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The impact of different process gas compositions on the inactivation effect of an atmospheric pressure plasma jet on *Bacillus* spores

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ABSTRACT

Atmospheric plasma provides the advantages of high microbial inactivation that can be performed under ambient conditions. It is consequently regarded as potential alternative to traditional food preservation methods. In this study we systematically tested the influence of argon as plasma carrier gas with admixtures of oxygen (0–0.34 vol.%) and nitrogen (0–0.3 vol.%) towards its emission intensity of UV-C light, excited OH and N_2-species and atomic oxygen. A mixture of argon, 0.135 vol.% oxygen and 0.2 vol.% nitrogen emitted four fold more UV photons than pure argon. However, sporicidal effects on *Bacillus atrophaeus* (3.1 $\log_{10}$) and *Bacillus subtilis* spores (2.4 $\log_{10}$) were found for pure argon plasma, which were similar as compared to the sporicidal effect of the plasma with highest UV-emission. To distinguish lethal effects caused by emitted UV-light and reactive species, UV-sensitive mutant spore strains (PS578 and FB122) were exposed to plasmas with different UV-emission intensities and a significant impact of UV-light on the first phase of spore inactivation was confirmed. *Industrial relevance:* As an efficient method for the inactivation of microorganisms at low temperatures and atmospheric pressure, plasma is already commercially used for the sterilization of medical devices. The results presented in this study could be useful for a process optimization regardless if the plasma is applied for food preservation or surface decontamination. Especially the impact of emitted UV photons from the plasma on the first inactivation phase of endospores attached to surfaces, depicts a high potential of such plasmas for a rapid spore inactivation.

1. Introduction

The formation of highly resistant endospores by stressed vegetative cells belonging to class *Bacilli* or *Clostridia* results in perfect vehicles for the spoilage of food and/or infection of humans. Spores alone are not hazardous in stored food or during food consumption, but they can cause foodborne diseases after outgrowth. After the sporulation, the matured spore has a well-structured multilayer morphology with several mechanisms to withstand multiple environmental stress conditions, like wet and dry heat, desiccation, irradiation and chemical agents (P. Setlow, 2007). Consequently, spores are perfectly adapted to survive on the surface of dry food products like herbs and spices (Sagoo et al., 2009; Vij, Ailes, Wolyniak, Angulo, & Klontz, 2006) or in a food production line (Faille et al., 2013).

Several pasteurization and sterilization methods are applied to increase the microbial safety of herbs and spices, which include the treatment with wet steam, fumigation with ethylene-oxide or γ-irradiation (Mckee, 1995). However, all of these methods have disadvantages with regard to sensorial properties, consumer safety or acceptance. Consequently, there is a need for alternative low-temperature pasteurization and sterilization processes for the surface of dry foods. A promising process with the potential to fulfill these requirements is a cold atmospheric pressure plasma treatment.

In general, plasma is a gas containing free electrons, ions and neutral particles, which can be further categorized by its thermodynamic properties into thermal and non-thermal plasmas (Schlüter et al., 2013). Thermal plasmas are characterized by the existence of a thermodynamic equilibrium between neutral particles, ions and electrons. The temperature of these plasmas is usually above 6000 K under atmospheric pressure (Moreau, Orange, & Feuilloley, 2008). In contrast the temperature of nonthermal plasmas is much lower and can be directly applied to thermal sensitive surfaces. In nonthermal plasmas the electron temperature can reach several 10,000 K, whereas the gas temperature can be close to ambient temperature. The term "cold plasma" is defined for treatment temperatures below 70 °C (Schlüter et al., 2013).

Nonthermal plasmas at atmospheric pressure can be generated by different plasma setups, whereas one of the most common is a plasma

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jet. Here the carrier gas (e.g. argon, helium, air or nitrogen) is passed through a nozzle in which an electric field with a high voltage difference is present. This voltage creates a strong force on orbiting electrons, which in turn provides energy for their escape, resulting in the ionization of atoms and/or molecules. The free electrons quickly collide with gas molecules providing excitation energy to create unique, highly reactive products (Keener, 2008) and the emission of UV light (Brandenburg et al., 2009) with antimicrobial effects. The concentrations in which the agents occur in the plasma depend greatly on the device setup, the operating conditions (gas type and power of plasma excitation) and the gas composition (Brandenburg et al., 2009; Roth, Feichtinger, & Hertel, 2010).

The inactivation potential towards bacterial spores of these nonthermal atmospheric plasmas was shown by many authors (Boudam et al., 2006; Brandenburg et al., 2009; Lassen, Nordby, & Grun, 2005; Moreau et al., 2008; Schnabel et al., 2012; van Gils, Hofmann, Boekema, Brandenburg, & Bruggeman, 2013), but the mechanisms leading to spore inactivation are still under investigation. The often described biphasic inactivation kinetics of various *Bacillus* spore strains on dry surfaces are presumably due to combined inactivation effects of the nonthermal plasma. Of special interest in this regard are UV and VUV photons, since they are known to induce DNA strain breaks (P. Setlow, 2007) or to damage other proteins in the cell (Philip et al., 2002).

UV photons with a wavelength ≤ 275 nm can break C—H and C—C bonds and might be responsible for the initial rapid inactivation (Boudam et al., 2006). However, larger quantities of UV and VUV photons are only emitted in low-pressure or vacuum plasma systems, which are in most cases not suitable for food treatments (Knorr et al., 2011). Further, most researchers claim that UV emission plays a minor role in the inactivation of microorganisms at atmospheric pressure, and the inactivation process is controlled by chemically reactive species (Knorr et al., 2011; Laroussi, 2005; Laroussi & Leipold, 2004; Pointu et al., 2005). The mechanisms involved are the intrinsic photodesorption and etching (Moisan, Barbeau, & Pelletier, 2001), which describe the degradation and erosion of outer structures of microorganisms, leading to cell death.

This study investigates the inactivation behavior of *Bacillus subtilis* spores and isogenic mutant strains, with increased sensitivity towards UV light, on glass surfaces to clarify the impact of UV emission from an atmospheric plasma jet on spore inactivation. Further a systematic modification of the plasma carrier gas composition towards a maximum amount of emitted UV photons was done to enhance spore inactivation and to optimize the process.

2. Material and methods

2.1. Nonthermal radio-frequency-driven plasma jet

The atmospheric pressure plasma jet used in this study was described in detail by Brandenburg et al. (2009). The jet consists of ceramic nozzle with an inner diameter of 7 mm. The radio frequency (RF, frequency 27.12 MHz and system power 30 W) is coupled via a matching network to the wolfram-needle-electrode, which is mounted in the center of the nozzle. A grounded ring electrode is placed at the nozzle outlet and a gas flow of 10 slm of argon was used to generate plasma from the tip of the needle electrode to the inner wall of the nozzle. The generated plasma expanded to the outside of the nozzle with a length of 15 mm. The plasma is found to be filamentary and a mixing of the argon gas with the surrounding air could be determined by optical emission spectroscopy for the pure argon plasma. To increase the emission of UV photons in the plasma, 0.1, 0.2 and 0.3 vol.% of nitrogen and 0 to 0.34 vol.% (increase in 0.01% steps) of oxygen were added to the argon gas until an unstable plasma ignition was visible.

2.2. Optical emission spectroscopy

For optical emission spectroscopy a Black-Comet-UV–vis spectrometer (StellarNet Inc., Tempa, USA), equipped with a F400-UV–vis-SR fiber optic and a quartz lens in the range from 190 to 900 nm, was used. This setup enabled the measurement of UV-C (190–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm) irradiation, as well as the spectral bands of OH radicals (309 nm) and atomic oxygen (777.5 nm). The distance between the detection lens and the nozzle outlet was 15 mm in axial and 10 mm in vertical direction. The spectrum was measured 10 times with an integration time of 100 ms. The average spectrum was base-line corrected and normalized using a self-written LabVIEW routine.

2.3. Measurement of UV dosage

The intensity of the emitted UV dosage for certain carrier gas compositions (Table 1) was further quantified with UV-Tec control strips (UV-Tec Messtechnik GmbH, Bergisch Gladbach, Germany). The strips were placed at different distances in axial direction to the nozzle outlet (Table 1) for different exposure times. After exposure the UV-dosage is directly shown by means of coloration of the UV-control-strips (sensitivity in the range λ: 250–420 nm). After measuring the affected area (Table 1) and quantifying the UV-dosage related to a defined exposure time, the UV irradiance in W cm^{-2} was calculated.

2.4. B. subtilis strains, spore preparation and purification

In this study we used a *Bacillus athropheus* strain (WIS 396/3), a *B. subtilis* strain PS832 (wild-type) (Paidhungat & Setlow, 2000) and two isogenic derivatives of the *B. subtilis* strain, namely FB122 (Douki, Setlow, & Setlow, 2005) and PS578 (Genest, Setlow, Melly, & Setlow, 2002). Spores of *B. athropheus* strain (WIS 396/3) are used as surrogates for *Bacillus anthracis* during chemical and UV- and γ-irradiation sterilization (personal communication Dr. Marshall Bundeswehr Research Institute for Protective Technologies and NBC Protection (WIS)). The strain FB122 (*sleB spo VF*) lacks the gene for encoding the cortex lytic enzyme sleB and is not able to synthesize dipicolinic acid (DPA) during sporulation. The strain PS578 ($\alpha^-\beta^-$), lacks the genes encoding the spore's two major α/β-type small acid soluble proteins (SASP), such that the α/β-type SASP level in PS578 spores is only ~25% of that in PS832 wild-type spores.

All spore strains were prepared at 37 °C on 2xSG agar plates without antibiotics, according to a method described elsewhere (Nicholson & Setlow, 1990; Paidhungat & Setlow, 2000). After sporulation the spores were harvested and cleaned by repeated centrifugation (3-fold at 5000 *g*), washed with cold distilled water (4 °C), and sonicated for 1 min. The purified spore suspensions contained ≥95% phase-bright spores and nearly no spore agglomerates, as was verified by a particle image analysis system (FPIA 3000, Malvern Instruments, Worcestershire, UK). The spores were then stored in distilled water in the dark at 4 °C.

Table 1
Experimental settings for UV-dosage determination with UV-control strips.

Carrier gas composition	Distance between nozzle and surface [mm]	Exposure time [min]	Irradiated area [cm^2]
Argon	10	1.5	0.2
	15	5	0.80
	20	15	1.77
Argon + 0.135% vol oxygen	10	1.5	0.2
	15	5	0.80
	20	15	1.77
Argon + 0.135% vol oxygen + 0.2% vol nitrogen	10	1.5	0.2
	15	5	0.80
	20	15	1.77