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Decontamination Effects of Low-temperature Plasma Generated by Corona Discharge
Part I: an Overview

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Abstract: The first part of this article is devoted to a minireview of basic terms of plasma physics and chemistry described in a way understandable even to nonspecialists in the field. Among the methods of generation of low-temperature plasma, the unipolar electric corona discharge in air at atmospheric pressure is described in more detail. Selected studies are mentioned that concern the decontamination effects of low-temperature plasma and the currently known mechanisms of its microbicidal action. The key part of this mechanism is the action of UV light and both charged and uncharged particles. The most important common mechanism seems to be different forms of reactive oxygen species and some methods of their determination are therefore briefly described.

Introduction

The term decontamination denotes a set of methods and procedures used to partially or completely destroy or remove microorganisms, i.e. viruses, bacteria and eukaryotic organisms, from the given location. Although a large number of decontamination methods is known and used, no universal method is available. Based on their efficiency, the decontamination methods are grouped into several categories. The highest level of decontamination is sterilization, which is required to eliminate all viable forms on a surface or inside a given system (for more details see, e.g., [1]).

One of the possible methods of decontamination or sterilization is the action of electric discharges and the plasma generated by them. The method is not yet frequently used in the practice but it is potentially important, especially for the decontamination or sterilization of heat labile or otherwise sensitive materials. The microbicidal properties of plasma and possibilities of their use have been the subject of many studies of foreign [e.g. 2–9] and Czech authors [e.g. 10–13]. Their results are discussed below.

An example of a practical application of this kind of decontamination is the apparatus called Plasmacluster Ion Generator [14], which relatively efficiently kills off bacteria in the air through the action of H^+ and O^{2-} produced by decomposition of the water vapour. Another application is the use of the Sterrad sterilization chamber that dates from 1993 and is based on a combined action of hydrogen peroxide vapours and the low-temperature plasma. A typical sterilization cycle of this system includes evacuation of the chamber and a subsequent injection of a measured amount of hydrogen peroxide whose vapours sterilize the surface of the objects placed in the chamber. An electromagnetic field at radio frequency 13.45 MHz is then applied on the chamber contents, in which the hydrogen peroxide vapour breaks apart, producing a low-temperature plasma cloud that generates ultraviolet light and free radicals. The sterilization process takes ca. 1 hour depending on the selected regime and the temperature does not exceed 45 °C. The process is highly efficient and cost-effective (see, e.g., [35]).

Low-temperature plasma as a decontamination agent has the following advantages:

- it does not raise the temperature of the decontaminated material,
- purchase costs are low and operational costs are negligible,
- no contamination of surface with chemicals occurs,
- the decontamination need not take place in a closed chamber,
- decontaminated objects are immediately ready to use,
- the process does not generate any side products,
- it presents no problems with storage of the decontamination agent.

The studies published so far on the subject are difficult to compare and reproduce since they use different types of discharges that generate plasma under different conditions, and make use of a variety of experimental parameters. In addition, the decontamination properties of the plasma or the plasma-generating discharges are rarely addressed in a sufficiently complex manner and are usually demonstrated on a single or a low number of tested microorganisms; the decontamination properties of the given method are often stated only qualitatively. Model organisms were frequently only vegetative forms of bacteria; when the much more resistant spores were tested; no comparison is often given with the effectiveness of the treatment in the vegetative forms. Elimination of resistant spores, especially bacilli and clostridia, represents a specific problem in decontamination. The possible mechanisms and causes of the decontamination action of different discharges have not yet been fully clarified despite intensive research, due to the complexity of physical and chemical processes taking place in the discharges. Among the potential mechanisms is the action of UV radiation, charged particles, free radicals or reactive oxygen species. The relative shares of these mechanisms in the final effect, their details or possible synergistic actions are not yet fully clear.

Our study aims at contributing to a complex characterization of the decontamination properties of low-temperature plasma generated by a corona discharge at atmospheric pressure. Its first part brings a concise overview of basic terms relevant in the field and some earlier results. The second part brings more recent results on the action of the corona discharge on a set of model microorganisms on different surfaces. The attendant problems and results are described in a way that should be comprehensible even to readers unfamiliar with plasma physics and chemistry. A more detailed treatise of the data gathered here can be found in [15].

Plasma

Plasma is by definition [16] a quasineutral gas formed by neutral as well as charged particles (ions and electrons) that exhibit collective behaviour. Ionization occurs when an electron gains sufficient energy for overcoming the attractive force of atom nuclei. Electron can gain this energy by, e.g., heating of a neutral gas to sufficiently high temperature of at least several thousand Kelvins. The plasma so formed, in which both electrons and ions have about the same temperature, is called thermal or isothermic plasma. The radiation emitted by thermal plasma has a continuous heat radiation spectrum. This plasma is found, e.g., inside stars, in the initial stages of a nuclear explosion, in an electric arc or in a lightning channel. In terms of sterilization, it represents an ultimate solution in which the contaminated object is incinerated at a very high temperature.

Ionization can occur also by other mechanisms, in particular by collisions of electrons in electron shell with free accelerated electrons or as a result of an external photoelectric effect. These processes give rise to plasma in which the temperature of ions and neutral atoms is considerably lower than that of electrons. This plasma is then called low-temperature, nonthermal or nonisothermic. Accelerated electrons can be supplied to the plasma from outside in the form of an electron beam, or the electrons can be accelerated directly inside the plasma volume by an electric field or electromagnetic radiation. The photons necessary to produce the photoelectric effect can also be provided from the outside or they can be emitted by the discharge itself. Nonthermal plasma emits the light at discrete wavelengths that correspond to the transitions of electrons in atom or molecule shells in both the visible and the ultraviolet (UV) part of the spectrum.

Due to the low heat capacity of the electron component, the application of nonthermal plasma on surfaces does not produce any considerable heating of the surface. High-energy electrons in a relatively cold gas produce a number of nonequilibrium chemical reactions called plasmachemical reactions. Even in a simple mixture of oxygen and nitrogen, the plasma is the site of hundreds of chemical reactions. The plasma chemical reactions proceed under the direct participation of free electrons and photons of the radiation emitted by the discharge.

In addition to the emission of UV radiation, an important property of the low-temperature plasma is the presence of high-energy electrons, which are highly reactive and cause numerous chemical and physical processes such as oxidation, excitation of atoms and molecules, production of free radicals and other reactive particles. This kind of plasma is most often generated by electrical gas discharge, especially microwave discharge, dielectric barrier discharge and corona discharge.

Microwave discharge is produced either in the absence of electrodes by ionizing electrons by external electromagnetic field, or by using metal electrodes inside waveguides or resonators. The amplitude of the electric component of the microwave radiation has to be sufficient to cause breakdown. The frequency of the driving electromagnetic field is in the gigahertz range. In view of the high frequency of the external field, no spark channels are formed.

The properties of different kinds of discharges and types of plasma are described in more detail, e.g., in [17, 18, 19]. The properties of the plasma produced by a unipolar corona discharge, which is the main subject of this study, are briefly described below.

Corona discharge

Corona discharge can be observed only in an inhomogeneous electrical field formed between two or more electrodes. At least one of the electrodes has to have a small curvature diameter (the active electrode or coronizing electrode). In an experiment, this electrode is usually realized as a point electrode (using e.g. a needle) or a thin wire. Around this electrode is formed the light-emitting part of the discharge which, in the case of a point electrode, has the form of a crown, giving the name to the discharge (the Latin *corona* = crown). This phenomenon, called St. Elmo's fire, has long been known to sailors since it has appeared at the end of ship's masts and spars during storms. The other electrode can be a plane electrode in the form of any planar conductor, or a conductor with an order-of-magnitude larger curvature radius than the corona-forming electrode. In this case, the discharge is called a unipolar corona discharge; it can be positive or negative depending on the polarity of the corona-forming electrode. The electrode system can also feature small curvature diameters of both electrodes; in this case, a bipolar corona is formed around both the cathode and the anode. On switching on the voltage, a high-intensity electric field is formed in the close vicinity of the electrode with a small curvature diameter. When this field attains sufficient intensity, local electron avalanches and a local breakdown are formed within it; they however cannot spread into the whole inter-electrode space. This close vicinity of the electrode, in which ionization takes place, is called the ionization region. It can be observed as a weakly lucent space around the point electrode (corona in the stricter sense of the word). The ionization region is surrounded by a dark outer region. The current in the outer region of a unipolar corona discharge consists predominantly of ions with the same charge.

Our work concerns solely the direct-current negative point-to-plane corona discharge whose typical configuration is schematically illustrated in Fig. 1. As shown in the figure, a small ionization region is formed around the point electrode; the high-intensity electric field triggers here avalanche ionization. Apart from the ionization region, the space between the electrodes contains a relatively large drift region with a low intensity of the electric field. The current in this region is conducted by ionized gas molecules; electrons and ions drift here and interact with neutral particles but lack sufficient energy to cause their ionization. The density of charged particles in this region is relatively low so that their mutual interactions are negligible. With the current of the order of microamperes and higher, the negative corona burns in a stable way. Negative coronas in an electronegative gas such as the oxygen-containing air are characterized by the appearance of current pulses with a frequency of hundreds to thousands kilohertz (named the Trichel pulses); this frequency is so high that the ion flux and the drift regions appear to be continuous.

The distribution of current density in a corona discharge is described by the Wartburg law (see e.g. [17, 18]) that was determined empirically and is mathematically described as follows:

$$j(r,d) = j_0 \cos^5 \Theta;\ \Theta < 65°;$$
$$j_0 \approx I / 2d^2$$

where $j(r,d)$ is current density, j_0 is corona axis current density, Q is the apex angle defined by the equation Q = r / d, I is the total corona current, r and d are radial and axial distances from the tip of the point electrode, respectively. The meaning of the symbols is also apparent from Figure 1.

The corona discharge burning at atmospheric pressure in air is one of the simplest, and thus well-reproducible, sources of low-temperature plasma. In contrast to other kinds of discharges, which usually require a construction of a complex device, it can be generated in a simple device with low first expenses and negligible operating costs. It is therefore suitable as a model for experimental research into the microbicidal properties of electric discharges.

Possible mechanisms of the microbicidal action of low-temperature plasma

Although the killing of microorganisms by low-temperature plasma is known (see e.g. [20]), it has not yet been satisfactorily studied and elucidated. The current explanations include the action of

- UV light and/or
- reactive particles, which can carry electric charge.

The most important among these particles are apparently various reactive oxygen species. Different assessments have been offered for the role of individual plasma components in the ultimate decontamination effect and the share and significance of individual mechanisms of this action.

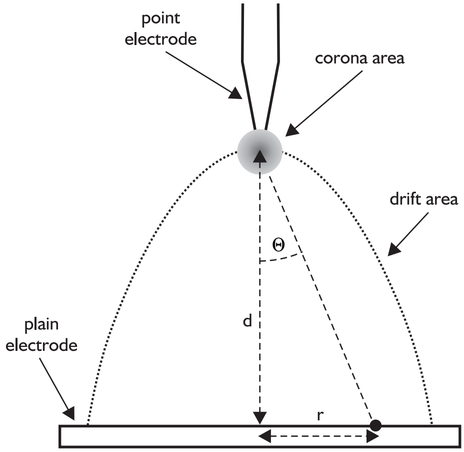

Figure 1 – Configuration of a direct-current negative point-to-plane type corona discharge.

Action of UV light

UV light is not considered an overly effective decontamination agent although its bactericidal properties are unquestionable. They are most apparent in the wavelength range of 250 to 280 nm, with a maximum effect around 260 nm. UV light acts only on irradiated surfaces. The mechanisms of its effect are taken to include its propensity to damage DNA and prevent thereby cell reproduction, cause damage to proteins, generate hydrogen peroxide in aqueous solutions including intracellular environment, and generate ozone in air. However, its efficiency and the contribution to the overall microbicidal effect of the plasma are the matter of contention. Some studies take UV light to be the prime cause of cell death; others assume its contribution to the microbicidal effect to be marginal. Thus, study [2] assumes that UV light is a dominant component in the cell-killing effect, and the same conclusion, at least for some microorganisms, was made in study [8]. On the other hand, comparison of the effect of UV light produced by an atmospheric pressure glow discharge with the same dose of UV light generated by another source, performed in [21], did not reveal any significant contribution of UV light to the microbicidal effects of low-temperature plasma. Study [22] documented the killing of some bacterial spores by a sufficient dose of UV light while according to [36] *Bacillus subtilis* spores are not inactivated even by a UV light intensity of 100 $\mathrm{J\ cm^{-2}}$.

Attempts at explaining these discrepancies included examination of the effect of composition of the atmosphere around the decontaminated objects. The results were again ambiguous. According to study [2], the suitable atmosphere is oxygen O_2 but not nitrogen N_2 which, in contrast to oxygen, does not emit photons with wavelengths shorter than 230 nm. Likewise, study [10] in which plasma was generated by a microwave device called surfatron stated that the sterilization effects were detectable after a shorter time period in an oxygen-containing atmosphere than in an oxygen-free atmosphere. Also study [6] showed that the decontamination efficiency significantly rises even at low oxygen concentrations in the discharge atmosphere. In contrast, experiments with cascaded dielectric barrier discharge [8] showed only a weak decontamination effect in an oxygen-containing atmosphere; a 15 s exposure of *Bacillus subtilis* spores in an atmosphere of $N_2{:}O_2 = 8{:}2$ reduced their count by an order of magnitude whereas in a pure nitrogen atmosphere the drop was 4 orders of magnitude with the same exposure. With the exception of this last study, the differences in the results of individual studies can be associated with the action of reactive particles described below.

Action of reactive particles

The important role of reactive particles in decontamination is indicated by the effect of the ambient atmosphere, especially the effect of oxygen. Study [6] documented a significant increase in decontamination efficiency when even low concentrations of oxygen were present in the discharge atmosphere. Significant can be also the action of other reactive atoms or molecules, e.g. NO_X. According to [23] the killing of microorganisms is strongly affected by charged particles/ions, whose charge concentrates on the surface of cell membranes and causes their disturbance or disruption. This effect should be especially strong with Gram-negative bacteria. Also study [9] attributed the bactericidal effect of the corona discharge in a nitrogen atmosphere to the action of unspecified ions. Particles considered to be most important for the microbicidal effect are mainly reactive oxygen species.

Oxygen is commonly found in the form of stable O_2 molecules. The reactivity of these molecules is not negligible, but it is not sufficiently high to permit measurable rates of spontaneous oxidation of most biological materials under normal life conditions. Since oxygen is a major component of air, all aerobic organisms are appropriately adapted to its presence. The factor of fundamental importance for the existence of life is the anomaly in the electron structure of the O_2 molecule, which in its basic low-energy state contains in the highest antibonding orbital two unpaired electrons with parallel spins. The spin multiplicity is therefore 3 (triplet), usually denoted as $^3O_2$. This contrasts with the basic state of the majority of other substances, which is a singlet state in which all electrons are paired and, according to the spin conservation law, their reactions with the triplet structure of oxygen are spin-forbidden. The reactions of $^3O_2$ with singlet molecules therefore have very large activation energy and proceed at a measurable rate only when conditions for bypassing the spin law, are met. The spin prohibition in fact forms the basis of our life in the current form since without it all organic matter would be rapidly oxidized by atmospheric oxygen to carbon dioxide and water.

Under certain conditions, oxygen can give rise to reactive forms that have a much higher reactivity than $^3O_2$.

1) Atomic oxygen O. This form of oxygen is found in the stratosphere where it is formed as a transient side product of ozone or peroxides splitting by UV light. It is also formed in electric discharges during the interaction of high-energy ions or electrons with oxygen molecules, or by decomposition of ozone. It is highly reactive and oxidizes rapidly other materials or reacts with other oxygen molecules to yield O_2 or ozone O_3.
2) Singlet oxygen $^1O_2$. Unlike the common $^3O_2$, this molecule is in an electron excited state in which all electrons are paired and spin multiplicity is 1 (singlet). It exists in two electron states denoted $O_2(^1\Delta_g)$ and $O_2(^1\Sigma_g)$. The lifetime of the longer-lived $O_2(^1\Delta_g)$ in water is ca. 4 μs, in other solvents it can persist up to milliseconds. Singlet oxygen is generated in a number of chemical, photochemical or photosensitized reactions; one of the physical processes yielding $^1O_2$ is the microwave discharge in oxygen atmosphere [24]. Singlet oxygen is highly reactive species since its reaction with most substances does not contradict the spin prohibition.

3) Ozone O_3 is a stable allotropic modification of oxygen. It is commonly found in the stratosphere in concentrations of 1 to 100 ppm at 15–50 km altitudes. It arises in electrical discharges in an oxygen-containing atmosphere – in nature due to lightning, in the laboratory it is usually produced by silent electrical discharges in so-called ozonators. Ozone is also generated by photochemical reactions (near germicide UV lamps, in photooxidative smog). It is a strong oxidant; in higher concentration, it is toxic, and an 8-hour exposure to a concentration of 0.1 ppm ozone is deleterious to humans. Its microbicidal properties are well known and ozone is sometimes used for water disinfection. In high concentrations (up to 1500 ppm) and with longer exposures (up to 4 hours) it is at least partially efficient even against bacterial spores [25, 26, 27].
4) Superoxide anion $O_2^{\bullet -}$ arises by reduction of triplet oxygen. The lifetime of $O_2^{\bullet -}$ does not exceed 5 μs. It is not too reactive but it can react with other atoms and molecules to produce the considerably more reactive hydroxyl radicals and singlet oxygen. A reaction of two superoxide anions and hydrogen yields hydrogen peroxide. It is formed in the cells by numerous biochemical reactions and aerobic organisms can eliminate it by the action of superoxide dismutase.
5) Hydroxyl radical $^{\bullet}OH$ is formed in the so-called Haber-Weiss reaction of hydrogen peroxide with superoxide. It is a very reactive particle with a lifetime of ca. 10^{-9} s. It is very dangerous for live cells, which cannot eliminate it enzymatically in contrast to the superoxide anion.

Among these particles, the role of ozone in the killing of microorganisms by plasma or electric discharge is largely indisputable, as stressed also in [20]. As shown e.g. in [3], another probable cause of the microbicidal action of the plasma is singlet oxygen $^1O_2$. Assertions about the potential role of other particles often lack experimental evidence.

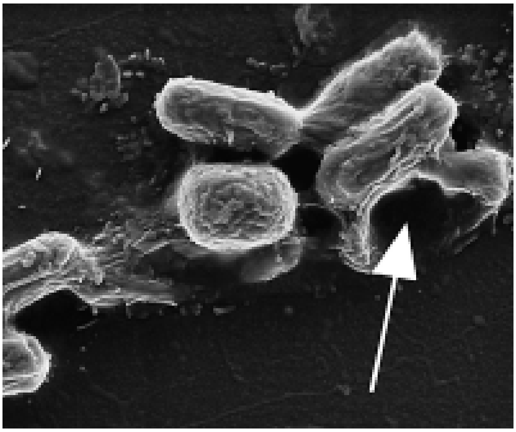

Figure 2 – Photograph of Bacillus subtilis spores from a scanning electron microscope. Mechanical damage to spore surface after exposure to a barrier discharge, caused by reactive particles, is denoted by arrow. Adapted from [8].

Various proposals of the mechanisms of bactericidal action of reactive particles can be found in the literature. According to [2] and [8], these particles most probably damage biological membranes, the bacterial cell wall and other biological structures by a mechanism similar to that occurring in the plasma etching of semiconductors. The picture from a scanning electron microscope given in Figure 2 shows *Bacillus subtilis* spores after an exposure to a barrier discharge. It clearly illustrates a mechanical damage to the spore surface caused by reactive particles and preventing further spore survival.

Charged particles, i.e. positive and negative ions, can mechanically damage cell envelopes. An experimental evidence of this mechanism is given in study [14], which documents protein cleavage in bacterial membrane under the action of H^+ and O^{2-} ions generated by water vapour decomposition. The result of this action is illustrated in the electron microscopic picture in Figure 3, which shows a bulge on the bacterial envelope caused by damaged cell membrane.

A sophisticated proposal of a combined mechanism of the microbicidal effect of plasma is described in [3]. It consists in a synergistic action of active oxygen and UV radiation, which occurs in three phases:

1. Destruction of the genetic material of the microorganisms by UV light.
2. Erosion of microbial surface atom-by-atom or by etching caused by reactive particles and supported synergistically by UV light.
3. Further destruction of unprotected genetic material by UV light.

These hypotheses notwithstanding, the mechanism of microbicidal action of plasma has not yet been completely elucidated. The methodology of the experiments that

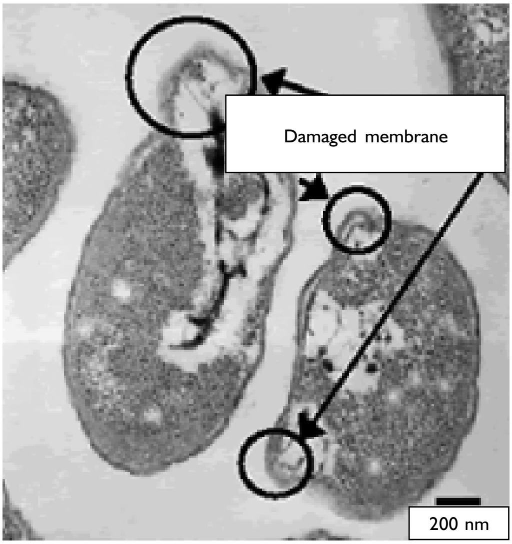

Figure 3 – Damage to cell envelopes by H^+ and O^{2-} ions from water vapour. Arrows denote damage caused by cleavage of bacterial membrane proteins. Adapted from [14], experimental details are not given.

form the basis of the above hypotheses is not standardized and different mechanisms can therefore play different roles under various sets of experimental conditions. The importance of different mechanisms can also vary depending on the microorganism in question.

Detection methods for reactive oxygen species

Any hypotheses on the participation of individual reactive particles in the ultimate decontamination effect have to be based on the determination of the presence and amount of these particles. The detection and proof of reactive oxygen species, which are obviously the most important for the microbicidal effect, make use of sophisticated methods such as electron paramagnetic resonance (EPR), photoaccoustic calorimetry, IR spectroscopy, luminescence methods, etc. An alternative is represented by simple methods based on the reactions of the reactive species in liquid phase with compounds forming characteristic primary or secondary products. The choice of suitable reactions is complicated e.g. by the short lifetime of most particles and the selectivity of the requisite reactions. These reactions should be sufficiently selective for different reactive oxygen species and permit their differentiation despite the fact that the chemical properties of individual reactive oxygen species are very similar.

One of the possibilities is the so-called iodide method used mostly for the detection of $^1O_2$ and other reactive oxygen species [28]. The method is based on the reaction of reactive oxygen species with iodide I^- in the presence of ammonium molybdate $(NH_4)_2MoO_4$ as catalyst. The reaction product is the triiodide anion I_3^- whose amount is proportional to the amount of reactive oxygen species and its yellow colour with an absorbance maximum at 287 or 351 nm permits a quantitative spectrophotometric determination. The method is very sensitive but its disadvantage is a low selectivity.

The diagnostics of oxidizing particles makes use of several reagents such as D_2O or NaN_3 in the iodide detection reagent for $^1O_2$. The lifetime of $^1O_2$ in D_2O is about 16 times longer than in H_2O [24, 29, 30] whereas NaN_3 is an important physical quencher of $^1O_2$ [31, 32]. The presence of D_2O instead of H_2O in the detection reagent increases the production of I_3^- whereas the presence of NaN_3 suppresses it. Other more selective organic substrates for $^1O_2$ oxidation that can be used as detection and diagnostic reagents include e.g. uric acid [33, 34]. It should be noted, however, that the high selectivity is compensated by an order of magnitude lower sensitivity as compared with the iodide reagent.

Conclusion

Low-temperature plasma can be generated by electric discharges of different types in gases of different composition. The simplest generation appears to be its production by means of a unipolar corona discharge in air at atmospheric pressure. Plasma has inhibitory to killing effects on different microorganisms.

This effect involves the participation of UV light and different uncharged and charged particles among which reactive oxygen species appear to be the most important. The decontamination effects of low-temperature plasma are well known but the mechanism or mechanisms of its action is/are not sufficiently known and the technological parameters of its practical use have not yet been sufficiently elaborated. Further research in this field is therefore useful and necessary.

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