

Theory and Practice of Hydrogen Peroxide Vapour Bio-decontamination

Introduction.

Hydrogen Peroxide Vaporised (HPV) has become the method of choice for many bio-decontamination requirements in the pharmaceutical, biomedical and healthcare sectors because it is reliable, rapid, leaves no residues and may be validated, yet there is considerable misunderstanding about how it works and the physical chemistry of the process. By understanding the physical chemistry it is possible to optimise the process and hence not only ensure that the process is repeatable but it is also possible to reduce cycle times to a minimum.

Method of generating the hydrogen peroxide vapour.



Before we examine the equations governing the hydrogen peroxide vapour it is first necessary to understand how this vapour is produced. In most commercial hydrogen peroxide generators, such as the Clarus® C, an aqueous solution of hydrogen peroxide is evaporated in such a way as to produce the same weight ratio in the vapour phase as in the source liquid. This vapour is transported to the chamber to be bio-decontaminated in a heated carrier gas, initially air, and vapours from the chamber are returned to the gas generator where further quantities of the aqueous solution are evaporated. The most commonly used aqueous solution of hydrogen peroxide is 30% w/w and is frequently evaporated into the heated carrier gas stream to produce hydrogen

peroxide concentrations of in excess of 3,000 ppm or 4.5 mg/l. Care must therefore be exercised to prevent condensation of these high concentrations in the delivery system to the chamber by heating the walls of the delivery pipes.

Physical Chemistry of the hydrogen peroxide and water vapour mixtures.

The equations governing equilibrium vapour pressures of hydrogen peroxide and water vapour mixtures were first published by Scatchard et al¹ and Keyes². These equations were used by Watling et al³ to analyse the time it would take during a bio-decontamination cycle to reach saturation and then to

¹ Scatchard G. et al. (1952), *J. Am. Chem. Soc.*, **74**, 3715.

² Keyes, F. G. (1947), *J. Chem. Phys.*, **15**, 602.

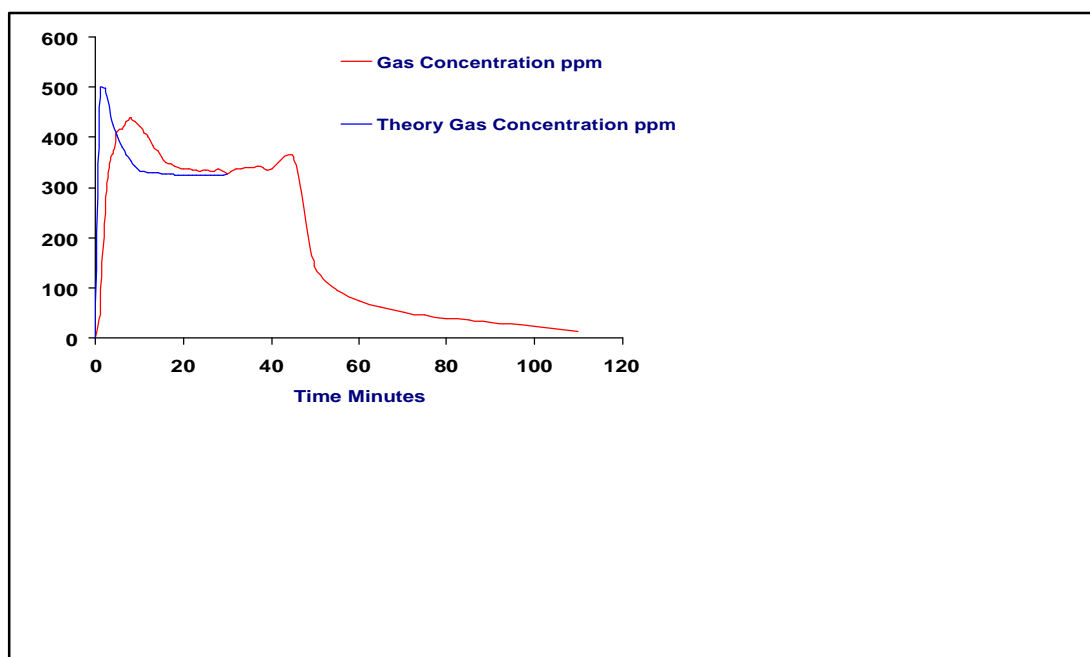
³ Watling D. et al. (2002), *PDA J. Sci. & Tech.* **56**, No 6, 291.

examine the theoretical equilibrium concentration of the condensate that forms and also the equilibrium concentrations of the hydrogen peroxide and water vapours.

This analysis assumed that any condensate and vapour are in equilibrium, and produced some interesting results. Hydrogen peroxide has a much lower vapour pressure than water and in an aqueous solution the vapour pressure of the hydrogen peroxide and water are both reduced because of the affinity of the water and hydrogen peroxide molecules for each other. However because the evaporation process used to generate the vapours produces the same weight ratios in the vapour phase as the source liquid the vapour concentration of the hydrogen peroxide produced by commercial generators is much higher than would be achieved in an equilibrium state at the same temperature.

Hence once saturated conditions have been reached and condensation occurs the concentration of the condensate may be expected to be at a higher concentration than the source liquid. If further quantities of aqueous solution are evaporated and delivered to the chamber condensation will continue to form, drawing from the vapour large amounts of hydrogen peroxide thus reducing the hydrogen peroxide vapour concentration. This reduced vapour concentration implies that the concentration of the condensate will also be reduced because the vapour and liquid are assumed to be in equilibrium. It should be remembered that our analysis assumes that the liquid and vapour phases remain in equilibrium. Eventually if large amounts of aqueous solution are evaporated and delivered to the chamber it may be expected that vapour concentrations in the chamber will tend towards the equilibrium vapour pressures of the source liquid at the chamber temperature.

The final part of the bio-decontamination cycle is the removal of the hydrogen peroxide from the chamber to a safe level; this is known as the aeration phase. This is generally achieved by passing clean air into the chamber and removing the peroxide by dilution, this process may be enhanced by passing the air/vapour mixture through a carbon filter to destruct the hydrogen peroxide vapour. At the start of the aeration phase a very interesting thing



happens, the hydrogen peroxide vapour concentration rises, despite the fact that clean air is being introduced into the chamber. This is because the condensate on the surfaces starts to evaporate, but because water has a much higher vapour pressure than hydrogen peroxide the water evaporates from the condensate faster than the hydrogen peroxide, thus increasing the concentration of the condensate. The increase in concentration of the condensate leads to a higher equilibrium vapour pressure of the hydrogen peroxide and hence an increase in the vapour concentration. The graph above was taken from a test on a small chamber and shows the theoretical and measured gas concentrations during a bio-decontamination cycle. The measured gas concentrations show similar characteristics to the theoretical but lag behind the theoretical values because of the response time of the instrumentation.

Deviation from the Theoretically predicted Vapour Concentrations

The equations used to predict the gas concentration during a bio-decontamination cycle using hydrogen peroxide vapour assume that the vapours and liquid are in equilibrium. In fact during the gassing phase of the cycle when condensation is forming and the aeration phase when it is evaporating the vapours and liquid cannot be in equilibrium.

Consider the condensation phase of the cycle. In an equilibrium state the number of molecules entering and leaving the liquid phase is equal, and hence the mass of condensate will remain constant. Conversely while condensation is forming the number of molecules entering the liquid phase must be greater than the number leaving and hence the vapour and liquid phases are not in equilibrium.

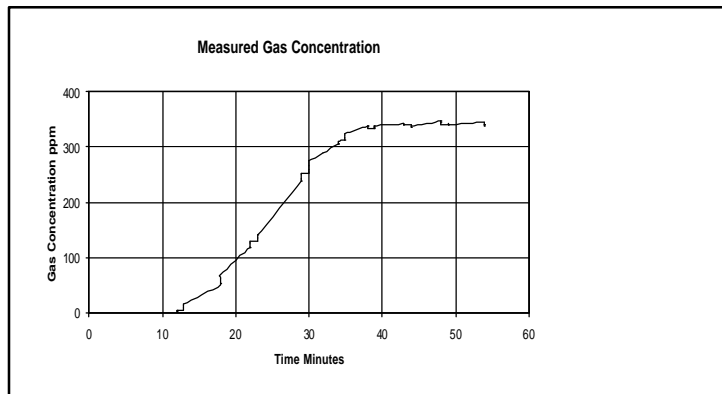
It follows that for condensation to form the vapour pressure must be greater than the equilibrium vapour pressure, but this is not the complete story. If the vapour is introduced into the chamber at a higher temperature than the walls of the chamber and hence at a higher temperature than any condensate, then that higher temperature will mean that the molecules may remain in the vapour state simply because they are at a higher temperature. It may therefore be expected that during the gassing phase of the cycle the gas concentration will be higher than the equilibrium value. The difference between the predicted equilibrium vapour pressure and the measured concentration will depend on the size of the chamber, the temperature of the supplied vapours, and the rate of delivery of the vapours. It is possible that this difference may be quite large and in small chambers has been observed to be several hundred ppm.

It may also be expected during the aeration phase that the vapour concentrations will be less than predicted from the equilibrium equations. At this stage of the cycle the liquid is evaporating and hence the condensate is giving up molecules and the reverse arguments apply to those during the formation of condensation, but there is no doubt that during the aeration phase the concentration of the condensate increases and this may add to the biological efficacy of the process.

Room Bio-decontamination

In larger volumes such as rooms there is likely to be a much closer relationship between the calculated equilibrium vapour concentration and the measured value, but it is unlikely that the initial peak in concentration predicted by the equations will be seen.

In the BIOQUELL Room Bio-Decontamination System (RBDS®) the hot



vapours are delivered into the room at high velocity by a rotating nozzle giving good distribution to all parts of the space. Within the plume of vapour delivered by the rotating nozzle the temperature of the vapours will fall generating an ever

changing mini-environment in which it is likely that some droplets will form at high liquid concentration. These droplets will re-evaporate as the plume spreads out providing the vapours within the room have not reached dew point. It follows that an instrument measuring the vapour concentration within the room will see values that increase until saturation is reached and then flatten out. The graph shows a typical gas concentration curve for room bio-decontamination.

Practice versus Theory

An understanding of the theory of the physical chemistry behind hydrogen peroxide bio-decontamination is very helpful when optimising the liquid evaporation rate, carrier gas flow, temperature of the delivered vapours and the time to reach condensation, for all of these parameters the equations give precise values. But because the equations assume that the vapours and condensate are in equilibrium there will always be a difference between observed and calculated vapour concentrations during a gassing phase of the cycle. This does not invalidate the use of these equations for this phase as the information derived from their use is a guide as to how the cycle may be expected to progress and will indicate when the process is not under control.

Because it is not possible to calculate directly the conditions during a gassing cycle BIOQUELL have developed some computer programmes to perform these iterative calculations. These calculators have been used to assist in designing gassing cycles and to optimise the conditions during bio-decontamination procedures.

Comparison with Formaldehyde

Formaldehyde gassing has for many years been the accepted process for bio-decontamination of microbiological safety cabinets, chambers and rooms, yet there has never been the same analysis of the physical chemistry of the process and hence optimisation and cycle development are much more difficult if not impossible. It also suffers from long exposure times, deposition of residues and the need for very high levels of humidity leading to heavy condensation, and more recently concerns have been expressed that it is carcinogenic.

It is interesting to note that although hydrogen peroxide vapour has only been commercially available since about 1990 that there is more efficacy data in the literature than there is for formaldehyde.

Conclusions and Summary

The theoretical work undertaken by BIOQUELL has led to a much better understanding of the physical chemistry of hydrogen peroxide and water vapours; this in turn has made it possible to improve the efficacy and reduce bio-decontamination cycle times. Vaporised hydrogen peroxide is now the process of choice in the pharmaceutical industry and has been proved to be effective in killing antibiotic resistant bacteria from surfaces in hospitals.

Much has been said and written about whether vaporised hydrogen peroxide bio-decontamination is a condensation or dry process, there can now be little doubt that it is most effective once micro-condensation has formed. Those that argue that it is a dry process must explain why it is more difficult to achieve a kill on surfaces that are hot. It would be expected that for a dry gas kills would be faster on hot surfaces as chemical reactions take place at a faster rate as the temperature rises, whereas if it is a condensation process it is predictable that kill is faster on colder surfaces where condensation forms more quickly.