Importance of the substrate nature to preserve microorganisms’ cultivability in electrostatic air samplers

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Abstract. Recent research shows that electrostatic precipitation is a gentle method to collect airborne microorganisms and preserve their cultivability. However, the corona discharge used to charge the particles and the high electric field used to capture them are known to have a germicidal effect. The present paper investigates this paradoxical situation. Vegetative cells of E. coli and B. subtilis and spores of A. fumigatus and B. subtilis were deposited on different media and subjected to electrostatic fields of different strengths and polarities for controlled time periods. Vegetative cells are inactivated on cultivation agar plates, but remain cultivable when exposed on a stainless steel electrode and transferred afterwards onto agar plates. For the investigated conditions, spores were not affected by the corona discharge. Further experiments with a pH indicator show that chemical reactions occur when an aqueous media is exposed to the discharge. Some of these reactions are likely to create hydrogen peroxide which is known to kill a broad range of microorganisms. It is therefore highlighted that collecting electrodes in electrostatic air samplers should rather be dry conductive media.

1. Introduction

Electrostatic precipitation used as a sampling method (sometimes named electrostatic sampling) is one of the most efficient ways to collect particles by charging the aerosols (charger stage) and collecting them due to electrostatics forces (collection stage). Recently it was shown to be a suitable method to collect bioaerosols and several devices were built to investigate their performance [1]. Compared to impaction, advantages of electrostatic sampling are lower impaction stress [2], pressure drop and power consumption [3]. Electrostatic sampling can also achieve cultivable bioaerosol concentration levels as much as nine times higher than the BioStage impactor [4] and five to ten times higher airborne allergen and toxin concentration levels than the BioSampler [5].

However, the DC corona discharges used in most electostatic samplers to charge airborne particles may be germicidal [6-9]. For instance, the atmospheric point-to-plane corona discharge can be used either to collect airborne particles on a culture medium with the purpose to keep bioagents alive [10] or to sterilize a culture medium [9]. In the field of electrostatic sampling, Yao et al. [11] developed a two stages electrostatic sampler and investigated the impact of high electric fields on microorganisms with several media and conditions. Unfortunately the authors restricted their study to the impact of the electric field that is applied in the collection stage of their sampler; the first stage, composed of a charger that is usually used to charge airborne particles prior to their collection, was not studied. This key point should not be omitted in such studies because the reactive species created by the charger are likely to be responsible for the germicidal effect. Direct exposure and remote exposure seem to be
both equally effective to kill microorganisms [6]. Since numerous samplers developed to collect bioaerosols include a charger stage, the present study investigates the effect of corona discharges on microorganisms. This study extends the conclusions of Yao et al. [11].

Vegetative bacteria of *E. coli* and *B. subtilis*, spores of *B. subtilis* and fungal spores of *A. fumigatus* were plated on solid media and exposed to the corona discharge in the configuration used by Julák et al. [9] and Sillanpää et al. [10]. To be relevant in the field of air sampling these media included solid culture media [2,4,10,12]. Stainless steel plates were also used to investigate the effect of the discharge over dry conductive media [1,13]. Additional experiments were carried out with a pH indicator gel to investigate the chemical impact of the discharge.

2. Material and Method

The corona discharge was generated from the tip of a needle-shaped electrode made of a hollow stainless steel tube ($D_{\text{ext}} = 0.5 \text{mm}; D_{\text{int}} = 0.25 \text{mm}$). The needle was placed vertically, 10 mm above the surface of the exposed plate. Positive and negative discharges were both investigated. A voltage of 10 kV was applied between the two electrodes with a DC power supply (HT 9, Sefelec) for 10 minutes.

Vegetative cells of *E. coli* (ATCC® 9627™) and *B. subtilis* (ATCC® 23857™) were grown in 250 ml of their culture medium during one night at 37°C and 30°C respectively. The resulting bacterial suspensions had concentrations of about $10^6 \text{CFU/µl}$. Endospores of *B. subtilis* (ATCC® 23857™) and fungal spores of *A. fumigatus* (ATCC® 1028™) were already in stock suspensions at a concentration of $10^7 \text{CFU/µl}$. Suspensions were spread out on plates without dilution: 200 µl was plated out on culturing plates; about 10 µl was deposited as little droplets on stainless steel plates and left few minutes at room temperature to dry (Protocols had to be slightly different since stainless steel plates cannot absorb the suspensions). The plates were immediately exposed to the corona discharge. Afterwards cells deposited on stainless steel plates were transferred to appropriate culturing plates. Microorganisms were cultivated overnight. Two Petri dishes were not exposed to the corona discharge and were used as a reference for normal growth.

Bromothymol blue was diluted in hot agar solution (0.5% in weight) to obtain moist solid media such as culture plates. The advantage of these new media is that they can reveal some chemical reactions: Bromothymol blue is yellow when the pH is below 6.0 and blue when the pH is above 7.8. These pH indicator media were exposed to the corona discharge as vegetative cells and spores were.

3. Results

Prior to the experiments on microorganisms, discharges were observed. It appeared as expected that the positive discharge is uniform while the negative discharge is composed of one or two tufts. Next it was verified that the properties of the plates were not altered or deteriorated by exposure to the discharge (surfaces were not altered by arcs and microorganisms can be cultivated onto agar plates previously exposed to a corona discharge).

![Figure 1](image1.png)

**Figure 1.** Pictures of the plates after cultivation. A continuous overlay of vegetative bacteria grew except in a zone right under the needle: a circular inhibition zone for the positive corona discharge; a comet-like inhibition zone for the negative corona discharge.
When culturing media were exposed to discharges of 10kV, a continuous overlay for both vegetative bacteria grew and formed a white layer except in a zone right under the needle (see Figure 1). The positive discharge led to a nearly circular inhibition zone and the negative discharge led to a comet-like inhibition zone of a larger surface area. This latter shape is similar to the inhibition zone observed by Scholtz and Julák [14]. The pictures show also that vegetative bacteria of *E. coli* are more resistant to the effects of the discharges than vegetative bacteria of *B. subtilis*. In this configuration, spores of *B. subtilis* and fungal spores of *A. fumigatus* were not affected by the germicidal effect of the discharge. The same vegetative bacteria were not affected by the discharge when they were exposed while being on stainless steel plates. Results obtained with the most sensitive bacteria, namely *B. subtilis*, are shown on Figure 2. The comet-like inhibition zone seen with the negative discharge is not visible here.

Exposure of pH indicator gels to the positive and negative discharges revealed yellow zones (acidic or discolored areas) and blue zones (basic areas) shown on Figure 3. The former correspond to the inhibition zones observed for vegetative bacteria when located in the central region of these gels. Basic areas do not seem to be correlated to an inhibition of the microorganisms’ cultivability. Also it should be noticed that pH change is not related to an inhibition of microorganisms’ cultivability when located next to the stainless steel counter electrode dipped into the gels.

4. Discussion
The present experiments show that both positive and negative corona discharges are lethal to vegetative bacteria on culture media. On the contrary, no adverse effect was observed with the same microorganisms on a dry conductive medium. Therefore, the mechanism responsible of the microorganisms’ death (or growth inhibition) is likely to be a chemical reaction with the substrate. Electric field strength, ultraviolet radiation and temperature are often considered as candidate to explain the toxicity of electrical discharges. But they seem to play a secondary role [6-7]. Moreover, these processes are independent of the nature of the exposed media so they cannot be responsible for the growth inhibition observed here. Also reactive species generated by the electrical discharge such as ozone do not seem to have a direct role.

The shape of the inhibition zones differs markedly. Exposure to the positive corona discharge resulted in an axisymmetric zone, while the negative discharge resulted in elliptical zones occupying a larger area. These differences were found to be related to the plasma region at the tip of the needle. The positive corona discharge is uniform and axisymmetric so the spot below is axisymmetric. On the contrary the negative one is composed of one or two tufts. Each tuft results in a comet-like inhibition zone.
Electrical current in culture media implies electrolysis. In that case, one electrode should accept electrons and the pH in the vicinity of the electrode should subsequently decreases. At the same time, the other electrode should donate electrons and the pH should subsequently increases. Experiments with a pH indicator confirmed this expected behavior. However microorganisms grew near the counter electrodes dipped into media regardless of the polarity of the discharge. The pH is therefore not directly related to the culture inhibition. Furthermore, the experiments with a negative discharge revealed that two distinct chemical reactions occur right under the needle (Figure 3): one increases the pH (blue area) while the other seems to decrease it or to discolor the dye (yellow areas). Only the last one is correlated with an inhibition of the microorganisms’ growth (or death).

The mechanism of the corona discharge in air is very complex and involves numerous chemical reactions [15]. However a mechanism may be suggested for the negative discharge. It is well-known that the electrons created in the avalanche leave the ionisation region and change into negative ions by attachment to electronegative molecules in the gas (such as O_2 molecules in air) and electron impact dissociation. According to Challenger et al. [16] and Richardson et al. [17] these negative ions combine with water by the following mechanisms:

\[ \text{O}_3^- + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{O}_2 + e^- \]
\[ 2\text{O}_3^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_3^- + \text{O}_2 \]

to create hydrogen peroxide that is highly toxic for microorganisms and that can degrade the dye. Meanwhile, electrolysis increases the pH right under the needle in this configuration (blue area). This mechanism is suspected to be responsible for the yellow and blue spots as well as the growth inhibition observed in the middle of the agar plate.

5. Conclusion
Electrostatic precipitators are very efficient samplers that can be used to collect biological airborne agents. But samples analysis requires to maintain the integrity of the microorganisms and this constraint add design rules: chemical reactions should be taken into account. The reported results suggest that dry electrodes prevent some chemical reactions that are harmful for microorganisms and so they preserve the cultivability.

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