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BACTERIAL BIOAEROSOL IN THE WAREHOUSING AREA OF FOOD INDUSTRY FACILITY

BIOAEROSOL BAKTERYJNY W STREFIE MAGAZYNOWEJ ZAKŁADU PRZEMYSŁU SPOŻYWCZEGO

Abstract: In food industry is observed increase of attention to step related with warehousing of food product and raw materials in the food processing chain. Microbial contamination of raw materials and food products due to warehouse errors could generate economical losses for the facility. The aim of study was estimation of the bacterial contamination in the bioaerosol from warehousing area of the selected food processing plant. Research materials were microorganisms isolated from the air in the food warehousing area of the selected food processing plant. Air analysis was performed in four closed spaces: warehouse of food products (MPI and MPII), technical warehouse (MT) and the warehouse of unit packages (MPJ). Analysis was carried out three times by Koch's sedimentation method. Petri dishes with PCA agar medium (BTL, Poland) were placed at a height of 1.3 m for 15 minutes. Samples were incubated at 30°C for 48 hours (PN-89/Z-04111/03). Results were given in cfu·m⁻³. Each room was analyzed three times in May, July and October. Presence of catalase-positive and catalase-negative strains was determined by biochemical tests with use of hydrogen peroxide in concentration of 3%. Gram-negative rods were divided into two groups: oxidase-positive and oxidase negative by using the reaction of tetramethyl-1,4-phenylenediamine dihydrochloride oxidation. Analysis showed that the most polluted bioaerosol was observed in first warehouse of products (MPI), where in May and October assessed number of bacteria was 1·10⁵ cfu·m⁻³. Similarly high numbers of bacteria were determined initially in a technical magazine (MT), which then has 4-fold decrease. In the second warehouse of product (MPII) and the warehouse of unit packages (MPJ) the air contamination of bacteria ranged from 3.7·10⁴ cfu·m⁻³ in MPII space to 2·10⁴ cfu·m⁻³ in MPJ space.

Keywords: food industry, bioaerosol, bacteria, storage area

Introduction

The food industry, in relation to the secondary product contamination caused by microflora associated with the production, suffers high economic losses resulting from a decrease in the quality of the final product. Both bacteria and filamentous fungi may pose a threat not only to the final product during storage but also to the health of workers [1]. The degree of exposure to the adverse impacts in a significant extent is dependent on the composition of bioaerosol in warehouses. Persistence of pathogens in food products depends on processing conditions, habitats of microorganisms and transmission of microbial cells and spores [2]. Olborska and Lewicki [3] suggest, that 70% of microbial contamination of finished product is caused by spreading of microorganisms in the air. High risk of the microbial transmission to food commodities leads to increased interest in monitoring of the food processing environment [4].

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Bioaerosol is heterogeneous mixture consisting of the biological materials including: viruses, bacteria, fragments of filamentous fungi and spores [1, 5, 6]. In addition to it, bioaerosol contain metabolic products of microorganisms such as endotoxins, enterotoxins, enzymes and mycotoxins. [6, 7]. Bioaerosol is composed of particles with diameters from 0.02 to 100 μm [6]. Individual bioaerosol fractions is characterized by various particles size. In the cause of bacteria, cells diameter range from 0.5 to 2.0 μm eg *Bacillus*, *Pseudomonas*, *Xanthomonas* and *Arthrobacter*. Size of fungal spores is related to isolated species e.g. *Aspergillus fumigatus* (3.5-5.0 μm), *Aspergillus niger* (3.0-0.5 μm), *Penicillium brevicompactum* (7-17 μm), *Cladosporium macrocarpum* (5-8 μm) *Epiccocum nigrum* (15.0-25 μm) and *Trichoderma harzianum* (2.8-3.2 μm) [8]. Small particles of diameter ranged from 0.1 to 5.0 are capable to resist in the air for hours and may be transfer at a large distance from their source [9]. Bacterial cells and spores transferred with airborne particles may pose source of contamination of processing surfaces and indirectly raw materials or final products [10].

The aim of the study was to evaluate the state of bacteriological bioaerosols in warehouses in selected food plant.

Materials and methods

The research material was the bacterial microflora isolated from the air of the storage area in the selected food plant. The following rooms were analyzed: products storage rooms (MPI, MPIO), technical storage room (MT) and primary packages storage room (MPJ). Samples were taken by settle plate method in triplicate. Petri dishes with PCA medium (BTL, Poland) were exposed on height of 1.3 meters by time of 15 minutes. Each room was analyzed three times in the months of May, July and October. Then, the sample was incubated at 30°C for 48 hours [11]. The number of bacteria in the air was calculated according to Polish Standard Omelianski's formula as modified by Gogoberidze (PN-89/Z-04111/03) and is shown in $\text{cfu}\cdot\text{m}^{-3}$ [12]:

$$X = \frac{z \cdot 100 \cdot 100}{\pi \cdot r^2 \cdot 0.2 \cdot t} \left[\text{cfu} \cdot \text{m}^{-3} \right]$$

where: X - the number of microorganisms in the 1 m^3 of air, z - the number of colonies in the Petri plate, r - diameter of Petri plate, t - time of Petri plate exposure.

Bacteria were divided into morphological groups using Gram staining method. Pure strains of bacteria after 48 hours incubation at 30°C were stained to isolate a group of Gram-positive and Gram-negative bacteria. Presence of catalase-positive and catalase-negative strains was determined by biochemical tests with use of hydrogen peroxide in concentration of 3%. Gram-negative rods were divided into two groups: oxidase-positive and oxidase-negative by using the reaction of tetramethyl-1,4-phenylenediamine dihydrochloride oxidation [13, 14].

Results and discussion

The analysis of composition of bioaerosols during the five-month study period showed that the number of bacteria in different storage areas remained almost unchanged. The highest difference was observed in the case of MT, which in May the number amounted to

$1 \cdot 10^5$ $\text{cfu} \cdot \text{m}^{-3}$, and during the subsequent measurements it was at the level of $3 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$. The number of bacteria also in the case of MPI was slightly different from the others, namely in July it amounted to $4.4 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$, and in May and October $1 \cdot 10^5$ $\text{cfu} \cdot \text{m}^{-3}$. In the remaining storage rooms, that are MPJ and MPII, the bacterial bioaerosol remained constant (Fig. 1). The level of microbial contamination of air was set by Interdepartmental Commission for Maximum Admissible Concentration and Intensities for Agents Harmful to Health in the Working Environment and should not exceed $5 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$ [15, 16]. According to guidelines, the number of bacteria in the bioaerosol was higher than the recommended level in cases of MT and MPI.

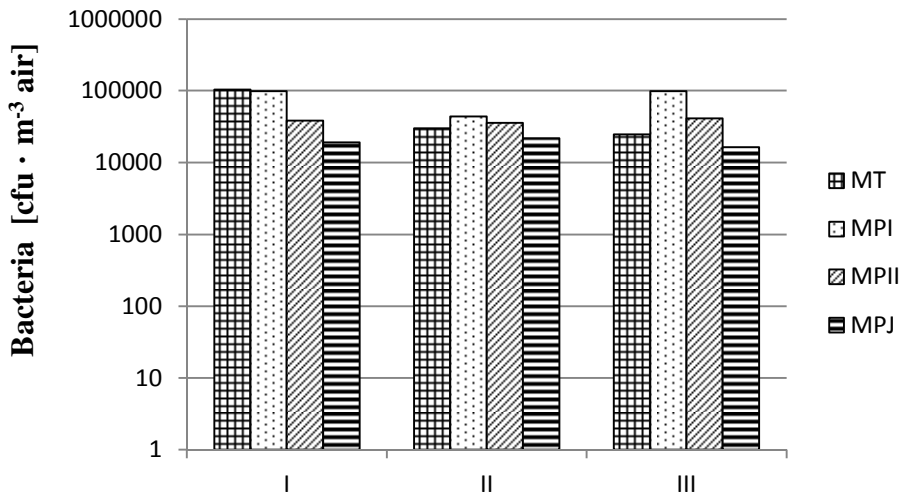


Fig. 1. Total number of bacteria [$\text{cfu} \cdot \text{m}^{-3}$] in bioaerosols from storage facilities of the food plant (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected I - in May; II - in July; III - in October

Table 1
The occurrence of each morphological groups of bacteria, depending on the storage room (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected in May, July and October [%]

			MT	MPI	MPII	MPJ
Gram-negative	Rods	May	12	55	50	25
		July	0	25	77	30
		October	22	30	9	50
Gram-positive	Bacilli	May	61	34	25	50
		July	91	19	0	30
		October	33	42	53	50
	Coccus	May	27	11	25	25
		July	9	56	23	40
		October	44	28	38	0

Morphological division showed that the Gram-negative bacteria dominated only in MPII. In MPI similar levels of Gram-positive and Gram-negative bacteria were recorded. In other storage rooms, most bacterial microflora were Gram-positive bacteria. Bacilli accounted for the largest population of bacterial bioaerosols in MT and MPJ. In MPI most frequently cocci and bacilli, on the MPII dominated bacilli (Table 1). Karbowska-Berent et al, in their study also noted a predominance of Gram-positive bacteria over the Gram-negative [15]. Predominance of sporulated bacteria may pose a threat for health of workers in the storage area. The group of bacilli contains species which may be cytotoxic. Species belonging to *Bacillus* genera may product high temperature resistant cytotoxic metabolites [17].

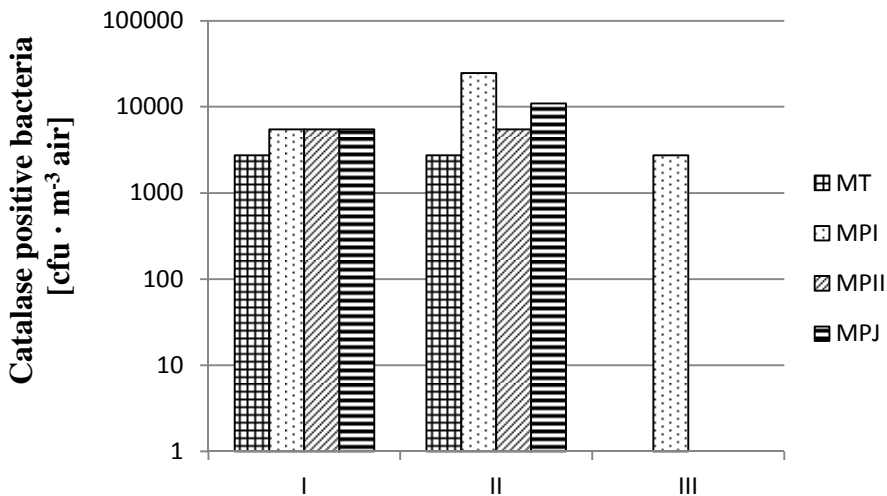


Fig. 2. Total number of catalase positive bacteria in bioaerosols from storage facilities of the food plant (MPI and MPII - products storage rooms, MT - technical storage room and the MPJ - primary packages storage room) in samples collected I - in May; II - in July; III - in October

Analysis of bacterial strains collected in May indicated that number of catalase-positive bacteria was at similar level in all storage areas. This group of bacteria accounted for 30% of the MPJ bacterial microflora of the air. In other areas the number of catalase-positive bacteria compared to the total number of bacteria was less than 15%. In July was an increase in the number of catalase-positive strains from $5.5 \cdot 10^3$ to $2.5 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$ in the case of first product storage area (MPI) and from $5.5 \cdot 10^3$ to $1.1 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$ in the warehouse of unit packages (MPJ). These group of bacteria accounted for 50% of the total number of bacteria in both warehouses. In October, catalase-positive bacteria occurred only in MPI and accounted for 3% of the total number of bacteria isolated from the first product warehouse (MPI) (Fig. 2, Table 2). Results showed that the highest rate of contamination of catalase-positive bacteria was observed in first warehouse of products (MPI) and storage room of units package. The contamination of

bioaerosol at a level higher than $1 \cdot 10^3$ cfu·m⁻³ may pose source of final product cross-contamination and may cause adverse human health effects. The group of catalase-positive cocci includes genus of *Staphylococcus*, which not only pose threat to human health but also may form biofilm on the food related surfaces [18]. Among the bacteria of this group the most dangerous pathogen of food is *Staphylococcus aureus*. Antibiotic-resistant strains of *S. aureus* are frequently isolated from food. In addition, some of these strains produce the thermostable, protease resistant and dangerous to human health enterotoxin. *Staphylococcus* species are often isolated from the air, dust, waste water, milk, food and equipment in the food plant [19].

Table 2

The ratio of catalase-positive to total number of bacteria, depending on the storage room (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected in May, July and October [%]

Month	MT	MPI	MPII	MPJ
May	3	6	14	29
July	9	56	15	50
October	0	3	0	0

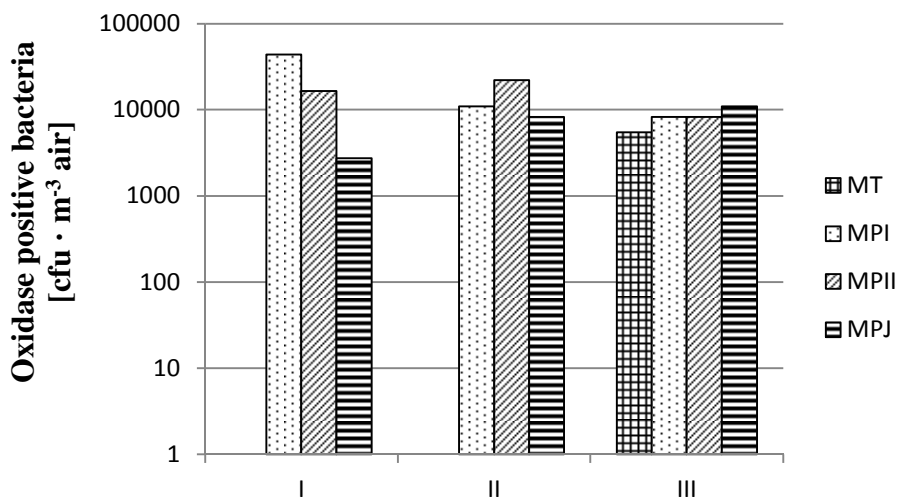


Fig. 3. Total number of oxidase positive bacteria in bioaerosols from storage facilities of the food plant (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected I - in May; II - in July; III - in October

In the samples collected in May the highest number of oxidase-positive bacteria in bioaerosol was $2.5 \cdot 10^4$ cfu·m⁻³ (MPI), and it accounted for 44% of the bacterial microflora. In the following months, number of oxidase-positive bacteria and its percentages compared to the total number of bacteria in the air in the MPI, were lower. In the case of MPII highest content of oxidase-positive bacteria was observed in July and it

amounted to $2.2 \cdot 10^4$ cfu·m⁻³. In this period also was noted the highest percent of oxidase-positive strains compared to total bacteria number and it amounted to 62%. Storage room MT was characterized by an increase in the number of oxidase-positive bacteria during the 5-month study period ranged from $2.8 \cdot 10^3$ cfu·m⁻³ in May to $1.1 \cdot 10^4$ cfu·m⁻³ in October. Number of oxidase-positive bacteria in relation to the total number of bacteria in bioaerosol of MT storage space increased from 14 to 67%. Oxidase-positive bacteria in MPT occurred only during the third collection trial, and their number was at a level similar to remaining rooms. Higher content of oxidase-positive bacteria in the air was observed in the summer months. This may be due to the higher intensity of production in these months. Group of oxidase-positive bacteria contain strains of the genus *Pseudomonas*, *Legionella* or *Campylobacter*. However, the oxidase-negative rods include a large group of pathogens contaminating food as *Salmonella*, *Shigella*, *Escherichia*, *Listeria* and *Yersinia* [19-22]. The biggest problem of air pollution from gram-negative is related to meat processing plants [23].

Table 3

The ratio of oxidase-positive to total number of bacteria, depending on the storage room (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected in May, July and October [%]

Month	MT	MPI	MPII	MPJ
May	0	44	43	14
July	0	25	62	37
October	22	8	20	67

Conclusions

- The composition of the bacterial bioaerosol remained at a similar level in each storage facility areas of the food plant.
- Gram-positive bacteria were the dominant group in most storage rooms.
- The results suggest that the bacterial bioaerosols can pose a potential threat to the final product.
- In case of the technical storage room in May and (MT) primary packages storage room (MPJ) in May and October the numbers of bacteria in the bioaerosol where higher than exceeds the guidelines set by the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment.
- Height number of sporulated bacteria in the air may generate a threat for the health of workers.

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BIOAEROSOL BAKTERYJNY W STREFIE MAGAZYNOWEJ ZAKŁADU PRZEMYSŁU SPOŻYWCZEGO

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Abstrakt: Branża spożywcza zwraca coraz większą uwagę na etap magazynowania surowców i produktów gotowych. Mikrobiologiczne zanieczyszczenie surowca czy produktu gotowego w wyniku błędów przy magazynowaniu może generować straty ekonomiczne dla przedsiębiorstwa. Celem pracy była ocena stanu bioaerozolu bakteriologicznego magazynów w wybranym zakładzie przemysłu spożywczego. Materiał badawczy stanowiła mikroflora bakteryjna wyizolowana z powietrza w strefie magazynowej wybranego zakładu przemysłu spożywczego. Analizie zostały poddane cztery pomieszczenia: magazyny produktów (MPI, MPIO), magazyn techniczny (MT) oraz magazyn opakowań jednostkowych (MPJ). Badania wykonano metodą sedymentacyjną Kocha w trzech powtórzeniach. Płytki Petriego z podłożem agarowym PCA (BTL, Polska) eksponowano na wysokości 1,3 m przez okres 15 minut. Następnie próby inkubowano w temperaturze 30°C przez 48 godziny (PN-89/Z-04111/03). Liczebność bakterii podano w jtk·m⁻³. W każdym pomieszczeniu dokonano analizy trzykrotnie w miesiącach maj, lipiec i październik. Równolegle przeprowadzono wstępne testy biochemiczne. Przy użyciu 3% nadtlenku wodoru bakterie podzielono na dwie grupy: posiadające i nieposiadające katalazy. Gram-ujemne pałeczki podzielono na oksydazo-dodatnie i oksydazo-ujemne przy zastosowaniu reakcji utleniania dichlorowodoru tetrametylo-1,4-fenylendiaminy. Największym stopniem zanieczyszczenia charakteryzował się pierwszy magazyn produktu gotowego (MPI), gdzie w maju i październiku oznaczono liczebność bakterii na poziomie 1·10⁵ jtk·m⁻³. Podobnie wysoką liczebność bakterii oznaczono początkowo w magazynie technicznym (MT), która następnie uległa 4-krotnemu obniżeniu. W drugim magazynie produktu gotowego (MPIO) i magazynie opakowań jednostkowych (MPJ) zanieczyszczenie powietrza bakteriami utrzymywało się na stałym poziomie 3,7·10⁴ jtk·m⁻³ w MPIO i 2·10⁴ jtk·m⁻³ w MPJ.

Słowa kluczowe: przemysł spożywczy, bioaerosol, bakterie, przestrzeń magazynowa