



K Y O T O
INSTITUTE OF
TECHNOLOGY



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SS 25

Spezialisierungsmodul

as part of an internship at Kyoto Institute of Technology (KIT)

Development and Ion-Optical Simulation of an Electron-Impact Ionization Time-of-Flight Mass Spectrometer

handed in by

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Friday 18th April, 2025

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Abstract

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List of Abbreviations

| | |
|---------------|-----------------------------|
| APP | Atmospheric Pressure Plasma |
|---------------|-----------------------------|

| | | |
|--------------------------|-------|-------------------------------|
| C. Sphaerospermum | | Cladosporium Sphaerospermum |
| DBD | | Dielectric Barrier Discharge |
| OES | | Optical Emission Spectroscopy |
| UV | | Ultraviolet |
| KIT | | Kyoto Institute of Technology |

1 | Introduction

2 | Scientific Context

There have been many recent studies on the use of plasmas for sterilization and its effects on microorganisms [10, 8, 16, 11, 20]. This chapter aims to provide an overview of the scientific context surrounding this topic as well as research on the interaction of fungi with radiation.

The use of plasma treatment in the medical field goes back to the 20th century. Its history is outlined well in [13]. While the first plasma treatments were used on skin their antimicrobial¹ properties were soon discovered. In the 1990s Mounir Laroussi demonstrated the use of DBD plasma for sterilization of surfaces [9]. Its first use focussed on the sterilization of medical instruments and equipment where removing bacteria and viruses is crucial. With this began many studies on the interaction of plasma with bacteria, and it has been shown that DBD plasma can very effectively be used for sterilization [20, 8]. With this many devices have been developed that are now used commonly in hospitals and laboratories [13]. Although sterilization has been studied extensively most of the studies have focussed on the deactivation of bacteria exclusively. Recent studies have started to investigate the interaction of plasma with fungi which is relevant for the food industry and the cleaning of environments [2]. While its efficacy has been proven well, it is not yet fully understood which processes carry responsibility for the inactivation of microorganisms especially on fungi. It is believed that the main mechanism of inactivation is the generation of reactive species in the plasma which can diffuse into the cell and damage DNA and proteins [8, 20]. A clear relationship between treatment time, proximity and inactivation rate has been shown.

Non-thermal plasma can however also radiate ultraviolet (UV) light and the electric field applied through DBD can also have an effect on the microorganisms. A recent study by Li, Yiqian and others [10] has researched the effects of the physical energy of the plasma on bacteria by separating samples with different materials transparent to radiation and electric fields and found them to have a significant effect. In this work a similar approach is taken to investigate the effects of the radiation on the fungus *C. sphaerospermum*. The basis for which

¹Inhibits the growth of microorganisms.

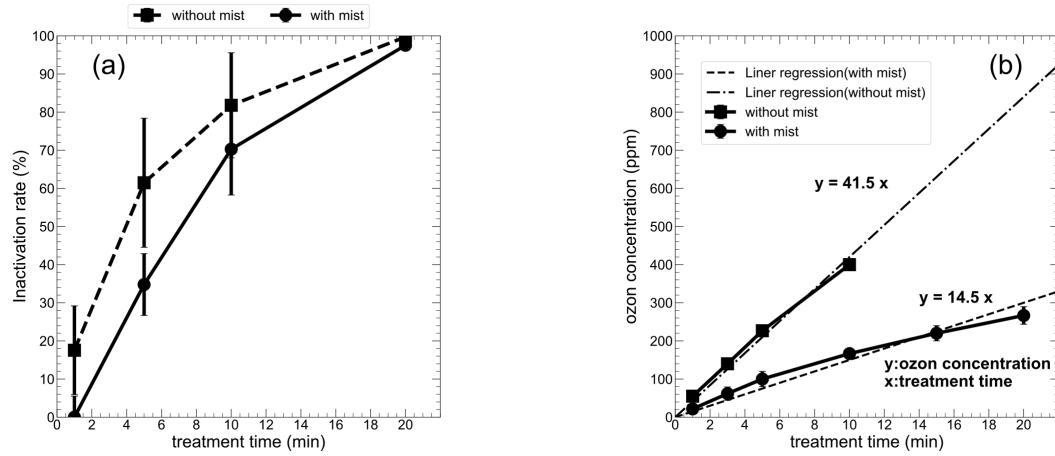


Figure 2.1: Results of the study by Tomoya Ohara and others [16]. (a) inactivation rates of *C.sphaerospermum* as a function of treatment time in the treatments without and with water mist, and (b) ozone concentration as a function of the time.

was formed by previous work by the department for plasma physics at the Kyoto Institute of Technology done by Tomoya Ohara and others [16] where this study continued. They showed that treatment times of 20 minutes are sufficient to inactivate the fungus by more than 99 % and measure the concentration of ozone and hydroxide ions. Figure 2.1 shows the results of their study.

According to prior studies moulds like *C. sphaerospermum* are a lot more resistant to UV radiation than bacteria [8].

3 | Theory and Methods

In this chapter the theoretical background and the methods used in this work are presented. This includes the collection of research on the fungal species *Cladosporium sphaerospermum*, as well as the atmospheric pressure plasma and the different processes that lead to the sterilization of the mould as they interact.

3.1 *Cladosporium sphaerospermum*

Cladosporium sphaerospermum (*C. sphaerospermum*) is a species of fungus. Fungi are a large group of eukaryotic organisms¹ that include yeasts, moulds and common mushrooms. It is a black mould that is commonly found in indoor environments, especially in areas that are damp or have high humidity. It was first described by a German mycologist, Albert Julius Otto Penzig, in 1886 from decaying citrus plant material in Italy [17]. *C. sphaerospermum* primarily reproduces asexually through the production of conidia, which are non-motile spores² formed at the tips of hyphae³. These spores are easily dispersed through the air, allowing the mould to rapidly spread into new environments. Moulds like *C. sphaerospermum* require humid conditions because moisture is essential for spore germination⁴. In dry environments, spores usually remain dormant [2].

¹Organisms whose cells have a membrane around their nucleus, which includes animals, plants and fungi.

²Spores not capable of movement.

³Long, branching structures that grow from their tips. They give moulds their furry appearance.

⁴The process by which a spore begins to grow new hyphae.

3.1.1 Growth and Morphology⁵

C. sphaerospermum has a darkly-pigmented mycelium⁶ that can appear black or dark green. The colonies of the mould are typically flat and have more of a powdery appearance than other moulds. It is typical for fungi of the *Cladosporium* family to have branching, tree-like hyphae on whose ends conidia are formed in chains. The spores themselves are round to oval in shape and measure a few micrometers in length. They are very resilient and can stay alive even in conditions not favourable for growth. Due to their small size they are invisible to the naked eye. In Figure 3.1 the morphology of *C. sphaerospermum* is shown.

In addition to a humid environment optimal conditions for the growth of *C. sphaerospermum* include a temperature of 25 °C. It is however a psychrophilic fungus⁷ and can grow at temperatures as low as -5 °C [17]. It nourishes through saprotrophic nutrition, which is the process of using decaying or dead organic matter as a source of nutrients. This is why it is commonly found in decaying plant material, where it was also discovered. The conversion of starch, cellulose and other compounds such as carbon dioxide provides the energy needed for growth.

3.1.2 Ecological Role and Occurrence

Like many fungi *C. sphaerospermum* plays an important role in the ecosystem as a decomposer. It breaks down dead organic matter, recycling nutrients back into the soil, where it thrives. This process is important as it fertilizes the soil and allows for the growth of new plants, enabling the life cycle. Because of its resilience to different conditions it is able to grow in many different environments which include anthropogenic places like indoor environments. In humid areas and on porous surfaces, such as wood or concrete walls it can build mycelium and produce new spores. The easily dispersed spores reach virtually everywhere and are even found in orbiting spacecraft [5].

While fungi don't perform photosynthesis and therefore do not convert CO₂ into oxygen they still contribute to the carbon cycle. By digesting plant material and binding carbon dioxide in their biomass they play an important role in storing carbon and reducing the amount of carbon dioxide released into the atmosphere when plants decay. Because of their abundance they are able to store large amounts and help build stable compounds for long-term soil carbon storage.

⁵The study of the structure of organisms.

⁶A network of branching hyphae.

⁷An organism that is able to grow in very low temperatures.

⁸The outer edge of a cell wall layer formed parallel to the spore surface.

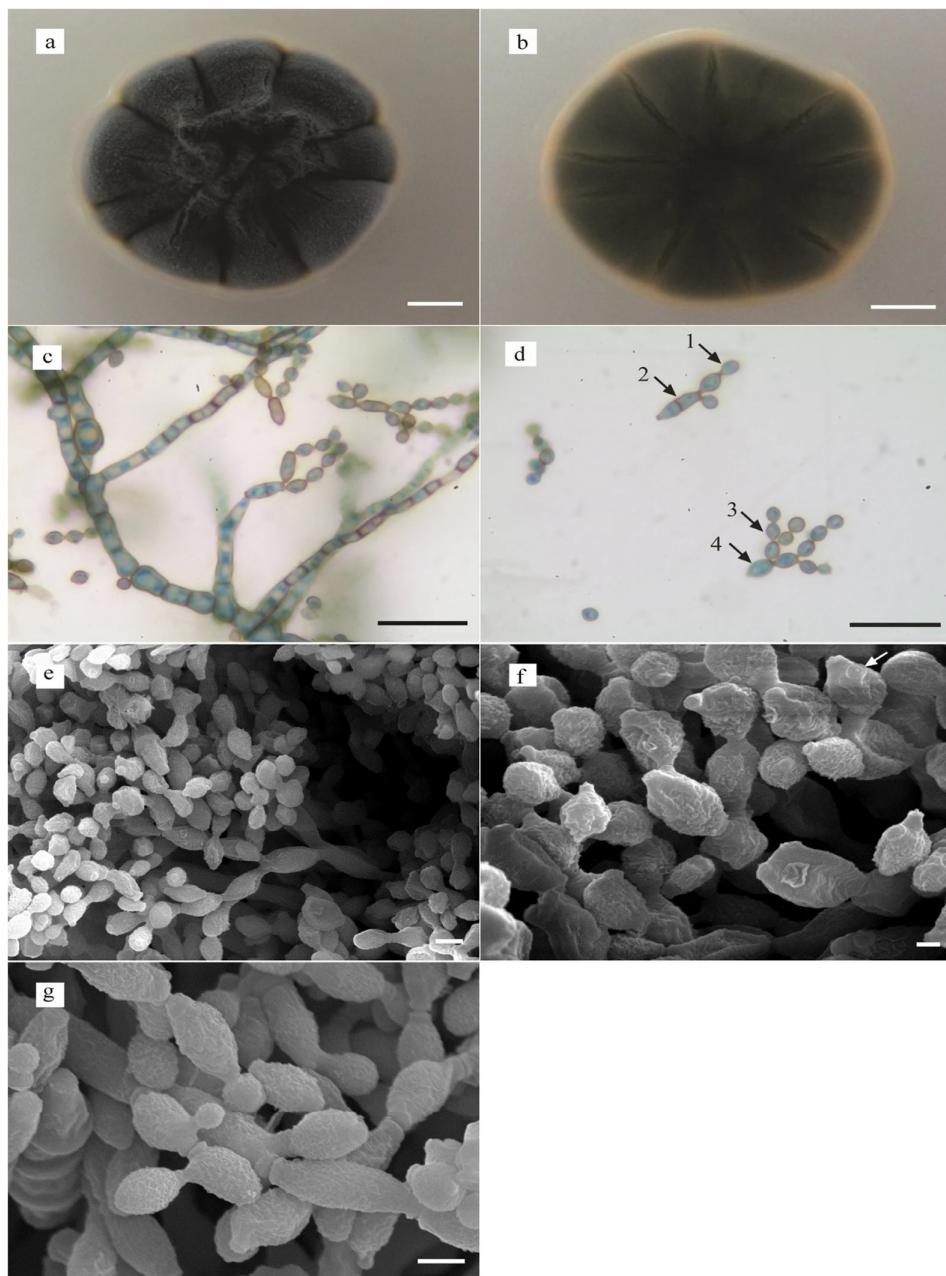


Figure 3.1: Morphology of *C. sphaerospermum* from [24]. Colonial morphology front (a) and reverse (b) of *C. sphaerospermum* UM 843 on SDA after 7-day incubation. Light micrograph showing conidia (d 2 and d 4). $\times 630$ magnification, bars 20 μm . Observation under scanning electron micrograph showing (e,f,g) conidiophores bearing conidium (e, $\times 2000$ magnification, bar 3 μm), pericinal rim⁸. (f, $\times 5000$ magnification bar 1 μm) and verruculose surface of conidia (g, $\times 5000$ magnification, bar 2 μm).

3.1.3 Effects on Human Health

Because of its ubiquity *C. sphaerospermum* surrounds humans in their daily lives. The small size of the spores allows them to be inhaled deeply into the lungs where they can lead to allergenic reactions as well as pathogenic infections⁹, particularly when the immune system is weakened. Compared to other airborne moulds it is however not considered a serious health risk as it does not produce mycotoxins¹⁰. Fungi in general can cause respiratory problems, especially when inhaling them in large quantities over a long time. They are however also essential in medical science as they are used to produce antibiotics, such as penicillin. While working with them in the lab clean benches can be used to provide sufficient ventilation but they generally don't pose any danger to a healthy person.

3.1.4 Response to Radiation

UV radiation is commonly used to sterilize surfaces and is very effective in killing most microorganisms like bacteria and fungi. *C. sphaerospermum* however shows a higher resistance to different types of radiation including UV and even high energy ionizing radiation. It is able to not only survive doses of ionizing radiation that would kill most other organisms but also to thrive in these conditions. This was discovered in 1990s as the fungus is able to grow in the high radiation environment of the destroyed Chernobyl nuclear power plant [5], even on the highly radioactive reactor walls. The mould does not only survive in these conditions but was proposed to be radiotrophic - a process where fungi potentially use ionizing radiation as a source of energy analogous to photosynthesis - growing faster.

Its tolerance to radiation both ionizing and non-ionizing is attributed to the pigment molecule Melanin. Pigment molecules such as Melanin absorb visible light and other radiation which can protect cells, can be used to absorb energy. It gives the mould its dark colour and protects its from UV radiation. Melanin is also able to absorb ionizing radiation and potentially convert it into chemical energy usable to the fungus.

To further research this discovery the mould was sent to the International Space Station in 2018 where it was exposed to cosmic radiation [14]. The study found that the mould was able to survive the radiation and even thrive in the microgravity environment while absorbing some of the energy. The results of the study suggest that *C. sphaerospermum* could potentially serve

⁹A pathogen is any organism that can cause disease in a host.

¹⁰Toxins produced by certain fungi that can cause severe disease in humans and animals.

as a highly sought-after solution for creating a self-replicating biological radiation shield or be otherwise useful in space exploration as discussed in [21].

3.2 Atmospheric Pressure Plasma

A plasma is a state of matter that consists of ionized gas and free electrons. The particles behave collectively in a highly dynamic way because they are coupled closely through electromagnetic fields. The state is also conductive and the plasma can be influenced by external electric and magnetic fields [4]. Although the particles carry charges locally, a plasma is electrically neutral when observed as a whole. Plasmas can be created by applying energy in the form of heat or electromagnetic fields to a gas. To ignite a plasma at low temperatures, a discharge is needed, the conditions for which - such as pressure, voltage and distance - are described by Paschen's law. This law states that it is generally easier to create a plasma in a low-pressure environment, as the mean free path (the distance between particle collisions) is larger, allowing the electrons to gain more energy before colliding with other particles, so they can exceed the ionization threshold. This is why many plasmas are created in vacuum chambers. However, with a sufficiently high voltage, it is possible to create plasma at atmospheric pressure also. Such a plasma is known as an atmospheric pressure plasma (APP). APPs have the advantage of being comparatively simple to handle as no vacuum vessel is required to contain them. This also makes it easier to bring them into the proximity of samples for various applications such as sterilization, surface treatment or material processing [4]. To sustain the plasma an alternating current in the Kilohertz range is used, so that the changing electromagnetic field is able to accelerate electrons continuously. The much heavier ions are accelerated only minimally in comparison. Although electron temperatures commonly reach around 10^4 K, it is possible to sustain a plasma at room temperatures if the energy provided is controlled [3]. Such plasmas are referred to as cold plasmas or non-thermal plasmas. Because of the high voltage required to ignite APPs arc discharges can occur between the electrodes which significantly reduce the plasma stability. This makes it very hard to predict their behaviour and would heat them up to much higher gas temperatures which needs to be prevented for many experiments. Dielectric barriers can be used to achieve a controlled discharge and energy transfer.

Many processes occur in a plasma besides ionization. The gas atoms are also frequently excited which leads to the emission of photons as these states are non-stable which gives plasmas their characteristic glow. Molecules can also be dissociated by collisions which leads to the formation of radicals and other chemical bonds that can have varying lifespans. The

radiation as well as the reactive species produced in the plasma allow the plasma to interact with surfaces and materials in its proximity.

3.3 Dielectric Barrier Discharge

To achieve a controlled non-thermal plasma at atmospheric pressure a dielectric barrier discharge (DBD) can be used. This means that the two electrodes that carry the high voltage to ignite and sustain the plasma are separated by a dielectric barrier. There are different configurations to realize this. Some setups use only one dielectric barrier at one of the electrodes while commonly both electrons - planar or cylindrical - are covered with a dielectric material. The dielectric barrier is a non-conductive material that prevents the formation of an arc discharge between the electrodes but can be polarized by the electric field. Often glass, quartz or ceramics are used for this purpose. While the plasma is ignited the dielectric barrier prevents the electrons from flowing freely between the electrodes and charges are built up on the surface of the dielectric. This stops the discharge very quickly and thereby limits the current flow, an alternating current is needed. By controlling the voltage and frequency of this current the discharge can be sustained in a stable way. The dielectric barrier also helps to create a uniform electric field similar to its use in capacitors.

DBD is used widely in different applications that require a non-thermal plasma like the semiconductor or medical industry. It is used for surface treatment, cleaning and sterilization, like in this work, where a cold plasma is needed that does not destroy the surfaces it interacts with. It is interesting

3.4 Optical Emission Spectroscopy

Optical emission spectroscopy (OES) uses the electromagnetic radiation emitted by a plasma to analyse its composition. This can be done non-intrusively meaning that the plasma does not need to be disturbed and remains unaffected by the measurement. That is a major advantage when working with small volumes of plasma like in this work where probes would significantly change the conditions [3]. OES measures a spectrum of the emitted light which gives the intensity at different wavelengths. Because the photons originate from distinct transitions between energy levels sharp peaks are observed in the spectrum. Their wavelength can be used to identify the species that are present in the plasma as well as the processes that occur.

This also allows an estimate of the plasma temperature and the concentration of the different gases in the plasma, although more information like cross-sections for the different processes is needed [3].

3.4.1 Boltzmann Plots

Boltzmann Plots are often used to derive the electron temperature T_e from a measured spectrum. They underlie the assumption that the probability for transitions follows a Boltzmann distribution, with the intensity of the emission line proportional to the Einstein coefficient for spontaneous emission. The relationship between intensity and temperature is given by:

$$\frac{I_j}{g_j} \propto A_{ij} \exp\left(-\frac{E_j}{k_B T_e}\right), \quad (3.1)$$

where I_j is the intensity of the emission line from an excited state j , g_j is the degeneracy of that state, E_j is the energy of the state, A_{ij} is the Einstein coefficient for spontaneous emission, and k_B is the Boltzmann constant.

When the intensities are normalized, the relative intensity is used, and the plot of $\ln\left(\frac{I_j}{g_j A_{ij}}\right)$ versus the energy E_j results in a straight line. The slope m of this line provides an estimate for the electron temperature T_e :

$$m = -\frac{1}{k_B T_e}. \quad (3.2)$$

However, it is also assumed that the plasma is in local thermal equilibrium which is not the case in non-thermal plasmas. The assumption that the electron temperature is equal to the gas temperature is not valid and the Boltzmann plot can not be used to get an absolute temperature value in this work [3].

3.4.2 Other methods

A very interesting study by Hiroshi Akatsuka [7] was able to show that the electron temperature and density can be derived from the OES spectrum of a non-equilibrium plasma. To do so the continuum spectrum of the plasma was measured and its absolute emissivity per wavelength

λ was calculated. The continuum spectrum is caused mainly by BREMSSTRAHLUNG emitted by decelerating electrons in the plasma [7]. It is much less intense than the peaks formed by electron transitions but is closely related to the electron temperature and density. The following relationship describes how the emissivity ε_λ and the intensity I_λ , found through OES, are related and the optical length through the plasma L is known:

$$I_\lambda = \int_0^L \varepsilon_\lambda(l) dl. \quad (3.3)$$

By then fitting an electron energy distribution function (EEDF) to the measured spectrum the electron temperature and density can be derived as fit parameters. The EEDF describes the distribution of the electron energy in the plasma and is a function of the electron temperature. A Maxwellian distribution and a Druyvesteyn distribution were used in the study to obtain accurate results [7]. While the results from this study are used as a reference for comparison the method itself could not be applied in this work because of limited resources and time.

In OES one needs to account for the spectrometer's spectral response, which is the sensitivity of the detector per wavelength. This is usually done by measuring a calibration spectrum and correcting the measured spectrum accordingly.

3.5 Sterilization Mechanism

APPs have been used for sterilization of surfaces in many studies as outlined in chapter 2. Although the plasma is not in direct contact with the sample two main mechanism have been found to be responsible for the sterilization effect. The first is the radiation emitted by the plasma, which can be UV or visible light. The second is the reactive species that are produced in the plasma and interact with the surface. Additionally, heat as well as electric fields can also play a role in the sterilization process. The exact mechanism is still subject to research and what process dominates depends on the specific setup and sample. This study aims to test and isolate the effects of the radiation and reactive species on the inactivation of *C. sphaerospermum*. The following sections will discuss what is known about the different processes and how contribute to the sterilization.

3.5.1 Reactive Species

As discussed in chapter 2 the main mechanism for the inactivation of microorganisms in prior studies appeared to be the reactive species produced in the plasma. Because of the high electron temperatures in the plasma some electrons gain enough energy to dissociate molecules and create radicals. When working with an APP the main gases present are oxygen and nitrogen as well as water vapour. The most important reactive species produced are hydroxyl radicals (OH) and ozone (O_3). Radicals are missing an electron and are therefore highly reactive chemicals. They are widely used in water treatment and sterilization because they can react with organic compounds and degrade them [22]. Studies such as [23] have demonstrated that the concentration of ozone can be correlated with the rate of microbial inactivation directly. These reactive species can penetrate cells and cause damage to vital biomolecules, including DNA and proteins. This leads to the inactivation of the microorganism as it is unable to reproduce or perform its normal functions and was proven to be effective against the spores of *C. sphaerospermum* [16]. By using chemical probing methods the concentration of reactive species can be measured. For example the concentration of OH radicals can be quantified by their fluorescence when they are reacted with a chemical probe such as sodium terephthalate (NaTA).

3.5.2 Radiation Effects

In addition to the radicals produced by the plasma it emits radiation that interacts with the sample. In APP visible light as well as UV light in the UV-A and UV-B range is typically emitted [3]. UV radiation is used a lot for sterilization and is standard in many clean benches used in laboratories but slightly higher frequencies in the UV-C range are used. A study by Li, Yiqian and others [10] found that plasma emitting UV-A and UV-B light can have a significant effect on the inactivation rate of bacteria when using a quartz-glass separation but that the shorter wavelengths are more effective.

The main reason why UV radiation is effective at killing microorganisms is its photochemical interaction with DNA. DNA is very susceptible to absorption of UV light which can alter its structure leading to the breaking of bonds and the formation of mutations [12]. DNA contains four bases: adenine (A), thymine (T), cytosine (C), and guanine (G). These are all aromatic molecules. Aromatic molecules are ring-shaped compounds with delocalized π -electrons, meaning their electrons are not confined to individual bonds but can move freely

within the molecule [12]. This makes them highly stable, but the electrons can efficiently be excited by UV light, especially around 260 nm. The transition

$$\pi \rightarrow \pi^*, \quad (3.4)$$

describes the excitation of an electron from a bonding¹¹ π -orbital to an anti-bonding¹² π^* -orbital. This significantly weakens the bonds in the molecule and can lead to the formation of dimers¹³. Fungi just like bacteria rely on the integrity of their DNA as it is the basis for their reproduction of cells, which makes any damage to the DNA highly effective in killing them.

As mentioned in section 3.1.4 *C. sphaerospermum* is more resistant to UV radiation than bacteria because it is a melanized fungus. Melanin helps to protect the DNA from UV radiation by absorbing some photons before they reach the DNA. This means higher doses are necessary to achieve the same inactivation rate.

¹¹A bonding orbital results from constructive interference of atomic orbitals, lowering electronic energy.

¹²An anti-bonding orbital results from destructive interference of atomic orbitals, raising electronic energy.

¹³In DNA, dimers can form when two adjacent bases bond abnormally due to UV exposure [12].

4

Experimental Setup

In this chapter the setup as well as the methods by which the *C. sphaerospermum* samples are prepared and treated are described. All experiments were conducted in the laboratory of the group for plasma physics at the Kyoto Institute of Technology led by Assoc. Prof. Kazou Takahashi. The work was done in collaboration with Shinano Kinoshita and based on previous research [16] conducted by Tomoya Ohara and her.

4.1 APP Setup

To create an atmospheric plasma a DBD is used. The setup consists of two pairs of isolated electrodes. The electrodes are separated by a dielectric material which prevents a continuous discharge. A diagram of entire setup is shown in figure 4.1 and a photograph of its implementation is shown in figure 4.2. The experiment is contained with a transparent box to prevent contamination and control the atmosphere around the plasma and mould. Through a tube dry or mist, generated by an ultrasonic mist generator, can be introduced into the chamber. While the effect of humidity has been studied in the past [16] it is not the focus of this work. Therefore, the mist generator is not used in experiments.

4.1.1 Dielectric Barrier Discharge

For the discharge four electrodes are used. They are coated in ceramics. Figures 4.3 and 4.4 show the exact discharge setup. The DBD is powered by a high voltage DC power supply that is then converted to an AC voltage with a blocking oscillator. An oscilloscope is used to measure the voltage and current of the discharge. Figure ?? shows the voltage and current signal of the DBD. The discharge area has a small volume and radiation is only emitted between the electrodes.

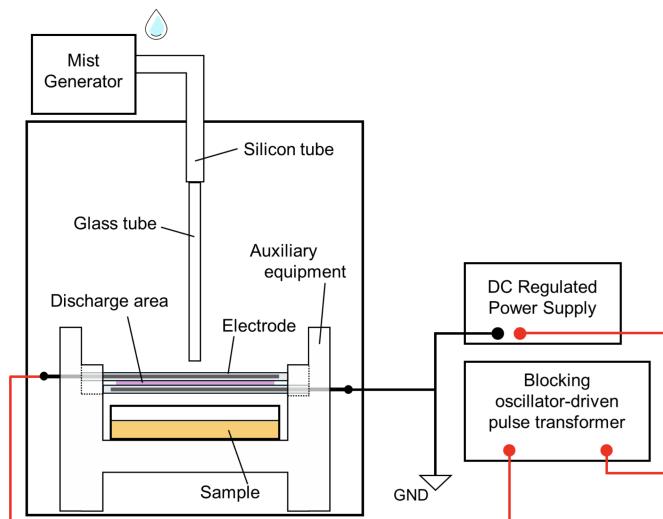


Figure 4.1: This figure shows the experimental setup. The APP is generated between two pairs of electrodes. The entire setup is placed in a box to prevent contamination. The sample is placed underneath the discharge area. A tube can provide water mist and dry air to control the humidity in the chamber. A power supply and a blocking oscillator provide an oscillating voltage to the electrodes.



Figure 4.2: Photograph of the experimental setup.

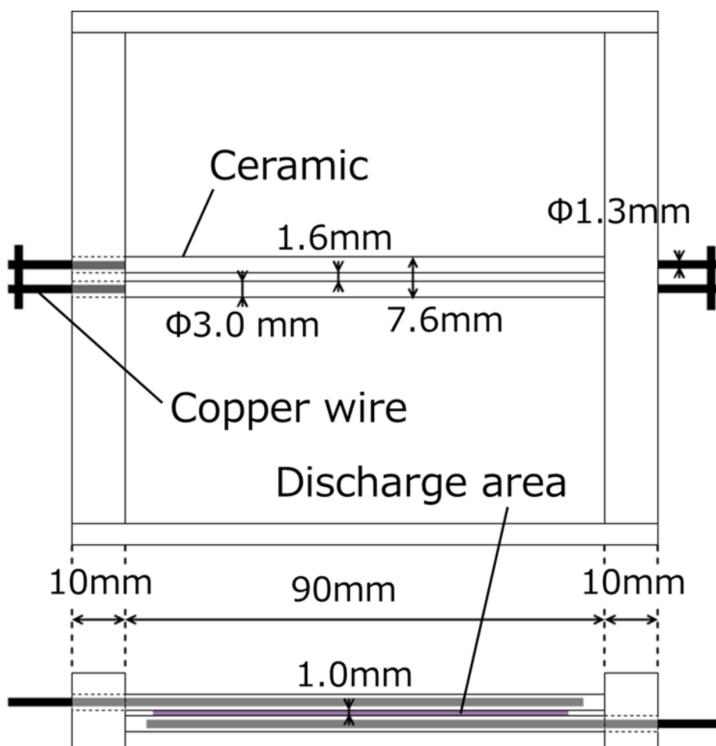


Figure 4.3: Technical sketch of the DBD setup. On the top the view from above the DBD setup is shown, on the bottom the side view. The APP is generated by a DBD with a frequency of 13.56 MHz and a voltage of 13 kV. This diagram has kindly been provided by Shinano Kinoshita.

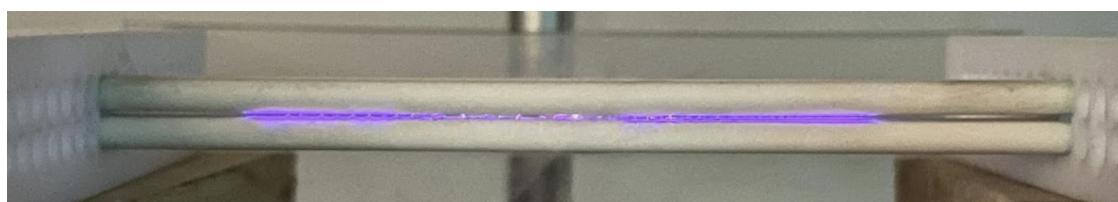


Figure 4.4: Image of an ignited plasma at atmospheric pressure between the electrodes.

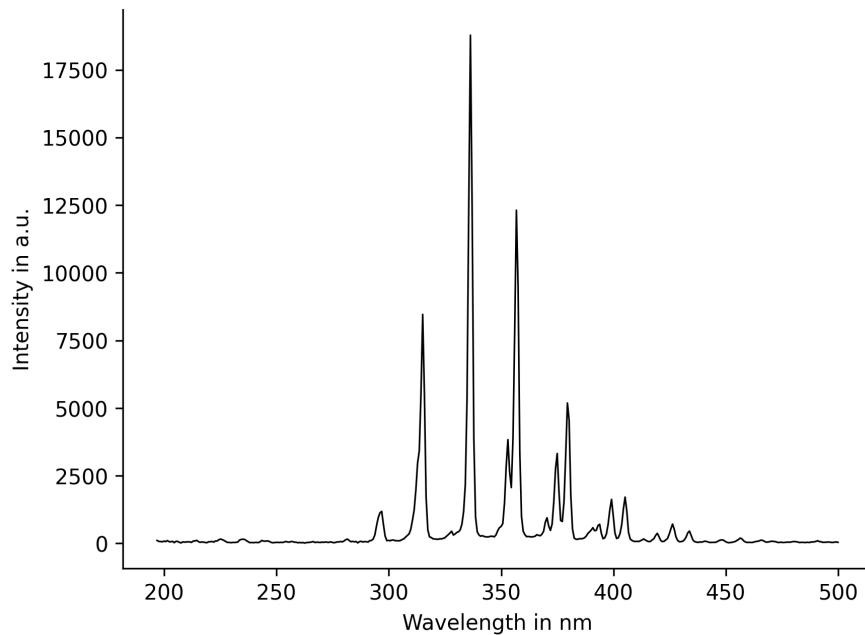


Figure 4.5: Raw OES spectrum of the plasma at atmospheric pressure with ambient air as the working gas. The peaks are analysed in section 5.1.

4.1.2 Measurement of the Plasma Spectrum

To measure the optical spectrum of the plasma a photonic multichannel analyser, the PMA-12 by Hamamatsu, is used. It is able to measure spectra down to 200 nm and up to 1200 nm. The spectrometer is connected to a computer via USB and the data is recorded with a software that can automatically correct for its spectral response. Figure 4.5 shows a spectrum of the plasma recorded with the spectrometer. It is later analysed in section 5.1.

4.2 Preparation of Samples

The samples are grown in Petri dishes on an agar medium. The medium is prepared in the lab beforehand. Table ?? shows its composition. To create new samples spores of the fungus *C. sphaerospermum* are collected from thriving colonies. They are dissolved in sterilized water and then scraped off the surface by using a cotton swab. The solution containing the spores is then diluted in two or three steps of 1:10 to reach a concentration of around 3.5×10^4 or 3.5×10^3 spores per ml. This is then verified using a cell counting plate under a microscope.

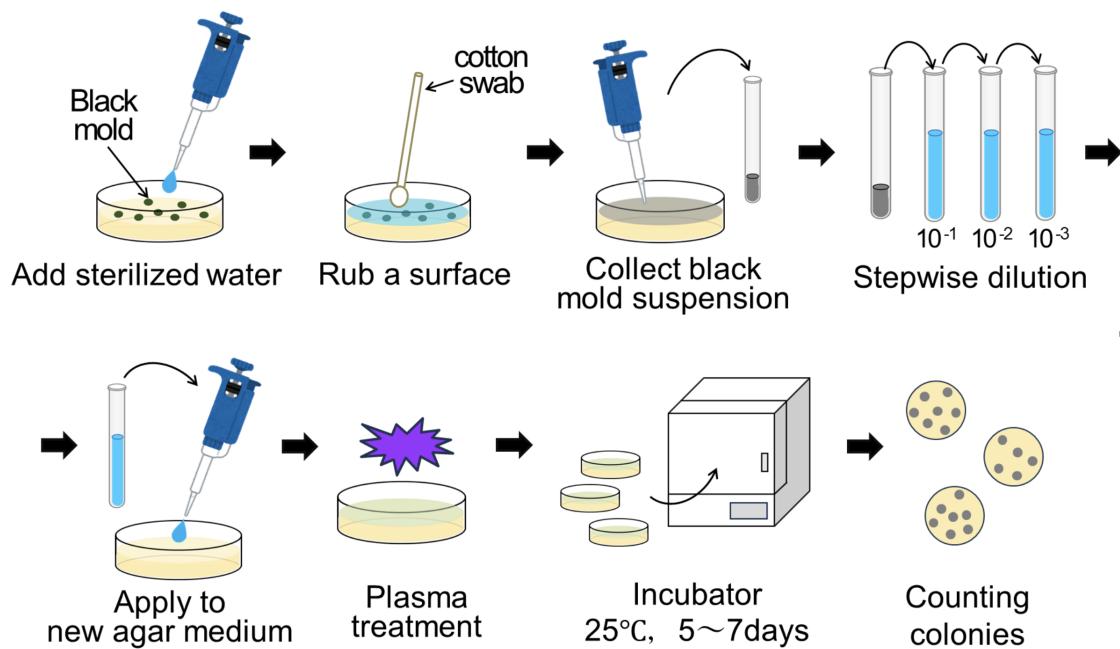


Figure 4.6: Diagram of the experiment process and the preparation of samples. The fungus is cultivated on an agar medium and stored in Petri dishes. Spores are collected by dissolving them in sterilized water and then their concentration is diluted in steps to the desired concentration. The samples are then treated with the APP and the inactivation rate is measured by counting the colonies on the agar medium.

$100 \mu\text{l}$ of the diluted solution are then spread on fresh agar media and are ready for treatment.

After treating the spores with plasma, UV or none the samples are incubated in an incubator for multiple days at their ideal growing temperature of 25°C . When the incubation period is over the samples are taken out and the number of colonies is counted to get an estimate of the inactivation percentile. Each experiment contains at least two control samples that are not treated and can be used as a reference.

4.3 UV Exposure

To test the effects of UV radiation on the samples before exposing them to the radiation emitted by the plasma, a UV lamp is used. The lamp is inside a clean bench to allow exposure to the UV light while preventing contamination. Figure 4.7 shows the spectrum of the UV lamp. It

emits light in the range of 200 nm to 450 nm with its main peak at 254 nm. The data sheet¹ of the lamp states that it emits radiation with an irradiance of $51 \mu\text{W}/\text{cm}^2$. This is measured at the radius of the cylindrical glass tube that encloses the lamp. To estimate the dose of radiation that the samples are exposed to, the effective irradiance I_{eff} at a given distance from the lamp must first be determined. For this, the distance r from the lamp to the sample is measured, and the irradiance is calculated using the formula:

$$I_{\text{eff}} = I_0 \cdot \frac{r_0}{r}, \quad (4.1)$$

where I_0 is the irradiance at the radius r_0 of the lamp. Here it is assumed that the lamp acts more like a thin line source of light than a point source, which only applies for short distances from the lamp. Once the effective irradiance is known, the dose D_{UV} can be calculated as:

$$D_{\text{UV}} = I_{\text{eff}} \cdot t, \quad (4.2)$$

where t is the time of exposure. This provides an estimate of the UV dose received by the samples during irradiation. In the setup used for the experiments the effective irradiance computes to $1.14 \mu\text{W}/\text{cm}^2$ at a distance of 58 cm from the lamp.

¹The lamp used is the sterilization lamp GL15 by Toshiba

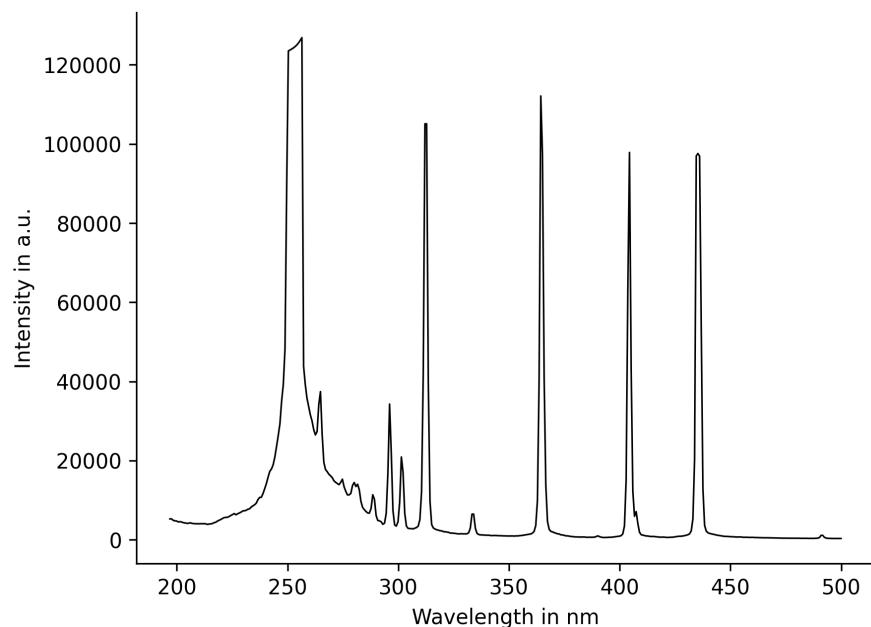


Figure 4.7: Spectrum of the UV lamp. The main peak is at 254 nm. The spectrum was measured with the same spectrometer from section 4.1.2.

4.4 Plasma Treatment

4.4.1 Full Plasma Treatment

4.4.2 Isolation of Radiation

To isolate the effects of the radiation emitted by the plasma from the reactive chemicals a transparent barrier is used. The barrier is made of quartz glass and is also transparent to visible light. It is 8 mm thick. To hold the barrier in place a mount is designed and 3D printed from PLA. The mount is designed to hold the barrier in place and to allow for easy removal.

To verify the transparency of the quartz glass, the spectrum of the UV lamp shown in Figure 4.7 is measured with and without the barrier. The results are found to be nearly identical in the range that is relevant for this experiment, so no correction is needed.

5 | Results

In this chapter the results of the experiments are presented. They are divided into three sections that were conducted consecutively to answer the research question that were posed in the introduction. The first section shows an analysis of the OES spectrum of the APP and confirms the plasma composition. The second section describes the effect of UV radiation had on the spores of *C. sphaerospermum* in the experiment. In the third section results of the APP treatment on the spores of *C. sphaerospermum* with and without reactive species are shown.

5.1 OES Analysis

In the following the OES spectrum of the plasma first shown in section 4.1.2 is analysed. To do so, the peaks of the spectrum are found using PYTHON and the SCIPY library. They are then compared to possible different species that could be present, and their wavelengths matched to identify the transitions. Figure 5.1 shows the spectrum with the peaks marked using [15, 18, 1]. The spectrum confirms the presence of the main species in the plasma, which are as expected mostly nitrogen, oxygen and water. Most oxygen is dissociated and makes new compounds with hydrogen or nitrogen leaving nitrogen molecules to be dominant in emission. Almost all visible peaks were found to correspond to the Second Positive System (2PS) of nitrogen. The 2PS is a system of transitions between the vibrational levels of the first excited state of nitrogen:



and consists of many bands. Figure 5.2 shows a plate of the 2PS of molecular nitrogen.

Using data from [6] listed in Table 1 (Appendix) and the OES spectrum of the plasma a Boltzmann plot is created. It is shown in Figure 5.3. By calculating the slope of the plot from relative intensities the electron temperature can be estimated. As mentioned in 3.4.1 this can not be used as an absolute measurement in this work, because the plasma is non-thermal and

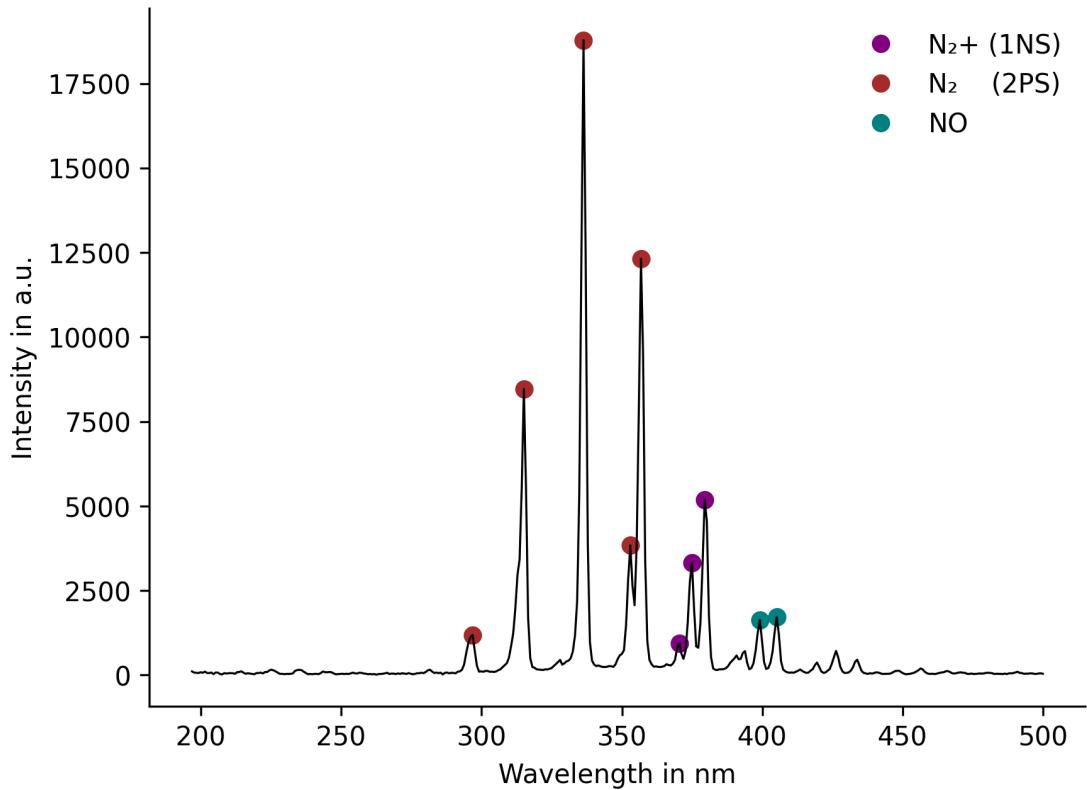


Figure 5.1: OES spectrum of the plasma with the peaks identified. The Second Positive System of molecular nitrogen is dominant.

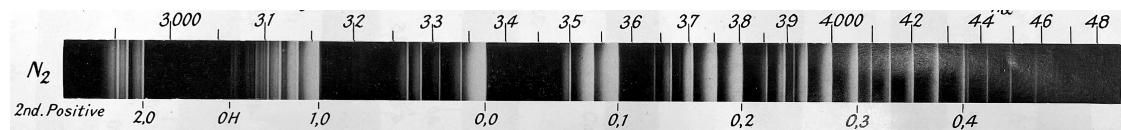


Figure 5.2: Plate of the Dinitrogen Second Positive System from [19]. The wavelengths are displayed above the photograph in Å.

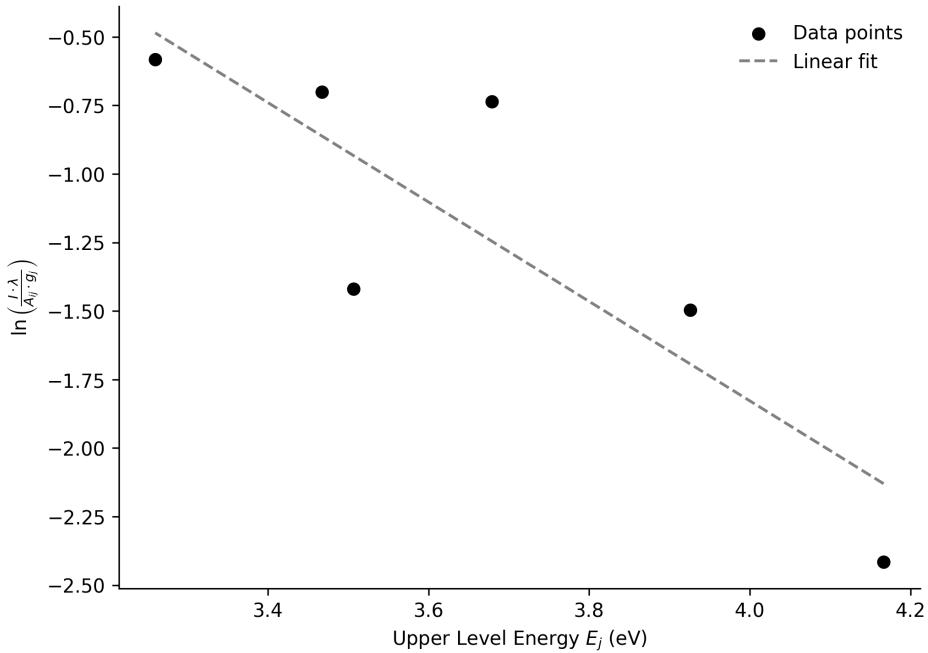
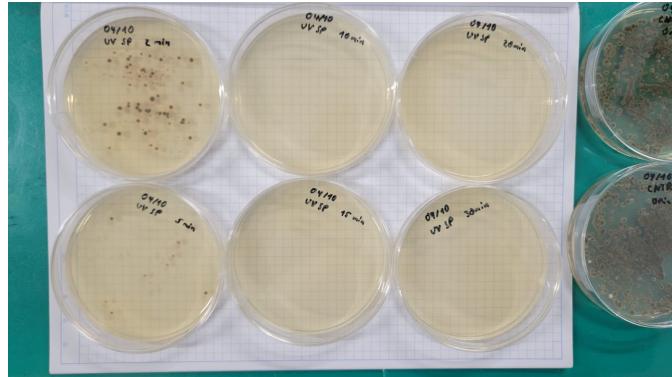


Figure 5.3: Boltzmann Plot to estimate electron temperature using data from [6] listed in Table 1.

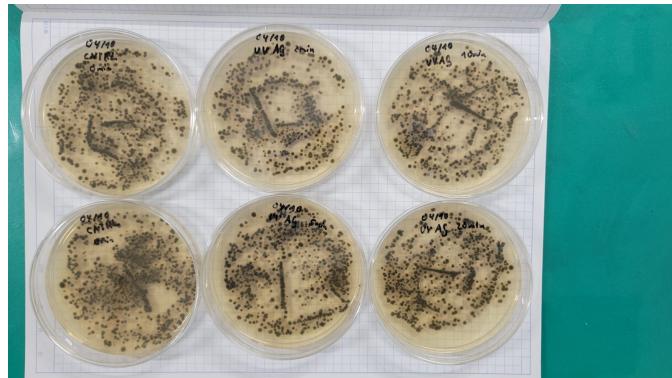
is not in a local thermal equilibrium. From the slope obtained by fitting the data a temperature value of around 6500 K is found which appears to be in the expected order of magnitude. It lies between the values (3365 K – 9168 K) that were found in [7] for an Argon plasma with a similar setup. This result also supports the correct identification of the observed emission peaks as transitions of the 2PS of molecular nitrogen.

5.2 Effect of UV Exposure

To confirm the effect of UV radiation on the spores of *C. sphaerospermum*, a control experiment is performed. First the spores are exposed to the UV lamp on agar medium for different times. After 15 min of exposure full deactivation was achieved. To control for the effect of the UV light on the agar medium, a second experiment is performed where the agar medium and spores are exposed to the UV light separately. To expose the spores they are instead put into the UV light in water and added to untreated agar media later. In Figure 5.4 the petri dishes after incubation are shown. Table 5.1 shows the number of colonies in a matrix. It becomes clear from this data that the UV light has no significant effect on the agar medium while it has



(a) Spore UV exposure



(b) Agar UV exposure

Figure 5.4: Petri dishes with spores (a) and agar (b) treated with UV light

a strong effect on the spores after an exposure time of 15 minutes. Using equation 4.2 the dose of UV radiation can be estimated and equates to 1.03 mJ/cm^2 .

Table 5.1: Number of colonies after UV exposure as a matrix. The results after 15 minutes are shown with an estimated dose of ca. 1.03 mJ/cm².

| # of colonies | Spores no UV | Spores UV |
|---------------|--------------|-----------|
| Agar no UV | >100 (cntrl) | 0 |
| Agar UV | >100 | 0 |

5.3 Effect of Plasma Treatment

6 | Conclusion

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Appendix

Table 1: Spectroscopic data for selected N₂ Second Positive System transitions. A_{ij} and E_j values from Gilmore and others (1992) [6].

| Wavelength in nm | Intensity in a.u. | A_{ij} in s^{-1} | g_j | E_j in cm^{-1} |
|------------------|-------------------|----------------------|-------|---------------------------|
| 297 | 1185.53 | 3.94×10^6 | 1 | 33606 |
| 315 | 8464.27 | 1.19×10^7 | 1 | 31665 |
| 337 | 18781.21 | 1.32×10^7 | 1 | 29671 |
| 353 | 3834.82 | 5.60×10^6 | 1 | 28284 |
| 357 | 12313.39 | 8.86×10^6 | 1 | 27966 |
| 380 | 5188.45 | 3.53×10^6 | 1 | 26290 |