Predictive Sterility Assurance

for

Aseptic Processing

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Introduction

Terminal sterilisation and aseptic processing constitute the two principal methods of manufacture of sterile pharmaceutical product. Of these two methods, aseptic processing has become more associated with sterility failure. Inevitably, this has led to aseptic processing coming under considerable scrutiny from Regulatory Authorities, none more so than the Food and Drug Administration. It is increasingly the expressed view of authorities that the sterility assurance of product processed by aseptic methods must be improved in order to reduce the risk of harm to the public (1).

Conventional methods to estimate sterility assurance for product prepared by aseptic processing are based upon 'medium-fill' studies. Typically, in such a study, sterile microbial growth medium is processed through the entire manufacturing and filling operation, followed by incubation of the final product and subsequent examination for bacterial growth (2). Routinely, at least 3,000 units are filled to detect a contamination rate of not more than one contaminated unit per thousand units filled. The resultant estimate of contamination level of 10^{-3} compares unfavourably with a Sterility Assurance Level (SAL) of 10^{-6} targetted for product processed by terminal sterilisation. However, it is often claimed that advanced aseptic processing technologies which eliminate or minimise human intervention can achieve contamination rates considerably lower than the usual target rate of 1 in 1,000. To test the validity of such claims, a new approach to estimating sterility assurance for aseptic processing beyond conventional use of medium-fill studies is essential.

Blow/Fill/Seal processing is a fully automated technology that is designed to be operated remotely when contained within a controlled environment. It is thus an ideal test system for examination of the relationship between the extent of product contamination by airborne micro-organisms during medium-fill studies and the level of airborne micro-organisms in the environment. Moreover, noting experimentally how changes in operating conditions influence the level of product contamination may well provide a better understanding of the manner in which processing factors impact upon microbiological quality of product. Given this understanding, prediction of sterility assurance for aseptic processing becomes a realisable goal.

This article describes a series of experiments (designated Study 2) that is a follow-up to earlier published work describing Study 1 (3). Together, these two studies are aimed at defining the exact form of the relationship between fraction of product contaminated and level of microbial challenge over an extensive range of challenge concentrations for Blow/Fill/Seal processing operating under a particular set of conditions. In addition, Study 2 was designed to examine whether the mode of air shower operation, variation in production cycle time and 'interventions' may produce discernible effects on the fraction of product contaminated.

Microbiological Challenge Studies - Design Considerations

Certain specific problems associated with the design of an airborne microbiological challenge test for aseptic processing may be considered under the following headings:

Nature and Production of the Microbial Challenge. In view of the diversity of micro-organisms present in the environment and the form in which they occur, it can be justifiably argued that more than one type or species of organism should be employed in challenge studies. However, in choosing any organism, due regard must be given to non-pathogenicity, ease of production and recognition, viability, average cell size and size distribution. Furthermore, an airborne challenge may consist of the micro-organisms contained within liquid droplets, located on solid particles or as a dispersion of discrete cells in air. For a given species of micro-organism, a dispersion comprising discrete cells is made up of the particles of the smallest possible dimension for that species and is very likely the most rigorous challenge that can be devised. Logically, such a dispersion is the challenge of choice. The production of a dispersion can be achieved by aerosolisation of a liquid suspension of cells into droplets of sufficiently small size that, on expansion into a gaseous fluid, instantaneous evaporation of the liquid droplet occurs, leaving the discrete cells suspended in the gas. Dispersions so formed are referred to as 'dry'. Nebulisers restricting droplet size and so giving rise to dry dispersions have been described (4).

Sampling of the Microbial Challenge. In order to estimate the concentration of the microbial dispersion used to challenge the process, there is a requirement to detect accurately, and with a high degree of precision, micro-organisms present in a known volume of dispersion. Quantitative detection is best achieved by making use of any one viable micro-organism, recovered from the dispersion, to produce a visible colony when incubated on solidified growth medium. In practice, the collecting device must be able to recover micro-organisms from air dispersions

containing a wide range of concentrations. The characteristics, specifications and performances of various devices for collecting micro-organisms have been detailed (5). An air sampler, based upon filtration, has proved particularly useful for sampling microbial challenges over a wide range of challenge concentrations.

Containment of the Microbial Challenge. In order to present the microbial challenge to the Blow/Fill/Seal machine in a controlled and reproducible fashion, it is necessary for the air dispersed micro-organisms to be contained within defined limits. However, all contained dispersions of particles in air undergo decay primarily due to the influence of gravity, i.e. particles fall out (6). A form of decay known as 'stirred-settling' occurs if sufficient air turbulence is created so that, even though the dispersion is decaying under the influence of gravity, generally a uniform distribution of particles exists throughout the contained volume. Under these conditions, for a given particle mass, the rate of decay is independent of particle concentration but inversely related to the height of the containment volume (6). To maintain dispersion concentration under stirred-settling conditions, it is then necessary to balance loss of particles from the dispersion through decay with controlled introduction of dispersed particles into the containment volume.

Microbiological Challenge Studies - Developmental Work

Laboratory based Studies. In order to develop the basic techniques to generate controlled microbial challenges, laboratory based studies have been carried out to investigate the production and containment of air dispersed micro-organisms.

A containment vessel of volume 0.13 m^3 was constructed from sheet

aluminium with the dimensions as shown in Figure 1. Four access ports were located in the chamber walls. Two of these ports were used in charging the chamber with dispersion; the inlet port was connected directly to a modified Collison nebuliser (4) and the outlet port exhausted to atmosphere via a filter to allow equilibration of pressure within the chamber during nebulisation and sampling. The two remaining ports were used for sampling purposes. An 'upward drift' electrically driven fan (~ 300 r.p.m) was positioned centrally on the base of the chamber.

Figure 2 shows the behaviour of air dispersed spores of *Bacillus subtilis* var. *niger* contained under stirred-settling conditions within this laboratory vessel. Dispersions at concentrations ranging from 10^1 to 2×10^6 spores dm^{-3} were generated by 30 min aerosolisation of spore suspensions at different concentrations ranging from 1×10^4 to 7×10^8 spores cm^{-3}. Following the initial 30 min charging of the vessel, the spore dispersions were held under stirred-settling conditions, without further nebulisation, and sampled at 60 min intervals over a 300 min period. It is clear from the figure that spore dispersions undergo decay with the rate of dispersion decay being constant over the 300 min period (half-life of 188 min) and essentially the same over a 10^5 fold change in spore concentration.

Figure 3 shows the behaviour of spore dispersions held within the laboratory vessel under stirred-settling conditions during the continuous aerosolisation of spore suspensions at different concentrations ranging from 1×10^4 to 7×10^8 spores cm^{-3}. It is evident from the figure that, for each of the four different

dispersions, the concentration of air dispersed spores attains a plateau level after around 60 min aerosolisation, and that this level is maintained throughout the subsequent period of nebuliser operation. Furthermore, the spore concentration at the plateau level is observed to be directly related to the spore concentration of nebulised suspension. These findings demonstrate the potential of employing containment conditions of stirred-settling, coupled with continuous introduction of dispersion to the contained volume, to generate microbial dispersions of fixed and controlled concentration.

Scale-up Studies. To develop the above laboratory based methods in order to allow generation and maintenance of controlled airborne microbial challenges over prolonged time periods in the environment surrounding a Blow/Fill/Seal machine, it has been necessary to carry out initially a number of scale-up experiments. This work was carried out employing a 'mobile cabin' modified to serve as a containment room (Figure 4); the volume of the cabin was 24.6 m^3, representing around a 190 fold increase in volume over the laboratory vessel. A sampling port was located on each of the two end walls of the cabin, Port 1 at a height of 0.5 m and, Port 2 on the opposite wall, at a height of 1.5 m. A multi-jet climbing column nebuliser (3) was positioned centrally within the cabin at a height of 1 m; a supply line to the nebuliser ensured that it could be recharged with spore suspension during operation. Four electrically driven 'upward-drift' fans were placed at floor level and located on diagonal axes 1 m from each corner.

Figure 5 shows the behaviour of a given spore dispersion held within the mobile cabin as a function of time; this particular dispersion was generated by

continuous aerosolisation of a suspension of 3×10^3 spores cm^{-3}. It is seen from the figure that, over an extended experimental period (0.5 to 9.25 h), spore concentration as estimated at the two distant locations of differing heights fall around a mean level of 6.5×10^2 spores m^{-3}. The constancy of spore concentration within the room throughout the 8.75 h experimental period provides strong evidence of the feasibility of producing controlled microbial challenges within the relatively large volume needed to enclose a Blow/Fill/Seal machine.

Material and Methods

Study Set-up. A previous communication (3) gave a detailed description of the experimental set-up used to generate, and to maintain over specified time periods, controlled airborne microbial challenges in the enclosed space surrounding an operating Blow/Fill/Seal machine. The test method, the basic test design and the Blow/Fill/Seal machinery used in the present work were identical to those described before.

The containment room (67.5 m^3) was sited at Weiler Engineering, Elk Grove, Arlington Heights, Chicago, a location where no sterile pharmaceutical production is carried out. Blow/Fill/Seal machine, ALP 624-017, was tooled to the same specification and mould configuration as those of ALP 624-015, the machine used in our previous work (Study 1). The configuration provided 24 moulded ampoules each with a 2 cm^3 fill volume. The operation of the machine was identical to that used in earlier work, namely production of 24 ampoules (1 cycle) every 12 s to give an overall production of 120 ampoules min^{-1}.

The air shower unit of machine ALP 624-017, developed to provide 'local

protection' of filling mandrels, was of the same basic design as previously described (3). Prior to commencement of the experimental work, the HEPA filter unit within the air shower assembly was DOP tested and rated as 99.99% efficient. By controlling the speed of the fan located in the unit, the velocity of air emerging from the air shower outlet, a slot running the length of the base of the shower enclosure, could be varied between 1.6 and 3.7 m s^{-1}; at a given fan speed, the velocity of emerging air was found to be constant over the entire length of the slot.

For each challenge test, the Blow/Fill/Seal machine was set-up according to the machine manufacture's protocol for 'medium-fill validation'. The fill medium used was heat sterilised Tryptone Soya Broth, tested to meet minimum USP fertility level. The medium was delivered to the point-of-fill after passing through two in-line liquid filters (nominal pore size 0.2 μm) that were tested for integrity at the end of each day's experimentation.

Microbial Challenges. Air-dispersed spores of *Bacillus subtilis* var. *niger* (NCIMB 8056) comprised the microbial challenge. Aerosolisation of an aqueous spore suspension at a predetermined concentration gave spores dispersed throughout the air of the containment room at a given challenge concentration over the range 3×10^2 to 3×10^6 viable spores m^{-3}. The test duration, during which the challenge spore concentration was maintained at a nominal level, varied according to concentration, the lower the concentration, the longer the duration, a longer test duration being required at low spore challenge concentration to allow detection of the frequency of ampoule contamination. In practice, test durations

ranged from 1 to 10 h. The concentration of spores in the air of the containment room was monitored intermittently during each challenge test by collecting spores present in sample volumes of air, drawn isokinetically from the room via three sampling ports (3).

Test Design. Irrespective of test conditions, the following activities were carried out throughout the duration of the test:

1. continuous operation of the Blow/Fill/Seal employing medium-fill
2. continuous aerosolisation of appropriate spore suspension
3. periodic sampling of the containment room air.

In practice, a minimum of four sampling operations, comprising replicate sampling at the three access ports, were carried out for each challenge test. As previously described (3), each port was positioned at a selected location on one of three walls of the containment room; the ports were E (east), S (south), and W (west). For a given challenge test, the spore challenge concentration is the mean value of spore concentration derived from individual estimates of concentration made at the different sampling locations during the test time.

Immediately after production, all ampoules were incubated at 30-35°C for 14 days so that contamination of ampoules could be assessed by appearance of visible growth. To allow measurement of fraction of product contaminated, expressed in terms of the ratio of number of contaminated ampoules to total number of ampoules produced, each individual ampoule was identified relative to time of production and filling location.

Results

Stability and Uniformity of the Spore Challenge. One challenge concentration, generated by nebulisation of a spore suspension containing around 6×10^7 spores cm^{-3}, has been chosen to illustrate the general findings when spore challenges were generated within the contained environment housing an operating Blow/Fill/Seal machine.

Figure 6 is a plot of the estimates of the concentration of spores in the containment room air sampled at the three different locations (E, S and W) against time covering the entire challenge period (530 min). At t = 0 min, aerosolisation of the spore suspension commenced and was maintained through the entire challenge period. At t = 30 min, and at regular intervals (~ 30 min) throughout the challenge period, estimates of spore concentration were made at the three different sampling locations. It is evident from Figure 6 that, at each sampling occasion, the three estimates of spore concentration fall within a 2-3 fold range; this relatively narrow range indicates active dispersal of spores throughout the air within the containment room to give rise to a reasonably homogenous distribution. It is also seen from the figure that spore concentration of dispersion was maintained at a fixed level (around 3×10^6 spores m^{-3}) over the challenge time extending from 30 to 530 min. As in earlier study (3), the first 30 min of aerosolisation was excluded from the challenge period as this period was utilised to establish the concentration of dispersed spores within the containment room.

Relationship between Fraction of Product Contaminated and Microbial Challenge Level. Figure 7 is a plot, on logarithmic scales, of fraction of

contaminated ampoules against spore challenge concentration for the Blow/Fill/Seal machine operating without the air shower functioning. The four closed points represent data generated in the present work (Study 2), whereas, the three open points represent previously reported data generated in Study 1 (3) with the identical machine specification and operating conditions employed. It is immediately evident that both sets of datum points fall around the same curve (the dashed line is the extrapolation of the curve to lower levels of product contamination). Datum points from both studies define comprehensively the form of the relationship between fraction of product contaminated and level of spore challenge concentration over a 50,000 fold range of spore concentration. Moreover, the datum points show that the linear portion of the curve defining the direct relationship is experimentally demonstrable, and holds over around a 7,000 fold change in challenge concentration.

Operation of Air Shower. Figure 8 gives data generated for the Blow/Fill/Seal machinery operating with the air shower at maximum setting (open points represent data generated in Study 1 and solid points represent data generated in Study 2); the uppermost curve and its extrapolate depicted in Figure 8 represent the common behaviour defined by data generated in both studies with air shower off (taken from Figure 7). These plots reveal that, for each study, there is a distinct relationship between fraction of product contaminated and spore challenge concentration with the air shower operating maximally. Furthermore, the two curves derived with air shower operating at maximum setting and the common curve derived with air shower off are in effect parallel. However, the two curves generated with air shower operating maximally are shifted downwards from that

seen without air shower operation, the magnitude of this shift being around 9 and 70 fold for machines employed in Study 1 and Study 2 respectively.

Variation in Cycle Time. Production using Blow/Fill/Seal technology is an incremental process. An increment (cycle) of production comprises container moulding, filling, sealing and discharging of product, with transfer of moulded container(s) from the moulding station to the filling station being achieved via a mould carriage which, at the end of the cycle, returns to the moulding station. In the present study, adjustment in cycle time was achieved through changing the period of time taken for the mould carriage to move from the moulding station to the filling station; the filling, sealing and returning of mould carriage elements of the cycle were held constant.

At cycle times of 12 and 14 s, determinations were made of the fraction of ampoules contaminated for the Blow/Fill/Seal machine (Study 2) with air shower both off and operating maximally. The upper solid line of Figure 9 is the curve, originally depicted in Figure 7, relating fraction product contaminated and level of spore challenge concentration for a cycle time of 12 s without the air shower operating, and the lower solid line (taken from Figure 8) is the curve for the same cycle time derived in Study 2 with air shower at maximum operation; these two lines are given for comparative purposes. The two datum points represent the findings obtained with a 14 s cycle time, the closed point for air shower off and open point for air shower on at maximum. Each datum point is seen to fall above the corresponding curve generated for a cycle time of 12 s; the magnitude of the displacement is small for air shower off but is around 20 fold for air shower operating.

Simulated Interventions. At a given spore challenge concentration (nominally 3×10^6 spores m^{-3}), two gross interventions were simulated through switching the air shower off from maximum setting, and, in so doing, compromising local protection of the filling mandrels, and then, after a fixed time period (5 or 15 min) switching the air shower back on to maximum. Figure 10 is a plot of fraction of contaminated ampoules observed in each successive 5 production cycles (around 1 min production) throughout the challenge period (330 min); the two sets of dashed lines delineate the boundaries of the two intervention periods of 5 and 15 min duration, designated X and Y respectively, and values in parenthesis give the overall fraction of ampoules contaminated during the experimental periods before intervention X, between interventions X and Y and after intervention Y.

It is seen from Figure 10 that, prior to the first intervention X, the fraction of ampoules contaminated, at production intervals of 5 cycles, falls within the range 0 to 0.13, the overall fraction of ampoules contaminated throughout this first period (test time 0 to 121 min) being 0.022. At the start of intervention X (test time 122 min) switching off the air shower is seen to have an immediate impact on the fraction of ampoules contaminated, the level increasing to a maximum of 0.28 (around a 13 fold increase above the mean fraction contaminated that existed prior to intervention X). At the end of intervention X (test time 127 min) restarting operation of the air shower is seen to bring about an immediate fall in the fraction of ampoules contaminated; within one 5 cycle increment of production (corresponding to 1 min), the fraction falls to a level within the pre-intervention range. For that period following intervention X up to start of intervention Y (test time 128 to 217 min), the overall fraction of ampoules contaminated is 0.020.

This level does not differ significantly ($p = 0.05$) from the level of 0.022 recorded prior to simulated intervention X, demonstrating that machine performance, in terms of fraction of product contaminated, returns fully to the pre-intervention level.

Figure 10 shows a similar behaviour for intervention Y, a duration of 15 min (test time 218 to 233 min). Again switching off the air shower (start of intervention Y) is observed to bring about an immediate increase in the frequency of ampoules contaminated; in this instance, the fraction contaminated attains a maximum level of 0.48 (around a 22 fold increase above the original pre-intervention level). On restarting the air shower (end of intervention Y), the fraction of ampoules contaminated is again observed to fall rapidly to pre-intervention levels. However, on this occasion, following the simulated intervention of 15 min, the minimum time required to achieve pre-intervention contamination levels is around 3 min. For the test period following intervention Y (test time 234 to 330 min) the overall level of fraction of ampoules contaminated is 0.026. Again, this level does not differ significantly ($p = 0.05$) from that recorded prior to the two simulated interventions, indicating full recovery of machine performance.

Discussion

The experimental approach underlying the present fundamental investigation of automated aseptic processing 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. To set spore

challenge concentration within the operating environment, 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 spore suspension at a predetermined rate and by regulating dispersion decay via stirred-settling conditions in the containment volume around a machine. Under these experimental conditions, controlled challenges of air-dispersed spores were generated over a wide range of spore concentrations through aerosolisation of spore suspensions at different spore concentrations. In practice, it has been possible to generate controlled challenges at spore concentrations extending over a 50,000 fold range for periods of time ranging up to 10 h.

The results of a second study in which an operating Blow/Fill/Seal machine has been challenged with air-dispersed spores have again demonstrated unequivocally that the quality of the microbiological environment surrounding the machine impacts upon the fraction of product contaminated. In general, for the Blow/Fill/Seal machine operating under a fixed set of machine conditions, there is a regular and definable relationship between the fraction of product contaminated and the level of airborne micro-organisms. For machine operating with air shower off, the constancy of the behaviour between Study 1 and Study 2 provides strong evidence that, under controlled conditions, Blow/Fill/Seal machine performance in respect of product contamination is highly consistent and reproducible. Overall, for air shower off, the relationship between product contamination and the spore challenge concentration has been comprehensively defined over a 50,000 fold range in spore challenge concentration. Moreover, the linear portion of the relationship is amenable to extrapolation providing a means for predicting air quality

under which the frequency of product contamination is low and acceptable (see dashed line on Figure 7). For example, for the particular mode of machine operation employed here, a level of one organism per cubic meter of air is predicted to provide a rate of product contamination of 2.3×10^{-7} (i.e. 1 contaminated ampoule in around 4.3×10^{6} ampoules produced).

Study 1 had previously shown that operation of an air shower around the filling mandrels reduced the probability of product contamination (3). In the present study, local protection is again seen to reduce the level of product contamination. However, for Study 2, maximal operation of the air shower is observed to bring about a 70 fold reduction in the level of product contamination for a given challenge concentration as represented by the common curve derived with air shower off as opposed to around a 9 fold reduction for air shower operation in Study 1. This difference between the two studies in the extent of protection afforded by the air shower suggests that, for a given machine type, the configuration and arrangement of the air shower is critical to the efficiency of local protection. Nonetheless, the different curves derived for air shower operation in the two studies appear regular and also amenable to extrapolation (lower two dashed lines on Figure 8). They provide, at an average challenge concentration of one organism per cubic meter of air, predicted contamination rates of around 2.6×10^{-8} and 3.5×10^{-9} for air shower operation in Study 1 and Study 2 respectively. It is worth noting that none of these predicted contamination rates for an average challenge of 1 spore per cubic meter or air, with or without air shower operation, could be assessed by any practicable medium-fill study. Furthermore, the above findings provide clear evidence that microbial contamination of product occurs

during the filling element of the Blow/Fill/Seal process.

The observed increase in rate of product contamination consequent upon an increase in cycle time (both for air shower off and on at maximum) is indicative that the length of time taken by the mould carriage to move from the Parison head (moulding station) to the filling station is a critical determinant of product contamination. Clearly, present data are limited and caution must be exercised in making definitive interpretations. Nonetheless, this behaviour is in keeping with a second mode of product contamination occurring before the filling process (i.e. during container moulding and/or transportation). It is also interesting to note the impact of changes in cycle time on the efficiency of local protection afforded by operation of an air shower around the filling mandrels. For the same air shower operating at maximum setting, around a 70 fold reduction in product contamination was recorded at a cycle time of 12 s as opposed to around a 10 fold reduction at a 14 s cycle time. This behaviour could be explained by that element of product contamination which occurs prior to filling increasing with increasing cycle time and, in so doing, reducing the apparent protection of the air shower operating around the filling mandrels.

Results of simulating gross interventions that compromise local protection of an air shower, achieved through switching the air shower off, have revealed that such interventions impact immediately upon the rate of product contamination. Equally, resumption of operation of the air shower was shown to impact immediately upon the level of product contamination. The rigor of the intervention, as controlled by the length of time when the air shower was not operated, also

influences the level of product contamination. Thus, for an intervention of 5 min duration, fraction of ampoules contaminated was observed to achieve a maximum of 0.28, whereas, for the intervention of 15 min duration, a maximum of 0.48 was recorded. Furthermore, the minimum recovery time required to achieve pre-intervention levels was less than 1 min following a 5 min intervention as opposed to around 3 min following a 15 min intervention. Clearly, both the establishment and the destruction of local protection afforded by the air shower are time functions. This behaviour is in keeping with local protection, provided by filtered air emerging from the air shower, being achieved in part through establishing a compartment of 'clean' air within which critical Blow/Fill/Seal operations are conducted. However, further experimental work is required to provide conclusive proof for the existence of such a compartment. The constancy of the rate of product contamination for the production periods prior to, between and after the two simulated interventions is also worthy of note. It provides further evidence that, under controlled conditions, Blow/Fill/Seal machine performance, in terms of microbiological quality of product is highly reproducible and predictable.

It is essential to recognise that the findings described above, and their interpretation, apply only to the particular test machinery operated under the specified conditions. Nonetheless, they demonstrate the potential of employing 'medium-fill' under conditions of controlled microbial challenge to rationalise the performance of Blow/Fill/Seal technology in aseptic processing. The findings reported here have shown that:

a) the fraction of product contaminated is determined by the microbiological quality of the Blow/Fill/Seal machine environment.

b) under fixed operating conditions, the relationship between the fraction of product contaminated and the level of airborne micro-organisms is regular, highly consistent and reproducible.

c) the protective shower of air around the filling mandrels reduces the frequency of product contamination; the efficiency of local protection is dependent upon air shower design and machine operating conditions.

d) the time taken for the mould carriage to move from the moulding station to the filling station is a critical determinant of the rate of product contamination.

e) 'interventions' that compromise local protection afforded by the air shower impact immediately and detrimentally upon the frequency of product contamination; however, the observed effects are fully reversible.

The work also serves to demonstrate that responses to controlled microbial challenges can provide an effective approach to estimating rates of product contamination for advanced aseptic processing. Furthermore, they allow prediction of operating conditions, including machine operating conditions and environmental quality, under which an acceptably low frequency of product contamination is attained.

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Figure Captions

Figure 1. Schematic representation of the laboratory containment vessel.

Figure 2. Stirred-settling decay of spore dispersions held within the laboratory vessel.

Figure 3. Spore concentration of dispersions within the laboratory vessel during continuous aerosolisation of suspensions at different spore concentrations.

(O) concentration of aerosolised suspension 1×10^4 spores cm^{-3}
(Δ) " " " " 9×10^5 " "
(□) " " " " 4×10^7 " "
(●) " " " " 7×10^8 " "

Figure 4. Schematic representation of modified mobile cabin.

Figure 5. Estimates of dispersion concentration within the mobile cabin generated by continuous aerosolisation of a suspension of 3×10^3 spores cm^{-3}; solid points represent estimates made at Port 1 and open points those made at Port 2.

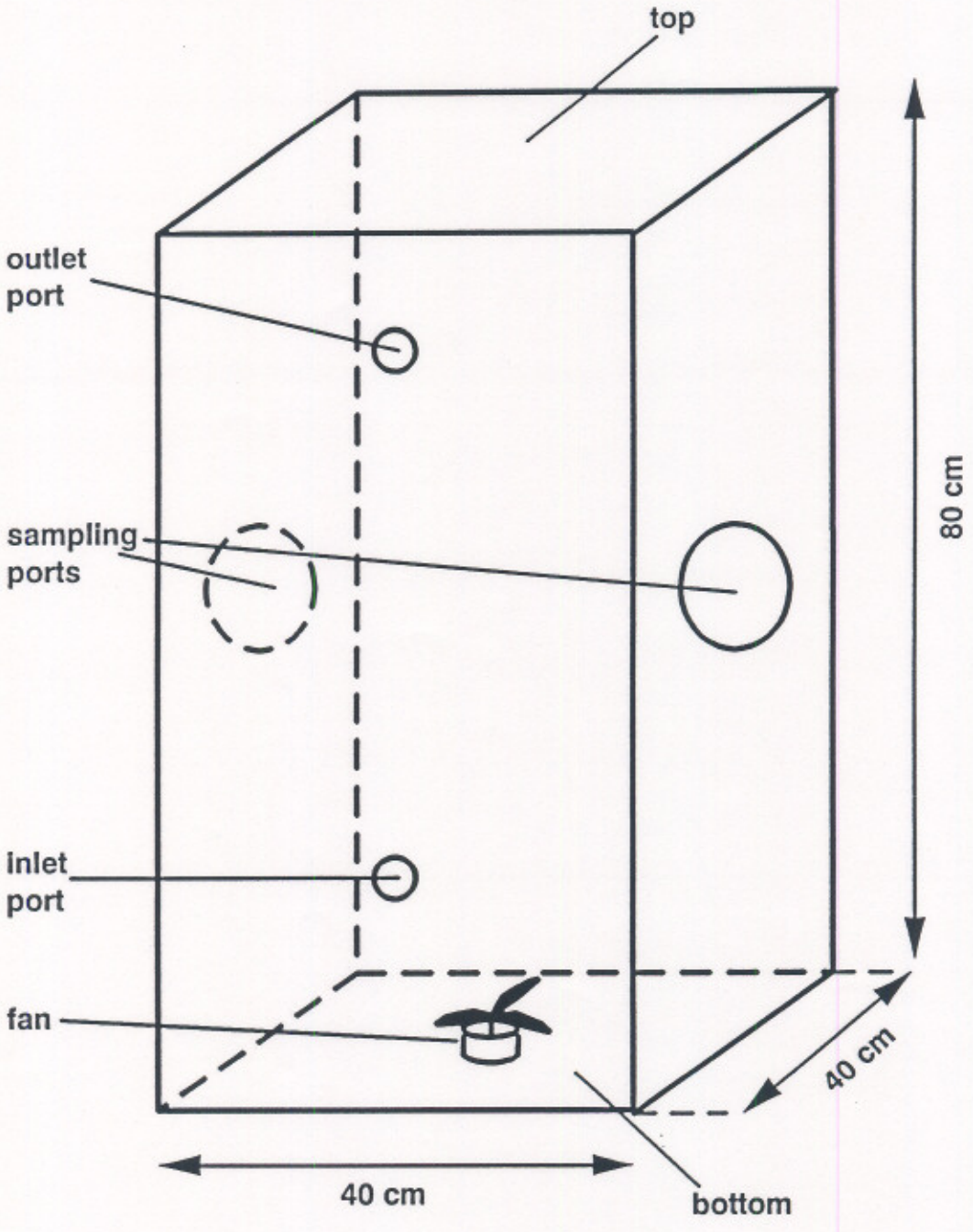
Figure 6. Estimates of dispersion concentration made at the three sampling ports of the containment room during aerosolisation of a suspension of 6×10^7 spores cm^{-3}.

Figure 7. Fraction of contaminated ampoules as a function of spore challenge concentration for the Blow/Fill/Seal machine operation without the air shower operating (dashed line is extrapolate of the curve).

Figure 8. The relationships between fraction of contaminated ampoules and spore challenge concentration for the Blow/Fill/Seal machine operating with the air shower at maximum setting for Study 1 and Study 2 (dashed lines are extrapolates of the two curves); the uppermost curve and its extrapolate are the common behaviour seen with air shower off (taken from Figure 7).

Figure 9. Datum points generated for the Blow/Fill/Seal machine functioning with a 14 s cycle time, the closed point with air shower off and open point with air shower on; the upper solid line is the curve for a cycle time of 12 s with air shower off (taken from Figure 7) and the lower solid line is the corresponding curve for 12 s cycle time with air shower operated maximally (taken from Figure 8).

Figure 10. Fraction of ampoules contaminated for increments of 5 production cycles throughout the experimental period covering the two simulated interventions (X and Y); the dashed lines delineate the boundaries of the two interventions of 5 and 15 min duration.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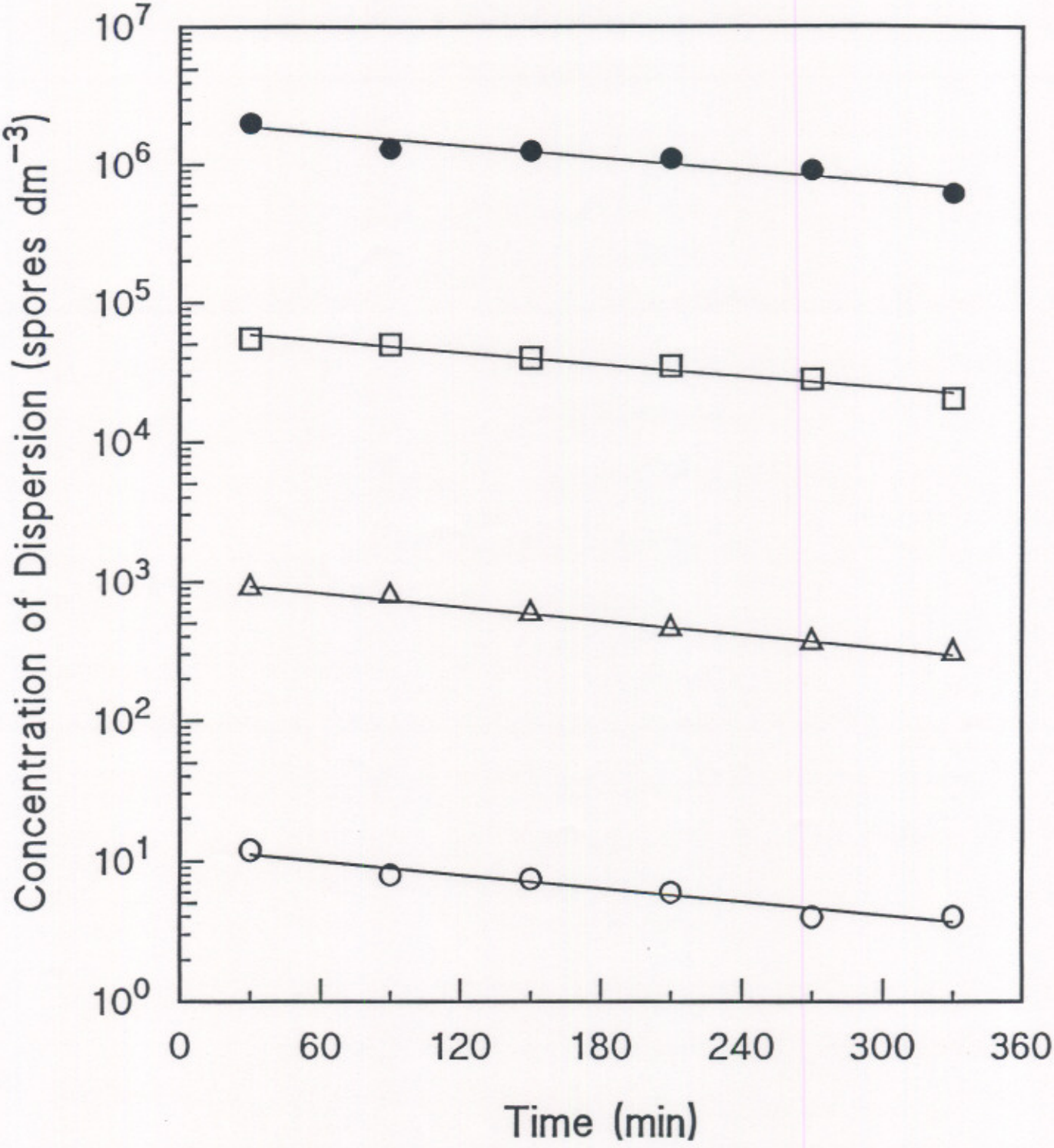
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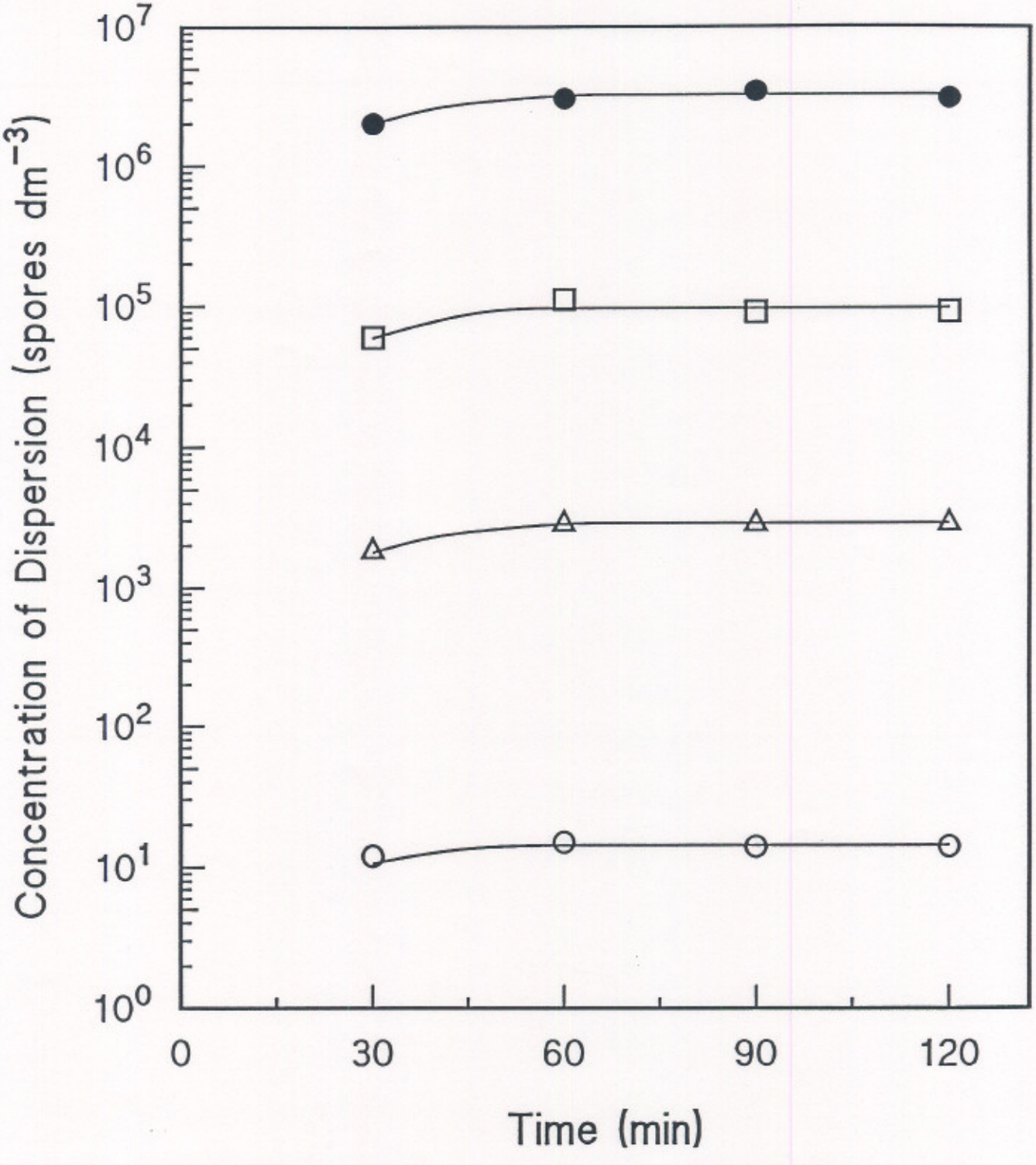
120
180
240
300
360
Time (min)


Concentration of Dispersion (spores dm^{-3})
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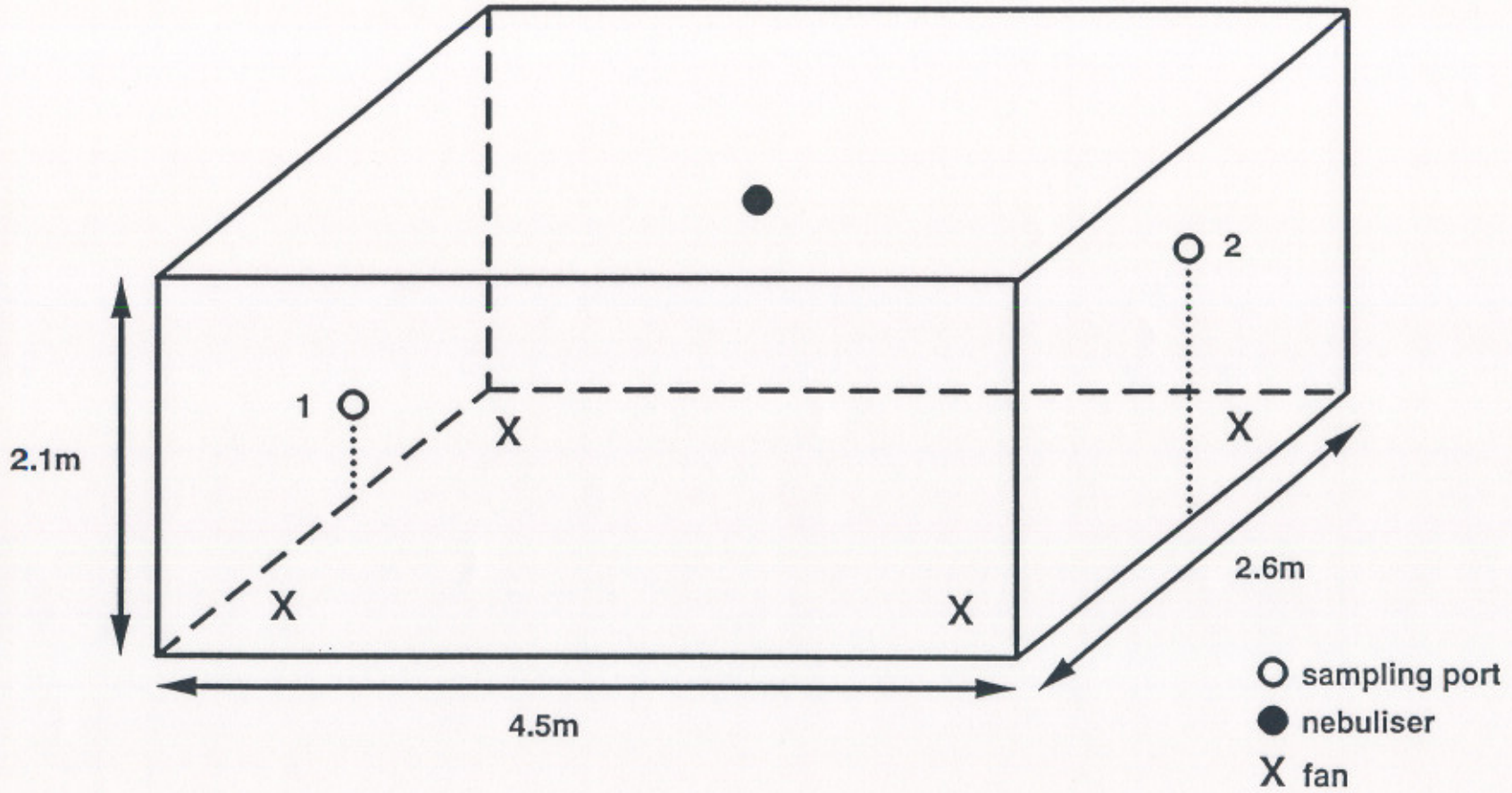
120
Time (min)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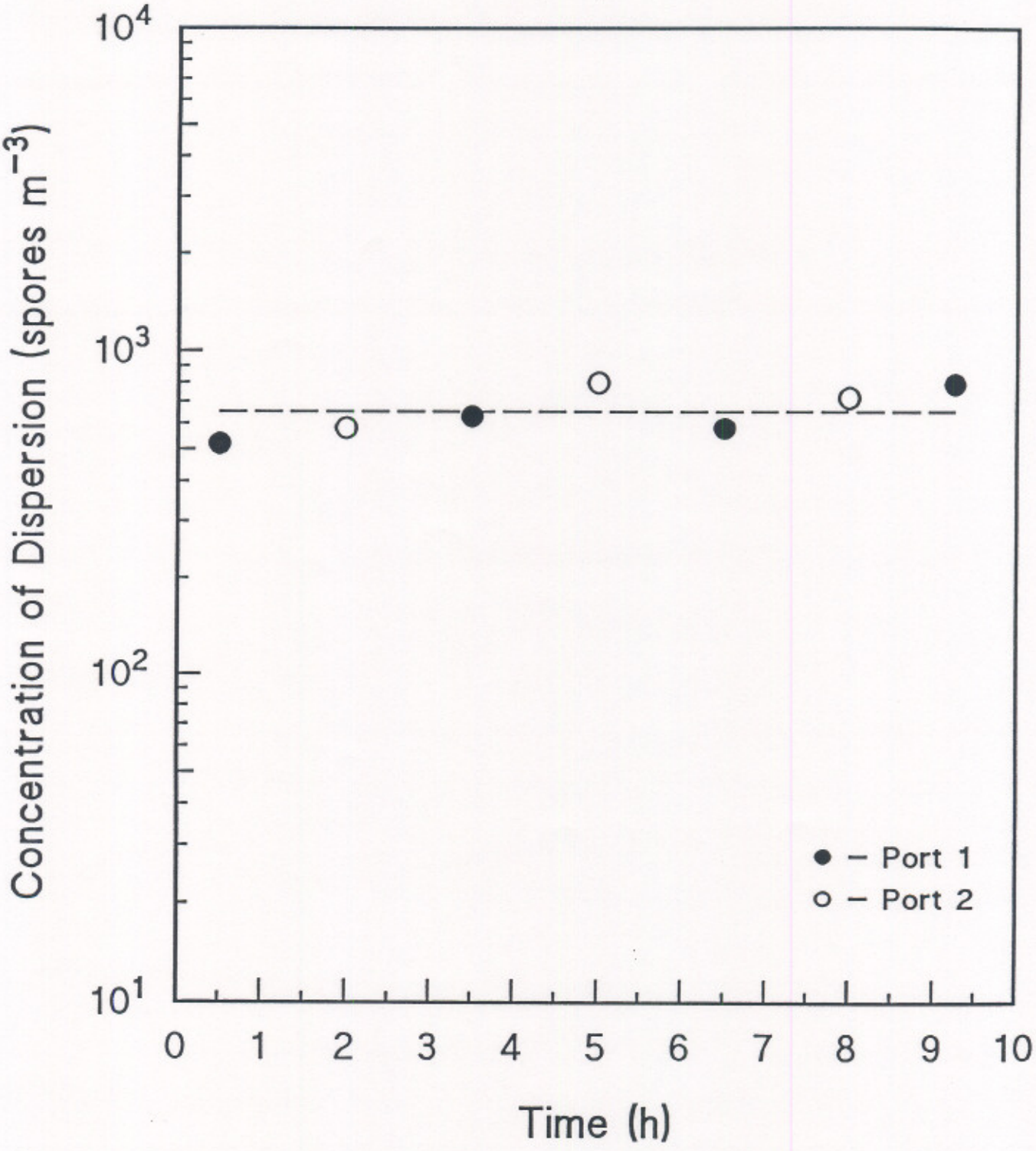
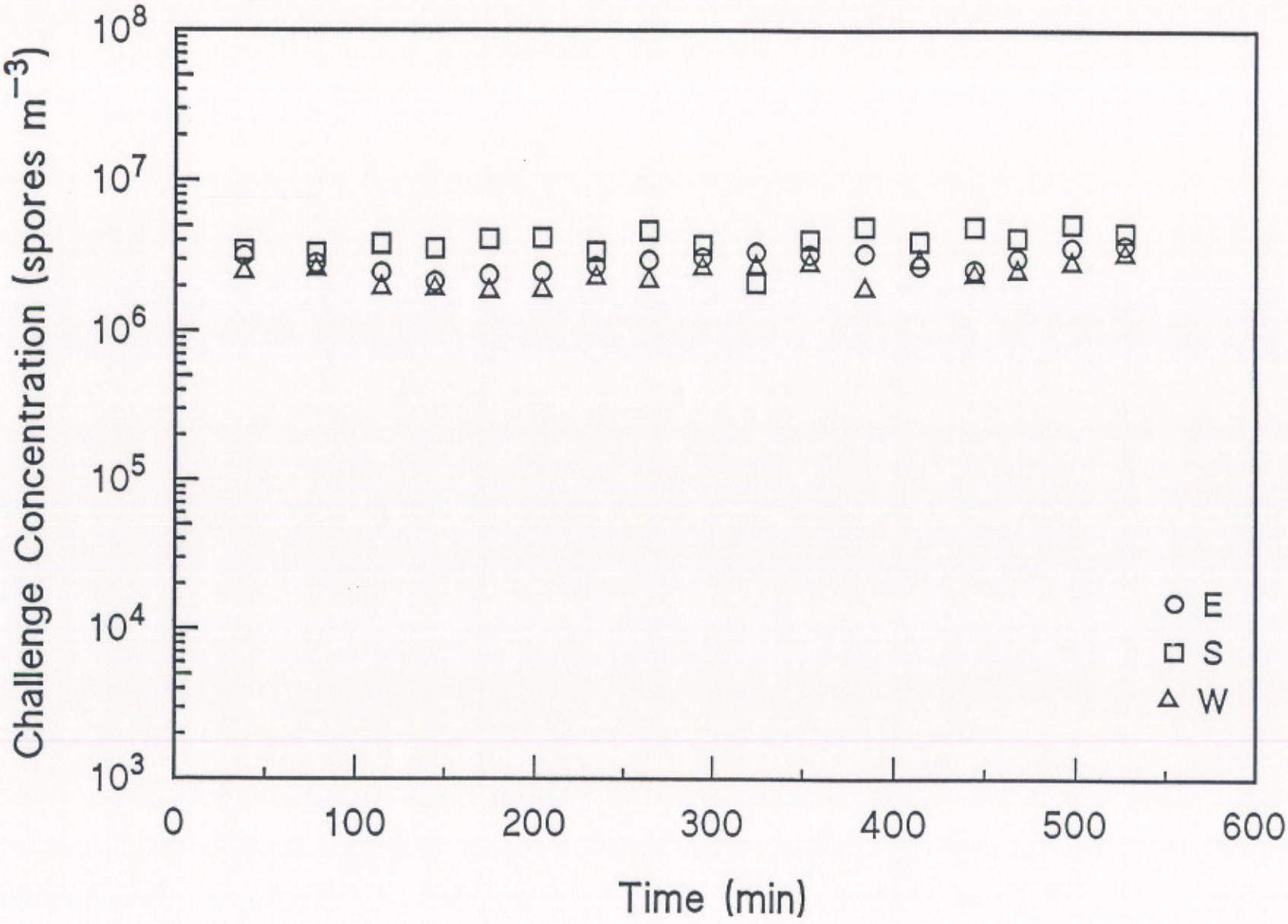
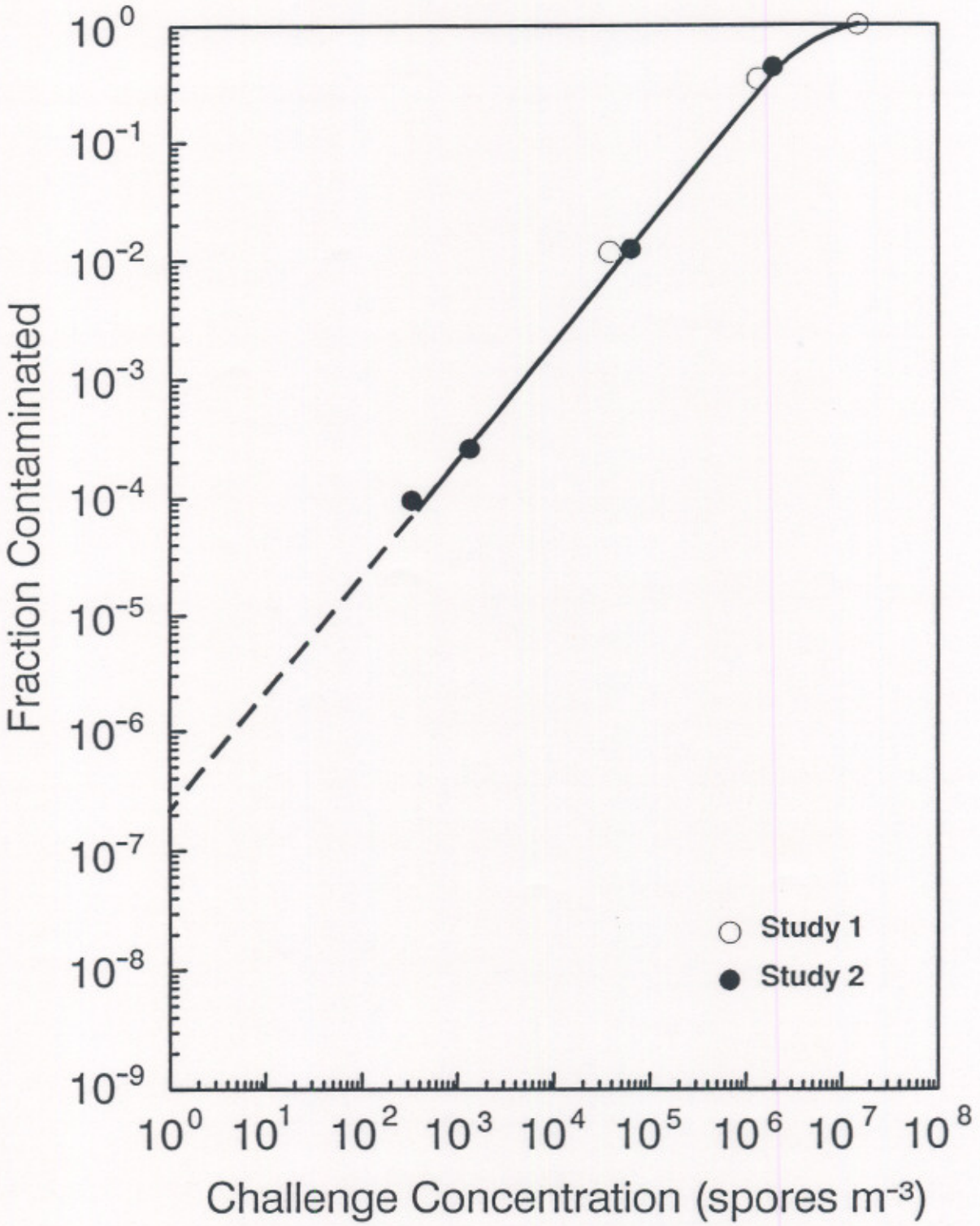
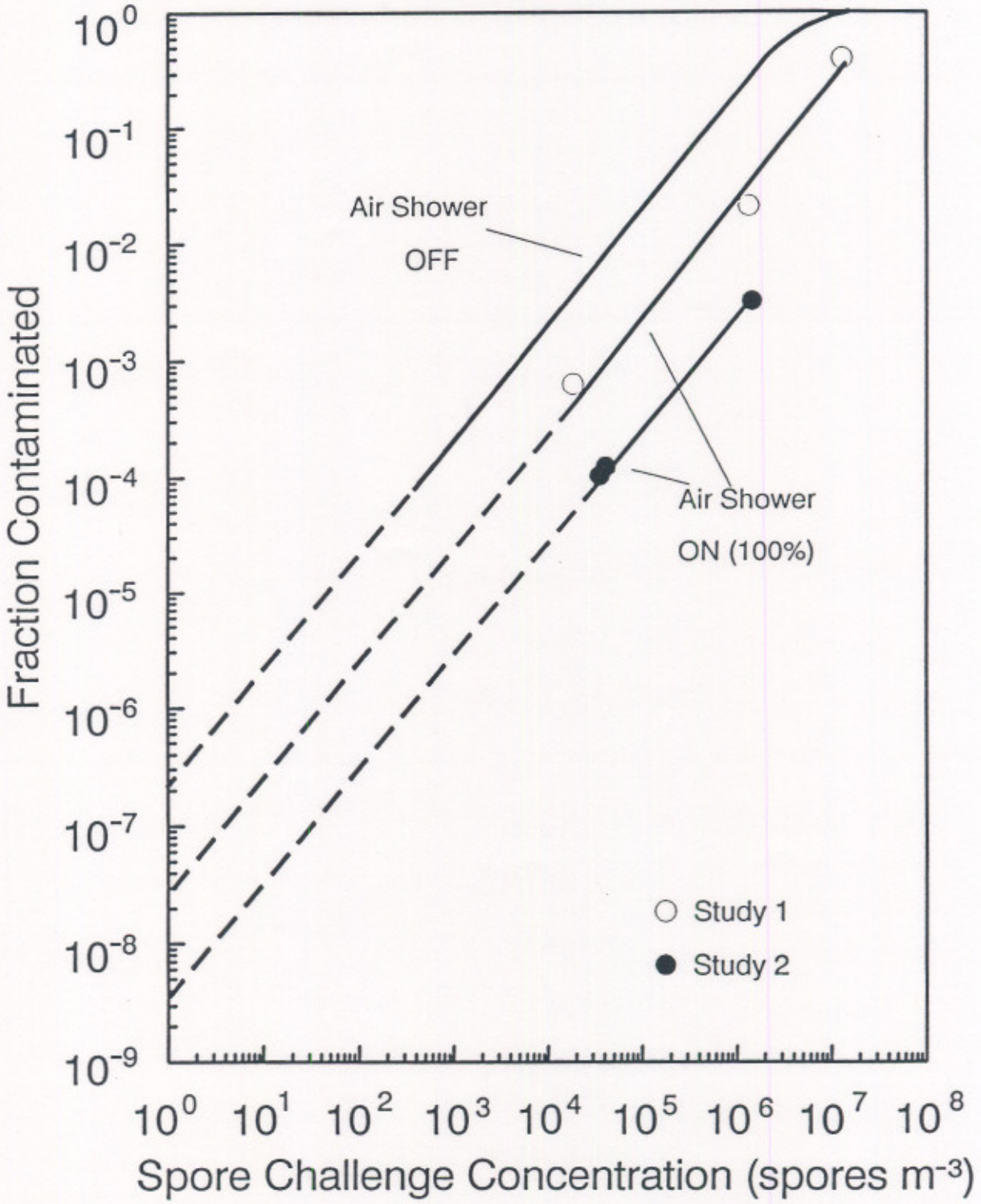
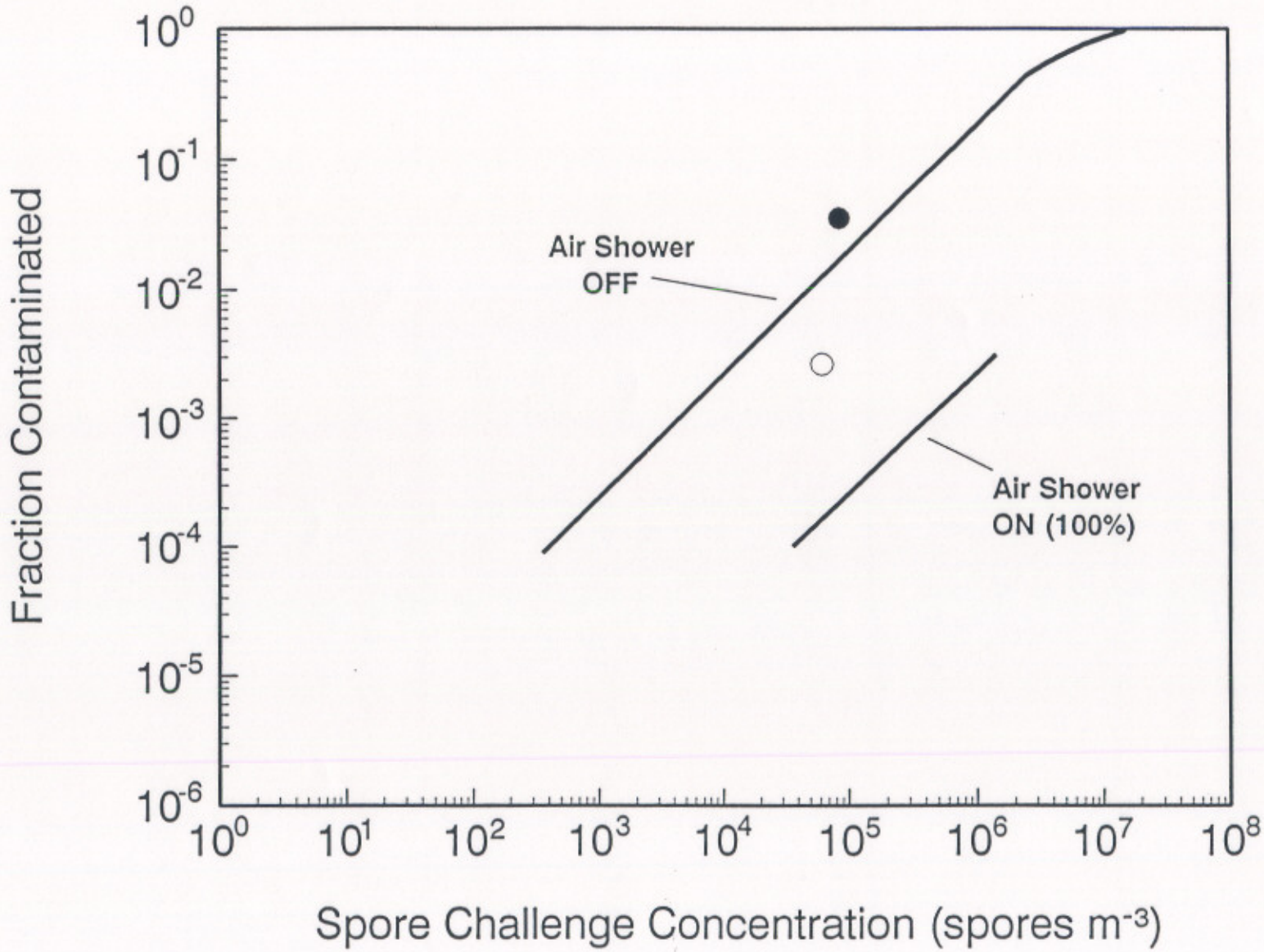
sampling port
nebuliser
X fan

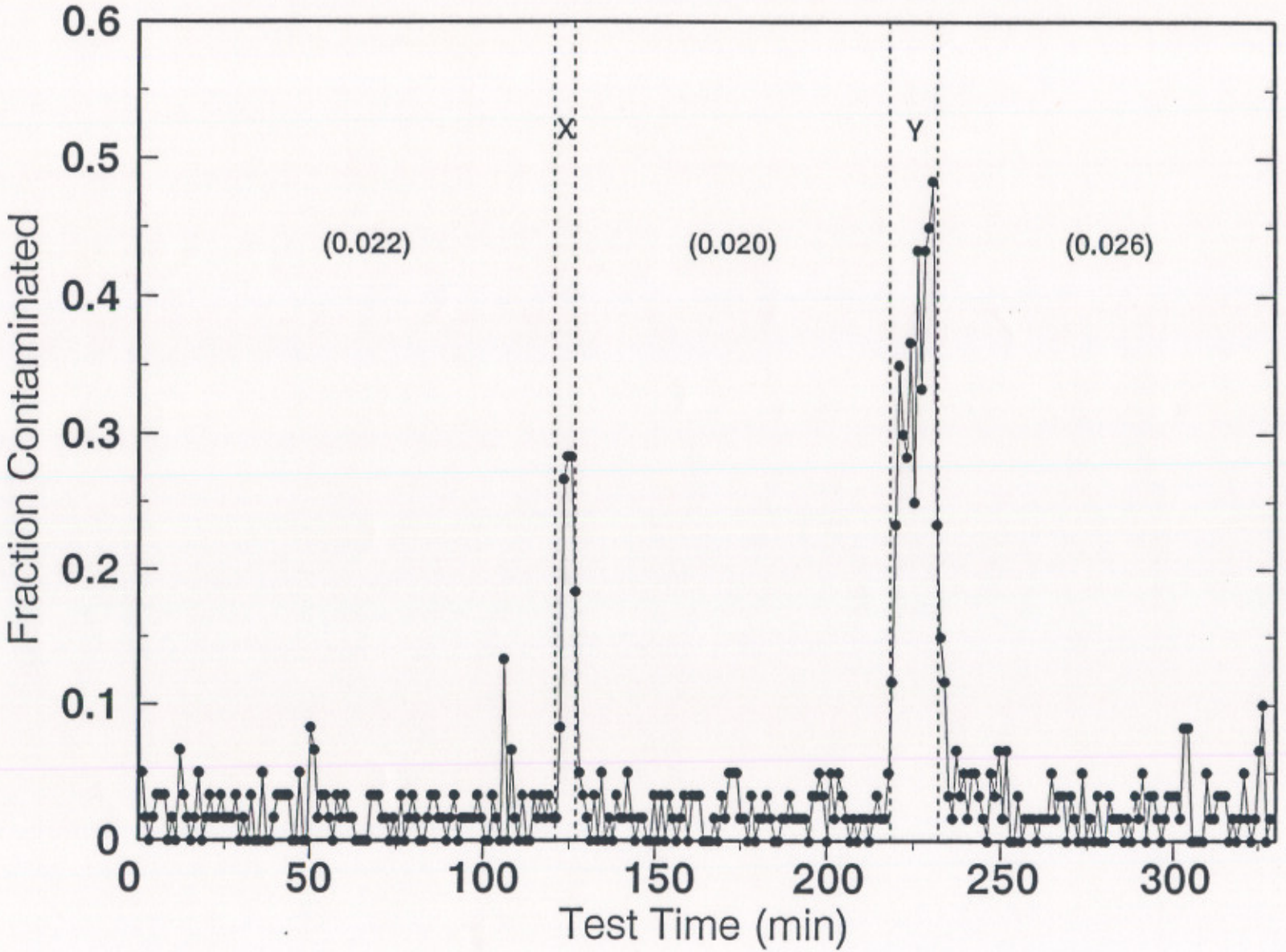

Concentration of Dispersion (spores m^{-3})
10^4
10^3
10^2
10^1
0
1
2
3
4
5
6
7
8
9
10
Time (h)
● – Port 1
○ – Port 2


Challenge Concentration (spores m^{-3})
10^8
10^7
10^6
10^5
10^4
10^3
0
100
200
300
400
500
600
Time (min)
E
S
W


Fraction Contaminated
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7} 10^{8}
Challenge Concentration (spores m^{-3})
Study 1
Study 2


Fraction Contaminated
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
Air Shower
OFF
Air Shower
ON (100%)
Study 1
Study 2
10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7} 10^{8}
Spore Challenge Concentration (spores m^{-3})


Fraction Contaminated
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
Air Shower
OFF
Air Shower
ON (100%)
10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7} 10^{8}
Spore Challenge Concentration (spores m^{-3})


0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction Contaminated
X
Y
(0.022)
(0.020)
(0.026)
0
50
100
150
200
250
300
Test Time (min)