



Review

Airborne contamination in the food industry: An update on monitoring and disinfection techniques of air

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ABSTRACT

Background: hygienic and safe production is a high priority in the food industry. During processing, food may be subjected to bio-contamination. Accordingly, preservation of overall quality by keeping a clean environment is a goal to pursue. Among microbial vectors, air is considered a contributing factor to cross-contamination.

Scope and approach: nowadays, in food plants emphasis is paid to the assessment of air bioload in view of prevention of recontamination. Normally, air entering a processing plant is chilled and filtered to remove undesired microorganisms from outside. Nevertheless, apart from clean-room environments, uncontrolled factors (processes, personnel, structures, etc.) contribute to the release of microorganisms in indoor environments, resulting in generation of bioaerosols highly variable within and among plants, and on a daily basis within the same plant.

Key findings and conclusions: this review focuses on the relevance of bioaerosol monitoring in the food industry, providing an update of air sampling techniques and methods of analysis in view to strengthen preventive hygienic actions. Disinfection procedures to minimize microbial counts in the air as additional safeguard to the standard chemical sanitation protocols are reviewed. Benefits and limitations of air treatment by chemical fogging, ozonation, uv irradiation or cold plasma are outlined. Air bioload monitoring and the implementation of subsequent air disinfection procedures are a feasible and a routinely exploitable strategy to satisfy hygienic requirements in food plants. Further research is required to face technical challenges and optimize the feasibility of some disinfection technologies for the real-world of food environments.

1. Microbial contamination of food: routes, vectors and factors limiting spreading

Food contaminants are classified as extraneous substances of either physical, chemical or biological origin. Microorganisms may be responsible for outbreaks of food-related illnesses or food spoilage. In a generic food facility, major routes of food recontamination by microorganisms are via surface contact, via personnel or via the air (Fig. 1) (den Aantrekker, Boom, Zwietering, & van Schothorst, 2003). Generally, the contribution of the first two routes is prevailing, but the importance of each means of contamination is also a function of the type of product or process. This review deals with items related to food contamination by air route. Employees can transfer microorganisms both directly (from their body to the food product) and indirectly (transferring contamination from one area/surface to another) (Aarnisalo, 2007). In this context, the Annex II of the European Regulation No 852/2004 on food hygiene (EC, 2004) takes into consideration the relevant role of employees, establishing their supervision

and instruction in food hygiene matters in relation to the work activity. Also exposure to contaminated surfaces has been identified as a major source of food contamination (Otto et al., 2011). Both food-contact (e.g., equipment, utensils, workbenches, conveyor belts) and no food-contact surfaces (e.g., drains, utility pipes, maintenance equipment, structures, and areas away from production such as hallways, entrances and welfare facilities) can collect microorganisms and other debris from employees, as well as from the air and other materials. These mutual interactions among above cited vectors can boost the microbial spread in a food facility (Fig. 1). In general, the low incidence and/or viability of pathogens in suspension in the air makes the route of air-to-food of low impact on foodborne diseases (Pérez-Rodríguez, Valero, Carrasco, García, & Zurera, 2008). Nonetheless, the recontamination by air is noteworthy for products such as beverages, refrigerated dairy and culinary products and products with very low viable counts, such as dried infant formulae (Reij, den Aantrekker, & ILSI Europe Risk Analysis in Microbiology, 2004). In high-risk areas, for instance after the last heat treatment before filling and packaging, the food product (e.g.,

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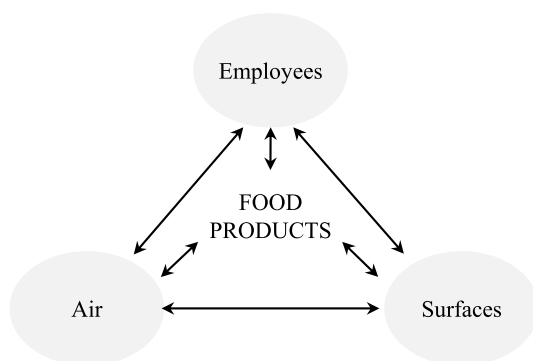


Fig. 1. Overview of major sources/vectors of microbial contamination and their interactions in the food industry.

beverages) is susceptible to recontamination. In dairy production facilities, spray drying and milling operations have been reported as a possible means of microbial transfer, making dissemination of pathogens through ventilation a probable event (Mullane, Whyte, Wall, Quinn, & Fanning, 2008). To counteract the risk of airborne biocontamination in the filling room, air filters should be changed on a regular basis, and a positive air pressure should be adopted (Lawlor, Schuman, Simpson, & Taormina, 2009). By modelling studies, den Aantrekker et al. (2003) carried out a quantitative estimation of the probability of product contamination via the air. Assuming settling velocities of microorganisms under the influence of gravity only, the authors took into consideration what-if scenarios to exemplify the determination of design criteria to control a specified contamination level. As a conclusion, both the type of product and processing conditions strongly influence the contamination level. Comprehensive approaches to model factory air movements have been described in literature and represent a contribution of research to improve the understanding and tackling of microbiological risks (Pérez-Rodríguez et al., 2008; Possas, Carrasco, García-Gimeno, & Valero, 2017).

Other factors can contribute to microbial transfer to food, namely, raw materials, ingredients, pests, water, processing conditions, packaging material, transport vehicles, plant design, poor zoning, open drains, as well as wet and dry cleaning operations by brushing, which often result in the generation of bioaerosols in the form of water droplets or dry dust (Ehaval, 2007; Marriott & Gravani, 2006). If cleaning and disinfection procedures are not performed in the correct manner, residues of organic and inorganic soils could remain, and subsequently food spoilage and pathogenic bacteria could create a suitable environment for biofilm development. In a wide range of food industries, biofilms have become challenging (Marino, Maifreni, Baggio, & Innocente, 2018). In the topmost layers of the biofilm, chunks of the extracellular polymeric substances, with the accompanying microbial population, can cross-contaminate other products, by the action of food or liquid passing over the surface (Marriott & Gravani, 2006). To the best of our knowledge, to date, detaching and air diffusion of above-mentioned substances have not been reported.

Generally, epidemiological data on common contamination routes and sources are scarcely described in the literature (Reij, den Aantrekker, & ILSI Europe Risk Analysis in Microbiology Task Force, 2004). Recent research in this area is focused to achieve greater insight into the mechanisms of microbial transfer and cross-contamination dynamics during food processing (Possas et al., 2017). Considering the complexity of parameters involved in microbial transfer, it is apparent that only an integrated approach may be effective to prevent or minimize food contamination. Hygienic design of equipment/structures and proper sanitation are factors limiting the microbial contamination in full compliance with legislation (EC, 2004; EN 1672-2, 1997). Good hygiene practices include also personal hygiene, zone separation, prevention of cross-contamination, use of purified water (Gurnari, 2015).

Additional actions in the management of food processing such as proper selection of ingredients, food storage conditions, plant maintenance and air filtration are efficient tools in view of keeping or improving food safety. The relative contribution of these factors is variable as a function of the food sector.

Food hygiene is currently defined as measures and conditions necessary to control hazards and ensure the safety of food at all stages of the chain (Codex Alimentarius, 2003; EC, 2004). It is realized through established prerequisite programs, including good manufacturing practices (GMP), good hygiene practices (GHP) and standard operating procedures (SOP), which contribute to make hazard analysis critical control point (HACCP) an effective system to control food safety (Byrne, Lyng, Dunne, & Bolton, 2008; Varzakas, 2016). Even with the best control measures in place, a food product may still pose a risk to the consumer (den Aantrekker et al., 2003). Thus, all means to reduce or prevent contamination and to improve the suitability for consumption are considered part of the hygiene concept. A proper management of air quality can mitigate the introduction of microorganisms throughout the production stream of a food product. Each food production facility should evaluate the presence of microorganisms in the site, sampling both surfaces and the air, through the implementation of an environmental monitoring program (EMP) necessary for the subsequent development of a food safety plan (FPS) (Pleitner, 2018). The developed EMP allows to evaluate the effectiveness of the microbial controls in place. Such activity is pivotal in a well-run company.

This review aims at highlighting the role of the airborne route in the microbial spreading in the food industry. The scope is to provide an overview on both bioaerosol monitoring, including air sampling techniques and methods of analysis, and on subsequent air disinfection procedures as a proactive strategy in addition to routine sanitation practices. The items covered in this review are addressed to food safety aspects. Studies related to the field of occupational health are outside of the scope. The major target readers are food business operators who can perceive the potential advantages in terms of food safety arising from the implementation of environmental control protocols.

2. What is a bioaerosol and why air monitoring is important?

The suspensions of microscopic solid or liquid particles in the air are defined as aerosols (Ferguson, Cumbrell, & Whitby, 2019). Those of major impact in the food sector are known as bioaerosols and consist of living substances with diameters up to 50 µm (Burfoot, 2016). These may include bacteria, mold spores and yeasts (Lee, 2011). Indeed, although rarely documented, phage contamination can also occur through aerosolization (Verreault et al., 2011). Viruses can be found on aerosol particles of various sizes, from the submicrometer range to tens of micrometers in aerodynamic diameter. Virtually all microorganisms present in bioaerosols are easily translocated by air currents, but their reproduction is uncommon in the air due to the lack of moisture and nutrients. Despite the sensitiveness to environmental conditions, also food pathogens can survive in the air, for instance in association with dust particles (Mullane, Whyte, Wall, Quinn, & Fanning, 2007). Additionally, contamination from airborne yeasts and molds can affect the quality and shelf life of a food product (Ehaval, 2007). The bioaerosol of the food industry is a mixture of many species of microorganisms including bacteria endospores and exospores (e.g., *Bacillus*, *Clostridium*), vegetative cells mainly of Gram positive bacteria (e.g., *Micrococcus*, *Staphylococcus*), molds (e.g., *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium*) as well as yeasts (e.g., *Saccharomyces*, *Torulaspora*, *Hanseniaspora*, *Pichia*) (Pérez-Martín, Seseña, Fernández-González, Arévalo, & Llanos Palop, 2014).

Aerosolized microorganisms may persist within droplets derived from the aerosolization of water spraying/splashing during food processing or the sanitation process. In these cases, microorganisms grow in a liquid medium, such as spilled product, rinse water or wastewater, which subsequently becomes aerosolized. Microorganisms may also be

suspended as such in the air after dissipation or evaporation or as “passengers” on solid dust particles (e.g., hair, clothing fiber, skin), which are dispersed in a food processing unit (Chang, Ting, & Horng, 2019; Heo, Lim, Kee, & Lee, 2017). Microorganisms in the air may settle on food products, equipment, containers and other food contact surfaces during handling (Brandl et al., 2014). Any point at which the food product is exposed to air is a possible route for airborne contamination. Combining the knowledge acquired under real situations in food factories and the use of computer models, Burfoot (2016) reported that the smaller the particle suspended in the air the greater the flight time and the distance it may travel. Indeed, the fate of airborne particles is quite complex and ruled by several mechanisms including: gravitational settling, Brownian diffusion, inertial impaction, direct interception (by, for example, van der Waal's forces) and electrostatic attraction (Da, Géhin, Havet, Ben Othmane, & Sollicec, 2015). The combination of above-mentioned parameters influences the aerodynamic behavior of particles affecting the success of the air sampling. Generally, the airborne particles most of interest in food environments are those containing bacteria with low-medium size (above 1 µm and below 20 µm) which can disperse easily around the generation area. By the way, aerosols in food plants have not been studied sufficiently to accurately generalize particle-size distribution. Generally, in high-care areas less than 1% of particles in the air will settle, and most of them will be removed by the filtration system. The contribution of airborne microorganisms to food contamination has been addressed (Chang et al., 2019; Chen et al., 2019; Shale & Lues, 2007). Burfoot and Brown (2004) reported that the ratio of microorganisms to total particles may range up to more than two orders of magnitude. For instance, these authors observed in different food factory environments that above-mentioned ratio was low (1–30,000) in periods of inactivity in a well-designed production area, whereas it reached high levels (about 1–200) near to employees during hand-washing and next to cleaning operations. To date, the awareness of the industry about the importance of the hygienic design, remarkably for the air handling system, is still low (Da et al., 2015). Nonetheless, overemphasis on the role of air as a source of food contamination should be avoided. Burfoot, Whyte, Tinker, Hall, and Allen (2007) quantified the contribution of airborne microorganisms to contamination of poultry carcasses undergoing processing in an evisceration room. The use of ultra-clean air provided by a high-efficiency particulate air (HEPA) unit reduced total aerobic counts on horizontal settle plates by 68-fold. Differently, after measurement by sponging, the use of ultra-clean air had no effect on the counts on carcasses. The latter resulted so heavily contaminated that the airborne bacteria in the evisceration room represented less than 1% of total number of bacteria on carcasses.

The food industry is aware that monitoring aerosols is becoming a must in standard quality-control practices. Generally, the primary focus is addressed to total viable microorganisms rather than total particle counts. Air monitoring can be included as a part of an HACCP system in the food industry (Beletsiotis, Ghikas, & Kalantzi, 2011). The role of bioaerosol monitoring consists in:

- being the basic step for prevention;
- implementing a pro-active action to minimize cross-contamination phenomena, which are major contributors in food-borne outbreaks;
- complying with legal requirements or guidelines stating that the air in food sector has to be controlled without specifying the methodology or minimum acceptable standards (Wray, 2011);
- finding the potential source of new contamination whenever any structural implementation has been introduced, and subsequently undertaking appropriate corrective measures;
- collecting epidemiological data, possibly with a view to set occupational exposure limits (Wirtanen, Miettinen, Pahkala, Enbom, & Vanne, 2002).

Information sources provided by the food legislator are quite

generic. The European regulation states the need to minimize airborne contamination and to avoid mechanical airflow from a contaminated area to a clean area (EC, 2004). Guidances, intended to assist food producers to meet the air quality and hygienic requirements of the food manufacturing process, are available. The European Hygienic Engineering and Design Group (EHEDG) supported the European legislation producing a guideline focusing on air handling systems installed in the food industry for air quality control (EHEDG, 2016).

3. Bioaerosol monitoring: air sampling techniques and methods of analysis

The assessment of air microbial load in the food industry is performed through the sampling of a representative amount of air and its subsequent analysis. Quantification and identification of bioaerosols is affected by several factors, such as the rate with which the result is required, the efficiency of sampling equipment, the ratio of total cell counts versus viability of cells in the sample, the particle size range selected as well as the analysis methods (Dybwad, Skogan, & Blatny, 2014). Once the reasons for carrying out sampling have been defined, the rate of relevance of above-mentioned parameters can be established. The samplers should apply minimum stress during air collection to reduce the impairment of the biological activity of the aerosol. In addition, during air sampling, different environmental parameters can cumulatively stress microorganisms affecting (through desiccation) their viability. In long-term (> 30 min) sampling of bioaerosols, especially for vegetative bacteria, the combination of controlled humidity and refrigerated temperature of air sampler should provide viability maintenance (Walls et al., 2017). The literature provides little information on the causative variables that lead to differing colony recoveries (Wirtanen et al., 2002). Through years, to monitor air in a consistent way, performance measurements for air samplers have been reported using several efficiency terms, including aspiration-, sampling-, recovery- and overall-efficiency (Dybwad et al., 2014). The sampler efficiency is described also by factors such as the design of the inlet, collection stage and choice of collection medium, which affect the viability of microorganisms. Generally, the collection efficiency is expressed as the 50% aerodynamic cut-off diameter, D_{ae50} (µm), i.e. the particle size collected to 50% diameter. The proper choice of a sampler with a D_{ae50} below the mean size of the particles being sampled is crucial for efficient collection. The performance information supplied with commercially available samplers is often limited to collection efficiencies, but data on sampling stress are not always provided. Summing up, the evaluation of air microbial load is not a trivial task. It can be performed through several sampling methods each with pros and cons (Table 1). Recently, Reponen (2017) reviewed the techniques of air sampling of microorganisms in generic environments providing a list of commercially available bioaerosol samplers. Both passive (settle plates) and active (using a sampling device) air sampling techniques can be adopted (Haig, Mackay, Walker, & Williams, 2016; Reponen, 2017). The former approach consisting in the exposure of agar plates to air for a certain period of time has been traditionally used. In this case, the collection is governed by gravitational force, which is related to the particle mass. Settle plates technique is not quantitative, and in high aerosol concentrations the uncountable numbers of colonies may represent a problem. Active bioaerosol sampling exploits different collection principles, such as impaction, impingement, cyclonic separation, filtration, thermal or electrostatic precipitation. A large number of commercial samplers is available on the market. Nevertheless, different results are obtained from different equipment in the same place, at the same time (Verreault, Moineau, & Duchaine, 2011). Properties and critical factors affecting the use of air samplers have been recently reviewed by Brown and Wray (2014). Data comparison is difficult because the type of the device is reflected in the biodiversity of the bioaerosol (Mbareche, Veillette, Bilodeau, & Duchaine, 2018). Dybwad et al. (2014) through a comparative evaluation of 9 different samplers

Table 1
 Pros and cons of air sampling techniques available for the food industry.
 Sources: H. M. L. Lelieveld, M. A. Mostert, & J. Holah, 2005. *Handbook of hygiene control in the food industry*. Oxford, UK: Woodhead Publishing Limited; Ljungqvist & Reinmüller, 2007; Verreault et al., 2011; Reponen, 2017.

Sampler	Air sampling	Pros	Cons	Use in real food industry
Settle plate	Passive	Easy and cheap device to monitor generic air bioload. No cell stress by reduced viability.	Qualitative method, based on collection by “fall out”. Biased to larger particles. Sensitive to air movements.	++
Impactor	Active	Multiple choice of devices (slit and sieves). Practical in industrial use. Information on size distribution. Used to recover viruses.	Cost of device.	++
Cyclone separator	Active	Available as portable hand-held instrument. Practical in industrial use. Less cell stressing than impaction methods.	Selective for large air particles. Tendency to higher counts than other air samplers.	++
Filter	Active	Not expensive. Simple to operate. Suitable for enumeration of molds and bacterial spores.	Possible stress by cell desiccation.	+
Impinger	Active	Used also to recover viruses. Useful for heavily contaminated air environments.	Impractical in industrial use. Sterilization of the device after each use.	+
Electrostatic precipitator	Active	Useful for collection of viruses or sensitive microbial strains. Compatible with analysis by polymerase chain reaction.	Possible loss of survivability. No literature in food sector.	-

+, frequent use; +, occasional use; -, not used.

(impactors, impingers, cyclones, electrostatic precipitators and filtration samplers) revealed significant differences in terms of cultivation-based biological sampling efficiencies and PCR-/microscopy-based physical sampling efficiencies as a function of the bioaerosol's stress-sensitivity and particle size. Typically, impaction is a common technique for the collection of airborne viable particles (Miettinen, 2016). In particular, there are two types of solid-surface impactors: slit samplers and sieve samplers, the latter being preferred. In a sieve sampler the air is drawn through a large number of small, evenly spaced holes drilled in a metal plate. Air particles impact on an agar surface located below the perforated plate. The Andersen sampler, a cascade-sieve impactor is likely the most-known device giving information on the size distribution of the microbiological aerosol. Liquid-using impactors, called impingers, are useful for sampling heavily contaminated air thanks to the dilution of the liquid sample for the subsequent culture growth analysis. Other instruments adopted in the food industry include centrifugal samplers based on cyclonic separation. In this case, air is pulled into the sampling unit and pushed outside thus impacting on a strip of nutrient agar. Such device is characterized by selectivity for large particles, which are likely to include viable particles. For this reasons the tendency is to exhibit higher counts than with other devices. A further type of active sampler, relying on filtration as a collecting mechanism, is the filter system, which is recognized to be suitable for the subsequent enumeration of mold or bacterial spores. Airborne microorganisms can be collected also through electrostatic precipitators following ionization and subsequent deposition in an electric field on a growth medium. The adoption of this technique resulted more efficient than other methods (such as impingers) for sensitive microbial strains e.g., *Pseudomonas fluorescens* (Miettinen, 2016). Each of the above-mentioned devices has limitations that the user should be aware of. To date, in the food industry settle plates and impactors, being simple and practical, are the most used devices for routine microbial air monitoring.

After collection, the air sample is analyzed through culture, microscopic, biochemical, immunological or molecular assays (Mbareche, Brisebois, Veillette, & Duchaine, 2017; Reponen, Willeke, Grinshpun, & Nevalainen, 2011). The choice of the analytical method relies on factors including cost, time required, sensitivity, specificity and the sampling method used. The selection is defined before air sampling is carried out. Traditionally, in the food industry culture-based methods prevail for enumerating the airborne microbial counts (Oppliger, 2014). Microorganisms collected by impaction are cultured directly, whereas following the use of filter systems the transfer to a culture medium is required. Usually, for surveys on the characterization of the airborne microbiota the selection of general media is preferred, because it favors the growth of a large diversity of species. The simultaneous isolation of both bacteria and fungi is not satisfactory using only one culture medium. In case of volumetric samplings, the concentration of cultivable airborne microorganisms is obtained by referring the colony forming units (CFU) to the volume of air sampled. The limitation of plate count method is that it reveals only a part of the microbial population. Some bacteria may be in an eclipsed state defined as viable but not cultivable (VBNC) as a response to stress conditions (Maukonen, 2007). Despite this disadvantage, plate count method is by far the gold standard in food microbiology. In addition to culture technique, also microscopic analysis is used to estimate the total number of microorganisms in an air sample, allowing enumeration of both cultivable and non-cultivable microorganisms. Direct microscopy is generally employed to identify fungi, exploiting the morphological characteristics of spores. Phase-contrast microscopy allows to count bacterial endospores due to their phase-bright appearance in contrast to darker vegetative cells. Recently, investigations focused on health effects following exposure to harmful bioaerosols, led to a demand for accurate and reliable monitoring systems (Choi, Kang, & Jung, 2015). Molecular techniques such as polymerase chain reaction (PCR) amplification of 16S rDNA, followed by its sequencing and DNA-DNA hybridization

allow to increase sensitivity and specificity, while decreasing the time required for analysis (Stetzenbac, Buttner, & Cruz, 2004). Indeed, in the food industry, the development of real-time continuous monitoring of microorganisms in the air would be important to verify the occurrence of undesired trends that are not always revealed with periodic samplings. Through years, the quantitative PCR ((q)PCR) developed in the medical research area for assessing total or species-specific airborne bacterial load. Besides, the use of (q)PCR is more suitable than other techniques for the analysis of air samples in the detection of phage genome (Verreault et al., 2011). In this case, various sampling devices can be used to recover airborne viruses. Nevertheless, it is still challenging to study viral aerosols using metagenomics mainly due to limited quantity of viruses in the air samples and due to the limited viral databases for viral metagenome library analysis (Behzad, Gojobori, & Mineta, 2015; Prussin, Marr, & Bibby, 2014). To date, the most common techniques to recover viruses are liquid and solid impactors as well as filters. An extensive compilation of studies (mostly experimental in controlled chambers) on the recovery of viral particles was carried out by Verreault et al. (2011). The (q)PCR technique is advantaged by the coupling to other molecular methods (like sequencing and DNA-DNA hybridization) to obtain information about the species diversity (Oppliger, Charrière, Droz, & Rinsoz, 2008). The sensitivity of (q)PCR is of different orders of magnitude higher than that of culture techniques. Moreover, it is able to amplify the DNA of VBNC cells. Nonetheless, given current available technologies, it is impossible to real-time monitor all the airborne biological agents and classify them to the species level (Yao, 2018). To date, in the food industry, despite the above discussed advantages, biochemical and molecular methods are not applied as routine techniques to monitor indoor microbial air quality.

4. Levels of air contamination in commercial food processing plants

The presence of microorganisms in the air of food facilities is predominantly accidental and is highly variable or transient, generally ranging from 10 to 10,000 CFU/m³ (Ehaval, 2007). Based on the assumption that it is impossible to keep microbial counts at zero level, information on bioaerosol is important to evaluate the risk on both product quality and/or shelf life and public health.

In processing plants producing pork, poultry, beef and dairy products, air has been recognized as a contributor to food contamination. In particular, environments such as slaughterhouses are potentially critical, because animals are a microbial source of contamination. Prendergast, Daly, Sheridan, McDowell, and Blair (2004) investigated the aerobiology of slaughter operations in two commercial beef abattoirs. Although quantitatively different, both of them showed a similar trend in counts within intraday processing, with lower levels before slaughtering (about 1 log₁₀ CFU/m³ of air). The authors observed differences in the aerial contamination among different sites in one abattoir. In this case, total viable counts differed significantly ($P < 0.001$) ranging from 1.79 up to 3.47 log₁₀ CFU/m³ of air in the zone collecting washed carcass (“clean area”) and in the exsanguination site (“dirty area”), respectively. This pattern was not observed in the other abattoir due to the different building design, which allowed to effectively reducing the penetration of airborne contamination from “dirty” to “clean” areas. In addition to what has been already mentioned, Pearce, Sheridan, and Bolton (2006) in a pork slaughtering plant enumerated about 1 log₁₀ cycle decrease of aerobic mesophilic bacteria from the “wet” room (bleeding site) to the “clean” room (chilling site). The authors pointed out the role of animals as a source of air contamination.

In a dairy plant, Beletsiotis et al. (2011) recovered as dominant fungal genera *Cladosporium* spp., *Penicillium* spp. And yeasts. Due to the absence of an air filtration unit and the overlapping in the relative microbial air contamination, the authors ascribed the indoor presence

of fungal contamination deriving from the outdoor environment. The aerobiology of commercial dairy environments was investigated also by Soldatou, Psoni, Tzanetakis, and Litopoulou-Tzanetaki (2006) through sedimentation technique and subsequent incubation. The authors isolated mainly micrococci and bacilli in two cheese factories making Feta cheese. Physiological and biochemical activities of abovementioned microflora were investigated too. The air contaminants exhibited acidifying and proteolytic activities potentially contributing to cheese ripening and flavor. More recently, Brandl et al. (2014) studied the bioaerosol in different sites of milk powder and powdered infant formula processing units of a dairy plant. As expected, due to the strict hygienic requirements of these environments, numbers of cultivable microorganisms were very low (< 100 CFU/m³ of air) during production in filling, bagging and final packaging zones in comparison with other industrial locations. Additionally, following measurements on particle sizes of air, through handheld laser particle counters, the authors found a high correlation between total airborne particles in the size range 1–5 μm and numbers of CFU. The authors concluded on the practical usefulness of a simple surveillance system based upon laser-mediated counting of airborne particles occurring in a specified size range. Simon and Duquenne (2014) referred on the airborne bioload, measured by an impactor sampler, in cheese-maturing cellars. Concentrations from 10³ to 10⁶ CFU/m³ and from 10⁴ to 2 × 10⁸ CFU/m³ were recorded for bacteria and fungi, respectively. Such levels resulted from 1 up to 5 log₁₀ cycles (brushing area) higher than those revealed in points of the plant considered uncontaminated. The authors concluded that throughout the process certain employees are exposed to high concentrations of airborne cultivable fungi.

Few studies focused on the composition of the microbiota present in the air of wineries, in particular on yeasts, both beneficial and spoilage ones (Ocón et al., 2013), and molds (Ocón et al., 2011). An in-depth study on the microbial ecology in the air of a winery was recently reviewed by Pérez-Martín et al. (2014).

Overall, above discussed investigations remark the large variability of microbial air counts in food commercial plants as a function of a range of factors, including the sector, the hygienic requirements of each zone of the plant, the design, as well as processing conditions. To date, the legislator does not impose any restriction on the number of airborne microorganisms being aware of the complexity of an ecosystem such as the air in the food industry. Nonetheless, the European Community Board (European Collaborative Action, 1996), in the context of the provision of healthy and environmentally sustainable buildings laid down a report on indoor air quality and its impact on man. In this document, the air of generic indoor environments (private houses, non-industrial workplaces and public buildings) was categorized in “very low” (< 50 CFU/m³), “low” (50–100 CFU/m³), “medium” (100–500 CFU/m³) and “high” (> 500 CFU/m³).

5. Air handling

The food environment is often wet and includes many sources of aerosols contributing to microbial contamination, especially in critical areas where the products are exposed to air for long periods. Different physical mechanisms affect the movements of airborne particles resulting in a greater difficulty to control their movements. Generally, proper implementation of air-handling equipment can ensure that a large part of the airborne particles does not come into contact with exposed foods. An approach to reduce air microbial load consists in the filtration of air entering a specific area. Besides filtration, also a heating, ventilation and air conditioning (HVAC) system is widely used. This equipment allows the desired management of temperature and humidity of air as well as the flow direction and the pressurization within a specific area allowing to control airborne microorganisms. The latter are not inactivated, but possibly accumulated on the filter surface and can proliferate in case of high humidity ($> 80\%$). Generally, an air flow of 1.5 m/s or greater is required to ensure maintenance of one-way

flow. Temperatures and relative humidity (likewise atmospheric gases, light, irradiation and surrounding organic material) are environmental factors associated with survival and growth of airborne microorganisms (Ijaz, Zargar, Wright, Rubino, & Sattar, 2016). Therefore, the control of these factors is desirable. To remove the heat load imposed by the processing environment (processes and people) and to provide employees with fresh air, 5–25 air changes per hour are considered sufficient. Proper ventilation removes also moisture released during processing and prevents condensation and the subsequent mold growth on surfaces. In addition, to prevent bioaerosol contamination within HVAC systems, it is crucial to have a good understanding of the mechanisms of particle deposition and the subsequent fouling rate (Da et al., 2015). In food manufacturing facilities, the use of computational fluid dynamics programs is a useful tool for prediction of airflow movements inside specific areas. This approach supports the correct placement of air ventilation systems enhancing good sanitary of food processing environments (Skåra & Rosnes, 2016).

6. Air disinfection

In general, to inactivate environmental bioaerosols, different microbial decontamination technologies have been investigated. These include carbon nanotube filter, ion emissions, UV irradiation and electrostatic field (Liang et al., 2012). In the air of a food facility, type and amounts of microorganisms can vary widely as a function of the site and on a day-to-day basis in the same environment (Masotti et al., 2019). To strengthen preventive measures against air bioload, in view of attaining the goal of providing a safe and a high quality product to the consumer, food business operators are interested in the adoption of additional approaches other than regular sanitation procedures. In particular, chemical fogging, ozonation and UV irradiation of the air are major commercially available solutions. These techniques are currently implemented in the pharmaceutical and clinical sectors, but far from being common in food processing environments. Each of these techniques is characterized by benefits and drawbacks to be properly evaluated for effective disinfection (Table 2). In the food industry a steady growing interest is arising in these additional disinfection practices to minimize cross-contamination from the air, especially in critical areas (e.g., filling, packaging). One prerequisite for their effective implementation is the application to closed environments.

Table 2
Pros and cons of disinfection techniques available in food industry for air treatment.

Sources: Burfoot et al., 1999; Marriott & Gravani, 2006; Pascual et al., 2007; Stanga, 2010; Krishnan et al., 2012; Cutler & Zimmerman, 2011; O'Donnell et al., 2012; Christ et al., 2016; Zhou et al., 2016; Yang et al., 2018; Chen et al., 2019; Masotti et al., 2019.

Disinfection technique	Pros	Cons	Use in food industry
Air filtration and UV irradiation	Disinfection efficacy of in-duct UV-C lamps.	Energy consumption. Increase of temperature of air supply. Fungi can escape UV radiation.	+ +
Chemical aerosolization	Wide spectrum of efficacy against microorganisms. Environmental friendliness (as a function of the agent used). Dry aerosol.	Time for aerosolization and chemical action. Sealing of treated environments. Controlled room re-entry, to avoid safety issues. Equipment material compatibility.	+
Ozone gas	Excellent antimicrobial activity. Production <i>in situ</i> . Immediate action. Auto-decomposition. Lack of residues on food.	Health and safety issues in case of uncontrolled room re-entry. Need of a gaseous ozone analyzer. Absence of personnel and food. Use of sealed environments. Corrosive to several soft metals and rubber. Cost of ozone generator.	+
UV irradiation	Discrete disinfection efficacy. No use of chemicals. Synergistic effectiveness when in tandem with other technologies (e.g., photocatalysis, air filtration).	Health effects due to the production of ozone as a by-product. Delivery of sufficient UV irradiation to large volumes of air. Influence of environmental conditions.	+
Cold plasma	Disinfection efficacy in air duct flow. Static purpose-built system or mobile unit.	Health effects due to the production of ozone as a by-product. Cost of cold plasma tubes. No up-scale for commercial applications. Lack of research data on air disinfection effectiveness in food environments.	-

+ +, frequent use; +, occasional use; -, not used.

7. Air disinfection by chemical fogging

Fogging or aerosolization is the dispersion of a liquid in the form of fine mist in the air. Aerosolized disinfectants have been applied since many years for therapeutic use in the healthcare sector (Otter, Yezli, Perl, Barbut, & French, 2013). Subsequently, this technique has been implemented also in food factories for decontamination of products (fruits and vegetables) (Oh, Gray, Dougherty, & Kang, 2005) or disinfection of surfaces in packaging or storage areas, process lines, cooling chambers (Holah et al., 1995). Fogging is also used to reduce the counts of airborne viable microorganisms deriving from low-care areas, people, structures, or formed as aerosols during cleaning procedures (Burfoot, Hall, Brown, & Xu, 1999). The ultrafine droplet size of the dry fog prevents it from easily falling onto surfaces, a desirable quality for area decontaminations (Krishnan et al., 2012).

This technique has been also used quite widely by chilled food manufacturers, especially in high-care environments such as salad, sandwich, ready meal and dairy processing. Typically, the process requires at least 15–30 min for fog dispersion and proper chemical action. Subsequently, to allow settling of suspended droplets, a period of 45–60 min is necessary to reenter the treated room. Various types of delivery systems of the disinfectant solution in the air in the form of fine mist are available (Brown & Wray, 2014). Either a static purpose-built system with strategically placed nozzles or, more commonly, a mobile unit can be adopted (Holah, 2011). Over the years, fogging automatic systems developed. The engineering of devices, in particular the type of nozzle, is of primary importance for the success of the treatment. Checking nozzles for clogging and gaskets for integrity are preliminary steps to take before the disinfection treatment. Fogging is generally categorized, on the base of droplet size, into atomization (or nebulization) and aerosolization (Stanga, 2010). The former term is used when droplets have a diameter > 30 µm. These sizes result in shorter settling times, undesirable moistened surfaces and reduced disinfecting activity. Typically, with aerosolization, droplets of disinfectant are no wider than 5 µm. Small sizes (within the range 0.5–5 µm) characterize droplets with non-wetting surface, longer suspension times and an electric charge as a consequence of friction during the aerosolization.

Fogging for air disinfection of food environments is a scarcely studied research topic (Bore & Langsrud, 2005). Burfoot et al. (1999) reported that in the chilled food industry, fogs were most effective when the diameter of droplets lied between 10 and 20 µm giving a uniform coverage and a reasonable settling time (45 min). Up to 3 log₁₀ cycle

reduction was measured in air microbial counts as well as on upward-facing surfaces, by using an active concentration of 2 mg/mL of a quaternary ammonium formulation. Smaller droplets allowed a good distribution, but the fog remained airborne for several hours, thus not allowing the entering of personnel in the working area. Bagge-Ravn, Gardshodn, Gram, and Fønnesbech Vogel (2003) in the slicing area of a salmon smokehouse evaluated the efficacy of peracetic acid-based fogging. After spread of a dense fog (mean droplet sizes of 15 µm) by a mobile unit, air was monitored by passive air sampling through settle plates exposed for 2 h in different spots of the room. The authors obtained a significant improvement of air hygiene level (expressed in terms of reduction of total aerobic counts). More recently, by test trials in dairy environments, Masotti et al. (2019) reported the effectiveness of hydrogen peroxide aerosolization in the inactivation of airborne microorganisms. The mist dispenser produced particles with diameters of 5–15 µm of aerosolized hydrogen peroxide. Weekly-based air treatments in cheese making and packaging rooms lasted 16 and 20 min, respectively, and were followed by 20 min of settling time to allow the aerosol decomposition. Following the post-treatment air sampling, microorganisms were almost absent during 5 weeks of investigation in the packaging room (< 10 CFU/m³), whereas in the cheese making area only a slight number of bacteria (63 CFU/m³) and molds (39 CFU/m³) were enumerated. The occurrence of these residual molds (mainly represented by *Cladosporium herbarum*, *Penicillium* spp. And *Alternaria alternata*) was ascribed to recontamination from outdoor air and failures in the facility design.

Overall, major output from the literature on fogging disinfection outlined the facts that *i*) this technique should not be considered as a substitute of the regular cleaning and disinfection procedures; *ii*) further research is required to comprehensively evaluate the impact of parameters such as type of chemical, relative humidity and temperature; *iii*) the success of the aerosolization is related to the design of the treated area.

8. Air disinfection by ozone

Ozone (O₃) is a gas acting as a strong oxidizing agent and biocide (Marriott & Gravani, 2006). It has a broad-spectrum antimicrobial power, being active against bacteria, fungi, viruses, protozoa and bacterial and fungal spores (Pascual, Llorca, & Canut, 2007). For this reason, ozone has been used for decades for water treatment. An extensive review on the principles of ozone treatment, the mechanism of action and applications in the food industry has been recently published (Brodowska, Nowak, & Śmigielski, 2017). In food processing environments the most advanced germicidal applications include food surface hygiene, sanitation of food plant equipment, treatment of food plant waste and reuse of waste water (Guzel-Seydim, Greene, & Seydim, 2004). Ozonation is performed after the cleaning step, because the germicidal activity is lost following its contact with residual organic material such as food debris. Several organizations and countries approved the use of ozone as antimicrobial agent for direct contact with drinking water and for food decontamination, including vegetables, fish, meat, poultry and dairy products (Brodowska et al., 2017; Christ, Savi, & Scussel, 2016; Tiwari & Rice, 2012). In recent years, ozonation has become more and more widely accepted as an eco-friendly “green” technology (O'Donnell, Tiwari, Cullen, & Rice, 2012). An increasing interest for ozone application resulted in the opinion of the Italian Ministry of Health (2010) endorsing the use of gaseous ozone for disinfecting empty cheese ripening and storage facilities. Portable ozone generators are now available. They have discharge units and fans to create the ozone at variable concentrations and catalytic converters to decompose ozone to oxygen after the treatment. Benefits related to the use of ozone consist in the easy access to hidden sites, being in the gaseous state. It has also the advantage of the absence of by-products, as it breaks down quickly into oxygen without leaving undesirable residues on either food or food contact surfaces. This technique allows

both to save water in comparison to the use of other biocides and to improve the quality of wastewaters, for instance by avoiding the presence of harmful chlorine compounds. Furthermore, ozone is generated *in situ* on demand without the need to store it. On the other hand, some disadvantages consist in the high capital cost (i.e., the corona discharge generator). Despite this, ozone treatment remains more cost-effective than alternative treatment techniques.

Most studies focused on the effectiveness of ozone in the aqueous phase (ozonated water) against foodborne microorganisms attached to food contact surfaces or for food decontamination (Baumann, Martin, & Feng, 2009; Brodowska et al., 2017; Cullen & Norton, 2012). Only few published reports are available on the use of gaseous ozone. In this case, the disinfection treatment is carried out in confined spaces, for long times (1–4 h vs 1–10 min of ozonated water; Pascual et al., 2007) generally overnight and in the absence of personnel. Ozone in the gaseous phase presents safety issues to humans, being a powerful irritant to the respiratory tract and a cellular poison that interferes with the ability of lungs to fight infectious agents (Marriott & Gravani, 2006). In the United States, the Occupational Safety and Health Administration (OSHA) recommends that ozone exposure must not be higher than 0.1 ppm by volume (the equivalent of 0.2 mg/m³ of air) under normal working conditions for 8 h daily, or 40 h a week without adverse effects. Exposure to ozone at 0.1–1.0 ppm causes irritation to eyes, throat and nose as well as headaches. High levels (from 1.0 ppm up to 100 ppm) result in asthma-like symptoms (Pascual et al., 2007). Therefore, efficient systems for the detection and destruction of residual ozone after the air disinfection treatment speed up its decomposition and are reasonably required for the safety of employees. Foreseeing the potential risks, a continuous ozone analyzer, triggering a general alarm as soon as the concentration of ozone exceeds 0.1 ppm in the atmosphere of the ozonation room, should be installed. The above-mentioned term “safety” also refers to the equipment and instrumentation. Ozone may interact with the equipment and all surfaces. Therefore, it is essential to take into consideration only ozone-compatible materials.

In the dairy field, in particular in cheese ripening rooms, ozone gas proved to be effective in reducing the viable numbers of mold spores in the air. Serra, Abrunhosa, Kozakiewicz, Venâncio, and Lima (2003) tested gaseous ozone treatments (overnight, during non-work time) for 20 weeks in a closed ripening room of unspecified cheese types. Ozone generated at a rate of 8 g/h for 12 h/d allowed obtaining a 10-fold reduction in the viable airborne mold loads to mean levels < 50 MPN/m³ of air. Differently, the treatment did not affect the number of mold spores and hyphae on food contact surfaces, due to the short half-life of ozone. On this basis, according to the authors, gaseous ozone is useful to reduce the sedimentation of airborne molds on cheese surface during ripening. Pinto, Schmidt, Raimundo, and Raihmer (2007), in the ripening room of extra-hard cheeses, carried out an environmental disinfection program consisting in the discontinuous generation of 0.48 mg of gaseous ozone per m³ of air. Following a 40-day trial, the authors observed 1.5 log₁₀ reduction of fungal viable counts in the air, meanwhile a lower but significant reduction was measured on cheeses surface (0.7 log₁₀ cycles). More recently, Masotti et al. (2019), investigated the effectiveness of air ozonation in the packaging room of a dairy factory over a 5-week period to reduce air contamination. The treatment realized overnight 3 h/d and for 3 d per week mainly resulted in the absence of microbial growth in 92% of air samplings, whereas the remaining ones were characterized by bioload levels < 20 MPN/m³. The authors underlined the usefulness of a periodic air ozonation as a practical solution to counteract unexpected spike levels of bioaerosol due to uncontrolled factors.

In general, before installing an ozone generator, an *ad-hoc* tailored study is recommended to take into consideration factors specific to any processing environment. This approach can allow designing a safe and efficient program of air disinfection contributing to the implementation of food safety management.

9. Air disinfection by UV radiation

Ultraviolet light in the frequency range 100–280 nm, categorized as UV-C, is an established means of disinfection. Radiation at short wavelengths (approximately 254 nm) allows inactivating microorganisms such as bacteria, viruses, protozoa, molds, yeasts and algae. This environmentally friendly technology is established to reduce microbial contamination in the public health field (hospitals, health care facilities, public shelters) and the pharmaceutical industry (Lee, 2011). In the food industry, UV-C irradiation is exploited to disinfect air, surfaces of plant, packaging materials, water as well as fruit and vegetables during post-harvest storage (Begum, Hocking, & Miskelly, 2009). The germicidal action mechanism consists in damaging deoxyribonucleic acid (DNA), thus rendering the microbes incapable of replicating (Kowalski, 2009). Microorganisms in the air are inactivated as a function of both the distance from the source of radiation and reflection. Lamps installed together with suitable coating materials (e.g., stainless steel and anodized aluminum) allow to reflect as much as 80% of the emitted radiation (Stanga, 2010). Currently, UV-C lamps used in air disinfection applications are low-pressure mercury vapor lamps. Innovation challenges consist in: *i*) lamp technology to develop more versatile and efficient lamps, *ii*) the use of nontoxic materials, in drivers and controls to adapt performance as a function of the need (e.g. occupied/unoccupied room) and *iii*) systems to warn in case of malfunction (Miller, Linnes, & Luongo, 2013). UV lamps prove to be very useful when coupled with high efficient air filters in air ducts and store rooms for seasoning, chilling and drying when foods cannot be removed (e.g., cheese, salami, Parma ham) (Stanga, 2010). UV energy is mainly applied after air passage through the HVAC air-handling ductwork (also called “in-duct” system) allowing an effective air microbial inactivation. Bacteria, viruses and molds that either grow or pass through the air handling system are reduced. In the real world of food environments, the irradiation at high intensities remains not accessible to personnel in the room. Lamp locations and air movement patterns within a room need to be considered for optimal disinfection. The inactivation of microorganisms is dependent on several parameters, including: *i*) the dose of radiation received (measured in J/m^2), which is the product of intensity (measured in W/m^2) and exposure duration (measured in s); *ii*) the wavelength of received radiation and *iii*) the microbial sensitivity to UV-C radiation (Reed, 2010). For instance, for 90% inactivation of *Aspergillus niger*, *A. flavus* and *Penicillium roqueforti* the required UV-C doses are 132, 60 and 13 J/m^2 , respectively (Begum et al., 2009). This species-dependent response is a function of the composition of conidia, which can be either thin-walled and with light pigmentation or dark-pigmented due to melanin. The latter component is photo-protective and increases the survival and longevity of fungal spores, whereas non-melanin compounds are less defensive against UV-C radiation (Kowalski, 2009). The susceptibility of airborne microorganisms is also a function of temperature and relative humidity. There is a substantial lack of information on air-based UV constants. Furthermore, environmental conditions are known to affect UV light. For instance, as relative humidity increases, UV light becomes less efficient (Cutler & Zimmerman, 2011). The delivery of the required UV dose uniformly and consistently to large volumes of air is a significant challenge given the current state of the technology. To date the UV inactivation of bioaerosols is considered an added value in comparison to the standard chemical sanitation protocol alone.

Most research studies on UV irradiation are dedicated to food decontamination and water purification (Begum et al., 2009). Investigations on air as the target medium are scarce (Miller et al., 2013). Cundith, Kerth, Jones, McCaskey, and Kuhlert (2002) reported that the use of wall-mounted germicidal air cleaning units, using a combination of UV light and electrostatically polarized low-density media filter, proved to substantially reduce the risk of microbial contamination of meat products in a small meat processing plant. Under the conditions described by the authors, after 18 h of filtration a reduction from 1 to

1.5 \log_{10} in airborne bacteria and molds was observed. In bakeries, UV lamps are used on bread slicing equipment to minimize contamination from airborne molds (Begum et al., 2009). Recently, Yang, Zhang, Nunayon, Chan, and Lai (2018) investigated the performance of UV irradiation through experiments evaluating exposure time, UV dose received and bacteria susceptibility. The authors confirmed that the ventilation duct UV germicidal irradiation system would potentially provide a supplementary solution for improving indoor air quality within mechanical ventilated/air-conditioned environments. Despite UV-C is an effective microbial inactivation means, a drawback limiting its application is the production of ozone, a molecule of concern for its healthy effects (Ryan, McCabe, Clements, Hernandez, & Miller, 2010).

10. Air disinfection by cold plasma

Air ionization is a decontamination technology primarily focused on liquids or surfaces (Arnold, Boothe, & Mitchell, 2004; Liang et al., 2012). Recently, this technique turned into the spotlight for the application in the food sector to reduce microbial contamination of food (Lacombe et al., 2015; Misra & Jo, 2017). Cold plasma has been recently investigated also for air sterilization (Liang et al., 2012; Zhou, Yang, Lai, & Huang, 2016). The principle of this technique consists in the passage of the air over an ionizing tube emitting high voltage discharge (in-duct system) resulting in positively and negatively charged ions, clusters of oxygen ions, oxygen-containing radicals, UV-C irradiation and a series of combined effects of these factors (Niemira, 2012; Zhou et al., 2016). These reactive chemical species attract naturally charged airborne micro-organisms, damaging their membranes, DNA and/or proteins. In addition, high-voltage electrical discharges result in the generation of ozone. Thus, monitoring schemes should be implemented to avoid the presence of excess ozone concentration in the treated room. Measures to remove the ozone should be evaluated if required. For the scale up to commercial treatment levels an optimization and a more complete understanding of these chemical processes is required. An additional aspect to take into account for practical considerations is the cost of cold plasma tubes and the decrease in the emission of ion species with time (Lai, Cheung, Wong, & Li, 2016).

The in-duct cold plasma system is very useful for disinfecting large quantities of air as it passes through the HVAC system before its recirculation. Obviously, this will only be useful for disinfection of contaminated air through the duct, but not at the sources, i.e. inanimate environmental surfaces (Lai et al., 2016). Despite recent appearance on market of cold plasma disinfection units for in-duct applications (Zhou et al., 2016), the limitation of this technology is the early stage of development and the variety and complexity of the necessary equipment.

11. Conclusions

In the course of time, the safety of food gained a high priority, because industry has been under pressure to deliver products minimally processed, more fresh in taste and appearance, with less preservatives and with prolonged shelf life. Thus, intervention strategies to control all vectors of food contamination should be pursued. Bioaerosols in a food facility may be potential contributors to food spoilage. Due to factory air movements, a complete environmental control is complex and almost impossible. In the design of new factories, proper planning in locating air inlets, extracts, doorways and processing equipment is of utmost importance to optimize air movements. The periodic monitoring of microbial levels in the air is useful to identify potential sources of contamination. Intervention should be taken to maintain a bioaerosol load consistent with the hygienic requirements of the food product. Through years, air disinfection techniques such as chemical aerosolization, ozonation and UV irradiation evolved providing a feasible and cost-effective solution for the decontamination of selected areas of the facility. Air decontamination can entail the benefit of reducing microbial settling on frequently touched or food contact surfaces, thus

preventing the risk of microbial spread. Furthermore, the implementation of a proactive approach based on scheduled air disinfection treatments would be an ancillary strategy, especially in case of inadequate hygiene of structures.

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