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The generation of aerosols by accidents which may occur during plant-scale production of micro-organisms

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SUMMARY

Experiments have been performed to simulate accidents which may occur during large-scale production of micro-organisms. Four types of accident, which were considered to be the most likely to result in the greatest hazard to health, were simulated using a bacterial model. The accidents were all concerned with faults occurring in the operation of the microbial fermenter. Gross contamination of surfaces occurred in all experiments, but only three types of accident produced a measurable aerosol.

INTRODUCTION

The hazards associated with the manipulation of micro-organisms are well recognized, and in most laboratories working techniques have been adopted which minimize the dissemination of these materials into the general environment. The aim of such techniques is the containment of the micro-organisms to specific areas of the laboratory in order to: (*a*) maintain experimental integrity and prevent cross-contamination of cultures, media, research animals, etc., and (*b*) reduce hazards to the health of laboratory workers.

Fortunately the majority of micro-organisms are quite harmless or must be absorbed in large numbers to produce disease in healthy persons, with the result that measures taken to protect the experiment will also protect the health of the worker. The relatively few micro-organisms which are highly infectious are well known and the use of them is restricted by various codes of practice to certain approved laboratories, wherein the prime consideration is the protection of health.

In these laboratories, very sophisticated techniques may be required to protect the worker while carrying out certain procedures, and it is necessary that each part of the work be analysed in advance, to determine the risks involved so that adequate protective measures may be designed. Reviews (Sulkin, 1961; Pike, 1976) have shown that a significant proportion of laboratory-acquired infections result from the inhalation of airborne particles or droplets, and even undetected aerosols may contribute to occupational illness (Laboratory Safety Monograph, 1978). Clearly, a vital consideration in risk assessment is the probability of the release of infectious particles from a laboratory procedure. In addition to the immediate risk of inhalation of the aerosol, further hazards to health may occur if the airborne particles are carried on air currents and settle on to working surfaces which are assumed to be clean. Several reports have described the risk of aerosol production during common laboratory procedures such as pipetting, inoculation of media,

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centrifugation and accidents (Kenny & Sabel, 1968; Dimmick, Vogl & Chatigny, 1973; Stern *et al.* 1974). The hazard is clearly increased when the micro-organism is present in high concentration and when it is able to retain its infectivity in the aerosol state.

Increasing industrial interest in the properties of micro-organisms now extends to such fields as ore leaching (Schwartz, 1977), vaccine production (Van Hemert, 1974) and animal feed supplies (Shennan & Levi, 1974) and this means that many test organisms are produced for investigation of their commercial potential. Such work inevitably requires that the organisms be available in large numbers, and if the fermentation process is carried out on a plant scale the risks to the health of workers may be considerable should the plant develop a fault. In order to assess the risk which might arise in such circumstances experiments have been carried out to measure the aerosol produced during accidental releases of bacterial suspension in batch culture production.

MATERIAL AND METHODS

Design of experiments

The most important item of plant in the production of bacteria is the fermenter or culture vessel. This is a sealed container made of stainless steel or glass in which the bacterial suspension is allowed to increase in concentration by self-replication, from about 10^4 cells/ml following inoculation, to some 10^{10} cells ml. The bacterial suspending fluid is an aqueous solution of essential ingredients required to promote growth. In the production of an aerobic bacterium the culture fluid would be continuously stirred and aerated by compressed air during the fermentation so the culture would also contain an additive to prevent foaming. The fermenter would therefore have connected to it pipework for supplying culture fluid, compressed air and anti-foam to the vessel, and for removing the compressed air (through bacteriological filters) and final bacterial suspension.

The range of possible accidents which might occur in such a process is clearly very great, and for this work it was decided to concentrate on four types of accident which were considered to be the most likely to result in the generation of a hazardous bacterial aerosol. The accidents were all concerned with the operation of the fermenter.

A. Failure of the filter which removes bacteria from the compressed air after it has passed through the culture. If the filter developed a hole or became completely dislodged bacteria-laden air would be released from the vessel into the plant room.

B. Failure in the supply of anti-foam. This would result in a build-up of foam which would eventually wet the air filter and degrade its efficiency. Air exhausted from the fermenter could then be contaminated with bacteria.

C. Failure in the pipework which carries the culture for further treatment. A breakage in this line would result in a massive spillage of bacterial suspension.

D. Breakage of the culture vessel, for example as a result of being hit by debris from a gas explosion. This might result in widespread air and surface contamination.

The capacities of plant-scale fermenters are typically up to 400 l, and the simulation of accidents involving this amount of bacterial suspension was precluded

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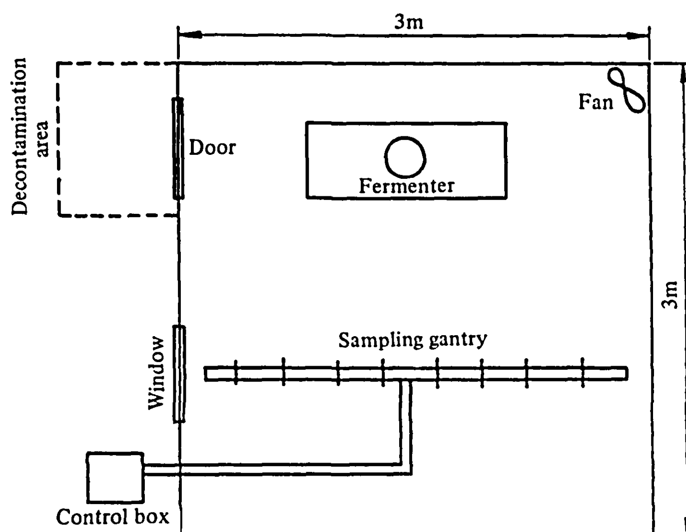

Fig. 1. Typical layout of experimental room.

on the grounds of expense. Instead a vessel of 1 l capacity was used for these experiments and this was charged with 0·5 l of bacterial culture simulant. This consisted of a suspension (10^{10}/ml) of spores of *Bacillus subtilis* var. *niger* (BG) previously cultured on a plant scale. This spore is extremely robust and is known to be quite stable in the aerosol state and has been used widely in aerobiology as a tracer and simulant.

Since aliquots of the same suspension were used for each experiment this had the advantage that the results were comparable without correction for the inevitable differences which arise in the aerostability of batch-grown cultures. Additionally the results quoted represent the 'worst case', since a fresh bacterial culture would contain mainly vegetative cells which are less resistant to stresses such as aerosolization.

Experimental method

The experiments took place in rooms of various sizes which were hermetically sealed, but provided with the means to carry out forced-air ventilation. Aerosols were thus contained in a known volume of air, which could be mixed to enable a representative sample to be taken. All operations were controlled remotely from outside the room, electrical cables, vacuum lines, etc. passing through a sealed duct to the control point. The contents of the room comprised only the fermenter mounted on a table, a large-bladed fan for mixing the aerosol and various air samplers attached to a gantry. A typical layout is shown in Fig. 1. A well-defined decontamination area was provided immediately outside the door and this was kept stocked with supplies of Chloros decontaminant, fresh water, protective clothing, foot-baths, brushes, towels, etc.

The procedure used was essentially the same for each experiment but with some differences, described later, to take account of the type of accident to be simulated. The procedure was as follows:

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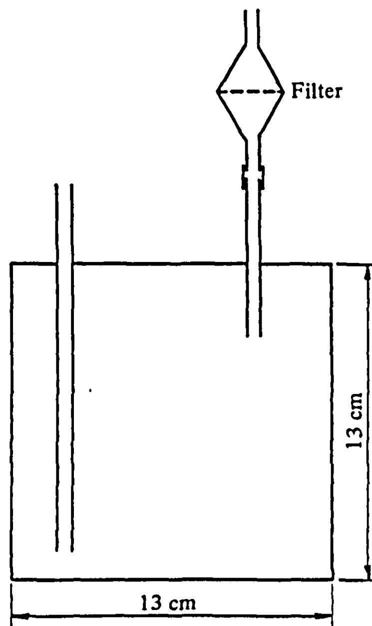

Fig. 2. Sketch of metal container.

(1) With the test room and equipment in a 'clean' state, i.e. decontaminated after any previous experiments, the sampling devices were loaded.

(2) In a separate room the fermenter was prepared, charged with 500 ml of BG slurry and then installed at the test site.

(3) When all personnel had evacuated the room, the ventilation system was closed down and sealed, and the doorway closed and sealed with adhesive tape.

(4) The experiment was then carried out: (*a*) the mixing fan was started; (*b*) after 5 min the 'accident' was simulated; (*c*) after successful completion of the simulation (observed through a window) the air in the room was mixed for a further 5 min before (*d*) the samplers were started, and run for a period of about 25 min.

(5) The room was ventilated for a sufficient time to provide 10 air changes before the door seal was broken.

(6) Two people, wearing full protective clothing (rubber boots, gloves, gowns and respirators) entered the room, and working together, removed the air samples in a way that avoided cross-contamination, i.e. one dismantled the sampers and held them so that the other could remove the substrates without contacting potentially contaminated surfaces.

(7) All obvious signs of surface contamination were treated with 10 % hypochlorite solution, the room sealed again and fumigated with formaldehyde vapour. After 16 h the room was ventilated to remove all trace of the vapour.

The first three types of accidents were simulated using the Porton fermenter (Herbert, Phipps & Tempest, 1965). This is a glass vessel of 1 l capacity which has bolted to it a stainless steel top incorporating several access ports to allow for sampling, removal of air, or the installation of thermometers, heaters, etc. Attached to the top is an electrically driven stirrer which operates in a baffle cage. Compressed air is passed down the shaft of the stirrer to aerate the culture. A drain plug is provided at the base of the vessel.

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The fourth type of accident, which involved the breakage of the fermenter, was simulated using glass and metal vessels. Two different vessels were used in these experiments since it was believed that the aerosol created on breakage would depend greatly on the fabric of the vessel. The glass fermenter was simply a length of tubing of similar dimensions to the Porton fermenter, which was sealed with rubber gaskets into the fermenter rig. The metal container was fabricated out of 0·43 mm tinplate. Fig. 2 shows its construction.

The four types of accident were simulated in the following manner.

Series A. Failure of the bacteriological filter

This was simulated by operating the Porton fermenter without a bacteriological filter in place. In the experiment the culture fluid was mixed and aerated (1 l air/min) for a period of 20 min, since it was considered that this was the maximum length of time that such a failure would remain undetected.

Series B. Failure in the supply of anti-foam

In the Porton fermenter, stirring and aeration of the BG suspension in the absence of anti-foam agent produced considerable foaming. When the bacteriological air filter was installed close to the fermenter it very quickly became wetted by the foam and BG suspension could be seen to penetrate. The simulation was also allowed to proceed for 20 min.

Series C. Failure in the culture-transfer pipework

This effect was produced by removing the drain plug of the Porton fermenter while in operation. By using an electromagnetic valve to control a vacuum line the plug was removed at a predetermined time. The result of removing the plug was that most of the BG suspension was expelled forcibly from the fermenter by the action of the stirrer and the compressed air. Large quantities of suspension fell 1 m to the floor.

Series D. Breakage of the fermenter

Breakage of the glass fermenter was achieved by striking it with a missile. For this purpose a 6 mm steel ball was propelled at the side of the vessel with compressed air controlled by an electromagnetic valve. This caused complete shattering of the glass fermenter resulting in visible, widespread contamination of the room.

Rupture of the metal container was caused by the detonation of an explosive charge. The container, holding 500 ml of BG suspension, was restrained against sandbags while, at a distance of 30 cm, a 50 g shaped charge of plastic explosive was detonated. For this experiment the air samplers operated outside the test room, air being withdrawn from the room via a 50 cm length of 7 cm diameter straight pipe.

Methods of sampling

In order to characterize the aerosol as fully as possible, air samples were taken with several types of sampler each having a high collection efficiency for particles in the respirable size range. Particles of this size present the greatest hazard to

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Table 1. *Analysis of BG released from fermenter vessels during simulated accidents*

Series	Expt	Room volume (m^3)	Volume of culture expelled (ml)	Culture concentration (cells per ml)	Total cells expelled	Cells in aerosol	Fraction of expelled cells in aerosol %
A. Filter failure	1	62	90	$5{\cdot}0\times10^{9}$	$4{\cdot}5\times10^{11}$	Nil	—
	2	62	100	$6{\cdot}0\times10^{9}$	$6{\cdot}0\times10^{11}$	Nil	—
	14	19	5	$8{\cdot}3\times10^{9}$	$4{\cdot}2\times10^{10}$	Nil	—
B. Anti-foam failure	3	19	5	$4{\cdot}2\times10^{9}$	$2{\cdot}1\times10^{10}$	$2{\cdot}4\times10^{5}$	$1{\cdot}1\times10^{-3}$
	5	19	50	$1{\cdot}0\times10^{10}$	$5{\cdot}0\times10^{11}$	$2{\cdot}2\times10^{6}$	$4{\cdot}4\times10^{-4}$
	6	19	75	$5{\cdot}5\times10^{9}$	$4{\cdot}1\times10^{11}$	$5{\cdot}8\times10^{6}$	$1{\cdot}4\times10^{-3}$
	8	19	30	$7{\cdot}1\times10^{9}$	$2{\cdot}1\times10^{11}$	$3{\cdot}9\times10^{5}$	$1{\cdot}9\times10^{-4}$
	9	19	20	$7{\cdot}0\times10^{9}$	$1{\cdot}4\times10^{11}$	$1{\cdot}1\times10^{7}$	$7{\cdot}9\times10^{-3}$
							Mean $2{\cdot}2\times10^{-3}$
C. Pipework failure	7	19	500	$3{\cdot}1\times10^{9}$	$1{\cdot}6\times10^{12}$	$3{\cdot}7\times10^{6}$	$2{\cdot}3\times10^{-4}$
	10	19	500	$5{\cdot}8\times10^{9}$	$2{\cdot}9\times10^{12}$	$3{\cdot}5\times10^{6}$	$1{\cdot}2\times10^{-4}$
	11	19	500	$4{\cdot}0\times10^{9}$	$2{\cdot}0\times10^{12}$	$1{\cdot}4\times10^{6}$	$7{\cdot}0\times10^{-5}$
							Mean $1{\cdot}4\times10^{-4}$
D. Glass vessel shatter	12	19	500	$7{\cdot}0\times10^{9}$	$3{\cdot}5\times10^{12}$	$1{\cdot}3\times10^{6}$	$3{\cdot}7\times10^{-5}$
	13	19	500	$7{\cdot}7\times10^{9}$	$3{\cdot}9\times10^{12}$	$1{\cdot}5\times10^{6}$	$3{\cdot}8\times10^{-5}$
							Mean $3{\cdot}8\times10^{-5}$
D. Metal vessel rupture	15	45	300	$7{\cdot}8\times10^{9}$	$2{\cdot}3\times10^{12}$	$1{\cdot}4\times10^{6}$	$6{\cdot}1\times10^{-5}$
	16	45	450	$5{\cdot}1\times10^{9}$	$2{\cdot}3\times10^{12}$	$4{\cdot}9\times10^{7}$	$2{\cdot}1\times10^{-3}$
	17	45	20	$7{\cdot}5\times10^{9}$	$1{\cdot}5\times10^{11}$	$2{\cdot}8\times10^{6}$	$1{\cdot}8\times10^{-3}$
	18	45	450	$7{\cdot}7\times10^{9}$	$3{\cdot}5\times10^{12}$	$1{\cdot}0\times10^{7}$	$2{\cdot}9\times10^{-4}$
							Mean $1{\cdot}1\times10^{-3}$

Note: experiments 1–14 used 500 ml culture fluid, experiments 15–18 used 450 ml culture fluid.

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health and may be carried on air currents to places remote from the site of any accident. The samplers used were as follows.

(1) *Cascade impactor*. This device (May, 1945) separates the aerosol sample into four different size ranges. From the total number of BG spores collected in each range it is possible to calculate the Mass Median Diameter (MMD) of the original aerosol. Particles were impacted on to glass slides which were coated with a gelatin/glycerol mixture. Samples were washed from the slides with a phosphate buffer, and after dilution aliquots of the suspension were plated out on tryptone agar for incubation and counting.

(2) *Multi-stage liquid impinger*. This sampler (May, 1976) separates the aerosol into three size ranges each intended to approximate to the size range which is most readily retained by one of the three major portions of the human respiratory system, the naso-pharynx, $> 6\ \mu m$ diameter, the bronchi, 6–3 μm diameter, and the alveoli, $< 3\ \mu m$ diameter. The liquid substrates from each stage were diluted in a phosphate buffer and incubated on tryptone agar.

(3) *Slit sampler*. By impacting particles on to a revolving agar plate this sampler (Bourdillon, Lidwell & Thomas, 1941) records rapid changes in bacterial aerosol concentration.

At least four samplers of each type were used and were operated sequentially over the 25 min sampling period. In some experiments, settle plates containing a nutrient agar were exposed to define the extent of surface contamination produced by the simulated accident.

In series D experiments, when an explosive charge was used to rupture the metal culture vessel, the test organism was exposed to additional stresses which could have affected its survival in air. These stresses were caused by the pressure wave from the explosion and aerosolization in the chemical products of the explosion. To determine whether survival had been affected by these stresses, known quantities of BG spores were attached to spiders' webs and exposed to the explosion and also to the explosion products throughout the sampling period. Bacteria attached to such micro-threads react to chemical and physical stresses as though in the aerosol state (May & Druett, 1968).

RESULTS AND DISCUSSION

For the experiments in Series A the Porton fermenter was operated without a filter in place, and as a result of the lack of back pressure the culture fluid bubbled out freely through the outlet port. Large volumes of culture fluid were expelled from the vessel in this manner and as a result gross contamination of surfaces around the fermenter occurred. Results from the settle plates showed that the contamination was restricted to the immediate vicinity of the fermenter and largely confined to the table and tray on which the experiment was sited. No contamination was found on settle plates which were exposed at distances greater than 1 m from the fermenter (Table 1).

In Series B experiments, which simulated a failure in the supply of anti-foam, when the filter became wetted by the foam culture fluid was forced through the filter in irregular spurts. Between 20 and 75 ml of culture fluid was expelled in experiments 5, 6, 8 and 9 (Table 1) and most of this was contained in the tray on

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Table 2. *Results of analysis of air samples taken during experiments in Series B – anti-foam failure*

Experiment		3	4	5	6	8		9	
Cascade impactor (cells per litre)	1	0	1	126	282	15	8	516	583
	2	0	1	58	268	0	12	458	401
	3	0	0	68	81	8	24	385	621
	4	0	0	123	205	4	9	402	355
Multistage liquid impinger (cells per litre)	1	25	0	74	228	10		605	
	2	15	0	82	339	21		361	
	3	24	0	91	287	24		398	
	4	15	0	104	260	19		367	
Slit sampler (particles per litre)	1	10	—	—	87	6		191	
	2	8	—	—	55	5		246	
	3	8	—	—	52	4		222	
	4	6	—	—	25	5		165	
	5	6	—	—		3		167	
Cells per particle		1·3	—	—	3·6	4·0		2·4	
Mass median diameter (μm)	—	—	—	< 1·0	1·3	1·6		1·1	

Table 3. *Results of analysis of air samples taken during experiments in Series C – pipework failure*

Experiment		7		10		11	
Cascade impactor (cells per litre)	1	81	98	238	194	87	72
	2	319	149	143	108	84	63
	3	131	139	106	96	40	43
	4	237	110	81	44	23	15
Multistage liquid impinger (cells per litre)	1	59		119		61	
	2	89		105		54	
	3	127		70		50	
	4	123		55		24	
Slit sampler (particles per litre)	1	—		9		7	
	2	—		12		16	
	3	—		11		15	
	4	—		7		13	
	5	—		2		9	
Cells per particle		—		15		5	
Mass median diameter (μm)		2·9		4·3		4·2	

which the fermenter rested. Settle plate results showed that there was widespread and uniform contamination of the floor with up to 40 particles/cm^2. In experiment 4 the filter was connected to the fermenter with a longer piece of tubing than usual and as a result the filter did not become wetted by foam and no culture fluid was lost.

Simulation of pipework failure in Series C was effected by removing the drain plug in the Porton fermenter. This caused the contents of the vessel to surge out under the action of the stirrer and compressed air supply, causing widespread visible contamination of the floor and walls of the room. The fermenter was empty after less than 1 min but the stirrer and compressed air supply were operated for a further 5 min.

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Table 4. *Results of analysis of air samples taken during experiments in Series D – shatter of glass vessel*

Experiment		12		13	
Cascade impactor (cells per litre)	1	87	69	90	109
	2	44	70	77	52
	3	37	37	72	68
	4	25	12	35	41
Multistage liquid impinger (cells per litre)	1	51		34	
	2	45		18	
	3	0		31	
	4	0		28	
Slit sampler (particles per litre)	1	11		10	
	2	14		11	
	3	15		7	
	4	11		11	
	5	8		10	
Cells per particle		5		7	
Mass median diameter (μm)		4·0		4·4	

Table 5. *Results of analysis of air samples taken during experiments in Series D – explosive rupture of metal vessel*

Experiment		15		16		17		18	
Cascade impactor (cells per litre)	1	33	25	604	945	13	20	152	153
	2	13	22	388	547	10	7	114	96
	3	11	16	359	434	13	13	39	25
	4	6	6	220	314	8	3	17	13
Multistage liquid impinger (cells per litre)	1	21		1703		151		370	
	2	18		843		91		237	
	3	31		54		39		86	
	4	28		58		57		39	
Mass median diameter (μm)		3·2		4·0		4·2		3·7	

In Series D, striking the glass vessel with a missile produced cracking of the glass and the vibration of the stirrer caused immediate collapse of the vessel into several pieces. Instantaneous loss of all culture fluid ensued and this was propelled on to the floor, walls and ceiling by the action of the stirrer, which continued to operate for a further 5 min. It was not possible to view the explosive rupture of the metal vessel as it took place. Upon later inspection, however, two of the vessels – those in experiments 16 and 18 – had been punctured by holes and crushed, with some tearing of the welded seams. None of the culture fluid from these vessels remained, and it appeared that most of this had seeped into the surrounding sandbags. In experiments 15 and 17 the vessels were crushed, with extensive opening of the seams. Quantities of culture fluid remained in both of these vessels. Results from the exposure of BG spores attached to micro-threads showed that the airborne survival of the spore was unaffected by the explosion or the explosion products. More than 90% of the BG survived these stresses.

The results of the analysis of the air samples taken during Series B, C and D are shown in Tables 2–5. The three experiments in Series A, in which failure of

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the bacteriological filter was simulated, produced no detectable airborne particle. Some decrease in aerosol concentration may be observed during the sampling period in Series C and D, and it may be that the peak concentrations produced by these instantaneous accidents occurred before the sampling period commenced.

Evidence gained from the settle plates and by visual examination of the room indicated that the major portion of the culture fluid expelled from the fermenter during the experiments was disseminated in large droplets which rapidly became attached to surfaces. Clearly, the processes simulated here were inefficient in creating the forces required to shear the test liquid into droplets small enough to constitute a stable aerosol. The absence of respirable-sized particles in Series A experiments was an unexpected finding in view of work by Blanchard & Syzdek (1972) which showed that bacteria could be aerosolized in jet drops from bubbles bursting in a bacterial suspension. An explanation may lie in the physical properties of the culture fluid, which was a viscous suspension of cells in a proteinaceous liquid. In a study into the causes of infection occurring during haemodialysis it was found (MRC, 1975) that massive spillages of infected blood and even bursting bubbles in foaming blood did not contribute significantly to the respiratory hazard.

Despite widespread and sometimes gross contamination of surfaces in the experimental rooms Table 1 shows that only a small fraction of the expelled culture was recovered as an aerosol. Measured by this fraction, failure in the supply of anti-foam (Series B) and the rupture of the metal culture vessel (Series D) created the greatest potential hazard. In Series B culture foam was forced through a fine filter to produce a significantly smaller-sized aerosol (MMD 1·0–1·6 μm diameter) than that produced in the other series (MMD 2·9–4·4 μm diameter). Comparison of the results from Series A and B highlights the dangers of operating an air filter which has become wet. More stable aerosol was produced in Series B than in Series A when no filter was used.

Dimmick, Vogl & Chatigny (1973) have attempted to quantify the risks from several laboratory procedures by the use of a 'spray factor'. This is defined as the aerosol output per min divided by the concentration of viable units per ml in the dispersed material. Calculations based on their own and other work showed that one of the most hazardous operations was the use of open blenders or homogenizers to which spray factors of 10^{-5} to 10^{-4} were assigned. The blending of infectious material is known to have been the cause of several cases of laboratory-acquired infections (Sulkin & Pike, 1951). Spray factors for the accidents simulated here in Series B, C and D ranged from 3×10^{-5} to 3×10^{-4}, and when account is taken of the increase in aerosol output which would result from extrapolation to plant size, these figures will increase significantly.

The respiratory hazard created by the types of accident simulated here will clearly depend upon the pathogenicity and aerostability of the cultured organism and upon factors connected with the design and scale of the plant. The risks from three of the accidents are likely to be at least as high as those from other activities which have resulted in respiratory infection. Widespread contamination of surfaces occurred in all the accidents and even those from which no measurable aerosol was recovered may result in a respiratory hazard if this contamination became aerosolized as a result of conditions existing in the plant.

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