International Journal of Occupational Safety and Ergonomics (*JOSE*) 2010, Vol. 16, No. 2, 275–280

Aspects of Tests and Assessment of Filtering Materials Used for Respiratory Protection Against Bioaerosols. Part II: Sweat in the Environment, Microorganisms in the Form of a Bioaerosol

Katarzyna Majchrzycka

Department of Personal Protective Equipment, Central Institute for Labour Protection – National Research Institute (CIOP-PIB), Łódź, Poland

Beata Gutarowska

Department of Biotechnology and Food Science, Technical University of Łódź, Łódź, Poland

Agnieszka Brochocka

Department of Personal Protective Equipment, Central Institute for Labour Protection – National Research Institute (CIOP-PIB), Łódź, Poland

The second part of the article presents the results of a study of antimicrobial activity of filter nonwovens with an addition of biocides, as a function of the presence of sweat in the environment and the method of microbe deposition on a nonwoven in the form of a liquid and a bioaerosol. At the same time, the filtration efficiency of nonwovens against microorganisms in the form of a bioaerosol was tested with the dynamic method. The results showed that the addition of sweat on the surface of a nonwoven resulted in an insignificant decrease of biological activity that still remained high. Moreover, an active nonwoven showed biostatic and biocidal activity only when microbes were deposited on the surface in the form of a solution. The nonwoven did not show any biological activity after deposition of microorganisms with the dynamical method in the form of a bioaerosol.

filtering material bioactive fibres bacteria, biostatic and biocidal activity sweat bioaerosol filtration

1. INTRODUCTION

Microorganisms are present in the air in the form of bioaerosols. Bioaerosols are biphasic or three-phase systems composed of the dispersing phase (the air) and dispersed liquid or solid (water vapour, dust particles, seeds, secretions from the human nasopharyngeal cavity, etc.). Bioaerosols ensure the survival of microorganisms in the air with unfavourable conditions: drought, lack of food, operation of light, UV radiation, etc. [1, 2].

Every environmental factor with a protective effect on microbes contributes to an increased survivability of microorganisms, even to their development. They are primarily airborne organic substances: proteins, sugars and lipids that not

Correspondence and requests for offprints should be sent to Katarzyna Majchrzycka, CIOP-PIB, ul. Wierzbowa 48, 90-133 Łódź, Poland. E-mail: <kamaj@ciop.lodz.pl>.

only have a protective effect on microorganisms, but also provide carbon sources that are necessary for feeding and gaining energy [3].

The microbes stopped in filter nonwovens during air filtration can survive for a long time, because they are stopped together with all bioaerosols with organic particles in the form of mucus, saliva droplets, etc. [1]. When respiratory protection half-masks are used, the filter material is wetted with water vapour and sweat.

To destroy the microflora stopped during filtration, disinfecting agents (biocides) are added to filter materials. Their efficiency and performance depend on a number of factors including their structure and the properties of the active agent, action time, concentration of the active agent, number of microbes in the environment, temperature, pH, the presence of impurities, surfactants, etc. [4].

The antimicrobial activity of nonwovens is a result of many factors. Part I of this paper (p. 263–73) discusses how the type of active agent, contact time and the type of microbes affect the activity of nonwovens. Part II investigates whether an addition of artificial sweat to a bioactive nonwoven has a protective effect on the microbes and whether it changes their survivability. In addition, the sensitivity of bacteria deposited on a nonwoven in the form of a bioaerosol was tested and compared with the variant where the bacteria was deposited in liquid form. The filtration of a bioaerosol with *Escherichia coli* and *Staphylococcus aureus* was also tested to confirm the filtration efficiency of bioactive materials against the microorganisms.

2. EXPERIMENTAL

2.1. Tested Materials

The study was carried out with nonwovens XII and XIII (Table 1, p. 264). Nonwoven XIII showed high antimicrobial biocidal activity (see Part I): 3.2–4.3 against *E. coli* and *S. aureus* after 6 h of incubation. Samples of materials were prepared according to the method described in Part I. Round, 8 cm in diameter, pieces were cut out of the materials for the dynamic method.

2.2. Microorganisms

E. coli and *S. aureus* were used in the tests (Table 2, p. 265). The microorganisms were activated on liquid TSB (tryptic soy bullion) medium at 37 °C for 48 h. Part I describes the preparation of an inoculum suspension. The density of the bacterial suspension ranged from 10^8 to 10^9 CFU/ml.

2.3. Media and Reagents

Two culture media were used: TSB to activate the bacteria prior to the preparation of the inoculum suspension; TSA (tryptic soy agar) to culture the bacteria in Petri dishes, after the contact of microorganisms with fabrics. A solution of physiological salt (0.85% NaCl) was used for dilutions and to wash out the microorganisms from fabrics. Artificial sweat was an acidic solution, its composition conforming with Standard No. EN ISO 105-E04:1996 [5].

3. TEST METHODS

3.1. Evaluation of Sweat Effect on Antimicrobial Activity of Nonwovens

The static method was used to study the effect of sweat on the antimicrobial activity of nonwovens (Part I). Artificial sweat (0.1 ml) and the inoculum suspension (0.1 ml) were both deposited on nonwovens XIII and XII (control). Each sample of material was inoculated three times, with three exposure times and incubated at 37 °C. The samples were taken at 0, after 4 and after 6 h from material inoculation.

3.2. Evaluation of Antimicrobial Activity of Nonwovens Against Microorganisms in the Form of a Bioaerosol (Dynamic Method)

The tests compared the development of antimicrobial activity of nonwovens depending on the form of deposited microorganisms. Bacteria *E. coli* and *S. aureus* were deposited on nonwovens as a liquid (an inoculum suspension) and sprayed as a bioaerosol. The activity of

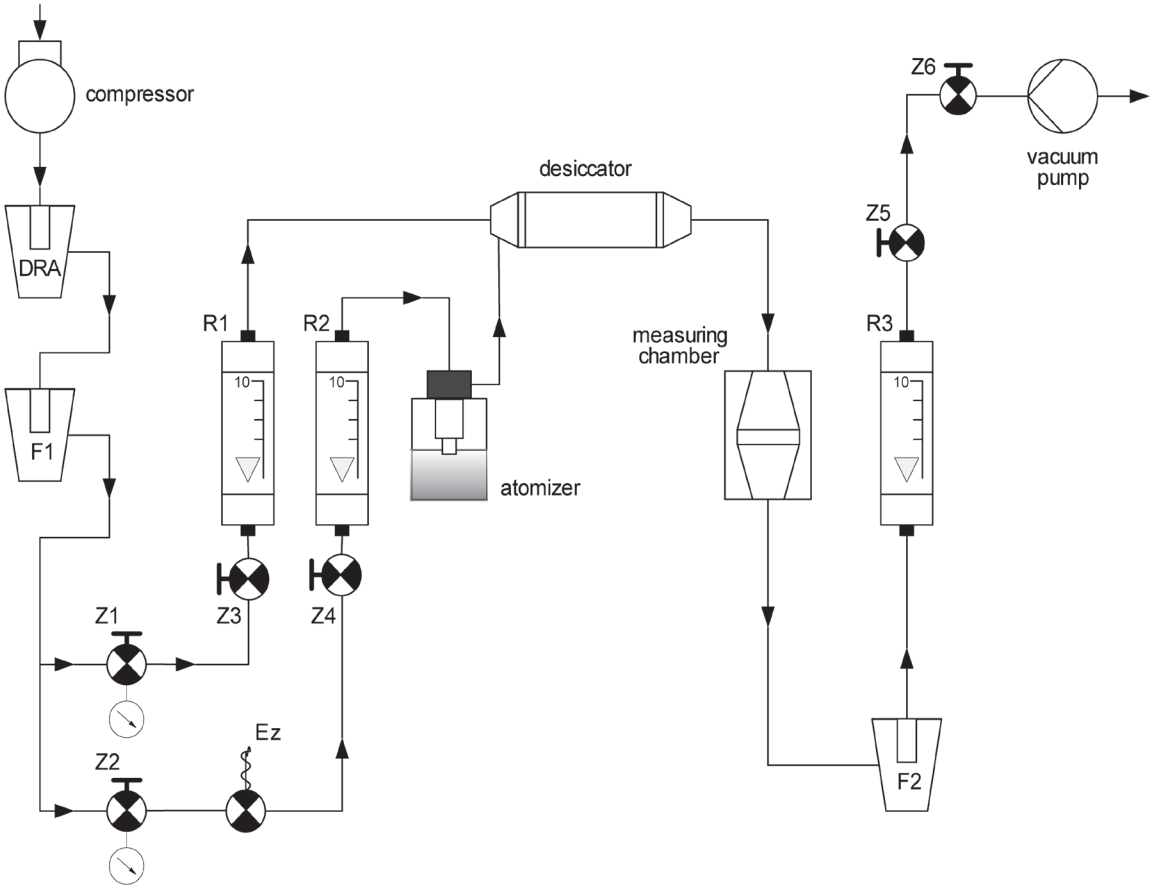

Figure 1. Experimental setup used for testing antimicrobial activity of filter materials (the dynamic method). *Notes.* ODW—cyclone separator; F1—air filter; Z1, Z2—pressure reduction valves; M1, M2—pressure gauges; Ez—electromagnetic valve; R1, R2, R3—airflow meters/rotameters; Z3, Z4—airflow fine adjustment valves; Z5—air suction fine adjustment valve; Z6—air suction preliminary adjustment valve; F2—output filter; DRA—oil filter.

nonwovens against microorganisms in the form of a liquid was tested with the static method (Part I). In another, dynamic, variant of the experiment, the microorganisms were deposited as a bioaerosol. The inoculum suspension prepared for the tests was a bioaerosol aerosolized in a system composed of an active filter nonwoven and a microbiological filter. The tests involved dynamic generation of the bacterial aerosol with an atomizer, mixing it with a stream of dry air and guiding the bacteria through a set of filters assembled within a tight system. Figure 1 illustrates the setup used to generate the bioaerosol and to guide it onto a two-filter set used in the dynamic method. The bioaerosol produced in tests moved at a flow rate of 30 L/min. The flow rate of the air, both supplied to the setup and drained from it, was controlled by a rotameter system. At the beginning of the experiment, the nebulisers (mist generators) created mist for 30 min, which was necessary to stabilize the aerosol in the device. Then, a set of two filters was installed for each test: a filter made of the tested nonwoven and a microbiological filter. The latter was used to analyse the number of microorganisms stopped in the filter material.

A gelatine Sartorius (Germany) microbiological filter with a diameter of 80 mm, pore diameter of 3 μm and 99.99% collection efficiency was used in the tests. A control test was also carried out; it consisted in spraying the bioaerosol on the microbiological filter for 20 min under experimental conditions (flow rate of 30 L/min, so that 600 L of the bioaerosol were plotted on the sample). It made it possible to achieve the total number of microbes in the air with a bioaerosol volume of 600 L. The results

were expressed in colony forming units per cubic metre (CFU/m^3) of the air. The number of the tested microorganisms in the bioaerosol ranged from 1.4×10^4 to 5.5×10^4 CFU/m^3.

Each time the experimental setup was switched on and the microorganisms sprayed for 20 min. After that time, the pump was switched off and the nonwoven filters were aseptically removed and placed in a sterile Petri dish. Then, the filters were stored for 4 and 6 h (since the bacteria were sprayed on the nonwoven) at 37 °C. The tests in time aimed at verifying how long the bacteria would be viable after they were sprayed on the filter material. Also, a sample at time 0 was taken, just after the bacteria were sprayed on the material. After appropriate time, like in the static method, the filters were washed out, the suspension was diluted and seeded on a solid TSA medium (the methodology of the static method; Part I). Each set of filters was analysed 4–6 times. After spraying, the microbiological filters used as the second ones in the set, were transferred into 10 ml of sterile physiological salt, warmed to 30 °C, dissolved, diluted and seeded on a TSA medium, as described in this paragraph. The biostatic and biocidal activity of nonwovens was calculated with the formula from Part I. The methods of calculating the bacterial survival index N_t and the statistical methods were discussed in section 4.3. in Part I.

4. RESULTS

4.1. Evaluation of the Biocidal Activity of Active Nonwoven With and Without an Addition of Artificial Sweat Against Bacteria *E. coli* and *S. aureus*

Tables 1–2 illustrate the bacterial survival index N_t, and the biological activity of nonwoven XIII with and without added sweat after 6-h exposure to bacteria.

Values of bacterial survival indexes on bioactive nonwoven with and without added sweat (Table 1) indicate a slightly higher bacteria survival in the presence of sweat. The tested nonwoven actively reacts to microorganisms, as during incubation the amount of surviving bacteria decreases significantly.

It has been found that after an addition of sweat on the surface of active nonwoven XIII its biological activity slightly decreased, still remaining on a high level (Table 2). The biocidal activity decreased by 0.23 for *E. coli* and by 0.43 for *S. aureus* on the nonwoven with added sweat, whereas the growth inhibition effect decreased by 0.05 and 0.13, respectively. The decrease in biocidal activity might have resulted from a protective effect of sweat on the microorganisms. However, statistical analysis with the *t* test showed that there were no statistically significant differences between N_t for both tested bacteria on

TABLE 1. Bacterial Survival Index N_t at 0 and After 6 h of Incubation With Bioactive Nonwoven XIII With and Without Sweat

	E. coli				*S. aureus*			
	0 h		6 h		0 h		6 h	
Nonwoven	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
XIII	1.000	0.206	0.000049	0.00000	1.000	0.505	0.000105	0.00000
XIII + sweat	1.000	0.464	0.000134	0.00000	1.000	0. 821	0.000256	0.00000

TABLE 2. Biostatic and Biocidal Activity of Nonwoven XIII With and Without Artificial Sweat After 6 h of Incubation With *E. coli* and *S. aureus*

	Biostatic Activity		Biocidal Activity	
Nonwoven	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
XIII	3.995	3.711	4.328	4.269
XIII + sweat	3.948	3.581	4.096	3.843

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

the nonwoven with and without sweat after 6 h of incubation ($p \leq .05$).

4.2. Evaluation of Biocidal Activity of Active Nonwoven Against Microorganisms *E. coli* and *S. aureus* as a Liquid and as a Bioaerosol

The experiment was carried out for two bacteria deposited on a nonwoven in the form of a solution (static method) and a bioaerosol (dynamic method). Tables 3–4 illustrate the bacterial survival index N_t, and the biostatic and biocidal activity of nonwoven XIII against tested bacteria deposited by aerolisolization of the suspension (dynamic method) and in the form of a solution (static method).

The results showed that bacterial survival indexes were higher for the aqueous suspension than for a bioaerosol (Table 3). The survival of bacteria placed on a nonwoven in the form of bioaerosol did not change during incubation with the material. Active nonwoven XIII showed biostatic and biocidal activity only if the microbes were deposited on its surface as a solution (Table 4). The nonwoven did not show any biological activity after dynamic deposition of microbes as a bioaerosol. Statistical analysis using the *t* test for both tested species showed that there were significant differences between N_t of bacteria after inoculation as a bioaerosol and an aqueous suspension and 6 h of incubation ($p \geq .05$).

4.3. Filtration Efficiency of Microorganisms on Active Filter Nonwovens

Filtration efficiency of tested nonwoven XIII and control nonwoven XII for bacteria was experimentally verified with the dynamic method. If was calculated for microorganisms as a ratio of the number of microorganisms stopped on the filter nonwoven to the sum of all the microorganisms that were present in the system during spraying. Figure 2 illustrates the results (as percentage). Both filter nonwovens XII and XIII filtered out *E. coli* and *S. aureus* with a high efficiency of 86–95%. Active nonwoven XIII had a slightly higher bacteria filtration efficiency (91–95%) than the control nonwoven without the addition of an active agent (nonwoven XII) (86–94%).

5. SUMMARY

The study indicates that sweat in the vicinity of antimicrobial textile materials does not essentially change their activity, which is satisfactory from the point of view of the users of protective half-masks. It was found that

TABLE 3. Bacterial Survival Index N_t at 0, After 4 and 6 h of Incubation of Bioactive Nonwoven XIII With *E. coli* and *S. aureus* as an Aerosol and as an Aqueous Suspension

	E. coli						*S. aureus*					
	0 h		4 h		6 h		0 h		4 h		6 h	
Form of Bioorganism	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***
Bioaerosol	1.000	0.334	0.480	0.425	0.495	0.266	1.000	0.504	0.779	0.170	0.738	0.111
Aqueous suspension	1.000	0.187	0.006	0.001	0.000	0.000	1.000	0.225	0.064	0.008	0.000	0.000

TABLE 4. Biostatic and Biocidal Activity of Nonwoven XIII Against *E. coli* and *S. aureus* Deposited in the Form of a Solution (Static Method) and a Bioaerosol (Dynamic Method) After 4 and 6 h of Incubation

	Biostatic Activity				Biocidal Activity			
	E. coli		*S. aureus*		*E. coli*		*S. aureus*	
Form of Bioorganism	**4 h**	**6 h**	**4 h**	**6 h**	**4 h**	**6 h**	**4 h**	**6 h**
Bioaerosol	2.227	1.914	1.301	3.559	2.600	4.285	1.334	3.652
Aqueous suspension	0.047	0.224	0.020	0.011	0.237	0.363	0.160	0.184

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

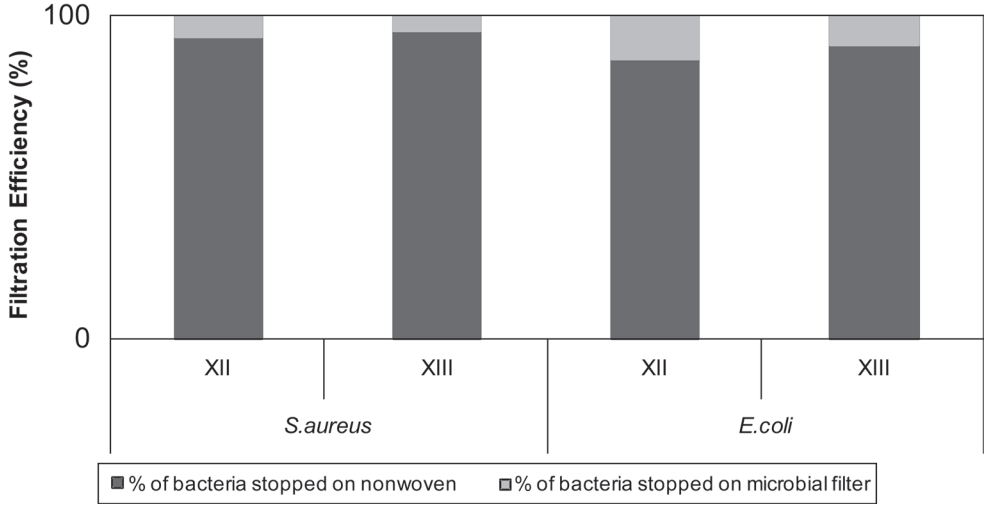

Figure 2. Effectiveness of filtration of nonwovens XII and XIII for *S. aureus* and *E. coli*

after adding sweat on the surface of an active nonwoven its biological activity decreased insignificantly, still remaining at a high level. A slight decrease in the activity may result from a protective effect of organic and mineral compounds in an artificial sweat solution (composed of histidine hydrochloride, sodium phosphate and sodium chloride).

The study brought surprising results with regard to a decrease in biological activity of the nonwoven after depositing microorganisms in the form of a bioaerosol as compared to a liquid suspension in physiological salt. A decrease in nonwoven activity against microorganisms placed on a material in the form of a bioaerosol may be caused by various stress factors, e.g., desiccation or shearing stress in the stream of the sprayed liquid.

The absence of nonwoven activity could result from low moisture content in the nonwoven after spraying the microorganisms in the air stream and during sample incubation. A moist environment probably activated the biocides in fibres (silver in the form of master basis, biocide 3 present in nonwoven XIII).

In a test of microbiological activity of nonwovens with the dynamic method one should verify if the textile material is suitably moist. In tests with the static method 0.1 ml of a suspension of microorganisms in physiological salt was deposited on the nonwoven. Such a volume of physiological salt in the sample of a polymer textile material of 4 cm^2 in surface area was sufficient to activate the antibacterial compounds. An explanation of the hypothesis requires additional studies of biological activity of nonwovens with an addition of various antimicrobial compounds incubated in different moisture conditions.

The active filter nonwovens tested in this study showed high filtration efficiency of 86–95% for bacteria *E. coli* and *S. aureus*. Nonwovens XII and XIII may be used in systems of half-masks as bioactive and filtering layers.

REFERENCES

1. Krzysztofik K. Mikroflora powietrza [Air microflora]. Warszawa, Poland: Oficyna Wydawnicza Politechniki Warszawskiej; 1992.
2. Libudzisz Z, Kowal K, Żakowska Z. Mikrobiologia techniczna [Technical microbiology]. Warszawa, Poland: PWN; 2007.
3. Schlegel HG. Mikrobiologia ogólna [General microbiology]. Warszawa, Poland: PWN; 1996.
4. Brycki B. Chemiczne inhibitory biodeterioracji [Chemical inhibitors of biodeterioration]. Ochrona przed korozją. 9s/A/2006:272–92.
5. European Committee for Standardization (CEN). Textiles—tests for colour fastness—part E04: colour fastness to perspiration (Standard No. EN ISO 105-E04:2009). Brussels, Belgium: CEN; 2009.