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# EFFICACY OF A NOVEL BIOFILTER IN HATCHERY SANITATION: I. REMOVAL OF AIRBORNE BACTERIA, DUST AND ENDOTOXIN

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**Abstract:** A novel biofilter containing organic, bentonite and halloysite media was applied for elimination of microbial pollutants from the air of an industrial hatchery. The concentrations of total mesophilic bacteria, Gram-negative bacteria, thermophilic actinomycetes, dust and bacterial endotoxin were determined in the air of hatchery during 2 months before installation of the biofilter, and during 6 months after installation of the biofilter, at the inlet and outlet ducts from each medium. Before installation of the biofilter, the concentrations of total mesophilic bacteria, Gram-negative bacteria, thermophilic actinomycetes, dust and endotoxin in the air were within the ranges of 0.97-131.2 × $10^3$ cfu/$m^3$, 0.0-34.4 × $10^3$ cfu/$m^3$, 0.0-0.02 × $10^3$ cfu/$m^3$, 0.37-4.53 mg/$m^3$, and 50.9-520,450.4 ng/$m^3$, respectively. *Enterococcus faecalis* and Gram-negative bacteria (*Acinetobacter* spp., *Escherichia coli*, *Enterobacter cloacae*, and other species) prevailed among bacterial species recovered from the air of the hatchery. A total of 56 species or genera of bacteria were identified in the air samples taken in the examined hatchery; of these, 11, 11 and 6 species or genera respectively were reported as having allergenic, immunotoxic and/or infectious properties The concentrations of total mesophilic bacteria, Gram-negative bacteria, *Enterococcus faecalis* and endotoxin found at the inlet duct of the biofilter after its installation were significantly smaller compared to those recorded before its installation ($p<0.05$). The concentrations of Gram-negative bacteria, *Enterococcus faecalis* and dust found at the outlet ducts of biofilter after its installation were significantly smaller compared to those recorded at the inlet duct of the biofilter ($p<0.01$). The concentrations of total meso-philic bacteria were also smaller at the outlet ducts of the biofilter compared to that at the inlet duct; however, the difference was not significant because of the massive growth of *Streptomyces* species in the biofilter's media which contaminated the outcoming air. In conclusion, the applied biofilter proved to be effective in the elimination of potentially pathogenic bacteria, dust and endotoxin from the air of the hatchery. The efficacy of the biofilter could be improved by the inhibition of the *Streptomyces* growth in the media of the biofilter.

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## INTRODUCTION

Occupations associated with animal breeding and processing of animal materials may release large quantities of organic dust and bioaerosols causing allergic and/or immunotoxic reactions and respiratory disease in the exposed workers and animals [5, 11, 12, 13, 15, 20, 21, 29, 30, 34]. So far, relatively little is known about the risk associated

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with exposure to bioaerosols in hatcheries. In the studies performed 20-35 years ago in the hatcheries of chicks and ducklings by a group from the Institute of Agricultural Medicine in Lublin [8, 9, 10, 36], large concentrations of airborne bacteria were found, exceeding up to 6 times the proposed Occupational Exposure Limit (OEL) value of $10^5$ cfu/$m^3$ [17]. In the same facilities, the concentration of dust exceeded up to twice the OEL value of 4 mg/$m^3$ [31], while concentrations of $NH_3$ and $H_2S$ were below the OEL values [8]. Thus, the bacterial pollutants of air were identified as a main occupational risk factor in the environment of hatcheries. Nearly half (45.4%) of the workers employed in hatcheries over 3 years reported occurrence of respiratory, conjunctival and skin symptoms associated with the performed work, mainly at the time of removing chickens from hatching boxes [8].

Various methods were applied for cleaning the air in hatcheries and hatching cabinets, including chemical disinfection with formaldehyde or hydrogen peroxide, ionization of air [28], improving ventilation and the use of different filters, including biofilters, cleaning the air inside the hatchery and the air leaving the hatchery building. The principle of biofilters is absorbing air impurities by microorganisms developing in various fillings – beds [1, 23, 27, 35]. A novel biofilter harbouring 3 different beds was recently designed and constructed by the authors from the University of Agriculture in Lublin.

The aim of the present work was to determine the levels of microorganisms, dust and endotoxin in the air of a modern hatchery, and to examine the efficacy of a novel biofilter in the removal of these pollutants from outgoing air.

## MATERIALS AND METHODS

**Examined facility.** The study was conducted at the big, industrial Poultry Hatchery in Dębówka, 20 km south of Warsaw, Poland. The hatchery has an annual output of 20-25 million Cobb and Ross meat hens, which represents 4% of the national production.

**The use of a novel biofilter.** The biofilter was designed and constructed by the authors from the University of Agriculture in Lublin. It measures 2.0 × 1.8 × 1.8 m, and includes the following components: a high pressure fan with a maximum capacity of 1,500 $m^3$/h; an air humidifier; and a biofiltration chamber. The biofiltration chamber is divided into 3 independent parts to facilitate the simultaneous assessment of biofiltration properties of 3 different fillings (media, beds). The depth of the filter medium was between 1.2-1.4 m. In this study, the following media were used: • organic medium containing 50% compost and 50% peat (OM); • organic-mineral medium containing 20% bentonite, 40% compost and 40% peat (BM); • organic-mineral medium containing 20% halloysite, 40% compost and 40% peat (HM) (Figs 1, 2).

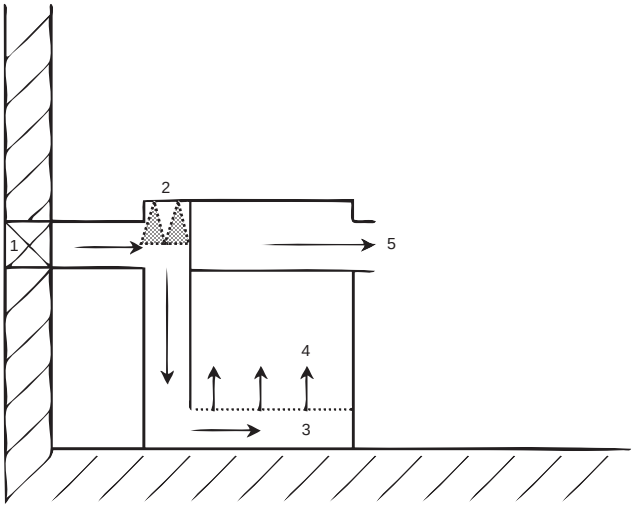


**Figure 1.** Schematic diagram of biofilter. 1 – fan, 2 – humidifier, 3 – air distribution, 4 – biofilter media, 5 – outlet gas.

**Table 1.** Physicochemical and microbiological properties of biofilter media.

| Determination | Biofilter media | | |
|---|---|---|---|
| | OM | BM | HM |
| Temperature [ºC] | 19.5 | 19.1 | 18.9 |
| Moisture [%] | 62.1 | 59.3 | 57.4 |
| pH | 6.2 | 6.7 | 6.7 |
| Concentration of bacteria (mesophilic + psychrophilic, cfu × $10^6$/g) | 612.8 | 221.9 | 296.0 |
| Concentration of fungi (cfu × $10^6$/g) | 2.6 | 2.3 | 4.2 |

Physicochemical and microbiological properties of the biofilter media tested throughout this study are presented in Table 1. The temperature of the filter material was determined using an electronic thermometer. The pH was measured using a pH meter (CP-104, Elmetron, Poland). Moisture content was determined gravimetrically. The concentration of bacteria was determined by dilution plating, using nutrient agar cultures incubated for 24 hrs at 37ºC, and for 72 hrs at 22ºC for counting of meso- and psychrophilic bacteria, respectively. The concentration of fungi was determined by dilution plating, using Sabouraud agar cultures incubated for 120 hrs at 26ºC.

The biofilter was installed in the examined hatchery on 5 March 2005 in the ventilation outlet of the hatching room (Fig. 2), which was equipped with 8 hatchers (AS-4H, Petersime, Zulte, Belgium) and 12 incubators (AS-4S, Petersime, Zulte, Belgium) with an input of 115,000 eggs each. The air was drawn through the inlet pipe situated behind the hatchers in the hatching room. The pipe crossed the wall to the main device located in the neighbouring room and the cleaned air came out through 3 outlet ducts, each corresponding to a particular medium (Fig. 2).

**Air sampling sites.** Air sampling was conducted at following 8 points (shown on Figure 2):

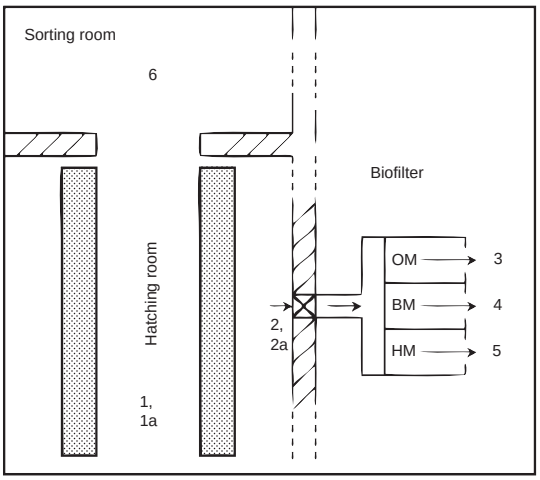


**Figure 2.** Place of collected air samples. 1-6 – sampling points. OM – organic medium, BM – medium with bentonite, 5 – medium with halloysite.

1) Middle of the corridor in the hatching room, before installation of biofilter, in 4 repetitions between January–February 2005.

1a) The same place as "1", after installation of biofilter, in 5 repetitions in the months of April, May, June, July and October 2005.

2) Behind the hatchers in the hatching room, before installation of biofilter, in 4 repetitions between January–February 2005.

2a) The same place as "2", at the inlet duct of biofilter after its installation, in 5 repetitions in the months of April, May, June, July and October 2005.

3) At the organic medium (OM) outlet duct of the biofilter after its installation, in 5 repetitions in the months of April, May, June, July and October 2005.

4) At the bentonite medium (BM) outlet duct of biofilter after its installation, in 5 repetitions in the months of April, May, June, July and October 2005.

5) At the halloysite (HM) medium outlet duct of biofilter after its installation, in 5 repetitions in the months of April, May, June, July and October 2005.

6) Middle of the sorting room where chicken were removed from hatching boxes, before installation of biofilter, in 3 repetitions between January – February 2005.

**Microbiological examination of the air.** Air samples were taken by use of a stationary AS-50 sampler (TWOMET, Zgierz, Poland), at the flow rate of 50 l/min. Polypropylene filters (FIPRO-50, Instytut Włókiennictwa, Łódź, Poland) with 50 mm diameter were used. Each sample was collected in duplicate, 1 for determination of the concentration and species composition of microorganisms, and the other 2 for determination of the concentration of dust and endotoxin.

The concentration of dust in the air was determined gravimetrically from the difference between weight of the filter measured before and after sampling.

The concentration and species composition of microorganisms in collected air samples were determined by dilution plating. The filters were extracted in 3 ml of sterile saline (0.85% NaCl) with 0.05% Tween 80, and after shaking, serial 10-fold dilutions were made. The 0.1 ml aliquots of each dilution were spread on duplicate sets of 3 agar media: blood agar for estimation of total mesophilic Gram-negative and Gram-positive bacteria, eosin methylene blue (EMB) agar (Merck, Darmstadt, Germany) for estimation of Gram-negative bacteria, and half-strength tryptic soya agar (Sigma, St. Louis, MO, USA) for estimation of thermophilic actinomycetes. The blood agar plates and EMB agar plates were subsequently incubated for 1 day at 37ºC, then 3 days at 22ºC and finally 3 days at 4ºC [9]. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria as possible. The tryptic soya agar plates were incubated for 5 days at 55ºC. The grown colonies were counted and differentiated and the data reported as cfu per 1 cubic metre of air (cfu/m$^3$).

Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manual [19, 39, 41] and Cowan & Steel [4]. Additionally, the selected isolates were identified with microtests: API Systems 20E and NE (bioMérieux, Marcy l'Etoile, France) and BIOLOG System (Biolog, Inc., Hayward, CA, USA).

The concentration of bacterial endotoxin in the airborne dust was determined by the Limulus amebocyte lysate gel tube test (LAL) [24]. The filters were extracted for 1 hour in 10 ml of pyrogen-free water at room temperature, heated to 100ºC in a Koch apparatus for 15 min (for better dissolving of endotoxin and inactivation of interfering substances), and after cooling, serial dilutions were prepared. The 0.1 ml dilutions were mixed equally with the "Pyrotell" Limulus reagent (Associates of Cape Code, Falmouth, MA, USA). The test was incubated for 1 hour in a water bath at 37ºC, using pyrogen-free water as a negative control and the standard lipopolysaccharide (endotoxin) of *Escherichia coli* 0113:H10 (Difco) as positive control. The formation of a stable clot was regarded as a positive result. The estimated concentration of endotoxin in dust (ng/mg) was multiplied per estimated concentration of dust in the air (mg/m$^3$) and the results reported as micrograms of the equivalents of the *E. coli* 0113:H10 endotoxin per 1 m$^3$ of air. To convert to Endotoxin Units (EU), the value in nanograms was multiplied by 10.

**Statistical analysis.** The data were analysed by Shapiro-Wilk test for distribution and Mann Whitney test, using STATISTICA for Windows v. 5.0 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

## RESULTS

**The concentration and composition of airborne bacteria in the hatchery before and after installation of biofilter.** The median concentrations of total mesophilic

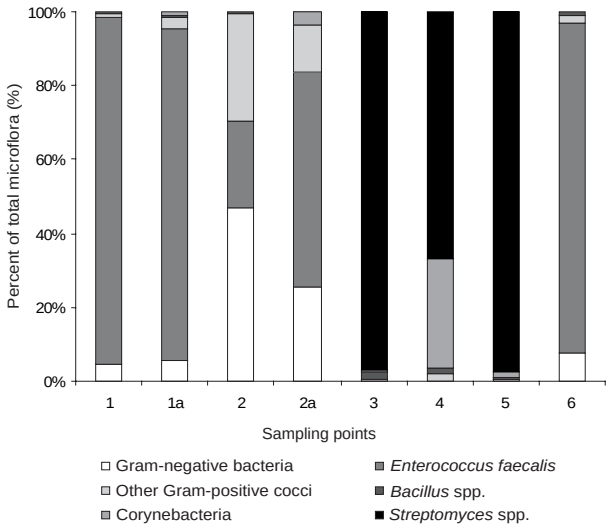


**Figure 3.** Composition of total mesophilic bacteria in the air of a hatchery.

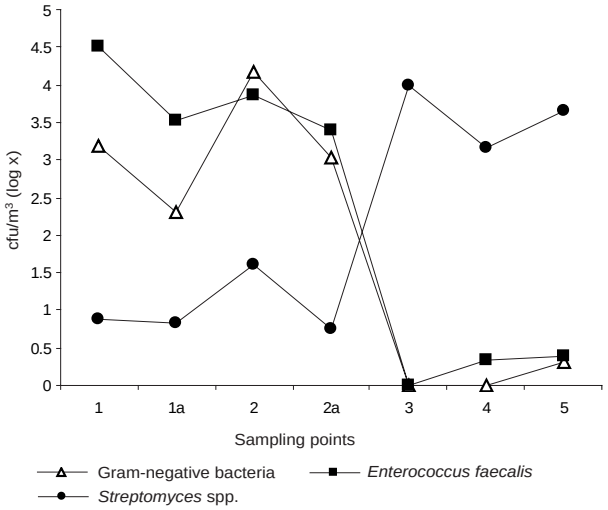


**Figure 4.** Main constituents of bacterial flora in the air of a hatchery before and after passing through biofilter (mean values).

bacteria in the hatchery air before installation of the biofilter ranged from 1.56-33.9 × $10^3$ cfu/$m^3$. After installation of the biofilter, the concentrations were smaller in the inlet air entering the filter (medians 0.7925-3.188 × $10^3$ cfu/$m^3$) and the smallest in the outlet air coming out the filter (medians 0.3525-0.72 × $10^3$ cfu/$m^3$). The difference between the concentrations measured at the inlet duct before and after installation of the biofilter was statistically significant ($p<0.05$), but no significant differences were observed between inlet and outlet ducts after its installation (Tab. 2).

Cocci belonging to the species *Enterococcus faecalis* and Gram-negative bacteria were the main components of

**Table 2.** Concentration of total mesophilic bacteria (grown on blood agar) in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 1.56 (0.97-131.2) | 5 | 0.7925 (0.032-16.564) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 16.83 (9.8-78.9) | 5 | 3.188 (0.762-9.85)# |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.3525 (0.05-46.755) |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.43 (0.13-5.0028) |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.72 (0.074-12.17) |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 33.9 (4.67-53.28) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu × $10^3$/$m^3$); ND = not determined. #value significantly smaller compared to that before installation of biofilter ($p<0.05$).

**Table 3.** Concentration of Gram-negative bacteria (grown on blood agar) in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January–February 2005 | | After installation of biofilter April–October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 0.105 (0.02-6.03) | 5 | 0.12 (0.0-0.72) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 7.41 (1.6-41.18) | 5 | 1.35 (0.1975-1.96)# |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.0 (0.0-0.0025)** |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.0 (0.0-0.0)** |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.0 (0.0-0.01)** |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 1.76 (1.26-3.94) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu × $10^3$/$m^3$); ND = not determined. #value significantly smaller compared to that before installation of biofilter ($p\leq0.05$). **value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.01$).

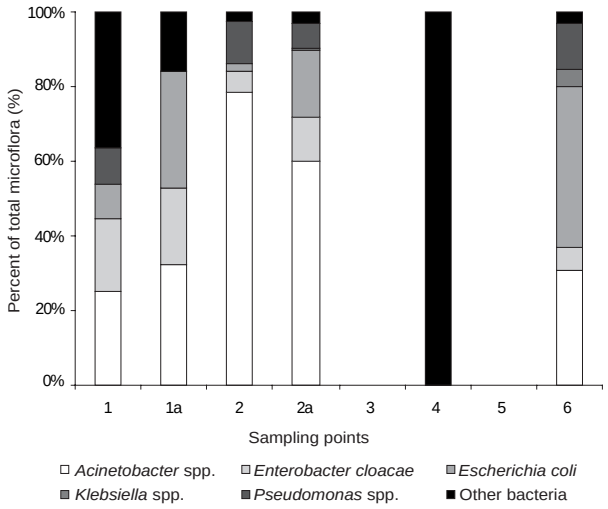


**Figure 5.** Composition of Gram-negative bacteria in the air of a hatchery.

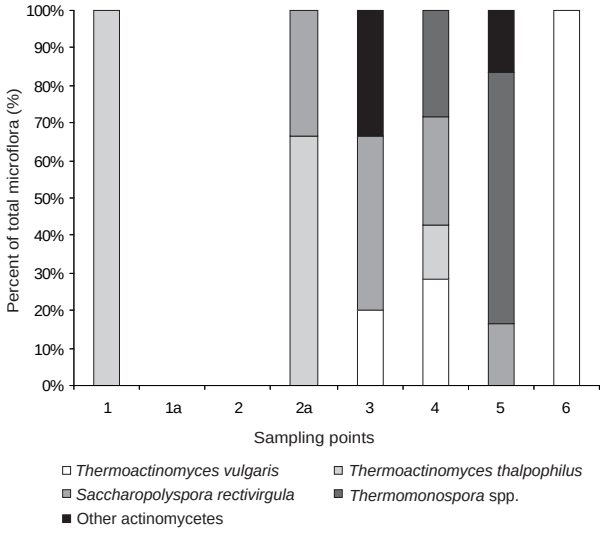


**Figure 6.** Composition of thermophilic actinomycetes in the air of a hatchery.

the airborne bacterial flora of the hatchery before installation of the biofilter, forming respectively 23.2-94.1% and 4.6-47.1% of the total blood agar count in the inlet air entering the filter. By contrast, in the outlet air coming out the filter, the dominant bacteria were *Streptomyces* strains, forming 66.9-97.5% of the total count (Fig. 3). The prevalence of *Enterococcus faecalis* and Gram-negative bacteria among airborne bacteria in the hatchery before entering the biofilter, and domination of *Streptomyces* strains in the air coming out the biofilter, are shown in Figure 4. The concentrations of airborne Gram-negative bacteria (grown on blood agar) and *Enterococcus faecalis* were significantly smaller at the inlet duct after installation of the biofilter compared to those before installation ($p<0.05$). Concentrations of these bacteria recorded at the outlet ducts of the biofilter (OM, BM, HM) were significantly smaller compared to those recorded at the inlet duct after installation of the filter ($p<0.01$) (Tab. 3-4). In contrast, the concentrations of airborne *Streptomyces* spp. were significantly greater at all outlet ducts of the biofilter, compared to that at the inlet duct after its installation ($p<0.05$) (Tab. 5). When the *Streptomyces* strains were subtracted from the total count of airborne mesophilic bacteria grown on blood agar, the numbers of mesophilic bacteria recorded at the outlet ducts of the biofilter were significantly smaller compared to that recorded at the inlet duct after its installation ($p<0.05$) (Tab. 6).

The median concentration of airborne Gram-negative bacteria grown on EMB agar was significantly smaller ($p<0.05$) at the inlet duct of biofilter after its installation ($0.205 \times 10^3$ cfu/m$^3$) compared to that recorded before installation ($2.17 \times 10^3$ cfu/m$^3$). Median concentrations of these bacteria noted at the outlet ducts of biofilter ($0.0 \times 10^3$ cfu/m$^3$) were significantly smaller ($p<0.01$) compared to that recorded at the inlet duct of biofilter after its installation ($0.205 \times 10^3$ cfu/m$^3$) (Tab. 7). The main constituents of the airborne Gram-negative flora of hatchery before installation of the biofilter and in the inlet air entering the filter were: *Acinetobacter* spp., *Escherichia coli* and *Enterobacter cloacae*, forming 25.2-78.3%, 2.1-43.2%, and 5.6-20.6% of the total EMB agar count, respectively (Fig. 5). No Gram-negative bacteria were noted at the organic

**Table 4.** Concentration of *Enterobacter faecalis* (grown on blood agar) in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 1.1 (0.75-124.51) | 5 | 0.5225 (0.012-15.24) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 6.65 (2.92-12.18) | 5 | 0.994 (0.268-5.35)$^{\#}$ |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.0 (0.0-0.003)$^{**}$ |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.0 (0.0-0.011)$^{**}$ |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.0 (0.0-0.0125)$^{**}$ |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 31.32 (3.0.-47.89) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu $\times 10^3$/m$^3$); ND = not determined. $^{\#}$value significantly smaller compared to that before installation of biofilter ($p \leq 0.05$). $^{**}$value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.01$).

**Table 5.** Concentration of *Streptomyces* spp. (grown on blood agar) in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 0.005 (0.0-0.02) | 5 | 0.004 (0.0-0.0175) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 0.002 (0.0-0.16) | 5 | 0.0 (0.0-0.025) |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.265 (0.018-45.6)$^{+}$ |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.085 (0.012-4.71)$^{+}$ |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.6325 (0.004-12.125)$^{+}$ |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 0.0 (0.0-0.03) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu × $10^3/m^3$); ND = not determined. $^{+}$value significantly greater compared to that recorded at the inlet of biofilter after its installation ($p<0.05$).

**Table 6.** Concentration of total mesophilic bacteria (grown on blood agar), after subtracting *Streptomyces* strains, in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 1.54 (0.97-131.2) | 5 | 0.7925 (0.032-16.56) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 16.83 (9.8-78.9) | 5 | 3.188 (0.762-9.825)$^{\#}$ |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.059 (0.0225-1.155)$^{*}$ |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.28 (0.01-2.8485)$^{*}$ |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.07 (0.045-0.3098)$^{**}$ |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 33.9 (4.67-53.25) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu × $10^3/m^3$); ND = not determined. $^{\#}$value significantly smaller compared to that before installation of biofilter ($p<0.05$). $^{*}$value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.05$). $^{**}$value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.01$).

**Table 7.** Concentration of Gram-negative bacteria (grown on EMB agar) in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 0.015 (0.0-6.15) | 5 | 0.0175 (0.0025-0.136) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 2.17 (0.74-34.4) | 5 | 0.205 (0.04-1.2725)$^{\#}$ |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.0 (0.0-0.0)$^{**}$ |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.0 (0.0-0.005)$^{**}$ |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.0 (0.0-0.0)$^{**}$ |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 0.58 (0.28-1.55) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu × $10^3/m^3$); ND = not determined. $^{\#}$value significantly smaller compared to that before installation of biofilter ($p\leq0.05$). $^{**}$value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.01$).

medium (OM) and halloysite medium (HM) outlets of the biofilter, while at the outlet of bentonite medium only trace amounts of *Pantoea agglomerans* (0.0025-0.005 × $10^3$ cfu/$m^3$) were recorded.

The median concentrations of thermophilic actinomycetes in the air of the examined hatchery were very small, ranging from 0.0-0.04 × $10^3$ cfu/$m^3$. No significant differences were observed between the concentrations noted before installation of the biofilter or in the inlet air entering the filter and those recorded in the outlet air coming out the filter (Tab. 8). The most common were *Thermoactinomyces* strains (*Th. thalpophilus*, *Th. vulgaris*), followed by *Saccharopolyspora rectivirgula* (Fig. 6).

**Identified bacterial species.** In the air samples taken in the examined hatchery, a total of 56 species or genera of bacteria were identified, of these, 11, 11 and 6 species or genera respectively were reported as having allergenic, immunotoxic and/or infectious properties [11, 14, 18, 20, 21, 34] (Tab. 9). These figures are certainly an underestimation, as

**Table 8.** Concentration of thermophilic actinomycetes (grown on tryptic soya), in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 0.0 (0.0-0.02) | 5 | 0.0 (0.0-0.0) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 0.0 (0.0-0.0) | 5 | 0.0 (0.0-0.02) |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.04 (0.0-0.07) |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.02 (0.0-0.02) |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.01 (0.0-0.04) |
| 6. Sorting room: manual removal of chickens from hatching boxes | 3 | 0.0 (0.0-0.02) | | ND |

N = number of samples; C = concentration of bacteria in the air (cfu × $10^3/m^3$); ND = not determined.

**Table 9.** List of bacterial species and genera identified in the air samples taken in the hatchery.

**Gram-negative bacteria:** ***Acinetobacter baumanii***[*+] (1, 1a, 2, 2a, 6), ***Acinetobacter lwoffii***[*+] (2a), ***Acinetobacter genospecies*** 9[*+] (2a), *Agrobacterium radiobacter* (1a, 2a), ***Alcaligenes faecalis***[*+] (2), *Brevundimonas diminuta* (2a), *Brevundimonas vesicularis* (2a), ***Chryseobacterium meningosepticum***[#] (1, 2a, 6), *Chryseobacterium tirrenicum* (2a), ***Citrobacter youngae***[+] (1a, 6), *Empedobacter brevis* (2a), ***Enterobacter cloaceae***[+] (1, 1a, 2, 2a, 6), ***Escherichia coli***[+#] (1, 1a, 2, 2a, 6), ***Klebsiella pneumoniae* ss *pneumoniae***[+#] (2a), ***Klebsiella* spp.**[+] (2, 6), *Leclercia adecarboxylata* (2, 2a, 6), ***Pantoea agglomerans***[*+] (synonyms: ***Erwinia herbicola***, ***Enterobacter agglomerans***) (2a, 4), *Pseudomonas pseudoalcaligenes* (2a), *Pseudomonas putida* (1, 2, 2a, 6), *Pseudomonas stutzeri* (1, 2), *Pseudomonas* spp. (1, 2, 2a, 6), ***Salmonella* spp.**[+#] (1a), *Sphingobacterium multivorum* (1a, 2a), *Sphingomonas paucimobilis* (2, 2a, 6), *Stenotrophomonas maltophilia* (2).

**Bacilli:** *Bacillus* spp. (1, 1a, 2, 2a, 3-6).

**Corynebacteria:** *Aureobacterium* spp. (4, 5), *Brevibacterium casei* (2a), *Brevibacterium otitidis* (1a, 2a), *Brevibacterium* spp. (1a, 3, 5), *Corynebacterium xerosis* (2a), *Corynebacterium* spp. (1a, 2a, 5), *Gordona sputi* (2a), *Jonesia denitrificans* (4), *Microbacterium laevaniformans* (4), *Microbacterium saperdae* (3-5), *Microbacterium terregens* (4), *Microbacterium testaceum* (4), *Microbacterium* spp. (4), *Rhodococcus* spp. (4).

**Gram-positive cocci:** *Dermacoccus nishinomiyaensis* (2a), ***Enterococcus faecalis***[#] (1, 1a, 2, 2a, 3-6), *Enterococcus faecium* (2a), *Micrococcus luteus* (2a, 3), *Micrococcus* spp. (1a, 2a, 3-5), *Pediococcus pentosaceus* (2), ***Staphylococcus aureus***[#] (2a), *Staphylococcus* spp. (1, 1a, 2, 2a, 3-6), *Streptococcus* spp. (4).

**Mesophilic actinomycetes:** ***Streptomyces albus***[*] (1, 1a, 2, 2a, 3-6), Streptomyces spp. (1a, 2, 2a, 3-5).

**Thermophilic actinomycetes:** ***Saccharopolyspora rectivirgula***[*] (synonyms: ***Micropolyspora faeni***, ***Faenia rectivirgula***) (2a, 3-5), ***Thermoactinomyces thalpophilus***[*] (1, 2a, 4), ***Thermoactinomyces vulgaris***[*] (3, 4, 6), ***Thermomonospora fusca***[*] (4, 5), ***Thermomonospora* spp.**[*] (5).

Sites of isolation are given in parentheses. The names of species reported as having allergenic, immunotoxic and/or infectious properties (see text) are in bold, marked as follows: [*]allergenic species; [+]immunotoxic species, [#]infectious species.

a part of bacterial strains could be identified only to generic level. The most of potentially pathogenic species were found among Gram-negative bacteria. Over 99% of Gram-negative strains were isolated from air samples taken before installation of the biofilter or in the inlet air entering the filter, while only single strains were isolated from the outlet air coming out the bentonite medium of the biofilter. In contrast, 8 out of 14 identified species and/or genera of corynebacteria were isolated only from the samples of the outlet air coming out the biofilter (Tab. 9).

**The concentration of dust and bacterial endotoxin in the air of the hatchery before and after installation of the biofilter.** The median concentration of airborne dust at the inlet duct of the biofilter after its installation (0.47 mg/$m^3$) was insignificantly smaller compared to those recorded before its installation (0.635-3.55 mg/$m^3$). Median dust concentrations at the outlets of the biofilter from bentonite and halloysite media (0.067 and 0.13 mg/$m^3$, respectively) were significantly smaller ($p<0.05$ and $p<0.01$, respectively) compared to that recorded at the inlet duct of the biofilter after its installation (0.47 mg/$m^3$) (Tab. 10). The concentration noted at the outlet of the biofilter from organic medium (0.067 mg/$m^3$) was also distinctly smaller compared to that recorded at its inlet duct after installation, but the difference did not attain significance level ($0.05<p<0.1$).

The median concentrations of airborne endotoxin in the inlet air entering the biofilter after its installation (4.168-4.4 ng/$m^3$) were significantly smaller ($p<0.05$) compared to those recorded before installation of the biofilter (52.7-364.25 ng/$m^3$). The median concentrations of airborne endotoxin at the outlets of the biofilter (0.508-2.1875 ng/$m^3$) were smaller compared to that recorded at its inlet duct

**Table 10.** Concentration of dust in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 0.635 (0.37-1.07) | 5 | 0.47 (0.14-1.07) |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 3.55 (1.53-4.53) | 5 | 0.47 (0.28-4.47) |
| 3. Outlet of biofilter (OM) | | ND | 5 | 0.067 (0.00067-0.4) |
| 4. Outlet of biofilter (BM) | | ND | 5 | 0.067 (0.0067-0.47)* |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.13 (0.067-0.2)** |
| 6. Sorting room: manual removal of chickens from hatching boxes | 2 | 4.28 (4.23-4.33) | | ND |

N = number of samples; C = concentration of dust in the air (mg/m$^3$); ND = not determined. *value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.05$). **value significantly smaller compared to that recorded at the inlet of biofilter after its installation ($p<0.01$).

**Table 11.** Concentration of bacterial endotoxin in hatchery air before and after installation of biofilter.

| Sampling point/period | Before installation of biofilter January – February 2005 | | After installation of biofilter April – October 2005 | |
|---|---|---|---|---|
| | N | C (median, range) | N | C (median, range) |
| 1, 1a. Corridor between incubators and hatchers | 4 | 52.7 (51.9-104.5) | 5 | 4.168 (0.52-45.2)# |
| 2, 2a. Inlet of biofilter behind hatchers | 4 | 364.25 (50.9-520,450.4) | 5 | 4.4 (1.06-41.7)# |
| 3. Outlet of biofilter (OM) | | ND | 5 | 2.1875 (0.052-41.7) |
| 4. Outlet of biofilter (BM) | | ND | 5 | 2.1 (0.020937-21.875) |
| 5. Outlet of biofilter (HM) | | ND | 5 | 0.508 (0.1417-21.875) |
| 6. Sorting room: manual removal of chickens from hatching boxes | 2 | 2073.55 (2065.4-2081.7) | | ND |

N = number of samples; C = concentration of endotoxin in the air (ng/m$^3$); ND = not determined. #value significantly smaller compared to that before installation of biofilter ($p<0.05$).

after installation (4.4 ng/m$^3$), but the difference was not statistically significant (Tab. 11).

## DISCUSSION

The median concentrations of airborne mesophilic bacteria recorded in the hatchery before installation of the biofilter ranged from 1.56-33.9 × 10$^3$ cfu/m$^3$, and were lower compared to those recorded by earlier authors which ranged from 8.3-627.0 × 10$^3$ cfu/m$^3$ [2, 6, 9, 10, 16, 25, 36, 40]. Similarly to the data reported by Dutkiewicz [9, 10] and Skórska [36], a relatively high median value (33.9 × 10$^3$ cfu/m$^3$) was noted in the sorting room during the removal of chickens from the hatching boxes. However, this value was 3-10 times lower compared to those reported by the cited authors 20-30 years ago [9, 10, 36]. This seems to indicate that the hygienic conditions in a modern industrial hatchery are much better than in older ones, and are not associated with a high occupational risk for the workers.

The domination of *Enterococcus faecalis* and common occurrence of *Acinetobacter* spp. in the air of the hatchery found in the present work was reported also by the earlier authors [9, 10, 36]. *Enterococcus faecalis* is a commensal developing abundantly in intestines of birds and mammals which could be facultatively pathogenic for man, causing endocarditis, urinary tract infections and sepsis [14, 18]. *Acinetobacter* strains produce endotoxins and allergens [37, 38] and may cause infections in debilitated persons. Besides the aforementioned species, at least 17 other species of bacteria detected in the air of the hatchery could be a potential cause of allergic, immunotoxic and/or infectious diseases.

The median concentrations of airborne mesophilic bacteria in the inlet air entering the biofilter after its installation were lower compared to those recorded before its installation and ranged from 0.7925-3.188 × 10$^3$ cfu/m$^3$. The lowest were median concentrations measured at the outlet ducts of the biofilter which ranged from 0.3525-0.72 × 10$^3$ cfu/m$^3$. The difference was mostly evident and significant in the case of predominant constituents of the airborne bacterial flora: *Enterococcus faecalis* and Gram-negative bacteria, which were reduced almost to zero level by passing through the biofilter, and were recovered in trace amounts only in single cases. By contrast, the median concentrations of *Streptomyces* spp. were significantly greater in the samples of air taken at the outlet ducts of the biofilter (0.085-0.6325 × 10$^3$ cfu/m$^3$) compared to the samples of inlet air (0.0-0.005 × 10$^3$ cfu/m$^3$).

As, so far, there are no internationally recognised Occupational Exposure Limit (OEL) values for bioaerosols, the results obtained in the present work could be compared only to the proposals raised by particular authors. The OEL value proposed by Malmros *et al.* for total mesophilic bacteria ($10 \times 10^3$ cfu/m$^3$) [26] was exceeded in 2 out of 5 medians, and in 7 out of 21 individual air samples collected in the hatchery before entering the biofilter, and in none out of 3 medians and in 2 out of 15 individual air samples collected at the outlet ducts of the biofilter. Another OEL value for mesophilic bacteria proposed by Górny & Dutkiewicz ($100 \times 10^3$ cfu/m$^3$) [17] was not exceeded in any of 5 medians and in 1 out of 21 individual air samples collected in the hatchery before entering biofilter, and in none of 3 medians and 15 individual air samples collected at the outlet ducts of the biofilter. The OEL value for airborne Gram-negative bacteria proposed by Clark [3] and Malmros *et al.* [26] ($1 \times 10^3$ cfu/m$^3$) was exceeded in 1 out of 5 medians, and in 5 out of 21 individual air samples collected in the hatchery before entering the biofilter, and in none of 3 medians and 15 individual air samples collected at the outlet ducts of the biofilter. Another OEL value for Gram-negative bacteria proposed by Górny & Dutkiewicz ($20 \times 10^3$ cfu/m$^3$) [17] was exceeded in none out of 5 medians and in 1 out of 21 individual air samples collected in the hatchery before entering the biofilter, and in none out of 3 medians and 15 individual air samples collected at the outlet ducts of the biofilter. Nowhere was the OEL value proposed by Górny & Dutkiewicz exceeded [17] for airborne thermophilic actinomycetes ($20 \times 10^3$ cfu/m$^3$).

The median concentrations of dust in the air of the hatchery before installlation of the biofilter and in the inlet air entering biofilter after its installation were in the range 0.47-4.28 mg/m$^3$. The median concentrations of dust in the outlet air coming out the biofilter were about 10 times lower and ranged from 0.067-0.13 mg/m$^3$. The Polish OEL value of 4 mg/m$^3$ [31] was slightly exceeded in 1 out of 5 medians, and in 4 out of 20 individual air samples collected in the hatchery before entering the biofilter, and in none out of 3 medians and 15 individual air samples collected at the outlet ducts of biofilter.

The median concentrations of bacterial endotoxin in the air of the hatchery before installation of the biofilter were in the range 52.7-2,073.55 ng/m$^3$, being the greatest at the removal of chickens manually from the hatching boxes. The values of 3 medians and 10 individual samples exceeded in all cases the OEL values proposed by the Dutch Expert Committee on Occupational Standards (DECOS) [7] (5 ng/m$^3$), and by Laitinen *et al.* [22] (25 ng/m$^3$). The values of 2 out of 3 medians and of 6 out of 10 individual samples exceeded the OEL values proposed by Clark [3] (100 ng/m$^3$), by Rylander [32] (100-200 ng/m$^3$), and by Malmros *et al.* [26] (100 ng/m$^3$), while values of 2 out of 3 medians and of 5 out of 10 individual samples exceeded the OEL values proposed by Górny & Dutkiewicz [17] (200 ng/m$^3$). At 3 out of 10 sampling points, airborne endotoxin occurred in large quantities of the order $10^3$-$10^5$ ng/m$^3$, posing a risk of respiratory disease in exposed workers [33].

The median concentrations of endotoxin in the inlet air entering the biofilter after its installation were in the range 4.168-4.4 ng/m$^3$. None of the values of 2 medians and values of 2 out of 10 individual samples exceeded the OEL values proposed by DECOS [7] and Laitinen *et al.* [22], while nowhere were the OEL values proposed by Clark [3], Rylander [32], Malmros *et al.* [26], and Górny & Dutkiewicz [17] exceeded. The median concentrations of endotoxin in the outlet air coming out the biofilter were in the range 0.508-2.1875 ng/m$^3$. None of the values of 3 medians and values of 4 out of 15 individual samples exceeded the OEL value proposed by DECOS [7], while only 1 out of 15 individual samples exceeded the OEL value proposed by Laitinen *et al.* [22]. Nowhere were the OEL values proposed by Clark [3], Rylander [32], Malmros *et al.* [26], and Górny & Dutkiewicz [17] exceeded.

Efficacy of biofiltration depends mostly on the quantity and activity of microorganisms developing in the media. Activity of microorganisms is conditioned by the physicochemical properies of filtration material. Monitoring of these parameters ensures a proper adsorption and biodegradation of the pollutants. In the present work, monitoring of the physicochemical properies of filtration media was conducted during entire study period and revealed stabilization of tested parameters. The mean temperature and humidity of all tested media were on similar levels, being within limits reported by many authors as an optimum [1, 23].

The problem of biological reduction of microbial pollutants has been addressed in only a few scientific studies. Irrespective of the kind of reduced factor, the authors of all studies reported the relationship between the type of applied filtration filling and the efficacy of the filtration. Schlegelmilch *et al.* [35] demonstrated the highest effectiveness of the removal of microbial pollutants (99%) for the filtration material containing coconut chips as a basic component. Martens *et al.* [27] examined efficacy of the cleaning of air leaving a piggery with the use of biofilters filled with various filtration materials, such as a mixture of peat and coconut chips, bark, and biocompost. The efficacy of endotoxin reduction by all tested fillings approximated 90%.

To summarize, the biofilter applied in this study proved to be effective for the elimination of potentially pathogenic bacteria, dust and endotoxin from the air of the hatchery. The most effective medium seems to be organic-mineral containing 20% halloysite, 40% compost and 40% peat. The efficacy of the biofilter was diminished by the release of mesophilic actinomycetes of the genus *Streptomyces* and corynebacteria that evidently proliferate in this medium. Although these bacteria are mostly not pathogenic for man and animals (except for *Streptomyces albus* which could be a cause of allergic alveolitis), the projected inhibition of their growth in the biofilter's media would improve the degree of microbiological purity of outcoming air.

In conclusion, the microbiological analyses proved that irrespective of the biofiltration efficacy and the quantity of microorganisms leaving the biofilter, nearly all pathogenic bacteria are retained by the filtration media. Thus, the air after biofiltration, in spite of containing some microorganisms, poses a greatly diminished health hazard.

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