Airborne Microbial Challenges of Blow/Fill/Seal Equipment: A Case Study

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RESEARCH ARTICLE

Airborne Microbial Challenges of Blow/Fill/Seal Equipment: A Case Study

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ABSTRACT: Controlled microbial challenges, comprising air-dispersed spores of Bacillus subtilis *var* niger, *have been generated within a containment room (around 54 m^3 in volume) housing a Blow/Fill/Seal machine. 'Stirred-settling' conditions were created throughout the room and the airborne spore challenge was monitored to ensure homogeneity within the room for extended periods of time. The Blow/Fill/Seal machine was set to fill 2 cm^3 ampoules with Tryptone Soya Broth at each of three airborne challenge levels of 10^4, 10^6 and 10^7 spores m^{-3} (about 10^2, 10^4 and 10^5 spores ft^{-3} respectively). A relationship has been established between the level of airborne micro-organisms in the machine operating environment and the extent of product contamination. This relationship allows prediction of operating conditions under which a level of sterility assurance, equal to that demanded of terminal sterilization, is attained. It is stressed that the findings apply only to the particular Blow/Fill/Seal machine and to the specific conditions of machine operation.*

Introduction

Blow/Fill/Seal (or Form/Fill/Seal) technology has gained wide acceptance within the Pharmaceutical Industry, offering the manufacturer unrivalled flexibility in the packaging and presentation of sterile liquid products; it can be used for the manufacture of products that are subsequently processed by steam sterilisation and for products produced sterile via aseptic processing. Blow/Fill/Seal machines carry out both the forming of the primary packaging and the subsequent filling operation. In operation, thermoplastic polymeric granulate is heat moulded to form the primary packaging as a single container or a row of several containers. The mould holding the containers is moved to a filling station where the appropriate volume of product is delivered. The containers are then sealed and moved to a trimming station where excess polymer is removed and the product discharged for subsequent labelling and secondary packaging. Detailed description of the technology of Blow/Fill/Seal is available in the open literature (1, 2).

The use of Blow/Fill/Seal technology in association with sterilisation by filtration has, in recent years, come under considerable scrutiny from the Regulatory Authorities, and none more so than the Food and Drug Administration. Concerns surrounding the possibility of product contamination by airborne particles, and in particular viable micro-organisms, have led to considerable debate about the quality of the environment within which the machine is housed. Manufacturers and users of the technology have argued that the unique processing qualities of Blow/Fill/Seal machines warrant separate and specific standards applied to them and not, as at present, have conventional BS 5295 or the U.S. Federal Standard 209D imposed (3).

The argument that Blow/Fill/Seal technology should be considered a unique and novel aseptic processing technique is based upon critical operations being conducted under localised protection of a 'shower' of filtered air. Such circumstances, it is believed, decrease the probability of product contamination and give a low and acceptable Sterility Assurance Level (4). However, given the localised protection of an air shower, the influence of the quality of the environment housing the Blow/Fill/Seal machine on the sterility assurance of product is unknown and the value of monitoring this environment has to be questioned.

The present work was designed to address concerns surrounding the microbiological safety of Blow/Fill/Seal technology. The objective was to seek a relationship between the level of airborne microorganisms in the environment and the product contamination rate of a Blow/Fill/Seal machine. To achieve this end, controlled challenges of microorganisms dispersed in air, at concentrations extending over a 1000 fold range, have been generated within a containment room housing a Blow/Fill/Seal machine producing ampoules filled with medium that supports the growth of the challenge micro-organisms; air dispersed micro-organisms, comprising a given challenge, were distributed throughout the containment room at a fixed concentration maintained over an extended test period.

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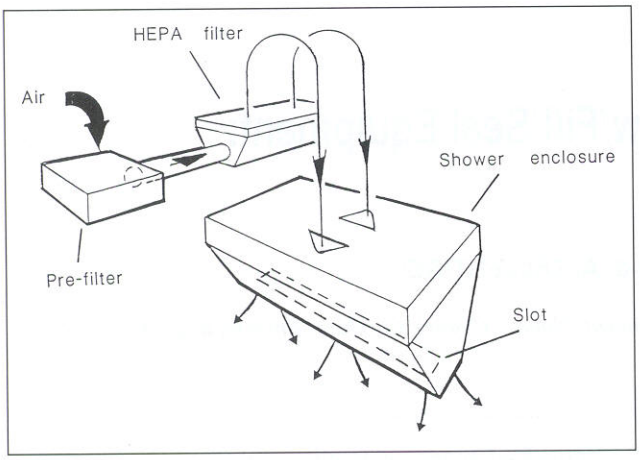

Figure 1—Air shower unit located over filling mandrels.

Materials and Methods

Blow/Fill/Seal Machine. A state-of-the-art Blow/Fill/Seal machine (ALP 624-015), designed to produce sterile liquid product via aseptic processing, has been used for test purposes. The machine has a twenty-four cavity mould with a 2 cm^3 fill volume; the filling mandrels are located within the protection of a shower of filtered air. Figure 1 is a diagrammatic representation of the air shower unit. At the core of the air shower is a HEPA filter unit (filter face area 30.5 × 30.5 cm) capable of filtering air to meet Class 10 conditions at the filter face, and therefore meeting the requirements of Federal Standard 209D. By control of the speed of the fan located in the unit, the face velocity of filtered air can be varied between 0.25 and 1.2 ms^{-1}. The total volume of the air shower, from HEPA face to enclosure exhaust is around 80 dm^3. Filtered air, originally drawn from the containment room, is forced downward through two parallel tubes and enters the shower enclosure deflected by two stainless steel plates. Air exhausts from the shower enclosure via a slot (3.7 × 40.5 cm) in which the filling mandrels are positioned. When the HEPA fan is operating maximally, an air velocity of 4.0 ms^{-1} was measured along the entire length of the enclosure slot.

For each level of microbial challenge, the Blow/Fill/Seal machine was set up according to the manufacturer's protocol for "media-fill validation." Filling lines were cleaned *in situ* and then sterilised by exposure to steam. Two liquid filters (nominal pore size 0.2 μm), located in series upstream of the fill tubes, were tested for integrity. A fill volume of 2 cm^3 was set and Tryptone Soya Broth, tested to meet minimum USP fertility level, employed as the fill medium. The rate of machine operation was fixed at 1 cycle (24 ampoules) per 12 s, giving a production rate of 120 ampoules min^{-1}. This rate was maintained throughout the present experimental work.

Containment Room. Control of the microbial challenge was achieved by containing it within a room housing the Blow/Fill/Seal machine. The room, measuring 5.54 × 4.52 × 2.77 m (69.5 m^3), is shown schematically in Figure 2. All services to the machine were remote, enabling its operation without the need for personnel to enter the room; product, conveyed on the de-flash product belt, was removed from the room via a tunnel incorporating a clean-up system to prevent release of airborne micro-organisms. Monitoring of the microbial challenge within the containment room was carried out through three ports, each positioned at a selected location on one of three walls of the room. Each port was on the vertical mid-line of its wall, at a height of 0.5, 1.2 and 2.2 meters for East, South and West walls respectively (see Fig. 2).

Test Organism. Endospores of *Bacillus subtilis* var. *niger* (NCIB 8056) were selected as the test organism; they exist as discrete cells, produce readily scoreable colonies, and are robust and thus able to withstand the physical stresses associated with manipulative procedures. Furthermore, the spores, which are elliptical in shape and around 0.3 μm^3 in volume, are highly stable when individually dispersed in air. Such discrete spores present a rigorous airborne challenge.

The source of the *B. subtilis* NCIB 8056 was a spore suspension obtained from the National Collection of Industrial Bacteria in 1977, and stored at 4°C since that time. Suspensions at concentrations of around 5×10^6, 5×10^7 and 5×10^8 spores cm^{-3} were produced for the present work. Surface growth from an isolated colony of the test organism, grown on TGY agar (Oxoid Ltd), was inoculated into MR-VP liquid medium (Oxoid Ltd). This subculture was incubated at 37°C for 8 hr, after which time it comprised a population of cells in logarithmic phase. The vegetative cell suspension was flooded onto the surface of a number of TGY agar plates and the plates incubated at 32°C for 7 days to provide essentially 100% sporulation. Spores were harvested by aspirating the surfaces of plates with sterile distilled water; the suspension, derived from pooling these aspirates, was filtered through a No. 3 sinter glass filter (nominal pore size range 15 to 40 μm) and subsequently washed, by centrifugation and resuspension three times in sterile distilled water. Appropriate aliquots of washed suspension were diluted to yield working suspensions of desired spore concentration.

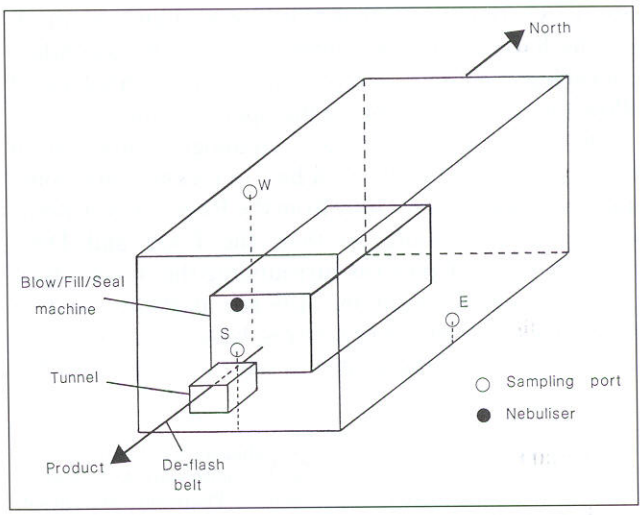

Figure 2—Schematic representation of the containment room indicating locations of sampling ports and nebuliser.

Microbial Challenges. A multi-jet climbing column nebuliser (patent pending) was used to generate air-dispersed spores at concentrations of around 10^4, 10^6 and 10^7 spores m^{-3} of air of the containment room by aerosolisation of aqueous spore suspensions at corresponding concentrations of 5×10^6, 5×10^7 and 5×10^8 spores cm^{-3}. The nebuliser was positioned within the room on the plane of the mid-line of the south wall, 1 m from that wall at a height of 2 m (see Fig. 2); the positioning of the nebuliser was not critical and the ultimate location was chosen to facilitate nebuliser servicing. However, the proximity of the nebuliser to port S is noted. Routinely, the nebuliser was charged with 400 cm^3 of the appropriate spore suspension and supplied with air at 1 bar (15 psi). Under continuous operation, the consumption rate of spore suspension was around 1 cm^3 min^{-1}. In practice, the nebuliser aerosolised the spore suspension into droplets of sufficiently small size (mean diameter around 4 μm) which, on expansion into the air of the containment room, evaporated 'instantaneously' to leave discrete spores dispersed in air. The spores were distributed throughout the containment room with the aid of four electrically driven 'upward drift' fans placed at floor level and located on diagonal axes 1 m from each corner.

The concentration of spores dispersed in the containment room was monitored by collecting spores present in a sample volume of 1.2 dm^3, drawn isokinetically from the room via each of the three sampling ports, on a 47 mm soluble gelatin filter (Sartorius, GmbH). Collected spores were recovered by dissolution of the filter in an appropriate aliquot of sterile distilled water and enumerated by surface plate counts on TGY agar. The gelatin filter (nominal pore size 3 μm) achieves essentially 100% collection efficiency for air-dispersed *B. subtilis* spores. A determination of the concentration of air-dispersed spores was made at each of the three sampling locations at intervals of 15 min.

Test Design. The Blow/Fill/Seal technology was challenged separately with each of three nominal concentrations of spores in the air of the containment room. For each concentration, the period of microbial challenge lasted a minimum of 135 min during which the following three activities were performed for the whole of the period:

1. continuous operation of the Blow/Fill/Seal machine employing medium fill
2. continuous aerosolisation of appropriate spore suspension
3. periodic sampling of the room air at each of the ports at intervals of 15 min.

Two distinct sequential test phases occurred during each microbial challenge; Phase 1, lasting a minimum of 90 min, when the air shower was not operated, and Phase 2, lasting at least 45 min, when the air shower was operated maximally. The disparity in duration of the two phases came about because the initial 30 min or thereabouts of Phase 1 was utilised to establish the level of spore concentration in the room air. For practical purposes, the starting point of the period of microbial challenge (0 min) corresponded to the commencement of aerosolisation of the particular spore suspension.

To allow product contamination, expressed in terms of the fraction of ampoules contaminated for each minute of production, to be related to production conditions, all broth-filled ampoules were identified relative to the time of production within the challenge period. Immediately after production, the broth-filled ampoules were incubated at 30–35°C for 14 days so that contamination of individual ampoules could be assessed by the appearance of visible growth.

Results

One challenge concentration, generated by aerosolisation of a spore suspension containing 5×10^8 spores cm^{-3}, has been chosen to illustrate the general findings of spore distribution within the containment room and of corresponding contamination rate of product flowing from the Blow/Fill/Seal machine.

Figure 3(a) is a plot of the estimates of the concentration of spores in the room air sampled at the three different locations (E, S and W) against time covering the entire challenge period. It can be seen that, for each sampling occasion, the three estimates fall within a 2–3 fold range. However, in all instances, estimates made at location S exceeded those made at E and W; a likely explanation of this observation is the proximity of the nebuliser to location S. Nonetheless, the relatively narrow range covering the estimates of spore concentration, observed on any one occasion at distant locations of differing heights within the room, indicates active dispersal of spores throughout the air in the room to give reasonably homogeneous distribution. Given this distribution of spores, the overall concentration in the room air, at any one occasion, is represented by the mean of the three estimates obtained at the different sampling locations. The solid diamonds included on Figure 3(a) are the individual values of this mean, corresponding to the 11 sampling occasions falling at regular intervals throughout the challenge period. At 15 min, the mean spore concentration was around 1.7×10^7 spores m^{-3} and this level was maintained throughout the remainder of Phase 1 (no air shower). In the present test, switch-on of the air shower occurred at 112 min (corresponding to commencement of Phase 2), and it can be seen that on the sampling occasion immediately following this time,

TABLE I
Overall Challenge Conditions Achieved in the Containment Room

Conc. Spores in Nebuliser (Spores cm^{-3})	Challenge Concentration (Spores m^{-3})	
	Phase 1 (Air Shower Off)	Phase 2 (Air Shower On)
5×10^6	$4.1 \times 10^4 \pm 6.6 \times 10^3$*	$1.9 \times 10^4 \pm 5.1 \times 10^3$
5×10^7	$1.4 \times 10^6 \pm 1.6 \times 10^4$	$1.3 \times 10^6 \pm 2.3 \times 10^5$
5×10^8	$1.7 \times 10^7 \pm 1.7 \times 10^6$	$1.3 \times 10^7 \pm 2.1 \times 10^6$

* Standard error

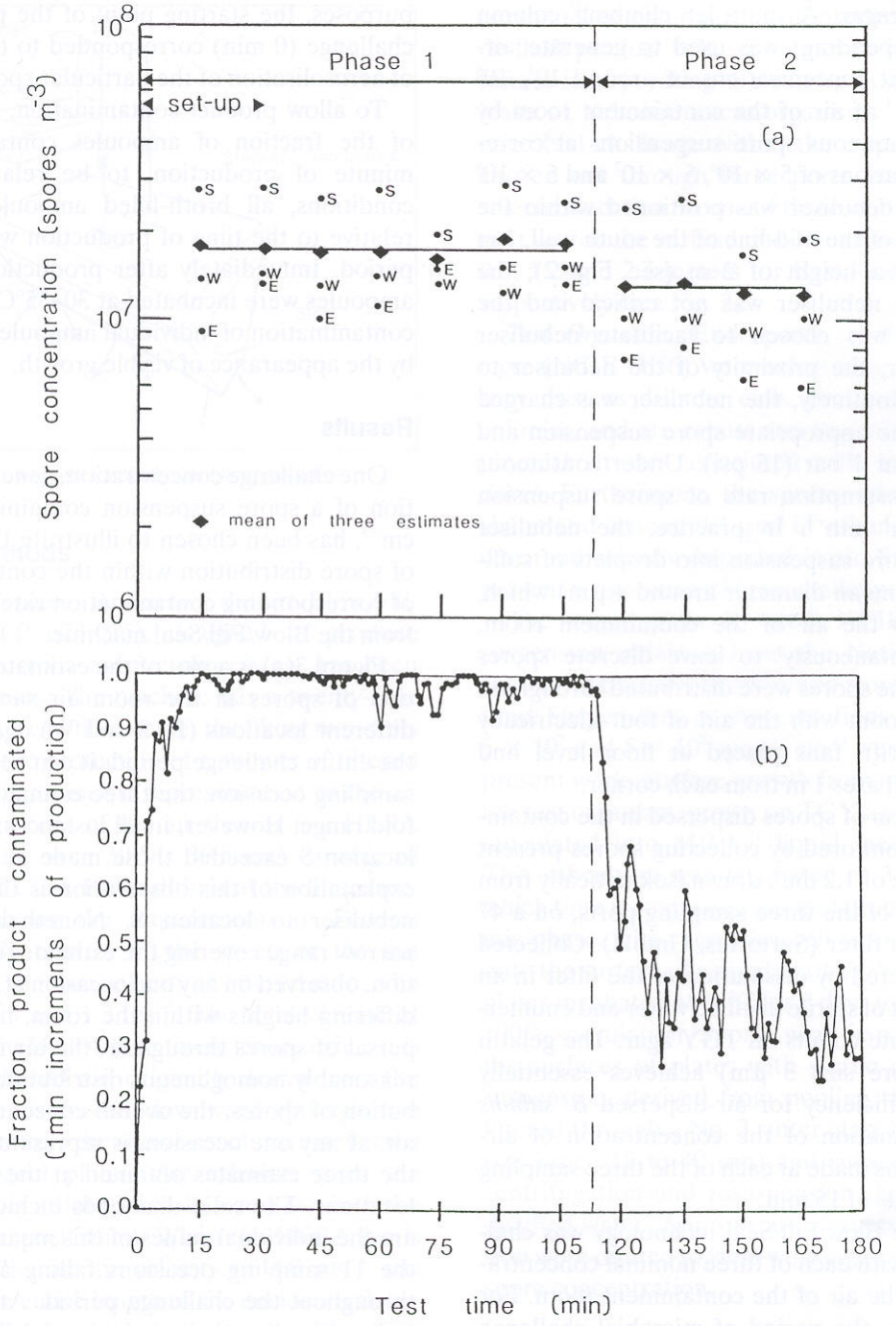

Figure 3—(a) Typical estimates of the concentration of air-dispersed spores present within the containment room during the 2 phases* of the challenge period. (b) Fraction of product contaminated corresponding to phases of the challenge period referred to above. *Vertical dashed line indicates the time when the air shower was brought into operation.

the mean spore concentration fell to a level around 1.3×10^7 spores m^{-3}. This lower level was maintained during the remainder of Phase 2 and it probably resulted from the removal of spores from the room air by the filtering action of the air shower.

Figure 3(b) shows the corresponding data for the fraction of contaminated product observed at minute intervals throughout the challenge period. It should be noted that outside the challenge periods, when no spores were detected in the air of the containment room, no product contamination was observed in 15,000 ampoules examined and, hence, at 0 min, zero product contamination is recorded on Figure 3(b). On introduction of air-dispersed spores into the room, product contamination was evidenced immediately with a fraction contaminated of 0.23 at 1 min. This fraction increased progressively during the set-up period to reach a level close to 1.0, which was maintained during the remaining period of Phase 1 (air shower off). On operation of the air shower (Phase 2), the fraction of contaminated product decreased rapidly to a level within the range 0.25 to 0.65 throughout Phase 2.

Data, similar to those shown in Figure 3, were generated for the other two spore challenges. Table I summarises the overall challenge conditions achieved within the containment room. It lists the mean concentration of air-dispersed spores, generated during each phase of the challenge period, for the three spore challenges; each mean value, and its associated standard error, was derived from individual estimates of concen-

tration of air-dispersed spores made at the three sampling locations during Phases 1 and 2 of the challenge period, excluding those estimates made during the initial 30 min set-up period of Phase 1. It can be seen from the table that controlled challenges were established over a 400 fold range of spore concentration for Phase 1 (air shower off) and a 600 fold range for Phase 2 (air shower on).

Figure 4 shows plots, on logarithmic scales, of the overall fraction of product contaminated against spore challenge concentration for the Blow/Fill/Seal machine operating with and without the protection of the air shower. The overall fraction contaminated is the ratio of the number of contaminated ampoules to the total number of ampoules produced during the particular challenge phase, again excluding from Phase 1 consideration of the initial 30 min set-up period. It should be noted that the total number of ampoules produced in any one phase was maximally 9500 and minimally 5600. The plots reveal that, for both modes of machine operation, there is a strong direct relationship between fraction product contaminated and spore challenge level, over the range of challenge levels examined. However, the curve describing the relationship with the air shower operating is clearly shifted downwards from that seen without operation of the air shower, the magnitude of this shift being around 10 fold for those portions of the curves that are linear.

Discussion

The experimental approach underlying the present fundamental investigation of Blow/Fill/Seal technology has been to establish, over extended time periods, controlled challenges of air-dispersed spores distributed throughout the environment within which the Blow/Fill/Seal machine operated. While the test design has a number of novel features, the key element has been the application of a basic aerobiological technique known as 'stirred-settling' (5). The principles of this technique are as follows:

> All aerosols, comprising particles dispersed in air, decay under the influence of gravity, i.e. particles fall out. Under the specific conditions of stirred-settling, in which air-dispersed particles are actively distributed throughout a volume by air turbulence, particle concentration remains homogenous throughout the volume even though the aerosol is decaying. Furthermore, the rate of aerosol decay is determined by the effective height of the air volume and by the particle mass, and is independent of the particle concentration.

To set spore challenge concentration within the containment room under conditions of stirred-settling, it was necessary to balance the rate of production and the rate of loss of air-dispersed spores. This balance was achieved by continuous aerosolisation of a suspension of spores at a predetermined rate during the initial 30 min set-up period and throughout the remainder of the challenge period. The independence of stirred-settling decay on particle concentration enabled spore challenges to be generated over a wide range of spore concentrations by simply varying the rate of production of air-dispersed spores through aerosolisation of spore suspensions at different spore concentrations. The closeness of the estimates of the concentration of air-dispersed spores made at different locations and at different times during the effective challenge period is a measure of the degree of success in achieving stirred-settling behaviour in a containment volume substantially in excess of that normally utilised. Furthermore, such behaviour was evident over a wide range of spore challenge concentration. In this first series of experiments, practical considerations dictated that the lower limit of spore challenge concentration be established at around 10^4 spores m^{-3}, but given the experience gained in this work it should be feasible to design a test employing challenges at least 10× lower than those used here.

Results of challenging an operating Blow/Fill/Seal machine with air-dispersed spores have provided, for the first time, unequivocal evidence that the level of airborne microorganisms in the machine environment has a profound effect on the level of product contamination. Under challenge conditions employed to date, the fraction of product contaminated was found to vary from less than 0.001 to close to 1.0, i.e. > 1,000 fold range. From the viewpoint of manufacturing operations, it is also important to note that product contamination was evidenced immediately on introduction of air-dispersed spores to the machine environment.

For each mode of machine operation, air shower off or air shower on, a direct relationship between fraction product contaminated and spore challenge concentration has been established. The parallel nature of the effective parts of the relationships demonstrates dramatically the impact of the air shower on the performance of the Blow/Fill/Seal machine. For a given level of spore challenge concentration, a 10 fold reduction in product contamination was seen with the air shower on as opposed to the air shower off. Equally, around a 10 fold

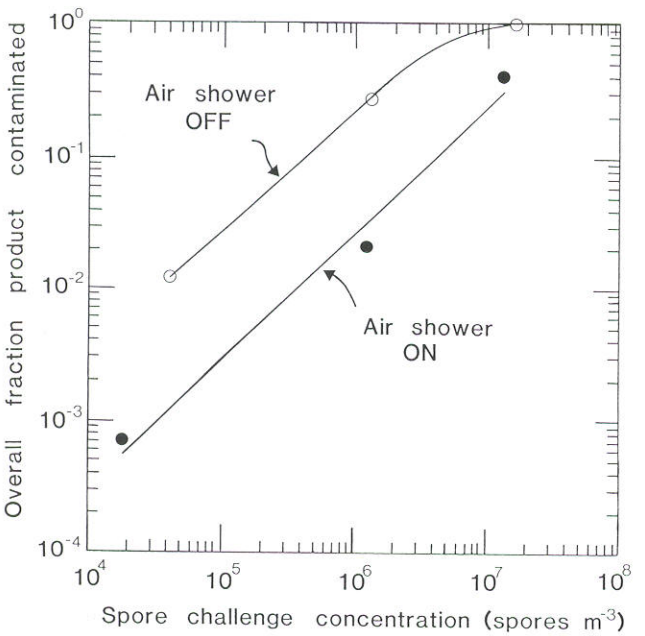

Figure 4—Fraction of product contaminated as a function of spore challenge concentration for the Blow/Fill/Seal machine operating with and without the protection of the air shower.

decrease in spore challenge was required with the air shower off over that for the air shower on to achieve the same level of product contamination. The linearity, exhibited by the lower portions of the curves shown in Figure 4, provides a reasonable basis for extrapolation of the curves to lower levels of fraction product contaminated. Such extrapolation has been undertaken in Figure 5, in which the solid lines are the curves of Figure 4 relocated on more extensive axes and the dashed lines are extrapolates of the curves. The resulting predictions relate the fraction of product contaminated and spore challenge concentration over levels of product contaminated that defy measurement. Such levels then have to be expressed in terms of the probability of product contaminated (left vertical axis) which is directly comparable to Sterility Assurance Level (right vertical axis). These extrapolations, while tentative, imply that a Sterility Assurance Level similar to that targeted for product given terminal sterilisation is achievable with properly controlled Blow/Fill/Seal technology.

It is essential to recognise that the findings described above, and their interpretation, apply only to the particular Blow/Fill/Seal machine and to the specific conditions of machine operation. The influence of machine design, together with variables associated with machine operation (e.g. production rate, ampoule geometry, fill volume, air shower setting, air supply to containment room), are unknown at this point in time. Nonetheless, this early series of investigations, constituting only a beginning to the overall fundamental studies needed, has major significance to the application of Blow/Fill/Seal technology in aseptic processing. The investigations show that:

a) the microbiological quality of the operating environment within which the Blow/Fill/Seal machine is housed is relevant to the level of product contamination,
b) a direct relationship exists between the extent of product contamination and the level of airborne microorganisms and this could allow prediction of operating conditions under which an acceptably low Sterility Assurance Level is attained,
c) operation of a protective air shower around the filling mandrels reduces the level of product contamination.

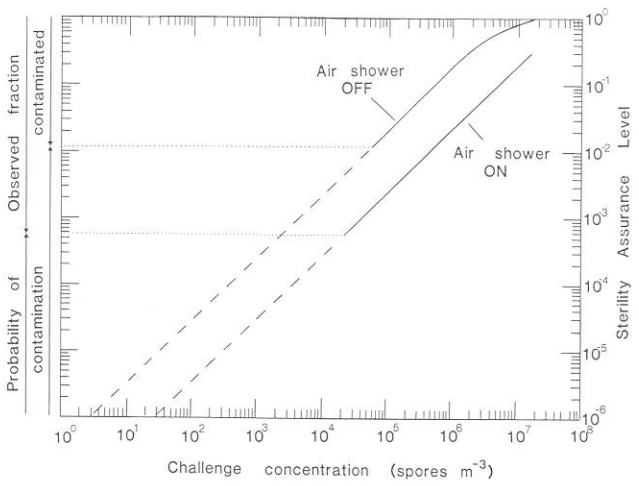

Figure 5—Extrapolation of experimentally derived curves given in Figure 4.

Assuming that, with time, it is possible to rationalise machine design and conditions of machine operation in relation to product contamination, it will be feasible to operate routinely Blow/Fill/Seal machines to a known, low Sterility Assurance Level. In other words, the opportunity exists with aseptic processing to meet a Sterility Assurance Level demanded of terminal sterilisation (i.e. 10^{-6}).

Acknowledgment

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