Etching of *Bacillus atrophaeus* by oxygen atoms, molecules and argon ions

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Abstract. The etching of spores of *Bacillus atrophaeus* by oxygen atoms, molecules and argon ions is investigated in a particle beam experiment. Thereby, the conditions occurring in an argon oxygen plasma are mimicked and fundamental inactivation mechanisms are revealed. It is shown that only the combined impact of argon ions and of O atoms or O$_2$ molecules causes significant etching of the spores. This is explained by the process of chemical sputtering, where an ion induced defect at the surface of the spore reacts with either the incident bi-radical O$_2$ or with an incident O atom. This leads to the formation of CO, CO$_2$ and H$_2$O and thus to erosion. This process is compared to the plasma etching of hydrocarbon thin films as an atomistic model system for the spore coat. It is shown that the etch rate in an inductively coupled argon oxygen plasma is only maximal if both, the electron density and thus the ion flux towards the surface and the O atom flux are optimized simultaneously.

1. Introduction
Sterilization is a key technology in the medical industry[1, 2, 3, 4]. Many sterilization methods are known to be efficient such as heat treatment, gamma radiation or exposure to toxic chemicals (ozone O$_3$, ethylene oxide C$_2$H$_4$O, or hydrogen peroxide H$_2$O$_2$). All these methods have in common that they impose a severe stress on the objects to be decontaminated: the wear of surgical instruments is significant and implants made from thermolabile polymers loose their mechanical integrity[5]. One technology that avoids these issues is plasma sterilization. In a plasma, a source gas is dissociated and ionized and reactive species such as electrons, ions, atoms, radicals and UV photons are interacting with the biological system to be inactivated. Typical sterilization plasmas are generated in argon with admixtures of nitrogen, hydrogen, or oxygen[3, 1, 2]. In such a process, the object itself can stay at rather moderate temperatures since the dissociation of the precursor gas is initiated in the plasma state. It has been proven that plasma sterilization can be very efficient in inactivating bacteria or pathogenic biomolecules[6, 7, 8].

The validation of a sterilization technique is usually based on the proof to inactivate endospores of bacteria. They are known to be a very resistant form of a biological system. In the case of plasma sterilization, it is postulated that incident UV photons, ions and chemical active species have a combined effect on endospores[3, 1, 2, 9, 10, 6]. It is generally believed that the inactivation itself is caused by UV photons, which penetrate deep into the cell and cause DNA strand breaks. Sterilization times of the order of seconds for inactivating isolated spores are sufficient at typical photon fluxes in low temperature
In almost all practical cases, however, the sterilization efficiency is largely reduced, because of shadowing of the UV radiation by multilayered stacks of spores or by biofilms in which the spores are embedded. Therefore, a significant plasma-induced chemical or physical etching of the biological systems is essential. Summarizing, an optimum plasma process needs to provide both, an intense UV-photon flux and a significant etching efficiency.

The etching of biological systems is often performed by adding hydrogen or oxygen to the discharge[11, 12]. In a recent paper, we studied the interaction of hydrogen atoms with endospores[13]. It was shown that the combined impact of ions and H atoms causes significant erosion, which becomes visible as a perforation of the endosporic shell. H atoms alone have no inactivation efficiency. Furthermore, we studied also the ability of oxygen atoms and oxygen molecules [14] to inactivate endospores of Bacillus atrophaeus. It is shown, that the impact of O atoms does not cause sterilization at room temperature. However, the simultaneous impact of O2 molecules or O atoms and of argon ions at around 200 eV causes a very effective etching and perforation of the spore coat. This is due to the process of chemical sputtering, where the bi-radical O2 or the O atom interacts with ion-induced defects at the spore surface leading to the formation of CO, CO2 and H2O and thus to etching. This result was deduced from beam experiments where the plasma treatment is mimicked using absolutely quantified beams of oxygen molecules, of oxygen atoms and of argon ions.

In this paper, we transfer the results from the beam experiments in [14] to plasma experiments using an inductively coupled plasma from argon - oxygen mixtures. To assess the efficiency of the chemical sputtering process, we study the plasma etching of thin hydrocarbon films as being a model system for the spore coat.

2. Experiment
The erosion of endospores of Bacillus atrophaeus is studied by exposing them to particle beams of oxygen molecules, of oxygen atoms, of argon ions and of combination of these. Thereby, the exposure of a biological system to an argon-oxygen plasma is mimicked. Spores of Bacillus atrophaeus (ATCC 51189) were used as biological samples, because they are known to be highly resistant against conventional H2O2 based sterilization methods. The spores were analyzed using scanning electron microscopy (SEM). Doped silicon is used as substrate. The substrates were coated with monolayers of endospores using a spraying technique.

A sketch of the particle beam experiment is shown in Fig. 1a. It consists of an exposition chamber containing the particle sources and a load lock. Three atom source and one ion source are implemented: (i) one oxygen source is based on a microwave plasma excited in an Evenson cavity[15]. Oxygen atoms are guided from the microwave cavity to the sample via a quartz tube with a length of 30 cm. The quartz tube has an S-bend shape to avoid direct line-of-sight from the microwave plasma to the samples. Thereby, any UV radiation from the plasma cannot reach the sample, which would otherwise be the dominant inactivation mechanism. (ii) A second oxygen and a hydrogen source are installed consisting of a small iridium/tungsten capillary, which is heated to 1850 K and to 2100 K to dissociate a flow of oxygen or hydrogen molecules, respectively. These sources are not used in the current experiment. (iii) The Ar+ ion beam is produced using a commercial plasma ion source (type Gen2 by Tectra GmbH)[16]. (iv) In addition, the samples are also exposed to stable oxygen molecules. In that case, a specific pressure of pure O2 has been adjusted and the arrival rate at the surface has been deduced from simple gas kinetics. Details on the sources and their calibration can be found elsewhere [14].

The etch rate of endospores is also assessed by etching hydrocarbon films in an inductively coupled low pressure plasma from mixtures of argon and oxygen. The plasma reactor consists of a stainless steel vessel with cylindrical shape (see Fig. 1a). A quartz window acts as bottom to separate an ICP antenna
Figure 1. Schematic of the experimental setups: (a) particle beam experiment with three atom sources and one ion source; (b) inductively coupled plasma with a two stage differential pumped mass spectrometer.

from vacuum. The antenna is operated at 13.56 MHz with powers up to 500 W. A ring shower as gas inlet is embedded within the chamber wall. The vessel is equipped with six additional ports to allow for diagnostic, pumping and pressure measurements. The chamber is evacuated with a turbo pump and rough pump, which are connected to the vessel via a butterfly valve. The gas pressure ranges between 0.1 and 20 Pa and gas flows from 1 to 50 sccm can be adjusted. The plasma volume is approximately 5 l. The samples are placed onto a glass carrier which is placed 2 cm above the bottom window. The samples are hard amorphous hydrogenated carbon films deposited in a separate capacitively coupled plasma from acetylene.

The electron density is analyzed using the Langmuir probe system APS3. A tungsten wire with a
The oxygen atom density is measured using a Balzers HiQuad Mass spectrometer QMG 700 being mounted inside a two stage differential pumping system on top of the reactor (see Fig. 1b). A mechanical flag is mounted directly in front of the ionizer in the second stage to allow a separate measurement of the background signal without a molecular beam component. This flag is manipulated externally via a mechanical feed through. Details of the mass spectrometry setup can be found in [20, 21].

The quantification of the oxygen atom density is performed using threshold ionization mass spectrometry (TIMS). For this, the electron energy in the ionizer is set to 15 eV and the count rate is measured. This rate is quantified by calibrating the signal using oxygen molecules at a given pressure of 10 Pa. By comparing the cross sections of oxygen molecules[22] and oxygen atoms[23] the count rate of O atoms is converted into a partial pressure of oxygen.

3. Results and Discussion

3.1. Exposure of B. atrophaeus to particle beams

Recently, Raballand et al. [14] studied the exposure of endospores of B. atrophaeus (ATCC 51189) to oxygen atoms, oxygen molecules and argon ions and combinations of these. She showed, that the exposure to an O atom flux \((j_O = 2.4 \times 10^{15} \text{ cm}^{-2} \text{s}^{-1})\) and to an oxygen molecules flux \((j_{O_2} = 1.5 \times 10^{17} \text{ cm}^{-2} \text{s}^{-1})\) for 240 min does not lead to a biological inactivation of the spores. The inefficiency of inactivation and etching of the spores by O atoms is explained as follows: the endosporic shell of Bacillus atrophaeus consists of a peptidoglycan and a protein layer, which can be regarded, on an atomistic level, as a specific type of hydrocarbon compound. The chemical etching of various hydrocarbon compounds by oxygen atoms becomes only effective above a substrate temperature of 400 K, as known from literature [24, 25]. Since the substrate temperature in the experiment stays at 300 K, the absence of any etching is conceivable.

The experiments by Raballand et al. [14] revealed, however, that the oxygen etching can easily be enhanced by an additional ion bombardment. Fig. 2 compares the SEM micrographs of endospores as being treated for 120 min in three different experiments:

- **Argon ions only** (Fig. 2a): endospores exposed to Ar\(^+\) ions at 200 eV \((j_{Ar^+} = 1.8 \times 10^{14} \text{ cm}^{-2} \text{s}^{-1})\) show only a slightly altered surface texture, as can be seen on the SEM micrograph. Apparently, Ar\(^+\) ion bombardment at 200 eV does not cause any significant erosion of the spore coat. This can be explained by the energy dependence of the physical sputtering yield \(Y(\text{Ar}^+)\) of hydrocarbon compounds by argon ions. The physical sputtering process becomes only significant for ion energies well above 200 eV[26].

- **Argon ions and oxygen molecules** (Fig. 2b): Endospores exposed to a simultaneous flux of argon ions \((j_{Ar^+} = 1.8 \times 10^{14} \text{ cm}^{-2} \text{s}^{-1})\) and oxygen molecules \((j_{O_2} = 4.5 \times 10^{15} \text{ cm}^{-2} \text{s}^{-1})\) exhibit, however, a very different appearance. Although the absolute size of the spores did not change noticeably, the spore coat became porous, showing deep etch channels. The high sputtering yield is explained by the simultaneous impact of ions and oxygen molecules leading to the process of...

\[\text{diameter of } 50 \, \mu\text{m} \text{ and a length of } 5 \, \text{mm is used to sample the plasma currents [17]. The probe is inserted into the vessel via a vacuum feed through and is positioned by a belt drive at the center of the discharge. An auxiliary stainless steel probe picks up the floating potential which is used to track an additive offset to the voltage ramp. Passive rf-compensation of the probe characteristics is maintained by a set of band-stop filters in the probe circuit and by capacitive coupling to the auxiliary probe. The current-voltage characteristics are at first numerically filtered as will be presented elsewhere [18]. The resulting second derivative is proportional to the EDF and the Druyvesteyn method is used to calculate electron density and mean electron energy [19].} \]
Figure 2. SEM micrographs of *Bacillus atrophaeus* exposed for 120 minutes in the beam experiment to (a) argon ions ($j_{Ar^+} = 1.8 \times 10^{14} \text{ cm}^{-2}\text{s}^{-1}$) at 200 eV, to (b) a combined flux of argon ions ($j_{Ar^+} = 1.8 \times 10^{14} \text{ cm}^{-2}\text{s}^{-1}$) at 200 eV and O$_2$ molecules ($j_{O_2} = 4.5 \times 10^{15} \text{ cm}^{-2}\text{s}^{-1}$), and to (c) a combined flux of argon ions ($j_{Ar^+} = 1.8 \times 10^{14} \text{ cm}^{-2}\text{s}^{-1}$) at 200 eV, O$_2$ molecules ($j_{O_2} = 1.5 \times 10^{17} \text{ cm}^{-2}\text{s}^{-1}$) and O atoms ($j_O = 2.4 \times 10^{15} \text{ cm}^{-2}\text{s}^{-1}$). Adapted from [14].

*chemical sputtering*[27]: impinging energetic ions are able to break bonds in the spore coat within their corresponding collision cascade. The additional flux of oxygen molecules is able to react with these open bonds. The repeated ion-induced bond breaking followed by their reaction with oxygen molecules leads, below the surface, to the formation of presumably CO, CO$_2$ and H$_2$O as volatile components. These reaction products diffuse to the surface and desorb[28]. As a result, etching occurs.
At first glance, it is surprising that the impact of stable ground state oxygen molecules is able to cause significant etching, if a simultaneous flux of ions impinges on the surface. However, O\(_2\) possesses a triplet ground state with two unpaired electrons, which makes it a so-called bi-radical. The electronic structure of O\(_2\) explains its reactivity: if an ion at the surface creates a defect, namely an open bond with an unpaired electron, the O\(_2\) bi-radical can easily react at this open site. The very same reaction occurs during the slow auto-oxidation of any polymer being exposed to ambient atmosphere\(^\text{[29]}\).

- **Argon ions and oxygen atoms and molecules** (Fig. 2c): The exposure of Bacillus atrophaeus to O atoms (\(j_O= 2.4 \times 10^{15}\) cm\(^{-2}\)s\(^{-1}\)), to O\(_2\) molecules (\(j_{O_2}= 1.5 \times 10^{17}\) cm\(^{-2}\)s\(^{-1}\)) and to argon ions (\(j_{Ar^+}= 1.8 \times 10^{14}\) cm\(^{-2}\)s\(^{-1}\)) is performed using the plasma ion source and the microwave source to dissociate 1% of a flow of 5 sccm O\(_2\) molecules. The spores are efficiently etched as indicated by the appearance of deep trenches and pores. The fast etching is again explained by the *simultaneous* impact of ions, of oxygen molecules, and of oxygen atoms leading to the process of chemical sputtering.

Summarizing, the experiments using particle beams revealed that a strong etching of the endospores occurs only if argon ions and O atoms or O\(_2\) molecules interact simultaneously with the spores. This is explained by the process of chemical sputtering as the combined impact of neutrals and ions. In the following, we test this observation in a plasma experiment using argon oxygen mixtures. The quantification of absolute plasma etch rates of endospores is, however, difficult since the etching leads to a perforation of the spore coat rather than to a layer-by-layer removal. Instead, we study the etching of hydrocarbon thin films as being a model system which resembles the stoichiometry of the spore coats on an atomistic level. Such a rough model system is able to produce comparable results, because *ion-induced etching* in a low pressure plasma does not strongly depend on the exact chemical nature of the materials to be etched. In contrast, a pure chemical erosion process relies very much on the exact nature of the surface groups and the definition of such a simple model system for the spore coat would not be possible.

### 3.2. Etching of hydrocarbon films in oxygen containing plasmas

An inductively coupled plasma at a pressure of 10 Pa and a power of 200 W is used to etch hydrocarbon films using various mixtures of oxygen and argon. The total flow has been kept at 10 sccm, while varying the oxygen admixture. Fig. 3a shows the electron density, as measured via Langmuir probes, and the oxygen partial pressure, as measured by threshold ionization mass spectrometry. It can be seen that the electron density decreases drastically with increasing oxygen admixture, whereas the oxygen atom concentration slowly increases before it reaches a maximum at an admixture level of 80 %. Such a variation of electron density and atomic oxygen concentration is typical for a plasma experiment where the power is kept constant. At increasing admixture of a molecular gas, more power is dissipated in rotational and vibrational excitation of the molecules and less in ionization. Consequently, the ionization degree and the electron density decreases sharply.

Within the very same parameter range, the etch rate of hydrocarbon films is also quantified, as shown in Fig. 3b. One can clearly see that the etch rate peaks already at a small oxygen admixture of 5%. At higher oxygen admixtures, the etch rate decreases again although the oxygen atom concentration is still increasing. This clearly illustrates that the plasma etch rate of the hydrocarbon films is not only linked to the oxygen atom concentration.

According to the process of chemical sputtering, the combined impact of ions and atoms is crucial for an efficient etch rate. Both fluxes, the ion fluxes and the O atom flux are proportional to the bulk densities...
Figure 3. Electron density $n_e$ and oxygen atom partial pressure $p_O$ in dependence of the admixture of oxygen to an argon inductively coupled plasma at a total pressure of 10 Pa, a power of 200 W and a total flow (argon plus oxygen) of 10 sccm. (b) Etch rate of a hydrocarbon film in the ICP plasma at the same process parameters (solid symbols). Product of $n_e$ and $p_O$ (open symbols) using the data in (a). The value for $p_O$ at an admixture of 5% O$_2$ has been derived by interpolation.

of electrons and O atoms. As a rough hypothesis, one might state that the etch rate is proportional to the product of ion flux and atom flux and thereby also proportional to the product of electron density and O atom density. This hypothesis is tested by plotting the product of oxygen partial pressure $p_O$ and electron density $n_e$ in dependence of the O$_2$ admixture (The missing value for $p_O$ at an admixture of 5% O$_2$ has been derived by interpolation). One can clearly see that the overall behavior of the etch rate is very well reproduced by the product $p_O \times n_e$. 
The maximum etch rate is observed at an admixture level of 5% O$_2$. This corresponds to an oxygen atom partial pressure around 0.2 Pa, which converts into a wall collision rate of $7.9 \times 10^{17}$ cm$^{-2}$s$^{-1}$ oxygen atoms according to standard gas kinetics assuming a gas temperature of 300 K. The electron density of $6 \times 10^{17}$ m$^{-3}$ at 5% O$_2$ admixture converts to an ion flux onto the surface of $1.64 \times 10^{17}$ cm$^{-2}$s$^{-1}$, as can be deduced using the Bohm criterion. This results in ratio of the atom to ion arrival rates of 4.8. A ratio significantly above one is usually required for the chemical sputtering process to be efficient, since the ion-induced defects at the surface needs to be quickly saturated by incident neutral species.

Similar observations on the overall etch rate in plasmas from oxygen - noble gas mixtures have been made by several authors: a small admixture of oxygen up to 10% to a noble gas is sufficient to reach the maximum etch rate [30, 31, 32]. Landkammer et al. [31] showed further that the chemical identity of the ions providing the kinetic energy input for chemical sputtering is irrelevant: the etch rate is proportional to the number of ion-induced defects within a thin surface layer with a thickness identical to the penetration length of the incident neutrals. Consequently, argon ions are rather efficient at 200 eV for chemical sputtering, because incident argon ion create defects near the surface, where they can easily react with incident neutrals. Lighter ions are in principle less efficient for chemical sputtering, since they create most of the ion-induced defects beyond the penetration length of the incident neutrals leading to a much smaller chemical sputtering rate. But, if the energy of the lighter ions is reduced and their flux is increased to create the same number of defects as argon ions, the same etch rate would result. The chemical reactivity is only dominated by neutral oxygen species [33]. These could be either oxygen atoms or oxygen molecules, depending on their absolute concentrations [34].

Summarizing one can state, that the optimum process parameters to reach an optimum etch rate in an oxygen containing low pressure plasma need to balance the ion density simultaneously with the O partial pressure. Only the combined impact of both species assures an optimum etch rate.

4. Conclusions

Spores of *Bacillus atrophaeus* were exposed to beams of Ar$^+$ ions, oxygen molecules and oxygen atoms. It is shown, that only the simultaneous impact of O atoms or O$_2$ molecules and of argon ions at around 200 eV causes a very effective etching and perforation of the spore coat. This is due to the process of chemical sputtering, where the bi-radical O$_2$ or the O atoms interact with ion-induced defects at the spore surface leading to the formation of CO, CO$_2$ and H$_2$O and thus to etching. This observation is directly supported in experiments using an inductively coupled plasma from argon - oxygen mixtures. The maximum etch rate is only reached if both the electron density (and thus the ion flux) and the O atom density are optimized simultaneously. The contribution of chemical sputtering to the efficiency of the plasma-induced inactivation of bacteria is of great importance for the development and validation of plasma sterilization techniques.

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