atmosphere

MDPI

Article

Development of a Novel Bioaerosol Chamber to Determine Survival Rates of Airborne Staphylococci

Marcus Clauß *, Stefan Linke, Christin Tautz and Sarah Bromann

Thuenen Institute of Agricultural Technology, Bundesallee 47, D-38116 Braunschweig, Germany; stefan.linke@thuenen.de (S.L.); christin.tautz@thuenen.de (C.T.); sarah.bromann@thuenen.de (S.B.)
* Correspondence: marcus.clauss@thuenen.de

Abstract: Large amounts of microorganisms are emitted from animal houses into the environment via exhaust air. To assess the potential risks, the spread of microorganisms can be simulated with computer models. Such modeling usually does not take into account die-off rates, since there are hardly any reliable data so far on how long microorganisms can survive in outdoor air. Previous studies were conducted almost exclusively in closed chambers and usually only took into account the influence of individual environmental factors such as temperature or humidity. Therefore, a novel bioaerosol chamber was developed to quantify the survival rates of Staphylococci specific to livestock under outdoor air conditions. For evaluation, the survival rates of *Staphylococcus xylosus* were determined as a function of temperature, relative humidity, ozone concentration, and global radiation. Survival rates decreased with increasing temperature, decreasing relative humidity, increasing global radiation intensity, and increasing ozone concentration. At 12 min in the airborne state, die-off rates of more than 90% were observed, especially at high global radiation levels > 400 W/m^2. The novel bioaerosol chamber enabled the investigation of the survival rates of airborne microorganisms over a certain period of time in a quasi-closed system and yet under real outdoor air conditions.

Keywords: bioaerosols; tenacity; *Staphylococcus xylosus*

Citation: Clauß, M.; Linke, S.; Tautz, C.; Bromann, S. Development of a Novel Bioaerosol Chamber to Determine Survival Rates of Airborne Staphylococci. *Atmosphere* **2022**, *13*, 869. https://doi.org/10.3390/atmos13060869

Academic Editors: Cinzia Perrino and Salvatore Romano

Received: 30 April 2022
Accepted: 23 May 2022
Published: 25 May 2022

1. Introduction

The air in animal housings contains large amounts of microorganisms that are emitted into the environment via exhaust air. These are suspected to negatively affect the health of humans and animals in the vicinity of livestock facilities [1]. Therefore, it is of great interest to know the dispersal distance of airborne microorganisms from livestock operations to assess potential risks. Characteristic for emissions from animal husbandry are bacteria from the family *Staphylococcaceae*. Bacteria of this family are often found in large numbers in the air of animal houses. Their detection is usually carried out by culture-based methods, i.e., the microorganisms are collected from the air, then grown on nutrient plates, and the grown colonies are counted as colony-forming units (CFU). Typical concentrations of Staphylococci are, for example, 10,000 CFU/m^3 in cattle farming, 100,000 CFU/m^3 in pig farming, and 1,000,000 CFU/m^3 in poultry farming [2]. In addition to the comparatively high concentrations in poultry farming, about 20% of the Staphylococci belong to Risk Group 2 and are therefore a health concern [3]. High concentrations of Staphylococci are also found in the emissions from poultry farms. Typical emission factors, i.e., the number of CFU that escapes into the environment via exhaust air per livestock unit (LSU) and second, are, for example, about 1,000,000 CFU/LSU*s as an annual average, with sometimes large differences between day and night of up to three powers of ten [4].

Computer models can predict the dispersion of Staphylococci and calculate the concentrations in the surroundings of livestock facilities [5]. For realistic dispersion modeling of microorganisms, further input parameters are necessary in addition to the prevailing meteorological conditions, orography, and emission factor. Here, particle size is particularly

relevant, since the aerodynamic diameter of the particles determines their sedimentation and thus how far they are carried via the air. For Staphylococci from poultry farming, it is known that they are more likely to be found on large particles. In emissions from chicken and turkey farms, only about 15% of Staphylococci were found in the dust fraction PM2.5, 20% in the dust fraction PM10, and 65% were larger than PM10 [2,6].

Another important factor that has been largely ignored in dispersion modeling is the survival rate of Staphylococci in the air. The survival of bacteria in the air depends on many factors. The most important is the tenacity of the individual species as well as the meteorological conditions of temperature, humidity, solar radiation, and the so-called "open air factor" (OAF). The latter describes a biocidal effect of outdoor air on microorganisms that is mainly caused by ozone–olefin reaction products [7]. A large number of studies have been conducted on the survival of microorganisms in the air over the last 50 years, but they show clear limitations. The main problem is that the studies have almost exclusively been conducted in closed systems [8]. The substances causing the OAF are extremely reactive and degraded within a few minutes on the surfaces in the test chambers used [9]. In addition, usually only the effects of temperature and relative humidity on the survival rates of airborne microorganisms have been studied. Data that also consider global radiation and OAF are virtually non-existent. However, it is known from individual studies that the survival rates of airborne microorganisms are also strongly influenced by OAF and global radiation [10–12]. If the survival rates of airborne bacteria are to be studied under outdoor air conditions, these factors must also be taken into account. However, the existing closed test systems are not suitable for this purpose. Therefore, a novel bioaerosol chamber was developed to study the survival rates of airborne microorganisms under real outdoor air conditions. The system was designed to maintain the OAF in the chamber as much as possible and also to allow the natural sunlight spectrum to enter the chamber without loss. The basis for the concept of a novel bioaerosol chamber according to Clauß et al. (2016) [13] was provided by the investigations of Hood (1971) [14] that a twelvefold air exchange per hour would be necessary to maintain the OAF in a closed system. From this, a concept was derived for a bioaerosol chamber consisting of a translucent foil balloon. This is continuously inflated by supplying fresh outside air in such a way that the chamber volume increases exponentially. The continuous supply of fresh air largely maintains the OAF inside the system. To evaluate the chamber, the meteorological factors affecting the survival rates of the model organism *Staphylococcus xylosus* in the air were determined. The data will be used in the future to help improve dispersal calculations for microorganisms.

2. Materials and Methods

The schematic structure of the test system is shown in Figure 1. The bioaerosol chamber consists of a 200 µm thick, UV-A and UV-B transparent greenhouse film (Lumisol clear AF, BayWa AG, München, Germany). In preliminary tests, a chamber with a volume of 8 m^3 and a test time of 14 min proved to be the most practicable. The foil chamber was made under the cleanest and least germy conditions possible before each trial. First, the greenhouse film was cut to the desired size of two pieces of 3.25 m × 3.70 m. Then, the two pieces of film were placed on top of each other, and the edges were taped with vapor barrier tape (Ursa Adhesive Tape SECO KP, GLOBUS hitseller GmbH, Völklingen, Germany) to form a closed-film balloon. Self-made eyelets were attached to the four corners of the foil chamber with pieces of the vapor barrier tape and a washer in each case, in order to attach them to the corners of the measuring platform with tensioning rubbers during later operation of the chamber. In this way, the chamber was secured against gusts of wind. Three ports made of PVC and fitted with silicone ring seals were connected to the foil chamber. A central port with a diameter of 100 mm, positioned centrally at the bottom, was used to fill and empty the chamber. The supply of fresh outside air from below also reduced premature sedimentation of the particles. The other two smaller ports were used to accommodate the sensor for temperature and relative humidity and as a passage for a PTFE tube for ozone measurement. The film was cut at the appropriate locations to accommodate

the ports. The three ports were disinfected with Bacillol® AF before installation and then covered with aluminum foil. The still-airless foil balloon with the installed ports was finally folded up to an approximately 1 m × 1 m square and was thus available for the upcoming experiments.

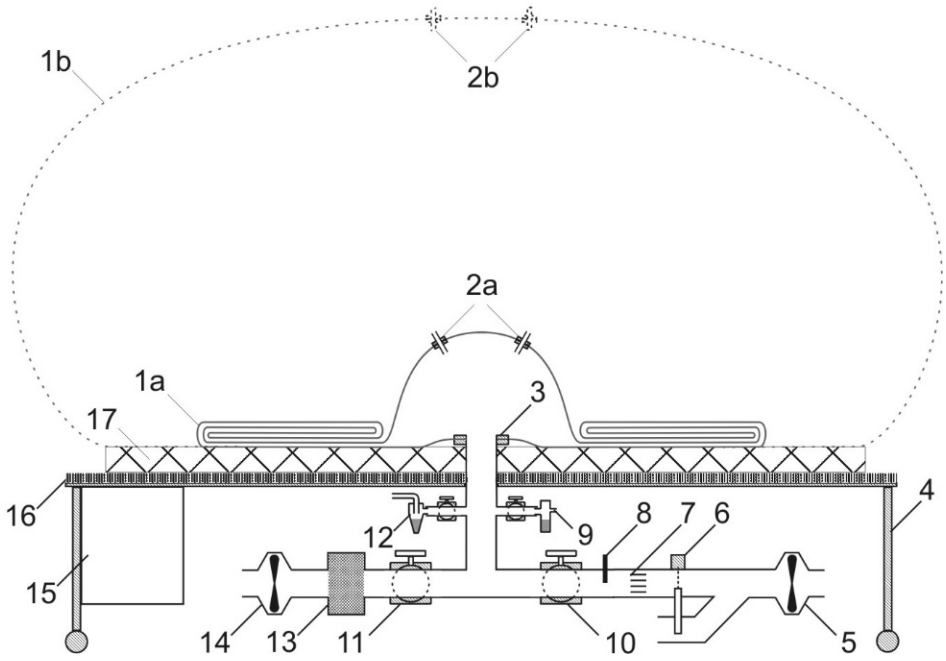

Figure 1. (1) Foil chamber (a: at the beginning of an experiment; b: fully inflated at the end of an experiment), (2) ports for different sensors (a: at the beginning of an experiment; b: fully inflated at the end of an experiment), (3) port for filling and emptying the chamber, (4) mobile measuring platform, (5) fan for filling, (6) stepper motor driven slide valve, (7) flow straightener, (8) hot wire anemometer, (9) bioaerosol generator, (10) ball valve, (11) ball valve 2, (12) bioaerosol collector, (13) HEPA filter (H14), (14) fan for draining, (15) measuring and control equipment, (16) artificial grass, and (17) spacer (wire mesh). (Figure created by author.)

The foil chamber was installed on a mobile rectangular measuring platform with the dimensions 3 m × 4 m edge length and 1 m height. This was made of lightweight aluminum profiles (item Industrietechnik GmbH, Solingen, Germany). To enable the chamber to be moved outdoors for the tests, it rested on 4 antistatic heavy-duty rollers. Between the chamber and the measuring platform there was artificial grass over the entire surface so that the light coming from the sun was not reflected from the aluminum surface of the platform from below into the chamber but was absorbed and scattered as naturally as possible, imitating a green surface. Between the artificial turf and the underside of the foil chamber, a wire cage about 10 cm high acted as a spacer to ensure under-ventilation of the foil chamber and minimize heating from below.

A PVC pressure line system was housed underneath the measuring platform. All associated connections were either glued airtight or are designed with O-ring seals made of silicone or Viton®. The system included two fans, pipe and filter systems for filling and emptying the chamber, and the measurement and control technology. Additionally connected to the piping system were a bioaerosol generator and a bioaerosol collector. Several ball valves were used to seal everything airtight. Additional safety was provided by a system of HEPA filters that prevented microorganisms from escaping into the environment. No filter was installed at the air inlet, since in preliminary tests various filter materials had lowered the ozone concentrations in the chamber to below 5% compared to the outside air.

The heart of the fresh air supply for filling the bioaerosol chamber was a mechanical pull gate valve in a Y-pipe section. In the zero position, one branch was closed, while the other was 100% open. A stepper motor was used to open the closed section while the open

section closed. The upstream fan always ran at full power, so that the supply of fresh air was controlled only by the position of the pull gate. This ensured a uniformly increasing course of the quantity of fresh air supplied, which was largely independent of changes in the back pressure, e.g., due to unfolding of the film chamber. To double the chamber volume per time unit, the fresh air supply was controlled by means of a PD control loop. The controller was implemented in software on an ATMega128 microcontroller (Atmel company, San José, USA). The flow velocity of the supply air (actual value) was measured via a hot-wire anemometer (HVAC miniature flow transmitter EE671-V3xDSx 0.2–10 m/s, E+E ELEKTRONIK GmbH, Bad Homburg, Germany) and compared with a setpoint. The setpoint was obtained from Equation (1) for a doubling of the air volume every 2.5 min.

$$w = 0.0212 \times e^{1.386 \times t} \quad (1)$$

w: setpoint.

t: time from start of the experiment (s).

An actuator value was calculated from the (control) difference. This served as the input parameter for the stepper motor that drives the slide valve. Every three seconds, the current-manipulated variable, setpoint, and actual value were recorded. Additionally measured and recorded every 3 s were humidity and temperature (humidity/temperature OEM sensor with Modbus interface type: EE071-HTPBAN1, E+E ELEKTRONIK GmbH, Bad Homburg, Germany), air pressure (TruStability® Board Mount Pressure Sensor HSCSRNN1.6BA2A3 0. 1600 mBar, Honeywell Deutschland Holding GmbH, Offenbach, Germany), differential pressure (TruStability® Board Mount Pressure Sensor HSCDRRN010ND3A3 ± 16 mBar, Honeywell Deutschland Holding GmbH, Offenbach, Germany), global radiation (Pyranometer CMP3, Adolf Thies GmbH & Co. KG, Göttingen, Germany), and ozone (ozone monitor APOA-370 with measuring point switch, HORIBA Europe GmbH, Oberursel, Germany).

Humidity (%), temperature (°C), and pressure (mbar) were measured both inside the chamber and outside. Additionally, the pumped volume (L) was given so that the chamber volume can be read. The global radiation (W/m^2) was measured only outside the chamber. The global radiation value inside the chamber was calculated by multiplying the value measured outside by 0.835 (transmission of the film). For safety, the pressure inside the chamber was continuously monitored. As soon as it exceeded a value of 0.8 mbar, an acoustic signal sounded as a warning, and from a pressure of 1.2 mbar, the supply of ambient air was automatically switched off.

Staphylococcus xylosus DSM 20266 were used as test bioaerosol. The strain was obtained from the DSMZ Braunschweig, Germany, and a permanent culture was established. For each experiment, some of the permanent culture was taken with a sterile inoculating loop, spread evenly on a plate of Casein Soy Peptone (CASO) Agar, and incubated at 37 °C for approximately 43 h. For the experiments in the bioaerosol chamber, the suspension of a pure culture of *S. xylosus* was prepared with a target concentration of 1×10^9 CFU/mL to 5×10^9 CFU/mL. For this purpose, 2 inoculation loops full of *S. xylosus* colonies were taken from the CASO agar plates and resuspended (vortexed) in 1.3 mL of sterile 0.65% NaCl solution in a 1.5 mL sterile reaction tube (Eppendorf). In total, 100 µL of this suspension was diluted (vortexed) in another tube with 0.9 mL of sterile 0.65% NaCl solution. A photospectrometer was used to check against pure 0.65% NaCl at a wavelength of 600 nm to see if the dilution of the suspension had an absorbance between 5.0–5.7. If lower or higher, either bacteria or sterile 0.65% NaCl solution was added to the initial suspension, and the 1:9 dilution was remeasured until the transmittance was in the target range. When the transmission was suitable, 1 mL of the initial suspension (not the dilution) was given into the bioaerosol generator. To determine the percentage of culturable cells of *S. xylosus* in the initial suspension, a dilution series was set up in sterile 0.65% NaCl and dilution levels 10^{-6}, 10^{-7}, and 10^{-8} (final dilution) were plated out in two parallels on CASO agar. Total cell counts were determined according to VDI 4253 sheet 4 [15] by transferring the liquid from the 10^{-3} dilution step to a 15 mL Falcon® centrifuge tube containing 1 mL of

37% formaldehyde solution, incubating for 2 h at room temperature, and then storing at 4 °C in the refrigerator until evaluation. With this procedure, on average approximately 35% (+/−20%) of the counted cells were culturable.

A medical inhalation device (Pari Sinus inhalation device with Pari LC Sprint Sinus nebulizer, blue nozzle, PARI GmbH, Starnberg, Germany) was used as bioaerosol generator. A volume of 1 mL of a freshly prepared suspension of *S. xylosus* was pipetted into the nebulizer and was thus available for the experiments.

A wet cyclone made of stainless steel was used as a bioaerosol sampler in all tests, which was structurally based on the collection head of the commercially available Coriolis®µ bioaerosol collector (Bertin Corp., Rockville, MD, USA). The air inlet was modified to allow an airtight connection to the chamber piping system. The original plastic cones of the Coriolis®µ were used as collection vessels. The exhaust air from the wet cyclone was filtered through a HEPA filter (H14). A "high volume" sample gas pump was used to generate the required volume flow of 300 L/min (MCZ Umwelttechnik, Bad Nauheim, Germany).

Before each experiment, the aluminum foil was removed from the ports of the foil balloon. The middle port was connected to the pipe system, the temperature/humidity sensor was installed in the two upper ports, and the PTFE line to the ozone device was inserted. All ball valves were closed during this process. The bioaerosol chamber was then moved outside under the open sky. To avoid premature heating of the chamber in the sun, it was covered with an opaque fabric tarpaulin until shortly before the start of the experiments. The bioaerosol generator was connected and filled with the test bioaerosol. The cyclone collector vessel filled with 15 mL of sterile 0.65% NaCl solution was installed.

Each experiment started with commissioning of the measurement equipment. On the piping system, the ball valve for fresh air supply was opened and all other ball valves remained closed. With a Labview interface installed on a notebook, the control program of the chamber was started, and all data were recorded from that point on. The stepper motor-driven pull valve moved to the start position, and the fan for the supply of outside air started until full speed was reached. Then, the process of inflating the chamber starts as the pull gate opens continuously with time the upper tube connecting to the chamber and at the same time closes the lower tube leading to the outside so that the amount of fresh air supplied to the chamber increases continuously. The fresh air supply was controlled in such a way that the chamber volume doubled per unit time (every 2.5 min) and thus increased exponentially in a uniform manner. After 1 min at a chamber volume of approximately 100 L, the ball valve on the bioaerosol generator was opened and the generator started. The test microorganisms were then aerosolized into the chamber for one minute. The bioaerosol generator was then turned off again. The test microorganisms now remained in the airborne state for approximately 14 min as the chamber volume steadily increased, resulting in a steady dilution in the air. As soon as the chamber was fully inflated and the differential pressure between the inside of the chamber and the outside rose above 0.8 mbar, an acoustic signal sounded and the ball valve for the supply of fresh air was manually closed. In addition, the supply air was automatically shut off at a pressure of 1.2 mbar.

This was followed immediately by the emptying of the chamber. For this purpose, the ball valve of the air outlet was opened and the fan for emptying the chamber was started. The chamber air thus passed through a HEPA filter to the outside. After about 10 s, the ball valve in front of the bioaerosol collector was opened and the collector was started. A sample was taken at 300 L/min for one minute from the air flow of the chamber air. The sample was processed immediately afterwards in the laboratory. After complete evacuation, the chamber was folded, disconnected from the piping system, the ports were dismantled in a tub containing disinfectant solution, and the resulting holes in the film were sealed airtight with vapor barrier tape. The chamber was then autoclaved (121 °C, 1 bar overpressure) and sent for recycling. The ports and the tubing system were disinfected with surface disinfectant Bacillol®AF and dried and were thus available for the next experiments.

Figure 2 shows the schematic diagram of an experiment with the 8 m^3 bioaerosol chamber as a function of chamber volume and time. After outside air flowed into the chamber for 1 min, the bacteria were aerosolized for 1 min. Inside the chamber, they are then in the airborne state for about 12 min. After the maximum chamber volume is reached, a part of the bacteria is collected again from the air with Coriolis® cyclone collector and so they are available for further evaluation.

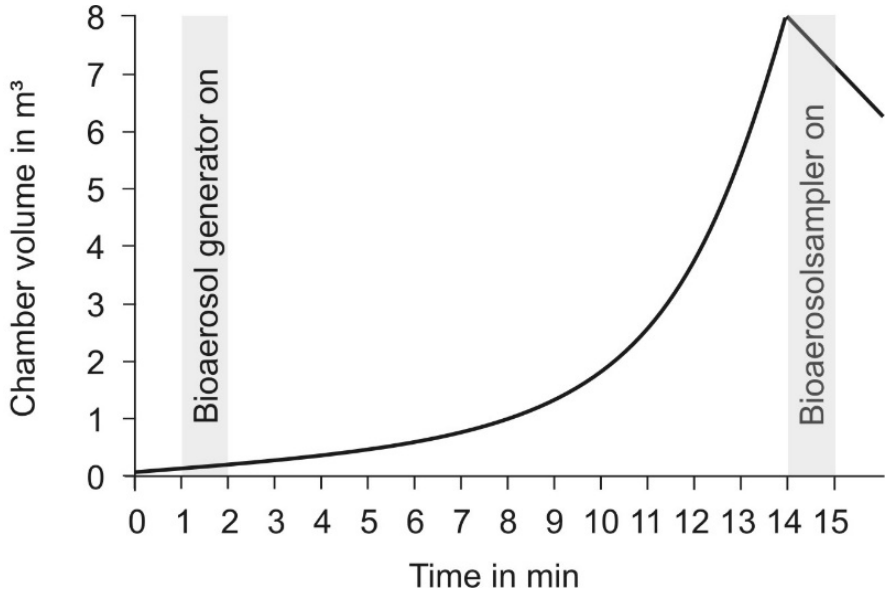

Figure 2. Schematic representation of the test procedure as a function of the chamber volume and the test time.

To calculate the number of microorganisms injected into the chamber, the residual amount of suspension in the nebulizer was determined gravimetrically and subtracted from the original amount. Since some of the 15 mL of collection fluid initially introduced was lost during sampling, the residual amount after collection was also determined gravimetrically.

For the determination of the cultivable fraction of Staphylococci in the samples, after preparation of a dilution series in sterile 0.65% NaCl solution, dilution levels 10^0 to 10^{-5} (final dilution) were plated out in two parallels on CASO agar. The inoculated plates were then incubated for 24 h at 37 °C, and the colonies grown were counted.

The remaining suspension from the Coriolis collection tube was transferred to a Falcon®Tube (15 mL) containing 1.3 mL of 37% formaldehyde solution, shaken well, fixed for 2 h at room temperature, and then stored in a refrigerator at 4 °C. The total cell count (TCC) was determined using the DAPI method according to VDI 4253 sheet 4 [15]. For the subsequent evaluation, the ratio of culturable cells to total cell count was determined in each case.

Excel was used for the evaluation of the data. The calculation of the respective survival rates (SR) as a function of the meteorological parameters is obtained from Equation (2). In each experiment, the ratio of total cell count (TCCT) and culturable cells (CFUT) was first calculated in the test bioaerosol (initial suspension or stable dust). After the experiments, the ratio of total cell count (TCCS) and culturable cells (CFUS) was also determined in the sample. Subsequently, the ratio in the test aerosol was divided by the ratio in the sample and multiplied by 100 to arrive at the survival rate in %.

$$SR\ (\%) = (CFUT\ /TCCT)/(CFUS/TCCS) \times 100 \quad (2)$$

CFUT: cultivable cells in the test bioaerosol.
TCCT: total cell count in the test bioaerosol.
CFUS: cultivable cells in the sample.

TCCS: total cell count in the sample.

3. Results

3.1. Conditions within the Chamber

The aim was to perform the experiments under realistic outdoor air conditions corresponding to the climatic conditions prevailing in our latitudes. In general, the novel bioaerosol chamber was very well-suited to study the survival rates of airborne microorganisms under realistic outdoor air conditions. Figure 3 shows the curves of temperature and relative humidity in the bioaerosol chamber during the experiments.

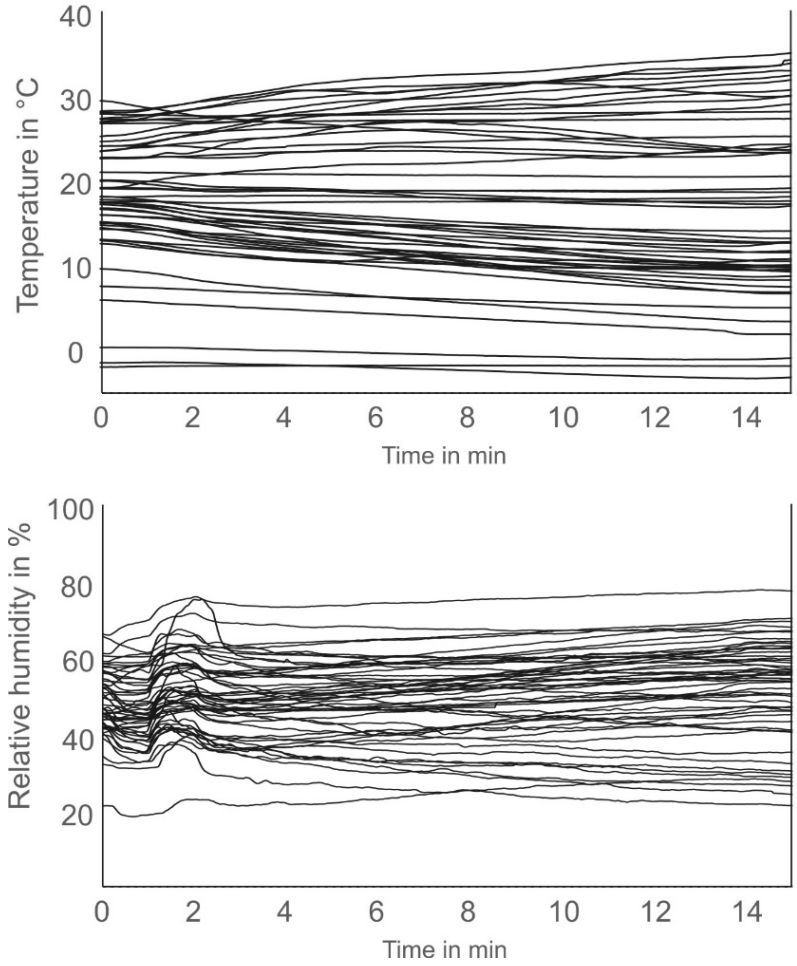

Figure 3. Course of temperature and relative humidity in the bioaerosol chamber during the 54 experiments.

Overall, the experiments took place at temperatures in the chamber ranging from approx. −3 °C to approx. 35 °C. Temperatures inside the bioaerosol chamber were always slightly higher than outside. The temperature differences in the chamber between the beginning and end of a 12 min experiment were on average 4.2 °C to a maximum of 11.4 °C. For comparison in the outdoor air, temperature differences between the beginning and end of an experiment were on average only 0.7 °C to a maximum of 4.8 °C. At medium outside temperatures, the temperature curves are relatively constant, and the temperature difference between the beginning and end of the tests is small. However, at low outdoor temperatures, the temperatures in the chamber continued to drop somewhat during the tests. In contrast, at high outdoor temperatures and strong solar radiation, the temperatures in the chamber increased significantly during the experiments.

Relative humidities within the chamber were approximately between 20% and 80% and were relatively constant, rising or falling during the tests somewhat depending on the

meteorological conditions in the outside air. Between the beginning and end of an experiment, the differences in the bioaerosol chamber were on average 12.7%. By comparison, the relative humidity in the outside air changed by only 4.2% on average. When the bioaerosols were sprayed into the chamber, there was also a slight increase of approx. 5–10%, but this quickly fell again.

Figure 4 shows the relative radiation intensity of the natural sunlight spectrum in the wavelength range of 320 nm–780 nm compared to the light spectrum in the chamber. The course of the two spectra is approximately the same, but the relative radiation intensity in the chamber was on average 11% lower (4%–16%, depending on the wavelength). Thus, the transmission of the foil for this wavelength range was 89% on average. In the UV range of 320 nm–400 nm, the transmission of the foil was 95%, and in the global radiation range of 310 nm–2800 nm it was 83.5%.

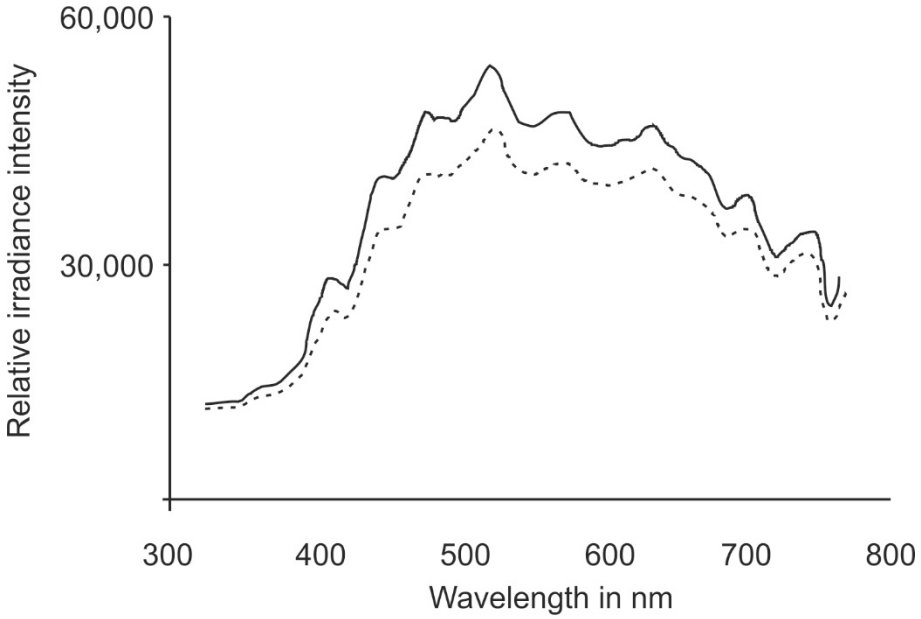

Figure 4. Measured relative irradiance of the natural sunlight spectrum compared to the light spectrum in the chamber.

For the experiments, global irradiance varied greatly throughout the year. The average irradiances during the experiments in the chamber ranged from 4.81 W/m^2 on a dull morning to 978 W/m^2 in summer with full sunlight. There were also frequent strong fluctuations during the individual tests; in an extreme case, a drop from 831 W/m^2 to 35 W/m^2 was measured when a dense cloud moved in front of the sun on an August day.

Overall, relatively low ozone concentrations from 0.002 ppm to a maximum of 0.045 ppm were measured in the outdoor air during the experiments. The measured values were confirmed by comparing the data with a weather station 300 m away of the German Weather Service (DWD). The ozone concentrations in the chamber were significantly lower in each case and between 0.001 ppm and 0.032 ppm. Compared to the outside air, the values in the chamber averaged about 60% despite the continuous supply of fresh air.

Despite the differences between chamber and outdoor air, it was possible to create conditions inside the bioaerosol chamber that approximate real outdoor air conditions and can be considered representative of climatic conditions in the mid-latitudes.

3.2. Microbiological Test

The novel bioaerosol chamber was very well-suited to study the survival rates of airborne microorganisms under realistic outdoor air conditions. During the twelve-minute

experiments, an average of about 80% of the bacterial cells inside the chamber was lost due to sedimentation or wall effects. However, enough were still present for evaluation.

Table 1 shows the mean values of temperature, relative humidity, ozone concentration, and global radiation for the 52 individual experiments in the chamber and the survival rates determined. The calculated survival rates range from 0.003% to 437.6%. Overall, about 1/3 of the survival rates are above 100%.

Table 1. Mean values of temperature, relative humidity, ozone concentration, and global radiation for the 52 individual experiments in the chamber and the survival rates determined.

Number of Experiment	Temperature (°C)	Relative Humidity (%)	Ozone Concentration (ppm)	Global Radiation (W/m^2)	Survival Rate (%)
1	25.2	59	0.018	173	30.7
2	25.6	45	0.019	290	5.2
3	31.6	34	0.020	512	0.2
4	19.3	62	0.013	65	19.8
5	24.1	62	0.009	160	2.1
6	18.8	61	0.013	72	166.6
7	15.2	77	0.003	51	20.9
8	18.4	50	0.012	208	11.1
9	15.6	60	0.005	90	27.9
10	14.7	54	0.002	63	78.9
11	11.8	51	0.008	57	42.2
12	13.2	49	0.009	86	100.5
13	15.0	53	0.006	87	144.4
14	13.8	56	0.011	23	155.7
15	15.4	62	0.007	17	137.7
16	14.2	56	0.019	40	20.2
17	10.6	57	0.010	29	99.1
18	11.5	43	0.005	37	72.9
19	13.4	54	0.012	24	437.6
20	13.1	50	0.017	168	295.4
21	12.1	52	0.022	112	159.6
22	11.6	43	0.017	124	134.9
23	11.1	43	0.024	76	296.1
24	10.9	66	0.016	84	138.1
25	11.8	54	0.018	181	50.5
26	11.2	53	0.023	171	130.9
27	6.5	63	0.012	4	154.4
28	30.4	32	0.026	693	1.8
29	23.2	39	0.038	817	0.1
30	18.0	69	0.016	96	31.0
31	36.7	25	0.016	359	76.3
32	26.1	40	0.022	135	37.8
33	28.9	34	0.022	517	0.2
34	31.3	26	0.026	684	0.003
35	28.6	48	0.016	484	21.8
36	30.7	42	0.018	431	10.1
37	27.5	37	0.022	549	0.2
38	30.1	31	0.027	668	0.004
39	29.5	48	0.071	457	14.4
40	27.6	54	0.026	5	223.6
41	18.6	60	0.016	143	175.9
42	23.6	48	0.019	302	67.1
43	22.6	55	0.011	442	27.9
44	25.3	45	0.017	442	12.2
45	19.4	62	0.009	4	87.8
46	21.2	57	0.008	4	57.7
47	9.9	56	0.001	27	197.6
48	6.7	67	0.001	30	128.2
49	−1.1	61	0.007	61	124.5
50	4.4	49	0.011	88	214.7
51	−1.8	54	0.014	144	14.9
52	0.2	48	0.019	181	50.4

In the following figures, the survival rates are plotted logarithmically against the individual parameters global radiation, temperature, relative humidity, and ozone concentration. Figure 5 shows the survival rates of *S. xylosus* as a function of global radiation. The individual values vary strongly in the range of two powers of ten. Nevertheless, an exponential decrease of the survival rate with increasing intensity of the global radiation can be clearly seen. From 500 W/m^2, at least a reduction of 90% takes place in 12 min. In the range up to 200 W/m^2, survival rates of more than 100% also occur mathematically.

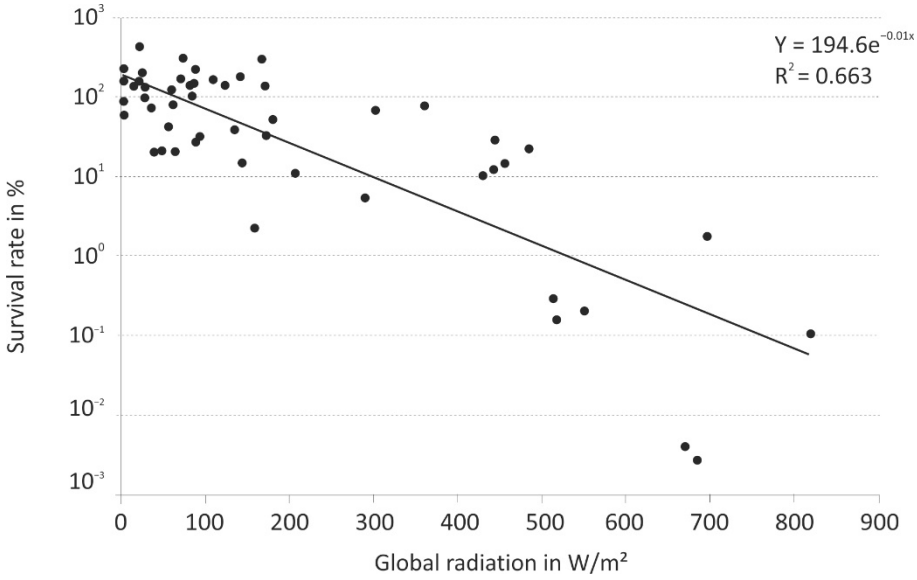

Figure 5. Survival rates of *Staphylococcus xylosus* after 12 min in the airborne state as a function of global radiation (n = 54).

Figure 6 shows the survival rates of *S. xylosus* as a function of temperature and global radiation. Here too, a decrease in survival rate with increasing temperature can be seen, with even larger fluctuation ranges. Especially from 20 °C to 35 °C, the survival rates decrease significantly. If the influence of global radiation is also taken into account, survival rates of less than 10% were only found at high global radiation values of over 400 W/m^2.

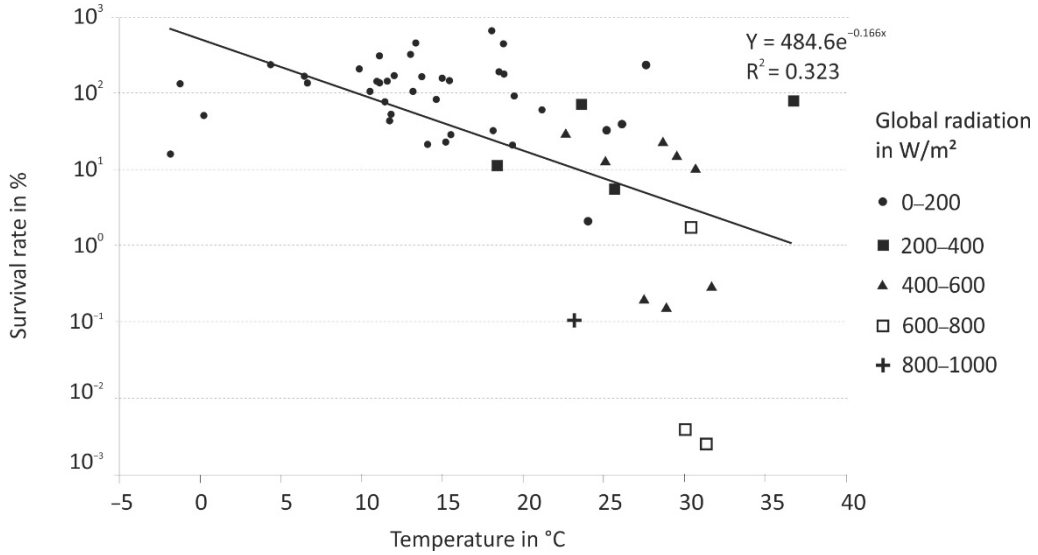

Figure 6. Survival rates of *Staphylococcus xylosus* after 12 min in the airborne state as a function of temperature and the influence of global radiation (n = 54).

Figure 7 shows the survival rates of *S. xylosus* as a function of relative humidity and global radiation. Here, the survival rate decreases especially at low relative humidity of <40%. As with temperature, survival rates < 10% are observed at global radiation levels >400 W/m^2.

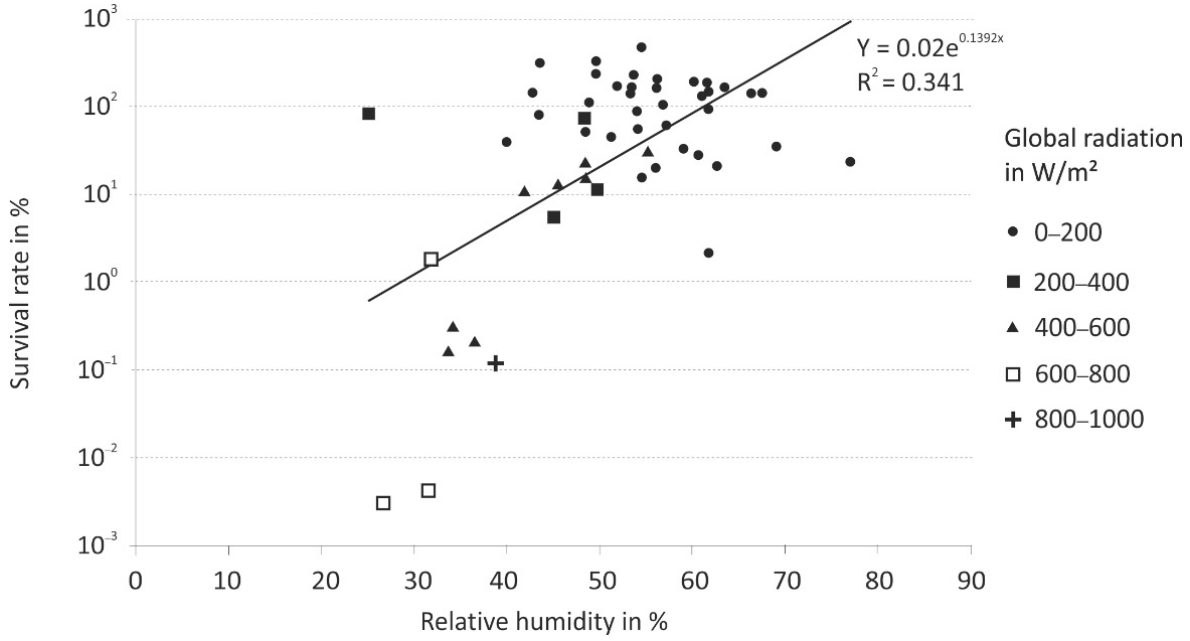

Figure 7. Survival rates of *Staphylococcus xylosus* after 12 min in the airborne state as a function of relative humidity and the influence of global radiation (n = 54).

Figure 8 shows the survival rates of *S. xylosus* as a function of ozone concentration and global radiation. Again, an exponential decrease tends to be seen with even greater variation. Especially above 0.025 ppm, the survival rate is significantly reduced. Again, survival rates of less than 10% occur primarily at global radiation values of >400 W/m^2.

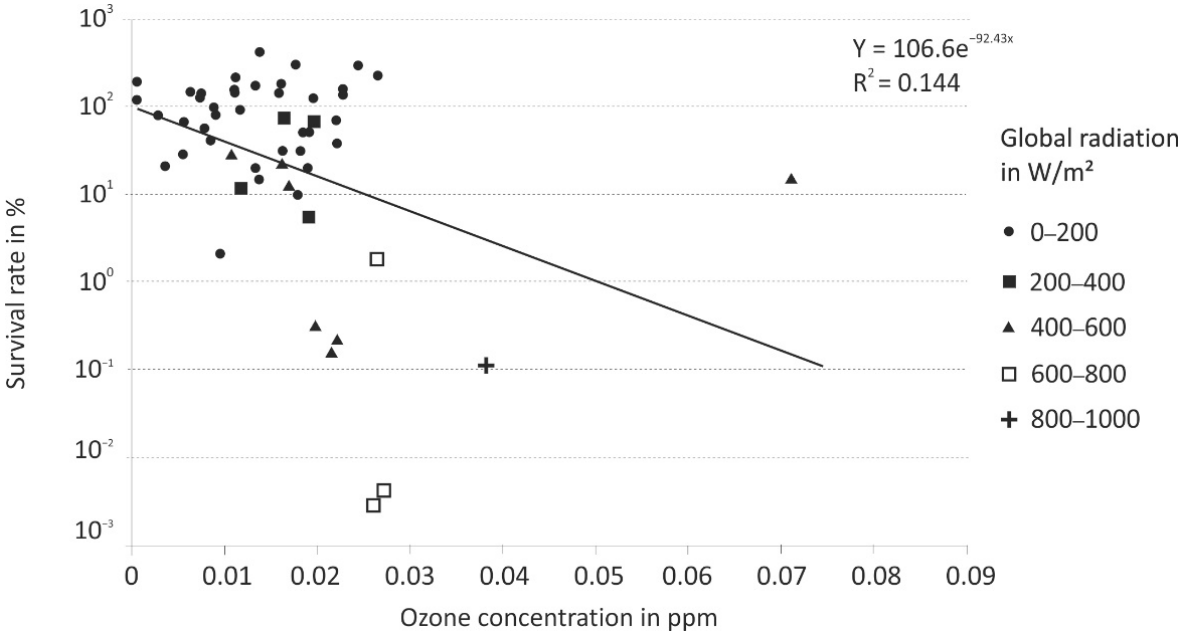

Figure 8. Survival rates of *Staphylococcus xylosus* after 12 min in the airborne state as a function of ozone and the influence of global radiation (n = 54).

In summary, under the given experimental conditions, global irradiance has the greatest influence on the survival rates of *S. xylosus* in the airborne state. At mean irradiances of >400 W/m^2, a reduction in survival rate of more than 90% can be expected in 12 min. For the other parameters, no clear dependence of the survival rates on temperature, relative humidity, and ozone could be determined in this period, also due to the strong scattering of the values, since the global radiation obviously acted synergistically there and had the greater influence.

4. Discussion

The results show a good applicability of the novel bioaerosol chamber, because for the first time all important meteorological factors influencing the survival rates of airborne microorganisms could be considered together. In particular, maintaining the ozone concentration inside the chamber was a challenge. Despite the concentration losses, there was still sufficient ozone, and thus presumably OAF, at 60% inside the chamber compared to the outside air to demonstrate an impact on the survival rates of the test microorganisms in subsequent studies. However, the results also underline the need for a continuous supply of fresh air if the OAF is to be taken into account.

Our results showed relatively large ranges of variation, making it difficult to quantify the effects of individual meteorological parameters. Moreover, about one third of the calculated survival rates were above 100%. One reason for this could be methodological differences in the determination of cell number by DAPI staining and the culture-based determination of CFU. Both methods also have a relatively high variability. The results should therefore be validated in further studies. The limitation of the test time to about 15 min in our 8 m^3 chamber was not a major restriction for our research question. In terms of dispersion modeling, at average wind speeds of 3 m/s, this corresponds to dispersion distances of about 3 km, which is sufficient for evaluating livestock housing in practice. However, our results showed relatively large ranges of variation, making it difficult to quantify the individual effects. One reason is certainly the relatively short time that the bacteria are in the airborne state compared to other studies. Authors such as Dinter and Müller (1988) [16] or Gannon et al. (2007) [17] have had their test microorganisms in static and rotating chambers for several hours or Handley and Webster (1995) [11] with the microthread technique for several days. If longer periods in the airborne state could be studied, perhaps the influence of meteorological conditions on survival rates would be clearer. In larger chambers with a volume of about 50 m^3, test times of up to 30 min were possible in preliminary tests. With longer test times, or longer phases of the airborne state, it may be possible to differentiate the influences of the various factors even better. However, larger chambers then come at the expense of manageability. The system is therefore rather unsuitable for investigating the long-term transport of bioaerosols.

Results from field studies already show that meteorological parameters such as temperature, humidity, or UV radiation negatively affect the survival of airborne bacteria [18–20]. Moreover, in the present study, meteorological parameters have been shown to have a significant impact on the survival rate of airborne bacteria, even during the short experimental period of 12 min. In addition, the dependencies of survival rates described by various authors when studying individual meteorological parameters in closed test chambers were also observed here. For example, that kill rates increase with increasing temperatures [21], low humidities reduce viability [22], or sunlight reduces survival rates [12]. There are also synergistic effects between the parameters. For example, relative humidity affects the germicidal effect of UV radiation [23].

In terms of dispersion calculations, this means that a strong reduction in survival rates of airborne Staphylococci due to weather is to be expected, especially on sunny, warm days. Especially in the case of global radiation, despite the large range of variation of two orders of magnitude, an exponential decrease in survival rate with increasing intensity was clearly evident. Thus, global radiation seems to be the most dominant parameter with respect to its influence on the survival rate of airborne *S. xylosus*. Based on the natural sunlight

spectrum, it is primarily the proportion of UV radiation that has the greatest influence on the survival of bacteria in the air [24]. Here, it is also to be considered that the portion of the UV radiation of the global radiation increases, e.g., with the height over N. N. or with lower latitudes. Therefore, the influence of global radiation would probably be even greater there than in the present experiments. In contrast, it has to be in mind that the test bacteria were mainly single cells in the air. Bacteria in real dust may be better protected from solar radiation than when they were exposed to radiation as single cells comparatively unprotected, as in the case of *S. xylosus*.

5. Conclusions

The novel bioaerosol chamber has proven itself in practice and enabled the investigation of the persistence of airborne microorganisms over a certain period of time in a quasi-closed system and yet under real outdoor air conditions. With regard to dispersion modeling, further studies are needed, e.g., with Staphylococci in real dust, to demonstrate the influence of global radiation on their survival rates. The novel bioaerosol chamber described in this work is suitable for these experiments. Quantification of the influences of global radiation, temperature, relative humidity, and ozone on survival rates of airborne Staphylococci, as required to improve dispersion models, is possible in principle based on the data collected in this study. However, further experiments should be conducted beforehand to validate the results due to the high variability.

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

Funding: This research was funded by the Saxon State Office for Environment, Agriculture and Geology (LfULG).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data of the measurements are included in the final report of the research project "Investigation of the tenacity of airborne microorganisms under outdoor air conditions as real as possible": https://publikationen.sachsen.de/bdb/artikel/39031 (accessed on 30 April 2022).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Douglas, P.; Robertson, S.; Gay, R.; Hansella, A.L.; Gant, T.W. A systematic review of the public health risks of bioaerosols from intensive farming. *Int. J. Hyg. Environ. Health* **2018**, *221*, 134–173. [CrossRef] [PubMed]
2. Clauß, M. Emission of Bioaerosols from Livestock Facilities—Methods and Results from Available Bioaerosol Investigations in and Around Agricultural Livestock Farming. Thünen Working Paper 138a 2020. Available online: https://www.thuenen.de/media/publikationen/thuenen-workingpaper/ThuenenWorkingPaper_138a.pdf (accessed on 30 April 2022).
3. Springorum, A.C.; Clauß, M.; Rieger, M.; Hinz, T.; Hartung, J. Mikrobielle Belastung der Luft in verschiedenen alternativen Haltungsformen für Legehennen. *Gefahrst. Reinhalt. Luft* **2018**, *75*, 331–335. (In German)
4. Clauß, M.; Springorum, A.C. Diurnale Schwankungen der Bioaerosolkonzentrationen in der landwirtschaftlichen Nutztierhaltung—Wie repräsentativ sind ausschließlich tagsüber durchgeführte Messungen? *Gefahrst. Reinhalt. Luft* **2018**, *78*, 353–360. (In German)
5. Van Leuken, J.P.G.; Swart, A.N.; Havelaar, A.H.; Van Pul, A.; Van der Hoek, W.; Heederik, D. Atmospheric dispersion modelling of bioaerosols that are pathogenic to humans and livestock—A review to inform risk assessment studies. *Microb. Risk Anal.* **2016**, *1*, 19–39. [CrossRef] [PubMed]
6. Clauß, M. Particle size distribution of airborne microorganisms in the environment—A review. *Appl. Agric. For. Res.* **2015**, *65*, 77–100.

7. Cox, R.A.; Ammann, M.; Crowley, J.N.; Griffiths, P.T.; Herrmann, H.; Hoffmann, E.H.; Jenkin, M.E.; McNeill, V.F.; Mellouki, A.; Penkett, C.J.; et al. Opinion: The germicidal effect of ambient air (open-air factor) revisited. *Atmos. Chem. Phys.* **2021**, *21*, 13011–13018. [CrossRef]
8. Springorum, A.C.; Clauß, M. Das Überleben von Bakterien im luftgetragenen Zustand—Ein Literaturüberblick zur Tenazität. *Gefahrst. Reinhalt. Luft* **2016**, *76*, 351–358. (In German)
9. Hood, A.M. Open-air factors in enclosed systems. *J. Hyg. Camb.* **1974**, *72*, 53–60. [CrossRef] [PubMed]
10. May, K.R.; Druett, H.A. A microthread technique for studying the viability of microbes in a simulated airborne state. *J. Gen. Microbiol.* **1968**, *51*, 353–367. [CrossRef] [PubMed]
11. Handley, B.A.; Webster, A.J. Some factors affecting the airborne survival of bacteria outdoors. *J. Appl. Bacteriol.* **1995**, *79*, 368–378. [CrossRef] [PubMed]
12. Paez-Rubio, T.; Peccia, J. Estimating Solar and Nonsolar Inactivation Rates of Airborne Bacteria. *J. Environ. Eng.* **2005**, *131*, 512–517. [CrossRef]
13. Clauß, M.; Huf, A.; Springorum, A.C. An Unconventional Bioaerosol Chamber for Tenacity Studies of Airborne Microorganisms under Almost Ambient Air Conditions. In Proceedings of the Bioaerosol Chamber Expert Meeting, Vienna, Austria, 14–15 April 2016.
14. Hood, A.M. An indoor system for the study of biological aerosols in open air conditions. *J. Hyg. Camb.* **1971**, *69*, 607–617. [CrossRef] [PubMed]
15. *VDI 4253/4*; Bioaerosols and Biological Agents—Determination of Total Cell Count by Fluorescence Analysis after Staining with DAPI; Beuth Verlag: Berlin, Germany, 2013.
16. Dinter, P.S.; Müller, W. The tenacity of bacteria in the airborne state. VI. Tenacity of airborne S. senftenberg. *Zbl. Bakt. Mikrobiol. Hyg. B* **1980**, *186*, 278–288.
17. Gannon, B.W.; Hayes, C.M.; Roe, J.M. Survival rate of airborne Mycobacterium bovis. *Res. Vet. Sci.* **2007**, *82*, 169–172. [CrossRef] [PubMed]
18. Harrison, R.M.; Jones, A.M.; Biggins, P.D.E.; Pomeroy, N.; Cox, C.S.; Kidd, S.P.; Hobman, J.L.; Brown, N.L.; Beswick, A. Climate factors influencing bacterial count in background air samples. *Int. J. Biometeorol.* **2005**, *49*, 167–178. [CrossRef] [PubMed]
19. Jones, A.M.; Harrison, R.M. The effects of meteorological factors on atmospheric bioaerosol concentrations—A review. *Sci. Total Environ.* **2004**, *326*, 151–180. [CrossRef] [PubMed]
20. Zhen, Q.; Deng, Y.; Wang, Y.; Wang, X.; Zhang, H.; Sun, X.; Ouyang, Z. Meteorological factors had more impact on airborne bacterial communities than air pollutants. *Sci. Total Environ.* **2017**, *601–602*, 703–712. [CrossRef] [PubMed]
21. Ehrlich, R.; Miller, S.; Walker, R.L. Relationship between Atmospheric Temperature and Survival of Airborne Bacteria. *Appl. Microbiol.* **1970**, *19*, 245–249. [CrossRef]
22. Müller, W.; Gröning, K. Die Tenazität von Bakterien im luftgetragenen Zustand. II. Mitteilung, Experimentelle Untersuchung zur Bestimmung der Absterbekonstante beta für Kokken. *Zbl. Bakt. Mikrobiol. Hyg. B* **1981**, *173*, 180–187. (In German)
23. Kaplan, R.W. Mutation und Tötung durch UV in Bakterien verschiedenen Wassergehalts. *Naturwissenschaften* **1955**, *7*, 184–185. (In German) [CrossRef]
24. Harm, W. *Biological Effects of Ultraviolet Radiation*; Cambridge University Press: Cambridge, UK, 1980.