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ABSTRACT

This literature review deals with air handling in controlled environments in the food industry. The concern to determine the importance of the airflow as a possible source of contamination is growing, because the airborne dust particles can introduce foreign matter including microbial contaminants into the products produced. The following criteria should be used to determine a sampling strategy: the sampling method, specificity and level of sensitivity required as well as the speed with which a result is required. The microbial particles are likely to be more important than the total particle counts, because they can cause infections or be responsible for allergenic and toxic illnesses. Important information needed from the sampling period includes: location and area of the site, date and time of sampling, test temperature and moisture conditions, functions of the ventilation system during sampling, personnel in the area, volume of air sampled, collection media used and incubation time and temperature used as well as sampler used.

A careful planning of the plant layout as well as the services are needed to be able to choose an optimal clean air solution. Common methods used to reduce viable microbial counts in the production facilities include filtration, chemical fogging, ozone and UV radiation. The air filters needed in most food-processing environment are of barrier type, in which dust particles and the majority of microbes are captured and retained. The degree of filtration required is dependent largely on the product being manufactured. Since most food production plants are located in industrial areas with heavy atmospheric pollution, consumers need to be assured that these pollutants are excluded from the foodstuffs they consume.The filtration must be efficient enough to eliminate bacteria, fungi and spores from the airstream. The primary air filters protect the mechanical items of an air movement system from gross contamination over years of operation. Secondary filters are employed to remove smaller particulates from the air to a level needed in the process. Tertiary filters offer the

best protection in installations where maximum particulate control is required. Some of the topics to be considered when choosing filters for the controlled environment are: cleanroom classification, number of air exchanges needed, classification of filters, efficiency needed, particles to be captured, filter material and type of filter. Filter header frames and cases are usually manufactured from galvanized mild steel or stainless-steel; only prefilters use card frames. The design, installation and sealing of a suitable filter-framing system is essential to guarantee complete filtration integrity. All filters must be compatible with the food-processing environment and employ nonmigrating media.

Clean air solutions should be considered in processes, where microbial inactivation, e.g. through termal sterilization or deep-freezing, is not feasible. In a well-designed controlled environment clean airflows help to reduce the airborne contamination rate. It must be taken into account that the personnel itself is one of the main sources in contaminating products produced if the procedures, accessories and garments used are inappropriate. The potential risk situations created by interaction between people, air movements and airborne contaminants are difficult to predict with common microbiological measuring methods. The limitation of risks concept, which includes visualization of air movements, particle challenge testing and calculation of the risk factor, presents a method by which the risks can be limited. In the review methods for assessing viable airborne microbes are given in Chapter 7. In Chapter 8 available reports on the microbial air quality in food processing areas have been presented. Clean air solutions in food processing have been reported in Chapter 10.

PREFACE

This literature review is a part of the activities in a project dealing with systems analysis in life management. The project aims at developing system analytical methods for life management and production safety of industrial systems. The case studies is related to the management of air handling in food and packaging material production. This review about air handling systems and clean air solutions will function as a basic source for facts to be used in the food and paper industries when improving or rebuilding the air handling systems in the processes. The methods used aim at better maintenance activities in the air handling system.

The work at VTT Biotechnology and VTT Industrial Systems has mainly been funded by the thematic funds for the VTT project ”Systems analysis in life management” on safety and operational reliability. The first version of this literature study was compiled during a prestudy on air quality in food processing ”Ilmanlaadun vaikutus elintarvikeprosessoinnissa”, which was funded by Tekes. This literature review has been written by Gun Wirtanen, Hanna Miettinen, Liisa Vanne, Seppo Enbom and Satu Pahkala. Our special thanks are due to Dr. Berit Reinmüller at the Royal Institute of Technology for valuable rewiewing of the manuscript and to freelance artist Antti Huovinen for painting the picture on the cover.

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1. INTRODUCTION

The expression air quality can have various meanings to people, depending on the context. In the context of 'sick-building syndrome', determination of air quality can be based on mould-spore levels in the indoor air or the hydrocarbon levels in our urban environment. For the food processor air quality is often synonymous with microbial purity or the absence of microbial particles in the air, which may come in contact with the food at critical times during the processing of the product (Hampson & Kaiser, 1995).

Competition for market share is currently intense in the food and beverage industry. A functioning quality assurance system is a must for every company aiming to increase its market share (Steinbeck, 1997; Schicht, 1999a). Quality- and health-conscious consumers strive for low-fat natural products free from preservatives and other foreign chemicals. Wholesalers and retailers require longer shelflives and guarantees of quality and higher-capacity production units (Todt, 1990; Steinbeck, 1997; Schicht, 1999b).

Changes in lifestyle pressure the industry to produce more precooked and prepacked meals. For the food-processing, dairy and beverage industries to achieve a world-class manufacturing environment, careful planning of plant layout, environmental control and services design is required (Smith, 1997). The process air coming into contact with food must be controlled. The quality of air within industrial buildings is controlled by many food manufacturers, and for some food manufacturers it is therefore necessary to impose additional controls on environmental air quality to reduce the contamination rate (Brown, 1996). New packing materials such as polyethylene terephtalate (PET) containers for fruit juices and mineral waters require substitution of the pasteurization process with low-temperature aseptic filling in cleanrooms (Schicht, 1999a, b). In the manufacturing of hygienic and storable products without intensive use of intermediate substances, it is essential to prevent the access of undesirable microbes into the product. The aseptic procedure, maintained from the processing of raw materials right up to the finished product within its package, has been employed increasingly and successfully in recent years. Cleanroom technology plays an important role in these new manufacturing processes. It enables the provision of defined environmental conditions, e.g. temperature and moisture content, in the process air. Moreover, and very important, post-

contamination of the product by microbes or other contaminants can be effectively prevented by air filtration or disinfection (Todt, 1990).

In this literature review, a survey of air handling in the pharmaceutical and food industries has been carried out. The aim here was to provide information and new ideas for food processors. The solutions used in pharmaceutical cleanroom technology have been explained in more detail, although it is obvious that cleanroom technology in a strict sense is not necessary in many areas of the food industry. However, these solutions can be applied to other processes with different demands for hygiene.

2. BIOAEROSOLS

2.1 Air as a carrier of particles

The ability of air to contain and transport liquids, solids and living substances, such as microbes, is frequently overlooked or forgotten. Typically, biological agents such as plant cells, pollen, algae, protozoa, bacteria, yeasts, mould spores and viruses originating from natural habitats can be found in the air (Parrett & Crilly, 2000).

There are a number of ways in which biological and microbiologial material may be made airborne, e.g. through wind, rain splash and releases from animals. Almost every human and animal activity can create bioaerosols. For example, sneezing and coughing cause bacteria to become airborne. Humans shed skin and bacteria into the air, and even wearing clothes does little to stop the process (Griffiths & DeCosemo, 1994). An aerosol can be defined as a suspension of microscopic solid or liquid particles in air or gas, such as smoke, fog or mist. The size of aerosol particles are generally in the range 0.5–50 µm (Figure 1). Particle size is the major factor influencing aerodynamic behaviour (Kang & Frank, 1989a). Ljungqvist and Reinmüller (1998) showed with a controlled

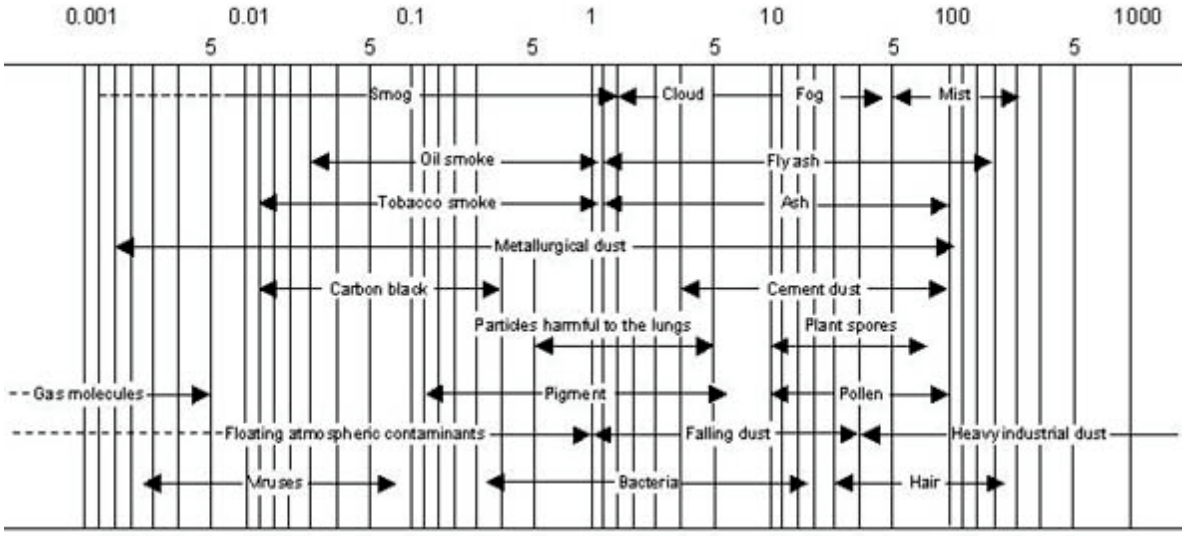

Figure 1. Particle size distribution (diameter as µm) of some atmospheric contaminants (Brown, 1996).

environment that the exposure situation with a human as a contamination source gives rise to the total number of airborne particles detectable with standard methods. The ratio between viable and nonviable particles ≥ 0.5 μm in this study was estimated to be 1:10 000. In industrial processes, airborne dust particles not only introduce foreign matter into the product, but also microbial contamination (Ljungqvist & Reinmüller, 1993, 1995a).

In annual studies of microbial concentrations in the atmosphere over Moscow (Vlodavets & Mats, 1958) and Montreal (Kelly & Pady, 1954) there were low winter and high summer airborne bacterial concentrations. The Moscow study explained the high bacterial concentration found in summer and low concentration in winter as correlated with the dusty summer dry period and the low winter and spring concentration as due to snow cover and rain, respectively (Vlodavets & Mats, 1958). The Montreal study had a midsummer trough for bacteria, which fungi did not have. The trough may result from the effect of high solar radiation and temperature and low humidity or a combination of both (Kelly & Pady, 1954).

The great majority (approx. 73–90%) of bacterial genera in the outdoor atmosphere are Gram-positive (Lighthart, 1997). Changes in bacterial composition have been observed over time, e.g. the levels of Gram-positive spore-forming bacteria were minimum (17%) and Gram-negative bacteria maximum (22%) during the night, while during daylight Gram-positives were maximum (35%) and Gram-negatives minimum (≤ 12%; Shaffer & Lighthart, 1997). Fungal bioaerosols have been observed to be highly dependent on weather conditions. The highest concentration of total colony counts appeared when the temperature was 25–30 °C, relative humidity (RH) 60–70%, and wind speed < 1 m/s (Lin & Li, 2000).

2.2 Microbial viability in the air

After becoming airborne, an organism may have a very short life, its stability being influenced by RH, temperature, oxygen levels, solar and ultraviolet (UV) radiation, and chemical factors. In general stability is lower where RH is low (Parrett & Crilly, 2000). High temperatures inactivate all pathogens, some more rapidly than others. In general, a number of different factors affecting microbes

can cumulatively stress them and affect their viability (Griffiths & DeCosemo, 1994). Important factors include organism species, growth conditions under culture, method of aerosol generation, sampling techniques and the airborne environment. Desiccation, radiation, oxygen, ozone and its reaction products as well as various pollutants can affect the viability of microbes (Griffiths & DeCosemo, 1994).

2.2.1 Growth phase

The growth phase affects the survival of microbes in an aerosol (Griffiths & DeCosemo, 1994). Brown (1953) found that the viability is minimal during the transition from stationary to logarithmic stages for *Escherichia coli*. Better survival rates from the stationary rather than from the logarithmic stages have been observed for both *E. coli* and *Serratia marcescens* (Goodlow & Leonard, 1961; Dark & Callow, 1973). There is little information available on the survival of microbes aerosolized from food environments. Stersky *et al*. (1972) showed that *Salmonella* New Brunswick survived much longer when aerosolized from skim milk than from distilled water.

Bacillus subtilis var. *niger* spores are widely reported to be aerostable. The viabilities of *Salmonella* Enteritidis and *Salmonella* Typhimurium were significantly better than those shown by aerosols of *Legionella pneumophila* and *Mycobacterium tuberculosis* studied for 2 h in air at 24 °C with 75% RH (McDermid & Lever, 1996). Yeasts are eukaryotes and are likely to be affected differently by aerosolization and sampling than bacteria. Bacterial spores survive better than the vegetative cells do. Microbes can mutate and adapt to changes in their growth environment, implying that it becomes very difficult to know if a given strain will respond in a consistent way to a stress factor applied over a long period of time (Griffiths & DeCosemo, 1994).

2.2.2 Oxygen and pollutants

Oxygen slowly kills most airborne microbes through oxidation (Kowalski & Bahnfleth, 1998). The toxic effect of oxygen is related to moisture content; it usually increases with the degree of desiccation, increasing oxygen con-

centration, time of exposure and also on whether the desiccation is caused by aerosolization or drying. It is important to remember that the combined effects of oxygen and RH should be considered when explaining losses in viability (Griffiths & DeCosemo, 1994). Airborne fungal contamination also correlates with air pollutants, e.g. ozone concentration (Lin & Li, 2000).

2.2.3 Dehydration

Airborne bacteria are subject to dehydration caused by evaporation of water from droplet-carrying microbes, as well as evaporation of cellular water. Dehydration of microbes causes osmotic stress and may result in decreased survival. Thompson *et al*. (1994) showed that the total recovery on an agar slide of viable *Pseudomonas fluorescens* was much higher at high than at low RH levels. The decrease in total recovery of microbes with increase in desiccation time was more pronounced at low RH levels of < 50% (Thompson *et al*., 1994). For yeast cells the survival was 4 times higher under high RH (> 70%) conditions compared with low RH (20–60%). The mould spores of *Penicillum* sp. were not affected by RH (Lin & Li, 1999b).

3. CLEANROOM TECHNOLOGY

3.1 Definitions

A cleanroom can be defined in many ways; the definition used here is that detailed in the 14644-1:1999 "Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness" standard. *A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room, and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary*. The objective of cleanroom technology in various cleanroom classes in the food and beverage industry is to ensure the control of biocontaminants in sensitive processes. Use of this technology should be considered in processes where microbial inactivation, e.g. through termal sterilization or deepfreezing, is not feasible. If critical process risks are identified due to exposure of the product to airborne microbes during processing or if severe sedimentation of airborne microbes can occur on critical process surfaces, cleanroom technology can be used to solve the problems (Schicht, 1999a; Whyte, 2001).

Cleanroom technology is an alternative to the use of preservatives when a long shelflife for products is desired. It is a gentle technology for protecting products with the additional advantage of not influencing their taste and consistency (Schicht, 1999a, b). The cleanroom concept will be a sound proposition as long as it offers the most attractive benefit-to-cost relationship. Both good design and proper operation must directly address the specific hazard situation of each case. It must be integrated into the hygienic chain of the entire operation, which always fails at the weakest link in the process (Schicht, 1999a).

In aseptic processes using cleanroom technology, the most critical steps are the final packing operations. For products that are either sterile or contain very low numbers of microbes there is a risk of recontamination during packaging, which can be minimized when using cleanroom applications of the right class. The shelflife of sensitive products such as yoghurts, desserts, soft cheese, presliced bread and fruit juices as well as small portions of chilled food can thus be increased (Schicht, 1999a).

The traditional pharmaceutical cleanroom technique employs highly filtered air free of microbes, appropriate airflow patterns, as well as enclosures separating the clean area from the surroundings. Personnel must use suitable cleanroom garments. They must also be well trained and motivated to work in a cleanroom environment. Appropriate cleaning procedures for equipment and surfaces need to be adopted and validated in the cleanroom processes. All personnel and material flows must be strictly controlled (Schicht, 1999b). In the food industry, applications of cleanroom technology for critical parts of the processes are increasing.

Cleanrooms can be constructed in all shapes and sizes, from special-purpose minienvironments to large expansive production facilities. They may be designed to protect both the product and the personnel. The level of cleanliness has traditionally been associated with method of ventilation, but it does not guarantee the ventilation performance because the airflow is extremely complex (Stribling, 2000). When designing a plant, special care should be taken to ensure a well-distributed flow of relatively dry, high-efficiency particulate air (HEPA) filtered air along walls, windows and ceiling. The RH near the relatively cold surfaces with condensation is thereby reduced. The outside and recirculated air is treated in separate air units for each production area. By means of these units, the air is heated or cooled, dehumidified, filtered and delivered (Todt, 1990). The performance of a cleanroom is defined by a set of complex interactions between the airflow, sources of contamination and heat, position of the air terminals and exhaust, as well as the objects occupying the space in question (Stribling, 2000).

Cleanliness classes as well as the number and size of particles in the various cleanroom classes and controlled environments are given in the standard ISO 14644-1:1999. A summary of these cleanroom classes is presented in Table 1 and Figure 2. Appropriate air cleanliness classes for food and beverage applications are dependent on the product manufactured. In contrast to the needs of the pharmaceutical industry, no guidance values have been established for food processing. Food industry personnel must choose the appropriate cleanroom application based on risk analysis procedures and results from their own process. For aseptic filling operations in the pharmaceutical industry, in which the product is exposed to the atmosphere and where sterility assurance levels must be met, a grade A (corresponds to ISO class 5 cleanroom) environment is required (Schicht, 1999b). A comparison of different standards is shown in Table 2.

Table 1. Selected airborne particulate cleanliness classes for cleanrooms and clean zones (ISO 14644-1:1999).

ISO classification	Maximum concentration limits (particles/m^3 of air) for particles equal to and larger than the sizes shown below					
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1 µm	5 µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
ISO Class 4	10 000	2 370	1 020	352	83	
ISO Class 5	100 000	23 700	10 200	3 520	832	29
ISO Class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO Class 7				352 000	83 200	2 930
ISO Class 8				3 520 000	832 000	29 300
ISO Class 9				35 200 000	8 320 000	293 000

Table 2. Comparison of cleanroom classifications defined in different standards (Gerbig &Houge, 1994; Winter & Holmgren, 2000).

FED STD-209D (Winter & Holmgren, 2000)	FED STD-209E (Winter & Holmgren, 2000)	ISO 14644-1 (1999)	EC-GGMP (at rest); Gerbig & Houge (1994)
1	M 1.5	~ Class 3	
10	M 2.5	~ Class 4	
100	M 3.5	~ Class 5	Grades A[a] and B
1 000	M 4.5	~ Class 6	
10 000	M 5.5	~ Class 7	Grade C
100 000	M 6.5	~ Class 8	Grade D

[a] = laminar flow bench

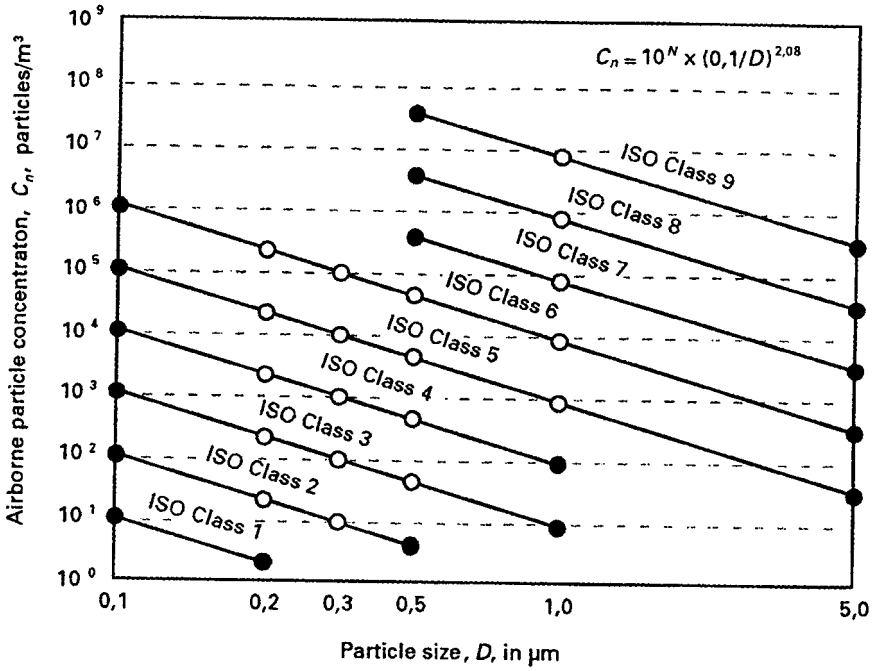

Figure 2. Graphical representation of ISO-class concentration limits for selected ISO classes.

3.2 Layout of cleanrooms

3.2.1 Cleanroom design

In a well-designed plant layout suitable airflows help to reduce the airborne contamination rate (Heber, 1997). Nothing apart from processing equipment of stainless steel cleanable in-place should be placed on the factory floor. These devices should be designed so that the floor beneath is easily accessible and cleanable (Holah & Timperley, 1999). Problems may occur if the air used to protect food and the equipment has been contaminated before flowing over the stored food. At no point in the factory should uncooked and cooked products be in the same process area, due to the possibility of cross-contamination through manual treatment, equipment or from the air (Worfel *et al*., 1996). Fitzpatrick (1994) suggested that plant design should therefore include a separate cleanroom

environment as a storage area for finished food products and prepared food components prior to filling and packing (Figure 3). Two common ways to influence airflow and minimize transfer of airborne microbes are to build separating structures, e.g. walls between 'clean' and 'dirty' areas or to separate 'clean' from 'dirty' areas by suitable airflows and adequate distances between the processes (Worfel *et al*., 1996). In a study by Worfel *et al*. (1996) the importance of plant layout and separation of different areas was shown clearly. The total airborne contamination during meat processing in 3 slaughtering facilities was measured. In the first plant the slaughter line was serpentine and circled back upon itself throughout the slaughter room. There was no dividing wall between the hide-on and hide-off working areas. The second plant had a modified straight-line rail layout with a dividing wall separating the hide-on and hide-off working areas. The third plant had a dividing wall separating the hide-on and hide-off areas and a dividing wall separating the stunning and bleeding area from the rest of the facility. The rail system in the plant was serpentine throughout the hide-off room (Worfel *et al*., 1996). The results indicated that a modified straight-line rail layout with dividing wall between the hide-on and hide-off

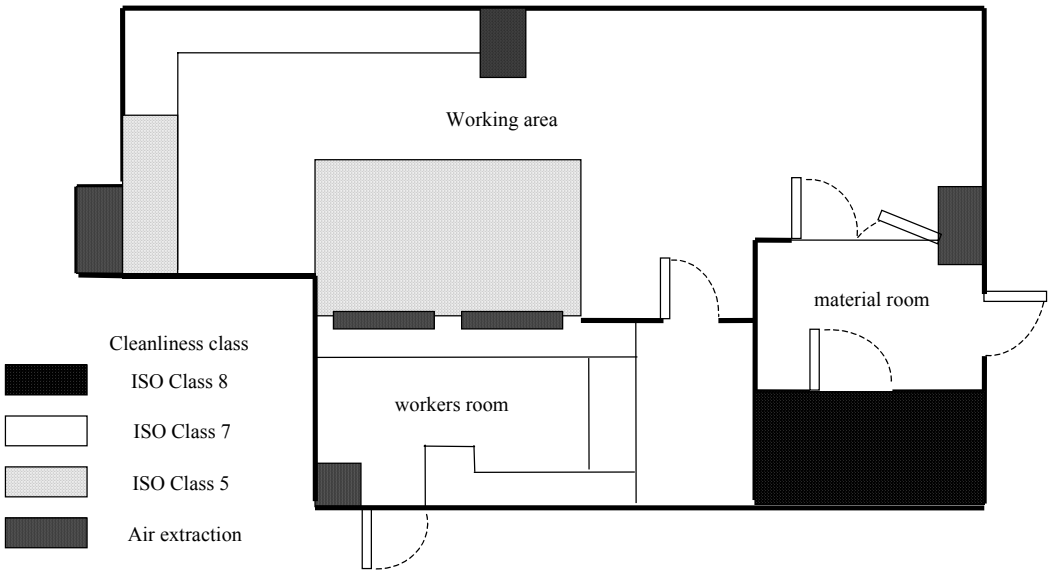

Figure 3. Sketch of cleanroom with ISO cleanliness classes at operational conditions (Havet & Hennequin, 1999).

areas was effective in reducing viable airborne microbial counts in the hide-off areas when positive air pressure was maintained in the slaughtering room. The results also showed that the highest viable airborne populations in the hide-off area were in the eviscerating and paunch-opening processes. Thus, the requirement for a dividing wall between the hide-on and hide-off areas in beef slaughtering/dressing plants is justified (Worfel *et al*., 1996).

3.2.2 Ceilings, walls and floors

Ceiling, wall and floor elements should comply with all relevant regulations concerning fire protection, sound and thermal insulation. Materials and surface finishes should meet all general requirements for their application. Particular attention should be focused on smoothness and effective sealing of utility services or other penetrations (ISO 14644-4:2001). Wall panels and framework are important factors in selecting wall systems used for the cleanroom. Each project should be evaluated on an individual basis based on its needs and requirements. Modular cleanroom walls are designed for flexibility, because upgrading, size requirements, equipment needs and degree of cleanliness needed often change rapidly. The use of modular cleanroom wall systems in construction can be an effective alternative to conventional construction (Kouri, 2000). Corridors and ceilings should be covered with food-grade plastic cladding for easy washdown. Extensive use of plastic coverings ensures that there are no awkward corners and cracks, which might otherwise allow bacteria to accumulate. The nonporous, high-impact, sealed floors are also crack-free and fitted with central drains allowing then to be hosed down and the water drained away (Birks, 1999).

3.2.3 Doors

Doors are subjected to all kinds of abuse from general wear and tear in high-traffic areas to continuous impacts from trolleys, vessels and equipment. Whilst the walls, floors and ceilings may be subjected to the same environmental conditions and have the same hygiene requirements as the doors, the operational requirements of doors will be very different. Many door systems in the market

are traditionally built mostly of timber and to a lesser extent of steel or aluminium (Cronin, 2000).

The choice of door material should be based on the environmental facts that take into consider e.g. how the door will be subjected to heat, light, radiation, moisture, weathering and microbial as well as organic contamination. Furthermore, security and aesthetics should be evaluated. These facts should be compared with the proposed operational and performance requirements, which cover resistance, cleanability, strength, durability, impact resistance and most importantly, frequency of operation of the door in service (Cronin, 2000). In addition to the careful attention focused on correct choice of the wall material and construction, the method of interfacing the door with the wall must be carefully considered. Glass-reinforced polyester (GRP) is one of the toughest and most suitable plastics for doors in the cleanroom environment. Its quality combined with surface durability, strength and resistance to water and chemicals helps users and specifiers to solve many maintenance problems associated with conventional door materials using e.g. moulded seamless structures as well as absence of steel and timber. A range of aluminium and stainless-steel adjustable frame systems has been developed by manufacturers to provide solutions for problems associated with the wall frame interface (Cronin, 2000).

No matter how durable the chosen door material is, additional door protection can maintain the appearance of the door and help to extend its life. Face protection can also be considered with a choice of materials. As an alternative to stainless-steel, polyethylene face protection is becoming increasingly popular in the cleanroom (Cronin, 2000). The pressure regimes found in cleanrooms in pharmaceutical manufacturing and packaging often create problems for correct operation of the doors. Even with careful modelling techniques to determine the airflow in a given situation, it will be difficult to predict the total input from the doors. Therefore, special attention in determining what effect airflow will have on door operation must be considered in the process layout (Cronin, 2000).

Cleanroom doors should have as few horizontal surfaces as possible. Particular attention should be focused on minimization of steps and ledges in the door surface. Thresholds should also be avoided. Consideration should be given to minimization of abrasion in the mechanical elements of the door, e.g. latches, locks and hinges, and also between the door and its frame. Pushplates, automatic

openings or appropriate door-swing direction should be considered where contamination transfer is of concern (ISO 14644-4:2001).

3.2.4 Laminar flow tunnels

For the production of sensitive products such as milk and milk-based items or even noncarbonated drinks, the food and beverage industry uses automatic or semiautomatic machines with ventilated tunnels providing laminar flow over the sensitive areas to give a classification of 1000 particles/ft^3 (comparable to ISO class 6 operational). These covered machines are themselves installed in rooms classified between 10 000 and 100 000 particles/ft^3 (comparable to ISO class 7 and 8 operational). One of the greatest problems is transferring food products and the packaging material into the process with the required microbiological quality (Fontcuberta, 2000). In these applications, particular attention is focused on the treatment of the packaging materials, e.g. bottles, stoppers, caps and sealing covers. Recontamination is avoided through sterilization of the incoming material in-place within the protected zone or through removing the outer protection cover before the material enter the packaging process (Fontcuberta, 2000). The containment barrier is a physical barrier, also referred to as a tunnel in the food and beverage industry. It separates the minienvironment around the product and the indispensable active machine elements from the ambient environment. It can be manufactured in flexible, e.g. polyvinyl chloride (PVC) film or rigid materials such as stainless-steel. The choice of material is based upon the criteria used for cleanability and ability to resist occurring elements in the process (Fontcuberta, 2000).

3.2.5 Equipment and material

Reinmüller (2000) stated that the air-conditioning equipment used especially in cleanroom environments should be placed in premises with adequate space and of specified hygienic standards. All installations should be accessible for inspection and the premises should be equipped with sufficient light to allow inspections and cleaning (Reinmüller, 2000). Air intakes should be protected against rain and snow and supplied with hygienic floor drains to carry away any water or melted snow. Whenever water is used in cooling towers, evaporating condensers and in humidifiers, the water quality must be controlled. If high RH

is required in one part of the building, the humidity should be added and controlled by a secondary system located close to the premises in question (Reinmüller, 2000).

All installations should be made of stainless steel and plastics, which are easily cleaned and are corrosion-resistant (Todt, 1990). The surface materials used should be hard, nonporous, smooth and cleanable (Reinmüller, 2000). All equipment must be designed to meet cleanroom and hygiene requirements. The media required for the process, e.g. steam, water and compressed air, must match the special requirements and undergo additional disinfection treatment if necessary (Steinbeck, 1997). Sterile gas and pure-water installations must be considered where appropriate along with other service connections (Morgan, 1992). It is equally important to consider all equipment and furniture going into the processing area covered by a certain cleanroom class. This should be compatible with the particle level specified and ideally should be non-shedding and easy to clean, with no surface defects that will entrap dirt and dust produced by work processes.

3.2.6 Changing rooms

All personnel working within a clean area should be aware of the necessary cleanroom protocols to be carried out to ensure the room performs to its design specifications during its lifetime (Morgan, 1992). According to ISO 14644-4:2001, changing rooms should be equipped with specialized airlocks for personnel entry to and exit from the cleanroom areas for sensitive processes. Separation of the personnel entering and leaving the cleanroom should be ensured via the changing room. The overpressure in the changing area should be approx. 15 Pa above the external pressure and 15 Pa below the cleanroom pressure, so that air flows from the cleanroom through the change area to the air-handling unit (Morgan, 1992).

Separate canteens for employees working in the uncooked and the cooked product areas can be used. Another solution is that each worker whenever entering the 'high care' area changes the coat and all other outer garments in a tiled cloakroom. The washroom area should be used before entering the food-processing area (Birks, 1999).

4. AIR HANDLING IN CLEANROOMS

4.1 Air distribution

Airborne contamination can be reduced by controlling airflow through proper ventilation systems and good facility design. Airflow and distribution should be designed and regulated so that air flows from finished product areas to raw ingredient receiving areas. Lutgring *et al*. (1997) examined bioaerosol concentrations in poultry slaughtering plants. The results indicated that airflow and location of processing operations affect the microbial levels of the entire process. To reduce running costs the cleanroom can be designed such that 70–90% of conditioned air is recirculated and reconditioned through the air-conditioning unit with the remainder coming from fresh air to provide comfort conditions within the room (Morgan, 1992). Different pieces of equipment may require air protection at specific points. For example, liquid filters may need protection directly around the nozzle, whilst seals may be best protected by a horizontal flow across the sealing head (Fitzpatrick, 1994).

To meet the air cleanliness requirements, it is sometimes sufficient to dilute the concentrations of airborne microbes in the room. The aim can be achieved by supplying necessary quantities of filtered air into the room by means of suitable air diffusers. The diffusers should supply the air with sufficient kinetic energy to guarantee uniform mixing with the room air. This air distribution method is referred to as a mixing type (Lehtimäki, 1998), and corresponds with that normally provided by all types of air-conditioning systems (Figure 4a). The mixing-type air distribution method may not always be powerful enough making the technique impractical in certain cases and expensive in others. Today, food companies are beginning to raise air standards at critical control points by using unidirectional or laminar airflow systems (Figure 4b). In unidirectional airflow, incidental contamination is constantly blown away from the product with an air velocity of 0.3–0.5 m/s. Radial or vector distribution technology provides a clean and dependable supply of air with minimal turbulence, while enhancing uniformity of direction precisely at the critical areas (Scholler & Kaiser, 1997). Thus, the air moves in parallel streamlines, and freely flowing microbes will be swept away by the airflow path (Schicht, 1999a). These solutions are used for limited areas where there is an increased risk of contamination. This technique has been successfully implemented in many industries such as microelectronics,

pharmaceutical manufacturing, medical, plastics, coatings, photography and automotive paint (Scholler & Kaiser, 1997).

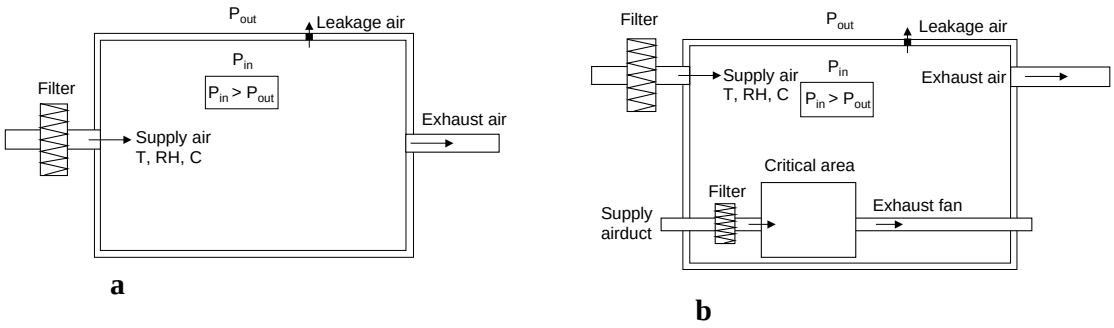

Figure 4. a) Mixing airflow and b) Unidirectional airflow (Lehtimäki, 1998).

4.2 Airflow and airflow velocity

The influence of airflow velocity on contamination control of the air based on different key characteristics e.g. ceiling height, internal heat load and internal particle generation has not been clarified thoroughly. Ogawa (2000) studied the dynamic state of the airflow in the workstation, using air velocity as the parameter in a computer-aided model. The author concluded that airflow characteristics in the workstation showed no significant changes while varying the velocity of supplied airflow. It can, however, be observed that the capacity for recovering cleanliness per unit of supplied air has a tendency to increase with airflow velocity. Although the movements of the operator can disturb the unidirectional airflow in the workstation, particles generated from the operator's head will be exhausted outside the station, without contaminating the equipment surface. It may as well be said that the risk of contamination becomes higher as air velocity is decreased. It was also discovered that the operator's motion affects airflow from inside the equipment area. Each system has different airflow velocities, depending on the process (Ogawa, 2000). The choice of vertical, horizontal or inclined airflow is dependent on the geometry of the objects to be protected as well as local hotspots and thermal currents produced by them, and upon the manipulations required during processing (Schicht, 1999a).

The airflow within the cleanroom area is an important factor in the precise control of air over the container into which food products are assembled. Other

factors affecting airflow in cleanrooms include shape and size of container, temperature of food, line speed, movement of people, complexity of plant, room geometry and position of inlet, outlet and exhaust points. The air may be forced through central HEPA filters directly into the cleanrooms or to separate distribution units producing laminar air flow (Fitzpatrick, 1994).

To guarantee the necessary stability of the displacement airflow in an industrial environment, the air velocities should be at least 0.3 m/s (Steinbeck, 1997; Schicht, 1999a). Therefore, displacement airflow is synonymous with the circulation of considerable quantities of air per metre-squared (m^2) of protected room area. In the interest of cost-effectiveness, this principle should be used as seldom as possible (Schicht 1999a). In the planning of a clean-air protection scheme, the areas protected with displacement airflow should be kept to a minimum according to the requirements in the process (Scholler & Kaiser, 1997; Steinbeck, 1997; Schicht, 1999a).

4.3 Air exchanges

The simplest cleanroom should have at least 15–20 air exchanges per hour, but preferably 20–50 since this will ensure that particles generated by the work process are swept away from the area (Morgan, 1992). Cleaner facilities require higher volumes of air exchange. In Class 100 particles/ft_3 (according to FED-STD 209D and comparable to ISO class 5 operational) facilities in which vertical laminar flow is required, the exchange rates are 400–600 per hour with an air velocity of 0.3 m/s. Alternatively, with a nominal flow rate of 0.5 m/s, 250 or more air exchanges per hour are needed (Morgan, 1992; Fitzpatrick, 1994). The clean air environment is strictly controlled for particle size, flow pattern and microbial load. Combined with laminar flow the room is cleaned quickly and particles can be prevented from settling onto food and equipment surfaces (Fitzpatrick, 1994).

4.4 Filtration

Air filtration is used to prevent particulate contaminants from entering the production line. The degree of filtration required is dependent largely on the product being manufactured, whether terminal sterilization occurs and whether

preservatives are used (Smith, 1997). Filtration must be efficient enough to eliminate bacteria, fungi and spores from the airstream (Smith, 1997). Aseptic handling is achieved by ensuring the process with laminar airflow that has passed through HEPA filters. If the food production plants are located in industrial areas with heavy atmospheric pollution, consumers need to be assured that these pollutants are excluded from the foodstuffs they consume (Smith, 1997; Souto & Fernandes, 2000).

The primary air filters protect the mechanical items of an air movement system from gross contamination over years of operation. The maintenance of clean dust-free air-handling equipment is a prerequisite in the food-processing industry (Brown, 1996). Secondary filters are employed to remove smaller particulates from the air to a level needed in the process. A rigid pocket- or cell-type filter will ensure that the selected level is maintained throughout filter life. To ensure overall installation efficiency it is necessary to install a leak-free filter-holding frame system (Brown, 1996). Tertiary filters offer the best protection in installations where maximum particulate control is required. The various types of filters are shown in Figure 5. These filters are usually HEPA or ultralow-penetration air (ULPA) filters. The European standard EN 1822 has classified HEPA and ULPA filters in the classes shown in Table 3 (H ~ HEPA and U ~ ULPA).

Winter and Holmgren (2000) published a filter guide for consulting engineers and contractors who design and construct cleanrooms for the pharmaceutical industry and also for the owners of these cleanrooms. It deals with filters in air-conditioning systems for cleanrooms and is meant to be a tool to help decision making on which type of filter can be used to meet the requirements of cleanrooms and how they should be tested. Topics to be dealt with are:

- cleanroom classification
- number of air exchanges
- filter materials
- classification of filters
- efficiency and particle size
- testing filters
- differential pressure across filters
- filter type to be used.

The air filters needed in a food-processing environment are usually of the barrier type, in which dust particles and the majority of microbes can be captured and retained in the filter media matrix. Filter header frames and cases are usually manufactured from galvanized mild steel or stainless-steel; only prefilters use card frames. The use of cellulose products should be avoided in tertiary filters. All filters must be compatible with the food-processing environment and employ nonmigrating media. The design, installation and sealing of a suitable filter-framing system is essential to guarantee complete filtration integrity. The final and highest grade of filter selected should be the last item in the air-handling system prior to the supply ductwork (Brown, 1996).

Table 3. Classification and requirements for HEPA and ULPA filters according to EN 1822 (Winter & Holmgren, 2000).

	Total value		**Local value**	
Filter classes	*Efficiency (%)*	*Penetration (%)*	*Efficiency (%)*	*Penetration (%)*
H 10	85	15	-	-
H 11	95	5	-	-
H 12	99.5	0.5	-	-
H 13	99.95	0.05	99.75	0.25
H 14	99.995	0.005	99.975	0.025
U 15	99.9995	0.0005	99.997	0.0025
U 16	99.99995	0.00005	99.99975	0.00025
U 17	99.999995	0.000005	99.9999	0.0001

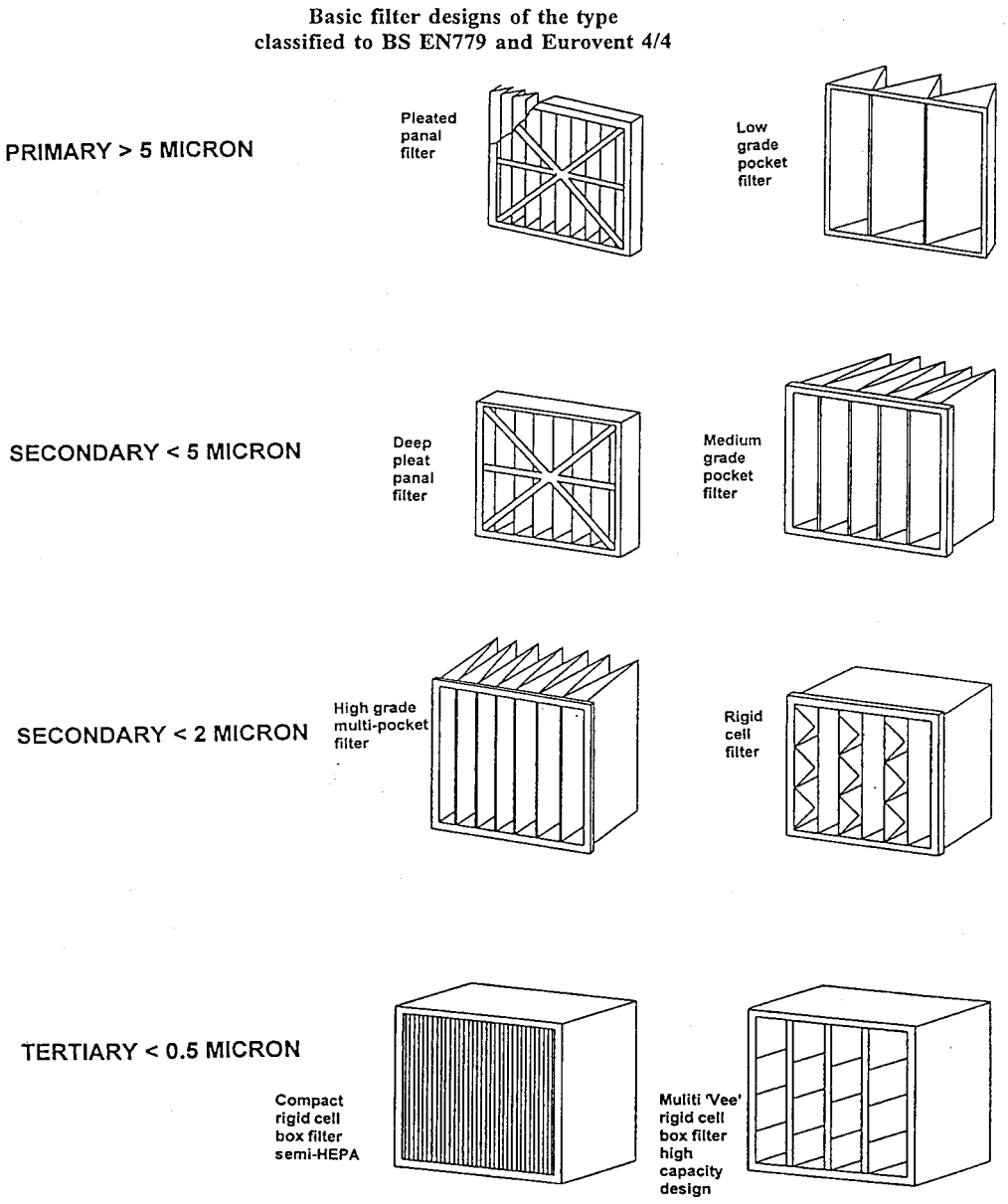

Figure 5. Basic filter designs of the type classified in BS EN779 and Eurovent 4/4 as well as EN1822 (Brown, 1996).

The design and performance of filters can vary widely, and careful comparison of various products is needed. Choosing filters for air-conditioning systems for cleanrooms is a very complex process. When designing and describing filters, special attention should be focused on the filter class and standard setting of requirements for the specific filter classes (Winter & Holmgren, 2000). In the food industry, the selection of air filters must be based on reliable functioning and cost-effectiveness. In most cases, use of filters of the finest grade is not necessary.

4.5 Process air

Air is an ingredient in many foods, e.g. aerated products, products moved using fluidized-bed technology or products produced by fermentation. Process air may also include modified atmospheric gases, which are in direct contact with the product. In-line cartridge filters of suitable quality should be used when air is supplied from compressors. These filters should be sited as near as possible to the machine and there should be adequate facilities for keeping them in a hygienic state. The requirements should also comply with air instrument pneumatics if it is vented into a high- or medium-care area (Brown, 1996). Furthermore, there may be special or additional requirements for the instruments, e.g. dry air or pressure control within tight limits.

4.6 Pressure difference

A method for minimizing contamination in food manufacturing is the creation of a constant, small, positive pressure difference. In practice, it is difficult to guarantee continuous overpressure, e.g. when doors are opened (Steinbeck, 1997). Consideration must be made for all the openings: doors, conveyors, piping, light fixtures, conduits, vents, drains and general construction (Scholler & Kaiser, 1997). When the product is transferred from the cleanroom atmosphere to the outside atmosphere the flow of air is always outwards, due to the positive pressure difference. This positive pressure also serves to protect the cleanroom integrity when personnel, equipment and materials are being taken into the food assembly room (Fitzpatrick, 1994).

An air-pressure control system is required to maintain a positive air pressure in areas where the final product is exposed to air to minimize the contamination rate. As an example, an overpressure of 45 Pa at the cleanest area, 30 Pa at a less clean zone, 15 Pa in the change area and 5–15 Pa in the facility room give a good pressure gradient (Morgan, 1992). To facilitate air-pressure control, the various stages of production at various levels of cleanliness need to be divided into separate compartments with efficient sealing, e.g. doorways or air locks between each compartment. The internal positive pressure in the production facility aids in preventing infiltration of contaminated ambient air into the airflow (Smith, 1997).

The pressure difference between the outdoor and indoor air caused by wind pressure can be much higher than between indoor spaces. Due to the limited capacity of air-conditioning systems, the pressure difference between indoor and outdoor air is in many cases negative, especially during windy weather. Therefore, the situation of clean production areas near the building envelope should be avoided.

Room pressure in facilities using common air supplies is difficult to maintain. A change in pressure of one room will affect the pressure in other rooms. To create a stable control system, a high level of system damping is often necessary. When a door is opened and the room pressure falls, the dampers open fully to try to maintain the room pressure. On closure of the door, the room pressure rises to excessive levels as the control system fails to close the damper down in time. This means that it needs time for the stability to return. During such overpressure the filters can be damaged or blown out, resulting in disruption of the cleanroom activities. The principle of door-flow protection, stipulating an airflow of limited but sufficiently high velocity through an open door is a prime feature of the Health Service standard 2025 for design of operating theatres. The technique used in hospitals to control operating room pressure prevents airborne contamination. The air-pressure stabilizers are passive, gravity-operated devices that translate from a closed to an open position over a very small pressure range. A combination of blade dynamics and low fraction bearings ensure very low hysteresis, making the control devices very sensitive (Gately, 2000).

4.7 Humidity and temperature

The temperature and humidity control required in the cleanroom is important. It is preferable to specify a wider control band so that both unit costs and running costs are reduced (Morgan, 1992). Too much water in the air generally causes humidity problems in the food industry. The most common method for dehumidifying air is to use a refrigeration coil for condensating the moisture of the air. After condensating, the air is normally reheated to the supply temperature; therefore, abundant energy is needed. Microbial growth in the condensate water generated in the cooling system is one of the main hygiene risks in this type of system (Brown, 1996).

Another way to solve moisture problems is to use an adsorption air dehumidification system i.e. a liquid desiccant cooling system (Hong *et al.,* 2000). In this system, the humid air is dried in a spraying tower by a hydrophilic absorbent liquid. A typical liquid is water-lithium chloride. Before spraying, the liquid is cooled. After leaving the dehumidification tower, the liquid must be heated and regenerated in a regeneration spraying tower by another airflow (e.g. outdoor air). Compared with condensating air dehumidification, the adsorption system is more energy-efficient especially if there are waste energy sources available. Microbiological problems with this system are smaller than those encountered when condensating air dehumidification (Hong *et al.,* 2000; Lehtinen, 2002).

In general, higher temperatures and higher RH are more conductive to microbial survival and growth. Temperatures and RH should be optimized to reduce the survival and growth of foodborne and airborne microbes (Lutgring *et al.,* 1997). Intrinsic properties of foods (pH, water activity etc.) or extrinsic ones, such as temperature and atmosphere, are usually known because they can be easily monitored, but the impact of RH of the air has rarely been studied. A major difficulty is to reach an equilibrium between the atmospheric RH and the water activity (a_w) of the product surface. When the product is placed in an airflow of known velocity, temperature and RH, the evolution of the a_W on the surface can be described by a model that combines water transfers inside the product and at the air/product interface (Olvera *et al.,* 1999).

Temperature and humidity levels for personnel comfort should be defined for these specific installations. A typical set range for RH is > 30% RH to < 65% RH. Specific guidance to adjust temperature specifications to cleanroom garments used is given in ISO 7730 (ISO 14644-4:2001).

4.8 Exhaust

At a typical food-processing plant, undesirable air is exhausted to the outdoors. Unfortunately, part of the discharged air often stays in the air-foil of the building, setting up a scenario in which exhausted contamination can partly re-enter the plant at another location. Roof exhaust stacks from heavily contaminated areas that do not have HEPA filters need to be high enough so that none of the exhausted air can be re-entrained. Exhaust stacks should be 1.3–2.0 times the height of the building, including any parapet or other roof equipment. Stronger winds increase the need for taller exhaust stacks. Unfortunately, the stacks in reality are usually short and the roof vortex can be equal to the building's height (Scholler & Kaiser, 1997). At locations where humidity originates from the process in larger quantities, it is locally exhausted with the air and expelled into the atmosphere by separate fans mounted on the roof (Todt, 1990).

With regard to the particle concentration, air supplies via twisted outlets are advantageous compared with locally installed laminar outlets, because the maximum particle concentrations are lower and the particle distribution more even (Sodec & Halupczok, 2000). The temperature difference has no significant influence on particle distribution. The maximum particle concentrations are only slightly affected through air exchange and volume flow rate by the laminar flow unit. In aerodynamic terms air supply via twist outlets is more effective than with laminar outlets in cleanrooms of ISO Classes 7 and 8. The main advantages are: lower maximum air velocities in the occupied zone, more even pattern of air velocities, more even indoor air temperature, prevention of low air temperatures under the outlets, less frequent peak particle concentration as well as more even distribution of particle concentration (Sodec & Halupczok, 2000).

5. PERSONNEL IN CLEANROOMS

5.1 Behaviour of personnel

Personnel are usually the predominant source of airborne microbes in cleanroom production areas (Schmitt, 2000). Personal hygiene must therefore be incorporated into the overall concept with care. The personnel must be properly trained. The workers must fully understand the procedures and the microbiological risks inherent in inappropriate actions (Steinbeck, 1997; Schicht, 1999a). It has been found that a slowly gesturing person generates approx. 500 000 particles/min and a rapidly gesturing person will generate 5 000 000 particles/min. Therefore, limited personnel movements and specific cleanroom garments are essential part of strict cleanroom requirements (Schmitt, 2000).

5.2 Cleanroom clothing

The main function of cleanroom garments is the protection of products manufactured in a cleanroom against fibres and particles released by humans from skin and garments. The textile of the garment itself is important; it must not be an additional source of particles released during use. The textile surface cannot be viewed as a typical surface such as walls or wafers because it is 3-dimensional. This third dimension is the most interesting with regard to cleanliness due to the continuous interaction between the outer and inner surfaces. Cleanroom garment fabrics usually act as surface filters, which means the barrier ability increases with longer test duration and increasing particle size. The barrier ability of the fabric itself is important, and also that of the cuffs and fastening. In practical use, particles and fibres not only pass through the cleanroom fabrics by air transport, but also through mechanical stress. In addition to the barrier function of a cleanroom garment system, wear comfort is also an important criterion (Schmeer-Lioe *et al.*, 2000).

There is a high need for garments that will diffuse increasingly fewer particles, especially after many washes. To achieve the best possible decontamination results, minimum residual contamination after washing in a gentle washing procedure is needed. From the operator's point of view there is a great demand for comfort and good antimicrobial and deodorizing performance over long

durations (Kuhl, 2000). Many types of clothing are available e.g. woven and nonwoven fabrics, that are either washable or reusable or disposable garments. The level of cleanroom class and particulate contamination permissible will determine which garments should be used and can vary from overshoes and coats to full masks and gowns (Morgan, 1992).

6. LIMITATION OF RISKS

Unequipped cleanrooms are generally not considered to be a problem. The situation may change dramatically when cleanrooms are equipped and run with machinery and operators in various processes with temperature differences. Optimal airflow patterns in clean zones can easily be disturbed, e.g. by machine-guarding measures or equipment design. The effect of these problems increases since inappropriate interference by operators contributes to a higher risk of airborne contamination. The predominant sources of contaminants within a cleanroom are people and machinery. The potential risk situations created by interaction between people, air movements and airborne contaminants are difficult to predict with common microbiological measuring methods (Reinmüller, 1999).

In a cleanroom the microbiological burden is usually very low (< 1 colony-forming unit (CFU)/sample), and it is difficult to achieve statistically significant results. It is extremely difficult to evaluate potential microbiological hazards, and too often microbiological assessment is based on poor microbiological data and on subjective conclusions from visual observations. The assessment should not be based on an intuitive unsystematic approach (Ljungqvist & Reinmüller, 1993, 1995b). Knowledge of the interaction between air movements and the dispersion of contaminants plays a vital role in the microbiological assessment of clean zones protected by unidirectional airflow. According to Stoke's law the settling velocity for a spherical particle of 20 μm and unit density (1g/cm^3) in air is less than 2 cm/s. This velocity is much less than that of air in a unidirectional flow (30–45 cm/s), which is commonly used in pharmaceutical production. This means that settling velocity plays an inferior role compared with the unidirectional flow velocity. However, contamination risk is not only dependent on the concentration of contaminants but also on their motion (Ljungqvist & Reinmüller, 1995b). The number of particles that pass a unit area per unit time characterizes the risk. Ljungqvist and Reinmüller (1993, 1995b) reported an application of the limitation of risks (LR) method. The advantage of this approach is the uncomplicated, immediate registration of results using particle counters. The concept, with visualization of air movements, particle challenge testing and calculation of the risk factor presents a method by which the risks can be limited. The LR method (Table 4) has successfully been used for

microbiological assessment in critical regions during aseptic processing of sterile drug products (Ljungqvist & Reinmüller, 1995b).

Table 4. Concept of the limitation of risks (Ljungqvist & Reinmüller, 1995b).

Step	Content
1.	*Visualize the main air movements* and identify critical vortex or turbulent regions by using the smoke technique.
2.	*Perform the challenge test* by placing the particle-counting probe in the critical area. During measuring, particles ≥ 0.5 µm/ft^3 (challenge level) are generated. The measurements should be carried out during simulated production activity with exaggerated human interference during the measuring periods.
3.	*Calculate the risk factor* defined by the ratio between measured particle concentration in the critical region and the challenge level in the surrounding air. If the risk factor is less than 10^{-4} (0.01%) during the challenge test, there should be no microbiological contamination during normal operational conditions from the air into the process, as suggested by the authors.

7. METHODS IN AIR-QUALITY ASSESSMENT

The air quality in cleanrooms should be monitored after intallations before normal operation and regularly during operation. To ensure proper conditions before operation starts, the air cleanliness should first be predicted by measuring airborne particulate cleanliness according to standard methods e.g. ISO 14644–1:2000 and 14644–2:2000. Normally, these measurements are carried out with a particle counter. If the cleanliness of the cleanroom meets the specifications, the next step is to begin bioaerosol monitoring.

7.1 Bioaerosol monitoring

Traditionally, microbial viability is understood as the ability to divide and multiply. Only viable microbes can cause infection, while both living and dead ones or their products can be responsible for allergenic and toxic illnesses. Therefore, methods for assessing both viable microbes as well as the total number of airborne microbes are highly needed (Thompson *et al.*, 1994).

The food industry is currently following the pharmaceutical and medical industries by recognizing that microbial monitoring of air is a must in standard quality-control practices. Many food producers now include bioaerosol monitoring as part of their Hazard Analysis Critical Control Point (HACCP) system (Parrett & Crilly, 2000). Bioaerosol monitoring is carried out traditionally for 3 principal reasons:

1) to meet legal requirements in complying with guidelines which often state that air quality may have to be monitored but do not specify the methodology or the acceptable limits to use,
2) to collect epidemiological data, possibly with a view to set occupational exposure limits and
3) for scientific interest to determine how the air affects the products processed.

Concern is growing in the food industry to determine the importance of the airborne route as a possible source of contamination (Griffiths & DeCosemo, 1994). The causes of elevated concentration of microbes in food industry air vary. One basic source is growth of microbes in a liquid medium, e.g. spilled

product, rinse water or wastewater, which then becomes aerosolized (Ren & Frank, 1992b). Any point at which the product is exposed to air is a possible route for airborne contamination. The following criteria should be used to determine a sampling strategy: the sampling method, specificity and level of sensitivity required as well as the speed with which a result is required. Furthermore, information on the importance of total cell counts versus viability of the cells in the sample as well as the particle size range needed put demands on the method used. When the reasons for carrying out sampling are fully understood, the correct weighting can be assigned to each of the above-mentioned criteria (Griffiths & DeCosemo, 1994).

It is likely that the overall level of microbes will require monitoring, although the detection of potential pathogens may also be required. The major concern is probably for the viable microbes contaminating the food products. Viable counts are therefore likely to be more important than the total particle counts. Sensitive methods may be required, since low levels must be detected as in the monitoring of pathogens or potential pathogens. In both the food and pharmaceutical industries the speed with which a result needs to be obtained is a question of economics (Griffiths & DeCosemo, 1994).

7.1.1 Air sampling

Aerosols exhibit complex aerodynamic behaviour resulting from a combination of physical influences that include Brownian motion, electrical gradient, gravitational field, inertial force, electromagnetic radiation, particle density, thermal gradients, hygroscopicity and humidity (Kang & Frank, 1989b). Generally, the performance of bioaerosol sampling devices is characterized by their ability to aspirate particles into the inlet, to transmit them through the sampler's interior and to collect them on the collection surface. In the case of viable microbial sampling, the performance of samplers must fulfil the stability of microbial viability as an additional component during sampling (Thompson *et al.*, 1994). The samplers must also collect a representative sample of the required fraction of bioaerosol with a minimum of stress, so that the biological activity of the aerosol is not too much impaired (Griffiths & DeCosemo, 1994). The efficiency of a sampler in collecting a particle of a given size is related to the air velocity in the impaction nozzle. Too low a velocity in the inlet will result in failure to collect the particles of interest. Too high a velocity results in a high

shear force and may cause serious damage to the microbes, thus decreasing their viable recovery. The larger the aerosol particles, the greater the over- or underestimation of the aerosol concentration is likely to be. If the sampler is not mounted on the axis of the ambient airflow, the measured aerosol concentration may be significantly different from that in the ambient air environment (Thompson *et al.*, 1994).

Samplers differ from each other in flow rate and optimal sample density, while optimal sampling times differ considerably from one sampler to another (Nevalainen *et al*., 1992). Furthermore, the ambient bioaerosol is a mixture of many species of microbes. Sporeformers and hardy species may mask the sample and impede the recovery of more sensitive organisms. Thus, sample analysis is severely limited since little information is available on the causative variables that lead to differing colony recoveries (Thompson *et al.*, 1994). Airborne contaminant counts are also dependent on the person operating the measurement device. The more stringent the air purity requirements, the greater the influence of air-sampling techniques (Meier & Zingre, 2000). Contamination from the sampler, especially in clean environments, will decrease if there is a waiting period set for 1–2 min after installation of and before starting the air sampler. If measurements are taken in a conventionally ventilated room, airborne contaminant counts may also vary greatly if people are moving around or if work is being performed (Meier & Zingre, 2000).

According to Parret and Crilly (2000), important information from the sampling period includes:

- location and area of the site
- date and time of sampling
- test temperature and moisture conditions
- functions of the ventilation system during sampling
- personnel in the area
- volume of air sampled along with sampling period
- collection media used and
- incubation time and temperature used.

The sampling time must be considered according to the process and aim of sampling. In the food industries, both the situation during processing and after cleaning procedures can be monitored. In a pharmaceutical cleanroom facility,

there was no relationship between sampling time and frequency of alerts. Sampling at any time during the aseptic filling operation gave a satisfactory measure of the microbial cleanliness in the cleanroom during the setup and aseptic filling operation (Cundell *et al*., 1998).

7.1.2 Efficiency of the air samplers

Sampler efficiency is a measure of how well the inlet of a sampler draws in particles without being affected by the particles' sizes, shapes, velocities and direction (Nevalainen *et al*., 1992). The total efficiency (TE) of a sampler is determined by several factors such as the design of the inlet, collection stage and choice of collection medium, which affect the viability of the collected microbes (Henningson & Ahlberg, 1994). TE can be divided into collection efficiency (CE) and preservation efficiency (PE). Nevalainen *et al.* (1992) determined PE as biological efficiency that refers to the ability of the sampler to maintain microbial viability and prevent cell damage during sampling. CE is usually expressed as the 50 % aerodynamic cutoff diameter (D_{ae50}) i.e. the particle size collected to 50% diameter (Henningson & Ahlberg, 1994). For efficient collection it is crucial to choose an impactor with a D_{ae50} below the mean size of the particles being sampled (Jensen *et al*., 1992). It is not possible to determine the PE of a sampler with the methods on hand. TE can be estimated as (Henningson & Ahlberg, 1994):

TE = CE * PE (1), where TE = total efficiency,
CE = collection efficiency,
PE = preservation efficiency

Nevalainen *et al*. (1992) determined CE as a measure of how well the inlet of a sampler deposits the particles without being affected by their physical properties. In ISO 14698-1:2000 CE was expressed as:

CE = IE * (1 - WL) * CSE (2), where IE = inlet efficiency,
CSE = collection stage efficiency
WL = sampler wall losses.

7.1.3 Sedimentation plates

Sedimentation also referred to as settle plate technique has traditionally been used in the food industry. The plates are easy, inexpensive and collect particles in their original state (Kang & Frank, 1989a); however, they have serious drawbacks. The ability of settle plates to collect airborne particles is governed by the gravitational force on the particle which decreases with a velocity dependent on its mass. Hence, settle plates are biased towards collecting larger particles and are sensitive to air movement (Griffiths & DeCosemo, 1994). The method is not quantitative and in high aerosol concentrations the uncountable numbers of colonies can be a problem (Holah *et al*., 1995a). In addition, microscope slides coated with agar can be exposed and particles counted using a microscope. This technique is only used for total particulate counts (Kang & Frank, 1989a).

7.1.4 Impactors and impingers

Impaction is the most common technique for collection of airborne viable particles (Ljungqvist & Reinmüller, 1998). In impactors the inertial forces are used to collect particles. The inertia of a particle is determined by its mass and velocity. In the collection stage of impactors the airstream is forced to change direction, and particles with too high a level of inertia are impacted on either a solid or liquid surface. Liquid-using impactors are called impingers (Henningson & Ahlberg, 1994). The aerodynamic particle diameter of the target aerosol is one of the most important physical factors determining CE of the sampler of inertial devices (Li & Lin, 1999). Impactors with several collection stages, i.e. cascade impactors, give information on the size distribution of the aerosol (Henningson & Ahlberg, 1994).

In 1958 a cascade-sieve impactor, the Andersen sampler, was developed and is now probably the best-known sampler for microbiological aerosols (Andersen, 1958). It is one of the few samplers with a solid collection surface that gives information on the size distribution of the microbiological aerosol (see Figure 6). The sampler collects airborne particles onto a series of nutrient agar plates at a flow rate of 28.3 l/min (Griffiths & DeCosemo, 1994). Each sieve has successively smaller holes. This causes increased particle velocity as air flows through the apparatus. Large particles impact at the initial stage and small particles follow the airflow until accelerated sufficiently to impact at a later stage

(Kang & Frank, 1989a). Detection of microbes relies on their ability to grow following sampling (Griffiths & DeCosemo, 1994). The CE of the Andersen sampler was found to be greater than 90% for particles with a D_{ae50} of < 2.5 μm. This efficiency was not affected by air velocity; at air velocities < 1 m/s, particles as large as 20 μm D_{ae50} were collected with a near 100% efficiency. The efficiency, however, fell rapidly with increased air velocity. The CE of the Andersen sampler is more affected by air velocity than by particle size (Griffiths *et al.*, 1993; Upton *et al.*, 1993). The calculated D_{ae50} value is 0.57 μm (Nevalainen *et al.*, 1992). The Andersen 6-stage sampler is often used as a reference sampler (Henningson & Ahlberg, 1994). The 2-stage Andersen sampler is used to collect and separate respirable from nonrespirable particles.

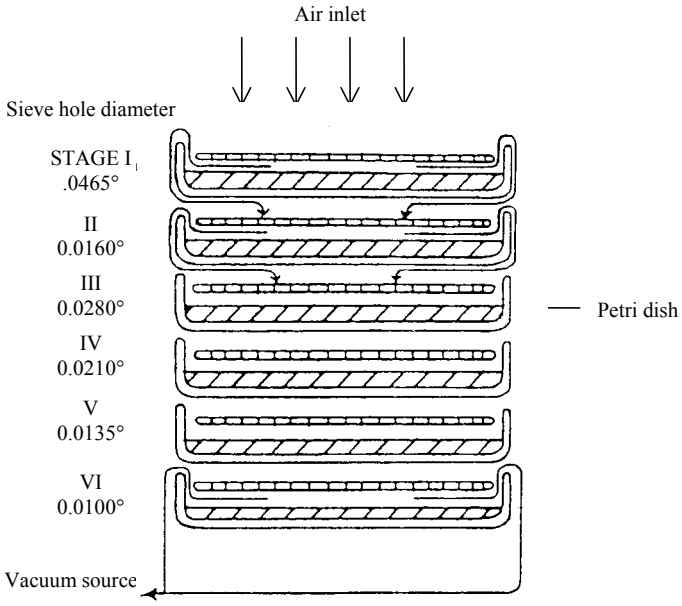

Figure 6. Diagram of 6-stage Andersen (Andersen, 1958).

There are 2 types of solid-surface impactors: slit samplers and sieve samplers. The slit sampler usually has a 0.2–1.0 mm-wide tapered slit, which produces a jet stream when the air is sampled by vacuum (Kang & Frank,1989a; Ljungqvist & Reinmüller, 1998). The slit sampler may have a turntable for rotating the agar plate so that aerosol particles are evenly distributed on the agar surface (Kang & Frank, 1989a). The velocity of the air varies according to the slit width of the air sampler used. A linear velocity of 20–50 m/s is typical, since particles with a

minimum diameter of 0.5–1.0 μm do not follow the deflecting stream of air but impact against the collection surface. The smaller the size, the higher the velocity needed in the impaction (Ljungqvist & Reinmüller, 1998). Sieve samplers are operated by drawing air through a large number of small, evenly spaced holes drilled in a metal plate. The particles are impacted on an agar surface located below the perforated plate (Kang & Frank, 1989a). The impaction velocity is dependent on the size of the perforations, distance to the impaction surface and performance of the vacuum pump (Ljungqvist & Reinmüller, 1998). When the concentration of viable particles in an aerosol is high, one sieve hole may allow more than one viable particle to pass through, resulting in the formation of a single colony from 2 or more viable particles. This inaccuracy can be corrected by reducing sampling time or by using either the microscopic method or a positive-hole method for enumeration (Kang & Frank, 1989a). Normally the positive-hole correction tables are included in each commercial sieve sampler.

The surface air system (SAS) sampler is a portable, single-stage sieve sampler that collects particles onto a contact plate or standard Petri plate. The sampler operates with a flow rate of 180 l/min (Griffiths & DeCosemo, 1994). The calculated D_{ae50} value is 1.45 μm (Nevalainen *et al*., 1992). The microbial air sampler MAS-100 (Figure 7) is an impactor that aspirates air either horizontally or vertically through a perforated plate with 400 holes 0.7 mm in diameter (Meier & Zingre, 2000). The resulting airstream containing particles is directed onto the agar surface of a standard Petri plate. The impaction speed of the airborne microbes on the agar surface is approx. 11 m/s, which according to the manufacturer corresponds to stage 5 in the Andersen sampler. This speed guarantees that all particles over 1 μm are collected. At an aspiration volume of 100 l/min and with 400 holes 0.7-mm-diameter holes serving as a catch for the lid, the MAS-100 attains a collision speed of 10.8 m/s. The calculated D_{ae50} value is 1.62 μm (Meier & Zingre, 2000) or 1.72 μm (Li & Lin, 1999). Air samplers with a D_{ae50} of less than 2 μm should theoretically be able to precipitate practically any airborne microbiological contaminant-carrying particle (Meier & Zingre, 2000).

Figure 7. The MAS-100 air sampler is an impactor.

Impingement methods are highly efficient for particles greater than 1 μm when high jet velocities are used (Kang & Frank, 1989a). The all glass impinger-30 sampler (Figure 8) is a widely used high-velocity impinger (Kang & Frank, 1989a; Griffiths & DeCosemo, 1994). The sampler operates by drawing aerosols through an inlet tube curved to simulate the nasal-passage respiratory infection potential of airborne microbes (Nevalainen *et al*., 1992). The jet is held 30 mm above the impinger base and consists of a short piece of capillary tubing designed to reduce cell injury (Kang & Frank, 1989a). The calculated D_{ae50} value is 0.31 μm (Nevalainen *et al*., 1992).

When the sampler is used for recovering total amount of airborne microbes from the environment, the curved inlet tube should be washed with a known amount of collection fluid after sampling, since larger particles (i.e. diameter > 15 μm) are collected on the tube wall by inertial force (Kang & Frank**,** 1989a). The agglomerated microbes are separated into suspension, which increases the CFU number (Lin & Li, 1999a). The bubbles, rising through the liquid, entrain previously collected particles and create new aerosols by bursting at the liquid-

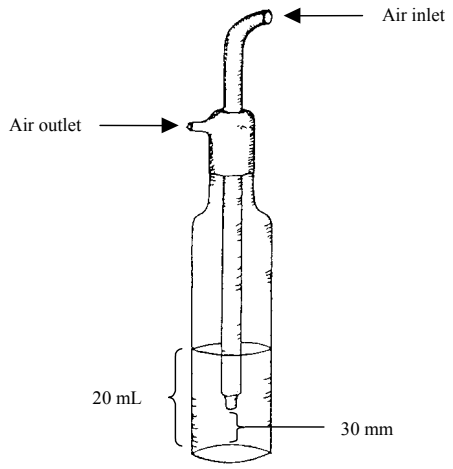

Figure 8. AGI-30 impinger (Cox, 1987).

air surface when the impingers operate at a high-level collection fluid and sufficiently high sampling flow rate. The number of reaerosolized particles increased as sampling time increased (Lin *et al*., 1997). The theoretical overall inlet sampling efficiency including wall loss is close to 100% for 1-μm particles and is significantly reduced for 5-μm and larger particles (Willeke *et al.*, 1992). Hydrophobicity may play an important role in the CE of AGI-30 impingers. Spores from many fungi are hydrophobic and may be lost because they float to the surface of collection suspensions and are reentered into the exit airflow (Grinshpun *et al*., 1997; Lin & Li, 1999a). The sampling time and flow rate influence the CE of hydrophobic spores. The impinger is inexpensive and simple to operate, but viability loss may occur due to the amount of shear forces involved in collection. Another limitation is that the glassware should be sterilized before each sampling (Kang & Frank, 1989a). Another application of this principle, the multistage liquid impinger has 3 stages that are intended to correspond to the principal deposition sites in the human respiratory system: the upper respiratory tract, the bronchioles and the alveoli (Griffiths & DeCosemo, 1994). Impingement is useful for sampling heavily contaminated air, since the liquid samples can be diluted to the appropriate level for subsequent growth culture analysis (Lin *et al*., 2000).

Overnight mailing on ice of a viable bioaerosol sample of AGI-30 peptone liquid did not significantly alter the quantification of microbes collected during winter. During summer the concentration of mesophilic bacteria increased in the samples.

7.1.5 Centrifugal samplers

Centrifugal samplers have a propeller that pulls air into the sampling unit and pushes the air outward to impact on a tangentially placed strip of nutrient agar set on a flexible plastic base. Particles in the incoming air may be thrown out of the airstream by centrifugal force to be caught against the peripheral surface (Ljungqvist & Reinmüller, 1998). Centrifugal samplers do not generate high-velocity jet flow during sampling, and less stress is imposed on airborne microbes compared with impaction methods. These samplers are simple and easy to operate and can rapidly sample a high volume of air, resulting in more representative sampling (Kang & Frank, 1989a). They, however, demonstrate an inherent selectivity for larger particles and, since larger particles are more likely to include viable particles, there is a tendency towards higher counts than with other types of air samplers (Ljungqvist & Reinmüller, 1998). The principle of centrifugal samplers is shown in Figure 9.

The Reuter centrifugal sampler (RCS) is a portable hand-held instrument, much used in the biotechnology and food industry (Griffiths & DeCosemo, 1994). Air from a distance of at least 40 cm is sucked into the sampler by means of an impeller. The air enters the impeller drum concentrically from a conical sampling area. The air leaves the sampling drum in a spiral outside the cone of entering air (Kang & Frank, 1989a). An agar strip is inserted into the drum around the impeller blades. The sampler gives no indication of the size of particles (Griffiths & DeCosemo, 1994). In some investigation the cutoff size of the sampler has been found to be above 3 µm, so single cells pass through the sampler (Clark *et al.*, 1981; Macher & First, 1983).

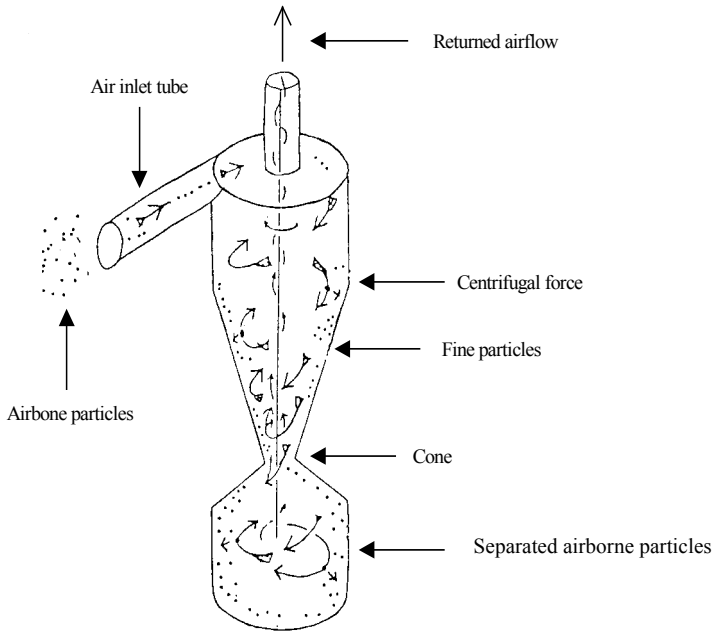

Figure 9. Centrifugal sampler (Cox, 1987).

7.1.6 Filter systems

Several different collecting mechanisms in filtration (impaction, interception and diffusion) are active. Usually a single filter is used and all particle sizes are collected with no partitioning into size fractions (Henningson & Ahlberg, 1994). Battery-powered personal samplers with filters have long been used in the occupational hygiene field to collect respirable and inhalable dusts (Griffiths & DeCosemo, 1994). In general, the filters are inexpensive and simple to operate (Kang & Frank, 1989a). The air filtration apparatus (Figure 10) consists of cellulose fibre, sodium alginate, fibre glass, gelatine membrane filter or synthetic membrane filters mounted in an appropriate holder and connected to a vacuum source through a flow rate controller (Kang & Frank, 1989a). Microbes can be removed by washing off from the filter to carry out total number rather than viable number enumeration (Griffiths & DeCosemo, 1994). The suspension can also be assessed with appropriate bacteriological techniques. Membrane filters can also be directly placed on an agar surface for incubation. The filter methods

are good for enumerating mould or bacterial spores (Kang & Frank, 1989a). One of the main disadvantages of using filters in collecting microbes is that they do not protect cells when large volumes of air pass over the filters causing desiccation (Griffiths & DeCosemo, 1994). Shortening of the sampling period for this method may reduce this stress (Kang & Frank, 1989a).

Figure 10. The MD8 equipment is based on the air filtration technique.

The gelatine filter membrane (Figure 11) is composed of gelatine foam designed to prevent vegetative microbes from being inactivated by desiccation when air is drawn through the filter (Parks *et al*., 1996). The gelatine membrane is water-soluble so that it can easily be diluted for plating or be solubilized on top of a nutrient agar, resulting in bacterial colonies on the agar surface (Kang & Frank, 1989a). If gelatine filters are to be placed on agar plates, then the plates should be carefully dried beforehand, because wet plates can make colony counting difficult. The gelatine filters do not melt into the agar if they are incubated at low temperature. Therefore, the filter plates should first be incubated at 26 °C for 1 h to enssure melting of the gelatine filter into the agar (Parks *et al*., 1996). The large number of pores present in these membranes allows a large volume of air to be sampled during a short time (Kang & Frank, 1989a). Prolonged storage of the filters before assay causes death of sensitive vegetative bacteria (Parks *et al*., 1996). Yeast and mesophilic bacterial concentrations have been observed to

decrease significantly after mailing with the nuclepore filtration and eluation method, while concentrations of mould and thermophilic bacteria remained steady (Thorne *et al*., 1994). The material of the membrane does not significantly affect the amount of cells, since equivalent numbers of bacteria and yeasts could be collected on nitrocellulose, polyamide and gelatine filters analysed with 4',6-diamidino-2-phenylindole (DAPI) counting (Neef *et al*., 1995).

Figure 11. A geatine membrane filter unit used in the MD8 filtration equipment.

7.1.7 Particle samplers

Optical particle counters are based on laser-light scattering by a single particle and are widely used for measuring the concentration and size distribution of airborne particles. The crucial response characteristics of a particle counter include the sizing accuracy, counting efficiency and detection limits (Yoo *et al*., 1996). The detection limit of the smallest detectable particles is a crucial characteristic of the counter. Light-scattering counters are usually calibrated by means of polystyrene latex (PSL) spheres. Due to the differences between the optical properties of the calibrated PSL spheres and actual particles, errors in the results may occur (Yoo *et al*., 1996). Optical counters on the market include laser diffractometers, phase-doppler systems, intensity-deconvolution systems, and laser-particle interation system/image analyzers (Anon., 2001). Other techniques used in particle counters include the electrical mobility techniques used in electrical aerosol analysers and differential mobility analysers as well as light detection and ranging (LIDAR) technology, which uses light waves in the same way that radar uses radio waves (Anon., 2001).

7.1.8 Conclusions regarding samplers

In Tables 5a and 5b microbiological air samplers, collection methods and a few comments on the methods are presented. In the food industry, the settle plates and impactor- or centrifugal-type samplers, e.g. SAS, MAS or RCS, are the most commonly used air-sampling methods in routine monitoring. The Andersen impactor gives the most reliable results, but it is less practical for routine use. The RCS sampler is convenient to use but does not quantitatively recover very small viable particles. The filtration methods may not be optimal for counting vegetative cells due to the stress it places on cells through dehydration during sampling. A glass impinger is inexpensive and simple to operate, but viability loss may occur due to the amount of shear forces involved in collection (Kang and Frank, 1989c, d). Data obtained using the AGI-30 must be used with caution in food-processing environments containing large viable particles, because the agglomerated microbes will be separated into suspension, which increases the CFU number (Kang & Frank, 1989d; Lin & Li, 1999a).

Table 5a. Methods including commercial air sampling devices used in collection of air for microbiological sampling.

Sampling principle	Sampler	Collection surface	Comments	$D_{ae50\ (\mu m)}$	References
cascade sieve impactor	Andersen 6-stage	agar in petri dishes	reliable results, information on size distribution, impractical in industrial use	0.57	Andersen, 1958; Nevalainen *et al*., 1992
	Andersen 2-stage	agar in petri dishes	impractical in industrial use		Nevalainen *et al*., 1992
sieve impactor	Andersen 1-stage	agar in petri dish		6.61	Nevalainen *et al*., 1992
	MicroBio MB2	contact plate			Parret & Crilly, 2000
	Surface Air System, SAS	contact plate	practical in industrial use	1.45	Griffiths & DeCosemo, 1994; Nevalainen *et al*., 1992
	MAS-100	agar in petri dish	practical in industrial use	1.62–1.72	Meier & Zingre, 2000; Li & Lin, 1999
slit impactor	Casella MK II	agar media		0.67	Bourdillon *et al*., 1941; Nevalainen *et al,* 1992
	Burkard Personal Sampler			2.52	Nevalainen *et al*., 1992
centrifugal airstream	cyclones	wet or dry surface		> 3	Henningson & Ahlberg, 1994
	Reuter centrifugal sampler; RCS	special agar strips	less stress to microbes than in impactors, selectivity for larger particles, practical in industrial use		Kang & Frank, 1989b; Clark *et al*., 1981; Macher & First, 1983

Table 5b. Methods including commercial air sampling devices used in collection of air for microbiological sampling.

Sampling principle	Sampler	Collection surface	Comments	$D_{ae50\ (\mu m)}$	References
impinger	all glass impinger, AGI-30	collection fluid	efficient for collection of bacteria and yeast; impractival in industrial use, glass	0.31	Kang & Frank, 1989a, d; Nevalainen *et al*., 1992; Griffiths & DeCosemo, 1994
	multistage liquid impinger	collection fluid	stainless steel		Griffiths & DeCosemo, 1994
	BioSampler	collection fluid	more genttle and efficient (for spores) than AGI-30, impractical in industrial use		Willeke *et al*., 1992; Lin *et al*., 2000;
filtration	Sartorius MD8	gelatine filter	efficient for spores, decreased desiccation rate of microbes, practical in isolators and for big sampling volumes		Kang & Frank,, 1989a, d; Parks *et al*., 1996
gravitation	settle plates	agar in petri dishes	simple to use, not quantitative, unreliable		Griffiths & DeCosemo, 1994
ionization	electrostatic precipitation	filter, collection fluids, agar media	mechanically complex; collection of microorganisms on charged surfaces		Griffiths & DeCosemo, 1994; Mainelis *et al.*, 1999
temperature gradient	thermal precipitation				Griffiths & DeCosemo, 1994
specific bindings	biosensors		specific, future development; at the moment impractical in industrial use		Anon., 2001

Lower levels of total bacterial recovery were observed in filter samplers than impinger samplers. This was primarily due to the higher biological stress occurring during the sampling process of filtration. Sampling flow rate did not significantly affect bacterial recovery. Sampling time related to dehydration effect did play a role in bacterial recoveries, especially for sensitive strains. The impingers are likely to perform better than the filtration methods for sampling airborne bacterial microbes (Li *et al*., 1999). Fungal spores are more reliably collected with filtration than impingement. Impingement is suitable for yeast bioaerosols (Lin & Li, 1999a). The nucleopore filtration method was recommended for moulds and thermophilic organisms rather than the AGI-30, but for yeasts and mesophilic bacteria the AGI-30 method was more favourable (Thorne *et al*., 1994).

Each of the proposed monitoring methods has limitations that the user should be aware of. Monitoring effectiveness is dependent to a great extent on the monitoring methods used and the nature of the aerosol present (Kang & Frank, 1989a). Food plant aerosols have not been studied sufficiently to accurately generalize about particle-size distribution. Particle density is influenced by RH and particle composition. It can be stated that an effective separation volume varies more due to the mass of the particle than to the particle size. Microbial air results measured with different sampler types are strongly influenced by the sampling environment and the results are sometimes contradictory. The choice of instrument is clearly dependent on the experimental parameters and the secondary means of identification (Neef *et al*., 1995). Airborne counts recorded from different sampling techniques are not directly comparable but would be expected to show similar trends in level variation (Holah *et al*., 1995a).

7.2 Bioaerosol assay methods

7.2.1 Culturing techniques

Culturing of microbes directly or through a broth on solid agar media with incubation for a certain period and temperature is the traditional method for enumerating the microbial count in the air sample. The culture technique is easy to use and requires no specialized equipment for sampling. The total population of microbes in a sample can, however, be severely underestimated, if assessment is carried out with this type of method (Griffiths & DeCosemo, 1994). Microbes

in the air may lose their ability to form colonies and still be viable, i.e. be nonculturable. In cases where the airborne microbes are nonculturable, data on CFUs does not describe the actual microbial population (Heidelberg *et al.*, 1997). Epifluorescent microscopic methods for counting the total amount of microbes showed that there was wide variation in the culturability of microbial cells; the culturable amount varied from 0.02% to 10.6% (Lighthart, 1997).

In the development of methods that utilize culture techniques as part of the assessment criteria, growth conditions, diluent and culture plate media must be standardized to be able to estimate microbial counts in the air (Griffiths & DeCosemo, 1994). Procedures other than plate counting are also needed to enumerate microbes in the indoor aerosol samples (Heidelberg *et al*., 1997). The culture technique is useful for direct identification of certain pathogens or spoilage microbes in the food-processing air, e.g. spoilage moulds on specific agars (Lund, 1996). Collection of microbes in the fluid is used in the impingers. The selection of a liquid collection medium is dependent upon the particular organisms to be isolated. In quantitative studies a medium that will minimize both multiplication and death of the organism must be employed (Kang & Frank, 1989a).

The usefulness of organic compounds e.g. di-, tri- and polysaccharides, sugar alcohols, proteins, polypeptides, organic acid salts, antibiotics, chelating agents and culture supernatant fluid in enhancing cell survival has been investigated. The majority of the most effective organic additives are sugars or polyhydric alcohols (Griffiths & DeCosemo, 1994). Marthi and Lighthart (1990) showed that addition of an organic compound, e.g. betaine, to the impinger buffer significantly increased the colony-forming abilities of the airborne microbes. The compound acted in some cases as a protective agent maintaining the culturability of some bacteria. Heidelberg *et al*. (1997), however, showed that the effect could be the opposite in other cases, due to strain variability against betaine. Thus, protective agents in routine monitoring may introduce a bias, protecting the culturability of only some parts of the microbial population.

7.2.2 Fluorescence and microscopy techniques

Microscopy is a method with which the total count of microbes as well as morphological data on the microbes can be obtained. The microscopical method is relatively simple and rapid. Automatic counting and size evaluation in the assessment of airborne microbes by means of image processing of fluorescence microscopy data reduces analysis time (Griffiths & DeCosemo, 1994). Fluorescence microscopy can be applied in the evaluation of airborne microbes harvested e.g. on filters (Kildesø & Nielsen, 1997) and in impinger liquids (Terzieva *et al*., 1996). However, manual focusing of the microscope is needed due to the impurities, e.g. larger particles, present in the sample (Kildesø & Nielsen, 1997). Phase-contrast microscopy is particularly useful for counting bacterial endospores as they appear phase-bright against the darker vegetative cells (Griffiths & DeCosemo, 1994). The fluorochromes attach to particular cell components, e.g. proteins, nucleic acids, and coenzymes, which fluoresce when excited with light of a suitable wavelenght (Griffiths & DeCosemo, 1994). The addition of UV fluorescence capability into aerosol counters offers a way to distinguish biological particles from most organic and inorganic particles (Seaver *et al*., 1999). Viable staining methods have been applied for the detection of viable microbes (Terzieva *et al*., 1996, Hernandez *et al.*, 1999). The actual viable count may be underestimated when assessed by this technique because microbes injured in the sampling stage may recover later on (Terzieva *et al*., 1996). Understanding of the fluorescence effect on the cell viability, the presence of non-biological particles and the interferences from mixtures has not yet been achieved (Brosseau *et al*., 2000). Technical problems related to fluorescence microscopy include the low-contrast and low-light intensity rendering difficulties in automatic image processing (Kildesø & Nielsen, 1997).

7.2.3 ATP bioluminescence

Adenosine triphosphate (ATP) is present both in microbial cells and food ingredients and can be measured using the luciferase enzyme complex found in fireflies. The light output of a sample is directly proportional to the amount of ATP present. The detection limit of the method is about 10^4 cells (Wirtanen, 1995). This method is non-specific, i.e. it measures the ATP content of the microbial population in the sample as a whole (Griffiths & DeCosemo, 1994). The ATP content in airborne cells, which are stressed through assessment, can

be altered by the effect of aerosolization and this can also affect the detection level of the method. The total concentration of adenylate within the cells may give a better estimate of cell concentration (Griffiths & DeCosemo, 1994). Application of a fully automated ATP-monitoring system AutoTrac for continuous checking for microbial contamination in air is being developed (Brady, 1999).

7.2.4 Molecular methods

Molecular biology detection methods include polymerase chain reaction (PCR) and gene probes (Griffiths & DeCosemo, 1994). The PCR analysis method permits the detection of DNA regardless of the metabolic state of the cells. The method may therefore be orders of magnitude more sensitive than culture techniques. Applications of this method for the quantitation of airborne organisms are still under development. PCR-based techniques allow the detection and identification of microbes at a group or species level within a background of other microbes. The specificity, sensitivity, and reduced processing time of this technique are suitable for the detection of small amounts of target microbial cells in a sample (Alvarez *et al*., 1995). Specialized equipment and skilled personnel are, however, required for successful applications (Griffiths & DeCosemo, 1994). The air samples may also contain compounds inhibitory to the amplification assay, e.g. high concentration of nontarget DNA (Alvarez *et al*., 1995). In many applications, pre-enrichment of the sample is needed.

Nucleic acid hybridization has been applied for the detection and identification of microbes in bioaerosols. Each hybridization format is suitable for different aerosol concentrations. It is possible to identify a fast-growing airborne organism within 24 h using colony-hybridization technique. Colony hybridization can be used to detect amounts as low as even 1 CFU, whereas whole-cell hybridization requires a substantially high aerosol concentration, e.g. $> 5 \times 10^4$ CFU, from filtered air samples (Neef *et al*., 1995).

7.3 Aerosol generation methods

The need to control bioaerosol quality is due to the characterization of bioaerosol-monitoring methods based on particle size, biological content and concentration. Bioaerosols can be produced from either liquid suspension or from dried material (Griffiths & DeCosemo, 1994) in which the liquid is either transported through jets and dispersed from vibrating needles, vibrating orifices or spinning disks, or by exposing the liquid surface to high-velocity airstreams (Green & Lane, 1964; Hinds, 1982). According to Griffiths and DeCosemo (1994), aerosol generation is best achieved using a liquid suspension/atomizer technique. A development of the basic atomizer concept confines the droplet-producing jet so that the aerosol is delivered through a tube referred to as a nebulizer (Griffiths & DeCosemo, 1994). Glass atomizers have been found to be very effective for the distribution of large cells, e.g. *S. cerevisiae*, while nebulizers are effective in distributing small cells, e.g. *E. coli* and *B. subtilis* var. *niger* (Griffiths & DeCosemo, 1994). In the preparation of suspensions, phosphate-buffered water is preferred to distilled-deionized water to minimize osmotic shock and associated viability loss in the sample. The phosphate buffer as well as the individual species substantially affect the aerodynamic size distribution of the bioaerosols (Johnson *et al*., 1999). The production of monodisperse bioaerosols has been found to be better achieved using tubes of air or electrically driven spinning top disk generators, vibrating needle generators and vibrating orifice generators (Griffiths & DeCosemo, 1994). The number of microbes in the droplets can be 0, 1 or more. It is normally determined using droplet-to-particle size rations and particle concentration (Hayakawa, 1964; Mercher *et al*., 1968). The sizes of the aerosol particles are determined by the concentration of salts used in the suspension and the operating parameters selected for the aerosol generator (Griffiths & DeCosemo, 1994).

7.4 Effect of aerosol generation on cell viability

The quantitative loss of viability due to the process of aerosol generation is related to many equipment parameters, and to the species and state of the microbe. It is likely that the liquid used to make up the stock suspension, and the initial culturing procedure, may greatly affect the initial viability of the system. The residence time of the organisms in the supporting liquid prior to aerosol generation is also likely to affect its viability. The size of the microbe or the

spore can greatly affect the performance of the aerosol generator (Griffiths & DeCosemo, 1994). Factors influencing the survival of microbes in an atomizer-aerosol generator include the shear stress of the airstream, impaction onto the surfaces of the nebulizer, concentration effect in which volatile liquid constituents are removed from the suspensions, agitation of the microbes, desiccation of aerosol droplets containing microbes, possible destructive oxidation effects when the aerosol particles are exposed to the air and the bacteriostatic effects of metals in contact with the suspended bacterial cells (Rosebury, 1947).

8. AIR-QUALITY IN SOME FOOD PROCESSES

The level of microbes in the air varies significantly in different food factories. Bacteria are more common than yeasts and moulds in the food-processing air, and the levels of bacteria and fungi are normally related. The highest microbial levels occur in factories handling products derived from the soil, e.g. vegetables. Cleaning, freezing and weighing operations readily release particles, especially when processing contaminated material (Holah *et al*., 1995a).

Bioaerosols can be found in all work environments but only some of them cause diseases. In the food industry, increased concentrations of certain yeasts, moulds and bacteria can be hazardous both for the products manufactured and for the employees. In a study presented by Sheehan and Giranda (1994), Gram-negative bacteria were isolated during production with concurrent sanitation, whereas only Gram-positive cocci could be found from the air when only production was performed. From a food industry perspective, increased concentrations of microbes in the air during sanitation may decrease the shelflife of the foods being processed concurrently. Food industry hygienists are therefore becoming more concerned over the role of air as a vector of food contamination. The risk of airborne contamination has been evaluated by interpreting air exchanges between neighbouring areas and air movement at critical points. An optimized air exchange rate can reduce or even eliminate contamination risk. Exposure to both normal and elevated levels of microbes in the bioaerosol should be sampled to determine baseline counts at various points in the different product lines (Burfoot *et al*., 1999; Xu & Burfoot, 1999).

8.1 Dairies

In 4 dairy plants a significant increase in microbial content of the air was found when compared with the air in plants without processing activity. The drains, water spraying and personnel activity were associated with significant increases in the total count as well as staphylococcal count in the air (Ren & Frank, 1992a).

The mean aerosol particle sizes from dairy processing plant environments ranged from approximately 4.3 to 5.3 μm. In addition, a more heavily contaminated dairy processing environment contained larger aerosol particles (Kang & Frank,

1989b, d). Both the high total viable count and the high proportion of viable count in the total aerosol observed highlight the importance of personnel as a major source of biological aerosols in dairy plants and the need to control aerosols in critical areas of the plant such as filling and packaging areas (Ren & Frank, 1992a, b). The majority of viable aerosol particles in milk-processing air were bacteria, whereas in the idle ice-cream room, the majority were moulds (Kang & Frank, 1989a).

Many studies have shown that viable aerosol counts vary from 1.2 to 3.0 log CFU/100 l air in dairies producing fluid milk and from 1.0 to 2.3 log CFU/100 l air in ice-cream plants (Hedrick & Heldman, 1969; Cannon, 1970; Radmore *et al.*, 1988; Kang & Frank, 1989a; Ren & Frank, 1992a, b). Usually, there are more microbes in the milk-filling areas than in the processing or storage areas. The variation of viable counts in the air from different areas of a dairy plant and ice-cream plant are presented in Table 6. In these studies, a significant positive correlation was found between the concentration of viable cells and total particle counts measured by a laser particle counter. Air sampling was carried out with an Andersen 2-stage sampler, using a sample volume of 100 l (Ren & Frank, 1992a, b).

Hedrick and Heldman (1969) stated that if the air in a dairy plant contains more than 2.15 log CFU/100 l air the air quality is poor. Radmore *et al.* (1988) proposed that good-quality air in a dairy is indicated by a viable count less than 1.3 log CFU/100 l air.

8.2 Breweries and beverage production

Henriksson and Haikara (1991) examined microbial loads in the air of filling areas in 10 breweries and one soft drink factory. The total airborne bacterial count measured with an SAS impactor was an average of 3.6 log CFU/m^3. Furthermore, anaerobic bacteria e.g. the lactic acid bacteria (LAB), *Pectinatus* and *Megasphaera* were also found in the aerosol near the beer-filling machine. High temperature and RH explained a significant part of the high microbial counts. The construction and location of the filling machines in the hall also influenced the microbial count (Henriksson & Haikara, 1991). Airborne microbes in beverage operations can originate from drains, warmers, pasteurizers and cooling tunnels as well as air-handling systems.

Table 6. Viable counts in different areas of production.

Fluid milk plant[1)]	Viable count log CFU/100 l	Ice-cream plant[2)]	Viable count log CFU/100 l
Raw milk storage	2.0	Pasteurized mix storage	2.3
Milk processing area	2.3	Processing area	2.1
Milk filling area	2.4	Filling area	2.3

1) Ren & Frank, 1992a

2) Ren & Frank, 1992b

Remus (1996) stated that the common routes through which microbes can contaminate the air: solid particles e.g. dust, skin fragments, hair, droplets originating from the atomization of liquids in beverage fillers and natural propagation i.e. sporulation. In the maintenance programme it is recommended that the air-handling system as well as the cooling coil be periodically cleaned and sanitized. In breweries, modern air-handling systems with HEPA filters are installed after the heating or cooling coil and before or after the blower. The purpose of the filters is to remove suspended dust as well as microbes to reduce the off-flavours and contamination of the products.

Laplace *et al.* (1998) carried out studies to determine the effect of indigenous microflora in the air when producing traditional French cider. Batches of apple cider were processed under 2 separate conditions, one in a traditional cider-manufacturing cellar and the other under laboratory conditions. In both methods alcoholic fermentation was achieved mainly by *Saccharomyces cerevisiae*, leading to a similar alcoholic fermentation profile in both batches. In the cider made in the traditional cellar *Candida lusitaniae* was present for the first 14 d of fermentation. The traditional cider also contained a wide range of LAB including *Leuconostoc oenos,* whereas only a few LAB species were present in the laboratory sample. Malolactic fermentation occurred in the traditional cider but not in the laboratory cider, and the sensory quality of the laboratory cider was therefore poor. This study showed that the air in the fermentation cellar significantly affects on the quality of the end product. There should be no unfavourable contaminants in the air, but in this process the air also functions as

a source of desirable LAB. If a cleanroom application were to be planned it should be placed at the correct stage in the process, thus allowing desirable LAB but no contaminants into the fermentation tanks (Laplace *et al.,* 1998).

8.3 Bakeries

In bakeries, air can act as an efficient vector of dust and microbes causing contamination of surfaces and products. The most common types of airborne contaminants are fungi and spore-forming bacteria. In conventional bakery buildings, the cooling lines for baked products are either built in a circle around the production rooms or as a freezer. If the product is not protected, there is a risk of air-mediated postcontamination.

Airborne fungi can cause huge losses in bakery products and raw materials. Jain (2000) found that the incidence of fungi fluctuated with the season. A total of 19 fungi were isolated from a large bakery in India. The predominant fungal forms belonged to species of *Aspergillus, Cladosporium, Fusarium, Penicillium* and *Rhizopus.* The highest fungal count recorded in this study was 3.4 log CFU/m^3 and the highest spore count was 3.3 log CFU/m^3 (Jain, 2000).

8.4 Meat and poultry production

In the poultry industry airborne microflora is dominated by spherical bacteria, and the viable bacteria consist mainly of *Staphylococcus* spp. and *Micrococcus* spp. The concentrations of viable fungi represent approx. 0.01% of the total viable count. The principal fungal genera have been identified as *Penicillium, Aspergillus, Paecilomyces, Geotrichum* and *Rhizopus* (Nielsen & Breum, 1995). Few studies are available on the air quality in chilling areas of poultry-processing plants. The microbial flora measured with the MD8 sampler in a study by Fries and Graw (1999) consisted mainly of streptococci, micrococci, staphylococci, corynebacteria, bacilli, lactobacilli and enterobacteria originating from the carcasses, water and soil. Invisible aerosols in the poultry process have been shown to contain up to 6.3 log CFU/ml. The higher total viable counts beneath the conveyor indicated the impact of the carcasses as well as the direction of the airflow towards the carcasses from the ceilings. The hygienic levels of the aerosol samples from the 2 plants studied were quite different. One

of the plants had a poor building design with unsatisfactory airflow. The airborne microbial load in the 2 plants varied from 2.2 log CFU/m^3 to 3.2 log CFU/m^3. The air samples were found to contain primarily micrococci and Gram-positive irregular rods (Fries & Graw, 1999). Ellerbroeck (1997) sampled the air in chillers with the 6-stage Andersen impactor and found that the mean load was 3.3 log CFU/m^3 in a dry-chilling system and 4.2 log CFU/m^3 in a chilling system using water sprays. Rahkio and Korkeala (1997) used the 2-stage Andersen sampler and showed that there was no difference in the airborne bacterial loads in a slaughterhouse between those determined in the morning and in the afternoon. The microbial load of the air is influenced by the building design and airflow system. Therefore, further research should be performed on the 2 above-mentioned factors as well as inspection of the chilling machinery (Fries & Graw, 1999). Aerial dispersion of microbes occurring in the chillers appears to cross-contaminate carcasses. This can occur during air-chilling of poultry both with and without water sprays incorporated (Mead *et al.*, 2000). In an aerobiological study of freezers and coolers carried out with an SAS impactor in the meat-processing plant 3 ranges were established as guidelines for microbial quality: in areas in range A with ≤ 100 cfu/m^3 the air quality was considered excellent, range B with a microbial load from 100 to 300 cfu/m^3 had intermediate air quality and range C with > 300 cfu/m^3 poor air quality (Al-Dagal *et al.*, 1992).

9. AIR DISINFECTION METHODS IN THE FOOD INDUSTRY

The use of high care or cleanroom technology to prevent recontamination of prepared food products, e.g. salads, sandwiches and convenience food, is increasingly being applied in food-processing areas (Holah *et al.*, 1995a). Clean air is provided through HEPA filters; other methods used to reduce viable microbial counts in the air include chemical fogging, ozone and UV radiation.

9.1 Disinfectant fogging

Disinfectants are commonly applied as fogs in the chilled-food industry. Disinfection is an additional safeguard, not a substitute for cleaning (Holah *et al.*, 1995b). Fogging has been shown to be effective in reducing viable airborne microbes. Burfoot *et al.* (1999) showed that fogging reduces the number of viable cells effectively on upward-facing surfaces but not on vertical or downward-facing surfaces. Better spray action can be achieved by locating the spray nozzles near the target and directing the jet towards the target. The use of more aggressive disinfectants e.g. peracetic acid or aldehyde formulations can also increase the disinfection effect on vertical surfaces. Fogging performed using an active concentration of 2 mg/ml of a quarternary ammonium formulation resulted in a 4-log reduction in microbial counts on wall and floor materials as well as in the air (Burfoot *et al.,* 1999). The concentration of chlorine in fogs must be very high e.g. 500 μg/ml to be effective, while a concentration of 10 μg/ml already causes discomfort for personnel working in the facilities. The best fogging effect was achieved using fog droplets with a diameter of 10–20 μm (Holah *et al.*, 1995b). When the droplets are smaller the fog produced gives a uniform coverage. The fog, however, remains airborne for several hours, which also prevents people from working in the area after the fogging period if the ventilation is not effective enough; large droplets do not disperse well. Other research studies point out that fogs consisting of phenols, quaternary ammonium compounds and iodofors are ineffective for air disinfection. Dascher *et al.* (1987) stated that both chemical fogging and UV disinfection were ineffective. Sometimes fogging was very effective killing 100% of the test microbes, while in other cases no destruction was observed (Holah *et al.,* 1995b). They concluded from the aerobiology cabinet studies that

fogging was uncontrollable and ineffective compared with other disinfection methods, e.g. application of ozone and use of UV radiation.

9.2 Ozone disinfection

In the ozonation experiments carried out in an aerobiology cabinet exposure of 4 µg/ml for 5–10 min reduced the number of airborne *P. aeruginosa* significantly with 2–4 log units. Holah *et al.* (1995b) found some variation in the amount of ozone generated between the trials, which partly explained the differences between the results in the replicate trials. Some synergism could be observed between ozonation and UV radiation in these trials, but further investigation is needed to draw firm conclusions. Both ozonation and UV radiation were predictable and controllable disinfection methods based on the 2–3-log-units reduction in airborne counts needed for effective disinfection.

9.3 Ultraviolet disinfection

McClean (1991) stated that the UV disinfection is well established in the brewing, potable water and pharmaceutical industries. Shortwave UV radiation (UVC, 254 nm) has been shown to reduce the microbial load both in air and on hard surfaces free of organic residues (Bintsis *et al.,* 2000). In general, bacterial cells are more sensitive than spores or fungal cells and algae are considerably more resistant against UV radiation than fungi (Scholte, 1996). The irradiation dose needed for the inactivation of 90% of *Bacillus subtilis* spores in a population is 120 Ws/m^2, whereas a dose of 1320 Ws/m^2 is needed for *Aspergillus niger* mould and 3600–6000 Ws/m^2 for algae (Anon., 1992). A combination of HEPA-filtered laminar air and UV radiation has been suggested for air-handling treatment in production units handling sensitive foodstuffs (Shah *et al.*, 1994). Microbial air quality in cold stores and egg-hatching cabinets has also been improved using UV radiation units (Bintsis *et al.,* 2000). To ensure total contamination control, UV radiation systems are used for airflow into sterile environments. The microbes are deactivated as they are exposed to the UV source (McClean, 1991). The outcome of the UV radiation experiments was easier to control than that of chemical fogging. Important factors related to the fallout rate of the bioaerosol. The UV radiation could reduce the airborne microbial counts by 4 log units. The effect can also be improved using 2–4 lamps and mirrors in the radiation unit (Shah *et al.*, 1994; Holah *et al.,* 1995b).

The best position for use is immediately prior to the point at which air is to be used, because the UV treatment has no residual effect (McClean, 1991). Further work on air disinfection methods is needed to improve both the disinfection treatment itself and the monitoring methods used in the process (Holah *et al.,* 1995b).

Fungal contamination of sausages during ripening was efficiently reduced using shielded UV lamps in the salami-ripening chambers (Papa *et al.,* 1995). Bodmer (1999) showed that UVC radiation is beneficial in disinfection of air in meat-processing plants; the treatment destroyed bacteria, yeasts and moulds, as well as viruses. UV systems can be used to disinfect displacement air for pressurizing tanks or pipelines holding perishable fluids. Immersible UV treatment systems have been designed to fit in the tank head airspace and disinfect the air present (McClean, 1991). During manufacture of aseptically filled dairy products, UV sterilization has been applied to both foil caps and cartons (Kuse, 1982; Nicolas, 1995). The combination of UV radiation and chemical fogging showed synergistic effects, giving lower residual counts for the combined treatment than for each treatment separately (Holah *et al.,* 1995b). Bintsis *et al.* (2000) suggested that the use of UVC radiation technology will increase in air disinfection equipment with improved technology solutions.

10. CLEANROOM APPLICATIONS IN FOOD AND DRINK PROCESSES

Packaged cooked-meat products are high-risk items intended for consumption without further cooking. To assure the safety of the facilities where these types of products are produced, the facilities should comply with EC legislation. This means that the air temperature should be below 12 ºC and cross-contamination routes should be prevented (van Zuijlen, 1995). New technological applications, e.g. cleanroom applications should be used to prevent microbial contamination of products. The advantage is that the shelflife of products produced in correctly operating facilities is extended and no pathogens occur in the products. When building new facilities all options should be evaluated. The other choice in producing prepacked sliced meat, apart from packaging in a cleanroom environment, is a second pasteurization after packaging. The disadvantages of an added heating process are reduced taste and texture of the product. Cleanroom application in these types of production lines is, however, challenging, because it is very difficult to guarantee no recontamination when products are packed manually (van Zuijlen, 1995).

In the examples presented below a high level of air cleanliness and almost absolute protection against airborne microbes were ensured. The protection requirement was met economically, regarding both investment and operation expenses. Operationally, such cleanroom systems address the specific hazard situation of each case. Nevertheless, they are, nothing but an element in the hygienic chain of the entire process, which always fails at its weakest link (Schicht, 1999b). Therefore, process hygiene should be handled throughout the process, even though cleanroom applications have been installed.

10.1 Cooked-meat product line

In the design process of a high-care production line for cooked-meat products with packaging under cleanroom conditions the choice of a suitable level for air cleanliness, sufficient air treatment equipment and adequate clothing as well as planning of the facilities and training of personnel should be handled at an early stage. An essential part of the entire process is planning of a cleaning and disinfection programme fulfilling cleanroom praxis (van Zuijlen, 1995). The

price of a laminar system and air cleanliness is much higher than the price of a minimal turbulent system. In many cases laminar flow of the treated air in food handling is not needed, because the production area is designed to avoid all contact between cooked and uncooked products. In building and equipment design the conditions required for cleaner areas include a smooth finish for walls and ceilings, smooth epoxy floors, no horizontal surfaces, all pipes above the false ceiling, airtight walls and ceilings, doors and windows fitting tightly, floors sloped away from the high-care area, no drains in the room, chillers of hygienic design, separate storage place for cleaning and technical equipment and separate entrances for personnel and packaging material, as well as lockers for materials (van Zuijlen, 1995).

The total high-care area in meat production described by van Zuijlen (1995) consisted of a room for removing products from the moulds, a room for grilling products before packaging, a prechilling room after the cooking cabinets, a final chilling room of the products before packaging, a room for removing the secondary wrapping of the packaging material, a packaging room where the products are cut and packaged, a transport locker to transport used cooking trolleys out of the cleanroom and a changing room with a cupboard for cleaning and technical equipment. It is important to choose between dry- and wet-cleaning in the high-care area. In a food process carried out in a cleanroom, where dust particles are the main hazard in the process, dry-cleaning should be used since wet-pressure-cleaning produces aerosols (van Zuijlen, 1995). Steam hoses should not be used in chilled areas because they cause condensation. The air filters should also be prevented from becoming wet. In a high-care area the quality of performance is established by measuring microbes at different places. The microbial quality of the air was assessed both during production as well as before and after production. Furthermore, the microbial load on the products, clothing of personnel as well as production and cleaning equipment was assessed at various times. Van Zuijlen (1995) stated that it is not possible to achieve cleanroom conditions immediately when the area is commissioned. Six months after starting up the cleanroom process line, a situation close to that of the target goal was reached, and the most important goals in achieving the set targets were development of an effective cleaning procedure as well as solving various technical problems associated with the equipment.

10.2 Sausage production line

Thermal processing of smoked sausages requires rapid cooling from 55 ºC to 15 ºC to prevent microbial growth. Georgieva and Akterian (1998) investigated a controllable hydroaerosol cooling process for smoked, scalded sausages. The hydroaerosol medium consisted of droplets (size: 100–200 μm) dispersed in moist air. The cooling intensity of the hydroaerosol process was as muchas 3.8 times higher than for cooling under conventional conditions. It can enhance the food safety of sausages, but the use of these types of cooling techniques also requires a cleanair application to ensure the air quality used in cooling. Müller (1997) described humidifying systems and ventilators with variable airvolume flows for use in meat industry processing. An efficient air-management system can help to ensure that the entire handling process is performed under hygienic conditions. It is also very important to have sufficient room to change filters to maintain hygiene.

10.3 Yoghurt-manufacturing line

In the milk-processing industry clean compressed air has been used in yoghurt production to keep the aerobic starter cultures uncontaminated. Already, slight contamination can destroy entire batches either in the fermentation or storage phases, where much excess air is used. Kronsbein (1996) reported that in a German dairy the compressed air for the storage tanks was filtered through a biologically inert, hydrophobic filter. In this dairy the filter units were placed in the air inlets of the tanks to avoid infiltration of contaminants, e.g. bacteriophages, bacterial spores and vegetative cells as well as fungi, into the products.

10.4 Butter production line

The quality of cream for buttermaking is satisfactory when there are no fungi or coliforms in the cream; furthermore the total CFU count must be less than 1000 CFU/ml. The water used in the butter process should have a microbial count less than 10 CFU/ml when incubated at 37 ºC. Contamination of butter from the air can therefore be a problem, especially in batch manufacturing and packaging processes, where the butter may be exposed to air for long periods. The air in the

butter-packaging rooms normally carries mould spores originating from the outside air, from contaminated surfaces and from the ceilings (Murphy, 1990). The growth of moulds is partially dependent on the contamination degree of the air and on the cleanliness of the equipment. Air contamination can be especially serious in unsalted or lightly salted butter, since moulds develop more easily in such products. The butter-processing area should be held under slight positive pressure and ventilated with sterile filtered air. Other cleanroom applications can also be installed in the department to increase butter quality (Murphy, 1990).

10.5 Cheese production line

To achieve continual product quality, cleanroom technology is increasingly used in dairies. New technologies, e.g. cleanroom applications with filtered, moistened and strictly monitored airflows, ensure freedom from foreign spores (Todt, 1993). Todt (1990) illustrated the integration of cleanroom technology into air-conditioning systems at a Swiss soft cheese production line. Installation and optimization of the new air-conditioning plant and production conditions proved to be favourable for the cheese process. The main requirements specified for the new installations were:

- reduction of condensation on walls, ceilings and windows
- no dead zones in airflow distribution
- no infiltration or intake of undesirable black mildew or fungus spores with particle sizes larger than 2.5 μm
- energy consumption during operation to be minimized
- air-conditioning plant and ducting must be cleanroom-compatible, e.g easily cleaned and disinfected
- almost fully automatic, computerized controls.

All the air supplied to the buildings was cleaned at the air-treatment unit with EU 6 prefilters and EU 11 HEPA filters (see Table 3). In the EU 6 filters 90% or more of particles larger than 5 μm are removed, whereas the EU 11 filters retain at least 95% of 0.3-μm particles and 100% of those having a diameter of more than 1 μm. Infiltration of undesirable spores through leaks in the building walls and windows was also prevented by maintaining the air pressure in the rooms at a value slightly above atmospheric (Todt, 1990).

The strategy of working with HEPA-filtered air and of avoiding condensation on surfaces reduced the growth of black mucor mildew, which was a major spoilage of the product. Air conditioning provided more constant temperatures in the cheese production area and, consequently, lower variations in the water content of the cheese. The more uniform climatic conditions and continuous extraction of incident moisture improved working conditions in the production area. The occurrence of black mucor mildew was reduced to such an extent that production rejects were no longer caused by it. The efforts and costs involved for inspection and monitoring, cleaning and chemical disinfection were greatly reduced (Todt, 1990).

In cheese production a typical situation for the brine and hurdle room is high localized humidity release. Large quantities of water vapour emanate from the open brine bath and the washing machine (steam cleaning). The recirculated/outside air-treatment unit is similar to that for the cheese production line. An additional recirculated air-treatment unit has been installed to provide higher rates of air dehumidification. The supply air is likewise fed in via short stainless-steel ducts and long throw jets. The exhaust air is collected directly above the washing machine and expelled into the atmosphere by fans mounted on the roof. Through this solution, large amounts of water vapour were eliminated from the system (Todt, 1990). This procedure required less energy than dehumidification and recirculation of the air. The recirculated air is extracted via an extract air grille near the brine bath and returned to the air-treatment plant. The air-conditioning system in the brine bath/hurdles room functions at a constant outside air/circulated air ratio (Todt, 1990). The fans can also in this case be operated in 2 modes to save energy. Under normal conditions they run at the lower speed. When the setpoint values are exceeded or the cleaning mode is run, the fans are switched to high-speed operation. The pressure in the brine bath/hurdles room was slightly less than that in the other parts of the cheese production line and in the drying cellars, and slightly above outside atmospheric value. The cheese is left for a relatively short time in this area and the contamination risks were less severe than in cheese production or in the drying cellar (Todt, 1990). The use of cleanroom applications is important especially in manufacturing of soft cheese, where large open brine baths are used. The air-conditioning systems provide savings in the process, exceeding the installation and running costs because the intervals between the cleaning procedures can be altered and the use of disinfectants can be reduced substantially (Todt, 1993).

10.6 Cheese-drying and -ripening cellars

The moisture in cheese is reduced in a short time under controlled conditions. The air velocity in the cheese-storage areas must be kept extremely low (< 0.12 m/s) and equally distributed throughout the entire room. The conditions necessary for drying should be ensured in each room by air-handling units (one in each) for both recirculated air and outside air installed in an adjacent machine room. In the drying cellars, the treated air is transported through plastic ducts and supplied to the rooms via special supply air outlet grilles. Two thirds of the recirculated air was extracted through vertical ducts from above the floor and one third from underneath the supply air ducts near the ceiling. This air was returned to the air-treatment plant via the plastic duct system. To maintain a small excess pressure in the drying cellars, a constant proportion of 7% outside air was added to the recirculated extracted air. The supply air flows into the room in such a manner that the inlet velocity is reduced by high induction. Hence the cheese on the drying hurdles was mainly surrounded with secondary air having extremely low velocities (< 0.1 m/s). After the drying cycle the cheese was normally transferred to the ripening cellar, where the temperature was slightly reduced and the RH increased (Todt, 1990).

A cleanroom was employed for the ripening of soft cheese. Exact requirements were specified for the application: air cleanliness corresponding to pharmaceutical room grade C, a chill-room temperature of 7–8 °C, RH of 92–98% and an air velocity of less than 0.12 m/s. The walls and ceiling are of stainless-steel, and the floor is constructed of durable earthenware tiles. The HEPA-filtered supply air was ejected from circular ducts, allowing for easy cleaning. Microbial contamination of the cheese loaves during their long exposure in the maturing room was effectively prevented, due to the optimized thermal and air cleanliness conditions, well organized operational procedures and a highly trained staff. The maintained air exchange rate guaranteed particularly uniform thermal conditions throughout the room, while inside the cheese-carrying racks there were optimal conditions for maturing of the product. Exact requirements had to be met during component assembly of chilled food portions. Here, unidirectional airflow at air velocities of 0.3–0.45 m/s was employed for reducing the changes in food recontamination by airborne microbes during the assembly operation (Schicht, 1999a).

10.7 Still-water production

The Italian mineral water industry began in 1991 to develop new methods for providing a quality product. The task, however, proved to be rather difficult, since still-water products were prone to severe mould contamination. The first measures carried out on still-water were focused on the following fundamental objectives (Sabatini, 2000):

- contamination control for the line between the sterilizer and capper for airborne particles
- control of the ozone level in the working areas
- definition of cleanliness requirements, which the cap suppliers should meet
- maintenance of the defined standard for cap cleanliness and improvement in tanks for proper sterilization of devices
- control of biological pollution for operators through functional and physical barriers
- introduction of environmental sterilization cycles based on vapour agents combined with biological control for surfaces and air
- training of personnel working in the process line and management
- implementation of schedules for management, control and maintenance.

10.8 Beverage bottling

Utilization of PET containers for beverages, e.g. fruit juices and mineral water, has made the application of cleanroom areas urgently needed in bottling. These containers do not withstand the temperatures associated with pasteurization. If preservatives are to be avoided, then an aseptic filling operation is needed. According to Schicht (1999b) the filling equipment was located in a cleanroom of ISO Class 7 (ref. Table 2). The critical process steps of bottle rinsing, filling and closing are performed in a compact clean area with unidirectional airflow in an ISO Class 5 cleanroom. The filling operation is fully automatic and no operator interference in the critical area is possible without stopping the filling process (Schicht, 1999b).

10.9 Packaging of baguettes and rolls

A German bakery employs cleanroom technology on a large scale in its 3 manufacturing facilities for baguettes and rolls. After the baking process, the products are cooled in a cooling tower, in which overpressure is maintained with ultrapure sterile air. The products are then conveyed to the primary packing stations. The entire conveying and packing is maintained at ISO Class 7 air cleanliness. The conveyor belts and the packing stations where the product is exposed are maintained at an ISO Class 5 air cleanliness level by means of unidirectional airflow of ultraclean air. In this process the PVC lamella curtains separated the ultraclean areas from the surroundings. The operators control the process from the outside and interfere in the critical areas only in case of an emergency (Schicht, 1999b).

11. CONCLUSIONS

11.1 Bioaerosols

In the food-processing careful planning of the plant layout as well as the services and the environmental control is required so that products can be safely produced, because airborne dust particles can introduce foreign matter including microbial contaminants into the products. After becoming airborne, an organism may have a very short life, its stability being influenced by RH, temperature, oxygen levels, solar and ultraviolet (UV) radiation, and chemical factors. The growth phase affects the survival of microbes in an aerosol. Bacterial spores survive better than the vegetative cells do and both *E. coli* and *S. marcescens* better from stationary than from logarithmic stages. *Salmonella* New Brunswick showed to survive better when aerosolized from skim milk than from distilled water. Yeasts are eukaryotes and are likely to be affected differently by aerosolization and sampling than bacteria. Oxygen slowly kills most airborne microbes through oxidation. Airborne bacteria are also subject to dehydration, which causes osmotic stress in the cells and may result in decreased survival.

11.2 Cleanroom technology

Defined environmental conditions in the process air can be achieved using cleanroom applications. The use of cleanroom technology in the food and beverage industry is to ensure the control of biocontaminants in sensitive processes. This technology should be considered in processes, where microbial inactivation, e.g. through termal sterilization or deep-freezing, is not feasible. It is also an alternative to preservatives when a long shelflife of products is desired. Appropriate air cleanliness classes for food and beverage applications are dependent on the product manufactured, which should be chosen using risk analysis for the process planned or reconstructed. Appropriate cleaning procedures for equipment and surfaces need to be adopted and validated in the cleanroom applications installed in the food industry.

In a well-designed plant layout suitable airflows help to reduce the airborne contamination rate. Two common ways to change the airflow and minimize the transfer of airborne microbes are to build walls between 'clean' and 'dirty' areas or to separate 'clean' from 'dirty' areas by suitable airflows and adequate

distances between the processes. Special attention on the door operations and on airflow must also be considered in the process layout. Problems may occur especially if the air used to protect food is contaminated before flowed over the product. Recontamination can be avoided through removing the outer protection cover before the materials enter the process.

The surface materials used should be hard, nonporous, smooth and cleanable and all equipment must be designed to meet cleanroom and hygiene requirements. All installations should be accessible for inspection and the premises should be equipped with sufficient light to allow inspections and cleaning. Ceiling, wall and floor elements should comply with all relevant regulations concerning fire protection, sound and thermal insulation. Attention has been focused on smoothness and effective sealing of utility services or other penetrations. Cleanroom doors should have as few horizontal surfaces as possible. Thresholds should also be avoided. Operational and performance requirements covering resistance, cleanability, strength, durability, impact resistance and frequency of operation of construction materials should be evaluated.

11.3 Air handling in cleanrooms

Airborne contamination can be reduced by controlling airflow through proper ventilation systems and good facility design. Airflow and distribution should be designed and regulated so that air flows from finished product areas to raw ingredient receiving areas. The risk of contamination becomes higher as air velocity is decreased. Simple cleanroom should have at least 15–20 air exchanges per hour. In the food industry, the selection of air filters must be based on reliable functioning and cost-effectiveness. In most cases, use of filters of the finest grade is not necessary. All filters must be compatible with the food-processing environment and employ nonmigrating media. The degree of filtration required depends on the product being manufactured, whether sterilization or preservatives are used. The air filters needed in a food-processing environment are usually of the barrier type, in which dust particles and the majority of microbes can be captured and retained. An air-pressure control system is required to maintain a positive air pressure in areas where the final product is exposed to air to minimize the contamination rate. An overpressure of 45 Pa at the cleanest area, 30 Pa at a less clean zone, 15 Pa in the change area and 5–15 Pa in the facility room give a good pressure gradient. The internal

positive pressure in the production facility aids in preventing infiltration of contaminated ambient air into the airflow. The influence of airflow velocity on contamination control of the air based on different key characteristics in the design e.g. shape and size of container, temperature of food, line speed, movement of people, complexity of plant, room geometry and position of inlet, outlet and exhaust points. The temperature and humidity control required in the cleanroom is important. Too much water in the air generally causes humidity problems in the process and microbial growth in the condensate.

11.4 Personnel in cleanrooms

Personnel are usually the predominant source of airborne microbes in cleanroom production areas. The workers must fully understand the procedures and the microbiological risks inherent in inappropriate actions. The main function of cleanroom garments is the protection of products manufactured in a cleanroom against fibres and particles released by humans from skin and garments. Various types of clothing are available e.g. woven and nonwoven fabrics, that are either washable or reusable or disposable garments. In addition to the barrier function of a cleanroom garment system, wear comfort is also an important criterion. Cleanroom garment fabrics usually act as surface filters, which means the barrier ability increases with longer test duration and increasing particle size. There is a high need for garments that will dissipate increasingly fewer particles, especially after many washes.

11.5 Limitation of risks

The potential risk situations created by interaction between people, air movements and airborne contaminants are difficult to predict with common microbiological measuring methods. The assessment should not be based on an intuitive unsystematic approach. Knowledge of the interaction between air movements and the dispersion of contaminants plays a vital role in the microbiological assessment of clean zones protected by unidirectional airflow. The limitation of risks (LR) concept, with visualization of air movements, particle challenge testing and calculation of the risk factor presents a method by which the risks can be limited. The LR method has successfully been used for microbiological assessment in critical regions during aseptic processing of sterile drug products.

11.6 Methods in air-quality assessment

Many food producers include bioaerosol monitoring as part of their Hazard Analysis Critical Control Point (HACCP) system to meet legal requirements, to collect epidemiological data and to determine the effect of air on the products processed. In the food industry the concern to determine the importance of the airborne route as a possible source of contamination is growing. Methods assessing both viable and total number of airborne microbes are therefore needed. The following criteria should be used to determine a sampling strategy: the sampling method, specificity and level of sensitivity required as well as the speed with which a result is required. The air cleanliness of isolated processes should first be assessed according to procedures given in standard methods using a particle counter. The microbial particles are likely to be more important than the total particle counts, because they can cause infections or be responsible for allergenic and toxic illnesses. The sampling time must be considered according to the process and aim of sampling. Other important information needed from the sampling period includes: location and area of the site, date and time of sampling, test temperature and moisture conditions, functions of the ventilation system during sampling, personnel in the area, volume of air sampled, collection media used and incubation time and temperature used as well as sampler used.

The efficiency of a sampler in collecting a particle of a given size is related to the air velocity in the impaction nozzle. Too low a velocity in the inlet will result in failure to collect the particles of interest. Too high a velocity results in a high shear force and may cause serious damage to the microbes, thus decreasing their viable recovery. Sedimentation or settle plate technique, impactors, impingers, centrifugal samplers and filtration are being used in monitoring the microbial quality of the air. Optical particle counters on the market include laser diffractometers, phase-doppler systems, intensity-deconvolution systems, and laser-particle interation system/image analyzers. Other techniques used in particle counters include the electrical mobility techniques used in electrical aerosol analysers and differential mobility analysers as well as LIDAR technology.

The ability of settle plates to collect airborne particles is governed by the gravitational force on the particle which decreases with a velocity dependent on its mass. The method is not quantitative and in high aerosol concentrations the uncountable numbers of colonies can be a problem. Impaction using either slit or

sieve samplers is the most common technique for collection of airborne viable particles. Sieve samplers are operated by drawing air through a large number of small, evenly spaced holes drilled in a metal plate and the particles are impacted on an agar surface located below the perforated plate. Liquid-using impactors are called impingers. Centrifugal samplers are simple and easy to operate and can rapidly sample a high volume of air, resulting in more representative sampling, though they show inherent selectivity for larger particles. Air filtration apparatus based on cellulose fibre, sodium alginate, fibre glass, gelatine membrane filter or synthetic membrane filters mounted in an appropriate holder collect the particles through a flow rate controller connected to a vacuum source. The gelatine filter membrane is composed of gelatine foam designed to prevent vegetative microbes from being inactivated by desiccation when air is drawn through the filter. The gelatine membrane is water-soluble so that it can easily be diluted for plating or be solubilized on top of a nutrient agar, resulting in bacterial colonies on the agar surface. The filter methods are good for enumerating mould or bacterial spores. Each of the proposed monitoring methods has limitations that the user should be aware of. Airborne counts recorded from different sampling techniques are not directly comparable but would be expected to show similar trends in level variation. In assessing the bioaerosol content the culturing technique of microbes directly or through a broth on solid agar media is the traditional method for enumerating the microbial count in the air. It is useful for direct identification of certain pathogens or spoilage microbes in the food-processing air, e.g. spoilage moulds on specific agars. Microscopy is a method with which the total count of microbes as well as morphological data on the microbes can be obtained. ATP is present in microbial cells and the light output of a sample is directly proportional to the amount of ATP present in the airborne cells, which can vary due to the stress caused by the sampling method used. Fully automated ATP-monitoring systems with continuous checking for microbial contamination in air is being developed. Molecular biology detection methods, e.g. PCR, gene probes and nucleic acid hybridization enable the detection of DNA regardless of the metabolic state of the cells.

11.7 Air-quality in some food processes

The highest microbial levels occur in factories handling products derived from the soil, e.g. vegetables. Cleaning, freezing and weighing operations readily release particles, especially when processing contaminated material. In the food

industry, increased concentrations of certain yeasts, moulds and bacteria can be hazardous both for the products manufactured and for the employees. From a food industry perspective, increased concentrations of microbes in the air during sanitation may decrease the shelflife of the foods being processed concurrently. The hygienists are becoming more concerned over the role of air as a vector of food contamination.

11.8 Air disinfection methods in the food industry

Clean air is provided through HEPA filters. Other methods used to reduce viable microbial counts in the air include chemical fogging, ozone and UV radiation. Disinfectants are commonly applied as fogs in the chilled-food industry, where the disinfection should be seen as an additional safeguard, not a substitute for cleaning. The use of more aggressive disinfectants e.g. peracetic acid or aldehyde formulations can also increase the disinfection effect on vertical surfaces. The concentration of chlorine in fogs must be very high e.g. 500 μg/ml to be effective, while a concentration of 10 μg/ml already causes discomfort for personnel working in the facilities. The best fogging effect was achieved using fog droplets with a diameter of 10–20 μm. In aerobiology cabinet studies fogging was uncontrollable and ineffective compared with other disinfection methods, e.g. application of ozone and use of UV radiation. Some synergism could be observed between ozonation and UV radiation. A combination of HEPA-filtered laminar air and UV radiation has been suggested for air-handling treatment in production units handling sensitive foodstuffs.

11.9 Cleanroom applications in food and drink processes

Cleanroom applications should be used to prevent microbial contamination of products intended for consumption without further heat treatment. The advantage is that the shelflife of products produced in correctly operating facilities is extended without using additives. Furthermore, a high level of the process hygiene must also be kept throughout the process, where cleanroom applications have been installed.

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Title

Clean air solutions in food processing

Abstract

This literature review deals with air handling in controlled environments in the food industry. The concern to determine the importance of the airflow as a possible source of contamination is growing, because the airborne dust particles can introduce foreign matter including microbial contaminants into the products produced. The following criteria should be used to determine a sampling strategy: the sampling method, specificity and level of sensitivity required as well as the speed with which a result is required. The microbial particles are likely to be more important than the total particle counts, because they can cause infections or be responsible for allergenic and toxic illnesses. Important information needed from the sampling period includes: location and area of the site, date and time of sampling, test temperature and moisture conditions, functions of the ventilation system during sampling, personnel in the area, volume of air sampled, collection media used and incubation time and temperature used as well as sampler used.

A careful planning of the plant layout as well as the services are needed to be able to choose an optimal clean air solution. Common methods used to reduce viable microbial counts in the production facilities include filtration, chemical fogging, ozone and UV radiation. The air filters needed in most food-processing environment are of barrier type, in which dust particles and the majority of microbes are captured and retained. The degree of filtration required is dependent largely on the product being manufactured. Since most food production plants are located in industrial areas with heavy atmospheric pollution, consumers need to be assured that these pollutants are excluded from the foodstuffs they consume.The filtration must be efficient enough to eliminate bacteria, fungi and spores from the airstream. The primary air filters protect the mechanical items of an air movement system from gross contamination over years of operation. Secondary filters are employed to remove smaller particulates from the air to a level needed in the process. Tertiary filters offer the best protection in installations where maximum particulate control is required. Some of the topics to be considered when choosing filters for the controlled environment are: cleanroom classification, number of air exchanges needed, classification of filters, efficiency needed, particles to be captured, filter material and type of filter. Filter header frames and cases are usually manufactured from galvanized mild steel or stainless-steel; only prefilters use card frames. The design, installation and sealing of a suitable filter-framing system is essential to guarantee complete filtration integrity. All filters must be compatible with the food-processing environment and employ nonmigrating media.

Clean air solutions should be considered in processes, where microbial inactivation, e.g. through termal sterilization or deep-freezing, is not feasible. In a well-designed controlled environment clean airflows help to reduce the airborne contamination rate. It must be taken into account that the personnel itself is one of the main sources in contaminating products produced if the procedures, accessories and garments used are inappropriate. The potential risk situations created by interaction between people, air movements and airborne contaminants are difficult to predict with common microbiological measuring methods. The limitation of risks concept, which includes visualization of air movements, particle challenge testing and calculation of the risk factor, presents a method by which the risks can be limited. In the review methods for assessing viable airborne microbes are given in Chapter 7. In Chapter 8 available reports on the microbial air quality in food processing areas have been presented. Clean air solutions in food processing have been reported in Chapter 10.

Keywords
food industry, bioaerosols, monitoring, cleanrooms, controlled atmospheres, air quality, microbes, sampling, disinfection, food processing

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