

Microbial activity levels in atmospheric bioaerosols in Qingdao

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Abstract Bioaerosols are an important subgroup of atmospheric aerosols, and microorganisms are a major component of bioaerosols. Microbial activity can affect climate change, human health and environmental processes. We developed a diacetate (FDA) hydrolysis method to measure microbial activity in bioaerosols. A large number of preliminary experiments were used to optimise the experimental conditions and guarantee the applicability of this assay to environmental bioaerosol samples. The level of microbial activity in the atmosphere in the Qingdao coastal region was measured using the FDA method in May and from September to December 2012. The microbial activity in bioaerosols varied from 10.7 to 85.8 ng/m³ sodium fluorescein, with an average of 30.2 ng/m³. The microbial activity in bioaerosols displayed monthly variations, with the highest value in May. However, the activity showed no significant correlation with temperature, relative humidity and wind speed. Thus, further study is needed to elucidate the major factors and mechanisms that affect microbial activity in the atmosphere.

Keywords Bioaerosols · Microbial activity · FDA hydrolysis · Meteorologic factors

1 Introduction

Bioaerosols are a significant group of atmospheric aerosols that represent an average of 30 % of number concentration of atmospheric particles (Matthias-Maser and Jaenicke 1995; White et al. 1999). Airborne microorganisms are major contributors to bioaerosols and can serve as both cloud condensation nuclei (Bauer et al. 2003; Möhler et al. 2007) and ice condensation nuclei (Möhler et al. 2008; Bowers et al. 2009; Pratt et al. 2009; Delort et al. 2010). In addition, respiratory diseases such as pneumonia, rhinitis and asthma, as well as death, can be caused by exposure to airborne microorganisms (Liao and Luo 2005; Abdel-Hameed et al. 2009; Xu and Yao 2011). This is especially true for those who are physiologically sensitive to microbes (Dales et al. 2004; Peternel et al. 2004; Kim and Kim 2007). To understand the impact of bioaerosols on climate and public health, a number of investigations into airborne microorganisms in these particles have been conducted (Hua et al. 2007; Polymenakou et al. 2008). Most studies have investigated either the concentration of airborne microorganisms (Fang et al. 2007; Griffin et al. 2007) or the microbial communities in bioaerosols (Bowers et al. 2009, 2011; Jeon et al. 2011). Hara and Zhang (2012)

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presented a quantitative investigations of airborne bacterial cells coupled with LIVE/DEAD BacLight assay in southwestern Japan, and indicated that the bacterial viability in long-range transported dust was considerably lower than that in non-dusty air, probably because most bacteria could not survive during their long-range transports. However, few studies have focused on microbial activity. This omission may be due to the greater difficulty of determining microbial activity in bioaerosols relative to soil and water samples. This difficulty is due to extremely low concentrations of microorganisms in the atmosphere because it is not a suitable habitat (Peternel et al. 2004; Abdel-Hameed et al. 2009; Li et al. 2011).

Microbial activity is a measure of the physiological processes performed by microorganisms. This activity represents the level of microbial metabolism and is used in biological tests to determine the effect of various pollutants on environmental quality (Megharaj et al. 2000; Thavamani et al. 2012). Microbial activity is not equivalent to microbial biomass, although these two metrics do correlate to some extent (Stubberfield and Shaw 1990). Researchers generally analyse the concentrations of microbes but ignore their viability (Griffiths and DeCosmo 1994). However, compared with the concentration and community composition of airborne microorganisms, microbial activity can be a more accurate and direct indicator of the potential hazard to public health. In atmospheric cloud water samples, 76 % of the bacteria are metabolically active and can thus affect climate, human health and environmental processes (Kourtev et al. 2011). Actively metabolising cells can alter the chemical composition of clouds by transforming organic carbon (Amato et al. 2007a, b) or nitrogen (Hill et al. 2007) in cloud water. Therefore, microbial activity can indirectly affect cloud formation, atmospheric chemical processes and global climate change. Determining the microbial activity in bioaerosols is very important for understanding the impact of these particles on global climate change, atmospheric microbial pollution and human health.

The level of microbial activity depends on biological, chemical and physical factors, as well as the nutritional status of the living environment. Zheng et al. (2009) explained that environmental factors (such as CO₂, O₃, temperature and soil management) can restrain, promote or maintain the activities of soil microbes. For atmospheric microbes, temperature and

humidity are thought to be the major factors affecting the quantity of viable microbes (Maier et al. 2010). However, the major factors and mechanisms affecting microbial activity in the environment remain unclear. Many methods to investigate microbial activity in soil and water samples exist (Green et al. 2006; Maier et al. 2010). These include respiration assays, radioactive labelling of cell macromolecules, measuring adenosine energy charges and enzymatic activity assays. Within this latter category, the fluorescein diacetate (FDA) hydrolysis method is a simple, rapid and sensitive enzymatic activity assay. Unlike other methods, FDA hydrolysis is directly proportional to the level of microbial activity in environmental samples (Gumprecht et al. 1995; Battin 1997; Zablotowicz et al. 1998). Fluorescein diacetate [3', 6'-diacetylfluorescein (FDA)] is a colourless compound and is widely used to measure microbial activity in soil and water samples (Fontvieille et al. 1992; Bjurman 1993). FDA can be hydrolysed by many enzymes including proteases, lipases and esterases that are secreted by living bacteria and fungi (Adam and Duncan 2001; Green et al. 2006). The product of this hydrolysis conversion is fluorescein, which can be measured by fluorospectrophotometer, as a fluorescent substance. However, little is known about its potential utility of investigating atmospheric bioaerosols, most likely due to the low level of atmospheric microorganisms.

In a coastal region, bioaerosol concentration and characteristics are also affected by the ocean except for geographical and climatic factors. However, global information on bioaerosols, especially coastal bioaerosol, is very limited. Qingdao is on the shore of the Yellow Sea, and the bioaerosols in this region have special characteristics due to the influence of ocean. Therefore, it is important to study the microbial activity in coastal region to understand the impact of ocean on characteristics of bioaerosols and evaluate the impact of bioaerosols on the atmospheric environment and human health. To know the level of microbial activity in bioaerosols and try to establish the relationship between activity and environmental factors, the FDA hydrolysis method was developed to measure the microbial activity in bioaerosols in this study, and the level of microbial activity in the atmosphere of the Qingdao coastal region was detected using this method. The influence of temperature, relative humidity and wind speed on microbial activity was also investigated.

2 Materials and methods

2.1 Sampling site

As shown in Fig. 1, the sampling site was located on the roof of an academic building on the Laoshan campus of the Ocean University of China ($36^{\circ}16'N$, $120^{\circ}50'E$, 9 m above the ground) in the Qingdao coastal region, which is 7.0 km inland. The samplers were placed 1.5 m above the roof. The trees and grass around the sampling site account for 50 % of the total area. The bioaerosol samples were collected in May 2012 and also from September to December 2012, and the sampling began at 8:00 am during the observational period. The sampling of bioaerosols was performed at 10-day intervals from September to December 2010, and was at 3-day intervals in May 2012. Meteorological parameters such as temperature, relative humidity, wind speed and wind direction of Laoshan District, close to the sampling site, were downloaded from the Qingdao Meteorological Administration (<http://qdqx.qingdao.gov.cn/zdz/ystj.aspx>).

2.2 Sample collection

The bioaerosol samples were collected on polycarbonate membranes (with pore size of $0.22\ \mu\text{m}$) using a

six-stage culturable microorganism FA-1 cascade impactor (Applied Technical Institute of Liaoyang, China) with a flow rate of $28.3\ \text{L}\ \text{min}^{-1}$ for 30 min. The aerosol particle sizes were fractionated into six stages: >7 , 4.7–7.0, 3.3–4.7, 2.1–3.3, 1.1–2.1 and $0.65\text{--}1.1\ \mu\text{m}$ (Li et al. 2011). The membranes were autoclaved at $121\ ^{\circ}\text{C}$ for 15 min before the sampling. Two parallel samples were collected on each sampling day.

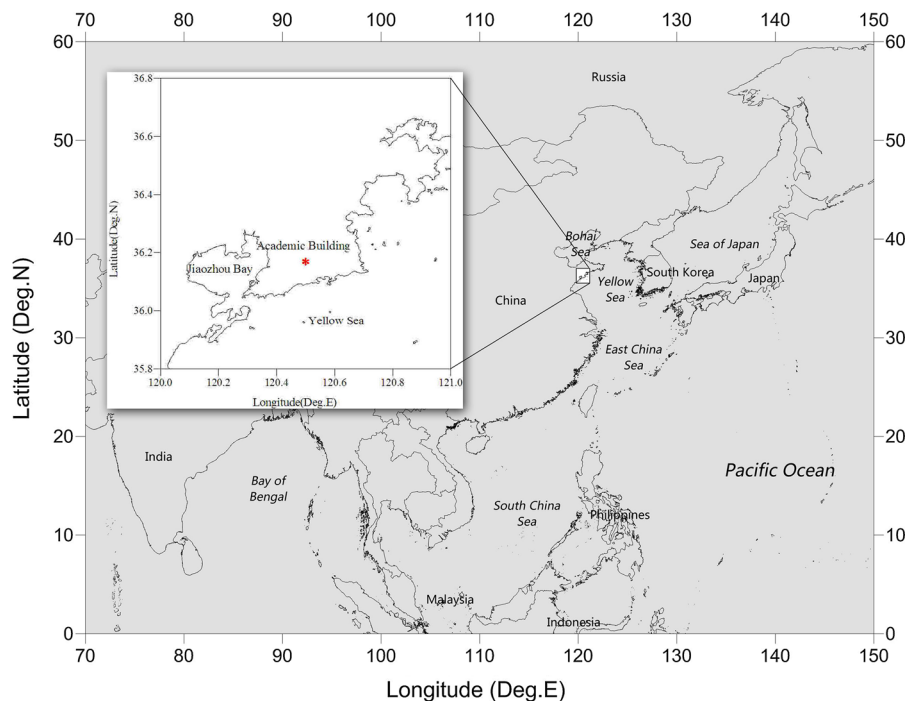
In May 2012, two replicate bioaerosol samples were collected every 3 days to determine the applicability of the method. The standard deviation (SD) of duplicate samples was calculated to investigate the precision of the established method.

2.3 Pre-treatment method

2.3.1 Initial method

Various experimental factors of the FDA hydrolytic activity in bioaerosols were studied. These included testing the elution medium, FDA substrate concentration, membrane coating materials, reaction temperature, reaction time, termination method, termination time, sampling duration and standard curve. All optimisation procedures were based on the

Fig. 1 The location of the sampling site



following preliminary method we had developed in earlier experiments. All appliances used in this study were autoclaved at 121 °C for 15 min.

For the initial method, bioaerosol samples were collected on polycarbonate membranes using a six-stage culturable microorganism FA-1 cascade impactor with a flow rate of 28.3 L min⁻¹ for 30 min. After bioaerosol samples were collected, the sample membranes were put into a 100-mL conical flask with 20 mL physiological saline. The flask was then shaken at 30 °C for 30 min at 150 r/min to separate the adherent microbes from the membranes. Then, the 200 µL FDA solution was added to the extract liquid to start the hydrolysis reaction. The reaction was proceeding at 30 °C for 120 min in the dark. To prevent ongoing hydrolysis in an uncontrolled reaction, 1 mL chloroform/methanol (2:1 v/v) was added to simultaneously stop the reaction of both samples and blanks. This also guarantees the reliability of the measurements. The blank membrane was pre-treated following the same procedure with bioaerosols samples.

2.3.2 Optimisation procedures

To determine the optimum experimental procedure, the bioaerosols were collected in an outdoor environment (Fig. 1). After being autoclaved at 121 °C for 15 min, the beef extract-peptone medium was inoculated with collected airborne microbes and cultivated at 37 °C with shaking at 120 rpm for 24 h. The cells were harvested by filtration and washed twice with sterilised physiological saline. The cells were then resuspended in sterilised physiological saline. The concentration of microbial cells was calculated to be 10⁹ cells mL⁻¹ by epifluorescence microscopy. All of the microorganism groups used in this study were prepared by diluting the above microbe-containing solution into different concentrations.

To choose a suitable elution medium to transfer the microorganisms from the membranes into solution, the influence of pH 7.6 phosphate buffer and physiological saline on groups of microorganisms was studied. Swisher and Carroll (1980) showed that FDA exhibits its maximum hydrolysis rate at pH 7.6. Furthermore, at this pH, no spontaneous FDA hydrolysis is observed (Adam and Duncan 2001).

To choose an appropriate FDA concentration, the influence of varying FDA concentrations from 0 to

800 µg/mL on microorganisms was studied. FDA has low solubility in water and other polar solvents (Breeuwer et al. 1995). Therefore, the choice of concentration was based on the fact that the elution solution became cloudy when FDA concentrations are higher than 800 µg/mL.

Due to mechanical stress and dehydration, microorganisms may lose activity during the collection and deposition of particles (Griffiths and DeCosemo 1994; Mandrioli 1998). To reduce the loss of viability and maintain the activity of airborne microorganisms during sampling, a coating material for the membrane that would maintain microorganism viability (Sorokulova et al. 2012) without interfering with subsequent measurements was necessary. The coating materials can reduce the loss of microorganism activity because of its viscosity, which can absorb the impact and pressure of microorganism hitting on the membrane. Considering their low toxicity to microorganisms, their solubility in water and the solution viscosity of the materials, glycerol, sodium alginate and polyethylene glycol (PEG) were chosen as potential coating materials. The influences of the polycarbonate membranes coated with glycerol, sodium alginate or PEG-4000 on control and microorganism groups were studied.

To determine the optimum reaction temperature, four groups of parallel samples were collected on 4 days under similar weather condition. Each group included two parallel samples collected by two samplers at the sampling site simultaneously, and the average microbe activity of the two parallel samples was used to determine the optimum reaction temperature. The similar sample collection was performed for succedent optimisation of reaction time and termination solvent. At temperatures higher than 30 °C, considerable spontaneous FDA hydrolysis occurred (Breeuwer et al. 1995; Green et al. 2006), affecting the accuracy of results (Guilbault and Kramer 1964). Considering these points, the influence of temperatures ranging from 20 to 35 °C was investigated.

To obtain the optimum reaction time, the reaction time ranging from 0 to 180 min was investigated. Previous studies have found a positive relationship between the amount of product produced and reaction time (Deng and Tabatabai 1994). However, Green et al. (2006) suggested that the reaction time should not be too long because microbial proliferation increases with extended time.

A termination solvent was needed to simultaneously stop the reaction of samples and blanks prior to analysis to avoid possible deviations. According to the soil assay (Adam and Duncan 2001; Green et al. 2006), acetone and chloroform/methanol (2:1 v/v) were chosen as termination solvents for the FDA hydrolysis reaction in bioaerosol samples.

If the sampling duration was too short, the concentration of microorganisms would be too low to measure. Conversely, a long sampling duration could cause a loss of microorganism viability due to impaction and pressure during sampling, which would result in artificially low microbial activity (Chen 2005; Xu et al. 2011). To determine the appropriate sampling duration, we collected 16 samples in 4 days. Four samples with different sampling durations (20, 30, 40 and 50 min) were collected using four samplers on each day.

The procedure for measuring microbial activity was determined according to the experimental results, which are discussed in Sect. 3.

2.4 Measurement of activity

Sodium fluorescein salt was used as a standard to assess FDA hydrolysis in bioaerosol samples because it releases the same yellow-coloured fluorescein acid as FDA. Additionally, the concentration of sodium fluorescein was calculated and used as an indicator of the level of microbial activity in bioaerosols. Similar to the reaction conditions of bioaerosol samples, the standards were placed at 30 °C for 150 min in the dark.

After the reaction was terminated, the fluorescence intensity of samples and blanks was measured using fluorescence spectrophotometry ($\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 530$ nm). The results from the experimental samples were corrected by subtracting the blank intensity from the measured values.

3 Results and discussion

3.1 Development of the method for measuring activity

3.1.1 Elution medium

When the fluorescence intensity varied from 0 to 4000, a good linear correlation was observed between the

fluorescence intensity and the microorganism concentration in both pH 7.6 phosphate buffer and physiological saline. The background absorbance of pH 7.6 phosphate buffer was as high as 58.288. Bioaerosols contain few microorganisms, and their resulting fluorescence intensity, which represents microbial activity, was less than 10 in our preliminary experiments. Therefore, the fluorescence intensity of the pH 7.6 phosphate buffer was too high to give a reliable measurement of microbial activity. However, the background fluorescence intensity of physiological saline was only 0.7141, and physiological saline is suitable for microorganism survival. Because of these factors, physiological saline was chosen as the best elution medium for the microbes on the membranes.

3.1.2 FDA substrate concentration

Figure 2a shows the results from FDA concentrations ranging from 0 to 800 $\mu\text{g/mL}$. We found that the fluorescein peaked at 100 $\mu\text{g/mL}$ FDA. The influence of FDA at concentrations of 0 to 100 $\mu\text{g/mL}$ on microorganisms is presented in Fig. 2b, which shows a positive correlation and a peak value of 100 $\mu\text{g/mL}$. According to these two series of experiments, 100 $\mu\text{g/mL}$ FDA was chosen as the best substrate concentration.

3.1.3 Coating materials

Our results showed that glycerol had an inhibitory effect on fluorescein in both groups, as indicated by the downward trend observed in the plot in Fig. 3a, b. Thus, glycerol did not maintain microbial activity and was not suitable as a membrane coating material. Similar to results in the control groups, the fluorescein concentration observed in the microorganism groups increased along with the sodium alginate concentration (Fig. 3c, d). Membranes coated with sodium alginate yielded results that would not reflect the true microbial activity in bioaerosols. Therefore, sodium alginate was not suitable for use as a coating material. The influence of PEG on fluorescein was studied using two different concentration groups. C0, C20 and C30 % were considered the low-concentration group. Figure 3e suggests that the 20 and 30 % PEG solutions had no significant influence on the fluorescein concentration observed in the control groups. The high-concentration group, which included C0', C40 and

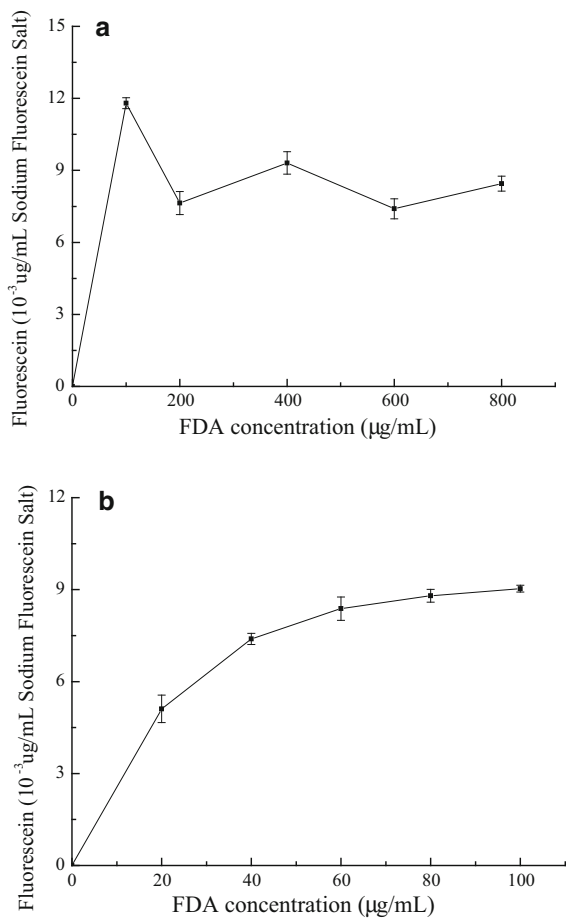


Fig. 2 Influence of concentrations of FDA on microorganisms. **a** 0–800 µg/mL, **b** 0–100 µg/mL

C50 %, had an influence similar to the low-concentration group. In addition, as indicated in Fig. 3f, neither high nor low PEG concentrations caused significant variations in the microorganism groups. These results indicated that PEG had no significant effect on the fluorescein signals of blank or microorganism groups. Therefore, the PEG solution could be used as a coating material without affecting the measurement of true microbial activity levels in bioaerosol samples. Considering viscosity and the ability to form the PEG film, a concentration of 40 % was chosen for use in the subsequent experiments.

3.1.4 Reaction temperature

Figure 4a shows that the fluorescein concentration increased significantly as the temperature rose from 20 to 30 °C and then remained stable or slightly decreased

as the temperature rose from 30 to 35 °C. This phenomenon may be ascribed to spontaneous FDA hydrolysis and the inhibition of enzymatic activity in microorganisms at temperatures higher than 30 °C. Considering the risk of spontaneous hydrolysis and the esterase activity discussed above, 30 °C was chosen as the optimum reaction temperature.

3.1.5 Reaction time

Figure 4b shows the relationship between fluorescein concentration and reaction times ranging from 0 to 180 min. The fluorescein concentration rose as reaction times increased from 0 to 150 min. However, after 150 min, the fluorescein concentration began to decrease. To find the best reaction time, we studied the reaction from 110 to 160 min. As indicated in Fig. 4c, the fluorescein concentration reached a relatively steady state from 140 to 160 min. In the end, 150 min was chosen as optimal reaction time to limit the risk of microbial proliferation while ensuring sufficient FDA hydrolysis.

3.1.6 Reaction termination

As shown in Fig. 5a, fluorescein concentration continued to increase following the addition of acetone, indicating that the acetone failed to terminate the hydrolysis reaction. A 2:1 volumetric ratio of chloroform/methanol also did not fully terminate the reaction but did halt it for approximately 40 min (Fig. 5b). This result was similar to that of Adam and Duncan (2001), who proposed chloroform/methanol (2:1 v/v) for terminating FDA hydrolysis in soil samples. Therefore, we concluded that a 2:1 ratio of chloroform/methanol (v/v) was efficient enough to stop hydrolysis and keep the fluorescein stable for up to 30 min, which provided sufficient time to measure a large number of samples while still ensuring consistency of the results. In addition, chloroform can facilitate the extraction of fluorescein by solubilising cell membranes. Therefore, a 2:1 ratio of chloroform/methanol (v/v) was chosen as the termination solvent, and 30 min was chosen as the effective termination time.

3.1.7 Sampling duration

As shown in Fig. 6, the fluorescein concentration reached its peak at a sampling duration of 40 min. This

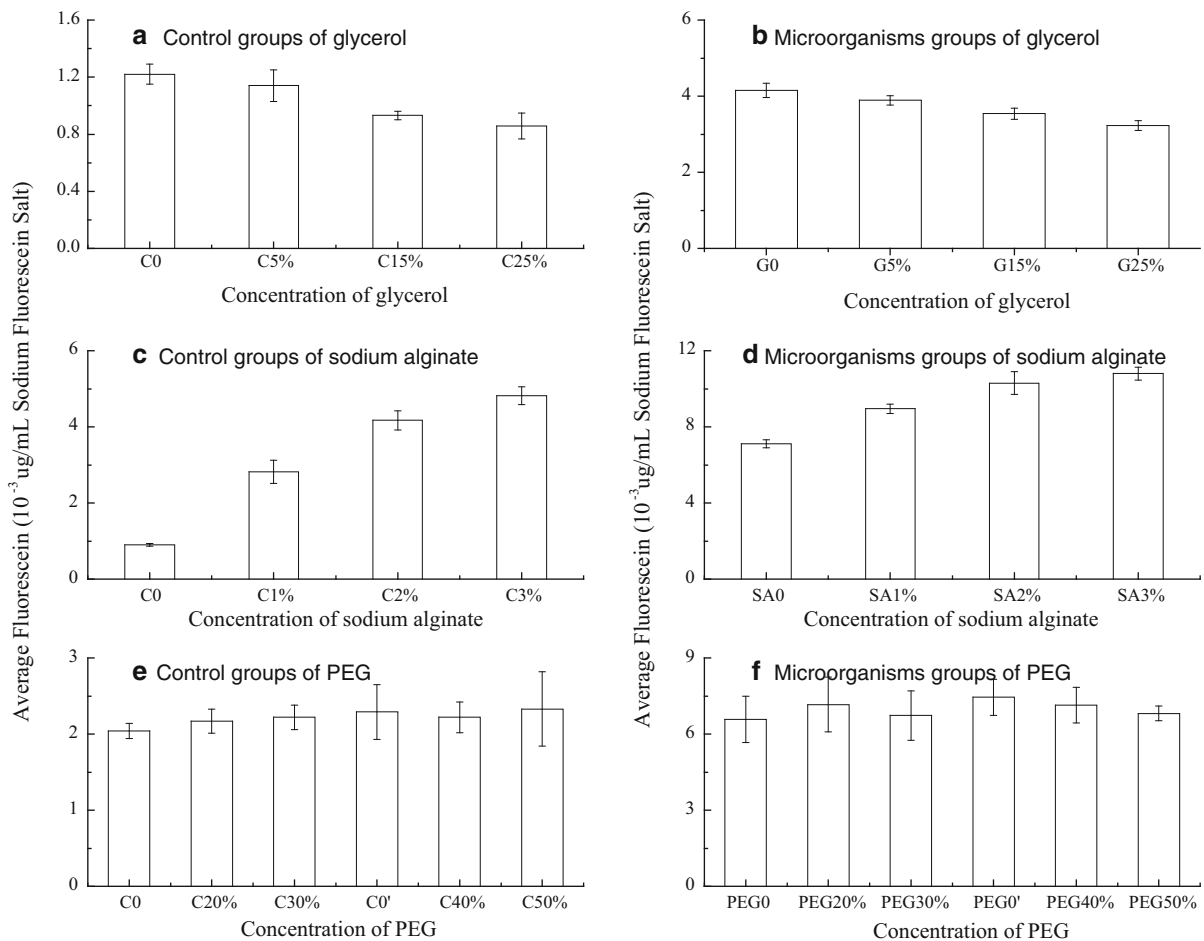


Fig. 3 Influence of different concentrations of coating material on control and microorganisms groups. **a** Control groups of glycerol, which contained 200 mL sterilised physiological saline and different concentrations of glycerol; **b** microorganism groups of glycerol, which added microorganisms to the same conditions as in the control groups; **c** control groups of sodium alginate, which contained 200 mL sterilised physiological saline and different concentrations of sodium alginate;

d microorganism groups of sodium alginate, which added microorganisms to the same conditions as in control groups; **e** control groups of PEG, which contained 200 mL sterilised physiological saline and different concentrations of PEG; **f** PEG microorganisms groups of PEG, which added microorganisms to the same conditions as in control groups; Concentration of glycerol, sodium alginate and PEG were shown in as a percentage concentration

phenomenon may be ascribed to the damage suffered by microorganisms in bioaerosols due to continuous stress and mechanical impact during long sampling periods. To collect samples with high microbial activity and ensure that measurements reflected the true level of microbial activity, 40 min was chosen as the optimal sampling duration.

3.1.8 The optimised method

According to the experiments described above, we develop a method to measure the total microbial

activity in atmospheric bioaerosols using FDA hydrolysis. After the pre-treatment as shown in Fig. 7, the fluorescence intensity of samples and blank are measured by fluorescence spectrophotometry, and the concentration of sodium fluorescein is calculated using the sodium fluorescein standard curve ($R^2 = 0.995$). The total microbial activity in bioaerosols is represented by the concentration of sodium fluorescein.

To determine the applicability of measuring microbial activity in bioaerosols using FDA hydrolysis, parallel samples were collected every 3 days from

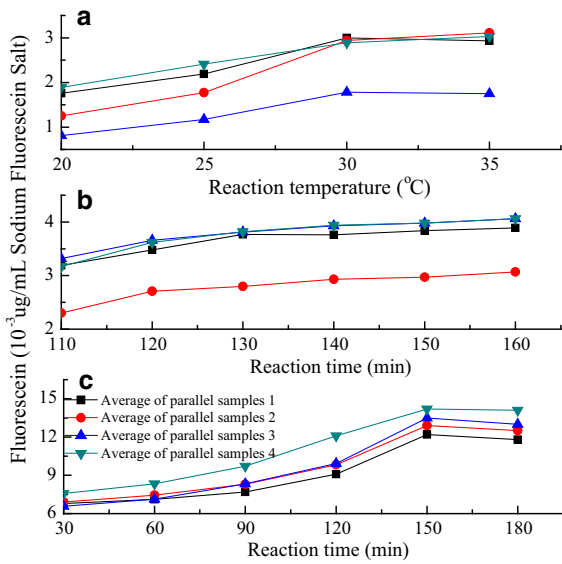


Fig. 4 Effect of reaction temperature and reaction time on microbial activity. **a** Reaction temperature, **b** reaction time of 0–180 min, **c** reaction time of 110–160 min

May 2, 2012, to May 29, 2012. The average fluorescein concentration of 20 blank samples was $(0.276 \pm 0.099) \times 10^{-3} \mu\text{g/mL}$. The SD of the two parallel samples ranged from 0.10 to 0.35, and the average SD was 0.20, which indicates high precision of the established method.

3.2 Microbial activity level in bioaerosols from the Qingdao coastal region

3.2.1 Monthly variation in activity

To calculate the level and monthly variation in microbial activity in bioaerosols, we collected samples in the Qingdao coastal region in May and from September to December 2012. The microbial activity in the bioaerosols, which varied greatly, was represented by the average of parallel samples. Figure 8 shows that the microbial activity ranged from 10.7 to 85.8 ng/m^3 sodium fluorescein during the sampling period and that the average level of microbial activity was 30.2 ng/m^3 sodium fluorescein.

Table 1 showed monthly averages of microbial activity in bioaerosol samples and meteorological information. In May 2012, the microbial activity was as high as 44.3 ng/m^3 sodium fluorescein, which was much higher than in the other months. The activity in

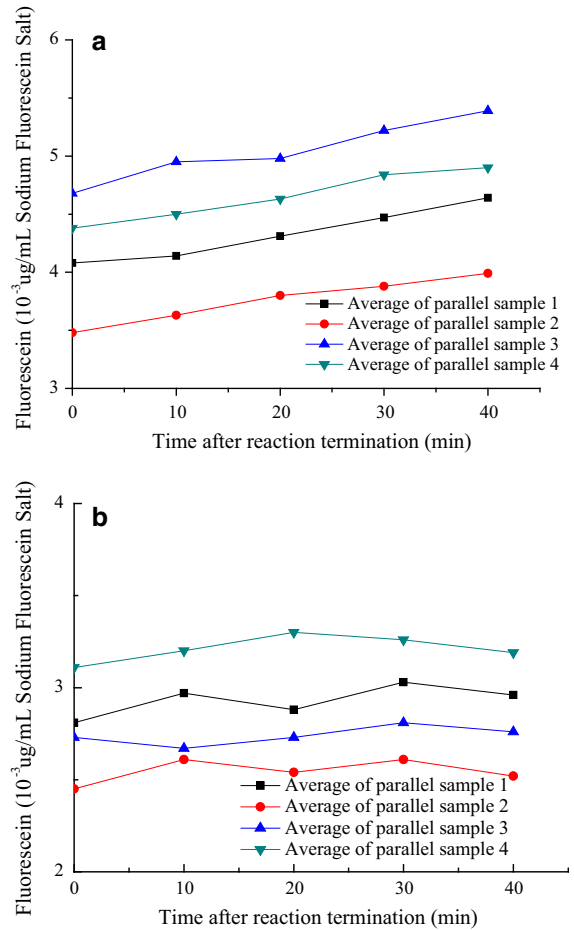


Fig. 5 Fluorescein concentration with variation in time after addition of termination solvent was added into the solution. **a** acetone, **b** chloroform/methanol (2:1 v/v)

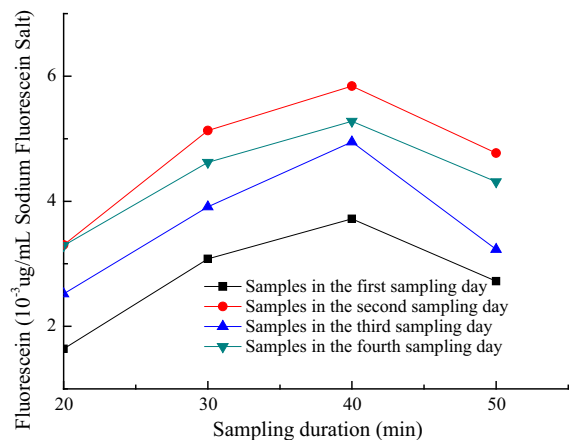


Fig. 6 Changes in fluorescein concentration variation with variations in sampling duration

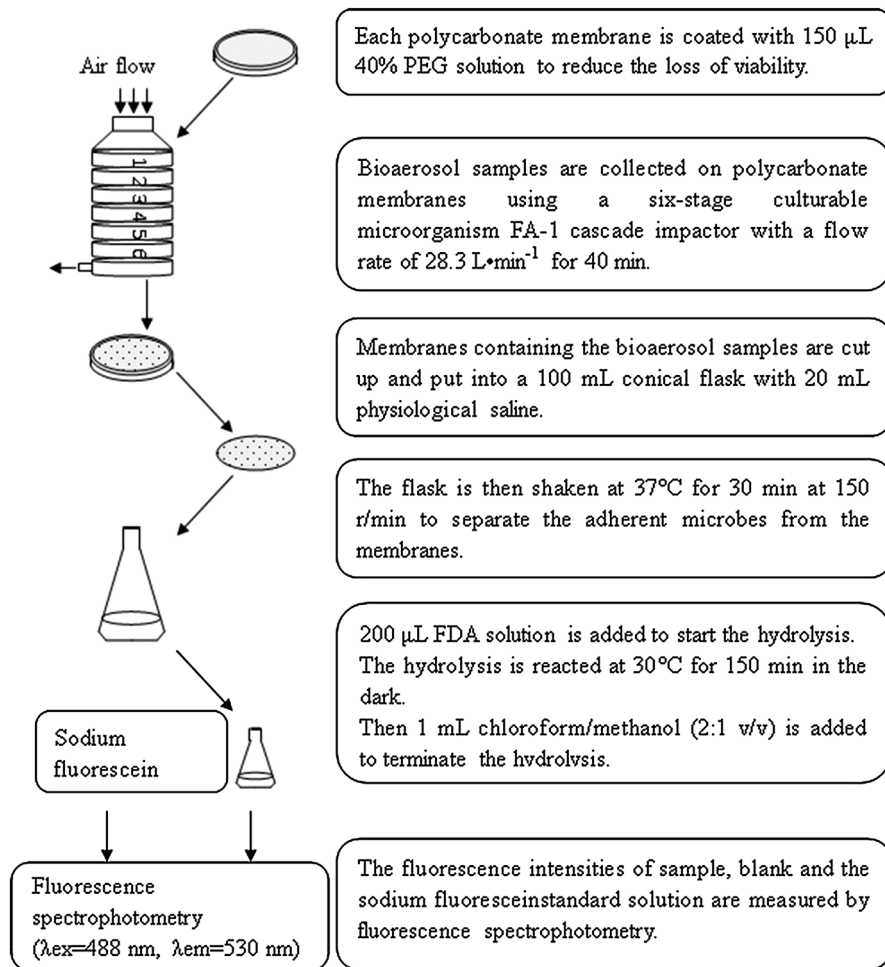


Fig. 7 The optimised method to measure the total microbial activity in bioaerosols using FDA hydrolysis

October showed a higher value, as well. The activities in November and December were similar, while the lowest activity was measured in Sep. Previously, it has been claimed that a positive correlation exists between the concentration of airborne microorganisms and the atmospheric temperature and that the appropriate temperature for bioaerosol microbes to survive and propagate is between 20 and 30 °C (Fang et al. 2004; Li et al. 2011). Many studies have found that microorganisms are more likely to survive and spread at a low relative humidity (Bowers et al. 2012; Tong and Lighthart 2000; Li et al. 2011). The average relative humidity (RH) was similar in these months, but the temperature varied greatly. As shown in Table 1, we found that the temperature was approximately 17 °C in May and October. We concluded that the high microbial activity levels observed in May

and October were due to the suitable temperature and humidity. Moreover, variations in wind between these 2 months will result in the presence of microbes from different sources. In May, more suspended particles are present, including pollen, catkin, and others, because many plants grow and blossom out in the spring. As a result, there are likely more sources of microbes in May compared to the other months included in the sampling period. These factors are conducive to the growth and propagation of microbes, and result in an increase in microbial activity. Furthermore, high wind speeds in the spring are advantageous for the spread microbes in the atmosphere. Together, these factors likely account for the high microbial activity observed in May. In Qingdao, leaves begin to fall from the trees in October, increasing the concentration of microbes in the

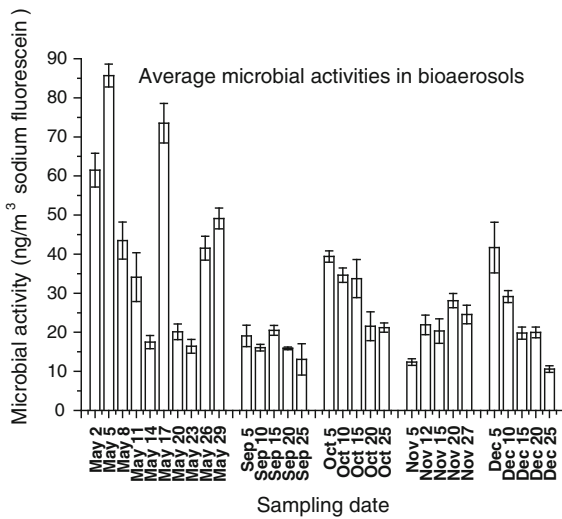


Fig. 8 Average microbial activity in bioaerosols in Qingdao from May to Dec., 2012

atmosphere. The microbial activity most likely increased in October due to suitable temperatures, RH, abundant microbes, large numbers of suspending particulates and sufficient nutrients. As the temperature gradually dropped in November, the microbial activity was concomitantly reduced. In September, the average temperature was 22 °C, but the microbial activity was low. This result may be ascribed to other meteorological parameters such as solar radiation and strong ultraviolet radiation (Fuzzi et al. 1997; Tong and Lighthart 1998).

The highest microbial activity was observed on May 5. The temperature on May 5 was moderate (21.2 °C), and the relative humidity was low (57 %), providing suitable conditions for microorganisms. The activity showed a high value of 73.5 ng/m³ sodium fluorescein on May 17, a little lower than the highest value on May 5 (85.8 ng/m³ sodium fluorescein). On May, a large amount of catkin material was floating in the atmosphere, providing plentiful organic matter. Additionally, the weather was cloudy on May 5, which meant that the relative intensity of radiation from the sun was weak and the UV damage to the microorganisms was low (Tong and Lighthart 1998). All of the environmental conditions were suitable for microorganisms to survive and propagate in the atmosphere, leading to high microbial activity in bioaerosols. The high value appeared on May 17 as the meteorological condition ($T = 19.8$ °C, $RH = 37$ %) was similar to May 5. This high level of activity may be detrimental to environmental quality and trigger respiratory disease more easily. The microbial activity was the lowest on December 25. According to the meteorological parameters on December 25, the temperature was as low as -5.9 °C, which inhibited the viability and propagation of microorganisms.

3.2.2 Impact of meteorological parameters on activity

To understand the impact of meteorological parameters on microbial activity in bioaerosols, we investigated the

Table 1 Average microbial activity and sampling information during the sampling period in 2012

Sampling date	T (°C) 08 a.m.	RH (%) 08 a.m.	WS (m/s) 08 a.m.	Activity	SD
May 2–May 29, 2012					
Range	10.20–22.20	37–97	1.50–5.40	44.3	3.44
Average	17.17	77.3	3.01		
Sept. 5–Sept 25, 2012					
Range	20.30–22.90	64–75	2.00–3.40	16.9	1.84
Average	21.98	70	2.64		
Oct. 2–Oct 25, 2012					
Range	15.60–19.70	49–75	1.40–5.60	30.1	2.61
Average	17.88	66.4	3.64		
Nov. 5–Nov 27, 2012					
Range	3.50–5.90	42–76	2.40–7.00	21.4	2.14
Average	5.16	63.6	4.30		
Dec. 5–Dec 25, 2012					
Range	-5.90–2.80	38–92	1.60–8.90	24.2	2.35
Average	-0.78	64.6	4.80		

T, RH and WS represent temperature, relative humidity and wind speed, respectively; Activity expressed by the ng/m³ sodium fluorescein; SD represents standard deviation of the two parallel samples

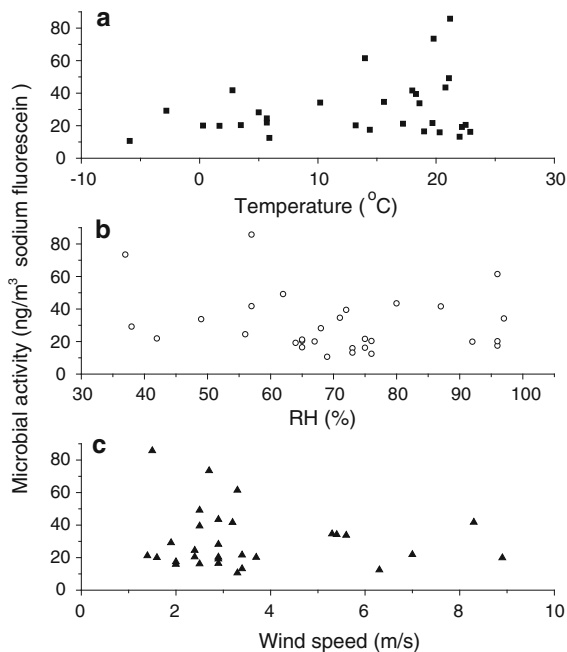


Fig. 9 The correlation of microbial activity in bioaerosols with temperature (a), relative humidity (b) and wind speed (c)

correlation of activity with temperature, relative humidity and wind speed. As shown in Fig. 9, we found that activity increased with rises in temperature during the study period. However, there was no statistically significant correlation between temperature and microbial activity ($n = 30$, $P > 0.05$). Temperature is an important environmental factor that affects many aspects of microbial physiology. In general, microbes grow at higher rates with higher temperatures and stop growing when temperatures rise beyond the critical point (Slonczewski et al. 2009). During our study, the highest atmospheric temperature was 23 °C, below the temperature at which critical enzymes or cell structures fail. At cold temperatures, microbial growth slows because enzymatic processes become too sluggish and fluidity of the cell membrane decreases (Slonczewski et al. 2009). Therefore, the microbial activity becomes lower at temperatures below 0 °C, as was the case for the sample collected on December 25. Many microbial species are present in the atmosphere, and each microbial has its own optimum and threshold temperatures that define the limits of growth. This may explain the poor correlation of temperature with activity we observed.

Li et al. (2011) found that airborne bacteria and marine fungal spores might be more abundant in

relatively low RH. The microbial activity will decrease greatly if the environment is too dry due to cessation of metabolism (Zhou and Gao 2000). As shown in Fig. 9, we found that microbial activity was not statistically correlated with relative humidity ($n = 30$, $P > 0.05$). Our results suggest that activity was not affected by relative humidity when the environmental RH was in a range of 37–97 %.

We found that the activity decreased gradually when wind speed increased. However, microbial activity showed no significant linear correlation with speed ($n = 30$, $P > 0.05$). Li et al. (2011) found that wind velocity does not significantly affect culturable airborne microbes, but total airborne microbes (including both culturable and non-culturable microbes) increase when the wind velocity rises. It follows that the influence of wind speed on the atmospheric microbial population is complicated. High wind speeds will be helpful for bringing microbes from the soil surface to the atmosphere; however, the microbial concentration will decrease due to increased deposition velocity of atmospheric particulates under high wind speed.

The atmosphere is an inhospitable climate for microorganisms mainly because of desiccation stress, which results in a limited time frame in which microbes can remain biologically active (Maier et al. 2010). To know the low level of activity is caused by low abundance of microorganisms or the influence of meteorological factors, the abundance of total amounts of airborne microorganisms and living microbial cells needs to be studied in future.

4 Conclusions

The microbial activity level in bioaerosols can be successfully measured using FDA hydrolysis. The microbial activity in bioaerosols displayed monthly variations, most likely related to meteorological factors. Microbial activity in bioaerosols was not significantly correlated with temperature, relative humidity and wind speed. However, the temperature and RH are very important to microbial growth, so the following may explain the weak correlation observed: first, the activity we measured was the summation of activity from all species of airborne microbes, each of which had its own specific maximum growth temperature and the other environmental conditions. Mixing of these species most likely made it difficult

to find correlations between activity and environmental factors. Secondly, the sampling time was <1 year, and the data collected was limited. Therefore, the influence of environmental factors on microbial activity, as well as the mechanisms by which this influence occurs, should be studied further.

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