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Biocidal Agent for Modification of Poly(lactic acid) High-efficiency Filtering Nonwovens

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Abstract
The article presents research on the development of modified poly(lactic acid) (PLA) melt-blown nonwovens for construction of respiratory protection devices (RPD) against biological hazards. Due to this specific application of nonwovens, a new biocidal agent was developed which fulfils two basic criteria. The first one is the efficient destruction of microorganisms harmful for people, and the second is no negative influence on compost activity in the process of BIO-nonwoven biodegradation. The biocidal agent was composed of biologically active chemical compounds, which were permanently imbued into the perlite matrix. The modified poly(lactic acid) (PLA) melt-blown nonwovens were tested for basic filtering parameters with the use of standard model aerosols as well as bioaerosols. A microbiological study was performed to prove the antimicrobial activity of the modified nonwovens against Gram-positive and Gram-negative bacteria. It was shown that modified PLA at low concentrations did not eliminate microorganisms that dwell in organic compost, and that the time of BIO-nonwoven biodegradation was 56 days. Survival of microorganisms after 2 hours of contact with the BIO-nonwoven was zero %, which means that the nonwovens tested were characterised by activity at the highest level, 3rd,, towards Gram-positive and Gram-negative bacteria. At the same time, the effectiveness of the BIO-nonwovens towards model aerosols (sodium chloride and mist of paraffin oil) was at a level that would ensure obtaining a 2nd class of protection of the filtering RPD.

Key words: *biocidal agent, poly(lactic acid), melt-blown nonwoven, respiratory protective devices (RPD).*

Introduction

Filtering materials used in the construction of respiratory protection devices (RPD) should fulfil all requirements concerning protection and usage parametres included in the European Norms (EN) and also any additional criteria connected with the prospective usage of such equipment. Equipment dedicated to protection against biological threats is such a case. It is vital that it has to block bioaerosol particles effectively, but also should be biocidal towards microorganisms throughout the time of it being used. The latter of the criteria listed stems directly from the confirmed ability of microorganisms to survive for a long time in polymer nonwovens that are used in air purification (filtration) [1 - 3].

Sulphur dioxide was the first biocidal compound, used for several centuries as the main agent that prevented illnesses. The next groundbreaking compound was when salts and oxides of heavy metals were applied, e.g. mercury, lead, arsenic, copper and iron, followed by compounds of phenol used in the disinfection of premises and operating theatres. Currently there are several millions of compounds that are synthesised, at least a few thousand of which present good biocidal activity. Due to great, and still growing by some 4% annually, demand for biocidal compounds, there has been a necessity to use them properly so as to minimise the adverse effects. The basic document introducing the idea of uniform documentation for active substances and biocidal products within the European Union countries and also for Switzerland, Norway and Iceland was 98/8/WE Directive of the European Parliament and the Board concerning introducing biocidal products onto the market [4], which is now modified by Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products [5].

The first step that results from the Directive was the registry of active substances, followed by the evaluation and registration of biocidal products that included registered active substances. The registry of active substances is done at the EU level, whereas registration of biocidal products is performed at that of member states. The registration of new active substances is very expensive and forecasts for the future show a further tightening of these procedures. Due to that fact, the best solution is to make use of the active substances already registered while working out new biocidal agents. Research into the development of new biocidal agents should thus be concentrated on maximising their efficiency by creating mixtures of several active substances with the synergy effect. It is also important to take precautions as the synergy effect may also occur in relation to toxicity and be hazardous to environment. Thus it must be demonstrated for every biocidal product introduced for use that this effect does not occur. It is also important to note that permanent use of the same active substance can cause the increasing resistance of microorganisms. Therefore effective biocidal formulations should contain at least one active substance and some other supporting compounds to avoid an increase in the resistance of bacteria [6].

There are several solutions for the use of various biocidal agents in materials designed for the construction of RPD. The first reports concern the use of triclosan [7] that was incorporated into fibres from poly(vinyl) chloride (PVC), from which air filtering materials were later produced. Toxicological studies proved that this biocide may pose a hazard connected with the possibility of producing highly harmful dioxanes. This prompted a search for other solutions that would minimise the potentially harmful influence of this biocide on the human organism. For instance, a USA patent presents an invention comprising nontoxic, environmentally-friendly triclosan-containing antimicrobial filters [8]. The idea of this invention was based on creating two systems of filtering layers, the outer of which included triclosan, whereas the other one, in contact with the skin, absorbed the particles of triclosan released. There have also been works carried out on other biocidal agents, for example in a USA patent it was iodinated resin [9], and in a Polish solution, biocidal agent Sanitized® was used [10], applied onto the nonwoven in the finishing process (bath, spraying). Another solution was equipment called Bioprotectives (filters and filtering half-masks), worked out on the basis of nonwovens produced using PP polypropylene fibres modified with a biocidal agent called Bioperlite [11,12] and then applying the melt-blown technique [13 - 15].

Progress in the development of biodegradable materials and research into how they could be applied in equipment protecting a man from hazards was the driving force that provoked further works on the development of bioactive materials. Article [16] described the electrospinning processing of PLA and the influence of silver nanoparticles on the morphology and microstructure of nonwoven membranes thus produced. PLA was electrospun from a chloroform solution and a filamentary and granular morphology was obtained, the filaments having an average diameter of 1.25 μm. When silver nanoparticles were incorporated, the filament diameter was reduced to an average of 0.65 μm and the density of beads was also decreased. Also electrospun PLA membranes loaded with sepiolite fibrillar particles functionalised with silverand copper (ATP) were described in [17]. The concentration of ATP in the membrane surface was used to assess the active biomass content, showing a significant decrease in silver and copper functionalized sepiolite in relation to to PLA. Recently a novel heterocyclic N-halamine acetate homopolymer has been synthesised [18]. Homopolymer was coated onto PLA melt-blown nonwoven fabric, and the surfaces took on biocidal properties after exposure to a diluted sodium hypochlorite solution. The coatings were quite stable versus UVA and florescent light exposure. Moreover they exhibited long-term shelf-life stability and were rechargeable when oxidative chlorine on the surfaces was partially exhausted after three month's storage. Additionally works were carried out aimed at working out a technological condition for the production of melt-blown nonwovens from biodegradable polymers and their application in filtering half-masks [19]. These works were further continued towards the modification of PLA fibres with the biocidal agent Bioperlite during the time of fleece formation [12, 20]. While establishing the content of Bioperlite, no criteria were applied connected with its susceptibility to being biodegradable in organic compost or the negative influence of PLA properties, which contributed to the continuation of works towards the modification of its chemical content. High expectations are currently placed on biocides, such as a wide range of activities, stability, low toxicity and biodegradability. At the same time, it was important that the biocide worked immediately at low concentration and caused no changes in the properties of a product that is in contact with. The economic aspect was relevant as well, since products that are to be used for the protection of workers against hazards, should not significantly influence labour costs. Considering all of the above, a new and cheap biocidal agent was prepared designed for the modification of polymer materials, PLA in particular. All aspects were considered, including technological and functional ones, connected with the application of filtering materials that include this agent in the construction of RPD's.

The basic aim of the study was to show that the biocidal agent established for modifying filtering materials with PLA used in the construction of RPD's meets technological and performance requirements in relation to its functionality, and at the same time not inhibit the biodegradation of these materials in the environment of organic compost.

Experimental

Materials

Polymers

Melt-blown nonwovens were made from PLA (United Kingdom). The polymer's characteristics are presented in ***Table 1***.

Biocidal agent

A biocidal agent worked out by the authors was used in the modification of melt-blown nonwovens. It was created on the grounds of previous experiments on the "Bioperlit" agent (anhydrous mixture of perlite and active substances) based on invention [12]. The need to modify "Bioperlit" (mostly active substances) resulted from the necessity to work out such a biocidal agent that would, on the one hand, ensure the biocidal effect while using respiratory protective equipment, but on the other, not have a negative effect on compost microorganisms.

A substrate for the biocidal agent was perlite. It was necessary to fulfil the condition that all active compounds included in the biocidal agent also met the requirement of EU Directive 98/8/EC [4] and complement Regulation (EU) No 528/2012 [5], complying with the condition of active compounds not being harmful to potential users of respiratory protection equipment. Apart from this basic condition, during the selection of active compounds that would be included in the biocidal agent, also criteria connected with affinity for liquids and permittivity were considered. Thanks to this, effective adhesion of microbiocides was ensured on the surface of hydrophilic perlite, Based on which, aliphatic polyamines and quaternary ammonium salts were selected. On the basis of these active compounds and the synergism of polyamine and quaternary ammonium salts, a biocidal agent was worked out that included the following ingredients:

- N-3-aminopropyl-N-dodecyl-1,3-propanediamine (Germany),
- N,N-didecyl-N-methyl-N-poly-(oxyethyl) ammonium propionate (USA),
- N,N-didecyl-N,N-dimethylammonium chloride (Poland),
- monoethanoloamine (Poland),
- ethylene glikol (Poland),
- glicerol (Germany),
- acetic acid (Poland),
- water (Poland).

The total content of active compounds in the formulation was 28.0%.

In order to evenly incorporate the biocidal agent onto perlite with a grain size no bigger than 50 μm, the final concentration of active compounds (AC) was determined in the modified perlite (AC%), and subsequently an appropriate amount of perlite (M_{perlit}) and biocidal agent

Table 1. *Characteristics of PLA polymer used in creating melt-blown nonwovens; * MFI (melt flow index) according to producer's data, ** MFI according to PN-EN ISO 1133:2002, temp. 210 °C, 2.16 kg. Tests were carried out at the Centre of Molecular and Macromolecular Studies - PAN, Lodz, Poland.*

Polymer type	Producer/Supplier	Flow index MFI*, g/10 min	Flow index MFI**, g/10 min
PLA 6202 D	NatureWorks, LLC	15 - 30	75.16 ± 6.72

(M_{PB-1}) was prepared according to the formula:

$$AC(\%) = 28\ M_{PB-1}/(M_{perlit} + 0.28\ M_{PB-1}) \quad (1)$$

The amount of biocidal agent (M_{PB-1}) calculated was dissolved in a tenfold higher amount of propanol-2 ($10 \times M_{PB-1}$). Subsequently, while still stirring, a proper amount of perlite (M_{perlit}) was slowly added to the solution prepared. The total amount of the product obtained that took the form of thick suspension was placed in a closed mixer for at least 4 hours at a temperature of 25 °C. The final product was transferred to a vacuum evaporator. The solvent was evaporated at a temperature of 35 - 40 °C under a reduced pressure of below 0,5 mmHg. The residue was dried at a temperature of 20 ± 2 °C under normal pressure for 48 hours and then under lower pressure (< 1.0 mmHg), at a temperature of 20 - 30 °C, for 24 hours. After completing the drying process, the modified perlite underwent homogenisation. The biocidal agent prepared in this way was marked with the symbol PB-1. The content of biocidal agent and its preparation method was submitted to the patent office as an invention [21].

Preparation of BIO-nonwovens

Biodegradable PLA polymer granulate placed in the extruder was melted at a temperature of 270 - 280 °C in zones 1 and 2 of extrusion. A polymer melt flow of 4 g/min was introduced into the fibre-head, heated to a temperature of 210 - 230 °C, which was followed by extrusion through holes in the spinning nozzles. Polymer was blown in the strong flow of hot air at a temperature of 270 - 300 °C. Microfibres created in this way were then stretched between the head and receiving device and additionally activated electrostatically (with the use of corona discharge) at a voltage of 30 kV. The distance between the mesh receiving device and the head was 300 mm.

Biocidal agent PB-1 was dosed in a controlled way into the head, and centrally and symmetrically into the zone where fibres were produced. The solution consisted of introducing the biocidal agent into the stream of polymer and permanently combining it with the PLA polymer by applying an originally worked out construction of the fibre-head, described in detail in [13] and patented in [14]. The biocidal agent was added to the molten polymer in the amounts of 5% or 10% by weight relative to that of dry PLA granulate. During the tests, the following marking of BIO-non-woven variants were used:

- sample 0 – PLA without biocidal agent,
- sample 1 – PLA with 5% of biocidal agent,
- sample 2 – PLA with 10% of biocidal agent.

Testing methods

Biocidal agent

- *Impact of compost*

Microbiological tests were carried out at the Laboratory of the Institute of Biopolymers and Chemical Fibres (IBWCh, Łódź, Poland). Standard compost produced by the Department of Composting Plant at Łódź Municipal Services was used in the tests.

Microbilogical studies of the PB-1 biocidal agent were performed only in order to check whether it had a destructive effect on microorganisms included in the compost. Should such a phenomenon occur, it could have some influence on the biodegradation of nonwoven modified with this agent. Concentration values used in the study were selected bearing in mind the worst possible scenario. If it does not influence the quality of compost at high values, it would not do so at low concentration values either.

Microbiological tests were based on the evaluation of the efficiency of the biocidal agent at four concentrations: 10, 15, 20 and 30% towards microorganisms present in the compost (biocidal activity and the growth inhibition zone).

- *Durability and influence of the PB-1 agent on PLA fibres.*

In order to illustrate the thickness of fibres and evenness of the biocidal agent spread in the structure of PLA nonwovens, a picture was taken using a scanning electron microscope - VEGA 5135 MM Tescan (United Kingdom). So as to assess the durability of the biocidal agent and its influence on PLA fibres, elementary analysis was applied, as well as spectroscopy (FTIR) in infrared and ultraviolet.

BIO-nonwoven

Determined elements:

- Surface mass according to PN-EN 29073-1:1994,
- Index of penetration of paraffin oil mist according to EN 13274-7: 2008,
- Index of penetration of sodium chloride according to EN 13274-7: 2008,
- Index of penetration of paraffin oil mist in time according to EN 13274-7: 2008,
- Value of air flow according to EN 13274-3:2001,
- Biodegradability in the compost environment according to a research procedure of the accredited Biodegradability Laboratory at IBWCH based on norms: PN-EN 14045:2005, PN-EN 14 806:2010 and PN-EN ISO 20 200:2007 entitled: ”Determination of the degree of degradation of plastics in simulated conditions of composting on a laboratory scale”
- Survival of organisms in contact with biocidal agent carried out according to methodology described in work [20]. Studies were carried out at the Laboratory of Biological Hazards in the Department of Chemical, Powder and Biological Hazards of the Central Institute for Labour Protection – National Research Institute (CIOP-PIB, Łódź, Poland).

Due to the fact that nonwovens were assessed considering their use as material in constructing respiratory protection equipment of different protection classes, tests were performed on one and three folded layers of PLA non-woven modified with biocidal agent PB-1.

Results and analysis

Biocidal agent

The results are shown in Figure 1.a - 1.e. During the research into the influence of PB-1 biocide at a concentration of 10% on microorganisms present in compost, no inhibition zone was observed on the growth of microorganisms around the wells filled with the solution of this agent. Inhibition of the growth of microorganisms was noted for a PB-1 concentration of 15%. This activity increased together with an increase in concentration (20 and 30%). That is why, during the modification of filtering nonwovens, it was necessary to optimise the concentration of biocidal agent PB-1 as a too high content of it included in the filtering material could have provoked the phenomenon of growth inhibition for microorganisms in compost and, as a result, worsen its quality.

In order to illustrate the thickness of fibres and the evenness of the spread of

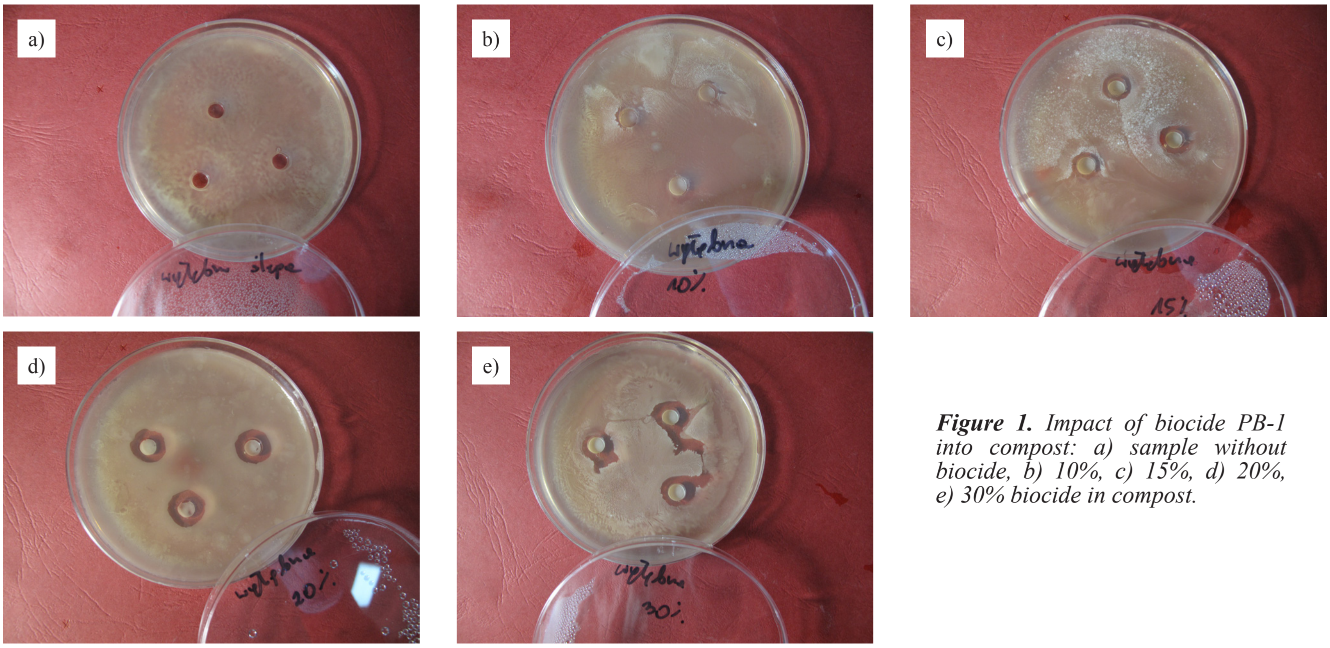

Figure 1. Impact of biocide PB-1 into compost: a) sample without biocide, b) 10%, c) 15%, d) 20%, e) 30% biocide in compost.

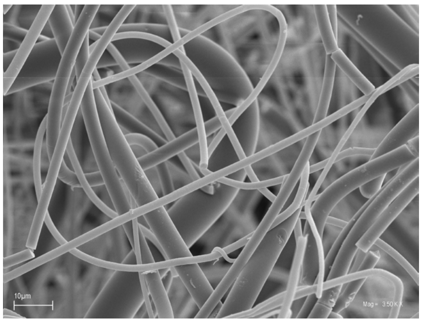

***Figure 2.** View of the surface of pure filtering nonwoven without a biocidal agent (sample 0).*

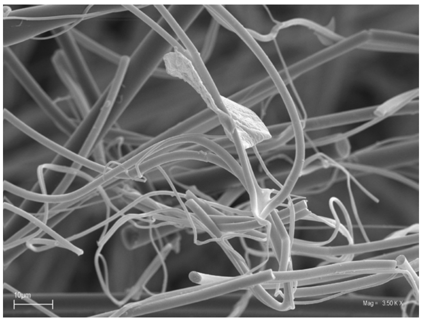

***Figure 3.** View of the surface of filtering nonwoven with biocidal agent at 5% (sample 1).*

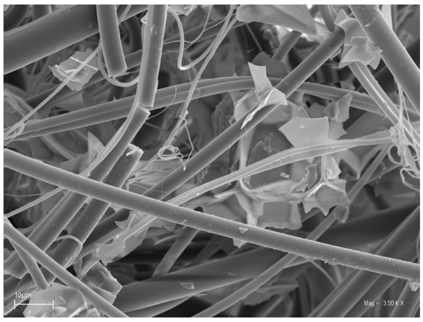

***Figure 4.** View of the surface of filtering nonwoven with biocidal agent at 10% (sample 2).*

the biocidal agent in the structure of PLA nonwovens, pictures were taken using a scanning electron microscope - VEGA 5135 MM Tescan (***Figures 2 - 4***).

The total amount of active substances in the modified PLA was determined by elemental analysis. In the starting formulation of PB-1, the nitrogen content was 3.26%. As can be seen from ***Table 2***, the average content of nitrogen in the bio nonwovens produced is 0.048% and 0.14% for samples 1 and 2, respectively, corresponding to 0.41% and 1.2% of the average content of active substances in the modified PLA.

***Table 2.** Results of elemental analysis for samples investigated.*

Sample	Average content of nitrogen, %	Average content of active substances, %
0	0.000	0.00
1	0.048 (SD 0.002)	0.41(SD 0.01)
2	0.140 (SD 0.003)	1.20 (SD 0.01)

FTIR spectra of samples 0, 1 and 2 are presented in ***Figure 5.a,*** see page 52, (range of 3500 - 450 cm^{-1}) and ***Figure 5.b*** (extended range of 1900 - 1300 cm^{-1}). Two fragments, A and B, of samples 1 and 2 were investigated by FTIR to check the even layout of the biocide in the modified material. A symmetric stretching vibration band of the carbonyl group, $\nu_{c=o,}$ in pure PLA was observed at 1751 cm^{-1}. The position of this band was the same in the modified PLA (***Figure 5.b***), clearly indicating that biocidal agent PB-1 did not change the structure of PLA. Some changes in FTIR spectra of the modified PLA in comparison to virgin PLA were observed in the ranges of 3500 - 3200 cm^{-1} & 3000 - 2800 cm^{-1} and in the "finger print" area. These changes were assigned to the stretching vibration bands of the N-H group of amines, which were components of PB-1, as well as stretching and deformation bands of CH_2 and CH_3 groups from hydrocarbon chains of biocidal substrates. Those bands gave mainly qualitative information, with no analytical applications.

The analysis performed showed that the interaction of the active substance of PB-1 deposited on perlite is primarily of a physical nature. Such intereactions do not cause any decrease in the biocidal activity of the formula. The strong adhesion of active substances to perlite did not allow an uncontrollable release of the agent from perlite, which has a fundamental meaning. The biocidal agent introduced into PLA material using the melt-blown method is connected with the nonwovens by means of adhesive forces. As can be seen from the SEM images (***Figures 3, 4***), the biocidal agent is woven into the fibres, which – on the one hand – makes it impossible for it to be released, but on the other , active substances deposited on perlite are free to react with microorganisms. Due to it being not an easy method of introducting a biocidal agent into PLA

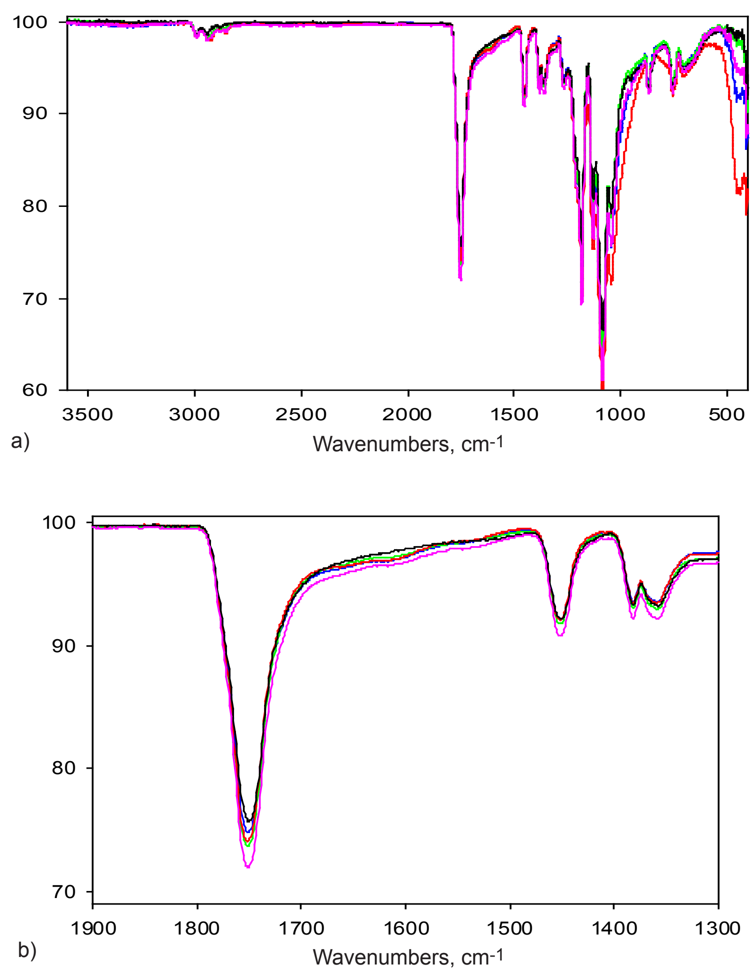

Figure 5. *FTIR (ATR) spectra of PLA nonwoven samples: (a) pure, and (b) modified with PB-1 biocide;* ***Legend****: sample 0, PLA nonwoven with no modification – black line; sample 1, modified PLA nonwoven – red line (fragment A), blue line (fragment B); sample 2, modified PLA nonwoven – pink line (fragment A), green line (fragment B).*

material, its spread therein was uneven, which was confirmed by the results of elementary analysis. These results only prove the presence of active substances in the material. Precise determination of the amount of active substances in the modified nonwoven is possible to determine only on the basis of the mass of the biocidal agent added into the granulate mass of PLA.

FTIR spectra confirmed a lack of strong interactions of active substances with the PLA material, which was evidence that PLA properties did not change after its modification. It is an extremely important conclusion concerning the technological process of obtaining nonwovens using the melt-blown method; especially when it comes to producing highly efficient filtering materials designed to be used in individual protection of a person against harmful aerosols. The filtering properties of these materials are heavily dependent on the possibility of obtaining nonwovens of a diametre of 1 μm and good susceptibility to static electricity using the method of corona discharge. Thus there is a fear that a change in the reological characteristic of the polymer material is a result of mixing in the biocidal agent, which could adversely affect the assumed filtering properties of bio nonwoven.

Addtionally slight changes in the intensity of bands in the ”finger print” region were observed, connected with the presence of functional groups of active substances. Due to the local heterogenity of the material, caused both by the thickness of PLA fibres and the quality of the biocidal agent introduced, these changes could not have been analysed quantitatively. Similar to the case of elementary analysis, FTIR spectra confirmed the presence of active substances in the modified PLA samples, with no possibility to determine them precisely.

To check the purity of virgin PLA as well as modified samples, UV spectra of aqueous extracts were also measured in the range of 200-900 nm. Results showed no chromophore groups in the samples investigated.

BIO-nonwoven

Relevant properties of the filtering nonwovens produced can be stated lub (ascertained). On the one hand the filtering nonwovens have the ability to efficiently block particles of aerosols. On the other hand they have in fluence on a drop in air pressure after passing of particles of aerosol through the filtering material, which obviously transfer directly into air flow resistance. These parametres were established using norms that are standards for evaluation of respiratory protection equipment, which remain in accordance with the 89/686/EWG Directive [22, 23]. Structural parametres of the nonwovens are presented in ***Table 4*** at the 3rd minute of the measurement, and in ***Figure 5*** as a change in the filtration index during the time of loading the BIO-nonwovens with the test aerosol, due to the fact that the concentration of biocidal agent PB-1 used (10% or 15%) influenced neither the filtering properties nor structural parame-

Table 4. *Results of tests on the index of penetration of paraffin oil mist, aerosol of chloride sodium and resistance of air flow – time of every test was 3 minutes.*

	1 layer				3 layers			
	paraffin oil mist		NaCl aerosol		paraffin oil mist		NaCl aerosol	
	penetration, %	resistance, Pa	penetration, %	resistance, Pa	penetration, %	resistance, Pa	penetration, %	resistance, Pa
Average	36.13	23.43	17.69	28.44	4.14	84.06	1.15	103.63
Standard deviation	1.67	1.80	1.03	1.21	0.56	6.52	0.15	4.46
Variance	2.78	3.24	1.07	1.46	0.31	42.52	0.02	19.85

tres of the nonwovens. In ***Table 3*** there are average values of parametres obtained from ten measurements, with no distinction of samples marked with symbols 0, 1 and 2.

The tests performed showed that the nonwovens produced were characterised by uniformity in terms of thickness and surface mass. As for the filtering index, for both sodium chloride and paraffin oil mist, the deposit on the layers of the nonwovens caused a radical drop in penetration of the aerosols and, as a consequence, an increase in the efficiency of filtration. This is an important indication as far as designing filters and filtering half-masks is concerned. A very good result was also achieved in terms of the change in the index of filtration of paraffin oil mist in time. In this case (***Figure 6***), slight changes were observed in this parameter, which means that for the whole period of use, the equipment should maintain its initial protective class. The same tendency was observed for the value of air flow resistance. The data obtained concerning filtering indexes guarantee the possibility of obtaining at least the 2nd protection class in the filtering respiratory protection equipment (filters [22] or filtering half-masks [23]).

Table 3. Results of tests on the thickness and surface mass of the nonwovens.

	Thickness, mm		Surface mass, g/m^2	
	layers		layers	
	1	3	1	3
Average	0.71	2.13	45.20	135.73
Standard deviation	0.09	0.07	4.26	2.91
Variance	0.01	0.01	18.17	8.50

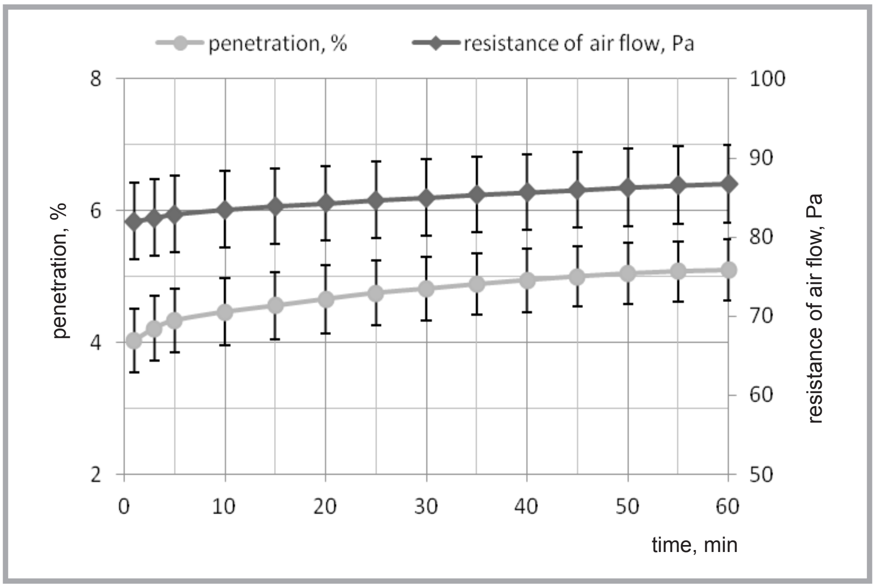

Figure 6. Index of paraffin oil mist penetration and air flow resistance in time.

The incubation period of the samples tested were carried out at a temperature of 58 ± 2 °C, which made it possible for microorganism growth at higher temperatures . Measurement of the moisture content was determined in each case prior to the beginning of the biodegradation process. The humidity of the compost used during the process of biodegradation was controlled at defined intervals and maintained at a level of 48.9%. The first observations of changes took place after 1 week of initiating the process. The frequency of taking samples was after 1, 4, 8, 12, 16, 20 and 24 weeks of the biodegradation process. Determining the overall amount of microorganisms was performed at the accredited Laboratory of Microbiology IBWCh (N = 4,3×10^7 cfu/g).

For samples marked as PLA (pure) and PLA+PB-1 (three repetitions each), after 1 and 4 weeks of incubation, no mass loss was noticed due to the lack of complete cleaning of samples of compost remnants. A probable factor influencing the phenomenon observed was the high hydrophobicity of the sample tested and its transition from the stable form into ”biofilm”. After 8 weeks (56 days) of the process, total biodegradation of the samples tested was obtained (mass loss = 100%).

In order to assess the results of susceptibility of the nonwovens to biodegradation, one of the alternative requirements included in the EN 13432:2000 standard [24, 25] was applied, used in the evaluation of wrappings to be composted. For any material to be acknowledged as biodegradable in tests on the ability to decompose during biological processing, a final decomposition of this material should take place (no more than 10% of a dry mass of sample remains in a sieve with a diametre of mesh > 2 mm) over a time period no longer than 3 months of samples undergoing the composting process. PLA nonwovens produced in this way and modified with biocidal agent PB-1 fulfilled this requirement amply.

The efficiency of microorganism growth inhibition was assessed with respect to the control, being the number of microorganisms obtained in the sample just after grafting (time 0). Results of microorganism survival (*S. aureus* and *P. aeruginosa*) with reference to various times of incubation are presented in ***Figures 7.*** and ***8***, respectively.

On the basis of the tests performed, it was shown that at a minimum concentration of biocidal agent PB-1 used for both *S.aureus* and *P. aeruginosa* during a time of incubation at a temperature of 37 °C, after just 2 hours there appeared a significant reduction in the number of microorganisms (survival 0%). Biocidal properties in relation to Gram-positive and Gram-negative bacteria were obtained at a similar level. So as to assess the biocidal abilities of the nonwovens, criteria of their activities were established using normative settings in order to determine the biostatic and biocidal effect of the disinfectants against bacteria on the basis of the PN-EN 1276:2000/Ap1:2001 standard [26]. Any activity below a 0.5 value is considered low, which means a three-fold decrease in the number of microorganisms. Due to the contact with PLA nonwoven modified with biocidal agent PB-1, the survival of microorganisms dropped to zero, and after just 2 hours of contact it was assumed that the nonwovens are characterised by activity against Gram-positive and Gram-negative bacteria at the highest level - the 3rd.

On the basis of complex research with reference to prospective application of bio-nonwovens, it was stated that biocid-

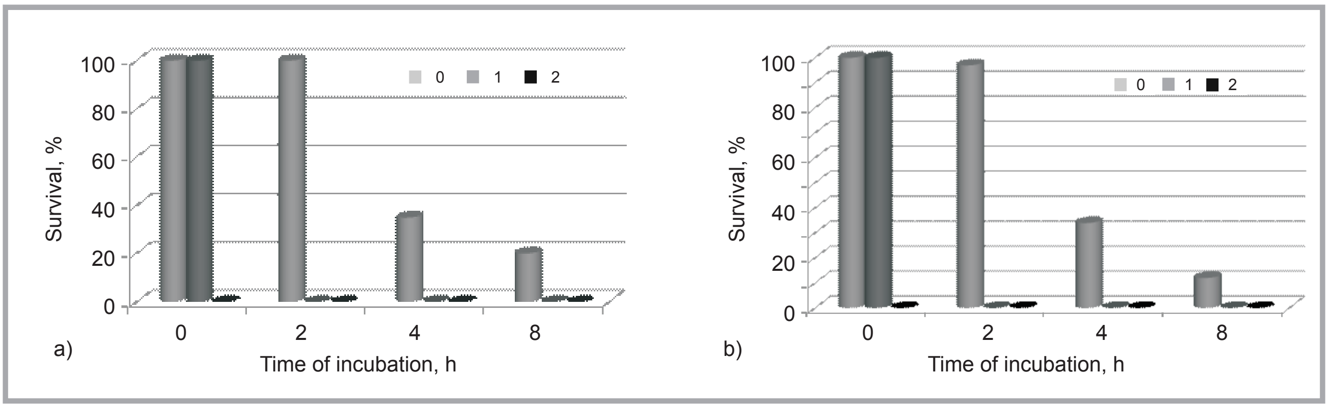

Figure 7. *Results of survival tests for: a) S. aureus and b) P. aeruginosa in time.*

al agent PB-1 introduced into PLA fibres works efficiently even at low concentration in relation to the polymer mass. It is of particular importance to emphasise its immediate efficiency with high biological activity against Gram-positive and Gram-negative bacteria. This fact was confirmed by the drop in the number of *S. aureus* and *P. aeruginosa tested* to zero after just 2 hours of contact with the bio non-woven. This property was ensured thanks to appropriate introduction of the biocide into the material of PLA fibres, which is illustrated in ***Figures 3*** and ***4***, and confirmed by studies described in [13, 20]. By comparing the results obtained concerning the survival of microorganisms with those described in other works [8 - 11], it may be assumed that biocide PB-1 ensures efficiency at the same or even higher level. Studies carried out at the same time showed there was no negative influence of the biocidal modifier on the PLA fibre structure, which was immensely important from the standpoint of the possibility of ensuring high efficiency of the bio non-woven's filtration. It was shown that from the point of view of filtration efficiency towards the model aerosols (sodium chloride and paraffin oil mist), bio non-woven may become the basis for constructing protective equipment (filters and filtering half-masks) at the 2nd level of protection class [22, 23].

This results in blocking at least 96.0% of aerosol particles in the filtering material. Also the short time of biodegradation of the bio nonwovens prepared was confirmed. Obtaining such an effect was provided by selecting the content of active substances of biocidal agent PB-1 and from the effect obtained on the basis of synergy between aliphatic polyamines and quaternary ammonium salts in particular, which gives an effect similar to the one described in [18].

Conclusions

When using biocidal agents, one needs to be guided by competence and sensibility as they may, while helping to obtain the property desired, also cause unintentional damage. That is why it is important to select these substances for a given application considering the properties of microorganisms that are expected in the environment where the biocidal agent is to be used, as well as the conditions of its application, such as time, temperature, humidity and contact with the skin. In fact, it should become a rule to obtain the assumed efficiency of biocidal products at a minimal concentration of the biocidal agent. On the basis of research into the survival of the microorganisms tested (*P. aeruginosa* and S.*aureus),* the biodegradability of BIO- nonwoven in organic compost and research into the durability of combining it with PLA material, it was stated that the PB-1 biocidal agent established fulfills the requirements for producing nonwovens designed for constructing respiratory protective equipment against bioaerosol. At the same time, due to the wide range of efficiency, minimal influence on the quality of polymer material and products made from it as well as a lack of a negative influence on the natural environment were demonstrated. The authors suggest that it can replace other biocides commonly used in the textile industry (e.g. triclosan).

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