Bactericidal Effects of the UV Radiation Generated by a Negative Corona Discharge

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Abstract. The UV radiation is one of the non-thermal atmospheric pressure plasma agents which can cause bacterial inactivation. In this paper, which shows results of experiments performed for the diploma thesis, authors compare the effects of the UV radiation generated by negative corona discharge (the indirect plasma treatment) on 2 types of microorganism: gram-negative bacteria Escherichia coli and brewer’s yeasts Saccharomyces cerevisiae.

All measurements were performed for two microorganism concentrations. The experimental setup was modified in a way to compare the impact of UV radiation and reactive species and the UV radiation only.

Keywords
Negative corona discharge, plasma decontamination, microorganism inactivation, NTP, UV radiation, plasma agents.

1. Introduction

Non-thermal atmospheric pressure plasma (NTP) is being investigated for biomedical applications for about 20 years and is still a hot topic not only in a highly specialized community. NTP are present in a variety of applications: decontamination of heat-sensitive materials, wound healing, food packaging sterilization, etc.

Plasma can trigger a complex response of microorganisms that may cause their death. This response can be initiated mainly by different plasma agents: charged particles, reactive species and UV radiation. It is no doubt that inactivation of microorganisms is caused by synergetic effect of all plasma agents. The role of each agent separately is a topic of great interest nowadays. A present study is dedicated mainly to the role of UV radiation generated by the negative corona discharge.

When a cell of a microorganism is affected by UV light the whole incident energy of the photon is absorbed by a nuclei acid of the cell. The replication ability is discontinued by forming of the thymine dimers. The irreversible changes can be caused when the intracellular repairing system is impaired [1, 2].

An effect of UV radiation generated by different discharges was studied for several working gases (9O₂, N₂/O₂, air, etc.). The result differs according to the electrical parameters, discharge type and working gas. Many authors agree that the effects caused by exposure to UV radiation in ambient air surroundings don’t play an important role in cell inactivation of microorganisms [3–7]. However, authors who work with synthetized air (N₂/O₂ mixture) declare a major role of UV radiation on microorganism inactivation [8–11].

<table>
<thead>
<tr>
<th>Working gas</th>
<th>Discharge type</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂/O₂</td>
<td>DBD (2.5 kV, 5kHz)</td>
<td>Major role</td>
<td>Eto et al. [8]</td>
</tr>
<tr>
<td>Air</td>
<td>Corona discharge (DC, 15 kV)</td>
<td>Not major role</td>
<td>Timoshkin et al. [3]</td>
</tr>
<tr>
<td>Air</td>
<td>DBD (35 kV, 12 kHz)</td>
<td>Not major role</td>
<td>Fridman et al. [4]</td>
</tr>
<tr>
<td>Air</td>
<td>DBD (20 kV, 2.2 kHz)</td>
<td>Not major role</td>
<td>Laroussi and Leipold [5]</td>
</tr>
<tr>
<td>Air</td>
<td>APPJ (DC, 2 kV)</td>
<td>Not major role</td>
<td>Kolb et al. [6]</td>
</tr>
<tr>
<td>Air</td>
<td>Glide arc (12 kV, 50 Hz)</td>
<td>Major role</td>
<td>Burlica et al. [7]</td>
</tr>
</tbody>
</table>

Tab. 1. The role of UV radiation in inactivation process of microorganisms.

2. Materials and method

2.1 Inoculum

Experiments were performed on gram-negative bacteria Escherichia coli (Czech Collection of Microorganisms, Brno, CZ) and on brewer’s yeasts Saccharomyces cerevisiae (CCM).

Escherichia coli is a gram-negative bacteria which occurs in the digestive tract (intestines) of people and animals. It perform an important part of the healthy human microflora, but at the same time there are E. coli pathogenic stains that are the reason of diarrhea, dysentery pyelonephritis, and the hemolytic-uremic syndrome. E. coli...
is one of the most studied bacteria and its genome is already well known. This bacteria is widely used in microbiological laboratories as a model organism [12].

*Saccharomyces cerevisiae* is a special type of yeast which is used during the preparation of the wine, beer and also for baking. Yeasts are eukaryotic and are usually used as a model organism of human cells [13].

Bacteria were grown on a Mueller-Hinton agar and brewer’s yeasts were grown on Sabouraud Dextrose agar (both were purchased from Oxoid, Brno, CZ).

### 2.2 Antibacterial assay

A concentrated mixture of a physiological saline solution and a microorganism was prepared to use with concentration about 10⁷ or 10⁸ colony forming units (CFU) in 1 ml. This solution was diluted with the physiological saline solution up to seven times in ratio 1:10, so the concentration 100 cfu/ml was obtained. Cultivating medium was prepared in Ø9 cm Petri dish. 1 ml of solution was uniformly deposited on an agar surface so the *E. coli* and *S. cerevisiae* concentration was 10², 10³, 10⁴ cfu per Petri dish.

Contaminated Petri dishes were exposed to a negative corona discharge in two experimental setup modifications. The first one was set in order to perform an indirect plasma treatment, when both UV light and reactive species were driven to agar surface. And the second setup modification was performed to expose the agar surface with UV radiation only.

Negative corona discharge parameters were set a follows: U = 12 kV; I = 400 μA; d = 4 mm (distance between the electrodes). The exposition time and microorganism concentration is shown in Table 2.

Treated Petri dishes were put to a thermostat and heated to 36.7 °C for overnight cultivation. The size of treated area was evaluated and the final concentration of microorganism was calculated with an agar disk method. The obtained results were compared and decontaminated Petri dishes were excluded.

All tests were done on triplicate.

<table>
<thead>
<tr>
<th>Concentration (cfu/ml)</th>
<th><em>Escherichia coli</em></th>
<th><em>Saccharomyces cerevisiae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV</td>
<td>UV + RS</td>
</tr>
<tr>
<td>10²</td>
<td>-</td>
<td>3, 5 min</td>
</tr>
<tr>
<td>10³</td>
<td>10, 20 min</td>
<td>3, 5, 7, 10 min</td>
</tr>
<tr>
<td>10⁴</td>
<td>10, 20 min</td>
<td>3, 5, 7, 10 min</td>
</tr>
</tbody>
</table>

*Tab. 2. The treatment duration for both microorganisms.*

### 2.3 Experimental setup

The negative corona discharge was generated by a simple point-to-plane electrode system (Fig. 1). This system used the high voltage supply UTES HT 2103. The point electrode was made from the injection needle which was bent to 60° angle to establish better stability and more power of discharge. The plane electrode was realized with the copper sheet in which the 9 mm hollow was made. Such electrode configuration let the UV light and reactive species (RS) pass through the hollow, while the charged particles were caught by the hollow electrode.

3 mm thin silica glass plate was used to separate UV radiation from reactive species. The plate was placed right over the Petri dish under the hollow plane electrode.

In the second case instead of silica glass plate a 3 mm plastic plate with a gap in the center was used. The plastic plate was placed on the Petri dish to conserve the same distance between the plane electrode and the Petri in order to expose a treated surface to UV radiation and reactive species.

The polarity of the plane electrode was positive and polarity of the point electrode was negative, the conical negative corona discharge was induced between electrodes.

![Fig. 1. Experimental setup: ampermeter A, voltmeter V, power supply HV. A point metallic electrode realized with a bent needle with a bent angle 60°. 1 plane copper electrode with 9 mm gap, 2 silica glass plate or 3mm plastic plate with a gap in the centre and 3 Petri dish with bacteria or yeasts.](image)

### 3. Results and discussion

Two different microorganisms were exposed to non-thermal plasma generated UV radiation and reactive species and to plasma generated UV radiation only.

No effect of UV radiation was observed neither for *E. coli* nor for *S. cerevisiae*. No significant inactivated area was observed on the surface of the treated agar. The negative result of the treatment might be caused by low power of the generated UV radiation and a big distance between the corona discharge and the surface of the
cultivated medium (3 mm silica glass and about 3 mm of air under the glass plate).

Fig. 2. Inactivation area size evaluated for \textit{S. cerevisiae}. The area of Petri dish is approximately 65 cm$^2$.

Fig. 3. Number of colony forming units left in the surrounding area for \textit{S. cerevisiae}.

Fig. 4. Inactivation area size evaluated for \textit{E. coli}. The area of Petri dish is approximately 65 cm$^2$.

Fig. 5. Number of colony forming units left in the surrounding area for \textit{E. coli}.

Positive results of inactivation is observed for indirect plasma treatment with all its agents. Inactivated areas size were increasing with increasing time of treatment for both microorganisms. The inactivation area depended on the concentration of bacteria and yeasts (see Figure 2 and 3 for \textit{S. cerevisiae} and Figure 4 and 5 for \textit{E. coli}).

The final concentration of bacteria on the Petri dish in the surrounding decreased with time.

4. Conclusion

The experiment with the negative corona discharge showed that UV radiation did not play a major role in inactivation of microorganisms. The power of the discharge is low and it does not produce a large amount of UV radiation to inactivate significant amount of cells. But at the same time the synergetic effect of UV radiation and reactive species play a significant role in microorganisms inactivation.

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References


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