Rate of $O_3$ production in DBD chamber and its effect on spore surface modification

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Introduction: Direct or indirect exposure to non-thermal atmospheric pressure plasma (NAPP) can inactivate microbes and cancer cells. As observed in scanning electron microscopic (SEM) images of microorganisms, plasma exposure causes irreversible outer wall damage. Reason for this outer wall destruction is not clear yet but various reactive oxygen and nitrogen species (RONS) produced in the discharge zone and afterglow region are considered to be the main chemical etchants. In ambient air, due to the ease of production of RONS, NAPP generated in He with $O_2$ as an admixture gas has gained research attention. In this experiment we have used He as main feed gas. Keeping the gas flow rate and applied voltage constant, $O_3$ admixture ratio is varied. $O_3$ production within dielectric barrier discharge (DBD) chamber was measured. $O_3$ density within DBD chamber is not constant but depends on the $O_2$ admixture ratio in the feed gas channel. $O_3$ density in the DBD chamber is maximum for the feed gas composition He + 1.5% $O_2$. Spores spread on a glass slide was exposed to plasma constituent within the chamber for direct plasma exposure and also treated remotely in an identical adjacent chamber connected with it. Difference of surface morphology for this two treatment mode is observed by FE-SEM imaging technique. Surface morphology of spores undergone direct plasma exposure is significantly different than the spores’ undergone remote treatment.

Experiments: 10 kHz high voltage power source ($V_{max} = 20$ kVpp, pulse duration $\tau = 23$ $\mu$s) was used to generate NAPP in DBD chamber ($Volume = 350$ cm$^3$) made of polypropylene ($\varepsilon = 2.5$). Parallel electrode configuration was used to generate plasma with electrode dimension $90 \times 130$ mm$^2$. Feed gas flow (2 slm) was controlled by two (100 sccm and 5 slm) mass flow controller and the applied voltage was 18 kVpp. 50 $\mu$l of B. lichenformis spore solution (spore concentration $10^7$ /ml) was spread on glass slide and air dried overnight before putting it in the discharge chamber.

Result: Collisions with He* (19.84 eV) with $O_2$ molecules dissociates them to form singlet O or O$^+$ or produces $O_3$ via 3 body collision process $O + O_2 + M \rightarrow O_3 + M$, where M is O, $O_2$, or He. Figure 1 represents the temporal variation of $O_3$ density in DBD chamber. $O_3$ density rapidly increases to its maximum value around 30 seconds after discharge initiation and then gradually decreases. $O_3$ density is minimum for pure He discharge and reaches to its maximum for He + 1.5% $O_2$ feed gas composition in this experimental set up. Figure 2 presents the FE-SEM images of B. lichenformis spore. Multilayer coating protects spores from oxidative stress and cell lytic enzymes. For pure He discharge, $O_3$ density is minimum, yet after 5 minutes of direct plasma exposure surface layer of spores breaks down and both inner coating and outer coating suffers irreversible damage [Figure 2(b)]. For the feed gas composition He + 1.5% $O_2$, $O_3$ density is maximum but after 15 minutes of remote plasma treatment where spores outer layer were bombarded by these $O_3$ molecules, no surface modification is observed in FE-SEM images [Figure 2(c)]. In direct plasma treatment spores are exposed to periodic variation of electric field in the discharge zone, short lived charged ions and molecules, singlet O and $O_3$ while in the remote treatment facility spores are only exposed to long-lived molecules such as $O_3$. Cells exposed to periodic variation of intense electric field suffers dielectric break down but exact reason for this difference is not clear yet.

![Figure 1: Temporal variation of $O_3$ density in DBD chamber.](image1.png)

![Figure 2 FE-SEM image of spores on slide (a) control, (b) pure He direct exposure for 5 minutes, and (c) remote plasma exposure for feed gas composition He + 1.5% $O_2$ for 15 minutes.](image2.png)