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Modelling the Viability of Microorganisms of Poly(lactic Acid) Melt-Blown Nonwoven Fabrics for the Use of Respiratory Protection

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Abstract
A variety of harmful microorganisms deposited in the work environment is the reason why it is becoming more and more common to use filtering respiratory protection equipment. To fulfil its protective role against biological hazards, it should not only ensure high efficiency of filtration but also present biocidal properties. Due to the fact that in laboratory studies confirming the biocidal properties of equipment it is possible to use only a limited number of testing organisms, using microbiological prognosis models seems a promising direction. The article presents a method for assessing the performance of filter materials with biocidal properties using a simple model describing the dependence of the viability of Gram - positive and Gram - negative bacteria in the biocidal structure of the material. A comparison of the results of theoretical predictions and experimental results confirmed that the model can be used as a tool for the approximate evaluation of the properties of these materials. Poly(lactic acid) (PLA) was used when validating the model melt-blown nonwovens for the construction of respiratory protection devices (RPD) against biological hazards.

Key words: *viability, poly(lactic acid), melt-blown nonwoven, respiratory protection, bioaerosol filtration efficiency.*

Introduction

There are many reports concerning the benefits of using respiratory protective equipment (RPD) against biological hazards [1 - 5]. Therefore in many research centres connected with personal safety at the workplace there are works carried out which are directed at achieving different goals. One of them is mastering the protective properties of filtering materials used in constructing individual or group protection equipment. In order to do so, new polymer materials [6 - 8], new techniques of modification [9 - 14] and innovative technologies are used in forming nonwovens [15 - 22]. At the same time, laboratory development of methods applicable when confirming the protective efficiency of new materials and filtering equipment is being carried out. The basic criterion when evaluating the credibility of these methods should be the highest possible compliance of measuring methods with the conditions of using the equipment at work places predicted. A number of laboratory methods have been elaborated which include the evaluation of such parameters as the efficiency of filtration against biological and non-biological aerosols [23 - 26], the viability of microorganisms stopped in filtering nonwoven [27 - 31], and the total efficiency of equipment, i.e. Protective Factor [32].

As for improving materials used in constructing filtering equipment for protection against bioaerosol, there is a dominating trend to provide these materials with biocidal features. This results from the fact that knowledge in the field of phenomena taking place in filtering materials after a longer time, e.g. in ventilating installations, is more and more common [33 - 35]. This knowledge creates a new approach to nosocomial infections and multiple application of individual equipment and the ways it is utilised. In the context of using respiratory protection equipment and to minimize the danger of infection with *Mycobacterium tuberculosis,* systematic research was carried out concerning the viability of microorganisms in filtering materials. It was shown that there is [36] a significant growth in the colonies of microorganisms after 5 days of storing grafted materials at a relative humidity of (85% RH). There was a particular growth of *Mycobacterium abscessus* by 20%, *Staphylococcus epidermidis* by 61% and *Bacillus subtilis niger* by 98%. These results were used to elaborate a recommendation concerning single- or multiple use of traditional respiratory protection equipment, but at the same time they became an impulse to intensify technological works on new bioactive filtering materials. It is important for these materials to present both high efficiency of filtration and the ability to quickly destroy microorganisms deposited in the equipment during use at the same time. As far as assessment of survival is concerned, microbiological methods were used based on the cultivation of microorganisms after their contact with the biocidal material. These are methods that require long-term studies and measuring workplaces that would be constructed especially for this purpose. That is why it is so important to elaborate an approximate way of evaluation of filtering materials with biocidal properties using simple model dependencies.

In order to do so, prognostic microbiology was considered, which is commonly used in the process of controlling the growth of microorganisms in food [37, 38]. Microbiological prognosis makes it possible to forecast the development, viability or inactivation of microorganisms in food. It uses mathematical elements and the principle that the reaction of microorganisms under certain conditions is repetitive. This environment may be determined through selection of parametres such as time, temperature, pH, activity towards water and accessibility to organic compounds that are the medium helping live organisms to develop. Accuracy of forecast concerning the behaviour of microorganisms depends not only on the level at which an accepted mathematical model is adjusted but also on proper determination of the specificity of the product tested. Primary, secondary and tertiary models may be used in forecasting. Establishing the basic parameters characterising the tempo of microorganism growth under constant environment conditions is possible with the use of primary models. In

the case of including changeable environmental factors, resulting for instance from the conditions in which the product is stored, it is necessary to apply secondary or tertiary models in the form of software designed to stimulate the growth of microorganisms. Each of the prognostic factors requires validation by comparing the values forecasted and observed in microbiological tests of actual products.

A primary model was suggested in the work so as to evaluate the index of microorganism viability in bioactive filtering nonwoven designed for constructing respiratory protection equipment. The immutability of features was assumed in time and the element forecasted was the speed of microorganisms' decay under the biocidal agent anchored in fibres of filtering material.

The aim of this work was to show a procedure evaluating the efficiency of destroying microorganisms during any contact with bioactive filtering material. Model validation was performed using laboratory research into the viability of microorganisms in PLA melt-blown nonwovens modified with a biocidal agent in such a way so as the particles of the biocidal agent would only be partly emerged in the material of the fibre [32].

Theoretical evaluation

due to the fact that most biocydes used in modifying filtering nonwovens affect the cells of microorganisms during chemical reactions, this type of reaction was assumed during modelling. This allowed to use the dependencies established by Madsen, Nyman and Chick [39]. They elaborated a mathematical model for chemical disinfection on the basis of an analogy between the microbial inactivation process and first-order reaction kinetics. The model has formed the basis of most subsequent investigations. The rate of chemical deactivation of microbiological material present in an form of bioaerosol particles deposited inside a filtering material may be described by the first order equation :

$$\frac{dN}{dt} = -k_d N \qquad (1)$$

In this equation N is the instantaneous number (concentration) of live bacteria and cells in a sample, and k_d is the cell death rate coefficient. The survival of biological cells P_k within a filtering material with the biocidic agent introduced is a function of time and is defined as:

$$P_k(t) = \frac{N(t)}{N_0}; P_k(t) = \langle 1, 0 \rangle \qquad (2)$$

The number of live cells as a function of time can be obtained by the integration of (1):

$$N(t) = N_0 \cdot \exp(-k_d \cdot t) \qquad (3)$$

where N_0 is the initial number of live cells deposited within the filter structure expressed in cfu.

Due to the fact that forecasting the viability of microorganisms in filtering material is for when it is designed to be used in respiratory protection equipment, the specificity of this fact was also included; especially the fact that the number of particles blocked in these materials during the flow of bioaerosol in the inhaling phase of the user depends on the efficiency of the nonwoven's filtration. This influences the N_0 value and the initial number of live cells deposited within the filter structure. The efficiency of filtration heavily depends on the filtration parameters of the nonwoven, especially on its porosity. From the point of view of the properties of aerosol, the most important parameters influencing the efficiency of filtration are the size and shape of particles. While establishing the efficiency of filtration of nonwovens, it was assumed that the changeable factor of the system fibre – the aerosol particle deposited, will be the nonwoven's porosity. From the same perspective, calculations were carried out for the spherical particle of paraffin oil mist with a diametre of d_p=0.3, assumed to be the best particle penetrating respiratory system. Selecting a non-biological particle of aerosol results from the fact that it was proven that from the point of view of filtering mechanisms, the viability of particles is of no importance. [23, 40]. The efficiency of filtration of the nonwoven layer η for paraffin oil mist particles with a diametre of d_p=0.3 μm used in the tests, was established from the following equation:

$$\eta = 1 - P = 1 - \exp\left(\frac{-4(1-\varepsilon)E_\Sigma L}{\pi D_f}\right) \qquad (4)$$

where, E_Σ means the total efficiency of an individual fibre, including the assumed mechanisms of deposition, L the thickness of the filtering layer, and D_f is the average diameter of fibres. For the paraffin oil mist particles used in the tests, diffusion effects are of importance in deposition mechanisms as well as in inertial and direct engagement ones. The efficiency of filter deposition on a fibre as a result of diffusion effects may be derived from the following equation:

$$E_D = 2 \cdot Pe^{-2/3} \qquad (5)$$

where, Pe is the Peclet number: $Pe = df/D$. The efficiency of particle deposition on a fibre as a result of inertial mechanisms was established on the basis of the following equation:

$$E_I = \frac{J \cdot Stk}{2 \cdot Ku^2} \qquad (6)$$

where, $J \equiv \left(29.6 - 28 \cdot \alpha^{0.62}\right) \cdot R^2 - 27.5 \cdot R^{2.8}$,

$$Stk = \frac{\rho_p d_p^2 C_c U_0}{18\, \eta D_f} \qquad (7)$$

and C_c is the Cunnigham correction factor.

The effects of particle deposition on a fibre connected with direct engagement may be calculated from the following equation:

$$E_R = \cdot \qquad (8)$$
$$= \frac{1}{2\,Ku}\left[2(1+R)\cdot \mathrm{h}\,(1+R) - (1+R) + \frac{1}{(1+R)}\right]$$

where, $R = \frac{d_p}{D_f}$ and

$$Ku = -\ln(\alpha)/2 - 3/4 + \alpha - \alpha^2.$$

It was checked whether the forecasts created with the use of the simple model of prognosis suggested describe the phenomenon of microorganism viability in bioactive filtering material in a reliable manner. Validation of the forecasting model was carried out using a graphic method and applying theoretical calculations and results of microbiological studies of PLA melt-blown nonwovens [32].

Material and methods

Filter media and particles

Bioactive nonwoven

Melt-blown nonwovens were used in the study, made from poly(lactic acid) polymer (PLA) modified with a biocidal agent. Nonwovens were formed from PLA polymer 6202 D, produced by NatureWorks (USA), LLC. The melting temperature of the polymer was 160 - 170 °C, and the polymer flow index remained at the level of 15 - 30 g/10 min at the temperature of 210 °C. (based on information from the producer). Nonwovens were produced using the melt-blown method. A biocidal agent was used in

the modification of PLA fibres, and lyophilised bioperlite was added at this stage [10, 14, 17], consisting of perlite with a grain diameter no bigger than 100 μm and with the biocidal agent applied on its surface. The biocidal agent was dosed centrally to the head and symmetrically to the zone of fibre production [10, 17]. The idea of the solution was based on introducing the biocidal agent into the stream of blown PLA polymer and coupling it permanently but partially with the nonwovens produced, composing the filtering material. In this way the likelihood of the majority of Bioperlite particles being able to negatively affect bacteria captured by the filtering structures (fibres) from the stream of flowing air was increased. Fibres created in this way were then stretched between the head and the receiving device and additionally activated electrostatically (with the use of corona discharge) at a voltage of 30 kV. Research connected with elaborating the above Bioactive PLA nonwoven technology was described in [32]. Variations in the created nonwovens used in validation of the forecasting model of viability of microorganisms in bioactive filtering nonwoven are presented in ***Table 1***.

The average diametre (d_f) of PLA fibres was around 1.5 - 2.0 μm. For the surface density of the four types of nonwovens measured, a dependence was established between the average porosity ε [-] and the thickness of the nonwoven layer (*L*). PLA density ranged between 1210 - 1430 kg/m^3. An average value of 1340 kg/m^3 was assumed for the measurements. For the filtering nonwovens prepared, surface densities and their thickness were established, and then their porosity ε was calculated. The porosity calculated for two thicknesses of the bioactive nonwovens are contrasted in ***Table 2***. Just the measurement of the thickness of a highly porous material was vitiated by an error which influenced the values of the porosity of the nonwovens calculated.

Microorganisms

Two strains of bacteria were used: *Pseudomonas aeruginosa* – rod- shaped gram negative bacteria with a diameter of 0.5 - 0.8 μm by 1.5 - 3.0 μm, aerodynamic diameter of 0.8 μm. and *Staphylococcus aureus* – cocci gram-positive bacteria with a diametre between 0.8 and 1.2 μm. The average aerodynamic diameter was 1.0 μm. Strains of bacteria came from the American Type Culture Collection (ATCC), stored according to international standards in the form of frozen lyophilisate of active cells from a 24-hour cultivation on TSB scaffolding (Caso Bulion, tryptic-soy broth, pH 7.3, of Merck with a 3% additive of yeast extract).

Table 1. *Characteristic of PLA melt-blown nonwoven used in validating a forecasting model of microorganism viability.*

No	Nonwoven content	Corona discharge	Surface mass, g/m^2
1	PLA + Bioperlite	+	96.0 ± 1.92
2	PLA		124.0 ± 3.72
3	PLA + Bioperlit		134.0 ± 2.68
4	PLA		90.0 ± 1.80
5	PLA + Bioperlit	-	106.0 ± 5.30
6	PLA		118.0 ± 2.36
7	PLA		96.0 ± 1.92
8	PLA +Bioperlit		134.0 ± 2.68

Table 2. *Porosity (ε) and packing degrees (α = 1 - ε) of the bioactive PLA filtering nonwovens.*

No	L = 1, mm	L = 2, mm	L = 1, mm	L = 2, mm
	α, -	α, -	ε, -	ε, -
1	0.07	0.03	0.93	0.97
3	0.10	0.05	0.90	0.95
5	0.08	0.04	0.92	0.96
8	0.10	0.05	0.90	0.95

Testing methods

The method of carrying out research into the viability of microorganisms in the bioactive filtering nonwoven was performed according to microbiological procedures described in [32]. Samples of bioactive nonwovens (filter with a diametre of 80 mm) were not sterilised prior to the microbiological tests for fear of changing their physico-chemical properties. Only their microbiological purity was checked prior to the tests. The number of microorganisms in the fibre was statistically insignificant in relation to the test results. The samples tested were stored on sterile, disposable Petri dishes, Rodac type. Control samples of the nonwovens (with no biocidal agent) and bioactive nonwovens were grafted with the bacteria tested as a result of a 15-minute exposure to the flowing bioaerosol. The bioaerosol moved at a volumetric flow rate of 30 l/min. The place and method of studying phenomena connected with the bioaerosol impact of filtering materials in conditions simulating using RPD were described in detail in [32]. The samples were secured against drying by adding 0.1 ml of sterile distilled water onto the edges of a Rodac dish where grafted samples of the nonwovens were placed and then sealed outside with a strip of parafilm. Control samples of the nonwovens and the bioactive ones to be used in microbiological tests were taken immediately after taking them out of the measuring system (marked as 0) and after 2, 4 & 8 hours from bioaerosol having stopped passing through the samples tested. Following that, the samples were transferred to containers of 200 ml volume with sterile saline at 99.9 ml volume so as to rinse bacteria from the nonwoven, and shaken for 15 minutes in a water bath at a temperature of 37 °C on a shaker type *Water Bath Shaker 357,* at a frequency of rotation of 150 c.p.m. Then every sample was diluted in sterile saline until the following dilution was obtained: 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, cultivated at 0.1, and 1 ml of the diluted sample was placed onto a sterile Petri's dish. The sample was poured over with a semi-liquid TSA medium (Caso Agar, tryptic – soy agar with polysorbate 80 and lecitin, pH 7.3, of *Merck*), mixed and left to settle. Bacteria cultures were incubated at a temperature of 37 °C for 24 hours, after which time the colonies were calculated. From the results obtained, the average was calculated for every bacteria strain tested and for each hour of exposure. There were 5 repetitions of tests. For each time of contact between a bacteria strain and the samples of nonwovens, bacteria survival was determined (*P*) as the number of bacteria grown that had survived contact with the bioactive nonwoven compared to the number of bacteria grown on the non-bioactive nonwoven. This number oscillated around 2.6×10^7 cfu/ml for *P. aeruginosa* and 6×10^7 cfu/ml for *S. aureus*.

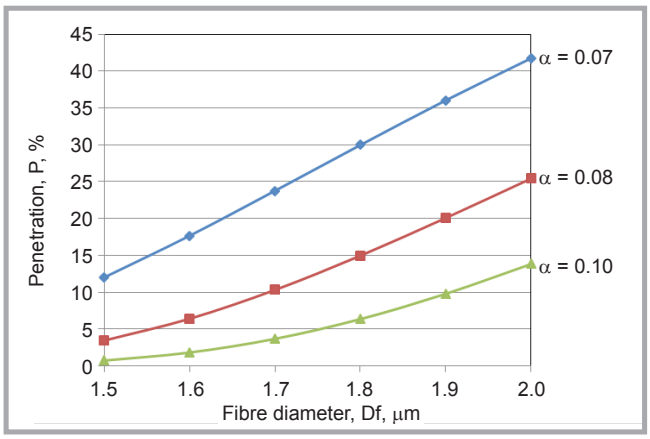

Figure 1. *Calculation of values of the coefficient of penetration for bioactive PLA nonwoven of thickness L = 1 mm for different packing degrees α.*

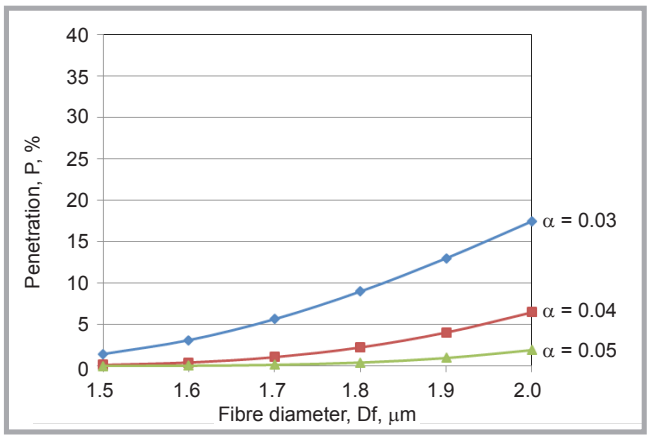

Figure 2. *Calculation of values of the coefficient of penetration for bioactive PLA nonwoven of thickness L = 2 mm for different degrees of packing α.*

$$P = \frac{N_0 - \overline{N}}{N_0} \times 100 \qquad (8)$$

where,

P – bacteria survival in %,

N_0 – average number of bacteria in the control sample without the biocidal agent,

N – average number of bacteria grown on the bioactive nonwoven.

Results and analysis

Including such mechanisms of deposition as diffusion, inertia and direct engagement, calculations were performed concerning the value of particle penetration through the layers of the nonwoven of thickness 1 mm and 2 mm for the assumed coefficients of packing of the layers within the range of 0.03 and 0.10. The results of calculations are presented in ***Figures 1*** and ***2*** (for the apparent speed of airflow U = 0.5 m/s).

Bioaerosols of two bacteria strains were used in the tests: Cocci bacteria of *S. aureus* that had the shape of rods with an aerodynamic diameter of $d_1 = 0.80$ μm and *Staphylococcus aureus* bacteria that had the shape of a sphere, with a diameter of $d_2 = 0.5$ - 1.5 μm (1.0 - 1.3 μm) and density that equalled that of water. Bioaerosol particles that were deposited on the test nonwoven underwent the same deposition effects as particles of paraffin oil mist. Therefore the value of penetration was assumed to be the same for both bacteria tested. Even though in laboratory tests electret and non electret bioactive PLA nonwoven were used, calculations did not include electrostatic charges. The electric state of nonwovens produced with the use of an electric field was manifested by a lower value of the penetration coefficient; however, it was assumed that it would not have a significant influence on the result of microorganism viability. Gravity effects were also not included in the calculations.

The quantity of the live bacteria detected within the filtering material under testing showed a gradual decrease in time. The reduction in the live population of bacteria was caused by the action of the biocides introduced earlier to the filter structure. The biocide molecules realized from the bioperlite particles gradually killed the live population of bioaerosol deposited within the filter structure. The process of disinfection of the filter samples with the help of the biocide used proceeded at various rates depending on the type of microorganisms applied and on the initial way of preparation of the filtering material. Experimental values of the survival of *Staphylococcus aureus* were compared with those calculated, shown in ***Figures 3*** and ***4***. ***Figure 3*** shows a graphic validation of the elaborated forecasting model of microorganism viability in PLA nonwoven that underwent activation in a corona discharge field. ***Figure 4*** presents the results of calculations and experiments in relation to the case where there is no electret PLA nonwoven.

Experimental values of the survival of *Pseudomonas aeruginosa* were compared with those calculated, shown in ***Figures 5*** and ***6***. ***Figure 5*** presents a graphic validation of the elaborated forecasting model of microorganism viability in PLA nonwoven that underwent activation in a corona discharge field. ***Figure 6*** presents the results of calculations and experiments in relation to the case where there is no electret PLA nonwoven.

The graphs in ***Figures 3 - 6*** present the results of a graphic method of validation of forecasting models according to the rules described in [37, 38]. This method is frequently used for simple microbiological forecasting as it provides a quick evaluation of microorganism growth in constant environmental conditions assumed. Thus it needs to be emphasised that the method described should only be used when evaluating the biocidal properties of materials which were not used and when performing a qualitative comparative analysis of new materials. It does not include the dynamic conditions that take place inside filtering materials while they are being used in respiratory protection equipment; especially changes in humidity, temperature, pH, interactions between microorganisms, accessibility of nutrients, and many other environmental factors. In the case of a dynamic model, it would be necessary to validate it mathematically using an accuracy factor A_f and bias factor B_f [37]. While comparing the results of microbiological forecasts obtained for food [38] with those presented in ***Figures 3 - 6***, it can be concluded that satisfactory compliance with experimental results was obtained in relation to *Staphylococcus aureus,* regardless of whether the nonwoven was or was not activated in the corona discharge field. In the case of *Pseudomonas aeruginosa,* there was a quick dieback of microorganisms in contact with the bioactive nonwoven. This phenomenon occurred at the first moment of of contact between the biocide and microorganisms (after 2 hours). Therefore the curves presented in ***Fig-***

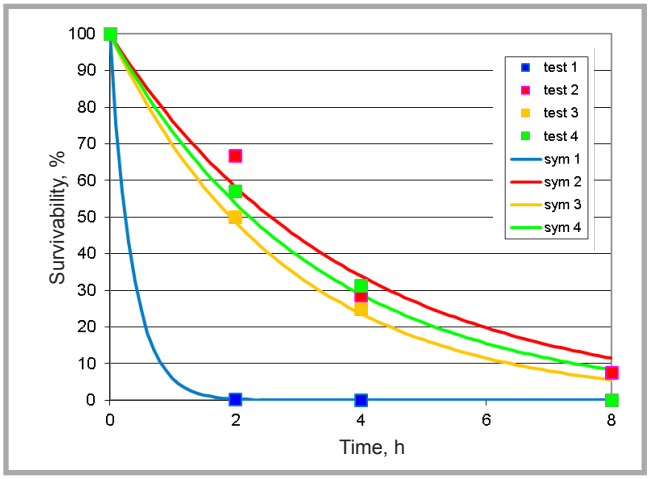

***Figure 3**. Results of survival values for electrets PLA nonwoven (tests 1 - 4) together with calculated survival lines (sym. 1 - 4) for Staphylococcus aureus.*

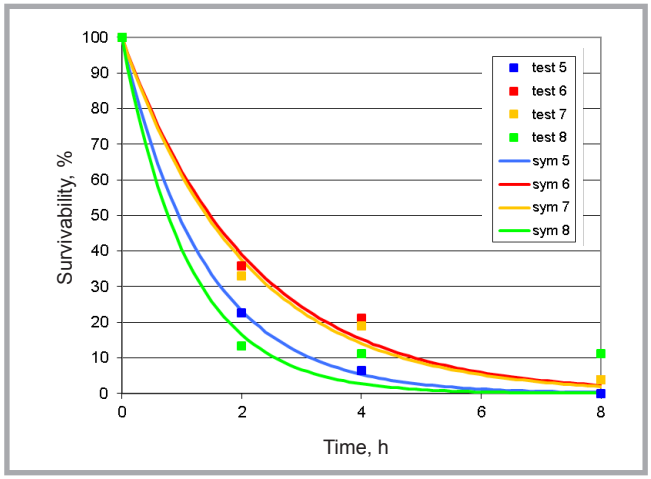

***Figure 4.** Results of survival values for non-electret PLA nonwoven (tests 5 - 8) together with calculated survival lines (sym.5 - 8) for Staphylococcus aureus.*

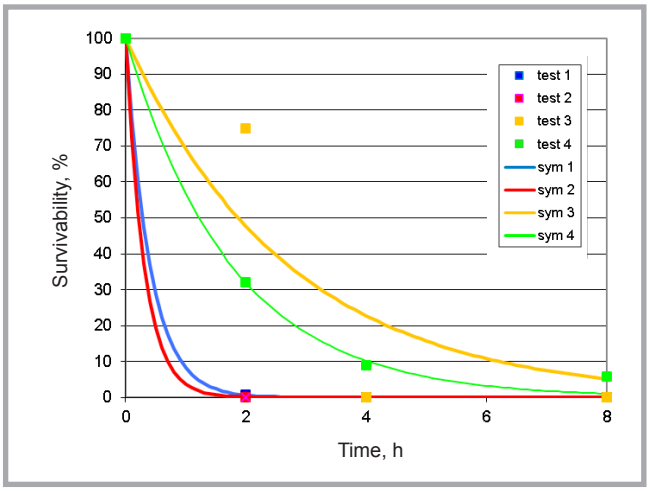

***Figure 5.** Results of survival values for electrets PLA nonwoven (tests 1 - 4) together with calculated survival lines (sym. 1 - 4) for Pseudomonas aeruginosa.*

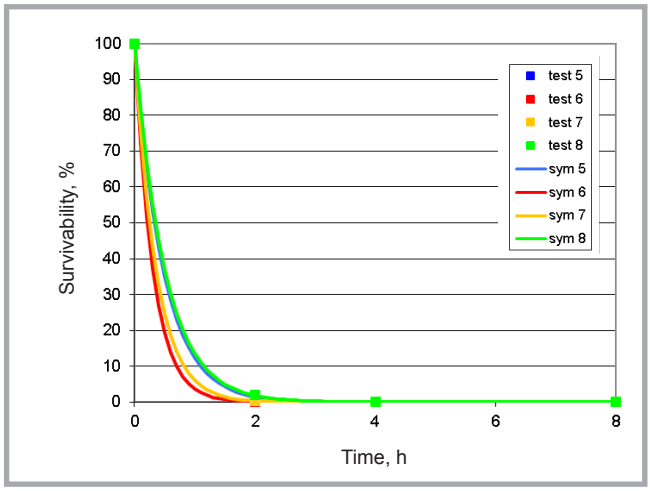

***Figure 6.** Results of survival values for non-electret PLA nonwoven (tests 5 - 8) together with calculated survival lines (sym. 5 - 8) for Pseudomonas aeruginosa.*

ures 5 - 6 overlap, which makes it impossible to interpret them correctly. In order to establish the compliance of the values of viability of *Pseudomonas aeruginosa* forecasted with the actual measurements, it would be necessary to carry out these tests in shorter intervals. This leads to the important conclusion that in the phase of designing experiments used while validating forecasting models, it is necessary to determine the sensitivity of the microorganisms tested to the disinfection conditions in bioactive filtering nonwovens. It needs to be emphasised that microbiological forecasting models are a new tool used particularly in ensuring the safety of food. The quick development of these methods is predicted, however, with particular emphasis placed on artificial neuron nets. It is assumed that they will meet requirements concerning the dynamics of the behaviour of microorganisms in food, with particular emphasis placed on the interaction that takes place between them. Thus it will be possible to carry out further works aimed at developing the model suggested to forecast the viability of microorganisms in bioactive filtering materials. This work also fits with the general tendency to model the efficiency of respiratory protection equipment in relation to the conditions of its use forecasted. To date, these works have been carried out in the area of filtering half-masks designed to be used in protection against flu virus type A (H1N1) [41].

Death rate constants k_d, which describe the survival of bacteria according to ***Equation 3***, were calculated for all tests performed with the use of the least squares method. Values of the death constants calculated are presented in ***Table 3*** for *Staphylococcus aureus* and ***Table 4*** for *Pseudomonas aeruginosa* (see page 112).

The Pseudomonas aeruginosa species showed great sensivity to the filer biocidic agent applied within the filter. Exact values of the death constant k_d were hard to find. In ***Figure 7*** (see page 112) there are death constant values calculated for all the tests performed for both bacteria species and for all filtering material samples.

The viability of both bacteria strains in similar test conditions shows general differences resulting from the different susceptibility of these species to contact with PLA bioactive nonwovens. *Staphylococcus aureus* proved less sensitive to the biocidal agent applied, which means that this strain of bacteria is more resistant to contact with the biocidal agent present inside the nonwoven. At the

Table 3. *Values of death rate constant (k_d) calculated for Staphylococcus aureus.*

No	Nonwoven content	Corona discharge	k_d, 1/hour
1	PLA + Bioperlite	+	0.31
2	PLA		2.82
3	PLA + Bioperlite		0.73
4	PLA		0.49
5	PLA + Bioperlite	-	0.36
6	PLA		0.27
7	PLA		0.90
8	PLA + Bioperlite		0.47

Table 4. *Values of death rate constant k_d calculated for Pseudomonas aeruginosa.*

No	Nonwoven content	Corona discharge	k_d, 1/hour
1	PLA + Bioperlite	+	0.57
2	PLA		2.50
3	PLA + Bioperlite		2.10
4	PLA		2.80
5	PLA + Bioperlite	-	0.37
6	PLA		3.30
7	PLA		2.00
8	PLA + Bioperlite		3.30

same time, high values of the death rate coefficient for *Pseudomonas aeruginosa* are evidence of rapid population extinction of this bacteria strain in the fibre structure. After two hours, the viability of *Staphylococcus aureus* was around 50% or more, for *Pseudomonas aeruginosa* - less than 40%, and for a further 4 hours of viability these values were around 30% and less than 20%, respectively. A reflection of these dependencies are lower values of the death coefficient k_d established for *Staphylococcus aureus* in the range between 0.27 and 0.73 (except for sample No 1, where $k_d > 1.0$). The viability coefficient for *Pseudomonas aeruginosa* had a high value of $k_d > 3.0$ for five samples of the nonwovens, and for the rest they ranged between 0.50 and around 2.0. This confirms the fact that *Pseudomonas aeruginosa* is highly sensitive to contact with Bioperlite, with its population undergoing rapid extinction. Comparing samples of the nonwovens with and without Bioperlite, a clear difference can be observed in the values of death rate coefficients established. These coefficients for samples No 1 and 3 with Bioperlite were higher than for samples No 2 and 4 without Bioperlite. A similar dependency can also be seen for the values of death rate coefficients for samples 5 - 8, which with with Bioperlite have slightly higher values of death rate coefficients than samples 6 and 7 without Bioperlite.

Conclusions

1. Almost in any case of filter samples, despite the preparation procedure, *Pseudomona aerugiosa* showed greater sensitivity to the test conditions and indicated higher death rate coefficients.
2. The presence of the biocide within the filter structure did not indicate a clear influence on bacteria survival.
3. The performance of the filter samples charged with the use of corona discharge only partly improved the bioactivity of the filters, which can be observed for both bacteria species in the case of the samples 1 and 3 and samples 5 and 7.
4. Evidently the death rate coefficient did not depend on the surface density of the filter samples.

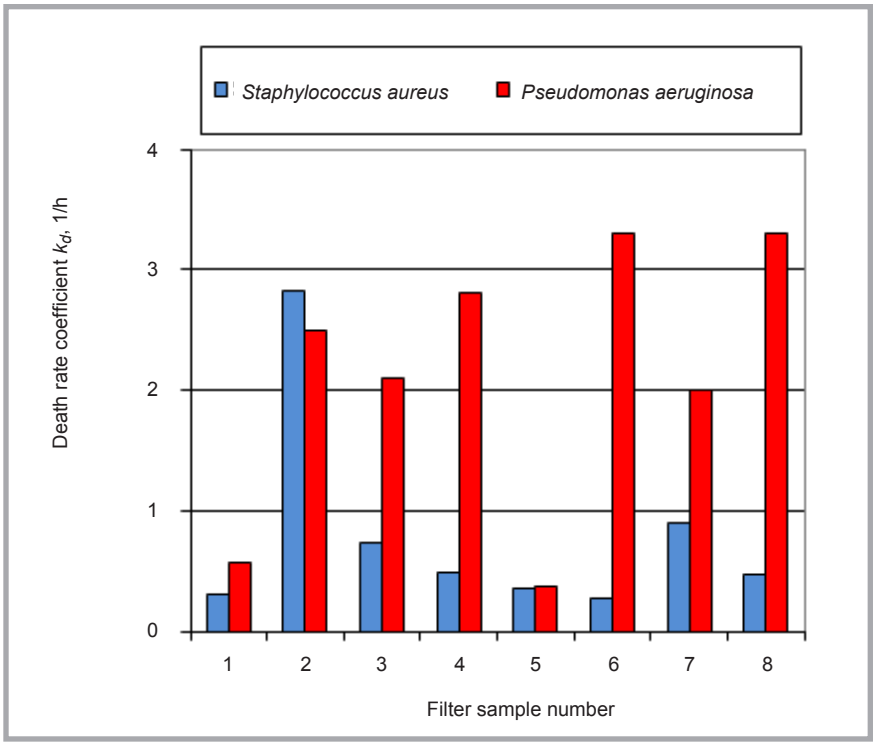

Figure 7. *Comparison of death rate coefficients established from experimental data for both bacterial species and for all filter samples.*

Summary

The simple model forecasting the viability of microorganisms in filtering nonwoven may be recommended when carrying out a qualitative evaluation of new bioactive filtering materials. It needs to be stressed that limitation of use may occur when there is a quick dieback of microorganisms in contact with the biocidal agent included in the nonwoven. This model does not include the dynamics of processes that take place in bioactive materials during use in conditions that expose harmful bioaerosol. In this area, further improvements are necessary. The use of neuron nets is a promising direction.

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