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SHORT COMMUNICATION

# Survival of Bacteria in Respiratory Protective Filters

EWA B. MIAŚKIEWICZ-PĘSKA* and TOMASZ SŁOMCZYŃSKI

Department of Biology, Faculty of Building Services, Hydro and Environmental Engineering,
Warsaw University of Technology, Warsaw, Poland

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Abstract

The aim of this study was to estimate the susceptibility of *Staphylococcus hominis* bacteria to the bacteriostatic agent in respiratory protective filters. Four types of filter media of different characteristics were tested. The number of bacteria was estimated by a culture-based method. It was proved that in treated filters the number of *S. hominis* was significantly reduced, even below the detection limit, while in untreated material bacteria were able to grow and multiply up to 100-fold within 8 hours. There was no correlation between filter structure and changes in the number of bacterial cells.

K e y w o r d s: respiratory protective filters, biostatic agent, treated filters

Coal excavation is still an activity that needs people involvement. Miners working underground are exposed to many environmental factors which cause harm to health or life. The presence of fumes, mists, gases such as carbon monoxide and dioxide, nitrogen monoxide, hydrogen sulphide and dust of different particle fractions requires the use of different preventive and protective measures (Wells, 1998). One of them is the reduction of dust particle generation using water spray systems.

Additionally, coal miners are obliged to use individual protective respiratory equipment, *i.e.* respiratory protective masks or respirators. The presence of dust particles of the respirable fraction in concentration exceeding limit values causes such serious diseases as "black lung", coal workers' pneumoconiosis, and chronic obstructive pulmonary disease (Donoghue, 2004). Lung illnesses are among the most serious of all diagnosed occupational diseases in the Polish mining industry in recent years (Labour Protection Council, 2007).

The application of respiratory protective masks is regulated by relevant standards, for example EN 136 or EN 140 and the Regulation of the Minister of Economy (EN 136:1998/AC:2003; EN 140:1998/AC:1999; Regulation of the Minister of Economy, 2002).

Air filters used in protective masks applied by coal miners are exposed to relatively high temperature and humidity. It was noticed that the relative humidity of the exhaled air exceeds 95%. Moreover, high amount of water is perspired by the coal workers – even more than 1litre of sweat per hour (Gierlotka, 2002). It was proved that at favourable temperature and humidity and in the presence of available source of carbon and energy bacteria deposited on the filters are able to colonize the filter material (Hugenholtz and Fuerst, 1992).

The aim of the research work was to study the influence of biostatic agent on bacterial growth in air filters used by coal miners in personal protective equipment under conditions similar to those occurring underground.

Respiratory protective filters classes P1 and P2 of different structure and characteristics were used in the studies (see Table I). They are applied in protective, dust absorbing masks used by coal-miners. The filters were treated with a biostatic agent (silver salts), added by the manufacturer after the production process. Untreated filters were used as a control. Treated filters were marked as $A_t$ (treated A filter), $B_t$, $C_t$ and $D_t$. Filters A, C, D consisted of three layers. The surface layer-coarse filter contained thick fibres of polyamide (PA), polyethylene (PE), polypropylene (PP) (substance 150–200 g/m$^2$). The next one, main filter of high efficiency, was made of pure PP fibres of the diameter below 1 µm (substance 60–100 g/m$^2$). The third, PP layer was a main filter cover (substance 15 g/m$^2$). Filters signed as B were made of PE and PA fibres and had only one layer (substance 200 g/m$^2$).

The bacterial strain used in the study was the Gram-positive *Staphylococcus hominis* which is a harmless

* Corresponding author: E. Miaśkiewicz-Pęska, Department of Biology, Faculty of Building Services, Hydro and Environmental Engineering, Warsaw University of Technology, Warsaw, Poland; **e-mail: ewa.miaskiewicz@is.pw.edu.pl**

Table I
Characteristics of filters tested.

| Filter class | Filter kind | Nr of layers | Woven material | Substance (g/m$^2$) |
|---|---|---|---|---|
| P1, P2 | A, $A_t$ | 1 – surface layer | PA[a], PE[b], PP[c] | 150–200 |
| | | 2 –main filter | PP | 60–100 |
| | | 3 – main filter cover | PP | 15 |
| P1 | B, $B_t$ | 1– main filter | PE, PA | 200 |
| P2 | C, $C_t$ | 1 – surface layer | PA, PE, PP | 150–200 |
| | | 2 – main filter | PP | 60–100 |
| | | 3 – main filter cover | PP | 15 |
| P1, P2 | D, $D_t$ | 1 – surface layer | PA, PE, PP | 150–200 |
| | | 2 – main filter | PP | 60–100 |
| | | 3 – main filter cover | PP | 15 |

[a] PA – polyamide, [b] PE – polyethylene, [c] PP – polypropylene

commensal on human skin. The strain was identified with the API Staph biochemical tests (bio-Merieux). In order to simulate conditions in which filters are used by coal miners a suspension of the bacterial cells was made in 0.1% solution of peptone ($10^5$–$10^6$ cfu/ml), samples of treated and untreated filters were inoculated with the sprayed bacterial suspension and incubated under static conditions (no air flow) at 39 ± 1°C and relative humidity (RH) 97 ± 2%. The number of bacteria was estimated by a culture-based method at the beginning of the experiment and after 8, 24 and 48 hours. Fragments of filters were measured and weighed and, then placed in 100 ml of 0.08% sterile sodium pyrophosphate solution containing 0.0005% of polyoxyethylenesorbitan monooleate (Tween 80). Before and after incubation bacteria were washed out of the filters within 40 min in a water bath shaker, plated on nutrient agar and incubated at 37°C for 2 days. The number of bacteria was given as cfu/cm$^2$ (colony forming units per cm$^2$ of the filter). Additionally, *S. hominis* growth in filters within first 8 hours was characterized by generation time.

It was observed that in A treated filters total decay of bacteria appeared within 8 h of incubation. In untreated filters bacteria were present within the whole time of the experiment (48 h). In P1 filters number of bacteria increased over fourfold within 8-hour incubation, from $2.3 \times 10^4$ cfu/cm$^2$ to $1.0 \times 10^5$ cfu/cm$^2$ and eightfold after 24 h. Generation time of *S. hominis* was 3 h 15 min. In P2 filters the number of bacteria decreased slightly within 8 h from $1.7 \times 10^5$ to $1.2 \times 10^5$ cfu/cm$^2$ and stayed at the same level to the end of the experiment. Initial humidity of A filters was 66–67% and $A_t$ 68–75%.

In B treated filters the number of *S. hominis* was reduced 1.6 times after 8-hour contact with antimicrobial agent. After 24 h only 50 cfu/cm$^2$ were found.

In untreated filters the number of bacteria increased 100-fold and reached $1.3 \times 10^5$ cfu/cm$^2$ within 8 h. Generation time was 2 h 24 min. Initial humidity of B filters was 80%, $B_t$ 85%.

C treated filters did not sustain bacterial growth; the number of *S. hominis* in $C_t$ systematically decreased. The most significant reduction of bacterial cells was observed within 8 h (from $2 \times 10^3$ to 62 cfu/cm$^2$). After 48 h bacteria were not detected in the filtering material. In untreated C filters the number of bacterial cells maintained at the level of $10^4$–$10^5$ cfu/cm$^2$. Initial humidity of C filters was 88% and $C_t$ 75%.

In the case of D treated filters bacteria were detected only 8 h after filters inoculation but their number was significantly reduced (approx. 2–3-fold). In untreated filters the number of *S. hominis* stayed at the level $10^4$ cfu/cm$^2$ (P1, P2). The highest increase in bacterial number (approx. 6-fold) was noticed in P1 untreated filters within the first 8 hours of incubation. *S. hominis* generation time reached 3 h 8 min. Initial humidity of D filters was 85% and $D_t$ 81%.

The studies indicated that *S. hominis* is able to not only survive but also multiply in untreated material. Sufficient water content (66–88%), favourable temperature (39 ± 1°C) and available nutrients stimulated bacterial growth in filters A, B and D. The most significant increase in number of bacterial cells was noticed in B filters, 100-fold, and in A and D filters (P1) 4.3-fold and 6-fold, respectively. Only in untreated C and D (P2) filters the number of *S. hominis* decreased (1.3 and 1.1-fold, respectively) but still remained at a high level, $10^3$–$10^4$ cfu/cm$^2$. Generation time for *S. hominis* in untreated filters ranged from 144 to 195 min when water content was 80% and 68–75%, respectively. The generation time in the experiment was much longer than observed in laboratory cultures grown under optimum conditions where it usually reaches 20–25 min. Probably, this was an effect of incubation temperature (39 ± 1°C) which was higher than the optimum (35 ± 1°C) and availability of peptone as the only source of nutrients.

The phenomenon of bacterial growth in untreated woven filters was studied by Karwowska *et al.* (2003). They observed that bacterial strains of different Gram characteristics (*Pseudomonas fluorescens*, *Micrococcus roseus*, *Bacillus subtilis*) multiplied in air filters, which was correlated with the increase in water content in the material. Also Kemp *et al.* (2001) observed significant growth of microorganisms in untreated air filters during the first 2 weeks of the experiment duration.

The application of biostatic agent resulted in significant reduction of bacterial cells in the filters tested. In the case of $C_t$ the number of *S. hominis* decreased 32 fold, from $2 \times 10^3$ cfu/cm$^2$ to 62 cfu/cm$^2$ within 8 hours and bacteria were not detected in filters after 48 hours. In $A_t$ filters bacteria were not found after 8 hours. This was correlated with the lowest water content (68–75%) if compared to $D_t$ and $B_t$ filters: 81 and 85%, respectively. In $D_t$ filters the number of bacteria decreased below the detection limit within 24 hours.

All of the filters tested were made of both hydrophobic – PP, PE and slightly hydrophilic polymers – PA (moisture absorption 4.5%). Nevertheless, the hydrophobic properties of fabrics were not a protection against bacterial growth. For this reason antibacterial treatment has been introduced by manufacturers. The most popular antimicrobial agent is silver. Silver ions react with thiol groups in vital enzymes inactivating them or interact with DNA (Matsamura *et al*., 2003). The efficacy of $AgNO_3$ to inactivate Gram-positive and Gram-negative bacteria in air filters were described by Miaskiewicz-Peska and Lebkowska (2011). Nevertheless, it was proved that silver particles were released from filtering materials, regardless of whether they were water or air filters (Foss Manufacturing, 2004), (Miaskiewicz-Peska and Lebkowska, 2011).

Recently, in the era of nanotechnology a trend of application of antimicrobial nanoparticles is being observed. Rai *et al.* (2009) described use of silver nanoparticles in textile impregnation against microbial growth and cited Duran *et al.* (2007), who reported such treated cotton fabrics activity against pathogenic *Staphylococcus aureus* bacteria. Nevertheless, there are more and more reports concerning health implications of nanoparticles and their harmful impact on the aquatic ecosystems (Łebkowska and Załęska-Radziwiłł, 2011; Marcato and Duran, 2011). It was proved that these particles can be released from textiles and access the human body by inhalation or contact with the skin. Moreover, their nanoscale size provides a very large surface area, which can cause the generation of oxyradicals (Moore, 2006).

The results for the pairs of filters (treated and untreated with biostatic agent) tested in the research revealed the effectiveness of antimicrobial finishing. Undoubtedly, the information is of benefit to filter manufacturers – they gained proof that the application of biostatic agent reduces bacterial growth under the conditions the filters are designed to be used. Nevertheless, the application of antimicrobial nanoparticles in personal respiratory masks should be preceded by hazard identification, followed by appropriate steps to eliminate harmful impact on human health and the natural environment.

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