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REVIEW ARTICLE

Low Temperature Atmospheric Pressure Plasma Sources for Microbial Decontamination

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Abstract. The aim of this article is to provide a survey of plasma sources at atmospheric pressure used for microbicidal treatment. In order to consider the interdisciplinary character of this topic an introduction and definition of basic terms and procedures is given for plasma as well as for microbicidal issues. The list of plasma sources makes no claim to be complete, but to represent the main principles of plasma generation at atmospheric pressure and to give an example of their microbicidal efficiency. The interpretation of the microbicidal results remain difficult due to the non standardized methods uses by different authors and due to the fact that small variations in the set up can change the results dramatically.

1. Introduction

Microbial decontamination denotes the decomposition or removal of microorganisms, including endotoxins, fungi, viruses and even prions. One aspect is the microbicidal treatment, defined as the inactivation of microorganisms with the purpose to prevent infections. The inactivation of microorganisms and the removal of biological hazardous contaminants is generally of great interest not only for the conditioning of surgical instruments but also plays a substantial role in the entire field of life science (Moreau et al., 2008; Wan et al., 2009). This varies from microbicidal treatment of food or food containing enclosures, the prevention or cure of infectious diseases, the bleaching of teeth, the treatment of finger nails for improved adhesion, the installation of appropriate hygiene strategies, the sterilization of spacecrafts up to the use of plasmas sources for medical applications like wound disinfection or stimulus for wound healing (Wang et al., 2009; Morfill, Shimizu, Steffes and Schmidt, 2009; Weltmann et al., 2008a; Lee et al., 2010; Cooper et al., 2007; Kaemling et al., 2005; Weltmann et al., 2010). Nevertheless, the sterilization of medical devices is still a main topic. Due to the design of new processes, techniques and instruments, the invention or improvement of modern medical devices as well as the initiation of stringent hygienic standards in the field of life science, the requirements and restrictions for sterilization processes continuously grow. In particular, the increasing application of complex and expensive medical devices like endoscopes or central venous catheters require innovative sterilization methods, that fulfill all performance requirements for such high tech instruments (Ruddy and Kibbler, 2002). In general an optimal sterilization process is effective, fast in process, cost-efficient, nontoxic and nonhazardous for the staff, the operator and the patient, environmentally friendly, energy efficient and does not stress the sterilized device or the containing materials.

Low temperature plasmas generated at atmospheric pressure consist of a variety of microbicidal active agents and are likely to become appropriate tools for microbial decontamination. Due to the absence of costly vacuum facilities atmospheric pressure plasma sources (APPS) are easily adaptable to even complex devices and conventional processes. Hence, their development and characterization is in the focus of research for more than two decades and until now the entire potential of APPS is unforeseeable yet.

In this review article an overview of APPS capable for microbial decontamination is given along with recent research results considering their microbicidal efficiency. Thereby, the main focus is to give an overview with regard to the manifold geometries of plasma sources and concepts of plasma ignition which are applicable for microbial decontamination. So the reader on the one hand will be informed about different possible setups invented in the last 10 years and on the other hand can choose a suitable plasma source for his special technical problem. Hence, the paper is organized as follows: Conventional sterilization methods and terms and definitions are introduced in section 2. Section 3 discusses low temperature atmospheric pressure plasmas and their effective components for microbial decontamination. Subsequently, different arrangements for the

generation of atmospheric pressure plasmas are presented and compared considering the applicability and microbicidal efficiency in section 4. A brief summary is given at the end of this article.

2. Terms and definitions

In order to discuss results and achievements of plasma based microbial decontamination processes, a consistent terminology is necessary. Hence, different microbial and process related terms are subsequently defined. The term microorganism includes apart from themselves in a narrower sense, cellular and non-cellular biological agents, which are capable of replication or of transferring genetic material (WHO, 1999). The european standard *EN 12740* additionally includes biological agents, which cause infections, allergies or toxic effects. Therefore, viruses, viroids, parasites, cells from plants and animals, pyrogens, prions and plasmids also belong to the term microorganism. Against this background microbial decontamination means the decomposition or removal of contaminating microbial species, including microorganisms, pyrogens, fungi, viruses and prions as written before. The microbicidal treatment, defined as the intention to destroy microbes, prevent their development, or inhibit their pathogenic action, is a collective term for disinfection, sterilization, including cleaning as well as aseptic and antiseptic activities. They all have different definitions and levels of microbicidal efficiency, which can be seen in table 1. The international standard *ISO 11139* defines inactivation as the loss of ability of microorganisms to grow and/or to proliferate. Whereas disinfection defines a state of living or dead material in which it is not longer able to cause infections in humans. The term disinfection is additionally devided into three levels (Rutala, 1996). Low level disinfection should be able to kill most bacteria, some viruses and some fungi, but cannot be relied on to kill resistant microorganisms like tubercle bacilli or bacterial spores. Intermediate disinfection inactivates *Mycobycterium tuberculosis*, vegetative bacteria, most viruses and fungis, but it does not necessarily kill bacterial spores. High level disinfection can be expected to inactivate all microorganisms, with the exception of high numbers of bacterial spores. Commonly, the term disinfection is used for a decrease of microbial count, which typically not results in sterility. Sterility is defined as state of being free from viable microorganisms (*ISO 11139*). This is an absolute condition, but since microbicidal efficiency is mostly an exponential decay function of stress, the quality of a microbial control process can be expressed as the ability to meet a probability-type endpoint (Pflug, 2007). In this context a sterility assurance level (SAL) is defined as the probability of a single microorganism occurring on an item after sterilization (Mosley, 2008; Ph. Eur. 6.0, 2008). For example, if the endpoint specification of the process is one non-sterile unit in one million units, then the probability that any one unit is non-sterile is 1×10^{-6}. The international standard *ISO 14937* with general requirements for the sterilization of health care products advices to extent the sterilization process by extrapolation to this SAL. Therefore, the nature of the inactivation kinetics effected by the sterilizing agent has to be known and taken

Table 1. Definitions of commonly used terms associated with microbial control processes.

Term	Definition	Lit.
Microorganism	Any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material	WHO (1999)
Biological Decontamination	Removal or neutralization of a contaminating microbial substance	AHMD (2008)
Antimicrobial	Tending to destroy microbes, prevent their development, or inhibit their pathogenic action	AHMD (2008)
Inactivation	Loss of ability of microorganisms to grow and/or multiply	*ISO 11139*
Disinfection	Process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects	Rutala et al. (2008)
Sterility	State of being free from viable microorganisms	*ISO 11139*
Sterility Assurance Level (SAL)	Probability of a single viable microorganism occurring on an item after sterilization	*ISO 11139*
Sterilization	Validated process used to render a product free from viable microorganisms	*ISO 11139*
Cleaning	Removal of contamination from an item to the extent necessary for further processing or for intended use	*ISO 11139*
Asepsis	Activities that lead to a state of being free of living pathogenic microorganisms	AHMD (2008)
Antisepsis	Destruction of pathogenic organisms on living tissue to prevent infection	AHMD (2008)

into account. This approach is best suited to sterilizing agents that demonstrate a first-order exponential decay inactivation kinetic, e.g. hot steam in an autoclave. However, especially for the characterization of non-thermal microbicidal processes the SAL concept is controversial discussed in literature, since these processes mostly do not show the typical exponential decay (von Woedtke and Jülich, 2001; von Woedtke et al., 2008). Against sterilization, where decomposed biological substances could remain on the surface, cleaning removes all contaminations from an object. Hence, an ideal cleaning does not require further disinfection or sterilization. All activities that lead to a state of being free of active pathogenic microorganisms, like sterilization or excellent cleaning, are activities for asepsis. In contrast, antisepsis are activities for the destruction of pathogenic organisms on living tissue with the aim to prevent infections. Here, only a reduction of infections-causing contaminants is aspired to reduce the risk of infection, sepsis or putrefaction.

3. Low temperature atmospheric pressure plasmas for microbial decontamination

3.1. Plasma classification

Low temperature plasmas can generally be subdivided into thermal and non-thermal plasmas (Tendero et al., 2006). Thermal plasmas are in a local thermal equilibrium (LTE) state. The temperature of electrons in the plasma is equal or near equal to the heavy particle temperature, although the photon radiation distribution may be well below the blackbody level. An example for thermal plasmas are arc plasmas (Nehra et al., 2008). Those plasmas are recently used for spraying, cutting and welding

applications, thermal plasma chemistry and waste destruction as well as for circuit breakers (Kogelschatz, 2004). Owing to the high temperature of those plasmas the direct plasma application is mostly limited to heat resistant materials. However, using thermal plasmas in remote mode, where the plasma itself does not reach the object, but the plasma produced radiation, radicals and chemical compounds, enables the treatment of even heat sensitive materials. This opens up new fields of application, like biological decontamination of polymers by means of thermal plasmas as described in section 4.4.

In contrast, non-thermal plasmas exhibit a moderate neutral gas temperature, which is either exactly or close to room temperature and therefore by orders of magnitudes lower than the electron temperature. These plasmas can be generated if most of the coupled energy is transmitted into the electrons of the plasma. Consequently, ions and neutral gas atoms gain only a little energy and stay cold. Due to this inequality between the heavy particle and electron energies those plasmas are classified as non-equilibrium or non-LTE plasmas. Non-LTE plasmas are capable for the treatment of thermolabile materials and moreover are easily adaptable to even complex geometries. Hence, they are widely used in a broad spectrum of applications ranging from low temperature plasma chemistry, decomposition of gaseous pollutants, light sources, surface modification to medical sterilization and microbial decontamination (Kogelschatz, 2004; Becker et al., 2005; Foest et al., 2005; Ehlbeck et al., 2008). But also emerging application areas like plasma healthcare can be approachable by the usage of non-LTE plasmas (Stoffels, 2007; Kong et al., 2009; Morfill, Kong and Zimmermann, 2009; Lloyd et al., 2010; Laroussi, 2009; Fridman et al., 2008). Thus, plasma treatment of cancer cells (Kim, Kim, Park, Jeon, Seo, Iza and Lee, 2009; Vandamme et al., 2010; Zhang et al., 2008), living human cells (Tümmel et al., 2007; Stoffels et al., 2008; Yonson et al., 2006), prevention of nosocomial infections (Morfill, Shimizu, Steffes and Schmidt, 2009) and the therapy of infected wounds (Fridman et al., 2008; Isbary et al., 2010) as a few examples are in the ongoing research focus.

Beside LTE and non-LTE plasmas translational plasmas can be classified as an optional third group. Strictly speaking, translational plasmas are non-LTE plasmas, but their gas temperature is with values of some thousand kelvin much higher than the temperature of typical non-LTE plasmas. Hence, the field of application for translational plasmas is almost identical to the application area of LTE plasmas. An example of a translational plasma is the microwave driven discharge as it is described in section 4.4.

3.2. Active plasma agents

Due to the complex physical and chemical processes inside a low temperature plasma, a multiplicity of different biological active agents are produced in dependence on the adjusted parameters like gas composition, flow rate, moisture, temperature and excitation properties. These agents are radicals and chemical products e.g. N_xO_y, atomic oxygen (O), ozone (O_3), hydroxyl (OH), reactive oxygen (ROS) and nitrogen

species (RNS), high energy UV radiation, radiation in the visible and infrared spectral range, charged particles, alternating electric fields, heat as well as physical and chemical etch processes. Especially the combination of different agents makes plasma attractive, because it is almost impossible for pathogens to develop resistance against these different kinds of plasma stress factors.

In recent years many investigations have been made in order to identify the role of single plasma agents for the microorganism inactivation process (Moisan et al., 2001; Laroussi and Leipold, 2004; Boudam et al., 2006; Brandenburg et al., 2007; Gaunt et al., 2006). Here, UV radiation and highly reactive species such as O, OH and NO_x are identified as process relevant components, whereas heat plays a minor role. Moreover, Dobrynin et al. (2009) conclude that both, positive and negative plasma ions play a key role in the interaction between biological organisms and plasma. Additionally, it was shown that pulsed electrical fields (PEF) have an effect on biological cells, too (Schoenbach et al., 2008; Schilling et al., 2008). Due to the fact, that VUV radiation is dominantly absorbed in ambient air at atmospheric pressure this kind of radiation plays a minor role when the plasma treated microorganisms are surrounded by air. However, several sources combine an effective VUV generation with an exhausting VUV transmitting gas flow (Lange et al., 2009). Thus, the air around the microorganisms was replaced by the process gas (e.g. noble gases) so that VUV radiation can now reach the probe and inactivate the microorganisms. An example for the strong influence of VUV radiation is seen in figure 1 where spores of *Bacillus atrophaeus* were inactivated using an atmospheric pressure plasma jet (kinpen09, neoplas tools GmbH) with a 5 slm pure argon gas flow rate (Lange and von Woedtke, 2010). To separate the influence of plasma generated VUV and UV radiation from the microbicidal effect of the plasma jet a chamber with changeable windows was used. For the transmission of UV radiation a fused silica (SiO_2) window and for VUV+UV radiation a magnesium fluorid (MgF_2) window was applied, whereas pure plasma treatment was done without a window. Inside the chamber the microbial load ($10^4 - 10^5$ spores of *Bacillus atrophaeus* on a circular area of $0.79\,mm^2$) was arranged. The plasma jet was placed outside the chamber in a distance of 8 mm from the load and the microbial load was exposed for a 5 minute duration. The most significant microbicidal agent produced by this source was indeed VUV radiation (2nd continuum of the argon excimer (Ar_2^*) at $\sim 126\,nm$). UV radiation emitted by excited OH molecules at 308 nm contributes only a little to the spore inactivation. The difference between UV and VUV effected inactivation is due to the high VUV intensity, which exceeds the UV intensity by a factor of six. Other agents like heat or reactive species produced in the effluent of this plasma jet configuration are not crucial for the inactivation of *Bacillus atrophaeus* spores. Therefore, spores treated with the effluent plasma have nearly the same inactivation level as spores treated with VUV+UV radiation.

However, for the entirety of plasma sources it is difficult to conclude, which plasma components are the most effective ones. In fact, process relevant agents must not be identical for different kinds of plasma sources and need to be identified in each case and
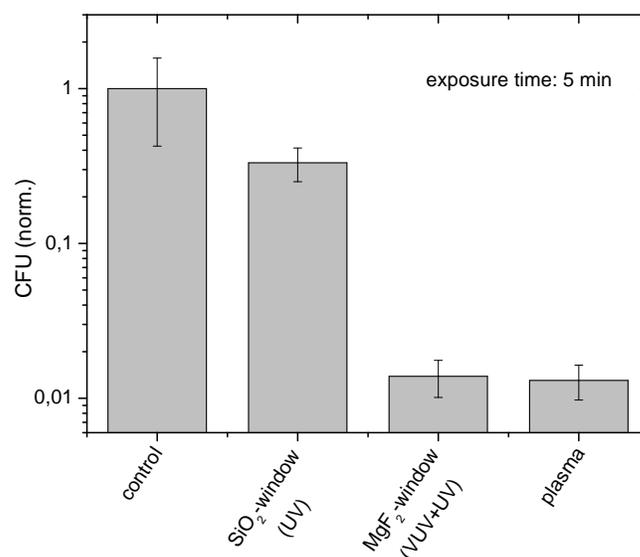
Figure 1. Inactivation of *Bacillus atrophaeus* spores by applying separately the plasma jet, the UV and the VUV+UV radiation generated by the atmospheric pressure plasma jet. The inactivation results (colony forming units of surviving spores) are normalized on the mean value of the control (Lange and von Woedtke, 2010).

may have specific synergistic effects.

3.3. Microbiological verification of plasma effectivity

3.3.1. Verification methods The evaluation of the plasma-induced microbicidal effect is usually done by proliferation assays. Here, the absence of augmentable microorganisms is detected and expressed by microbial inactivation rates. Therefore, the plasma treated bacteria have to be suspended in growth media, diluted in decimal steps and plated on agar afterwards. The augmentable microorganisms form colonies which can be counted. Using this method, the experimental detection limit has to be taken into account. If at least one colony occurs on the agar plate a statement on the proliferation ability and viability can be given. However, if no colony forms the microorganisms are not able to proliferate which does not necessarily mean that all microorganisms are dead or inactivated. To proof the viability of the treated microorganisms many tests exist in form of viability assays (e.g. BacTiter-Glo, Live/Dead*BacLight*). In these assays, different molecules of the microorganisms are labeled with fluorescent agents. This is generally accomplished by a dye exclusion technique. The bacteria with intact membrane are able to exclude the dye, while bacteria with damaged membrane take up the coloring agent. This allows a differentiation by fluorescent signals between healthy and non living microorganisms (LaFlamme et al., 2004). Proof for demolished membranes or other morphological disorders can also be given by electron microscopy (Laroussi et al., 2003) or atomic force microscopy (Pompl et al., 2009; Hähnel et al., 2010a; Kuo et al., 2006). However, only severe morphological changes can be observed with these two microscopic

methods.

3.3.2. Type of microorganisms and loads Different international standards and requirements for the characterization of a sterilizing agent and the development, validation and routine control of a sterilization process are given, e.g. *ISO 14937*. Most of them require identification and investigation of the most resistant microorganisms for the tested agents. In the beginning the American Environmental Protection Agency (EPA) required tests to be made with two types of aerobic and anaerobic spores e.g. *B. atrophaeus* and *Clostridium sporogenes*, while the current standard procedures for checking autoclaves and ethylene oxide sterilizers are based on the inactivation of *B. atrophaeus* spores, *B. coagulans* spores, *C. sporogenes* spores and *G. stearothermophilus* spores (Boucher, 1985; *ISO 17665*, International Standard, 2006).

Generally, microbial control processes should be tested for a variety of microorganism types to ensure process effectivity. This applies especially for plasma-based microbial control processes, because plasmas typically consist of more then one sterilizing agent. Hence, different types of microorganisms with their specific metabolic and morphologic properties show different sensitivities against plasma stress (Hury et al., 1998; Gadri et al., 2000). Examples for such properties are the thickness of cell walls or its chemical composition, the structure of membranes, DNA protection by core structures, the production of duration forms or the ability of aerobic or anaerobic respiration (Madigan and Martinko, 2005). For this reason, plasma-based microbial control processes should be tested with more than one microbial test organism, e.g. *B. atrophaeus* and *B. pumilus*. Other possible microorganisms are *A. brasiliensis*, *S. aureus* or *E. coli*. The international standard *ISO 14937* recommends the use of test loads consisting of microorganisms that have a high resistance to the sterilizing agent, that are present on the materials of construction and in the environment in which the product is manufactured, that are likely to be present in the environment of use or present on a reusable medical device as a result of its prior use on a patient and that cover a broad range of types (e.g. aerobic and anaerobic Gram positive and Gram negative bacteria, spores, mycobacteria, fungi including sporing forms, yeasts, parasites, and viruses). A selection of different microbial test organisms and their field of application is given in table 2. Moreover the requirements also claim the investigations of Gram-positive and Gram-negative vegetative bacteria, such as *S. aureus* and *E. coli* (e.g. to prove the water quality). Furthermore investigations of additional organic (bovine serum albumin or blood) and inorganic (salt) loads are required.

The comparability of different sterilizing methods is rather difficult, due to variations in inactivation kinetics, physical parameters, microorganisms and loads. Therefore, it is necessary to standardize investigation procedures for the ability of comparative reduction factors and thus inactivation efficacy. In experimental methodology, a round robin test is a test performed independently several times. This can involve multiple independent scientists performing the test with the use of the same method in different equipment, or a variety of methods and equipment. There are

Table 2. Selection of different microbial test organisms and their field of application.

Agent	Specification	Microorganism	Form	Gram stain	Comment	Lit.
heat	wet	*Geobacillus staerothermophilus*	spore	+	bioindicator for autoclaving	Spicher et al. (1996) Ph. Eur. 6.0 (2008)
	dry	*Bacillus atrophaeus*	spore	+	indicator for hot air	Kerkulek (1975) Ph. Eur. 6.0 (2008)
radiation	UV	*Bacillus pumilus*	spore	+	bioindicator for radiation	Prince (1976)
		Aspergillus brasiliensis	fungi	n/a	common used fungi	Muranyi et al.
	γ	*Deinococcus radiodurans*	vegetative	+	most resistant bio-indicator for γ-radiation	Anderson et al. (1956)
		Bacillus pumilus	spore	+	bioindicator	Ph. Eur. 6.0 (2008)
gas	$C_2H_4O^*$	*Bacillus atropheaus*	spore	+	bioindicator	*ISO 11138* Ph. Eur. 6.0 (2008)
	$CH_2O^\star$	*Geobacillus staerothermophilus*	spore	+	bioindicator	*ISO 11138*
	$H_2O_2^\diamond$	*Bacillus atrophaeus*	spore	+	bioindicator	Andersen et al. (2006)

*ethylene oxide, *formaldehyde, $^\diamond$hydrogen peroxide

different reasons for performing a round robin test. For example, if a new method of analysis has been developed, a round robin test involving proven methods would verify whether the new method produces results that agree with the established method or not. In plasma setups the aim of the comparative tests could be to devise a system for quantification of inactivation efficiency. Therefore, following should be standardized when analyzing the plasma effects. On the one hand the type of test object and used contamination and on the other the proof of the residual contamination (Köhnlein et al., 2008; von Woedtke et al., 2008). On that basis, complex processes with different variables can be evaluated by reduction factors. Routinely round robin tests are done by accredited test laboratories after *ISO 17025* or national admission procedures. Besides the investigation of the reduction factors it is essential to understand and to establish an analytical relation between the reduction factors and their dependency on the process relevant parameters (e.g. exposure time, plasma characteristics). Only this allows to provide a reliable estimate of the SAL achieved at given process parameters, since a low SAL (e.g. 1×10^{-6}) required in practice cannot be gained directly by measurement.

4. Atmospheric pressure plasma sources

In the following section strategies and setups for the generation of low-temperature atmospheric pressure plasma used for microorganism decontamination are presented. Consequently, only plasma sources that are reported to be effective against biological contaminants are reviewed here. For clarity, the plasma sources are classified by the excitation frequency and electrode configuration as it is done by other authors before knowing well that other classification schemes can be applied, too. The resulting

groups of discharges are coronas (DC or pulsed), dielectric barrier discharges (from low frequency to several MHz), atmospheric pressure plasma jets (from DC to some GHz) and microwave driven plasmas in the GHz range. Many of the presented discharge geometries can be miniaturized down to a scale of several hundred micrometers. Plasmas generated at these scales are denoted as microplasmas or microdischarges (Becker et al., 2006; Iza et al., 2008). Beside other interesting fields of application those plasmas can be used for microbial decontamination, too. Here, valuable results have been achieved in recent years (Rahul et al., 2005; Becker et al., 2005). However, this review is rather focused on the normal scale plasmas. Readers, who are interested in microplasmas are recommended to study the listed references.

4.1. Corona discharges

Corona discharges are applicable for a variety of different industrial applications such as surface treatment or the removal of volatile organic compounds as it is necessary for gas cleaning (Fridman et al., 2005). Another application is the collection of airborne microorganisms by electrostatic precipitation (Mainelis, 1999). Furthermore, coronas are applied to generate ozone for water disinfection (Shin and Sobsey, 2003). In the last years coronas have also been studied for the purpose of biological decontamination as it is described in this section.

A corona discharge usually appears near sharp electrode geometries like points, edges or thin wires where the electric field in the electrode vicinity is sufficiently large to accelerate randomly produced electrons up to the ionization energy level of surrounding gas atoms or molecules (Raizer, 1997). Thus, ionization and luminosity is mainly located at the sharp electrode. The typical electrode geometry is a sharp curved electrode arranged counterpart to a flat one (point to plate geometry) as it is displayed in figure 2 but also cylindrical configurations are applied (Pekárek, 2010). Coronas can be operated in DC or pulsed mode, where the pointed electrode can have a negative or positive potential. Usually, negative dc corona discharges are used for microbial inactivation although microbial inactivation has also been shown for different pulsed modes (Cramariuc et al., 2008).

Scholtz et al. (2010) studied the microbicidal effect of a negative DC corona in ambient air using a pin to plate geometry (see figure 2a). High voltages up to $10\,kV$ and currents up to $0.5\,mA$ were adjusted. On the anode an agar plate or a dish with aqueous suspension is located in which different types of microorganisms, including yeasts, vegetative bacteria and bacterial spores, were plasma treated. As a result bacteria in liquid suspension are completely inactivated within $5\,min$ of exposition, whereas the exposition up to $30\,min$ is necessary for the yeasts.

Bussiahn et al. (2010) presented a intermittent negative DC corona discharge using an arrangement similar to the one shown in figure 2b denoted as hairline plasma. A pointed hollow needle electrode is fed with argon gas at a flow rate of $0.5\,slm$ and connected to a negative high voltage in the range of $1 - 14\,kV$. Between the cathode and
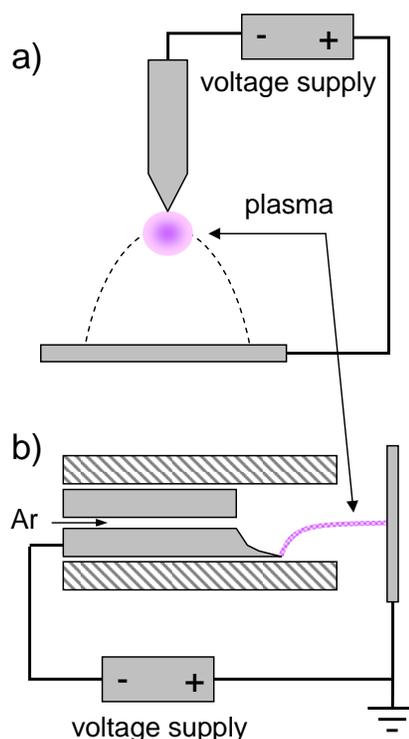
Figure 2. Point to plate electrode arrangements for generating a negative DC corona discharge. a) Plasma is generated at the point of the negative electrode. b) A thin plasma channel occurs between a pointed negative electrode and a grounded one due to a sufficient argon flow (according to Scholtz et al. (2010) and Bussiahn et al. (2010)).

the anode, which consists usually of biological material, an intermittent plasma with a temperature of $\sim 300\,$K develops. This discharge produces ns-short current pulses that have a repetition frequency of $\sim 1.8\,$kHz and an amplitude of several hundred mA. The radial extension and the length of the plasma are $30\,\mu$m and up to $1.5\,$cm, respectively. The microbicidal efficiency of this source was demonstrated generally for the gram negative bacteria *E. coli*. Furthermore the ability of the hairline plasma to enter small cavities is impressively shown in figure 3.

4.2. Dielectric Barrier discharge

Opposite to the small spot treatment with coronas, the dielectric barrier discharge (DBD) is an ideal plasma source vor treatment of extensive surfaces. Furthermore, the DBD is an alternating current discharge in non-thermal equilibrium. It is typically generated between two electrodes, whereas at least one dielectric limits the discharge current. The distance of the electrodes is from μm up to cm depending on the used process gas and operating voltage. Typical electrical operation parameters are in the range of some kV ignition voltage from line frequency to several MHz and power consumption of some W per dm^2 electrode area. For high power input the gas temperature reaches up to $150\,°$C. In figure 4 schematic pictures of the most common
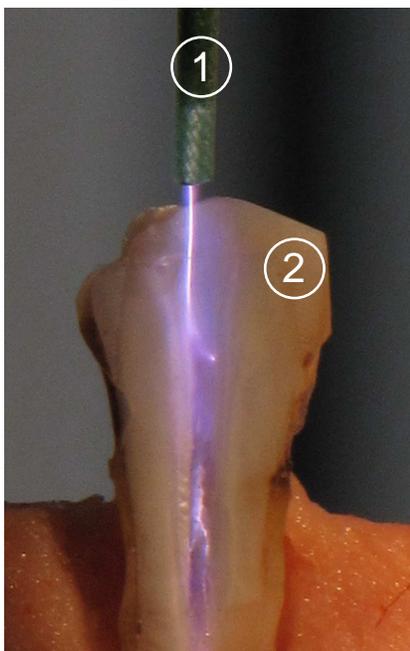
Figure 3. Application of the hairline plasma source (1) to a prepared root canal of a human tooth (2) according to Bussiahn et al. (2010).

electrode setups are shown. There are a variety of different electrode geometries and
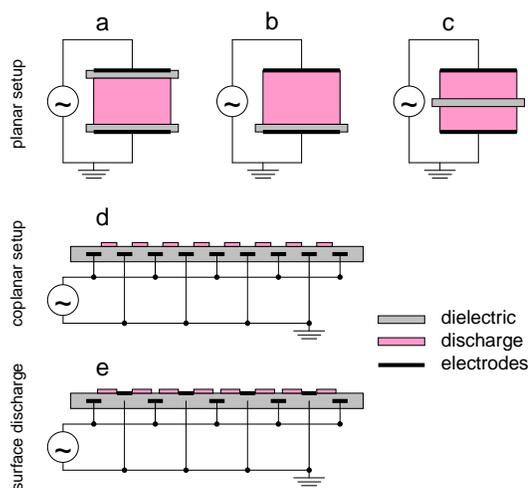
Figure 4. Typical electrode arrangements for DBDs. a) planar with dielectric at both electrodes, b) planar with only one electrode covered with dielectric, c) planar with dielectric in the discharge gap, d) coplanar setup where both electrodes are embedded inside the dielectric, e) setup for surface discharge generation with one electrode embedded in dielectric (according to Wagner et al. (2003).

setups for DBD. Depending on the application, it is possible to use planar or coplanar arrays with different geometries like curved, coaxial or twisted electrodes. According to the setup a volume or a surface discharge is generated. Thereby, two discharge modes

have to be discerned, diffuse and filamented. In the filamentary mode many small discharge channels are generated along the electrode area. In contrast, the diffuse mode consists of a uniform discharge. Crucial parameters for the operation mode are used process gases and the electrical operation of the discharge.

The major advantage of the DBD is due to the effortless discharge ignition. Nearly every combination of gases can be used, from noble gases over air or water vapor up to special admixtures of precursors are applicable. Furthermore, the gas flow of down to 100 sccm and less is comparatively low, whereby the DBD is especially interesting for industrial applications. Another advantage is the manifold adaptability due to the different electrode geometries. Hence, a homogeneous discharge can be ignited over several meters with nearly no limitations. A disadvantage is the high ignition voltage of 10 kV or more in some extend depending on the restricted electrode gap, wherefore certain precautions or isolations are essential.

For a brief review of possible prospects of DBDs some selective applications and setups with attention to the manifold geometries in combination with microbicidal efficiency will be presented below.

Polak et al. (2010) showed a special setup to generate a gas discharge inside a long and flexible tube for the use as biopsy channels in endoscopes by means of dielectric barrier discharge. To provide an extended electric field along the tube 2 electrodes are equidistantly twisted around the tube with 2 mm inner diameter. The electrodes are located inside the tube wall, whereby the interior tube is not disturbed by foreign material and the outer side is electrically insulated towards peripheral devices (figure 5a). For special electrode geometries the working voltage is at about 7 kV with 3 kHz working frequency. With this called bifilar helix discharge setup a uniform gas plasma could be ignited along a 5 m tube with inner diameter of 2 mm for various gas mixtures of He, Ar, O_2, N_2 (figure 5b). Preliminary results concerning the microbicidal efficiency were
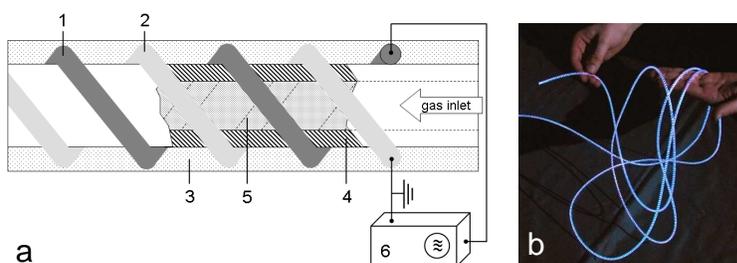
Figure 5. a) Schematic illustration of bifilar helix discharge setup to generate a DBD inside a long tube. 1) powered electrode, 2) grounded electrode, 3) outer tube, 4) inner tube, 5) gas discharge, 6) power supply. **b)** Mechanical manufactured tube (length: 5 m) with flat wire electrodes (Polak et al., 2010).

achieved using a *B. atrophaeus* spores solution mixed with 0.3 % bouvine serum albumin (BSA). Therefore, a special contamination procedure for long tubes was developed. It

was be demonstrated that a gas mixture of 1.5 slm‡ argon and 200 sccm forming gas composed of 95 % N_2 and 5 % H_2 leads to a reduction factor of more than $4 \log_{10}$ for an initial microorganism concentration of 10^6 CFU/ml and 10 min exposure time.

Fridman et al. (2006) introduced a plasma source specially designed for human skin treatment called Floating Electrode Dielectric Barrier Discharge (FE-DBD). This setup works with one powered electrode embedded in quartz glass, the treated surface is used as the second virtual electrode. Due to the high dielectric constant of the quartz at the powered electrode this setup is safe and can be used for human body treatment. So one is able to work in direct contact to the human body with distances in the range of some millimeters. Bacteria for quantitative analysis of sterilization were obtained by transferring some of skin flora from a patient with normal skin flora onto a blood agar plate. After 24 h at 37 °C in air incubator the grown colonies were transferred from agar surface into a sterile container and diluted with purified sterile water. 1 ml und 20 µl were pipetted onto agar and left to dry in a class I biological safty hood for 3 h or 5 min respectively. After plasma treatment they were spread over the agar plate by a sterile swab. The results indicate a complete inactivation of 10^7 CFU within 10 s of plasma treatment. Within 15 s even 10^8 CFU can be inactivated.

A methode for indirect sterilization of microorganisms is presented by Venezia et al. (2008). They used the afterglow of a PlasmaSol apparatus (PlasmaSol Corporation). This apparatus consists of a plasma-generating electrode powered with 30 ± 1 W imbedded into a sterilizing container . As reagent gas a mixture of 1 % ethylene, 50 % oxygen and 49 % nitrogen at a flow rate of 1 L/min was used. The gas was humidified on the way to the electrode by passing through a bubbler, which results in a concentration of 0.025 ± 0.005 g H_2O per liter of gas. As a control for lethal activity 10^6 CFU *Bacillus atrophaeus* spores (ATCC9372), 10^8 CFU Staphylococcus aureus (ATCC25923) and a huge variety of other microorganisms suspended in trypticase soy broth were pipetted on a 8×12 mm stainless steel disc. Afterwards the discs were placed inside the sterilizing container for plasma treatment. They can show that 2 min of plasma exposure time inhibit a $5 \log_{10}$ reduction down to the detection limit for *B. atrophaeus*. Within 10 min of treatment time almost all bacteria in dry or wet environment could be reduced about $5 \log_{10}$.

Hähnel et al. (2010b) used the remote impact of a surface DBD in ambient air for inactivation of microorganisms (figure 6a). Therefore, a special electrode geometry based on that shown in figure 4e was invented. With 10 kV sinusoidal ignition voltage, 2 kHz frequency in pulsed mode with 1 Hz and a maximum of 500 ms plasma on-time the mean gas temperature of the discharge was kept closed to 300 K. The microbicidal efficiency was tested with *B. atrophaeus* spores at varying relative process gas humidities. The results show a strong dependence on the humidity. As for 30 % humidity a maximum of $1 \log_{10}$ reduction could be reached, for 60 % relative air humidity all bacteria were killed. This is a reduction factor of $4 \log_{10}$ for 10^5 CFU/ml initial concentration after 150 s

‡ In this review the unit of the gas flow rate is generally denoted as it is done in the accordant reference.
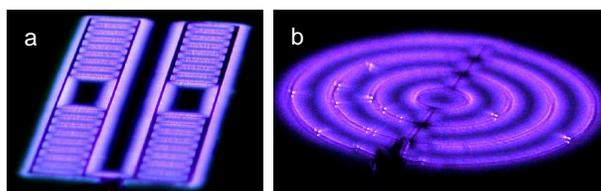
Figure 6. Various surface discharge electrodes made of circuit board materials for indirect plasma treatment of test strips (a) and liquid samples (b). (Hähnel et al., 2010b; Oehmigen et al., 2010)

plasma treatment. Additional pulse length variations indicate an exponential correlation between the plasma on-time and reduction rate. In fact, 5 min plasma treatment with 300 ms on-time per second led to a reduction factor of $5 \log_{10}$.

Oehmigen et al. (2010) used a similar surface DBD specially designed to fit into 60 mm diameter petri dish (figure 6b) to analyze the impact of an indirect discharge to microorganisms in solution. It could be shown that depending on the treatment time the pH-Value of the solution changed down to 2.78 for 0.85 % NaCl solution, whereas phosphate buffered saline solution (PBS) stayed at pH 7 even after 30 min plasma exposure. This could be concluded to the formation of NO_x inside the discharge. Hence, acidification of non-buffered solution was interpreted as a consequence of the formation of nitrous acid (HNO_2) and nitric acid (HNO_3). Subsequent microbial tests with *B. atrophaeus* spores exhibit correlative behavior. While all bacteria solved in 0.85 % NaCl solution after 5 min plasma treatment are inactivated ($6.5 \log_{10}$), the bacteria solved in PBS are subjected to a reduction of maximum $3 \log_{10}$ for 15 min exposure time.

Gadri et al. (2000) used an one atmospheric uniform glow discharge plasma (OAUGDP) in direct or remote mode to inactivate various microorganisms. The discharge is operated in air with a maximum of 14 % humidity. The different plasma modes are generated in 2 different setups. The first setup consists auf 2 parallel electrodes shown in figure 4a where the treated samples are situated on the bottom electrode. The second setup for remote mode consists of multiple plasma panels as displayed in figure 4e arranged in way that the air flow has to go a serpentine pathway along the active zones of the plasma panels. The microbial inactivation results of *E. coli* K12 cells in direct mode with 10 kV rms, 7 kHz and initial loading of 6×10^6 cells show complete inactivation of the cells within 25 s plasma exposure time. The remote mode setup exhibits for the same operational parameters similar effects, where 25 s plasma treatment result in about $6 \log_{10}$ reduction at 3×10^8 CFU initial concentration. Additional results for other microorganisms are shown in table 3. The microbicidal efficiency was supposed to the plasma generated chemical active agents like ozone, monatomic oxygen, superoxide, hydroxyl and nitric oxide.

Leipold et al. (2010) presented a special setup to decontaminate rotating cutting tool used for slicing in the meat industry by the means of DBD. The cutting tool is a disc with shallow cones, 450 mm diameter and a rotating speed of 3.6 s per revolution.

For Plasma treatment of both sides of the cutter two equal DBDs were used whereas the cutter works as the grounded electrode (figure 7). The water cooled DBDs have an electrode dimension of 100×100 mm and a distance to the surface of the circular knife between $2-4$ mm. Electrical working parameters are 21.7 kHz, 10.4 kV zero-peak voltage and power consumption of about 360 W. By pulsing the discharge with a duty cycle of 1:100 or 1:200 the consumed power could be reduced down to 3.6 W or 1.8 W respectively. The reduction efficiency of the discharge was tested with 240 μl *L. innocua* (as biological indicator for *L. monocytogenes*) with 5×10^7 CFU/ml initial concentration directly sprayed onto the knife. A significant increase of inactivation of about $4-5 \log_{10}$ could be achieved between 68 s and 170 s operation time and 360 W, for 1.8 W a reduction of even $3.5 \log_{10}$ for a 300 s process (37 s exposure time) are feasible.
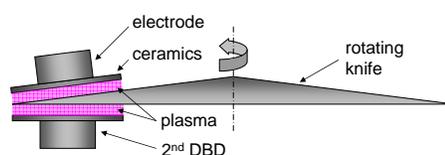

Figure 7. Sketch of the experimental setup for plasma surface decontamination of a circular knife used for slicing in meat industry (according to Leipold et al. (2010)).

Eto et al. (2008) describes a setup to decontaminate samples stored inside Tyvek packaging. Therefore they used an electrode arrangement similar to that shown in figure 4e built in a flexible sheet-like configuration to fit any shapes of Tyvek packaging and apply it to the inner sterilization of wrapped biological indicators. The DBD has dimensions of about 40×40 mm with a 2.5 kV and 5 kHz powered stainless steel electrode grid and is put directly on the permeable Tyvek side of the packaging. The contaminated bowl-shaped stainless steel discs are situated inside the Tyvek packaging with the contaminated side towards the discharge or upside down. *Geobacillus stearothermophilus* spores with initial population of 3×10^6 were used as test organisms for different mixtures of oxygen and nitrogen as process gases. The measurements show a maximum inactivation rate for 50 % N_2 and 50 % O_2, whereas a total inactivation of all microorganisms after 15 min irradiation time can be achieved. Furthermore no difference was detected whether the contaminated disc side is at the top in direction towards the discharge or the other way around. This is assumed to correlate with the formation of ozone, which has a concentration of 80 ppm. Thus, the generated chemical species are most effective. Furthermore, typical atmospheric conditions with air and 64.4 % humidity were used as process gas. A complete inactivation could be observed after only 5 min of plasma treatment. This result is associated with the combination of ozone and OH-radicals produced in the discharge afterglow.

Another setup for disinfection of goods inside closed packages is presented by Schwabedissen et al. (2007). They used a surface DBD similar to figure 4e where two powered electrodes in the kHz range are glued on the outer side of the closed package and a third electrode capacitively coupled to the outer electrodes is situated on

the inner wall of the package (see figure 8). With about $8\,\mathrm{kV}$ peak to peak voltage a discharge is formed between the inner electrode and the outer powered electrodes with about $2-4\,\mathrm{W}$ discharge power. The use of ambient air inside the package results in the formation of ozone as disinfecting agent. With the addition water vapor various
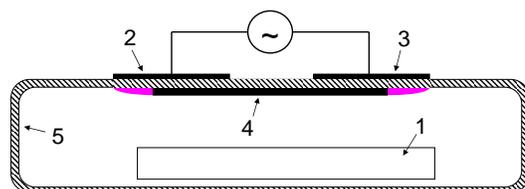

Figure 8. Scheme of PlasmaLabel concept with the goods to be disinfected (1) inside the closed package (5), two powered electrodes outside the package (2 and 3) and an inner electrode (4) (according to Schwabedissen et al. (2007)).

biological active species and radicals e.g. H_2O_2, nitric HNO_3 and nitrous HNO_2 acids, N_2O_4 and N_2O_5 are formed. Microbicidal efficiency of the PlasmaLabel discharge was tested in a polystyrol petri dish with a paper strip of *B. subtilis* (ATCC9372) inside. Initial concentration was more than $10^6\,\mathrm{CFU}$. The results show a reduction of $4\,\log_{10}$ within $10\,\mathrm{min}$ ozone exposure time. Additional practical test were performed with cherry tomatoes and strawberries packed into a rigid plastic container and sealed hermetically. While the untreated tomatoes were covered with mildew after 14 days storage at room temperature, the treated ones showed no effect. The same behavior could be observed for the strawberries.

The first commercialized plasma based process at atmospheric pressure is the Tip-Charger system (CerionX, Inc., Pennsauken, USA). Based on a dielectric barrier discharge the system cleans and sterilizes liquid transfer devices such as pipette tips, cannulae and pin tools. TipCharger cleaning stations can substitute traditional solvent-based wash methods in automated liquid handlers in biopharmaceutical and diagnostic industries. Unlike washing procedures, no liquid waste is generated and the plasma treatment does not simply dilute contaminants, but removes them. This allows the reuse of disposable pipette tips (Kurunczi, 2005).

A promising concept close to commercialization is the cascaded dielectric barrier discharge (CDBD) which combines UV radiation and direct plasma treatment (Heise et al., 2004). Therefore, the discharge gap consists of two separated parts, one is filled with an excimer gas for efficient UV emission. The second gap is flushed with a discharge gas forming reactive species (e.g. air). With this setup a fast reduction of viable cells by more then four orders of magnitude is possible within few seconds.

4.3. Atmospheric pressure plasma jet

Another frequently used plasma sources are the Atmospheric pressure plasma jets (called APPJ after Schütze et al. (1998)). These are non-thermal capacitively coupled plasma

sources typically operated in the radio frequency range, e.g. at 13.56 MHz or 27.12 MHz. In general, they consist of 2 electrodes (see figure 9) in different arrangements. This includes coaxial and special ring electrode setups, but also single electrode configurations with a virtual grounded electrode are established. The distance of the electrodes is in the range of some mm, whereas the exposure distance to the contaminated surface is rather in the cm range. Depending on the used process gases and electrode gaps typical
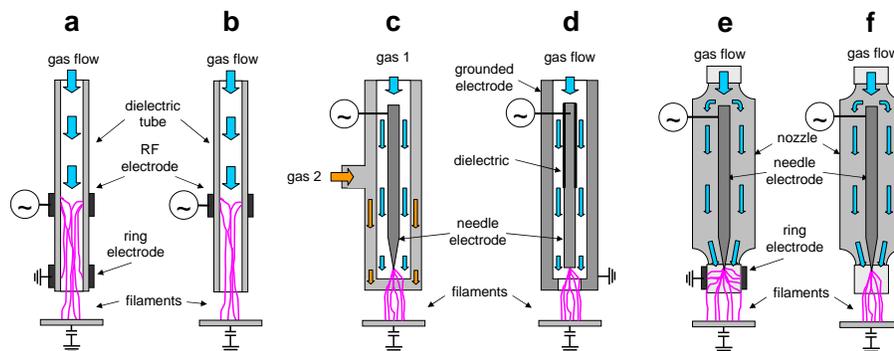

Figure 9. Principle designs for atmospheric pressure plasma jets a) using 1 powered and 1 grounded ring electrode, b) without grounded ring electrode, c) as combination of 2 tubes whereas the inner tube is streamed with a noble gas for discharge ignition and the outer tube with a precursor, d) composed of two coaxial electrodes with a dielectric in between e) consisting of an inner RF driven needle electrode and a grounded ring electrode, f) without grounded ring electrode.

ignition voltages of about some 100 V up to kV are applicable, which results in up to 500 W consumed power. Typical process gases are noble gases like helium or argon with gas flow rates up to some slm, but also difficult ignitible precursor admixtures can be used. Due to the use of noble gases even VUV radiation can be applied on the substrate under atmospheric conditions. The process temperature is based on the high gas flow and low power consumption about 350 K in general, but with special electrical input signals (e.g. burst mode) the temperature can be decreased down to nearly room temperature (Weltmann et al., 2009). APPJs can be utilized in direct or in remote mode, however most applications work in direct mode.

The major advantage of APPJs are the small plasma dimensions in combination with the ability of penetrating into narrow gaps with high aspect ratio (Weltmann et al., 2008b). This makes APPJs particularly interesting for applications at complex geometries with micro structured cavities or capillaries. Likewise, the small dimensions of the blown out discharge are advantageous for the precise treatment of sensitive spots. Thus, APPJs are often used for biomedical applications (Lee et al., 2010; Daeschlein et al., 2010). In parallel, the small spot size is a disadvantage for a homogeneous treatment of large areas, too. Therefore, a purpose-built configuration composed of an array of APPJs or a special process control has to be used (Foest et al., 2005; Ehlbeck et al., 2008; Weltmann et al., 2008b). Another advantage of APPJs for microbial inactivation is the high etch rate in the range of up to $18\,\mu\mathrm{m}/\mathrm{min}$ for polymers and

nearly $2\,\mu\mathrm{m}/\mathrm{min}$ for metals and metal oxides (Fricke et al., 2010; Jeong et al., 1998). On the other side in some extend the high gas flow of more than $10\,\mathrm{slm}$ is unfavorable, especially if noble gases are used. Furthermore, APPJs are limited in the use for complex 3-dimensional objects. Solutions exist, but they are not economical because of the high gas consumption (Brandenburg et al., 2008). Following, an overview of the past 10 years of microbial inactivation by the means of APPJs will be given.

Herrmann et al. (1999) used a coplanar APPJ setup as shown in figure 9d for decontamination of biological and chemical warfare agents. The inner electrode is coupled to a radio frequency generator with $13.56\,\mathrm{MHz}$, the outer electrode is grounded and has a $8\,\mathrm{mm}$ diameter at the exit. The used RF power was $250\,\mathrm{W}$, whereby an effluent gas temperature of $150\,^{\circ}\mathrm{C}$ could be measured. As process gas Helium with $92\,\mathrm{slm}$ and $0.72\,\mathrm{slm}$ oxygen admixture was utilized. Used microorganisms for determining the microbicidal efficiency of the discharge were *B. globiglii* spores as being variation for *B. anthracis* spores. About $10^{7}\,\mathrm{CFU}$ of these spores were suspended in $10\,\mu\mathrm{l}$ of water and inoculated onto a glass coupon forming a $\sim 5\,\mathrm{mm}$ diameter spot after drying. The decontamination curves show an inactivation of all microorganisms after $30\,\mathrm{s}$ exposure time. The curves are additionally compared with hot gas of $175\,^{\circ}\mathrm{C}$ blowed through the APPJ without plasma ignition. After $120\,\mathrm{s}$ hot air treatment only about $2.5\log_{10}$ could be inactivated. Further examinations were done by cooling the RF electrode with water to decouple the temperature effect and the effect of the discharge itself. This results in a significant increase of about $2\log_{10}$ decontamination for temperatures lower than $100\,^{\circ}\mathrm{C}$.

Laroussi et al. (2006) present a plasma pencil designed as shown in figure 9a. The dielectric tube has a $2.5\,\mathrm{cm}$ diameter and is $12\,\mathrm{cm}$ long, the 2 copper electrodes are separated by a $0.5 - 1\,\mathrm{cm}$ gap. To ignite the plasma, $5\,\mathrm{kV}$ square voltage pulses with a repetition rate of $1 - 10\,\mathrm{kHz}$ were applied, whereby a total power consumption of about $15\,\mathrm{W}$ could be measured. Used process gases were helium and small admixtures of oxygen with $1 - 10\,\mathrm{l}/\mathrm{min}$ flow rate. Preliminary experiments for inactivation of bacteria were carried out using Petri dishes contaminated with about $10^{6}\,\mathrm{CFU}/\mathrm{ml}$ *E. coli* on Agar. The distance between the Petri dish and the pencil was about $3\,\mathrm{cm}$ and 2 gas mixtures (helium and helium $+\,0.75\,\%$ oxygen) were compared by visually estimating the inactivated region. This results show an equal small area of inactivation for both gases after $30\,\mathrm{s}$ of exposure time. By raising the treatment time to $120\,\mathrm{s}$ the diameter of the complete inactivated area is enlarged about five times for helium $+\,0.75\,\%$ oxygen and about 3 times for pure helium.

Sladek and Stoffels (2005) invented a non-thermal plasma source called "plasma needle". The electrode arrangement is similar to figure 9f with a $4\,\mathrm{mm}$ inner diameter Perpex tube as nozzle. The $0.3\,\mathrm{mm}$ needle is driven by a RF-Frequency of $13.05\,\mathrm{MHz}$ and up to $350\,\mathrm{mW}$ power are dissipated inside the discharge. In a distance of $1\,\mathrm{mm}$ from the needle this power results in a process temperature ranging from nearly room temperature up to $70\,^{\circ}\mathrm{C}$. The used process gas was helium at a flow rate of $2\,\mathrm{l}/\mathrm{min}$. The inactivation experiments were carried out using $100\,\mu\mathrm{l}$ of *E. coli* (Cat. no. 69825)

with initial concentration of about 1×10^8 CFU/ml plated on a petri dish filled with nutrient agar. The results show an inactivation of $4.5 \log_{10}$ and 6 mm void size within 10 s exposure time for 180 mW and 1 mm distance. For 60 s treatment time the void size reaches its maximum with 10 cm and about 1.3×10^5 CFU could be inactivated.

Lim et al. (2007) used an APPJ with a coaxial electrode configuration comparable to figure 9d. The inner electrode has a stepped structure partially covered by a dielectric tube on the top of the electrode. The electrodes are about 12 cm long and have a outer diameter of 1.2 cm without dielectric and 1.41 cm with dielectric for the powered electrode and an inner diameter of 1.45 cm for the grounded electrode. The APPJ is operated at a frequency of 13.56 MHz, input power = 130 W and flow rates of 10 slm argon or helium with 0.25 % admixture of oxygen. The inactivation rate was determined using 0.01 ml of *Bacillus atrophaeus* spores (*B. subtilis var. niger*, ATCC9372) solution with an initial concentration of 2×10^9 spores/ml deposited on a glass sample. The measurements show a drastic difference between Ar + O_2 and He + O_2. While the treatment with helium as background gas takes 180 s for $2 \log_{10}$ the argon plasma exposure results in a total inactivation of $7 \log_{10}$ reduction for 35 s. Additional variation of the exposure distance for Ar + O_2 mixture reveals a complete inactivation after 35 s for 5 mm distance, 90 s for 10 mm und only a $3 \log_{10}$ reduction for 180 s exposure time and 15 mm distance.

Another experiment with a configuration shown in figure 9e for inactivation of microorganisms on simulated wound environment was carried out by Daeschlein et al. (2010). The pin type center electrode has a diameter of 1 mm and the quartz tube has an inner diameter of 1.6 mm. As process gas argon flows through the capillary with a flow rate of 8 l/min. The APPJ is electrical driven with 1.5 MHz in the range of $1 - 5$ kV. Depending on the gas flow rate and the applied voltage, the discharge has a length up to 12 mm and a temperature in the discharge center of about 50 °C. The tested species were typical wound colonizators, like methicillin-sensitive *Staphylococcus aureus* (ATCC1924), *Pseudomonas aeruginosa* (ATCC15422), *Enterococcus faecium* (ATCC6057), *Candida albicans* (ATCC10231) and a clinical isolated β-hemolyzing *Streptococci* of the Lancefield serogroup A. Therefore, the soluted microorganisms are plated on blood agar and afterwards the non-visible growth of about $2 - 3 \log_{10}$ CFU/plate initial concentration was treated with the APPJ in 7 mm distance following 3 meandric lines for a total exposure time of 6 min. The treatment of the five test species resulted in different reductions. For *P. aeruginosa* 19 among 20 agar plates were totally free of viable microorganisms, the treatment of HSA *Streptococci* showed a complete inactivation for 18 of 20 agar plates. For *S. aureus* and *C. albicans* at 8 among 20 agar plates no surviving microorganism could be detected, whereas for *E. faecium* no agar plate could be completely inactivated.

Ehlbeck et al. (2008) and Weltmann et al. (2008b) show a setup for inactivation of catheters. The APPJ is configured as shown in figure 9e, but at the end of the nozzle a T-type quartz tube is arranged, so catheters can be guided via the apertures at both sides of its vertical part figure 10. The generated discharge completely surrounds the

outer surface of the catheter. The APPJ is operated at a frequency of 27.12 MHz, with a power of 20 W and gas flow rate of 20 slm argon with and without admixture of 0.25 % air. The inactivation efficiency was tested by dividing the catheter into 6 sections and
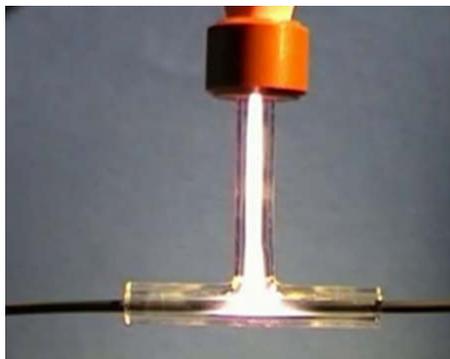

Figure 10. Schematic illustration of catheter treatment with moveable t-type APPJ (Ehlbeck et al., 2008).

contaminating each section with vegetative *Staphylococcus aureus* solution. The 6th section was kept as control, whereas the other sections were treated with the T-type discharge. It reveals a $5 \log_{10}$ reduction for pure argon and a complete inactivation of $6 \log_{10}$ for argon with 0.25 % air admixture. Additionally, the dependency of the amount of inactivation cycles was tested and no increase of efficiency could be detected.

The same team investigated the inactivation of spot-contaminated samples of *S. aureus*, *E. coli* and *B. atropheaus* with more than $3 \times 10^6 \, \text{MOs/cm}^2$ by means of an APPJ operated with a pure argon flow rate of 20 slm (Brandenburg et al., 2007; Brandenburg et al., 2009). Here, a reduction of about seven orders of magnitude is reached in about 10 min for *S. aureus*. For *B. atrophaeus* spores and *E. coli* five orders of magnitude within 12 min and seven orders of magnitude in 7 min are obtained, respectively. Furthermore, the microbicidal efficiency of UV and VUV radiation was evaluated. Therefore, the samples where covered with a quartz window (UV transparent) and a MgF2 window (VUV+UV transparent). The distance between the window and the contaminated sample was below 0.6 mm. It was shown, that direct plasma treatment (without shielding) gives the highest reduction of microorganisms. By shielding the samples the reduction is about 1-2 orders of magnitude lower. In contrast to the results of Lange and von Woedtke (2010) (see figure 1) no difference between UV and VUV+UV radiation with regard to the microbicidal treatment is obtained in this work. Hence, the intensity of the VUV radiation reaching the contaminated sample must be very small compared to the UV intensity. This is probably due to the small air gap between the samples and the shielding window where VUV radiation is absorbed. This effect did not occur in the setup of Lange and von Woedtke (2010) since the samples were arranged in a chamber filled with pure argon.

Perni et al. (2008) present a special setup for disinfection of cut fruit surfaces. Therefore, an electrode arrangement similar to figure 9b is utilized. The discharge

system includes a ceramic tube with inner diameter of 1.5 mm and a concentric ring-shaped powered electrode wrapped around the ceramic tube. As grounded electrode a downstream disc electrode placed 1 cm below the nozzle is used. The discharge is operated with an AC power supply of 8 kV and an excitation frequency of 30 KHz. The process gas consists of Helium with a flow rate of 5 l/min and oxygen admixture of 25 ml/min. The temperature of the samples remained below 30 °C. The microorganisms selected for study were a nanopathogenic strain of *Escherichia coli*, *Listeria monocytogenes* and two species of spoilage organisms: *Gluconobacter liquefaciens* and *Saccharomyces cerevisiae*. After cultivation in melon (*Cucumis melo* var. reticulatus) or mango (*Magifera indica*) juice they were deposited on the appropriate cut fruit discs. With an initial concentration in the range of 6-7 $\log_{10}$ the dependency of the exposure time was analyzed. The results show a 2 $\log_{10}$ reduction for *G. liquefaciens* on mango after 10 s exposure time, a 2.5 $\log_{10}$ reduction for *E. coli* and *L. monocytogenes* after 30 s and a 2.5 $\log_{10}$ reduction for *S. cerevisiae* after 40 s exposure time. The results for the cut melon surface show no significant difference.

4.4. Microwave driven discharges

Compared to DBDs microwave (MW) driven discharges are generated without electrodes. The general function of these sources is based on the MW absorption by electrons. Subsequently, the electrons gain kinetic energy to finally ionize the heavy particles by inelastic collisions. This results in an augmentation of electron concentration combined with an increase of power consumption. The electron temperature in the formed discharge core is about 2×10^4 K and the electron density reaches values of 3×10^{21} m^{-3} (Jasinski et al., 2002). In figure 11 the typical setups for generation of microwave driven gas discharges are shown.

The MW are generated by a magnetron and are guided to the process chamber by wave guides or coaxial cables. The magnetrons are working in the GHz frequency range, typically at 2.45 GHz. Typical consumed power is in the range of some W up to kW. The wave guide is directly coupled to a special discharge head or a resonator. These devices are specially designed to induce peaking of field intensity in the center of the resonator or at the tip of the discharge head. Depending on the consumed MW power neutral gas temperatures between room temperature and up to some thousand Kelvin can be reached (Uhm et al., 2006). Hence, the MW discharge can not be clearly assigned to thermodynamical equilibrium or non-thermodynamical equilibrium (translational plasma). Subject to the neutral gas temperature und the material of the contaminated surface the MW discharge is operated in direct or in remote mode. In direct mode a variety of agents reach the surface and induce the bacterial necrosis, whereas in remote mode only the chemical species and radicals are effective.

The major advantage of MW driven discharges is the electrode-less setup, so the discharges are torch-like and easy to handle. Additionally, they can be ignited in air environment, even with special admixtures, precursors or water vapor. The gas
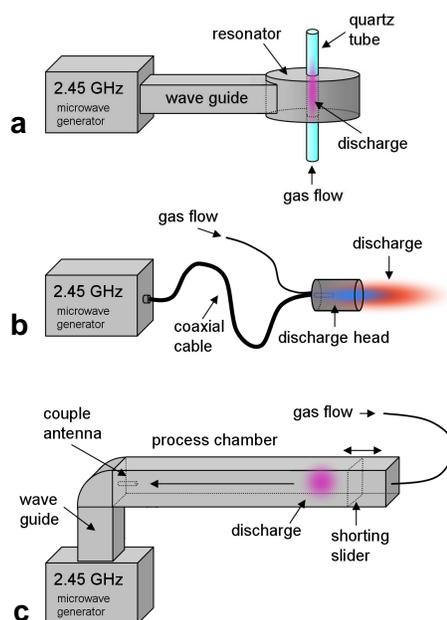
Figure 11. Typical setups for microwave driven gas discharge generation at atmospheric pressure. a) Resonant cavity plasmas using different kinds of resonators (e.g. round or cylindrical) to induce peaking of field intensity in the center of the resonator, b) Free expanding microwave plasma torches with a particular discharge head, c) autonomous through the chamber moving discharge.

fluxes are in a moderate range of some slm. Depending on the used discharge gas a high amount of reactive species can be produced (e.g. N_xO_y, O^3, O_2^-, OH) (Uhm et al., 2006). Obtainable concentrations are 2750 ppm for NO and 400 ppm for ozone (Kühn et al., 2010). By reducing the MW power down to 85 W and less the discharge operates at nearly room temperature (300 K) and thus is capable for direct treatment of thermolabile materials (Shimizu et al., 2008). The disadvantage of MW driven discharges is the spatial limitation. For direct decontamination of large areas an array of discharges has to be used, similar to the arrangement of atmospheric pressure plasma jets. Alternatively, the remote mode can be utilized. Following, a brief review of possible setups for inactivation of bacteria by means of microwave driven discharge will be given.

(Lai et al., 2005) described a setup analogue to figure 11b. The microwave was guided through a wave guide towards one or more discharge heads build from spark plugs. To induce a peak in the field intensity an additional electrical voltage synchronized to the microwave was applied, since the microwave field was too low to initiate discharge by itself (2.45 GHz, 700 W). The torch was operated in direct mode with distances of $3-5$ cm to the contaminated surface and exposure times of $2-20$ s were applied. The used process gas was air at 0.393 l/s gas flow. To contaminate the surface 30 µl *B. cereus* (ATCC No. 11778) spores solution with an initial concentration of about 3.5×10^7 CFU/ml was inoculated on glass coupons. For the three tested exposure distances a linear correlation between inactivation rate and treatment time could be

found. At an exposure distance of $3\,\mathrm{cm}$ $5\,\mathrm{s}$ treatment results in a $2.5\log_{10}$ reduction, whereas after $10\,\mathrm{s}$ a $5\log_{10}$ reduction could be observed. For same inactivation rates at higher treatment distances (4 and $5\,\mathrm{cm}$) about $13\,\mathrm{s}$ and respectively $20\,\mathrm{s}$ were sufficient.

Sato et al. (2007) used a conventional atmospheric pressure microwave plasma source (ADTEC Plasma Technology Co.Ltd) similar to the setup shown in figure 11a with a coaxial cable as wave guide. The interior diameter of the quartz tube is $10\,\mathrm{mm}$, the dimensions of the cavity are $80\,\mathrm{mm}$ in diameter and $150\,\mathrm{mm}$ in hight. The discharge was operated at $2.45\,\mathrm{GHz}$, $400\,\mathrm{W}$ input power and argon flow rates of 1, 3 and $5\,\mathrm{slm}$. The exposure distance between the nozzle exit and the contaminated surface was set between $30\,\mathrm{mm}$ and $200\,\mathrm{mm}$, which results in an exposure temperature of $323\,\mathrm{K}$ to $383\,\mathrm{K}$. As contaminated samples membrane filters with *E. coli* concentrations up to 10^5 CFU/ml were used. The results show about $1\log_{10}$ reduction for $5\,\mathrm{slm}$ argon flow and $120\,\mathrm{s}$ treatment time at $353\,\mathrm{K}$ (exposure distance $= 165\,\mathrm{mm}$). For $600\,\mathrm{s}$ a total inactivation of nearly $3\log_{10}$ reduction can be achieved. By decreasing the exposure distance to $124\,\mathrm{mm}$ and respectively increasing the temperature to $383\,\mathrm{k}$ about $2\log_{10}$ reduction within $120\,\mathrm{s}$ and complete inactivation after $600\,\mathrm{s}$ is obtainable.

A setup for indirect treatment of contaminated surfaces is presented by Shimizu et al. (2008). They also used a plasma torch comparable to figure 11b but developed a special electrode configuration for plasma ignition with 6 stainless steel electrodes placed inside an aluminum cylinder. Hence, a discharge is ignited between every electrode and the grounded cylinder. To minimize toxic byproducts the torch was operated with argon gas ($99.998\,\%$ purity) at $2.2\,\mathrm{slm}$. The microwave power was $85\,\mathrm{W}$ with a frequency of $2.45\,\mathrm{GHz}$, whereby the gas temperature in the vicinity of the torch is about $500\,\mathrm{K}$ rapidly decreasing with higher distance to the torch (at $17\,\mathrm{mm}$ distance about $300\,\mathrm{K}$). The microbicidal efficiency was verified using agar plates with a diameter of $88\,\mathrm{mm}$ that are brushed with approximately $0.5 \times 10^6\,\mathrm{CFU}/\mathrm{cm^2}$ *E. coli* (ATCC No. 9637). For distances of $20\,\mathrm{mm}$ between the torch and the plates the inactivation rate for different treatment times was evaluated measuring the diameter of inhibition zone. The results show an increasing diameter for raising exposure times. While after $1\,\mathrm{min}$ a $3\,\mathrm{cm}$ diameter is visible, after $4\,\mathrm{min}$ an area of about $6\,\mathrm{cm}$ diameter is decontaminated.

In this review another setup similar to figure 11b for indirect treatment of bacteria contaminated glass bottles is presented (figure 12). The used microwaves have a frequency of $2.45\,\mathrm{GHz}$ and the consumed power is in the range of $1.2\,\mathrm{kW}$. Accordingly, the gas temperature is about $4000\,\mathrm{K}$ at a gas flux of $13\,\mathrm{slm}$ air or air $+ 20\,\%$ humidity. The distance between the torch and the contaminated bottles is about $25\,\mathrm{cm}$ connected via a metal tube. The gas temperature at the end of the metal tube is $150\,^\circ\mathrm{C}$. In contact with the bottles the gas temperature is further cooled down, so inactivation because of heat can be obviated. The contamination of the glass bottles was achieved by spraying $100\,\mu\mathrm{l}$ *B. atrophaeus* spores solution with $1 \times 10^7\,\mathrm{CFU}/\mathrm{ml}$ into the bottles. Since the main decontamination process is induced by plasma chemistry the discharge is ignited for $7\,\mathrm{s}$, the reactive gas is introduced into the glass bottle and left there for 2, 5, 10, 15, 30, 45 and 60 minutes. The results are additionally compared with the inactivation achieved by

a mixture of air and NO gas without discharge, where the NO concentration is adjusted equal to the concentration generated by the plasma (see figure 13). Apparently, there
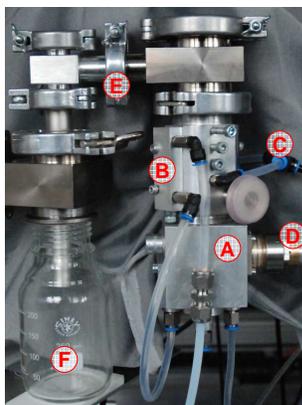

Figure 12. Microwave setup for inactivation of glass bottles. A) Microwave torch, B) cooling system, C) gas inlet, D) coaxial connector for power input, E) metal tube, F) contaminated 250 ml glass bottle.
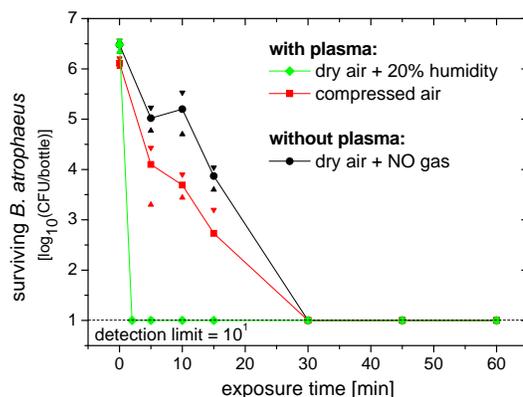

Figure 13. Surviving rates of *B. atrophaeus* spores treated with different exhaust gases for various exposure times in comparison with a mixture of air and NO gas without discharge ignition (minimum value ▲, maximum value ▼).

is only a small difference between exhaust plasma gas from air and the air and NO mixture without discharge ignition. After 10 min exposure time about $1 \log_{10}$ reduction for mixture of air and NO and nearly $2.5 \log_{10}$ reduction for the air plasma gas has been achieved. After 30 min there is a complete inactivation of $5 \log_{10}$ with a detection limit of $1 \log_{10}$ for both gases. In contrast, the admixture of 20 % humidity shows a drastic change in inactivation dynamics. All microorganisms could be inactivated within only 2 min exposure time. To clarify whether the effect is allocated to NO_x, H_2O_2, (R-)OH radicals or other plasma products remains for future investigations.

Furthermore, microbial inactivation in PET-bottles is demonstrated by Brandenburg et al. (2008). They developed a self propagating microwave-driven discharge at

2.45 GHz and power up to 1.7 kW in usual air (see figure 14). The device consists of a wave guide connected to the process chamber and an ignition device mounted on a moveable lance (Ehlbeck et al., 2003). To align the process chamber geometry to the magnetron frequency a shorting plunger is used. The lance with the ignition pin is guided into the bottle, the microwave field is applied and a discharge is ignited at the bottom of the bottle. After the ignition the lance is moved to its origin and the discharge propagates upwards through the bottle. This was repeated 3 times, whereby one cycle takes about 550 ms, so 1.6 s for the entire process. Prior to that, the PET-bottles are sprayed with a solution of *E. coli*, *S. aureus* or *A. brasiliensis*. After the treatment the bottles were rinsed and the resulting solution was analyzed by membrane filtration. The results show a $6.8 \log_{10}$ reduction for *E. coli*, $5.1 \log_{10}$ for *A. brasiliensis* and $6.7 \log_{10}$ for *S. aureus*.
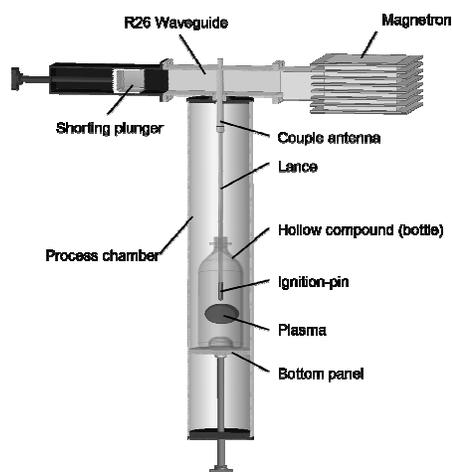

Figure 14. Device for the treatment of PET-bottles by means of propagating microwave-driven air plasma.

4.5. Comparison

Different atmospheric pressure plasma sources are applicable for microbial decontamination as it is described above. For clarity, an assortment of recent inactivation results obtained with corona discharges, dielectric barrier discharges, atmospheric pressure plasma jets and microwave discharges is displayed in table 3. Additionally, information about the appendant experimental conditions like exposure time, process gas, type of used microorganism, initial contamination and environmental condition, in which the microorganisms are plasma treated, are given. All kind of discharges produce considerable inactivation results within exposure times ranging from 10 s to 1800 s. Even in wet or liquid environments reduction factors of ≥ 6 are reported for some experiments, which are equal to some reduction factors gained in dry environment.

Generally, it has to be mentioned that up to now the comparison of inactivation results for different experimental conditions is virtually impossible. Hence, it is difficult

to adapt the conclusions made for one special experimental setup to another, even if the same kind of discharge is used. This lack of comparison is probably due to sensibility of living biological material against small environmental changes. Moreover, the active species generated by the discharge are also strongly effected by the surrounding conditions. E.g. its difficult to reproduce results achieved with an air-discharge, without knowing the humidity, the exact pressure or the gas temperature of the air. Furthermore, the interaction between the discharge and the microbial probe depends on experimental condition as well. This also includes the contamination of the samples, where a crucial difference between spot and spray contamination could be observed (Schneider et al., 2005).

To allow a comparison of different decontaminating plasmas standardized microbiological test procedures must be developed in future work. A major step in this direction would be the establishment of round robin tests between different institutions in order to adjust and standardize microbiological verification methods. Moreover, in publications the detailed description of used plasma parameters and environmental conditions is at least of the same importance for the comparison of different research results.

Despite valuable discoveries made in the past, until now the complex mechanisms that lead to microorganism inactivation are not completely revealed and further research along with the use of more sensitive analyzing methods must be done. One promising method is the use of proteomics (Landsberg et al., 2010), which is a tool for analyzing global control of protein stability, the protein interaction network, protein secretion or post-translational modifications of proteins (Hecker and Völker, 2004). In contrast to common used proliferation assays this tool offers a more detailed insight into the plasma-treated microorganisms and may help to answer pestering questions.

From the use-oriented point of view, each discharge type has its advantages and disadvantages depending on the technical problem to be solved. For this reason, the only meaningful comparison of different discharges is in direct combination with the industrial application. Since there are lots of different possible applications, the comparison is confined towards the technical process parameters of the different sources, so one has a short overview, which source is suitable for the corresponding application (table 4). However, the listed values are based on the reviewed articles and therefore limited to some extend.

5. Conclusion

This review shows the large variety of atmospheric plasma sources used for microbicidal treatments. The special kind of the decontamination task is as manifold as the plasma sources. The varying plasma parameters and therefore the different composition of microbicidal agents in combination with the complex microbial test methods using different microorganisms and procedures leads to an inhomogeneous picture. Main reason for that is the major role of chemistry for the microbial reduction efficiency at

Table 3. Assortment of recent inactivation results achieved with different atmospheric pressure plasma sources. Furthermore, appendant experimental conditions are given.

discharge type	RF	expos. time [s]	gas	MO	N_0 [cfu]	MO environment	Lit.
Corona[r]	> 4.5	300	O_3	Norwalk Virus	-	liquid	
	5	300	O_3	Poliovirus	-	liquid	Shin and Sobsey (2003)
	> 7	300	O_3	coliphage MS2	-	liquid	
Corona[d]	5	300	air	*Aeromonas*	1×10^5	u	Cramariuc et al. (2008)
	5	1200	air	E. *coli*	1×10^5	u	
	> 6	120	air	E. *coli*	5×10^6	liquid	
	> 6	1800	air	C. *albicans*	1×10^6	liquid	Scholtz et al. (2010)
	> 6	240	air	S. *epidermidis*	1×10^6	liquid	
	*	160	Ar	E. *coli*	u	agar, wet	Bussiahn et al. (2010)
DBD[r]	≥ 4.5	24	air	E. *coli*	8×10^6	PP, dry	
	≥ 6	22	air	S. *aureus*	1×10^7	PP, dry	Gadri et al. (2000)
	≥ 4	240	air	B. *subtilis*[s]	1×10^6	paper, dry	
	≥ 5	120	[1]N_2/O_2	B. *atrophaeus*[s]	1×10^6	steel, dry	Venezia et al. (2008)
	≥ 4	300	[2]air	S. *atrophaeus*[s]	1×10^5	PE, dry	Hähnel et al. (2010b)
	4	600	air	B. *subtilis*	$> 10^6$	paper, dry	Schwabedissen et al. (2007)
	> 6	900	N_2/O_2	G. *stearo-thermophilus*[s]	3×10^6	in Tyvek, dry	Eto et al. (2008)
DBD[d]	≥ 6	30	air	E. *coli*	1×10^7	PP, dry	Kelly-Wintenberg
	≥ 6	30	air	S. *aureus*	1×10^7	PP, dry	et al. (1998)
	≥ 4	120	air	B. *subtilis*	3×10^4	spacecraft mat., dry	Cooper et al. (2007)
	≥ 5	360	He	yeast	5×10^7	culture dish	Jin et al. (2006)
	5	340	air	L. *innocua*	5×10^7	steel, dry	Leipold et al. (2010)
	6	300	air	E. *coli*	1×10^7	physiol. saline, liquid	Oehmigen et al. (2010)
	> 4	600	Ar/F	B. *atrophaeus*[s]	1×10^6	PTFE, dry	this work
	7	10	air	human skin flora	1×10^7	agar, wet	Fridman et al. (2006)
	3.3	40	Ar	E. *coli*	2.5×10^6	PDMS, dry	Weng et al. (2009)
APPJ[d]	4.3	420	Ar	B. *atrophaeus*[s]	2×10^6	flat PE, dry	Brandenburg et al. (2007)
	3.8	240	Ar	E. *coli*	6×10^5	flat PE, dry	
	2.7	120	Ar	MSSA	7×10^2	agar, wet	
	4.0	120	Ar	P. *aeruginosa*	8×10^2	agar, wet	Daeschlein et al. (2010)
	2.0	120	Ar	C. *albicans*	1×10^2	agar, wet	
	4.6	420	Ar	B. *atrophaeus*[s]	4×10^6	flat PE, dry	Weltmann et al. (2008b)
	3.6	120	Ar	E. *coli*	4×10^5	flat PE, dry	
	7.8	180	Ar	E. *coli*	6.8×10^7	petri dish, wet	XU et al. (2009)
	6.8	180	Ar	S. *aureus*	1.2×10^8	petri dish, wet	
	6	30	[3]Ar/O_2	B. *atrophaeus*[s]	2.2×10^6	glass, dry	Lim et al. (2007)
	2.5	180	[3]He/O_2	B. *atrophaeus*[s]	2.2×10^6	glass, dry	
	2	10	He/O_2	G. *liquefaciens*	$> 10^6$	fruit, wet	Perni et al. (2008)
	2.5	30	He/O_2	E. *coli*	$> 10^6$	fruit, wet	
	*	30	He/O_2	E. *coli*	10^6	agar, wet	Laroussi et al. (2006)
	4.5	10	He	E. *coli*	10^7	agar, wet	Sladek and Stoffels (2005)
	> 6.5	60	[4]He/O_2	E. *coli*	u	glass, dry	Kim, Chung et al. (2009)
	5	120	Ar	M. *luteus*	1×10^5	nutrient broth	Yu et al. (2007)
	6	180	Ar	E. *coli*	1×10^6	nutrient broth	
	6	180	He	E. *coli*	1×10^7	distilled water	Ikawa et al. (2010)
	7	30	[5]He/O_2	B. *globigii*	1×10^7	glass, dry	Herrmann et al. (1999)
MW[r]	5	120	[6]air	B. *atrophaeus*	10^6	glass, dry	this work
MW[d]	*	240	Ar	E. *coli*	5×10^5	agar, wet	Shimizu et al. (2008)
	5	10	air	B. *cereus*	10^6	glass, dry	Lai et al. (2005)
	3	600	Ar	E. *coli*	10^5	membrane filter, dry	Sato et al. (2007)

[1]humidified and 1 % ethylene admixture, [2]at 60 % humidity, [3]0.25 % O_2 admixture, [4]0.5 % O_2 admixture, [5]0.8 % O_2 admixture, [6]20 % humidity, *general microorganism reduction is shown, [d]direct plasma treatment, [r]remote plasma treatment, [s]spores, F: forming gas (95 % N_2+5 % H_2), MO: microorganism, MSSA: methicillin-sensitive S. *aureus*, PDMS: polydimethyl siloxane film, PE: polyethylene, PP: polypropylene, RF: reduction factor ($\log_{10}(N/N_0)$), u: unspecified

Table 4. Used parameter ranges for the different discharge types reviewed in this article.

parameter	corona	DBE	APPJ	MW
temperature [K]	∼300	∼300	300 ... 430	300 ... 4000
voltage [kV]	1 ... 14	1.5 ... 10.4	1 ... 5	
frequency	DC, pulsed DC	2 ... 21.7 kHz	1 kHz ... 27.12 MHz	typ. 2.45 GHz
power [W]	50	1.8 ... 360	15 ... 250	85 ... 1700
flow rate [slm]	0 ... 0.5	0 ... 1.7	8 ... 92	1 ... 23.5
used gases	Ar/air	Ar/air/humidity	He/Ar/O$_2$/air	Ar/air/humidity

atmospheric pressure opening a wide field of process parameters. In consequence, each microbicidal plasma treatment has to be carefully adapted to the specific task. This seems to be the reason for the up to now limited utilization of plasma decontamination processes in industrial applications. Currently, only few systems for very specific applications are commercially available.

Another problem is that the existing chemical disinfection and sterilization processes are cheap and effective. So plasma processes have to be even more time and cost efficient or have to occupy niches such as sterilization of endoscope channels.

To overcome these difficulties more interdisciplinary research especially between physicist and biologist is needed. Due to the required high amount of reliability the application of professional engineering methods is desired.

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