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Comparison of pulsed corona plasma and pulsed electric fields for the decontamination of water containing *Legionella pneumophila* as model organism

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ABSTRACT

Pulsed corona plasma and pulsed electric fields were assessed for their capacity to kill *Legionella pneumophila* in water. Electrical parameters such as in particular dissipated energy were equal for both treatments. This was accomplished by changing the polarity of the applied high voltage pulses in a coaxial electrode geometry resulting in the generation of corona plasma or an electric field. For corona plasma, generated by high voltage pulses with peak voltages of +80 kV, *Legionella* were completely killed, corresponding to a log-reduction of 5.4 (CFU/ml) after a treatment time of 12.5 min. For the application of pulsed electric fields from peak voltages of −80 kV a survival of log 2.54 (CFU/ml) was still detectable after this treatment time. Scanning electron microscopy images of *L. pneumophila* showed rupture of cells after plasma treatment. In contrast, the morphology of bacteria seems to be intact after application of pulsed electric fields. The more efficient killing for the same energy input observed for pulsed corona plasma is likely due to induced chemical processes and the generation of reactive species as indicated by the evolution of hydrogen peroxide. This suggests that the higher efficacy and efficiency of pulsed corona plasma is primarily associated with the combined effect of the applied electric fields and the promoted reaction chemistry.

1. Introduction

Legionella pneumophila are Gram-negative bacteria that were first described in 1979 after an outbreak of pneumonia among members of the American Legion. The elongated non-spore forming aerobic microorganisms with a length of 2–5 μm proliferate in amoeba but can also replicate within alveolar macrophages. Although 15 serogroups of *Legionella pneumophila* are confirmed, serogroup 1 (sg1) is most frequently associated with severe infections [1]. Legionellosis can traditionally be distinguished in two clinical pictures. One is described as Legionnaires' disease (named after the first observed outbreak) causing severe pneumonia. The other is the so called Pontiac fever whose etiopathology is rather moderate, flue-like and most of all self-limiting. The difference to Legionnaires' disease is the lack of pneumonic symptoms [2,3].

In modern societies *Legionella* often persist in water tanks, cooling systems or air conditioning systems causing a severe respiratory disease when contaminated water or aerosol is inhaled by human beings. Several countries reported an increase in cases of legionellosis [4,5]. The Department of Epidemiology (Atlanta, USA) investigated data provided by the Center of Disease Control (CDC) for the years 1990 to 2005. They recognized an increase of 70% from 1310 cases in 2002 to 2223 cases in 2003. Two years later the rate of new infections increased to 12,000 in 2005.

Additional efforts are needed to develop highly efficient disinfection systems to reduce Legionella species in water containing environments [6].

To eradicate *Legionella* several physical and chemical disinfection methods have been described including thermal treatment (superheat-and-flush or instantaneous heating-system), copper/silver ionization, UV-light or hyperchlorination. However, these disinfection methods have limitations [7]. Thermal treatment has disadvantages such as high costs and duration because only temperatures above 60 °C for extended times lead to an almost complete killing of *L. pneumophila*. UV-light is only recommended in combination with superheat-and-flush to provide comprehensive protection. Additionally, prefiltration is necessary to prevent accumulation of chalk residues on the quartz sleeves housing the UV source, which otherwise would

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decrease UV light emission. Chlorine is highly corrosive and can cause severe plumbing damage. A coating of the pipe system is necessary. This, however, cannot eliminate leakage completely. Furthermore, it was demonstrated that *Legionella* is rarely sensitive to chlorine [7,8]. Thus, more advanced disinfection methods are necessary and motivate the development of new treatment techniques such as pulsed electric fields (PEF) and pulsed corona plasma, respectively. Both of them have already proved to be effective, bio-compatible and environmental friendly [9].

Potential applications of PEF treatment are food processing, medical treatment or water treatment [10–14]. If parameters like pulse polarity, conductivity and electrode shape are adjusted correctly, alternatively non-thermal, i.e. corona plasma, can be formed. It has been demonstrated that non-thermal plasma generated directly in water does have a variety of physical and chemical effects known to be effective for pollutant degradation, bacterial killing, including the killing of spores [9,15–20]. Beside the occurrence of strong electric fields, ultraviolet radiations, shockwaves and probably most importantly chemical reactive species such as hydroxyl radicals and hydrogen peroxide (H_2O_2) are generated by the plasma [21]. Nevertheless, processes responsible for bacterial killing with pulsed corona plasma are not fully understood. Especially the role of the electric field in comparison to effects mediated by the plasma itself is unclear. Therefore it is still ambiguous which of the methods is more efficient and causes a higher log-reduction of colony forming units (CFU), requires less time and/or less energy.

Differences between pulsed corona plasma and PEF were already compared previously although in different experimental setups. Slightly higher decontamination efficiency was found for plasma when *Escherichia coli* was used as model organism. Corona plasma was generated in a wire to plate geometry, applying pulses of 600 ns at a repetition rate of 0.1 Hz and with peak voltages of 120 kV. The results were correlated to PEF-treatments conducted in a plate-to-plate setup for an applied homogenous pulsed electric field of 80 kV/cm with pulse durations of 60 ns, 300 ns and 2 μs. In this setting shorter pulses in sub-microsecond range appeared to be more effective than longer pulses [22,23].

Comparative studies were also performed using *Pseudomonas fluorescens* as a model microorganism. Plasma was formed in a needle to plate system when applying pulses of 20 kV with a duration of 6 μs. Air or nitrogen could be bubbled through the needle to enhance energy efficiency. When plasma was applied directly to water, it was found to be more energy efficient than PEF-treatment, which was conducted in plate-to-plate geometry for a homogeneous field of 66 kV/cm and a pulse length of 150 μs [24].

Although the plasma was not generated directly in water a further study showed that the combination of plasma and PEF treatment had synergistic killing effects dependent in which order the methods were applied. Using a plasma jet close to the liquid surface first and afterwards PEF treatment led to an almost complete killing of *Staphylococcus aureus*. Pulsed electric fields were applied with a plate to plate configuration using peak voltage of 3 kV, pulse duration of 100 μs and a repetition rate of 1 Hz [9].

However, all these studies were facing the problem that plasma source and PEF source were not directly comparable due to two different experimental setups for either the application of plasma or the electric field.

In this study two different methods were compared for their effect on the viability of pathogenic *Legionella* in water. An experimental setup was established, which allowed the generation of plasma and pulsed electric fields, respectively.

All experiments were performed with the same experimental setup and an equal peak voltage of about 80 kV. Almost the same amount of energy in either the plasma or PEF treatment was delivered per discharge or pulsed field. This was accomplished by changing the polarity of the applied short high voltage pulses, which resulted either in the generation of corona plasma or an electric field only. This allowed a direct comparison on the effectiveness and differences in killing mechanisms for both methods.

2. Materials and methods

2.1. Electrical setup

A coaxial electrode geometry was used for plasma and PEF treatment, respectively. For a more robust electrode design two twisted tungsten wires (W-005135/13, Goodfellow, Huntingdon, England) with a diameter of 0.05 mm each (pure, uncoated) were aligned in the middle of a glass tube and served as high voltage electrode. The glass tube had a length of 67 mm and a diameter of 34.5 mm. Ground electrode was a metal mesh of stainless steel that was fixed on the inner wall of the class tube. High voltage electrode was replaced after each experiment to establish the most comparable conditions for all experiments. The assembled reactor was holding a volume of 68 ml. Positive or negative high voltage pulses could be applied to the center electrode by a 6-stage Marx-bank with a repetition rate of 20 Hz. The setup was described previously in more detail [19,25]. An inherent advantage of this setup is the possibility to create either a pulsed corona plasma using positive polarity high voltage pulses (Fig. 1) or just a pulsed electrical field using negative polarity high voltage pulses.

During application of positive high voltage pulses in a wire to cylinder or needle to plate system, a strong electric field is located close to the surface of the high voltage electrode (wire). Although the field weakens over distance, electron avalanches result in streamer propagating to the outer electrode (metal mesh) forming a plasma. Negative polarity uses to be less attractive for electron avalanches. If streamers formed at all, they are significantly shorter than with a positive polarity [26,27]. The described mechanism can be employed to develop an experimental setup (pulse width, reactor chamber, conductivity) in which only a positive discharge is formed, even when negative pulses with the same peak voltage were used.

Pulses applied by the Marx-bank are characterized by short rise times of about 20 ns, a peak voltage of 80 kV and an exponential decay resulting in pulse lengths (FWHM) of about 140 ns for positive (plasma) and approximately 240 ns for negative (PEF) polarity (Fig. 2).

Although pulse length for PEF treatment is increasing, the calculated pulse energy is almost similar for both polarities. This can be explained by current flows that compensate for differences in applied voltages. Beyond 400 ns energy dissipated in pulses applied for plasma and PEF

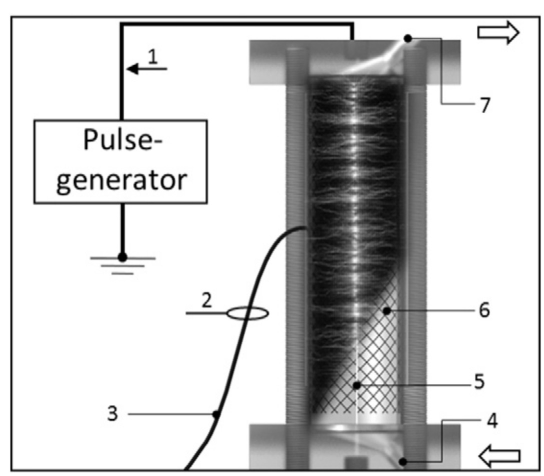

Fig. 1. Pulsed corona plasma in coaxial geometry with increased exposure time. (1) voltage measurement, (2) current measurement, (3) ground connection, (4) bottom connector to peristaltic pump, (5) tungsten high voltage electrode, (6) ground electrode (stainless steel mesh), (7) upper connector to peristaltic pump. Arrows indicate the flow direction of *Legionella* suspension. Positive or negative high voltage pulses were applied to the center electrode from a 6-stage Marx-bank with a repetition rate of 20 Hz. Conductivity of treated suspension was adjusted to 60 μS/cm. A flow rate of 140 ml/min was maintained by a peristaltic pump, which was placed before the setup with a pushing flow from the bottom to the top as indicated by arrows.