Biological Aerosols: A Review of Airborne Contamination and its Measurement in Dairy Processing Plants

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ABSTRACT

Processing plant air is a source of post-pasteurization contamination of dairy products. Little is known about the extent to which biological aerosols contaminate pasteurized products, however evidence indicates that air within a packaging area is a critical control point for both pathogens and spoilage microorganisms. Consequently, it is important to understand the characteristics of biological aerosols, learn how to control their occurrence, and discover practical and valid monitoring methods. Methods used for monitoring viable particles in air include the use of sedimentation plates, impingers, slit and sieve impactors, filters, and centrifugal samplers. Each of these methods has limitations on its usefulness for dairy plant air monitoring. Microorganisms are often injured due to the stresses of the aerosolized state and consequently may not grow on selective media. Sampling methods such as impingement and filtration which subject the organisms to additional stress may cause sufficient injury to prevent growth on non-selective media. However, gentler collection methods such as centrifugal samplers may not generate enough force to collect the smallest viable particles. Aerosols are generated within the dairy plant by worker activity, sink and floor drains, water spraying, and air conditioning systems. Environmental sanitation, air filtration, air flow control, and control over personnel cleanliness and activity are useful control measures. The adoption of “clean room” design principles for a packaging area will aid in controlling biological aerosols in new dairy processing plants.

BIOLOGICAL AEROSOLS

An aerosol can be defined as a suspension of microscopic solid or liquid particles in air or gas such as smoke, fog or mist. Biological aerosols include bacteria, yeasts, molds, spores of bacteria and molds, viruses and pollen. The size of aerosol particles generally range from 0.5 to 50 μm. Particle size is the major factor influencing aerodynamic behavior.

Aerodynamic behavior of aerosols

Aerosols exhibit complex aerodynamic behavior 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. Some of these physical forces such as gravitational field, electrical gradient, inertial force and thermal gradients are taken advantage of in aerosol sampling. Behavior of microbiological aerosols is governed by both physical and biological attributes. The physical factors control where, how and in what quantities the particles reach a particular landing site. Molecular motion, gravitational, thermal and electrostatic fields play important roles as does humidity. Inertial forces and fluid dynamics are primary influences in the landing process while interactions with electromagnetic radiation can be utilized for particle sizing, observation and analysis. This topic has been reviewed by Cox (20).

Biological injury resulting from the aerosol condition

The most important biological attribute of an aerosol is the degree of sublethal or lethal damage to the biological particles dispersed in the air. Biological stresses occur during aerosol generation (artificial or natural), aerosol dispersal, and aerosol collection or landing. These stresses are generally assumed to be sublethal when considered individually.
but when combined with other environmental stresses such as dehydration, hydration, irradiation, oxidation from oxygen or ozone, and effects of various pollutants, the result is often lethal. Stersky et al. (68) measured the inactivation of *Salmonella newbrunswick* aerosolized under various conditions. Distilled water dissemination in the first 20 min resulted in D-values ranging from 41 min at 21°C and 30% relative humidity (RH) to 206 min at 10°C and 90% RH. Skim milk dissemination at 10°C resulted in D-values ranging from 245 min to 404 min at 90% and 30% RH and at 21°C from 164 min to 470 min. Aerosolization is stressful for most vegetative cells so additional stresses from collection procedures and growth media must be minimized. Aerosolized organisms have been subjected to mechanical or physiological damage which reduces recovery on selective media. Stersky and Hedrick (66) tested the growth inhibition of various combinations of selective media on airborne bacteria. The ratio of *Escherichia coli* colonies on violet red bile (VRB), desoxycholate (DES), MacConkey (MAC) agar with overlay, standard plate count agar (SPC) overlay on VRB, and SPC overlay on DES to the colonies on SPC was less than 4%. Eosin methylene blue agar showed excellent recovery (122%) compared to SPC. Tergitol and endo agar recovered 23 and 40% respectively. Recovery of *Psedomonas* spp. on modified selective media was greater than that of coliforms. Recovery rates of airborne *Salmonella newbrunswick* ranged from <1% on *Salmonella-Shigella* agar to 118% on MAC/SPC. Recovery rates were somewhat improved by impinging onto SPC agar followed by overlaying with the selective media. Recommended non-selective media for overall microbial recovery include trypticase soy agar, brain-heart infusion agar and Mueller-Hinton agar. These media may also be fortified with blood to neutralize anti-microbial compounds that may be carried into the sampler (44).

Collection fluids are used for some types of aerosol samplers (liquid impingers). The selection of a liquid collection medium is dependent upon the particular organism being isolated. In quantitative studies a medium must be employed which will minimize both multiplication and death of the organism. The common collection media include buffered gelatin (71), phosphate buffer (48,75), 2% peptone water (22), nutrient broth (25) and gelatin-milk broth (46).

Factors involved in experimental techniques

Research on biological aerosols often includes generation, storing and collection of aerosols. Some factors in addition to sublethal injury which will influence experimental results are as follows: Strain of microorganism. Vegetative cells are more susceptible than spores to aerosol stress. There may be substantial strain variation for any given species. Growth conditions. The growth medium and growth phase influence susceptibility of the microorganism to aerosol stress. Aerosol generation. The degree of shear stress influences viability, especially for vegetative cells. If high viability aerosols are to be generated artificially, a low shear force aerosol generator such as spinning top, vibrating needle, or Berglund-Liu vibrating orifice aerosol generator should be chosen (20). Two fluid (air-liquid) atomizers (e.g. Collision atomizer (19)) were first choice dispersers in the past, but they impose high shear stress. Aerosols behave differently when generated by wet and dry methods. Aerosol particle size. Particle size continuously changes during aerosol storage and collection. Size may decrease through evaporation and collision, and may increase through agglomeration and absorption. Spray fluids. When the growth medium and the spray fluids are different, the spray fluid may affect viability. Stersky et al. (68) found that *Salmonella* which were aerosolized from skim milk had greater D-values than those aerosolized from distilled water. Aerosolization of coliforms with skim milk as opposed to distilled water resulted in growth of more colonies on selective media (66). Spent culture fluids, di- and tri-saccharides and the polyhydric alcohols, sorbitol and inositol provide the best protection from aerosol generated forces (20). Aerosol storage. In order to study the fate of microbes in aerosol, the aerosol should be stored for an extended period of time under specific conditions such as known relative humidity, irradiation, etc. Special techniques or apparatus such as the vertical wind tunnel (24), microthread apparatus (51), or rotating drum (30) are required. Aerosol collection methods. Collection methods greatly influence the recovery of viable particles. A discussion of the principles applications, advantages and limitations of these methods follows.

**AEROSOL SAMPLING METHODS**

Methods for sampling airborne microorganisms are basically the same as the methods used to sample dust and other airborne particulates. Existing samplers have been modified for the recovery of living biological agents so that the viability of the microorganism is preserved without permitting growth. Dimmick and Akers (23) state “Ideally, an aerosol sampler for microbiological assay should be capable of counting the total number of living airborne particles in a unit volume of air, as well as determining the number of viable units per particle and the size of the particles containing such units. However, this presupposes that 100% of the airborne cells, living or dead, can be physically separated from air without killing them during or after sampling.” Such a sampler has not yet been designed (44).

Most of positive samplers (impingers, impactors, filters, etc.) need vacuum for sampling air. The vacuum pump exhaust must be isolated from the area being sampled, as it may cause erroneous results. Seven types of commercially available aerosol samplers are listed in Table 1.

**Sedimentation methods**

The exposure agar plate and microscopic slide exposure methods rely on the force of gravity (if the microorganism containing particles are greater than 10 μm) and air currents (all sizes by random chance) to deposit particles on a non-selective or selective agar surface. Results
TABLE 1. Commercial sources of aerosol samplers.

**Impingers**

- **All-Glass Impinger 30 and Pre-Impinger;** Ace Glass, Inc., P.O. Box 688, Vineland, NJ 08360.
- **Midget Impinger with Personal Air Sampler;** Supelco Inc., Supelco Park, Bellefonte, PA 16823-0048.
- **May 3-stage Glass Impinger;** A. W. Dixon Co., 30 Anerly Station Road, London S.E.20, England.

**Impactors (slit type)**

- **Casella single slit and four slit sampler;** BGI Incorporated, Air Sampling Instruments, 58 Guinan Street, Waltham, MA 02154.
- **Mattson-Garvin air sampler;** Mattson Garvin Company, 130 Atlantic Drive, Maitland, FL 32751.
- **New Brunswick STA Air Sampler;** New Brunswick Scientific Company, Inc., P.O. Box 986, 44 Talmadge Road, Edison, NJ 08817.

**Impactors (sieve type)**

- **Andersen 6-stage, and 2-stage samplers;** Andersen Samplers, Inc., 4215-C Wendell Drive, Atlanta, GA 30336.
- **Ross-Microban sieve air sampler;** Ross Industries, Midland, VA 22728.
- **Personal Particulate, Dust, Aerosol Collector;** SKC Inc., 334 Valley View Road, Eighty Four, PA 15330.

**Filtration samplers**

- **Millipore membrane filterfield monitor;** Millipore Corporation, Bedford, MA 01730.
- **Gelman membrane filter air sampler;** Gelman Sciences Inc., 600 S. Wagner Road, Ann Arbor, MI 48106.
- **MSF 37 monitor;** Micro Filtration Systems, 6800 Sierra Court, Dublin, CA 94568.
- **Satorius MD8 Air Sampler;** Satorius Filters Inc., 30940 San Clemente St., Bldg., D, Hayward, CA 94544.

**Centrifugal samplers**

- **RCS Centrifugal sampler;** Folex-Biotest-Schleussner, Inc., 6 Daniel Road East, Fairfield, NJ 07006.

**Electrostatic precipitation samplers**

- **General Electric electrostatic air sampler;** General Electric Co., Lamps Components & Technical Products Div., 21800 Tungsten Road, Cleveland, OH 44117.

**Thermal precipitation samplers**

- **Thermal precipitator, hot wire;** Casella London Ltd., Regent House, Britannia Walk, London N1 7ND.

are obtained as cfu or particles/min. Particle size distribution may be obtained by direct microscopic examination. The 15th edition of Standard Methods for the Examination of Dairy Products (14) classifies sedimentation as a Class D method and recommends 15 min exposure of standard size (90 mm diam) Petri plates containing Standard Methods Agar or a selective medium. After exposure, plates are incubated according to the appropriate procedure. 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.

**Limitations.** Sedimentation methods are easy, inexpensive, and collect particles in their original state. The major disadvantages are their inability to measure airborne microorganisms quantitatively, i.e., number of viable particles/cu ft, and the relatively long sampling period that is required. Viable aerosol counts by this method are not at all, or only weakly correlated with the counts determined by other quantitative methods (61). Air movement will influence the deposition of the particles so that particle-size distribution may indicate a greater number of large particles than is actually present.

**Impinger methods.** Impinger methods use a liquid (simple salt solutions, with additives such as proteins, antifoam, or antifreeze) for collection. When the air is dispersed through the liquid, particles in the air are entrapped. Quantitation of airborne microorganisms is accomplished by diluting and plating the collection fluid or by using a membrane filtration plating technique when the expected microbial load is low. In high velocity liquid impingers, air is drawn through a small jet and is directed against a liquid surface with the resulting collection of suspended particles in the liquid.

**All Glass Impinger-30 sampler.** The All Glass Impinger-30 (AGI-30, Ace Glass Inc.) sampler is a high velocity impinger widely used for air sample collection (Fig. 1). The jet is held 30 mm above the impinger base and consists of a short piece of capillary tube designed to reduce cell injury. The AGI-30 sampler operates by drawing aerosols through an inlet tube curved to simulate the nasal passage (20). This makes it especially useful for studying the respiratory infection potential of airborne microorganisms. The usual sampling rate is 12.5 L/min. When it is used for recovering total airborne microorganisms from the environment, the curved inlet tube should be washed with a known amount of collecting fluid after sampling since large particles (i.e., over 15 μm diam) are collected on the tube wall by inertial force.

Impingement methods are highly efficient for particles greater than 1 μm when high jet velocities are used. This is a Class B method in the 15th edition of Standard Methods for the Examination of Dairy Products (14).

**Limitations.** The impinger is inexpensive and simple to operate, but viability loss may occur due to the amount of shear force involved in collection. The air stream approaches sonic velocity when particulates impinge on the collection fluid, resulting in almost complete collection of suspended particles; however, this condition tends to cause the destruction of vegetative cells (1) or may result in overestimation due to the dispersion of dust particles and the breaking up of clumps of bacteria (61). Another limitation is that the glassware should be sterilized before each sampling. Also, the apparatus is easily broken.

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Impaction methods. Impaction usually involves the collection of microbial aerosols on an agar surface, but dry or coated surfaces may be used for special purposes such as particle size determination. An impactor consists of an air jet that is directed over the impaction plate so that particles collide with and stick onto the surface. There are two types of impactors, slit samplers (e.g. Casella slit sampler) and sieve samplers (e.g. Andersen multistage sieve sampler, Fig. 2).

This is also a Class B method in the 15th edition of Standard Methods for the Examination of Dairy Products (14).

Slit sampler. The slit sampler usually has a tapered slit which produces a jet stream when the air is sampled by vacuum. The slit sampler may have a turn-table for rotating the agar plate so that aerosol particles are distributed evenly on the agar surface. Some slit samplers have a timing device on the turn-table which allows continuous monitoring of airborne viable particles count (e.g. New Brunswick STA sampler). These samplers will collect particles which are greater than 0.5 μm in size (44).

Sieve sampler. Sieve samplers are operated by drawing air through a large number of small, evenly spaced holes drilled in a metal plate (sieve). The suspended particles are impacted on an agar surface located a few millimeters below the perforated plate. There are single stage (e.g. Ross Microban sampler) and multistage sieve samplers (e.g. Andersen sampler). A multistage sieve sampler consists of a series (2, 6 or 8) of stacked sieves and plates each with 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 air flow until accelerated sufficiently to impact at a later stage (Fig. 2.B).

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 single colony from two 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. The microscopic method involves counting particles through a dissecting type microscope before colonies merge. The "positive hole" methods, designed for the Andersen 2-stage and 6-stage sampler, are essentially a count of the jets which delivered viable particles to the Petri plates. This count is converted to a viable particle count by the use of the "positive hole" conversion tables (4,47).

The multistage sieve sampler provides particle size distribution information. The usefulness of this information in plant sanitation programs has yet to be determined.

Limitations. Usually, impaction methods give higher particle recovery than other methods (27,69,70). Impaction results in low sampling stresses and after collection sample manipulation is not required. Multistage sieve samplers are cumbersome to handle and are expensive. The exact volume of agar must be poured into all plates aseptically so that the gap between the sieve and agar surface meets the manufacturer's specification. The inside of the sampler and even the outside of pre-poured agar plates should...
be maintained sterile until sampling, as they can contribute to contamination.

Filtration methods. Filters are widely used for aerosol sampling due to low cost and simplicity of operation. The air filtration apparatus consists of cellulose fiber, sodium alginate, glass fiber, gelatin membrane filter (GMF, pore size 3 μm) or synthetic membrane filters (pore size 0.45 or 0.22 μm) mounted in an appropriate holder and connected to a vacuum source through a flow rate controller (e.g., critical orifice). After a fiber filter is used, the whole filter or a section of it is agitated in a suitable liquid until the particles are uniformly dispersed. Aliquots of the suspension are then assayed by appropriate bacteriological techniques. Membrane filters can either be treated similar to fiber filters or directly placed on an agar surface and incubated.

Gelatin membrane filtration method. The gelatin membrane is water soluble so that it can easily be diluted for plating or be solubilized on top of a nutrient medium resulting in bacteria colonies that are easily counted. However, this hygroscopic property causes difficulties in sampling due to swelling of the membrane when the relative humidity is over 90% (64). The large number of pores present in these membranes allows a large volume of air to be sampled during a short time (2.7 L of air/min/cm²/500 mm water column).

Limitations. Filtration methods are good for enumerating mold or bacterial spores. They may not be effective for counting vegetative cells because of the stress of cell dehydration produced during sampling (26). The shorter sampling times used in gelatin membrane filtration may reduce this stress.

Centrifugal methods. Centrifugal force can be used to propel aerosol particles onto a collection surface. When the aerosol is spun in a circular path at high velocity, the suspended particles impact on the collecting surface by a force proportional to the particle’s velocity and mass. Centrifugal samplers do not generate high velocity jet flow during sampling, so less stress is imposed on airborne microbes as compared to impingement and impaction methods. Centrifugal samplers are simple and easy to operate and may be less expensive than impactor types. Generally, centrifugal samplers can rapidly sample a high volume of air resulting in more representative sampling.

Limitations. Some devices may not generate sufficient centrifugal force to propel small particles onto the collection surface. The recovery efficiency of these samplers depends on the particle size being sampled and the amount of centrifugal force generated.

Biotest Reuter centrifugal air sampler. The Reuter centrifugal air sampler (RCS sampler, Biotest Diagnostics Co.) is battery operated, portable, light in weight (2.5 lb.) and convenient to use (Fig. 3). A plastic strip containing a culture medium lines the impeller drum. Air from a distance of at least 40 cm is sucked into the sampler by means of an impeller. Air enters the impeller drum concentrically from a conical sampling area. It is set in rotation, and the aerosol impacted by centrifugal force onto the agar surface. Air then leaves the sampling drum in a spiral outside the cone of entering air. After the sample has been taken, the agar strips are incubated and the colonies counted. The sampler has a self-timer for sampling from 30 s to 8 min. The actual sampling rate is 280 L/min. However, the manufacturer has published an effective sampling rate or separation volume of 40 L/min (4 μm particles, a value derived from an attempt to reconcile the actual number of viable particles collected from an air sample with measurements involving airflow direction, air velocity and available collecting surface area. Clark et al. (18) indicated the effective sampling volume of the RCS sampler will vary widely depending on aerosol particle size. Consequently, the results obtained by using this sampler must be interpreted with considerable caution. Macher and First (49) measured the collection efficiency of RCS sampler and found improved efficiency with increasing particle size. Particles larger than 15 μm are almost 100% collected, those in the 4 to 6 μm range are collected at 55 - 75% efficiency and particles smaller than 1 μm pass through the sampler without significant retention. Although RCS sampler does not accurately estimate total viable particle concentration, Placencia and Oxborrow (58) recommended this sampler for good manufacturing practices investigations. These investigators found that the RCS sampler will collect more viable particles than a slit sampler and it could detect the difference in the environmental quality of each medical device manufacturing facility tested. In addition, the RCS sampler effectively detects various types of microorganisms (58).

Electrostatic precipitation methods

Aerosol particles can be ionized and collected on either a positively or negatively charged surface. Electrostatic precipitators employ various solid collection surfaces such as agar or glass. During ionization of the air sample, oxides...
of nitrogen and ozone are produced which may be toxic to microorganisms. These samplers have a high sampling rate, high collection efficiency, and low resistance to airflow. They are mechanically complex and must be handled carefully. Although several electrostatic precipitators are manufactured specifically for sampling microbial aerosols, they are not widely used for this purpose (74).

**Thermal precipitation methods**

Thermal precipitation methods recover particles based on thermophoresis principles in which particles move away from a hot surface toward a colder surface by a force proportional to the temperature gradient. These samplers can be used for the determination of particle size distribution, though they are more effective when collecting small particles (less than 1 \( \mu m \)). However, they are not in common use since precise adjustments are required and air sampling rates are quite low (300-400 ml/min). The aerosol particles are usually collected on a glass coverslip or electron microscope grid, and are subsequently sized and counted microscopically (74).

Additional information on aerosol samplers can be found in the following references (1,3,20,23,31,35,50,69,74).

**Comparison studies on aerosol samplers**

Millipore and absorbent cotton samplers more efficiently recovered mold spores than the AGI-30 sampler (65). Fields et al. (27) recommended the use of the membrane filter field (MF) monitor for estimating airborne microorganisms after comparing it with the Reyniers slit air sampler for microbiological laboratory and clean room environment testing. These results showed that a significantly higher number of microorganisms were recovered by the Reyniers slit sampler with a high degree of consistency. The MF sampler detected 79% of the concentration measured by the Reyniers slit sampler and the types of microorganisms identified from both sampling methods were similar. Chaigny (17) concluded that .4 \( \mu m \) Millipore filter as an air sampler of small particles may be somewhat more efficient than the AGI-30 sampler, but viable recovery will usually be lower except in the case of bacterial spores or fungi. Similarly, the Litton (LVS) large volume electrostatic sampler is from 40 to 70% as efficient as the AGI-30 sampler, but it has a sampling rate approximately 100 times greater than the AGI-30 sampler.

The Andersen 2-stage impactor was more effective than the May 3-stage glass impinger for recovering *Escherichia coli* from aerosol in a waste water plant environment (75). Curtis et al. (21) compared the Andersen 8-stage and 2-stage air samplers for recovery of viable organisms. They found that the 2-stage disposable air sampler gave lower values for airborne bacterial colony-forming particles than did the 8-stage viable air sampler in either a swine barn or a classroom. When Lembke et al. (46) tried to devise a method to determine the precision of the AGI-30 sampler and the Andersen 6-stage air sampler over a wide range of aerosol concentrations inside a municipal solid-waste recovery system, they found a high degree of variability associated with both types of air sampling devices. They indicated that slippage of particles from one stage to another stage, particle fragmentation or agglomeration, and wall losses in Andersen 6-stage air sampler, and processing technique in AGI-30 sampler may account for some of the variance. An Andersen 6-stage sampler, a Casella slit sampler, an AGI-30 sampler, and a filter sampler with gelatin membrane filters or ordinary membrane filters were tested for collection efficiency with a bacterial aerosol in laboratory experiment, in field experiments, and in experiments with skin fragment sampling (48). The Andersen sampler gave the highest bacterial counts in all environments tested. The slit sampler gave significantly lower counts only in the aerosol experiments and in one of the field experiments. The filters performed efficient sampling in skin fragment experiments only.

Radmore and Lück (61) compared exposure plate, liquid impinger and gelatin membrane filtration (GMF) methods. Air counts determined by exposure of agar plates were not at all, or only weakly correlated with the counts determined by two other methods. The relationship between the liquid impinger and GMF methods were also not very consistent (r=0.75). At levels above 1000 microorganisms/m³, the impinger method yielded counts up to 6 times higher than the GMF method. They explained this was probably a result of the dispersion of dust particles and the breaking up of chains and clumps of bacteria during the bubbling of air through the impingement liquid.

In comparative studies of airborne microbial recovery rate (22,58,59), the RCS sampler was found to be significantly more efficient than a slit sampler or a liquid impinger. The RCS sampler samples air the shape of a sphere with a diameter of 1.3 ft—representing an air volume of about 1.2 cu ft versus only 0.5 cu ft dimension of air sampled by the slit sampler (22).

Comparison studies of air sampling devices indicate that there is often no obvious choice of the correct sampler to use. A multistage sieve sampler such as the Andersen may be most efficient at viable particle recovery but it is not suitable for taking repeated sampling on a routine basis and requires a vacuum source. Filter samplers work well for quality control monitoring of molds and bacterial spores, but bacterial recovery is questionable, depending on the extent of dehydration that occurs during sampling. In addition, a vacuum source is required. The RCS sampler is convenient to use, creates its own air flow and recovers bacteria as well as molds. Even though the RCS sampler does not recover the smallest viable particles, it is still useful for determining relative air quality on a routine basis. Slit samplers may not be as convenient to use as the RCS sampler, especially if a vacuum source is required. However, slit samplers are more efficient at recovering small particles.

**SAMPLING AND MEASUREMENT STANDARDS**

*Standard Methods for the Examination of Dairy Products*

The 15th edition of the Standard Methods for the
Examination of Dairy Products (14) lists no Class A standard method for testing the microbiological quality of air in dairy environments, though there are methods designated as Class D and B. Favero et al. (26) introduced air sampling strategies and various air sampling methods in “Compendium of Methods for the Microbiological Examination of Foods.” They pointed out that the first and the most important decision is whether air sampling at any level is required. If it is, then quantitative and qualitative guidelines should be established which relate numbers and types of microorganisms per volume of air to critical levels of product contamination.

**NASA air cleanliness standards**

Favero et al. (26) also suggested that the NASA air cleanliness standards may be used as a reference point after experiments to determine suitability. The “NASA Standards for Clean Rooms and Work Stations for the Microbially Controlled Environment” (54) defines three air cleanliness classes (Table 2). According to the standards, the collection methods must conform to “Standard Procedures for the Microbiological Examination of Space Hardware (NHB 5340.1 or revisions thereof)” which specifies use of a slit sampler.

**Federal standard 209C**

Federal standard 209C for “Clean Rooms and Work Station Requirements, Controlled Environment” establishes standard classes of air cleanliness for airborne particulate levels in clean rooms and clean zones. These classes are based only on particle enumeration and place more emphasis on small particles which are not necessarily viable (29). This standard is not useful for food plant applications.

**Standard reference samplers**

Brachman et al. (9) recommended the AGI-30 sampler as a standard reference sampler because of historical use, economics, availability and its simple design. On the other hand, the American Conference of Governmental Industrial Hygienists Committee on Bioaerosols (2) used the Andersen multistage air sampler as the reference sampler for its committee activities and reports. In the pharmaceutical industry, the slit sampler is the most widely used device for monitoring sterile manufacturing and quality control environments (1).

**BIological AEROSOLS IN DAIRY PROCESSING PLANTS**

Research on the importance of biological aerosols in food processing environments is limited and has dealt primarily with the dairy industry. Most research since Olson and Hammer’s (56) initial contribution has been related to the numbers, types, and sources of airborne microorganisms in the dairy plant (12,16,37,38,39,45,55,57,70). Little research has been reported on the relationship of product quality to air quality (5,11,13,57) or control of airborne microorganisms (8,36,38,40,56,67). Although investigators have used different methodology, their studies indicate that bacteria, yeasts and molds are continuously falling from the air in the dairy plant environment. It is evident that microbial contamination of dairy products and equipment from the air is to be expected under normal operating conditions.

**Types and populations of microorganisms**

Data on levels of microorganisms in dairy plants obtained using various methods is presented in Table 3-6. Large variations in viable particle levels are found even when similar samplers and locations are compared. These variations are due in part to differences in facility design, air flow, personnel activity and degree of environmental sanitation.

Sunga et al. (70) used both a Casella slit sampler and an Andersen (6-stage sieve) sampler to test dairy processing areas. Both samplers produced similar results with about 68% of the total determinations producing bacterial counts of over 30 organisms/5 cu ft and a very low population of probable Staphylococcus spp., coliforms and yeasts.

**TABLE 2. NASA air cleanliness classes**

<table>
<thead>
<tr>
<th>Class, English (Metric) System</th>
<th>100</th>
<th>10,000</th>
<th>100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3.5)</td>
<td>(350)</td>
<td>(3500)</td>
<td></td>
</tr>
<tr>
<td>Max. No. of .5 μm and larger</td>
<td>100</td>
<td>10,000</td>
<td>100,000</td>
</tr>
<tr>
<td>Particles per cu ft (per liter)</td>
<td>(3.5)</td>
<td>(350)</td>
<td>(3500)</td>
</tr>
<tr>
<td>Max. No. of 5 μm and larger</td>
<td></td>
<td>65</td>
<td>700</td>
</tr>
<tr>
<td>Particles per cu ft (per liter)</td>
<td>(2.3)</td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td>Max. No. of Viable Particles</td>
<td>0.1</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>per cu. ft. (per liter)</td>
<td>(0.0035)</td>
<td>(0.0176)</td>
<td>(0.0884)</td>
</tr>
<tr>
<td>Avg. No. of Viable Particles</td>
<td>1,200</td>
<td>6,000</td>
<td>30,000</td>
</tr>
<tr>
<td>per sq. ft. (per M²) per week</td>
<td>(12900)</td>
<td>(64600)</td>
<td>(323000)</td>
</tr>
</tbody>
</table>

1NASA standards for clean rooms and work stations for the microbially controlled environment (54).

2Statistically unreliable except when a large number of samplings is taken.

TABLE 3. Levels of microorganisms found in air of dairy plants by exposure agar plate method using 90mm plate (data adjusted to 15 min exposure).

<table>
<thead>
<tr>
<th>Locations</th>
<th>Bacteria range (mean)</th>
<th>Yeasts range (mean)</th>
<th>Molds range (mean)</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market milk areas</td>
<td>1.8-185.8 (46.5)</td>
<td>0-6.3 (1.1)</td>
<td>0.5-75.5 (1.1)</td>
<td>56</td>
</tr>
<tr>
<td>Butter areas</td>
<td>4.5-129 (41.6)</td>
<td>0-5.3 (1.1)</td>
<td>0.8-71.5 (1.1)</td>
<td>16</td>
</tr>
<tr>
<td>Cheese areas</td>
<td>3-171.5 (36.2)</td>
<td>0-10.8 (1.1)</td>
<td>N.D.</td>
<td>45</td>
</tr>
</tbody>
</table>

1N.D. = Not determined.

2Maximum counts.
TABLE 4. Levels of microorganisms found in air of pasteurized milk processing areas using quantitative sampling methods.

<table>
<thead>
<tr>
<th>Sampler</th>
<th>Bacteria range (mean)</th>
<th>Yeasts range (mean)</th>
<th>Molds range (mean)</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casella</td>
<td>66-334 (195)</td>
<td>12-181 (70)</td>
<td>51-293 (145)</td>
<td>33</td>
</tr>
<tr>
<td>Casella</td>
<td>141-3143 (1105)</td>
<td>0-4462* (1088)</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Casella</td>
<td>0-141 (8)</td>
<td>0-127 (30)</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Andersen</td>
<td>0-7 (6)</td>
<td>92-205 (138)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andersen</td>
<td>(3260±5283)*2</td>
<td>(1812±3814)*12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Yeasts and molds count.

Low levels of *Staphylococcus* spp. and coliforms detected.

Non-molds count.

Expressed as mean±standard deviation.

Gelatin membrane filtration method.

Figure 4. Viable particle size distribution of total bacterial counts from various food packaging areas (70). The ranges of particle size collected on each stage are: stage 1; > 9.2 μm stage 2; 5.5-9.2 μm stage 3; 3.3-5.5 μm stage 4; 2.0-3.3 μm stage 5; 1.0-2.0 μm stage 6; <1.0 μm.

Cannon (12) reported on the viable particle counts of air samples from fluid milk plants with the use of the Andersen sampler. He obtained a mean count of 92.3/cu ft of non-mold colonies and 51.3/cu ft of mold colonies. From non-mold colonies, 25% of these were Gram negative rods and 24% of cultures grew in tryptase soy broth in 5 d at 10°C.

In subsequent study, Cannon (13) sampled the air in the milk processing areas of 10 dairy plants and found an average of 32 non-molds and 14 molds/10 L. The bacteria isolated were primarily micrococcii, Gram-negative rods (excluding coliform), bacilli, and corynebacteria. He also found a few streptococci, coliform and lactobacilli. Twenty-five percent of the isolates grew at 10°C in 5 d indicating refrigerated spoilage potential.

Radmore and Lück (61) analyzed 209 samples from 31 dairy factories. An average of 12.5% of the total count consisted of thermophilic organisms and 4 of the 209 air samples contained *Enterobacteriaceae*. None of the samples produced viable *Staphylococcus aureus*.

Olson and Hammer (56), and Cannon (12) concluded that bacteria were the most numerous and yeasts the least numerous in processing plant air. On the other hand, Heldman et al. (37) reported that molds were the most numerous with yeasts still being the least numerous. Microbial populations can vary widely within and among plants.

TABLE 5. Levels of microorganisms found in air of cheese processing areas using quantitative sampling methods.

<table>
<thead>
<tr>
<th>Locations</th>
<th>Sampler</th>
<th>Bacteria range (mean)</th>
<th>Yeasts range (mean)</th>
<th>Molds range (mean)</th>
<th>Y&amp;M 1 range (mean)</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottage cheese</td>
<td>Casella</td>
<td>109-242 (158)</td>
<td>13-212 (62)</td>
<td>133-2361 (786)</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Casella</td>
<td></td>
<td>(62)</td>
<td>133-2361 (786)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casella</td>
<td>N.D.</td>
<td>0-7 (1.4)</td>
<td>7-318 (57)</td>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Andersen</td>
<td>N.D.</td>
<td>0-113 (37)</td>
<td>7-127 (126)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andersen</td>
<td>565-3037 (258)*3</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Cheese areas</td>
<td>slit sampler</td>
<td>24-7-2354 (986)</td>
<td>N.D.</td>
<td>35-3496 (1056)</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Casella</td>
<td>106-24791 (1683)</td>
<td>N.D.</td>
<td>35-3496 (1056)</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4086)</td>
<td>N.D.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GMF 5</td>
<td>(2244)</td>
<td>N.D.</td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(133)</td>
<td>N.D.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(701)</td>
<td>N.D.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Yeasts and molds count.

No data available.

Total viable particles count.

Lactobacilli on 2% plain agar, over which a layer of selective acetate agar medium.

Gelatin membrane filtration method.

S.

JOURNAL OF FOOD PROTECTION, VOL. 52, JULY 1989
TABLE 6. Levels of microorganisms found in air of other dairy processing areas using quantitative sampling methods.

<table>
<thead>
<tr>
<th>Locations</th>
<th>Sampler</th>
<th>Bacteria range (mean)</th>
<th>Yeasts range (mean)</th>
<th>Molds range (mean)</th>
<th>Y&amp;M range (mean)</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Casella</td>
<td>102-371 (218)</td>
<td>10-182 (85)</td>
<td>119-1951 (486)</td>
<td>141-918 (434)</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Casella</td>
<td>388-4662 (1596)</td>
<td>0-28 (7.8)</td>
<td>7-388 (88)</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Andersen</td>
<td>0-14 (8.5)</td>
<td>106-212 (131)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry milk areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Casella</td>
<td>283-2119 (1095)</td>
<td>106-2966 (1021)</td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>GMF3</td>
<td>(1766)</td>
<td>(802)</td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>2911 (542)</td>
<td>(589)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Production</td>
<td>1274 (2296)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice cream areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Casella</td>
<td>353-883 (579)</td>
<td>141-565 (297)</td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Andersen</td>
<td>0-14 (2.1)</td>
<td>0-42.4 (25.4)</td>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-14 (8.5)</td>
<td>14-113 (65.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condensed milk areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GMF3</td>
<td>(221)</td>
<td>(94.6)</td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Production</td>
<td>(1117)</td>
<td>(925)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Packaging</td>
<td>(13.0)</td>
<td>(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Storage tank</td>
<td>(50.5)</td>
<td>(20.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy factories</td>
<td>slit sampler</td>
<td>18000 (15)</td>
<td>(94.6)</td>
<td></td>
<td></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>RCS6</td>
<td>10-15000 (3)</td>
<td>(20.5)</td>
<td></td>
<td></td>
<td>63</td>
</tr>
</tbody>
</table>

1Yeasts and molds count.  
2Low levels of Staphylococcus spp. and coliforms detected.  
3Gelatin membrane filtration count.  
4Maximum counts.  
5Total viable particles count.  
6Biotest RCS centrifugal air sampler.

(12, 16, 56, 70), and on a day to day basis within the same plant (37).

Rossmore et al. (63) frequently isolated species of Pseudomonas, Serratia, Klebsiella, Sarcina, Micrococcus, Staphylococcus, Fusarium, Aspergillus, Rhisopus, Penicillium, Chladosporium, Candida, Kluyveromyces, Rhodotorula and Saccharomyces from the air of dairy plants.

Size distribution of viable particles

Since aerosol sampler performance depends on the size of particles being sampled, information on particle size distribution in dairy plants is useful. Sunga et al. (70) determined the size distribution of viable particles recovered from a dairy processing environment. These data are summarized in Fig. 4. Forty-four percent of total viable airborne bacteria were in the particle size range of 2.0-5.5 μm. Fifty-eight percent of the molds were in the range of 1.0-3.3 μm.

Eleven percent of non-molds and 2.4% of molds were found in particles less than 1 μm. It is apparent from these data that samplers which do not recover the smallest particles (i.e., RCS sampler) will often underestimate the total amount of air contamination. Whether this underestimation is of quality control significance has not been determined.

Factors affecting airborne microbial types and populations

The airborne microbial population within a dairy plant exhibits no distinct seasonal variation in type or number (16, 56). Also machine activity has no apparent effect on airborne microbial counts (37).

Cerna (16) concluded that the major factor affecting airborne microbial populations in different dairies is the presence of workers, their numbers and activity. Heldman et al. (37) also reported an association between airborne bacteria counts and worker activity. One experiment of Hedrick et al. (33) showed that a medium sized man confined to a 5 cu ft area and inactive, shed, with a uniform soiled by 4 h of normal wear, 110 bacteria colonies/ 5 cu ft; without clothes, 20 colonies; and immediately after a shower (without clothes), 3 colonies. Additional human contributions to airborne populations may be attributed to coughing, sneezing, speaking or exhaling (35). Drains add large numbers of bacteria and a few yeasts and molds to the...
air during flooding, especially after being idle overnight (33). The general quality of plant sanitation, the location of the dairy, quality of ventilation and degree of personal hygiene were found by Cerna (16) to be important factors in determining processing plant air quality. Hedrick et al. (33) indicated that the cleanliness of the storage area and precautions of unpacking supplies in processing and packaging areas are also important.

Perry et al. (57) studied airborne contamination of cheese with lactobacilli. They selectively isolated and identified similar lactobacilli from the air and cheeses. “Cheese types” of lactobacilli were found in the air some months before and after the cheese was made, which indicates contamination of the air from other sources within the dairy. Naylor and Sharpe (55) compared possible sources of lactobacilli contamination and concluded that air was the major source.

**Routes of airborne product contamination**

Any point at which product is exposed to air is a possible route for airborne contamination. Air for mixing raw milk followed by a Moseley keeping quality test (52) for estimating the air quality in a cottage cheeses processing room area. His data indicate that poor air quality was the cause of unsatisfactory shelf-life. In another study, the shelf-life of cottage cheese negatively correlated (r=-0.642) with the viable particle counts of cheese processing room air. The coefficient of determination indicated that air contamination contributed approximately 40% to the variation in shelf-life (11).

Cannon (13) did not find a relationship between airborne microbial populations and keeping quality of the packaged milk. This was probably because the contamination of milk from sources other than air was sufficient to overshadow any airborne contamination.

A special packaging system, the ‘long-life machine’ (designed by Ex-Cell-O Co., Walled Lake, MI), encloses the filling chamber with fitted glass fiber covers and is designed to eliminate the need for a defoamer. This system protects products from airborne contamination and may extend the shelf-life of whole milk by 7 d. The SPC for whole milk run on this machine did not reach 20,000 cfu/ml for 18 d, whereas the same milk packaged by a standard machine exceeded 20,000 cfu/ml after 12 d (43). This data shows the extent to which airborne contamination may influence the shelf-life of product made from high quality raw milk.

**Air quality and shelf-life of products**

Angeline (5) used exposed plates of pasteurized skim milk followed by a Moseley keeping quality test (52) for estimating the air quality in a cottage cheeses processing room area. His data indicate that poor air quality was the cause of unsatisfactory shelf-life. In another study, the shelf-life of cottage cheese negatively correlated (r=-0.642) with the viable particle counts of cheese processing room air. The coefficient of determination indicated that air contamination contributed approximately 40% to the variation in shelf-life (11).

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**TABLE 7. Recommended maximum levels for air in various processing situations based on data from a Casella slit sampler.**

<table>
<thead>
<tr>
<th>Types of product</th>
<th>Standard Plate Yeast &amp; Mold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk and cream</td>
<td>1.8</td>
</tr>
<tr>
<td>Butter</td>
<td>3.6</td>
</tr>
<tr>
<td>Dried milk</td>
<td>2.8</td>
</tr>
<tr>
<td>Cultured milk and cream and cottage cheese</td>
<td>1.8</td>
</tr>
<tr>
<td>Ripened cheese</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Proposed guidelines of air quality in dairy processing and packaging areas**

Mossel (53) developed the following formula for calculating acceptable viable particle levels:

\[
\text{Limit (microorganisms per cu ft)} = \frac{P}{100} \times \frac{N}{V}
\]

where P is what one regards as a significant percentage increase in the count of microorganisms being considered; N is the geometric mean of the acceptable level of microorganisms in the food per g, and V is total volume of air (cu ft) passing over or through 1 g of the food in the course of processing. The usefulness of this formula has not been determined.

Hedrick (32) recommended the maximum levels of viable particles for air in various processing situations based on feasibility as well as desirability (Table 7).

Radmore et al. (62) proposed air quality guidelines based on data collected from a simulated filling operation. This approach may be valuable, but the usefulness of the guidelines has not been demonstrated. In addition, these proposed guidelines are based upon limited data with a high degree of variability.

**Importance of microbial air quality**

Two of the most important objectives of dairy processing are maintenance of product safety and acceptable shelf-life. These objectives are closely related since they are strongly influenced by post-pasteurization contamination. Potential sources of post-pasteurization contamination include air, the filling machine, improperly cleaned equipment and worn equipment as important possibilities. Air is usually considered the least important of these sources. Hedrick and Heldman (34) concluded that airborne contamination was of most importance in the manufacture of cultured milk products, followed by powder milk, cheese, market milk, ice cream and butter manufacturing in decreasing order of importance. More recently, the ice cream industry is emphasizing the control of air quality as means of preventing contamination of product with *Listeria*. The FDA and Milk Industry Foundation / International Ice Cream Association (28) recently issued guidelines for controlling environmental contamination in dairy plants. They indicate
that airborne contamination is strongly suspected as a vehicle for pathogens entering into products.

An important part of the FDA Dairy Initiative Inspections has been the sampling of finished products for the presence of Listeria, Yersinia, Salmonella, and Campylobacter. None of these pathogens can survive the pasteurization process. As of 1986 the FDA found 26 plants which produced contaminated products (72). Among the 26, eight contained pathogenic Listeria in their packaged products. Since these potential pathogens do not survive pasteurization, these plants probably have post-pasteurization contamination problems (6,7,72). Dirty fillers and aerosols have been reported as the source of pathogen contamination (72).

In one study (12), about 25% of non-mold colonies from the air of a fluid milk plant were Gram negative rods and 24% of total colonies isolated could grow in 5 d at 10°C. Though the origin of these Gram negative rods is not clear, their presence in the air of the packaging area is of both public health and product shelf life significance. Plakhotya (60) found that Salmonella in an aerosol condition could survive up to 4.5 h, indicating the potential for airborne dissemination.

Controlling air quality in food processing areas

Aerosols of bactericidal or viricidal agents have been used in dairy industry for controlling airborne bacteria and bacteriophage. These agents have included various forms of chlorine, glycol, alcohols and quaternary ammonium compounds (8,10). Fogging must be limited because of potential health effects on exposed workers. For example, a chlorine fog of 500 ppm will reduce airborne microflora, but over 10 ppm causes excessive human discomfort (32). In addition, fogging will be less effective if the source of the aerosol is not controlled. Aerosols often originate from unclean surfaces as previously discussed.

Ultra-violet radiation can be used to decrease airborne microflora, but its use is also limited by worker safety.

The use of laminar air flow with HEPA (high efficiency particulate air) filters is highly recommended at critical points (35,36,40). HEPA filters remove 100% of particles that are greater than 0.3 μm in size and consequently will remove all viable bacteria.

Stersky et al. (67) used a bipolar-oriented electrical field to reduce the level of viable aerosolized microorganisms. They found that a field of 14K to 20K volts reduced the mean airborne population by 31 to 59% during regular forced ventilation, depending upon the microbial species.

Vickers (73) concluded that the adoption of design conditions used in 'clean air' rooms and environmentally controlled work stations for dairy packaging and other critical hygiene areas is required to ensure production of safe dairy products with long shelf-life. These design conditions include physical separation of critical and non-critical hygiene areas, the adoption of airlocks, pressurization of critical hygiene areas, and use of HEPA filters. Also, heating, ventilating and air conditioning (HVAC) systems should be designed for easy cleaning and must be adequately maintained (28). The engineering aspect of air flow control within a dairy processing plant are discussed by Heldman and Hedrick (35). New dairy processing facilities are being designed and operated using these clean room concepts (42).

Considering the potential importance of the problem, there is little published research on air quality in modern food processing plants. Additional study is needed to propose reasonable air quality guidelines in dairy and food processing plants and to recommend appropriate monitoring methods. Closer attention to air quality at critical processing points is required to reduce the risk of product contamination, but little information is available on the extent of the current problem.

ACKNOWLEDGMENTS

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