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MICROBIOLOGICAL AIR CONTROL OF FOOD INDUSTRY ENTERPRISES: RELEVANCE, REGULATORY DOCUMENTS AND RESEARCH METHODS

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Keywords: *contamination, air, food products, maximum permissible level, pathogenic bacteria*

Abstract

A review of regulatory documents on air control was carried out; approaches to air sampling at food industry enterprises and methods of air disinfection were considered. It has been established that air is one of the important aspects in ensuring the quality and safety of food products. Air is a transport medium for pathogenic and spoilage microorganisms. The concentration of microorganisms and the area of their distribution in the air of industrial premises are influenced by the technological features of the products being manufactured and the design of the enterprise. The transfer of microorganisms at food industry enterprises occurs due to bioaerosols that are formed during high-pressure washing. The use of air filters allows reducing the level of microorganisms in the indoor air. Microbial air monitoring should be carried out during the technological process at critical control points (CCP). To assess air, it is advisable to select those microorganisms that cause spoilage of manufactured products or affected their safety. Passive and active air sampling methods have certain limitations. There are various methods of air disinfection, the main ones being fogging, ozonation and UV irradiation. The choice of the method should be made based on the characteristics of a particular enterprise. In Russian regulatory documents, microbiological indicators when assessing air at food industry enterprises are limited to total microbial count, yeast and mold count, while in foreign practice, the choice of indicators is based on those microorganisms that caused spoilage of finished products released from a particular enterprise. It is necessary to develop modern integrated approaches to ensure air control at food industry enterprises and establish regulatory documents on microbiological indicators and their permissible levels.

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Introduction

Air monitoring for microbial contamination, which is included in HACCP risk analysis system, is recognized as an important aspect of product quality control at food industry enterprises [1].

Air flows, particularly in industrial premises, transfer suspended droplets of liquid, solids and microorganisms (bacteria, spores, molds, yeasts, phages), which together represent aerosols [2] or bioaerosols [3] up to 50 μm in diameter [4,5]. Bioaerosols transport almost all microorganisms in indoor air. Although their reproduction in the air is difficult; they can survive in it by settling on dust particles [6]. The air itself does not promote the growth of microorganisms and acts only as a supporting medium or carrier until they settle on the surface of objects.

The purpose of this article is to establish the relevance of microbial air monitoring at food industry enterprises. To do this, it is necessary to review the regulatory documents on air control at food industry enterprises, analyze modern approaches to air sampling in industrial premises, and also focus on modern methods of air treatment.

Materials and methods

The object of the study was the publications of domestic and foreign scientists on the microflora of air at food industry enterprises and methods of air treatment, as well as regulatory documents establishing rules, general principles or characteristics of air at food industry enterprises. The data search was carried out in the ScienceDirect, Google Scholar, eLibrary and other open-source electronic databases. Combinations of keywords were used, such as microbiological composition of air, bioaerosols, microorganisms in the air, air disinfection, maximum permissible level. Keywords were used in English and Russian. In addition, the search for related articles was carried out using citation chains. Non-peer-reviewed, uninformative and duplicate sources, as well as those not related to the topic of research, were excluded from the search results.

Air microflora at agricultural enterprises

At food industry enterprises, bioaerosols may contain various microorganisms, including spores of *Bacillus* spp. and *Clostridium* spp., gram-positive *Micrococcus* spp. and *Staphylococcus* spp., molds *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp., as well as yeasts *Saccharomyces* spp., *Torulaspora* spp., *Hanseniaspora* spp., *Pichia* spp. [7].

Microorganisms that are found in food raw materials, in moisture on equipment or in wastewater, become aerosols during food production or during washing process (spraying or splashing water) [8,9]. Then aerosols are transferred by air flows to objects located inside the enterprises (food products, raw materials, industrial environment objects) [10]. The smaller the size of an aerosol particle suspended in the air, the longer it stays in the air flow and the longer it may travel [3,11]. Brandl et al. [10] conducted a study to measure the concentration of aerosol particles, as well as the concentration of bacteria, yeast and molds in the air (at least 100 liters were sampled) of a milk powder processing plant. A correlation was established between the number of particles and the number of viable cells in the air. Bacterial counts were highly correlated with the total number of airborne particles of 1 to 5 μm, suggesting that a simple control system based on airborne particle counts could be implemented. The number of cultivated microorganisms on average was less than 100 CFU per 1 m^3 of air, but in the areas of finished product filling and packaging their number was higher. Based on the identification of the isolated bacteria, they were represented by 4 main types: *Firmicutes* (41%), *Acinobacteria* (28%), *Proteobacteria* (26%) and *Bacteroidetes* (5%). The dominant bacterial genera were *Staphylococcus* and *Bacillus*. Molds were also found, represented by *Penicllium cammberti, Penicillium glabrum* and *Scropulariopsis brevicaulis*. In conclusion, the authors indicated that the microbial composition of the bioaerosols was typical for this production.

Pearce et al. [12] assessed the concentration of *Escherichia coli* and *Salmonella* spp. in the air of a pig slaughter plant. An impactor type sampler was used to take air samples (volume of at least 100 liters). According to the data obtained, the concentration of microorganisms in the air increased during the slaughter of animals. Before the slaughter, the contamination was 1.58 to 2.49 lg CFU/m^3, and after 11 hours of slaughter, values of 2.74 to 3.61 lg CFU/m^3 were obtained. The lowest concentration of microorganisms was observed in the air of refrigeration chambers, and the highest was in the area of carcass bleeding and scalding. However, *Escherichia coli* counts in the air decreased as work progressed. At the same time, the lowest counts of *Escherichia coli* also remained in the refrigerators. *Salmonella* spp. were detected only in three air samples taken from the scalding area and the evisceration area.

Prendergast et al. [13] conducted a comparative assessment of microbial air contamination of two cattle slaughter plants with different designs. At both enterprises, the smallest number of microorganisms, i. e. about 1.0 lg CFU/m^3 of air, was observed before the start of the work process. On the slaughter line with a straight single-section structure, a decrease in air contamination was observed from the "dirty" to the "clean" zones. However, airborne contamination at the slaughter line with a winding two-section structure showed the opposite trend. For example, at the skinning site, the total microbial contamination of the air at the first enterprise was 3.49 ± 0.29 lg CFU/m^3, and at the second enterprise it was 3.03 ± 0.29 lg CFU/m^3, while the air contamination at the site, which immediately follows the carcass wet processing, decreased to 1.79 ± 0.29 lg CFU/m^3 and to 2.78 ± 0.29 lg CFU/m^3, respectively.

The results of studying the air of industrial premises show great variability in microbial contamination depending on a number of factors, such as the type of raw materials processed, design of industrial premises, manufacturing technologies and hygienic requirements. As a rule, less than 1% of aerosols settle in rooms with a high level of hygiene, because most of them are removed by the ventilation system and retained by filters [14,15]. Particles of 1 to 20 μm, which are easily dispersed directly around the aerosol generation zone, are of particular concern.

Regulatory documents on air control

In Russia and other countries, there is not enough information about the maximum permissible levels (MPL) of microorganisms in the air of food industry enterprises during the technological process.

The document developed in 1995 [16], "The procedure for sanitary and microbiological control in the production of meat and meat products" provides only permissible levels of mold content in refrigeration chambers at meat industry enterprises.

To assess air quality of industrial premises at fish and marine invertebrates processing enterprises, "Instructions for sanitary and microbiological control of food production from fish and marine invertebrates" was developed in 1991 [17]. According to this document, to assess the sanitary state of air in industrial premises, two indicators are standardized: total microbial count and mold count. To determine them, it is proposed to use two methods of air sampling: sedimentation and aspiration. The same document provides the MPL of standardized indicators for each method.

As a part of production control, in workshops for the production of pasteurized canned foods, air condition is determined based on total microbial count and mold count, as well as the presence of coliforms in 1 m^3 of air. This is reflected in the "Instructions on the procedure and frequency of monitoring the content of microbiological and chemical pollutants in meat, poultry, eggs and their processed products" developed in 2000 [18].

"Instructions for sanitary and microbiological control of carcasses, poultry meat, poultry products, eggs and egg products at poultry and poultry processing enterprises" [19] developed in 1990 also reflects the requirements for

the microbiological state of air in industrial premises during poultry processing and establishes three indicators: total microbial count (TMC), mold and yeast count.

The sanitary condition of the air in refrigeration chambers was determined by the total number of molds and the number of *Cladosporium* and *Thamnidium*, which contribute to the spoilage of meat products, especially meat. However, SP 4695–88 "Sanitary rules for refrigerators" [20] expired in 2021. Instead, in terms of sanitary and epidemiological requirements for refrigeration equipment (refrigeration chambers) in relation to product safety and requirements for the processes of its production, storage, transportation, sale, operation, application (use), the following documents have been established: SanERR2.3/2.4.3590–20 "Sanitary and epidemiological requirements for the organization of public catering" [21], SP 2.3.6.3668–20 "Sanitary and epidemiological requirements for the conditions of operation of retail facilities and markets selling food products" [22], SP 2.4.3648–20 "Sanitary and epidemiological requirements for organizations of education and training, recreation and health improvement of children and youth" [23]. However, none of these documents contain requirements for the microbiological quality of air in refrigeration chambers. Also, instead of SP 4695–88, in relation to sanitary and microbiological methods for studying refrigeration equipment (refrigeration chambers), according to the Decree of the Government of the Russian Federation N1850 dated November 16, 2020 [24], methodological recommendations MR4.2.0220–20 "Methods of sanitary and bacteriological investigation of microbial contamination of environmental objects" [25] may be applied. However, these MR only apply to the assessment of washouts. Thus, the documents introduced to replace SP 4695–88 do not contain information on assessing the air of refrigeration chambers.

In 2022, MR2.3.0279–22 "Recommendations for the implementation of production control over the compliance of manufactured products with standards, technical regulations and specifications" was developed [26]. However, this document did not reflect the control of the sanitary condition of air in industrial premises.

Among foreign sources, we can highlight the "Guidelines on air handling systems in the food industry — Air quality control for building ventilation" [27], which was developed by the European Hygienic Engineering & Design Group (EHEDG). In this guide, the authors draw attention to the importance of monitoring the state of air at food industry enterprises, which includes physical factors such as temperature, humidity, as well as biological factors, i. e. the number of microorganisms. However, there are no recommendations on methods of air sampling and the study of microorganisms, as well as on their permissible levels in indoor air. Another document that addresses the topic of assessing air quality in food industry is the environmental management guide developed by 3M [28]. The authors consider air as one of the important routes for fungal spore transfer and recommend monitoring its quality using the sedimentation method. Guided by this document, the manufacturer should know that sampling points and frequency are indicated in the production control program and they are specific for each enterprise. Taking a closer look at the issue of the pathogenic microorganism spread at the enterprise by aerosol, the authors concluded that a more appropriate strategy for solving the problem would be to identify the sources and locations of aerosol formation rather than monitoring the air for the presence of pathogens in aerosols.

Air sampling methods

The stage of air sampling for microbiological assessment is also important. Air sampling methods may be divided into two categories: passive and active ones.

Passive method

The passive or sedimentation method is based on the ability of microorganisms under the influence of gravity and under the influence of air flow (together with dust particles and aerosol droplets) to settle on the surface of the nutrient medium in open plates. The number of microorganisms present is measured in CFU/m^2/t, where t is a unit of time. It is known that small and light particles remain in the air longer than large and dense ones. In addition, if the air flow rate exceeds the rate of deposition, the particles will remain suspended for indefinite period. Even indoors, air flow is subject to slight temperature fluctuations, so the volume of air in a passively collected sample will be unknown. The combination of these and other factors has a significant impact on the representativeness of the sample obtained by the passive method [29].

Active method

In the active sampling method, air sampler physically draws a predetermined volume of air and passes it through a particle collection substrate, which may be a liquid, solid medium, or a nitrocellulose membrane. The number of microorganisms present in the sample is measured in CFU/m^3 of air.

There are several types of active samplers, with the most popular being impactors, impinger and electrostatic samplers.

Impactors

Inertia of particles is used to facilitate collection. The air sample is passed through a series of nozzles that direct the air with particles toward a plate containing a dense nutrient medium positioned perpendicular to the nozzle outlet. The plate deflects air flows by 90°, while part of the air passes by through the space between the plate and the walls of the device. Particles with sufficiently low inertia are carried away by air flow and do not settle on the nutrient medium. However, particles with higher inertia cannot follow the 90° curve of the air flow and, under the influence of centrifugal force, hit the dense nutrient medium or

membrane filter. Thus, the efficiency of particle capture by an impactor primarily depends on the diameter and density of particles, the diameter of the nozzle, and also on the air flow rate [29]. One of the main advantages of using impactors for air sampling to identify microorganisms is their ease of use. For example, after taking air samples, plates with a dense nutrient medium are transferred to a thermostat without intermediate steps. However, the collision of microorganisms with a dense nutrient medium may harm them, including loss of cultivability [30,31] and even loss of membrane integrity [32], which reduces the proportion of culturable microorganisms. However, ease of use and extensive reference information make agar-based impactors the preferred tool in many studies [33].

Impinger

Impingers direct the flow of air containing particles through nozzles that exit into a chamber containing liquid. When particles in air come out of the nozzles, they enter the collection chamber. The distance from the nozzle outlet to the liquid surface, together with the air flow rate, influences the diameter of the particles collected. The collection of airborne microorganisms into the liquid prevents the collected particles from drying out, but the shear forces in the air, combined with the turbulence caused by forcing air into the chamber, may cause them to lose viability. This bioefficiency (the ability of the sampling device to maintain the viability of the bioaerosol during and after sampling) may also be reduced by evaporation, re-aerosolization (loss of previously collected particles), and particle adhesion to internal walls of the collection chamber [34,35].

Other sampler types

A special type of bioaerosol impactors are fungal spore traps. Most of these impactors are disposable devices with a single circular nozzle or slit that directs airborne particles toward a glass slide with an adhesive surface. After sampling, such impactors are disassembled and the slide is examined under a microscope.

A less popular sampling method is electrostatic deposition. Upon entering an electrostatic sampler, bioaerosol particles are electrically charged and then pass through an electric field, where they are separated from the air flow and deposited on charged plates. Despite active research on the natural charge of bioaerosol particles, the efficiency and design of electrostatic precipitators, there is concern that the electric field affects microbial viability. Therefore, more extensive research is needed for their widespread implementation in industrial practice [36,37].

Air sampling to control pathogenic microorganisms

Pearce et al. [12] conducted a study aimed at isolating *Salmonella* from air samples taken at different stages of pig slaughter and processing. Passive and active (manual impactor) sampling methods were used. Samples were collected on non-selective agar (PCA). The procedure for enriching microorganisms was carried out by adding agar from a plate to a buffered peptone solution with further incubation at a temperature of 37 °C for 24 hours. After incubation, an aliquot of the enriched culture of 0.1 cm^3 was transferred to 10 cm^3 of Rappaport Vassiliadis (RV) medium and incubated at a temperature of 42 °C for 24 hours. After the incubation, the RV medium was swabbed onto brilliant green agar (BGA) and then incubated at 37 °C for 24 hours. The grown black colonies were determined as *Salmonella* using biochemical and serological tests. Pathogenic microorganisms (*Salmonella* spp. and *Listeria* spp.) found in the air of food industry enterprises as a part of aerosols [38] are very often subject to significant stress, leading to their damage and/or death.

In the other work, the researchers collected air samples from several cattle, pig and sheep slaughter and processing plants. As in the previous case, the impaction and sedimentation methods were used, and the target indicators were *Salmonella* and *Listeria*. To detect *Salmonella*, air sampling was carried out in parallel on two nutrient media: non-selective PCA agar and selective BGA agar [38]. PCA enrichment procedure was carried out in the same way as by Pearce et al. [12], except that the volume of the buffered peptone solution was 200 cm^3. BGA agar plates were incubated with air sample at 37 °C for 24 hours. After incubation, typical colonies were selected and cultured on BGA and XLD agars and incubated under the same conditions. In both cases, after incubation, typical colonies were selected, transferred to MacConkey agar and incubated under the same conditions, followed by colony identification using API 20E biochemical tests. To detect *Listeria*, air sampling was carried out in the same way as for detecting *Salmonella*. LSA agar with selective additive SR140 was used as a selective medium. The contents of non-selective media plates were transferred to Listeria enrichment broth (LEB) and incubated at 30 °C for 48 hours, after which they were passaged onto LSA agar. Selective medium plates were incubated under the same conditions. After incubation, typical colonies were selected and determined as *Listeria* using API Listeria biochemical test and Listeria test kit.

In a study by Dobeic et al. [39], in order to detect *Listeria* in the air of slaughterhouses, polycarbonate filters were used as a substrate for collecting cells of microorganisms of this genus, placed on the bottom of plates and soaked in 2 cm^3 of the primary enrichment Fraser broth with half the concentration of antibiotics. The moistened filter material served as a trap for dust particles, aerosol and possible bacterial cells. The samples were delivered to the laboratory within several hours. Before incubation, an additional 8 cm^3 of Fraser broth with half the concentration of antibiotics was added to each plate and the contents were gently shaken. Cultures were incubated at 30 °C for 24 hours. Subsequently, 0.1 cm^3 of inoculum was transferred into 10 cm^3 of the secondary enrichment Fraser broth with a full concentration of antibiotics. Using a loop for subculture, inoculum was also taken from the primary enrich-

ment medium onto selective media: ALOA agar or Palcam agar, followed by incubation at 37 °C for 24 to 48 hours. The same procedure was repeated with the culture obtained in secondary medium after 48 h of incubation. Up to five representative colonies of *Listeria* spp. grown on ALOA and Palcam agars were transferred to blood agar to determine hemolytic activity. Final identification was made using the Listeria API kit.

Air purification and disinfection methods

Modern ventilation systems are designed to ensure pure air both entering and leaving industrial premises. Separate air filtration systems should be used to reduce the risk of cross-contamination between different production areas. Any ventilation systems must have filters and insulated panel casing consisting of a frame and various fixed and removable access panels. Air treatment is achieved using HEPA filters. Primary air filters protect the mechanical elements of the air flow system from heavy contamination throughout many years of operation. Secondary filters are used to remove fine particles down to levels necessary to maintain process hygiene. Rigid cellular filter ensures that the selected level of air purity is maintained throughout the entire life of the filter. To ensure the overall efficiency of the system, it is necessary to use a sealed filter mounting. Tertiary filters provide the best protection in units where maximum particle control is required. These are typically HEPA filters or ultra-low penetration air (ULPA) filters [16]. The required degree of filtration largely depends on the technology of the product being manufactured. For example, the presence of HEPA filters reduces the number of molds indoors by 30 times [11]. Maintaining the purity of input-outlet equipment is mandatory for its effective functioning at food industry enterprises [17]. The required efficiency of input-outlet equipment in the ventilated area should be set in accordance with the maximum permissible level (MPL) of microorganisms. In the air of industrial premises, the species composition of microorganisms and their numbers may vary greatly [40]. To provide consumers with safe and high-quality products, the manufacturer must be interested in the effectiveness of regular cleaning and disinfection procedures. However, due to the increasing resistance of microorganisms to various disinfectants, there is an urgent need to introduce additional approaches to air disinfection, in particular, fogging, ozonation and UV irradiation.

Fogging method is based on spraying a disinfectant to form an aerosol with a given particle size. Various commercial systems are available on the market, both static, which are integrated into the premises' communications network, and the most commonly used, mobile. The effectiveness of this method of air disinfection using various disinfectants based on quaternary ammonium compounds [41], peracetic acid [42], hydrogen peroxide [41] has been confirmed by a number of scientific studies.

Ozonation is based on the use of ozone gas. Ozone is a strong oxidizing agent; it has an antimicrobial effect and is effective against bacteria, fungi, viruses and protozoa. As for the spectrum of action, each microorganism is inherently sensitive to ozone. Bacteria are more sensitive than yeasts and molds. Gram-positive bacteria are more sensitive to ozone than gram-negative microorganisms, and spores are more resistant than vegetative cells. In the US, the permissible level of ozone exposure in the workplace is 0.1 ppm, as adopted by the Occupational Safety and Health Administration (OSHA). This is the concentration at which a person may be continuously exposed to ozone under normal operating conditions for 8 hours per day or 40 hours per week without any adverse effects. The short-term exposure limit is 0.3 ppm, which means exposure lasting less than 15 minutes no more than 4 times per day with intervals of at least 1 hour between each short-term exposure. Safety aspects must always be taken into account, especially when ozone gas is used in refrigerating chamber, rooms or enclosed spaces. In such situations, it is necessary to accurately control the concentration at various critical points and establish appropriate safety intervals before opening to avoid risk to human health. Ozone is a toxic gas that must be monitored in the workplace when used to disinfect equipment and installations. A wide range of ozone sensors are available to monitor its levels. These are typically UV analyzers equipped with a cell that measures concentrations from 0.1 to 100 ppmv, which trigger an alarm as soon as the ozone concentration rises above 0.1 ppm [43]. Ozone treatment is performed after washing procedures, since its bactericidal activity decreases in the presence of residual organic compounds. Portable units are used to form ozone from atmospheric oxygen. Ozone interacts with surfaces and equipment, so before use, it is necessary to ensure that the materials used at the enterprise are resistant to ozonation. The effectiveness of ozonation is confirmed by a number of scientific studies. Thus, Serra et al. [44] revealed a 10-fold reduction in airborne viable molds in a cheese ripening chamber when ozonation was used for 20 weeks. Masotti et al. [40] assessed the effectiveness of ozonation in a dairy packaging facility over a period of 5 weeks. The authors found that there was no growth of bacteria and fungi in 92% of air samples taken after air treatment with ozone 3 days a week for 3 hours.

UV is capable of destroying molecular bonds in DNA and thereby inactivating microorganisms. Short-wave UV radiation (254 nm) has been shown to reduce microbial load both in the air and on solid surfaces free of organic residues [45]. The effectiveness of UV irradiation depends on many different parameters, such as intensity, exposure time, lamp location and air flow patterns.

The disinfecting ability of UV is well known and widely used in medical and veterinary practice, as well as in the disinfection of air, surfaces and instruments [46]. The microbial status of the air in egg incubation cabinets has also been improved using UV light installations [45]. UV light has been shown to be able to reduce airborne microbial counts by 4 log units [47].

The susceptibility of airborne microorganisms to UV radiation depends on temperature and relative humidity. For example, as relative humidity increases, UV radiation becomes less effective [48]. Uniform distribution of the required UV dose in large volumes of air is a major challenge given the current state of technology [49]. Today, UV inactivation of bioaerosols is considered an additional method to standard cleaning and disinfection procedures.

Conclusion

Based on a review of regulatory documents on air control at food industry enterprises, consideration of modern approaches to air sampling in industrial premises, as well as modern methods of air treatment, the following conclusions were made.

It has been established that air is one of the important aspects in ensuring the quality and safety of food products. Air is a transport medium for most pathogenic and spoilage microorganisms. The concentration of microorganisms and the area of their distribution in the air of industrial premises are influenced by various factors, including the technological features of the products being manufactured and the design of the enterprise. The transfer of microorganisms at food industry enterprises occurs due to bioaerosols that settle on the surface of equipment, finished products or raw materials. The formation of bioaerosols is caused by procedures involving the use of water or air under high pressure. The use of air filters allows reducing the level of microorganisms in the indoor air. Microbial air monitoring should be carried out during the technological process at critical control points (CCP). To assess air, it is advisable to select those microorganisms that cause spoilage of manufactured products or affected their safety.

Existing air sampling methods, both passive and active, allow the collection of air samples, but with certain limitations. The passive (or sedimentation) method makes it possible to capture larger particles settling under the influence of gravity. To use the active method based on forced particle settling, a special device is necessary.

There are various methods of air disinfection, the main ones being fogging, ozonation and UV irradiation. These methods have both a number of advantages and a number of disadvantages, so the choice should be made based on the characteristics of a particular enterprise.

Currently, in Russian regulatory documents, microbiological indicators when assessing air at food industry enterprises are limited to total microbial count, yeast and mold count. At the same time, in foreign practice, the choice of indicators is based on those microorganisms that caused spoilage and influenced the safety of finished products released from a particular enterprise.

Thus, we believe that it is necessary to develop modern integrated approaches to ensure air control at food industry enterprises and establish regulatory documents on microbiological indicators and their permissible levels.

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