VALIDATION

Factors Influencing Hydrogen Peroxide Gas Sterilant Efficacy

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There are three (3) major processing parameters that affect the inactivation of microorganisms by H_2O_2 gas. They are sterilant concentration, exposure time, and percent saturation, the latter being influenced by the temperature and/or humidity level in the enclosure. The presence (or absence) of air or other inert gases does not affect the ability of H_2O_2 gas to inactivate organisms; however, it can assist in, or obstruct, the delivery of the sterilant. In a flow through system, the air acts as a carrier to deliver the sterilant vapor to the sites to be decontaminated.

Selectively increasing the concentration of the sterilant has been shown to directly increase the microbial inactivation rate, as is the case with other sterilants. The maximum allowable (non-condensing) H_2O_2 gas concentration can be increased by raising the temperature of the enclosure and/or by using a more concentrated aqueous sterilant, as shown in Figure 1. The tradeoff here, of course, is that it takes time to heat and then cool an enclosure prior to use and additional handling precautions are recommended as the aqueous H_2O_2 concentration is increased. Pharmacia (Kalamazoo, MI) was able to successfully utilize both features in a large filling isolator application where an automated CIP system and hot air drying provided consistent surface temperatures of around 35°C.[1,2] H_2O_2 gas concentrations of 1.7 mg/liter were obtained during the Pharmacia studies when 50 wt. % H_2O_2 was simultaneously flashed vaporized into the infeed, filling, and outfeed sections of the isolator. An exposure time of less than 60 minutes was required to consistently sterilize numerous biological indicators under such conditions.

Flash vaporization differs from natural evaporation in that it produces a mixture of H_2O_2 and water vapor that has substantially the same weight percent composition as the multi-component liquid.[3] The validity of calculations for estimating the maximum allowable H_2O_2 gas concentration in air at a given temperature and humidity (Figure 2) was questioned by Marcos-Martin, *et al.*[4] who stated that "condensation is a phenomenon that cannot be avoided." Studies conducted by STERIS Corp. (formerly AMSCO) validated that flash vaporized H_2O_2 gas could exist in the vapor state at high concentrations within an isolator and that the physical state (i.e. vapor or condensate) at various surface temperatures were predictable.[5]

An increase in exposure time will also result in a corresponding increase in microbial inactivation if all other process parameters remain constant. A linear regression analysis of *G. stearothermophilus* spore survivors at varying exposure times did not show an initial lag phase or tailing in kill rate that has been observed for aqueous H_2O_2.[6] Rather, the sporicidal activity of H_2O_2 gas against various lots of *G. stearothermophilus* spore suspensions when dried on stainless steel was found to follow first order kinetics.

Increasing the temperature and/or decreasing the background humidity in an enclosure without increasing the H_2O_2 gas concentration tends to decrease the overall microbial inactivation rate by reducing the percent saturation of the gas. Percent saturation is the ratio of the actual concentration within an enclosure to the dew point concentration. A 3-fold variance in D-value can be seen in Figure 3 where 1.6 mg/liter of H_2O_2 gas was tested in an isolator under varying degrees of water vapor content.[7] The need for moisture in inactivating bacterial spores is not unique to this technology as 10-fold and 120-fold variances in D-value have been documented for ethylene oxide and heat processes, respectively.[8,9] This factor, although not necessarily controllable in a typical isolator, can be accounted for during the validation process, which will be discussed later in the chapter.

The concentration of H_2O_2 gas within an isolator will be reduced by its' reaction with various surfaces that the molecules come in contact with. Decomposition, absorption and/or adsorption tend to keep the isolator concentration below the theoretical inlet concentration, although losses from the vaporization process (<5%) also contribute to the difference. In addition, exceeding the dew point will lead to condensation of the sterilant on cooler surfaces, which will lower the gas concentration in warmer areas of the isolator. With the exception of condensation, all of these factors remain constant from one (1) decontamination cycle to another; therefore, they can also be accounted for during cycle development and validation.

Figure 1. Dewpoint Gas Concentration for Various Temperatures Upon the Flash Vaporization of Different Aqueous H_2O_2 Solutions into a 10% RH Air Stream

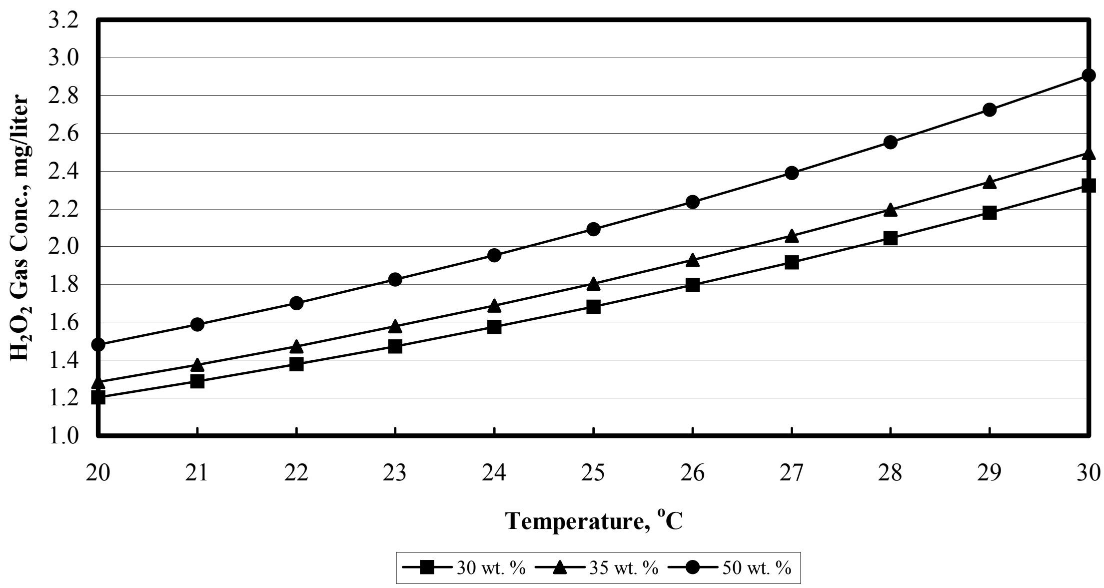


Figure 2. Effect of Temperature and Humidity on Dew Point Gas Concentration for Flash Vaporized 35 wt. % H_2O_2 Solution

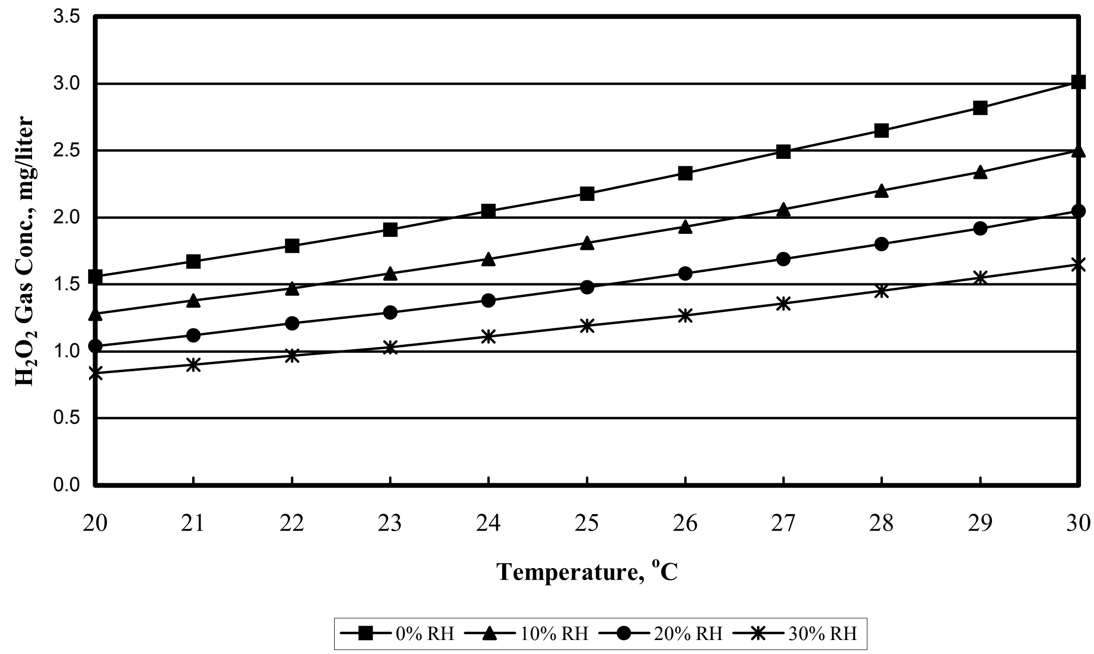
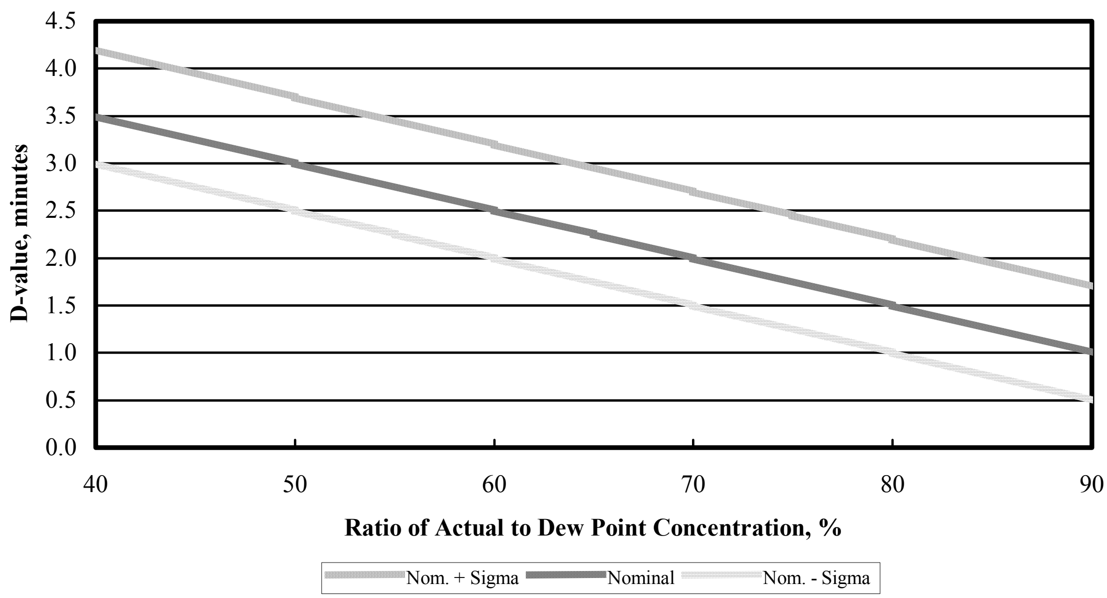


Figure 3. Effect of Percent Saturation on H_2O_2 Gas D-values When Tested at 1.6 mg/liter (Best Fit Data Obtained from VHP1000 Cycle Development Guide)



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