working principle of nonthermal plasma surface dielectric barrier discharge

Two copper electrodes are used, one as the HV electrode and the other as the ground electrode, separated by a dielectric layer. The voltage source is 20 Volts from the DC power supply, which is then transformed into high voltage using a transformer. The schematic diagram of the device can be found in Fig. 1. In this study, different gas sources were used, namely free air, oxygen, and nitrogen, which flowed at a flow rate of 1 L/min. The distance between the plasma source and the sample is 3 mm. OES is used to observe the reactive species produced by plasma, characterized by the intensity's magnitude at specific wavelengths.

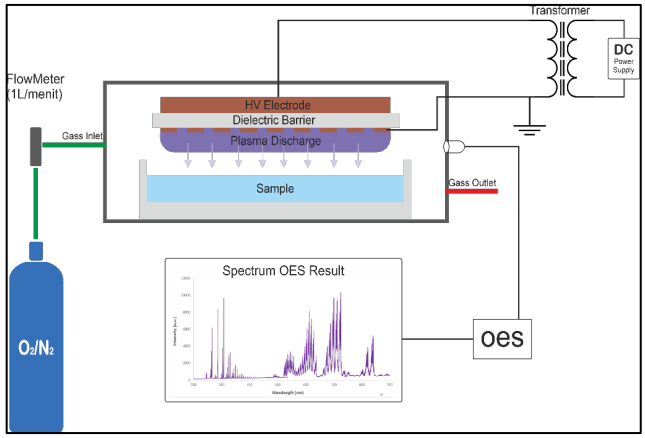


Figure 1. Schematic of surface dielectric barrier discharge nonthermal plasma

E. coli bacteria sample preparation

E. coli bacterial isolates aged for 24 hours were diluted by serial dilution to 10^{-6} dilutions by one ose of bacterial isolates were homogenized with 9 mL of sterile physiological NaCl (10^{-1} dilution). 1 mL of the 10^{-1} dilution of bacterial suspension was homogenized with 9 mL of sterile physiological NaCl (10-2 dilution). And so on until the 10^{-6} dilution. The resulting dilution was treated using a surface dielectric barrier non-thermal discharge plasma.

Surface dielectric barrier discharges nonthermal plasma treatment.

Nonthermal plasma treatment uses a surface dielectric barrier discharge system with various gas sources, namely free air, oxygen, and nitrogen. Oxygen and nitrogen gas flowed at a rate of 1 L/min. After the gas flowed for 1 minute, the plasma was turned on, and the bacterial treatment was carried out for 120 seconds with a treatment distance of 3 mm for each gas source. After completion of the treatment, the bacterial samples were incubated at 37 ^{0}C for 24 hours by placing the petri dish in an inverted position. Counting the number of colonies in each treatment. Each experiment was repeated three times.

Optical emission spectroscopy

Optical emission spectroscopy (OES) was used to characterize reactive plasma species and to analyze plasma composition, which could explain the relationship or mechanism between reactive spaces formed in plasma and the ability to inactivate bacteria (Wiegand et al., 2013). The optical emission spectra were measured using Aurora 4000 at a wavelength of 200 to 1000 nm with an integration time of 5000 ms and three repetitions of the spectrum capture and then averaged to obtain the optical emission spectrum from the plasma. The emission spectra were analyzed qualitatively to determine the chemical species at each wavelength peak and then analyzed using the National Institute of

Standards and Technology's atomic spectrum database and previous journal publications to identify chemically active species (Sarangapani et al., 2016).

Data analysis

The data obtained based on variations in gas sources, namely free air, oxygen, and nitrogen on the number of bacterial colonies resulting from non-thermal SDBD plasma treatment, were tabulated, and analyzed using ANOVA using SPSS 26 software. If p-value <0.05 H_0 was accepted, source variation The gas used in the SDBD non-thermal plasma treatment affects the number of bacterial colonies.

Result and Discussion

The nonthermal plasma spectrum of the surface dielectric barrier discharge of the optical emission spectrometer (OES)

The results of the non-thermal SDBD plasma spectrum obtained using OES can be seen in Figure 2.

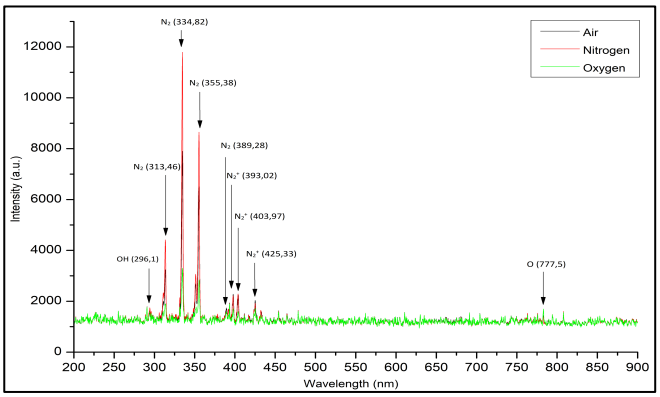
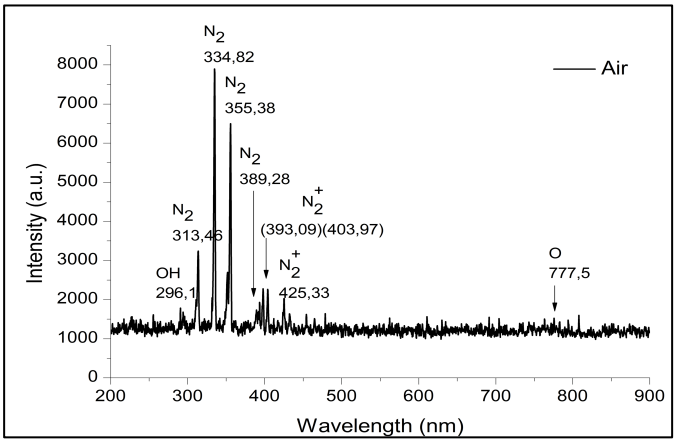


Figure 2. Combined spectrum of air, oxygen, and nitrogen gas sources

Spectrum results obtained spectrum differences in each use of gas sources. This difference in spectrumindicates the concentration of the reactive species produced. The results of the spectrum differences can be seen in Figure 3.



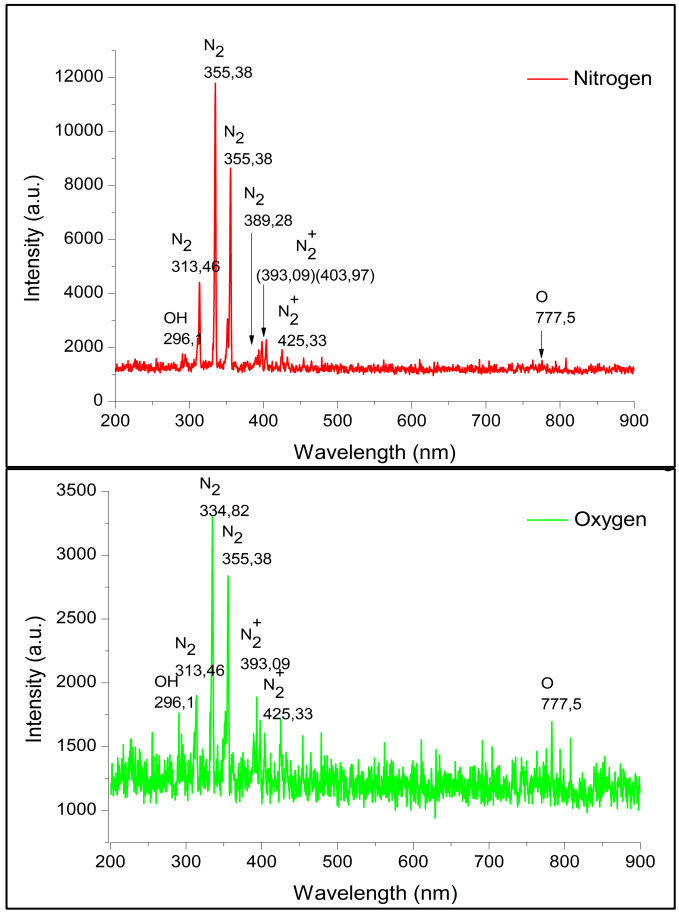


Figure 3. SDBD nonthermal plasma spectrum of each gas source

The spectrum results obtained using OES make it possible to determine the reactive species generated from SDBD nonthermal plasma by looking at the intensity peaks at specific wavelengths. Optical emission from the second positive system N_2 at a wavelength of 315 to 380 nm and the first negative system N_2^+ at 390 nm to 435 nm, can be observed in nonthermal plasma SDBD for all gas sources, namely air, oxygen, and nitrogen (Zhou et al., 2015). The second positive system of N_2 and the first negative system of N_2^+ are formed by the collision of excitation electrons and the ionization of the ground state of the N_2 molecule. Reactive emission of OH radicals was also obtained at a wavelength of 296,1 nm and O at a wavelength of 777,5 nm corresponding to the transition of oxygen atoms O ($3p^5P$-$3s^5S$). O is a chemically reactive species that can kill microorganisms and can be easily converted into ROS, such as OH, $^1O_2$, superoxide anions (O_2^-), H_2O_2, and ozone (O_3) (Zhang et al., 2013). The formation of this species is caused by the energetic collision of electrons with nitrogen and oxygen molecules present in the surrounding air. The reactive species produced in the gas phase plasma cannot react directly with the cells because of the protection provided by the nutrient solution. Reactive species can initiate various chemical

reactions at the gas-liquid interface and form many primary and secondary reactive species (Wang et al., 2017). OH radicals can be formed by dissociating electron collisions with molecules that diffuse into the plasma. Meanwhile, the energetic collision of electrons with O_2 molecules can lead to the formation of O radicals. The recombination process of O and H radicals can also contribute to the formation of OH radicals. Similarly, the excited nitrogen species originate from dissociating nitrogen molecules in the surrounding environment or gas source. (Adhikari et al., 2021; Hosseini et al., 2018; Kaushik et al., 2022; Misra et al., 2015; Naz et al., 2021; Pourbagher et al., 2021; Rezaei et al., 2014; Wang et al., 2017; Zhou et al., 2015).

Effectiveness of inactivation of E. coli bacteria as a result of SDBD nonthermal plasma treatment using a variety of gas sources.

Non-thermal plasma treatment with various gas sources used three variations of gas sources, namely air, oxygen, and nitrogen, with a fixed distance and time of treatment, 3 mm and 120 seconds. The treatment results obtained that the variation of the gas source used during non-thermal plasma treatment affected the colony growth. the results of colony growth can be seen in figure 5. Air and oxygen gas sources can inactivate bacteria, namely 6.33 x 10^7 CFU/ml and 6 x 10^7 CFU/ml. The number of colonies resulting from treatment using air and oxygen gas sources was much lower than the number of colonies in control, which was 409 x 10^7 CFU/ml. At the same time, the treatment results used a nitrogen gas source that is 41.67 x 10^7 CFU/ml. These results indicate that the air and oxygen gas sources used for SDBD non-thermal plasma treatment are more effective than SDBD non-thermal plasma treatments using nitrogen gas sources. The complete results of the number of colonies in each treatment as well as the graph of the effect of treatment with variations in gas sources can be seen in table 1 and figure 4.

Table 1. Number of colonies resulting from SDBD non-thermal plasma treatment

Gas Source	Number of colonies (x 10^7 CFU/ml)		
	I	II	III
Control	410	418	399
Air	7	2	10
Nitrogen	11	6	1
Oxygen	23	52	50

Note; *p < 0,05

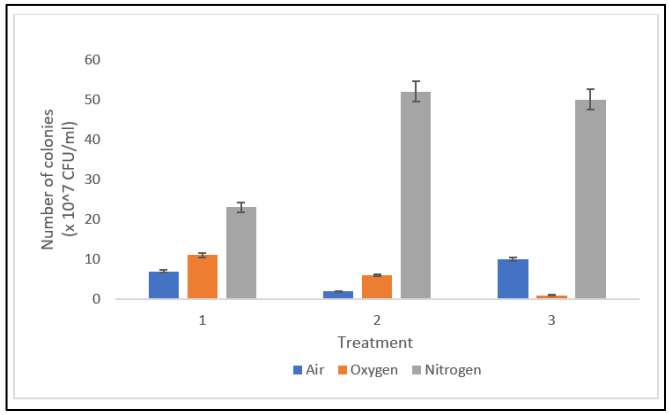


Figure 4. Graph of the number of colonies resulting from SDBD nonthermal plasma treatment (p < 0.05)

The results of the non-thermal plasma treatment against E. coli bacteria show that non-thermal plasma can decontaminate bacteria, and the gas source used has an essential role in decontaminating bacteria. According to (Zhou et al., 2015), inactivation of bacteria compared to N_2 and He gas sources, plasma treatment using air and oxygen gas sources can kill bacteria more efficiently. The difference in results is due to the reactive species formed from the source of the type of gas used. Using oxygen or air as a carrier gas is more efficient at killing bacteria. This is also supported by research from (Belgacem et al. 2017) (Belgacem et al., 2017) and (Carré et al., 2018), which showed that the highest bacterial inactivation rate was obtained using oxygen as a plasma reactor gas. So oxygen becomes raw material in forming reactive oxygen species (ROS), which are effective agents of inactivating bacteria in non-thermal plasma (Amalda et al., 2020).

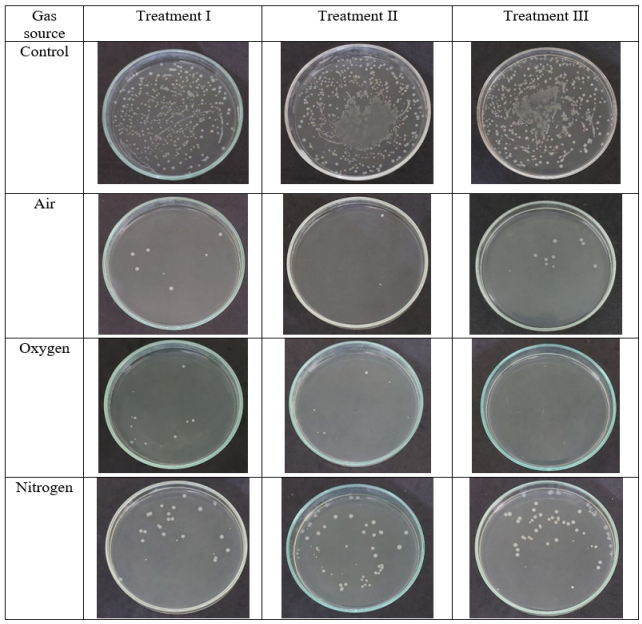


Figure 5. Picture of the number of colonies resulting from SDBD nonthermal plasma treatment with various gas sources.

When accumulated, ROS cause oxidative stress resulting in protein, DNA, and lipid damage, and even cell death (Akter et al., 2020; Feng & Wang, 2020). High oxygen levels will lead to the formation of ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), reactive hydroxyl radicals (OH), and high Ozone (O_3) (Feng & Wang, 2020). OH can cause protein breakdown, reduce ATP, and lead to low energy levels in bacterial cells. In addition, OH can break the phosphodiester bond in the DNA molecule, which causes DNA breakdown and destroys lipid moieties in the plasma membrane (Amalda et al., 2020; Gaur et al., 2021). The O_2^- radicals that are also formed directly or indirectly damage proteins, DNA, and lipids, by helping the formation of more reactive radical molecules that ultimately lead to low cell viability and cell death (Amalda et al., 2020; Feng & Wang, 2020). At the same time, O_3 can interfere with cellular respiration. Protein molecules that make up bacterial cell membranes are known to be susceptible to oxidation processes, so that non-thermal plasma treatment on bacteria can cause erosion that results in the rupture of bacterial cell walls (Akter et al., 2020; Amalda et al., 2020; Fallon et al., 2020; Feng & Wang, 2020; Gaur et al., 2021). These results prove that ROS reduction while using a nitrogen gas source causes no significant reduction in the number of colonies. Furthermore, proves that N_2, which is the main component of free air and the dominant peak in the spectroscopic results, does not play an essential role in the sterilization of SDBD nontermal plasma (Mastanaiah et al., 2013).

Conclusion

The results of research that has been carried out regarding the identification of the effect of reactive oxygen species and reactive nitrogen species (RNS) on bacterial inactivation using non-thermal plasma SDBD with variations of gas sources, namely air, oxygen, and nitrogen, show that the results of treatment using oxygen and air gas sources are more effective in inactivating bacteria E. coli which was marked by a reduction in the number of colonies after treatment, namely 6 x 10^7 CFU/ml and 6.33 x 10^7 CFU/ml. Compared to the control, which is 409 x 10^7 CFU/ml. Meanwhile, the treatment results using a nitrogen gas source are 41.67 x 10^7 CFU/ml. This shows that oxygen is essential as a gas source for plasma formation. The higher the concentration of oxygen used in plasma formation, the more ROS formed, so the more influential the inactivation of bacteria produced. The results of this study indicate that ROS are reactive species that play an essential role in the inactivation of bacteria resulting from SDBD non-thermal plasma treatment.

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References

Adhikari, B. C., Lamichhane, P., Lim, J. S., Nguyen, L. N., & Choi, E. H. (2021). Generation of reactive species by naturally sucked air in the Ar plasma jet. *Results in Physics*, *30*, 104863. https://doi.org/10.1016/j.rinp.2021.104863

Ahn, H. J., Kim, K. Il, Hoan, N. N., Kim, C. H., Moon, E., Choi, K. S., Yang, S. S., & Lee, J. S. (2014). Targeting cancer cells with reactive oxygen and nitrogen species generated by atmospheric-pressure air plasma. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086173

Akter, M., Yadav, D. K., Ki, S. H., Choi, E. H., & Han, I. (2020). Inactivation of infectious bacteria using nonthermal biocompatible plasma cabinet sterilizer. *International Journal of Molecular Sciences*, *21*(21), 1–14. https://doi.org/10.3390/ijms21218321

Amalda, E. C., Alhamidah, F., Oktanella, Y., & Khuzain, M. (2020). Kajian Artikel: Potensi Plasma Non Termal Sebagai Kandidat Terapi Mastitis Subklinis. *VITEK : Bidang Kedokteran Hewan*, *10*(November), 1–9. https://doi.org/10.30742/jv.v10i0.41

Belgacem, Z. Ben, Charpentier, E., Le-bras, F., Maho, T., Robert, E., Pouvesle, J., Polidor, F., Gangloff, C., Boudifa, M., & Gelle, M. (2017). Innovative non-thermal plasma disinfection process inside sealed bags : Assessment of bactericidal and sporicidal effectiveness in regard to current sterilization norms. *PLoS ONE*, *12*(6), 1–18.

Carré, G., Charpentier, E., Audonnet, S., Terryn, C., Boudifa, M., Doliwa, C., Belgacem, Z. Ben, Gangloff, S. C., & Gelle, M. P. (2018). Contribution of fluorescence techniques in determining the efficiency of the non-thermal plasma treatment. *Frontiers in Microbiology*, *9*(SEP), 1–12. https://doi.org/10.3389/fmicb.2018.02171

Dezest, M., Bulteau, A. L., Quinton, D., Chavatte, L., Bechec, M. Le, Cambus, J. P., Arbault, S., Nègre-Salvayre, A., Cleâment, F., & Cousty, S. (2017). Oxidative modification and electrochemical inactivation of Escherichia coli upon cold atmospheric pressure plasma exposure. *PLoS ONE*, *12*(3), 1–18. https://doi.org/10.1371/journal.pone.0173618

Dubey, S. K., Parab, S., Alexander, A., Agrawal, M., Achalla, V. P. K., Pal, U. N., Pandey, M. M., & Kesharwani, P. (2022). Cold atmospheric plasma therapy in wound healing. *Process Biochemistry*, *112*(June 2021), 112–123. https://doi.org/10.1016/j.procbio.2021.11.017

Fallon, M., Boyle, M., Kennedy, S., Daniels, S., & Humphreys, H. (2020). Cold atmospheric plasma, the removal of blood from steel and its effect on staphylococcal biofilm formation. A pilot study. *Clinical Plasma Medicine*, *19–20*(March), 100104. https://doi.org/10.1016/j.cpme.2020.100104

Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes*, *12*(1). https://doi.org/10.1080/19490976.2020.1801944

Gaur, N., Kurita, H., Oh, J. S., Miyachika, S., Ito, M., Mizuno, A., Cowin, A. J., Allinson, S., Short, R. D., & Szili, E. J. (2021). On cold atmospheric-pressure plasma jet induced DNA damage in cells. *Journal of Physics D: Applied Physics*, *54*(3). https://doi.org/10.1088/1361-6463/abb8ab

Hati, S., Mandal, S., Vij, S., Minz, P. S., Basu, S., Khetra, Y., Yadav, D., & Dahiya, M. (2012). Nonthermal plasma technology and its potential applications against foodborne microorganisms. *Journal of Food Processing and Preservation*, *36*(6), 518–524. https://doi.org/10.1111/j.1745-4549.2011.00619.x

Hosseini, S. I., Mohsenimehr, S., Hadian, J., & Ghorbanpour, M. (2018). *Physico-chemical induced modification of seed germination and early development in artichoke (Cynara scolymus L .) using low energy plasma technology Physico-chemical induced modification of seed germination and early development in*. *January*. https://doi.org/10.1063/1.5016037

Kaushik, N., Mitra, S., Baek, E. J., Nguyen, L. N., Bhartiya, P., Kim, J. H., Choi, E. H., & Kaushik, N. K. (2022). The inactivation and destruction of viruses by reactive oxygen species generated through physical and cold atmospheric plasma techniques: Current status and perspectives. *Journal of Advanced Research*. https://doi.org/10.1016/j.jare.2022.03.002

Lopes, B. B., De Paula Leite Kraft, M. B., Rehder, J., Batista, F. R. X., & Puzzi, M. B. (2013). The interactions between non-thermal atmospheric pressure plasma and ex-vivo dermal fibroblasts. *Procedia Engineering*, *59*, 92–100. https://doi.org/10.1016/j.proeng.2013.05.098

Lotfy, K. (2020). The impact of the carrier gas composition of non-thermal atmospheric pressure plasma jet for bacteria sterilization. *AIP Advances*, *10*(1). https://doi.org/10.1063/1.5099923

Lotfy, K., Al-Qahtani, S. M., Al-Harbi, N. A., El-Absy, K. M., Bu Shulaybi, F. A., Alali, S. A., & Mashtoly, T. A. (2022). Influence of Non-Thermal Plasma on the Quality and Nutritional Content of Palm Dates. *Applied Sciences*, *12*(17), 8587. https://doi.org/10.3390/app12178587

Martines, E., Zuin, M., Cavazzana, R., Gazza, E., Serianni, G., Spagnolo, S., Spolaore, M., Leonardi, A., Deligianni, V., Brun, P., Aragona, M., Castagliuolo, I., & Brun, P. (2009). A novel plasma source for sterilization of living tissues. *New Journal of Physics*, *11*, 8–16. https://doi.org/10.1088/1367-2630/11/11/115014

Mastanaiah, N., Banerjee, P., Johnson, J. A., & Roy, S. (2013). Examining the role of ozone in surface plasma sterilization using dielectric barrier discharge (DBD) plasma. *Plasma Processes and Polymers*, *10*(12), 1120–1133. https://doi.org/10.1002/ppap.201300108

Misra, N. N., Keener, K. M., Bourke, P., & Cullen, P. J. (2015). Generation of In-Package Cold Plasma and Efficacy Assessment Using Methylene Blue. *Plasma Chemistry and Plasma Processing*, *35*(6), 1043–1056. https://doi.org/10.1007/s11090-015-9638-5

Naz, M. Y., Shukrullah, S., Rehman, S. U., Khan, Y., Al-Arainy, A. A., & Meer, R. (2021). Optical characterization of non-thermal plasma jet energy carriers for effective catalytic processing of industrial wastewaters. *Scientific Reports*, *11*(1), 1–13. https://doi.org/10.1038/s41598-021-82019-4

Nupangtha, W., Kuensaen, C., Ngamjarurojana, A., Chomdej, S., & Boonyawan, D. (2021). A surface dielectric barrier discharge non-thermal plasma to induce cell death in colorectal cancer cells. *AIP Advances*, *11*(7), 15–17. https://doi.org/10.1063/5.0053501

Patinglag, L., Melling, L. M., Whitehead, K. A., Sawtell, D., Iles, A., & Shaw, K. J. (2021). Non-thermal plasma-based inactivation of bacteria in water using a microfluidic reactor. *Water Research*, *201*(February), 117321. https://doi.org/10.1016/j.watres.2021.117321

Pourbagher, R., Abbaspour-Fard, M. H., Sohbatzadeh, F., & Rohani, A. (2021). In vivo antibacterial effect of non-thermal atmospheric plasma on pseudomonas tolaasii, a causative agent of Agaricus bisporus blotch disease. *Food Control*, *130*(March). https://doi.org/10.1016/j.foodcont.2021.108319

Rezaei, F., Abbasi-firouzjah, M., & Shokri, B. (2014). *Investigation of antibacterial and wettability behaviours of plasma-modified PMMA films*. *085401*. https://doi.org/10.1088/0022-3727/47/8/085401

Sarangapani, C., Misra, N. N., Milosavljevic, V., Bourke, P., O'Regan, F., & Cullen, P. J. (2016). Pesticide degradation in water using atmospheric air cold plasma. *Journal of Water Process Engineering*, *9*, 225–232. https://doi.org/10.1016/j.jwpe.2016.01.003

Wang, Y., Cheng, C., Gao, P., Li, S., Shen, J., Lan, Y., Yu,

Y., & Chu, P. K. (2017). *Cold atmospheric-pressure air plasma treatment of C6 glioma cells : effects of reactive oxygen species in the medium produced by the plasma on cell death*. *June 2018*. https://doi.org/10.1088/2058-6272/19/2/025503

Wiegand, C., Beier, O., Horn, K., Pfuch, A., Tölke, T., Hipler, U. C., & Schimanski, A. (2013). Antimicrobial impact of cold atmospheric pressure plasma on medical critical yeasts and bacteria cultures. *Skin Pharmacology and Physiology*, *27*(1), 25–35. https://doi.org/10.1159/000351353

Xu, D., Liu, D., Wang, B., Chen, C., Chen, Z., Li, D., Yang, Y., Chen, H., & Kong, M. G. (2015). In situ OH generation from O2-and H2O2 plays a critical role in plasma-induced cell death. *PLoS ONE*, *10*(6), 1–14. https://doi.org/10.1371/journal.pone.0128205

Zhang, Q., Liang, Y., Feng, H., Ma, R., Tian, Y., Zhang, J., & Fang, J. (2013). A study of oxidative stress induced by non-thermal plasma-activated water for bacterial damage. *Applied Physics Letters*, *102*(20). https://doi.org/10.1063/1.4807133

Zhou, R., Zhang, X., Bi, Z., Zong, Z., Niu, J., Song, Y., Liu, D., & Yang, S. (2015). Inactivation of Escherichia coli cells in aqueous solution by atmospheric-pressure N2, He, air, and O2 microplasmas. *Applied and Environmental Microbiology*, *81*(15), 5257–5265. https://doi.org/10.1128/AEM.01287-15