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Study of an RF cold atmospheric pressure plasma jet: mass spectrometry and bacteria inactivation

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Abstract

In the past few years, the bactericidal effect of atmospheric pressure plasmas have gained significant attention in dermatology and for application like the sterilisation of heat sensitive medical devices such as endoscopes, dentist and surgical tools. Previous research performed at the TUe by van Gils et al has shown that plasma induced liquid chemistry is important in the inactivation of bacteria in a liquid. A chemical model was developed to predict the time depend chemical compounds induced by the plasma due to a constant production of nitric oxide (NO), ozone (O$_3$) and hydroxide (OH) in the liquid phase. This model was fitted to species measured in the liquid phase. HNO$_2$, ONOO$^-$ and H$_2$O$_2$ were found in quantities that could explain the bactericidal effect of a plasma treatment. This motivated a study of bacteria inactivation by a plasma jet at different axial positions, aiming to find the necessary threshold concentrations of plasma produced species. The gas phase reactive neutral species, liquid species, UV and ion fluxes are measured in order to find a correlation between these parameters and the bactericidal effect of a plasma. This work has focussed on two issues: (1) the determination of the absolute surface densities of plasma produced NO and O$_3$ species to remove the necessity of the assumptions in the model, and (2) the distance dependent bactericidal effect of the plasma treatment. A molecular beam mass spectrometry study has been performed to measure the absolute NO and O$_3$ surface densities. A procedure has been developed to circumvent the large changing background signal of the mass spectrometer which complicates absolute calibration of NO under the experimental conditions of the RF plasma jet. This method enables the MS to detect sub ppm NO and O$_3$ concentrations. A systematic study of the production of NO and O$_3$ shows that an increase in plasma dissipated power and admixing air results in an increase of the NO density in the plasma jet. The admixing of O$_2$ decreases the NO production. The O$_3$ production of the plasma jet is increased by admixing O$_2$ and air into the plasma, increasing the power and decreasing the duty cycle. To explain the bactericidal effect of a plasma, distance dependent biological measurements are combined with diagnostics of NO, O$_3$, H$_2$O$_2$, UV and ion flux, and the pH in the liquid. O$_3$ and NO surface densities and ion fluxes are obtained with the mass spectrometer. H$_2$O$_2$ densities in the liquid are measured with a colorimetric method. The UV flux is measured with a spectrometer. It is shown that a synergistic effect of either H$_2$O$_2$ and pH and/or the pH dependent HNO$_2$ concentration is responsible for the distance dependent effect of a bacteria inactivation with a plasma jet.
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6 Conclusions
1 Introduction

In the past few years the bactericidal effect of atmospheric pressure plasmas have gained significant attention [1]. Possible applications for these plasmas are found in in dermatology [2] and the sterilisation of heat sensitive medical devices like endoscopes, dentist tools [3], surgical tools [4] and even medical treatments [5]. At the TU/e in collaboration with the burn wound center, the potential of plasmas in the treatment of burn wounds is considered. Patients with severe burn wounds have a compromised immune system, and are therefore more susceptible to infections by bacteria. The most common infection of burn wound patients is caused by \textit{P. aeruginosa}. Avoiding these infections will lead to better healing of the burn wounds and lower the mortality rate [6, 7]. Previous research has shown promising results for the use of atmospheric plasmas to successfully reduce the number of \textit{P. aeruginosa} bacteria [8, 9, 10]. Several configurations of atmospheric plasmas have been used to investigate the mechanisms behind bacteria inactivation [10, 11, 12]. The mechanisms of bacteria inactivation by a radio frequency atmospheric plasma jet has been investigated by van Gils et al [13]. In that work, a plasma jet was investigated using diagnostics for power, gas temperature, UV flux and ion production. A chemical model has been used to analyse the liquid chemistry. Treatments on bacteria and cells were performed and bacteria inactivation was investigated. Heat, UV flux, electric field and ion flux of the plasma jet were determined not to contribute to bactericidal action of the plasma jet. The plasma was reported to induce inactivation of bacteria by the changes in induced chemistry. Further, it was suggested that the pH plays an important role in the liquid chemistry as it determines the equilibria of various possible bactericidal chemical species. It is reported that the concentration of these bactericidal species on their own are not sufficient to inactivate bacteria, therefore synergistic effects are expected. This work focuses on determining the nitric oxide (NO) and ozone (O$_3$) density produced by the plasma jet. These are two of the three gas phase species that are believed to alter the main liquid phase chemistry in the model developed by van Gils et al [13]. The density of NO and O$_3$ are measured with a Molecular Beam Mass spectrometer (MBMS). It is of utmost importance to obtain absolute densities of these species to evaluate the plasma induced liquid chemistry and bactericidal action of the plasma. Treatments on \textit{P. aeruginosa} have also been performed in the course of this work. Measurements on bacteria inactivation are linked to the mass spectrometry measurements and several other plasma parameter like UV flux, H$_2$O$_2$ concentration and pH are obtained.

1.1 Mass spectrometry

Mass spectrometry (MS) is a diagnostic method with many advantages over other diagnostics for the analysis of atmospheric plasmas. MS is capable of measuring neutral atoms, molecules, positive ions, negative ions, excited species and their kinetic energy at a plasma surface interface with a time resolutions down to microseconds. MS does not have the limitations of other commonly used optical methods, which are normally either applied at a minimum distance from a surface or require e.g. certain minimal absorption path lengths which cannot always be arranged for in the case of plasma jets. Being able to measure both atomic and molecular neutrals as well as ions without major changes to the settings of the device is also not possible with optical techniques. With the MS, the sampled species have to be extracted through a small orifice into a differential pumping system to allow for a minimum amount of collisions before the species are analysed by the mass analyser. This construction allows the determination of surface densities but makes absolute calibrations of species densities more complex. The calibrations depend on the sampling geometry, gas composition and the sensitivity of the detector. To this end, independent calibrations with known densities of species have to be performed. The large dimension of the pumping stages and the fixed position of the orifice are mayor restrictions on the sampling position. The capability of measuring many species also has a drawback: the signal
of several species can overlap and interfere with each other.

1.2 Atmospheric pressure plasma jet

When a DC voltage of sufficient amplitude is applied across two electrodes, a plasma is created. The process to sustain the plasma is due to the electric fields which accelerates the electrons. Which upon collisions with atoms and molecules create further ionisation. The energy of the electrons is transferred to gas molecules and atoms by elastic and inelastic collisions. As the energy transfer from the electric field to the electrons is more efficient than the transfer between electrons and neutrals under certain conditions. It is possible to alter the balance between the energy of electrons and neutrals. It is thus possible to create a plasma with a gas temperature close to room temperature, while the electron temperature is of the order of a few eV. This leads to a reactive electron induced chemistry at room temperature. For biomedical applications cold, non-equilibrium atmospheric pressure plasmas are necessary and can be generated in the following ways:

- The plasma can be created between two electrodes covered with an insulator (dielectric), a dielectric barrier discharge (DBD). The insulator prevents a large electrical current (and thus a large increase in temperature) between the electrodes.
- Micro plasmas have a size in the order of µm up to a few mm. Due to the small size of the plasma, the heat loss to the surroundings is large and the gas temperature can be kept low.
- The use of atomic gasses decreases the electron neutral energy transfer due to their low elastic collision cross section.
- A gas flow between the electrode enhances the cooling of gas molecules in the plasma.
- Pulsed plasmas can be created with a pulsed modulated power source. The time constant of the gas heating in a gas discharge is in the order of 100 ns to 1 µs. A nano second pulsed plasma can thus prevent significant gas heating.

An atmospheric pressure plasma jet (APPJ) is a combination of the above mentioned configurations. The plasma is created inside a small glass tube which is flushed with a noble gas. The glass tube transports the plasma created species outside the active plasma region. The application of this APPJ is therefore not bound to the configuration of the electrodes. Many different APPJ designed are being developed all over the world. The TUE developed a plasma jet enabling the user to measure the plasma dissipated power. The plasma is created with a sharp needle electrode inside the glass capillary. The local high electric field leads to a low ignition voltage, allowing to test several configurations with the same jet. Due to the design several configurations of the second electrode can be investigated, a ring electrode for a cross field jet or the jet can be adapted with a plate electrode in front of the jet (linear field jet), with a hole in the centre for the gas to flow through. The feed gas can be helium or argon. The jet can be driven by MHz or kHz power which can even be time modulated. The configuration of the APPJ used in this thesis is further discussed in chapter 2.

1.3 Bacteria inactivation

The human consists of three layers: the epidermis, dermis and subcutaneous fat (see figure 1.1). The outer epidermis layer (stratum corneum) is continuously refreshed from the deeper epidermis layers. Keratinocyte cells are formed in the deepest epidermis layers. As these keratinocytes
mature, they form the stratum corneum on the outermost epidermis layer. A first degree burn wound can be recognised by the formation of blisters, mostly between the epidermis and dermis. The dermis contains the blood vessels, lymph vessels, hair follicles, fibroblasts and nerves. The dermis is held together by a protein called collagen, made up by fibroblasts. This layer gives skin flexibility and strength. A burn wound that penetrates to the dermis is often classified as a second degree burn wound. The subcutaneous fat layer (subcutis) is the deepest layer of skin, consisting of a network of collagen and fat cells to protect the body and help to conserve the heat of the body. A burn wound that penetrates to the subcutis is often classified as a third degree burn wounds.

Figure 1.1: Schematic cross section of the human skin, showing the depth of injury for first second third degree burn wounds. [14]

Burn wounds are susceptible to infection, even more so if a large area of skin is damaged. Healing skin mostly consists of moisture, coagulated blood and dead skin cells. These circumstances are also ideal for the bacteria to grow, and an ideal place for infections. A good indication of a burn wound infection can be attained by determining the bactericidal concentration. A burn wound infection is indicated with a concentration of $10^4$ [15] or $10^5$ [14] CFU/g of tissue. CFU is the amount of Colony Forming Units.

*P. aeruginosa* is a common bacteria found in burn wound infections. It is intrinsically resistant to antibiotics and disinfectants. *P. aeruginosa* can form a bio film that shields the bacteria, making them less susceptible to topical treatments. Unless otherwise specified, the *Pseudomonas* strain used in this thesis is PAO1.

### 1.4 Motivation

This research is continuing the work performed by van Gils et al [11]. They investigated the mechanisms of bacterial inactivation in the liquid phase induced by a non-touching RF cold atmospheric pressure plasma jet. Heat, ions, UV-flux, electric fields and gas flow do not have prominent direct effect on the bacteria inactivation for their experimental conditions. Any influence of these components on the inactivation of bacteria is therefore due their influence in the liquid chemistry. The liquid chemistry is mainly induced by the reactive neutral species. A 1D time depend fluid chemistry model has been developed to analyse the possible bactericidal effect of several individual chemical component. The model assumes a constant flux of reactive neutral species. This motivated a study of bacteria inactivation at different axial positions and the measurement of gas phase reactive neutral species, liquid species concentration, UV, and ions fluxes at these axial positions. Van Gils et al used the OH, O$_3$ and NO as fitting parameters in their liquid model obtain the liquid chemistry. In this work we measure the NO and O$_3$ densities by mass spectrometry and H$_2$O$_2$ in the liquid phase to indicate the OH flux at different densities. Recent results in literature suggest an important effect of the gas flow pattern on treatments by plasma jets. To this end, we studied the effect of the flow pattern.
of the effluent with mass spectrometry. In general, mechanisms of plasma bio interaction are not completely understood nor are plasma properties quantified. In this work we perform a systematic study of the production of NO and O$_3$ as a function of different plasma parameters, such as air/O$_2$ admixture and plasma power.

1.5 Outline

The components of the mass spectrometer, and the diagnostics used for H$_2$O$_2$ and UV flux measurements are presented in chapter 2. The used methods and calibrations are described in chapter 3. The results of the measurements are presented in chapter 4. We analyse the effect of bacteria inactivation by the plasma in chapter 5.
2 Experimental setup

The different setups used in this work are being discussed in this chapter. The first section explains the principle of operation of a molecular beam mass spectrometer (MBMS) and reviews the used components. The used configuration of the plasma jet is shown in the second section. The positioning of the plasma jet on the MS is presented in the section 2.3. The last few sections show the materials/setups used for the O$_3$ calibration, the measurement of the UV irradiation and H$_2$O$_2$ concentrations in the liquid phase, pH and the configuration and equipment used for the treatment of bacteria with a plasma jet.

2.1 Molecular beam mass spectrometry (MBMS)

To gain insight in the radicals and ions produced by the plasma jet, molecular beam mass spectrometry (MBMS) has been used. MBMS is a technique suitable for identifying the chemical composition of a gas sample at atmospheric pressure.

A MS measurement is based on the manipulation of ion trajectories by electric and magnetic fields. Precisely manipulating the ion trajectories includes that collisions with other gaseous species have to be avoided. A collision can cause the ions/atoms to react, neutralise, scatter and change the composition of the gas. A mass spectrometer must, therefore, be operated in vacuum with pressures typically below $10^{-2}$ Pa. The ion mean free paths should be longer than their trajectories in the MS. Even lower operation pressures are required for the detection of the electrons via a second electron multiplier (SEM). The mass spectrometer must, therefore, be placed inside a differentially pumped vacuum chamber.

The principle of a MBMS is shown in figure 2.1. Particles or ions created by the plasma are extracted into the MS. During operation of a MBMS the gas is sampled through a small orifice or capillary in a vacuum chamber. The large amount of collisions a capillary makes sampling through a capillary not suitable for measuring reactive species and ions, and therefore a small orifice is used. In the vacuum chamber the gas is diluted by lowering the gas pressure. For the detection of neutral molecules the molecules have to be ionised. The created ions are selected regarding their energy and their mass over charge ratio (m/z). The selected ions are measured by a detector typical yielding a signal in counts per second (c/s).

The signal measured with the detector consist of a molecular beam signal and a background signal. The beam signal originate directly from sample orifice, whereas the background signal is determined by the background density/pressure in the MS chamber. Particles accounting for the background signal have undergone several collisions with the chamber wall. For an accurate determination of particle densities in the process chamber, the beam signal must be separated from the background signal.
Figure 2.1 is a simplified sketch of the components of the MBMS used in this thesis. The gas enters the vacuum chamber through a small orifice. A 50 µm sample orifice is used as a smaller orifice tends to block easily by for example dust particles. After three different pumping stages the pressure reduces to the desired low pressure. A three pumping stage setup is used to enlarge the signal to background ratio as observed by Singh et al [16]. Pressure in the vacuum stages are reduced with a Pfeiffer HiPace 300 turbo pump, which are connected to a BOC Edwards XDS10 mechanical pump. The pressure in the first stage is measured using an Edwards APG-L-NW16 gauge (1 bar to 10^{-4} mbar). The pressure in the second and third stage are measured using an Edwards AIM-S-NW25 gauge (10^{-4} to 10^{-8} mbar). The pressure in the first stage is in the order 10^{-1} Pa, second stage in the order 10^{-4} Pa and in the last stage a pressure 10^{-5} Pa is achieved under steady state operation of the device. To be able to detect ions generated by the plasma, a positive or negative potential is applied to the first two skimmers using two Delta Elektronica DC power supplies. A potential of ±10 V is applied on the first skimmer and a potential of ±40 V is applied on the second skimmer. The MS is equipped with a beam block (shutter) in the second stage as shown in figure 2.2.
2.1.1 Molecular Beam

Passing the orifice, the atoms enter the first of three pumping stages. Due to the large pressure drop in the first pumping stage the gas expands rapidly. This free jet expansion lowers the density of the gas and at some point the density is low enough so that the atoms do not collide with each other. The flow changes from a collisional to a collisionless, free molecular flow. A molecular beam is a directed beam of atoms or molecules with no collisions between the particles. A molecular beam is extracted from the free molecular flow via a hollow cone with a circular opening (skimmer) between the first and second pumping stage. These skimmers have an aerodynamic design to minimise disturbances to the flow. If the pressure drop is high enough, a supersonic flow is achieved. In a supersonic flow the beam expands and the flow velocity increases to supersonic values. Information of the boundary conditions downstream of the flow travels at the speed of sound. If the expansion goes faster than the speed of sound the boundary conditions are not felt upstream of the supersonic beam expansion. At some point the beam over expands and shock waves occur to compensate the over expansion. The position of the shockwave ($x_m$) can be estimated by [17]

$$\frac{x_m}{d} = 0.67 \sqrt{\frac{P_0}{P_b}}. \tag{1}$$

Dependent on the pressure in the first stage, there are 2 different approaches to extract a molecular beam as shown in figure 2.3. At pressure ratios ($P_0/P_b$) up to $10^5$ the position of the mach disk seems to depend on the square root of the pressure ratio.
Figure 2.3: Schematic illustration of expansion of a free jet expansion (a) into a chamber with significant background pressure and with formation of shock waves and (b) into the chamber with negligible background density and continuous transition into the molecular flow regime. Picture taken from [17].

If the pressure in the first stage is for example 50 Pa with a 50 µm orifice, the position of the mach disk is \( x_m \approx 1.5 \) mm behind the orifice. To extract a molecular beam the position of the first skimmer should be upstream of this shock wave in the so called zone of silence. The position of the skimmer should be within the first 1.5 mm behind the orifice to avoid disturbances originating from the shock waves. With the skimmer in the zone of silence an aerodynamic design of the skimmer is recommended to minimise the effect of the disturbance of shock waves.

The pressure in the first stage of the used MBMS is measured to be in the order of \( 10^{-1} \) Pa, 2-3 orders of magnitude lower as in the previous discussed approach with a pressure of 50 Pa in the first stage. At this low pressure in the first stage, no mach disk is observed. The beam expansion can be divided into two different regions. A continuum flow regime can be observed directly behind the sampling orifice. This continuum flow gradually changes in a collision-less free molecular flow. No shock waves occur and the position of the skimmer should be placed in the free molecular flow. The transition region is indicated by the quitting surface \( x_q \) a can be estimated with the following formula [18]:

\[
\frac{x_q}{d} \approx \frac{M_{\infty}}{C_1}^{1/(\gamma-1)},
\]

where \( d \) is the diameter of the sampling orifice, \( M_{\infty} \) is the final Mach number, and \( C_1 \) is 3.2, 3.6, or 3.9 for an adiabatic index \( \gamma \) of 5/3, 7/5, and 9/7, respectively. In the case of atmospheric air sampled through an orifice of 50 mm diameter, the location of the quitting surface is approximately at \( x_q = 1.05 \) mm downstream of the sampling orifice. The first skimmer is positioned 5 mm downstream of the orifice, in the molecular flow regime.

The relative composition of atoms in a free jet expansion can change. The processes that contributes to this change in chemical composition is called composition distortion [17, 19, 20].
For example, radial diffusion due to the strong radial pressure gradients of the free jet expansion discriminates atoms with respect to their mass. Heavier species do not diffuse outward as quickly as the species with a lower mass. This effect causes an enrichment of the heavier species in the center of the streamline which can be estimated using Sherman’s formula [21]. Other examples of composition distortion are interference of the MB with a skimmer or chemical processes due to the drop in temperature and pressure in the expansion. The effect of enrichment of heavier species can induce an error as large as a factor 4 difference in MS signal depending on the main collision partner as measured in [22]. This factor 4 originates from a flow with helium or air as a main collision partner of N$_2$O. Helium and air have a different collision cross section, mass and thermal properties. A N$_2$O$_2$ and argon molecule have more similar thermal, mass and cross sectional properties. The effect of composition distortion is expected not to be as crucial as with helium-air mixtures.

2.1.2 ioniser

![Diagram of ioniser and corresponding parameters](image)

Figure 2.4: The ioniser and the corresponding parameters in the MS software.

After the three pumping stages the molecular beam enters the ioniser. In the ioniser, electrons are being produced by running a current (I$_{\text{emission}}$) through a filament (see figure 2.4). The potential (V$_{\text{electron energy}}$) creates an electric field in which the produced electrons are accelerated towards the molecular beam to ionise the neutrals.
2.1.3 Bessel box

![Diagram of a Bessel box]

Figure 2.5: Schematic overview of the Bessel box. Ions with too low energy (2) or too high energy will not pass the Bessel box. Only ions within a certain energy range will pass the Bessel box (3).

After ionisation, the ions enter a Bessel box energy analyser (BBEA) [23, 24]. A Bessel box energy analyser selects the ions as function of their energy. A BBEA separates the electrons from the ions and makes sure no photons reach the detection of the MS. A BBMA has a cylindrical shape with a disk inside (see figure 2.5). Applying an electric field to the cylinder and the disk deflects the charged species. The trajectories of these ions are described by Bessel functions, hence the name Bessel box. The advantage of the Bessel box is its cheap and simple design and minimal field distortion near the slits. The disadvantage of the Bessel box is the complex shape of the field, which does not allow a simple description of the focusing and dispersion relations. Finally, the transmission efficiency of the Bessel box is only around 10% [17].

2.1.4 Quadrupole mass selector

![Diagram of a quadrupole mass selector]

Figure 2.6: Quadrupole mass selector. Only ions with a certain value of m/z have a stable trajectory and pass the QMS. Figure taken from [17].

If the ions are separated from the electrons and radiation, they enter a linear quadrupole mass selector (QMS). A QMS consists of 4 rods parallel to the flow of the molecular beam. Each opposite rod is connected to the same potential. This potential consist of DC and AC component and results in a changing electric field. The trajectory of a passing ion in this changing electric
field is described by the so-called Mathieu’s differential equations and depends on the mass over charge ratio. An ion with a m/z ratio in an unstable region starts to oscillates perpendicular to the flow direction and will oscillate out of the boundaries of the mass selector. Ions with a specific m/z range can be selected via the DC and AC component. Only the selected ions with the proper m/z ratio pass through the QMS towards the detector.

2.1.5 Secondary electron multiplier

![Secondary electron multiplier diagram]

Figure 2.7: Schematic representation of the secondary electron multiplier with the adjustable voltage parameters

After selecting ions with a specific m/z value, the ion signal is enhanced by a Secondary Electron Multiplier (SEM). If an ion hits the wall of the multiplier, secondary electrons are released from the wall. A potential is applied and the secondary electrons are accelerated towards the other wall producing more and more electrons. Depending on the potential used to accelerate the electron, amplification factors in the order of $10^6$ – $10^8$ are being reached. This large amplification factor and a fast response time are the advantages of using a SEM over other detectors like a Faraday cup. Ions that hit the wall, contaminate the surface, leading to a decrease in amplification factor over time [25]. This implies that a calibration of the MS should be performed on a weekly basis. Another disadvantage of the SEM is the mass/velocity dependence gain of the SEM [26] [27].

2.2 Plasma jet

The atmospheric plasma jet used in this work consist of a concentric needle electrode ($d=1$ mm) in a glass tube within a plastic enclosure. A schematic representation of the plasma source is shown in figure 2.8. The glass tube has an inner diameter of 1.65 mm and an outer diameter of 3 mm. The second ring electrode with a length of 5 mm is placed at roughly 2 mm before the end of the glass tube and connected to ground. The needle of the plasma jet is connected via a matching circuit to a RF power amplifier (Amplifier Research 75W, 5-250MHz). The input of this power amplifier is a wave generator (Agilent 33220A) operating at 13.56 MHz. A second wave generator of the same type was used to apply 20 kHz pulsing to the RF signal. The gas flow of the plasma jet is controlled by Brooks 5850E mass flow controllers. In this work an argon flow feed gas has been used with possible admixtures of dry air and O$_2$. 
The power is measured by measuring the voltage with a 1:100 Tektronix P5100 voltage probe and the current with a Pearson 2877 current probe. The signals are recorded with an Agilent Technologies DSO 1024A, 200MHz, 2 GSa/s oscilloscope. To calculate the total power the voltage and current signals are corrected for the relative time delay ($\tau_{\text{shift}}$) in the probe cables.

$$P_{\text{total}} = \frac{1}{T} \int_0^T U(t) I(t + \tau_{\text{shift}}) dt$$  \hspace{1cm} (3)

The total dissipated power ($P_{\text{on}}$) is measured when the plasma is on, consisting of the plasma dissipated power and the matchbox dissipated power. The matchbox dissipated power has been measured by calculating the dissipated power without plasma ($P_{\text{off}}$) as a function of the current. The plasma dissipated power is calculated by subtracting the matchbox dissipated power from the total power at the same current.

$$P_{\text{on}} = P_{\text{total}} - P_{\text{off}}$$

This method is more thoroughly described in a previous published paper by Hofmann et al [28] and is based on the fact that the major power loss in the matching box is due to the resistance of the coil in series with the plasma.
2.3 MBMS and plasma jet

A schematic representation of the plasma jet aligned at orifice of the molecular beam mass spectrometer is shown in figure 2.10. The position of the plasma jet in the x y and z direction can be adjusted via micrometer screws.

![Schematic drawing of the plasma jet](image)

Figure 2.10: Schematic drawing of the MS adapted with a silicon suction ring to pump off the argon in the cup around the orifice and a cut of 96-well plate to mimic the flow pattern of the plasma jet used for bacteria treatment. The position of the jet is adapted with micrometer screws.

In order to investigate the influence of the gas flow, the MS is equipped with a suction ring. The suction ring is a silicon tube (Ø = 9 mm) with five holes drilled into the tube. The tube is bend and placed at the bottom of the cup surrounding the orifice. To mimic the flow of a bacteria treatment in a well plate, 9 wells are cut out in square shape (see figure 2.11). The height of the well is adjusted to be 8 mm. The bottom of the center well is completely removed to allow sampling through the orifice.

![Example of a 96 well plate](image)

Figure 2.11: Example of a 96 well plate. A well has a diameter of 8.5 mm and a height of 11 mm. The red square indicates a cut well plate.

2.4 MBMS and vacuum chamber

The MBMS can be equipped with a vacuum chamber. The volume of the vacuum chamber is approximately 4 liter. The vacuum chamber can be pumped down to a pressure of 250 Pa and filled with Brooks 5850E mass flow controllers. The pressure is measured with a MKS INSTRUMENTS 112A pressure transducer. The overpressure valve opens at a pressure of 350 Pa above the pressure outside the vacuum chamber.
Figure 2.12: Schematic picture of the mass spectrometer equipped with a vacuum chamber. The vacuum chamber can be evacuated and filled with different mixtures of Ar/air.

2.5 Absolute Calibration

For the absolute calibration of NO, an argon bottle with a premixed NO concentration of 203±1 ppm NO from Linde gas is available.

The absolute calibration of O₃ mass spectrometer measurements is performed by the determination of the absolute density of O₃ in the gas phase by a UV absorption measurement. A polymethylmethacrylaat absorption chamber with an absorption path length of 8 mm has been used as shown in figure 2.13. A Roithner UVtop250-HL-T039 with the emission at 254 nm, corresponding to a strong absorption of O₃ is used as light source. The light from the LED is guided through the absorption chamber via 2 lenses. The light is collected in a UV fiber and guided to a Ocean Optics HR2000 spectrometer. Silicon tubes are being used to minimise the decomposition of ozone at the wall of the tubes [29]. The O₃ density which is produced by a remote plasma jet has been measured with a tube directly from the jet to the MS and via the absorption chamber to the MS. With the same plasma jet settings no change in O₃ signal is detected. This indicates a minimal influence of the tubes and absorption chamber on the O₃ density.
Figure 2.13: Schematic representation of the calibration setup used for determining the ozone density. The ozone is produced with the Tue plasma jet with a ring electrode. The ozone absorption at 254 nm is measured in the absorption cell with a spectrometer. The UV is produced by a LED. The gas with a measured concentration $O_3$ is guided to the MS to perform a simultaneous measurement.

2.6 $H_2O_2$

The $H_2O_2$ in the plasma activated solution is determined with a colorimetric method using ammonium metavanadate. The reaction of $H_2O_2$ and metavanadate results in the formation of peroxovanadium cations which produce a red orange colour with a maximum absorbance at 450 nm. The advantage of using ammonium metavanadate is that it is sensitive to $H_2O_2$ in the liquid but shows little to no effect from nitrate, chloride or ferric iron [30]. A Roithner LED 450-06 produced light at 450 nm and is used as light source for the absorption measurement. The liquid detection solution is placed in a cuvette that has been placed in a cuvette holder. The absorption signal has been measured with an Ocean Optics HR2000 spectrometer.

2.7 UV irradiance

The UV light has been measured with an Ocean Optics HR2000 spectrometer. For the absolute calibration a deuterium lamp with a known spectral irradiance (W/m/nm) at a fixed distance in the wavelength range 200-400 nm has been used.

2.8 pH

A VWR Symphony SB70P pH meter has been used for measuring the pH of the plasma treated solutions.

2.9 Bacteria inactivation

For measurement on bacteria the PA01 strain of $P. aeruginosa$ is used in the bacteria treatment experiments. Before the bacteria can be used for a plasma treatment the bacteria need to multiply. To allow good bacteria multiplication the bacteria are shaken at 167 rpm in a Heidolph Unimax 1010 and the temperature is kept at 37°C by a Heidolph Incubator 1000. After multiplication the concentration of the bacteria is measured by an Thermo scientific Helios Epsilon.
The Helios Epsilon measures the absorption of a laser. A 0.9% saline solution has been used for treatment of bacteria. As a buffered saline solution, *Gibco Dulbecco’s Phosphate* buffered saline is used. After treatment, the bacteria are plated and put in in a conditioned chamber at 37° with 5 ppm CO to allow good bacteria multiplication. The treatments of bacteria are done in 96-well plate in a flow cabin with a down flow of 0.31 m/s. The experiment on bacteria that are presented in this thesis have been performed in collaboration with the Association of Dutch Burn Centres, in their laboratory in Beverwijk.
3 Experimental procedures and calibration methods

3.1 Mass spectrometry

The most common way to operate the MS is in Residual Gas Analyser (RGA) mode. During the RGA mode the molecules are ionised in an internal ionisation source via electron impact ionisation

\[ M + e^- \rightarrow M^+ + 2e^- \]  

Electrons are produced by driving a current through a filament. An electric field accelerates the ions towards the molecular beam with enough energy to overcome the ionisation threshold energy of an atom. If the electrons have enough energy they ionise an atom or a molecule, a positive charged ion is produced in the collision. The cross section of electron impact ionisation is zero below the threshold energy and increases with increasing electron energy to reach a maximum for most atoms, between 50 to 100 eV. After the maximum value the cross section slowly decreases. An electron can have significant more energy than necessary for a single ionisation process and therefore cause the production of multiple ionised ions

\[ M + e^- \rightarrow M^{z+} + (z + 1)e^- \]  

or lead to dissociative ionisation,

\[ AB + e^- \rightarrow A^+ + B + 2e^- \]  

An ionisation by energetic electrons (typically 70 eV in RGA mode) leads to all three above processes. These processes produce a specific number of fragment ions in addition to the ion from the observed parent molecule. This specific pattern of ionisation is called the cracking or fragmentation pattern of a molecule. Cracking patterns of a lot of molecules can be found in tables for an electron impact energy of 70 eV [31]. Due to the (near) maximum cross section and known cracking pattern at this electron energy, a commonly used ionisation energy is 70 eV. Problems in the interpretation of the mass spectra start to arise if cracking patterns of different molecules start to overlap with each other. For example NO\(^+\) yields a signal at a value of 30 amu/z. N\(_2\) has also an isotope with a mass at a value of 30 amu/z. The N isotope with a mass of 15 amu has an abundance of roughly 0.4%. In spite this small abundance, due to the large amount of N\(_2\) this isotope with a signal at 30 amu/z can be an important factor when measuring NO within our experiment conditions. The expected abundance of the N\(_2\) isotope with a mass of 30 amu is 10 ppm or less. This is in the same order of magnitude as the expected NO concentration of 4 ppm [32]. To distinguish between contribution of these signals at 30 amu/z, the ratio between the parent peak of N\(_2\) at 28 amu/z and the contributing peak from the N\(_2\) isotope at 30 amu/z can be used.

Figure 3.1 shows an example of a mass spectrum with the mass spectrometer operating in RGA mode.
Figure 3.1: Example of a RGA mass scan during an 1.5 slm Ar flow directed at the orifice at a distance of 12 mm between electrode and the orifice.

An Ar plasma flow is directed at the orifice. Clearly visible are the peaks of Ar (40 amu/z) its isotopes (38 and 36 amu/z) and some double ionised Ar atoms (18, 19 and 20 amu/z). Air peaks like O\(_2\) (32 amu/z), N\(_2\) (28 amu/z) CO\(_2\) (44 amu/z) and H\(_2\)O (18 amu/z). An example of overlapping cracking patterns is shown at 18 amu/z. The signal of H\(_2\)O overlaps with the signal of Ar\(^{2+}\) isotope.

For post processing the values of a mass peaks are integrated and multiplied by the step size of the mass scan.

**Electron emission current** A maximum in the MS signal as function of the electron emission current in the ioniser has been reported by Agarwal *et al* [33]. At high emission current there is an onset of the space charge in the ioniser. This space charge shields the electric field and the electrons are not accelerated fast enough towards the molecular beam. In figure 3.2 the MS signal is measured as a function of electron emission current. The same trend as reported in [33] has been observed. The electron energy in our measurement is 70eV instead of values around 16eV used in the paper of Agarwal *et al*.
Figure 3.2: Mass spectrometer signal as function of emission current, both measurement series are normalised on the signal at 150 µA.

Below the maximum there is a linear increase in MS signal as function of electron emission current. If the current is higher than the saturation point the signal decreases, indicating an onset of space charge accumulation in the ioniser.

Figure 3.3 shows the signal at 30 amu/z at an emission current of 3 and 125 amu/z during a measurement with the shutter (which blocks the molecular beam) in place or not. A change in emission current does not have an effect on the signal with the shutter in and out.

Figure 3.3: Mass spectrometer signal at 30 amu/z during a lab air measurement for an emission current of 3 and 125 µA.

The fluctuations in the points with a higher emission current are smaller as with lower emission currents. For measuring O₃ and NO the emission current is set to 125 µA. This reduces the fluctuations and because the signal is not in the saturation regime no extra fluctuations are expected from an onset of the space charge. Due to the large density of N₂, O₂ and Ar, these signals are measured at an emission current between 1 and 3 µA to avoid saturation of the secondary electron multiplier.
Secondary electron multiplier To minimise the effect of drift in the signal during a change in the sensitivity of the secondary electron multiplier (SEM) [25] an optimum multiplier voltage needs to be derived. The MS signal is measured as a function of the multiplier voltage. A scan of the lab air for the signal at 14 amu/z (N$_2^+$,N$^+$) and not at 28 amu/z to avoid saturation of the SEM.

![Graph of signal MS at 14 amu/z vs. Multiplier voltage](image)

Figure 3.4: Mass spectrometer signal as function of multiplier current

Figure 3.4 shows an increasing MS signal if the multiplier voltage is increased. Around a multiplier voltage of 2200 V the signal starts to level off. To minimise the effect of a drift in the multiplier voltage, the multiplier voltage is set to value of 2600 V on the plateau of the MS signal.

Absolute density calibration Due to the composition distortion in the supersonic beam expansion and species dependent response of the MS because of the difference in ionisation cross section and mass dependence of the gain of the SEM it is almost impossible to theoretically calculate the absolute concentration from the detected signal. Therefore a calibration should be performed to measure absolute densities. The absolute signal of the MS changes over time due to processes like contamination of the filament, clogging of the orifice, drift in the SEM sensitivity and quadrupole transmittance drift [25]. To detect a drift in the sensitivity a lab air scan is performed and the pressures in the pumping stages are checked for consistency. In addition the N$_2$ (28 amu/z) and O$_2$ (32 amu/z) signals are recorded for every measurement. A change O$_2$ and n$_2$ signal is an indication that the MS has to be calibrated again.

In the remainder of this chapter, the procedures to measure air, NO and O$_3$ densities are described together with their respective calibration method. An overview of used MS settings and calibration methods used in this chapter is shown in table 1.

3.1.1 Air

For the calibration of air a vacuum chamber is attached on top of the MS. Argon with purity of 99.999% and dry air has been used to make different Ar/air mixtures. A schematic picture of the calibration setup for air is shown in section 2.4. The mass spectrometer is absolutely calibrated for the air signal by filling the vacuum chamber on top of the MS with different Ar air mixtures. First the vacuum chamber is pumped down to a pressure of roughly 250 Pa. After the chamber is evacuated, the tubes are flushed and the chamber is filled with Ar air mixtures. If an overpressure of 350 pa above atmospheric is reached the overpressure value opens and the
Table 1: Overview of used MS settings and calibration methods used in this chapter.

<table>
<thead>
<tr>
<th>subject</th>
<th>Calibration gas</th>
<th>Electron energy [eV]</th>
<th>emission current [µA]</th>
<th>Ionisation method</th>
<th>MS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air calibration</td>
<td>dry air</td>
<td>70</td>
<td>1</td>
<td>impact ionisation</td>
<td>vacuum chamber</td>
<td>20-24</td>
</tr>
<tr>
<td>NO calibration</td>
<td>Ar NO</td>
<td>70</td>
<td>125</td>
<td>impact ionisation</td>
<td>vacuum chamber</td>
<td>24-25</td>
</tr>
<tr>
<td>NO calibration</td>
<td>He NO</td>
<td>0 - 12</td>
<td>125</td>
<td>Electron attachment</td>
<td>vacuum chamber</td>
<td>25-28</td>
</tr>
<tr>
<td>NO calibration</td>
<td>Ar NO</td>
<td>7-25</td>
<td>125</td>
<td>threshold ionisation</td>
<td>vacuum chamber</td>
<td></td>
</tr>
<tr>
<td>NO background</td>
<td>Ar, air, Ar plasma</td>
<td>3-10</td>
<td>125</td>
<td>impact ionisation</td>
<td>Plasma jet, suction ring and well plate</td>
<td>28-32</td>
</tr>
<tr>
<td>NO calibration</td>
<td>Ar NO</td>
<td>70</td>
<td>125</td>
<td>impact ionisation</td>
<td>Plasma jet, suction ring and well plate</td>
<td>32-36</td>
</tr>
<tr>
<td>O$_3$ calibration</td>
<td>Ar-O$_2$ plasma</td>
<td>70</td>
<td>125</td>
<td>impact ionisation</td>
<td>Plasma jet, suction ring, O$_3$ absorption chamber, spectrometer</td>
<td>36-38</td>
</tr>
</tbody>
</table>
flow rate is reduced approx. 0.2-0.4 slm. The volume of the vacuum chamber is 4 l and therefore the system is left to settle for 10 to 20 minutes. After the system has been settled the percentage of air is calculated. The percentage of air enrichment in the Ar flow is calculated with the signal of Ar (40 amu/z), O$_2$(32 amu/z) and N$_2$ (32 amu/z), see equation 7.

\begin{equation}
\text{(7)}
\end{equation}

The air and argon signal during settling of the system signals after the chamber has been filled with only Ar are shown in figure 3.5. The ratio O$_2$/N$_2$ in atmospheric air is 78/21=3.7. To check if the ratio of the N$_2$ and O$_2$ signal correspond to air, the O$_2$ signal is multiplied by 3.7. As shown in figure 3.5 the O$_2$·3.7 signal overlaps with the N$_2$ signal. This shows that using only the largest peaks for O$_2$ and N$_2$ at 28 and 32 amu/z gives a reliable value for the amount of air.

![Figure 3.5: Settling of the MS signal after the chamber has been filled with argon and no air admixture.](image)

Figure 3.5: Settling of the MS signal after the chamber has been filled with argon and no air admixture.

During a period of 10 minutes the signal of Ar increases while the air signal decreases. During the evacuation and filling of the chamber with Ar, air leaks into the chamber. During overpressure the excess air in the chamber is blown out of the chamber resulting in a decreasing air signal and increasing Ar signal. The time constant of the changing signals in figure 3.5 are in the same order as expected at a 0.2-0.4 slm flow rate though a 4L chamber.

For different percentage of air/Ar flows the percentage of air is calculated via equation 7. The results of this calibration measurement are shown in figure 3.6.
The relation between the premixed air concentration ($s_a$) and the measured air concentration ($s_m$), obtained by the ratios of the signals at in formula 7 is

$$S_a = 0.96 \cdot S_m + 1.42$$

(8)

The 95% confidence interval is indicated with the red line. The error bars indicate the fluctuation in the raw signal of the percentage of air. As the line $S_a=S_m$ is in the confidence interval, no extra calibration is used to determine the percentage of air.
3.1.2 NO

A calibration procedure for NO, based on the same principle as the calibration in air, is presented which yields a significant and unacceptable inaccuracy. This result motivates the investigation of other calibration methods which are discussed in this section.

To perform the calibration of the MS for NO, the MS is equipped with the vacuum chamber on top as presented in section 3.1.1. The MS is set in RGA mode which implies ionisation of the molecular beam via electron impact ionisation. The same procedure is used as with the air calibration. A calibrated Ar-NO bottle from Linde gas with $203 \pm 1$ ppm NO is diluted with Ar. For different NO concentrations, the signal of the MS is plotted against the known amount of NO. The mass spectrometer is calibrated using the RGA mode,-ionisation of the molecules via impact ionisation. Figure 3.7 shows the signal of Ar (40 amu/z), $N_2$ (28 amu/z), $O_2$ (32 amu/z) and NO (amu/z 30) as function of time during one measurement.

![Figure 3.7](image)

Figure 3.7: Signal of Ar (40 amu/z), $N_2$ (28 amu/z), $O_2$ (32 amu/z) and NO (30 amu/z) as function of time during one measurement. At 10 minutes the vacuum chamber is evacuated and after that filled with an Ar-NO mixture which contains 10 ppm NO ($2.5 \cdot 10^{20}$ m$^{-3}$). The system is left to relax for 60 min. to reach a steady state.

After 10 minutes the vacuum chamber is evacuated from a previous measurement. This coincides with the drop in Ar and NO signal around 10 minutes. After the chamber is evacuated the tubes are flushed and the chamber filled with an Ar with a NO density of 10 ppm ($2.5 \cdot 10^{20}$ m$^{-3}$) mixture. The $N_2$ and $O_2$ signal increased if the chamber is evacuated and filled. This increase is due to the fact the chamber is not completely air tight and a small amount of air is flowing in the chamber. From the moment the pressure in the chamber is at 350 Pa above atmospheric pressure, the overpressure valve opens and due to the continuous flow (0.2 l/min) the air signals decays exponentially to zero. This indicates that during the overpressure the air concentration is decreased and no contamination form the surround air is entering the chamber. The Ar signal is stable but the signal at 30 amu/z is time dependent with a longer time constant than the time constant of the gas composition change in the chamber (i.e. 4 hours). To correct for this changing background signal, the vacuum chamber is filled with Ar before and after each measurement serie. The background obtained in the pure argon chamber is subtracted from the total signal. This background can decrease or increase during the measurements and has an order of magnitude equivalent to a signal of roughly 20 ppm ($5 \cdot 10^{20}$ m$^{-3}$) NO.
Figure 3.8 shows the results of the NO calibration. The maximum error in the flow controllers are estimated to be 2% of the full scale. Due to the low flow of the flow controllers during overpressure the maximum error is estimated to be 10%. The error can be as large as 40 ppm if a value if a MS signal of $8.5 \times 10^4$ is measured (see figure 3.8). This large error at high NO concentrations and the large changing background with a long time constant makes this calibration method not suitable for determining the NO production from a plasma jet.

**Electron attachment**  Electron impact ionisation is often not suitable for fragile molecules as impact ionisation dissociates the molecule. Electrons with an energy typical below the impact ionisation threshold can very efficient attach to molecules via electron attachment. Electron Attachment Mass Spectrometry (EAMS) can under these conditions be performed. Electron attachment can be less destructive for molecules and lead to a lower production of secondary ion fragments. Electron attachment is a resonant process that lead to distinct peaks in the electron attachment cross section as a function of the electron energy (see figure 3.9). The cross section reaches there maxima for different molecules at a different value of the electron energy. And can also be used for identifying molecules with overlapping cracking patterns. For electron attachment a third body reaction is necessary to ensure energy and momentum conservation.

$$AB + M + e^- \rightarrow AB^- + M \quad (9)$$

The electron attachment in the MS takes place at low at a pressure in the order of $10^{-5}$ Pa. At this pressure a third body reactions are slow and a negative ion will be produced through dissociative electron attachment.

$$AB + e^- \rightarrow A^- + B \quad (10)$$

Due to the dissociative electron attachment the signal from NO will results in a O$^-$ ion at 16 amu/z. This signal will overlap with the O$^-$ produced via dissociative electron attachment of O$_2$. 

![Graph showing the relationship between integrated MS signal at 30 amu/z (in 10$^4$ c/s) and concentration NO (in ppm). The line shows the calculated linear trend. The error bars indicate the fluctuation in the raw signal for determining a measuring point and a 10% error from the uncertainty in the mass flow controllers. The maximum error in the flow controllers are estimated to be 2% of the full scale.](image)
From the cross section data published by Rapp et al [34] the total number of ions as function of the electron energy is plotted in figure 10. The cross section profiles of O₂ and NO partially overlap. NO will change the peak of the total signal. The calibration of EAMS is performed with a premixed He-NO bottle (linde gas) containing 500 ppm NO. The same procedure to prepare the vacuum chamber as for the air calibration is used. MS is equipped with the vacuum chamber see figure 2.12 and evacuated before the chamber is filled with a mixture of helium and NO.

Two electron energy scans at 16 amu/z during EAMS of helium and helium with 500 ppm NO (figure 3.10). Although the peak of the signal does not shift, the signal with He-NO is higher. The background signal (only helium) is subtracted from the signal of helium with 500 ppm NO to obtain the signal which should correspond to NO. The results of the subtraction for three different NO concentration are shown in figure 3.11.
At high NO concentrations a significant contribution of NO is clearly visible. At lower concentration of NO the contribution of NO seems to disappear and the background signal due to impurities in the MS seems to overrule the NO signal. Detection of the NO signal becomes even harder if used for measuring the NO production in a Ar plasma jet operating in ambient lab air. The background signal at 16 amu/z will be much larger due to the O\(^-\) originating from the O\(_2\) in the air. Due to the large overlapping background signal electron attachment cannot be used to measure NO.

**Appearance potential**  Appearance Potential Mass Spectrometry (APMS) is a technique that exploits the difference in ionisation potential to distinguish between overlapping signals. The cross sections of the NO and N\(_2\) isotope with a signal at 30 amu/z as function of the electron energy are shown in figure 3.12.

The difference in the ionisation threshold can be used to distinguish between two molecules
with the same mass. The use of appearance potential mass spectrometry to measure the absolute density of atoms is explained in [33]. This technique can also in principle be used to measure excited states of molecules and atoms. Excited states have a different ionisation potential as atoms in the ground state. Appearance potential is performed with the MS equipped with the vacuum chamber. The same procedure to prepare the vacuum chamber as for the air calibration is used. The vacuum chamber is evacuated and filled with an Ar and an Ar-NO mixture containing 13 ppm NO.

![Graph](image_url)

**Figure 3.13:** APM spectrum for 30 amu/z in AR and Ar containing 13 ppm NO.

The signal of Ar and 13 ppm NO is larger as just with Ar. This larger signal can indicate that the (change in) background is much larger as the signal from NO or it indicates that the background signal is NO produced in the MS. The fact that no change in slope of the NO\(^+\) APM spectrum is detected at a concentration of 13 ppm NO, makes this methods not suitable for measuring NO in the plasma jet.

From the comparison of different techniques in previous paragraphs electron impact ionisation at 70 eV is the most promising method which allows to detect an NO concentration of 2.5 ppm. However for an accurate absolute calibration it is necessary to consider accurately the changing background in time.

**Background NO** In this paragraph the results of a more detailed study of the time varying background signal at 30 amu/z is presented.

The background is investigated using the plasma jet on the MS adapted with a well plate and a suction ring (with a flow rate of 30 slm) as discussed in section 2.3. The signal at 30 amu/z while switching on a plasma with a continuous power of 0.4W is shown in figure 3.14. Switching the plasma on, decreases the signal at 30 amu/z instead of the expected increase due to the NO production of the plasma jet. A change in beam component has a time constant in the order of several milliseconds [36] and a jump in the signal due to the NO production of the plasma jet is expected. The decrease in signal has a much larger time constant, and therefore the decrease in signal is not related to a decreasing in beam component but due to a slow change in background. Due to the low 0.4 W plasma power not much NO is produced and the change in background is larger as the signal from the produced NO.
Simultaneously with the signal at 30 amu/z, the N\textsubscript{2} and air signals are measured at 28 and 32 amu/z. The result is shown in figure 3.15.

Turning the plasma on changes the gas flow of the jet and therefore the mixing of air in the Ar flow. If the plasma is turned on, the air signal decreases at the position of the orifice. This decreasing air signal, changes the background signal at 30 amu/z.
Figure 3.16: Signal at 30 amu/z after a 4 hour 1.5 slm Ar flow of the plasma jet at 4 mm distance between electrode and orifice to prevent significant air entrainment. The MS is turned off from 230 upto 240 min. In addition the response of an air flow and switching back to argon is shown.

Figure 3.16 shows the NO signal after the system was able to stabilise for approximately 4 hours. At the start of the measurement the signal at 30 amu/z had a value of $12 \times 10^3$ c/s. After approximately 4 hours the signal settles at a value of $4 \times 10^3$ c/s. The MS is turned off during a constant argon flow from 230 upto 240 min. After the MS is turned on again there are some minor changes in the signal as observed when the MS is turned on. This indicates that the electronics/heating is not contributing a lot in the changing background. At 245 min. the argon flow is replaced by an air flow. The jump in signal indicates a beam component at 30 amu/z originating from air. This indicates an overlapping cracking pattern of air with NO. The increasing background during the air flow directed at the orifice indicates that if the beam component is changed the background starts to settle again. At t=250 min. the flow is switched from an air to Ar flow. The MS has to settle for several hours to get at the same MS signal of $4 \times 10^3$ c/s at the beginning of the measurement. The signal at 30 amu/z seems to be related to switching from an Ar to an air flow. Air seems to have a beam component at 30 amu/z. The changing beam component also changes the background and the MS has to settle again.

**Cracking pattern of air** To link the possible beam component at 30 amu/z from air, different Ar-air mixtures are used as flow of the plasma jet. The plasma jet is placed close to the orifice (i.e. 4 mm) to ensure no admixing of the surrounding air into the flow. An Ar flow is alternated with an Ar-air mixture. The raw data of a measurement serie is shown in figure 3.17.
The signal at 28 amu/z corresponding to N\textsubscript{2} shows a clear step function if the flow is switched from an argon to an Ar-air flow. Signal at 30 amu/z has a fast and a slow response. The fast response of the signal at 30 amu/z indicated at beam component of air at 30 amu/z. The magnitude fast response seems to be larger if switched from an Ar to an Ar-air flow. The slow response of the signal at 30 amu/z indicate the settling of the background. As the background is feed by the molecular beam, a change in beam component results in a slow changing background. As the signal at 28 amu/z has a much larger beam to background ratio compared to the signal at 30 amu/z, this changing background is not an issue. The beam component is estimated by using the drop in the signal if switched from an Ar-air flow to an Ar flow. The results are presented in figure 3.18.

Figure 3.17: Signal at 30 and 28 (N\textsubscript{2}) amu/z while using the jet, and switching from an Ar flow to Ar with air admixture.

If the drop in signal at 30 amu/z is related to the N\textsubscript{2} isotope with a mass of 30 a linear is expected between the signal at 28 and 30 amu/z as the ratio of the N\textsubscript{2} isotopes is not expected.
to change. Measurement performed on 2 different days show that the measurements are not reproducible.

Although the measurements are not reproducible an estimation can be made of the error due to the change in air signal. Figure 3.15 shows that the \( N_2 \) signal reduces approximately \( 1 \cdot 10^5 \) c/s if the plasma is turned on. From figure 3.18 (NO signal approximately 300 c/s) and the final calibration presented in figure 3.21, the jump in signal at 30 amu/z results in an overestimation of the NO concentration of approximately 0.75 ppm (1.7\( \cdot \)10\(^{19}\) m\(^{-3}\)). At a higher continuous plasma power (2.6 W, 20 mm) the decrease seems to be negligible. Although there is no clear relation found between the power/distance and the decrease in background, the change in background is more than a factor two less at power above 2 W. Therefore the error due to the background is estimated to be much lower as 1.7\( \cdot \)10\(^{19}\) m\(^{-3}\) and therefore negligible.

**Beam block**  The MS is equipped with a shutter that enables to block the molecular beam and to measure the background. Figure 3.19 shows the NO signal during a plasma on and off measurement with the shutter in and out at a plasma power of 0.4 W. If the shutter blocks the beam, the signal drops immediately because the beam component is blocked. However the pressure in the pumping stages changes and therefore also the background signal starts to settle at a different value. The fast changing background also makes it hard to extrapolate the changing background at the point the shutter was out to extract a beam component. As a consequence the shutter is not used to correct for the background and extracting the beam component.

![Figure 3.19: Signal at 30 amu/z during plasma on and off measurements with a continuous 1.5 slm argon plasma power of 0.4 W at a distance of 14 mm between electrode and orifice.](image)

**Absolute NO calibration**  It has been shown that the background of the signal at 30 amu/z has a large background which changes on a time scale of hours while the beam component changes within a millisecond for calibration and measure the NO production of a plasma jet. This large difference in time constant is used to minimise the influence of the changing background. If the plasma is switched off and only an argon flow is present, the signal at 30 amu/z will drop. This drop in signal is used as a signal for the NO concentration. For calibrating the MS for the NO signal, the MS is adapted to with the jet as described in section 2.3. The suction ring (30 slm flow rate) and the adapted well plate are installed around the orifice. The jet is placed at a distance of approximately 3 mm between the orifice and the exit of glass tube. At this distance no surrounding air is mixed into the flow. An Ar-NO flow with various concentrations of NO is obtained by dilution of a premixed Ar-NO bottle (203±1 ppm NO, Linde gas) with argon by 2
mass flow controllers. The NO signal during a calibration measurement is shown in figure 3.20. At \( t=0 \) a pure Ar flow is applied. After roughly 30 minutes the flow is switched from a pure Ar flow to an Ar flow with \( 3.4 \times 10^{20} \text{m}^{-3} \) (13.6 ppm) NO. The initial increase in signal is rather slow. This is due to the fact the tubes were not flushed and it took some time for the NO to mix into the Ar flow. After about 20 minutes the flow is switched back to a pure Ar flow. The drop in signal is used as a measurement of the NO concentration in 3.21. Notice the changing background on an hour time scale. The trend of the background is not disturbed by the fact the flow is switched from Ar to Ar-NO.

![Graph showing the change in signal over time](image)

Figure 3.20: Example of the raw data at 30 amu/z used for an NO calibration. The increase in signal is due to the various premixing of NO to the argon flow. The value in the graph are the calculated NO densities in units of \( 10^{19} \text{m}^{-3} \).

The result of the NO calibration is a linear relation between the NO concentration/density and the MS signal, see figure 3.21. The lowest detected NO signal is 0.2 ppm. This is the lowest NO mixture that could be made within the range of the used mass flow controllers.

![Graph showing the NO calibration curve](image)

Figure 3.21: NO calibration curve obtained from data as shown in figure 3.20. The measurements are performed on two different days.
A linear fit is used to determine from the data presented in figure 3.21 MS signal at 30 amu/z $(S_{NO} \text{ [c/s]})$ the NO number density $(n_{NO} \text{ [m}^{-3}\text{]})$.

$$n_{NO} = 7.36 \cdot 10^{16} \cdot S_{NO}$$ (11)

The error in the absolute NO density is estimated by the error in the linear fit coefficient which equals $1 \cdot 10^{15} \text{ m}^{-3}/(\text{c/s})$ or 1.3%. The measurement under the calibration conditions has a detection limit of approximately 0.1 ppm ($2.5 \cdot 10^{18} \text{m}^{-3}$).

To measure the NO production of a plasma jet, the plasma jet is axially aligned at the orifice of the MS as shown in section 2.3. The MS is equipped with a well plate and the suction ring with a flow rate of 30 slm. Because the plasma jet sometimes needs a spark, created by an externally inserted wire near the plasma needle to ignite the plasma, the change in signal from plasma off to on is not used. Instead the change in signal if the plasma is turned off is used as measurement for the NO concentration.

Figure 3.22: Integrated MS signal at 30 amu/z during a NO measurement at a distance of 20 mm between electrode and orifice and a continuous 1.5 slm Ar plasma power of 2.6 W.

Figure 3.22 is an example of NO production at higher plasma power of 2.6 W. The jump in signal is clearly visible and used as signal for the plasma produced NO. With the calibration curve presented in figure 3.21 the NO density is calculated. The fluctuations in the signal at 30 amu/z are estimated to be 0.5 ppm ($1.25 \cdot 10^{19} \text{m}^{-3}$) Although the detection limit of the calibration is in the order of 0.1 ppm, the detection limit of the NO produced by a plasma seems to be around 1 ppm. The reason for this change in detection limit is most likely large changing background as can be seen in figure 3.14.

The over estimation due to a change in background is estimated to be much lower as $1.7 \cdot 10^{19} \text{m}^{-3}$ and therefore negligible. The error bars in the measured NO concentration presented in this thesis consists of a systematic error 2.3% from the calibration and an error of $1.25 \cdot 10^{19} \text{m}^{-3}$ due to fluctuation in the raw signal at 30 amu/z.

**Composition distortion**  The calibration of NO is performed in argon. The plasma produced NO is measured in argon admixed with surrounding air. The composition distortion in the molecular beam expansion of the MS due to a change in the main collision partner has been presented in section 2.1.1. The change in main collision partner from argon to air does not have a significant effect on the measured NO density as shown in figure 3.23.
Figure 3.23: Relative NO signal obtained for different Ar/air mixtures with a constant NO density admixed equal to $3.4 \times 10^{19}$ m$^{-3}$. 
3.1.3 O$_3$

An absolute calibration the MS for O$_3$ is obtained by an absorption measurement. A sketch of the setup used for the ozone calibration is shown section 2.5. The O$_3$ concentration (N) is determined with the Lambert-Beer law:

$$\frac{I_a}{I_0} = \exp(-\sigma \cdot N \cdot L).$$

(12)

The absorption cross section ($\sigma$) of O$_3$ has a maximum of $1.147 \cdot 10^{-21} \text{m}^2$ at 254 nm [37]. This wavelength and absorption cross section is used to determine the concentration O$_3$. Although the spectrometer measures a broad spectrum (due to the broadband LED) only the signal at 254 nm is selected. The signal of the MS and the signal of the spectrometer during the calibration measurement are respectively shown in figure 3.24 en 3.25. At t=0 there is only an Ar flow. This spectrometer signal is used as I$_0$ signal for the upcoming absorption signal. Next the plasma jet is switched on and the Ar/O$_2$ plasma starts to produce O$_3$. The O$_3$ fills the absorption chamber and absorbs the light at 254 nm. The light signal decreases and the stable absorption signal is used as I$_a$ to obtain the O$_3$ density. After the absorption chamber a silicon tube guides the ozone to the orifice of the MS. The jump in O$_3$ signal at 48 amu/z is used at MS signal. The O$_2$ concentration of the Ar flow and the power of the plasma jet is adjusted to change the O$_3$ production of the plasma jet.

![Figure 3.24: Signal of the MS during an absorption measurement for 3 different concentrations of O$_3$. The O$_3$ densities are given in units $10^{20} \text{m}^{-3}$.](image)

Figure 3.24: Signal of the MS during an absorption measurement for 3 different concentrations of O$_3$. The O$_3$ densities are given in units $10^{20} \text{m}^{-3}$. 
The results of the calibration measurement are presented in figure 3.26. A linear relation between the measured O$_3$ signal and the calculated O$_3$ concentration from the absorption measurement is found. The error bar of 2·10$^{20}$ m$^{-3}$ indicates the reading error of the MS and spectrometer signal.

The relation between the number density O$_3$ ($n_{O_3}$) and the integrated MS signal ($S_{ms}$) is approximated by the linear fit through the origin in figure 3.26.

$$ n_{O_3} = 4.39 \cdot 10^{17} \cdot S_{ms} $$

The error in the absolute O$_3$ density is estimated by the error in the linear fit coefficient of $1.23 \cdot 10^{16}$ m$^{-3}$/c/s or an error of 2.3%. The calibration method has a detection limit, estimated from fluctuation in the signal of 1 ppm ($2.5 \cdot 10^{19}$ m$^{-3}$).
3.1.4 Ions

To extract the ions and measure ions, a positive or negative voltage is applied to the first and second skimmer. The first skimmer is biased by a voltage of ±10 V and the second skimmer by a voltage of ±40 V. The electric field will accelerate the ions through the orifice in the MS. An example of positive ions is shown in figure 3.27. Figure 3.28 shows an example of negative ion scan.

Figure 3.27: Example of a mass scan with a negative voltage on the skimmer with a continuous 1.5 slm argon plasma power of 1.1 W, at a distance of 5 mm between electrode and orifice. The MS is equipped with the suction ring with a 30 slm flow rate and no well plate.

Figure 3.28: Example of a mass scan with a positive voltage on the skimmer with a 1.5 slm argon plasma power of 4 W and 20% DC, at a distance of 5 mm between electrode and orifice. The MS is equipped with the suction ring with a 30 slm flow rate and no well plate.

3.2 H₂O₂ in the liquid phase

The setup used for the H₂O₂ measurement in the liquid phase is sketched in figure 3.29. First 100 µl saline solution is treated in a 96-well plate. After treatment the plasma activated liquid is mixed with 900 µl ammonium metavanadate in a cuvette in a cuvette holder. The absorption
coefficient used to determine the H$_2$O$_2$ concentration is 283 L/(mol·cm) \[30\]. This absorption is used with the Lambert beer law to calculate the H$_2$O$_2$ concentration. The obtained H$_2$O$_2$ concentration is corrected for the ten fold dilution and the evaporation of the liquid during the treatment.

![Diagram](figure3.29.png)

Figure 3.29: After treating the saline solution with the plasma jet, the plasma activated solution is pipetted into the ammonium metavanadate. The metavanadate changes colour if H$_2$O$_2$ is present in the plasma activated water. An absorption measurement determines the H$_2$O$_2$ concentration.

### 3.3 UV irradiance

The radiation of the plasma jet is measured with an optical fiber spectrometer. The optical fiber is connected to a lens which is coaxial aligned to the flow of the plasma jet. Between the lens and the plasma jet a metal orifice with a diameter of 100 μm is placed. The spectrometer is calibrated with an absolutely calibrated deuterium lamp with a known spectral irradiance in units (W/m$^2$/nm) at a fixed distance of 13 cm from the lamp.

![Diagram](figure3.30.png)

Figure 3.30: Overview of the UV measurement performed on a plasma jet. For calibration the plasma jet is replaced with an absolutely calibrated deuterium light source.

The calibration lamp is directed at the orifice and the spectrum is used to calibrate the radiation collected from the plasma jet. For the calibration the plasma jet is replaced with the calibration lamp. An example of a calibrated emission spectrum is shown in figure 3.31.
3.4 Bacteria inactivation

Bacteria (strain PAO1) are routinely cultured on Luria Broth (LB)-agar plate at 37°C. One colony is suspended in 5 ml LB and cultured at 37°C, 167 rpm in a Heidolph incubator. After approximately 6 or 18 hours, the bacterial concentration is estimated with a Thermo scientific Helios Epsilon by measuring the light absorption of bacteria. A value of 1A is calibrated to correspond to a value of $2.3 \cdot 10^9$ CFU/ml. CFU/ml is the amount of Colony Forming Units per ml. The bacteria solution is then spun around at 10,000 rpm for 2 minutes. The bacteria form a pellet at the bottom so the LB medium is easily replaced with a 0.9% NaCl solution. The osmotic value of physiological saline solution is similar to that in cells or bacteria. The saline solution has an initial pH of 5.64 to 5.91. The solution is not buffered because treatments of buffered solutions were not effective until the buffer was depleted. Bacteria were diluted to $10^7$ CFU/ml for the plasma treatment. This was checked by plating dilutions on LB agar. 100 µl of the bacterial suspension is pipetted in a micro titre plate and treated with an atmospheric plasma jet.
Figure 3.32: After plasma treatment the treated bacteria in the liquid is diluted. The diluted solutions are pipetted on 2 agar plates and after a day in conditioned chamber at 37°C and 5 ppm CO₂, the bacteria colonies are counted.

After the plasma treatment, the treated bacterial suspension is added to 900 l phosphate buffered NaCl solution. A buffered solution is used to neutralise the pH and bactericidal effects after the plasma treatment. Bacterial dilutions of up to $10^5$ times are plated on two agar plates (100 µl) for more reliable measurements. Plates are incubated at 37°C, the CFU are counted the next day. The antibacterial effect of the treatment was quantified by the log reduction, $\log(N_t/N_c)$, where $N_t$ is the number of viable cells after treatment and $N_c$ the number of viable cells in the control samples. Bacteria counts between 30 and 300 CFU on a plate are used a viable counts.
4 Results and discussion

This chapter presents the results of the air concentrations, NO densities, O\textsubscript{3} densities and ion fluxes measured in the argon jet effluent with the mass spectrometer. In addition the results of H\textsubscript{2}O\textsubscript{2} concentrations in the plasma treated saline solution, UVA and UVB irradiance and the results of plasma treatments on bacteria are presented. The diagnostics which have been used and the calibrations and experimental procedures are described in detail in chapter 2 and 3.

4.1 Air concentration

The calibration described in chapter 3 has been used to investigate the effect of the mixing of surrounding air. The effect of the presence of a well plate, the flow rate of the suction ring, and effect of the position of the jet on are presented in this section without the presence of plasma. The influence of the presence of a plasma on the air admixture in the Ar effluent is also discussed.

The effect of the position of the plasma jet in the horizontal x-y plane is investigated by measuring the percentage of air in the Ar flow for different positions in this horizontal plane.

Figure 4.1: Air concentration as function of the position of the plasma jet in the horizontal plane, in an 1.5 slm Ar flow at 13 mm distance between electrode and orifice with a well plate and a 30 slm flow through the suction ring. The minimum air concentration is equal to 5% at position 0,0 which corresponds to the case when the axis of symmetry of the jet is aligned with the sample orifice of the mass spectrometer. The measurement point are indicated with the black dots.
Figure 4.2: Air concentration as function of the position of the plasma jet in the horizontal plane, in a 1.5 slm Ar flow at 13 mm distance without a well plate and a 30 slm flow through the suction ring. The minimum air concentration is equal to 27% at position 0,0 which corresponds to the case when the axis of symmetry of the jet is aligned with the sample orifice of the mass spectrometer.

A radial cross section of the measured air concentrations with and without the well plate is shown in figure 4.1 and figure 4.2 respectively. If the position of the plasma jet not axially aligned to the center of the orifice, the percentage of air increases as more surround air mixes into the argon flow. Figure 4.1 and 4.2 also indicates that the on-axis air entrainment of the plasma with a well plate is lower as without a well plate. To get an idea of the error in the mixing of the surrounding air due to the positioning of the plasma jet, the jet has been aligned 3 times by minimalising the N\(_2\) (air) signal at 28 amu/z. The minimum amount of air entrainment is assumed to correspond to the center of the plasma jet. The variation of calculated percentage of air during the alignment (3 times) is in the order of 1%.

The effect of the axial distance to the well plate on the mixing of the surround air is shown in figure 4.3.
Close to the orifice no entrainment of surrounding air is observed. At a distance of 30 mm between the electrode and the orifice of the MS, the air entrainment increase upto an air concentration of 50-60%. The increase of air entrainment percentage of air in case of the well plate is higher than without a well plate. The lower air concentration with the well plate can be explained by the local enrichment of Ar due to the flow pattern as schematically sketched in figure 4.4. The change in trend in the admixing of surrounding air into the Ar flow at 25 mm can be explained with the local Ar enrichment. If the flow nozzle is further away, the beam flow will widen and the significant amount of air will be already mixed into the Ar flow before it reaches the well plate.
Because Ar will fill the area around the sampling orifice of the mass spectrometer, the effect of the suction ring is determined by changing the flow through the suction ring and measure the percentage of air with and without a well plate. The plasma treatment on bacteria are performed in a flow cabin with a downward flow of 0.31 m/s. To get a similar downward flow a pumping volume of 90 slm is necessary. The rota meter that has been used to measure the flow through the suction ring, can measure flows upto 30 slm of air.

Figure 4.5 shows percentage of surrounding air as function of pumping volume. If the suction
is not used (0 slm), the cup surrounding the orifice is filled with argon, as argon is heavier than air. As the pumping volume is increased, more Ar is pumped from the cup and more ambient air is mixed into the argon flow. Ideal would be to use a pumping volume of 90 slm to mimic the flow conditions while treating the bacteria with plasma. The value of surrounding air concentration approaches a constant value of approximately 30% with a well plate at the maximum range of the rotameter. The value 'max.' in the pumping volume is air concentration obtained with the pump directly attached to the suction ring and no rotameter in between. The pumping volume is assumed to be much higher as 30 slm. The air concentration does not change much in comparison with a pumping volume of 30 slm. Therefore in this thesis a pumping volume of 30 slm is used to pump the Ar from the cup surrounding the orifice and to mimic the flow as used during the treatment of bacteria with the plasma jet.

Van Gessel et al measured spatially resolved air densities in the effluent with plasma on and off using Raman scattering. The presence of a plasma increases the mixing of surrounding air. The same plasma settings are used to measure the N\textsubscript{2} signal as function of the axial distance as shown in figure 4.6.

As observed by van Gessel et al the air density increases in the presence of a plasma. Figure 4.7 shows the N\textsubscript{2} signal in the presence of two different powers at a fixed distance. At 2.6 W an 20 mm a decrease in signal has been observed and at 0.4 W and 11 mm the same decrease in N\textsubscript{2} has been observed as with a 0.4 W plasma at 15 mm. It should be noted that this N\textsubscript{2} signals are obtained with the use of a well plate. The well plate significantly increases the complexity of the flow pattern. The effect of the plasma on the flow pattern has not been no further research has been done on the effect of the presence of a plasma on the flow pattern.
Figure 4.7: \( \text{N}_2 \) signal for plasma on and off. The distance between electrode and orifice is 15 mm and the plasma powers are without duty cycle. The MS is equipped with a well plate and a suction ring with a flow rate of 1.5 slm.

The flow is locally enriched with argon if the plasma jet operates above a well plate as observed in figure 4.3. The flow changes in the presence of a plasma results in a lower air signal. The surroundings the orifice is filled with argon due to geometry of the mass spectrometer. In all the measurements of this work. A suction ring with a 30 slm flow is used to ensure that the flow condition of the plasma jet during bio treatments is approached and a steady state value of the argon/air mixing is obtained which does not depend significant on small variations in the flow.
4.2 NO

The NO densities reported in this section are obtained for the condition that the plasma jet axis of symmetry is aligned to the orifice of the MS. The method presented in chapter 3.1.2 has been used to determine the absolute NO density. The effects of the axial distance, power, duty cycle, amount of premixing air and O\textsubscript{2} to the air jet, well plate, flow rate of the suction ring on the NO concentration are presented in this section. In addition a comparison between the NO densities measured with the MS and NO densities measured by the independent technique of laser induced fluorescence (LIF) to validate the measurement is shown.

The dependence of the axial distance of the NO concentration at two different continuous plasma power is shown in figure 4.8. The NO density does not change with increasing distance at a continuous power of 2.6 and 3.6 W in the range 14-24 mm. The estimated plasma lengths are 6 and 8 mm for respectively 2.6 and 3.6 W.

![Figure 4.8: NO density as function of the distance for 2.6 and 3.6 W continuous plasma power and a 1.5 slm argon flow. The MS is equipped with the well plate and the suction ring with a flow rate of 30 slm](image)

In figure 4.9 the NO density as function of the plasma dissipated power is shown. Increasing the power increased the NO production. Gessel \textit{et al} \cite{38} reports

\begin{equation}
N + O_2 \rightarrow NO + O \tag{14}
\end{equation}

\begin{equation}
N + OH \rightarrow NO + H \tag{15}
\end{equation}

as the main production reactions of NO in cold atmospheric plasma jets. Increasing the power increases the production of N and OH and via the main production reactions 14 and 15 more NO is produced. Increasing the power also increases the temperature of the plasma. The reaction rates of equation 14 and 15 depend on the temperature \cite{39}. An increase in gas temperature also increases the NO production rate.
Figure 4.9: NO density as function of continuous plasma dissipated power and a 1.5 slm Ar flow at a distance of 16 mm between electrode and orifice. The MS is equipped with the well plate and the suction ring with a flow rate of 30 slm.

The effect of premixing air into the Ar flow on the NO density is shown in figure 4.10. A NO density of approximately $7 \cdot 10^{19} \text{ m}^{-3}$ is reached if 4% air is premixed to the Ar flow. Adding air to the flow shortens the visible plasma length as the plasma is quenched in the effluent by the air.

Figure 4.10: NO density for different admixture of air into the 1.5 slm Ar plasma with a 2.6 W continuous power. The distance between electrode orifice is kept at 17 mm. MS is equipped with well plate and suction ring with a 30 slm flow rate.

When O$_2$ is premixed into the argon flow instead of air, the NO concentration is below the NO detection limit of 1 ppm. In this case, a large amount of O and O$_3$ will be produced (see section 4.3). As O will be abundantly produced and the reaction $O + N_2 \rightarrow NO + N$ is too slow at the low temperatures of the plasma jet, NO will not be formed significantly. In addition any formed NO will be quickly oxidised [38] by O or O$_3$:

$$NO + O + M \rightarrow NO_2 + M$$ (16)
\[ \text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2. \quad (17) \]

The effect of the duty cycle on the measured NO density is shown in figure 4.11. The NO density clearly increases with increasing plasma power. A small increase with reducing duty cycle at constant averaged power is found. This could be explained by the fact that the instantaneous plasma power increases and as such also the precursors of NO formation i.e. the atomic N density is expected to increase.

![Figure 4.11: NO density as function of power and duty cycle with a 1.5 slm argon flow, the MS is equipped with the suction ring and well plate.](image)

The measured NO density as function of distance measured with LIF and with the MS are presented in figure 4.12. The decrease in NO density in the effluent can be explained via reaction of NO with the plasma produced O\(_3\) (see equation 17) or the reaction of NO with the plasma produced OH

\[ \text{NO} + \text{OH} + \text{M} \rightarrow \text{HNO}_2 + \text{M} \quad (18) \]

or the reaction of NO with HO\(_2\):

\[ \text{NO} + \text{HO}_2 \rightarrow \text{NO}_2 + \text{OH}. \quad (19) \]

The NO\(_2\) and HNO\(_2\) can convert further to species like HNO\(_3\) and N\(_2\)O\(_5\).
Figure 4.12: Comparison of the NO density measured with LIF and MS, as function of distance with a 3.5 W plasma, 20% duty cycle, 1slm Ar, premixed with 2% air, LIF data from [38], MS equipped with the suction ring with 30 slm flow and no well plate. The MS measurements are done on a plate with a downwards flow, the LIF measurements are performed with the jet pointing upwards.

A 10% error in the LIF signal is estimated from errors in the fit of the spectral lines of NO and the error in the measured temperature to calculate the NO density. Plasma produced species like NO$_2$ can create NO in the MS via dissociative ionisation while using impact ionisation. This could explain the higher NO density measured with the MS in comparison with LIF. LIF is more selective as the NO in a single vibrational level in the ground state is being measured. The cracking pattern of NO$_2$ has its main peak (relative intensity = 1) at 30 amu/z (same as NO). The second peak of NO$_2$ can be found at 46 amu/z with a relative intensity of 0.4.

The signal of NO and the expected signal at 30 amu/z from NO$_2$ as a function of distance is presented in 4.13. The signal of NO$_2$ has been measured at 46 amu/z and multiplied with 2.5. The rather constant NO signal (compared to the decrease in figure 4.12 as function of the distance) is probably due to misalignment of the plasma jet but that does not influence the ratio between NO and NO$_2$. 
The NO signal from dissociative ionisation of NO\textsubscript{2} signal is not a major (5 to 10\%) contributor to the total NO signal. Other overlapping cracking patterns of HNO\textsubscript{2}, HNO\textsubscript{5} and H\textsubscript{2}O\textsubscript{5} could still affect the NO signal, however cracking patterns are not available in literature. The effect is expected to be small. The LIF measurements are performed with the plasma jet pointing upwards in free flow as the MS measurements are done with the plasma jet pointed downwards on a plate. These two different configurations lead to different flow patterns and contribute to the deviation in measured NO densities.

For the effect of the surrounding air entrainment on the measured NO density of the plasma jet the suction ring is used. Using the suction ring with a flow of 30 slm will increase the concentration of surrounding air from approximately 10 to 30\%, see section 4.1. Using a 30 slm pump flow decreases the NO concentration as shown in figure 4.14. The surrounding humid air will introduce additional destruction reactions like reaction 18 and 19 and result in lower NO concentrations. Using no suction ring also enhances the accumulation of NO in the surroundings of the orifice.
Day 1 suction ring flow rate 30 slm
Day 2 suction ring flow rate 30 slm
Day 1 no suction ring flow rate

Figure 4.14: NO density as function of distance of at a plasma power of 3.5W 20% duty cycle 1 slm argon with 2% air flow with the suction ring on and off, no well plate.

The effect of the well plate on the NO density is shown in figure 4.15 and 4.16. The well plate seems to have a minor effect on the NO density. A big change in surrounding air concentration with or without the presence of a well plate or not is shown in section 4.1. The increase in air density due to the well plate is only local and downstream of the active plasma region, and does not have the same effect as premixing air into the argon flow or an Ar enrichment of the surrounding air further upstream of the flow.

Figure 4.15: NO density as function of distance with and without the well plate at the sampling orifice of the MS from a 2.6 W continuous argon plasma power, 1.5 slm argon flow 30 slm suction ring flow.
Figure 4.16: NO density as function of distance with and without the well plate at the sampling orifice of the MS from a 3.6 W continuous argon plasma power, 1.5 slm argon flow 30 slm suction ring flow.

The most important results of this section are summarised below:

- The NO density does not depend on the distance at a continuous power of 2.6 and 3.6 W.
- An increase in average plasma dissipated power results in an increase in measured NO density.
- Premixing air and premixing O\textsubscript{2} are respectively increasing and decreasing the NO density.
- Air admixing in the effluent has a much less effect on the NO production compared to the admixture of air in the Ar flow through the jet.
4.3 $O_3$

In this section the dependence of the $O_3$ density as function of the axial distance, plasma dissipated power, duty cycle, amount of premixing air and $O_2$ into the argon flow, are being discussed. The $O_3$ concentrations are obtained on the axis of symmetry of the plasma jet. The measurements are performed with a well plate attached to the sampling orifice and a 30 slm flow rate is used for the suction ring. The (calibration) method presented in chapter 3.1.3 has been used to determine the absolute $O_3$ density.

The effect of the plasma dissipated power and the axial distance on the $O_3$ density is shown in figure 4.17. The $O_3$ concentration increases with increasing distance in the far effluent of the plasma jet. At a distance of approximately 20 mm the $O_3$ concentration reaches its maximum. Increasing the power increases the $O_3$ concentration.

Figure 4.17: $O_3$ density as a function of the distance for different powers of a continuous Ar plasma.

The production and destruction rates of $O_3$ of a 6.5 W argon plasma jet have been investigated by Zhang et al [40]. The main production reaction for the formation of $O_3$ is the reaction with $O$:

$$O + O_2 + M \rightarrow O_3 + M.$$  \hspace{1cm} (20)

The $O$ is produced via reaction:

$$O_2 + e \rightarrow 2O + e.$$  \hspace{1cm} (21)

The plasma jet is a rich source of $O$ and it is shown by Zhang et al [40] that the large $O$ density in the core of the plasma jet destructs the $O_3$ produced at this location. That causes an increase in the $O_3$ density with increasing axial distance which coincides with the drop of the $O$ density at the edge of the plasma plume. As the temperature is rather low, the life time of the $O_3$ is rather larger outside the active plasma which leads to a constant $O_3$ density for distances between 20 and 30 mm as shown in figure 4.17.

The effect of the duty cycle on the $O_3$ concentration is shown in figure 4.18. The $O_3$ density increases with decreasing duty cycle and increasing power.
Figure 4.18: \( \text{O}_3 \) density as function of average RF power and duty cycle with the plasma tip located at the edge of the well plate, plasma tip 8 mm from orifice. Using a 1.5 slm argon flow. The black points indicate the measuring points.

With increasing power the \( \text{O} \) density will increase and as such the production in the far effluent of \( \text{O}_3 \) is also expected to increase. The reduction of the duty cycle leads to an increase in instantaneous plasma power and thus temporally higher \( \text{O} \) densities. The smaller the duty cycle the higher the increase of the \( \text{O} \) density. In addition the plasma off time, allows that all \( \text{O} \) is recombining in \( \text{O}_2 \) and \( \text{O}_3 \) via \(^{[40]}\)

\[
\text{O} + \text{O} + \text{M} \rightarrow \text{O}_2 + \text{M} \tag{22}
\]

and

\[
\text{O} + \text{O}_2 + \text{M} \rightarrow \text{O}_3 + \text{M} \tag{23}
\]

This will also increase the \( \text{O}_3 \) production compared to a continuous plasma.

To enhance the \( \text{O}_3 \) production, air is premixed in the Ar feed gas. The effect of premixing of air on the \( \text{O}_3 \) production is shown in figure 4.19. A larger admixture of air results in a higher \( \text{O}_3 \) production. The \( \text{O}_2 \) from the air enhances the production of \( \text{O} \) in the plasma active region together with the presence of \( \text{O}_2 \) the \( \text{O}_3 \) production is increased. Particular in the case of the 2% air admixture case, a maximum \( \text{O}_3 \) concentration as a function of the axial distance is found (4.19). The important \( \text{O}_3 \) destruction reaction in the far effluent is according to \(^{[40]}\) the reaction of \( \text{O}_3 \) via reactions with \( \text{O}_2 \):

\[
\text{O}_2 + \text{O}_3 \rightarrow \text{O}_2 + \text{O}_2 + \text{O} \tag{24}
\]
Figure 4.19: O$_3$ density as a function of the distance for different air admixtures with a RF plasma power of 1W, 20% duty cycle, 1.5 slm Ar flow rate. The measurements are performed with a well plate attached to the sample orifice of the mass spectrometer and a suction ring with a flow rate of 30 slm.

The effect of premixing pure O$_2$ is shown in figure 4.20. Premixing O$_2$ into the Ar flow increases the O$_2$ density more as premixing air as more O$_2$ is available for the production of O and O$_3$.

Figure 4.20: O$_3$ density as a function of the distance for different O$_2$ admixtures with a plasma power of 1W and 20% DC

In conclusion, the most important results of this section are summarised:

- The O$_3$ density reaches a maximum value in the far effluent of the plasma.
- Increasing the plasma power and premixing air/O$_2$ increases the O$_3$ production.
- The O$_3$ increases at the same average power dissipation when the duty cycle is decreased.
4.4 Ions

In this section, the ion fluxes as a function of the axial distance are presented. For the ion measurements, a 30 slm flow rate through the suction ring is used but no well plate is attached to the orifice. An example of a positive and negative ion mass scan is shown in section 3.1.4. The signal of several positive ions clusters as function of the distance is shown in figure 4.21. With increasing distance the formation of water ion clusters is enhanced and the total ion flux reduces due to ionic recombination processes.

![Figure 4.21](image1.png)

Figure 4.21: Integrated signal of the positive ion peaks as function of the axial distance with a continuous RF plasma power of 1.1W with a 1.5 slm Ar flow. The MS is equipped with the suction ring (30 slm) but without the well plate. Plasma length is approximately 3 mm.

The integrated positive ion signal as function of the axial distance is shown in figure 4.22.

![Figure 4.22](image2.png)

Figure 4.22: Total positive ion counts as function of the distance at 1.1W continuous plasma power, 1.5 slm Ar flow. MS equipped with suction ring with a flow rate of 30 slm, no well plate has been used. The plasma length is approximately 3 mm.

When taking into account dissociative electron-ion recombination which has a typical rate ($r_d$)
of $10^{-13}$ m$^3$ s$^{-1}$ [41], a flow velocity of $\sim 10$ m/s (1.5 slm, φ= 1.65 mm) and distance of roughly 10 mm the decrease in ion density ($n$) due to electron-ion recombination can be calculated (assuming quasi-neutrality $n_i=n_e$) via [42]:

$$
\frac{n_t}{n_0} = \frac{1}{1 + n_0 \cdot t \cdot r_d} \quad (25)
$$

After 10 mm the expected ion density is calculated to decrease 3 orders of magnitude. The observed decrease in ion signal is estimated to be in the same order of magnitude. The calculation is made assuming a constant flow velocity along the stream line. A flow model [43] of the flow pattern of a plasma jet indicate a decreasing in velocity along the streamline. This decrease in velocity will decrease the ion flux to the liquid surface even further. The formation of ion water clusters can also have an effect on the lifetime of ions.

Gessel et al [44] measured the electron density with Thomson scattering at 0.5 mm axial position, radially in the center. The electron density of a similar jet as used in this thesis with an 1 slm argon flow, ring electrode, 3.5 W power has been estimated to be in the order of $10^{19}$ m$^{-3}$. The positive ions signal decrease approximately 3 orders of magnitude over a distance of 10 mm. Assuming a distance of 10 mm between electrode and the surface of the liquid, the estimated positive ion density is in the order of $10^{16}$ m$^{-3}$.

Negative ions have been measured on a 4 W plasma with 20% duty cycle and a plasma length of approximately 9 mm as it the negative ion density was below the detection limit in the case of 1.1 W. The MS signal as function of the distance is shown in figure 4.23. Just as the positive ion flux an exponentially decay with respect to the distance has been observed.

![Integrated negative ion signal vs. Distance electrode orifice](image)

Figure 4.23: Total positive ion counts as function of the distance at 4W plasma power with 20 % duty cycle, 1.5 slm Ar flow. MS equipped with suction ring with a flow rate of 30 slm, no well plate has been used. The plasma length is approximately 9 mm.

### 4.5 H$_2$O$_2$

The H$_2$O$_2$ concentrations presented in this section are obtained using the method described in section 3.2. The H$_2$O$_2$ concentration is measured as a function of treatment time and axial distance from the jet.

The results of the H$_2$O$_2$ measurements are shown in figure 4.24. A decrease in H$_2$O$_2$ concentration has been observed with increasing distance. H$_2$O$_2$ can be created both in the gas and
liquid phase through OH as follows[13];

\[
\text{OH} + \text{OH} + \text{M} \rightarrow \text{H}_2\text{O} + \text{H}_2\text{O}_2 + \text{M.}
\]  

(26)

An explanation for the decreasing \( \text{H}_2\text{O}_2 \) concentration with increasing distance is the decreasing OH density. Increasing the treatment time increases the total OH flux to the surface of the liquid and therefore the \( \text{H}_2\text{O}_2 \) concentration in the liquid.

![Figure 4.24: \( \text{H}_2\text{O}_2 \) concentration as function of treatment time and axial distance of the jet to the liquid. The values are corrected for evaporation during the treatment and performed with a 1.1W continuous RF power, and a flow rate of Ar of 1.5 slm.]

**4.6 UV irradiance**

The irriadiance measurements presented in this section are measured using the method described in section 3.3. The spectrometer is calibrated and the UV irradiance is measured as function of the distance. The UVB (280-315 nm) and UVA (315-400nm) irradiance as function of the distance is shown in figure 4.25. The UVA and UVB irradiancie both decrease as a function of the axial distance.
4.7 Bacteria inactivation

The influence of the treatment distance and the treatment time on bacteria inactivation is being presented in this section. The results of bacteria inactivation due to a plasma jet treatment are obtained using the method described in section 3.4.

A continuous argon plasma dissipated power of 1.12±0.05 W is used to inactive bacteria with a plasma jet. An initial bacteria concentration of 2.1 to $6\cdot10^6$ CFU/ml has been used in the treated solution. The visible length of the plasma effluent is approximately 3 mm. The distance from the electrode to the surface of the treated liquid is used as the treatment distance.

<table>
<thead>
<tr>
<th>Distance</th>
<th>Log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.22</td>
</tr>
<tr>
<td>15</td>
<td>0.63</td>
</tr>
<tr>
<td>14</td>
<td>3.61</td>
</tr>
<tr>
<td>13</td>
<td>6.85</td>
</tr>
<tr>
<td>12</td>
<td>6.85</td>
</tr>
<tr>
<td>11</td>
<td>6.85</td>
</tr>
</tbody>
</table>

Table 2: Example of a plasma treatment on bacteria for different distances at a plasma power of 1.1W and a treatment time of 75 sec. The bacteria are in a stationary grow phase and the bacteria concentration in the treated liquid is $7\cdot10^6$ CFU/ml.

An example of a plasma treatment as function of the distance is shown in table 2. During a distance scan a sharp transition is observed from a log reduction below 1 and a log reduction in the order of 6-7 corresponding to no CFU on the agar plates at all. If the plasma is at a distance which is too far from the liquid the bacteria survive the treatment with the plasma jet. Decreasing the distance increases the bactericidal component delivered to liquid and bacteria are inactivated. The transition in bacteria inactivation is not a smooth but a sharp transition. This indicates that if the bactericidal component induced by the plasma in the liquid reaches a certain threshold the bacteria in the liquid are inactivated. At 14 mm the log reduction is between 1 and 6. This distance between a complete bacteria kill and a log reduction below 1 is indicated as the 'transition distance'.

The initial bacteria concentration in the treated liquid has been reached after a multiplication
time of approximately 4 hours (in the morning) or 16 hours (overnight). Bacteria that have been multiplying for 4 hours are assumed to be in a more logarithmic growth phase. Bacteria that have been multiplying overnight are in a more stationary growth phase. The bacteria that have been multiplying for 16 hours are more saturated, indicated by black spots in the bacteria suspension. If the bacteria are in a saturated grow phase, the bacteria seems to be less affected to the plasma treatment as shown in in figure 4.26. The growth phase of the bacteria will change during a plasma treatment, reducing the reproducibility of the measurements.

![Graph showing transition distance vs. treatment time for logarithmic and stationary growth phases.](image)

**Figure 4.26:** Transition distance with bacteria in a stationary and more logarithmic grow phase with a RF 1.1 W continuous plasma power, and a 1.5 slm Ar flow.

The transition distance as a function of the initial bacteria concentration in the treated liquid is shown in figure 4.27.

![Graph showing transition distance vs. initial bacteria concentration](image)

**Figure 4.27:** Transition distance as function bacteria in the liquid before a plasma treatment at 1.1W 1.5 slm Ar

The transitions distance does not seem to be influence by the initial bacteria concentration. Figure 4.28 shows the transition distance of 3 measurement series performed on several days.
with bacteria in approximately the same growth phase and the same plasma settings. The linear relation indicates that the transition distance shifts 4 mm up if the treatment time changes from 60 to 105 seconds. If the dose of the bactericidal component is assumed to be linear with the treatment time the bactericidal component of the plasma increases a factor 2 if the distance is changed from 16 to 12 mm.

![Graph showing transition distance as function of distance and treatment time](image)

**Figure 4.28:** Transition distance as function of distance and treatment time with bacteria in approximately the same growth phase and an initial bacteria concentration in the treated liquid of 2.1 to 6.7 \( \cdot \) 10^6 CFU/ml.

The results shown in figure 4.28, show a change in transition distance up to 3 mm for the same measurement performed on different days. The resolution of 1 mm in the changing distance will be contributing to this variation. The growth phase of the bacteria can change during a treatment serie. This will also contribute to the reproducibility of the measurements.
5 Mechanisms of bacteria inactivation

This chapter an analysis of the chemistry responsible for bacteria inactivation is given. The analysis of the direct plasma induced chemistry is based on correlations found between the species densities and UV irradiance as reported in chapter 4. The direct plasma induced effects are reported in section 5.1 while the secondary induced liquid phase chemistry is analysed in section 5.2.

5.1 Direct plasma induced chemistry

The mechanisms of bacteria inactivation are analysed by comparing the distance dependence of the bactericidal effect of the plasma jet, found in 4.7, with the distance dependence behaviour of the possible contributing bactericidal components of the plasma or induced by the plasma: NO and O$_3$ density, ion and UV flux, pH and H$_2$O$_2$ density in the liquid phase. The linear trend in transition distance as discussed in section 4.7 indicates that the bactericidal component of the plasma jet is less dominant if the distance is increased. If the bactericidal component is proportional to the treatment time, the component responsible for the bactericidal effect should decrease with a factor of approximately 2 if the distance is enlarged from 11 to 17 mm.

5.1.1 NO and O$_3$

The NO density measured with the MS at a 1.1 W continuous power Argon plasma is close to the detection limit of the MS. NO measurements at 2.6 W and 3.6 W indicate a close to constant NO density as function of the distance, as shown in section 4.2. Assuming a constant NO density as function of the distance the NO density at 1.1 W is estimated to be a constant value of 3.29·10$^{19}$ m$^{-3}$ ($\approx$1.3 ppm). The NO density in the gas phase does not depend on the distance at low continuous plasma power. Therefore the NO density is not responsible for the distance dependent bactericidal effect of the plasma jet. The minimum estimated lethal NO dose in the gas phase is reported in literature to be in the order of 40-200 ppm [45, 46]. Although these NO densities are obtained using different conditions (longer exposure time, buffered solution) it is unlikely that the NO in gas phase is directly responsible for the inactivation of bacteria. The formation of reactive nitrogen species (RNS) due to chemical reactions in the liquid phase can however be important in bacteria inactivation [13]. The role of RNS in the inactivation of bacteria will be discussed in section 5.2.4.

The distance dependent O$_3$ density of a 0.95 W continuous argon plasma with no duty cycle is shown in figure 4.17. The O$_3$ density increases with increasing distance. The maximum estimated O$_3$ concentration in the liquid is estimated with a 1D chemical model by Gils et al [13]. With an estimated gas phase O$_3$ density of 7·10$^{18}$ m$^{-3}$, the maximum predicted O$_3$ concentration in the liquid is 0.0006 mM. The maximum measured O$_3$ at 0.95 W continuous power argon plasma is approximately (2·10$^{19}$ m$^{-3}$) at a distance of 16 mm (maximum distance used in plasma treatment of bacteria). The measured O$_3$ density is a factor 3 higher as the estimated value used in the liquid chemistry model. If a 3 times higher O$_3$ density in the gas phase induces a 3 times higher O$_3$ concentration in the liquid phase, the concentration O$_3$ in the liquid phase is 0.0018 mM. The minimum inhibitory concentration (MIC), i.e. the lowest concentration needed to inhibit bacteria growth, of O$_3$ is reported in literature to be [13] 0.0046 - 0.1 mM O$_3$ in the liquid. The estimated O$_3$ concentration is still below the estimated MIC value.

Henry’s law can predict the ozone concentration in the liquid ($c_{\text{liquid}}$) with a known ozone pressure ($P_{O_3}$) in the gas phase.

\[
c_{\text{liquid}} = P_{O_3} \cdot k_H
\]  

(27)
The Henry’s law constant $k_H$ of $O_3$ is in the order of $0.01 \text{ m·kg}^{-1}\text{·bar}$. Due this low solubility of ozone the concentration of $O_3$ in the liquid is even lower. Due to the low $O_3$ density and increasing $O_3$ density with increasing distance while the bactericidal activity decreases with increasing distance, $O_3$ only is not a key bactericidal component of the plasma jet. The possible inactivation of bacteria due to secondary chemical reactions with $O_3$ is being discussed in section 5.2.2.

5.1.2 Ions

The positive ion signal decays exponentially as a function of the distance as shown in section 4.4. The actual density of ions reaching the liquid surface at 10 mm distance is calculated to be in the order of $10^{16}\text{m}^3$. Three orders of magnitude lower than the measured reactive species such as $O_3$, NO. As a result neutral induced liquid chemistry will be dominant over ion induced chemistry.

5.1.3 UV

The UVA and UVB irradiance as function of the distance is shown in section 4.6. As discussed in a previous published paper of van Gils et al [13] a UVC and UVB irradiance in the order of $10^{-6}\text{W·m}^{-3}$ is no sufficient to induce a direct bactericidal effect. An absolutely calibrated UV spectrum from 200 to 400 nm of a $1.4\pm0.1\text{ W}$ argon plasma from the same plasma setup as used in this work is presented in the paper of van Gils et al [13]. The spectral irradiance of the UVC spectrum is even lower as the UVA an UVB spectrum and therefore not likely to have a bactericidal effect. It should be noted that the second continuum of the argon excimer ($\text{Ar}_2^*$) [47] at 126 nm is found to be the dominant cause of VUV radiation in a (similair) 20W, 20 slm argon jet. Lange and von Woedtke [48] measured an enhanced bactericidal effect of a plasma treatment due to VUV radiation. VUV with a wavelength smaller than 180 nm is completely absorbed by liquid layers which are thicker than 100 $\mu\text{m}$ [13]. Therefore any effect of VUV on bacteria is due to the possible production of reactive species such as OH at the liquid surface.

Although the UV radiation is too weak to have a directly bactericidal effect, the decay of UVA and UVB is in the same order as expected for the bacterial component. It cannot be a priori excluded that UVA and UVB induced secondary liquid chemistry could be involved in the inactivation of bacteria. The effect of UVA and UVB flux on the liquid chemistry is analysed in section 5.2.1.
5.1.4 pH

Figure 5.1: pH of the liquid as function of the treatment distance 1.1 W continuous 1.5 slm Ar plasma power with a treatment time of 20 min. of 2 ml saline solution.

The saline solution used for the bacteria treatment is a non buffered solution. Therefore acidification will occur due to a plasma treatment as shown in figure 5.1. The pH meter requires a minimum amount of 2 ml solution to operate and therefore the pH measurements are performed in 2 ml saline solution. The treatment time has been scaled with the volume of the liquid. A 20 min. plasma treatment of 2 ml saline solution corresponds to a treatment time of 1 min. in 100 µl. Ikawa et al [49] calculated the pH from the NO$_2^-$ and NO$_3^-$ concentration. The acidification of the liquid due to a plasma treatment is expected to be caused by the reactive nitrogen species (RNS) produced by the plasma jet. The NO$_x^-$ concentration can therefore be estimated if the pH is known.

$$\text{pH} = -\log([\text{H}^+]) \approx -\log([\text{NO}_x^-])$$

(28)

The pH of the liquid decreases if the treatment distance is decreased. An important production reaction of H$^+$ is the reaction with NO and OH [13]

$$\text{OH} + \text{NO} \rightarrow \text{NO}_2^- + \text{H}^+.$$

(29)

The decreasing pH with increase distance can not be explained via a change in NO density as NO in the gas phase is measured to be constant with increasing distance. As being discussed in section 5.1.5, the OH production in the liquid is expected to decrease with increasing distance. If OH is the limited production factor in equation 29, the pH decreases with increasing distance if the OH production is decreased.

Bacteria in LB (no plasma treatment) with an artificial low pH of 4, does not lead to bacteria inactivation. The pH alone is thus not responsible for the bactericidal effect of a plasma treatment. However the pH determines the equilibria of several various possible bactericidal species like HNO$_2$ and the MIC value of for example H$_2$O$_2$. These two effect are discussed in section 5.2.3 and section 5.2.4.
5.1.5 \( \text{H}_2\text{O}_2 \)

The \( \text{H}_2\text{O}_2 \) measurements in the liquid phase are shown in section 5.1.5. The \( \text{H}_2\text{O}_2 \) is produced in the liquid in three ways that can explain the distance dependent \( \text{H}_2\text{O}_2 \) production in the liquid. (1) \( \text{H}_2\text{O}_2 \) can be produced in the gas phase and absorbed in the liquid. If the gas phase \( \text{H}_2\text{O}_2 \) density decreases with increasing the distance, the \( \text{H}_2\text{O}_2 \) production will also decrease as a function of the distance. (2) As noted in section 5.1.3 VUV can not be neglected in the liquid chemistry. A significant flux of VUV on water can lead to significant dissociation of \( \text{H}_2\text{O} \) producing \( \text{OH} \) at the liquid surface. A quantum yield of 1 at 123.6 nm (corresponding to the \( \text{Ar}^* \) excimer radiation wavelength) is reported [13]. The main production reaction of \( \text{H}_2\text{O}_2 \) in the liquid phase is the reaction with \( \text{OH} \):

\[
\text{OH} + \text{OH} \rightarrow \text{H}_2\text{O}_2. \tag{30}
\]

The distance dependent \( \text{H}_2\text{O}_2 \) could also indicate the distance dependent \( \text{OH} \) production of the \( \text{OH} \) via the decreasing in VUV flux. (3) \( \text{OH} \) is formed in the plasma. The plasma produced \( \text{OH} \) in the gas phase will be absorbed in the liquid to form \( \text{H}_2\text{O}_2 \). The distance dependent behaviour of the \( \text{H}_2\text{O}_2 \) concentration in the liquid phase indicate a decreasing gas phase \( \text{OH} \) flux with increasing distance. R. Mensink \textit{et al.} [50] determined the \( \text{OH} \) density with LIF on a commercial available plasma jet; the kINPen, a similar jet as used in this work. The \( \text{OH} \) concentration decreases a factor 2 if the distance from the nozzle is increase from 2 to 6 mm.

The relative decrease with increasing distance of the \( \text{H}_2\text{O}_2 \) concentration is similar as the bactericidal component is expected to behave. If the threshold of the bactericidal effect of \( \text{H}_2\text{O}_2 \) is for example 2.5 mM the bacteria are inactivated above this concentration. Below this concentration the bacteria stay alive. Using a longer treatment time shifts the distance up at which the critical concentration \( \text{H}_2\text{O}_2 \) is reached. The MIC value of \( \text{H}_2\text{O}_2 \) has been tested by adding \( \text{P. aeruginosa} \) to different \( \text{H}_2\text{O}_2 \) solutions. The \( \text{P. aeruginosa} \) bacteria are incubated in the \( \text{H}_2\text{O}_2 \) solutions overnight at a temperature of 37\(^\circ\)C. The minimal inhibitory concentration (MIC) value of \( \text{H}_2\text{O}_2 \) on PAO1 has been determined by using different \( \text{H}_2\text{O}_2 \) solutions with 5000 CFU in the solution. The \( \text{H}_2\text{O}_2 \) concentration start at 6\% and the \( \text{H}_2\text{O}_2 \) concentrations are diluted in 2 fold. After an overnight incubation at 37\(^\circ\)C growth of the bacteria have been plated on agar and incubated overnight. At a \( \text{H}_2\text{O}_2 \) concentration of 490 mM no bacteria growth has been observed. This is 2 order of magnitude more as the maximum measured \( \text{H}_2\text{O}_2 \) concentration of 6 mM. It should be noted that the pH of the liquid during bacteria treatment is expected to be significantly lower as for the diluted \( \text{H}_2\text{O}_2 \) suspensions. Although the measured \( \text{H}_2\text{O}_2 \) concentration is below the MIC value a synergistic effects with pH and other species could still contribute to secondary chemistry which may lead to bactericidal effects. The \( \text{H}_2\text{O}_2 \) related chemistry will be analysed in section 5.2.3.

None of the components that have been discussed in this section are on their own responsible for the bactericidal effect. The induced secondary chemistry plays as stated before, an important role. Therefore synergistic effects and the influence of secondary liquid chemistry is discussed in the next sections.
5.2 Liquid phase chemistry

As concluded in the previous section, synergistic effects and secondary liquid chemistry can be important in the mechanisms of bacteria inactivation. Therefore the UV, O\textsubscript{3} and H\textsubscript{2}O\textsubscript{2} induced chemistry is analysed in this section.

5.2.1 UV

Liquid chemistry can be induced by the UVA and UVB flux. An UV flux of 2·10\textsuperscript{-5} W/m\textsuperscript{2}, a photon energy at 310 nm of 6.4·10\textsuperscript{-19} J (4eV), treatment time of 1 min, surface of 1 cm\textsuperscript{2} yields the following number of photons reaching the water surface.

\[ n_{\text{photons}} = \frac{2 \cdot 10^{-5} \cdot 1 \cdot 10^{-4} \cdot 60}{6.4 \cdot 10^{-19}} = 1.8 \cdot 10^{11} \]  

(31)

to the liquid surface. Assuming that the UV light is completely absorbed in 100 µl, the density of the produced species can reach a maximum number density of 1.8·10\textsuperscript{15}/L equivalent to a molar concentration of 3·10\textsuperscript{-9} M. At this concentration none of the by Gils et al [13] investigated components are bactericidal. Therefore UV induced chemistry is not likely to be the dominant bactericidal component of the plasma jet under the present conditions. However, the liquid chemistry induced by VUV can play an important role in the inactivation of bacteria, as discussed in section 5.1.3.

5.2.2 O\textsubscript{3}, O\textsubscript{2}\textsuperscript{-} and HO\textsubscript{2}

The amount of plasma produced ozone is not directly bactericidal as shown in section 5.1.1. An increasing O\textsubscript{3} density can decrease the bactericidal component in the liquid. The reaction of the possible bactericidal component O\textsubscript{2}\textsuperscript{-} with O\textsubscript{3}

\[ \text{O}_3 + \text{O}_2^- \rightarrow \text{O}_2 + \text{O}_3^- \]  

(32)

is an important O\textsubscript{2}\textsuperscript{-} (1.6·10\textsuperscript{9} M\textsuperscript{-1}s\textsuperscript{-1}) destruction reaction [13] in the liquid chemistry.

Ikawa et al [49] suggest super oxide (O\textsubscript{2}\textsuperscript{-}) and hydroperoxyl (HO\textsubscript{2}) as bactericidal component in the liquid as the bactericidal effect seems to occur at a pH of 4.8.

\[ \text{HO}_2 \leftrightarrow \text{O}_2^- + \text{H}^+ \quad \text{pK}_a = 4.8 \]  

(33)

Acidification decreases the O\textsubscript{2}\textsuperscript{-} concentration, therefore O\textsubscript{2}\textsuperscript{-} does not play an important role in the inactivation of bacteria. Acidification increase the HO\textsubscript{2} density. The change in HO\textsubscript{2} density with a pH can be estimated with the following formula:

\[ \text{pH} = \text{pK}_a + \frac{[\text{O}_2^-]}{[\text{HO}_2]} \]  

(34)

A pH changes from 3.55 to 3.65 increases the HO\textsubscript{2} concentration by approximately 7%. This is lower as the expected decrease for the bactericidal component. Although acidification increases the HO\textsubscript{2} concentration the maximum expected HO\textsubscript{2} concentration in the liquid is estimated to be 10\textsuperscript{-10}mM [13] for the experimental conditions described in the current work. Due to this low concentration and distance dependent behaviour of OH\textsubscript{2} is it unlikely that HO\textsubscript{2} plays an important role in the inactivation of bacteria.

5.2.3 H\textsubscript{2}O\textsubscript{2}

Van Gils et al [13] reported a MIC value of H\textsubscript{2}O\textsubscript{2} in the range from 0.15 mM to 83 mM depending on the pH of the liquid. Although the MIC values are specific for every strain, it is an indication that MIC value is strongly depended on the pH. The pH, and therefore the MIC
value, will be lower in plasma activated saline. Synergistic effects of the pH and the H_2O_2 can therefore play an important role in the inactivation of bacteria. H_2O_2 [51, 52] has a pK_a value of 11.6 so no pH dependent decomposition of H_2O_2 is expected.

However the presence of H_2O_2 can enable the production of bactericidal species like the formation of peroxynitrous acid from nitrous acid[53]:

\[
\text{H}_2\text{O}_2 + \text{HNO}_2 \rightarrow \text{HNO}_3 + \text{H}_2\text{O}. \tag{35}
\]

HNO_2 production can be enhanced with the presence of H_2O_2 via the balance equation

\[
3\text{HNO}_2 \rightarrow 2\text{NO} \text{HNO}_3 + \text{H}_2\text{O}_2. \tag{36}
\]

The effect of reactive nitrogen species like HNO_2 and HNO_3 is discussed in section 5.2.4.

The distance dependent H_2O_2 concentration depends on the OH concentration. However, OH in the liquid enables (in the presence of NO) also the production of HNO_2 and NO_2^- [13] via;

\[
\text{OH} + \text{NO} \rightarrow \text{NO}_2^- + \text{H}^+ \tag{37}
\]

\[
\text{H}_2\text{O} + 2\text{NO}_2 \rightarrow \text{HNO}_2 + \text{NO}_3^- + \text{H}^+ \tag{38}
\]

\[
\text{H}_2\text{O} + \text{NO}_2 + \text{NO} \rightarrow 2\text{HNO}_2. \tag{39}
\]

OH also enables the formation of ONOOH in the presence of O_3 [13]

\[
\text{OH} + \text{O}_3 \rightarrow \text{O}_2 + \text{HO}_2 \tag{40}
\]

\[
\text{HO}_2 + \text{NO} \rightarrow \text{ONO}^-. \tag{41}
\]

and ONOO^-

\[
\text{H}_2\text{O}_2 + \text{OH} \rightarrow \text{O}_2^- + \text{H}_2\text{O} \tag{42}
\]

\[
\text{O}_2^- + \text{NO} \rightarrow \text{ONOO}^-. \tag{43}
\]

with the availability of NO in the liquid. If the OH density is the limiting factor in the production of HNO_2 and ONOOH, the distance dependent behaviour can be explained by the OH concentration as limiting factor in the production of in the production of HNO_2 and ONOOH. The effect of ONOOH and HNO_2 is discussed in section 5.2.4.

5.2.4 ONOOH/ONOO^- and HNO_2/NO^-

As shown in section 5.1.4 The pH of the liquid decreases from 5.1 (untreated saline solution) to a value of 3.65 at 16 mm and a value of 3.55 at 10 mm treatment distance. This pH corresponds to the equivalent NO_x^- (x=2,3) concentration of 0.2 mM at 16 mm and 0.3 mM at 10 cm.

The equilibrium of ONOOH/ONOO^- is determined by the following balance reaction:

\[
\text{ONOOH} \leftrightarrow \text{ONOO}^- + \text{H}^+ \text{ pK}_a = 6.8. \tag{44}
\]

With formula (34), a pK_a of 6.8 [52], a pH of 3.6 and a NO_2^- concentration of 0.25mM the ONOOH concentration is estimated to be 12.8mM. The following balance reaction,

\[
\text{HNO}_2 \leftrightarrow \text{NO}_2^- + \text{H}^+ \text{ pK}_a = 3.4 \tag{45}
\]

is responsible for the equilibria of HNO_2/NO_2^- . With formula (34), a pK_a of 3.4 [51], a pH of 3.6 and a NO_2^- concentration of 0.25mM the ONOOH concentration is estimated to be 1mM.

Van Gils et al [13] estimated the MIC value of HNO_2/NO_2^- to be 0.2mM at a pH of 3.3 and 30mM at a pH of 5.0 and for ONOOH/ONOO^- a MIC value of 0.25 mM at a pH of 7.0. The
calculated absolute concentrations are in the same order as the MIC values. Therefore RNS are possible bactericidal components.

Treatment of the *P. aeruginosa* in buffered solutions has been less effective in the inactivation of bacteria as with treatments in non-buffered solution. This indicates that pH dependent equilibria are important in the inactivation of bacteria. The equilibrium of ONOOH/ONOO$^-_2$ is determined by equation (44). With a pK$_a$ of 6.8 [52], the ONOOH concentration will be enhanced by the acidification of the plasma treated saline solution. The change in ONOOH concentration due to a change in pH can be estimated with formula 34. The ONOOH concentration increased with 3% if the pH changes from 3.65 at a treatment distance of 16 mm to a pH of 3.55 at a treatment distance of 10 mm. This is lower as the expected (factor two decrease for the bactericidal component.

Equation (45) is responsible for the equilibria of HNO$_2$/NO$^-_2$. With a pK$_a$ of 3.4[51], the HNO$_2$ concentration will be enhanced by the acidification of the plasma treated saline solution. The HNO$_2$ concentration increased with 60% if the pH changes from 3.65 at a treatment distance of 16 mm to a pH of 3.55 at a treatment distance of 10 mm. This is in the range of expected factor 2 decrease of the bactericidal component of the plasma jet. Therefore the HNO$_2$ concentration is expected to play an important role in the inactivation of bacteria.

5.3 Conclusion

- UV radiation(200-400 nm), O$_3$ and ion related chemistry play do not play an important role in the inactivation of bacteria due to a plasma treatment.

- H$_2$O$_2$ and NO alone do not play an important role in the inactivation of bacteria due to a plasma treatment.

- Acidification lowers the MIC value of H$_2$O$_2$. Due to acidification the MIC of H$_2$O$_2$ can be in the same order of magnitude as the measured H$_2$O$_2$ concentration in the liquid phase. The distance depend behaviour of a plasma treatment can be explained via decreasing VUV flux or decreasing OH, H$_2$O$_2$ density in the gas phase. Therefore a synergistic effect of H$_2$O$_2$ is expected to play a role in the in activation of bacteria.

- The pH of the liquid decreases if the treatment distance is decrease. Although the decrease in pH is small (3.55 at 16 mm treatment distance to 3.65 at 10 mm treatment distance), due to a pK$_a$ value of 3.8 for the ratio HNO$_2$/NO$^-_2$, a small change in pH has a significant effect on the HNO$_2$ concentration. The change in NO$^-_2$ concentration is in the same order as the expected bactericidal component of a plasma treatment. The HNO$_2$ concentration is estimated from the pH of the liquid, and is in the same order of magnitude as the reported MIC value of NO$^-_2$. Therefore HNO$_2$ is expected to play an important role in the inactivation of bacteria.
6 Conclusions

This chapter summarises the main conclusions that are drawn from the measurements performed in the course of this work. The MS has been absolutely calibrated for NO and O\textsubscript{3} measurements and used to characterise the NO and O\textsubscript{3} production of a RF atmospheric plasma jet. Further, an analysis of the plasma induced chemistry and correlations found between the measured species densities, UV and the observed distance depend bactericidal effect of a plasma treatment is presented to determine the key chemical pathways of bacteria inactivation in a plasma treated liquid. The results and conclusions can be summarized as follows:

- The MS has been absolutely calibrated for NO with an argon bottle with a known NO concentration (see section 3.1.2). A large time varying background signal for NO has been observed. The difference in time scales between the beam (below the sampling rate of 100 µs) and background signal (≈ 4 hours) has been exploited to detect NO concentrations down to 0.27 ppm (6.8 \times 10^{18} \text{m}^{-3}). Detection of even lower NO densities are expected to be possible but is beyond the scope of this work.

- For the absolute calibration of O\textsubscript{3}, ozone has been produced by a plasma jet. The produced O\textsubscript{3} is guided to the MS and a UV absorption measurement has been performed to measure the O\textsubscript{3} density (see section 3.1.3). Although this calibration has been performed at a O\textsubscript{3} density above 8 ppm (2.1 \times 10^{20} \text{m}^{-3}), the plasma produced O\textsubscript{3} densities of 0.4 ppm 1\times 10^{19} \text{m}^{-3} have been detected.

- The MS is used to obtain NO and O\textsubscript{3} produced surface densities with an accuracy of approximately ±1.25\times10^{19} \text{m}^{-3} (see section 4). The NO density is overestimated due to overlapping cracking patterns of plasma produced species like NO\textsubscript{2}, HNO\textsubscript{3} and N\textsubscript{2}O\textsubscript{5}. The over estimation of the NO density due to NO\textsubscript{2} is approximately 10%. Air seems contribute to the NO signal as well, the error is estimated to be below 0.75 ppm ±1.9\times10^{19} \text{m}^{-3}. The plasma jet produced NO densities obtained with the MS are compared with LIF measurements and are in good agreement (less than a factor three) with each other.

- An increase in average plasma dissipated power results in an increase of the measured NO density (see section 4.2). Premixing air is increasing the NO density, while premixing O\textsubscript{2} is decreasing it, as shown in section 4.3.

- As shown in 4.1, the geometry of the well plates where the bacteria treatments are performed has a significant effect on the surface air concentration in the Ar effluent of the jet. Differences as large as 30% are found. Nonetheless, the effect on the NO densities is much smaller than in the case where O\textsubscript{2} and air are added to the Ar flow through the jet.

- The O\textsubscript{3} density reaches a maximum value in the far effluent of the plasma. Increasing the plasma power, decreasing the duty cycle and premixing air/O\textsubscript{2} increases the O\textsubscript{3} production (see section 4.3).

- As shown in section 5, the liquid chemistry induced by UV, gas phase reactive species like NO, O\textsubscript{3}, OH and ions are on their own not sufficient to explain the bactericidal effect induce by the plasma jet. The synergistic effect of either pH and HNO\textsubscript{2} or H\textsubscript{2}O\textsubscript{2} and pH are the main contributors to the bactericidal effect induced by the liquid chemistry.
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References


