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Inactivation effects of electrostatic field on 
*Bacillus subtilis*

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**Abstract**

In order to study the inactivation effects of electrostatic field of electret films on *Bacillus subtilis*, a plane–plane electrode system was used to simulate the electric field of the electret films and the viability of *B. subtilis* affected by electrostatic field for different applying durations was investigated. It was found that the survival ratio of *B. subtilis* can be considerably affected by the field and duration. It was also found that the viability of bacillus decreases with the increase of the duration. In addition, the comparative survival ratio (CSR) of *B. subtilis* decreases to 35% even during a short duration as the applied field reaches an enough high value of more than 15 kV/cm. These indicated that the uniform field inactivated the viability of *B. subtilis* availability. Based on the inactivation effect of the applied field on the *B. subtilis*, the effectiveness of charged polypropylene films on the inactivation of *B. subtilis* was measured and discussed.

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**Keywords:** Electrostatic field; Inactivation; Survival ratio; Electret

1. **Introduction**

Severe acute respiratory syndrome, as the major new infectious disease of the century, spread around the world last year. Since the disease badly endangers the
health of people, it is important to find effective means to prevent and eliminate the virus [1]. Thus, the Natural Science Foundation of China started a research project to study the effectiveness of electret filter on the purpose.

Electret filters have been widely used in industrial application such as particulate respirators and air controllers. The charged fibers shape electrostatic field which improves the filtration efficiency by the electrostatic attraction of particles and microorganism [2]. For more than one hundred years, scientists have reported that direct and alternating electrical currents can kill or inhibit the growth of bacteria and yeast [3]. Many efforts have been made to study the inactivation of microorganism by pulse electric field [4–5]. However, we lack database on the inactivation mechanism of bacteria and virus by electret filters which are of stable value to the electrostatic field.

In this paper, plane–plane electrode system was used to study the inactivation effect of electrical field on microorganisms. Bacillus subtilis as the experimental microorganism was dealt with different applying fields and actuation durations. The polypropylene (PP) films, known as one kind of electret media, were charged and used to deal with B. subtilis directly. Comparison between the inactivation effects of both the electrostatics field and charged electret films on the viability of B. subtilis was studied.

2. Experiments

2.1. Samples and setup

A stainless-steel plane–plane electrode system was used to provide uniform electrostatic field. One of the electrodes was linked to positive high voltage while the other was grounded.

_B. subtilis_ was supplied by Food Science & Nutritional Engineering College of China Agricultural University. It was prepared by inoculating a loop of bacteria from a mother dish into 100 cm³ of nutrition broth. The nutrition broth was placed in a constant temperature incubator of 37°C for 16 h before the experiments. The PP films, with 32 mm diameter and 80 μm thickness, were charged by needle-plane electrode system for 3 min under room conditions and the net charge density was measured by an electrostatic charge meter. As the PP film has good hydrobolicity, the _B. subtilis_ suspension was not easy to cover the PP surface uniformly, some changes were made to modify the wettability. The changes had not affected the conductivity and pH value of bacterial solution.

2.2. Procedures

There were three key processes in the experiments. (1) Put diluted _B. subtilis_ suspension on the glass slice or PP film, and make them uniformly cover the slice or film; (2) Place the glass slice between the plane electrodes with or without applied voltage for some duration. The glass slice was put into sterile water and the solution
was shaken for a certain period of time. Four replicates of each sample were plated. The same process was carried out for PP films, except that no voltage was applied. (3) All plates were incubated at 37°C for 24 h. The plate count method was used to count the number of colonies and compare the survival ratio. All experiments were done under room conditions.

3. Results and discussion

3.1. The natural survival ratio of B. subtilis

After the B. subtilis were placed on the grounded electrode for different durations of 0, 10, 30 s and 1–60 min without the applying field, each of the samples was taken out and incubated. The natural survival ratio (NSR) is defined as the fraction of the number of viable colonies after being placed for a period of time in the number of viable colonies when the placed time is 0 s. Experiments were performed repeatedly and all results were expressed as mean values. Fig. 1 shows the NSR of B. subtilis changed along with the placed duration in the case without the applying field. It can be found that the NSR decreases with the increase of the placed duration. When the placed duration is 30 min, the NSR is about 50%. With a further increase in the placed duration, the B. subtilis solutions on the glass slice can be air dried and the nutriments are consumed; thus, the death of a large amount of organisms made the continuation of experiments difficult.

3.2. The comparative survival ratio of B. subtilis after applying voltage

Fig. 2 shows the comparative survival ratio (CSR) of B. subtilis applied with different voltages and actuation duration. The applied electrical fields ranged from 5 to 20 kV/cm with different actuation durations of 5–20 min. To obtain the

![Fig. 1. The NSR of Bacillus subtilis without applying field.](image-url)
inactivation effects of electric field, check experiments of samples without applying voltage were carried out at the same time. After the plates were incubated at 37 °C for 24 h, the colony number was counted and the CSR was calculated. The CSR was defined as the fraction of the viable colony number after voltage was applied in the viable colony number from check experiment. According to this definition, the influences of natural decease of *B. subtilis* on the inactivation effect of applied field can no doubt be eliminated. It was found that with the increase of the actuation duration, the CSR decreased at any given field. The CSR is kept at about 35%, if the applying field is larger than 15 kV/cm. Fig. 3 typically shows the photos of the *B. subtilis* with and without an applying field of 20 kV/cm for 20 min. This further indicates that the applied field affects the viability of *B. subtilis*.

Taking the natural decay rate into account, the total survival ratio (product of the NSR and the CSR) of *B. subtilis* would be no more than 20% when the electrical field intensity reached 15 kV/cm and the actuation duration reached 20 min.

3.3. The inactivation effects of charged electret films on *B. subtilis*

Charged electret films can shape electrostatic field which is dependent on the surface charge density. The surface electrostatic field (*E*, kV/cm) at the centre of the films can be calculated [6]:

\[ E = \frac{\rho_s}{2\varepsilon_0\varepsilon_r}, \]  

where \( \rho_s \) is the surface charge density (\( \mu \text{C/m}^2 \)), \( \varepsilon_0 \) is the permittivity of free space and \( \varepsilon_r \) is the relative permittivity of atmosphere. So Eq. (1) can be deformed as

\[ E \approx 5.65 \times 10^{10} \rho_s. \]  

![](image)  

Fig. 2. The CSR of *Bacillus subtilis* after applied field.
In order to verify the effectiveness of electret films, the charged PP film with a charge density of 12 mC/m² was spread with *B. subtilis* suspensions for a period of time. The check experiments of samples dealt with uncharged PP film were also carried through at the same time. The viability of *B. subtilis* varied along with the actuation duration is shown in Fig. 4. Similar to the result shown in the case of an applied field with 5 kV/cm in Fig. 2, the CSR of *B. subtilis* of charged PP films decreases with the increase of actuation duration as shown in Fig. 4. According to Eq. (2), the surface charge density of PP film was about 12 mC/m² corresponding with $E$ of 7 kV/cm. Thus the electrical field shaped by electrodes used in our experiment correlates well with the one shaped by the electret films. This indicates that the charged PP films show the same inactivation effect on *B. subtilis* as the plane–plane electrode system does. In

![Fig. 3. Photos of colonies of *Bacillus subtilis* with and without applying field for 20 min; (a) bacteria without applied field, (b) bacteria with applied 20 kV/cm field.](image)

![Fig. 4. The CSR of *Bacillus subtilis* dealt with charged PP film.](image)
other words, the electret films can effectively inactivate the bacteria. The experiments indicated that when the field is larger than 15 kV/cm, the CSR of *B. subtilis* is about 35%. According to Eq. (2), if $E$ is about 15 kV/cm, then $\rho_s$ is 27 $\mu$C/m$^2$. Further work, however, should be carried out in the manufacture of higher charge density PP films to improve the inactivation effect and this can be encouraged by the fact that $\rho_s$ of electret films arrived at 30 $\mu$C/m$^2$ without difficulty [7].

4. Conclusions

(1) The NSR of *B. subtilis* decreases with the increase of placed time. When the time is longer than 30 min, the NSR is lower than 50%.

(2) The uniform electrostatic field generated by plane–plane electrodes inactivated the viability of *B. subtilis* availability.

(3) The CSR decreases with the increase of actuation duration.

(4) The CSR of *B. subtilis* decreases to 35% even during a very short duration as the applied field reach to an enough high value of more than 15 kV/cm.

(5) The total survival ratio of *B. subtilis* is no more than 20% when the electrical field reached 15 kV/cm and the actuation duration reached 20 min.

(6) The charged PP films show the same inactivation effect on *B. subtilis* as the plane–plane electrode system does. The electret films can effectively inactivate the bacteria.

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