

Factors affecting the activity of ethylene oxide

The bactericidal activity of ethylene oxide is proportional to the partial pressure of gas in the reaction chamber, time of exposure, temperature of treatment and level and type of contamination. At room temperature, the time taken to reduce the initial concentration of cells by 90% can be very slow. For this reason elevated temperatures of 50–60 °C are recommended and these result in greatly increased rates of kill. Concentrations of ethylene oxide between 500 and 1000 mg L⁻¹ are usually employed. Relative humidity has a most pronounced effect, as at very high humidities ethylene oxide may be hydrolysed to the much less active ethylene glycol. This is borne out by the observation that the gas is 10 times more active at 30% RH than at 97% RH. The optimum value for activity appears to be between 28% and 33% RH. Below 28% RH the alkylating action of ethylene oxide is inhibited due to lack of water. The degree of dehydration of cells greatly influences activity and it may not be possible to rehydrate very dry organisms simply by exposure to increased RH. The RH value chosen in practice is usually between 40% and 70%.

Microorganisms may be protected from the action of ethylene oxide by occlusion within crystalline material or when coated with organic matter or salts. *B. subtilis* var. *niger* spores dried from salt-water solutions are much more resistant to the gas than are suspensions dried from distilled water.

Biological indicators used to test the efficacy of ethylene oxide treatment employ spores of *B. subtilis* dried on to suitable carriers, such as pieces of aluminium foil.

Formaldehyde

Formaldehyde (H.CHO) in its pure form is a gas at room temperature, with a boiling point of -19 °C but readily polymerizes at temperatures below 80 °C to form a white solid. The vapour, which is extremely irritating to the eyes, nose and throat, can be generated either from solid polymers such as paraformaldehyde or from a solution of 37% formaldehyde in water (formalin). Formalin usually contains about 10% methanol to prevent polymerization.

As with ethylene oxide, formaldehyde is a very reactive molecule and there is only a small differential in resistance between bacterial spores and vegetative cells. Its bactericidal powers are superior to those of

ethylene oxide (concentrations of 3–10 mg L⁻¹ are effective) but it has weak penetrating power and is really only a surface bactericide. It is also more readily inactivated by organic matter. Adsorbed gas is very difficult to remove and long airing times are required. Its mechanism of action is thought to involve the production of intramolecular crosslinks between proteins, together with interactions with RNA and DNA. It acts as a mutagenic agent and an alkylating agent, reacting with carbonyl, thiol and hydroxyl groups. In order to be effective, the gas must dissolve in a film of moisture surrounding the bacteria. For this reason, relative humidities in the order of 75% are required. Formaldehyde used in conjunction with low-temperature steam is a very effective sterilization medium.

Peracetic acid

The toxic nature of ethylene oxide and formaldehyde has prompted the search for further gaseous sterilants. Peracetic acid has been widely used as an aqueous solution but its use in the gaseous phase is more limited. It is a liquid at room temperature, requiring heat treatment to vaporize. Although it is highly active against bacteria (including mycobacteria and endospores), fungi and viruses, it is rather unstable and is damaging to certain materials such as metals and rubber.

Hydrogen peroxide

Hydrogen peroxide is similar to peracetic acid in that it is a solution at room temperature and must be heated to generate the gaseous phase. The main attraction of hydrogen peroxide as an antimicrobial agent is the fact that its decomposition products are oxygen and water. Most work on the antimicrobial properties of hydrogen peroxide has been carried out on aqueous solutions where it has been shown to have a good range of activity, including against bacterial spores. The biocidal efficacy of the vapour phase is less than that in solution and is influenced by environmental conditions.

Chlorine dioxide

Chlorine dioxide is a gas at room temperature but is primarily used in aqueous solution where it has