Polish Journal of Environmental Studies Vol. 12, No. 1 (2003), 57-61

Viability of Bacteria in Fiber Filters as a Result of Filter Humidity

E. Karwowska., E. Sztompka , M. Łebkowska*, E. Miaśkiewicz - Pęska

Faculty of Environmental Engineering, Institute of Environmental Engineering Systems,
Department of Environmental Biology,
Warsaw University of Technology, ul. Nowowiejska 20, 00-653 Warsaw, Poland

Received: 20 May, 2002
Accepted: 12 August, 2002

Abstract

Research on the influence of filter humidity on bacterial survival in filter media was carried out. The results indicate that an increase in water content up to 93% of weight stimulated bacterial growth. When filter humidity was stable, the number of tested bacterial groups reached $10^6 – 10^7$ cfu/cm^2 and did not change much within 105 days of the experiment. Strains of *Pseudomonas* dominated – their percentage in humidity 93% of weight was 92,3%. If water content in filters was not sufficient (13% of weight), the number of bacteria dropped within the first 14 days to 10^2- 10^4 cfu/cm^2 and Gram positive endospore--forming rods were found as dominants.

Keywords: polyester filters, filter humidity, bacterial aerosol.

Introduction

Air is the environment which transports organic and inorganic particles, gases, and living microorganisms– viruses, bacteria (including *Actinomycetes*), fungi and algae. They come from the soil, water, wastewater and other kinds of wastes. Fungal spores, bacterial endospores, fragments of cells as well as pollens may cause allergenic reactions such as fever, infections of the respiratory tract and even asthma. Typical examples of such agents are spores of *Bacilli*, conidia of *Actinomycetes* and representatives of bacterial species – *Arthrobacter* and *Brevibacterium* [1].

Microorganisms can also have toxic and immunogenic influence on people and animals.

Among all bacterial cells present in bioaerosols Gram negative rods such as *Pseudomonas, Enterobacter, Alcaligenes, Flavobacterium* and *Cytophaga* are regarded as dominants. Representatives of *Salmonella* and *Legionella* species are also indicated. Rods of *Pseudomonas* are most frequent in the aerosols generated in wastewater treatment plants, humidifiers and in metal working machines. If the cutting fluids get into the atmosphere when metal working machines work, oil mist is produced**.** This can contain microorganisms such as *Pseudomonas* aeruginosa – an agent causing infections of the respiratory tract [1].

Representatives of Gram positive rods - *Staphylococci* and *Streptococci* are components of aerosols originating from wastewater and present in hospitals causing infections of the upper respiratory tract and skin.

Bacterial cells are capable of endotoxin production which are termostable lipopolisaccharides of high molecular weight. They are part of the bacterial cell wall giving, together with proteins and phospholipids, heteropolimers. Particles of 30-50 μm are present in organic dust emitted during treatment of grain, herbs and cotton.

Endotoxin producing bacteria are the most numerous in dust present in animal farming facilities where the concentration of endotoxins can reach up to 2000 ng/mg of dust while for example in a typical school there are only 0.16 ng/mg [1].

*Corresponding author

Flanningan [2] indicated that lipopolisaccharides and peptidoglycan isolated from bacterial cell walls caused the release of histamine in tissue cultures. Similar effects were obtained for the components of fungal cell wall.

Representatives of mold species such as *Aspergillus, Penicillium, Fusarium, Mucor, Alternaria, Stachybotrys* and *Trichoderma* are the most numerous in bioaerosols. Spores and hyphea of molds cause infections, allergies and asthma. Numerous species of fungi produce mycotoxins - metabolites of a low particle weight which negatively influence immune mechanisms, disturb the function of the nervous system and can also act in a mutagenic and carcinogenic way. More details concerning mycotoxins are described by Zyska [3].

Lately, more and more attention is being paid to Sick Building Syndrome (SBS). It is not a typical illness but is associated with minor indoor air quality in buildings equipped with air conditioning and a ventilation system. SBS is the term to describe psycho-physical discomfort such as mental fatigue and irritation, headaches, infections of the respiratory tract all of which go away some time after leaving the sick building**.** Microorganisms multiply in the reservoirs and cooling coils in filters and on the inner surface of the ductwork [1].

Hugentholtz et al. [4] have indicated that the number of bacteria growing in water remaining for a long time in ventilation and air conditioning systems may reach up to 10^7 cfu/ml when material deposited in the ductwork and reservoirs contain 10^6 cfu /cm^2. Bacteria multiplying in these systems belong to the following genera: *Pseudomonas, Flavobacterium, Bacillus, Corynebacterium, Blastobacter, Alcaligenes, Staphylococcus, Spirillum, and Vibrio* [4].

Insulation materials, gypsum walls, and wallpaper often serve enough nutrients for bacteria and fungi to let them grow. Humidity and the presence of organic matter which serves as a source of carbon and energy enable microbes to grow [5]. Bacterial and fungal glucans are considered causes of SBS effect. Rylander [6] proved that even small concentrations of (1→)-β-D-glucane in office air cause difficulties in breathing.

Filtration is one of the common methods of separating aerosols from the atmospheric air. It is applied in ventilation and air conditioning systems in houses, offices, hospitals, food plants**,** pharmaceutical processing plants, and in surgery masks. Separation of air contamination inside buildings requires proper installations and filters of high collection efficiency. It should be pointed out that microorganisms can multiply in filters and then become reintroduced into the air. Air-conditioning systems can thus also be a source of microbial contamination in the air. The most frequently used filters are made of synthetic or glass fiber and replaced cellulose and cotton.

Capacities of microbial growth inside filter material and its dynamics are not well recognized. Changes in the filter structure and efficiency caused by microbial activity are not clear.

The majority of research on microbial multiplication in filters has led to the conclusion that unused filters, stored in low air humidity (<70%), do not provide good conditions for microorganisms to grow. However, it was proved that retained dust could stimulate growth of fungi inside filter material when relative humidity exceeds 70% [5].

Studies on bacterial survival in synthetic and glass fiber filters show a significant decrease in survival within 1h when relative air humidity ranged between 30 and 60% [5]. This depended on the filter media and bacterial strain applied, e.g. Gram positive *Micrococcus* luteus was more resistant to harmful environmental conditions than Gram negative rod - *Escherichia coli*.

The research work was designed to test bacterial capacity of survival and multiplication in filters made of polyester under the following humidity conditions:

- without additional humidification,
- with constant filters humidity,
- whether or not filter humidity was different at the beginning of the experiment.

Table 1. Changes in quantity of microorganisms in filter media without additional humidification.

Date	Day of experiment	Number of bacteria in filters (cfu/cm^2)	Standard deviation (cfu/cm^2)	Coefficient of variation (%)	Average number of bacteria in 1 cm^2 of filter
20.03	0 (t_o)	3.8×10^4 2.4×10^4 1.8×10^4 1.9×10^4	0.9×10^4	37.2	$(2.5 \pm 0.6) \times 10^4$
22.03	2	4.8×10^4 6.4×10^3 5.2×10^4 7.2×10^3	2.5×10^4	88.0	$(2.8 \pm 1.5) \times 10^4$
26.03	6	5.8×10^2 9.6×10^2 3.8×10^3 4.2×10^3	1.9×10^3	78.8	$(2.4 \pm 1.1) \times 10^3$
2.04	14	2.2×10^2 9.9×10^2 6.3×10^2 2.0×10^2	3.8×10^2	73.8	$(5.1 \pm 2.5) \times 10^2$

Materials and Methods

The suspension of the following strains: *Pseudomonas fluorescens, Micrococcos roseus* and endospore producing strain of *Bacillus* was aerosolized. The suspension was prepared as follows: bacteria growing on the agar nutritive medium were washed out and suspended in sterile tap water after 48h of incubation at 26°C. Volume of the solution was 2 dm^3. The numbers of bacteria in 1ml of the solution was estimated in the Helber chamber. The suspension was then aerosolized in the test chamber by means of a nebulizer.

After 15 minutes the number of bacteria in the air reached about $3x10^5$ colony forming units (cfu)/m^3, which was estimated by means of an impactor. A sampling time was 5, 10, 15, 20 seconds, sampling rate 1.1m^3/h and nutrient agar was used as a medium. The proportions of the tested strains in the aerosol were as follows: strains of *Pseudomonas* - 68%, *Bacillus* - 20% and *Micrococcus* – 12%.

During aerosolization, filters were placed 15 cm from the nebulizer for 10 seconds. Then, filters were placed in sterile Petri plates and incubated at 26°C. Humidity was kept on the appropriate level by a sprayer filled with the sterile distilled water.

Quantitative analysis of bacteria present in filter was carried out by plating on nutrient agar medium. The research work was carried out on 4 filters in parallel. Bacteria were washed out by the following method: the filter was placed in 0.1% sodium pirophosphate solution and rinsed by agitation for 40 min at 120 rpm. Plated bacteria were incubated at 26°C for 48h.

Results

The first stage of the research work relayed on the analysis of bacterial survival in filter media without additional humidification. Results showed that the average number of bacteria decreased within 14 days from $2.5x10^4$ cfu/cm^2 to $5.1x10^2$ cfu/cm^2 (Tab.1). Immediately after application of bacterial aerosol, filter humidity was 13% of weight. It was observed that additional humidification up to 50% of weight caused a rapid increase in the number of bacteria within 4 days giving finally $7.6x10^4$cfu/cm^3.

Research of the second stage relayed on the application of bacterial aerosol together with sterile water in t_0 to reach humidity in the range of 13-133% of weight. These filters were then incubated for 7 days at 26°C. As a result, the number of bacteria increased from 5.3 x 10^3 cfu/cm^2 for 13% to 4.5 $x10^6$ cfu/cm^2 for humidity 93% of weight. For a further increase in humidity the number of bacteria did not change and was 8.2 x 10^5 cfu/cm^2 (Tab.2).

Qualitative analysis shows that Gram negative rods, especially strains of Pseudomonas, increased in number when humidity was between 13-93%. Their percentage for humidity 13% was 33% and for humidity 93% reached 92.3%. It also appeared that, in the above-mentioned range of humidity, the percentage of Gram positive bacteria – *Bacillus* sp. and *Micrococcus* sp. decreased (Tab.3). In filters where humidity was as high as 133% of weight, the percentage of Gram positive rods and Gram negative rods were on a similar level to that for humidity 53%.

Research on the third stage was carried out for 105 days; humidity was held at the level of 50% of weight.

The results showed rapid increase in number of bacteria within 4 days after the experiment had begun – from 1.7 $x10^4$ to 1.4 $x10^7$ cfu/cm^2. The number of bacteria held the same level – 3.5- 5.2 $x10^7$cfu/cm^2 within 33 days of the studies. The decrease in the number – $2x10^6$ cfu/cm^2, was observed in the 49th day of research. A similar number was in the 105th day (Tab.4).

Qualitative analysis showed that the percentage of Gram positive and Gram negative rods remained stable during the studies and reached 80-90% and 3-8%, respectively.

Table 2. The significance of the primary filter humidification for the number of bacterial microflora.

Additional humidification (g H_2O/filter)	Filter humidity (% of weight)	Number of bacteria in each filter (cfu/cm^2)	Standard deviation (cfu/cm^2)	Coefficient of variation (%)	Average number of bacteria in 1cm^2 of filter
0	13	$2.6 x10^3$ $8.0 x10^3$ $3.0 x10^3$ $7.7 x10^3$	$2.9 x10^3$	54.9	$(5.3±1.7) x10^3$
0.65	53	$7.2 x10^5$ $2.4 x10^5$ $2.2 x10^5$ $3.1 x10^5$	$2.3 x10^5$	63.1	$(3.7±1.4) x10^5$
1.3	93	$2.9 x10^6$ $5.5 x10^6$ $5.9 x10^6$ $3.8 x10^6$	$1.4 x10^6$	31.3	$(4.5±0.8) x10^6$
1.95	133	$2.3 x10^5$ $1.0 x10^6$ $6.4 x10^5$ $1.4 x10^6$	$5.0 x10^5$	61.1	$(8.2±3.0) x10^5$

Table 3. Percentage of groups of microorganisms in filters of different humidity.

Filter humidity (% of weight)	Percentage of groups of microorganisms (%)		
	Gram negative rods	Gram positive rods	Micrococci
13	33.3	52.4	14.3
53	77.9	19.5	2.6
93	92.3	3.1	4.6
133	72.0	14.0	13.0

Table 4. Changes in number of bacteria in filter media with constant filter humidity – around 50% of weight.

Date	Day of experiment	Number of bacteria in filters (cfu/cm_2)	Standard deviation (cfu/cm_2)	Coefficient of variation (%)	Average number of bacteria in 1cm^2 of filter
17.05	0 (t_o)	2.2×10^4 1.6×10^4 4.2×10^3 2.7×10^4	9.8×10^3	56.8	$(1.7 \pm 0.6) \times 10^4$
21.05	4	1.3×10^7 1.4×10^7 9.8×10^6 1.8×10^7	3.4×10^6	24.7	$(1.4 \pm 0.2) \times 10^7$
31.05	14	4.8×10^7 2.2×10^7 2.0×10^7 5.2×10^7	1.7×10^7	47.4	$(3.5 \pm 1.0) \times 10^7$
5.06	19	1.7×10^7 7.8×10^7 3.8×10^7 2.9×10^7	2.6×10^7	65.3	$(4.1 \pm 1.6) \times 10^7$
19.06	33	5.9×10^7 4.9×10^7 4.0×10^7 6.0×10^7	0.9×10^7	18.1	$(5.2 \pm 0.6) \times 10^7$
5.07	49	2.4×10^6 2.4×10^6 1.8×10^6 1.4×10^6	0.5×10^6	24.5	$(2.0 \pm 0.3) \times 10^6$
29.08	105	4.6×10^6 4.0×10^6 3.4×10^6 3.7×10^6	0.5×10^6	13.0	$(3.9 \pm 0.3) \times 10^6$

Table 5. Percentage of groups of microorganisms in filter media of constant humidity – around 50% of weight.

Date	Day of experiment	Percentage of groups of microorganisms		
		Gram negative rods	Gram positive rods	micrococci
17.05	0 (t_o)	84	8	8
21.05	4	85.7	5.7	8.6
31.05	14	79.3	3	17.7
19.06	33	79	7	14
29.08	105	91	5.2	4.7

Percentage of bacteria of Micrococcus species changed slightly up and down (Tab.5).

Conclusions

The results of research led to the following conclusions:

1. The number of bacteria decreased together with the decrease of filter humidity.
2. Water content in a filter on which bacterial solution was aerosolized was of great importance for multiplication. An increase in filter humidity up to 90% of weight was a stimulating factor for growth of microflora.
3. The applied bacteria differed in ability to survive in different humidity. When water content in the filter increased, percentage of endospore-forming rods also went up.
4. Constant filter humidity had a stimulating effect on microbial multiplication (especially during the first 4 days). The number of bacteria reached stable, high level (10^6-10^7) up to 105th day of experiment; percentage of tested bacterial groups did not change much.

References

1. ŁEBKOWSKA M. Bioaerosols in indoor air. Chłodnictwo i klimatyzacja, **11-12**, **2000**.
2. FLANNIGAN B. Microbial aerosols in buildings: origins, health implications and controls. Conference papers " Microbial degradation and corrosion of technical materials" Łódź **2001**.
3. ZYSKA B.: Microbiology of indoor air in buildings**.** Conference papers "Problems of indoor air quality in Poland '99. Warsaw University of Technology Publishing House, Warsaw, **2000**.
4. HUGENHOLTZ P., FUERST J.A.: Heterotrophic bacteria in air-handling system. Appl. Envir. Microbiol., **58**, 12, **1992**.
5. MAUS R., GOPPELSRODER A., UMHAUER H.: Viability of bacteria in unused air filter media. Atmospheric Environment, **31**, 15, **1997**.
6. LACEY J., DUTKIEWICZ J.: Bioaerosols in occupational lung disease. J. Aerosol Sci., **25**, 8, **1994**.