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Evaluation of a New Bioactive Nonwoven Fabric for Respiratory Protection

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Abstract
Legal regulations concerning protecting workers threatened with biological factors in the workplace and the global threat of terrorist attacks influence the need to master the properties of protective equipment and the methods of its evaluation. The article presents an approach to nonwovens with biocidal properties designed for respiratory protection devices (RPD) against bioaerosols. It was assumed that these materials should fulfil two basic criteria – high efficiency of filtration against bioaerosols and have the ability to destroy microorganisms blocked in the nonwoven. An experimental setup that enabled to control the flow of bioaerosol by a sample of nonwovens was created, making it possible to also evaluate the efficiency of filtration by applying a particle counter. Microorganisms with an aerodynamic diameter of ≤ 1.0 μm and various shape were selected for the study, all belonging to two aerobic types of gram positive (+) and gram negative (-) bacteria. The measurement was based on bioaerosol flow through a filter with a diameter of 80 mm, at a volumetric flow rate of 30 l/min, for 15 minutes. Tightly sealed filters were stored for 2, 4 and 8 hours at a temperature of 37 °C. In order to evaluate bacteria survival after contact with the bioactive nonwoven, they were rinsed and shaken for 15 minutes in a shaker at a frequency of rotation of 150 c.p.m. After dilution in sterile saline, microorganisms were seeded on a sterile Petrie's dish. They were later incubated at a temperature of 37 °C for 24 hours, after which time the colonies grown were counted. Applying the method discussed, the efficiency of filtration against aerosol was confirmed as well as the bioactivity of melt-blown nonwovens made from poly (lactic acid PLA) modified with a biocidal compound.

Key words: *respiratory protective devices, bacterial survival, filtering efficiency, bioaerosol, nonwovens' biocidal activity, poly(lactic) acid air filters, protection against bioaerosols.*

Introduction

The basic rule of creating safe working conditions and non-work life is to eliminate dangers or minimise their outcomes. As for biological hazards transferred through the air, this means not only efficient inhaled air filtration and cleaning off of biological particles, but also the destruction of the inhibition of their development while the protective equipment is used, including in particular RPD [1]. This results primarily from the fact the nonwovens, which is the basis of such equipment, may cause a so-called "secondary source of infection" [2 - 4]. This phenomenon may be the outcome of the detachment of particles of pathogenic microorganisms that colonise the nonwoven and their migration inside the material. This process is fostered by the dynamic flow of breathing air. Additionally the unfavourable microclimate inside the RPD (higher humidity and air temperature) has no influence on the prolonged life of microorganisms or their endospores [2, 5, 6]. The problems listed urge scientists and producers of nonwovens to constantly improve these materials, especially permanent improvement of the efficiency of blocking microorganisms in the filtering material and their further decontamination. In this respect, the author has conducted her research into the modification of polypropylene (PP) and polycarbonate (PC) melt-blown nonwovens. Results of these works have been patented, published and implemented as so-called bio-protections of the respiratory system [7 - 9]. The need to continue research into this matter is connected with an even broader application of polymers from renewable sources in filtering materials [10] instead of petroleum polymers.

At the same time, evaluation of the protective properties of nonwoven and RPD is problematic with respect to ensuring an efficient barrier against biological factors [11 - 13]. Despite numerous researches into phenomena related to the filtration of bioaerosols through filtering materials, no unified methodology of the research and evaluation of RPD has been worked out in Europe and worldwide, the reason for which being that there is a wide variety of biological particles that may pose a threat to man. Bioaerosols include bacteria, viruses, fungi, algae, and dust mites. In addition, biological products such as pollen, endotoxins, proteins, and animals excreta form aerosols. All of the forms of bioaerosol listed may negatively influence human life as they have the ability to incubate, grow, multiply, and produce toxic substances. It should be stressed that in the case of exposure to inhaling bioaerosol, the negative influence on human life depends on the number of viable particles, while in the case of the non-bioaerosols the amount of the mass of exhaled particles is important. Due to this fact, RPD against bioaerosol must be tested for its efficiency against biological particles and the range of its applications should relate solely to the group of microorganisms tested. At the same time, it should be emphasised that using standard methods [14, 15] in evaluating the efficiency of filtering materials and RPD with the use of non-biological aerosols is helpful in classifying equipment and is also a sign of the efficiency of filtration towards bioaerosols expected.

As was mentioned before, there are no specified standard methods that could be applied in research on the efficiency of filtration. However, the rule applied by a number of scientists in this area has reflected phenomena occurring during the dynamic flow of bioaerosol through filtering materials in laboratory conditions [16 - 24]. In order to do so, it is crucial to simulate the dispersion of microorganisms in the closed measuring system and mixing them with a stream of clean air. As a result of the flow of the bioaerosol

thus created through the filtering material, the deposition of particles on the nonwovens take place. From the point of view of filtration phenomenon, the efficiency of deposition depends on the speed of flow, size and shape of particles, and on the parameters that are directly related to the filtering material, such as the thickness of fibres, porosity and the presence of electrostatic powers of attraction. In the case of research into filtration efficiency, it is important that the question of particle detection be solved as well.

Numerous works concerned research aimed at confirming the efficiency of medical equipment (surgical masks) that protect a patient against large drops of bioaerosol that come from health care workers through speaking, coughing or sneezing [16, 19, 20]. For instance, it was shown [16] that using surgical masks lowers the risk of *Mycobacterium tuberculosis* infection by 2 to 4 times, while in the case of using highly effective HEPA filters, it is lowered by 45.5 times. That research confirmed that surgical masks do not provide enough protection against sub-micrometer-size bioaerosols, which triggered a series of research into the evaluation of the usefulness of the RPD standard. Other research [19 - 22] concerned equipment of an average and high protection class (P2 and P3 according to [25, 26])and usually compared the efficiency of filtration against biological and non-biological aerosols. In all experiments, good correlation between the results of the DOP aerosol (Dispersed Oil Particles) tested was observed as well as microorganisms as for the same size of particles. Interesting results were observed in work [20], where results of the penetration of *Pseudomonas fluorescents* and *Streptococcus salivarius* (*S. salivarius)*were compared with those for the spheric particle of paraffin oil.

As a result of these works, it was clearly stated that the result of penetration depended on the shape of the particle. Penetration for spheric particles of *S. salivarius* and paraffin oil remained at the same level regardless of the type of respiratory protective equipment tested. As for a particle of oblong shape, an increase in efficiency was observed for each of the types of protection tested, respectively. In work [21] a testing setup was described that included a particle counter used in determining the efficiency for various ranges of particle sizes. The results obtained were similar to previous observations together with confirmation as model considerations that assumed that the filtering mechanism is identical for biological and non-biological particles. Similar conclusions were obtained by a group of American scientists from the University of Minnesota [19], who analysed the phenomenon of bioaerosol filtration for various values of aerosol velocity and air humidity, with two detection methods: using a particle counter and breeding colonies for the live biological particles. As for the volume concentration of the flow rate, two values were applied: 85 l/min (heavy working conditions) and 45 l/min (normal working conditions). It was confirmed that the result of the penetration of bioaerosols was influenced mostly by the velocity and type of equipment, whereas no significant influence of humidity was noted. A high correlation was obtained between the results of penetration with the use of a particle counter and breeding colonies of microorganisms on agar broth. In conclusion, the notion was presented that the manner of measurements of the efficiency of filtering materials towards bioaerosols should result from their future application. In the case of analysis of the ability to deposit particles, their size and shape are of basic importance. While in the study of the phenomena of bioaerosol particle reaction to the filtering material or re-emission phenomena, it is crucial for the research to be carried out with the use of viable bioaerosols.

Apart from the efficiency of filtration against microorganisms, evaluation of microorganism survival while in contact with material of biocidal activity is of the essence. As far as this is concerned, the application of quality or quantity methods could be considered. Quality methods serve to evaluate the bioactivity of textiles that contain biocidal components (biocides), allowing to correctly assess their biostatic activities. Methods belonging to this group are usually based on placing a textile sample on agar broth with breeding bacteria. Then bacteria growth is observed under and around the sample. If the textile product applied (fabric or hosiery) contains an effective biocide, an inhibition zone will be observed for bacteria under and around the sample, and there will be the so-called 'halo effect'. Coverage of the inhibition zone is determined in milimetres. Usually it is assumed that the positive effect of the bacteriostatic activity of a fabric is observed when there is no increase in the number of microorganisms under the sample and the inhibition zone of bacteria around the sample is 1 - 2 mm. In reality, the coverage of the inhibition zone depends not only on the efficiency of the bactieriostatic compound applied, but also on its solubility and diffusion coefficient. Using methods of this group, it is difficult to assess more subtle differences in the efficiency of various biocides and the methods of imbuing fibres with them.

Quantity methods are applied when evaluating activity that reduces the population of microorganisms. Thus they may be used to determine the quantity of bacteriocidal reactions of textiles. Bacterial strains bred under certain conditions and time in solutions, with and without a sample, undergo counting and reduction rating. Unfortunately comparing the results of antibacterial activity evaluation of textiles made according to different norms is very difficult or even impossible. In the case of quality methods this is due to the fact that various strains and substrate thicknesses are used, the actual weight of fabric and the subjectivity of bacteria growth evaluation under the sample. In the case of quantity methods, the cause of differences in assessment may be differences in procedure, concentration or microorganism dilution as well as different bacteria strains used during the test. In the case of nonwovens, applying a quality method of evaluation of bioactive properties seems problematic. Nonwovens are characterised by significant porosity and their field of contact with the surface layer of the agar broth will be small. Also with the assumption that bio-aerosol flows through the filtering material during the inhale-exhale phase, it should be expected that microorganisms will penetrate inside the nonwoven and evaluating merely the external layer will be subject to substantial error. For all the reasons above, it is quantity methods that are preferred, based on rinsing microorganisms deposited in the filtering material, incubating them under defined conditions and then counting using microscopic methods [27, 28].

The aim of the research presented is to highlight an approach for the evaluation of nonwovens designed for RPD against bioaerosol and confirm the possibilities of achieving high efficiency of filtration and biocidity of nonwovens made from thermoplastic polymers from renewable sources.

Experiments

Materials

Innovative melt-blown nonwovens from poly(lactic acid) (PLA) were used in the study, modified with a compound with biocidal properties. Melt-blown nonwovens were created at the experimental workshop of the Central Institute for Labour Protection – National Research Institute (CIOP-PIB) [9]. The polymer characteristic is presented in ***Table 1***.

To modify PLA fibres, a biocidal compound based on invention [29] was used.

The selection of the biocide compound resulted from technological and health conditions. It was necessary to ensure a permanent connection of biocide particles with the thermoplastic polymer at the stage of fibre formation. This is achieved by an appropriate granulation formulation vehicle biocide - perlite, whose particle size - about 100 μm - can be partially embedded in fibres of a diameter of about 1 - 5 μm. Another very important criterion for the selection of the biocide was to ensure that the preparation of biocidal compounds was considered harmless to humans according to Directive 2012/16/EU of the European Commission, amending Directive 98/8/EC of the European Parliament and Council to include hydrochloric acid as an active substance, which at the same time can damage gram positive and gram negative cell membranes, typical risks borne by inhalation.

It comprises perlite with a grain diameter no bigger than 100 μm, with a biocidal compound applied on its surface. The content of the compound by weight of biocidal mass (Bioperlite) was as follows:

- 5 - 25% by weight of N,N-didecyl-N,N-dimethyl chloride,
- 1 - 10% by weight of N-benzyl-N-dodecyl-N,N-dimethyl chloride,
- 0.5 - 15% by weight of N,N-bis(3-aminopropyl)-N-dodecylamine,
- 0.1 - 10% by weight of sodium of 2-phosphobutane-1,2,4-tricarboxylic acid,
- 0.5 - 4% by weight of glicerine,
- 20 - 80% of lower aliphatic alcohol (favourably 2-propanol),
- 5 - 30% by weight of demineralised water.

The content of active substances was between 2 and 8 % by weight and for perlite between 92 and 98% of the total mass of the compound. Bioperlite was obtained by spraying dry perlite with a solution of the biocidal compound with the content listed above. The manner of introducing the biocidal compound into polymer fibres was described in patent [30]. Parameters of the process of creating nonwovens are presented in ***Table 2***.

In order to improve the efficiency of bioaerosol filtration in the nonwoven, corona discharge (with a discharge of 30 kV) was applied additionally. Filtering characteristics of the nonwovens obtained are presented in ***Table 3***, A standard method [14] was applied to evaluate the efficiency of filtration based on measuring the penetration of model particles of paraffin oil mist. Air flow resistance through the filtering material (so-called breathing resistance) was determined using norm [15], which is normally used to evaluate RPD.

The study was carried out for two series of nonwovens (1-4) and (5-8), differing in the mass per unit of the PLA base non-woven, with the same % content of biocidal modifier. In order to compare, nonwovens with no corona discharge were also produced. For each variation of the nonwoven, 20 measurements were done concerning filtering and structural parameters.

The total amount of biocidal substance introduced into the PLA fibres through Bioperlite was determined on the basis of elementary analysis. It was established that the average amount of biocidal substance in 4 samples of nonwovens containing 10% of Bioperlite was 0.86% by weight per active substance. The amount of active biocidal substance adsorbed on the surface of the nonwovens was determined using the spectrophotometric method in UV. It was established that under the conditions of extraction performed, 48% of the biocidal substance contained in the nonwoven underwent desorption. On the basis of the analyses carried out, it may be stated that 52% of the biocidal substance is permanently attached to the fibres, whereas the rest may undergo desorption.

Table 1. *Characteristic of polymer PLA used in making melt-blown nonwovens; *) MFI according to PN-EN 14704-1:2006 at temperature 210 °C.*

Polymer type	PLA 6202 D
Producer/Supplier	NatureWorks, LLC
Melting temperature, °C	160-170
Flow index MFI*, g/10 min	15-30

Table 2. *Parameters of the process of creating melt-blown nonwovens from PLA modified with Bioperlite.*

Temperature of the I extrusion zone, °C	270
Temperature of the II extrusion zone, °C	270
Air temperature, °C	270
Head temperature, °C	210
Airflow, m^3/h	8.8
Polymer flow, g/min	4.0
Distance between the receiving device and the head, mm	300

Test methods

Bioactive properties of nonwovens applied in RPD is a term defined by the author in research projects realised earlier that were connected with working out bio-protections for the respiratory system [31]. The covers complied with two criteria of evaluation: efficiency of filtration towards biological particles and inhibiting the development or destroying of microorganisms deposited in the filtering material during the use of equipment. The original methodology of research into the bioactivity of nonwovens includes bioaerosol flow through the nonwoven, which stimulates the conditions of using RPD as well as microbiological evaluation for various times of incubation for microorganisms stopped inside the nonwoven.

Table 3. *Characteristics of melt-blown nonwovens with PLA and nonwoven PLA modified with Bioperlite.*

No	Nonwoven content	Electrostatic activation	Mass per unit, g/m^2	Paraffin oil mist penetration, %	Air flow resistance, Pa
1	PLA+Bioperlite	+	96.0 ± 1.92	3.5 ± 007	222.0 ± 11.10
2	PLA	+	90.0 ± 1.80	5.9 ± 0.18	213.0 ± 10.65
3	PLA+Bioperlite	-	106.0 ± 5.30	7.8 ± 0.31	223.3 ± 6.69
4	PLA	-	96.0 ± 1.92	9.5 ± 0.48	217.0 ± 10.85
5	PLA+Bioperlite	+	134.0 ± 2.68	1.8 ± 0.05	307.0 ± 15.35
6	PLA	+	124.0 ± 3.72	4.8 ± 0.14	253.0 ± 12.65
7	PLA+Bioperlite	-	134.0 ± 2.68	5.4 ± 0.16	296.0 ± 10.76
8	PLA	-	118.0 ± 2.36	7.0 ± 0.35	250.0 ± 12.50

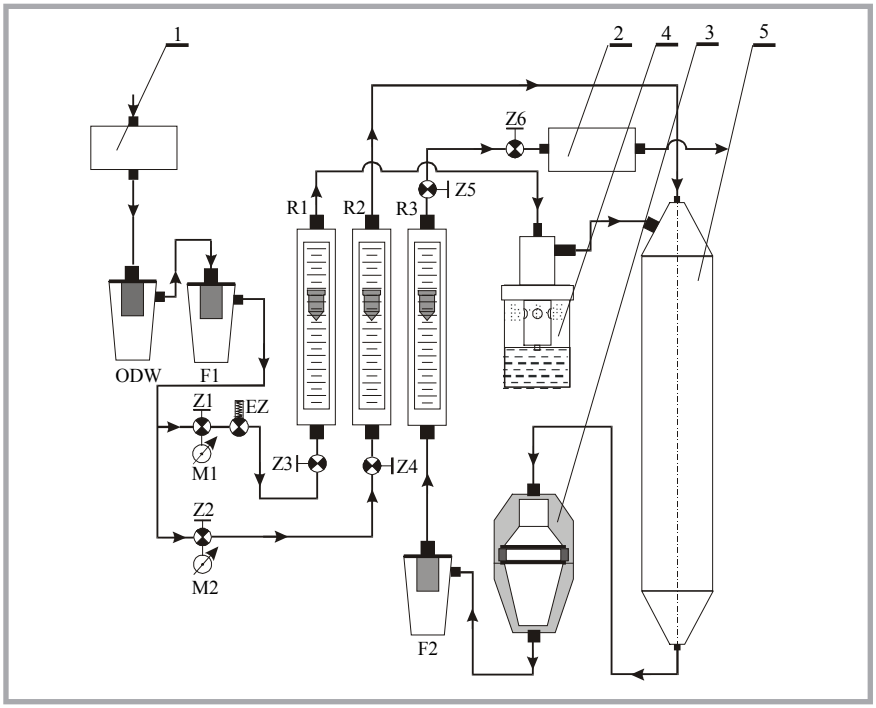

Figure 1. *Idea diagram of an assembly for determining the efficiency of nonwoven used in construction of respiratory protective equipment; 1) compressor, 2) vacuum pump, 3) test chamber, 4) collision atomizer, 5) drier.* ***Notes****. ODW- cyclone separator; F1 – air filter type AHF 04/0.01 µm; Z1, Z2 – pressure reduction valves; M1, M2 - manometers; EZ - electrovalve; R1, R2, R3 – rotameters; Z3, Z4 - airflow fine adjustment valves; Z5 – air suction fine adjustment valve; Z6 - air suction preliminary adjustment valve; F2 - output filter type ACF 04/0.003 ppm.* ***Source****: from author's own sources*

Experimental setup

A diagram of the experimental setup is presented in ***Figure 1***.

Tests were carried out at the Department of Chemical, Dust and Biological Hazards at CIOP-PIB. In order to ensure safety in the workplace, the experimental setup was placed in a fume hood. In order to ensure air purity in the measurement system, cyclonic separation and an oil filter were applied, which removed particles with a diameter of 0.01 µm. Behind the measuring chamber, a filter was placed with a precision of filtration of 0.003 p.p.m. so as to prevent microbiological contamination of valves, the flow metre and vacuum pump. A collison atomiser was used for spraying bioaerosol [14]. To detect the number of bio-aerosol particles that went through the filter (penetration), an optical particle counter (OPC) was used, model Grimm 1.109 (Grimm Aerosol Technic, Germany). The range of particles measured was between 0.3 and 20 µm.

The construction of the measuring workshop makes it possible to apply various testing microorganisms in terms of their shape and properties, which makes it possible to obtain bioaerosols with different characteristics concerning the dispersion of particle size and different concentrations. It is also possible to regulate the bioaerosol flow rate (simulation of a minute lung ventilation) and carry out tests at different times of bioaerosol flow through the nonwoven sample.

Testing methodology

The microorganism solution was placed in a Collison atomiser, where it was sprayed, followed by mixing with a stream of dry air. In order to stabilise the testing conditions and evaluate the microorganism concentration, bioaerosol was placed in the measuring system for 30 minutes with no nonwoven attached. The appropriate measurement was based on bioaerosol flow through the nonwoven sample (filter with a diameter of 80 mm) at a flow rate of 30 l/min for 15 minutes. After this time, the filters were placed on a sterile Petrie's dish. Then tightly sealed filters were stored for 2, 4 and 8 hours at a temperature of 37 °C. Also tests of samples that were directly taken out of the measuring system were done (in the so-called 0 time). In order to evaluate the survival of microorganisms after their contact with bioactive PLA nonwoven , microorganisms were rinsed from each of the samples tested and shaken for 15 minutes in a water bath (temperature of 37 °C) on a shaker with a rotation frequency of 150 c.p.m. Then the sample was diluted in sterile saline until dilutions of 10^{-5}, 10^{-6}, 10^{-7} & 10^{-8} were achieved and seeded on the sterile Petrie's dish. The sample was poured with semi-liquid agar broth TSA (Caso Agar, tryptic soy agar) with an additive of polisorbate 80 and lecitin, pH = 7.3, from Merck, Poland), then mixed and left to set. Then they were incubated at a temperature of 37 °C for 24 hours, after which time all colonies grown were counted. From the results obtained, the average for each microorganism for every given hour of exposure was calculated.

In order to check the efficiency of filtration of biodegradable nonwovens towards microorganisms, particle concentration was established in front of and behind each filter tested using an OPC particle counter, model Grimm 1.109 (Grimm Aerosol Technic, Germany). 10 repetitions were performed for each variation of incubation time.

To test the bioactivity of PLA nonwovens, microorganisms with an aerodynamic diametre of ≤ 1.0 µm were selected, but of different shapes and belonging to two aerobic Gram positive (+) and Gram negative (-) bacteria. The necessity to carry out tests in relation to both types of microorganisms results from their different sensitivity to biocidal substances (biocides) for bacteria that are Gram positive (sensitive) and Gram negative (resistant). Considering the potential ability to effectively block particles of bioaerosol that are harmful to health in the filtering material of RPD, it was of the essence to apply in tests particles that most deeply penetrate the respiratory system as the so-called "worst case". The maximum depth of penetration for particles moving in the stream of air in the respiratory system depends mainly on their aerodynamic diameters. And thus, particles with an aerodynamic diameter of [31]:

- < 0.65 µm reach the region of alveoli,
- 0.65 – 1.1 µm reach the region of pulmonary bronchioles,
- 1.1 – 2.1 µm reach the region of final bronchi,
- 2.1 – 3.3 µm reach the region of secondary bronchi,
- 3.3 – 4.7 µm reach the region of the trachea and primary bronchi,
- 4.7 – 7 µm reach the region of the throat,

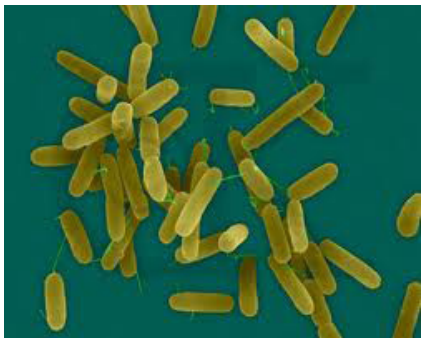

Figure 2. *P. aeruginosa – aerobic bacteria (Gram negative rod);* ***Source****: http://www.pseudomonas.com/ (3 Nov 2012).*

- 7 – 11 μm penetrate the channels of the nasal cavity.

Taking into account the above criteria in the evaluation of bioactive PLA nonwovens, *Pseudomonas aeruginosa* (*P. aeruginosa)* was used as an example of a Gram negative rod (***Figure 2***) and *Staphylococcus aureus* (*S. aureus*) as an example of Gram positive granuloma (***Figure 3***).

P. aeruginosa is a type of aerobic bacteria (Gram negative rod) with a size of 0.5 - 0.8 μm by 1.5 - 3.0 μm and aerodynamic diameter of 0.8 μm. Bacterium lives mainly in soil and water and on the surface of plants, rarely on animal skin, and occasionally it may be isolated from the human skin of people with appropriate purity of the immunological system. It is an opportunistic bacterium (i.e. causing contamination only in people with decreased immunity) both for humans and plants, and one of the most dangerous and most important microorganisms causing nosocomial infections. *S. aureus* can be found in the nasopharyngeal cavity and on human and animal skin. Contamination with bacteria takes place particularly frequently among hospital workers, which is of significance for spreading nosocomial infections. Staphylococci produce thermoresistant enterotoxin only in contaminated food. Staphylococcus toxin is very resistant to high temperature and is not destroyed even in the process of boiling for 30 minutes. The optimal temperature for its development is 37 °C. Poisoning with staphylococci has a short incubation period – 2 hours on average. Staphylococci that do not produce spores are easily killed during heating, whereas enterotoxin produced by *S. aureus* is resistant to this. Poisoning with *S. aureus* spread mostly as a result of direct contact. Less of importance in transmitting contamination is the role played by air, but this is significant in case of lung inflammation or large burns that come in contact with staphylococcus. Recently it has also been shown that *S. aureus* spread through air may be increased if the carriers have a viral respiratory system infection. This viewpoint creates an additional argument justifying selecting this bacterium for tests.

Bacteria strains came from the American Type Culture Collection (ATCC). They were stored according to international standards in the form of frozen lyophilizate of active cells from a 24-hour breed on TBS (Caso Bulion, tryptic soy broth, pH 7.3, from Merck, Poland, with a 3% yeast extract). 0.1 ml of defrosted lyophilizate was placed onto 50 ml of a sterile TBS base in Erlenmayer tubes of 200 ml and incubated for 24 hours at a temperature of 37 °C in order to produce bacteria in the stationary growth phase when metabolic activity and susceptibility to stress during transformation into aerosol are at their lowest level. Then the colony was centrifuged at a speed of 3500 r.p.m. for 10 minutes so as to separate cells from the base. Bacteria were suspended in 600 ml of saline (0.85% NaCl). The suspension was then moved to a sterile atomiser and connected to the apparatus system. A control culture was made using a magnetic mixer to check the number of microorganisms in the suspension. The number of microorganisms remained at the level of 2.6×10^7 cfu/ml for *P. aeruginosa* and 6×10^7 cfu/ml for *S. aureus*.

Samples of bioactive PLA nonwovens were not sterilised for fear of changing their physico-chemical properties. The microbiological purity of the nonwovens was checked. The number of microorganisms in the nonwovens was low and did not statistically influence the test results.

Indices of research analysis results

The following were marked during tests:

1) index of efficiency of filtration (*S*) of the test microorganisms calculated according to the following dependency:

$$S_{\%} = \frac{N_0 - N_k}{N_0} \times 100, \% \qquad (1)$$

where:

S – efficiency of filtration (1-P) in %,

N_o – number of microorganisms in front of the filter

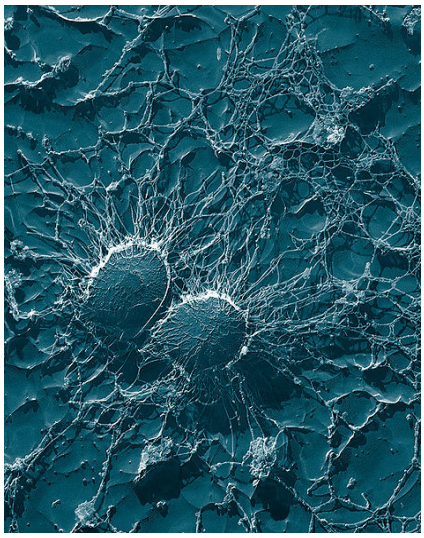

Figure 3. *S. aureus – aerobic bacteria (Gram positive granuloma);* ***Source:*** *http://pl.wikipedia.org/w/index.php?title=Plik:Staphylococcus_aureus,_50,000×,_USDA,_ARS,_EMU.jpg&filetimestamp=20060722190735#file (3 Nov 2012).*

N_k – number of microorganisms behind the filter.

2) index of bacteria survival (*P*) i.e. the number of microorganisms which survived contact with the bioactive material as opposed to the initial number grafted on the nonwoven in time t = 0.

$$P = \frac{N_0 - \overline{N}}{N_0} \times 100 \qquad (2)$$

where:

P – bacteria survival in %,

N_0 – average number of microorganisms grafted on the sample of non-woven material,

$\overline{N}$ – average number of microorganisms at a given time.

A statistical significance test was applied in the analysis of results – test F (analysis of variance ANOVA). A system with repeated measurements was applied as measuring the dependent variable was performed numerous times for samples of nonwovens from one batch.

Results

Efficiency of filtration

Results of tests on bioactive PLA nonwoven filtration against two microorganisms: *S. aureus* and *P. aeruginosa* are presented in ***Table 4***.

Results of tests point to very good results that were obtained for the efficiency of stopping microorganisms by PLA non-

Table 4. Efficiency of filtration of biactive nonwoven PLA.

Variation (charge)	*S. aureus*		*P. aeruginosa*	
	Average value of filtration efficiency, %	Standard deviation	Average value of filtration efficiency, %	Standard deviation
1 (+)	99.34	0.39	97.36	3.78
3 (-)	96.13	0.58	94.96	1.07
5 (+)	98.11	1.22	99.26	0.48
7 (-)	97.64	0.74	97.62	0.17

woven modified with Bioperlite. Efficiency of filtration remains at the level of 94.96 and 99.34. For the mass per unit of the nonwovens produced, the same dependency was obtained. Nonwovens charged electrostatically obtained higher values of filtration efficiency (lower index of penetration for the aerosol tested). At the same time it needs to be emphasised that all variations of bioactive PLA nonwovens produced achieved satisfactory results from the point of view of requirements set for RPD [25, 26]. Statistical analysis was carried out maintaining similar aerodynamic diametres. Due to the different number of cells of *P. aeruginosa* and *S.aureus* that flowed onto the non-woven sample tested, in the statistical analysis no output data were compared, only indices of a number of microorganisms. These indices were calculated considering the number of microorganisms (*P. aeruginosa* or *S. aureus*) in time 0 (cfu/sample) in relation to the value determined during the time of bioaerosol flow through the measuring system without the test sample (jtk/sample). On the basis of the statistical analysis carried out it was estimated that the shape of microorganisms while maintaining similar aerodynamic measurements had no statistical influence on the number of bacteria stopped on bioactive PLA nonwovens. This conclusion concerns the case when particles of bacterium *S. aureus* that have the shape of granuloma have cells with a size of between 0.5 – 1.5 µm, and when bacterium of *P. aeruginosa* that has the shape of a rod is characterised by measurements between 0.4 – 0.7 × 1.0 – 3.0 µm.

Referring to literature [11 - 13] that concerns phenomena to do with the filtration of biological particles, it needs to be stressed that obtaining a significant difference in the efficiency of filtration towards biological particles is part of current discussions about appropriate assessment of nonwovens applied in protection against aerosol. It is of particular importance to take a decision as to which shape of microorganisms is most representative in evaluation of protective (barrier) properties of RPD. So far, various results have been obtained in this area. In work [34], it was put forward that particles with the shape of a sphere (polystyrene latex) penetrate the filtering material better as opposed to *Mchelone* particles, which have the shape of a rod, assuming that they have similar aerodynamic diameters. At the same time, from conclusions presented in work [20], it is clear that this dependency is the opposite as it is the length to width ratio of bacteria that is of the essence. This view was shared by the researchers in [35] who carried out tests with the use of *Bacillus subtilis* (a rod) and *Staphylococcus epidermidis* (a sphere). However, it was suggested that the aerodynamic diameter of microorganisms may not be the best parameter to assess the penetration of non-spherical particles, which is similar as in the case of research into the penetration of asbestos particles through filtering materials, where the course of the phenomenon is determined by the fibre length and not the aerodynamic diameter. These discrepancies confirm the view presented in the introduction to this work. In research into bioprotections, it is necessary to apply particles with a size and shape that is distinct or typical for the forecasted conditions of the protective equipment's use. Therefore designing new filtering materials should be preceded by the characterisation and analysis of hazards against which the filtering equipment shall protect. At the same time, in order to confirm the protective efficiency of such equipment, it is necessary to apply microorganisms with the same characteristics as far as the size and shape of particles are concerned.

Bacteria survival in contact with bioactive PLA nonvowen

The efficiency of inhibiting the growth of microorganisms was evaluated in relation to the control i.e. the number of microorganisms obtained in the sample just after grafting (time 0). The results of tests on the survival of the test microorganisms in relation to the different times of incubation are presented in ***Figure 4,*** respectively.

Criteria of nonwoven bioactivity were set using normative recommendations [32] for marking the biostatic and biocidal effect of disinfectants in relation to bacteria. Level 3 was recognised as high, meaning a 1000 times decrease in the number of microorganisms. On the basis of the analysis carried out, it was shown that for both *P. aeruginosa* and *S. aureus,* in the time of incubation at a temperature of 37 °C within as little as two hours, the phenomenon of growth inhibition occurs

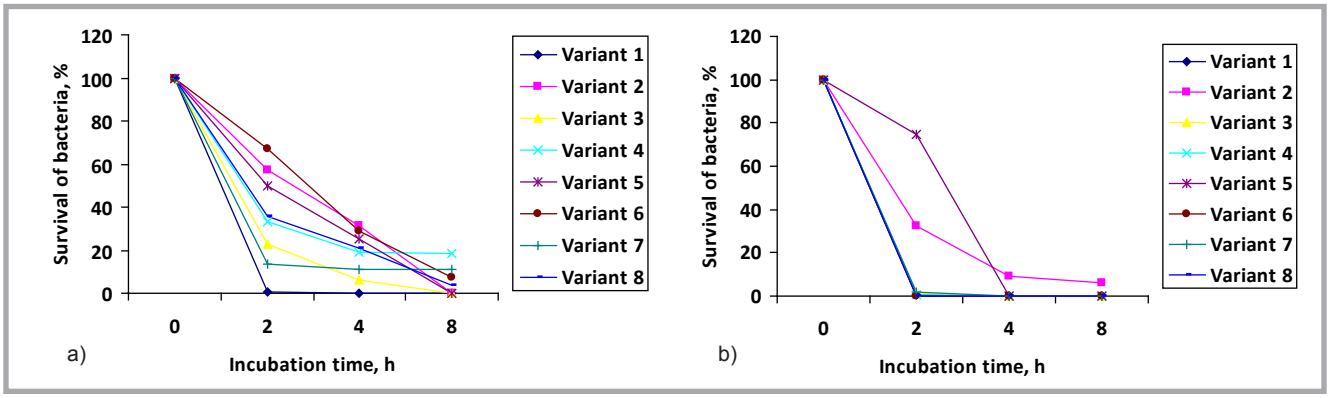

Figure 4. Results of tests on survival of: a) S. aureus and b) P. aeruginosa in time.

for the microorganisms ($p < 0.01$), i.e. the time of incubation causes a lowering of the number of populations of microorganisms studied and achieving of the 3rd level of efficiency. For *P. aeruginosa* no relevant differences between the nonwovens used in tests were noted (charged and not charged electrostatically), which means that biocidal properties in relation to gram negative bacteria were obtained at a similar level for biocidal nonwovens activated and not activated in the field of corona discharge. In the case of *S. aureus*, the best results were obtained for variations of PLA nonwovens with Bioperlite activated in the field of coronary discharge. An exception is nonwoven with a mass per unit of 134 g/m^2. As for the survival of microorganisms, this was evaluated to be much worse than for nonwoven with a lower mass per unit, both for Gram positive and Gram negative bacteria, which may result from the worse distribution of bioperlite modifier in the nonwoven volume and, therefore, less contact of the biocidal preparation with the cell membrane of a bacterium. At the same time, it needs to be emphasised that PLA nonwovens with no bioperlite were not as good a breeding ground for microorganisms as was in the case of nonwovens with PP. After 4 hours of contact with bacteria, despite no biocidal agent, a significant reduction in the population of microorganisms occurred. The explanation for this phenomenon requires further research, particularly in the context of broader usage of polymers from renewable sources whose structure and properties are radically different to those of synthetic polymers used thus far in the production of nonwovens, which are the basis of RPD construction.

Conclusion

1) All variations of nonwovens produced from bioactive PLA polymer achieved satisfactory results from the point of view of requirements for RPD. In the case of electret nonwovens, the efficiency of filtration was higher in comparison with nonwovens with no electrostatic activation.
2) On the basis on the statistical analysis carried out, it was presented that for both *P. aeruginosa* and *S. aureus*, during the time of incubation at a temperature of 37 °C, after as little as 2 hours, the phenomenon of growth inhibition occurs for microorganisms. No significant influence of the activation process was established in the field of corona discharge on the biocidal properties of PLA nonwovens modified with bioperlite.
3) Due to increasing awareness concerning environmental protection as well as requirements of legislative bodies, production, usage and disposal, products made from traditional petroleum polymers are looked down on more frequently. A solution to that problem may be the development of substitute products based on renewable sources. The results of research obtained in this project point to the great potential of the use of biodegradable polymers as substitutes for traditional solutions applied in the construction of RPD against bioaerosols.

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