CASPR TECHNOLOGIES

EXECUTIVE BRIEF

Fighting the Monkeypox virus and other non-tested or novel pathogens: use of hydrogen peroxide as a biocide

Hydrogen peroxide possesses many properties that render it particularly useful as a sterilant and disinfectant; it is colourless and odourless and ultimately decomposes to harmless water and oxygen [1].

Although hydrogen peroxide can be applied either as a liquid or as a vapour for disinfection purposes, there is recent evidence to show that its mode of action in vapour form may be quite different from that in aqueous solution, and that the vapour is capable of bringing about more intensive oxidation of a range of biological macromolecules than do aqueous solutions of hydrogen peroxide, even at concentrations 10,000 times inferior [2].

Vapour-phase H_2O_2 is a more potent oxidizer of protein than liquid-phase H_2O_2, even at concentrations 10,000 times inferior

The interest in **environmentally friendly, non-toxic and degradable yet potent** biocides, has never been so high. Oxidizing agents like hydrogen peroxide (H_2O_2), are increasingly used in a number of medical, food and industrial applications but also in environmental ones.

In the medical arena, oxidizing agents are particularly useful for hard surface disinfection and the high-level disinfection of medical devices. Their main advantages are their **broad-spectrum activity,** which includes **efficacy against bacterial endospores**, their **lack of environmental toxicity** following their complete degradation. H_2O_2 is particularly interesting for its application in liquid but also vaporized form for antisepsis and for the **disinfection of surfaces** and medical devices and for room fumigation (the so-called deep clean) [3].

Mode of action of hydrogen peroxide as a biocide

Hydrogen peroxide is extensively used as a biocide, particularly in applications where its decomposition into non-toxic by-products is important. For quite some time, it was believed that the Fenton reaction leading to the production of free hydroxyl radicals is the basis of hydrogen peroxide action and evidence exists for this reaction leading to oxidation of DNA, proteins and membrane lipids in vivo.

However, investigations of DNA oxidation suggest that the oxidizing radical is the ferryl radical formed from DNA-associated iron, not hydroxyl. Investigations of protein oxidation suggest that selective oxidation of certain proteins might occur, and that **vapour-phase hydrogen peroxide is a more potent oxidizer of protein than liquid-phase hydrogen peroxide**. Few studies have investigated membrane damage by hydrogen peroxide, though it is suggested that this is important for the biocidal mechanism.

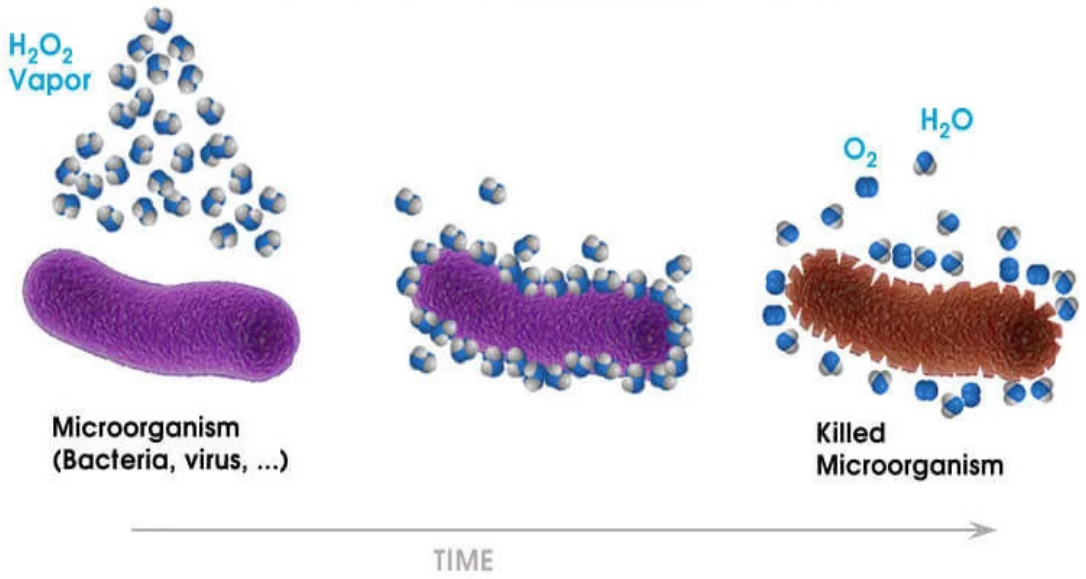
Hydrogen peroxide has been shown to inactivate a wide variety of infective biological agents ranging from both the vegetative cells and spores of bacteria and fungi (Rij and Forney, 1995; Rogers et al., 2005; Hall et al., 2008), protozoa and their cysts (Coulon et al., 2010), viruses (Pottage et al., 2010) and even prions (Fichet et al., 2007).

Oxidizing agents have been shown to react strongly with thiol groups in enzymes and proteins, DNA and the bacterial cell membrane [4].

Oxidative biocides, like gaseous H_2O_2, are proposed to have multiple targets within a cell as well as in almost every biomolecule; these include peroxidation and disruption of membrane layers, oxidation of oxygen scavengers and thiol groups, enzyme inhibition, oxidation of nucleosides, impaired energy production, disruption of protein synthesis and, ultimately, cell death [4].

Gaseous H_2O_2 interacts with amino acids (like cysteine, methionine, histidine, glycine, tryptophan and lysine), proteins (like BSA and aldolase), enzymes (tested on Alkaline phosphatase), RNA and DNA [2].

H_2O_2 interacts with amino acids, proteins, enzymes, RNA and DNA, /..../ ultimately leading to cell death



Further evidence clearly shows the gaseous H_2O_2 considerably outperforming the liquid system in terms of protein oxidation and their known antimicrobial activities [2].
We speculate that, the reason why gaseous H_2O_2 is more effective than liquid H_2O_2, is because this gas has higher kinetic energies and is uncharged, it can surround and penetrate the three-dimensional protein structures more easily, oxidizing buried cysteine residues and breaking vulnerable bonds between subunits. In contrast, fully dissolved (liquid) biocides might not be able to penetrate three-dimensional structures, although this may be facilitated by formulation effects (formulation being a mixture of chemicals in addition to the biocide).

We have not found, so far, one family of pathogens capable of resisting the exposure to gaseous hydrogen peroxide. Some pathogens will be affected faster or slower, depending on their structure.

Sources

[1] *Vamosvigyazo, 1981*

[2] *Finnegan et al., 2010*

[3] *Ezra Linley et al., 2012*

[4] *Denyer SP et al., 1998*