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Aspects of Tests and Assessment of Filtering Materials Used for Respiratory Protection Against Bioaerosols. Part I: Type of Active Substance, Contact Time, Microorganism Species

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This paper presents the results of a study on antimicrobial activity of polymer filter nonwovens produced by needle-punching or melt-blowing with an addition of disinfecting agents. The first part of the paper discusses how the biocidal activity of nonwovens is a function of the active agent added to the nonwovens, the duration of the contact of microorganisms with nonwovens and the type of microorganisms. The types of fibres and disinfecting agents had a considerable effect on the biocidal activity of nonwovens. The biocidal effect of nonwovens increased with the duration of their contact with microorganisms. Fibre activity differed considerably depending on the species of the microorganism. The microorganisms most sensitive to biocidal activity of the active filter nonwoven were S. aureus*,* M. flavus *and* E. coli*. There were no biocidal effects on spore-forming bacterium* B. subtilis*.*

filtering material bioactive fibres microorganisms biostatic and biocidal activity contact time

1. INTRODUCTION

In recent years there has been a growing interest in textile products made of bioactive chemical fibres. Disinfecting agents (biocides) are integrated into the structure of the fibre or they are attached to their surface; they are released after contact of microorganisms with the surface of the fibre [1, 2].

The biocides used in the textile industry represent various groups of chemical compounds, e.g., aldehydes (HNA), diphenols (Triclosan), quaternary ammonium salts (Sanitized), halogen combinations, metal ions (Ag, Zn, Cu), metal oxides (titanium oxide), natural and synthetic dyes with biocidal properties, and even antibiotics [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. Manufacturing

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bioactive fibres involves durable binding of biocides with fibres. The manufacturing process consists of introducing metal particles into the zeolites added to the fibres during their formation, attaching antibiotics through graft copolymerization or by processing existing fibres, e.g., coating fibres with films or incorporation into fibres [2, 11].

Bioactive fibres have numerous applications: in hospitals, in the military, in some public utilities and in daily life. They can be used in the production of hospital bed linen, mattresses, blankets, dressings, socks, underwear and protective clothing [12, 13]. The production of filter nonwovens for respiratory protection against bioaerosols is a new area of application [14]. It is necessary to use bioactive fibres in filtering half masks for respiratory protection for workers exposed to biological agents, e.g., pathogenic bioaerosols. Thanks to their antimicrobial properties, bioactive filter materials reduce or eliminate the causes of epidemic diseases and intrahospital infections; they also decrease the risk of allergies. Filtration and biocidal activity of textile materials used in the production of half masks against airborne, including pathogenic, microflora has not been assessed yet. Bioactive filter materials are used in various conditions; therefore, it is important to study their efficiency as modified by factors related to nonwovens, microorganisms and the environment.

This study evaluated antimicrobial activity of needle-punched and melt-blown filter nonwovens in their use in the manufacturing of filtering half masks. The nonwovens used in this study had various chemical composition and active ingredients. The study included examination of growth inhibition effects and biocidal effects for different contact times of those nonwovens and microorganisms. The efficiency of the nonwovens against diverse microflora was also assessed.

TABLE 1. Nonwovens Used in this Study and Their Characteristics

Nonwoven	Description	Remarks
I	melt-blown nonwoven; polypropylene*	control for II, III, IV
II	melt-blown nonwoven; polypropylene-biocide (50 mg Ag/m^2)	
III	melt-blown nonwoven; polypropylene-biocide (75 mg Ag/m^2)	
IV	melt-blown nonwoven; polypropylene-biocide (100 mg Ag/m^2)	
V	needle-punched nonwoven; polypropylene + polyacrylonitrile	control for VI, VII
VI	needle-punched nonwoven; polypropylene-biocide[1] + polyacrylonitrile	
VII	needle-punched nonwoven; polypropylene-biocide[2] + polyacrylonitrile	
VIII	melt-blown nonwoven; polypropylene*	control for IX, X, XI
IX	melt-blown nonwoven; polypropylene-biocide (nanosilver 50 ppm in the form of alcoholic solution)	
X	melt-blown nonwoven; polypropylene-biocide (nanosilver 250 ppm in the form of alcoholic solution)	
XI	melt-blown nonwoven; polypropylene-biocide (nanosilver 750 ppm in the form of alcoholic solution)	
XII	needle-punched nonwoven; polypropylene + acrylic fibres	control for XIII, XIV, XV
XIII	needle-punched nonwoven; polypropylene-silver (in the form of master batches) + acrylic fibre-biocide[3]	
XIV	needle-punched nonwoven; polypropylene-silver (deposited on fibres) + acrylic fibre-biocide[3]	
XV	needle-punched nonwoven; polypropylene + acrylic fibre-biocide[3]	
XVI	needle-punched nonwoven; polyester + bicomponent BICO[4]	control for XVII, XVIII
XVII	needle-punched nonwoven; polyester-biocide[5] + bicomponent BICO[4]	
XVIII	needle-punched nonwoven; polyester-biocide[6] + bicomponent BICO[4]	

Notes. 1, 2—fibres with added biocides from Texal (Poland); 3—acrylic fibres with added biocide from Polymir (Belarus); 4—Huvis (Korea); 5—polyester fibres from Boryszew (Poland), biocide from Huvis (Korea); 6—polyester fibres from Trevira (Poland); *—the same fibres, different date of production.

2. EXPERIMENTAL

2.1. Tested Materials

Table 1 lists the nonwovens used in the study. Tests were carried out with samples of fabrics; 2 × 2 cm squares were cut and placed in sterile Petri dishes. The fabrics were then inoculated with a suspension of microorganisms. Prior to the tests, the nonwovens were also assessed for microbiological purity. Squares of nonwovens (4 × 4 cm samples) were transferred into a solution of physiological salt (0.85% NaCl) to wash out the microorganisms from the material and were shaken for 15 min; the samples were seeded in a sterile Petri dish, and poured over with half-liquid TSA (tryptic soy agar) and Sabouraud media. The purity was acceptable, and the number of microorganisms in input fabrics did not affect the final result.

2.2. Microorganisms

Table 2 lists the microorganisms used in the tests. They were activated on 150 ml of liquid TSB (tryptic soy bullion; bacteria), Sabouraud (yeasts) and MEA (malt extract agar) slant (moulds) media in 250-ml Erlenmayer flasks at 37 °C for 48 h (bacteria, yeasts) or at 27 °C for 72 h (moulds). After incubation, the inoculum suspension of microorganisms was prepared. The bacteria and yeast cells were separated from the liquid medium by centrifugation (at g = 910 for 10 min) followed by suspension in 150 ml of physiological salt. The mould spores were washed out from the slants using a salt solution with Tween 80. Cell density in inoculum suspensions was determined with a microscope (Thom chamber) and breeding by seeding on appropriate microbiological media. The density of bacterial suspensions was in the 10^8–10^9 CFU/ml range, there were 2–4 × 10^6 CFU/ml of yeast and mould cells in the inoculum.

2.3. Media and Reagents

The following culture media were used: TSB was used to activate bacteria prior to preparation of the inoculum suspension; TSA was used to culture bacteria in Petri dishes after their contact

TABLE 2. Description of the Microorganisms Used in Tests

Microorganisms	Source	Description
Escherichia coli	American type culture collection ATCC 10536	reference strain, bacterium, Gram-negative rod, considerable resistance to biocides
Pseudomonas aeruginosa	culture collection of ITFiM PŁ Łock	reference strain, bacterium, Gram-negative rod, high resistance to disinfectants, pathogen (purulent dermatitis and suppurative inflammation of organs), intrahospital infections
Klebsiella pneumoniae	culture collection of the Polish Academy of Sciences, Wrocław	reference strain, bacterium, Gram-negative rod, pathogen (pneumonia), airborne, intrahospital infections
Staphylococcus aureus	American type culture collection ATCC 6538	reference strain, bacterium, Gram-positive coccus, pathogen (purulent dermatitis, pneumonia), airborne, frequently carried in nasopharyngeal cavity, intrahospital infections
Micrococcus flavus	culture collection ITFiM PŁ Łock, strain isolated from indoor air	bacterium, Gram-positive coccus, very often isolated from air, saprophyte not dangerous to health, high resistance to UV and disinfectants
Bacillus subtilis	culture collection ITFiM PŁ Łock, strain isolated from indoor air	bacterium, Gram-positive spore-producing bacillus, very frequently occurring in natural environment (air, soil), saprophyte not dangerous to health
Candida albicans	culture collection ITFiM PŁ Łock 0001	yeast-like fungus, potentially pathogenic, ubiquitous in human environment (mucosae, air, skin)
Aspergillus niger	culture collection ITFiM PŁ Łock 0439	mould fungus, test species for resistance testing of various technical materials, saprophytic species commonly occurring as air microflora
Penicillium chrysogenum	culture collection ITFiM PŁ Łock, strain isolated from indoor air	mould fungus, very often isolated from air, saprophyte not dangerous to health

Notes. ITFiM PŁ—Institute of Fermentation Technology and Microbiology, Technical University of Łódź, Poland.

with fabrics; Sabouraud (a liquid medium) was used to activate yeasts (*Candida albicans*) prior to preparation of the inoculum suspension; a solid medium was used to culture yeasts on Petri dishes after their contact with fabrics; MEA 7°Blg (a slant medium) was used to activate the moulds (*Aspergillus niger, Penicillium chrysogenum*) prior to preparation of the inoculum suspension and in the form of mould culture plates after their contact with the fabrics. Physiological salt was used for dilutions and to wash out microorganisms from fabrics.

3. TEST METHODS

All textiles were inoculated with 0.1 ml of the inoculum suspension of microorganisms. Each material sample was inoculated three times, with three durations of incubation; they were incubated at 37 (bacteria, yeasts) or 27 °C (moulds) in a sterile Petri dish. The samples were taken at 0, after 4 or after 6 h from material inoculation. The samples of materials were subsequently transferred into 100 ml of physiological salt to wash out the microorganisms from the fabric and they were shaken for 15 min in a water bath. The samples were diluted in sterile physiological salt and seeded in a sterile Petri dish. Subsequently they were poured over with a half-liquid TSA (bacteria), Sabouraud (yeasts) or MEA (moulds) medium, mixed and left to cool. Next, the samples were incubated at 37 for 48 h (bacteria, yeasts) or at 27 °C for 72 h (moulds). After that time all grown colonies were counted. On the basis of the results, a mean value for each microorganism was calculated for each duration of incubation and each textile material (the tests were carried out three to five times, depending on the sample and the duration).

4. METHODS OF CALCULATIONS

4.1. Biostatic and Biocidal Activity

The antimicrobial activity was calculated from the results presented as a mean number of microorganisms per test (CFU/test) for a given duration of exposure. The antimicrobial activity of the tested nonwovens was described with two parameters, (a) the biostatic effect, i.e., the inhibition of microorganism growth and (b) the biocidal effect. For the purpose of the present study, the criteria for the activity of nonwovens were established using normative regulations for determining biostatic and biocidal effects of disinfectants for bacteria and fungi as provided by Standards No. EN 1276:2009 [15] and EN 1650:2008 [16]. In short, the activity below 0.5 was regarded as low and meant a threefold decrease in the number of microorganisms; whereas, the activity of 3 or more for fungi and bacteria was regarded as high and meant a thousandfold decrease in the number of microorganisms.

The biostatic (inhibiting) activity was calculated with the formula

$$\textit{biostatic activity} = \log A/B \tag{1}$$

where A—number of microorganisms per test after exposure time t with the control nonwoven, B—number of microorganisms per test after exposure time t with the bioactive nonwoven.

The biocidal activity was calculated with the formula

$$\textit{biocidal activity} = \log C/B \tag{2}$$

where C—number of microorganisms per test after time $t = 0$ with the control nonwoven, B—number of microorganisms per test after exposure time t with the bioactive nonwoven.

4.2. Microorganisms Survival Index

Because the microbiological analyses for the tested textile materials were not done at the same time and because microorganisms themselves are characterized by their specific growth physiology, to compare the obtained data the microorganisms survival index N_t was calculated with the following equation

$$N_t = \frac{N}{N_0}, \tag{3}$$

where N_0—number of microorganisms on the sample of the textile material for time $t = 0$, N—number of microorganisms on the sample of the textile material for time t_n.

4.3. Statistical Methods

Statistical calculations were done with arithmetic mean (*M*), standard deviation (*SD*) [17]; whereas, significant differences between groups of data were analysed with the *F* (ANOVA), LSD and Dunnett's tests [18]. Statistical analyses were done with Statistica version 6.0.

5. RESULTS

5.1. Evaluation of Antimicrobial Activity of Filter Nonwovens

On the basis of the results obtained in examinations of the number of *Escherichia coli* and *Staphylococcus aureus* during their exposure on filter nonwovens (potentially bioactive nonwovens and control nonwovens), N_t (Table 3) and biostatic and biocidal activities (Table 4) were calculated for bioactive nonwovens. Table 5 presents the results of statistical analyses of surviving microorganisms.

Table 3 shows that during incubation the index of microorganism survival on various nonwovens (N_t) decreased until the 6th hour of incubation time. For nonwovens XIII–XV, the decrease in N_t was high after 4 and after 6 h for both *E. coli* (N_t = 0.000–0.001) and *S. aureus* (N_t = 0.000).

It was found that the biostatic activity of all tested nonwovens was considerably lower than their biocidal activity (Table 4). The bactericidal effect increased with time, after 6 h the biocidal activity of the tested nonwovens was significantly higher as compared to 4-h incubation. In view of the lower biostatic activity, the differences in the growth inhibition effect for different incubation times were not noticeable, like for the biocidal activity. It was concluded that some needle-punched nonwovens with potentially bioactive properties did not show any antimicrobial effects, as proven by the negative values of biostatic activity, which means that in these nonwovens the number of bacteria grew during their exposure, as compared to the control nonwoven (without an active agent). They were nonwovens III, IV, VII, IX, X, XI. Those nonwovens were composed of polypropylene fibres with an addition of silver in various forms and concentrations, and were mainly made with melt-blown technology. Melt-

TABLE 3. Microorganism Survival Index N_t at 0, After 4 and After 6 h of Incubation With Bioactive Nonwovens

Non-woven	N_t of *E. coli*						N_t of *S. aureus*					
	0 h		4 h		6 h		0 h		4 h		6 h	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
I	1.000	0.354	0.719	0.221	0.650	0.106	1.000	0.221	0.676	0.031	0.396	0.031
II	1.000	0.131	0.598	0.262	0.443	0.072	1.000	0.025	0.658	0.062	0.342	0.037
III	1.000	0.095	0.818	0.216	0.412	0.296	1.000	0.146	0.662	0.122	0.371	0.012
IV	1.000	0.019	0.951	0.149	0.761	0.278	1.000	0.088	0.637	0.221	0.322	0.22
V	1.000	0.290	0.709	0.120	0.659	0.150	1.000	0.165	0.968	0.018	0.942	0.012
VI	1.000	0.138	0.892	0.125	0.442	0.228	1.000	0.157	0.736	0.176	0.458	0.176
VII	1.000	0.046	0.827	0.106	0.552	0.250	1.000	0.210	0.707	0.034	0.573	0.120
VIII	1.000	0.125	0.926	0.102	0.862	0.138	1.000	0.045	0.920	0.123	0.826	0.071
IX	1.000	0.000	0.626	0.038	0.461	0.130	1.000	0.233	0.708	0.066	0.475	0.011
X	1.000	0.009	0.907	0.121	0.894	0.103	1.000	0.198	0.807	0.148	0.429	0.062
XI	1.000	0.009	0.838	0.142	0.161	0.018	1.000	0.257	0.765	0.203	0.501	0.061
XII	1.000	0.226	0.823	0.119	0.604	0.001	1.000	0.360	0.927	0.119	0.897	0.085
XIII	1.000	0.187	0.006	0.001	0.000	0.000	1.000	0.225	0.064	0.008	0.000	0.000
XIV	1.000	0.079	0.005	0.001	0.000	0.000	1.000	0.039	0.065	0.013	0.000	0.000
XV	1.000	0.222	0.009	0.000	0.001	0.000	1.000	0.130	0.088	0.005	0.000	0.000
XVI	1.000	0.062	0.815	0.105	0.701	0.065	1.000	0.084	0.895	0.081	0.312	0.046
XVII	1.000	0.042	0.799	0.056	0.501	0.012	1.000	0.045	0.556	0.096	0.438	0.001
XVIII	1.000	0.061	0.610	0.102	0.225	0.056	1.000	0.026	0.689	0.100	0.296	0.048

TABLE 4. Biostatic and Biocidal Activity of Nonwovens Against *E. coli* and *S. aureus* After 4 and After 6 h of Incubation

	Biostatic Activity				Biocidal Activity			
	E. coli		*S. aureus*		*E. coli*		*S. aureus*	
Nonwoven	4 h	6 h	4 h	6 h	4 h	6 h	4 h	6 h
II	0.297	0.384	0.121	0.087	0.441	0.571	1.242	1.526
III	–0.022	0.232	–0.004	0.044	0.121	0.419	1.117	1.484
IV	–0.070	–0.016	0.051	0.132	0.074	0.171	1.173	1.572
VI	0.138	0.437	0.016	0.038	0.152	0.456	0.179	0.385
VII	–0.091	0.420	–0.023	–0.116	–0.077	0.438	0.140	0.231
IX	–0.493	–0.253	0.010	0.337	–0.690	–0.632	0.186	0.186
X	–0.493	–0.304	0.000	0.191	–0.690	–0.683	0.176	0.176
XI	–0.453	0.446	0.019	0.117	–0.650	0.067	0.195	0.195
XIII	2.227	1.914	1.301	3.559	2.600	4.285	1.334	3.652
XIV	2.277	1.512	1.342	3.420	2.650	3.882	1.375	3.513
XV	2.141	1.114	1.166	3.106	2.514	3.484	1.199	3.199
XVII	nt	0.183	nt	0.317	nt	0.370	nt	0.925
XVIII	nt	0.366	nt	0.270	nt	0.553	nt	0.878

Notes. □—low activity, ▒—average activity, ■—high activity; nt—not tested.

TABLE 5. Statistical Analysis of the Survival of *E. coli* and *S. aureus* on Bioactive Nonwovens Versus Control Nonwoven After 6 h of Incubation

	E. coli			*S. aureus*		
Nonwoven	*F* Test	LSD Test	Dunnett's Test	*F* Test	LSD Test	Dunnett's Test
II	*ns*	nt	nt	+	+	+
III	*ns*	nt	nt	+	+	+
IV	*ns*	nt	nt	*ns*	nt	nt
VI	*ns*	nt	nt	*ns*	nt	nt
VII	*ns*	nt	nt	*ns*	nt	nt
IX	ns	nt	nt	*ns*	nt	nt
X	*ns*	nt	nt	*ns*	nt	nt
XI	*ns*	nt	nt	ns	nt	nt
XIII	+	+	+	+	+	+
XIV	+	+	+	+	+	+
XV	+	+	+	+	+	+
XVII	*ns*	nt	nt	*ns*	nt	nt
XVIII	*ns*	nt	nt	*ns*	nt	nt

Notes. +—statistically significant at $p \geq .05$; nt—not tested.

blown nonwovens II, III, IV containing silver in various concentrations showed medium biocidal activity against *E. coli* and *S. aureus*, which was revealed after 6 h of incubation (Figures 1–2). The activity was higher for *S. aureus* bacteria. Polyester needle-punched nonwovens XVII and XVIII interacted with medium biocidal activity, but only against Gram-positive *S. aureus*, while polypropylene needle-punched nonwovens interacted against Gram-negative bacterium *E. coli*. The nonwovens mentioned here differed in the biocide component. High levels of bacteriostatic and bactericidal activity were found for needle-punched nonwovens XIII, XIV, XV. These materials showed both bacteriostatic and bactericidal effects against bacteria. The results of their activity against *S. aureus* were noticeable after 4 h and increased after 6 h of incubation; however, this phenomenon was not observed for biostatic activity against *E. coli*. The biocide used in these nonwovens gave only a biocidal effect for *E. coli*, but did not inhibit its

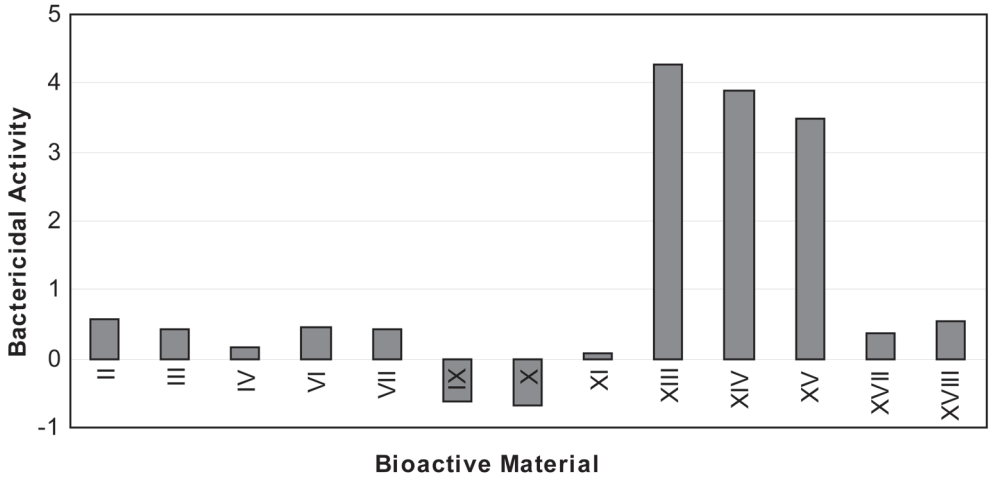

Figure 1. Bactericidal activity of bioactive materials after 6 h of incubation with *E. coli*.

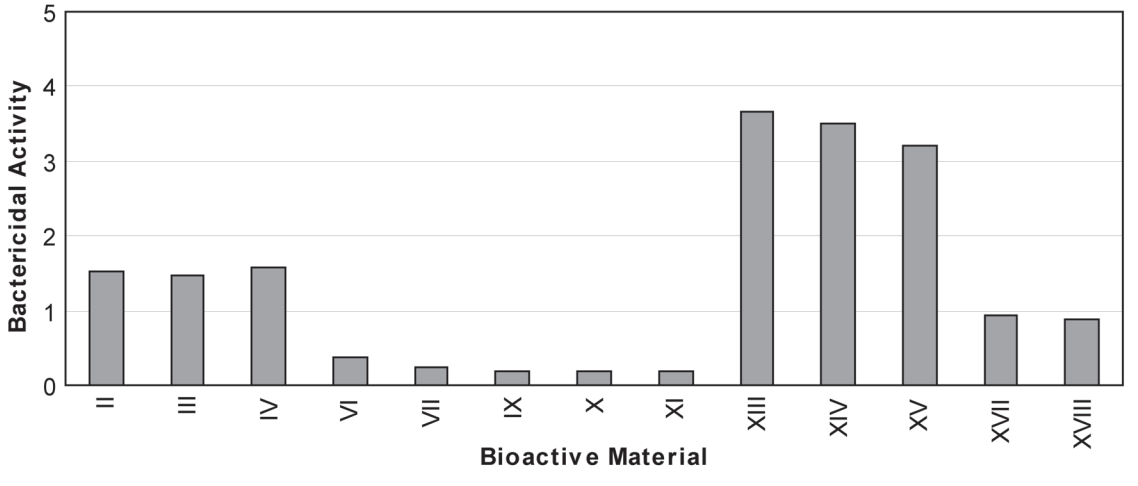

Figure 2. Bactericidal activity of bioactive materials after 6 h of incubation with *S. aureus*.

growth. The nonwovens contained two types of bioactive fibres, polypropylene with silver that had been deposited in the form of master batches, and another type of acrylic bioactive fibres manufactured by Polymir (Belarus), which could enhance the antimicrobial effect.

The evaluation of bacteriostatic activity of the tested nonwovens against *E. coli* and *S. aureus* showed that nonwovens XIII, XIV, XV were biologically the most active ones. Nonwoven XIII was the most active among them (biocidal activity of 3.6 for *S. aureus* and 4.3 for *E. coli*).

For most samples there were no statistically significant differences for bacteria survival on bioactive nonwovens and their control during the time of incubation with microorganisms (Table 5). For nonwovens XIII, XIV and XV, there were statistically significant differences both during incubation of *E. coli* and *S. aureus*. There were statistically significant differences for nonwovens II, III, IV, but only for *S. aureus*. Furthermore, there were statistically significant differences for bacteria survival at 0 and after 6 h of incubation on nonwovens VI and VII.

5.2. Evaluation of Biostatic and Biocidal Activity of Nonwoven Filters Against Various Microorganisms in the Air

The purpose of this experiment was to compare the antimicrobial activity of a highly antimicrobial nonwoven (nonwoven XIII) against different microflora. The following criteria were used in selecting microorganisms:

- high isolation frequency in the air (typical air microflora includes *A. niger, P. chrysogenum, Micrococcus flavus, Bacillus subtilis, S. aureus*);

- various sensitivity to disinfectants (*B. subtilis, A. niger, P. chrysogenum, Pseudomonas aeruginosa, Klebsiella pneumoniae* are highly resistant; *E. coli, S. aureus* are sensitive*)*;
- potential pathogenicity and transferability by the air (*K. pneumoniae, S. aureus, C. albicans*).

Nine species of microbes were selected and incubated on control and bioactive nonwovens for 6 h; then they were checked for the number of microorganisms that survived the incubation with nonwovens in experiments condition. Tables 6–8 present the N_t survival index, the biostatic and biocidal activity and the statistical analysis of the differences in survival.

The microorganisms survival index N_t decreased to zero after 6 h of incubation for *E. coli, S. aureus, M. flavus, C. albicans*; it stayed at a low level for *P. aeurginosa, K. pneumoniae, P. chrysogenum* and *A. niger*. For *B. subtilis* N_t remained at a high level of 0.55 (Table 6).

Nonwoven XIII had a bioactive effect on all tested microorganisms except *B. subtilis*, against which both the growth inhibition effect and the biocidal effect were lower than the assumed criterion (Table 7). *B. subtilis* belongs to highly resistant microorganisms because of its spore formation (Figure 3).

TABLE 6. Microorganism Survival Index N_t for Various Microorganisms After Incubation With Bioactive Nonwoven XIII

	N_t After 0 h		N_t After 6 h	
Microorganisms	***M***	***SD***	***M***	***SD***
Escherichia coli	1.000	0.107	0.000	0.000
Pseudomonas aeruginosa	1.000	0.219	0.001	0.000
Klebsiella pneumoniae	1.000	0.258	0.001	0.000
Staphylococcus aureus	1.000	0.205	0.000	0.000
Micrococcus flavus	1.000	0.146	0.000	0.000
Bacillus subtilis	1.000	0.239	0.550	0.348
Candida albicans	1.000	0.099	0.000	0.000
Aspergillus niger	1.000	0.144	0.036	0.006
Penicillium chrysogenum	1.000	0.176	0.004	0.001

TABLE 7. Biostatic and Biocidal Activity of Nonwoven XIII After 6 h of Incubation With Various Microorganisms

Microorganisms	**Biostatic Activity**	**Biocidal Activity**
Escherichia coli	3.995	4.328
Pseudomonas aeruginosa	2.858	3.164
Klebsiella pneumoniae	2.734	3.290
Staphylococcus aureus	3.711	4.269
Micrococcus flavus	3.703	3.981
Bacillus subtilis	0.413	0.423
Candida albicans	2.161	3.681
Aspergillus niger	1.449	1.569
Penicillium chrysogenum	2.299	2.417

Notes. □—low activity, □—average activity, ■—high activity.

TABLE 8. Statistical Analysis of the Survival of Microorganisms on Bioactive Nonwoven XIII Versus Control Nonwoven

	Statistical Test		
Microorganism	***F***	**LSD**	**Dunnett's**
Escherichia coli	+	+	+
Pseudomonas aeruginosa	*ns*	nt	nt
Klebsiella pneumoniae	*ns*	nt	nt
Staphylococcus aureus	*ns*	nt	nt
Micrococcus flavus	*ns*	nt	nt
Bacillus subtilis	*ns*	nt	nt
Candida albicans	*ns*	nt	nt
Aspergillus niger	+	+	+
Penicillium chrysogenum	+	+	+

Notes. +—statistically significant at $p \geq .05$; nt—not tested.

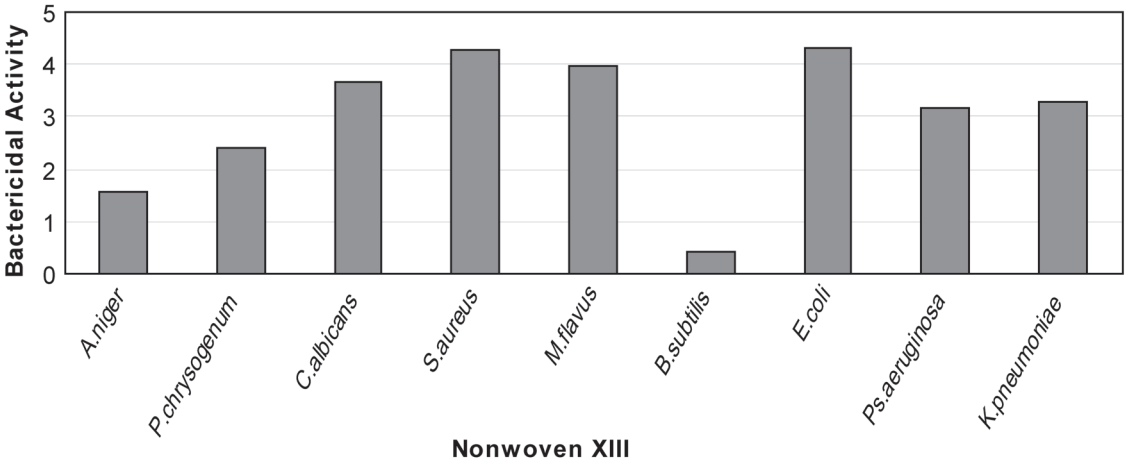

Figure 3. Bactericidal activity of nonwoven XIII against microorganisms.

Needle-punched nonwoven XIII had both a biostatic and a biocidal effect on all the microbes, but the biocidal effect was always higher. The tested nonwoven displayed high bactericidal performance and medium fungicidal properties.

The microorganisms that were most sensitive to biocidal effect of nonwoven XIII included Gram-positive coccus *S. aureus* and *M. flavus*, and Gram-negative rod *E. coli*. The nonwoven also displayed high biocidal activity against other pathogenic Gram-negative rods, *P. aeruginosa, K. pneumoniae* and yeasts *C. albicans*.

The microbes resistant to the biocide contained in nonwoven XIII were moulds *A. niger, P. chrysogenum*, for which the nonwoven showed medium fungicidal activity at the level of 1.5–2.4.

Statistical analysis of the survival of various microorganisms on bioactive nonwoven XIII carried out with *F*, LSD and Dunnett's tests showed that after 6 h of incubation time there were statistically significant differences for *E. coli, A. niger* and *P. chrysogenum*, as opposed to the control sample (Table 8). For *C. albicans, M. flavus, S. aureus, P. aeurginosa* and *K. pneumoniae* there were statistically significant differences between their survival at the beginning of incubation and after 6 h. When the survival of those microorganisms on the tested and control nonwovens was compared, statistically significant differences were found with LSD and Dunnett's tests, whereas the *F* test did not reveal such differences. The low values of survival index N_t after 6 h could have had some influence on the results of the analysis. It was unambiguously stated, however, that there were no statistically relevant differences between the survival index of *B. subtilis* on the tested and control nonwoven during incubation.

6. SUMMARY

The results of this study show that bioactive filter materials with added active agents have various antimicrobial activity, depending on the type of fibres, contact time and type of microbes. The efficiency of microorganism growth inhibition primarily depends on the type of the active agent present in the fibres.

The evaluation of bacteriostatic and bactericidal activity of the tested nonwovens against *E. coli* and *S. aureus* showed that needle-punched nonwovens XIII, XIV, XV had the highest biological activity. Those nonwovens contained propylene fibres with silver added and acrylic fibres with biocide 3 (the name and composition of the biocide was not revealed by the manufacturer). The most bioactive nonwoven XIII displayed high bactericidal and medium fungicidal performance. The nonwoven contained two types of bioactive agents, which distinctly increased its activity.

The addition of preparations with silver ions is not always satisfactory, in spite of numerous literature reports that such nonwovens show good microorganism growth inhibition performance [13, 14, 19, 20, 21]. This primarily depends on the form of silver; in our study nonwovens with silver in master batch form were the most active.

Nonwovens II, III, IV, IX, X, XI (with silver) were not very active because of the silver content in their fibres and the short contact time of the active agent with the microorganisms during the test (6 h).

The active agent should be placed just under the surface of the fibre. A biocide on the surface of the fibre or too deep in the structure of the fibre can be easily removed or does not ensure suitable contact with the microorganisms. Therefore, in such cases microscope analyses are necessary to determine the way the biocides are placed and the size of silver particles. Textiles with nanoparticles of silver and copper have greatest antimicrobial efficiency [13, 19, 20, 22]. The duration of contact is also very important; in our experimental conditions this was determined in real conditions in which masks are used. Contact time of a bioactive nonwoven is a factor contributing to a reduction in microorganisms; the longer the contact time, the more efficient the reduction, which has been confirmed in tests; the biocidal effect of nonwovens increases in line with the contact time with microbes. High efficiency for up to 6 h of use is a significant requirement for filter nonwovens to be used in half-masks.

The biostatic activity of all tested needle-punched and melt-blown nonwovens was lower than their biocidal activity. The nonwovens had a rather more destructive effect on bacteria cell metabolism than inhibited their growth. This is understandable as most often silver, which has a biocidal effect, was the active agent in the fibres [14, 23].

The low efficiency of some nonwovens with added biocides indicates the necessity to carry out a microbiological evaluation and to select suitable preparations for microorganism growth inhibition.

The selection of microorganisms for testing active nonwovens also seems very important. The study indicates diverse sensitivity of microorganisms to the biocidal effect of active nonwovens. The microorganisms with high viability in environmental conditions that are harmful for them (bacteria from the *Bacillus* genus, moulds) displayed high resistance also under contact conditions with bioactive filter nonwovens.

If there is a risk of the presence of pathogenic bacteria in the air (e.g., in a hospital environment), additional tests of antimicrobial efficiency of bioactive nonwovens should be carried out with test strains such as *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Our study showed a high biocidal efficiency of nonwoven used against those pathogens.

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