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Interpretation of Tailing Phenomenon of Survivor Curve and Inactivation of Virus by Plasma Exposure

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There are two questions difficult to answer. One is the tailing phenomenon of survivor curve of plasma sterilization and the other is whether plasma cluster[R] can inactivate bacteria and virus.

Tailing is the phenomenon illustrated in Figure 1 [1]. Many physics researcher interpreted it as being done by Moison [2], which several different kinetics may co-exist. The author deny their interpretation and considers it is due to clumping phenomenon of biological indicator (BI, Figure 2) [3] because penetration depth of gas plasma is less than 1 μm and the thickness of each bacterial spore of BI is around 1 μm. Therefore if clumped, the second layer of BI by plasma exposure will be protected with the first layer. First layer will be inactivated with first order equation as straight line of survivor curve (Figure 2), but the second or third layer delayed inactivation by protection of the first layer, so the survivor curve tailed and as a whole it is not straight line (Figure 1) [4].

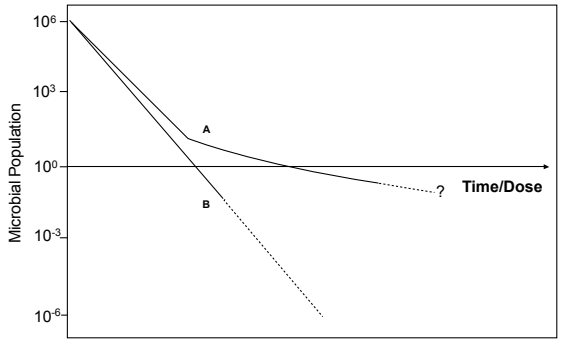

Figure 1: Rate of microbial inactivation on exposure to sterilization processes. In this case, the test microorganism (generally bacterial spores) at a starting population of 10^6 CFU (colony forming units) is exposed to the sterilizing agent under two conditions (A and B). The number of microorganisms can be determined over contact time using a direct-enumeration method. In process A, "tailing" is observed, which may not allow the extrapolation of the kill curve to a defined probability of survival (known as an SAL, sterility assurance level). In process B, the kill curve is linear, allowing extrapolation (dotted line) to an SAL of 10^{-6}.

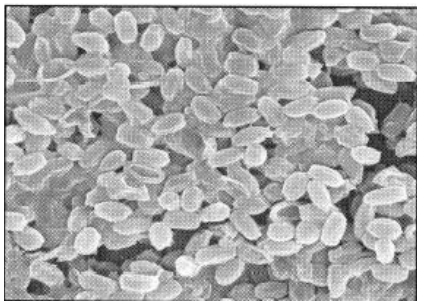

Figure 2: SEM (scanning electron microscopy) photo of *Geobacillus stearothermophilus* ATCC 7953 inoculated on SUS.

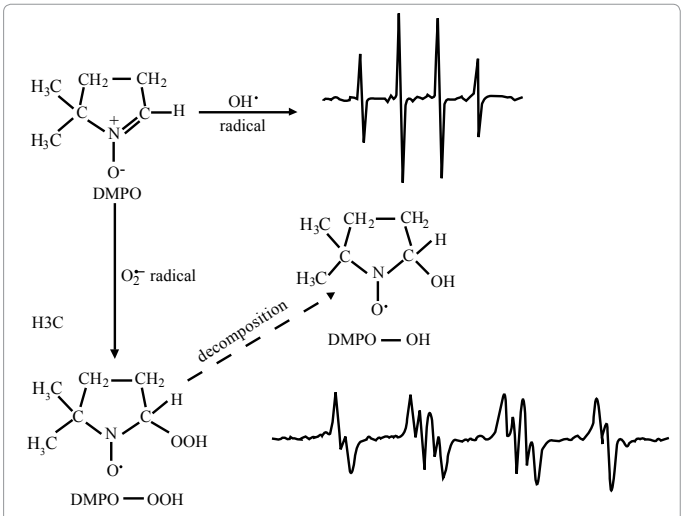

Figure 3: Reactions of DMPU with O_2^{-} and OH· radicals. The ESR spectra of DMPO-OH and DMPO-OOH adducts are shown diagrammatically to illustrate the key difference them. The ESR spectrum of DMPO-OH adduct has 4 lines and DMPO-OH adduct has 12 lines.

The next question is whether plasma cluster[R], nano-e[R] and so on can inactivate bacteria and virus.

The major component of plasma exposure is OH radical. The life period of OH radical is 10^{-6} second and the flight distance is 30 cm/0.01 second, so only 0.003 cm can fly during life period. It is hard to analyze OH radical even though the use of radical trapping agent such as DMPO (Figure 3) because the life period of OH radical is quite short and additionally it is quite hard to set DMPO within the equipment because plasma reaction will interfere with the presence of DMPO.

OH radical can react another OH radical at the kinetics rate of 5×10^9/M/second and produce hydrogen peroxide (H_2O_2). The re-produced H_2O_2 ($OH\cdot + OH\cdot \rightarrow H_2O_2$) can then be exposed Fenton Reaction as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot \quad K=76/M/second$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + OH\cdot \quad K=4.7\times10^3/M/second$$

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Radical	Spore Log Reduction
$O^{\cdot}$	2.2
$OOH^{\cdot}$	1.9
$OH^{\cdot}$	3.8
$H^{\cdot}$	1

Table 1: Comparison of sterility strength among $O^{\cdot}$, $OOH^{\cdot}$, $OH^{\cdot}$, and H.

So, the produced H_2O_2 is changed to $OH^{\cdot}$ by Fenton reaction and produced $OH^{\cdot}$ may react once again with $OH^{\cdot}$ to produce H_2O_2 again. As this reaction continuously occurs, so OH radical is quite reactive to microorganisms (Table 1). As bacteria have Cu and Fe in their body, Fenton reaction can easily occur. On the contrary, virus has not, so Fenton reaction does not occur. This means virus is not inactivated by Fenton reaction. To confirm this, it is necessary to determine Cu and Fe in the virus body by using ICP or ion chromatography-MS.

HEPA filter may trap virus with carrier, but not sure virus without carrier.

Bacteria charged with minus, so by charging minus, bacteria cannot into the interior of minus charged protection. On the contrary, enveloped and non-enveloped virus charged plus and minus, so hard to protect with charging [5].

As cluster^{R}, nano-e^{R} and so on promote PR of virus inactivation, but it is not always correct from the standpoint of science.

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