How to Reduce the Emission of Microorganisms from a Biofilter Used to Treat Waste Gas from a Food Industry Plant

Adam Muszyński, Agnieszka Tabernacka * and Monika Załęska-Radziwiłł

Faculty of Building Services, Hydro and Environmental Engineering, Warsaw University of Technology, Nowowiejska 20, 00-653 Warsaw, Poland; adam.muszynski@pw.edu.pl (A.M.);
monika.radziwill@pw.edu.pl (M.Z.-R.)
* Correspondence: agnieszka.tabernacka@pw.edu.pl; Tel.: +48-22-234-5943

Abstract: The aim of the study was to assess the bioaerosol removal efficiency by a semi-technical scale combined biofilter used to treat waste gas from a food industry plant. Two types of biofilter beds were tested: stumpwood chips and pine bark (CB) and stumpwood chips, pine bark and compost (CBC). Two types of membranes (covering the surface of the bed) were examined as the second stage of treatment: Pro Eko Tex UV (M1) and Pro Eko Tex UV 6 (M2). A conventional open biofilter (without membranes) was an emitter of microorganisms. There was no statistically significant difference between the number of bacteria emitted from CB or CBC beds, but fungal concentration was three times higher in gas treated by the CBC bed. The use of the membranes as the second stage of gas treatment significantly reduced the bacterial emission (74–78%) from the biofilter regardless of the bed and the membrane tested. The M1 membrane was also efficient in fungi removal from the treated gas by 80–97%. However, the M2 membrane could have been slowly colonized by fungi and have become an additional emitter of fungi in the system.

Keywords: bioaerosol emission; membranes; combined biofilter

1. Introduction

The development of new industries, as well as the combustion of fuels for the heat and energy, result in the emission of various pollutants to air, especially xenobiotic compounds posing a serious public health risk. Waste gases contain numerous organic compounds, including volatile fatty acids, aldehydes, ketones, alcohols, and inorganic compounds such as hydrogen sulphide and ammonia. Some of them are precursors of photochemical reactions leading to the transformation of compounds into more toxic forms, including mutagenic, teratogenic or cytotoxic. Their emission deteriorates the quality of the ambient air and poses a real threat to human health and life.

The reduction of emissions can be achieved through the use of appropriate gas treatment technologies, among which biotechnological methods are of great interest. They involve the filtration of contaminated air and sorption of pollutants in the water phase and/or on the support medium (carrier). The pollutants then undergo biochemical transformations carried out by microorganisms inhabiting the bioreactors. The advantages of this technology are the high efficiency of removing biodegradable substances, relatively low costs, selectivity, and the lack of waste products [1–3]. The most important factors deciding for the use of biotechnological waste gas treatment are the bioavailability and the biodegradability of air pollutants. Waste gases must also be free from dust and components that are toxic to microorganisms, such as heavy metals or acid vapours, and their temperature must not inhibit the metabolic activity of microorganisms.

Various bioreactor configurations have been applied to treat waste gases, including bioscrubbers, biotrickling filters, continuous stirred tank bioreactors, airlift bioscrubbers, dual liquid phase systems, external loop airlift bioreactors, membrane bioreactors, rotating drums, and two-stage bioreactors [4–10]. However, biofilters are the most common
reactors used for biological waste gas treatment due to the easy operation, high efficiency, and relatively low costs. It is a bioreactor filled with natural organic material (biofilter bed), which is a carrier for microorganisms. Pollutants are adsorbed from the waste gas to the carrier and then biodegraded by microorganisms which colonize the biofilter bed. Typical materials used in biofilters include compost from municipal waste or green waste, bark, leaves, heather, brushwood, wood particles, peat, soil, and dehydrated activated sludge [1,2,10–12]. Mixtures of peat and heather, peat and spruce branches, bark and compost or compost and wood were also used as filter bed. The advantage of using natural materials is the presence of macro- and micronutrients, necessary for microorganisms growth. Furthermore, these materials are inherently inhabited by a wide variety of microorganisms and usually additional bioaugmentation of biofilters is not necessary. However, addition of synthetic (polystyrene foam) or inert carriers (ceramics, perlite, glass beads) ensure better porosity and lower gas flow resistance through the biofilter bed [2,13].

The most frequently detected bacteria and archaea in biofilters include Alpha-, Beta-, Gamma-, and Deltaproteobacteria, Actinobacteria, Firmicutes, Verrucomicrobia, and Crenarchaeota. Fungi detected in biofilters belong mostly to two large divisions Basidiomycota and Ascomycota (e.g., Exophiala oligosperma, Exophiala lecanii-corni, Paecilomyces sp., Scedosporium apiospermum, Sporothrix variecibatus, Aspergillus sp.) [2,14]. Growth of different microbial taxa in a biofilter bed not only depends on the type of gas pollutants and carrier composition, but also on the operational parameters, such as the waste gas temperature, substrate mass loading rate, and volumetric loading rate. As a result, microbial communities in the biofilter differ even in very similar biofilter configurations treating waste gases with the same main pollutants [15–20].

The wide variety of microorganisms inhabiting the biofilter materials include also pathogens or potential pathogens such as E. coli, Shigella sp. or Enterobacter sp. Moreover, many of the fungi present in a biofilter bed, such as e.g., Aspergillus fumigatus can produce spores and mycotoxins. The potentially pathogenic organisms, spores, and toxins can be released to the treated gas as a secondary pollution and have a detrimental effect on human health and the ecosystem [14,21–23]. Therefore, a biofilter should be considered as a potential emitter of bioaerosol containing microorganisms that could be harmful and dangerous for human health and life. The generation and emission of bioaerosols from the biofilter beds is not yet well documented, particularly when biofilters are operated under various conditions, such as fluctuating temperatures, type of biofilter bed, various residence times, and organic loadings.

Ottengraf and Konnings [24] tested full scale biofilters filled with compost-polystyrene particles or with only compost to treat waste gases from oriental food processing, flavour and fragrance production, and domestic wastewater treatment plant. The number of moulds in the treated gas was higher than in the waste gas for the biofilters filled with compost-polystyrene particles. However, none of the tested biofilter reduced the concentration of bacteria, and these microorganisms were even emitted from biofilter beds despite the fact that they were not detected before filtration in waste gas from the oriental food processing and domestic wastewater treatment plant.

Sanchez-Monedero et al. [25] observed the emission of mesophilic bacteria and Aspergillus fumigatus moulds, while they analysed the operation of seven different biofilters packed with compost and used them to treat waste gases from composting plants. The total number of mesophilic bacteria in treated gas varied from $2 \times 10^3$ to $8 \times 10^4$ cfu/m$^3$ and the number of A. fumigatus was from $10^2$ to $1.2 \times 10^3$ cfu/m$^3$. Similarly, Chmielowiec-Korzieniowska et al. [26] proved that a biofilter can be a source of bioaerosol emission. The researchers analysed the concentration of airborne bacteria in the air extracted from the hatchery and indicated that there was a significant difference in bioaerosol composition before and after the waste gas treatment. Enterococcus faecalis and Gram-negative bacteria constituted up to 100% of all microorganisms in the waste gas, while in the treated gas Streptomyces strains were the dominant ones, forming 66.9–97.5% of the total bacteria. Differences in bacterial diversity in the waste gas from a swine fattening-finishing room and
gas treated by the percolating biofilter were also observed by Vyskocil et al. [27]. Authors noted that more Proteobacteria were present in the waste gas. The culturable and total bacteria and archaea emissions ranged from 333 to $2.3 \times 10^5$ cfu/m$^3$, 975 to $2.4 \times 10^5$ E. coli equivalent, and 896 to 5579 Methanosarcina mazei equivalent in 1 m$^3$.

One of the measures that could be undertaken to reduce bioaerosol emissions is the application of a membrane as the second stage of waste gas treatment. The use of membranes to separate organic vapor from contaminated gases has been investigated in recent years [19]. The mechanism of VOC removal from polluted waste gases on the membrane is based on the adsorption process. However, in the case of bioaerosol another mechanism plays a far more important role. Bioaerosol emissions are reduced due to membrane filtration. This removal of microorganisms is caused by the physicochemical interactions between the membrane and microorganisms, and by the sieving effect [28,29]. The main advantages of this method are low energy consumption and a small area required for the process. However, to the best of our knowledge, the use of membranes for waste gas treatment has only been tested for the removal of organic and inorganic contaminants. The application of a membrane as a second stage of waste gas treatment to remove bioaerosol from gases after biofiltration, as proposed in this study, is a completely novel concept.

The aim of the study was to assess the bioaerosol removal efficiency (both bacterial and fungal) by a pilot combined biofilter in a semi-technical scale used to treat waste gas from a food industry plant. Two types of biofilter beds and two types of membranes were tested, the effect of microbial elimination or emission from the biofilter was examined by comparing the obtained results with the use of descriptive statistics and was statistically verified.

2. Materials and Methods

2.1. Biofilter

The research was carried out on a semi-technical scale in a food industry plant located in eastern Poland manufacturing high-quality animal and vegetable fats. The tested two-stage membrane biofilter was connected to the installation for the extraction of process gases from lard and vegetable fats production and was operated to alleviate the odour nuisance (detailed description of fat processing can be found in an article by Lelicińska-Serafin et al. [30]). A biofilter was installed and operated to treat only part of the process gases emitted from one of the production lines (side-stream treatment). The biofilter could be used as a conventional open biofilter or as a combined biofilter with a membrane covering the surface of the biofilter bed (the second stage treatment) (Figure 1a). It was equipped with a fan, a scrubber, automatic regulation and measurement of gas flow, temperature and humidity control systems, and an installation for the distribution of waste gas and leachate drainage. The membrane fastening system provided proper sealing, and a sliding shelf for sampling was installed on top of the biofilter (Figure 2). The dimensions of the active surface of the biofilter were $1.32 \times 3.00$ m, and the height of the bed was 1.1–1.2 m.

The biofilter was operated at an average flow rate of 378 m$^3$/h (in the range of 322–399 m$^3$/h), which resulted in an EBRT of 45 s (in the range of 41–66 s) and an average surface and volumetric load of 94.8 and 82.5 m$^3$/((m$^2$ × h)) (in the range of 62.4–101.5 m$^3$/((m$^2$ × h)) and 54.2–88.3 m$^3$/((m$^3$ × h)), respectively). The concentration of VOCs in waste gas was in the range of 780–2890 ppb. The average pressure drop was $379 \pm 13$ Pa and $596 \pm 84$ Pa in the open biofilter filled with CB and CBC material, respectively. The M1 and M2 membranes generated an additional pressure drop of 59–64 Pa and 28–63 Pa, respectively.
Two types of materials were used as the biofilter bed (first stage of treatment): Stumpwood chips and pine bark (CB), and stumpwood chips, pine bark, and compost from green waste (CBC) (Table 1). CB was a 1:1 mix of pine bark and stumpwood chips with a grain diameter of 20–80 mm, whereas CBC was a mix of stumpwood chips with pine bark (50%, 1:1 ratio) with compost (50%). Two different membranes (trade names Pro Eko Tex UV and Pro Eko Tex UV 6, respectively) were also subsequently tested (second stage of treatment): More permeable and thinner (M1) and less permeable and thicker (M2) (average area

**Figure 1.** (a) Scheme of the pilot-scale combined biofilter equipped with a fan, a scrubber, an automatic regulation and control unit, and an installation for the distribution of waste gas and leachate drainage. Biofilter bed materials (CB—mix of stumpwood chips and pine bark; CBC—mix of stumpwood chips, pine bark, and compost from green waste) and membranes (M1 or M2) were tested alternatively. (b) Location of sampling points (1, 2, 3) of treated gases above the biofilter bed and the membranes—top view.

**Figure 2.** A pilot-scale combined biofilter examined for the treatment of waste gases from a food industry plant located in eastern Poland.
weight 400 ± 1 g/m² and 474 ± 3 g/m², respectively). Membranes consisted of 3 layers—2 outer and 1 middle functional layer made of PS and +ePTFE, respectively. Their average air permeability was 17.8 and 3.9 mm/s, respectively, while average watertightness was 199 and >2000 cm H₂O, respectively. Detailed parameters of biofilter bed materials and properties of membranes, as well as operational parameters of the biofiltration process, can be found in articles by Lelićinska-Serafin et al. [30] and Rolewicz-Kalińska et al. [31]. Six configurations of the biofilter, which were tested in this study, are presented in Table 2.

Table 1. Parameters (mean values and ranges in parentheses) of the biofilter bed materials: Stumpwood chips and pine bark (CB), and stumpwood chips, pine bark, and compost from green waste (CBC) [30,31].

| Parameter                        | CB (%) | CBC (%) |
|----------------------------------|--------|---------|
| Total organic matter (% d.m.)    | 86.0 (85.0–87.5) | 45.0 (40.6–47.8) |
| Total moisture content (%)       | 63.4 (60.6–66.3) | 46.8 (42.7–50.5) |
| pH                               | 6.77 (6.75–6.79) | 7.44 (7.27–7.70) |
| Specific surface (m²/g)          | 0.55 (0.37–0.67) | 1.67 (1.53–1.80) |
| Substitute diameter (mm)         | 37.1 (34.8–39.6) | 8.7 (6.7–9.9) |

Table 2. Configurations of the biofilter tested during the experiment.

| Biofilter Bed                    | Membrane       | Configuration |
|----------------------------------|----------------|---------------|
| stumpwood chips and pine bark (CB)| none           | CB            |
|                                  | Pro Eko Tex UV (M1) | CB + M1      |
|                                  | Pro Eko Tex UV 6 (M2) | CB + M2      |
| stumpwood chips, pine bark, and compost from green waste (CBC) | none           | CBC           |
|                                  | Pro Eko Tex UV (M1) | CBC + M1     |
|                                  | Pro Eko Tex UV 6 (M2) | CBC + M2     |

2.2. Gas Sampling

Three types of gas samples (waste gas, treated gas and ambient air) were taken using MAS-100 (Merck KGaA, Darmstadt, Germany) and SAS Super ISO (VWR) impactors following the manufacturers’ instructions. Waste gas samples (gas entering the biofilter) were taken at the inlet to the biofilter. Treated gas samples were taken from 3 different points located directly above the biofilter bed (CB and CBC configurations) or above the membranes (CB + M and CBC + M configurations) (Figure 1b):
- Point 1 was located symmetrically (centrally) in relation to the longer walls of the biofilter, but 50 cm from the shorter wall of the biofilter;
- Point 2 was located in the middle of the biofilter (at the same distance from all walls);
- Point 3 was located symmetrically (centrally) in relation to the longer walls of the biofilter, but 250 cm from the shorter wall of the biofilter.

The samples of treated gases were collected with the application of a shield (hood 0.7 × 0.7 × 1 m in height) that eliminated the interfering effects of environmental conditions (ambient air). Ambient air samples (the test background) were collected 5 m from the biofilter and 1.5 m above the ground level upwind. For each configuration, two series of sampling were performed with an interval of 2–3 weeks, each series of sampling was carried out in seven repetitions.

2.3. Microbiological Analyses

The number of culturable bacteria and fungi in gas samples was determined using tryptone-soya agar (TSA) and rose bengal chloramphenicol agar (RBC) after 48 h and 6 days incubation at 26 °C, respectively. Studies [25–27] show that bioaerosol in treated gas after biofiltration mainly contains microorganisms inhabiting the biofilter bed. As testing microorganisms found in high concentrations in outlet treated gases was a priority in our
research, we decided to use the incubation temperature that was relevant to the conditions in the biofilter bed. The results are presented as colony-forming units (cfu) in 1 m$^3$ of gas.

2.4. Statistical Measures and Methods

Standard statistical comparisons and graphing were made in Microsoft Excel. Results are presented in a box and whisker charts—the bottom and top of each box are the first and third quartiles, the band inside the box and the cross marker are the median and the mean, respectively, the whiskers represent the minimum and maximum values of each data set. Results that are numerically distant from the rest of the data (outside of 1.5 times the interquartile range above the third quartile and below the first quartile) are presented as outliers. As the outliers had a large impact on the mean values, the results of the different stages of the experiment (biofilter configurations) are compared based on the median values. The mood’s median test was used to test the null hypothesis that the medians of two series from two different stages of the experiment were identical. Pearson’s chi-squared test was performed at the significance level of 0.05, corresponding to the $\chi^2_{\text{crit}}$ value of 3.8.

3. Results and Discussion

Concentrations of bacteria and fungi in the waste gas, treated gases, and the ambient air are presented in Figures 3 and 4, respectively.

Figure 3. Total number of bacteria [cfu/m$^3$] in the waste gas, gases treated by a conventional biofilter (only bed) and combined biofilter (bed + membrane M1 or M2), and the ambient air. Types of bed materials: CB—mix of stumpwood chips and pine bark; CBC—mix of stumpwood chips, pine bark, and compost from green waste. The band inside the box—median; $\times$—mean value; $\circ$—outliers.
Figure 4. Total number of fungi [cfu/m$^3$] in the waste gas, gases treated by a conventional biofilter (only bed) and combined biofilter (bed + membrane M1 or M2), and the ambient air. For a detailed legend, see Figure 3.

The concentration of bacteria in waste gas from the food processing plant varied during the experiment from 12 to 6040 cfu/m$^3$ with median values 240 and 500 cfu/m$^3$ during testing CB and CBC beds, and was slightly higher than the total number of bacteria in the ambient air at the same time (142 and 200 cfu/m$^3$, respectively). The total number of fungi in waste gas varied from 0 to 1680 cfu/m$^3$, with medians 20 and 391 cfu/m$^3$ in the first and second part of the experiment (CB and CBC bed materials testing, respectively). These values are comparable to or lower than the total number of fungi in the ambient air at the same time (80 and 1410 cfu/m$^3$, respectively). The microbiological contamination of waste gases in this study was significantly lower than for dairy, piggery, and poultry food processing ($10^5$–$10^6$ cfu/m$^3$), presented by Mirskaya and Agranovski [32], Lutgring et al. [33], and Millner [34]. Lues at al. [35] showed that the total number of fungi in waste gas from the chicken-slaughtering facility can be also higher than presented in this study, reaching up to $10^4$ cfu/m$^3$.

The comparison of microbial concentrations in waste gas and treated gases in this study demonstrates that the conventional open biofilter (without membranes) is an emitter of both bacteria and fungi. The total number of bacteria in the treated gas from the open biofilter was higher compared to the waste gas introduced in the biofilter for both CB and CBC materials. The Mood’s test (Table 3) indicated that there were no statistically significant differences between the medians of bacterial number emitted from CB and CBC beds of the biofilter. However, a large variation in the emission of bacteria from the CB biofilter bed was detected during the experiment, which shows that this material is more heterogeneous and generates a greater variability of the emitted bacterial bioaerosol in comparison with the CBC bed (Figure 3). On the contrary, the fungal emission was more varied for the CBC biofilter bed (Figure 4). Moreover, the median concentration of fungi in the gas treated by the CB open biofilter was 1580 cfu/m$^3$, while in the case of CBC material it was almost three times higher (4520 cfu/m$^3$). Statistically significant differences between the fungal concentration in gas treated by CB and CBC biofilters were also confirmed by the Mood’s median test (Table 4). It should be noted that the number of fungi in the treated...
gas from the conventional biofilter is over one order of magnitude higher compared to the raw waste gas.

Table 3. Heatmap showing results of the Mood’s median test (p-values and χ² in parenthesis), performed to test the null hypothesis that the medians of two series of bacterial concentration in treated gases/ambient air were identical. Significance level of Pearson’s chi-squared test was $\alpha = 0.05$, corresponding to $\chi^2_{crit} = 3.8$. Statistically significant ($p < 0.05$, $\chi^2 > 3.8$) differences are highlighted in red. Bed materials: CB—mix of stumpwood chips and pine bark; CBC—mix of stumpwood chips, pine bark, and compost from green waste; CB/CBC + M1/M2—combined biofilter with CB/CBC bed and M1/M2 membrane.

| Configuration          | CB      | CB + M1 | CB + M2 | Ambient Air (CB) | CBC       | CBC + M1 | CBC + M2 |
|------------------------|---------|---------|---------|-----------------|-----------|----------|----------|
| CB + M1                | 0.00 (10) |         |         |                 |           |          |          |
| CB + M2                | 0.00 (22) | 0.24 (1.4) |         |                 |           |          |          |
| Ambient air (CB)       | 0.02 (5.8) | 0.04 (4.1) | 0.18 (1.8) |                 |           |          |          |
| CBC                    | 0.51 (0.4) |         |         |                 |           |          |          |
| CBC + M1               |         | 0.17 (1.9) |         | 0.00 (26) |           |          |          |
| CBC + M2               |         | 0.44 (0.6) |         | 0.00 (42) | 0.00 (10) |          | 0.00 (9.4) |
| Ambient air (CBC)      | 0.03 (4.6) |         |         | 0.00 (48) | 0.00 (10) | 0.00 (9.4) |          |

Table 4. Heatmap showing results of the Mood’s median test (p-values and χ² in parenthesis), performed to test the null hypothesis that the medians of two series of fungal concentration in treated gases/ambient air were identical. For the detailed legend, see Table 3.

| Configuration          | CB      | CB + M1 | CB + M2 | Ambient Air (CB) | CBC       | CBC + M1 | CBC + M2 |
|------------------------|---------|---------|---------|-----------------|-----------|----------|----------|
| CB + M1                | 0.00 (32) |         |         |                 |           |          |          |
| CB + M2                | 0.00 (>>4) |         |         |                 |           |          |          |
| Ambient air (CB)       | 0.00 (29) |         | 0.06 (3.6) |                 |           |          |          |
| CBC                    | 0.00 (27) | 0.04 (4.1) | 0.63 (0.2) |                 |           |          |          |
| CBC + M1               |         | 0.00 (31) |         | 0.00 (>>4) |           |          |          |
| CBC + M2               |         | 0.90 (0.01) | 0.00 (>>4) |                 |           |          |          |
| Ambient air (CBC)      | 0.00 (>>4) |         |         | 0.00 (12) | 0.03 (4.8) | 0.00 (23) |          |

Differences in bioaerosol emission between various biofilter beds, treating waste gases from biowaste composting processes, were also observed by Schlegelmilch et al. [36]. They showed that the biological systems designed for odour control are able to successfully reduce bioaerosol emissions, but the final result depended on the system configuration and operation. The number of bacteria in treated gases varied from $1.0 \times 10^3$ cfu/m$^3$ (coconut fiber) to $4.0 \times 10^7$ cfu/m$^3$ (coke-compost mixture) and the biofilter itself could act both as a source for microbial emissions originating from the filter bed and as an emission reduction point. Moreover, the composition of microbial population in treated gases was changing while passing the treatment system and the tested biofilters were able to retain potentially pathogenic microorganisms. Ferguson et al. [37] showed that biofilters filled with hardwood and western red cedar chips can be effectively used to remove (up to 92 and 95%, respectively) methicillin-resistant *Staphylococcus aureus* (MRSA) from the waste gas of a swine building.

Esquivel-Gonzalez et al. [38] investigated the emission of bioaerosols from biofilters during the treatment of toluene vapours. They proved that bioaerosol emission depended on the bed material—perlite generated a lower bioaerosol emission (up to $7 \times 10^7$ cells/m$^3$) and was more efficient in waste gas treatment (removal of toluene vapours up to 60%), whereas only 40% of pollutants were eliminated from the gases by a biofilter packed with tezontle, and the bioaerosol emission was as high as $1.3 \times 10^8$ cells/m$^3$. On the other hand, Frederickson et al. [39] showed that biofilters can be quite efficient in reducing bioaerosols emission, but concentrations of bacteria and fungi in the treated gases were highly dependent on the type of biofilter bed. The authors compared the performance of two biofilters filled with pine chips and wood chips, which were applied to treat waste gases from a composting facility. Despite the fairly effective removal of fungi, including
Aspergillus fumigatus moulds, the content of all bacteria and Gram-negative bacteria was still high at $10^4$ and $10^3$ cfu/m$^3$, respectively.

Vyskocil et al. [27] carried out an extensive research using both culture-dependent and molecular biology analyses to track changes in microbial concentrations and populations both captured and emitted by the percolating biofilter treating air from a swine fattening-finishing room. Results showed a reduction by 14.4% of culturable bacteria. The qPCR analysis showed a 75.0% decrease of the total number of bacteria, including reduction of coliphages (25.6%), Enterococcus (76.1%), and Escherichia coli (40.9%).

In this study, two membranes (M1 and M2) were tested as a second stage of the waste gas treatment to check if the microbial emission from both materials (CB and CBC), which were used as a biofilter bed, could be reduced. The modification of a conventional biofilter to a combined biofilter resulted in a significant decrease of bacterial emission for both membranes, as shown by the results of the Mood’s median test (Table 3). The average reduction of the bacterial emission was 76% for CB material and 74% for CBC (calculation for the median) when the M1 membrane was applied. Similar effects were observed for the combined biofilter with the M2 membrane. Reductions of bacterial emissions were 78% and 74% for CB and CBC biofilter beds, respectively (calculation for the median). The Mood’s median test showed no statistically significant differences between the concentration of bacteria in treated gases when M1 and M2 membranes were used in combination with the same biofilter bed (CB or CBC) and when the same membrane (M1 or M2) was used with different biofilter beds (CB and CBC). However, it is also worth mentioning that despite the use of membranes as the second stage of waste gas treatment, the total number of bacteria in the treated gases in all tested biofilter configurations was still twice as high as in the ambient air.

The high improvement in biofiltration efficiency was also observed in the case of fungi when using any of the tested membranes in combination with the CB material (Figure 4). The effect of reduction of fungi emission was similar for M1 and M2 membranes (97 and 95% as calculated for medians, respectively). There were no statistically significant differences between the total number of fungi in treated gases, as demonstrated by the Mood’s median test (Table 4). A slightly lower reduction of fungal emission (80%) was observed in the case of CBC material with the M1 membrane as the second stage of the treatment. The descriptive statistics of the data and the results of the Mood’s median test indicated that the content of fungi in the gases treated by the combined filter with the CBC bed and M1 membrane was also significantly lower than in the ambient air. However, the concentration of fungi was 20 times higher than in the case of gases treated by the combined biofilter with the same M1 membrane, but with the CB bed (Figure 4).

Surprisingly, the application of M2 membrane with the CBC bed has not resulted in the expected reduction of the fungal emission from the combined biofilter. The wide interquartile range (4000–8580 cfu/m$^3$) and high maximum value of fungal concentration (16,200 cfu/m$^3$) reflect the greater variation of the data than in the case of other tested configurations of the combined biofilter (Figure 4). The median value (5110 cfu/m$^3$) was similar to that obtained for gases treated by the conventional open biofilter without membranes (4520 cfu/m$^3$), and even significantly higher than in the ambient air (1410 cfu/m$^3$), as shown by the Mood’s median test (Table 4). It was also significantly higher than in the case of the combined biofilter with the CB bed and M2 membrane. One possible explanation for the fungal emission from the CBC + M2 combined biofilter may be the effect of fungi colonizing the outer surface of M2 membrane and, in the last stage of the experiment with the CBC bed, this thicker membrane became an additional emitter of fungi. It can be concluded that the expected effectiveness in fungal elimination from waste gases by the M2 membrane is achieved only in the first period of its use, when the membrane is not colonized by fungi. However, the negative effect of the decrease in efficiency with the time of use was definitely smaller for the thinner M1 membrane. The emission of fungi was successfully reduced throughout the whole period of testing this membrane, with both CB and CBC beds in the combined biofilter.
Some literature data indicate that membranes may be used to reduce bioaerosol emissions [40]. Kühner [41] used semi-permeable membranes to optimize open window composting processes proving that membrane covers were very effective in reducing bioaerosol emissions from the composting piles, regardless of the microbial species. However, the application of membranes as a second stage of waste gas treatment is a novel concept and to the best of our knowledge it has never been used to reduce bioaerosol emissions from biofilters.

The research described in this paper was a part of a larger scientific project. Its main goal was to determine the feasibility of using a combined biofilter with a membrane to reduce the odour nuisance of waste gases from the food industry. Lelicińska-Serafin et al. [30] and Rolewicz-Kalińska et al. [31] showed that the VOCs removal efficiency from waste gases in the combined biofilters ranged from 88 to 99% depending on the type of material of the biofilter bed and membrane. The application of membranes improved the efficiency of waste gas treatment in all the analysed cases by 7–9%. The treatment efficiency was more stable when the CBC material was used as the biofilter bed and the highest effectiveness (96–99%) was obtained by combining the CBC material with the M1 membrane. The authors concluded that the selection of material for the biofilter bed should be based on the parameters that are important for the biofiltration process—with a particular consideration for the specific surface, which plays a substantial role in the sorption process. The choice of the membrane should be determined by its permeability (to ensure the effective treatment) and the values of flow resistance (to eliminate the risk of gas leakage without the treatment). Our study shows that in addition to the above aspects, the ability to reduce the emission of microorganisms from the biofilter bed and resistance to colonization by fungi are also important factors in the selection of membranes. However, further research is needed to thoroughly investigate the possible colonization of membranes and to explain the environmental factors favouring this process, as well as the susceptibility of the membrane fabric to colonization by fungi.

4. Conclusions

A conventional open biofilter (without membranes), which is used to treat waste process gases from the food industry, is an emitter of both bacteria and fungi. The content of microorganisms in the treated gas was significantly higher than in the waste gas from the production, regardless of the tested biofilter bed (stumpwood chips and pine bark or stumpwood chips, pine bark, and compost), the latter being an exceptional source of fungal bioaerosol.

The use of a membrane as a second stage of treatment in a combined biofilter significantly improves the microbiological indicators of the treated gas emitted from the biofilter bed. The combined biofilter reduces the risk of emission of potential pathogens entering the ambient air, which may pose a threat to human health and the environment. However, membranes need to be microbiologically monitored on a regular basis in order to avoid a situation when they are colonized with moulds and become an additional emitter of fungi in the system.

Author Contributions: Conceptualization, A.T. and A.M.; methodology, A.T. and A.M.; validation, M.Z.-R.; investigation, A.T. and A.M.; resources, A.T., A.M., and M.Z.-R.; data curation, A.M.; writing—original draft preparation, A.T. and A.M.; writing—review and editing, A.M. and M.Z.-R.; visualization, A.M.; supervision, M.Z.-R.; project administration, A.T.; funding acquisition, A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Regional Development Fund operated by the National Centre for Research and Development under the Smart Growth Operational Programme 2014–2020 under the priority axis IV: “Increasing the scientific and research potential” in the Measure 4.1. “R&D activity”, Sub-measure 4.1.2 “Regional science and research agendas” in the frame of Project Contract No. POIR.04.01.02-00-0019/16; url: https://biozin.wordpress.com/, accessed on 23 May 2021.
26. Chmielowiec-Korzeniowska, A.; Tymczyna, L.; Skórka, C.; Sitkowska, J.; Cholewa, G.; Dutkiewicz, J. Efficacy of a novel biofilter in hatchery sanitation: I. Removal of airborne bacteria, dust and endotoxin. *Ann. Agric. Environ. Med.* 2007, 14, 141–150. [PubMed]

27. Vyskocil, J.M.; Létourneau, V.; Girard, M.; Lévesque, A.; Duchaine, C. Reduction of bioaerosols emitted from a swine confinement building by a percolating biofilter during a 10-month period. *Atmosphere* 2019, 10, 525. [CrossRef]

28. Jeong, S.; Naidu, G.; Leiknes, T.O.; Vigneswaran, S. Comprehensive Membrane Science and Engineering. In *Membrane Biofouling: Biofouling Assessment and Reduction Strategies in Seawater Reverse Osmosis Desalination*, 2nd ed.; Drioli, E., Giorno, L., Fontanovana, E., Eds.; Elsevier: Oxford, UK; Waltham, MA, USA, 2017; pp. 48–71.

29. Klein, G.M.; Meier, J.; Kottke, V. Fouling in membrane apparatus: The mechanisms of particle deposition. *Food Bioprod. Process.* 1999, 77, 119–126. [CrossRef]

30. Lelińska-Serafin, K.; Rolewicz-Kaliriska, A.; Manczarski, P. VOC removal performance of a joint process coupling biofiltration and membrane-filtration treating food industry waste gas. *Int. J. Environ. Res. Public Health* 2019, 16, 141–150. [CrossRef]

31. Rolewicz-Kaliriska, A.; Lelińska-Serafin, K.; Manczarski, P. Volatile organic compounds, ammonia and hydrogen sulphide removal using a two-stage membrane biofiltration process. *Chem. Eng. Res. Des.* 2021, 165, 69–80. [CrossRef]

32. Mirskaya, E.; Agranovski, I.E. Sources and mechanisms of bioaerosol generation in occupational environments. *Crit. Rev. Microbiol.* 2018, 44, 739–758. [CrossRef] [PubMed]

33. Lutgring, K.R.; Linton, R.H.; Zimmerman, N.J.; Peugh, M.; Heber, A.J. Distribution and quantification of bioaerosols in poultry-slaughtering plant. *J. Food Protect.* 1997, 60, 804–810. [CrossRef] [PubMed]

34. Millner, P.D. Bioaerosols associated with animal production operations. *Bioresour. Technol.* 2009, 100, 5379–5385. [CrossRef] [PubMed]

35. Lues, J.F.R.; Theron, M.M.; Venter, P.; Rasephei, M.H.R. Microbial composition in bioaerosols of a high-throughput chicken-slaughtering facility. *Poultry Sci.* 2007, 86, 142–149. [CrossRef] [PubMed]

36. Schlegelmilch, M.; Herold, T.; Streese, J.; Hensel, A.; Stegmann, R. The potential to reduce emissions of airborne microorganisms by means of biological waste gas treatment systems. *Waste Manag.* 2005, 25, 955–964. [CrossRef]

37. Ferguson, D.D.; Smith, T.C.; Donham, K.J.; Hoff, S.J. The efficiency of biofilters at mitigating airborne MRSA from a swine nursery. *J. Agric. Saf. Health* 2015, 21, 217–227.

38. Esquivel-Gonzalez, S.; Aizpuru, A.; Patrón-Soberano, A.; Arriaga, S. Characterization of bioaerosol emissions from two biofilters during treatment of toluene vapours using epifluorescence microscopy. *Int. Biodeterior. Biodegrad.* 2017, 123, 78–86. [CrossRef]

39. Frederickson, J.; Boardman, C.P.; Gladding, T.L.; Simpson, A.E.; Howell, G.; Sgouridis, F. Evidence: Biofilter Performance and Operation as Related to Commercial Composting; Environment Agency: Bristol, UK, 2013.

40. Kummer, V.; Thiel, W.R. Bioaerosols–sources and control measures. *Int. J. Hyg. Environ. Health* 2008, 211, 299–307. [CrossRef]

41. Kühner, M. Kompostierung Unter Semipermeablen Membranen; Stuttgarter Berichte zur Abfallwirtschaft 78, Bielefeld, Schmidt, 2001. Available online: https://katalog.ub-tu-braunschweig.de/vufind/Search/Results?lookfor=%223-503-05788-9%22&type=ISN&sort=year (accessed on 23 May 2021).