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Master Thesis

Comparison of different sampler performances in a bioaerosol chamber with three different organisms

Submitted by

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Affidavit

I hereby declare that I have authored this master thesis independently, and that I have not used any assistance other than that which is permitted. The work contained herein is my own except where explicitly stated otherwise. All ideas taken in wording or in basic content from unpublished sources or from published literature are duly identified and cited, and the precise references included.

I further declare that this master thesis has not been submitted, in whole or in part, in the same or a similar form, to any other educational institution as part of the requirements for an academic degree.

I hereby confirm that I am familiar with the standards of Scientific Integrity and with the guidelines of Good Scientific Practice, and that this work fully complies with these standards and guidelines.

Vienna, October 2022

Lena PIGLMANN (*manu propria*)



Preface

The research presented in this master thesis was conducted in the framework of the project:

“Comparison of bioaerosol samplers in three different bioaerosol facilities to establish common protocols and procedures and identify differences in the chamber setup”

The experiments for this thesis were done from 2019 – 2021 at the AIT Austrian Institute of Technology, Center for Health and Bioresources, Unit Bioresources, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria.

The other project partners are:

- The Aerosol Metrology Laboratory, Institut National de Recherche et de Sécurité, Vandœuvre-lès-Nancy Cedex, France
- The Bioaerosols Research Laboratory, Institut universitaire de cardiologie et de pneumologie de Québec – Université Laval, Chemin Sainte-Foy, Québec, Canada

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Abstract

The investigation with a bioaerosol chamber enables the study of organisms by the imitation of realistic environmental conditions in a sterile and safe manner. This master thesis has focused on the comparison of two different aerosol sampling devices, the SKC BioSampler and the 37mm closed face air sampling cassette, containing a polycarbonate membrane filter. In three experimental settings they were compared regarding the sampling efficiency of *Penicillium chrysogenum*, *Geobacillus stearothermophilus* and the bacteriophage PhiX174. The aerosolization process was accomplished in the bioaerosol chamber CCB3000 with a Liquid Sparging Aerosolizer. The organisms were first analysed in a mixed suspension without aerosolization, then in aerosolization and sampling experiments for each organism individually as well as for a mix of all three. By micro- and molecular-biological analysis methods, the CFU/m³, PFU/m³, spores/m³ and copy number/m³ were calculated. Both sampling devices were able to generate reliable and consistent values for all organisms and concentrations steps. However, the SKC BioSampler sampled significantly higher values throughout all experiments. In those with *Penicillium chrysogenum* alone, the filter cassette reached 32.43% – 59.79% of CFU/m³ compared to the SKC BioSampler. In case of *Geobacillus stearothermophilus* it was 45.33% – 57.20% of CFU/m³, whereas for the bacteriophage PhiX174 it ranged between 7.62% to 59.11% of PFU/m³. The molecular-biological analysis resulted in similar percentage ranges, if the two sampling devices are compared regarding the copy number/m³. When all three organisms were aerosolized as a mix, the filter cassette delivered even lower efficiency values. In summary it can be said, that the SKC BioSampler is preferable to the filter cassette regardless of the species or concentration which is sampled. Above all, the simple handling, and the minimization of damage to the collected particles speak in favour for the SCK BioSampler.

Kurzfassung

Experimente mit Hilfe einer Bioaerosolkammer ermöglichen es Organismen durch die Imitation von realistischen Umweltbedingungen auf eine sterile und sichere Art zu erforschen. Diese Masterarbeit legt ihren Fokus auf den Vergleich von zwei Aerosolsammelgeräten, den SKC BioSampler und eine 37mm closed-face Luftsammelkassette, welche einen Polykarbonat-Membran-Filter enthält. Durch drei verschiedene experimentelle Methoden werden sie anhand der Sammeleffizienz von *Penicillium chrysogenum*, *Geobacillus stearothermophilus* und der Bacteriophage PhiX174 verglichen. Der Aerosolisierungsprozess wurde in der Bioaerosolkammer CCB3000 mit einem Liquid Sparging Aerosolizer ermöglicht. Die Organismen wurden als eine gemischte Lösung ohne eine Aerosolisierung untersucht, danach in Aerosolisierungs- und Sammelexperimenten für jeden Organismus einzeln und für einen Mix aus allen drei. Durch mikro- und molekularbiologische Analysemethoden wurden die KBE/m³, PBE/m³, Sporen/m³ und Kopienzahl/m³ berechnet. Beide Sammelgeräte lieferten zuverlässige und konsistente Ergebnisse für alle Organismen und Konzentrationsstufen. Dennoch konnte der BioSampler in allen Experimenten signifikant höhere Werte erreichen. In den Versuchen mit *Penicillium chrysogenum* allein, erreichte die Filterkassette 32.43% – 59.79% der KBE/m³ verglichen mit dem BioSampler. Im Fall von *Geobacillus stearothermophilus* waren es 45.33% – 57.20% der KBE/m³ und für die Bakteriophage ergaben sich 7.62% - 59.11% PBE/m³. Ähnliche Prozentspannen ergaben sich beim Vergleich der Sammelgeräte in Bezug auf erreichte Kopienzahl/m³. Als die drei Organismen in einem Mix aerosolisiert wurden, erreichte die Filterkassette noch niedrigere Effizienzwerte. Zusammenfassend kann gesagt werden, dass der BioSampler der verwendeten Filterkassette vorzuziehen ist, unabhängig davon welcher Organismus oder welche Konzentration gesammelt wird. Vor allem die einfache Handhabung und der geringe Schaden an den Partikeln, sprechen für den SKC BioSampler.

1. Introduction

Aerosols can be found ubiquitous in nature, are transported by air and their size ranges from 0.001 to 100 μm (Georgakopoulos et al., 2009). This small size and mass can lead to the persisting of those aerosols for long periods of time and the easy transporting over long distances (Brown & Hovmøller, 2002). An aerosol that is made of material that has biological origins, is called a bioaerosol (Burge, 1995). Bioaerosols are very complex particles regarding their composition and contain most of the times viable or dead microorganisms, like fungi, bacteria or viruses, or organic compounds derived from them (Brandl & Mandal, 2011). Furthermore, they can contain fragments of organisms, for example dander or excrements from small insects. Those individual components can aggregate into higher-order structures and then form very complex micro-aggregates with organic and inorganic substances, which makes it very difficult to examine these structures analytically (Konlechner et al., 2013; Eduard et al., 2012; Bulski, 2020).

Airborne microorganisms can lead to health problems when they are present in high concentrations in indoor environments (Stetzenbach et al., 2004). Above all, the respiratory tract is an absorption path for such particles (Linsel, 2001). Allergies or asthma, as well as chronic lung disorders and hypersensitivity pneumonitis are potential diseases, which can be caused by the exposure to bioaerosols. Additionally, infections or the uptake of toxic compounds of fungi could result in health problems (Douwes et al., 2003). The effects depend not only on the duration of exposure and intensity, but also on the microbial composition and the health status of the affected patient (Bardana Jr., 2003).

Even though it is known that the bioaerosol exposure can cause severe health problems or spread infectious diseases, it is still not clear which specific biological agent of a complex bioaerosol is connected with the development of symptoms and diseases (Clark, 2019). The assured detection of bioaerosols and the evaluation of bioaerosol-dependent health risks is still challenging (Méheust et al., 2014). Most of the microorganisms in bioaerosols need specific environmental conditions to grow and propagate, which can be influenced by factors like the temperature or relative humidity (Stetzenbach et al., 2004). Therefore, it is important to analyse these bioaerosols under controlled and realistic conditions, in a sterile and safe manner. This is possible with a bioaerosol chamber, which allows the production of bioaerosols with a defined and consistent particle count over a long period of time (Konlechner et al., 2013). In a bioaerosol chamber environmental conditions like the temperature and the humidity can be simulated to study organisms, and especially pathogens, in more details.

1.1. Aim of the study

The successful monitoring of bioaerosols requires the development and validation of efficient bioaerosol samplers. Not all particles can be sampled with the same method, especially because the bioaerosol behaviour is depended on the particle size and therefore determines the size selectivity of the sampler (Cox et al., 2020). Due to these technical limitations, there is at the moment no bioaerosol sampling device that can be used for the whole spectrum of microorganisms found in the air (Kulkarni et al., 2011).

Currently there are mainly three different methods used in sampling devices namely, impaction, liquid-based and filtration (Brandl & Mandal, 2011). Impactors use solid media to collect bioaerosols by the deposition of the particles through the influence of gravity (H. R. An et al., 2004). Those devices are often fitted with a fan that transfers the air through a perforated template or a narrow slit onto the agar plate (Brandl & Mandal, 2011). Impingers are a liquid-based sampling method, which operates by accelerating particles through a narrow inlet placed at a defined distance from the bottom of the flask, filled with liquid. By producing a pressure drop in the impinger, the air is forced to enter through this inlet into the sampling device (Verreault et al., 2008). An example for this method would be the SKC BioSampler, which is capable of producing a swirl in the collection flask to remove the particles from the inner wall into the liquid (SKC, Inc., n.d.). Another liquid-based aerosol sampling method is the usage of a cyclone, where air is also transported into a collector cone that is filled with buffer. This results in the formation of a vortex, and because of the centrifugal force it is capable of trapping the particles onto the inner walls. Thereby the particles are divided from the air and concentrated in the collection liquid (de Sousa et al., 2020). This liquid-based sampling methods are preferably used for DNA or RNA based analysis. The collection of bioaerosols with filter-based methods, however, has the advantage of simplicity and effectivity of collecting particles of all sizes and is therefore often used to sample particles for culture-independent analysis (Lindsley, 2016). Filters collect the bioaerosols through impaction, electrostatic capture, interception or diffusion (Cox et al., 2020). The membrane filter can be made of glass fibre, polyvinylchloride, polycarbonate or cellulose acetate as well as gelatine (Brandl & Mandal, 2011). In addition to these commonly used sampling methods, alternative ones have been described, for example gravity sampling, electrostatic precipitation, or condensation techniques (Ghosh et al., 2015).

The aim of this study was to determine which sampling method would be best suited for the collection of three different organisms. The SKC BioSampler and the 37mm closed face air filter cassette were compared in their efficiency of sampling *Penicillium chrysogenum*, *Geobacillus stearothermophilus* and the bacteriophage PhiX174. This master thesis was done as a part of the project **“Comparison of bioaerosol samplers in three different bioaerosol facilities to establish common protocols and procedures and identify differences in the chamber setup”**.

This project was established in cooperation of the AIT Austrian Institute of Technology GmbH, Center for Health and Bioresources, unit Bioresources, Tulln, Austria with the Aerosol Metrology Laboratory, Institut National de Recherche et de Sécurité, Cedex, France, and the Bioaerosols Research Laboratory, Institut universitaire de cardiologie et de pneumologie de Québec – Université Laval, Chemin Sainte-Foy, Québec, Canada. The aim of this project was to compare different bioaerosol facilities and additionally to find out, if the comparison of different bioaerosol sampling devices would lead to uniform results.

1.2. Organisms

The aim of the organism selection was to find three different organisms that could be analysed at the same time. Together with the two other partner laboratories it was decided to choose one representative of each of the taxa fungi, bacteria, and virus. Especially important for the decision was, that the organisms do not negatively influence each other in the steps of cultivation and analysis. Each of the selected organism has different culture conditions regarding temperature, medium and duration of growth and therefore they can be analysed and evaluated without inhibiting the growth of each other.

1.2.1. *Penicillium chrysogenum*

Penicillium chrysogenum belongs to the genus of *Penicillia*, which contains more than 300 different species and is assigned to the ascomycetes. *Penicillia* owe their name to the structure of their aerial mycelium with a dense and brush-shaped arrangement of conidiophores, build-up of long, parallel spore chains of small, unicellular, round or oval conidia (Ksoll & Sorhage, 2012). These conidia are asexual spores which are responsible for the asexual reproduction of the fungi and can be released by wind, water, or animals. The habitat of *Penicillium chrysogenum* is indoors in stored food and damp building materials and in areas that are humid (Mokobi, 2020). Additionally, they are widespread in the nature and can live in the ground, in the grain of higher plants and because they are able to degrade cellulose, they can also live in dead plant parts. The macro-morphology of *Penicillium chrysogenum* is characterized as fast growing yellow to blue-green colonies with a white border. They often have a velvet-like and radially grooved surface (Ksoll & Sorhage, 2012). Alexander Fleming described it as a white fluffy mass that later turns green and then black (Mokobi, 2020).

The life cycle starts with the spore germination, where the spores swell through the uptake of water, germinate, and then grow a germinal thread. This germinal thread develops over time into a multicellular hypha and because of the transport of cytoplasm and substances for the cell wall synthesis by vesicles the fungi can grow on its hyphen tips. At first growth is supported by the supply of storage materials in the spore, but after some time nutrients are taken up from the medium. When the hypha is long enough, it is divided by septa which are perforated to enable the passing through of cytoplasm and transport vesicles. Branches are formed starting from the individual hyphen and in turn develop into hypha themselves by the apical tip growth, whereby they form the reticulate mycelium of *Penicillia*. Depending on their distance from the apical tip, and therefore their age, the cells of a hypha perform different tasks and because of this cell differentiation one generally speaks of heterogeneity of the fungal hypha. There are three regions which can be differentiated. The first are growing regions with apical cells on the hypha tip. Then there are non-growing regions with subapical cells, which are separated by septa.

Those are important for the transport, the nutrient uptake and they contain the organelles for the reactions of the penicillin-biosynthesis. The last classified region is found on the basis of the hypha and serves to maintain the cell pressure through the increased formation of vacuoles and therefore enables the transport of cytoplasm. This complex morphology creates special requirements for the technical implementation of industrial production processes and has led to numerous studies on the relationship between different process parameters (Ksoll & Sorhage, 2012; Schmitz, 2014).

The filamentous fungi typically generate energy through chemo-organo-heterotrophism, which means that they breakdown organic compounds through fermentation or aerobic and anaerobic breathing. These organic substances are also important as a source of carbon for building cell structures. The genus of *Penicillia* has been widely studied for its industrial production of the secreted antibiotic penicillin, which was discovered by Alexander Fleming. Also, many other natural products with biotechnological and pharmaceutical applications are produced and studied. Especially *Penicillium chrysogenum* is known as one of the most famous representatives of its genus and its biogenic performance has been significantly increased over several decades of classical strain improvement by selecting mutants (Guzmán-Chávez et al., 2018; Schmitz, 2014).

Even if the fungus has a very low pathogenicity potential, there are also incidences of opportunistic infections in immune-compromised persons. Symptoms of these can include pulmonary infection, for example pneumonia, localized granulomas and fungus balls, and systemic inflammations. They can also induce asthma and allergies by activating histamine to the epithelial lining of the lungs causing asthmatic symptoms (Mokobi, 2020).

1.2.2. *Geobacillus stearothermophilus*

The species of *Geobacillus stearothermophilus* was classified as *Bacillus stearothermophilus* until 2001. It belongs now to the genus *Geobacillus* and was first described in 1920. The genus includes a group of thermoresistant spore-forming bacteria, which are obligately thermophilic and are aerobic or facultative anaerobic using oxygen as an electron acceptor (Kotzekidou, 1999; Wells-Bennik et al., 2019). The vegetative state of *Geobacillus stearothermophilus* is rod-shaped and is not able to move (Niwin Flier, 2017). It grows on agar media plates as whitish to yellowish, round and single colonies which appear rather mucous than firm. The organism forms ellipsoidal spores that distend the sporangium. It is a gram-positive bacterium with a wide range of growth temperature from 40-75°C, but its optimum lies between 55-65°C (Burgess et al., 2017; Kotzekidou, 1999).

Spores are formed when the environmental or nutritional conditions are disadvantageous. They have a number of receptors embedded in the inner membrane that continuously monitor the environment and as soon as the conditions are favourable again, the vegetative cells are reactivated. The structure of the spores differs greatly from that of the vegetative cells and when viewed microscopically they appear as highly refractive structures. The bacterium has a permeable cytoplasmic membrane made of phospholipids and proteins, which is additionally surrounded by a peptidoglycan layer. The spores, on the other hand, have a more complex and multi-layered shell. The structural differences cause the increased resistance of spores to environmental influences such as dryness and high temperature, to chemicals and UV rays. Present as a spore, the organism can survive for several decades (Niwin Flier, 2017).

The heat resistance of the spores can be influenced by general sporulation conditions, for example temperature, cultivation medium and the chemical state of the bacterial spore. There is a wide heterogeneity within the species caused by a range of DNA base composition and a diversity of the phenotypic characters. The starch hydrolysis is positive and the reduction of nitrate to nitrite is necessary for surviving. The pH for successful growing should be at least 5.2, but the optimum range lies within 6.0 - 8.5, and most strains do not grow at NaCl concentrations over 4% (Burgess et al., 2017; Kotzekidou, 1999; Wells-Bennik et al., 2019).

The spores can be found ubiquitously in nature, they are common inhabitants of soil, desert sand, arctic waters, and hot springs, but also in food and compost. *Geobacillus stearothermophilus* is the main microorganism of beet sugar (Kotzekidou, 1999), it is included in the usual microflora of cocoa bean fermentation as well as of cocoa powder. It is a common contaminant of dairy products and can be isolated from milk powder, dried soups, and vegetables. Especially the heat resistance can be problematic because it enables the surviving of canning and sterilization processes and even ultra-high temperature treatments.

Additionally, the spores can germinate in the finished product and emerging vegetative cells can multiply and lead to spoilage, especially when it is exposed to elevated temperatures for prolonged periods of times. The spoilage results from the conversion of saccharides to organic acids without any gas formation and from the production of lipases and protease (Burgess et al., 2017; Head et al., 2008; Kotzekidou, 1999; Wells-Bennik et al., 2019).

Therefore, the spores of *Geobacillus stearothermophilus* are often used as biological indicators to validate the exposure of a product to a heat sterilization process (Head et al., 2008; Kotzekidou, 1999). Although the presence of *Bacillus* spores is not clinically relevant for humans or animals, they are an indication of an insufficient sterilisation process which can lead to the survival of pathogens (Niwin Flier, 2017). Endospores are currently used for controlling steam sterilization with a processing temperature of 121°C or higher. A long thermal processing time or severe processing conditions are needed to achieve commercial sterility (Head et al., 2008; Kotzekidou, 1999).

There are also other biotechnological purposes for the *Bacillus* genus, for example it produces a wide range of thermostable enzymes which are industrially used. It also presents a lot of advantages for the isolation of intracellular enzymes with a very good cell yield and the majority of the enzymes are rather resistant to the action of other protein denaturants (Burgess et al., 2017; Kotzekidou, 1999). Another potential usage of the bacterium is in the production of sustainable energy, like biofuels and the generation of bioactive molecules used as therapeutics. This is enabled by the robustness and the acceptance of adverse environmental conditions, which leads to an easy grow to very high densities (Kumar et al., 2013). Additionally important is the insect pathogen *Bacillus thuringiensis*. During sporulation the bacterium expresses *cry* genes, which enable the production of toxic crystal proteins (Peng et al., 2019). At first *Bacillus thuringiensis* was widely used as a pesticide, nowadays plants have been genetically engineered to contain those *cry* toxins and different insect-pest-resistant *Bt* crops have been established, for example corn, potato, and cotton as well as tobacco (Abbas, 2018).

1.2.3. Bacteriophage PhiX174 and its host

The PhiX174 bacteriophage belongs to the family of Microviridae bacteriophages and was first isolated in 1935. It has a small genome size with 5.386 nucleotides and is a small icosahedral and tailless virus with a maximum diameter of 320 Angstrom. The DNA is circular and single stranded, and the genome encodes four structural proteins: the DNA pilot protein H, the capsid protein F, the genome-associated protein J and the spike protein G. The exterior of the bacteriophage is composed of 60 F proteins forming the capsid, which is decorated by 12 spikes on the vertices, each containing five G proteins. Those two protein types consist both of eight anti-parallel β -strands which build up a β -barrel motif. On the other hand, the interior of the capsid possesses 60 copies of the J protein and 10-12 copies of the H protein, which emerges from the capsid as a tube on the cell surface. Although the DNA is small, it encodes 12 genes by using overlapping reading frames. These genes are among other molecular factors responsible for the DNA replicating, scaffolding and lysis functions (Christakos et al., 2016; Michel et al., 2010; Sun et al., 2017).

While tailed bacteriophages use their preformed tail for transporting their genomes into a host bacterium by host cell recognition, absorption, and penetration of the bacteria cell walls, it is still not completely solved how tailless bacteriophages perform those functions. The PhiX174 binds to the lipopolysaccharides of bacterial cells through its pentameric spikes. After the dissociation of the spikes, there are conformational changes in the major capsid proteins, which cause DNA ejection through preformed tubes consisting of viral H proteins. This mechanism enables the bacteriophage to protect its DNA conduit until a specific target is identified (Sun et al., 2017).

The PhiX174 possesses a non-pathogenic status and is therefore used in many experiments and diverse applications. In those included is the development of novel imaging reagents, the selection of peptide or protein binders through phage display, the production of biosensors and for antigen display platforms for serological assays. It is also used in epitope mapping, bio-nanoconjugate applications and vaccine development. The most common host of PhiX174 is *Escherichia coli*, and it can therefore be used as an indicator of viral or fecal contamination in aquatic environments. *Escherichia coli* possesses a specific rough lipopolysaccharide that is recognized by the coliphage receptor and enables the phage adsorption. The DNA replication, however, can be supported by different *Escherichia coli* strains and related bacteria, for example *Pseudomonas aeruginosa*. Therefore, it can be concluded that the limiting step of an infection with PhiX174 is not the replication or lysis, but the entry of the bacteriophage into the host cell (Christakos et al., 2016; Michel et al., 2010).

Escherichia coli belong to the bacterial family of Enterobacteriaceae, which are a member of the gamma-proteobacteria, containing 40 genera and 200 species. First found and described in 1885 by Theodor Escherich, the bacterium is nowadays one of the best studied organisms in the world. It is a gram-negative rod bacterium, which cannot build spores and is not acid resistant.

Escherichia coli is on the one hand one of the most important pathogens, because it is responsible for a wide spectrum of diseases in humans and animals. On the other hand, it is the most common inhabitant of the gastrointestinal tracts and lives in a mutually beneficial association with hosts, including humans and warm-blooded animals. The facultative anaerobic bacterium can produce energy by aerobic respiration if oxygen is present and can also switch to mixed-acid fermentation in the absence of oxygen. The presence of *Escherichia coli* in the intestine leads to a continuous training of the mucosal immune system and promotes the production of the secretory immunoglobulin A. Because of the easy handling, its ability to grow under aerobic and anaerobic conditions and the availability of the complete genome sequence it is an important host organism in biotechnology and therefore widely used in the industrial and the medical area, as well as in the field of recombinant DNA technology (Allocati et al., 2013; Reichardt, 2010).

1.3. Bioaerosol system

To investigate different microorganisms, it is necessary to study them under controlled and defined conditions. These conditions can be produced in bioaerosol test systems, comprising of an aerosol generator, a chamber, control or monitoring devices and sampling equipment.

1.3.1. Bioaerosol Chamber CCB3000

The laminar-flow-based bioaerosol test system used in this study, is able to generate a homogenous and constant supply of aerosolized cells. These aerosols are of a known concentration and defined culture ability and are collected by different biological sampling devices positioned in the chamber. It is possible to use the bioaerosol chamber for the comparison of amount and viability of the microbial cells before, during and, with a Gas Washing Bottle, after the aerosolization process. An advantage of the chamber, in contrast to the natural environment, is the homogenous distribution of cells and the constant ratios in culture ability. Additionally, each bioaerosol sampler can be placed on a predefined position inside the chamber and therefore the biological sampling efficiency can be quantified (Pogner et al., 2019; Unterwurzacher et al., 2018).

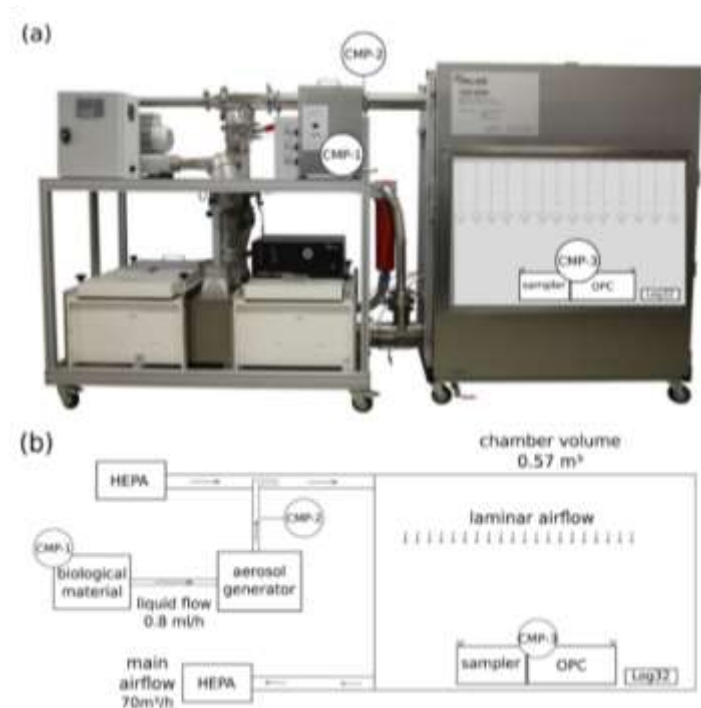


Figure 1 shows the bioaerosol test system with its main components and control and monitoring points (Pogner et al., 2019).

Figure 1: Bioaerosol test system with main components and control and monitoring points (CMP). (a) CMP-1 symbolizes the Starting Suspension, CMP-2 the Gas Washing Bottle, CMP-3 the different sampling devices and an optical particle counter (OPC), which is used for the evaluation of particle size distribution and particle enumeration. The Log32 is a data logger for temperature and relative humidity. The dashed arrows inside the chamber symbolize the laminar airflow. The OPC and Samplers are placed on a perforated metal plate to avoid any disturbance of the laminar air flow. In this master thesis a different aerosol-generator than shown in the picture was used. (b) schematic representation of the aerosol chamber system, with directions and volumes of air, aerosol, or liquid flow. The liquid flow was 1.66 ml/h in this thesis. (Taken from Pogner et al 2019)

The CCB 3000 consists of two units. The first one is used for the preparation of airflow and for the aerosol production. It also contains HEPA filter units (H13) to ensure clean incoming and outgoing air and a coalescence filter unit for the liquid separation during the cleaning and disinfection phase. The other unit of the chamber contains the testing space with an interior space of 0.72m² and a height of 79cm resulting in a total volume of 0.57m³. It can be combined with different components depending on the experimental setup. Different aerosol generators can be attached to the chamber for injecting aerosols into the main airflow. Before the turbulent main airstream heads to the testing part of the chamber it is converted into a laminar airflow from the top of the chamber to the bottom. To avoid turbulences at the point of sampling, two perforated metal plates at the top and at the bottom are mounted in the chamber. This assembly ensures an evenly distribution of particles with an aerodynamic diameter of 10nm, which is typical for virus-particles, up to a diameter of 30µm, typical for pollen. Samplers and other devices are placed on the bottom of this metal plate. Underneath, the airstream leaves the chamber again and is cleaned by a subsequent HEPA filter. The volume flow rate and therefore the laminar airflow inside the chamber can be adjusted between 20 and 100 m³/h. Konlechner et al., 2013 showed that the operation of measuring and collecting equipment has no significant influence on the constancy of the bioaerosol concentration and the particle distribution in the chamber. To avoid any contaminations from the environment, the chamber is operated with a slight negative pressure, which can be regulated through the disc valve of the exhaust air (Konlechner et al., 2013; Pogner et al., 2019; Unterwurzacher et al., 2018).

1.3.2. Liquid Sparging Aerosolizer

In this study a Liquid Sparging Aerosolizer (LSA, CH Technologies) was used, which is specifically designed for low air pressure aerosolization of sensitive and delicate microorganisms (Figure 2). It

minimizes physical damage to the organisms because it does not use sheering and impaction processes to nebulize the spore suspension. The underlying mechanisms is the transportation of spore suspension onto the porous disk (Diameter 2.54cm, stainless steel, 0.2 μm pore size) of the LSA by a peristaltic pump (Peristaltic Pump P-1, Ge Healthcare Bio-Sciences AB, Sweden). On this disk it forms a thin suspension film, and the air is sparged through the disk into the film. This process results in the breakage into bubbles that subsequently burst and release particles into the air. The sparging air stream is able to catch these released particles and carry them to the outlet (Mainelis, 2005).



Figure 2: Transport of the starting suspension in the shake flask, standing on a magnetic stirrer, to the LSA by the usage of a peristaltic pump. The aerosolized particles in the LSA are then transported to the chamber by the compressed air, used for the aerosolization process.

The LSA can produce particles in a low concentration range down to several hundred particles per m^3 . By the usage of a magnetic stirrer, the organism suspension is slowly stirred to keep the suspension homogenous throughout the whole experiment. The airflow of compressed air passing through the LSA was set in previous experiment with the bioaerosol chamber between 250 L/h and 1000 L/h. The majority of the particles impinge the glass, and the liquid droplets are not captured by the air stream. They are collected at the bottom of the LSA and play no further role in the aerosolization process. To determine the fraction that is nebulized and reached the main air stream through the outlet, the weight of the reservoir with spore suspension was determined before and after aerosolization (CH Technologies, n.d.; Konlechner et al., 2013; Pogner et al., 2019; Zhen et al., 2014).

1.3.3. Aerosol Sampling Devices

In this study, two methods for bioaerosol sampling were used and compared to each other.

1.3.3.1. 37mm Closed Face Air Sampling Cassettes



The 37mm closed face air sampling cassettes (Zefon International, Nr. 738PC-PP) are fully assembled, already containing the polycarbonate Track-Etch 0.8 μ m membrane filter and were operated using a Gilian 5000 pump (Sensidyn), placed inside the chamber. The pump is connected via a plastic tube to one side of the cassette, whereas it enables the sampling of particles through an inlet opening on the other side. The inlet and the outlet opening can be both closed again after the sampling process by associated plugs.

Figure 3: 37mm closed face air sampling cassettes – containing a polycarbonate Track-Etch membrane filter

1.3.3.2. SKC- BioSampler

The BioSampler Impinger (SKC Inc.) is a three-piece glass sampler, which includes an inlet section, and outlet section and a collection vessel (Figure 4). The pieces can be separated to allow a complete sterilization process by autoclaving. While the inlet section is placed facing to the inside of the chamber, the outlet section is connected to a vacuum pump (Edwards RV3), standing outside of the chamber. The pump is connected to the BioSampler through tubing and a conduit in the chamber wall. In contrast to the all glass impinger (AGI) the BioSampler has three nozzles inside the collection vessel. Through the airflow build up by the pump, the nozzles are causing the collection solution to swirl upward on the inner wall and thereby it gently removes the collected particles. This swirling motion builds up only a few bubbles and can minimize the re-aerosolization of the particles. Additionally, the three tangential

nozzles eject particles at an angle to the sampler's inner wall which limits the bouncing of particles and preserves aggregates of organisms (SKC, Inc., n.d.).



Figure 4: BioSampler Impinger – SKC BioSampler, on the top of the three-piece glass sampler is the inlet section, followed by the outlet section and on the bottom is the collection vessel, which is filled with buffer solution.

2. Material and Methods

The key components of the experimental part of the study are the cultivation and subsequent aerosolization and sampling of the three chosen microorganisms by two different techniques. Afterwards, the sampled material should be analysed using micro- as well as molecular-biological analysis methods. Different media and cultivation protocols as well as different types of analysis were used to test the efficiency of the two sampling devices and to be able to compare them for each of the three organisms.

2.1. Organisms

The three organisms were chosen in consultation with the two partner groups, each choosing one organism and making it available to the other laboratories. This process ensures that all experiments were carried out with the same starting organisms and that the results can therefore be compared. The following list gives details about their origin.

- The *Penicillium chrysogenum* was obtained as an environmental isolation by an air sampling process and stored at -80°C in the strain-collection of the Austrian Institute of Technology. (ID: AIT_FC1127)
- The *Geobacillus stearothermophilus* was obtained from Bioquell HPV-BI, Biological Indicator Type: 6-log *Geobacillus stearothermophilus* ATCC 12980 and was delivered on 2019-10-31.
- The bacteriophage PhiX174 and the bacterial host strain C (13706) were obtained by the Félix d'Hérelle Reference Center for Bacterial Viruses from the Université Laval (Québec, Canada).

In order to keep the following chapters clear and uniform, the following abbreviations are used:

- P. chryso for the *Penicillium chrysogenum*
- G. stearo for the *Geobacillus stearothermophilus*
- PhiX174 for the bacteriophage PhiX174

2.2. Culture and Harvest

To be able to analyse the chosen organisms in the bioaerosol chamber, detailed culture and harvest protocols have been established to ensure a consistent implementation. All protocols for the used media are described in detail, with the exact components and weights stated.

2.2.1. *Penicillium chrysogenum*

To successfully culture the *Penicillium chrysogenum* strain, 100µL of a cryostock were plated out onto Malt extract agar (Merck; mix 48g/L and sterilize by autoclaving) and grown for 10 to 14 days at 28°C. To grow enough spores, it is important that the fungi get enough air and are not stored upside down. Cryostocks were made by harvesting a culture grown on solid medium in a plate with a spatula used to sharp the fungi off the agar plate. All the material was collected in 1x PBS 0.01% Tween buffer (7.68g/L NaCl, 0.192g/L KCl, 1.3824g/L Na₂HPO₄, 0.2304g/L KH₂PO₄ mixed with 100µL/L Tween20 (Roth) sterilized by autoclaving). It was shortly mixed with a Vortex Genie 2 (Roth) and afterwards diluted in a 1:1 ratio with 40% Glycerine 1x PBS (Glycerine (Roth) is diluted with 1xPBS to a final concentration of 40%). Cryostocks were stored at -80°C.

The plates were controlled under the microscope for enough mature spores and afterwards they were harvested. All the material of one plate was scraped off with a spatula and collected in a sterile 15mL tube filled with 5mL of the buffer 1xPBS 0,01%Tween. The suspension was then mixed for 5 minutes with a Vortex Genie 2 at 2700 rpm. After that, it was filtrated twice with an autoclaved 10mL syringe (Braun) filled with sterilized glass wool (VWR), to get rid of agar pieces and other residues like mycelia. The spore suspension was then diluted with the 1xPBS 0.01%Tween buffer to a concentration which was countable in the microscope (magnification: 40x) with a Neubauer improved C-chip (In Cyto C-Chip, n.d.). This counted concentration of the fungi was later used to calculate the germination rates for each experiment.

To reach the desired concentrations for the experiments, different amounts of plates were harvested. With one optimal culture grown on solid medium in a plate, around 1×10^7 spores/mL can be reached. If the cultures on the media plates did not grow evenly and the spore count in the microscope was too low, additional plates were harvested, mixed with the already prepared suspension, and counted again. If the concentration was too high, it was diluted with the buffer 1x PBS 0.01% Tween and counted afterwards.

2.2.2. *Geobacillus stearothermophilus*

With the original material from Bioquell, cryostocks could be made by plating a small amount of the bacteria-powder mixed with 100µL 1xPBS buffer onto a SVIII medium plate (10g/L peptone, 5g/L yeast extract, 5g/L meat extract, 1.3g/L K₂HPO₄, 0.1g/L MgSO₄, 0.6g/L Sucrose, 15g/L Agar-Agar mixed and sterilized by autoclaving) and let it grow for 3 days at 50°C. Cryostocks were made by harvesting the culture grown on solid medium in a plate in 1xPBS and then diluted in a 1:1 ratio with 40% Glycerine 1xPBS and stored at -20°C. Additionally some of the cryostocks were stored at 4°C to use them for the overnight cultures needed for the experiments to avoid any big temperature differences.

Plate culture for the mixing experiments

For the cultivation of the bacterium, 12 SVIII medium were plated with 100µL of the cryostock and grown for 3 days at 50°C. Afterwards the plates were harvested with a spatula, transferring material from each plate in 1mL of 1xPBS, and the NaBr Gradient protocol (Table 1: Preparation protocol with defined quantities for all ingredients for the NaBr solutions) was carried out. After the last washing step of the pellets with 1xPBS, they were dissolved in 30mL 1xPBS.

Liquid culture for the aerosol chamber experiments

For the liquid culture of *Geobacillus stearothermophilus*, 100µL of a cryostock was grown over night in 25mL TSB (17g/L Tryptone, 3g/L Peptone, 2.5g/L Glucose, 5g/L NaCl, 2.5g/L K₂HPO₄ mixed and sterilized by autoclaving) at 50°C and 180rpm.

After shaking the overnight culture for approximately 16-18 hours, the optical density at a wavelength of 600nm (OD₆₀₀) was measured with cuvettes (Brand, 1.0mL macro) in a photometer (Bio-Rad Smart Spec™ Plus Spectrophotometer) and expected around 0.6 to 0.8. The entire 25mL of the culture were centrifuged for 5 minutes at 3000 rpm (Eppendorf Centrifuge 5810R) and the pellet was resuspended within 2mL 1x PBS. The OD₆₀₀ was measured again in a 1:10 dilution with 1xPBS and should be between 0.35 and 0.40. To produce higher volumes for the planned experiments the volume of the overnight culture, and therefore the volume of the cryostock, TSB and 1xPBS were upscaled.

2.2.3. Bacteriophage PhiX174

For the cultivation of the host bacterium and the bacteriophage PhiX174 some preliminary considerations had to be made. The bacterial host strain was stored in a freezer at -80°C and cryostocks can be made by mixing an overnight culture (for example: $100\mu\text{L}$ in 20mL TSB, shaken for 12 hours at 37°C and 180rpm) with 40% glycerol (Roth) in a 1:1 ratio. Those can be stored in sterile tubes each containing 1mL at -20°C . The host strains might become resistant to the bacteriophages; therefore, it is better to never use an old culture which might have changed or been contaminated. This information as well as the following culture protocol was provided from Nathalie Turgeon from the partner laboratory at Université Laval.

To gain a good titer of the bacteriophages, it was necessary to perform two successive phage amplifications. The first amplification was started from the original frozen stock stored at -80°C , while the second amplification was started from the first one. Because the phages and the host strain can mutate, the original stock of the phage culture is the best. Therefore, it is important to keep track and label the date of all amplifications and it should be avoided to perform third and fourth amplifications. The suspension of the first amplification can be stored in sterile tubes each containing 1mL at 4°C .

To grow the bacteriophages, a culture of the host bacterium was needed first. For that $100\mu\text{L}$ cryostock of the bacterial host strain was grown overnight in 25mL TSB at 37°C and a shaking rate of 180rpm for approximately 16 hours. At the next morning, the OD_{600} of the culture was measured in a 1:10 dilution with TSB with cuvettes (Brand, 1.0mL macro) in a photometer (Bio-Rad Smart SpecTM Plus Spectrophotometer) and was expected around 0.30 to 0.35. To make sure that the bacteria were in the exponential growth phase when the bacteriophages are added, $500\mu\text{L}$ of the overnight culture were heavily diluted within 50mL TSB and shaken again at 37°C and 180rpm . When the OD_{600} reached a value of 0.1 – 0.2, which took about one hour, $50\mu\text{L}$ suspension of the first amplification of the phages were added and the culture was shaken again at 37°C and 180rpm . Now every hour the OD_{600} was measured and should continuously get lower. In theory the OD should decrease to 0, but it often stagnates at 0.05 or lower. As soon as the OD was in that low range the culture can be used for the upcoming experiments. The culture needed to be centrifuged for 15 minutes at 4000rpm (Eppendorf, Centrifuge 5810R) and then filtrated through a 10mL syringe (Braun) and a $0.45\mu\text{m}$ syringe filter (Roth, Rotilabor nylon) to get rid of the remaining bacteria and obtain the phage lysate.

To control the growth of the phages and to prove that there are no contaminations or mutated host bacteria a negative and a positive control were needed. While the negative control was 5mL TSB in a sterile 15mL tube, the positive control was 5mL of the heavily diluted overnight culture of the host bacterium in a sterile 15mL tube. Both tubes were also shaken at 37°C and 180rpm and the OD_{600} was measured every hour. The OD_{600} of the negative control should always be close to zero because nothing should grow in it to make sure that the TSB medium is not contaminated.

The OD₆₀₀ of the positive control should increase more and more because it does not contain any phages and the bacteria can therefore grow exponentially without getting killed by them. The OD₆₀₀ of the positive control should in any case overtake the one of the actual phage culture, to prove that the phages actually kill the bacteria and grow in the culture.

For the planned experiments the volume of the needed phage lysate was higher, therefore all values of solutions were upscaled and calculated beforehand.

2.3. Optimization of *Geobacillus* Culture Methods

To establish the best cultivation conditions for the *Geobacillus stearothermophilus* some experiments were done beforehand.

2.3.1. Culture Conditions

At first different media were used to culture the bacterium overnight and were compared via OD₆₀₀ measurements and observations with the microscope. Additionally, each medium, described in the following list, was prepared as agar plates, and used to cultivate the bacterium on the plates at 50°C for three days for the comparison of the growth.

- TSB (see 2.2.2)
- SVIII (see 2.2.2)
- LB (10g/L Tryptone, 5g/L Yeast extract, 10g/L NaCl are mixed and sterilized by autoclaving)
- TGB (17g/L Tryptone, 3g/L Peptone, 2.5g/L K₂HPO₄, 5g/L NaCl mixed, pH around 7.3, sterilized by autoclaving, after cooling down 10mL/L Glycerine and 10g/L Sodium Pyruvate are added)

Secondly three different preparation protocols were compared to obtain a higher cell or spore concentrations. Each protocol was carried out with all different media and the final suspensions were observed in the microscope.

2.3.2. Spore Preparation Protocols

Difco Sporulation Protocol

1. The bacteria were grown overnight at 50°C and 200rpm in the respective medium.
2. The OD₆₀₀ of the overnight cultures were measured, and the cultures were diluted with the respective medium to obtain an OD₆₀₀ of around 0.1-0.2. The cells were then grown again until reaching an OD₆₀₀ of 0.8.
3. Of each culture 10mL were centrifuged at 4000rpm for 5 minutes, afterwards the pellets were each washed with 5mL 1xPBS and re-suspended in 5mL Difco Sporulation Medium (8g/L Bacto nutrient broth (Difco), 10mL/L 10% (w/v) KCl, 10mL/L 1.2% (w/v) MgSO₄ x H₂O, 1.5mL/L 1M NaOH with a final pH of 7.6, are mixed, autoclaved and cooled down to 50°C.

Then: 1mL/L 1M Ca(NO₃)₂, 1mL/L 0.01M MnCl₂, 1mL/L 1mM FeSO₄ were added) and let grown for 24 hours at 50°C and 200rpm.

4. The overnight cultures are treated with lysozyme to kill the cells and leave the spores purified. For that, 1mg/mL Lysozyme (Roche) was added, and the samples were incubated again at 50°C for 20 minutes. Afterwards they were washed 3 times with 1xPBS (in between centrifuged for 3 minutes at 4000rpm) and finally re-suspended in 10mL 1xPBS.

NaBr Gradient Spore Purification Protocol

(By Christian Laflamme and Nathalie Turgeon, updated February 24, 2015)

1. The NaBr (Sigma) solutions were prepared (
2. Table 1) and the density of every solution was checked by measuring the weight of 1mL. Afterwards they were sterilized by autoclaving.

Table 1: Preparation protocol with defined quantities for all ingredients for the NaBr solutions

Density (g/mL)	NaBr quantity (g)	Water quantity (mL)	Final volume (mL)
1.5	30	35	43
1.4	25	38	45
1.3	20	41	46
1.2	15	45	50
1.1	7.5	50	51.5

3. In a 10mL sterile tube a NaBr gradient was prepared by first adding 1mL of the NaBr solution 1.5g/mL density. Afterwards a 3mL syringe (Braun) and a needle (Braun, Sterican) were used to add 1mL of each of the other gradient layers in the following order: 1.4g/mL, 1.3g/mL, 1.2g/mL, 1.1g/mL and 1.0g/mL (water). It was important to place the needle on the side of the tube to make sure, that the solution is running slowly down to the top of the gradient.
4. With the usage of a new sterile syringe and needle, 1mL of the spore suspension was added on the top and the tube was centrifuged at 2400g at room temperature for 45 minutes (Eppendorf, Centrifuge 5810R).
5. The tube was carefully removed from the centrifuge, and the solution should be separated into three different phases. They were removed one at a time and placed in different 1.5mL tubes. Especially the bottom layer should contain the most concentrated spores. Each sample was washed 5 times with 1mL 1xPBS to remove the NaBr (in between centrifuged at 13.000g for 1 minute (Eppendorf, Centrifuge 5425)) and the pellets were each re-suspended in 1mL 1xPBS.

Additional Purification Protocol

The final solutions of the NaBr protocol were treated with 1mg/mL lysozyme and incubated at 50°C for 20 minutes. Afterwards they were washed again 3 times with 1mL 1xPBS and finally re-suspended in 1mL 1xPBS.

2.3.3. Evaluation of sporulation efficiency

To check the concentration of the *Geobacillus stearothermophilus* spores and estimate the ratio of spores to cells, the staining procedure according to RAKETTE (listed in the Microbiology Handbook of MERCK 1996/1997) was used. All obtained suspensions of the three preparation protocols were stained and examined under the microscope.

1. To get rid of fatty residues, the glass slide is lead through a flame.
2. Two drops of the sample were brought onto to glass slide, and it was left to dry.
3. The sample was fixated by leading the glass slide again through the flame.
4. The glass slide was covered with 5% malachite green (Sigma, 0.5g in 10mL water), hold into the flame for 20 seconds and left to be absorb for 30 seconds.
5. The sample was washed carefully with water for 30 seconds.
6. The glass slide was covered with safranin O-solution (Sigma) and hold into the flame for 30 seconds.
7. As the last step, the sample was washed carefully with water and dried.

2.3.4. Evaluation of aerosolization efficiency

Beside the staining of the *Geobacillus stearothermophilus* spores and cells, the suspensions obtained by different protocols were also analysed in the bioaerosol chamber. Three different protocols were chosen to be tested in the chamber and the suspensions were observed separately by aerosolization and the usage of the gas washing bottle.

1. overnight culture in TSB
2. cultivated on SVIII medium agar plates
3. SVIII medium agar plates harvested and treated with the NaBr sporulation protocol

2.4. Bioaerosol Chamber Settings

To ensure a consistent aerosol production and dispersion in the bioaerosol chamber, different settings had to be established in the beginning. All of them were set the same throughout all the different experiments, to make sure that the gained concentrations are reliable and comparable. To produce aerosols an aerosol generator is connected to the chamber and the aerosolized particles are transported into the chamber with an air stream. The settings for the Chamber CCB3000, the aerosol generator and the Gas Washing Bottle can be found in the following table.

Table 2: Summary of the device setting in the bioaerosol chamber experiments

Device	Parameter	Value
Bioaerosol Chamber CCB3000	Flow rate	70 m ³ /h
	Flow pressure	190 mbar difference pressure
Aerosol Generator LSA	Air flow	750 L/h
	Liquid flow	1.66 mL/min
Gas Washing Bottle GWB	Collection time	40 minutes
	Sampling liquid	40mL of 1x PBS 0.01% Tween

2.5. Sampling Devices

To sample bioaerosols in the test-system, the collection of the airflow directly after generation was done with a gas washing bottle and in the testing chamber two sampling devices with different sampling methods were positioned.

2.5.1. Control Devices

The aerosolization process may have an influence on the cell viability and therefore a potential loss of culture ability. To monitor this, the samples were measured in the initial suspension mixture and the aerosolized particles were collected directly after the generator into a 500mL gas washing bottle (DURAN DWK – LifeSciences). The gas washing bottle is directly connected to the air outlet of the LSA, allowing the aerosolized particles to be forwarded into the buffer solution inside the bottle (Figure 5). On the other side, another tube is connected directly via a plastic hose to the chamber (Pogner et al., 2019).



Figure 5: The Gas Washing Bottle (GWB) is directly connected to the LSA and the bioaerosol chamber. Therefore, the aerosolized particles are forwarded from the LSA into the buffer solution inside the GWB and then into the chamber.

After sampling with the gas washing bottle, it was removed from the set up and the LSA was directly connected to the bioaerosol chamber, allowing the particles to be forwarded into the sampling space. Each following experiment started with a seven-minute pre-run to achieve stable conditions within the chamber before the beginning of the sampling process.

It is important to control and monitor bioaerosol generation, particle counts and physical parameters such as temperature or relative humidity to gain an overview over the experimental conditions. An optical particle counter (Portable Aerosol Spectrometer, GRIMM 11-C) was used to monitor the steady production of aerosols (Figure 6). It enumerates the particles and evaluates the particle size distribution in 31 channels. It can count particles even in low numbers, by using a high-volume isokinetic sampling process and measures particles in the size from 0.253 - 35.15 μm . For measuring the temperature and the relative humidity during the whole time of every experiment, a Datalogger Log 32 (DOSTMANN Electronic GmbH) was used (Figure 7).



Figure 6 (Left): Optical particle counter – Portable Aerosol Spectrometer, GRIMM 11-C

Figure 7 (Right): Datalogger Log32 – Dostmann Electronic GmbH

2.5.2. 37mm Closed Face Air Sampling Cassettes

The sampling cassettes were operated via a Gilian 5000 pump with a volume flow of 3.5 L/min. The pumps were placed inside the chamber, directly connected to the filter cassettes via a plastic tube. The seven minutes delay and sampling time of 60 minutes can be programmed in the pump's operation conditions, and they start automatically. After sampling for one hour in the bioaerosol chamber, the filter cassettes were taken off the pumps and filled with 10mL of the buffer 1xPBS 0,01% Tween using a 10mL sterile syringe (Braun) and a needle (Braun, Sterican Ø80 x 120mm, 21G x 4 ¾"). To suspend the sampled particles, the filter cassettes were shaken for 20 minutes at 2000 rpm with a Vortex Genie 2 (Roth). The suspension was recovered with the syringe and needle and transfer into sterile tubes. This suspension was used to proceed to all the different analysis, as well as to take aliquots of 1mL for storage at -80°C and at -20°C (Protocol from (Simon et al., 2013)).

2.5.3. SKC BioSampler

While the inlet section is placed facing to the inside of the chamber, the outlet section is connected to a vacuum pump (Edwards RV3), with a sampling flow rate of 12.5 L/min. The pump is placed outside the chamber, to enable the activation of the sampling devices individually without opening the chamber and thereby avoiding disturbance of the laminar airflow.

Sampling was performed for 20 minutes in 20mL 1xPBS 0.01% Tween. The sampling was simultaneously to the filter cassette and in the middle of its sampling time, resulting in following procedure: 20 minutes only the filter cassette, 20 minutes cassettes and BioSampler, 20 minutes only the filter cassette.

In contrast to the filter cassettes, no additional processing step was necessary with the BioSampler. Before the suspension was obtained with a pipette and transferred into a sterile tube, it was important to shake the sample within the BioSampler properly. This ensured that no particles have sedimented on the ground of the glass beaker. The obtained suspension was used for all the different analysis and also to take aliquots of 1mL for storage at -80°C and at -20°C.

2.6. Analysis

The suspensions collected in the sampling devices, as well as the starting suspension (STS) and those obtained from the gas washing bottle (GWB) were qualitatively and quantitatively analysed by using microbiological and molecular-biological protocols. The obtained data were compared and possible correlations between the different sampling devices were calculated.

2.6.1. Colony Forming Units (CFU) or Plaque Forming Units (PFU)

2.6.1.1. *Penicillium chrysogenum*

All obtained suspensions (STS, GWB, Filter cassette, BioSampler) were counted through the microscope with the use of a Neubauer improved C-Chip (In Cyto C-Chip, n.d.). If there were too many spores to count, the suspension was diluted with the buffer 1xPBS 0.01% Tween. After counting, the spore count was calculated, and an appropriate concentration for plating was chosen to reach between 30 to 120 expected colonies per plate. The correct concentrations were plated in triplets on malt-extract-agar plates and grown at room temperature until the colony forming units were counted after three days.

2.6.1.2. *Geobacillus stearothermophilus*

To cultivate the bacteria, the suspensions from all devices were plated onto SVIII agar; the plates were closed with tape and grown at 50°C for two days, before counting the CFUs. The plates were stored upside down to avoid dripping of condense water onto the plates. To make sure that the plates were countable, the suspensions were diluted with the buffer 1x PBS and for all obtained suspensions (STS, GWB, Filter cassette, BioSampler) two or three different concentrations were plated in triplets.

2.6.1.3. Bacteriophage PhiX174

To cultivate the bacteriophages an addition of the host bacteria was needed. The suspensions obtained in the experiments were diluted with the phage SM buffer (5.8g/L NaCl, 2g/L MgSO₄ x 7H₂O, 50mL/L 1M Tris-HCl pH=7.5, 5mL/L 2% Gelatine mixed and sterilized by autoclaving) in 1:10 dilution steps to obtain a final concentration which would result in 10 – 80 plaques per plate. 100µL of the suspensions was mixed with 100µL of a bacterial host overnight culture and 4mL of soft agar (17g/L Tryptone, 3g/L Peptone, 2.5g/L Glucose, 5g/L NaCl, 2.5g/L K₂HPO₄, 7.5g/L Agar-Agar mixed and sterilized by autoclaving, stored at 50°C) in a glass eprouvette.

The gained suspension was mixed for a few seconds with a vortex device and poured onto TSA plates (17g/L Tryptone, 3g/L Peptone, 2.5g/L Glucose, 5g/L NaCl, 2.5g/L K₂HPO₄, 15g/L Agar-Agar mixed and sterilized by autoclaving) and left for a couple of minutes to let the soft agar solidify. The plates were grown at 37°C for one day before the plaques could be counted.

The suspensions from the devices were plated in three different concentrations, each in triplets, and additionally for each device an approximate titer was done. For that, 100µL of the host overnight culture was mixed with 4mL of soft-agar and poured onto a TSA plate. The soft agar was left to solidify and then the plate was divided into 8 zones. A serial dilution, of the suspensions to be tested, in steps of 1:10 was prepared from 10⁻¹ until 10⁻⁸ using the phage SM buffer. After this, 5µL of each dilution was dropped on a separate zone of the agar plate. It was important to avoid the moving of the drops and the production of aerosols, otherwise there would be plaques all over the plate. The plates were also grown at 37°C and after one day, the titer can be estimated by reading the latest dilution where plaques are visible.

2.6.2. Molecular Biology

2.6.2.1. DNA Isolation

For the molecular-biological analysis of *Penicillium chrysogenum* and *Geobacillus stearothermophilus* the DNA was isolated before further analysis. Two slightly different protocols were used for this step.

The samples of the experiments with the bioaerosol of each organism were isolated with the Qiagen DNeasy PowerSoil Kit with some modifications (Modifications established by (Unterwurzacher et al., 2018)). At first 1000µL of the sample was mixed with 600µL Power Beat Solution and 60µL C1 Solution in a 1.26g lysing Y-Matrix (MP Biomedicals) containing sterile tube. In the following step the cell disruption was performed for 60 seconds at 6.5 m/s within a FastPrep-24 (MP Biomedicals). The samples were heated at 65°C for 10 minutes and then the cell disruption and the heating step were repeated. The rest of the protocol was executed as described by the manufacturer, starting at step 5 by centrifuging the tubes.

The samples of the main experiments were isolated with the Qiagen DNeasy PowerSoil Pro Kit with also some additional changes (Modifications established by INRS). At the beginning 500µL of the sample was mixed with 800µL of the solution C1 within a PowerBead Pro Tube, which was spinned down briefly beforehand to ensure that the beads have settled at the bottom. The solutions were mixed by vortexing the tube briefly. Then the cell disruption was performed for 30 seconds at 6.5m/s with a FastPrep-24 (MP Biomedicals) After a 30 second pause, the cell disruption was repeated with the same settings. After these additional steps, the protocol of the kit was followed, starting at the centrifuging at step 3.

For the analysis of the bacteriophages, the samples were used directly, without any additional preparations. All samples were quantified using real-time PCR.

2.6.2.2. Real-time quantitative Polymerase-chain-reaction

The real-time quantitative Polymerase-chain-reaction (qPCR) is used for the detection and quantification of microorganisms and is known to be highly sensitive, specific, and fast. In comparison to the end-point PCR it doesn't require post-amplification manipulations and more accurate template quantification is possible over a wide dynamic range. It limits the chance of contamination and if proper controls are used, it seems to be the most accurate and reliable technique for the enzymatic amplification of specific DNA sequences (Postollec et al., 2011).

The principle of quantitative PCR is based on conventional PCR that uses two oligonucleotide primers that hybridize to opposite DNA strands and flank the region of interest in the target DNA. In the repetitive series of amplification cycles the template nucleic acid is denatured, annealed with the primers, and extended to generate a complementary strand by the usage of a DNA polymerase. This process results in the exponential accumulation of a specific fragment whose termini are defined by 5' end of the primers (Atawodi et al., 2010; Postollec et al., 2011).

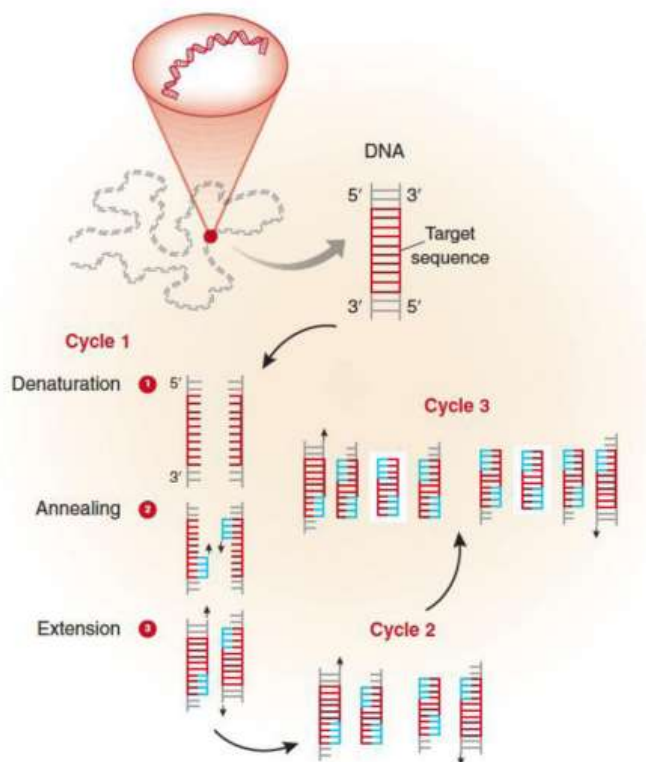


Figure 8: Schematic Representation of the different steps in a conventional PCR (Garibyan & Avashia, 2013).

With the usage of a qPCR, the increase of amplification products can be monitored at every cycle using a fluorescent reporter. This increase is plotted against the cycle number to generate the amplification curve. The cycle threshold value C_q can be calculated with this curve because it corresponds to the initial concentration of target nucleic acid. The C_q value serves as a basis for absolute or relative template quantification (Atawodi et al., 2010; Postollec et al., 2011).

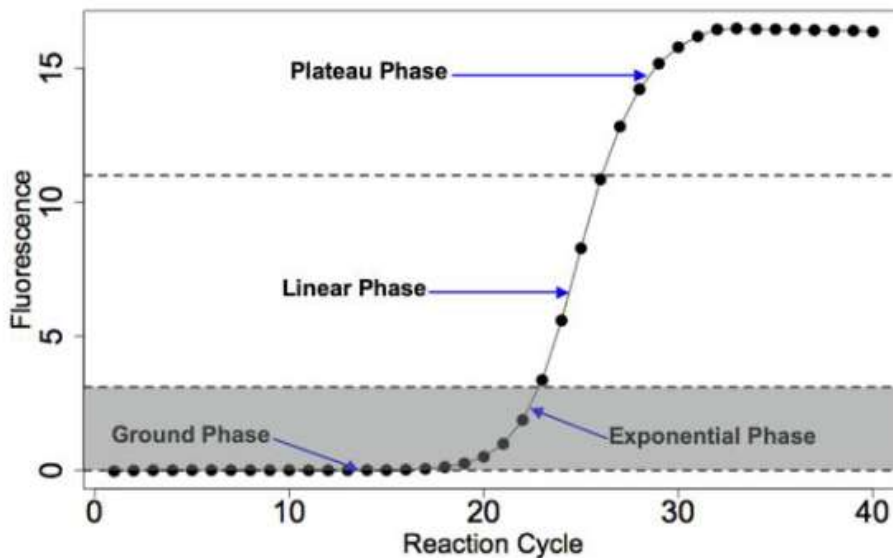


Figure 9: Schematic representation of a qPCR amplification plot with the four different phases of a PCR. The approximate boundaries of each phase are represented by dashed lines, whereas the bottom dashed horizontal line resembles the ground phase. The exponential phase is shown in grey (Page & Stromberg, 2011).

As it can be seen in Figure 9, the qPCR is in general build up by four major phases: the ground phase, exponential phase, linear phase and the plateau phase. During the beginning of the qPCR in the ground phase, the fluorescence emission at each cycle hasn't risen above the background signal, which allows the system to calculate the baseline fluorescence. In the exponential phase, the fluorescence signal reaches a level where it is significantly higher than the background levels. Once the linear phase begins, the qPCR reaches the optimal amplification period and if the reaction conditions are ideal, the number of target DNA copies approximately doubles every cycle. During the plateau stage, the reaction components are limited, and the fluorescence intensity reaches a level where it can no longer be used for calculations (Wong & Medrano, 2005).

The absolute quantification is generated by the comparison of the C_q values with a standard curve generated from amplification of known amounts of the target genes, so called standards.

2.6.2.3. Probe-based qPCR

For this master thesis, a probe-based qPCR method has been used for the quantification of the bacteriophages and the bacteria. This type of real-time PCR is conventionally a tri-oligonucleotide system because the probe is the third oligonucleotide. This improves the ability to detect a single transcript copy with higher sensitivity. Additionally, the probe-based qPCR has the advantage of a low background fluorescence.

The system is based on the usage of a probe, that is a sequence specific and short oligonucleotide capable of binding within the region limited by the primers. It also contains a fluorescent dye on one end and a quencher on the other end. The role of the quencher is to absorb the fluorescence emitted by the fluorescent dye and therefore inhibit the release of a detectable fluorescence signal. The quencher and the fluorescent dye are responsible for the formation of the fluorescence resonance energy transfer pair. In the beginning, the fluorescence emission is not detected because the quencher molecule is close enough to prevent it. However, after extending from the upstream primer, the polymerase reaches the 5' end of the probe and the probe is hydrolysed by the polymerase exonuclease activity. This process results in the disruption of the fluorescence dye and quencher pair and since the quencher cannot inhibit it anymore, a fluorescence signal is released. This signal is then detected in the same way as in a conventional qPCR and the cycle threshold value C_q can be calculated identically (Nagy et al., 2017; Wong & Medrano, 2005).

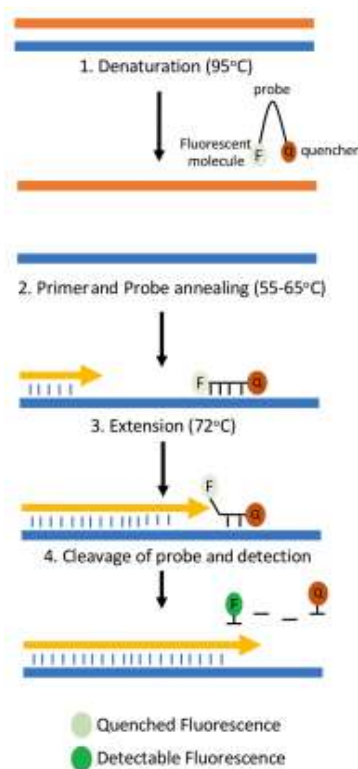


Figure 10: Schematic representation of the probe-based qPCR system with a fluorescent dye and a quencher (Adams, 2020).

2.6.2.4. Standards for qPCR – Quant it

To quantify samples with qPCR, standards with known concentrations are needed to establish a standard curve for comparing the different samples and for calculating the specific values. In this project, standards from plasmids (Bacteriophage PhiX174 and *Geobacillus stearothermophilus*) and from PCR products (*Penicillium chrysogenum*) were used.

To produce plasmid standards, an *E. coli* possessing a specific plasmid (contributed by Nathalie Turgeon, Université Laval) for the PhiX174 bacteriophages and one for the *Geobacillus stearothermophilus* were cultivated. Overnight cultures in 10mL TSB with 10µL Ampicillin and 3µL sample were produced and grown at 37°C at 180rpm. In the next morning, the cultures were each centrifuged for 10 minutes at 4000rpm (Eppendorf, Centrifuge 5810R) and the gained pellet was used for isolation with the Gene JET Plasmid Miniprep Kit (Thermo Fisher Scientific) following the protocol. The gained standards were stored at -20°C.

For *Penicillium chrysogenum* a standard curve in the qPCR was generated by using a PCR product, slightly larger than the region used for quantification. This standard was obtained by growing the fungi on a malt-extract-agar plate for two weeks at 28°C. The spores were harvested into a sterile tube and the DNA was isolated following the protocol of the Dneasy Plant Mini Kit (Qiagen). The DNA was used as a sample for a PCR, using a final reaction volume of 15µL. The solution consisted of 7.5µL GoTaq Polymerase (Promega), 0.75µL of each forward and reverse primer (NS31 and ITS4), 5µL of PCR grade water (Sigma) and 1µL sample. The cycling conditions included an initial denaturation step at 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C for 60 seconds and 72°C for 30 seconds. Afterwards the PCR product was purified with the QIAquick PCR Purification Kit Protocol (Qiagen) following the protocol with no additional modifications. The purified sample was stored at -20°C and used for the establishment of a standard curve.

For the determination of the plasmid and PCR-product concentration and the calculation of the copy number, the high sensitivity Quant-iT dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific) was used. The samples were analysed in duplicates by following the protocol with one additional change in the used volumes, where instead of 200µL buffer and 10µL standards or sample only 20µL buffer and 1µL standard or sample are used.

The quantification was accomplished within a 384-well plate and the CFX384 Real time system (Bio-Rad C1000 Touch thermal cycler) at an excitation/emission maximum of approximately 502/523nm and a temperature setting at 25°C.

On basis of the results of the Quant-iT, the concentration of the standards was calculated and dilution series, in 10-fold steps, for standard curves in the qPCR of the bioaerosol samples, were produced.

2.6.2.5. Analysis with qPCR

All samples were analysed in the CFX384 real time system (Bio-Rad C1000 Touch thermal cycler). The detailed primer sequences can be found in Table 3: Names, sequences, and targets of all used primers.

The final reaction volume for the analysis of *Penicillium chrysogenum* was 5µL, consisting of 2.5µL GoTaq qPCR (Promega) and each of 0.25µL FungiQuant forward and reverse primer (Sigma) and 2µL sample. The cycling conditions included an initial denaturation step at 95°C for 3 minutes, followed by 40 cycles of 95°C for 10 seconds, 55°C for 20 seconds and 72°C for 25 seconds and after each cycle the fluorescence was read.

For *Geobacillus stearothermophilus* the final reaction volume was 10µL, including 5µL IQ Supermix Buffer (BioRad), 0.065µL of the Taqman 16S Probe 10µM (Sigma), and 0.125µL of each 16S forward and reverse primer 50µM (Sigma), mixed with 3.81µL water (Sigma) and 1µL sample. The cycling conditions included an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of 95°C for 20 seconds and 62°C for 60 seconds and after each cycle the fluorescence was read.

The Bacteriophages PhiX174 were analysed with a final reaction volume of 10µL, consisting of 4.6875µL IQ Supermix Buffer (BioRad), 0.1875µL PhiX174 Probe 10µM (Merck), 0.09375µL of each PhiX174 forward and reverse primer 50µM (Sigma), mixed with 2.53125µL water (Sigma) and 2.5µL sample. The cycling conditions included an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of 94°C for 15 seconds and 60°C for 60 seconds and after each cycle the fluorescence was read.

Table 3: Names, sequences, and targets of all used primers

Primers	Sequence 5' - 3'	Target	Reference
NS31	TTGGAGGGCAAGTCTGGTGCC	Fungi	Liu et al., 2012
ITS4	TCCTCCGCTTATTGATATGC	Fungi	
16S forward	GGTAGTCCACGCCGTAAACG	Bacteria	Nathalie Turgeon, Université Laval
16S reverse	GACAACCATGCACCACCTG	Bacteria	
Taqman 16S Probe 10µM	TTCGCGTTGCTTCGAATTAAACCAC	Bacteria	
PhiX174 forward	ACAAAGTTTGGATTGCTACTGAC	Virus	
PhiX174 reverse	CGGCAGCAATAAACTCAACAGG	Virus	
PhiX174 Probe 10µM	CTCTCGTGCTCGTCGCTGCGTTGA	Virus	

2.7. Data Analysis

For the data analysis all obtained values from the microbiological and molecular-biological analysis were used. The calculation of specific values is necessary to be able to compare the results of the experiments and to gain an overview of the efficiency of the different sampling devices.

2.7.1. Calculations

2.7.1.1. Values of samples / m³

After counting the colony forming units on the plates and including the dilution and plated value, the Colony Forming Units value is given as CFU/mL, which is used for the following calculations.

$$\frac{CFU}{sample} = \frac{CFU}{mL} * Samplevolume [mL]$$

$$\frac{CFU}{L} = \frac{(CFU/sample)}{(Sampletime [min] * Pump FLOW Rate [L/min])}$$

$$\frac{CFU}{m^3} = \frac{CFU}{L} * 1000$$

The values for spores/m³ and Plaque Forming Units/m³ are calculated the same way as for CFU/m³.

2.7.1.2. Germination ratio

$$Germination\ rate = \frac{CFU/mL}{Spore\ count/mL}$$

2.7.1.3. Copy number/m³

To calculate the copy number of DNA in the samples (STS, GWB, Filter cassette and BioSampler), various steps must be taken to get from standards, over Cq values to the values for the samples.

1. Calculating the copy number of the standards

Measuring the standard samples with the Quant it protocol to calculate the concentration of the standard samples. The Quant it Kit includes control samples with different concentrations, which deliver a control curve with a function of the form $y = k * x + d$.

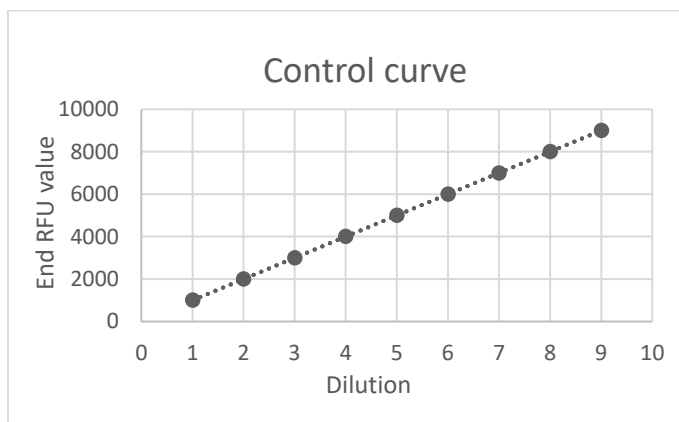


Figure 11: simplified graphic representation of the coordinate system for the control curve of the Quant it kit protocol

By measuring the samples with the Quant it protocol, the relative fluorescence units (RFU) can be determined. The RFU is a unit of measurement used in different analysis methods, which are based on fluorescence detection. The height of the RFU value depends on the amount of DNA a sample contains, meaning it will be higher, the higher the quantity of amplified DNA is.

In this function $y = k * x + d$ the measured end RFU values of the standard samples can be substitute for y and the x value can be calculated and results in the concentration of the sample in ng/ μ L.

$$\text{Concentration } x \text{ [ng}/\mu\text{L]} = \frac{\text{End RFU}(x) - d}{k}$$

To calculate the copy number/ μ L for the standard samples, the number of basepairs for the standard must be known. To determine the approximate mass of the whole double-stranded DNA molecule of the standard, the number of basepairs must be multiplied by the average molecular mass of one base pair, which equals 660 g/mol.

After calculating the concentration in nmol/μL, the copy number/μL can be determined by multiplying with the Avogadro's number $6.022 \times 10^{23} \text{ mol}^{-1}$. The Avogadro constant is a proportionality factor, which relates the number of constituent particles with the amount of substance in a sample.

$$\text{Concentration}_{\text{Standard}} [\text{nmol}/\mu\text{L}] = \frac{\text{Concentration } x [\text{ng}/\mu\text{L}]}{(\text{Basepairs}_{\text{Standard}} * 660) [\text{ng}/\text{nmol}]}$$

$$\frac{\text{Copy number}_{\text{Standard}}}{\mu\text{L}} = \text{Concentration}_{\text{Standard}} [\text{nmol}/\mu\text{L}] * 6.022 \times 10^{14} [\text{nmol}^{-1}]$$

2. Performing qPCR with standards and samples to obtain the Cq values (see 2.6.2.5)
3. Calculate copy number of samples based on Cq values of the standards and samples

First a standard curve is calculated from the Cq values obtained from the standards. This curve has a function of the form $y = k * x + d$.

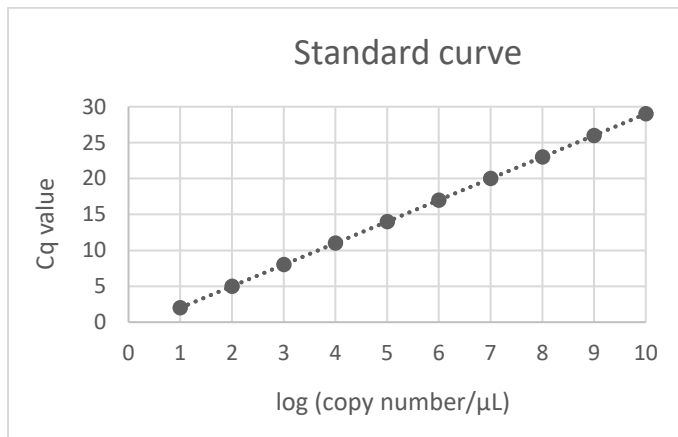


Figure 12: simplified graphic representation of the coordinate system for the standard curve of the qPCR measurement

Now the Cq values from the samples which should be examined can be entered into the equation as the y value and the x value can therefore be calculated.

$$x_{\text{Sample}} = \frac{Cq_{\text{Sample}} - d}{k}$$

Because the x value is still the log (copy number/ μL) it must be converted:

$$\frac{\text{Copy number}_{\text{Sample}}}{\mu\text{L}} = 10^{x_{\text{Sample}}}$$

To obtain the copy number per sample and m^3 further calculations are necessary:

$$\frac{\text{Copy number}}{\text{mL}} = \frac{\text{Copy number}}{\mu\text{L}} * 1000$$

$$\frac{\text{Copy number}}{\text{Sample}} = \frac{\text{Copy number}}{\text{mL}} * \text{Sample volume [mL]}$$

$$\frac{\text{Copy number}}{L} = \frac{(\text{Copy number/sample})}{(\text{Sample time [min]} * \text{Pump Flow Rate [L/min]})}$$

$$\frac{\text{Copy number}}{\text{m}^3} = \frac{\text{Copy number}}{L} * 1000$$

2.7.1.4. Ratio between the efficiencies of the SKC BioSampler and the filter cassette

To compare the efficiency of the two different sampling devices, the ratio in percentage is calculated for the CFU/ m^3 and PFU/ m^3 , the Spores/ m^3 as well as the copy number/ m^3 . Those calculations have been done for every repeat of one concentration separately, meaning only simultaneous samplings in the same atmosphere are compared, and then a mean value of them is calculated. By calculating the ratios separately, the ratio is more precise, because not all repeats have been done with the same starting suspension concentration and therefore the outcomes of the experiments can differ slightly. For the comparison the SKC BioSampler was set as a reference and the value of the filter cassette was compared to it. The experiments with the bioaerosol of each organism have been done in duplicates and therefore only Ratio₁ and Ratio₂ are calculated, whereas the experiments with the bioaerosol of the mixed organisms were accomplished with triplicates.

$$Ratio_1 = \frac{\text{Value of the filter cassette}_{\text{first repetition}}}{\text{Value of the SKC Biosampler}_{\text{first repetition}}}$$

$$Ratio_2 = \frac{\text{Value of the filter cassette}_{\text{second repetition}}}{\text{Value of the SKC Biosampler}_{\text{second repetition}}}$$

$$Ratio_3 = \frac{\text{Value of the filter cassette}_{\text{third repetition}}}{\text{Value of the SKC Biosampler}_{\text{third repetition}}}$$

$$Ratio [\%] = \frac{Ratio_1 + Ratio_2 + Ratio_3}{3} * 100$$

2.7.2. Graphics

All graphics are made with Microsoft Excel.

2.8. Experimental Designs

2.8.1. Evaluation of the starting suspension

To gain an overview on the different concentrations and the interactions of all three organisms the first experiment was done without using the bioaerosol chamber. The starting suspension contained in this set up of equal proportions of the three respective organisms. Each organism was cultured and harvested following its protocol and the suspensions were mixed. To obtain a linear concentration gradient the starting suspension was diluted until 10^{-5} with the respective buffer for each organism. The suspensions were then plated in triplicates on each of the respective media to cultivate each organism on its own. The suspensions were also analysed using qPCR to obtain the molecular-biological data and for the analysis of *Penicillium chrysogenum* they were also counted under the microscope.

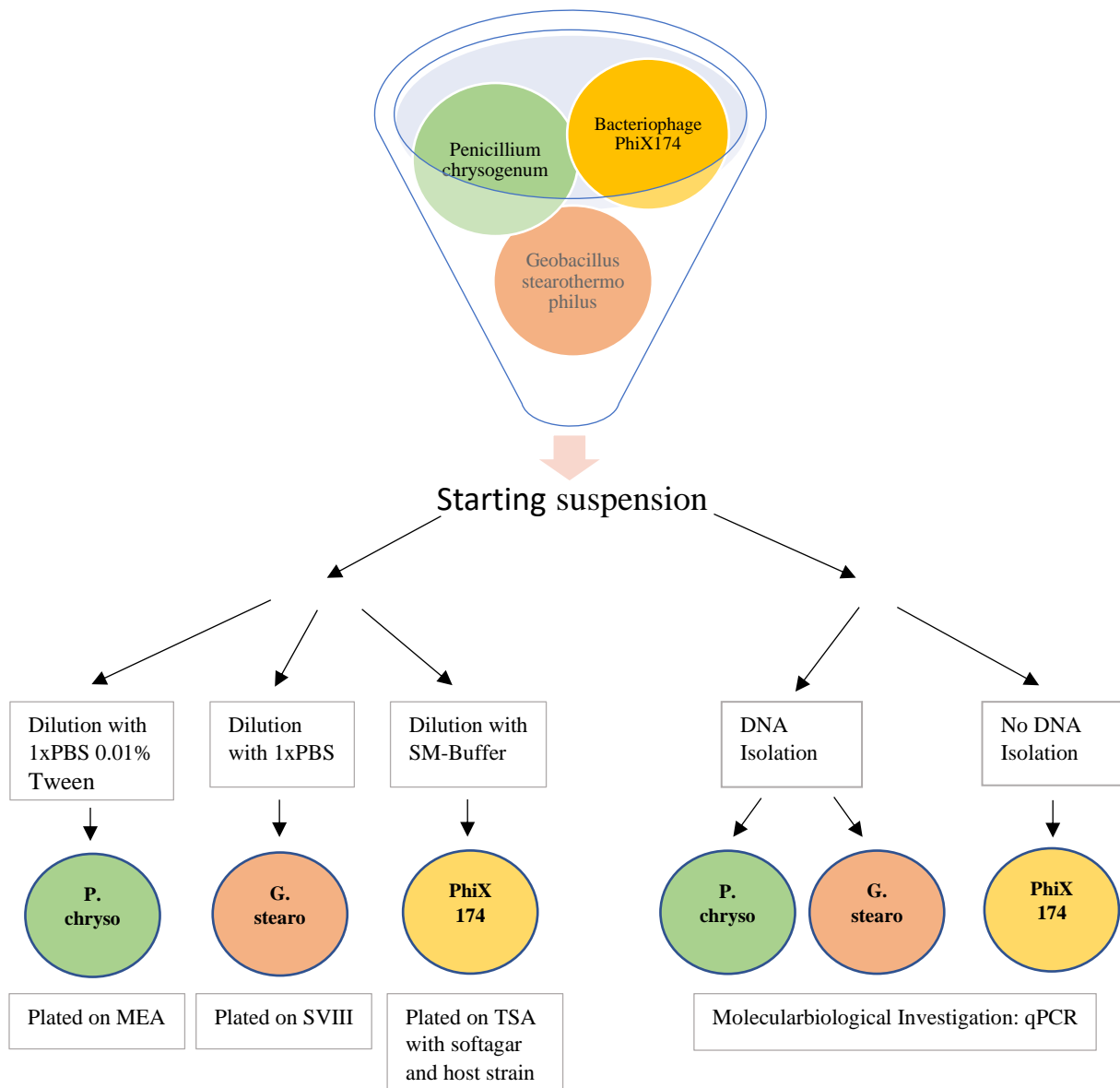


Figure 13: Composition of the starting suspension and following the steps of diluting and plating the different organism

Table 4: Evaluation of the starting suspension experiment - data

Organism	harvested material	Spore count / OD₆₀₀
P. chryso	2 cultures grown on solid medium in plates	1.288 x 10 ⁷ spores/mL
G. stearo	12 cultures grown on solid medium in plates	
PhiX174	30mL of phage lysate	0.058

2.8.2. Bioaerosol of each organism

The next step was to study the bioaerosols of each organism on its own. Therefore, the three organisms were individually aerosolized and sampled in the bioaerosol chamber in three different concentrations, which were each carried out in duplicates. To be able to check the repeatability, additionally in each experiment two SKC BioSamplers and two filter cassettes were used simultaneously. With the usage of these individual experiments, the overall performance of the organisms in the bioaerosol chamber can be observed. Additionally, the correct settings can be established, and an overview of the needed concentrations can be gained.

Table 5: Bioaerosol of each organism experiment - data of *Penicillium chrysogenum*

P. chryso	Date	STS spore count	STS CFU	Germination rate
1.1.	2020-08-17	5.56 x 10 ⁶	3.30 x 10 ⁶	0.59
1.2.	2020-09-18	7.88 x 10 ⁶	4.30 x 10 ⁶	0.55
2.1. and 2.2.	2020-08-24	1.03 x 10 ⁷	5.17 x 10 ⁶	0.50
3.1. and 3.2.	2020-09-17	7.11 x 10 ⁷	3.67 x 10 ⁷	0.52

Table 6: Bioaerosol of each organism experiment – data of *Geobacillus stearothermophilus*

G. stearo	Date	OD₆₀₀	STS CFU
1.1. and 1.2.	2020-12-22	1.387	1.36 x 10 ¹⁰
2.1. and 2.2.	2020-12-23	1.300	4.47 x 10 ¹⁰
3.1. and 3.2.	2020-12-29	1.799	9.50 x 10 ¹⁰

Table 7: Bioaerosol of each organism experiment – data of bacteriophage PhiX174

PhiX174	Date	OD₆₀₀	STS PFU
1.1.	2020-08-20	0.067	1.71 x 10 ⁹
1.2.	2020-08-25	0.084	1.39 x 10 ⁹
2.1. and 2.2.	2020-09-03	0.030	4.72 x 10 ⁹
3.1.	2020-08-18	0.022	1.54 x 10 ¹⁰
3.2.	2020-08-19	0.088	1.83 x 10 ¹⁰

2.8.3. Bioaerosol of mixed organisms

In the final experiments all three organisms were mixed and then studied in the bioaerosol chamber combined to obtain the desired comparison between the different sampling devices. In addition, different interactions between the three organisms can be observed and analysed, therefore three different concentrations were performed, each in triplicates. To be able to check the repeatability, additionally in each experiment two SKC BioSampler and two filter cassettes were used simultaneously.

Table 8: Starting material for the bioaerosol mix experiments

	Date	Spore count	OD₆₀₀	OD₆₀₀	CFU	CFU	PFU
		P. chryso	G. stearo	PhiX174	P. chryso	G. stearo	PhiX174
1.1.	2021-01-05	2.144 x 10 ⁷	1.421	0.025	1.82 x 10 ⁷	4.20 x 10 ⁸	4.83 x 10 ⁸
1.2.	2021-01-12	1.911 x 10 ⁷	1.417	0.043	1.64 x 10 ⁷	2.65 x 10 ⁷	4.65 x 10 ⁸
1.3.	2021-01-13	1.933 x 10 ⁷	3.431	0.020	1.38 x 10 ⁷	2.82 x 10 ⁷	5.23 x 10 ⁸
2.1.	2021-01-12	5.555 x 10 ⁷	1.538	0.050	4.50 x 10 ⁷	5.67 x 10 ⁷	6.23 x 10 ⁸
2.2.	2021-01-14	5.000 x 10 ⁷	3.828	0.055	4.27 x 10 ⁷	4.67 x 10 ⁷	5.97 x 10 ⁸
2.3.	2021-01-19	5.333 x 10 ⁷	3.987	0.035	4.80 x 10 ⁷	3.33 x 10 ⁷	9.00 x 10 ⁷
3.1.	2021-01-13	7.666 x 10 ⁷	3.817	0.047	5.77 x 10 ⁷	1.53 x 10 ⁹	3.87 x 10 ⁹
3.2.	2021-01-14	8.000 x 10 ⁷	4.000	0.062	6.20 x 10 ⁷	1.47 x 10 ⁸	1.17 x 10 ⁹
3.3.	2021-01-19	9.555 x 10 ⁷	3.891	0.031	7.93 x 10 ⁷	1.25 x 10 ⁸	1.27 x 10 ⁹

3. Results and Discussion

After all experiments have been carried out successfully in the concentrations levels and repetitions already specified, the data was evaluated and interpreted. In the following chapter the three categories of experiments are presented and discussed individually for each organism.

3.1. Evaluation of the starting suspension

3.1.1. *Penicillium chrysogenum*

The graph for the starting suspension experiment regarding *Penicillium chrysogenum* shows the dilution of the STS against the colony forming units/mL (blue) and the spores/mL (grey). The spore count was determined only for the undiluted starting suspension in a dilution of 1:100, because of the low analytical limit with the Neubauer improved C-chip (In Cyto C-Chip, n.d.). Therefore, the values for the diluted suspensions are estimated, based on the 1.29×10^7 spores/mL counted in the undiluted suspension. With increasing dilution of the starting suspension, the CFU/mL and spores/mL decrease, which means that a linear trendline describes the course well. The undiluted starting suspension shows the highest number at 5.60×10^6 CFU/mL, which decreases with each dilution step until it reaches to lowest value at 36.67 CFU/mL. The copy number values (orange) are also shown best by a linear decrease, starting at 1.70×10^8 copy number/mL for the undiluted suspension and decreasing until 3.14×10^5 copy number/mL for the highest one. There are two trendlines shown for these values, where one only includes the first four values of the data set.

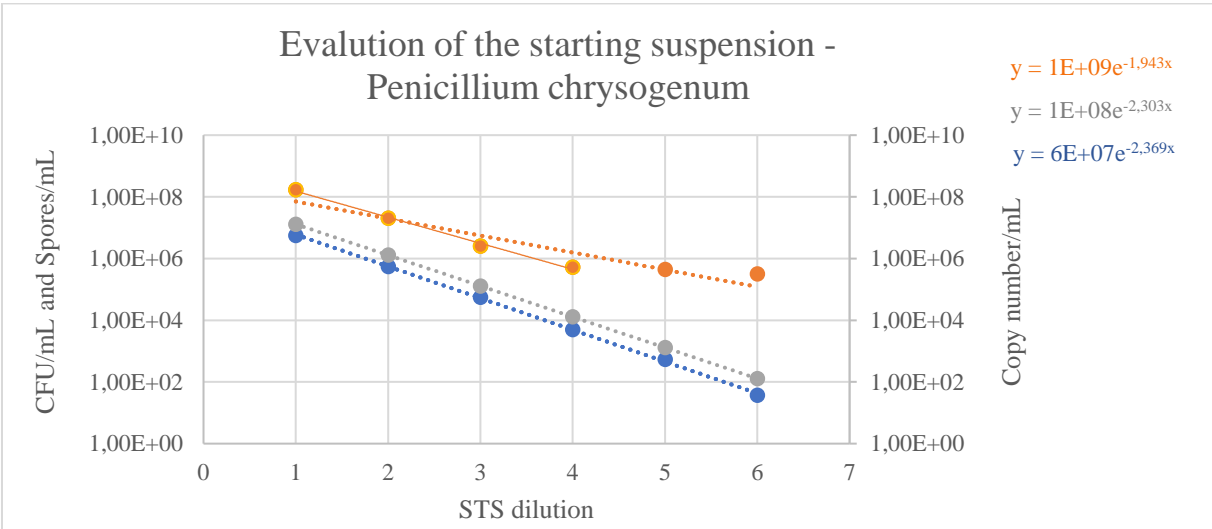


Figure 14: Correlation of the starting suspension dilution with the CFU/mL (blue) and spores/mL (grey) as well as the copy number/mL (orange) for the *Penicillium chrysogenum*.

3.1.2. *Geobacillus stearothermophilus*

The results of the starting suspension experiment for *Geobacillus stearothermophilus* are shown in the following graph. The STS dilution is displayed against the CFU/mL (blue) starting at the highest value of 2.06×10^8 CFU/ml for the undiluted suspension and decreasing until 2.13×10^3 CFU/ml for the last dilution step. Additionally shown is the graph of the STS dilution against the copy number/mL (orange). The undiluted suspension shows a value of 1.10×10^{11} copy number/mL which consistently decreases to 2.98×10^7 copy number/mL for the highest dilution. Both trend lines are represented by a linear decrease, because of the decreasing values of CFU/mL and copy number/mL with an increase of the STS dilution.

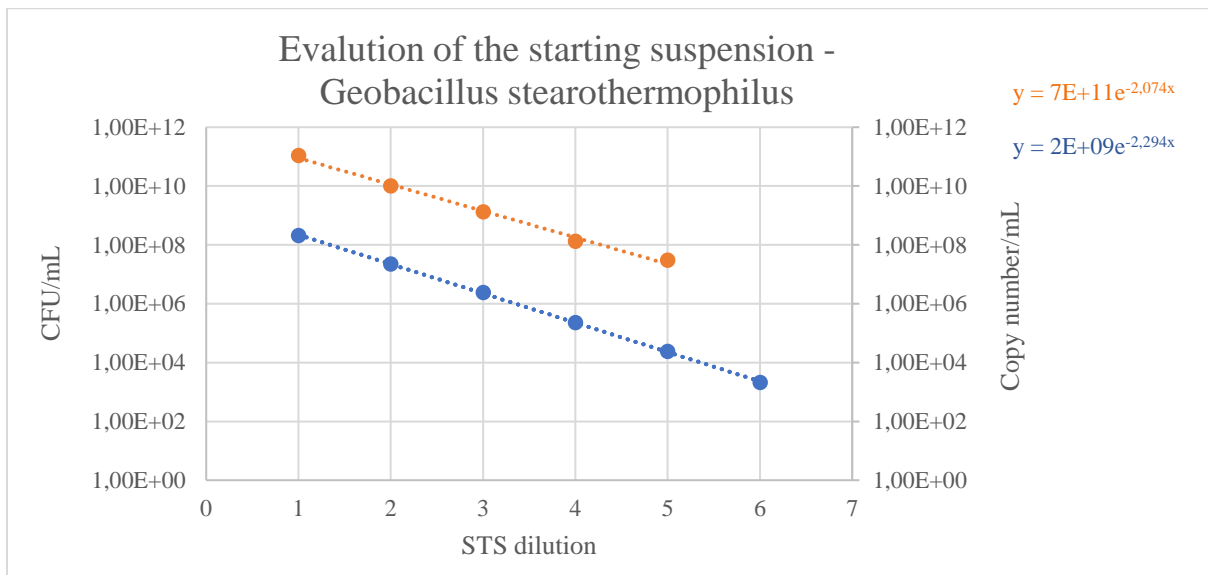


Figure 15: Correlation of the starting suspension dilution with the CFU/mL (blue) and the copy number/mL (orange) for the *Geobacillus stearothermophilus*.

3.1.3. Bacteriophage PhiX174

The bacteriophage PhiX174 was also analysed in the evaluation of the starting suspension and the calculated values are represented in a graph showing the STS dilution displayed against the PFU/mL (blue), starting at the highest value of 1.90×10^9 PFU/ml for the undiluted suspension and decreasing until 1.76×10^4 PFU/ml for the highest dilution step. Additionally shown is the graph of the STS dilution against the copy number/mL (orange). Although the first measurable Cq value was already 18.13, the values of the undiluted suspension and the first dilution step could not be determined. Therefore, the graph starts at the value of 9.73×10^8 copy number/mL for the third dilution step and consistently decreases to 2.95×10^5 copy number/mL for the last dilution step. Both trend lines are represented by a linear decrease.

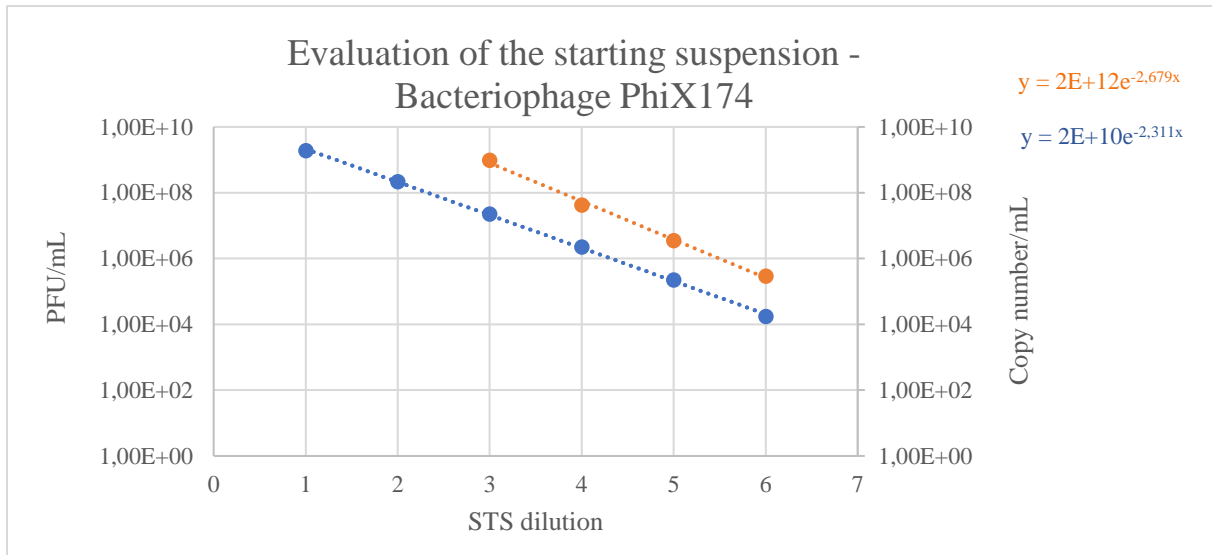


Figure 16: Correlation of the starting suspension dilution with the PFU/mL (blue) and the copy number/mL (orange) for the bacteriophage PhiX174.

3.1.4. Discussion

The evaluation of the starting suspension of all three organisms shows a consistent decrease of the CFU/mL or PFU/mL with every dilution step, which can be well described with a linear decreasing trendline.

In the case of *Penicillium chrysogenum*, the function of the copy number/mL is not as parallel to the CFU/mL function as the ideal would be. This ideal would suggest, that one colony forming unit can be paired with a constant value of copy numbers, resulting in a constant ratio of CFU to copy number (Morpeth et al., 2014). Especially the last two concentration steps delivered higher numbers in the molecular-biological analysis with the qPCR than they would be expected to. Maybe some interactions, which enhanced the values of the qPCR, happened or there are some disruptive factors in the not so heavily diluted suspensions by which the analysis gets inhibited. If those two higher values are not considered, and the trendline does only include the first four values, the two functions of CFU/mL and copy number/mL seem rather consistently parallel and can therefore describe the relationship of colony forming units to copy numbers more precise.

For *Geobacillus stearothermophilus* the qPCR reached its lower detection limit at the highest dilution step, but the CFU/mL and copy number/mL still show consistent parallel functions, meaning that each colony forming unit incorporates a similar amount of copy numbers.

The analysis of the copy number/mL for the bacteriophage PhiX174 has resulted in only four values, because the first two suspensions with the highest concentrations were above the upper detection limit of the qPCR. The remaining values do show a parallel relationship of plaque forming units and copy numbers.

3.2. Bioaerosols of each organism

The experiments with the bioaerosol of each organism have been performed with two SKC BioSampler and two filter cassettes. The graphs below show for each bar the mean value of these simultaneously taken duplicates (n=2).

3.2.1. *Penicillium chrysogenum*

Colony forming units and spore counts

For the experiments *Penicillium chrysogenum* was analysed in three different concentrations, each done in duplicates, resulting in 6 samples for each sampling method. In addition to the CFU/m³ values, each suspension of the sampling devices has been counted under the microscope with a Neubauer improved C-chip, to be able to calculate the spores/m³.

The initial starting suspension had a concentration of 5.55×10^6 and 7.88×10^6 spores/mL for the duplicates of concentration 1, resulting in 3.30×10^6 and 4.30×10^6 CFU/mL. The SKC BioSampler reached in these experiments the values of 9.54×10^3 and 4.17×10^3 CFU/m³. The filter cassette was able to sample significantly lower and more fluctuating concentrations of 3.10×10^3 and 3.97×10^2 CFU/m³. The spore counts in the first repeat were 1.41×10^6 and 1.59×10^5 spores/m³ for the SKC BioSampler and the filter cassette. The samples of the second repeat could not be counted due to the low concentrations, which were under the lower detection limit of the Neubauer improved C-chip.

The second concentration was set at a starting suspension concentration of 1.033×10^7 spores/mL, leading to 5.17×10^6 CFU/mL. Both repeats of concentration 2 have been done with the same starting suspension. The sampling with the SKC BioSampler resulted in similarly consistent spore counts of 5.59×10^6 and 5.63×10^6 spores/m³, leading to 3.99×10^4 and 1.61×10^4 CFU/m³. The filter cassette delivered equal values of 1.48×10^4 and 1.33×10^4 CFU/m³. However, the spore counts were, compared to the SKC BioSampler, significantly lower at 6.07×10^5 and 6.60×10^5 spores/m³.

The highest concentration was started with 7.11×10^7 spores/mL, leading to a concentration of 3.67×10^7 CFU/mL and this starting suspension was used for both repeats. The sampling concentrations of the SKC BioSampler were 8.41×10^3 and 4.70×10^3 CFU/m³. Despite this fluctuation, the spore counts resulted in rather consistent values of 4.86×10^6 and 4.26×10^6 spores/m³. The filter cassette reached values of 3.36×10^4 and 2.98×10^4 CFU/m³ and therefore being able to sample higher concentrations than the SKC BioSampler. Nevertheless, the spore counts contradict this, given the concentrations of 4.23×10^5 and 4.23×10^5 spores/m³. Apart from that, both sampling devices could not achieve a constant increase in spores/m³ and only the filter cassette was able to reach slightly increasing CFU/m³ values for the three concentrations.

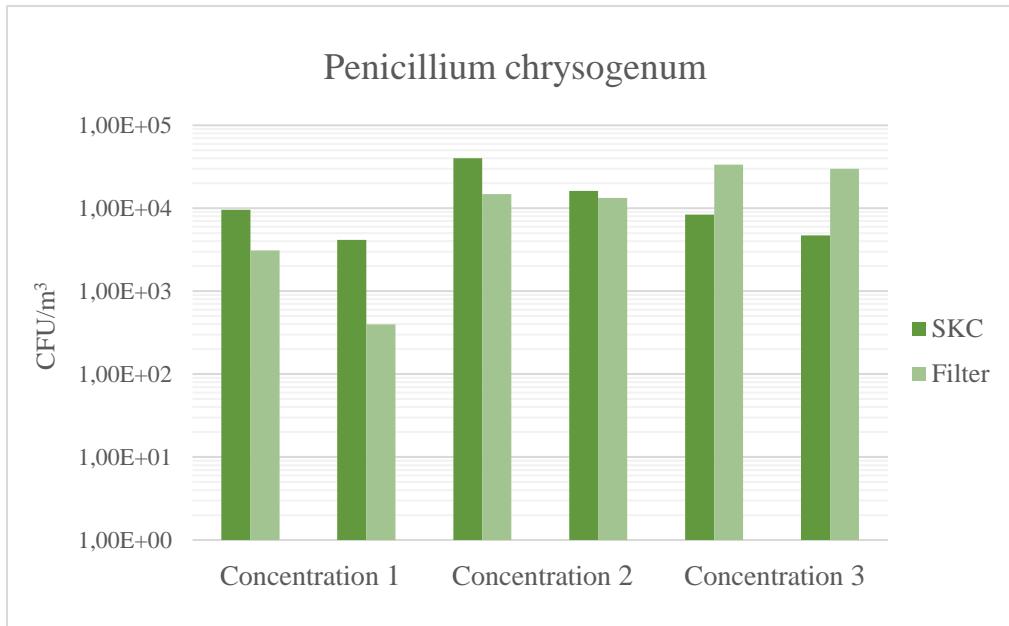


Figure 17: Values of reached CFU/m³ for the three different concentrations in the experiments with the bioaerosol of *Penicillium chrysogenum*.

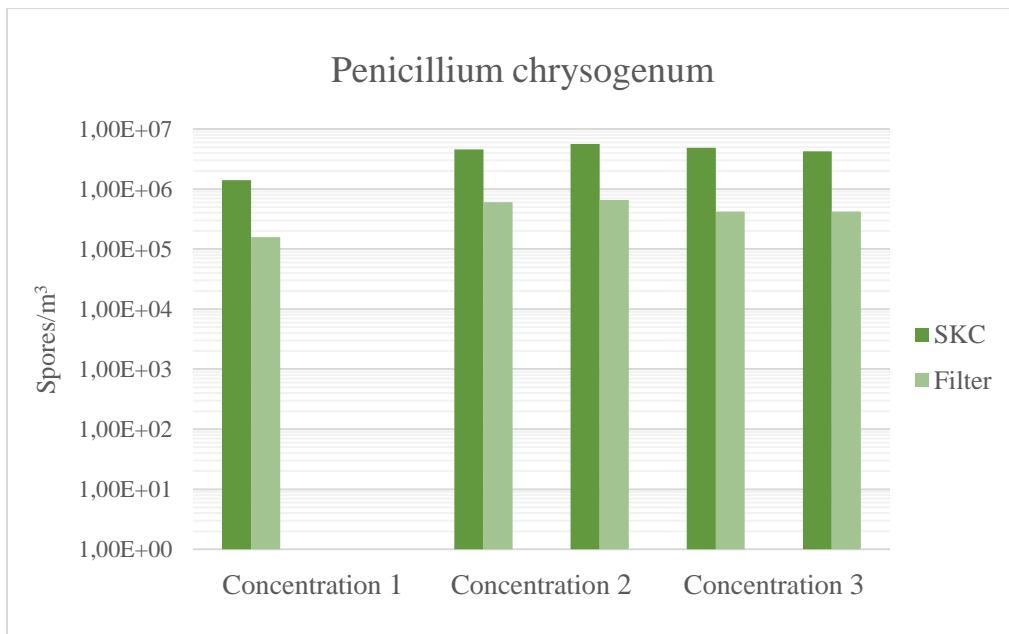


Figure 18: Values of reached spores/m³ for the three different concentrations in the experiments with the bioaerosol of *Penicillium chrysogenum*.

Copy numbers

Beside the microbiological analysis, also a molecular-biological analysis has been done with each suspension of the sampling devices and the values of the copy number/m³ were determined. The starting suspension of the first repeat with concentration 1 accomplished a value of 2.74×10^8 copy number/mL. It led to a sampling value of 3.02×10^7 and 1.86×10^7 copy number/m³ with the SKC BioSampler and the filter cassette, respectively. The second repeat of concentration 1 did not deliver any values in the qPCR below a Cq value of 35 and can therefore not be considered for the analysis and the graph. The second concentration step was set at a starting suspension of 1.96×10^8 copy number/mL and resulted in values of 2.00×10^7 and 1.98×10^7 copy number/m³ in the duplicates with the SKC BioSampler. The filter cassette was able to reach 1.98×10^7 and 6.60×10^6 copy number/m³. The third concentration step was started with a concentration of 1.59×10^9 copy number/mL. Comparable to the values of the first and the second concentration levels, the duplicates of this experiment led to concentrations sampled by the SKC BioSampler of 5.94×10^7 and 2.67×10^7 copy number/m³, whereas the filter cassette reached again lower concentrations of 2.82×10^7 and 1.60×10^7 copy number/m³.

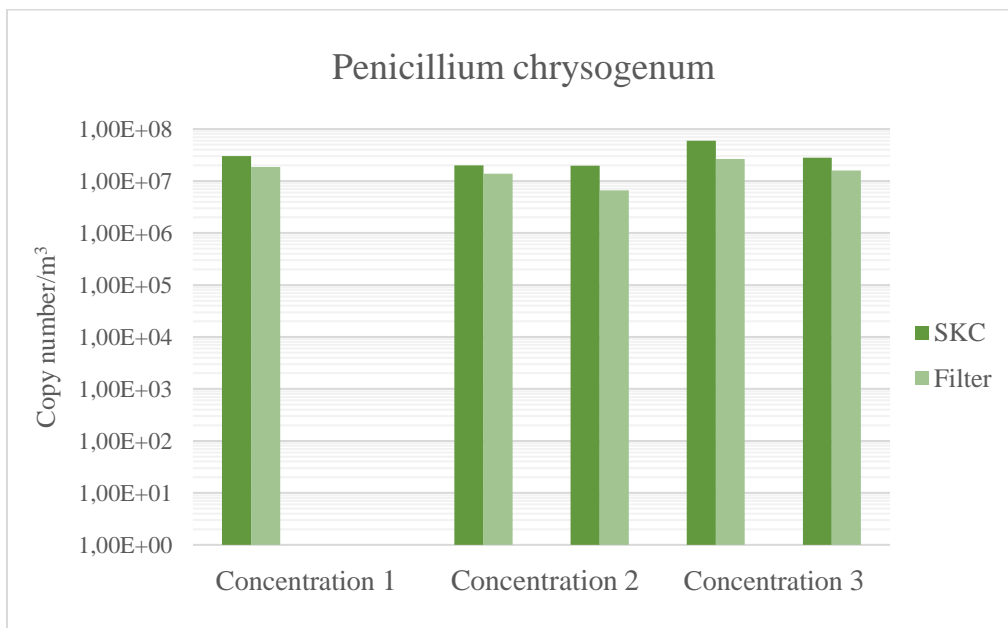


Figure 19: Values of reached copy number/m³ for the three different concentrations in the experiments with the bioaerosol of *Penicillium chrysogenum*.

Discussion

The values of sampled CFU/m³ of *Penicillium chrysogenum* do not show a consistent increase for the three concentration steps, if sampled with the SKC BioSampler (Figure 17). The filter cassette shows a better rise of the sampled concentrations, but there is still an outlier at the second repeat of concentration 1. These inconsistent gradients could have been caused by the aerosol generator LSA, as it reaches a plateau in generation efficiency after a certain concentration in the starting material (Mainelis et al., 2005).

The values of the colony forming units in the duplicates seem to match each other consistently, if both repeats of one concentration step have been carried out with the same starting suspension as it was the case with concentration 2 and 3. The deviation of the repeats of concentration 1 can result from the different starting suspension concentrations.

Although the SKC BioSampler was able to reach higher sampling values than the filter cassette in the first two concentrations, it does seem like there was a problem in the experiments with the highest starting concentration. There is no clear answer to why the CFU/m³ values differ that much from the other results, even though all experiments were done with identical settings and all calculations have been checked if they are correct. Additionally, the values of spores/m³ of those experiments would suggest higher results (Figure 18: Values of reached spores/m³ for the three different concentrations in the experiments with the bioaerosol of *Penicillium chrysogenum*.. Therefore, the reduced CFU/m³ values could have been caused by problems in the growth of the colonies or by negative influences on the cultivability during the sampling process. Another abnormality is, that the number of counted spores seems to remain constant even though different starting suspension concentrations were used for the experiments (Figure 18: Values of reached spores/m³ for the three different concentrations in the experiments with the bioaerosol of *Penicillium chrysogenum*. A reason for this circumstance could be the lower detection limit of the C-Chip, which only delivers reliable values with concentrations over 10⁴ cells/mL (In Cyto C-Chip, n.d.). The concentrations of the collected suspensions, regardless of which sampling device was used, were lower in all experiments and could hardly be counted under the microscope, which is why the values of the spores are rather unreliable.

Similar to the values of the spores/m³, also the copy numbers seem to be rather consistent for all three concentrations steps (Figure 19: Values of reached copy number/m³ for the three different concentrations in the experiments with the bioaerosol of *Penicillium chrysogenum*. The lack of an increase in the values could be explained by the fact that also cells that have already died can trigger a signal in the qPCR. The value of the copy number thus includes both living and dead cells and can therefore exceed the values of the colony forming units, which are only influenced by living cells (Àlvarez et al., 2013). Additionally, it could be, that the cells have been damaged in the aerosolization and sampling process, which can influence the process of the DNA isolation (Hey Reoun An et al., 2006).

In order to make a comparison of the two sampling devices possible, the values are summarized again in Table 9. It also includes the ratio between the two devices, whereby it is indicated in each which share the filter cassette has achieved compared to the SKC BioSampler.

In the example of CFU/m³ the filter cassette could only achieve 32.43% of the performance of the SKC BioSampler in the first concentration step. Apart from the outlier in concentration 3, the filter cassette could not deliver as high values as the SKC BioSampler, whether it is for the CFUs and spores or for the copy numbers.

Table 9: The table for *Penicillium chrysogenum* summarizes the values of CFU/m³ and spores/m³ as well as the copy number/m³ for the three different concentrations and the two different sampling devices. The values given in the table are the mean values of the duplicates of each concentration for each sampling devices (n=4). It also shows the ratio between the two devices, whereby it is indicated in percent which share the filter cassette has achieved compared to the SKC BioSampler.

Concentration	Device	CFU/m ³	Ratio CFU/m ³	Spores/m ³	Ratio Spores/m ³	Copy number/m ³	Ratio Copy number/m ³
1	SKC	9,54E+03		1,41E+06		3,02E+07	
1	Filter	3,10E+03	32,43%	1,59E+05	11,22%	1,86E+07	61,46%
2	SKC	2,80E+04		5,11E+06		1,99E+07	
2	Filter	1,41E+04	59,79%	6,33E+05	12,47%	1,03E+07	51,56%
3	SKC	6,55E+03		4,56E+06		4,38E+07	
3	Filter	3,17E+04	517,35%	4,23E+05	9,32%	2,14E+07	50,82%

3.2.2. *Geobacillus stearothermophilus*

Colony forming units

The *Geobacillus stearothermophilus* was analysed in the experiments with the bioaerosol of each organism in three different concentrations, which each had been performed in duplicates to be able to gain reliable and comparable values. In the first experiment the bacterium reached 1.36×10^{10} CFU/mL in the starting suspension, which was used for both repeats of concentration 1. The SKC BioSampler sampled in these duplicates 2.66×10^4 and 3.21×10^4 CFU/m³. The filter cassette was able to deliver slightly lower concentrations of 1.35×10^4 and 1.46×10^4 CFU/m³. The second concentration was set at a starting suspension concentration of 4.47×10^{10} CFU/m³. Both repeats of concentration 2 have been done with the same starting suspension. The SKC BioSampler reached similar values in the duplicates of 3.43×10^4 and 3.73×10^4 CFU/m³. The filter cassette delivered values of 1.60×10^4 and 1.65×10^4 CFU/m³, therefore both sampling devices reached similar concentrations as with concentration 1. The highest concentration was started with a concentration of 9.50×10^{10} CFU/mL. This starting suspension was also used for both repeats of the concentration 3 experiments, leading to sampling concentrations of 4.56×10^4 and 5.00×10^4 CFU/m³ with the SKC BioSampler. The filter cassette reached again slightly lower values of 2.71×10^4 and 2.75×10^4 CFU/m³. Despite the three increasing concentrations of the starting suspension, the two devices could not achieve a significant increase in the sampled concentrations.

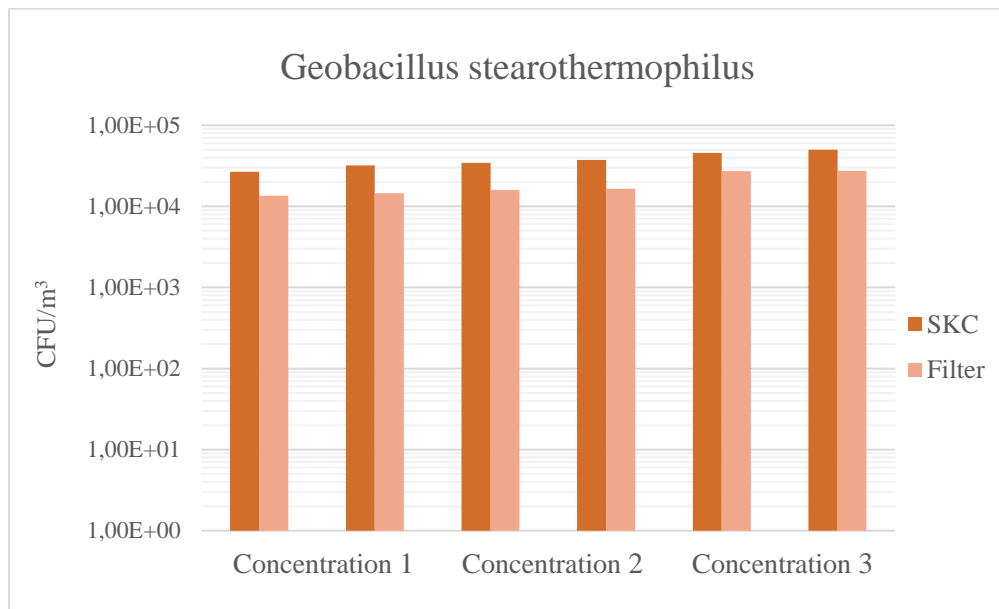


Figure 20: Values of reached CFU/m³ for the three different concentrations in the experiments with the bioaerosol of *Geobacillus stearothermophilus*.

Copy numbers

Like the bioaerosol experiments with the other two organisms, also each sampled suspension of the bacterium was analysed molecular-biologically with a qPCR to determine the values of the copy number/m³. The starting suspension of concentration 1 accomplished a value of 1.32×10^{11} copy number/mL. This starting suspension led to sampling values of 3.92×10^{10} and 2.20×10^9 copy number/m³ with the SKC BioSampler and 3.11×10^9 and 6.90×10^8 copy number/m³ in the filter cassette. The second concentration was set at a starting suspension of 3.72×10^{11} copy number/mL and resulted in values of 3.06×10^{10} and 1.20×10^9 copy number/m³ in the duplicates with the SKC BioSampler. The filter cassette reached lower, but also strongly fluctuating values of 7.73×10^9 and 4.62×10^8 copy number/m³ for the second concentration. The third concentration was started with a concentration of 2.45×10^{11} copy number/mL. The duplicates of this experiment led to concentrations sampled with the SKC BioSampler of 4.85×10^{10} and 9.71×10^9 copy number/m³, whereas the filter cassette reached values of 5.31×10^9 and 3.79×10^{10} copy number/m³. Both sampling devices were not able to deliver comparable values in the duplicates and there is no consistent increase in sampled concentrations.

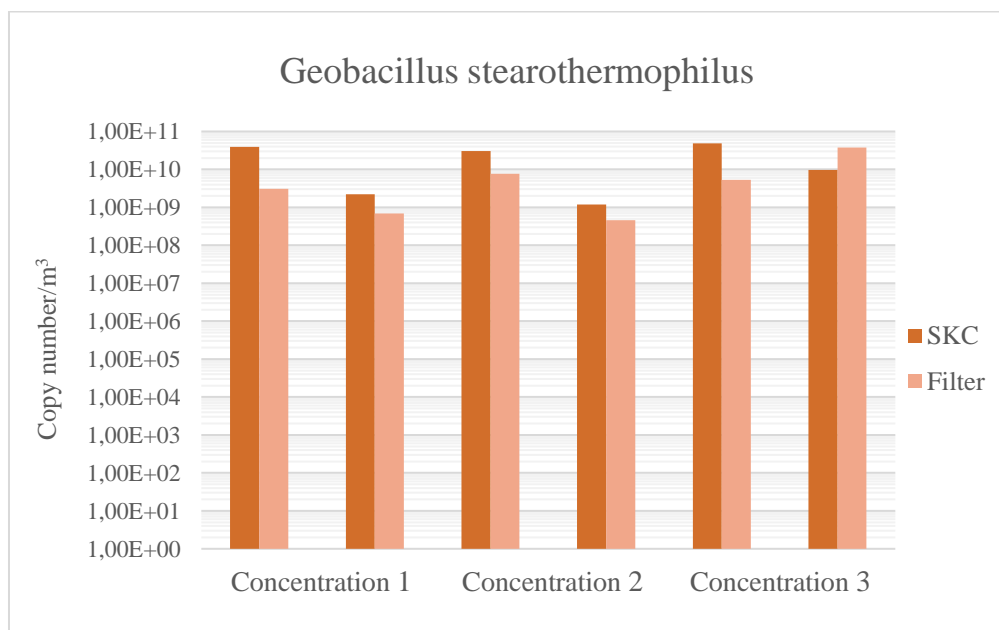


Figure 21: Values of reached copy number/m³ for the three different concentrations in the experiments with the bioaerosol of *Geobacillus stearothermophilus*.

Discussion

In the experiments with the bioaerosol of *Geobacillus stearothermophilus* rather consistent sampling concentrations are reached with both sampling devices, regardless of the starting suspension concentration (Figure 20: Values of reached CFU/m³ for the three different concentrations in the experiments with the bioaerosol of *Geobacillus stearothermophilus*.. Like the experiments with *Penicillium chrysogenum* those circumstances can be caused by the upper efficiency limit of the aerosol generator. Nevertheless, the duplicates of all concentrations delivered very similar CFU/m³ values for both the SKC BioSampler and the filter cassette. The values of the molecular-biological analysis with the qPCR however seem more inconsistent (Figure 21). These fluctuations can mainly be seen in the duplicates of the concentrations 1 and 2, where the values of the second repeats are lower than in the first repetition. Since the starting suspension experiment could show that the qPCR with the bacterium can deliver reliable values, it is assumed that the settings of the analysis are correct but may be subject to occasional fluctuations. If those fluctuations are not considered, the values of the copy number/m³ tend to be the same for all concentration levels and both sampling devices and are therefore comparable with the values of the CFU/m³.

The values of all experiments are summarized in Table 10: The table for *Geobacillus stearothermophilus* summarizes the values of CFU/m³ as well as the copy number/m³ for the three different concentrations and the two different sampling devices. The values given in the table are the mean values of the duplicates of each concentration for each sampling devices (n=4). and give an overview of the efficiency of the filter cassette compared to the SKC BioSampler. It was able to reach approximately 50% of the values achieved with the SKC BioSampler if the CFU/m³ values are compared. If the values of the copy number are considered, an outlier can be seen in the third concentration step, because there the filter cassette reached a higher value in the second repetition.

Table 10: The table for *Geobacillus stearothermophilus* summarizes the values of CFU/m³ as well as the copy number/m³ for the three different concentrations and the two different sampling devices. The values given in the table are the mean values of the duplicates of each concentration for each sampling devices (n=4). It also shows the ratio between the two devices, whereby it is indicated in percent which share the filter cassette has achieved compared to the SKC BioSampler.

Concentration	Device	CFU/m ³	Ratio CFU/m ³	Copy number/m ³	Ratio Copy number/m ³
1	SKC	2,94E+04		2,07E+10	
1	Filter	1,40E+04	48,04%	1,90E+09	20%
2	SKC	3,58E+04		1,59E+10	
2	Filter	1,62E+04	45,33%	4,10E+09	31,96%
3	SKC	4,78E+04		2,91E+10	
3	Filter	2,73E+04	57,20%	2,16E+10	200%

3.2.3. Bacteriophage PhiX174

Plaque forming units

The settings in the experiments with the bioaerosol of the bacteriophage PhiX174 are similar to those of the other two organisms and it was therefore analysed in three different concentration steps, each performed in duplicates. In the first repeat of concentration 1, the initial starting suspension concentration was 1.71×10^9 PFU/mL. The second repeat was started with 1.39×10^9 PFU/mL. The SKC BioSampler reached consistent values of 1.49×10^5 and 1.01×10^5 PFU/m³. The filter cassette was able to sample slightly lower concentrations of 3.59×10^4 and 4.51×10^4 PFU/m³. The second concentration was set at a starting suspension concentration of 4.72×10^9 PFU/m³. Both repeats have been done with the same starting suspension. The SKC BioSampler was able to achieve an increase in sampled concentrations and delivered values of 3.31×10^6 and 4.03×10^6 PFU/m³. Similar values have been delivered by the filter cassette, which reached only slightly lower values of 2.33×10^6 and 1.92×10^6 PFU/m³. The third concentration was started with 1.54×10^{10} in the first repeat of the experiment and 1.83×10^{10} PFU/mL in the second repetition. These starting suspensions led to rather inconsistent sampling concentrations of 1.06×10^7 and 1.83×10^6 PFU/m³ with the SKC BioSampler. The filter cassette reached lower, but also fluctuating values of 6.22×10^5 and 1.71×10^5 PFU/m³ and was therefore not able to deliver increasing sampling concentrations for the three experiments, despite increasing starting suspension concentrations.

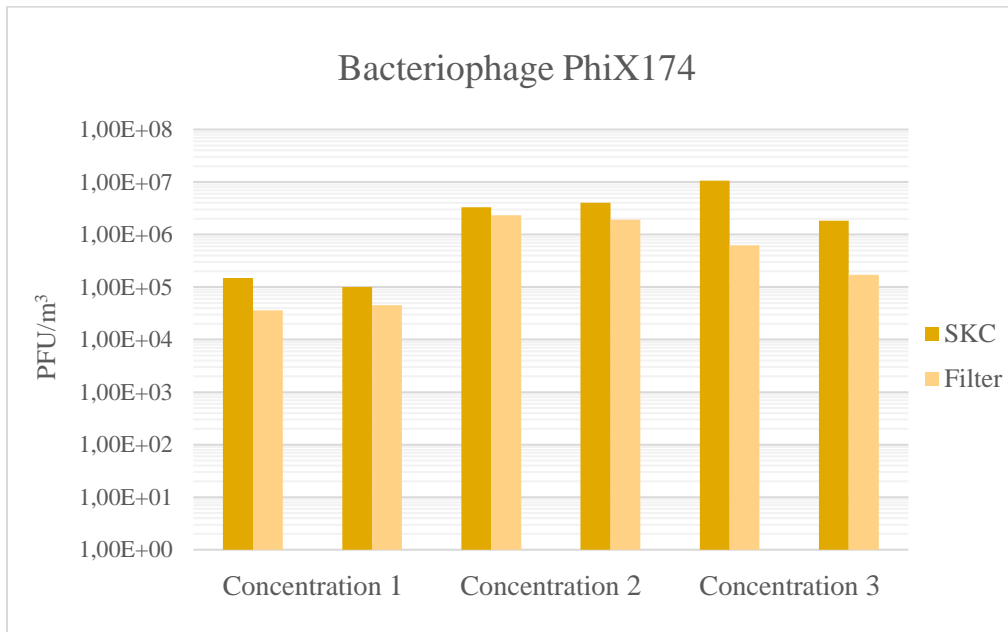


Figure 22: Values of reached PFU/m³ for the three different concentrations in the experiments with the bioaerosol of the bacteriophage PhiX174.

Copy numbers

Beside determining the plaques-forming-units, the bacteriophage PhiX174 was also analysed in a molecular-biological aspect with the usage of a qPCR. This allows the calculation of the copy number/m³ values for all obtained suspensions of the two different sampling devices. The starting suspensions of the duplicates with concentration 1 reached a value of 8.38×10^6 and 1.37×10^8 copy number/mL. Those led to strongly fluctuating values of 9.67×10^4 and 5.52×10^6 copy number/m³ with the SKC BioSampler. The filter cassette delivered lower, but also inconsistent values of 1.71×10^4 and 3.23×10^6 copy number/m³. The second concentration was set at a starting suspension of 5.21×10^8 copy number/mL and resulted in fluctuating values of 7.33×10^6 and 4.55×10^7 copy number/m³ in the duplicates with the SKC BioSampler. The filter cassette was able to reach 2.32×10^6 and 2.70×10^6 copy number/m³ and did therefore not deliver an increase in sampled concentrations. The third concentration was started with 1.48×10^8 copy number/mL in the first repeat and 8.31×10^8 copy number/mL in the second. They led again to rather inconsistent concentrations sampled with the SKC BioSampler of 2.83×10^7 and 1.88×10^8 copy number/m³. The filter cassette delivered not only fluctuating values of 4.08×10^6 and 8.43×10^7 copy number/m³ but sampled again also lower concentrations than the SKC BioSampler.

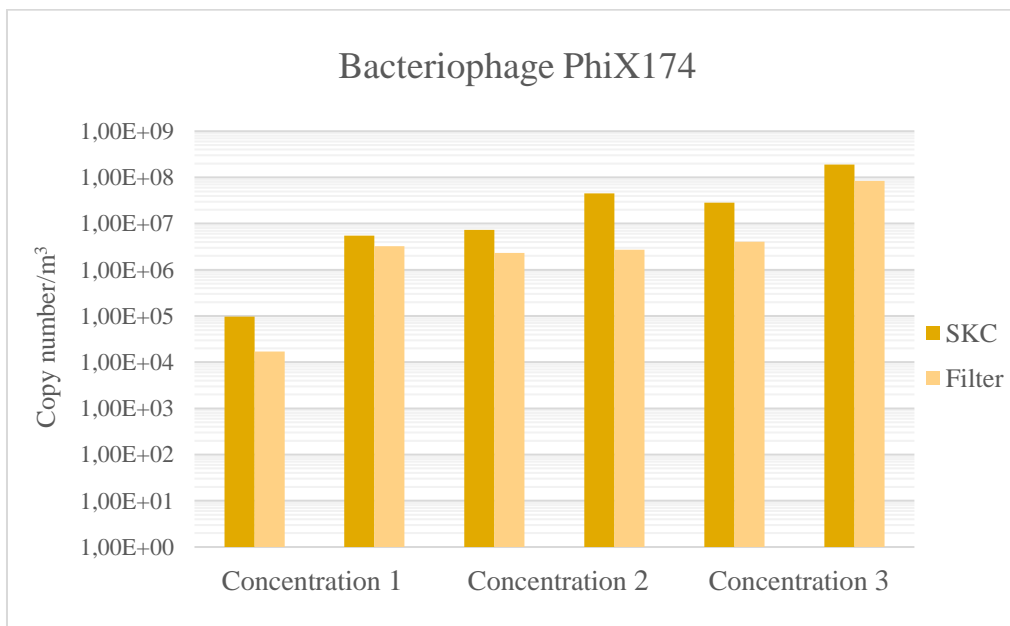


Figure 23: Values of reached copy number/m³ for the three different concentrations in the experiments with the bioaerosol of the bacteriophage PhiX174.

Discussion

The experiments with the bioaerosol of the bacteriophage resulted in increasing values of PFU/m³ for the three concentrations with the SKC BioSampler and the filter cassette (Figure 22: Values of reached PFU/m³ for the three different concentrations in the experiments with the bioaerosol of the bacteriophage PhiX174.. There is only one fluctuation of both sampling devices in the second repeat of concentration 3, although both repeats were done with similar concentrations of the starting suspension. This suggests that there were problems with the activities of the phages, maybe due to temperature differences or other negative influence during the aerosolization and sampling process. If this experiment is not considered, both devices show comparable values in the duplicates. However, the values of the copy number/m³ do show strong fluctuations in the repeats of all concentration steps and both sampling devices (Figure 23: Values of reached copy number/m³ for the three different concentrations in the experiments with the bioaerosol of the bacteriophage PhiX174. These differences can also be seen in the copy number/mL values of the starting suspensions of the three concentration steps. Because the starting suspension experiment did deliver reliable values in the analysis with the qPCR, those differences seem to be inevitable fluctuations in the analysis itself. Nevertheless, an increase of the values can be seen with increasing starting concentrations.

For the comparison of the two sampling devices the values of PFU/m³ and copy number/m³ are summarized and the ratio of the filter cassette efficiency compared to that of the SKC BioSampler is calculated (Table 11: The table for the bacteriophage PhiX174 summarizes the values of PFU/m³ as well as the copy number/m³ for the three different concentrations and the two different sampling devices. The values given in the table are the mean values of the duplicates of each concentration for each sampling devices (n=4). Overall, the SKC BioSampler did deliver higher values of plaque forming units as well as regarding the copy number.

Table 11: The table for the bacteriophage PhiX174 summarizes the values of PFU/m³ as well as the copy number/m³ for the three different concentrations and the two different sampling devices. The values given in the table are the mean values of the duplicates of each concentration for each sampling devices (n=4). It also shows the ratio between the two devices, whereby it is indicated in percent which share the filter cassette has achieved compared to the SKC BioSampler.

Concentration	Device	PFU/m³	Ratio PFU/m³	Copy number/m³	Ratio Copy number/m³
1	SKC	1,25E+05		2,81E+06	
1	Filter	4,05E+04	34,34%	1,62E+06	38,07%
2	SKC	3,67E+06		2,64E+07	
2	Filter	2,13E+06	59,11%	2,51E+06	18,79%
3	SKC	6,23E+06		1,08E+08	
3	Filter	3,97E+05	7,62%	4,42E+07	29,58%

3.3. Bioaerosol of mixed organisms

The experiments with mixed bioaerosols were also performed with three concentrations, three samplings each and with two SKC BioSamplers and two filter cassettes in each sampling. The graphs below show the mean value of these simultaneously taken duplicates for each bar (n=2).

3.3.1. *Penicillium chrysogenum*

Colony forming units and spore counts

For the experiments, all organisms were mixed and aerosolized simultaneously. For the analysis of *Penicillium chrysogenum* it was additionally necessary to count the spores of the starting suspension under the microscope with a Neubauer improved C-chip, to calculate the starting concentration. In addition, each suspension of the sampling devices has been counted, to calculate the spores/m³.

The first *Penicillium chrysogenum* experiment of concentration 1 was started with 2.14×10^7 spores/mL, afterwards the second and third repeat of this concentration were done with comparable spore counts of 1.91×10^7 and 1.93×10^7 spores/mL. The triplicates resulted all in approximately 1.50×10^7 CFU/mL. They led to constant CFU/m³ sampling concentrations with the SKC BioSampler between 1.22×10^4 and 1.46×10^4 CFU/m³, as well as more fluctuating spore counts of 3.14×10^6 , 7.17×10^5 , and 8.28×10^5 spores/m³. A similar result can be seen with the filter cassette, which delivered values of approximately 4.00×10^3 CFU/m³, and spore counts between 2.91×10^5 and 8.17×10^5 spores/m³.

The second concentration was accomplished with three similar starting suspension values of approximately 5.50×10^7 spores/mL, resulting in 4.50×10^7 CFU/mL. In the first experiment the SKC BioSampler was able to sample 5.52×10^4 CFU/m³, which is considerably lower than the values sampled in the second and third repeat, 5.86×10^5 and 5.25×10^5 CFU/m³. In case of the spore counts however, the results were rather the opposite. The first and second repeat delivered similar values of 7.42×10^5 and 6.82×10^5 spores/m³, whereas the third repeat led to a significantly higher concentration of 5.93×10^6 spores/m³. Despite this, the SKC BioSampler was at least for the CFU/m³ values able to achieve an increase in sampled concentrations compared to the experiments with concentration 1. The filter cassette collected fewer germinating particles, given the sampled concentrations between 1.03×10^4 and 5.40×10^4 CFU/m³. But, compared to the SKC BioSampler, the filter cassette was able to deliver consistent spore count values for all three experiments, at approximately 2.38×10^5 spores/m³.

The last concentration was started with values between 7.66×10^7 and 9.55×10^7 spores/mL, leading to CFU/mL concentrations between 5.77×10^7 and 7.93×10^7 CFU/mL. In contrast to the similar values of the second and third repeat with the SKC BioSampler, which reached 5.31×10^5 and 8.61×10^5 CFU/m³, the sampler was only able to collect 5.13×10^3 CFU/m³ in the first experiment.

This fluctuation can also be seen in the spore counts, although not quite as strongly. The values were between 7.29×10^5 and 6.81×10^6 spores/m³. The filter cassette, however sampled in the three repeats comparable values between 3.83×10^4 and 5.01×10^4 CFU/m³, and spore counts between 1.72×10^5 and 4.32×10^5 spores/m³. Both sampling devices were not able to achieve a significant increase in sampled concentrations compared to the experiments with concentration 2, despite increasing starting suspension concentrations.

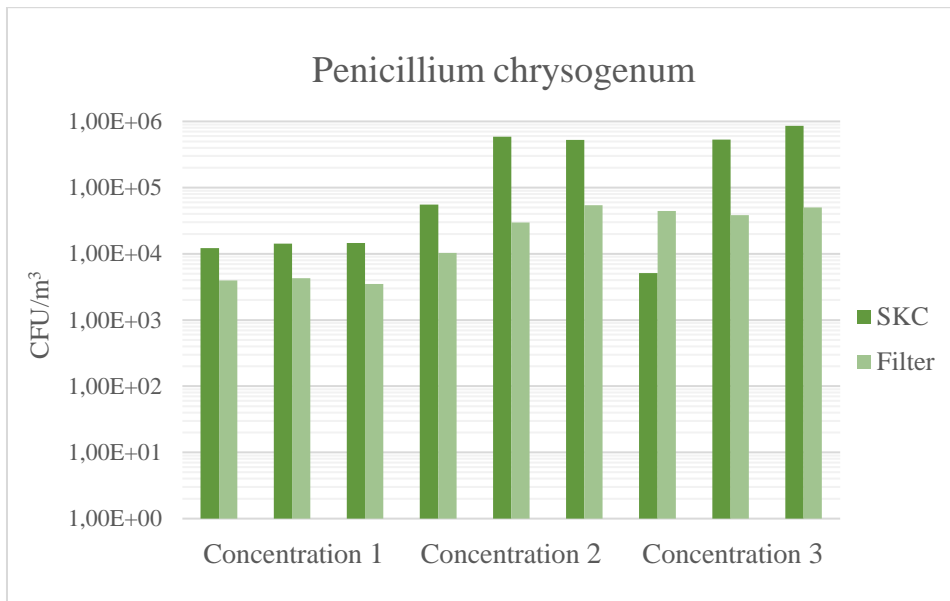


Figure 24: Values of CFU/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments with the bioaerosol of the mixed organisms.

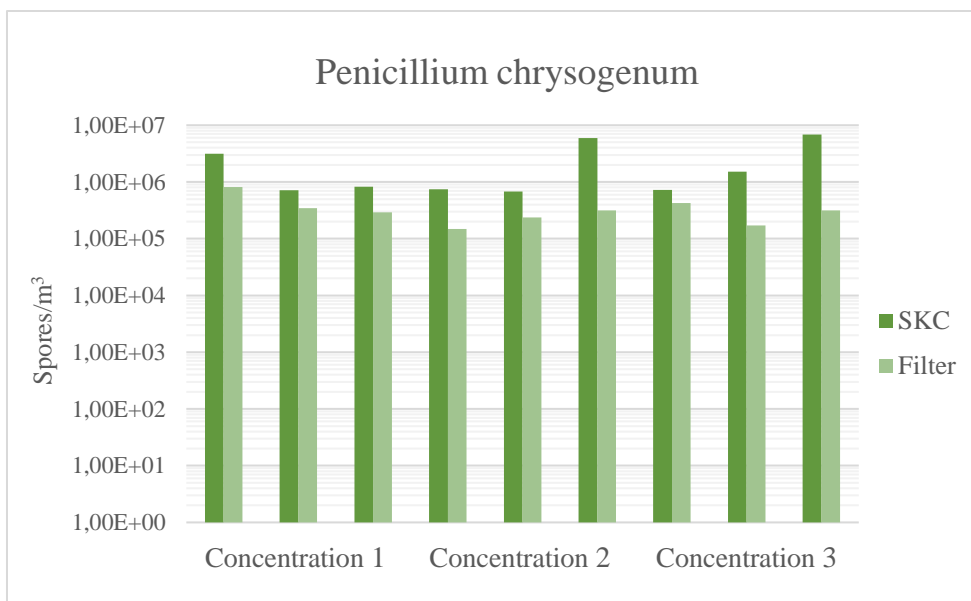


Figure 25: Values of spores/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments with the bioaerosol of the mixed organisms.

Copy numbers

Like the experiments with the bioaerosol of each organism on its own, the organisms were also analysed with a molecular-biological method to be able to calculate the copy number/m³ values of all sampled suspensions. The three experiments of concentration 1 resulted in similar values between 3.47×10^9 and 3.81×10^9 copy number/mL for the starting suspensions. These three repeats with the SKC BioSampler led to rather fluctuating concentration values between 4.74×10^8 and 6.57×10^9 copy numbers/m³. The filter cassette delivered also inconsistent values between 1.20×10^9 and 8.40×10^9 copy number/m³. The second concentration was done with a starting suspension of 8.85×10^9 copy number/mL in the first repeat, as well as 5.83×10^9 and 1.06×10^{10} copy number/mL in the second and third repetition. The experiments delivered non uniform values for the SKC BioSampler between 9.94×10^8 and 1.66×10^{10} copy number/m³. The filter cassette collected also rather scattering values for all three repeats, which were between 7.41×10^8 and 7.34×10^9 copy number/m³. The last concentration was carried out with copy number/mL values of the starting suspensions between 5.59×10^9 and 1.19×10^{10} . The SKC BioSampler was able to reach similar values in the first and third repeat, being at approximately 1.00×10^{10} copy number/m³, whereas the second repeat delivered a lower value of 1.95×10^9 copy number/m³. The sampling with the filter cassette delivered strongly different values of 1.99×10^{10} , 4.80×10^7 , and 1.18×10^9 copy number/m³. In Figure 26 it can clearly be seen, that both sampling devices were not able to achieve a consistent increase in sampled concentrations for the three different concentrations of the starting suspension.

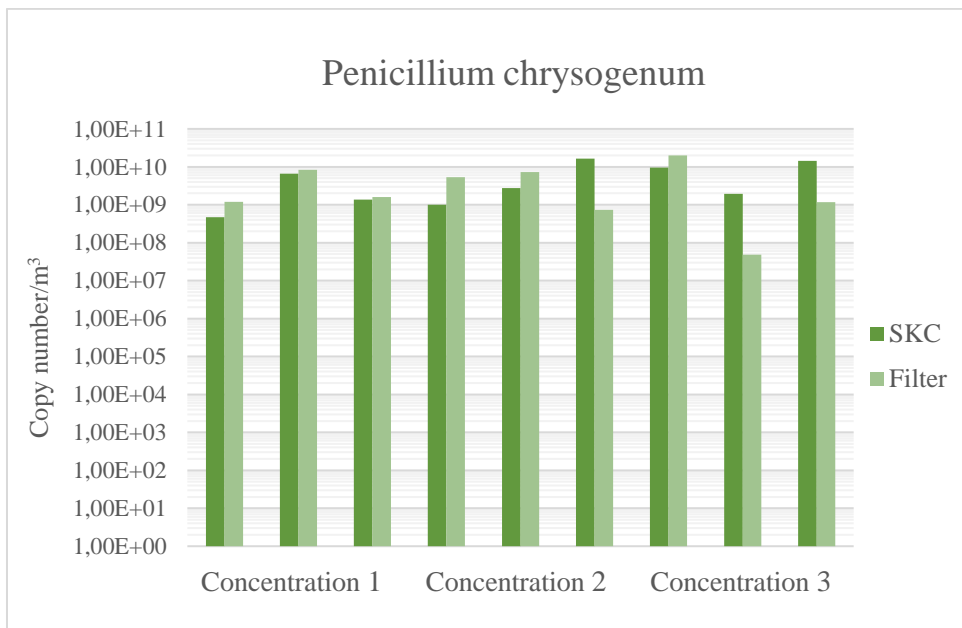


Figure 26: Values of copy number/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments with the bioaerosol of the mixed organisms.

Discussion

In the experiments with the bioaerosol of the mixed organisms, *Penicillium chrysogenum* delivered in the three concentrations relatively constant increasing values of sampled CFU/m³ (Figure 24: Values of CFU/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments with the bioaerosol of the mixed organism). However, the first repetitions of concentrations 2 and 3 should not be included here, because especially the SKC BioSampler collected significantly lower values than in the other repetitions. Since the starting suspensions of all repeats did have comparable concentrations, those fluctuations could be from problems in the growth of the colony forming units. Apart from these outliers, both the SKC BioSampler and the filter cassette were able to sample similar concentrations values in the repetitions of all concentration steps. The smaller increase in sampled CFU/m³ from concentration 2 to concentration 3 could be due to the maximal efficiency of the aerosol generator, which has already been discussed in the experiments with the bioaerosol of each organism.

The values of collected spores/m³ are higher if sampled with the SKC BioSampler, but still all experiments show fluctuations in themselves and rather similar values for all concentrations (Figure 25: Values of spores/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments). This could be influenced by the lower detection limit of the Neubauer improved C-Chip, which, as already stated, only delivers reliable values if the concentrations are above 10⁴ spores/mL. The average number of spores counted in the experiments was however less than that.

The molecular-biological analysis with the qPCR resulted in strong fluctuations (Figure 26: Values of copy number/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments), which can be also seen in the different values of the starting suspensions used for the same concentration step, even though they did have similar concentration values of CFU/mL and spores/mL. The reasons for those high differences between the repetitions and for the rather similar values of all concentration levels are discussed later (3.4.4).

The values of all experiments are summarized to give an overview for the comparison of the two sampling devices (Table 12). The values of the three repetitions of each concentration have been used to calculate a mean value of CFU/m³, spores/m³ as well as of the copy number/m³. The ratio of the efficiency of the filter cassette compared to that of the SKC BioSampler has been only calculated for the colony forming units and the spores, because the values of the copy numbers are strongly fluctuating and cannot provide any well-founded information for comparing the sampling devices. The ratio value of CFU/m³ for the third concentration has been calculated with and without the outlier of the first repetition and therefore resulted in very different percentages of 293% and 6.5%. Apart from this experiment, the SKC BioSampler has reached overall higher values of colony forming units and spores in all three concentrations.

Table 12: Summary of the gained values of CFU/m³ and spores/m³ as well as the copy number/m³ for the three different concentrations of *Penicillium chrysogenum* in the experiments with the bioaerosol of the mixed organisms. The values given in the table are the mean values of the triplicates of each concentration for each sampling devices (n=6). It also shows the ratio between the two devices, whereby it is indicated in percent which share the filter cassette has achieved compared to the SKC BioSampler. * value was calculated without the outlier in the first repeat of concentration 3

Concentration	Device	CFU/m ³	Ratio CFU/m ³	Spores/m ³	Ratio Spores/m ³	Copy number/m ³
1	SKC	1,37E+04		1,56E+06		2,80E+09
1	Filter	3,92E+03	29%	4,84E+05	36%	3,74E+09
2	SKC	3,89E+05		2,45E+06		6,77E+09
2	Filter	3,13E+04	11%	2,34E+05	20%	4,46E+09
3	SKC	4,66E+05		3,02E+06		8,56E+09
3	Filter	4,43E+04	293% // 6.5% *	3,04E+05	25%	7,03E+09

3.3.2. *Geobacillus stearothermophilus*

Colony forming units

The *Geobacillus stearothermophilus* was also analysed in three different concentrations, each done in triplicates. The first experiment of concentration 1 was started with 4.20×10^8 CFU/mL, the second and third repeat with approximately 2.70×10^7 CFU/mL. Because of the higher starting concentration in the first repeat, the SKC BioSampler reached a higher value of 1.55×10^4 CFU/m³ than in the other two, which were both at approximately 8.60×10^2 CFU/m³. The filter cassette delivered more consistent, but also lower values between 3.97×10^2 and 7.49×10^2 CFU/m³. The second concentration was done with starting suspensions of approximately 4.50×10^7 CFU/mL. The SKC BioSampler was able to sample comparable values between 1.10×10^3 and 1.90×10^3 CFU/m³. The filter cassette collected less particles, given the identical sampling concentrations in the first and second repeat of 4.76×10^2 CFU/m³ and the value of 7.14×10^2 CFU/m³ in the third. The last concentration was done with a concentration of 1.53×10^9 CFU/mL in the first repeat, and with lower values of approximately 1.30×10^8 CFU/mL in the other two. Despite those different starting concentrations, the SKC BioSampler delivered rather similar values between 1.57×10^4 and 3.09×10^4 CFU/m³ for the three experiments. The filter cassette, however sampled comparable values of approximately 3.30×10^3 CFU/m³ in the first two repeats and a higher value of 1.00×10^4 CFU/m³ in the third. Therefore, both sampling devices were able to sample higher concentrations than in the experiments with concentration 2.

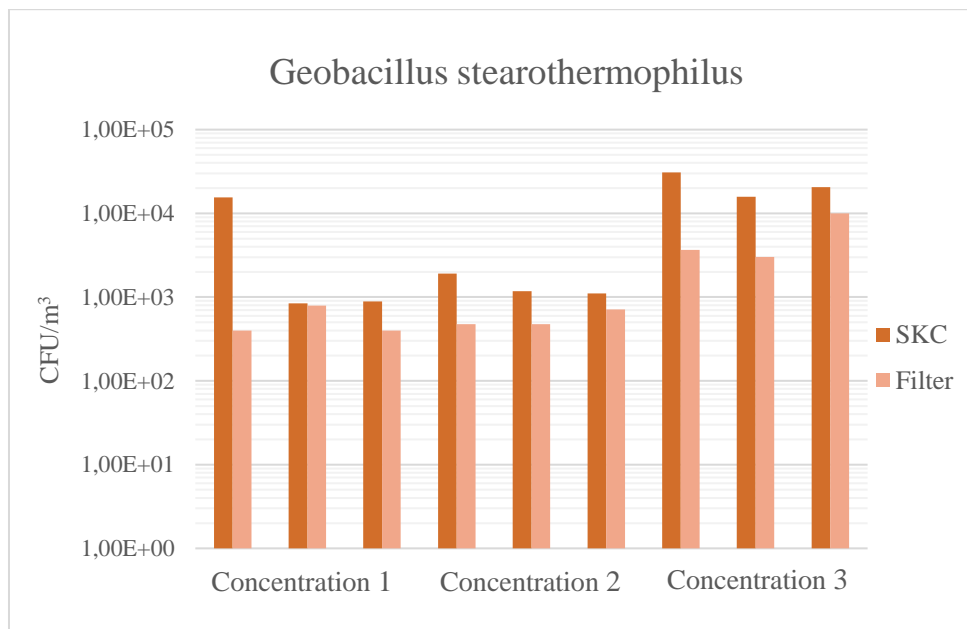


Figure 27: Values of CFU/m³ for the three different concentrations of *Geobacillus stearothermophilus* in the experiments with the bioaerosol of the mixed organisms.

Copy numbers

To calculate the copy number/m³ values of all sampled suspensions of the bacterium, they were analysed with the molecular-biological method of using a qPCR. The three experiments of concentration 1 were started with values between 8.19×10^9 and 1.17×10^{10} copy number/mL. The first repeat with the SKC BioSampler led to a value of 4.70×10^7 copy number/m³, whereas the other two repeats delivered more comparable values of approximately 6.00×10^9 copy number/m³. The filter cassette gave rather scattered values between 2.20×10^8 and 6.22×10^9 copy number/m³. The second concentration was done with three starting suspensions of similar copy number/mL values at approximately 2.00×10^{10} . The experiments delivered non uniform values for the SKC BioSampler between 2.61×10^7 and 1.24×10^9 copy number/m³. The filter cassette collected more comparable values for the first and second repeats, which were 1.11×10^{10} and 7.86×10^9 copy number/m³, but the third repeat could only reach 2.05×10^8 copy number/m³. Despite those fluctuations, the filter cassette was able to sample higher concentrations than the SKC BioSampler. However, both sampling devices did not deliver consistently increasing values compared to the experiments with concentration 1. The last concentration was carried out with copy number/mL values between 3.54×10^{10} and 6.27×10^{10} . The SKC BioSampler sampled again scattered values between 6.73×10^7 and 4.87×10^9 copy number/m³. The sampling with the filter cassette resulted also in inconsistent, but still higher values between 2.95×10^8 and 5.55×10^9 copy number/m³.

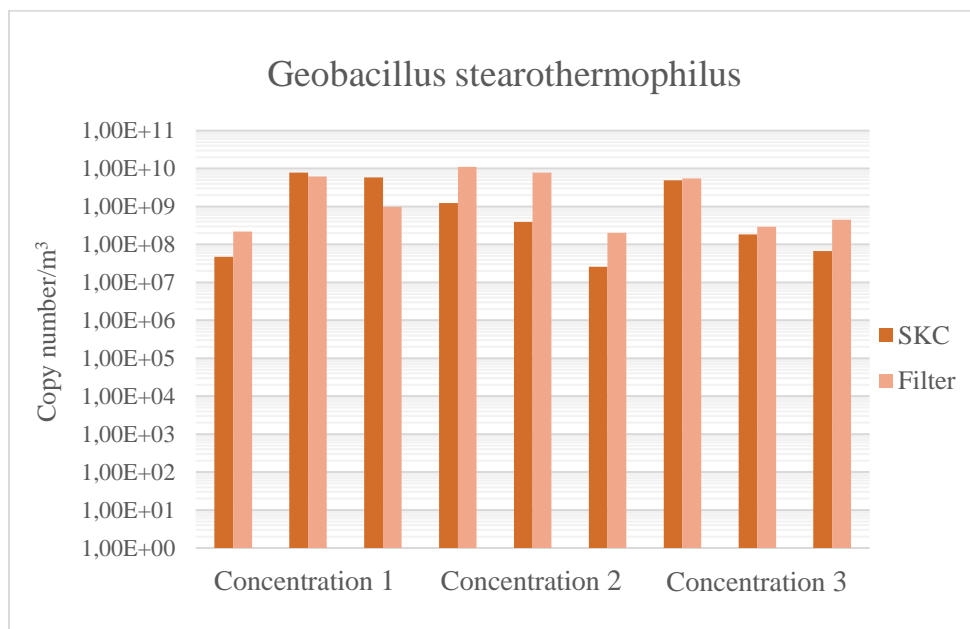


Figure 28: Values of copy number/m³ for the three different concentrations of *Geobacillus stearothermophilus* in the experiments with the bioaerosol of the mixed organisms.

Discussion

As the starting material was not counted, as for the *Penicillium* spores, there were some problems with reaching the correct concentration of the starting suspension in the experiments with *Geobacillus stearothermophilus*. This can especially be seen in the first repeat of concentration 1. Therefore, the SKC BioSampler did deliver here much higher results of CFU/m³ than in the other repetitions (Figure 27: Values of CFU/m³ for the three different concentrations of *Geobacillus stearothermophilus*, but it is unclear why the filter cassette did not. It could result from agglomerates in the SKC BioSampler, which then get divided into smaller parts (Cox et al., 2020). Additionally, the starting suspension concentrations for the second concentration were not much higher than for the first, resulting in only slightly increased CFU/m³ values, regardless of which sampling device was used. The problems with reaching the correct starting suspension concentration could be explained by the fluctuating growth of the overnight culture. Even though the OD₆₀₀ was measured before starting the experiments, and suspensions with too high optical densities were diluted, it is still difficult to say how many bacterial cells are still viable. The reason for this difficulty is, that the optical density is also influenced by dead cells and cell fragments (Eppendorf, 2015) and also by changes of the pH or the temperature (Francois et al., 2005). Especially with the *Geobacillus stearothermophilus* there are fluctuations in the concentration of culturable cells in overnight cultures because it can also form endospores if there is a lack of nutrition or other unfavourable growth situations. Those spores survive harsher settings but can need different culture conditions than vegetative cells of the bacterium to grow and form colonies (Wells-Bennik et al., 2019). In the third concentration, which was done with significantly higher starting suspension concentrations, there was an increase in the values of sampled CFU/m³ with both devices. Additionally good to mention is, that the repetitions of each concentration were able to deliver comparable values, if the starting suspension concentrations were also similar.

In the analysis of the copy number/m³ strong differences can be seen between the repetitions and an increase of the values for the three concentrations, as it can be seen for the CFU/m³, is missing (Figure 28: Values of copy number/m³ for the three different concentrations of *Geobacillus stearothermophilus*. The molecular-biological analysis did not deliver reliable values, leading to the assumption that there were similar problems as with the qPCR of *Penicillium chrysogenum*.

The results of the SKC BioSampler and the filter cassette are summarized for a better overview and comparison of the two sampling devices (Table 13: Summary of the gained values of CFU/m³ and the copy number/m³ for the three different concentrations in the of *Geobacillus stearothermophilus* in the experiments with the bioaerosol of the mixed organisms. The values given in the table are the mean values of the triplicates of each concentration for each sampling devices (n=6). The ratio of the sampling efficiency from the filter cassette compared to that of the SKC BioSampler is calculated for the values of CFU/m³, but not for the copy number/m³ as they didn't follow the increase of concentrations and didn't fit to the CFU values. For the first repeat of concentration 1, the ratio has been calculated with

and without taking the outlier into consideration, resulting in values of 47% and 69%. In summary it can be said, that the SKC BioSampler was able to reach higher concentrations of colony forming units in all experiments, especially with higher starting suspension concentrations.

Table 13: Summary of the gained values of CFU/m³ and the copy number/m³ for the three different concentrations in the of *Geobacillus stearotherophilus* in the experiments with the bioaerosol of the mixed organisms. The values given in the table are the mean values of the triplicates of each concentration for each sampling devices (n=6). It also shows the ratio between the two devices, whereby it is indicated in percent which share the filter cassette has achieved compared to the SKC BioSampler. *Value was calculated without the outlier in the first repeat of concentration 1

Concentration	Device	CFU/m ³	Ratio CFU/m ³	Copy number/m ³
1	SKC	5,76E+03		4,59E+09
1	Filter	5,29E+02	47% // 69%*	2,48E+09
2	SKC	1,39E+03		5,52E+08
2	Filter	5,56E+02	43%	6,38E+09
3	SKC	2,24E+04		1,71E+09
3	Filter	5,56E+03	27%	2,10E+09

3.3.3. Bacteriophage PhiX174

Plaque forming units

Like the other two organisms, the bacteriophage was analysed in three different concentrations, each carried out in triplicates to be able to accomplish a comparability and reliability of the values. The three experiments of concentration 1 were all started with approximately 5.00×10^8 PFU/mL, leading to consistent sampling concentrations between 1.34×10^5 and 2.10×10^5 PFU/m³ with the SKC BioSampler. The filter cassette delivered more fluctuating values between 2.50×10^4 and 1.17×10^5 PFU/m³. The second concentration was done with starting suspensions between 5.97×10^8 and 9.00×10^8 PFU/mL. In the three experiments the SKC BioSampler was able to sample comparable values of approximately 5.00×10^5 PFU/m³. The filter cassette collected less particles, given the sampled concentrations between 6.34×10^4 and 2.06×10^5 PFU/m³. It was therefore, in contrast to the SKC BioSampler, not able to sample higher concentrations than in the experiments with concentration 1. The last concentration was started with a concentration of 3.87×10^9 PFU/mL in the first repeat, and with 1.17×10^9 and 5.27×10^9 PFU/mL in the other two. In contrast to the results of the first and third repeat with the SCK BioSampler, which resulted in values of approximately 6.00×10^6 PFU/m³, the sampler was only able to collect 6.13×10^5 PFU/m³ in the second experiment. The filter cassette sampled significantly lower values between 7.90×10^4 and 4.05×10^5 PFU/m³.

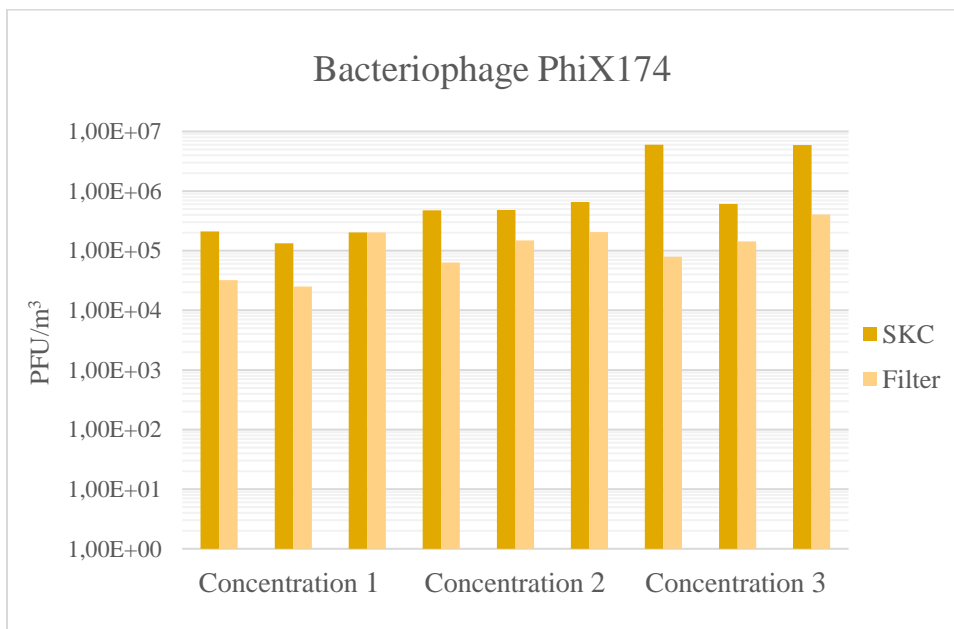


Figure 29: Values of PFU/m³ for the three different concentrations of the bacteriophage PhiX174 in the experiments with the bioaerosol of the mixed organisms.

Copy numbers

As already described, the sampled suspensions were analysed with a molecular-biological method to be able to calculate the copy number/m³ values for the experiments. The three experiments of concentration 1 were started with values between 7.56×10^7 and 9.30×10^8 copy number/mL. The first repeat with the filter cassette led to a value of 1.01×10^8 copy number/m³, whereas all other sampled suspensions did not show any values in the qPCR. The second concentration was accomplished with three starting suspensions of similar copy number/mL values at approximately 4.00×10^9 . The experiments with concentration 2 delivered, similar to the first concentration, almost no values, except for one of the SKC BioSampler and one of the filter cassette, reaching 8.27×10^{10} and 2.86×10^7 copy number/m³. The last concentration was done with copy number/mL values between 2.98×10^9 and 5.64×10^9 . The only value which could be determined was in the first repeat with the filter cassette, sampling a concentration of 3.29×10^9 copy number/m³.

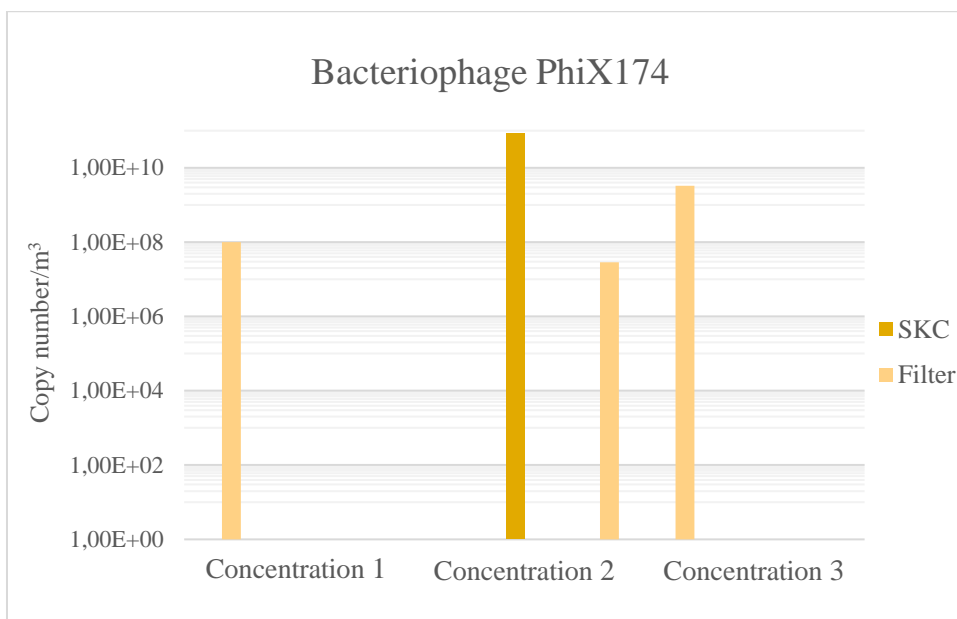


Figure 30: Values of copy number/m³ for the three different concentrations for the bacteriophage PhiX174 in the experiments with the bioaerosol of the mixed organisms.

Discussion

The experiments with the bioaerosol of the mixed organisms did deliver reasonable values for the PFU/m³ of the bacteriophage PhiX174, showing a consistent increase with each concentration and a good comparability of the repeats (Figure 29). However, there are some fluctuations, seen in the last repeat of concentration 1 with the filter cassette and in the second repeat of concentration 3 with the SKC BioSampler. Because the concentrations of the starting suspensions were similar in the repetitions and the differences are only seen with one sampling device, those outliers could result from problems in the growth conditions. Since the results of the other two organisms do not show any fluctuations for these experiments, there should have been no problem with the sampling process itself.

The molecular-biological analysis with the qPCR was not able to deliver results for most of the sampled suspensions (Figure 30: Values of copy number/m³ for the three different concentrations for the bacteriophage PhiX174). The starting solutions did result in reasonable copy number/mL values, but also showed some differences in the first concentration even though the concentrations of plaque forming units were similar. Nevertheless, the values of the PFU/mL and copy number/mL from the starting suspensions were comparable to those from the experiments for the evaluation of the starting suspension. Therefore, the samples were within the measurable range and the results can be rated as meaningful.

The protocol for the molecular-biological analysis was verified based on the experiments for the evaluation of the starting suspension and provided there reliable results for the mixed organisms. In addition, the copy number of the bacteriophages could be determined in the experiments with the bioaerosol of each organism, so the qPCR also works when the bacteriophages are aerosolized. Only in the experiments with the bioaerosol of the mixed organisms, the analysis could no longer deliver any results. Although values were obtained for the other two organisms, they also showed very strong fluctuations and rather unreliable values. All in all, these results indicate that something changes as soon as the three organisms are aerosolized and sampled in a mix.

Therefore, this analysis cannot be used to make any assumptions about the efficiencies of the sampling devices nor to compare them with each other. Therefore, only the ratio of the PFU/m³ was calculated and is summarized together with the total PFU/m³ values of all experiments in Table 14. As an overall result, the SKC BioSampler was able to achieve higher sampling concentrations than the filter cassette.

Table 14: Summary of the gained values of PFU/m³ for the three different concentrations of the bacteriophage PhiX174 in the experiments with the bioaerosol of the mixed organisms. The values given in the table are the mean values of the triplicates of each concentration for each sampling devices (n=6). It also shows the ratio between the two devices, whereby it is indicated in percent which share the filter cassette has achieved compared to the SKC BioSampler.

Concentration	Device	PFU/m³	Ratio PFU/m³
1	SKC	1,82E+05	
1	Filter	5,81E+04	31%
2	SKC	5,36E+05	
2	Filter	1,39E+05	25%
3	SKC	4,19E+06	
3	Filter	2,09E+05	11%

3.4. Summary of Comparisons

For the comparison of the two sampling devices, the different analyses are summarized. The values from the evaluation of the starting suspension are shown in red, the CFU/mL or PFU/mL are plotted against the copy number/mL values, because it was not analysed in the bioaerosol chamber and therefore no values per m³ can be calculated. The experiments with the bioaerosol of each organism (solo) and with the bioaerosol of the mixed organisms (mix) are colorized in blue and lilac and presented by plotting CFU/m³ or PFU/m³ against copy number/m³. The values of the SKC BioSampler are displayed in darker colours than the values of the filter cassette. All three concentrations are shown in the figure, either done in duplicates for the solo or in triplicates for the mixed experiments. Additionally presented are the values of the Gas Washing Bottle for the solo experiments in light green and for the mixed experiments in dark green by plotting the CFU/mL against the copy number/mL. The values of the starting suspension for each experiment were plotted the same way and are shown in yellow for the experiments with the bioaerosol of each organism (solo) and in brown for the experiments with the bioaerosol of the mixed organisms (mix).

3.4.1. *Penicillium chrysogenum*

All experiments with *Penicillium chrysogenum* are summarized in Figure 31. The evaluation of the starting suspension shows two outliers in the first two values, because of the too high values in the qPCR. Especially for the experiments with the bioaerosol of the mixed organisms, the values of the qPCR are scattered and therefore the different concentrations cannot be told apart. In the experiments with the bioaerosol of each organism the expected increase is seen and is only because of the efficiency limit of the aerosol generator not as strong as expected. The higher concentration values, which were achieved with the SKC BioSampler in the experiments with the bioaerosol of the mixed organisms, can be explained by the fact, that they were carried out with a higher starting suspension concentration. The values of the starting suspensions show a consistent increase and also fit very well with those of the evaluation of the starting suspension regarding the ratio between CFU and copy number. The values of the other experiments as well as those of the Gas Washing Bottle, however, do not really fit the linear increasing function.

Shown with the same type of representation and assignment, are the values of all experiments in spores/mL or spores/m³ against copy number/mL or copy number/m³ (Figure 32: Comparison of the different analyses in the evaluation of the starting suspension (red) as well as the solo (blue) and mixed (lilac) experiments. The graph shows the values of the spores/m³ and the copy number/m³ for the SKC BioSampler (darker colours) and the filter cassette (lighter colours) in the three different concentrations. Additionally presented are the values of the GWB for the solo (light green) and for the mixed experiments (dark green) and the values of the starting suspension for the solo (yellow) and mixed experiments (brown). The values from the evaluation of the starting suspension, the GWB and the STS are shown in spores/mL

and copy number/mL. For the experiments with the bioaerosol of each organism the consistent linear increase of the sampled concentrations and the comparability of the repeats are recognizable. Additionally, the higher sampling efficiency of the SKC BioSampler can be seen.

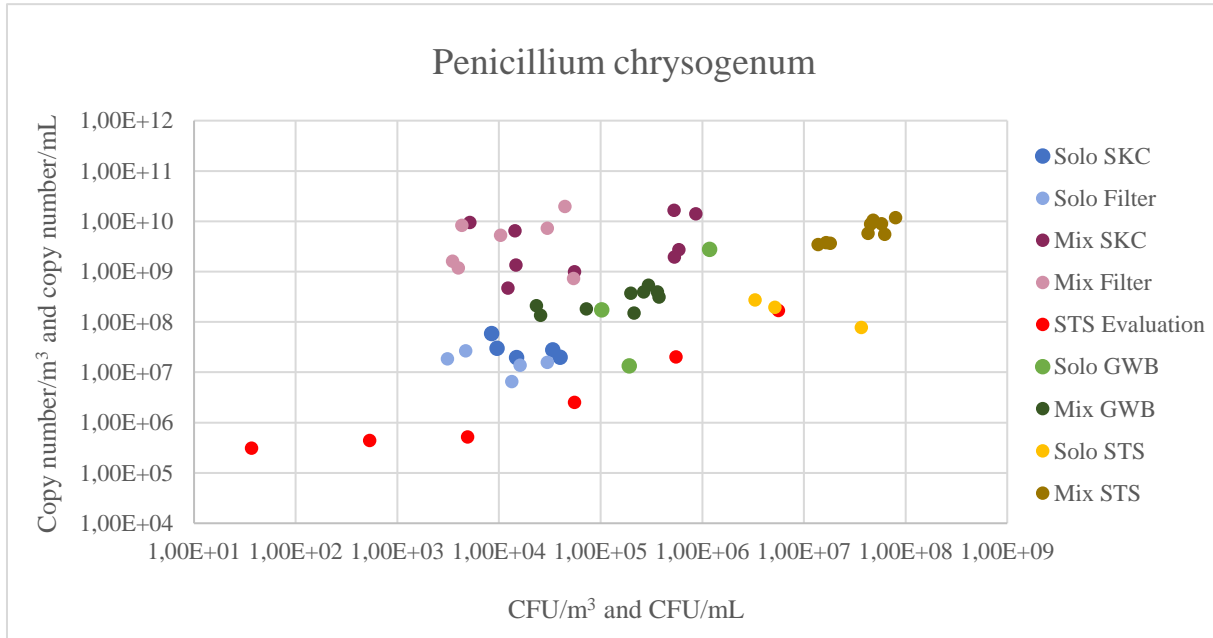


Figure 31: Comparison of the different analyses in the evaluation of the starting suspension (red) as well as the solo (blue) and mixed experiments (lilac). The graph shows the values of the CFU/m³ and the copy number/m³ for the SKC BioSampler (darker colours) and the filter cassette (lighter colours) in the three different concentrations. Additionally presented are the values of the GWB for the solo (light green) and for the mixed experiments (dark green) and the values of the starting suspension for the solo (yellow) and mixed experiments (brown). The values from the evaluation of the starting suspension, the GWB and the STS are shown in CFU/mL and copy number/mL.

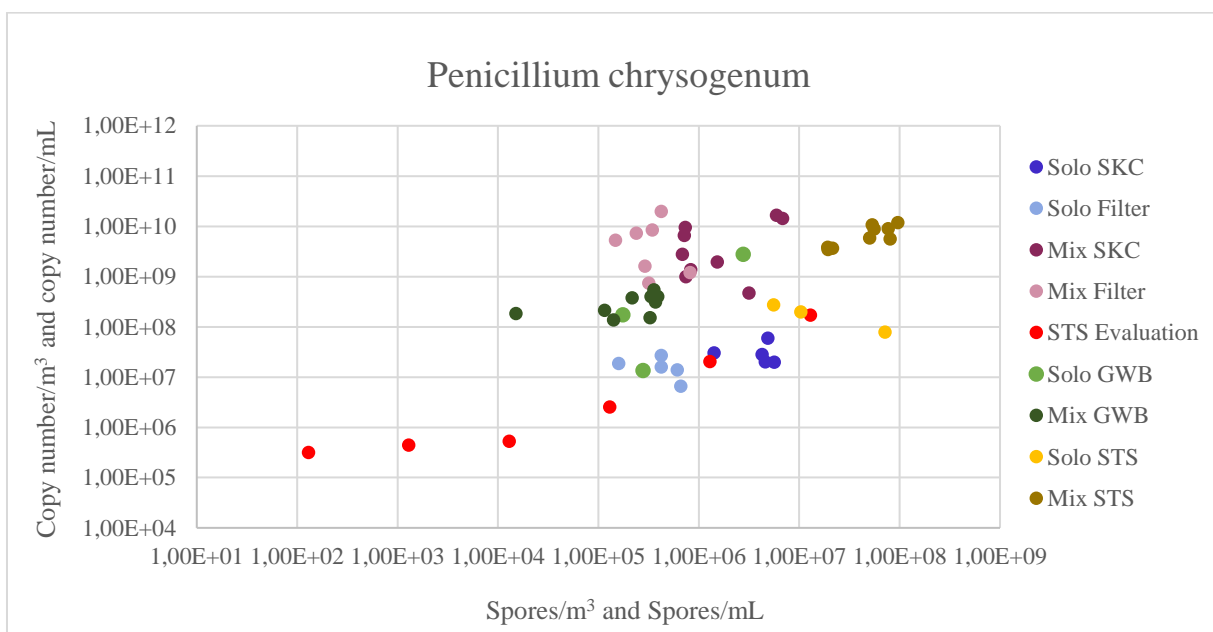


Figure 32: Comparison of the different analyses in the evaluation of the starting suspension (red) as well as the solo (blue) and mixed (lilac) experiments. The graph shows the values of the spores/m³ and the copy number/m³ for the SKC BioSampler (darker colours) and the filter cassette (lighter colours) in the three different concentrations. Additionally presented are the values of the GWB for the solo (light green) and for the mixed experiments (dark green) and the values of the starting suspension for the solo (yellow) and mixed experiments (brown). The values from the evaluation of the starting suspension, the GWB and the STS are shown in spores/mL and copy number/mL.

3.4.2. *Geobacillus stearothermophilus*

Like the *Penicillium chrysogenum*, the values of all experiments with the *Geobacillus stearothermophilus* are summarized for the comparison of the different sampling devices. The values for the mixed experiments are not valuable for a comparison of the two sampling devices, because of the strong fluctuations of the copy number values. The experiments with the bioaerosol of each organism, however, do show a consistently higher efficiency of the SKC BioSampler. The three different concentration steps can be seen in those values, but rather in an increase of copy number/m³ than in one of the CFU/m³. The values of the starting suspensions and those of the Gas Washing Bottle show a consistent linear increase and fit therefore very well with the evaluation of the starting suspension. Nevertheless, if this linear function is compared to the values of the SKC BioSampler and the filter cassette, there is a strong divergence.

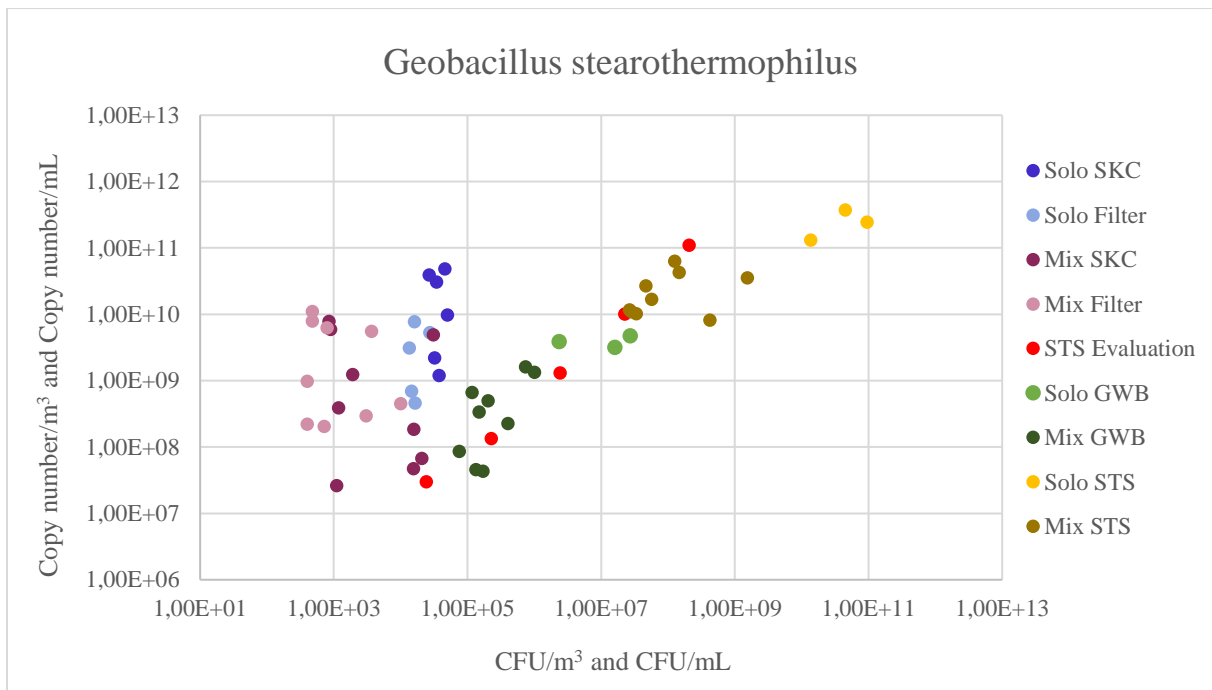


Figure 33: Comparison of the different analyses in the evaluation of the starting suspension (red) as well as the solo (blue) and mixed experiments (lilac). The graph shows the values of the CFU/m³ and the copy number/m³ for the SKC BioSampler (darker colours) and the filter cassette (lighter colours) in the three different concentrations. Additionally presented are the values of the GWB for the solo (light green) and for the mixed experiments (dark green) and the values of the starting suspension for the solo (yellow) and mixed experiments (brown). The values from the evaluation of the starting suspension, the GWB and the STS are shown in CFU/mL and copy number/mL.

3.4.3. Bacteriophage PhiX174

In contrast to the other two organisms, the values for the sampled suspensions of the bacteriophage are only shown for the evaluation of the starting suspension (red) and the experiments with the bioaerosol of each organism (blue). This is because it was not possible to determine the molecular-biological values of copy number/m³ with the qPCR in the experiments with the bioaerosol of the mixed organisms. Even though the repetitions do not fit that well together, which is caused by the fluctuations in the copy number values, the different concentration steps can be seen clearly. Also recognizable in the aerosol experiments is, that the SKC BioSampler was able to accomplish higher values. Additionally, in comparison to the other organisms, the results of the aerosolized samples fit to the results of the evaluation of the starting suspension regarding the ratio between PFU and copy number.

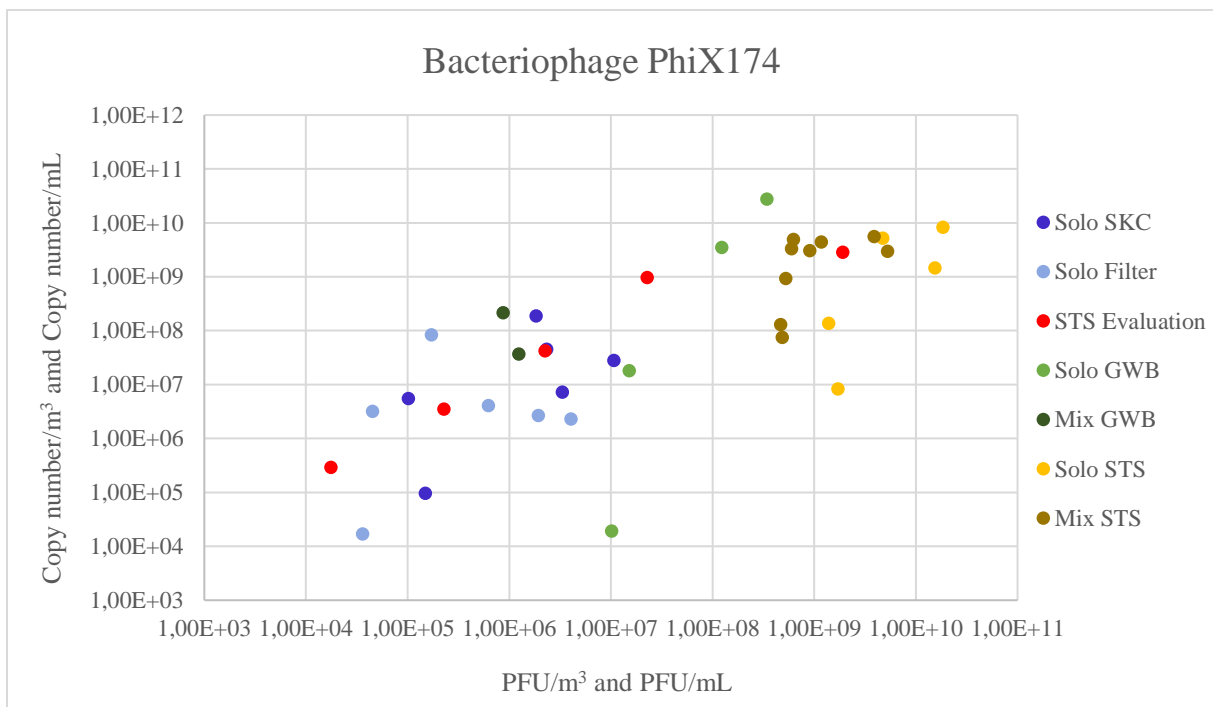


Figure 34: Comparison of the different analyses in the evaluation of the starting suspension (red) as well as the solo (blue) experiments. The graph shows the values of the PFU/m³ and the copy number/m³ for the SKC BioSampler (darker colours) and the filter cassette (lighter colours) in the three different concentrations. Additionally presented are the values of the GWB for the solo (light green) and for the mixed experiments (dark green) and the values of the starting suspension for the solo (yellow) and mixed experiments (brown). The values from the evaluation of the starting suspension, the GWB and the STS are shown in PFU/mL and copy number/mL.

3.4.4. Conclusio

In the direct comparison the experiments with bioaerosols containing the individual organisms did deliver more reliable and consistent values for the different concentrations and their repeats than the ones with the bioaerosols of the mixed organisms. Overall, the values of the mixed experiments show strong fluctuations in the qPCR, by which the copy number/m³ values become erratic. Therefore, the different starting suspension concentrations do not result in increasing sampling concentrations of copy number/m³ and do not show comparable results in the repeats, regardless of which sampling device was used.

Especially for the bacteriophage most suspensions of the mixed experiments were not able to deliver a value in the qPCR at all. The efficiency of the molecular-biological analysis is strongly dependent on the DNA isolation protocol, as already stated in other experiments with the bioaerosol chamber CCB3000 (Unterwurzacher et al., 2018). Also reported was, that mechanical damage can happen to sensitive microorganisms during the aerosol collection, which then could affect the efficiency of the DNA isolation (Hey Reoun An et al., 2006). However, this would not apply to the experiments of this study, because the suspensions of the bacteriophages had not undergone the DNA isolation protocol. It might be, that the three organisms influence each other in the aerosolization or sampling process and therefore decrease the efficiency and accuracy of the qPCR. This would explain why the evaluation of the starting suspension, which was in the same concentration range as the solo and mixed experiments, did deliver reliable values of copy number/mL, although the three organisms were also mixed, but not aerosolized yet. An additional problem, which could have also affected the analysis in the experiments is, that the qPCR is influenced by dead cells and inactive bacteriophages and can therefore deliver a much higher value than only viable cells would accomplish (Álvarez et al., 2013).

The Gas Washing Bottle proves that the cells survived the general aerosolization process well. It yielded high values in all experiments and therefore verifies, that the cells are viable and culturable after the aerosolization process. The results of the colony and plaque forming units of the different analyses are therefore reliable but cannot be compared with the values of the copy number, due to the strongly fluctuating qPCR values.

Overall, it can be summarized that the SKC BioSampler was able to sample higher concentrations of all three organisms, regardless of the starting suspension concentrations and if the organisms have been aerosolized separately or simultaneously. Those results coincide with other research projects on collection devices. Li et al., 2018 stated a similar conclusion in experiments with the influenza virus, where they compared the performance of three bioaerosol samplers, using the SKC BioSampler, a gelatine filter and a glass fiber filter.

Another comparison study of four aerosol samplers was accomplished with the SKC BioSampler, a compact cascade impactor, Teflon filters and gelatine filters (Fabian et al., 2009). They reported, that the SKC BioSampler recovered and preserved the infectivity of the virus much better than the other samplers.

Because of the well-established performance characteristics, and the minimal damage to sensitive microorganisms, the SKC BioSampler was also already used as a reference sampler to evaluate other sampling devices (H. R. An et al., 2004). Additionally, the results of the filter cassette in the experiments with the three different organisms are comparable with other published studies, which stated that it did not work as efficient as other sampling devices. Uhrbrand et al., 2018 tested different air samplers and filter materials for the collection and recovery of airborne human norovirus. They described significantly lower recovery of aerosolized murine norovirus with a 3- piece closed-face Millipore cassette than with other sampling devices. In the comparison of different filter types, they tested four different materials and found a significantly higher recovery of the virus by using nylon filters than by using polycarbonate filters. In an aerosolization study with *Legionella pneumophila* different sampling devices were compared, among them were a filter cassette containing a polycarbonate filter and different liquid based sampling methods (Chang & Chou, 2011). Results show, that even though the filter cassette had sampled higher values of total-cells-collected, the efficiency of obtaining viable and culturable cells was more appropriate by the BioSampler.

4. Summary

The research of aerosols is becoming more and more important, especially in the light of communicable diseases or allergies. It is central to be able to examine the pathogens precisely and to create controlled and realistic conditions for this. The investigation with a bioaerosol chamber enables the imitation of those environmental conditions in a sterile and save manner and to study fungi, bacteria and viruses more closely.

This master thesis has focused on the comparison of two sampling devices, to choose the most efficient collection method of viable and culturable cells from different organisms. In three different experimental settings, the SKC BioSampler and the 37mm closed face air sampling cassettes, containing a polycarbonate membrane filter, were compared regarding the sampling efficiency of *Penicillium chrysogenum*, *Geobacillus stearothermophilus* and the bacteriophage PhiX174. The aerosolization process was accomplished with the usage of the Liquid Sparging Aerosolizer into the bioaerosol chamber CCB3000. At first, the organisms were analysed in a mixed suspension and without the usage of the bioaerosol chamber. Determined were the CFU/mL and PFU/mL values of each of the suspensions and the copy number/mL values were calculated by the usage of a qPCR. Those experiments proved that the organisms did not influence each other in the culturing process or in the determination of the molecular-biological data. As a second step, each organism was aerosolized and sampled in the bioaerosol chamber separately in three different concentrations, each done in duplicates. To be able to compare the efficiencies of the two sampling devices, all suspensions were analysed with micro- and molecular-biological methods and the CFU/m³, spores/m³ and PFU/m³ as well as the copy number/m³ were calculated. In the last experimental setting, all organisms have been mixed, aerosolized and sampled simultaneously in three different concentrations, each carried out in triplicates. The analysing methods for all suspensions were like those of the solo experiments.

In summary it can be said that the overall set-up of the experiments has worked well. Also, the repetitions of the individual experiments mostly fit well together, if one considers that the initial concentrations of the starting suspensions always had slight deviations. Additionally, the SKC BioSampler and the filter cassette delivered both generally reliable values of CFU/m³ and PFU/m³ in the solo and mixed experiments and for all organisms and concentrations. Nevertheless, it must also be mentioned, that there were repeatedly outliers in the different experiments. Especially in the molecular-biologically analysis with the qPCR, there were great difficulties and high standard deviations for the experiments with mixed organisms. Overall, it can still be said, that the SKC BioSampler did sample significantly higher values than the filter cassette throughout all experiments.

For *Penicillium chrysogenum*, the filter cassette reached in the solo experiments between 32.43% – 59.79% of CFU/m³ compared to the SKC BioSampler. In case of *Geobacillus stearothermophilus* it was 45.33% – 57.20% of CFU/m³, whereas for the bacteriophage PhiX174 the efficiency reached from 7.62% to 59.11% of sampled PFU/m³, depending on the concentration level. The ratios of the copy number/m³ were comparable to those of the CFU and PFU for all three organisms.

In the experiments with the bioaerosol of the mixed organisms the filter cassette delivered even lower efficiency values, reaching only 6.5% – 29% of CFU/m³ of *Penicillium chrysogenum* compared to the SKC BioSampler, depending on the different concentration level. The ratio of collected CFU/m³ for the *Geobacillus stearothermophilus* was between 27% and 47%, whereas for the bacteriophage PhiX174 it was 11% - 31% PFU/m³. The qPCR did not deliver reliable values in the mixed experiments for all three organisms and therefore the copy number/m³ values are not used for the comparison of the two sampling devices. Additionally, also the sampled spores/m³ values are rather not meaningful because of the low concentration levels, which did not enable a trustworthy count with the Neubauer improved C-Chip. Especially the problems with the qPCR, when all three organisms were mixed, aerosolized, and sampled together should be studied in more detail to find out if they influence each other during the molecular-biological analysis or if there are other difficulties during the sampling process.

Above all, the simple handling of the collection device and the minimization of damage to the sampled particles speak in favour of the SKC BioSampler. By sampling the particles with a liquid-based strategy it is much easier to cultivate and analysis the suspension. The closed face air sampling cassette contains a polycarbonate filter, which means that it must be washed with buffer solution to regain the sampled particles. In this washing and shaking process, it is not certain that all the particles can be detached from the cassette housing and the filter itself. In addition to this more complex processing of the collected particles, the sampling time in the aerosol chamber in this study was one hour for the filter cassette, whereas the SKC BioSampler only collected for 20 minutes. In summary of this master thesis, it can be said, that the usage of a SKC BioSampler is preferable regardless of the species or concentration which is sampled.

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