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Inactivation of Microorganisms with Neon Plasma Jet at Atmospheric Pressure

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A dielectric barrier discharge (DBD) plasma jet of neon (Ne) at atmospheric pressure and room temperatures was arranged under a high frequency ac power supply. We achieved to obtain an atmospheric pressure glow-like discharge in this study. Not only have the effects on various microorganism been reported, but also atmospheric pressure plasma using Ne has been introduced. The microorganism inactivation by means of the non-thermal plasma were studied with eight kinds of typical microorganism, i.e., *Esherichia coli, Micrococcus luteus, Staphylococcus aureus, Salmonella typhimurium, Bacillus subtilis, Listeria monocytogenes, Candida albicans and Candida glabrata*. Treatment time was selected as 60 s. According to the Ne emission spectra of the plasma jet and the inactivation results of microorganism after the plasma treatment, it can be discussed that the reactive species in the Ne plasma had an important role in the inactivation of microorganism.

Keywords: Atmospheric pressure glow-like discharge, Plasma jet, Non-thermal plasma, Inactivation, Microorganism.

The importance of atmospheric pressure plasma jet devices has been known issue in science and technology for a long time. Recently, the atmospheric pressure discharge sources of noble gases have been developed[1]. These sources present more convenient alternative to potentially replace plasmas devices at low pressures for the known applications and to create new fields. Atmospheric plasmas provide a cheaper solution for applications than low pressure plasmas. Vacuum pump is not needed for atmospheric systems and shorter chamber length is enough to obtain plasma. The important subject is design of electrodes for atmospheric pressure plasmas. Atmospheric pressure plasmas can be generated using different geometries and high frequency [2]. Electrodes could be sorted to several different variations for capacitively coupled. Every type of electrode sequence represents different discharges for different aims and power supplies. This system allow us to control the processes that take place in the experiment during its applications in specific fields for physics.

Cold plasmas can be generated using with corona discharge, glow discharge, dielectric barrier discharge methods. Our plasma generated here can be described as the dielectric barrier discharge. It will also ensure advantages us to know the ability of the DBD for the future works. It is known that these types of plasma can be used in various applications like surface modification, inactivation of organisms etc. AC high voltage power supplies are used producing atmospheric plasmas to apply on developing semiconductors[3-5].

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Plasma technology can be especially considered in biomedical and commercial applications as microbial decontamination and sterilization areas. Non-thermal atmospheric plasma is used for some applications including decontamination of contaminated surfaces, improvement of food safety, material surface treatment, and sterilization of medical instruments[6, 7]. Some studies in literature include the data and results of the same microorganism for different powers, voltages and the measurement distances. The effects of direct and indirect application have been also investigated. Lately, the several recent studies research inactivation systems working with battery[8-14]. Researches have been started about sterilization at low temperatures in the 1990's. Quick surface and stuff sterilization solutions those are less damaging were wanted[15]. One inner and outer electrode system and two outer electrode system are generally used for bacteria inactivation[16]. Traditional methods of inactivation of bacteria are used thermal treatment, chemical treatment or radiation. These methods are not suitable for all areas. For this reason, the cold plasma technologies produce less toxic waste, less time consuming. Also microbial decontamination can be defined as the decomposition or removal of microorganisms[17] and cold plasma technology has a potential to impact the sterilization purposes; in particular, inactivating of microorganisms. In literature, the air plasma, argon discharge, helium discharge, helium–oxygen mixture and helium–nitrogen mixture plasmas are used for the inactivation[8-13, 18–22]. Microbial inactivation made by plasma is usually affected by several factors. There are four inactivation factors involved in the destruction of pathogens and chemical agents: heat, ultraviolet radiation, reactive neutral species and charged particles [23-24]. Characterizations of the inactivation process reported in literature have been studied on various bacteria. One of these studies is about the inactivation efficacy of atmospheric cold plasma (ACP) and *Salmonella typhimurium, Listeria monocytogenes* and *Escherichia coli* in lettuce tissue have been considered[25]. Plasma inactivation effects have been studied for *B. stearothermophilus* and *B. cereus* vegetative cells and spores. Atmospheric pressure cold plasma effect on the inactivation of *Escherichia coli* in fresh produce has been investigated[26]. Another study is worked by different gases and application. In these studies, the effects of helium (He) and argon (Ar) plasma treatments on inactivation of foodborne bacteria and meat microbiota are determined[24]. In addition to the bacterial inactivation studies, the fungal inactivation is detected. In several papers report the plasma fungicidal ability against *Candida albicans*[27-29].

MATERIALS AND METHODS

Bacterial strains and bacterial suspensions preparation

Six bacterial strains and two fungal strains are used in this study. *Esherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Candida albicans* and *Candida glabrata* have been obtained from the microbiology stock culture of the Microbiology Laboratory of Department of Biology, Faculty of Science.

Microbial inactivation was detected using the spread plate method. First, the experiment glass petri dishes were sterilized and then a thin layer (5mm) of Nutrient Agar was poured into each plate. Each plate was cooled until it becomes the solid. Plates were stored in plastic bags in fridge.

Esherichia coli, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimirium*, *Listeria monocytogenes*, *Candida albicans* and *Candida glabrata* were cultured in Nutrient broth before inoculation for 24 h at 37 °C. Optical density of bacterial cultures was measured using a spectrophotometer at 600 nm. Approximately inoculum 10^8CFU/ml was extended onto plates. Plasma treatment of the plate was applied. The surface layers of plates were exposed to Ne atmospheric plasma jet. The treatment time was chosed as 1minute. Plates were incubated overnight after plasma treatment for 24 h at 37 °C. Next day, the antimicrobial activity zones were detected in order to check the efficiency of bacterial inactivation using cold plasma treatment.

Experimental setup

Our system has the main body made by quartz glass and two electrodes coupled on it. The quartz glass tube has 15 mm outer diameter and 190 mm length. The electrodes have been specially built from copper properly for measurements for

quartz glass. Each electrode has 15 mm inner diameter and the driven electrode and ground electrode have 40 mm and 30 mm lengths, respectively. Driven and ground electrodes have been separated with 20 mm aperture. A fixing stand is also designed. Wood stuff is used for the lack of electrical interaction. Gas flow rate is controlled by using M+W Instruments Mass-Stream D6300 series flowmeter. ELES HV-711GK5 is the main power supply of the system. The power supply (the ac power supply was specially built) provides 10–25 kHz frequency and 4–20 kV input voltage adjustments. Large crocodiles are attached with proper cables for the electrodes. Ocean Optics HR2000+ is used for the emission spectra of Ne plasma jet. Fluke CNXt3000 thermocouple is also used to measure the initial temperature of the plasma. Schematic diagram of the system is presented in Fig. 1. Here the plasma temperature is measured from the jet region. Neon gas is sent by head of the glass tube to the system. 15 kHz frequency and 8kV input voltage have been selected and the flow rate is fixed to 3.0 lt/min. In this study, petri dish is located at 1 cm and 3 cm distances away from the discharge tube for all types of microorganism. Bottom of the petri dish is selected as the reference point to adjust the distance and then the plasma is applied to the microorganism for 1 min along.

The emission spectra of the discharge are collected after the plasma is obtained. When the spectra are investigated, in addition to Ne peaks, it is seen that there are the peaks of nitrogen and oxygen because of atmospheric pressure. The spectrum graph can be seen in Fig. 2. The temperature for the first moment of the plasma generation has been recorded as 39.1 ^{o}C.

RESULTS

Esherichia coli and *Salmonella typhimurium* are used as a gram negative bacterial representatives. *Staphylococcus aureus*, *Micrococcus luteus, Listeria monocytogenes* and *Bacillus subtilis* have been used gram positive representatives. In addition to fungal inactivation, *Candida albicans* and *Candida glabrata* are selected. Inactivation of these microorganism are crucial. Candida species are the microorganism exhibiting planktonic unicellular form, filamentous growth or complex multicellular structure is observed mainly in the infected tissues. We have shown that non-thermal plasmas can effectively inactive the strains of *Candida* species including *C. albicans* and *Candida glabrata.* It is well-known that *Listeria monocytogenes* cause disease in humans and animals. It is foodborne pathogens. Due to the ability to grow at refrigerator temperature, in particular, *Listeria monocytogenes* is an important issue for the ready to eat foods[30]. *Salmonella typhimurium* is a pathogenic bacteria. Salmonella species also cause agents of typhoid fever and diarrheal diseases in humans[31]. *Bacillus subtilis* has endospore. This endospore formation provides a means to ensure long-term survival in the environment[32]. Candida species are fungal organisms and cause to most common fungal infection in humans Candidiasis[33]. Bacterial spores are more resistant than vegetative cells to physical and chemical treatments. Destroying spores by exposure to plasma is possible. This can be result of UV photons passing through the spore-protecting coats and damaging the DNA. Gram-negative, gram-positive bacteria and yeasts have also been inactivated by using plasma obtained with dc power supply in literature[34]. Antimicrobial effects of cold plasma can be detected and measured by inhibition zones on agar plates (See Figures 3-9). One of the variable for each measurement is the distance, which was taken into account in experiment. The experiments have been done for two different distances as 1 cm and 3 cm. Effects of the distance are easily visible. The short application distance as 1 cm is more effective than 3 cm distance almost for all types. The results for all application distances, microorganism types and zone diameters observed here are represented in Table 1.

DISCUSSION

In this study, the antimicrobial effect of plasma treatment has been examined against a range of microorganisms commonly implicated in foodborne pathogens and pathogens caused human infections. Experimental variables can be taken into account as microorganism type, gas type, flow rate, distance to sample, treatment time and application frequency and potential. In our experiments, the constant frequency, input voltage

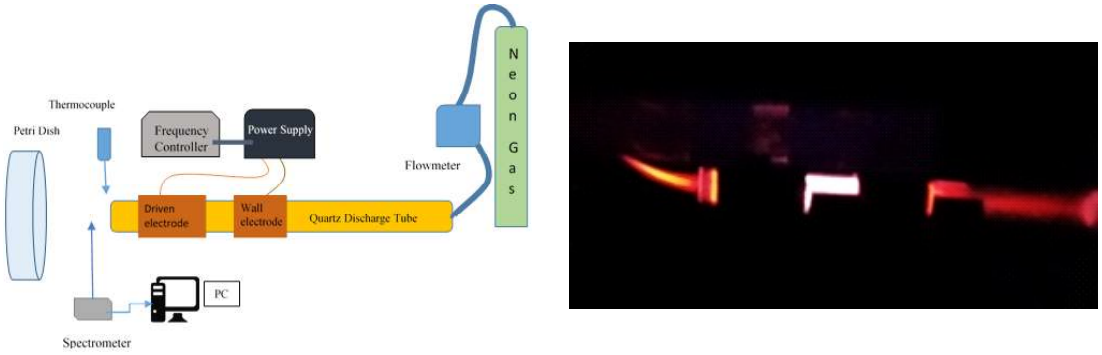


Fig. 1. (a) Schematic diagram of system and (b) image of atmospheric pressure plasma jet

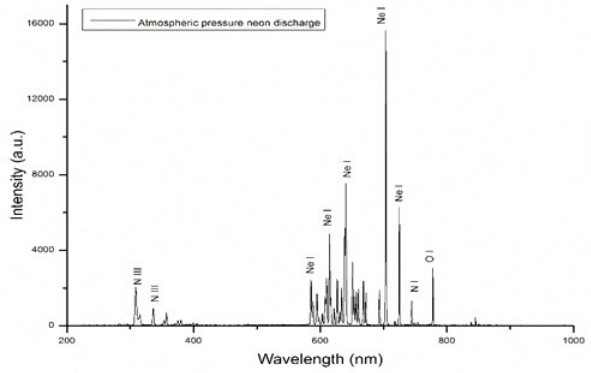


Fig. 2. The emission spectra of Ne plasma jet at the atmospheric pressure

Table 1. Zone diameters according to the application distances for 8 types of microorganism with atmospheric plasma jet of Neon at 8 kV–15 kHz

Bacteria Type	Application Distance(cm)	Zone Diameter (cm)
Esherichia coli	1	3
(ATCC25922)	3	2
Micrococcus luteus	1	2,1
	3	1,5
Staphylococcus aureus	1	1,4
	3	1
Salmonella	1	0,9
typhimurium	3	0,9
Bacillus subtilis	1	1,5
(NRS744)	3	1
Listeria monocytogenes	1	1,6
(ATCC7644)	3	1,5
Candida albicans	1	1,9
(90028)	3	1,7
Candida glabrata	1	2
	3	1,8

and gas flow rate for the different kinds of microorganism are used. The use of neon (Ne) gas and many types of microorganism are the main differences and novelty for this study. The aim of this study is to investigate microorganism inactivation of Ne plasma treatment against microbial species.

All of the process parameters studied showed a strong influence on the atmospheric cold plasma inactivation rate of bacterial and fungal species. Distances (1 cm and 3 cm) are used in experimental set up. It is seen that the application distance as 1cm is more effective almost for all microorganisms. For the first time, the atmospheric pressure glow-like discharge of Ne is used in this system. Results of plasma treatment using Ne have a positive inactivation effect for all types of microorganisms. Traditional sterilization and decontamination methods use heat, radiation, chemicals or steam. Our results indicate that this innotive technology may be used as an alternative

sterilization method as it is in many different areas such as biomedical and industrial applications. Potentially, cold plasma offers advantages over traditional methods, such as being more cost effective and time efficient, and producing less toxic products.

In conclusion, Ne atmospheric jet plasma treatment is very effective against microorganism

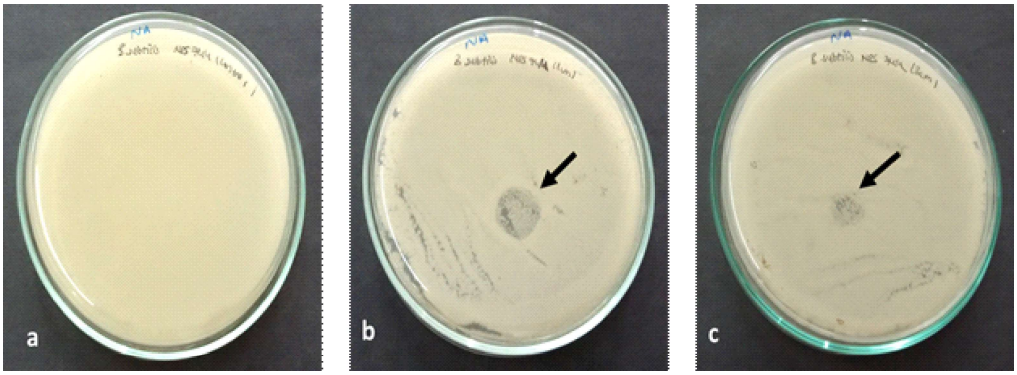


Fig. 3. *Bacillus subtilis* inhibition zones after plasma treatment. (a) Positive control (b) From 1cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone.

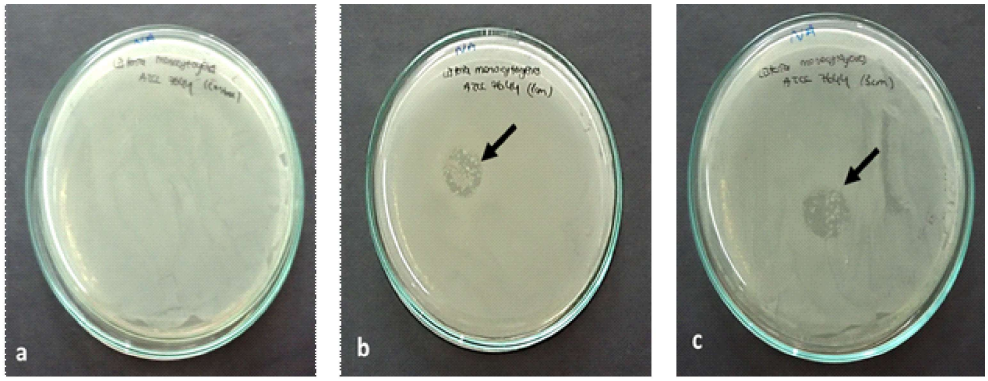


Fig. 4. *Listeria monocytogenes* inhibition zones after plasma treatment. (a) Positive control (b) From 1 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone

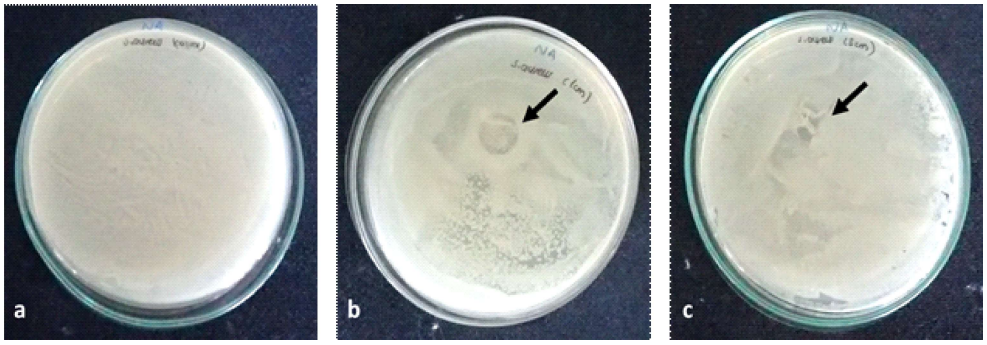


Fig. 5. *Staphylococcus aureus* inhibition zones after plasma treatment. (a) Positive control (b) From 1 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone

as *Esherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Candida albicans* and *Candida glabrata* within 60 s of treatment. Our early results showed that Ne plasma treatment has positive inactivation effects on gram negative and positive bacteria, endospore forming bacteria, human pathogens, foodborne pathogens and fungal microorganisms. The effects of electric field, heat and UV photons have an important role on microorganism inactivation with plasma treatment. It seems to be effective and promising alternative using Ne plasma in the inactivation of microorganism.

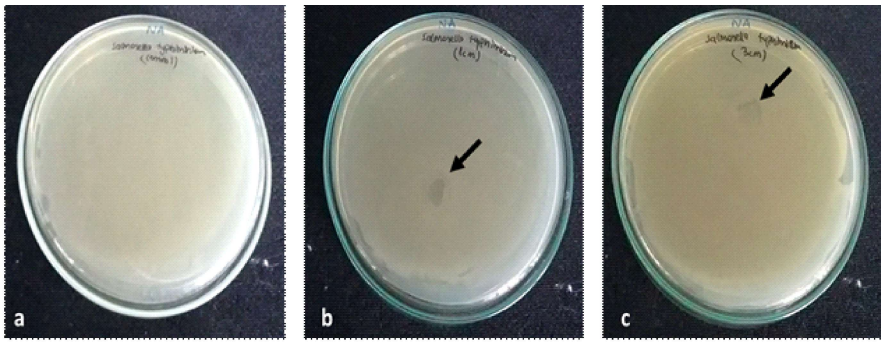


Fig. 6. *Salmonella typhimurium* inhibition zones after plasma treatment. (a) Positive control (b) From 1 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone

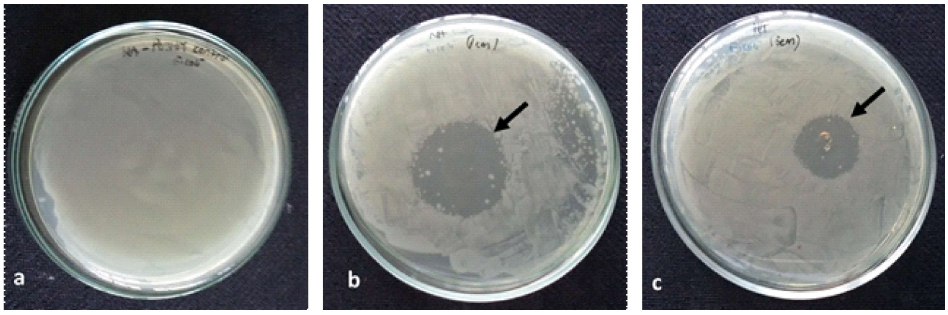


Fig. 7. *Esherichia coli* inhibition zones after plasma treatment. (a) Positive control (b) From 1 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone

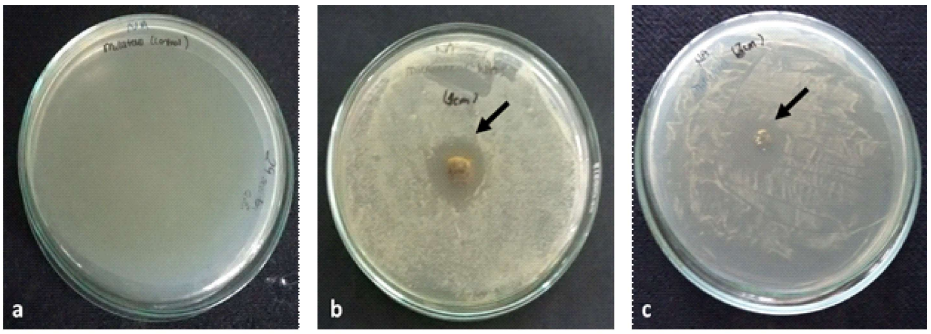


Fig. 8. *Micrococcus luteus* inhibition zones after plasma treatment. (a) Positive control (b) From 1 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone

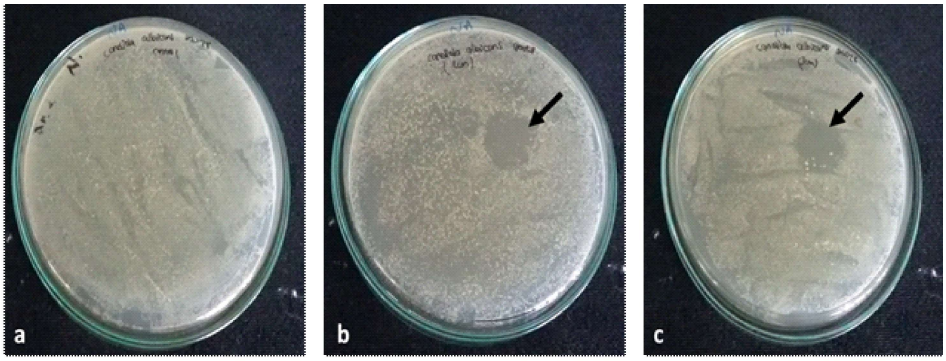


Fig. 9. *Candida albicans* inhibition zones after plasma treatment. (a) Positive control (b) From 1 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone. (c) From 3 cm distance of plasma treatment, an area at the centre of the agar plate is inhibition zone

According to criteria mentioned above, the future studies can be needed to compare the types of media, the different representatives of microorganism, gas type (or plasma composed a mixed gas), different gas rates and amount of time required for inactivation etc.…

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