The Collection Method for Crop Fungal Spores Based on an Efficient Microfluidic Device

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ABSTRACT

Nowadays, the airborne fungal spores have always taken an important role in the spread of crop fungal diseases and caused great concern. In this study, a novel efficient microfluidic chip for enriching the airborne fungal spores directly from gas flow was developed, which has better portability and cost-efficiency. The chip consists of three parts: half-wave pretreatment channel, inertial impactor, and low-pressure collection chamber. During the collection process, the particles were arranged in the radial position of the half-wave pretreatment channel based on their sizes, then separated by the inertial impactor and collected in the low-pressure collection chamber. The size distribution of the collected target was calculated by image process and recognition. The results show that the cut-off sizes of the proposed microfluidic device was found to be 4.83 µm (first-stage) and 0.98 µm (second-stage), respectively. The sharpness of the first and the second-stage collection efficiency curves were 1.31 and 1.79 respectively. The bounce effects and re-entrainment of particles can be eliminated by the low-pressure collection chamber without any silicon oil or grease. The collection reproducibility is acceptable. As a result, the proposed microfluidic chip can realize the crop fungal spore collection efficiently and can be used to improve the development of real-time crop fungal diseases monitoring technology.

Keywords: Crop fungal spores; Microfluidics; Separation performance; Pretreatment.

INTRODUCTION

Nowadays, crop fungal disease has become a big concern (Nugaeva et al., 2005; Chen et al., 2018). Free state crop fungal spores, especially rice false smut spores, can infect the rice floral and cause rice false smut under suitable temperature and humidity conditions (Akhmadeev et al., 2016). In addition, the rice false smut is also harmful to human beings and can cause many diseases, such as respiratory illness (Gratton et al., 2011; Li et al., 2017) and aflatoxin poisoning (Umemura et al., 2014). Therefore, an increasing need for accurate and in-time monitoring of crop fungal spores has emerged.

The conventional method of fungal spore recognition is the image recognition method (Antkowiak et al., 2008; Akhmadeev et al., 2016), which provides a basis for the intelligent and automated detection of crop diseases. Secondly, the weighing method has been used to complete high precision weighting detection of microparticles, and the minimum detection scale has reached the microgram level (Chan et al., 2014). Thirdly, the application of polymerase chain reaction (PCR) in the detection of crop pathogenic fungal has been very mature (Sireesha et al., 2018). However, the advantages of these methods are limited in fungal spore detection due to the existence of a large number of impurities. Therefore, an efficient separation and collection method is necessary for improving the detection accuracy.

In the impurities removing process, different kinds of crop fungal spores have different shapes and size distributions. For example, rice false smut spores were almost spherical and have a size distribution of 1–5 µm, while the rice blast spores are pyriform and the size distribution is 6–14 µm. There are also some other objects defined as impurities existing in the real detection environment, which size distribution ranges from 20 nm to 100 µm. Nowadays, the impact principle for specific particle size collection has been developed perfectly (Sing et al., 2018; Wada et al., 2016). The working principle is quite simple (Djoumi et al., 2018) and can be described as below. Particles larger than a threshold size will be collected by the impaction plate due to the sufficient inertia, while particles smaller than the threshold size will continue moving following the airflow. In this section, the threshold, which is called the cut-off size with a collection efficiency of 50%, was determined by the
Structural Design of the Microfluidic Device

Fig. 1 shows the schematic diagram of the proposed microfluidic chip structure, which composes of half-wave pretreatment channel, inertial impactor, and low-pressure collection chamber. In the design of pretreatment channel, one inner sidewall (as shown in Fig. 1(A), labeling 2) called half-wave structure was varied (from 0 µm to 500 µm, interval 50 µm), and the half-wave spacing between each half-wave was fixed at 300 µm. To ensure the pretreatment effects of spores, all half-wave structure with the same radius was repeated twice. Meanwhile, the other inner sidewall (as shown in Fig. 1(A), labeling 1) was designed as a plane.

In this study, a novel microfluidic device, which consists of pretreatment channel, inertial impactor, and the low-pressure collection chamber, was proposed to collect the crop fungal spores. Both numerical analyses and experiments were performed to obtain optimum design parameters and evaluate the feasibility of the microfluidic device. In the working process, the collected bioaerosol particles were firstly arranged in a radial position by the pretreatment channel to achieve a satisfying sharpness. Then, an inertial impactor was used to separate the collected bioaerosol particles in terms of their sizes. The low-pressure collection chamber was used to collect the bioaerosol particles to eliminate the bounce effects and re-entrainment of particles without silicon oil or grease. Therefore, the proposed microfluidic device can be directly used as the collection part of the real-time detection equipment of crop fungal diseases.

Principle of Airborne Spore Collection

When particles suspended in the gas flow were injected into the traditional inertial separation system, some particles with enough momentum can cross through the streamlines and be separated. However, the others which have insufficient momentum will flow away following the deflected airflow. This behavior of the particles in a curved channel can be characterized by the Stokes number (Rader et al., 1985),

$$\text{stk} = \frac{\rho_p d_p^2 C_c V}{9 \mu W}$$  \hspace{1cm} (1)

where $d_p$ is particle size (m), $\rho_p$ is the particle density (1000 kg m$^{-3}$), $\mu$ is the air viscosity ($1.81 \times 10^{-5}$ N·s·m$^{-2}$), $V$ is the air velocity at the microfluidic device inlet (m s$^{-1}$), $W$ is the nozzle width (m). $C_c$ is the Cunningham slip correction factor, based on particle size, which can be obtained by the following Eq. (2) (Rader et al., 1985):

$$C_c = 1 + 0.5 Kn_p [2.34 + 1.05 \exp(-0.195 Kn_p)]$$  \hspace{1cm} (2)

where $Kn_p$ is particle Knudsen number, which is defined as $2\lambda/d_p$, where $\lambda$ is the mean free path of the air molecule (m).

Fig. 1. The schematic diagram of the proposed microfluidic chip: (A) Three-dimensional structure, in this figure, 1 and 2 represent the plane and half-wave structure, respectively. 3 represents the nozzle, which was used to improve the velocity of particles. 4 represents the low-pressure collection chamber. (B) The image of a two-stage microfluidic device. A part of the pretreatment has been amplified, R and L represent the half-wave channel radius and the half-wave spacing, respectively.
In addition, $d_{50}$, the cut-off size which yields a 50% collection efficiency at each impaction stage, can be rearranged by Eq. (1):

$$d_{50} = \sqrt{\frac{9 \rho W \pi \sigma}{\rho_p C V}}$$

(3)

where $stk_{50}$ represents that the Stokes number corresponds to 50% particle collection efficiency. The numerical $d_{50}$ at each stage was 5.11 (first stage) and 0.99 µm (second stage), because the target bioaerosol element, referring to rice false smut spore, has a size distribution from 1 to 5 µm. Relatively large bioaerosols were removed at the first low-pressure collection chamber, the target bioaerosols were collected at the second stage, and small bioaerosols less than 1 µm are through the microfluidics.

**Principle of Half-wave Pretreatment Channel and Low-pressure Collection Chamber**

In the pretreatment process, the particle trajectory was determined by the combination of inertia force and radial drag force. As shown in Fig. 2(A), some particles with the same sizes were injected into the channel at different positions when they reach the first-stage impactor, the particles near the inner wall will escape following the deflected airflow due to the insufficient inertia. However, particles near the outer wall are different, they can be easily collected because of the light effects of deflected gas flow. Fig. 2(B) shows the particle trajectories of two different size particles when they start from the same position. Due to the strong inertia force and radial drag force, the magnitude and direction of moving particles are constantly changing when particles pass through the half-wave pretreatment channel. In this process, small particles have a greater tendency to follow the gas flow, the larger particles may appear a significant offset in the radial direction. Therefore, particles with different sizes can be arranged in the radial position from the plane to the half-wave structure. With the radial position and velocity distribution coupling, the large particles and collected target collection efficiency can be improved in the first low-pressure collection chamber and second low-pressure collection chamber, respectively. However, when the particle size is very large, the above rule will be not applicable because larger particles will remain their original trajectories when the inertia force was much stronger than the radial drag force.

Then, the drag force will play a dominant position under a certain flow velocity when the spores collected by the low-pressure collection chamber (Hinds et al., 1999). According to the drag force equation, in this motion, particles with different sizes will experience different drag forces, which means that spores and impurities will realize a secondary separation after the inertial separation. In addition, particles begin to settle in a vertical direction when collected by the low-pressure collection chamber. During this process, different substances have different shapes, so the accelerations that reach the same settling velocity are different and result in the difference in horizontal displacement. Therefore, the spores and impurities with the same aerodynamic diameter can also realize the further separation in the low-pressure collection chamber.

In this study, the particle size range of rice false smut spore is between 1 and 5 µm, the offset is increasing during this size range. The trajectories are derived from the integration of force balance on Newton’s second law of motion (Liu et al., 2015). The impact force was influenced by several factors including the half-wave channel radius, the half-wave spacing, the velocity of the particles and the particle size of the particle. The general function of the longitudinal offset ($L_f$) in the half-wave channel can be expressed by the following Eq. (4):

$$L_f = L(R, L, V, Kd_p^2 + c)$$

(4)

where $R$ is the half-wave channel radius (m), $L$ represents the half-wave spacing (m), $V$ is the air velocity (m s$^{-1}$). $K$ is the particle motion coefficient, $c$ is the initial position existed in the inlet of the proposed microfluidic device.

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**Fig. 2.** (A) The trajectory of the particle in the collection area. The width of the inertial impactor inlet was recorded as $W$, the width of the inertial impactor outlet was recorded as $W_1$, the chord length of the low-pressure collection chamber was recorded as $W_2$. (B) The sketched view of the motion mechanism: the small particles closely follow the streamline, while large particles appear a significant offset.
Numerical Analysis
A numerical analysis was conducted by using commercial computational software (COMSOL Multiphysics). In each simulation, 100 identical particles were evenly distributed in the microfluidic chip inlet. After calculating the flow field, the discrete phase model (DPM) with the Lagrangian approach was used to simulate the particle trajectories.

1. The number concentration of the airborne spores which collected by the microfluidic device was low. So, the flow field was not distributed by the particles (Kang et al., 2012).
2. The density and aerodynamic diameters of the particles were 1.05 g cm\(^{-3}\) and 0.5–8 µm, respectively. The inlet flow rate was 12.5 mL min\(^{-1}\).
3. The walls were set to no-slip. During the simulation, the particle collisions with microfluidic device walls were inelastic, then, the particles adhered to the wall irreversibly, was used to calculate the particle losses.
4. The traditional impaction was replaced with a low-pressure collection chamber, which means that the particle bounce and re-entrainment did not exist neither in the numerical analysis nor experiments.

Evaluation of Fungal Spore Collection Efficiency
The collection efficiency of given particle size can be calculated as Eq. (5) (Ding et al., 2000; Lee et al., 2006),

\[
\eta (d_p) = \frac{\eta_i}{\eta_i + \eta_j} \times 100\%
\]

where \(\eta_i\) represents the number of the particles collected in the low-pressure collection chamber and \(\eta_j\) represents the number of the particles passing through the corresponding individual stage with the airflow. Then, the particle losses of a given particle size is defined as the ratio of the number of particles outside the low-pressure collection chamber to the total number of particles, and it can be evaluated by measuring the collection efficiency except the low-pressure collection chambers (Kang et al., 2012). The sharpness (Lee et al., 2006) of the experimental collection efficiency curve at each low-pressure collection chamber can be calculated using Eq. (6),

\[
\sigma = \frac{d_{50.1}}{d_{50.9}}
\]

where \(\sigma\) is the geometric standard deviation (GSD), \(d_{50.1}\) and \(d_{50.9}\) are the particle sizes with collection efficiencies of 84.1% and 15.9% at each stage, respectively (Hinds et al., 1999).

MATERIALS AND EXPERIMENT
Spore Sample Preparation
The rice false smut spore suspension used in the experiment was provided by China national rice research institute and the laboratory character number of the fungal spore is DQ1701. The rice false smut spore suspension was cultured through liquid shake on the MS mediums. Then, the concentration of the rice false smut spores is about \(4.2 \times 10^8\) per milliliter according to microscopic examination, and 10 mL rice false smut spore suspension was taken during the experiment.

Microfluidic Device Fabrication
The microfluidic chip was fabricated using a conventional soft-lithography process. Firstly, Auto CAD 2014 was used to draw the microfluidic chip structure and use the film to make a mask. Secondly, SU-8, a negative photoresist, was spin-coated at rotational on a Si wafer to yield a layer height of 100 µm. Exposing the photoresist by ultraviolet lithography, and using a particular developing solution (1-methoxy-2-propyl acetate, Microchem) to develop the SU-8 photoresist pattern to yield a master mold. Finally, polydimethylsiloxane (PDMS) was poured over a petri dish and cured in an oven at 65°C for 1 h, and bonding the PDMS layer and substrate together.

Experimental Procedure
Fig. 3 shows a schematic diagram of the experimental platform, including the aerosol generator, the microfluidic device, and the measurement system. The rice false smut spore suspension was put in the aerosol generator (24 Jet Collision, BGI Collision), to maintain the particle integrity and biological activity due to the low pressure. Then, compressed air was filtered by a HEPA and entered the aerosol generator at 2 atm for atomizing bioaerosols. A diffusion dryer was placed behind the biological aerosol generator to remove the moisture of the aerosol stream. A \(^{210}\)Po neutralizer was installed behind the diffusion dryer to remove the electrical charge of the rice false smut spores. The flow rate in the microfluidic device was limited to 12.5 mL min\(^{-1}\) by rotameter (6–60 mL min\(^{-1}\), measurement uncertainty is less than 0.66%) and the excess flow was vented to the atmosphere directly. In addition, the outlet of the proposed microfluidic chip was connected by an enrichment chip (only an inlet, a collection area and an outlet to collect the particle which flows away from the microfluidic chip). Finally, we removed the PDMS layer of the microfluidic chip in the laboratory to make the microchannels and collected bioaerosols exposed directly, so the collected particles can be observed by a scanning electron microscope (SEM, Hitachi, S-3400N) after the gold sputtering treatment. Then, the images of the particle collection area were taken by SEM, which were used to calculate the size distribution of rice false smut spores based on Image Processing and Recognition. The whole experiment was carried out for two minutes and repeated three times.

RESULTS AND DISCUSSION
In this section, to seek for the optimal length of the half-wave spacing, different values (from 4 µm to 50 µm) of the half-wave spacing was used to simulate. During the simulations, particles with different sizes (4 µm, 4.5 µm, 5 µm) were applied. As shown in Fig. 4, as the length of the half-wave spacing (L) increases, collection efficiency is continuously increased when L is
less than 300 µm, and the collection efficiency tends to be a constant when \( L \) exceeds 300 µm. Therefore, there is an obvious relationship between collection efficiency and the length of half-wave spacing, and 300 µm was selected as the optimal parameter.

Figs. 5(A) and 5(B) show the variation collection efficiency obtained from numerical parametric study based on the jet area width \((W = 500 \mu m)\) of the inertial impactor, the chord length \((W_2)\) of the low-pressure collection area and the outlet width of the inertial impactor \((W_1, W_2)\) are shown in Fig. 2(A)). As shown in Fig. 5(A)), 50% collection efficiency was achieved when the ratio of \( W_2/W \) is 1.6. In addition, as presented in Fig. 5(B), the particle loss rate gradually decreases as the \( W_2/W \) ratio increases, therefore, the ratio of the jet area width to the outlet width of the separator \((W_1/W)\) is 1.5. Therefore, the parameters of the first-stage of designed microfluidic device were set at \( W = 500 \mu m, W_1 = 750 \mu m, W_2 = 800 \mu m, \) and the parameters of the second-stage were set at \( W = 140 \mu m, W_1 = 210 \mu m, W_2 = 224 \mu m.\)

As shown in Fig. 6(A), taking the radius of low-pressure collection chamber as x-axis, the ratio of particles impact in the wall of the low-pressure collection chamber and particles collected by the low-pressure collection chamber is indicated in the y-axis of the graph. The result shows that the ratio decreased as the radius of the low-pressure collection chamber increased. When the radius equals to 0.875 mm, the ratio approaches to 0. Therefore, the radius of the low-pressure collection chamber was finally set to 1 mm. Fig. 6(B) shows the distribution of particles in the second low-pressure collection chamber, it shows that the particles will become smaller when they are farther away from the inlet of the low-pressure collection chamber, which is consistent with the simulation results. As a result, particles do deceleration in the low-pressure collection chamber and stop moving orderly according to their sizes in general trend. Most importantly, particle bounce and re-entrainment problems were eliminated by replacing the impaction plate with a low-pressure collection chamber without any silicone oil or grease. This design can also reduce the change of a factor of the spores and the possibility of clogging. In addition, the in-situ detection could be realized and it is convenient for us to detect the false smut spore with image processing and recognition.
Fig. 6. (A) Different radius in low-pressure collection chamber corresponds to different ratios. (B) The particle distribution in the second low-pressure collection chamber. The direction of the yellow line arrow shows the flow direction.

Fig. 7. (A) The collection efficiency of rice false smut spores at each stage. A and B curve represent the simulation and experimental results of the second low-pressure collection chamber, C and D curve represent the simulation and experimental results of the second low-pressure collection chamber. (B) The relationship between the particle loss rate and particle aerodynamic diameter.

Fig. 7(A) shows the collection efficiency curve of aerosolized particles at each stage. The experimental cut-off sizes of each stage were 4.83 µm (first-stage) and 0.98 µm (second-stage) respectively, which are slightly different from the simulation results. The relationship between particle loss rate and particle aerodynamic diameter is shown in Fig. 7(B), which indicates the particles loss rate in the microfluidic chip is below 38%. According to the results, the particle losses mainly occurred at two positions: the half-wave pretreatment channel and the acceleration nozzle before the second-stage. During the experimental process, when particles flow in the half-wave pretreatment channel, particles with larger sizes can easily impact the half-wave channel surface due to larger inertia force, which will result in a relatively small cut-off size. In the acceleration region between the first-stage and the second-stage, many particle losses due to the high aspect ratio of the channel (Kang et al., 2014). The sharpness of the first and the second-stage collection efficiency curves were 1.31 and 1.79 respectively. The low-pressure collection chambers were visualized by using SEM. Figs. 8(A) and 8(B) show the deposited particles at the first and second low-pressure collection chamber, respectively. The impurities were surrounded by the blue circle, and rice smut spores were surrounded by a yellow circle. The magnification of the images was 950× and 2000×, respectively.

CONCLUSION

In this study, a novel two-stage microfluidic device which consists of half-wave pretreatment channel, inertial impactor, and low-pressure collection chamber was proposed to collect the crop fungal spores in the airflow directly. The channel parameters including half-wave spacing, $W_1/W$ and $W_2/W$ were 300 µm, 1.5 and 1.6, respectively. The experimental cut-off sizes of the proposed microfluidic device were found to be 4.83 µm (first-stage) and 0.98 µm (second-stage), respectively. Accordingly, the sharpness of the first and the second-stage collection efficiency curves were 1.31 and 1.79 respectively. Particle bounce and re-entrainment can be eliminated by replacing the traditional impaction plate with...
a low-pressure collection chamber. The microscale device can be used as a portable detector because it is easy-integrating, high-precision, and cost-efficient. Therefore, the developed collection microfluidic device can be used for the crop fungal spore collection and lay a foundation for the development of real-time crop fungal diseases monitoring technology.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Fig. 8. (A) is the first low-pressure collection chamber image, particles are clearly visible under magnification of 950 times. (B) is a part of the second low-pressure collection chamber image, in which the rice false smut spores were magnified 2000 times.
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