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The potential of a new air cleaner to reduce airborne microorganisms in pig house air: preliminary results

Die potenzielle Entkeimung von Schweinestallluft mit einem neuen Luftreinigungssystem: erste Untersuchungsergebnisse

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Summary

There is a need for technical solutions to reduce the concentrations of bioaerosols in the air and in the exhaust air of livestock buildings. A prototype of an air washer combined with a UV-irradiation system was positioned in a commercial pig fattening unit to test its efficiency of reducing culturable airborne microorganisms. No significant reduction in airborne bacteria and fungi was observed when untreated air passed through the device. However, when the air washer or the UV-irradiation system was activated, the concentrations of mesophilic aerobic bacteria, methicillin resistant *Staphylococcus aureus* and mesophilic aerotolerant cocci were reduced significantly ($p < 0.01$). Washing the air reduced bacteria by 84 to 96% and the relative reduction due to UV-irradiation ranged between 55 and 90%. The highest relative reduction in airborne bacteria (90 to 99%) was detected when the air washer and the UV-irradiation systems were in simultaneous operation. The concentration of total airborne fungi was reduced significantly ($p < 0.05$) only when the air was washed and UV-irradiated. Although these preliminary results provided significant and comprehensible findings, long-term studies are required to assess the efficiency of the device in more detail. The combination of air washing and UV-irradiation seem to be a useful technique for abating airborne microorganisms within or emitting from piggery buildings. However, some technical problems remain, such as the deposition of particulate matter on the surface of UV-irradiators and the consumption of fresh water by the air washer. These issues must be resolved before the system may be implemented for general practice.

Keywords: bioaerosols, air washer, UV-irradiation, MRSA, AGI-30 Impinger

Zusammenfassung

Die Entwicklung von technischen Lösungen zur Reduktion von Bioaerosolen innerhalb eines Stalls oder in der Stallabluft gewinnt zunehmend an Bedeutung. In orientierenden Untersuchungen wurde ein Prototyp eines Luftwäschers mit anschließender UV-Einheit hinsichtlich seiner Luftentkeimungseffizienz in einem konventionellen Mastschweinestall getestet. Für die Luftkeimmessungen wurden Impinger vom Typ AGI-30 eingesetzt. Es konnte keine signifikante Abnahme von luftgetragenen Mikroorganismen beobachtet werden, wenn die unbehandelte Stallluft das Gerät passierte. Bei Aktivierung des Wäschers oder der UV-Einheit nahmen die Konzentrationen mesophiler Gesamtbakterien, Methicillin-resistenter *Staphylococcus aureus* und mesophiler aerotoleranter grampositiver Kokken signifikant ($p < 0,01$) ab. Die Wäschereinheit reduzierte die Bakterienkonzentrationen um 84–96 %, während die UV-Strahlung Reduktionen von 55–90 % erreichte. Bei gleichzeitigem Betrieb des Luftwäschers und der UV-Strahlung wurden relativ gesehen die höchsten Konzentrationsabnahmen (90–99 %) luftgetragener Bakterien beobachtet. Nur bei dieser Einstellung wurde ebenfalls eine signifikante Reduktion ($p < 0,05$) luftgetragener Pilze gemessen. Diese ersten

Ergebnisse zeigen, dass die Kombination von Luftwäscher und UV-Strahlung eine wirkungsvolle Maßnahme zur Entkeimung der Stallluft darstellen kann. Allerdings sind weitere Untersuchungen notwendig, um das System im Dauerbetrieb und bei unterschiedlichen Einstellungen, wie z. B. verschiedenen Volumenströmen, auf die effiziente Entfernung von Luftverunreinigungen hin zu prüfen. Technische Probleme, wie beispielsweise Staubablagerungen auf UV-Strahlern und ein derzeit noch deutlich zu hoher Wasserverbrauch, müssten vor einem kommerziellen Einsatz überwunden werden.

Schlüsselwörter: Bioaerosole, Luftwäscher, UV-Strahlung, MRSA, AGI-30-Impinger

Introduction, Material and Methods

The air in pig fattening units contains large amounts of bioaerosols, such as bacteria, fungi and endotoxins (Seedorf et al., 1998; Friese et al., 2012). Such bioaerosols are cause for threefold concern. First, high concentrations of bioaerosols may impair the respiratory health of animals (Bækbo and Nielsen, 1988; Robertson et al., 1990). Second, the health of animal caretakers may be compromised by these air pollutants. Third, harmful microorganisms may be transmitted via the exhaust air to neighbouring animal houses and residential dwellings, and could potentially cause infections to other animals or adverse health effects in humans (Omland, 2002; Millner, 2009; Dee et al., 2010).

Implementation of air treatment techniques on farms, which are primarily designed to reduce dust, ammonia and other odorants from the exhaust air of animal houses, is a possible option to prevent harmful bioaerosol emissions.

Martens et al. (2001) reported that biofilters connected to the outlets of piggery buildings reduced the number of airborne culturable bacteria by 70–95%. However, the reduction rate of different filters may vary substantially. For example, Tymczyna et al. (2011) showed that the efficiency of a biofilter strongly depends on the packing material. The reduction efficiency of two different package materials in the same biofilter differed by about 46% when mean bacterial counts were compared. Other air treatment techniques, such as acid scrubbers or biotrickling filters, may also be applied to efficiently reduce emissions from animal houses (Melse et al., 2009). Interestingly, some reports observed that end-of-pipe systems using biofiltration may also serve as bioaerosol emission sources (Seedorf and Hartung, 1999; Martens et al., 2001). Furthermore, bioscrubbers may be difficult to control, and the reduction of gases and aerosols may fail when the process within the bioreactor is disturbed (Melse et al., 2009). Hence, the development of abatement techniques that do not involve a biological step may become more important. Simple systems available in the market include water sprinkling systems or the use of biological additives in the air of pig barns. For example, Kim et al. (2006) measured mean reduction rates of about 53% and 51% for total bacteria and total fungi, respectively, in a confined swine house. Another technical solution could be the application of air washers equipped with ultraviolet (UV) irradiation systems, which are already used to kill bacteria and viruses in the air conditioning systems of office buildings, business premises and factories. Such systems are not commonly used in livestock production. Theoretically, these systems could be installed as end-of-pipe systems or as indoor systems. In addition to reducing emissions, the latter application might also contribute to improving animal health. Thus, a prototype consisting of an air washer and a UV-irradiation unit was designed by a German company, and tested in a commercial pig fattening unit. Total mesophilic aerobic bacteria, mesophilic aerotolerant cocci, methicillin resistant *Staphylococcus aureus* (MRSA) and total fungi (moulds and yeasts) were selected as airborne indicator organisms to assess the microbial reduction potential of this air cleaner.

The air cleaner (prototype) was developed by Hölscher & Leuschner Comp., Germany. The system consists of a controllable fan, an air washer (spraying 4.5 l H_2O per minute via three nozzles) followed by a droplet interceptor, a UV-irradiation unit with 16 UV-C lamps (TUV 30 W, UV-Technik Wümbach, Germany) and a second droplet interceptor, which acts as a radiation shield to avoid the release of harmful UV irradiation (Fig. 1). The height and depth dimensions of the device (0.68 m x 0.68 m) are similar to quadratic air ducts installed in several piggeries in Germany.

For the measurements, the device was placed horizontally at a height of 1.2 m above the floor level in the centre of an animal house containing 624 fattening pigs housed in 48 pens on a slatted floor. The air flow through the system was adjusted to approximately 6992 $m^3 \times h^{-1}$. The flow rate was determined according to the Verein Deutscher Ingenieure (VDI) guideline 4257 Part 1 (2010) by using a Testo 405-V1 anemometer (Testo AG, Germany).

Sampling was conducted on the 8th, 9th and 10th week of one fattening period. Two samplings (sampling day

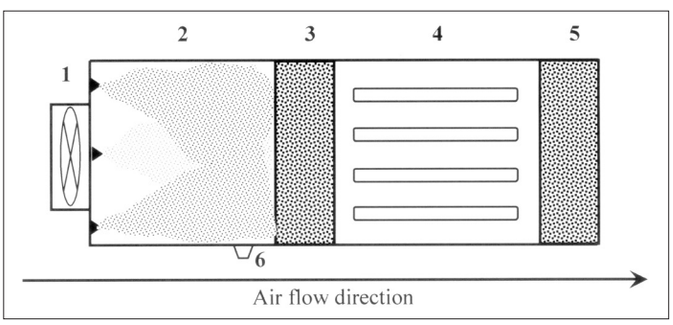

FIGURE 1: *Air cleaning device built from a fan [1], an air washing unit [2], a droplet interceptor [3], a UV-unit [4], a radiation shield [5] and a drain [6]. The device dimensions are: length = 2.6 m, width = 0.68 m and depth = 0.68 m.*

TABLE 1: *The concentrations and mean reduction of airborne microorganisms at the inlet and outlet of an air cleaning device tested at different device settings*

Air treatment (Sampling day)	Detected microorganisms	cfu/m³ inlet $\bar{x} \pm SD$	cfu/m³ outlet $\bar{x} \pm SD$	Reduction of $\bar{x}$ [%]
Washer + UV-C (1)	Mesophilic bacteria	366481 ± 109249	33034 ± 12208	91
	Aerotolerant cocci	178465 ± 60790	11686 ± 3691	93
	MRSA	781 ± 599	34 ± 30	96
	Fungi	1250 ± 938	481 ± 843	61
Washer + UV-C (1)	Mesophilic bacteria	280583 ± 149879	18862 ± 11756	93
	Aerotolerant cocci	104703 ± 22801	7877 ± 4008	92
	MRSA	906 ± 1207	18 ± 21	98
	Fungi	1163 ± 431	889 ± 770	24
Washer + UV-C (2)	Mesophilic bacteria	368841± 181917	9359 ± 4442	97
	Aerotolerant cocci	152140 ± 110236	3326 ± 92	98
	MRSA	687 ± 15	< 4	> 99
	Fungi	3848 ± 2413	272 ± 384	93
Washer + UV-C (3)	Mesophilic bacteria	314309 ± 66199	12214 ± 2042	96
	Aerotolerant cocci	82390 ± 9405	3970 ± 646	95
	MRSA	738 ± 453	4 ± 6	99
	Fungi	3011 ± 3455	347 ± 600	88
Washer + UV-C (4)	Mesophilic bacteria	23583 ± 4421	972 ± 891	96
	Aerotolerant cocci	7868 ± 2112	778 ± 891	90
	MRSA	75 ± 121	< 4	> 95
	Fungi	359 ± 311	389 ± 337	no reduction
Washer only (2)	Mesophilic bacteria	269275 ± 32959	25623 ± 2284	90
	Aerotolerant cocci	114348 ± 46557	4335 ± 2843	96
	MRSA	506 ± 153	41 ± 13	92
	Fungi	1033 ± 315	1084 ± 326	no reduction
Washer only (4)	Mesophilic bacteria	301944 ± 14046	43910 ± 17132	85
	Aerotolerant cocci	81946 ± 11177	13015 ± 983	84
	MRSA	372 ± 135	26 ± 5	93
	Fungi	931 ± 656	333 ± 289	64
UV-C only (2)	Mesophilic bacteria	102424 ± 8058	22753 ± 8148	78
	Aerotolerant cocci	41671 ± 5158	4565 ± 3377	89
	MRSA	261 ± 165	29 ± 25	89
	Fungi	4590 ± 1824	1541 ± 1240	66
UV-C only (4)	Mesophilic bacteria	241331 ± 133334	89269 ± 11067	63
	Aerotolerant cocci	43972 ± 21096	19888 ± 4580	55
	MRSA	280 ± 118	29 ± 11	90
	Fungi	1054 ± 25	321 ± 278	69
No treatment (3)	Mesophilic bacteria	333267 ± 96646	349594 ± 47984	no reduction
	Aerotolerant cocci	114236 ± 92872	79171 ± 16330	21
	MRSA	627 ± 596	324 ± 317	48
	Fungi	1983 ± 628	3011 ± 3455	no reduction
No treatment (3)	Mesophilic bacteria	336859 ± 71247	120353 ± 26752	64
	Aerotolerant cocci	79352 ± 19376	25010 ± 6476	68
	MRSA	375 ± 353	215 ±160	53
	Fungi	1128 ± 726	160 ± 278	86
No treatment (4)	Mesophilic bacteria	357500 ± 130338	261875 ± 3248	27
	Aerotolerant cocci	57259 ± 7605	78543 ± 25600	no reduction
	MRSA	289 ± 125	255 ± 160	12
	Fungi	903 ± 313	1418 ± 1060	no reduction

1 and 2) were conducted in the 8th week, one in the 9th (sampling day 3) and one (sampling day 4) in the 10th week.

Airborne bacteria and fungi were sampled simultaneously at the air inlet and outlet of the device by using three AGI-30 impingers (Ace Glass Inc., Vineland, N.J.) on each side. Impingers were positioned on either side of the centre of the air flow, at a distance of 0.5 m from the device. The air velocity at the sampling point behind the outlet was 4.2 m/s, which allowed air sampling to be almost isokinetic (VDI guideline 4252 Part 3, 2008). Samples were taken at different device settings listed in Table 1. The sampling time was 20 minutes. The air flow through the impingers (12.5 l min-1) was controlled before and after the end of the sampling time with a flow meter 044-14G from Analyt-MTC (Germany). One impinger sample from the outlet on the second sampling day was excluded because of inconsistent air flow. The device was initiated 10 min before each sampling event. If UV-light was used, the lamps were wiped clean with a cloth before the device was initiated, to avoid contamination by dust particles deposited during previous trials influencing the results.

Airborne mesophilic bacteria and airborne MRSA were quantified, as described by Schulz and Hartung (2009). To define the number of aerotolerant cocci, 0.1 ml of an impinger solution and a tenfold dilution were plated on Azide blood agar base supplemented with 5% sheep blood (Oxoid, Germany). The plates were incubated at 36°C for 48 h in an atmosphere with 95% air and 5% CO_2. Subsequently, typical colonies of suspected aerotolerant cocci were counted. Overall, 29 of these colonies were selected at random (from 15 different impinger samples at the inlet and from 14 different impinger samples at the outlet) and were analysed using microscopy, the catalase test and the Gram reaction. Gram positive and catalase negative cocci were subcultured and identified by the Rapid-Strep32-Test of bioMérieux (bioMérieux, France). The sample size was sufficient to detect bacteria other than aerotolerant cocci, with a prevalence above 10% at a given probability of 95% (Martin et al., 1987). Moulds and yeasts were quantified by plating out 0.5 ml aliquots of each impinger solution on DG-18 agar (Oxoid, Germany). The plates were incubated at 25°C, and the colonies were counted after five and seven days. The maximum number of counted colonies was used for the calculations. The number of airborne colony forming units per m^3 (cfu × m^{-3}) was calculated using the equation of Lin et al. (1999).

Statistical differences between the number of microorganisms measured at the inlet and the outlet of the air cleaning device were calculated using the U-test (Mann and Whitney, 1947). The NPAR1WAY procedure of the SAS software, version 9.3 (SAS Institute Inc., USA), was used to perform the test.

Results and Discussion

The mean concentrations and standard deviations (SD) of twelve samplings are summarized in Table 1. When untreated air passed through the device, no significant reduction in bacteria or fungi was

detected (see also Tab. 2). On four different sampling days, a clear reduction of the number of airborne bacteria was observed in air that passed through the device when it was washed and UV irradiated. The reduction rate in the arithmetic mean ($\bar{x}$) of the different bacteria groups ranged from 90% (aerotolerant cocci) to 99% (MRSA). For each of the three different bacteria groups, a highly significant reduction was recorded (Tab. 2). The reduction of fungi was also significant (Tab. 2). However, on one sampling day no difference was detected in the number of fungi at the device inlet and outlet.

By only washing the air, a significant reduction was measured for different bacteria groups, which was calculated from six samples at the inlet and six samples at the outlet. The mean reduction of bacteria groups ranged from 84% to 96%. From the same samples, no significant difference was observed for fungi (Tab. 2).

When air was treated with just UV irradiation, there was a significant reduction in bacteria. However the reduction was less effective when compared to the mean reductions of the other treatments. Furthermore, comparison of six samples from non-irradiated and irradiated air showed no significant effect on fungi.

All 29 colonies selected from different samples and grown in an enriched CO_2 atmosphere were identified as aerotolerant Gram positive and catalase negative cocci. Identification by the Rapid-Strep32-Test resulted in 19 records of *Aerococcus viridans* (ten from inlet, nine from outlet), six records of *Streptococcus acidominimus* (two from inlet, four from outlet), two records of *Leuconostoc* spp. (from inlet) and one record of *Gemella morbillorum* (from inlet). One culture could not be identified to the species or genus level.

The results of this preliminary study show that treating pig house air with the new air cleaner device clearly reduced the amount of different bacteria groups present in the air. Although the number of measurements was limited, because the aim of this preliminary investigation was to determine the effectiveness of the design, the device inlet and outlet readings were significantly different. The counts of airborne mesophilic bacteria and MRSA in untreated air were within the range of other studies (Seedorf et al., 1998; Friese et al., 2012). It is well known that these concentrations could be strongly influenced by factors such as the housing system, the stocking density, the animal activity, the air exchange rates, the management and the sampling method (Thorne et al., 1992; Seedorf and Hartung, 2002). An example for such a strong influence is the low concentration of microorganisms at the inlet of the device on sampling day four (Tab. 1, washer and UV-C in operation). In this situation measurements were conducted after feeding while most of the pigs lay on the floor and showed only a low activity. Probably this had a strong impact on the number of airborne bacteria within the barn. However, no decrease of the reduction efficiency was observed when air with more than a tenfold higher bacteria concentration was treated on sampling days one, two and three.

The volume of air that passed through the air cleaner was comparable to the median summer air rate of the air ducts installed at the investigated animal house. Under these conditions, the most efficient treatment was a combination of air washing and UV irradiation, which reduced the amount of airborne bacteria by 90 to 99%. Washing the air alone also seems to have the potential to reduce airborne bacteria by about one log step. This is probably because most airborne bacteria in pig houses are attached to dust particles or occur in large aggregates (Clauss et al., 2011). It is therefore easier to wash out these airborne bacteria. Particles load with bacteria may have also been impacted within the droplet interceptor before being drained off with the waste water. Another factor that may have reduced the number of bacteria could have been the adsorption of particles to the wet droplet interceptor. This might explain the reduction in mesophilic bacteria, aerotolerant cocci and MRSA during the second experiment with untreated air on sampling day three, which was conducted after the air washer had been active. In general, air washing did not reduce the total fungi count significantly. Most of the cultivated fungi were moulds, which build spores with hydrophobic surface layers (Bayry et al., 2012). It is possible that these spores do not bind to water droplets. Furthermore, unlike bacteria, spores may have been actively released into the animal house from their sporangia; hence they were not necessarily attached to other dust particles. This would explain the less effective and partly insignificant reduction in the amount of fungi by the device. In general, mould spores are relatively resistant to UV irradiation, and the UV intensity within the tested device is not sufficient to kill fungal spores (Kowalski et al., 2002). However, the calculated average UV-C intensity within the UV-unit (2 mW sec/cm^2) in combination with the air velocity should have been sufficient to kill airborne bacteria and yeasts (Kowalski et al., 2002). The lethal

TABLE 2: *Significant differences in microbial concentrations at the inlet and outlet of the air cleaner. Results of the Mann-Whitney U-test*

Air treatment	Sample size n_1, n_2*	Microorganisms	p (two-sided)
Washer + UV-C	15, 14	Mesophilic bacteria	< 0.001
		Aerotolerant cocci	< 0.001
		MRSA	< 0.001
		Fungi	< 0.05
Washer only	6, 6	Mesophilic bacteria	< 0.01
		Aerotolerant cocci	< 0.01
		MRSA	< 0.01
		Fungi	> 0.05
UV-C only	6, 6	Mesophilic bacteria	< 0.01
		Aerotolerant cocci	< 0.01
		MRSA	< 0.01
		Fungi	> 0.05
No treatment	9, 9	Mesophilic bacteria	> 0.05
		Aerotolerant cocci	> 0.05
		MRSA	> 0.05
		Fungi	> 0.05

* n_1 number of impinger samples taken at the inlet; n_2 number of impinger samples taken at the outlet

effect on yeasts probably explains the lower amount of fungi that were counted, because, at times, we observed higher proportions of yeast colonies on the DG-18 agar. To verify this observation, we recommend the need to distinguish between the number of yeast and moulds in future experiments. The lethal effect on bacteria obviously contributed to the total decline in all bacteria groups when the air washer was combined with UV irradiation. However, more investigations are necessary to demonstrate the significant effects of different air treatments. For example future experiments should evaluate the effects at different air flows and higher UV-C intensities as well as detect particle sizes, to assess the effect of the impaction processes within the droplet interceptor.

In addition to the total mesophilic bacteria and MRSA, aerotolerat cocci were used as indicator bacteria to determine the reduction efficiency. Identification of aerotolerant gram positive and catalase negative cocci confirmed that 90% or more of the suspected colonies belong to this group. Interestingly, the most frequently identified species was *Aerococcus viridans*, which is associated with arthritis, pneumonia and meningitis in pigs (Martin et al., 2007). In addition, the identified species *Streptococci acidominimus* belong to the a-haemolytic streptococci which are frequently associated with pneumonia in pigs (Palzer et al., 2008). It was not the priority objective to detect infectious agents but considering that most of the identified aerotolerant cocci and MRSA are potentially harmful bacteria we conclude that the tested air cleaner has the potential to reduce airborne infectious agents efficiently. It would be interesting to observe whether the continuous operation of one or more of these air cleaners in a pig barn could reduce the number of airborne microorganisms in the whole air space of a building, and whether such a reduction would improve the health of pigs. In this context accompanying factors, such as the climate and dust, as well as the endotoxin and ammonia concentrations, should also be assessed within the barn, because these factors can be associated with respiratory diseases in pigs (Stärk, 1999). Another option would be the use of the air cleaner as an end-of-pipe technique. Recent research has shown that pathogens and opportunistic microorganisms emitted from pig barns may be transmitted to adjacent barns, or may contaminate the vicinity of barns (Dee et al., 2010; Schulz et al., 2012). Hence, this technique may be useful in reducing or even eliminating the transmission of harmful microorganisms. However, some technical problems must be resolved before such a system can be installed on commercial farms. First, the water consumption of the device is too high compared to other systems (Hahne, 2006). For instance, tests should be conducted to determine whether airborne microorganisms may be sufficiently reduced by circulating water that is renewed at regular intervals. In addition, the use of other materials (i. e. pumps, nozzles) might also help reduce water consumption of the air cleaner. Second, it was observed that particulate matter deposited on the quartz tubes of the UV irradiators, even after operating the systems with the air washer unit. Hence, if the tubes were not regularly cleaned, this would reduce the level of UV irradiation if the system was operated over longer time periods. Therefore, the quartz tubes should be periodically (automatically) cleaned to maintain UV intensity. Third, the investment and operational costs must be calculated per animal house to assess how economic the air cleaner is after optimizing the system. Without an acceptable cost-performance ratio it will be difficult to introduce the system into the market.

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