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Antimicrobial Activity and Filtration Effectiveness of Nonwovens with Sanitized for Respiratory Protective Equipment

Abstract
The objective of the study was to optimise the production of bioactive filtration nonwovens with Sanitized® T 99-19, containing quaternary ammonium salts, by evaluating different production technologies (melt-blowing, needle punching), methods of biocide incorporation (bath, spraying), biocide concentration, and conditioning. The antimicrobial activity of nonwovens was tested against different microorganisms, from culture collection and workplaces, using the method AATCC 100. It was shown that the biological efficiency of nonwovens rose when the concentration of Sanitized was increased from 0.7% to 2%. Furthermore, higher biological activity was found in nonwovens subjected to a bath than in those which underwent spraying. The conditioning process did not significantly affect the antimicrobial activity of the nonwovens tested. As compared to melt-blown nonwovens, the needled variety were more efficient against both collection strains and those isolated from workplaces. Thus both types of nonwovens may be used for the production of bioactive half-masks protecting the respiratory tract of workers exposed to microorganisms.

Key words: *bioactive half-mask, antimicrobial nonwovens, Sanitized T9919, microorganisms.*

Introduction

Workers may be exposed to harmful physical, chemical, and microbial agents at their workplaces. The microorganisms present in bioaerosols may cause infectious diseases, allergies, and other occupational diseases [1].

In the European Union, the protection of workers against hazards related to exposure to biological agents is regulated by Directive 2000/54/EC [2], which points to risks faced by people working in food production plants, agriculture, healthcare facilities, in clinical, veterinary, and diagnostic laboratories, in places where there is contact with animals and/or animal products, as well as in refuse disposal plants and sewage purification installations.

The products primarily used to ensure respiratory protection against bioaerosols are air-purifying respirators, which are considered personal protection equipment. Bioactive air-purifying respirators are expected to exhibit high antimicrobial activity and to perform the same functions as traditional filtration equipment.
For many years now, research centers and industrial facilities have been studying nonwovens containing biocides imparting antibacterial and antifungal properties to textile products [3, 4]. Such textiles are produced by the addition, during the manufacturing process, of biologically active chemical substances such as chitosan, silver compounds, quaternary ammonium salts, imidazole, thiazole, dyes, antibiotics, metal oxides, and other compounds [5 - 9]. Nonwovens containing biocides are used in medicine (hospital underwear and bedding), in the external environment (ropes, tents, military uniforms), in hygienic products (textile footwear, insoles, socks, stockings), filtration materials, etc. [10 - 12]. There are many potential applications of nonwovens modified with antimicrobial agents; however, little research has been done on the use of this type of material for the production of air-purifying respirators designed for the protection of the respiratory tract of workers exposed to environments with high microbiological contamination [13-15]. There are some biocidal products available on the market which can be used in the textile industry in accordance with Directive 98/8/EC, such as silver ions and quaternary ammonium salts (QACs) [16-17]. One of the formulations used for finishing textiles coming in contact with the skin is Sanitized® T 99-19 manufactured by Clariant International Ltd. The QACs interact with the microbial cell wall, destabilising metabolic processes as well as preventing growth and reproduction [18,19]. From an industrial point of view, filtration nonwovens can be easily sprayed with or bathed in a biocidal solution. The key element of manufacturing antimicrobial nonwovens is the selection of an appropriate concentration of the formulation. At the same time, one should also bear in mind the requirements imposed on air-purifying respirators by the relevant standards.

Manufacturers of antimicrobial filtration equipment often face problems related to the fact that their products lose antimicrobial properties as a result of conditioning conducted in accordance with Standard EN 149:2001+A1:2009. Exposure to dry air at 70 ± 3 °C for 24 h may hamper the antimicrobial effects of respirators [20]. Furthermore each filtration class (FFP1, FFP2 and FFP3) requires particulate filtration of adequate efficiency, measured as the penetration rate. Thus the addition of an antimicrobial substance must not deteriorate the filtration efficiency of respirators.

It should be noted that all previous studies on the biological activity of filtration materials have been conducted exclusively

Gutarowska B, Skóra J, Nowak E, Łysiak I, Wdówka M. Antimicrobial Activity and Filtration Effectiveness of Nonwovens with Sanitized for Respiratory Protective Equipment.
FIBRES & TEXTILES in Eastern Europe 2014; 22, 3(105): 120-125.

Table 1. *Characteristics of nonwovens tested: „-" – without biocide; nc – without conditioning.*

Nonwoven	Polymer	Concentration of Sanitized T99-19 in nonwoven, %	Method of applying a biocide	Conditioning, temp., time
A0	polyester	Control for A1 - A8	-	nc
B0	polypropylene	Control for B1 - B4		
A1	polyester	0.670 ± 0.0005	spraying	
A2		0.670 ± 0.0005		70 °C, 24h; 30 °C, 24h
A3		2.040 ± 0.004		nc
A4		2.480 ± 0.007		70 °C, 24h; 30 °C, 24h
A5		0.630 ± 0.001	bath	nc
A6		0.720 ± 0.001		70 °C, 24h; 30 °C, 24h
A7		2.210 ± 0.002		nc
A8		2.120 ± 0.006		70 °C, 24h; 30 °C, 24h
B1	polypropylene	0.640 ± 0.010	spraying	nc
B2		0.660 ± 0.040		70 °C, 24h; 30 °C, 24h
B3		1.710 ± 0.752		nc
B4	polypropylene	0.870 ± 0.389	spraying	70 °C, 24h; 30 °C, 24h

on culture collection strains. Therefore it seems necessary to test the antimicrobial activity of personal protection equipment against strains isolated from workplaces, which may display different sensitivity due to their ability to adapt to their specific environmental conditions.

The aim of the study was to evaluate the antimicrobial activity of bioactive filter nonwovens with the addition of preparation Sanitized® T9919 containing quaternary ammonium salts by evaluating different production technologies (melt-blowing needle punching), methods of biocide addition (bath or spraying), biocide concentration, and conditioning. A comparative evaluation was conducted using culture collection strains as well as strains isolated from workplaces. Furthermore, the filtration efficiency of melt-blown nonwovens was examined using the paraffin oil mist test.

Materials and methods

Tested nonwovens

The filtration materials analysed included needled nonwovens (100% polyester) and melt-blown nonwovens (polypropylene) (Filter-Service Sp. z o.o., Poland). We examined different concentrations of the water solution of formulation Sanitized® T 99-19 (0.7% and 2%), methods of biocide application (spraying or bath), types of bioactive nonwoven (needled or melt-blown) and the influence of conditioning on antimicrobial activity. Samples of bioactive nonwovens were prepared according to the proportions: dry nonwoven fabric (25 kg) was weighed and spread evenly, while Sanitized was weighed in an amount of 150 g or 1500 g and dissolved in 1 litre of distilled water at 40 °C. These solutions were sprayed onto the fabric or the material was bathed in them. In the spraying method, the solution of biocide was sprayed onto finished products using a spray gun. The Bath method consisted of immersion of the nonwoven sample in a biocide solution (0.7% and 2% of mass of fiber), as well as squeezing and drying at a temperature of 20 - 21 °C and RH = 45% with forced air circulation. This gave a final concentration of the biocide of 0.7% and 2% of the fibre mass. The Sanitized product content in the nonwovens after the biocide application process was determined using the weight method and is presented in ***Table 1.***

The formulation Sanitized® T 99-19 (Clariant International Ltd., Switzerland) contains active components tetraalkylammonium compounds (CAS 68424-85-1) in glycol ether (CAS 112-34-5).

Conditioning was conducted in a BD-53 incubator (Binder) in accordance with Standard EN 149:2001+A1:2009 [21]. A description of the materials tested is given in ***Table 1***.

Microorganisms

Antimicrobial activity of the nonwovens was tested against microorganisms from the American Type Culture Collection (ATCC) and National Collection of Agricultural and Industrial Microorganisms (NCAiM) *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* NCAIM 01644, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404. The biological efficiency of the nonwovens was also tested against potentially pathogenic microorganisms frequently isolated from workplaces in composting plants (*Bacillus pumilus*, *Aspergillus fumigatus, Cladosporium macrocarpum, Penicillium crustosum, P. simplicissimum*), museums (*Bacillus subtilis, Staphylococcus haemolyticus, Aspergillus versicolor*, *A. niger*, *Penicillium aurantiogriseum*, *P. janthinellum*, *P. corylophilum*, *P. commune*), and tanneries (*Candida parapsilosis, Cryptococcus albidus, Cladosporium cladosporioides, Penicillium chrysogenum, Rhodotorula glutinis*).

Evaluation of the nonwovens' antimicrobial activity

Antimicrobial activity of the nonwovens was tested using the modified quantitative method AATCC 100 (modification - time of incubation following from the specification of use of the protective half-mask - 8 hours is the maximum time) [22].

Evaluation of filtration properties

Filtering properties of the melt-blown bioactive and control nonwoven were evaluated in penetrating paraffin oil mist and air flow resistance according to the EN 149:2001+A1:2009 [21] standard. The concentration of paraffin oil mist aerosol was measured by a laser photometer (Lorenz, Germany). Results were expressed as the efficiency of filtration.

Mathematical calculations

The arithmetic mean and standard deviation for the quantity of microorganisms on the surfaces of the materials tested were calculated.

Antimicrobial effects of the fabrics were described with two parameters: (1) biocidal activity and (2) biostatic activity according to Gutarowska and Michalski (2009) [16]. The criteria of nonwoven activity were established using normative regulation for determining the biostatic and biocide effects of disinfectants against bacteria and fungi according to Standards EN 1276:2009 [23] and EN 1650:2008 [24]. A value below 0.5 was accepted as low and meant a min. threefold increase in the number of microorganisms. A value of 3 or more was regarded as high and meant a thousandfold or higher increase in the number of microorganisms.

Table 2. *Antimicrobial activity of modified nonwoven: M - mean., SD - standard deviation, nt - not tested, (-) lack of microbial activity, A_b - biocidal activity, A_s - biostatic activity, ■ a value below 0.5 was accepted as low and meant a min. 3-fold increase in the number of microorganisms, ■ a value of 3 or more was regarded as high and meant a 1000-fold or higher increase in the number of microorganisms, * significantly different from the control sample (One-Way ANOVA, $p < 0.05$).*

No.	Nonwoven	E. coli ATCC 10536			S. aureus ATCC 6538			B subtilis NCAIM 01644			C. albicans ATCC 10231			A. niger ATCC 16404		
		Number, cfu/sample	A_s	A_b	Number, cfu/sample	A_s	A_b	Number, cfu/sample	A_s	A_b	Number, cfu/sample	A_s	A_b	Number, cfu/sample	A_s	A_b
1a.	A0	M: 3.9×10^6 SD: 7.1×10^5	nt	nt	M: 2.3×10^6 SD: 1.7×10^6	nt	nt	M: 3.3×10^5 SD: 2.4×10^3	nt	nt	M: 1.2×10^5 SD: 7.0×10^4	nt	nt	M: 1.6×10^5 SD: 2.4×10^4	nt	nt
1b.	A0	M: 4.2×10^7 SD: 1.5×10^7	nt	nt	M: 1.3×10^6 SD: 3.2×10^5	nt	nt	M: 1.2×10^6 SD: 6.8×10^5	nt	nt	M: 2.5×10^5 SD: 9.3×10^4	nt	nt	M: 2.0×10^4 SD: 2.7×10^3	nt	nt
2a.	B0	M: 4.2×10^6 SD: 1.6×10^6	nt	nt	M: 3.9×10^6 SD: 1.7×10^6	nt	nt	M: 3.1×10^5 SD: 1.3×10^4	nt	nt	M: 1.2×10^5 SD: 9.2×10^4	nt	nt	M: 1.6×10^5 SD: 6.4×10^3	nt	nt
2b.	B0	M: 5.5×10^7 SD: 1.9×10^7	nt	nt	M: 1.8×10^7 SD: 4.8×10^6	nt	nt	M: 3.1×10^6 SD: 1.1×10^6	nt	nt	M: 2.3×10^5 SD: 1.2×10^5	nt	nt	M: 2.0×10^4 SD: 2.7×10^3	nt	nt
3.	A1	M: 8.3×10^6* SD: 7.6×10^6	0.70	-	M: 6.6×10^6* SD: 8.0×10^6	-	-	M: 1.5×10^6 SD: 9.4×10^3	-	-	M: 2.0×10^5* SD: 2.8×10^4	0.10	-	M: 1.5×10^4 SD: 7.8×10^3	0.13	1.03
4.	A2	M: 1.8×10^7* SD: 2.4×10^6	0.37	-	M: 1.2×10^7* SD: 1.5×10^7	-	-	M: 2.2×10^6 SD: 7.6×10^5	-	-	M: 2.7×10^5 SD: 5.7×10^4	-	-	M: 2.0×10^4 SD: 1.9×10^2	-	0.91
5.	A3	M: 3.6×10^6* SD: 3.0×10^6	1.06	0.03	M: 1.2×10^6 SD: 1.5×10^6	0.05	0.28	M: 1.4×10^5 SD: 2.5×10^4	0.93	0.36	M: 1.2×10^6* SD: 8.1×10^5	-	-	M: 2.0×10^4 SD: 3.9×10^3	0.01	0.91
6.	A4	M: 1.5×10^7* SD: 1.7×10^7	0.43	-	M: 1.8×10^4* SD: 1.5×10^4	1.86	2.10	M: 2.1×10^3* SD: 8.8×10^1	2.77	2.20	M: 2.3×10^5 SD: 1.1×10^5	0.04	-	M: 2.1×10^4 SD: 2.0×10^3	-	0.89
7.	A5	M: 1.8×10^7* SD: 2.4×10^6	0.36	-	M: 2.0×10^6* SD: 3.6×10^5	-	0.05	M: 3.7×10^5 SD: 1.4×10^5	0.51	-	M: 6.7×10^5* SD: 3.3×10^5	-	-	M: 2.3×10^4 SD: 3.3×10^3	-	0.84
8.	A6	M: 2.2×10^6* SD: 2.3×10^5	1.28	0.24	M: 1.9×10^6* SD: 9.9×10^5	-	0.08	M: 4.2×10^5 SD: 7.2×10^4	0.46	-	M: 1.9×10^5* SD: 1.9×10^4	0.12	-	M: 4.4×10^4* SD: 1.5×10^4	-	0.57
9.	A7	M: 6.1×10^2* SD: 3.1×10^2	4.84	3.81	M: 1.7×10^1* SD: 2.2×10^1	4.89	5.13	M: 1.1×10^5* SD: 1.1×10^2	1.03	0.47	M: 1.8×10^1* SD: 2.6×10^1	4.13	3.83	M: 1.5×10^2* SD: 1.9×10^2	2.14	3.05
10.	A8	M: 8.7×10^2* SD: 9.6×10^2	4.68	3.65	M: 2.3×10^2* SD: 3.2×10^2	3.75	3.98	M: 7.7×10^4* SD: 1.2×10^4	1.20	0.63	M: 2.2×10^1* SD: 2.2×10^1	4.05	3.75	M: 1.1×10^2* SD: 2.1×10^1	2.26	3.16
11.	B1	M: 4.8×10^7 SD: 1.3×10^7	0.06	-	M: 9.6×10^6* SD: 5.5×10^6	0.28	-	M: 8.1×10^6* SD: 5.1×10^6	-	-	M: 1.7×10^6* SD: 1.2×10^6	-	-	M: 2.0×10^4 SD: 4.6×10^3	-	0.88
12.	B2	M: 5.9×10^7* SD: 3.9×10^7	-	-	M: 1.6×10^7 SD: 1.4×10^7	0.05	-	M: 4.9×10^6* SD: 1.8×10^6	-	-	M: 6.3×10^5 SD: 5.2×10^5	-	-	M: 1.4×10^4 SD: 1.6×10^3	0.14	1.03
13.	B3	M: 1.7×10^7* SD: 3.1×10^6	0.50	-	M: 1.8×10^6* SD: 1.1×10^6	1.01	0.34	M: 1.2×10^7* SD: 1.1×10^7	-	-	M: 2.9×10^5 SD: 2.4×10^5	-	-	M: 1.7×10^4 SD: 2.2×10^3	0.08	0.97
14.	B4	M: 2.7×10^7* SD: 1.2×10^7	0.31	-	M: 1.2×10^7* SD: 1.7×10^6	0.18	-	M: 1.0×10^6 SD: 9.7×10^5	0.48	-	M: 2.8×10^5 SD: 1.5×10^5	-	-	M: 1.4×10^4 SD: 5.4×10^3	0.14	1.03

The efficiency of filtration (E,%) was calculated according to Majchrzycka et al., (2012) [15].

Differences between the number of microorganism in the bioactive nonwovens and control sample were analysed using One-Way Analysis of Variance (ANOVA). Differences were considered significant at $p<0.05$. All data were analysed using the computer program Origin 6.1.

Results and discussion

The nonwovens studied exhibited different biological activity depending on the nonwoven type, conditioning, biocide concentration, and the manner of their incorporation into the nonwovens (***Table 2***). Needled nonwovens were found to display stronger antimicrobial activity (biostatic from 0.01 to 4.9 and biocidal from 0.05 to 5.1) than melt-blown nonwovens (biostatic from 0.05 to 1.0 and biocidal from 0.3 to 1) (***Table 2***). Similar findings were reported by Majchrzycka et al. (2010), who studied melt-blown and needled nonwovens containing various active agents (silver and quaternary ammonium salts) [13, 14]. Also in their study the highest biological activity against *S. aureus* and *E. coli* was exhibited by needled nonwovens. According to Majchrzycka et al., the lower activity of melt-blown nonwovens is due to the insufficient migration of the active substance to the surface of the nonwoven, resulting in inadequate contact of the biocide with the microorganisms. However, in the present study, the biocide was incorporated by spraying or a bath, rather than directly introduced into the polymer, as in Majchrzycka et al. The type of nonwoven – needled and melt-blown may significantly alter the application efficiency of Sanitized biocide that was in the solution (QACs in the water). The uniformity of Sanitized biocide distribution for melt-blown nonwovens is different than for needled nonwovens. This is due to a more compact surface structure of melt-blown nonwovens; these properties allow only surface distribution of an aqueous biocide solution. In the case of needled nonwovens, the solution wets the entire volume of the product. In the bathing method, the biocide is distributed more evenly than in the spraying method. It should be noted, however, that the antimicrobial efficiency of the resulting product depends primarily on the concentration of biocide therein . As for biocides embedded on media, which have been investigated by Majchrzycka et al., [13, 14], the effect of their distribution on antimicrobial activity of the product is as strong as that of the biocide concentration. The uneven distribution and stronger hydrophobic qualities of melt-blown nonwovens caused much lower antimicrobial activity than those of needled nonwovens, which was confirmed by the results obtained in the present research.

In turn, it should be emphasised that the melt-blown nonwovens very efficiently filtered oil mist (87.6% to 89.3%) (***Table 3***). Their filtration efficiency decreased only slightly following biocide incorporation (63.7% – 79.4%) and conditioning (61.6% – 75.9%) (***Table 3***). The initial flow resistance in the nonwovens analysed ranged from 31.4 to 32.5 Pa. Both oil mist penetration and initial flow resistance conformed to the requirements of the relevant standard (EN 149:2001+A1:2009). Similar filtration

properties of bioactive materials used in respirators were described by Majchrzycka et al. (2012) [15]. Thus both types of materials should be used in the production of air-purifying respirators: melt-blown nonwovens, which exhibit good filtration properties, and needled nonwovens, which have high antimicrobial activity. For full characterisation of bioactive nonwovens used for respiratory protection against bioaerosols, investigations should be extended to check the filtration of microorganisms on the filter material. However, no legal standards for evaluating bioaerosol filtration efficiency have been developed so far (it is not known what microorganisms should be tested, nor what apparatus and research methodology should be used). The lack of legal standards in evaluating fabrics in terms of bioaerosol filtration and only a few innovative reports on this subject in the literature [14, 15] point to the need for continued research in this area.

Comparing the biological activity of the quaternary ammonium salts incorporated into needled nonwovens studied by Majchrzycka et al. (2010) and the activity of formulation Sanitized® T 99-19 examined in this work (***Table 2***), it was found that Sanitized® T 99-19 displays higher biostatic and biocidal activity (A_{st} = 5.2 for nonwoven A8 and A_b = 5.1 for nonwoven A7) than the compounds investigated by Majchrzycka et al. (2010) (A_{st} = 3.6, A_b = 4.3) [13, 14]. This finding is important from the point of view of the possibility to use this biocidal formula in the textile industry for the production of respirators. Previous publications have reported the use of formulation Sanitized® T 99-19 for finishing hygienic footwear materials (linings and interlinings) as well as every-day use fabrics [18, 19].

In our study it was found that the biological activity of the nonwovens against microorganisms increases with the concentration of Sanitized® T 99-19. At a concentration of 0.7%, the formulation did not inhibit the growth of the microorganisms studied. The biostatic activity of the needled nonwovens containing 0.7% Sanitized® T 99-19 ranged from 1 to 1.3, while its biocidal activity was 0.05 to 1. In turn, the needled nonwovens containing 2% Sanitized® T 99-19 exhibited biostatic activity ranging from 0.01 to 4.9 and biocidal activity of 0.03 to 5.1. Similar findings were reported by Kenawy et al. (2003), Han and Yang (2004), Shaozao et al. (2000), and Majchrzycka et al. (2012), who studied nonwovens containing active compounds at different concentrations and found that their effect on microorganisms rose with an increasing content of active groups [5, 7, 10, 15, 25].

We determined that the process of conditioning does not negatively affect the biological activity of nonwovens containing Sanitized® T 99-19 (***Table 2***). A pairwise comparison of nonwovens, out of which only one was conditioned (e.g., A7 and A8), revealed that they had a similar effect on the microorganisms studied. The unconditioned nonwoven A7 exhibited biostatic activity of 1.0 – 4.9 and biocidal activity of 0.5 – 5.1, while the biostatic and biocidal activity of the conditioned nonwoven A8 amounted to 1.2 – 4.7 and 0.6 – 4, respectively. Different results were obtained by Gutarowska et al. (2008), who examined the influence of conditioning on the efficiency of bioactive respirators [20]. They found that bioactive nonwovens containing active substances such as ketamine and silver

Table 3. *Filtration properties of melt-blown nonwovens tested: M – mean. SD – standard deviation. (-) – not tested (B0-control sample. B1. B3 – samples without conditioning).*

Nonwoven	Filtration eficiency, %			Air flow resistance, Pa
	Before biocide application	After biocide application	After conditioning	
B0	M:88.0 SD:1.3	-	-	M:31.5 SD:1.2
B1	M:87.9 SD:1.4	M:78.1 SD:0.5	-	M:32.1 SD:1.6
B2	M:87.6 SD:1.2	M:79.4 SD:2.7	M:75.9 SD:2.8	M:31.4 SD:1.2
B3	M:89.3 SD:0.8	M:63.7 SD:4.2	-	M:33.7 SD:1.0
B4	M:88.5 SD:0.9	M:64.9 SD:6.4	M:61.6 SD:5.4	M:32.5 SD:1.2

Table 4. *Activity of bioactive A8 nonwoven against microorganisms isolated from workplaces: M - mean., SD - standard deviation, A_b - biocidal activity. A_s - biostatic activity, (m)- museums; (c) – composting plants, (t) – tanneries, □ a value below 0.5 was accepted as low and meant a min. 3-fold increase of the number of microorganisms, □ a value of level 3 or more was regarded as high and meant a 1000-fold or higher increase in the number of microorganisms, * significantly different from the control sample (One-Way ANOVA, $p < 0.05$).*

No.		Nonwoven / Strain (source of isolation)	Number of microorganisms, cfu/sample				
			A0, t = 0 h	A0, t = 8 h	A8, t = 0 h		
			M. SD	M. SD	M. SD	A_s	A_b
1.	Bacteria	*Bacillus pumilus* (c)	M: 2.5 × 10^6 SD: 1.2 × 10^6	M: 2.8 × 10^7 SD: 4.1 × 10^7	M: 2.1 × 10^4 SD: 1.1 × 10^4	3.13	2.08
2.	Bacteria	*Bacillus subtilis* (m)	M: 3.2 × 10^6 SD: 2.6 × 10^6	M: 3.3 × 10^6 SD: 6.7 × 10^5	M: 3.7 × 10^{2*} SD: 7.6 × 10^1	3.96	3.94
3.	Bacteria	*Staphylococcus haemolyticus* (m)	M: 1.8 × 10^5 SD: 4.4 × 10^4	M: 2.8 × 10^7 SD: 9.6 × 10^6	M: 1.8 × 10^{2*} SD: 1.0 × 10^2	5.18	3.00
4.	Yeast	*Candida parapsilosis* (t)	M: 1.1 × 10^5 SD: 2.9 × 10^4	M: 1.6 × 10^6 SD: 9.3 × 10^5	M: 1.8 × 10^{2*} SD: 1.2 × 10^2	3.93	2.78
5.	Yeast	*Cryptococcus albidus* (t)	M: 1.8 × 10^4 SD: 4.8 × 10^3	M: 4.2 × 10^5 SD: 1.4 × 10^5	M: 1.1 × 10^{3*} SD: 3.6 × 10^2	2.58	1.20
6.	Yeast	*Rhodotorula glutinis* (t)	M: 5.5 × 10^4 SD: 6.0 × 10^3	M: 1.7 × 10^5 SD: 6.3 × 10^4	M: 4.2 × 10^{2*} SD 1.6 × 10^2	2.61	2.12
7.	Moulds	*Aspergillus niger* (m)	M: 2.2 × 10^5 SD: 1.2 × 10^4	M: 4.4 × 10^4 SD: 1.5 × 10^4	M: 3.4 × 10^4 SD 1.9 × 10^4	0.11	0.82
8.	Moulds	*Aspergillus fumigatus* (c)	M: 5.6 × 10^5 SD: 3.1 × 10^5	M: 1.7 × 10^5 SD: 8.4 × 10^4	M: 2.6 × 10^{2*} SD: 1.3 × 10^2	2.81	3.34
9.	Moulds	*Aspergillus versicolor* (m)	M: 1.6 × 10^4 SD: 3.1 × 10^3	M: 1.8 × 10^4 SD: 2.2 × 10^4	M: 1.6 × 10^{1*} SD: 1.6 × 10^1	3.04	3.00
10.	Moulds	*Cladosporium cladosporioides*(t)	M: 4.8 × 10^4 SD: 1.6 × 10^4	M: 2.0 × 10^4 SD: 9.5 × 10^3	M: 1.2 × 10^{4*} SD 5.3 × 10^3	0.23	0.61
11.	Moulds	*Cladosporium macrocarpum* (c)	M: 1.3 × 10^4 SD: 3.6 × 10^3	M: 1.6 × 10^4 SD: 6.4 × 10^3	M: 6.0 × 10^{4*} SD: 1.2 × 10^3	0.42	0.34
12.	Moulds	*Penicillium aurantiogriseum* (m)	M: 8.1 × 10^4 SD: 3.0 × 10^4	M: 4.9 × 10^4 SD: 1.4 × 10^4	M: 1.8 × 10^4 SD: 1.9 × 10^4	0.44	0.66
13.	Moulds	*Penicillium chrysogenum* (t)	M: 1.5 × 10^5 SD: 3.2 × 10^5	M: 9.4 × 10^3 SD: 1.0 × 10^3	M: 3.3 × 10^{2*} SD: 3.9 × 10^2	1.46	2.66
14.	Moulds	*Penicillium commune* (m)	M: 4.7 × 10^5 SD: 2.7 × 10^5	M: 1.4 × 10^5 SD: 4.0 × 10^4	M: 4.6 × 10^{4*} SD: 2.3 × 10^4	0.49	1.01
15.	Moulds	*Penicillium corylophilum* (m)	M: 3.2 × 10^5 SD: 2.3 × 10^5	M: 7.1 × 10^4 SD: 2.2 × 10^4	M: 2.8 × 10^{4*} SD 1.0 × 10^4	0.40	1.06
16.	Moulds	*Penicillium crustosum* (c)	M: 2.5 × 10^4 SD: 1.7 × 10^3	M: 4.8 × 10^4 SD: 1.3 × 10^4	M: 1.3 × 10^{3*} SD: 4.8 × 10^0	3.47	3.18
17.	Moulds	*Penicillium janthinellum* (m)	M: 6.01 × 0^4 SD: 2.3 × 10^4	M: 3.3 × 10^4 SD: 1.1 × 10^4	M: 1.6 × 10^{4*} SD: 1.0 × 10^4	0.31	0.57
18.	Moulds	*Penicillium simplicissimum* (c)	M: 2.7 × 10^5 SD: 4.2 × 10^4	M: 2.0 × 10^5 SD: 8.9 × 10^4	M: 3.0 × 10^{5*} SD: 2.0 × 10^4	0.83	0.95

nanoparticles exhibited much lower inhibitory and biocidal activity against collection strains than unconditioned textiles. Therefore the type of bioactive substance may be of importance here, as some of them may become more volatile and more readily degradable at an elevated temperature (conditioning at 70 °C). Such an effect was not found for Sanitized® T 99-19, which is based on quaternary ammonium salts.

Our analyses revealed that the manner of incorporation of the biocide into nonwovens has a significant influence on the antimicrobial efficiency of the products (***Table 2***). The nonwovens subjected to a biocide bath exhibited higher biological activity than those subjected to spraying. The highest biostatic and biocidal activity of the nonwovens sprayed with Sanitized® T 99-19 was A_{st} = 2.8 and A_b = 2.2 (A4), respectively. The biocide bath increased the antimicrobial efficiency of the nonwovens (the highest biostatic and biocidal activity amounted to A_{st} = 4.9 and A_b = 5.1, respectively, for nonwoven A7). Differences in nonwoven activity depending on the manner of biocide incorporation were also described by Majchrzycka et al. (2006) [26].

In our study, the various groups of microorganisms were found to exhibit different sensitivity levels to the most efficient bioactive nonwoven, that is, A8. The nonwoven showed the highest antimicrobial activity against bacteria (A_{st} = 3.1 – 5.2; A_b = 2.1 – 3.9), followed by yeasts (A_{st} = 2.6 – 3.9; A_b = 1.2 – 2.8) and moulds (A_{st} = 0.1 – 3.5; A_b = 0.3 – 3.3). Furthermore the biological activity levels of nonwoven A8 against collection strains and strains isolated from workplaces were compared (***Table 4***). The biostatic activity of A8 was more variable against the strains isolated from the environment (from 0.1 to 5.2) than against the collection strains (1.2 to 4.7). On the other hand, the biocidal efficiency of A8 was similar for the collection strains (0.6 to 4) and for the isolated strains (0.3 to 3.9). This is an important observation because biocides and biologically active nonwovens are tested against collection strains rather than strains isolated from workplaces. Żakowska (2006) argues that while evaluating the efficiency of a biocide or antimicrobial nonwoven, appropriate biological material should be used, that is, strains isolated from the places where a given biocide or bioactive nonwoven are meant to be used, in order for the results to be meaningful from a practical viewpoint [27]. In the present study, a comparison of different strains belonging to the same genera revealed that the nonwovens had high biostatic and biocidal activity against *Staphylococcus* (collection strain A_{st} = 3.8; A_b = 4; strain isolated from workplace: A_{st} = 5.2; A_b = 3) and the yeast *Candida* (collection strain A_{st} = 4; A_b = 3.8; strain isolated from workplace: A_{st} = 3.9; A_b = 2.8). Within *Bacillus*, the collection strain was not found to be very sensitive (A_{st} = 1.2; A_b = 0.6) as compared to the isolated strains belonging to the same genus (A_{st} = 3.1 – 4; A_b = 2.1 – 3.9). On the other hand, *Aspergillus* strains differed in terms of their sensitivity to the ATCC strain (A_{st} = 2.3; A_b = 3.2), depending on the species they belonged to (A_{st} = 0.1 – 3; A_b = 0.8 – 3.3). We determined which microorganisms were characterised by the greatest sensitivity to needled nonwoven A8, which was subjected to a bath of 2% Sanitized® T 99-19; these were *E. coli* ATCC (A_{st} = 4.7; A_b = 3.6) and *S. haemolyticus* isolated from a museum (A_{st} = 5.2; A_b = 3). In turn, the greatest resistance was shown by the mould *A. niger* isolated from a museum (A_{st} = 0.1; A_b = 0.8) and *C. cladosporioides* from a tannery (A_{st} = 0.2, A_b = 0.6). Kenawy et al. (2003) also observed that quaternary ammonium salts incorporated into nonwovens were more efficient against the Gram-negative *E. coli* strain than against the Gram-positive *B. subtilis* strain. They explained the lower sensitivity of *B. subtilis* in the presence of spores, which are more resistant to the effects of active substances [7]. A screening test of bioactive nonwovens led to the identification of the most efficient technology of manufacturing filtration nonwovens. Subjecting needled nonwovens to a bath of a 2% solution of Sanitized® T 99-19 guarantees high biological activity against a wide spectrum of microorganisms, as well as those isolated from workplaces. The fact of obtaining highly efficient nonwovens for the production of personal protection equipment will result in improving the safety of workers in many industries, including waste management, where they are exposed to harmful biological factors.

It is essential for application purposes to study the durability of the bioactive materials obtained, especially their antimicrobial properties. After half-a-year storage at a temperature of 20 °C and RH 45%, the antimicrobial activities obtained decreased by 18 - 27%. As for the durability of filtration efficiency, the manufacturer ensures that model respirators without the addition of a biocide shall maintain the filtering qualities for a period of three years from the date of manufacture and in storage conditions from 20 to 40 °C and at RH < 90%.

Conclusions

1. Needled nonwovens exhibit higher antimicrobial activity than melt-blown nonwovens.
2. The biological efficiency of nonwovens increases with increased concentration (from 0.7% to 2%) of the formulation Sanitized® T 99-19.
3. The process of conditioning of finished products (at 70 °C or 30 °C for 24 h) does not hamper the antimicrobial activity of nonwovens containing Sanitized® T 99-19.
4. It was found that a bath of biocide solution is a more effective method of improving the antimicrobial efficiency of nonwovens than biocide spraying.
5. Needled nonwoven subjected to a bath of 2% Sanitized® T 99-19 exhibits the highest antimicrobial efficiency against the bacteria *E. coli* and *S. haemolyticus* and the yeast *Candida albicans*, and the lowest efficiency against the moulds *A. niger* and *C. cladosporioides.*
6. Needled nonwoven subjected to a bath of 2% Sanitized® T 99-19 shows high biostatic and biocidal activity against both collection strains and those isolated from the environment within the genera *Staphylococcus* and *Candida*. Within the genus *Bacillus*, the collection strain was not found to be very sensitive as compared to other strains belonging to this genus, but isolated from workplaces. *Aspergillus* moulds isolated from the environment reveal different degrees of sensitivity as compared to the ATCC strain, depending on their species.
7. Filtration properties of the melt-blown nonwovens studied conform to Standard EN 149:2001+A1:2009, but due to their low antimicrobial activity, air-purifying respirators they should be complemented with needled nonwovens, which show high biostatic and biocidal properties.
8. The optimum method of the production of filtration nonwovens makes it possible to obtain air-purifying respirators that effectively reduce microbial threats in workplaces.

Acknowledgments

- *This study was realized within the project of the Polish National Center for Research and Development coordinated by the Central Institute for Labour Protection – National Research Institute, no. III.B.03 "Development of principles for evaluation and prevention of hazards caused by biological agents in the working environment using indicators of microbial contamination".*
- *The paper presented was written courtesy of Filter Service Sp. z o.o.. We would like to express our warm thanks to Dr Jacek Krzyżanowski, the President, for cooperation in the research as well as M. Sc. Remigiusz Jabłonski and M. Sc. Mateusz Tyburcy, the employees, for the preparation of tested materials..*

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Received 09.09.2013 Reviewed 19.11.2013