



Article Distribution Characteristics and Factors Influencing Culturable Bacterial Bioaerosols on a Dairy Farm in Northern China

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Abstract: Studying the distribution characteristics of bioaerosols and their interaction with the environment is crucial for dairy farms. The distribution of aerosols differs in dairy farming from farming of other livestock, and their sensitivity to environmental factors varies across sites. Field experiments were conducted in an intensive commercial dairy farm in Northern China to investigate the horizontal and vertical distribution of culturable bacterial bioaerosols. Concentration levels and particle size ranges were analyzed, and the impact of multiple environmental factors on culturable bacterial bioaerosols was assessed. Significant variations in culturable bacterial bioaerosol concentrations were observed across eight functional zones, ranging from 1.14×10^3 to 7.35×10^3 CFU/m³. Culturable bacterial bioaerosols exhibited consistent carrier distribution patterns across six different size ranges. Vertical analysis revealed significantly higher culturable bacterial bioaerosol concentrations at a 1 m height compared to 4 m (p < 0.05), while similar size distributions were observed at different heights of the same sampling location. The top three environmental factors influencing culturable bacterial bioaerosol concentrations were PM100 concentration, wind direction, and air temperature. This study provides insights into the distribution characteristics of culturable bacterial bioaerosols on dairy farms and their response to environmental factors. The findings serve as a reference for evaluating bioaerosol emissions and establishing daily disinfection management measures on dairy farms.

Keywords: culturable bacterial bioaerosol; farm scale; horizontal distribution; vertical distribution; environmental factors; sensitivity analysis

1. Introduction

China is the third largest dairy country in the world, and the population of dairy cows has grown rapidly in the last few decades [1,2]. By 2021, the share of dairy farming in China with a stock of 100 head or more reached 70%, an increase in 2.8% year-on-year [3]. The rapid growth of dairy farming has also brought about social concern for air pollutants such as bioaerosol. Bioaerosols in animal buildings consist of a complex mixture of organic dust, biologically active components (e.g., endotoxin), and microorganisms (e.g., bacteria, fungi) [4]. The emission and transportation of bioaerosols increase the risk of infection and threaten the health of animals and workers [5]. Therefore, it is important to know the fate of emitted bioaerosols in the surroundings and their dispersion in ambient air.



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Animals themselves and their faeces are the most important sources of bioaerosols in livestock farms [6]. Differing from swine or poultry, cows are typically free-ranging and housed in naturally ventilated buildings with free access to outdoor open lots. More than 50% of the excrement of cows is retained on the solid floor and exposed to the air [7]. This creates the convenience of bioaerosol formation and dispersion. As there is a large airflow rate and an unorganized airflow pattern in naturally ventilated animal buildings, bioaerosols in dairy houses normally show a lower concentration (average of 4.3 log CFU/m³) compared with confined swine or poultry houses [8]. However, this does not mean there is lower emission intensity in dairy houses. Inversely, the pollutants in dairy houses are mostly discharged to the outdoor environment and are more likely to be cross-diffused between different buildings or different functional areas (e.g., office area, feed processing area, animal living area). In scenarios such as dairy farming with open emission sources, obtaining the distribution characteristics and influencing factors of aerosols at the farm level is more crucial than at the barn level, particularly when large side-wall openings are used during summer and transitional seasons (spring, autumn).

Previous studies mainly focused on indoor air characteristics and bioaerosol influencing factors at the livestock- and poultry-house level [9–11]. For example, Li et al. [12] investigated the spatial and temporal distribution of bioaerosols in enclosed layer houses using field measurement. They summarized that bioaerosols in layer houses contain a large number of pathogenic bacteria that may infect poultry and humans, and their concentration is influenced by housing design, feeding patterns, and hygiene conditions. Islam et al. [13] assessed the influence of temperature and relative humidity on bioaerosol counts and airborne bacteria in tied-stall dairy houses. They found more significant positive associations between outdoor environmental temperatures and aerosol numbers rather than indoor temperatures and aerosol numbers. This further indicates the essentiality of investigating the relationship between outdoor environmental factors and bioaerosols on a farm scale to provide precise prevention and control measures for bioaerosols.

In this study, field measurements were conducted in a typical large-scale dairy farm in Northern China to explore the distribution characteristics of culturable bacterial bioaerosols on a farm scale. Impacts of multi-environmental factors on the culturable bacterial bioaerosol concentration and distribution were also analyzed to allow for a more comprehensive assessment of contamination and thus guide accurate biosecurity prevention on dairy farms. This study contributes to the understanding of culturable bacterial bioaerosol transport characteristics in livestock facilities and provides a reference for setting up blocking points for disease prevention and control on dairy farms.

2. Materials and Methods

2.1. Overview of the Experimental Dairy Farm

Field measurements were conducted on a typical commercial dairy farm with a stock quantity of 3000 cows in Beichen District, Tianjin, China (39.25° N, 117.05° E). Cows on the farm are milked three times a day (Starting time: 06:30, 14:00 and 22:00) and fed three times a day (Starting time: 06:30, 14:00 and 17:20). Figure 1 shows the layout of the experimental dairy farm. Defining the area with cow living facilities as production areas, there are two relatively independent production areas on the experimental farm, i.e., production area 1 and production area 2 (Figure 1). The first production area is located southwest of the dairy farm and is 320 m long on north–south side and 200 m long on the east–west side, and it kept about 1430 cows during the field measurements. This production area is equipped with one milking parlor and five dairy barns. The dairy barn is a double-sloped roof structure with a length of 180 m, a width of 30 m and an eave height of 4 m. It is naturally ventilated (combined with cooling fans in summer), has a solid floor, and is equipped with outdoor open lots. The second production area is located southeast of the dairy farm. This area is equipped with one milking parlor and four dairy barns, whose building structure is similar to those in the first production area. Manure in the dairy barn is collected by a suction truck twice a day (Starting time: 06:30 and 14:00) and transported

to the waste management area for further treatment. After dry and wet separation, the dry matters are processed to be bedding materials—they are tilled, turned and dried in the waste management area. The waste management area is located northwest of the dairy farm; it is 110 m long on the north–south side and 140 m long on the east–west side. The whole dairy farm is disinfected by mobile sterilizing vehicles three times a week.





2.2. Field Sampling and Counting

Field measurements were conducted to investigate the distribution characteristics of culturable bacterial bioaerosols on the dairy farm from September to October 2021. Culturable bacterial bioaerosols were sampled in both horizontal (15 October–18 October) and vertical (26 September-10 October) dimensions using the six-stage Anderson samplers (TISCH, TEI Corp., Akron, OH, USA) with disposable nutrient agar plates (every 1000 mL of nutrient agar contains: peptone 10.0 g, beef extract 3.0 g~5.0 g, sodium chloride 5.0 g, agar powder 12.0~14.0 g), and incubated for bacterial colony counting. The microclimate of dairy farm was collected synchronously using a set of environmental monitoring equipment (Figure 1) to record the factors including air temperature (T), relative humidity (RH), wind speed (V), wind direction (WD), total irradiance intensity (GHI), ultraviolet irradiance intensity (UV), suspended particulate matter concentration (PM1, PM2.5, PM10, and PM100), etc. The WD and V were collected continuously through three-dimensional ultrasonic anemometers (Wind master pro, Gill Corp., Learnington, UK) at a height of 10 m every 30 s. The GHI and UV were recorded using a total irradiation meter (MS-40, EKO Corp., Kyoto, Japan) and a UV irradiation meter (CUV5, K&Z Corp., Assen, The Netherlands) at a height of 2 m every 30 s. The PM1, PM2.5, PM10, and PM100 were recorded every 5 min using digital universal particle sensors (PMSX003N, Panteng Technology Co., Beijing, China; SDS198, Nuofang Technology Co., Jinan, China). The spatial variation in the concentrations and carrier size of culturable bacterial bioaerosols were analyzed in both horizontal and vertical dimensions, and the effects of multivariate environmental factors on the distribution of culturable bacterial bioaerosols were assessed on a farm scale.

2.2.1. Horizontal Sampling of Culturable Bacterial Bioaerosols

According to the different functions and potential pollution levels, the dairy farm is divided into different function zones, including the office area (OA), feed processing area (FP), production area (PA), and waste management area (WM). The PA was further divided into dairy barns (DBs), open lots (OPs), interfiled roads (IRs), and milking parlors (MPs) based on the daily production scene. Thus, culturable bacterial bioaerosols were horizontally sampled at background points (BGs) and the seven different points within the OA, FP, WM, DB, OP, IR, and MP sites. During the sampling work, according to the principle of the number of equipment and personnel (eight sets; four people), the distance between sampling points, and similar sanitary conditions, the eight sampling points were divided into two batches (the first batch: WM, OA, FP, IR; the second batch: WM, OA, FP, IR; second batch: WM, OA, FP, IR; second batch: OP, BG, DB, MP). Each batch is operated independently by four sampling personnel, sampling at the same time, and each sampling point is equipped with two instruments. After sampling, we wiped and disinfected the inner surface of the instrument with 75% alcohol, and the sampling personnel went to the second batch of sampling points closest to the sampling point (for example: after WM sampling, the sampling personnel went to the OP sampling point, $OA \rightarrow BG$, $FP \rightarrow DB$, $IR \rightarrow MP$). After checking that the inner surface of the sterilized device is completely dry, they sampled the second batch of sampling points at the same time. At the end of the sampling day, all instruments were centrally autoclaved. The background sampling point was located in the open area southwest of the dairy farm, and the linear distance from the dairy farm was more than 500 m. The sampling points within the site were set up in the center of each functional area as much as possible (Figure 1). The total bacterial and fungal counts during dairy farming were low compared to other livestock species, and the higher totals were found in the middle of the day [14]. In order to prevent the field production activities from affecting the sampling, the field workers' rest time at noon was selected as the period for the testing. Culturable bacterial bioaerosols were sampled at 11:30~12:30 every day over four consecutive days. According to the general principles of particulate bioaerosol sampling and analysis [15], the sampling height was 1.2 m from the ground, and each sample was taken for 5 min. Two replicates were sampled simultaneously at each sampling point, and they were incubated at 37 °C for 48 h. The average count of cultivable bacterial colonies after incubation was used as the foundational data for calculating the aerosol concentration at the corresponding sampling point.

2.2.2. Vertical Sampling of Culturable Bacterial Bioaerosols

To achieve representativeness of sampling, wasted time should be reduced. Considering the intensive labor requirements and limited time budget of farm-scale measurements, the first production area and waste management area were selected as typical areas for the vertical sampling of culturable bacterial bioaerosols. The movement of air gives impetus to the spread of bioaerosols [16]. In summer and transition seasons, the side-wall curtains normally are fully open in dairy barns. Biological aerosols in the house are dispersed through the windows and doors with the airflow. In the experimental farm, the height of the windowsill of dairy barn is 1 m, and its eave height is about 4 m. Thus, culturable bacterial bioaerosols were sampled at the heights of 1 m and 4 m to investigate vertical distribution. The sampling point was set at the midpoint of the boundary between the two surveyed areas (Figure 1). The vertical sampling of culturable bacterial bioaerosols was conducted between approximately 11:30 and 13:00 for 10 days in clear and rain-free weather at 16 sampling points. Similarly, two synchronous replicates were set for each sampling point, and each sample was taken for 5 min.

2.2.3. Incubation and Counting

Samples were stored in an insulated box (0 $^{\circ}$ C~10 $^{\circ}$ C) after collection and transported to laboratory for incubation within 1 h. Colony counting with a counter (Icount-30F, Xunshu Technology Co., Hangzhou, China) was conducted. The correction of colony numbers

and concentration calculations of culturable bacterial bioaerosols were performed using Equation (1) and Equation (2), respectively.

$$Pr = N \times \left(\frac{1}{N} + \frac{1}{N-1} + \dots + \frac{1}{N-r+1}\right)$$
 (1)

where Pr is the number of colonies after correction, CFU/m^3 ; N is the number of sampling holes at each level of the sampler; r is the actual number of colonies, CFU.

$$C = \frac{N_1 + N_2 + N_3 + N_4 + N_5 + N_6}{t \times F} \times 1000$$
(2)

where *C* is the concentration of culturable bacterial bioaerosols, CFU/m^3 ; N_x is the number of colonies after calibration of each sampler stage, x = 1, 2, 3, 4, 5, 6; *t* is the duration of sampling, min; *F* is the sampling flow rate of the sampler, 28.3 L/min.

2.3. Data Processing and Analysis Methods

Due to the large variation in and disobedience of normal distribution, the measured concentration of culturable bacterial bioaerosols is represented by the median (calculated from Equations (3) and (4)) and range (i.e., maximum and minimum) in this study. When analyzing the impacts on the concentration of culturable bacterial bioaerosols, the contemporaneous environmental parameters were represented by averaging the data for 5 min before and after the time point of sampling. In this case, the wind direction was processed using the trapezoidal averaging method [17] for the collected data, as in Equation (5). Data analysis and visualization were performed using Origin 2018, Excel 2019, SPSS 26.0, and Spyder 5 (Anaconda3).

When "n" is an odd number:

$$M_d = X_{(n+1)/2}$$
(3)

When "n" is an even number:

$$M_d = 1/2(X_(n/2) + X_((n+1)/2))$$
(4)

$$\bar{\theta} = \frac{1}{2(n-1)}(\theta_1 + \theta_n) + \frac{1}{n-1}\sum_{i=2}^{n-1}\theta_i$$
(5)

where M_d is the median; X is the numerical series; *n* is the data position in the array (Equations (3) and (4)) or number of wind angle (Equation (5)); $\overline{\theta}$ is the average of wind angle; θ_i is the wind angle at a certain sampling moment.

In this study, cluster analysis and a significance test were used to explore the distribution characteristics of culturable bacterial bioaerosols. A statistical model for culturable bacterial bioaerosol concentration was developed based on the random forest algorithm to analyze the impacts of environmental factors on culturable bacterial bioaerosols. In modeling, WD, V, T, RH, GHI, UV, PM1, PM2.5, PM10, PM100 and vertically sampled culturable bacterial bioaerosol concentration were extracted from each sampling point to build the data set. The ratio of training set and test set was 8:2, and the hyperparameters were tuned to be optimal. The predicted values of 10 sets of concentrations were obtained by separately controlling 10 sets of single environmental factor values as "0" and inputting them into the prediction model. Finally, the difference between the predicted and true values of each group of concentrations was calculated according to Equation (6). The difference between the groups was used as a measure of the magnitude of the effect of each environmental factor on the concentration.

$$E = \frac{\sum_{i=1}^{N} (P_i - R)}{Q} \tag{6}$$

where *E* is the value of the effect of environmental factors on the concentration, P_i is the predicted values of concentrations after controlling for a single environmental factor of "0", CFU/m³, *R* is the real values involved in modeling, CFU/m³; *Q* is the amount of data contained in the test set.

3. Results and Discussion

3.1. Horizontal Distribution on a Farm Scale

3.1.1. Concentration in Different Functional Zones

Table 1 shows the measures of culturable bacterial bioaerosol concentration in different functional zones during the horizontal sampling period. The measured farm-scale concentration was between 1.14×10^3 and 7.35×10^3 CFU/m³. This value meets the requirement of not more than 2×10^4 CFU/m³ in the specification [18]. Meanwhile, the background concentration of culturable bacterial bioaerosols was between 1.02×10^3 and 3.48×10^3 CFU/m³, significantly lower (p < 0.05) than those in FP, PA and WM but very close to those in OA.

Table 1. Culturable bacterial bioaerosol concentration in different functional zones during field measurements (Unit: $10^3 \cdot CFU/m^3$).

Regions	Med	Max	Min	Extreme Difference
Background point (BG)	2.02	3.48	1.02	2.46
Office area (OA)	2.55	3.42	1.25	2.17
Feed processing area (FP)	3.18	4.52	1.54	2.98
Milking parlor (MP)	3.33	4.27	1.83	2.44
Dairy barn (DB)	4.18	5.16	2.00	3.16
Open lot (OP)	2.92	4.12	1.14	2.98
Interfiled road (IR)	3.54	7.35	3.25	4.10
Waste management area (WM)	3.03	4.45	2.50	1.95

The waste management area was expected to be the most contaminated region with the highest culturable bacterial bioaerosol concentration. However, it was somewhat surprising that the measured concentrations of culturable bacterial bioaerosols in the waste management area were very close to those in the feed processing area and open lots, and much lower than those in dairy barns and on interfield roads (Table 1). Furthermore, the waste management area exhibited the least variation in culturable bacterial bioaerosol concentration, suggesting that its bioaerosol levels remained relatively consistent and were minimally influenced by environmental factors. This may be due to the good air exchange with the surrounding area in this relatively independent region, as well as the relatively stable emission of bioaerosols. Further analysis revealed that during the measurement period, the largest extreme difference in concentration was found on the interfield roads, successively followed by the dairy barn. The dairy barn exhibited the highest median concentration, followed by the interfield roads.

Figure 2 shows the cluster analysis of culturable bacterial bioaerosol concentrations in different regions. The results show that culturable bacterial bioaerosols on the interfield road were clustered into a single category; the background and office area were clustered into the same category; and the remaining five regions can be clustered into another category. When clustered into three categories, the remaining five regions can be further clustered into dairy barns and the other two categories. In cluster analysis, individuals clustered in the same category have great similarity. This indicates that there are similar characteristics of culturable bacterial bioaerosol concentrations for the different sampling regions clustered in the same category. Combined with the extreme difference and cluster analysis, it can be seen that the interfield road was the region most seriously contaminated by culturable bacterial bioaerosols on the farm, and it should be the priority area for daily disinfection. The interfield road is prone to turbulence due to the diffusion blockage of animal buildings. Moreover, dust raising would be caused by the daily activities of cows (e.g., migration for

milking), vehicles (e.g., TMR truck, manure suction truck), and workers. This may be why the culturable bacterial bioaerosol concentrations showed different characteristics on the interfield road compared with the other regions. Thus, according to the cluster analysis and measured culturable bacterial bioaerosol concentrations on the dairy farm, the air contamination level would be IR > DB > WM \approx MP \approx OP \approx FP > OA \approx BG. This gives a reference for daily disinfection events on the dairy farm. However, this contamination gradient does not represent the emission level of culturable bacterial bioaerosols. The concentration would be highly affected by the air exchange rate and the ease of diffusivity in different emission scenarios, among other things. Even with a very high emission rate, the concentration can be very low when the air exchange rate is large enough.



Figure 2. Cluster Analysis of culturable bacterial bioaerosol concentrations in different regions.

Daily variations in culturable bacterial bioaerosol concentration in different regions are shown in Figure 3. Overall, the daily concentrations in various regions exhibited a consistent variation pattern throughout the sampling period (15 October–18 October). There are large differences in the measured concentrations on different days in the same area. This may be because of the influence of environmental factors and the geomorphic complexity of the diffusion surface. The concentration of bioaerosols fluctuates throughout the day, even within the same day, as highlighted by Qian et al. [19]. This variability is primarily influenced by factors such as topography and environmental conditions. In their research, Sun et al. [20] found that atmospheric bacteria primarily originate from the ground, and their concentration significantly decreases with increasing altitude. This indicates a strong relationship between culturable bacterial bioaerosol concentration and topographical conditions. Ma et al. [21] discovered that the diurnal variation in bioaerosols is primarily governed by bacteria and exhibits a strong correlation with relative humidity (RH). Forde et al. [22] analyzed the emission characteristics and discussed the sources of fluorescent bioaerosols in winter and summer at four different locations in the United Kingdom. They found that bacteria were the predominant component, and that bacterial concentration exhibited a strong correlation with relative humidity (RH). Furthermore, in studies conducted by other researchers [23–25], it has been observed that various environ-



mental factors have an impact on bioaerosol concentration, although the significance of their influence may vary. Therefore, in bioaerosol studies conducted on dairy farms, the influence of environmental factors on bioaerosols is indispensable.

Figure 3. Daily variation in concentrations in different functional areas during the sampling period.

3.1.2. Carrier Size Distribution of the Sample

Carrier size is an important indicator to evaluate the characteristics and hazards of bioaerosols [26]. Bioaerosols retained in different stages of the six-stage Anderson sampler have different carrier sizes. The percentage of carriers in different sizes was obtained based on the ratio of counts in each stage of the sampler to total counts of the six stages.

Figure 4 shows the carrier size distribution of culturable bacterial bioaerosols in different functional zones. Generally, except the dairy houses, the number of cultivable bacterial bioaerosols was the highest at the F5 stage, and the lowest at the F6 stage. This suggests that most carriers of culturable bacterial bioaerosols on the dairy farm were in the size range of $1.1 \sim 2.1 \,\mu m$. The percentages of carrier size distributed in the F5 stage were 26.57% (FP), 17.84% (DB), 29.11% (BG), 29.40% (WM), 29.60% (OA), 26.68% (OL), 19.33% (IR), and 22.90% (MP), respectively, in different functional zones. Qian et al. [19] found that over 55% of culturable bacterial bioaerosols was carried by the coarse particles with particle sizes over 2.1 μ m, which is consistent with the results in this study. Culturable bacterial bioaerosols with carrier sizes over 2.1 μm (F1 to F4 stage) accounted for 67.49% (FP), 76.85% (DB), 58.35% (BG), 62.64% (WM), 57.96% (OA), 61.55% (OP), 64.95% (IR), and 66.33% (MP), respectively. Reports demonstrate that bioaerosols with a carrier size smaller than 5.0 μ m (F3 to F6 stages) can easily enter into the lungs [27]. Measured culturable bacterial bioaerosols with a carrier size smaller than 5.0 μm accounted for 62.66% (FP), 52.95% (DB), 67.60% (BG), 66.35% (WM), 71.84% (OA), 70.63% (OP), 68.00% (IR), and 71.23% (MP), respectively.

As shown in Figure 4, the size distribution of culturable bacterial bioaerosols in different regions generally followed the same variation pattern, except for the dairy barn and milking parlor. In the dairy barn and milking parlor, the different variations mainly occurred in the F3 and F4 stages (Figure 4). This may be because of the different environmental factors indoors and outdoors. Due to the influence of the envelope structure and animals on mass and heat transfer, indoor environmental factors such as RH and radiation significantly differ from those outdoors. Normally, RH indoors would be higher than RH outdoors. The rise in RH can promote an increase in aerosol particle size [28]. Similar size distributions were observed between the dairy barn and the milking parlor, as these two sites collected bioaerosol samples indoors. The rest of the area was sampled in the open air, and therefore maybe there are differences.



Figure 4. Size-specific airborne culturable bacterial loads at different locations on the dairy farm.

3.2. Vertical Distribution

3.2.1. Concentration Gradient

Considering the farm-scale horizontal distribution of bioaerosols and the representativeness of sampling, vertical concentration gradients were examined in the most contaminated regions, which were the manure management area and the production area 1. Table 2 shows the culturable bacterial bioaerosol concentrations measured at different heights of the two sampling areas. The measured culturable bacterial bioaerosol concentrations in the first production area and the manure waste management area were 4.50×10^2 -6.79 $\times 10^3$ CFU/m³ and 2.60×10^2 ~6.20 $\times 10^3$ CFU/m³, respectively. The highest concentrations in the two sampling areas were mostly concentrated in the order of 10^3 , while the lowest concentrations were mostly in the order of 10^2 . Field measurements conducted by Ru et al. [29] showed that the culturable bacterial bioaerosol concentration of a dairy farm in summer was 1.50×10^2 – 1.61×10^4 CFU/m³ (2.4×10^3 CFU/m³ on average) and mostly in the order of 10^3 . Sampling in this study was conducted in the season of autumn in China. Compared with the results in summer, there were significant differences between culturable bacterial bioaerosol concentrations in summer [29] and autumn (p > 0.05), and a larger extreme difference in culturable bacterial bioaerosol concentration was observed in autumn. This may be because it was rainier during the sampling period in this study. The RH was around 60-80% during sampling due to the influence of rainy weather; this was much higher than the RH in summer, which was around 40–60% [29]. Dust and bioaerosol carriers are reduced in rainy weather or at higher RH due to the increase in particle settlement [30]. This makes the concentration of bioaerosols decrease sharply. Long after the rain has stopped, the humid environment combined with the return of temperature will again lead to a large breeding of microorganisms and consequently to an increase in the concentration of culturable bacterial bioaerosols. Therefore, the cultur-

B-4 m C-1 m Sampling Area A-1 m A-4 m B-1 m C-4 m D-1 m D-4 m 6.79 Max 5.08 4.434.604.39 5.164.573.67 Production area Min 0.450.59 0.730.92 0.540.480.75 0.62 (26 September-10 October) 2.50 Med 2.001.81 2.002.192.371.87 1.46 6.20 3.37 6.12 4.39 5.56 3.55 3.88 5.92 Max Waste management area Min 0.41 0.42 0.26 0.48 0.59 0.67 0.61 0.54 (26 September–10 October)

1.67

able bacterial bioaerosol concentration in this study was more widely ranged than in the summer, and this resulted in a bigger extreme difference.

Table 2. Concentration in each sampling area (Unit: $10^3 \cdot CFU/m^3$).

Note: A, B, C, and D represent the four sampling points of south, west, east and north, respectively. A-1 m and A-4 m represent the sampling height of 1 m and 4 m at the sampling point of south.

1.46

1.14

1.92

1.29

There is a significant vertical gradient for culturable bacterial bioaerosol concentrations in both two sampling areas. Culturable bacterial bioaerosol concentration at the height of 1 m was significantly higher than that at the height of 4 m (p < 0.05). Compared with the production area, a much bigger vertical concentration difference was observed between 1 m and 4 m in the waste management area. This is similar to the findings related to the dairy farm of Ru et al. [29] in summer. However, research on urban bioaerosols [31] showed a trend in bioaerosol concentrations at low levels, which were slightly greater than those at high levels, though there was a very small difference at different heights. This may be due to the specificity of emitting sources in different emission scenarios. In livestock buildings, manure is a major emission source of bioaerosols [6]. Taking the waste management area as an example, it is more contaminated by manure and there is less diffusion as it is blocked by surroundings such as dry-wet separation and the air-cure treatment of dairy slurry. Moreover, microorganisms from different emission sources are race-specific. Therefore, a bigger vertical gradient of culturable bacterial bioaerosols was observed on the dairy farm, especially in the waste management area. Concentration gradient is one of the necessary conditions for the successful implementation of the micrometeorology technique to measure the fluxes of gaseous pollutants from open emission sources [32–35]. This vertical gradient can be used to estimate the emission rate of culturable bacterial bioaerosols from dairy farms.

3.2.2. Carrier Size

Med

2.47

1.14

To exclude systematic errors in sampling and colony counting, this study took the data of colonies of the same particle size at each sampling area for analysis. Table 3 shows the proportion of carriers of different sizes at the heights of 1 m and 4 m. Using the median size distribution, we recorded the variation in carrier sizes in different sampling locations (shown in Figure 5), and the size distribution of culturable bacterial bioaerosols in summer was also plotted according to the results in Ru et al. [29] to make a comparison.

As shown in Figure 5a, during the test period, the proportion of the number of different carrier sizes of cultivable bacterial bioaerosols at different heights in each sampling area has the same trend. A similar result was observed in the summer trial (Figure 5b) according to Ru et al. [29]. Correlation analysis demonstrates that there was a strong covariance (range from 0.916 to 0.973) between the three groups of occupancy profiles at 1 m in the waste management area and at 1 m and 4 m in the production area. The percentage of all levels of the samples at 4 m in the waste management area had a relatively weak correlation (range from 0.714 to 0.747) with the other locations. The topography of the waste management area is relatively open, and there are no trees or buildings in the vicinity to block it. Combining this with the boundary layer theory, bioaerosols at the height of 4 m were less affected by the ground-level emission source and more affected by carriers in the free stream of the background. Therefore, there is a relatively weak correlation for the size distribution between culturable bacterial bioaerosols sampled at 4 m in the waste management area and

1.49

the other sampled locations. Above all, we concluded that culturable bacterial bioaerosols in a certain location of the dairy farm had the same vertical size distribution, and there is a certain relationship between the vertical size distribution in different locations.

Comparing with the carrier size distribution in different functional zones (Figures 4 and 5a), similar distribution patterns were found between vertical and horizontal space in the sampling period. Both results show the trend that the F1 and F5 stages have the largest proportion and the F6 stage has the least proportion of culturable bacterial bioaerosols. This is different from the findings of other studies, as some research has shown that the culturable bacteria and fungi aerosols from chicken and pig manure emissions are mainly distributed within the particle size range of 0.65 to 1.1 μ m and 2.1 to 3.3 μ m [36]. This difference in results might be attributed to the specific livestock species involved in the studies. In addition to that, animal activity, ventilation, bedding materials, and floor types can also influence the particle size distribution [37]. Even under the same experimental conditions with the same livestock species during the same season, there are variations in experimental results compared to those presented in this paper [11]. Gao et al. [38] pointed out that the factors influencing the particle size distribution of bioaerosols inside livestock barns are complex and difficult to analyze independently. Even within the respirable particle size range (<5 μm), significant differences in bacterial aerosols can be observed, even among the same livestock species [39]. Therefore, further research is needed for the analysis of bioaerosol particle size distribution. In this paper, a comparative presentation of sampling test results from the same location during different seasons was conducted (Figure 5). Additionally, the particle size distribution in different functional zones was also shown (Figure 4). While compared with the size distribution in different seasons, different size distributions were observed in the same area (e.g., waste management area) in summer (Figure 5b) and autumn (Figure 5a). This suggests that the seasonal influence on the size distribution may be more significant than the spatial variation.



(a) Percentage of culturable bacterial bioaerosols of different carrier sizes (autumn)

Figure 5. Cont.



(b) Percentage of culturable bacterial bioaerosols of different carrier sizes (summer)

Figure 5. Proportion of culturable bacterial bioaerosols in different sizes (Data in (**b**) were from Ru et al. [29]). Note: The sampling area in autumn is the production area (PA) and waste management area (WM), and the sampling area in summer is the production area (PA) and farming area (FA). The sampling area is composed of the waste management area (WM) and production area 1 (PA) (depicted in Figure 1); WM1 represents 1 m of waste management area (similar hereinafter).

Usiaht of the Comple	Stage —	1	Production Are	a	Manure Treatment Area		
Height of the Sample		Med	Max	Min	Med	Max	Min
1 m	F1	23.04	28.30	29.63	22.93	11.63	22.58
	F2	14.66	22.31	12.96	12.74	13.25	9.68
	F3	14.66	12.93	14.81	13.38	12.85	12.90
	F4	17.28	16.15	14.81	19.11	22.68	19.35
	F5	20.42	12.07	18.52	22.29	21.95	22.58
	F6	9.95	8.25	9.26	9.55	17.64	12.90
4 m	F1	20.12	19.79	22.22	19.51	17.06	21.88
	F2	13.61	11.75	13.33	13.82	19.19	9.38
	F3	17.16	10.62	20.00	12.20	13.74	15.63
	F4	15.98	25.88	17.78	15.45	15.17	21.88
	F5	21.30	24.43	17.78	24.39	19.67	21.88
	F6	11.83	7.53	8.89	14.63	15.17	9.38

Table 3. The percentage of different carrier sizes at different heights (%).

The shift of seasons is usually shown in the change of environmental parameters. Combined with the environmental factors of the experimental farm, it was found that the wind factor has a certain influence on the carrier size distribution of culturable bacterial bioaerosols. When the wind speed is slow, size distributions between different sampling locations or different heights were more stable. Again, take the waste management area as an example. When the maximum wind speed during sampling was below 1.8 m/s (Figure 6a), size distributions at different heights or in different locations followed similar

variations, and culturable bacterial bioaerosols in the F5 stage $(1.1-2.1 \mu m)$ had the highest proportion (Figure 6c). However, due to the wind speed during sampling increasing to about 2.5–3.5 m/s (Figure 6b), the size distribution at different heights or in different locations varied considerably (Figure 6d). In this case, the wind direction during sampling was mainly from the southwest. Size distributions differed at 1 m and 4 m in the south and north sampling locations due to the dust-raising effect of wind (Figure 6d). The higher the wind speed, the more seriously the size distribution was affected in the sampling locations up and down wind. This is consistent with the results in summer from the literature [29].

Unlike the waste management area, the production area is surrounded by a fence and buildings with ridge over 4 m. The size distribution of bioaerosols at the different sample heights was relatively less affected by the wind speed. Taking the case at the wind speed of 4-4.5 m/s as an example, size distributions were similar at different heights in the same location but a little bit varied in different sampling locations (Figure 7). In this case, the wind direction during sampling was mainly from the northwest. The greatest proportion of culturable bacterial bioaerosols was distributed in the F5 stage for the sampling locations at the south and west boundary of production area, similar to the result when wind speed was below 1.8 m/s, as seen in Figure 6b. However, the F1 stage accounted for the greatest proportion of culturable bacterial bioaerosols at the sampling locations at the east boundary of the production area. This can be because of the dust-raising effects of wind as the culturable bacterial bioaerosols distributed in the F1 stage represent the carriers with a larger size of over 7.0 μ m.



(a) Wind rose map when prevailing wind speed is below 2.0 m/s.

Figure 6. Cont.

 $\begin{array}{c} 10 \\ 9 \\ 8 \\ 7 \\ 6 \\ 5 \\ 4 \\ 3 \\ 1 \\ 0 \\ 1 \\ 2 \\ 1 \\ 0 \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \\ 10 \\ \end{array}$

Frequency of wind: times



SE

SSE

(**b**) Wind rose map when prevailing wind speed is over 2.0 m/s.

S





Figure 6. Cont.

SW

SSW



(d) Size distribution when prevailing wind speed is over 2.0 m/s.

Figure 6. Examples of the size distribution of culturable bacterial bioaerosols at different heights in the waste management area in the case of varied wind environment during sampling.

3.3. Influence of Environmental Factors on Culturable Bacterial Bioaerosol

3.3.1. Environmental Factors during Experiment

The measured environmental factors on the dairy farm in the field measurements are shown in Table 4. During the whole sampling period, the wind direction in the production area was predominantly northwest and southeast (Figure 8a). The wind speed was concentrated below 4.5 m/s, with an average wind speed of 2.76 m/s. Wind direction varied more frequently when bioaerosols were sampled in the waste management area (Figure 8b). The prevailing wind speed was close to that in the production area, with an average wind speed of 2.74 m/s.

Table 4. The measured environmental factors on the dairy farm.

Environmental Parameters	Unit	Production Area	Waste Management Area
Temperature (T)	°C	24.82 ± 3.65	24.94 ± 3.84
Relative humidity (RH)	%	65.23 ± 15.84	64.56 ± 16.66
Wind speed (V)	m/s	2.76 ± 1.81	2.74 ± 1.76
Total irradiation intensity (GHI)	W/m ²	527.83 ± 201.76	516.38 ± 226.79
UV irradiation intensity (UV)	W/m^2	26.77 ± 8.61	25.85 ± 10.52
PM1 concentration (PM1)	µg/m ³	24.73 ± 12.01	2.09 ± 1.33
PM2.5 concentration (PM2.5)	$\mu g/m^3$	35.67 ± 18.05	6.95 ± 3.30
PM10 concentration (PM10)	$\mu g/m^3$	41.76 ± 21.90	8.76 ± 3.96
PM100 concentration (PM100)	$\mu g/m^3$	32.65 ± 16.39	6.16 ± 6.05







(b) Size distribution of measured culturable bacterial bioaerosol.

Figure 7. An example of the size distribution of culturable bacterial bioaerosols at different heights in the production area in the case of the high wind speed during sampling.



(a) Wind rose diagram of the production area during sampling.



(b) Wind rose diagram of waste management area during sampling.

Figure 8. Changes in wind environment during field measurements.

3.3.2. Influence of Environmental Factors on Culturable Bacterial Bioaerosol Concentrations

As mentioned above, culturable bacterial bioaerosol concentration and distribution are strongly influenced by environmental factors such as relative humidity, wind speed, wind direction, etc. For a more in-depth analysis of the influence of environmental factors, a statistical model employing the random forest algorithm was constructed to establish a relationship between the concentrations of culturable bacterial bioaerosols and environmental factors. This was accomplished by utilizing the data collected in the preceding sections. The mean square error (MSE) was 0.0153 for the developed statistical model, which indicates the good reliability of the model. The sensitivity analysis of a single environmental factor was conducted by controlling it as "0" in the model and evaluating the difference between the calculated value and the real value. Figure 9a demonstrates the importance of different environmental factors regarding their effect on culturable bacterial bioaerosol concentrations, based on the sensitivity analysis.



Figure 9. The influence of environmental factors on the culturable bacterial bioaerosol concentration and the results of hierarchical cluster analysis. (**a**) Sensitivity of culturable bacterial bioaerosol concentration on different environmental factors. (**b**) Results of hierarchical clustering analysis of environmental factors.

Results showed that the influence of environmental factors was ranked in the order of PM100 > WD > T > UV > PM1 > RH > PM2.5 > GHI > V > PM10 (Figure 9a). The concentration of particle matters (PM100), wind direction, and air temperature were the top three factors affecting the culturable bacterial bioaerosol concentrations on the dairy farm, with very similar importance. A hierarchical cluster analysis was then performed on the different environmental factors as most of their importance was very similar. A strong correlation was found between the concentrations of PM1, PM2.5, and PM10, as well as between GHI and UV, respectively (Figure 9b). Thus, the effects of the concentration of PM1, PM2.5, and PM10 can be represented by any one of them according to their accessibility in a real situation. The same is true regarding GHI and UV. As the effects of PM10 and PM2.5 can be replaced by PM1 and effects of GHI can be replaced by UV, the statistical model was re-developed using the remaining seven environmental factors. The MSE was 0.0153 after re-modeling, which is similar to the performance the model with ten environmental factors. This further indicates that the substituted environmental factors (PM2.5, PM10, and GHI) had the least effect on culturable bacterial bioaerosol concentration, compared to the remaining factors. These retained factors are consistent with the findings of studies analyzing the environmental factors affecting bioaerosols [29,40,41].

Zhen et al. [42] evaluated the influences of 11 environmental factors on the airborne bacterial communities in an urban city and concluded that meteorological factors (T, RH, V, total radiation, atmospheric pressure and vapor pressure) had more impact than air pollutants (PM2.5, O_3 , SO_2 , NO_2 and CO). We divided the measured environmental factors into two categories in this study, i.e., meteorological factors (T, RH, V, WD, etc.) and air pollutants (PM1, PM2.5, PM10 and PM100). The meteorological factors (mean impact value = 0.08903) showed a slightly stronger effect on culturable bacterial concentrations than the air pollutants (mean impact value = 0.08899). This is consistent with the results from Zhen et al. [42].

Regarding meteorological factors, air exchange rate, which can be reflected as the change in wind speed and wind direction, has a decisive effect on the morphology and concentration of bioaerosols [8]. Research from Zhong et al. [43] showed that microbial activity was negatively correlated with wind speed and that there is a correlation between wind direction and microbial concentration. At the same time, strong winds can also bring in exogenous bacteria or dilute the concentration of local bacteria [43–45]. Bioaerosols carried by different wind directions would bring in different sources of microorganisms with different compositions and microbial activity. Thus, the impacts of wind would be a comprehensive effect of wind speed, wind direction, the surface complexity of experimental sites, and the level of pollution in the surrounding area. The wind direction showed a greater effect on the culturable bacterial bioaerosol concentration compared to the wind speed in this study.

Air temperature was the third of the top factors affecting the culturable bacterial bioaerosol concentrations in this study. The increase in T promotes bacterial growth and accelerates the movement of convective air, thus increasing the diffusion of bacteria in the atmosphere [46]. Li et al. [47] and Mouli et al. [44] found that there was a significant positive correlation between T and bioaerosol concentration. In reality, changes in T are often coupled with the variation in RH. Zhen et al. [42] found that RH varied a lot due to the different frequencies of precipitation in summer and autumn, and the variation in RH has a strong influence on bioaerosols in different seasons. Rainwater will directly wash away airborne particles, reducing the number of bacteria carriers. In non-precipitation weather, higher humidity promotes the process of the dry deposition of bacteria carriers in the air. The carriers absorb water from the environment, increasing their weight and size, and eventually reaching a state of dry deposition. A study on environmental parameters affecting the survival status of airborne infectious agents was conducted by Tang et al. [48]. They found that RH affects the survivability of airborne bacteria and consequently affects the concentrations of bioaerosols. The effect of RH on bioaerosol concentration is controversial. It is widely believed that low humidity and dry environments can lead to a decrease in the metabolic and physiological activity of microorganisms, and an environment with high humidity will provide suitable conditions for the growth of bacteria [49]. However, Qi et al. [50] found that RH had nearly no effect on microbial activity in bioaerosols when it was in the range of 37–97%. Microorganisms from different emission sources may react differently to the varied RH. The influence of increased RH could be counteracted due to the different performances of the dry deposition of carriers and the survival of microorganisms in a multivariate coupled environment. This may be the reason for the controversial effect of RH on the concentration of bioaerosols. In this study, RH showed an effect less than T but greater than wind speed.

Solar radiation, especially UV radiation, can affect microbial activity. UV light at wavelengths of 200~320 nm in solar irradiation has a killing effect on microorganisms [51]. However, UV radiation at a wavelength below 290 nm (which had the strongest bactericidal effect) is filtered by the atmosphere, and other environmental factors can have an impact on the effectiveness of solar irradiation in killing microorganisms [52]. The increase in

ambient humidity can suppress the effect of UV irradiation on killing microorganisms in bioaerosols. When the relative humidity is higher than 75%, the killing efficiency decreases by 40% [53,54]. When the wind speed is high, some disinfection devices using the UV principle would lose their germicidal function [55]. In this study, UV radiation was the fourth of the top factors affecting culturable bacterial bioaerosol concentrations, and solar irradiation (GHI) had much less effect.

Particulate matter is the major carrier of bioaerosols. The effects of particulate matter on bioaerosols may be associated with the size of particles. Han et al. [56] found a very small correlation between PM2.5 concentration and the concentration of total bacteria in bioaerosols, while PM10 concentration showed a significant positive correlation with total bacteria in bioaerosols. This is because it is much easier for airborne bacteria to adhere to coarse particulate matter [47, 57]. The concentration of particulate matter is subject to the influence of diverse environmental parameters, with this impact also being connected to particle size. The acceleration of wind speed increases the settling rate of particulate matter [58]. Larger particles in air are also more susceptible to the effects of gravity [59]. There is a positive correlation between temperature variation and particle concentration. High temperatures can cause fine dust to be dispersed in the air from the environment. On livestock farms, the increase in temperature leads to an increase in livestock activity, which in turn affects the concentration of particulate matter [60,61]. The increase in humidity leads to a better adhesion between particles [62], which has the effect of dust reduction, thus affecting the concentration of particles. For these reasons, PM1, PM2.5, PM10, and PM100 showed different effects on culturable bacterial bioaerosol concentration in this study, although the measured particulate matters with different sizes demonstrate similar variation trends. The measured PM100 has abrupt changes in concentration compared to other particulate matter, and its concentration remained at a high level most of the time. Results showed that PM100 had the greatest influence on the culturable bacterial bioaerosol concentration relative to other factors.

4. Study Limitations and Future Research

This study investigated the culturable bacterial bioaerosols of a dairy farm in a typical transition season, which is expected to be suitable for microbial survival and have relatively high concentrations. Constrained by the existing monitoring instruments, the collection and cultivation of bioaerosols were carried out manually. This limitation impedes the ability to maintain continuous, prolonged sampling across multiple points throughout the entire dairy farm. Thus, the sampling and analysis of bioaerosols were only carried out during a fixed period every day that was least influenced by daily production. In future research, it is recommended to conduct sampling across the entire year and examine the diurnal variation where possible. Furthermore, it is worth monitoring bioaerosols both in the cowshed and outside the cowshed in different functional zones simultaneously. This will allow for a comprehensive understanding of the various characteristics of bioaerosols on the dairy farm.

This research investigated the effects of environmental factors under real conditions through field measurements. These environmental factors were subject to variability, beyond direct control, and could potentially be influenced by factors such as building occlusion (particularly in the case of wind speed and wind direction) or daily operations. Therefore, it is recommended to conduct laboratory experiments using environmentally controlled warehouses to study the effects of single or combined environmental factors on the source of cultivable bacterial bioaerosols on dairy farms. In this way, the adverse effects of complex interfering factors are eliminated. These experiments can be supplemented with data from on-site trials for analysis and discussion, aiming to accurately determine the mechanisms of how multiple environmental factors influence bioaerosols.

During the experimental data collection process, samples from different locations were sampled at the same time and were analyzed in combination with environmental factors. This will be of great help to the field research of bioaerosols in livestock and poultry farms. However, the existing commonly used monitoring methods are highly labordependent, requiring the continuous monitoring of large quantities, which is a limitation. Therefore, new tools or equipment to support automatic sequential sampling or even real-time monitoring are needed to enable efficient measurement at various sampling points simultaneously.

5. Summary

This study investigated the horizontal and vertical characteristics (concentration and size distribution) of culturable bacterial bioaerosols at the farm scale on an intensive dairy farm of Northern China. The influence and sensitivity of environmental factors on culturable bacterial bioaerosols was examined by developing a statistical model and clustering analysis. The following results were summarized in this study:

(1) Culturable bacterial bioaerosols were sampled in eight different functional zones to analyze their horizontal spatial variations in concentration and carrier size. The concentrations of culturable bacterial bioaerosols in different areas of the dairy farm had similar daily trends. The concentration varied widely, in the range of $1.14 \times 10^3 \sim 7.35 \times 10^3$ CFU/m³. The interfield road should be the priority area for daily disinfection. Culturable bacterial bioaerosols were mainly distributed in coarse particles, with the highest proportion of the carrier size being larger than 7.0 µm and between 1.1 and 2.1 µm. The different functional regions on the dairy farm showed the similar size distributions of culturable bacterial bioaerosols.

(2) In terms of vertical characteristics, the concentrations of culturable bacterial bioaerosols differed significantly between different heights. Culturable bacterial bioaerosol concentration at 1 m was significantly greater than that at 4 m (p < 0.05). However, there was a similar carrier size distribution for culturable bacterial bioaerosols at different heights at the same sampling location. Wind speed affected the carrier size distribution of culturable bacterial bioaerosols at different sampling locations.

(3) Sensitivity analysis was conducted to examine the effects of environmental factors on culturable bacterial bioaerosol concentrations on dairy farms. In this study, the importance of environmental factors in terms of their effect on culturable bacterial bioaerosol concentrations was listed in the order of PM100, WD, T, UV, PM1, RH, PM2.5, GHI, V, and PM10 in descending order of impact. The monitoring of PM2.5, PM10, and GHI could be removed in further field experiments as a substitution effect of PM1 and UV was found for them in this study.

The findings of this study can provide a priori knowledge for understanding the culturable bacterial bioaerosol farm-scale characteristics in the real situation of dairy farms and exploring the effects of environmental factors on culturable bacterial bioaerosols on dairy farms. They also provided evidence for guiding daily disinfection strategies and epidemic prevention on commercial dairy farms. In subsequent studies, it is suggested to investigate the effect of the multiple environmental factors on bioaerosols emitted from animal farms under controlled laboratory tests, as well as estimate the fluxes of bioaerosols from dairy through field measurements to evaluate their diffusion and the risk of contamination to the surroundings.

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