Environment Protection Engineering

Vol. 39 2013 No. 1

DOI: 10.5277/EPE130110

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PRELIMINARY TESTING OF A NEW BIOAEROSOL SAMPLER DEVELOPED FOR THE MEASUREMENTS OF LOW AND MEDIUM CONCENTRATION LEVELS OF AIRBORNE BACTERIA AND FUNGI

A new bioaerosol sampler developed for extremely low concentration levels of airborne bacteria and fungi has been described. The results of the pilot study of the prototype of this new sampler are also presented in this paper. During the field studies the concentration levels of bacterial and fungal aerosols were obtained using simultaneously the new sampler and the reference cascade Andersen 6-stage impactor, as well as the Air Ideal sampler. Although only a preliminary study has been carried out, the obtained data indicated that the assumed designing parameters for the new sampler are suitable and guarantee high collection efficiency of this instrument.

1. INTRODUCTION

Certain human pathogens seem to be significant causes of infection from indoor air, infecting otherwise healthy individuals [1–5]. Exposure to bioaerosols should be controlled in such indoor environments like kindergartens, elementary schools [6] and others, even underground railway stations [7]. On the other hand, airborne bacteria and fungi may be especially hazardous in clinics and hospitals where they may be the major factor in the increasing morbidity from respiratory diseases [8,9]. Some bacteria such as *Streptococcus pyogenes*, *Neisseria meningitidis*, *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis* are known to be transmitted predominantly by airborne droplets from infected people, and they may cause nosocomial infection [10]. It is interesting that many opportunistic bacteria found in the indoor environment pose

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a potential threat only to immunocompromised patients in hospitals. Therefore, it is necessary to assess the composition and concentration of airborne microorganisms in clinics and hospital buildings. In particular, bioaerosol monitoring in hospitals can provide information for epidemiological investigation of nosocomial infectious diseases, research into airborne microorganisms spread and control, monitoring biohazard procedures, and can be used as a quality control measure.

It is important to note that although direct measurement of the concentration of living airborne bacteria and fungi is extremely difficult, the commonly used substitute of the concentration of living microorganisms present in the air is the number of colony forming units in the volume of air – CFU/m^3.

Among the available sampler types, impactors are very popular due to their ability to collect microorganisms directly onto agar growth medium without the need for post collection sample processing [11, 12]. The number of colonies which appear some days after the sampled airborne bioaerosol particles are collected divided by the volume of sampled air gives the concentration of studied bioaerosol in CFU/m^3. The collection characteristics of an impactor, including its cut-size diameter, depends on its design parameters such as nozzle diameter, ratio of the jet-to-plate distance over the nozzle diameter (Y/W) and sampling flow rate. However, not only cut-size diameter but also viability is a critically important characteristic for both monitoring and controlling bioaerosols. Viability control of bioaerosols is essential to prevent aerial infection problems caused by viable airborne microorganisms. For cultural sampling, only organisms that are alive in the aerosol, that remain alive during sampling, and can grow under the provided conditions will be recovered. Unfortunately, shear and impaction forces that can occur during sampling can damage cells. Therefore, the key question is how to increase the culturable fractions obtained by the use of the existing impactors? One of the factors influencing the culturability of bioaerosols is the formation of a liquid layer around the bioaerosol particles via their condensation growth [13] but until now nobody knows how to use this information in bioaerosol sampling. More practical approach is to cover the agar surface with glycerol [14]. However, actually, the gentlest samplers are still those that impact particles onto a relatively soft surface at relatively low speeds, such as culture plate impactors with soft agar [2].

After impaction, the number of colonies, N, grew in the same points where viable bioaerosol particles were collected, and can be expressed by the product of average concentration, C_a, and the sampled volume, V, or the sampler flow rate, Q, multiplied by the sampling time, t [15]:

$$N = C_a Q t \tag{1}$$

Since it is extremely important to obtain the optimum number of colonies, before sampling the optimum sampling time should be estimated from the Eq. (1):

$$t = \frac{N}{C_a Q} \tag{2}$$

where C_a is now the expected (assumed) concentration of bioaerosol, while N is the optimum number of colonies which will grow after viable bioaerosol particles are collected. For the typical Petri dish $N = 50$ [15]. More generally, it can be written:

$$N = \delta A \tag{3}$$

where δ is the optimum surface density of a sample collected on the surface A.

Therefore, the optimum sampling time depends on the assumed/expected concentration level of airborne bacteria or fungi and on the flow rate being the most important parameter of the used sampler for bioaerosol. However, to collect the optimum number (for example, 50) of colony forming units (CFU) when the expected concentration of bioaerosol is very low, the flow rate or the sampling time should be significantly increased. Unfortunately, increasing the flow rate means the increase of the impaction stress of collected microorganisms. Too high velocity of collecting particles results in a high shear force which may cause serious damage decreasing their viable recovery. Also the second solution to keep the appropriate value of N, although seems to be promising, is significantly limited. It has been observed that increased sampling time resulted in decreased viability for aerosolized vegetative bacterial cells. As air flows over the nutrient agar surface of an impactor, the agar may lose water, resulting in a hard surface. Finally, the occurring loss of viability in the sampling process causes underestimates of the resulting concentration levels of living bioaerosol particles.

To keep most of collected bacteria or fungi alive after a relatively long sampling process, the impact of bioaerosol particles onto the rotating table [16] or onto moving surface [17] has been applied but in our opinion such instruments (having never become popular) are not appropriate for measurements of the extremely low concentration levels of airborne bacteria and fungi.

The aim of this work was to develop a new bioaerosol sampler (NBS) appropriate for the conditions/limitations discussed above, and to preliminary test its sampling efficiency.

2. DESCRIPTION OF THE NEW BIOAEROSOL SAMPLER

The idea of the new instrument was to use as the impaction plate a set of typical Petri dishes filled with agar and to expose each dish to the sampled air only for a short time not exceeding 10–20 min. Therefore, the measurement of very low concentration level of viable bioaerosol particles is possible, because the total sampling time can be very long (1 h or more) what is necessary to collect a sufficient number of bioparticles but, on the other hand, the agar surface in every Petri dish should be kept

soft. The concentration of measured airborne bacteria/fungi can be calculated summarizing the number of colonies appearing after some days of incubation in all the used Petri dishes and dividing this sum by the total volume of sampled air. In the case of a higher (normal) level of the bioaerosol concentration – the colonies found in each separate Petri dish can be treated as the result of one, separate sampling.

Fig. 1. Testing version (not final) of a new bioaerosol sampler with a split impactor:
1 – store box of Petri dishes, 2 – top of the impactor with 100 round nozzles,
3 – pump, 4 – bottom of the impactor, 5 – installation for transportation of Petri dishes
(Patent pending: No.: WIPO ST 10/C PL400378)

The testing version of the NBS is shown in Fig. 1. The picture has been taken during the phase of the preparation for sampling. The two main parts of the impactor, containing a head with 100 round nozzles (the top of the impactor) and the bottom part of this instrument, i.e. the impaction plate (on this plate the Petri dish filled with agar will be located), are separated at this moment. Five Petri dishes are located in a special, rotating store-box. In the second step a special holder will take the Petri dish (Fig. 2) and move it on the impaction plate. Next, the bottom part of the impactor will be shifted up to connect with its top part being ready for sampling. After sampling, the exposed Petri dish is transported back to its previous position in the store-box and another dish is transported to the impactor for the collection of sampled bioaerosol particles. All the steps, including sampling, are fully automated and not only the sampling time but also the time of starting and ending the measurement process can be selected.

The calculated cut-size diameter d_{50} for this sampler is 0.99 µm (for the flow rate Q = 0.02 m^3/min). The d_{50} is generally interpreted as the mass median diameter or count median diameter at which 50% of the mass or number of the aerosol particles is retrieved from the airstream. The collection efficiency of the impactor becomes 100% above d_{50} [18].

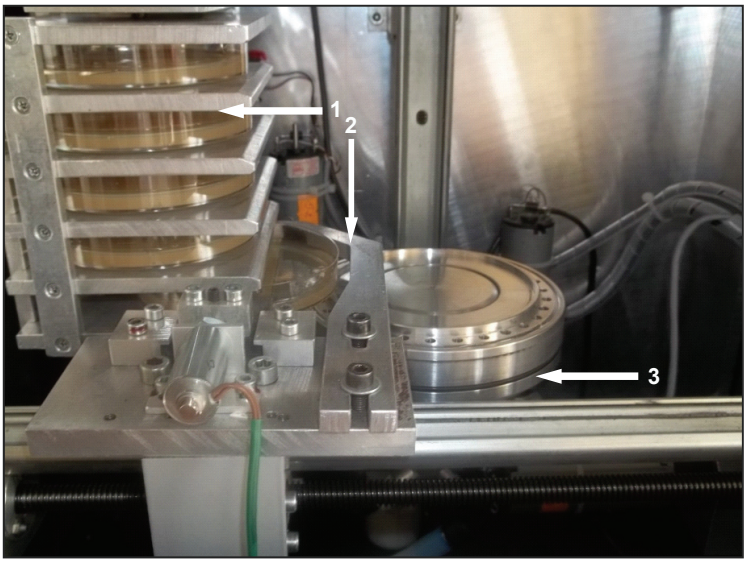

Fig. 2. The Petri dish transported from the store-box into the impactor
1 – store box of Petri dishes, 2 – holder for transportation of a Petri dish from the store box into the impactor, 3 – bottom of the ipactor

3. METHODS

During the field studies carried out both in an office and in a sterilized room, concentration levels of airborne bacteria and fungi were obtained using simultaneously the new sampler and two other commercial samplers. One of the commercial samplers is a multi-orifice cascade impactor, the Andersen 6-stage viable particle sizing sampler (And) [19], which is generally accepted as the standard instrument for viable bioaerosol particles. This impactor operates at a sampling flow rate of 0.0283 m^3/min. Each stage of this sampler contains 400 orifices with diameters ranging from 1.81 mm in the first stage to 0.25 mm in the sixth stage. The corresponding cut-off sizes for the six stages are 7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 µm. The other commercial sampler was one-stage impactor Air Ideal (AI), (bioMèrieux, Marcy l'Etoile, France) which operates at the sampling flow rate of 0.1 m^3/min. The reported cut-size diameter of this instrument is 3 µm.

In all three samplers microorganisms were collected on nutrient media (specific to either fungi or bacteria) in Petri dishes located on the stage/stages of the used impactors. Malt extract agar (MEA, 2%) was applied for fungi, with chloramphenicol added to inhibit bacterial growth. Trypcase soy agar (TSA) was used for bacteria, with cycloheximide as an inhibitor of fungal growth.

Additionally, the bacterial strains from one set of the airborne bacteria samples, collected simultaneously by the three samplers, were identified using the biochemical API test (bioMérieux, Inc., Marcy l'Etoile, France) and APILAB Plus (bioMérieux) software.

4. RESULTS AND DISCUSSION

The counts of airborne bacteria and fungi measured at the same location (mostly in the office room) with three bioaerosol samplers are shown in Tables 1–4.

Table 1

Concentration levels of airborne bacteria obtained in the office room by using the new bioaerosol sampler, Air Ideal sampler, and the 6-stage Andersen impactor

No.	Concentration of airborne bacteria [CFU/m^3]			NBS/And	NBS/AI
	Air Ideal (AI)	Andersen impactor (And)	New bioaerosol sampler (NBA)		
1	1137	1007	1110	1.10	0.98
2	290	286	390	1.36	1.34
3	1317	1374	1380	1.00	1.05
4	1577	1569	1840	1.17	1.117
5	1697	1569	1360	0.98	0.80
6	1410	1278	1300	1.02	0.92
7	1273	1250	1350	1.08	1.06
Averaged				1.10	1.05

The flow rate of the new aerosol sampler (NBS) was Q = 0.02 m^3/min. Previous measurements carried out for the higher flow rate (ca. 0.04 m^3/min and higher) showed that the concentration levels obtained by this new instrument were lower compared to the data obtained with the And impactor and AI sampler.

The actual data (Tables 1, 2) indicate that the NBS used in the office room provides concentration data comparable with these obtained by using the And impactor and the AI sampler. It is interesting to note that the bacteria concentration levels are, as a rule, even slightly higher comparing to results obtained with other samplers.

The concentration levels of airborne bacteria and fungi in the sterilized room (Tables 3, 4) look especially promising because the data obtained by the use of the NBS seems to be significantly higher compared to those obtained by using the And and AI samplers. However, due to the fact that only a small number of data was obtained, the only conclusion to be made is that application of the NBS for the precise determination of the culturable airborne bacteria and fungi in sterilized rooms and in not highly microbial polluted environments was a properly assumed target.

Table 2

Concentration levels of airborne fungi obtained in the office room by using the new bioaerosol sampler, Air Ideal sampler, and the 6-stage Andersen impactor)

No.	Concentration of airborne bacteria [CFU/m^3]			NBS/And	NBS/AI
	Air Ideal (AI)	Andersen impactor (And)	New bioaerosol sampler (NBA)		
1	480	470	374	0.80	0.79
2	123	162	170	1.05	1.38
3	457	413	370	0.90	0.81
4	637	596	590	0.99	0.93
5	600	505	640	1.27	1.07
6	537	661	560	0.85	1.04
7	413	421	390	0.98	0.94
8	90	97	100	1.03	1.11
Averaged				0.98	1.01

Table 3

Concentration levels of airborne bacteria obtained in the sterilized room by using the new bioaerosol sampler, Air Ideal sampler, and the 6-stage Andersen impactor

No.	Concentration of airborne bacteria [CFU/m^3]			NBS/And	NBS/AI
	Air Ideal (AI)	Andersen impactor (And)	New bioaerosol sampler (NBA)		
1	0	10	10	1.00	–
2	17	24	30	1.25	1.76
3	23	19	35	1.84	1.52
4	0	3	10	3.33	–
5	10	7	20	2.86	2.00
Averaged				2.06	1.75

Table 4

Concentration levels of airborne fungi
obtained in the sterilized room by using the new bioaerosol sampler,
Air Ideal sampler, and the 6-stage Andersen impactor

No.	Concentration of airborne bacteria [CFU/m^3]			NBS/And	NBS/AI
	Air Ideal (AI)	Andersen impactor (And)	New bioaerosol sampler (NBA)		
1	0	0	0	–	–
2	3	7	20	2.86	6.67
3	0	7	10	1.43	–

Table 5

Viable bacterial genera identified in samples
collected by three samplers in the office room

Bacteria	Percentage of species in total bacteria concentration [%]		
	Air Ideal	Andersen impactor	New bioaerosol sampler
Gram-positive cocci including:	35.0	39.1	36.6
Kocuria rosea	–	4.0	3.3
Micrococcus spp.	–	4.0	3.3
Staphylococcus capitis	7.0	–	–
Staphylococcus chromogenes	–	–8.6	3.3
Staphylococcus lentus	28.0	4.6	–
Staphylococcus sciuri		17.9	26.7
Nonsporing Gram-positive rods including:	35.1	41.7	50.1
Arthrobacter spp.	12.3	0.7	–
Brevibacterium spp.	22.8	31.1	43.4
Corynebacterium auris	–	8.6	6.7
Corynebacterium propinquum	–	1.3	–
Gram-positive rods, family *Bacillacae*, including:	14.1	5.9	3.3
Bacillus circulans	1.8	–	–
Bacillus mycoides	–	4.6	–
Bacillus pumilus	12.3	1.3	3.3
Actinomycetes, including:	12.3	6.7	3.3
Rhodococcus spp.	8.8	6.0	3.3
Streptomyces spp.	3.5	0.7	–
Gram-negative rods, including:	3.5	6.6	6.7
Pseudomonas spp.	3.5	6.6	6.7
Total	100.0	100.0	100.0

Table 5 shows the viable bacterial genera identified in samples collected by the samplers in the office room. It can be seen that *Brevibacterium* spp. and *Staphylococcus sciuri* were the predominant genus isolated from all the sampled tested. Although the relative concentrations of some species like Gram-negative and Gram-positive rods obtained by using the new sampler are higher compared to other results, further studies are needed for more precise conclusions. On the other hand, these data seem to be consistent with the result of Hirai [20] who found that Gram-negative rods, other than *Acinetobacter* spp. have been shown to lose their viability faster under drying conditions than the Gram-positive cocci.

Finally, it should be noted that future work to develop this new sampler, especially to reduce its size is needed. Actually the portability and ease of use of the microbial samplers for the biological exposure monitoring and assessment result in their increasing popularity [21–24].

5. CONCLUSIONS

The results demonstrate that the NBS retrieved the highest number of airborne culturable bacteria in the office room compared to Andersen and AI but these differences were not statistically significant. The concentration levels of the airborne fungi in the office room obtained by use of the three samplers were practically the same. The new sampler retrieved the highest number of airborne culturable bacteria and fungi in the sterilized room compared to both reference ones.

The measurements indicated that the assumed designing parameters for the new sampler are suitable and guarantee high collection efficiency of this instrument but the flow rate should not exceed 0.02 $m^3 \cdot min^{-1}$. The new sampler can be successfully used in the indoor environment with a low level of airborne bacteria and/or fungi.

ACKNOWLEDGEMENTS

This research was supported by the National Center for Research and Development, Warsaw, Poland, contract No. N R140012 06/2009.

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