MICROBIAL GROWTH IN THE NONWOVEN MATERIALS OF DUST-LOADED FILTERING FACEPIECE RESPIRATORS

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ABSTRACT

The paper aims at understanding the effect of dust type and concentration in the filtering nonwovens used in the construction of FFRs on viability of microorganisms. The experiments were performed using melt-blown polypropylene nonwoven typically used in the construction of FFRs as a high efficiency filtering layer and two types of dust collected at the composting and cement plant. Deposition of dust was performed in dust chamber in conditions simulating average and high dust concentration at the workplace. The survival of microorganisms in dust-loaded nonwovens was determined using microbiological quantitative method and the distribution of biofilm formed on the samples was analysed with scanning electron microscopy.

Key Words: microorganisms, filtering facepiece respirators, filtering nonwovens, organic dust

1. INTRODUCTION

In the EU one of the most wide-spread occupational health effects related to the exposure to biological agents are respiratory symptoms resulting from inhalation of bioaerosols. Those health effects range from acute mild and self-limiting to sever chronic and even life-threatening. To protect workers against those types of hazards the European Union has issued recommendations to Member States in the form of Directive 2000/54/EC of the European Parliament and the EU Council. Pursuant to the requirements of the Directive, the employer has a duty to take preventive measures to protect workers exposed to biological agents, which may include the use of personal protective equipment.

In the case of exposure to bioaerosols entering the human body by airborne route it is necessary to use a filtering respiratory protective devices such as filtering facepiece respirators (FFRs) constructed from few layers of filtering nonwoven materials of diverse properties. Recent research shows that, even with short-term use of such FFRs in the bioaerosol rich environment, favourable conditions for rapid growth of microorganisms on the filtering nonwovens can occur [1]. The viability of microorganisms on individual filtering nonwovens used in the construction of FFRs depends primarily on the kind of microorganism and the humidity levels. Moreover, model experiments performed on those materials revealed that dust deposited in the filtering material may be a good environment for microorganism growth [2]. On the other hand microorganism survival rate was proven to be negligibly impacted by the type of filtering nonwoven, as there was no obvious relationship between survival and nonwoven characteristics (mass per unit area, thickness and contact angle). It was also established that the composition of nonwovens did not significantly affect microorganism survival [3].

The aim of the study was to assess the influence of various types and contents of dust on multilayer nonwoven structures of FFRs on viability of microorganisms based on the model experiments performed in dust chamber. A modified AATCC 100-2004 method will be used to measure the survivability of microorganisms from the collection of pure cultures: *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus niger*. Moreover the distribution of biofilm formed in those three dimensional nonwoven structures will be analysed using scanning electron microscopy.

2. MATERIALS AND METHODS

2.1 Filtering Nonwovens

Polypropylene nonwoven typically used in the construction of FFRs as a high efficiency filtering layer was selected to test the survivability of microorganisms in the presence of dust. The nonwoven was prepared using melt-blown technique, in which molten polymer is extruded via specially designed nozzles and then blown into individual fibres by hot air. The temperatures of the extruder heating zone were in the range of 250 to 345°C and the temperature of air was approx. 310°C. Individual sheet of the nonwoven used within the study consisted of two thinner sub-layers of the nonwoven to simulate a multi-layer structure that is usually used in the construction or FFRs. The characteristics of the nonwoven are shown in table 1.

Table 1. Characteristics of multi-layer filtering nonwoven

Parameter	Value
Thickness, mm	1.7 ± 0.2
Mass per unit area, g/m^2	75.8 ± 9.5
Paraffin oil mist penetration, %	7.3 ± 1.9
Sodium chloride aerosol penetration, %	6.2 ± 1.9
Pressure drop, Pa	199 ± 31

2.3 Dust deposition

Two types of dust were selected for the study: dust HC with high microbial contamination and high carbon to nitrogen ratio (total number of microorganisms $1.38\times10^8 \pm 1.27\times10^7$ CFU/g; C:N = 98.64) collected from the waste homogenization hall of composting plant and dust LC, with low microbial contamination and low carbon to nitrogen ratio ($2.10\times10^3 \pm 1.77\times10^2$ CFU/g; C:N = 10.71) collected from the clinker transporting conveyor hall at cement plant. Detailed characterization of the studied dust types can be found in [4]. Prior to testing, dust samples were subsequently sterilized and dried as described in [2].
Samples of filtering nonwoven of 79 cm^2 surface area were placed in a sample holder of a dust chamber. There, air-dust mixture was passed through the sample with a constant volumetric flow rate of 95 L/min. The dust deposition time was selected in such a way that the mass of dust deposited corresponded to the mass of dust accumulating on the filtering layers of half-masks when used in workplaces under average (2 min) and high (4 min) dust concentrations. Pristine polypropylene nonwoven samples of the same dimensions were also prepared to serve as control variant.

2.4 Assessment of microorganisms survivability

The microorganisms obtained from American Type Culture Collection (ATCC) and National Collection of Agricultural and Industrial Microorganisms (NCAIM) and stored in the Pure Culture Collection LOCK 105. They were belonging to various taxonomic groups and having diverse growth physiology were used to study survival on selected nonwovens containing dust, i.e.: *E. coli* (ATCC 10536), *S. aureus* (ATCC 6538) and *B. subtilis* (NCAIM 01644) bacteria; *C. albicans* (ATCC 10231) yeasts and *A. niger* (ATCC 16404) moulds. Inocula of 1.9×10^6 - 8.7×10^9 CFU/ml density were obtained according to methodology described in [3]. After that, 25 μl portions of the inocula were placed on pristine and dust loaded nonwoven samples cut into 2×2 cm squares. They were then placed in sterile Petri dishes and incubated in climatic chamber at 30±2°C and 80% relative humidity. To determine microorganism survival on pristine and dust loaded nonwovens quantitative static method AATCC 100-2004 was used [5]. Test samples were taken immediately after incubation (t=0 h) and after 2, 4, 6, 8 12, and 24 h. The tests were performed according to methodology described in [3]. Microorganism survivability was calculated as a ratio of the number of microorganisms after a specific incubation time to the initial number of microorganisms.

2.5 Assessment of biofilm formation by scanning electron microscopy

To assess the development of microbial biofilm, we used pristine and dust-loaded samples with high dust concentration. They were inoculated with a mixed culture of all five microorganisms, in equal volumetric ratios, prepared as described in 2.4. After that the samples were stored in a climatic chamber for 7 days at 30 ± 2°C and 80% relative humidity. Analysis of biofilm development was carried out using scanning electron microscope (SEM) (HITACHI SEM SU8010, Hitachi High-technology Corporation, Tokyo, Japan) following a prior sputtering with gold (Q150T ES, Quorum Technologies, Lewes, UK).

3. EXPERIMENTAL RESULTS AND DISCUSSION

3.1 Microorganisms survivability

The survivability of selected microorganisms depending on dust type and content for subsequent incubation times is presented in the figure 1.

The survivability of microorganisms depended mostly on microorganism type. The highest values were observed for *B. subtilis* bacteria (N = 351-194250%) and the lowest for *A. niger* moulds (N = 101-465%). The survival rate of bacteria (*E. coli*, *S. aureus* and *B subtilis*) depended not only on the microorganism species, but also on the type of dust. For cement dust characterized with low microbial contamination and low carbon to nitrogen ratio a decrease of survivability was observed, while for highly microbiologically contaminated dust from the composting plant the survivability values were at approximately the same level as for the control sample. Only for *E. coli* a slight increase of survivability was observed for the samples loaded with low concentration of cement dust (from 881% to 1668% after 12 h of incubation). Both types of dust significantly decreased *C. albicans* yeast survival (from 595% for control sample to 39% for cement dust-loaded samples and 98 % for composting plant dust-loaded samples). Survivability of *A. niger* moulds was stable regardless of the dust type and content, only for the cement dust at high concentration a spike in survivability was observed after 12 h of incubation.

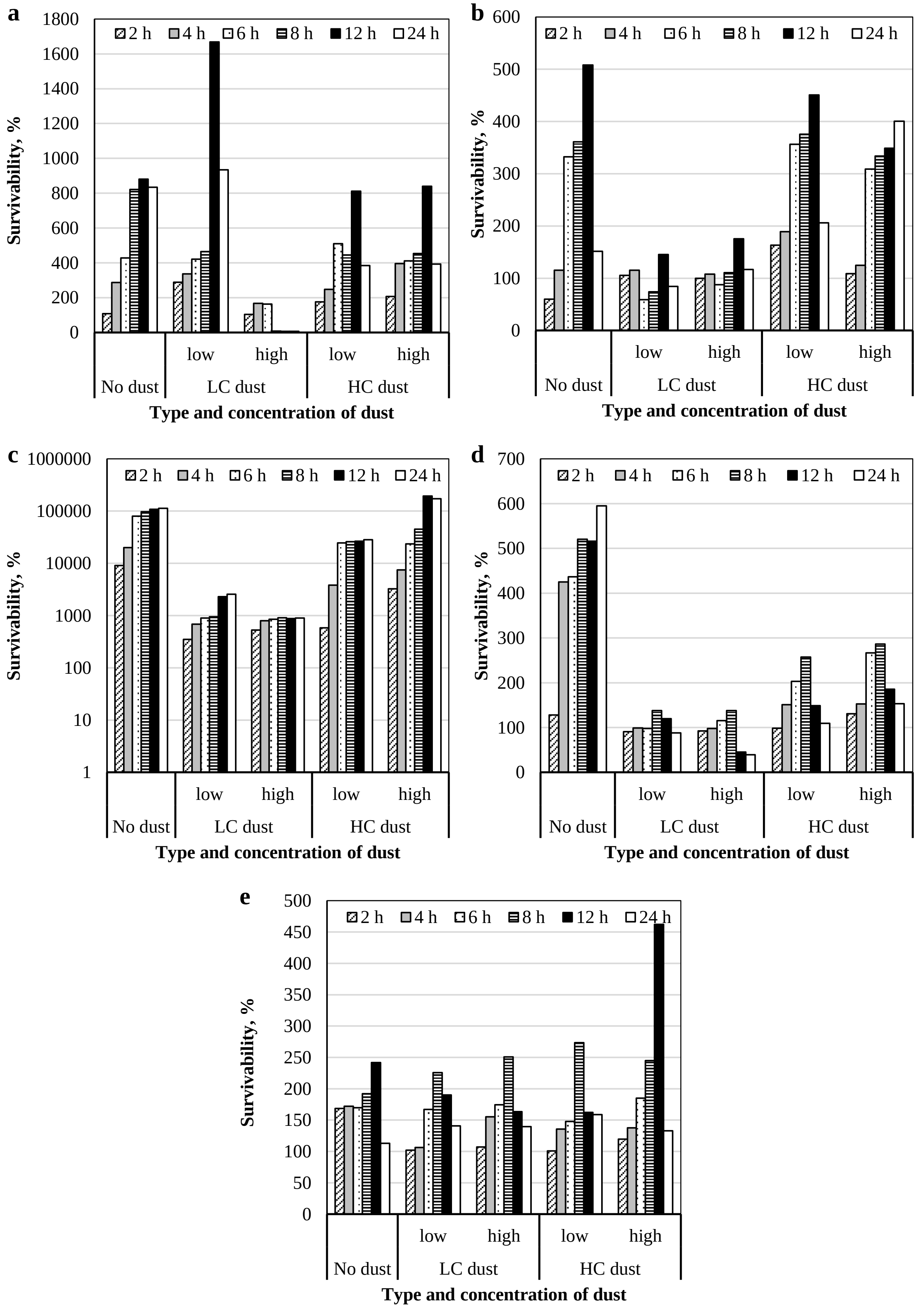

Figure 1. Survivability of microorganisms after subsequent incubation times for a) *E. coli*, b) *S. aureus*, c) *B. subtilis*, d) *C. albicans* and e) *A. niger*

3.2 Biofilm formation

Figure 2a shows pristine/non-loaded melt-blown filtering nonwoven. In all tested cases a biofilm covering single fibres (figure 2b), individual microbial cells coexisting with dust particles (figure 2c) as well as bacterial biofilm sticking fibres and dust together (figure 2d), were observed on SEM images. As previously described in [6], a biofilm that firmly sticks to the fibres may lower the porosity of the nonwoven causing increase in pressure drop and at the same time limiting the penetration of dust particles through the filtering material. On the other hand, in favourable flow conditions, fragments of biofilm may detach from the nonwoven surface, migrate within the filtering material, which in case of application into FFRs might constitute a source of secondary inhalation exposure for the worker.

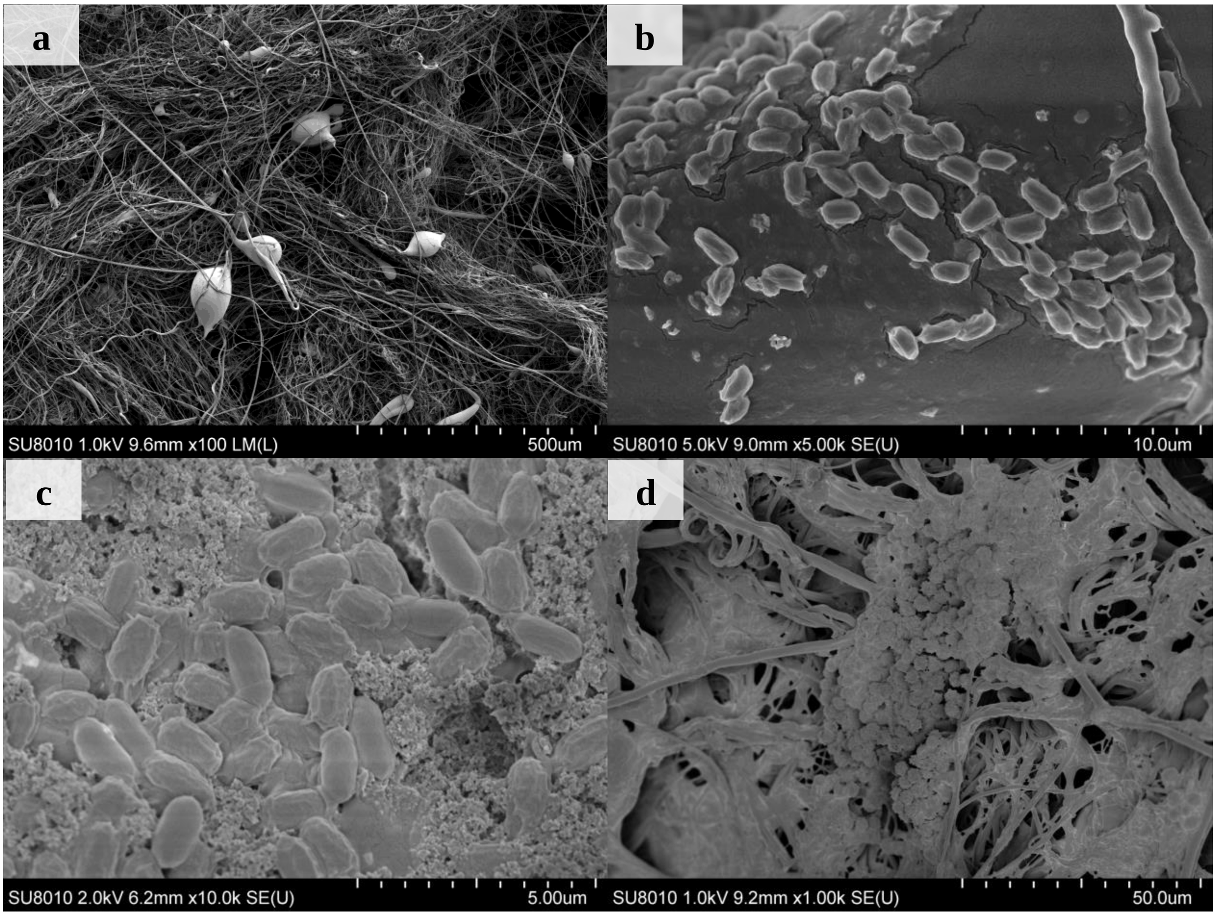

Figure 2. SEM images of (a) pristine melt-blown nonwoven (control sample) and (b-d) biofilms formed on dust-loaded nonwovens

4. CONCLUSION

Nonwoven used within the study consisted of two thin sub-layers of the basic nonwoven to simulate a multi-layer structure that is usually used in the construction or FFRs. Between the two sub-layers of the nonwoven an insulating air gap existed. This gap, however, did not inhibit the formation of biofilms. It is in agreement with previous research where no obvious relationship between microorganisms survival and nonwoven characteristics was observed. Multi-layer composition of nonwovens did not significantly affect microorganism survival.

The survivability of microorganisms depended mostly on microorganism type and type and content of deposited dust.

3. REFERENCES

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The presentation is based on the results of Phase IV of the National Program "Safety and working conditions improvement", financed in the years 2014–2016 in the field of research and development work by the Ministry of Science and Higher Education and the National Centre for Research and Development.