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_2 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 τ = 23 μs) was used to generate NAPP in DBD chamber (Volume = 350 cm^{-3}) made of polypropylene (ε_r = 2.5). Parallel electrode configuration was used to generate plasma with electrode dimension 90 × 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 kV_{pp}.
50 μl 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 fed 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 [Figure2(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.

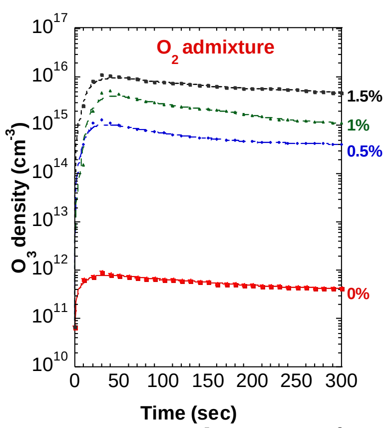

Figure 1: Temporal variation of O_3 density in DBD chamber.

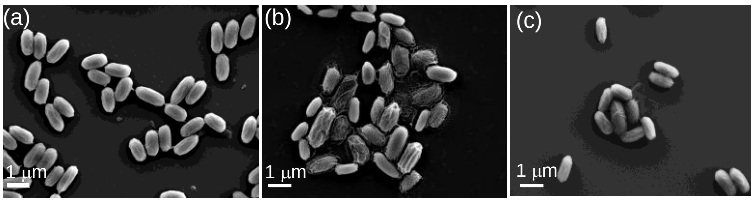

Figure 2 FE-SEM image of spores on slide (a) control, (b) pure He direct exposure for 5 minutes, and (c) remote plasma exposure for feed gas composition He + 1.5% O_2 for 15 minutes.