Role of UV Radiation, Solution Conductivity and Pulse Repetition Frequency in the Bactericidal Effects During Pulse Corona Discharges

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Abstract. Inactivation of bacteria Escherichia coli and Enterococcus faecalis by the pulsed corona discharge in liquid phase has been investigated. The reactor with point to plate geometry of electrodes was used for generation of the discharge in liquid phase. The effects of the solution conductivity and the pulse repetition frequency on the bacterial inactivation have been determined. Better efficiency of inactivation was observed for both types of bacteria with the increasing solution conductivity and the pulse repetition frequency since E. faecalis was more sensitive to these changes than E. coli. The role of UV radiation emitted by the electrical discharge in the overall bacterial efficiency was evaluated in dependence on the solution conductivity using UV light transparent spectrometric cell. It was determined that UV radiation contributes about 40% to the overall inactivation of bacteria by the discharge.

Introduction
Previous research has demonstrated that a high voltage pulse electrical discharges generated directly in the liquid phase initiate a variety of chemical and physical processes. These processes include a high electric field, intense ultraviolet radiation, overpressure shock waves and formation of various highly reactive chemical species such as radicals, molecular radicals and ions. It was shown that these processes are capable to efficiently destroy a number of organic compounds and cause serious damages to microorganisms present in the liquid phase [Sunka, 2001; Locke et al., 2006]. However, in comparison with destruction of organic compounds, where the degradation mechanism is attributed mainly to the oxidation by OH radicals, detailed mechanism of plasma-induced microbial inactivation is still largely not known. In principle, it is evident that, in addition to the chemical effects caused by produced reactive species (e.g. OH radical, atomic oxygen, ozone, hydrogen peroxide) plasma inactivation may be induced also by the heat, charged particles, high electric fields, UV photons and shock waves; all commonly present in the electrical discharge plasmas in liquid phase [Laroussi et al., 2003]. However, the contribution of individual processes is not known. In general, the inactivation effect of electrical discharges on microorganisms proceeds by cell wall damage (rupture) and/or by changes in the DNA. It may be expected that the former effect is caused mainly by chemical agents, heat, electric field (electroporation) and shock waves while the latter effect is caused mainly by UV radiation and electric field (apoptosis) [Schoenbach et al., 2007; Sunka et al., 2004].

Concerning the bactericidal effects of UV radiation, UV light (200–300 nm) with doses of several mWs·cm$^{-2}$ is known to cause lethal damage to cells. UV radiation affects the cells of bacteria by inducing the formation of thymine dimmers in the DNA. It suppresses replication of DNA that causes lethal effect to bacteria. Laroussi [2009, 2002] showed that atmospheric-pressure air plasma produces a few amount of UV with insufficient power densities, bellow 50 μW.cm$^{-2}$. Therefore, UV does not play a major role in the inactivation process by atmospheric-pressure plasma.

On the other hand, electrical discharges in liquid phase can emit significant intensity of UV light [Lukes et al., 2008]. Previous research obtained using the emission spectroscopy showed a radiation from the pulsed corona discharge in liquid phase in a wide range of wavelengths (200–1000 nm), which is dominated by the spectral lines of hydrogen (peaks at 434, 486, 656 nm) and oxygen atom (777 nm) and by emission from OH• radical (309 nm) [Sun et al., 1997; Sunka et al., 1999; An et al., 2007]. Consequently, [Lukes et al., 2008] have determined that pulse radiant power (190–280 nm) of the corona discharge in liquid phase could reach levels of the order of tens to hundreds of watts during the pulse, which corresponds to the UV radiation intensity of the order 0.1–10 mW.cm$^{-2}$ in dependence on solution conductivity. In the same paper, the first evaluation of the role of UV radiation on the lethal effects of the pulsed corona discharge in liquid phase was made. It was estimated that the UV radiation contributes about 30% to the overall inactivation of Escherichia coli.
In this work the role of UV radiation in the bacterial inactivation caused by pulsed corona discharge in liquid phase is investigated in more detail. The inactivation effect of pulsed corona discharge is studied in dependence on the solution conductivity (200 and 500 µS.cm⁻¹) and on the type of microorganism, *Escherichia coli* (gram-positive bacterium) and *Enterococcus faecalis* (gram-negative bacterium). In addition, the effect of pulse repetition frequency of applied power to the discharge on the inactivation of bacteria is determined.

**Experimental**

The reactor for generating pulsed corona discharge in liquid phase was described in detail previously [Sunka et al., 1999]. A needle to plate geometry of electrodes both immersed in a cylindrical glass vessel was used. The needle electrode was made from sharpened tungsten wire, which was almost totally insulated from surrounding liquid phase and grounded stainless steel electrode by Teflon® tube [Lukes et al., 2008]. Needle to plate distance was 52 mm. A pulsed high voltage applied to the needle was provided by a pulse power supply. All experiments were conducted with fixed applied voltage of 27 kV, pulse repetition frequency of 35 Hz (except of experiments dealing with effects of pulse repetition frequency) and charging capacitance of 7 nF. The mean electrical power \( P \) applied to the reactor was calculated from the applied voltage \( U \), charging capacity \( C \) and pulse repetition frequency \( f \) as \( P = f \cdot E_p \), where the pulse energy \( E_p \) was evaluated as the storage energy of the charged capacitor \( E_p = \frac{1}{2} \cdot C \cdot U^2 \). The reactor vessel was cooled during the whole experiment by a water circulating system to avoid heating of the solution and maintain isothermal conditions of about 16 °C.

Bacterial suspensions of *Escherichia coli* CCM 3954 (ATCC 25922) or *Enterococcus faecalis* CCM 4224 (ATCC 29212) were prepared by preculturing the bacteria cells in the growth medium. The suspensions were then carefully dispersed in NaCl solutions of non-zero conductivity (at least 50 µS.cm⁻¹) prepared from deionized water in order to avoid a burst of bacteria, which could be caused by osmotic shock in low conductive deionized water. After dispersion of bacteria the final solution conductivity was adjusted by NaCl to 200 or 500 µS.cm⁻¹. The total volume of bacterial suspension was 1250 ml. The number of bacteria cells in the solution was assayed by counting colony forming units (CFUs). Direct seeding method of 1 ml of bacteria suspension to Petri dishes (9 cm) was used. *E. coli* was cultivated on selective agar plates (MFC, HiMedia, Mumbai, India) at temperature of 43 °C for one day according to the instructions. *E. faecalis* was cultivated on selective agar plates (Slanetz-Bartley agar, HiMedia, Mumbai, India) at temperature of 37 °C for two days according to the instructions. The initial amount of bacteria was about 10⁵ CFU in 1 ml. The viability of the bacteria was determined as the ratio of the concentration of surviving bacteria to the total concentration.

Irradiation experiments of bacterial suspension in the cylindrical spectrometric cells (Starna Scientific Ltd., cell type 35, path length 10 mm, diameter 50 mm) were performed to quantify the contribution of UV radiation in the inactivation of investigated bacteria. We used the same experimental setup as in the batch experiments. To assess the role of UV radiation in bacterial inactivation by the discharge two cylindrical spectrometric cells of different light transmission were used. One of them was made from Pyrex glass (35/PX/10) with cutoff at \( \lambda = 280 \) nm and the other one was from Spectrosil Quartz (35/Q/10) with transmission below \( \lambda = 200 \) nm [Lukes et al., 2008]. Spectrometric cells were filled with bacterial suspension (volume of 17.1 ml), placed into the gap between discharge needle and grounded plate electrodes and irradiated by the light emitted from the discharge. The difference in the bacterial inactivation obtained in Pyrex and Quartz cell gave the contribution of UV radiation.

To determine contribution of UV light in overall inactivation of bacteria following assumptions was made [Lukes et al., 2008]:

a) discharge active zone was defined as the space between needle-plate electrodes, in which discharge (plasma) is formed and light from discharge is emitted.

b) the volume of active discharge zone (408 ml) was determined as \( V = \frac{1}{2} \cdot \pi \cdot d^2 \cdot h \), where \( d \) is diameter of discharge reactor (100 mm) and \( h \) is needle-plate electrode distance (52 mm).

c) the amount of the UV light emitted from discharge determined in the spectrometric cell is constant in whole active discharge zone and, thus, time constants of bacterial inactivation \( \tau_{UV} \) determined in the spectrometric cell can be used for all active discharge zone.

d) \( \tau_f \) is time constant obtained from inactivation of bacterial treated in total volume in the discharge reactor.

The contribution of UV light was then determined as

\[
UV\ contribution = \frac{1}{\tau_f} \times \frac{V_{DZ}}{V_T},
\]

where \( V_{DZ} \) is the volume of active zone (408 ml), \( V_T \) is the volume of the treated in the reactor (1250 ml).
Figure 1. Dependence of inactivation of *Escherichia coli* at different conductivity. (U = 27 kV, C = 7 nF, V = 1250 ml).

Results and discussion

Figure 1 shows the effects of pulse repetition frequency on the inactivation of bacteria of *E. coli*. *E. coli* concentration decreased exponentially with increasing time. The graph plotted dependence on time constants $\tau$. Time constants were obtained from equation for inactivation bacteria during pulse corona discharges in liquid phase: $\ln \tau = c_{CFU}/c_{CFU_0}$, where $c_{CFU_0}$ is total concentration of bacteria at the beginning and $c_{CFU}$ is concentration of surviving bacteria. Progression is steadily increasing with increasing frequency. Linear dependence bacterial inactivation on pulse repetition frequency was calculated subsequently: total applied energy $E_T$ was calculated from the time $t$ and mean electrical power $P$ as $E_T = t \cdot P$, mean electrical power is evaluated as $P = f \cdot E_p$. Consequently, there is apparent significant difference between conductivity of 200 $\mu$S.cm$^{-1}$ and 500 $\mu$S.cm$^{-1}$.

Next part of the paper is closely focused on the effect of solution conductivity and the contribution of the UV radiation from the discharge. Figure 2 shows effect of electric discharge on bacteria *E. coli* in the liquid of two different solution conductivities 200 $\mu$S.cm$^{-1}$ and 500 $\mu$S.cm$^{-1}$. Gradual decrease in survival bacteria was observed with increasing time of discharge treatment. Almost all bacteria were inactivated after 6 minutes. The slight difference in bacterial inactivation was observed in dependence on the solution conductivity. Amount of bacteria decreased faster in conductivity 500 $\mu$S.cm$^{-1}$ compared to 200 $\mu$S.cm$^{-1}$.

Figure 3 shows the experiment performed under the same conditions as in Figure 2, but the effect of UV light emitted from the discharge on bacteria irradiated in the Quartz spectrometric cell is shown. Spectrometric cell was filled with bacterial suspension, placed into the gap between the needle electrode and grounded electrode and irradiated by the light emitted from the discharge. Preliminary experiments performed with Pyrex spectrometric cell, (i.e., which do not transmit UV light) revealed no inactivation of bacteria [Lukes et al. 2008].

Thus, any other processes produced by electrical discharges than UV radiation did not influenced inactivation of bacterial suspension in Quartz spectrometric cell except of UV light. Thereby this allowed us to investigate just the role of UV light in the bacterial inactivation by the discharge. Figure 3 clearly shows that pulsed corona discharges emitted UV light with germicidal effect and that UV light contributed to bacterial inactivation of *Enterococcus faecalis* significantly.

Figure 4 shows effect of pulsed corona discharge on inactivation bacteria *Enterococcus faecalis* in liquid of the solution conductivity 200 $\mu$S.cm$^{-1}$ and 500 $\mu$S.cm$^{-1}$. It is apparent that after 6 min of exposure almost all bacteria were inactivated. However, there is the slight difference between inactivation of the same type of bacteria with different conductivity. Amount of bacteria decreased faster in conductivity 500 $\mu$S.cm$^{-1}$ compared to 200 $\mu$S.cm$^{-1}$.

Figure 5 shows the same type of experiment as in Figure 3 but with *Escherichia coli*. The effect of UV radiation on the inactivation of *E. coli* is again evident.

To clarify the role of UV light, Tables 1 and 2 summarize time constants obtained from experiments with *E. coli* and *E. faecalis*. Table 3 shows particular contributions of UV to inactivation of both types of bacteria. The greatest contribution was while *E. coli* was treated by pulsed corona discharge while solution conductivity was
500 μS.cm⁻¹. For other cases of solution conductivities, the contribution of UV light was of about 40%. It has been shown that the contribution of UV light during pulsed corona discharges is very important.

![Figure 2](image2.png)  ![Figure 3](image3.png)  ![Figure 4](image4.png)  ![Figure 5](image5.png)

Figure 2. Kinetics of inactivation of Enterococcus faecalis in liquid (U = 27 kV, C = 7 nF, V = 1250 ml).

Figure 3. Kinetics of inactivation of Enterococcus faecalis in spectrometric cell (U = 27 kV, C = 7 nF, V = 17.1 ml).

Figure 4. Kinetics of inactivation of Escherichia coli in liquid (U = 27 kV, C = 7 nF, V = 1250 ml).

Figure 5. Kinetics of inactivation of Escherichia coli in spectrometric cell (U = 27 kV, C = 7 nF, V = 17.1 ml).

Table 1. Time constants of bacterial inactivation by the discharge in spectrometric cell.

<table>
<thead>
<tr>
<th></th>
<th>Enterococcus faecalis</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time constant, 200 μS.cm⁻¹ [min]</td>
<td>0.92</td>
<td>0.689</td>
</tr>
<tr>
<td>Time constant, 500 μS.cm⁻¹ [min]</td>
<td>0.228</td>
<td>0.854</td>
</tr>
</tbody>
</table>

Table 2. Time constants of bacterial inactivation by the discharge in volume.

<table>
<thead>
<tr>
<th></th>
<th>Enterococcus faecalis</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time constant, 200 μS.cm⁻¹ [min]</td>
<td>0.387</td>
<td>0.282</td>
</tr>
<tr>
<td>Time constant, 500 μS.cm⁻¹ [min]</td>
<td>0.49</td>
<td>0.444</td>
</tr>
</tbody>
</table>

Table 3. Contribution of UV radiation in bacterial inactivation by the pulsed corona discharge.

<table>
<thead>
<tr>
<th></th>
<th>Enterococcus faecalis</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contribution of UV, 500 μS.cm⁻¹ [%]</td>
<td>39</td>
<td>51</td>
</tr>
<tr>
<td>Contribution of UV, 200 μS.cm⁻¹ [%]</td>
<td>42</td>
<td>40</td>
</tr>
</tbody>
</table>
From presented data follows that E. coli are durable than E. faecalis during pulse corona discharge in the liquid phase. There is probably important role of the composition of the bacterial cell wall. E. coli is a gram-negative bacterium and its cell wall is composed from murein layer and lipopolisacharides. E. faecalis is a gram-positive bacterium, which lacks outer membrane, but possess thicker murein layer. On the other hand, Laroussi [2003] have shown that the gram-positive bacteria Bacillus subtilis have more robust cell wall and its inactivation was slower than of E. coli under influence of atmospheric discharge plasma.

Conclusion

Results on the effects of pulsed electrical discharge on inactivation of bacterial microorganisms in liquid phase were presented. It was revealed that inactivation of bacteria depends on the solution conductivity and the pulse repetition frequency. With higher solution conductivity and higher pulse repetition frequency faster inactivation of microorganisms was obtained. It was shown that UV radiation emitted from the discharge contributes to the inactivation bacteria and its role increases with increasing solution conductivity. About 40% contribution of UV radiation to the overall inactivation of Escherichia coli or Enterococcus faecalis was estimated. Finally, better inactivation efficiency was determined for E. faecalis than for E. coli.

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References