NTNU
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Microbial Growth on Indoor Artificial Turf and Assessment of Suitable Disinfection Methods

Mikrobiell vekst på innendørs kunstgress og vurdering av egnede desinfeksjonsmetoder

Bachelor thesis

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Preface

This bachelor thesis is written by Chemical Engineering-students from the Department of Materials Science and Engineering at the Norwegian University of Science and Technology, and the project is done at a request from SIAT (Center for Sports Facilities and Technology).

The theme of the thesis is microbial growth on indoor artificial turf and disinfection methods. It has been very exciting, challenging and not least educational to work on this task. The work was carried out in the period 06.01.2021 - 20.05.2021.

We would like to express our gratitude to our supervisor Ina Merete Stuen, who has given us advice, follow-up and good feedback. We would also like to thank our external supervisor Bjørn Aas from SIAT who has been there for us and given us input throughout the project.

We want to thank Are Pedersen for all the help he gave us and for supplying us with a disinfection device and advice throughout the process, Flatåshallen that willingly let us use their hall for testing, and lastly Hege Sundgård for always helping us when we had questions regarding laboratory work. All work was carried out at Flatåshallen and at NTNU's Department of Materials Science and Engineering in the laboratory at Kalvskinnet.

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Abstract

Bacterial growth in artificial grass is a concern, particularly due to the latest generations of artificial turf (4G) contain organic infill instead of rubber granulate. The organic infill is more environmentally friendly but does not contain bacteria inhibitory substances and might even provide nutrition for the bacteria to grow. This might result in infections to its users, some even life threatening.

Therefore, microbial growth Flatåshallen's indoor turf was analyzed within a 4-week period after an initial testing, and disinfection methods were tested on turf pieces *in vitro*. This included taking samples from four areas of the pitch: area A (Middle of the penalty area), area B (Midway between the penalty box and halfway line), area C (On the midway line) and Area D (the sideline closest to the entrance). The samples were collected using a ESWAB 480CE within a 10x10 cm frame collecting 10 parallels from each area. The samples were analyzed with a spectrophotometer at 600 nm and inoculated at 37°C on tryptic soy agar (TSA) plates. Colony forming units (CFU) were counted two days after inoculation. The results for both methods showed that area D and A had the highest amount of contamination and were therefore chosen for the 4-week testing period.

During this period there were found averages of between 4 650 and 20 500 CFUs per dm^2 in the chosen areas. This is likely an underestimation due to the low amount of matter the swab picks up. Other factors that played a role in the uncertainty when collecting samples were the pressure applied during sample collection, that bacteria likely was not evenly spread out on the field and that the swab did not reach areas deep in the grass. Based on the results there was not an accumulation of bacteria over time, but probably a correlation between maintenance (brushing) of the grass and high contamination, likely due to stirring up existing microorganisms into the air and surface. In the CFU method there seemed to be yellow bacteria colonies dominating area A, while area D tended to have more variation. Fungal growth was also prevalent in the tests and seemed more dominating within area D than area A. The samples should have been diluted with transport media instead of water as the latter could lead to cell death and inaccuracy. The spectrophotometer was used at 420 nm, 500 nm, 600 nm and 660 nm in the 4-week period, and while the method had its limitations, it provided an indication of the total degree of contamination.

The disinfectant test was done twice *in vitro* with three chemical substances and UV light on individual grass offcuts. Samples from area A, B, C and D from Flatåshallen were inoculated in TSB and then separated from the media after four days. The bacteria culture was inserted into a water bath that the pieces were dipped in. Due to fungal growth in the first test, the process was repeated by isolating pure bacteria from the samples collected. The offcuts were soaked in ethanol and sodium hypochlorite to disinfect them between tests. The tests were done using hydrogen peroxide (1,5%), sodium hypochlorite (1,5%) and citric acid (1,5%), and these were sprayed on the grass with a collision nozzle with 1-2 m distance for 10 seconds. The UV light (6W, 254 nm) was swept across the piece for five seconds with a height about 10 cm. All disinfectant methods except UV-light resulted in a positive, but unsatisfying effect on both fungi and bacteria.

Sammendrag

Bakterievekst i kunstgress er et problem, spesielt på grunn av at nyere generasjoner av kunstgress (4G) inneholder organisk fyll istedenfor gummigranulat. Det organiske fyllet er mer miljøvennlig, men inneholder ikke bakteriehemmende stoffer og kan til og med tilføre næringsstoffer som vil øke bakterieveksten. Dette kan resultere i infeksjoner for dens brukere, noen til og med livstruende.

Derfor ble bakterieveksten i Flatåshallens innendørs kunstgressbane analysert over en firukersperiode etter initielle tester og desinfeksjonsmidler er testet på gressflekker *in vitro*. Her ble det tatt prøver fra fire ulike steder på banen: område A (i midten av straffefeltet), område B (midt mellom straffefeltet og straffeboksen), område C (midtlinjen) og område D (sidelinjen nærmest inngangen). Prøvene ble samlet med ESWAB 480CE som ble brukt innenfor en 10x10 ramme. Det ble samlet 10 paralleller fra hvert område. Prøvene ble testet på et spektrofotometer ved 600 nm og inokulert ved 37°C på tryptic soy agar (TSA) plater. Koloniformede enheter (CFU) ble telt etter to dager inokulering. Resultatene fra både spektrofotometer og CFU metoden viste at det var område D og A som retrospektivt hadde høyest grad av forurensning og de var derfor valgt å gå videre med i 4-ukers testen.

I denne perioden ble det gjennomsnittlig funnet mellom 4 650 og 20 500 CFU per dm^2. Dette tallet er sannsynligvis underestimert på grunn av den lave mengden stoff fanget opp av prøvepinnen. Andre faktorer som kan ha økt usikkerheten ved forsøket var trykket som ble brukt ved prøvetaking, at mengden bakterier sannsynligvis ikke vil være jevnt fordelt utover banen og at bomullsdotten ikke nådde områder dypt i gresset. Basert på resultatene var det ikke en akkumulering av bakterier over tid, men sannsynligvis en korrelasjon med vedlikehold (børsting) av banen og høy kontaminering, sannsynligvis på grunn av at det børstes opp eksisterende mikroorganismer til overflaten og luften. I CFU metoden var det ofte en gul bakteriekoloni som dominerte område A mens hadde område D mer variasjon. Soppvekst var også gjennomgående gjennom hele testperioden og var ofte observert på tester av område D. Prøvene burde ha blitt fortynnet med transportmedium istedenfor vann for å ikke føre til celledød, som vil skape usikkerhet i testen. Spektrofotometeret ble brukt ved 420 nm, 500 nm, 600 nm and 660 nm i 4-ukers perioden, og selv om metoden hadde sine begrensinger, ga den allikevel en indikasjon på den sammenlagte totale kontamineringen.

Desinfeksjonstesten ble gjort to ganger *in vitro* med tre kjemiske substanser og UV lys, brukt på individuelle gressflekker. Prøver fra område A, B, C og D i Flatåshallen ble inokulert i TSB og separert fra mediet etter fire dager. Bakteriekulturen ble tilsatt et vannbad som gressflekkene ble dyppet i. På grunn av soppvekst ved første test, ble prosessen gjentatt, men denne gangen ved å isolere ren bakteriekultur fra de første prøvene. Gressflekkene ble desinfisert ved å bade dem i etanol og klorin mellom testene. Testene ble utført ved å bruke hydrogenperoksid (1,5%), natriumhypokloritt (1,5%) og sitronsyre (1,5%) som ble sprayet på grasflekkene med en kollisjonsdyse ved 1-2 meter avstand på 10 sekunder. UV lyset (6W, 254 nm) ble sveipet over gressflekken på 5 sekunder med en høyde på 10 cm. Alle desinfiseringsmetodene utenom UV-lys virket å ha en positiv men utilfredsstillende effekt på både sopp og bakterier.

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Abbreviations

CFU	Colony Forming Unit
EPDM	Ethylene Propylene Diene Monomer
EPS	Extracellular Polymetric Substance
PCR	Polymerase Chain Reaction
qPCR	Quantitative (real-time) PCR
SBR	Styrene Butadiene Rubber
TPE	Termo Plastic Elastomer
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

Glossary

Absorption	How much light that is absorbed by an object or a solution
Antimicrobial agents	Substance that kills or inhibits growth of microorganisms
Aerobic microorganisms	A microorganism that utilizes oxygen for growth, and have an oxygen-based metabolism
Biofilm	Accumulation of microorganisms that act interdependently in order to stick to each other and a surface by forming a slimy protective matrix between them
DNA primer	A specific gene sequence that binds to a complementary sequence in a denaturised DNA and represent the starting point of the DNA replication
Epiglottitis	Condition that causes the inflammation and swelling of the epiglottis, a flap of tissue in the throat
Facultatively anaerobic microorganisms	A microorganism that can grow in both the presence and absence of oxygen
Fastidious microorganisms	Microorganisms in need of very specific care, has often complicated nutritional requirements
Hemocytometer	Plate with marked sections and specific volume used an aid to help count bacterial samples
Inert	Non-reacting
Nosocomial infection	An infection acquired in a hospital
Opportunistic bacteria	Bacteria that could work as a pathogen if given the opportunity by a weakened outer or inner immune system
Pathogen	Organisms such as bacteria, viruses and fungi that can induce illness
Planktonic bacteria	Bacteria living as individual organisms and not in colonies or biofilms
Sepsis	Condition where the body's reaction to an infection causes damage to its own tissues
Sinusitis	Sinus infection that causes swelling of the sinuses
Transmittance	How much light that goes through an object or a solution

1. Introduction

Artificial grass has since its inception in the mid-sixties been used in both sports fields and gardens and is a fundamental need for being able to practise sports in many countries of the world (1). Through the years the artificial turf used in sports has gone through several generations of change. In the beginning the fields were almost carpet-like because the grass was so short and did not contain any infill, but the grass has gotten higher and several infill types have been used in later years (2). For many years the use of rubber granules was preferred, but recently this has become a controversial option as several studies have shed a light on the environmental pollution and bad effects of microplastics, including several types of rubber granules (3-7). This has led to many new, environmentally friendly options being developed, but unfortunately it has been difficult to find an infill that does not affect the playing conditions that rubber granules provided, while also being easily available and affordable (8, 9). These types of infill are still not common in most of the world, even though there are usable candidates. One of these alternatives is a combination of sand and olive stones, which is currently being used in an indoor facility called Flatåshallen, located in Trondheim in Norway (10). Indoor facilities do however rarely have good ventilation and there are no methods in use today to clean or disinfect the pitches, even though these are used often, and blood, spit and other fluids often find their way onto the turf (11-13). In a time with much focus on disinfection and cleaning, it is uncommon to find indoor facilities where this is such a low priority as on artificial turf.

As with most surfaces, microorganisms might be found on this turf, but there are few investigations on how the conditions on artificial grass in indoor facilities with this type of infill fit microorganisms and how the presence of bacteria and fungi develop over time. The rubber infill which has been used contain a lot of toxic compounds such as heavy metal which may inhibit the growth and accumulation of microorganisms in the turf, but the environmentally friendly alternatives generally does not contain these toxic compounds (6, 8). This means that these types of infill potentially could be a better environment for bacteria and fungi than their predecessor. If the microbial presence is high enough, this could be health risk for the athletes. When sports are being practised on artificial turf it is common to obtain bruises and abrasions, which might provide microorganisms in the grass a pathway into the body and potentially lead to infections (14). Both bacteria and fungi could potentially have negative health effects if they have a notable presence in artificial grass (15, 16).

The Centre for Sport Facilities and Technology (SIAT) at NTNU is an establishment that are researching and studying topics regarding both the facilities where sports are practised and technology associated with sports, such as equipment used in Paralympic sports (17). Artificial grass is one of their focus areas, and as a part of their research they seek to find out how significant the microbial contamination is in Flatåshallen. It is important to find out if measures must be taken to irradicate the microorganisms in the grass to reduce the infection risks for the athletes. The objective of this project has therefore been split into two parts:

1. **How significant is the microbial presence in the artificial grass Flatåsen?**
2. **Which disinfection methods can be used for artificial grass?**

2. Theory

This literature review revolves around the relevant background information for the objective of this project. This includes the use of artificial grass, why fourth generation infill is being phased in, how the grass is being maintained, how bacteria grow and behave, the microbial presence in artificial turf and the infection risks associated these microorganisms. In addition to this, it also gives necessary information about the relevant testing methods, disinfection and some of the chemicals that are being used.

2.1. Usage and evolution of artificial grass in sports

Today artificial grass is used in sports fields all over the world and has in many countries replaced natural grass as the preferred turf. In a guide to artificial grass from 2015, the Norwegian Ministry of Culture estimated that an artificial grass field normally would have between 1500 and 2500 hours of use every football season, all depending on the weather conditions and the use of chemicals or heating systems to keep it operational during the winter (12). By comparison, a regular natural grass field only has about 150-250 hours of use. This difference is a consequence of long winters, little sunlight and rain. Rain is especially a big factor as muddy pitches make the playing conditions worse, increase the risk of ruining it and possibly increase the risk of injuries (2). Maintenance of natural grass also requires more resources and hours of work to keep the playing conditions good.

2.1.1. Evolution of artificial grass and the negative impact of rubber granules

Artificial turf was first used in sports in 1966, by the Houston Astros in their then new stadium, the Houston Astrodome (18). Initially they tried to use natural grass but the playing conditions quickly became so bad that they switched to artificial grass after a year. In Norway, artificial grass was first used in a football match in the northern city Harstad in 1977 and is now the dominant turf for football (19). These early fields used grass labelled as first-generation artificial grass, which was characterized by not having any kind of infill (2). The grass in this generation was usually short and made of nylon. It also did not look a lot like natural grass and had its disadvantages for the athletes. The grass did for example absorb a lot of heat and could therefore be an unpleasant playing field on warm days.

Later, second-generation turf was introduced with sand used as the infill (20). The grass fibres were now longer and the sand was used to keep the blades of grass upright. It was also able to dissipate some of the heat absorbed by the grass, but it could be painful for athletes making tackles or falling, as the friction between the turf and the athlete could result in painful abrasions and skin burns. In this version it also became more common to use polypropylene instead of nylon (2). The first two generations of artificial grass were not very suitable for sports such as football and rugby, as they did not alleviate shocks from the athletes' feet and were not suitable for using shoes with studs. Therefore third-generation of artificial grass was introduced, a type that did not result in as many abrasions as its predecessors (3). It became common to install a shock absorbent pad under the grass, but this is not the case for all third-generation artificial turfs. The layers and build of an artificial grass field with shock absorbent are shown in figure 1, from the sub-base to the grass fibres (21).

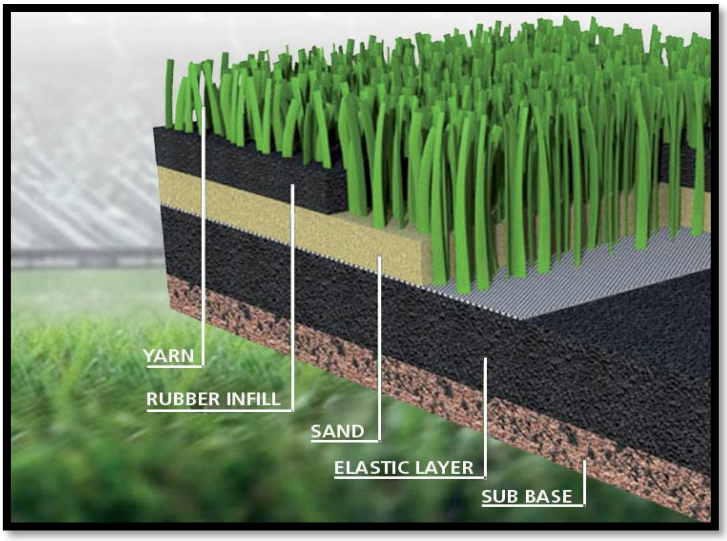

Figure 1. The layers of artificial turf in sports including the shock absorbent (elastic layers) that many fields use today (21).

The most significant difference in this generation was the use of rubber granules as infill (22). There are many alternatives as the granule, for example TPE, EPDM and SBR made from car tires (12). These changes in the grass have made the playing conditions better, but the infill has in recent years been a source of controversy as research has revealed the environmental and health consequences of these rubber granules. In Norway, it is estimated that over 1500 tons of rubber granules from sports fields every year end up in nature, which makes it one of the biggest sources microplastic pollution (4, 23). Rubber granules often contain a mixture of heavy metals and other organic compounds which in high concentrations might be toxic for life both in the ocean and on land. It might even have an impact on human health by moving along with the food chains (5, 6). There is a difference in the environmental impact of the alternative types of microplastics, as EPDM for example contain a smaller number of environmentally hazardous compounds than the plastic from car tires, according to a report by Norwegian company Byggforsk (24). These aspects have resulted in many proposals regulations of rubber granules, and the European Union is expected to set regulations within few years (4). Indoor sports fields also pose another problem, poor air quality partly caused by rubber granules (7). The amount of airborne dust might increase with use of certain types of rubber granules, especially SBR, according to a study by Norwegian Institute for Air Research (NILU) in 2005. Although they concluded that this did not result in any notable health hazards, except possibly for those who install the grass, the facilities had concentrations of airborne dust that exceeded the national recommendations for indoor facilities. Despite the conclusion that the health hazards are low, it is still important reduce the exposure to airborne dust, either by using other infill than SBR or bettering the ventilation (11).

This has led to the development of the fourth generation of artificial grass, where the main focus is to find a more environmentally friendly infill than the rubber granules or even make the turf without infill (3). Some sources define fourth generation artificial grass only as grass without infill, whereas others share the definition used here. Many brands also label their infill as either the fifth or sixth generation of artificial grass, but this is mostly for promotional reasons (2).

2.1.2. Infill options for 4G artificial grass

Rubber granules are gradually being replaced with environmentally friendly infills, but it has been difficult to find an infill that gives a good playing experience, is easily available, affordable and does not increase the chance of injuries (8). Still, some usable alternatives such as cork, olive stone and sand have been tried out, but most of these do not tick all the boxes that the ideal infill should do (4, 8). One option is to use sand and crushed olive stones, a combination currently used in Flatåshallen in Trondheim. The amount of infill used in this facility is 15 kg sand per m^2 and 2 kg crushed olive stones per m^2 (10).

2.1.3. Cleaning methods in use today

There are not many measures in use to keep the artificial turf clean (12). The Norwegian Ministry of Culture has in its guide to artificial grass only listed one cleaning method in its section on maintenance. This is an annually deep cleaning of the pitch done with a machine specially made for this purpose. The deep cleaning is performed to remove gum, snus, tape and leaves from the field. The culture department recommends doing this several times a year if necessary. Other maintenance methods as for example brushing the field with special made brushes are also recommended (25). These brushes can usually be attached to a vehicle and quickly brush an entire pitch, such as the brush in figure 2 (26). Measures like these might however be a problem in terms of microbial presence, as brushing of the field might stir up microorganisms, dust and other particles without removing them (27). There are no other common cleaning methods in use today (none stated in the Norwegian Ministry of Culture's guide), and this could potentially leave the pitches vulnerable to accumulation of organic material and microorganisms, as lack of cleaning generally leads to a higher rate of biofilm accumulation (28).

Figure 2. Example of how a brush for artificial turf could look. These are generally attached to a vehicle (26).

2.2. Bacterial behaviour and formation of biofilms

Bacteria does not normally exist as planktonic bacteria but tend to gather and form colonies. This happens in cycles where planktonic bacteria form colonies and grow together, and in the end disperse which lets the planktonic bacteria released from the colonies start the cycle again as they seek to form new colonies (29). When there is an accumulation of microorganisms, they can communicate by signal molecules to gather and create a community, in which they cooperate in order to create a protective structure (30). These structures are called biofilms, and provide the bacteria with physical protection, as well as protection against external factors such as UV-light, pH shifts and antimicrobials (31). Bacteria existing in biofilm can potentially be up to 1000-fold more resistant to antibiotic treatment than bacteria in a planktonic state. The biofilm often attracts different bacteria species enabling them to cooperate (30). This is a beneficial behaviour in nature, but in artificial environments created by humans it can cause problems. The biofilm's ability to protect the bacteria as well as enabling them to grow rapidly can be a danger to humans if the community includes bacteria that has a pathogenic effect on humans, such as *Staphylococcus aureus*, *Escherichia coli* and *Neisseria meningitidis (32, 33)*.

2.2.1. Biofilm occurrence and collective behaviour for efficiency and protection

Biofilms can be created by either one or multiple species (30). They can grow on both biotic and abiotic surfaces and usually occur in the area between air and liquid. This can often be a challenge as biofilms can form on several different surfaces, which means that many industries as such as food, medicine and many more have to spend money to remove the biofilms and to keep their equipment clean (34). Single specie biofilm is most common on medical implants and tissue in the human body, while multiple species biofilm is more common in nature.

Multi-species biofilm is advantageous because it enables the species to cooperate and exchange nutrients, metabolic components and remove toxic end products (30). They do so by using their primitive water canals created by EPS, an umbrella term for the polymeric components of the highly hydrated matrix that surrounds the bacteria in biofilms. EPS generally consists of proteins, polysaccharides, extracellular DNA glycolipids and more. The matrix is the term of the entire non-bacterial structure, and can have a slimy texture and makes up a large part of the biofilms, as it for some bacteria could consists of less than 10% bacteria and more than 90% matrix (35). The EPS acts as the building blocks of the matrix and is the main cause for protection from environmental stress such as UV-rays, rapid pH change, osmotic shock, and extreme dehydration (30). The biofilm is mostly created by metabolically similar species and can make them able to survive in climates they would not otherwise. They can even have a syntropic relationship, that requires mutual dependence to synthesize materials for energy.

Although different bacteria species can cooperate in a multi-specie biofilm, one of the most important interactions is the competition between them for the present nutrients (36). This also includes producing compounds that have a toxic effect on other bacteria, something *Pseudomonas aeruginosa* and S*treptococcus oligofermentas* are known to do in multi-species biofilms, with their victims being *Candida* and *Streptococcus mutans* respectively. When bacteria gather to create an interdependent biofilm community, they switch on genes that enable them to act this way (34). This makes biofilm-existing bacteria phenotypically different than bacteria that exist elsewhere.

2.2.2. Stages of biofilm creation

Biofilm-forming bacteria go through a cycle that begins and ends with planktonic bacteria seeking to form a colony (29). Bacteria prefers to establish themselves in biofilms, with only about 1% of the total bacterial population in the world being purely planktonic (34). For a biofilm to be formed a conditioning layer first must form on a surface. This consists of many different chemical compounds, both organic and inorganic, which helps the bacteria attach to the surface (31). After the conditioning layer is formed, there are five stages to the development of biofilms: reversible adhesion, irreversible adhesion, early maturation, maturation, and dispersion. A simplified illustration of this process can be seen in figure 3 where the two attachment-steps and the two maturation steps are combined (37).

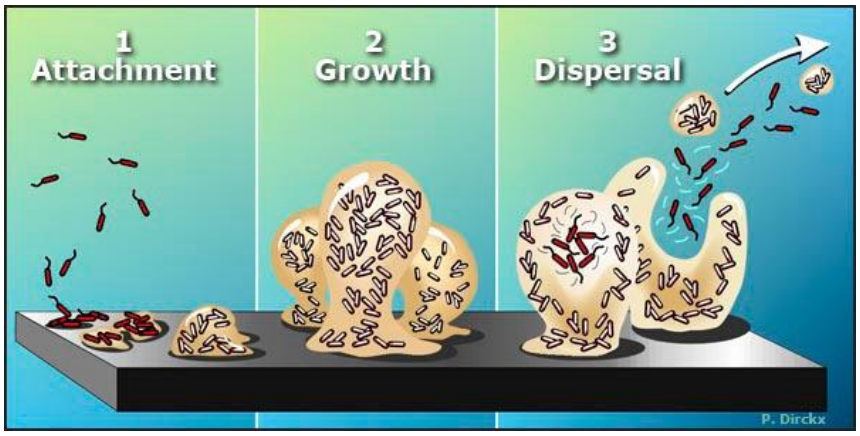

Figure 3. Simplified illustration of the main stages of biofilm development (37).

The first step of this process is the reversible adhesion, where planktonic bacteria are transferred to a surface and attached reversibly by weak van der Waals forces and other interactions (34). The attachment is mediated by pili, fimbriae and flagella, which in addition to EPS help adsorb the bacteria to the conditioned surface (31). There are also repulsive forces that work between the bacteria and the surface, which can cause dispersion and enables it to detach and move to other surfaces (34). Eventually the bacteria will attach permanently to the chosen area and to nearby bacteria, resulting in irreversible adsorption. In the maturation stage the biofilm will grow and change shape as the bacteria divide, often resulting in a mushroom-like form. Bacteria will multiply and the biofilm also attracts bacteria from the nearby area. In the distribution phase many bacteria die, which results in production of enzymes that break down polysaccharides. This results in the biofilm breaking apart and bacteria spread to other surfaces to make a new biofilm community. Biofilm creation happens continuously, and the different phases will usually intersect.

2.3. Microbial presence in artificial grass

The lack of cleaning and disinfection methods in artificial grass pitches, could lead to increased accumulation of microorganisms and biofilm in the turf (28). This might especially be the case on indoor surfaces, where the fields are screened from environmental factors. Bacterial accumulation is a potential problem for some users as it could result in dangerous infections (38). Therefore, it is interesting to study the basis of bacterial growth on artificial grass and what the origins of the bacteria are. When the origins of the bacteria are known, it is easier to investigate which pathogens can be found in the grass, how they infect a human body and which users are especially susceptible to these infections.

2.3.1. Microbial growth in artificial grass

Bacteria tend to grow together in colonies and form biofilms, and there is no reason to believe that microbial growth on artificial grass should be an exception as the bacteria could either form biofilms on the infill, on the grass fibres or on both sites, but this is an area with too few studies to state conclusively (30, 38). Especially in indoor facilities where the pitches are screened from external factors such as weather and other conditions that can inhibit growth, bacteria and fungi may be able to thrive, as many studies comparing general indoor and outdoor bacteria concentrations have shown (39, 40). These studies focus on airborne bacteria but give an indication of the concentration on the ground too. The bacterial concentration on indoor fourth generation artificial grass is not thoroughly studied, but there have been studies of other indoor surfaces for reference. In a study from 2009 at Ullevål University Hospital on hospital floors before mopping and another study on surfaces in office buildings in Belgium from 2005, the number of bacteria was between 300 and 500 CFU per dm^2 and from 4 to 4000 CFU per dm^2 respectively (41, 42). There are some potential problems for the microbes that could result in less growth on artificial grass, as microbial growth is restricted by factors such as temperature and the access to essential nutrients. The temperature in artificial grass indoors is often between 10 and 15 degrees Celsius, but information about nutrients in the grass could not be found (10, 43). On outdoor pitches the temperature of the artificial turf often rises to a level which inhibits bacterial and fungal growth (14).

Another factor that could impact the growth conditions is the choice of infill on the pitch (6). As the rubber granules contain organic compounds and heavy metals that could be hazardous, this could hinder or slow down the growth of microorganisms. This may not be the case for the fourth generation of artificial turf as the infill options that are used generally are organic and do not consist of hazardous compounds (8).

2.3.2. Possible sources of microorganisms in artificial grass

Microorganisms in artificial turf could have many origins and there is a big difference in the presence of bacteria in outdoor natural grass fields and artificial grass fields (38). In natural grass fields most of the bacteria are soil-related, whereas in artificial grass most bacteria come from external sources such as humans, animals, leaves and the natural microflora of the area. A study of American football fields using artificial turf, done in 2013 by the Weber State University concluded that the infill of the artificial grass serves as a possible habitat of microorganisms and that human activity increases the quantity of bacteria (13). This is mainly because of the transfer of spit, sweat and blood from humans to the grass. On outdoor surfaces microorganisms could come from both human and environmental sources, but this is not the case from indoor surfaces which are screened from the environment (44). The possible sources of microorganisms on indoor surfaces are among others human activity, materials in the

building, the ventilation and air conditioning. Studies have for example shown a correlation between increased human activity in indoor sports facilities and higher microbial presence in the air.

2.3.3. Common opportunistic bacteria in our normal flora

As studies concluded that among others, spit, sweat and blood were plausible sources of microorganisms in artificial grass, it is of interest to examine the pathogenic bacteria that are usually found in the skin, oral and upper respiratory tract flora, as these bacteria likely would be the ones that end up on the synthetic turf (13). Bacteria are common in our normal flora, but some of them act as opportunistic bacteria, which means that they can cause diseases if given the opportunity (16).

The skin flora is the habitat of some opportunistic bacteria, such as *Staphylococcus epidermis* and *Staphylococcus aureus* (45). The former makes up a large part of the skin flora and is a common nosocomial bacterium. Infections by this pathogen may be hard to treat as it is resistant to some antibiotics and can form biofilms on inert surfaces (45, 46). The other staphylococci, *S. aureus*, shares many of the qualities of *S. epidermis* as a pathogen by being a cause of nosocomial infections and being resistant to many antimicrobial agents (47). It can cause a wide range of disorders, some life-threatening, making it a very dangerous opportunistic pathogen. It can be found in between thirty to fifty percent of healthy adults and is even more likely to find in certain patients in hospitals.

Common habitats of other opportunistic pathogenic bacteria are the oral and upper respiratory tract flora. Among others, *Streptococcus pnemoniae, Haemophilus influenzae* and *Neisseria meningitidis* can be found here *(45). S. pnemoniae* is a pathogen that mainly affects the youngest or the oldest people and possesses the quality of being resistant to many antibiotics. This makes it difficult to treat, and infections could be the cause of sepsis, meningitidis, sinusitis and as the name suggests, pneumonia (48). *H. influenzae* could also be the cause of some of these diseases (49). Both sinusitis and pneumonia are believed to sometimes be the result of a *H. influenzae* infection, and in addition to these it is a major cause of acute epiglottitis and could sometimes be the cause of endocarditis, urinary infections and many more diseases. *N. meningitidis* is the main source of diseases like fatal sepsis and meningitidis and is an exclusive bacterium of humans (33). This has led to the development of meningococcus vaccines to decrease the likelihood of dramatic illness for people susceptible to infections.

These bacteria are only a few of the opportunistic bacteria that can be found in our normal flora, as more bacteria like these can be found in the stomach, conjunctiva and many other places in the body (45). Opportunistic bacteria generally require a host with a weakened immune response to cause a dangerous infection and this may be the case for some of the users of artificial grass sports fields, as a weakened immune system may have different causes, such as poor nutrition and alcohol consumption (16, 50).

2.3.4. Infection risks of playing on artificial turf

The human body has several means of protection from bacterial infections (51). Although many bacteria are inhabitants of the body, they rarely cause infections. The skin is one of the outer barriers of the body and limits bacterial growth by being a dry environment while also being colder and more acidic than what most bacteria prefer. These factors make it difficult for bacteria to colonise and grow. A functioning immune system combats the bacteria that does find its way into the body, which in turn reduces the number of bacteria able to cause dangerous infections (52).

Despite this, there may occur instances while performing sports on artificial grass where opportunistic bacteria get the chance to cause an infection (14). It is believed that bacteria could get access to infect the body through abrasions and wounds acquired on the field, and this is not too uncommon on artificial turf, as the legs figure 4 is an example of (53). Most sports are performed with much of the skin left bare, which in could result in the surface of the field being in direct contact with the skin of the athlete (14). Despite this, some studies have concluded that the transmission of the infectious bacteria more frequently happen as a result of physical contact between athletes, through bad sanitation or shared equipment.

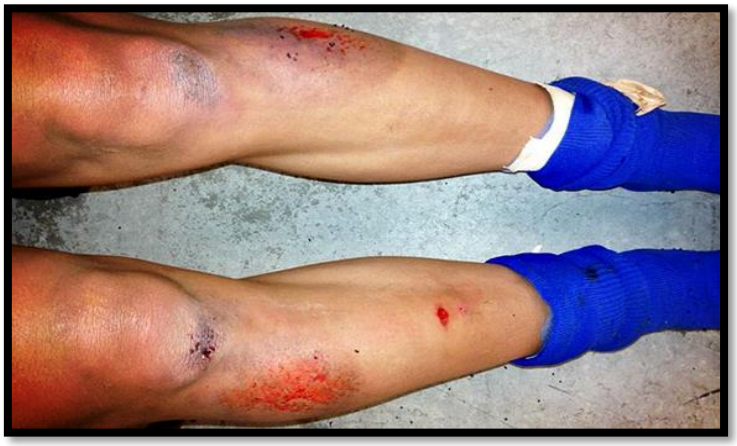

Figure 4. Abrasions and wounds acquired from activity on artificial turf. These types of injuries are very common (53).

If bacteria find its way into the human body it is the task of the immune system to fight the infection (52). People with weak immune systems or with underlying conditions that affect it, will be more susceptible to bacterial infections. There could be many reasons for an immune system to weaken, and some studies have discussed the possibility that heavy exercise can increase the infection risk (54). This means that athletes possibly leave themselves vulnerable after heavy training, and opportunistic bacteria in the artificial grass could exploit this opportunity.

2.4. Quantification methods for bacterial cultures

There are several methods of quantifying bacterial cultures, some only requiring basic laboratory such as the colony forming units (CFU) method or counting through microscopes (55, 56). Other methods require more expensive instruments such as a PCR, a flow cytometer, a spectrophotometer or other instruments that are not standard inventory on all laboratories (57, 58). Which method to use for quantification is decided by the purpose and accuracy needed for the experiment (59, 60). Some methods can only give rough estimations on the bacteria quantity while others cannot distinguish between viable and non-viable bacteria. Most of the methods that give very good results are often the ones requiring advanced equipment or need standard curves to give a reliable result (59). Since there are many methods available for quantification, it is essential to take a closer look at some relevant methods to know the restrictions and possibilities of each one.

2.4.1. Counting Colony Forming Units on agar plates

One simple method to estimate the number of bacteria is to inoculate a sample on an agar plate and count the number of colonies on the plate after a couple of days (55). The method does not require any expensive laboratory equipment and is simple to perform (61). Each colony represents one colony forming unit, which means that it is assumed to be the result of the growth of a single bacterium (55). This will result in an estimation of the number of live bacteria per millilitre of the original sample, as the non-viable bacteria will not be able to form colonies. The method may also require several dilutions of the original sample to get a countable number of bacteria on the plate, and the dilution factor must be taken into consideration when calculating the total number of bacteria.

There are several pros of using this method as opposed to other quantification methods, with one of the most significant ones being the fact that it does not count dead bacteria (55). In many cases it is of interest to know the number of viable bacteria, as dead bacteria do not work as pathogens nor make any other notable impact. There are some problems with this method connected to the uncertainty of the quantification (62). It is impossible to know if one colony on the plate is derived from a single bacterium. The colony could be the result of the growth more than one bacterium, which means the estimated number almost certainly will be much lower than the actual number of bacteria. One study from 2017 that compared the accuracy of this method to counting chambers and PCR methods, found that the colony counting method underestimated the bacteria concentration (CFUs per mL) by about two $\log_{10}$ (60).

2.4.2. Quantifying bacteria with a hemocytometer

Another way to quantify bacteria is by using a hemocytometer (56). A hemocytometer is a plate with several square sections that can contain a certain volume of bacteria suspension, and these squares are used as an aid to count the bacteria visually through a microscope. As seen in figure 5, there are nine major squares and one of them is marked with a blue outer rim (63). The four squares placed in the corners are often used for cell counting (56). The bacteria in each of them are counted, and added up to the total number of bacteria, before dividing with the number of squares counted. Then the dilution factor and volume of the hemocytometer is taken into consideration, and the total number of bacteria per millilitre is calculated. Cells located on two of the outer lines of each square, often the top and the right line, are not counted. After every count the hemocytometer is cleaned with 70% ethanol and dried off with a Kimwipe or a similar product (64).

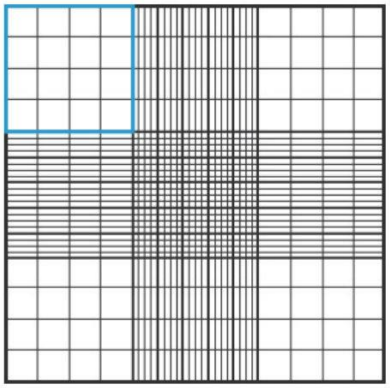

Figure 5. Possible layout of a hemocytometer. The blue lines represent one of the squares regularly used for counting. The other corner squares are also used, and the average number of cells per square is calculated (63).

In comparison to the CFU method, this method can give a more accurate number of total bacteria cells and does not rely on further cell growth (65). A bacteria sample can be taken with little preparation needed beforehand and analysed the same day, which makes the bacteria count more accurate than the CFU methods, where a colony can descend from more than one cell. It will still not give a perfect estimation of the total number of bacteria. The assumption that must be made in this method is that the average number of cells in one square of one part of the sample, will be the same for the entire sample, which often is not the case. The study that estimated the accuracy of the CFU method also estimated that the use of hemocytometers generally underestimated the cell concentration by about one $\log_{10}$ (60). This is a better result than doing a colony count on agar plates but worse than some other methods such as qPCR.

Another problem with this method is that it is not possible to distinguish between live and dead bacteria without the use of certain chemical stains (65, 66). This can easily be done with the addition of 3% Acetic Acid with Methylene Blue or Trypan Blue, but this can result in another source of error as it adds one more dilution to the process.

2.4.3. Optical Density of bacterial samples

The CFU method and counting chamber method rely on manual counting of colonies or bacteria cells, but there are alternatives to this (57, 67). One of these is to use the optical density of the sample to determine the concentration of bacteria. This can be done with a spectrophotometer, as higher concentrations of bacteria will result in more light scattering and less transmittance of light through the sample. The wavelength is usually set between 420-660 nm, mostly at 600 nm, but a measurement of the maximum absorbance of medium will give an indication of best wavelength to use for a specific analysis.

The optical density method is an indirect quantification method (68). To quantify the samples, a calibration curve of known bacteria concentrations must be made. Different bacteria will scatter and absorb light differently to one another, which means that every bacterium will have different calibration curves. Other variables that can impact the absorbance are the wavelength, the spectrophotometer and the growth medium. This means that an analysis of a sample containing an unknown composition of bacteria will be difficult to quantify accurately. A number could be estimated using McFarland standards, which are standards that give a rough estimation of the number of cells per millilitre by using specific volumes of 1% $BaCl_2$ and 1% H_2SO_4, but these estimations are generally not accurate and only applicable for a limited area of bacterial concentration (69). The composition of each McFarland standard and the estimated number of bacteria for each standard are shown in figure 6 (70).

McFarland standard no.	1.0% anhydrous $BaCl_2$ (ml)	1% H_2SO_4 (ml)	approximate bacterial density ($\times 10^8$) (CFU ml^{-1})
0.5	0.05	9.95	1.5
1	0.1	9.9	3.0
2	0.2	9.8	6.0
3	0.3	9.7	9.0
4	0.4	9.6	12.0

Figure 6. The McFarland scale showing the approximate number of CFU per mL based one the composition of barium chloride and sulfuric acid (70).

A problem with this method is the fact that the spectrophotometer is not able to differentiate dead and living bacteria, and it measures the absorbance and light scattering of pigments in the cells or substances elsewhere in the sample, such as the EPS of the biofilms (67, 71). This means that if the concentration of bacteria is calculated, the number will be higher than the actual concentration of living bacteria. The limit of detection is also a problem for the optical density method, as samples with low concentrations of bacteria will give small and undetectable changes in the absorbance, which gives a limited range of linearly correlation between number of cells and absorbance (71).

2.4.4. Quantification by real time PCR and flow cytometry

Two automated methods of quantifying bacteria are PCR and flow cytometry, which are reliable methods, but require expensive instruments that rarely are standard equipment in laboratories (58, 72). PCR is a method of amplifying the number of DNA in a sample by using a cycle of temperature changes, with nucleotides, primers and enzymes being used in the process (73). The DNA is first denatured, which results in two single stranded DNA for each original DNA in the sample. The next step is the addition of DNA primers specific to the desirable genes, followed by the addition of single nucleotides that correspond to the nucleotides in the original DNA. These processes are helped by specific enzymes, and after each cycle the number of DNA is doubled if the process is fully effective. The total number of DNA after *n* cycles will be given as in equation 1, given that the process is 100% efficient (58).

$$N_n = N_0 * 2^n \tag{1}$$

The original number of DNA can be found after a few cycles by using fluorescence (58). The fluorescence marks all the DNA strands and makes it possible to quantify it with the help of standards or controls. This is called real time PCR, often shortened qPCR as RT-PCR is the abbreviation of Reverse transcription PCR. In the initial cycles the level of fluorescence will be too low to detect, but after some cycles it will be a large enough amount to measure. Then the number of DNA strands and cycles are known, which means that equation 1 can be used to find the original number.

The pros of this method are that it is fast, effective, can easily identify specific gene sequences and can be used to both qualitative and quantitative analyses (58, 59). This can also be a disadvantage in some cases if you do not know which gene sequence to look for. Like the optical density method, qPCR cannot separate the dead cells from the living and the result will therefore not be able to give an indication of the current bacteria state.

Flow cytometry is a method that measures particles in a sample individually (74). The particles or bacterial cells in the sample are moving through the instrument in a narrow liquid stream until it passes a laser. This liquid flow ideally involves one cell passing the laser at a time, which makes it possible to analyse every cell individually with the laser beam. The light scattered by the particles is then detected and this information is used to analyse the particles. This makes flow cytometry able to analyse and determine the number of bacteria (75). One way of doing this is to quantify DNA in the cells by staining the nucleic acids with fluorochromes, but when doing this it is important to first remove RNA before the analysis by using the protein RNase. When the laser hits the cells, they emit lights in certain colours, depending on which fluorochrome is used, which can be detected and used to quantify the DNA. Another way of identifying and quantifying a particular bacteria cell in the sample is to use antibodies that bind to a specific membrane protein of the targeted bacteria.

Other advantages of using this method, is that the instrument also can sort the particles by size, as the light scattered in a forward direction at low angles is proportional with the size of the particle (74). It is also possible to use fluorescence, marked antibodies or other stains, to differentiate cells and estimate factors such as enzyme activity, membrane potential and pH (76). Most flow cytometers have the capability of measuring more than five cell parameters of each cell, and some instrument can even measure up to fourteen parameters, but this could require three or more lasers (74, 75).

2.5. General use of disinfection and relevant methods

Disinfection is a process that neutralizes or kills bacteria, fungi and viruses, but the process generally does not kill bacterial spores. Some methods of disinfection can kill some spores, but this is not the case for all methods (77). This makes disinfection important for the destruction of infectious and dangerous microorganisms, in foods and drinks, in addition on surfaces, some medical equipment and in many other circumstances (78, 79).

There are three levels of disinfection: High-level, intermediate-level and low-level disinfection (77). The difference between these levels is how much of the microorganisms that are killed. When using high-level disinfection methods almost all microorganisms are wiped out in addition to some bacterial spores. Intermediate disinfection is similar but does not include the killing of any spores, and low-level disinfection is defined as methods that only can destruct some bacteria, viruses and fungi.

Disinfection can be carried out by both physical and chemical methods (79). The physical methods include heat, radiation and filtration among others, while there are a number of chemical disinfectants in both gaseous and liquid form. The latter includes various types of disinfectants such as alcohols, aldehydes, chlorine compounds, oxidizing agents and acids. Chemical disinfection is often used when disinfection with heat is not possible.

2.5.1. Frequently used disinfection methods

The choice of disinfectant depends on multiple factors. The method has to be suitable with the product or surface that is being disinfected (79). Some surfaces cannot handle high temperatures and are therefore not suitable to heat disinfection, and some products might be difficult to treat with a chemical compound and therefore more suitable to heat disinfection. Another factor to consider is the efficiency of the method, as there in some cases might be enough to use a low-level disinfectant while other cases might require a high-level disinfectant. Lastly, it is important to consider safety as some effective disinfectants might be hazardous.

Heat disinfection is a simple disinfection method that can be used with the help of a washing machine, through boiling or a decontaminator (79, 80). As most bacteria species will die out in high temperatures, this principle can for example be used for the disinfection of water or products that can cope with high temperatures. The higher the temperature is, the faster the bacteria or microorganisms will die out.

Disinfection with ultraviolet light is a method that uses radiation to inactivate microorganisms (81). This type of radiation has less energy than gamma rays and X-rays, but more than visible light and radio waves. The wavelengths of UV-lights can be between 100 nm and 400 nm, but disinfection is often done with a wavelength of 254 nm which falls into the category of UVC-light. The radiation inactivates the microorganisms by damaging their DNA, which can make them incapable of performing the process that they need to survive. Although this type of disinfection is most suited for water, it can disinfect the air, surfaces and even entire rooms with the use of special made UV-lamps and apparatuses, such as hospital rooms like the one in figure 7 (82). There are some problems with UV-disinfection, especially when disinfecting surfaces with UV as microshadows and protective layers that absorb the light can make the disinfection less effective. There is also a possibility that the UV-light might damage grass fibres on artificial grass over time, due to the polymers absorb UV-light as heat (83). This will cause the polymer fibres to degenerate, gradually losing colour and shine, while also reducing stretchability, making the fibres more prone to breakage.

Figure 7. A hospital room being disinfected with pulsing UV-light (82).

In chemical disinfection a chemical agent in a liquid or gaseous state is used to disinfect an object or surface (84). There are many different chemical agents that can be used, and they are split into a few major categories according to their chemical nature. For example, are alcohols, acids, oxidizing agents and halogen-based compounds all examples of major groups.

Alcohols are some of the most common disinfectants, especially in the form of ethanol, and are often used to disinfect surfaces or for hand sanitation (85). They are often used in concentration of between 70% and 90% and kills microbes by denaturing proteins, a process that can cause cell lysis. Acids such as citric acid have an effect as a chemical disinfectant (84). High concentrations of acids may be harmful, and this makes acids less usable than alcohols yet still effective in lower concentrations. Acids usually target the bonds of the microorganism's nucleic acids and essential proteins to kill the microbe. Hydrogen peroxide is an example of an oxidizing agent, which is a group of chemical agents that denature proteins and lipids of bacteria and viruses and fungi to destroy them. They can be used in both low and medium concentrations (from 1% to >30%) based on what they are being used to disinfect. Halogen-based compounds as for example sodium hypochlorite, function about the same as alcohols, by denaturing proteins of the microorganism. They are used for a variety of microorganisms and are like acids usually effective in lower concentrations. Other examples of major groups of chemical agents worth mentioning are aldehydes, biguanides, alkalis, phenols and quaternary ammonium compounds.

2.5.2. Practical disinfection methods for artificial turf

Heat disinfection on artificial turf pose challenges as the grass fibres can melt at high temperatures, which makes finding an effective germicidal temperature and at the same time non-melting temperature very difficult, and it is difficult do in such a large scale as an indoor sports facility (86). When disinfecting large surfaces areas, using chemical disinfectants might be challenging as large amounts of liquid can be required to cover the entire area, which may both be expensive and difficult to acquire (87). Liquids may also struggle to reach all areas of the grass as it has a very rough structure with infill, which might contain organic matter and protect the microorganisms with its complex surface. UV-disinfection is an alternative with none of these problems as it does not melt the surface and is reusable, but there are some challenges (81). It is difficult for the UV-rays to reach all areas of the grass because of the rough structure and the infill of the grass (2). The effect of UV on artificial grass is therefore uncertain.

Some manufacturers in the market has started to address these challenges (88). This is done by patented technology called water mist or dry fog. Liquid is broken down to very small drops through patented collision nozzles, mainly in between 1 and 25 microns. The amount of liquid needed is not much and clean water is used, together with small parts of chemical disinfectants – typically around 1,5 to 3,5%. The task of the water is to transport disinfectant chemicals to the pathogens. From small amounts of water, you get a huge spread of the chemicals in a short time, covering all areas and relatively small usage of disinfectants. Since the droplet size is so small, it means low surface tension and that the fog likely will find the less accessible areas in the complex structure of the turf.

A company called Elergy AS has developed and tested a device for this specific purpose. This solution is specially made for disinfection with water mist. Elergy also has another purpose for this solution, dust suppression on artificial grass turf. In this project a special built solution for water mist for artificial grass turf was used, and figure 8 shows both a lab-scale prototype and a full-scale prototype which can be used for disinfecting an entire artificial pitch.

Figure 8. Prototypes of an apparatus that breaks down liquids to drops of down to less than ten microns. On the left is a handheld prototype that was used in this project and on the right is a full-scale prototype that could be used for a whole pitch.

2.6. Growth media for comprehensive bacteria cultures

Bacteria have both physical and chemical requirements to be able to grow and divide (89). Their physical requirements include temperature, pH, and osmotic pressure, while their chemical needs include macro elements, micro elements, growth factors and water. Macro elements make up the highest mass percentage of a bacteria cell, and includes carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus. Micro elements are only needed in small amounts and is necessary for the enzyme function within the bacteria cells. They often include elements such as iron, copper, molybdenum and zinc. These elements usually exist in tap water and is therefore usually not needed to be applied in growth media. Growth factors are organic compounds that is only needed in small amounts. Examples of growth factors include vitamins and amino acids. Bacteria can grow in vast different environments. Many bacteria species can only grow within an interval of 25-40 °C, and the temperature in the indoor artificial grass is mostly within 10-15 °C (10). What is most likely to exist in artificial grass is mesophile bacteria, with a growth range of 10-50 °C (89). The optimum temperature for most bacteria is about 37 °C and is therefore the preferred incubator temperature. There are different kinds of growth medium, but a complex medium can enable fast growth for a majority of bacteria species. Complex mediums are often based on extracts from plants, meat, yeast, or on denatured proteins from the same or different sources.

2.6.1. Contents and characteristics of Tryptic Soy Agar

Tryptic Soy Broth is a complex and rich medium that enables rapid but unspecific growth of most types of aerobic and facultatively anaerobic microorganisms. TSB is also able to grow some fastidious microorganisms (90, 91). It can be bought in pulverized form and it contains casein peptone, soya peptone, sodium chloride, dipotassium hydrogen phosphate and glucose. The peptones provide nitrogen, vitamins, and minerals. The sugars from soya peptone and glucose promote growth. Sodium chloride is applied to balance the osmotic pressure, and the dipotassium hydrogen phosphate acts as a buffering agent. TSB can be combined with agar to create Tryptic Soy Agar (TSA) plates, and it is well suited for plate counting. The common amounts of TSB and agar per litre medium are 30,0 g and 15,0 g respectively.

2.6.2. Nutrient Agar ISO, an alternative culture media for broad bacterial growth

Nutrient Agar ISO is an alternative to TSB. Nutrient Agar ISO is a generalized medium that enables growth of mesophilic aerobic bacteria. It can only grow less fastidious microorganisms than TSB, and is often used for testing wastewater, drinking water, milk, and other foods (92). It consists of bacteriological agar, peptone, and meat extract. Bacteriological agar is the solidifying agent, while beef extract and gelatin peptone provide vitamins, nitrogen, minerals, and amino acids for growth. The formula also conforms to a few regulations such as: International Organization for Standardization (ISO) and BAM.

3. Materials and methods

The laboratory work consisted of three main stages of testing. These were an initial testing of methods and sample collection, a main testing of bacterial contamination and a testing of disinfection methods on artificial grass. The equipment used for this work is summarized in table 1.

3.1. Equipment and chemicals

Table 1 gives an overview of the materials and chemical substances used in the project. The producer of each product is given, as well as the supplier and product number of the swabs that were ordered especially for this project.

Table 1. Summary of the equipment and chemicals that were used during the experimental part of the project, with the producer of each one and the producer and supplier of the ones that were especially ordered for the project.

Material/Chemical	Producer	Product number	Supplier
Agar	VWR International AS		
Autoclave (TOMY SX-700 E)	TOMY		
Bürker counting chamber	Marienfeld		
Chlorine (4% sodium hypochlorite)	Orkla		
Citric acid	Santa Maria AB		
ESWAB 480CE	Copan	4012538	St. Olav
Freebac – Clearoxyl 1,5% (1,5% H_2O_2)	Oxyl-Clean AS		
Incubator	Termaks AS		
Liquid spreader device (prototype)	Elergy AS		
Microscope (CH20BIMF200)	Olympus Optical Co.		
Nystatin dihydrate	AppliChem GmbH		
Spectrophotometer (Evolution 60S)	ThermoFisher Scientific		
Tryptic Soy Broth	VWR International AS		
UV-cabinet (SAFE 2020, 15W, 254 nm)	ThermoFisher Scientific		
UVG-54 Handheld UV Lamp (6W, 254 nm)	Analytik Jena US LLC		
Vortex (Combi-Spin FVL-2400N)	bioSan		

3.2. Methods

Initial testing involved collecting samples and analyzing them with the optical density method, cell counting with a hemocytometer and the CFU method. Over four weeks the main test was done with samples from two areas that was analyzed with the optical density method and the CFU method. This was followed by an *in vitro* examination of the effect of diluted hypochlorite, citric acid, hydrogen peroxide and UV light on individual contaminated grass patches.

3.2.1. Collection and preparation of bacterial samples

The samples were taken in Flatåshallen, an indoor football pitch in Trondheim. Initially, samples were taken from four different areas on this pitch. After this, two of these areas in Flatåshallen were chosen and tested for the rest of the project. The four initial areas of interest were inspired by FIFA's chosen field test positions, as stated in their handbook on the testing of football turf, as shown in figure 9 (93). In this figure, the numbered areas are the test areas as stated by FIFA, while the red marked areas are the ones used in this testing. Since the pitch in Flatåshallen was smaller than a full-sized football pitch, some of these areas were not tested, as they overlapped with another section. The following areas were chosen for collecting samples:

- Area A – Middle of the penalty area
- Area B – Midway between the penalty box and halfway line
- Area C – On the midway line
- Area D – The sideline closest to the entrance

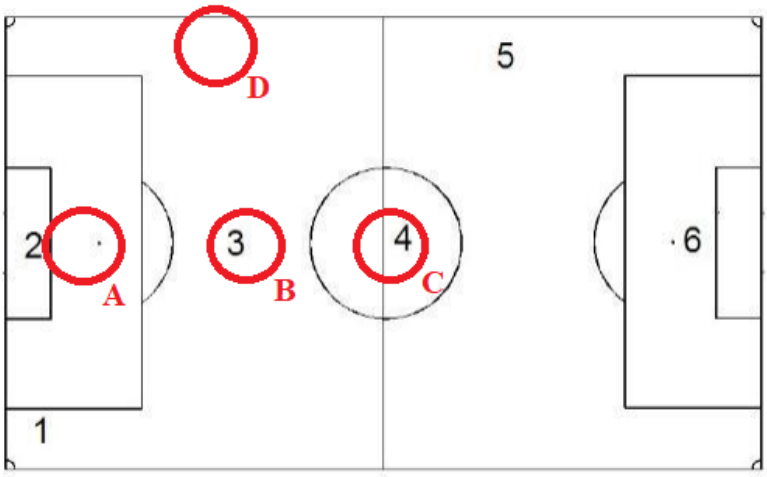

Figure 9. FIFA's field test positions (marked with numbers) and the field test positions used to collect samples in this project (marked with red rings and letters) (93).

Areas 2 and 6 were combined as area A, and 1 and 5 were combined as area D. All samples were collected from the same side of the field. In the initial testing, five parallels were sampled from each of the four areas. The rest of the tests were performed only in area A and D, but with 10 parallels instead of five.

For collecting the samples, the ESWAB 480CE was used, a package with a swab and a container with Amies transport medium (1 mL). The samples were taken by moving the swab in a certain pattern across the turf, in a restricted area of 10 cm x 10 cm. The pattern involved moving the swab from one side to the opposing side ten times in a winding pattern, first vertically and then horizontally, as shown in figure 10. The samples were then transferred to the laboratory where they were stored (4 ℃) and tested soon thereafter.

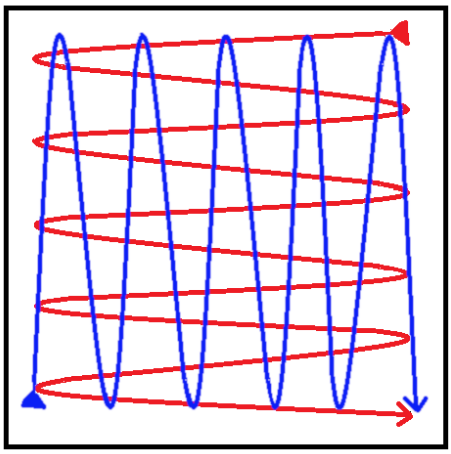

Figure 10. The pattern in which the swab was moved across the restricted area of the artificial turf.

The samples were prepared by vortexing the container of the samples (2 x 10 sec). This was followed by transferring bacterial suspension (0,5 mL) to a centrifuge tube which was then diluted with sterilized, distilled water (4,5 mL). The centrifuge tubes were then turned several times. All preparation and testing of bacterial samples were done on a disinfected bench and with sterile equipment.

3.2.2. Preparation of Trypticase Soy Agar (TSA) and liquid medium

The TSB medium was made with distilled water and TSB (30,0 g/L) and the solid medium also contained agar (15 g/L) (94). The agar and TSB were transferred to a pyrex bottle, and warm distilled water was added to the bottle and the solids were dissolved. The pH of the medium was then checked and regulated to about 7,3 before the bottle was marked and set to autoclavation (121 °C, 15 minutes). Then nystatin (60 mg/L) was added to both the TSA and TSB medium and petri dishes were filled to 1/3 of their volume with the TSA medium and cooled until the medium had solidified. The TSB medium was added to Erlenmeyer flasks (250 mL) with caps After this, the plates were stored (4 °C). In later parts of the project, nystatin was added to the agar plates after they had been made. Nystatin (120 mg) was dissolved in sterilized water (8,0 mL) and mixed. After the solution was homogenous, it was applied to the plates (100 µL per plate).

3.2.3. Counting CFUs on agar plates

After the samples were prepared, aliquots of the initial bacterial samples (0,50 mL) were applied to TSA plates and a glass rod was used to spread the suspension (61). After the bacterial suspension had settled, the plates were inoculated at 37°C for about two days, and the plates were controlled after 24 hours. After visible bacterial colonies had formed, the colonies on each plate were counted and noted. After the initial testing, one change was made to the method. The suspension volume applied to the plates was changed from 500 µL to 100 µL, and this volume was used for the remainder of the testing.

3.2.4. Quantification with a hemocytometer

A Bürker-chamber was used to quantify bacteria through a microscope. Bacterial suspension (100 µL, dilutions of 10X, 100X and 1000X) was pipetted from the samples to a centrifuge tube. A cover glass was put on the counting chamber and suspension was added (56). This was placed on under the microscope, which was set to 40X magnification and regulated to make the counting squares visible. A phase contrast device was also used to make the bacteria easier to see. The bacteria of each major corner square were counted and a calculation of the total number of bacteria was done.

3.2.5. Quantification by optical density

Both the initial samples and main samples were analysed with a spectrophotometer. After the samples were prepared and ready, aliquots of the samples (3 mL) were added to plastic cuvettes. An unused and sterile medium was handled the same way as the samples and used as a blank in the analysis (57). The samples were then analysed at 600 nm and the absorption of each sample was noted. In the main tests, the analysis was also performed at 420 nm, 500 nm and 660 nm.

3.2.6. Preparation of artificial turf in vitro

The artificial grass pieces were prepared by cutting out rectangles (25x33 cm) and putting each of them in a box that had been sterilized by ethanol. Each piece was filled with sand (1240 g), which was massaged in the to fill the bottom part of the turf (10). On top of the sand filling there was used crushed olive stones (165 g) which also were massaged in. A smaller offcut (25x22 cm) was filled with sand (825 g) and crushed olive stones (110 g).

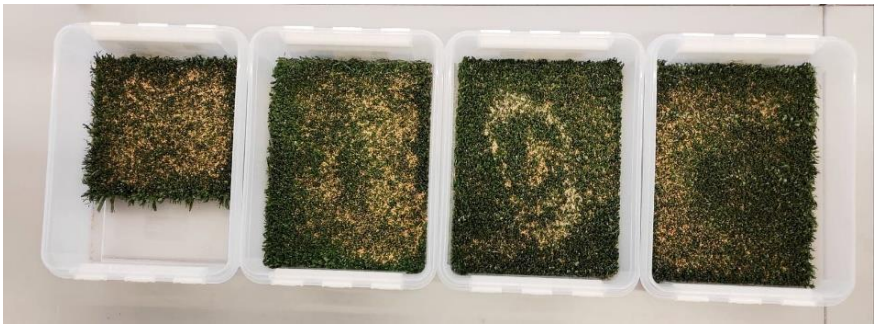

Figure 11. The artificial grass turf offcuts before inoculation. Measurements are from left to right: 25x22 cm and 25x33 cm for the rest.

3.2.7. Disinfection of in vitro artificial turf between testing

Before inoculation of bacterial cultures on the artificial grass offcuts they were disinfected. This was done by soaking the grass pieces in ethanol (70-90%) and massaging it into the grass fibres. This process was repeated, only this time with sodium hypochlorite (1%). The pieces were then set in a UV-light chamber for 60 minutes. Samples from the grass offcuts were inoculated on agar plates to control that the disinfection was successful.

3.2.8. Inoculation of bacteria on artificial turf

A total of four samples were collected from area A, B, C and D in Flatåshallen, and were transferred to the laboratory and vortexed (2x10 sec). Each sample (diluted in 1 mL of transport medium) were split into two parts (~0,5 mL) and was inoculated in separate Erlenmeyer flasks with TSB medium (250 ml) and nystatin (60 mg/L), resulting in a total of 8x250 mL bacteria culture and inoculated for three days. A sample of each set (100 µL) were placed on each their agar plate, which was incubated for a day and then examined. This part of the experiment was performed twice. The second time bacteria from Flatåshallen were isolated on agar plates and inoculated for four days in four Erlenmeyer flasks with only TSB medium (250 mL) instead of inoculating whole samples into Erlenmeyer flasks with both TSB medium and nystatin.

Bacterial suspension from each Erlenmeyer flask (2x50 mL) was pipetted over to centrifuge tubes and centrifuged (4200 rpm, 10 minutes). After the centrifugation, the excess medium was removed. The solid at the bottom of the tubes were then diluted with sterilized water and transferred to a box, shown in figure 12, containing sterilized water (6 L on the first try, 4 L on the second try). The artificial grass was then dipped in the box, left there for about ten seconds and taken out. The grass did contain a lot of suspension which had to be removed by tilting the grass offcut slightly as shown in figure 12.

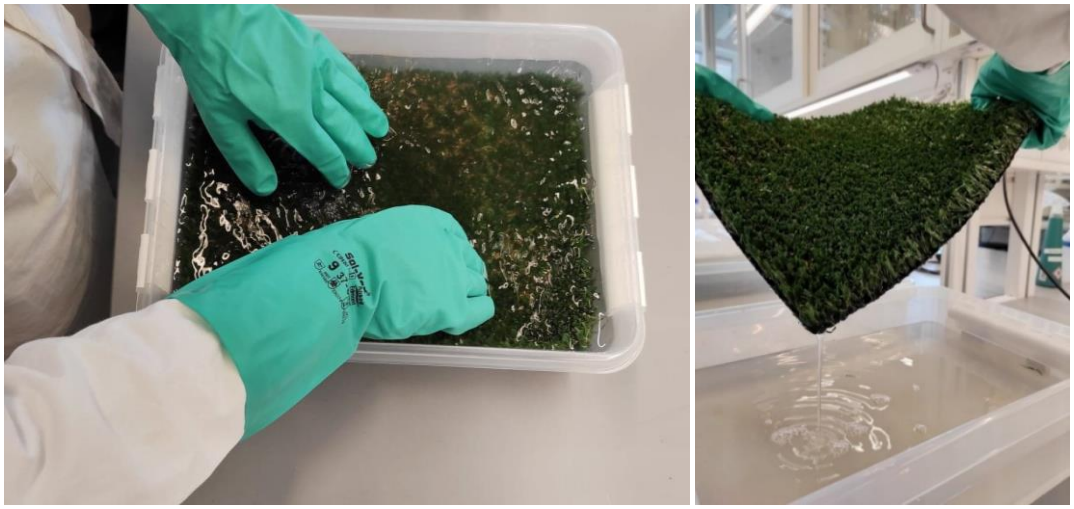

Figure 12. The inoculation of bacterial culture on the artificial grass. To the left is a picture from when the grass was lowered into the suspension and to the right is a picture from when the excess suspension was removed from the grass piece.

After most of the water had been removed, the artificial grass pieces were put back into their own boxes and lids were put on. They were then put into incubators at room temperature for two days in the first try, but in the second try the testing was done only a few hours after this process.

3.2.9. Disinfection of in vitro artificial turf

Before the disinfection, three samples were taken from each grass offcut in the same manner as the sampling in Flatåshallen in restricted areas of 8x8 cm. The eSwab was put into its belonging container with transport medium, and these containers were vortexed (2x10 seconds). Aliquots (50,0 µL) were taken from each container and transferred to Eppendorf tubes, and sterilized water (4,95 mL) was then added to each of these tubes. The tubes were shaken lightly and aliquots (500 µL) were taken from each of these tubes and transferred to new Eppendorf tubes. Sterilized water (4,50 mL) was also added to these, and the solutions were mixed. Aliquots of both the diluted and undiluted samples (100 µL) were inoculated on agar plates and spread with the help of glass rods with triangular ends. After the samples had settled on the plates, they were incubated at 37°C for two days after the first try and three days after the second try, with a check every day.

The disinfectants were prepared and adjusted to the desired concentration (1,0% first try, 1,5% second try). Citric acid (30,0 g first try, 45,0 g second try) was weighed out and diluted with distilled water (3 L). The sodium hypochlorite was prepared by diluting the original product (4%, 0,750 L first try, 1,125 L second try) with distilled water (2,250 L and 1,875 L respectively). The hydrogen peroxide solution was delivered in the desired concentration.

The grass offcuts were disinfected using two different methods. The chemical solutions of hydrogen peroxide, citric acid and sodium hypochlorite were spread out using a prototype of a liquid spreading device from Elergy AS. The solutions were put into a metal container which was connected to an air compressor and the collision nozzle spreading device. After the device was started, it got to run for about ten seconds before the artificial grass was placed in the fume cupboard. Then, the spreading device was moved manually across the fume cupboard and passed over the artificial grass for about four seconds. A picture from this process is shown in figure 13. At the second try, the spreading device was slightly adjusted, and therefore the process was altered. The spreading device was not moved across the fume cupboard but was set in one position and the runtime was increased to ten seconds. The UV disinfection was done by moving a handheld UV-ray over the surface of the artificial grass in about five seconds at a height of about 10 cm. Figure 14 is a picture taken during the UV disinfection.

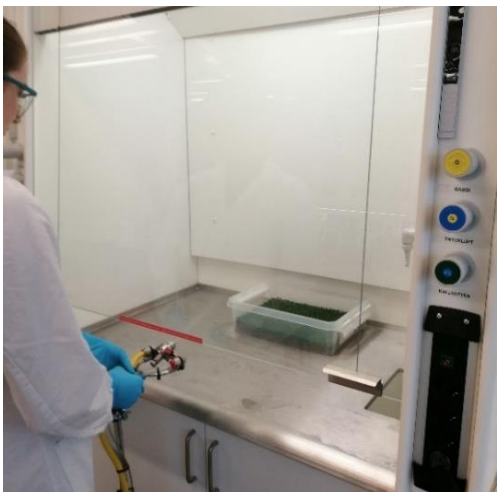

Figure 13. Picture taken midway through the process of the disinfection with the hydrogen peroxide solution.

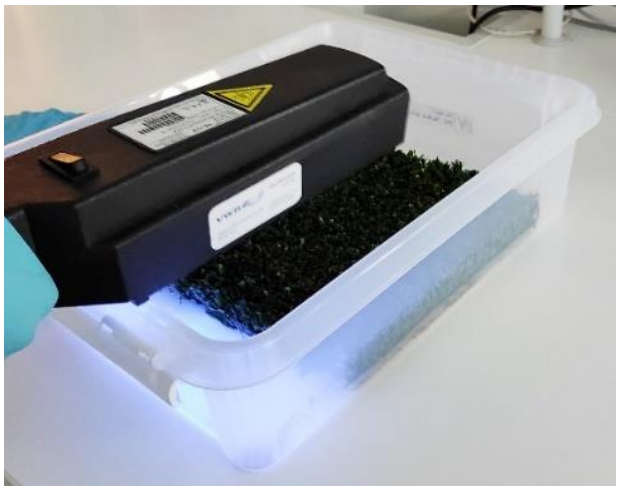

Figure 14. Picture taken during the disinfection with a UV-ray.

4. Results

The results from the initial testing, the analysis of microbial growth and the use of disinfection are presented in this section. This includes tables with results from each individual spectrophotometric analysis, the colony counts of agar plates and a figure displaying the maintenance and user activity during the test period.

4.1. Initial testing of bacterial samples

Before the main testing, an initial batch of samples were taken from four different areas on the field and then analysed with a spectrophotometer at 600 nm, inoculated on agar plates and observed through microscope with Bürker-chambers. The results from the spectrophotometric analysis of the samples from the four areas on the pitch are presented in section 4.1.1. and the observations made through the microscope and on the agar plates after cultivation are presented in section 4.1.2.

4.1.1. Bacterial presence on relevant areas of the pitch

The results of the optical density analysis are shown in table 2, which displays the absorption of each sample of the four areas, as well as averages and standard deviation. Sample 2 from area A is marked in red as this was not included in the calculations.

Table 2. Absorption measurements of the samples from area A, B, C and D. The A2 sample is written in red as it is not part of the calculation of average absorption and standard deviation.

Optical Density (Absorption)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Avg. Abs.	Standard dev.
Area A	0,035	0,006	0,022	0,030	0,031	0,030	0,005
Area B	0,028	0,014	0,033	0,016	0,023	0,023	0,008
Area C	0,007	0,011	0,014	0,022	0,015	0,014	0,006
Area D	0,037	0,034	0,037	0,036	0,044	0,038	0,004

4.1.2. Observations on inoculated agar plates and through microscope

Some of the plates inoculated with the bacterial samples contained growth of both fungi and bacteria, as for example on the plate shown in figure 15. This plate was contained the third sample from area B. Fungi was also observed on plates from other areas too, but some plates did not contain fungal growth at all.

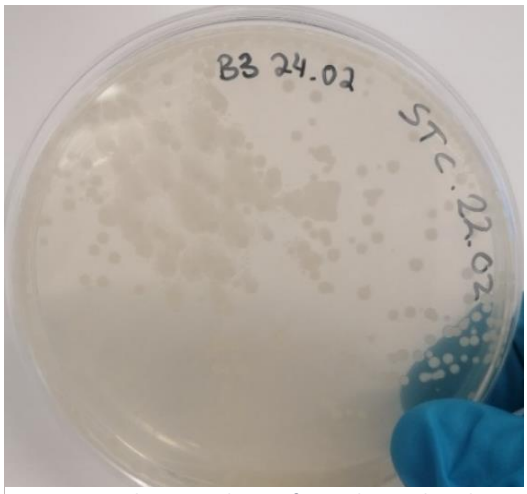

Figure 15. The agar plates of sample number three from area B, two days after inoculation.

The results of the bacterial growth are shown in figure 16. There was variation in each sample in both bacterial quantity and morphology, as some agar plates contained many different bacterial colonies and others contained almost no colonies.

Through the microscope both bacteria cells and other matter were observed, as shown in figure 17. The bacteria and other particles in the samples had similar appearances.

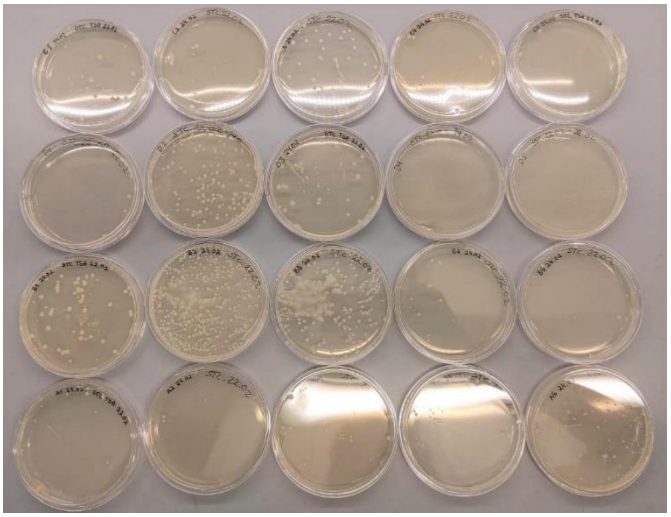

Figure 16. The agar plates two days after inoculation. Samples from the top left corner to the right: C1-C5, D1-D5, B1-B5 and A1-A5.

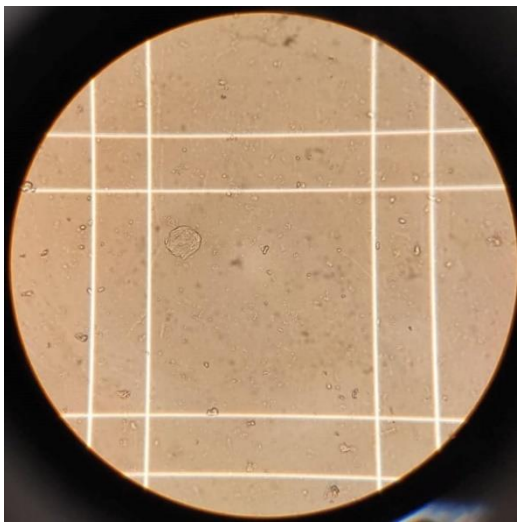

Figure 17. Observation of the bacteria sample in a Bürker chamber through a microscope.

4.2. Quantification and growth curves of bacterial samples

The sampling of the main tests from Flatåsen was done over the course of four weeks from March 3 to March 24 with exactly one week between every sample collection. The results from the CFU counts are presented in section 4.2.1., and the results from the spectrophotometric analyses are presented in section 4.2.2.

4.2.1. CFUs on agar plates

The amount of CFUs on each plate can be shown in table 3 and 4, as well as in figure 18. Fungal growth was more common in samples from area D than area A and the samples from both areas mainly consisted of four types of bacterial colonies in terms of appearance: bright yellow, milky white, pink beige and transparent grey. All these colonies were smooth and shiny except the transparent colonies which had a matte appearance.

The results in table 3 and 4 are presented with the number of isolated and bundled CFUs separately. The lowest average number of bacteria in the grass from one week of testing was calculated to be 8 550 bacteria per dm^2 in area A and 4 650 bacteria per dm^2 in area D, and the highest average in area A was calculated to be 20 500 bacteria per dm^2 and 7 700 bacteria per dm^2 in area D.

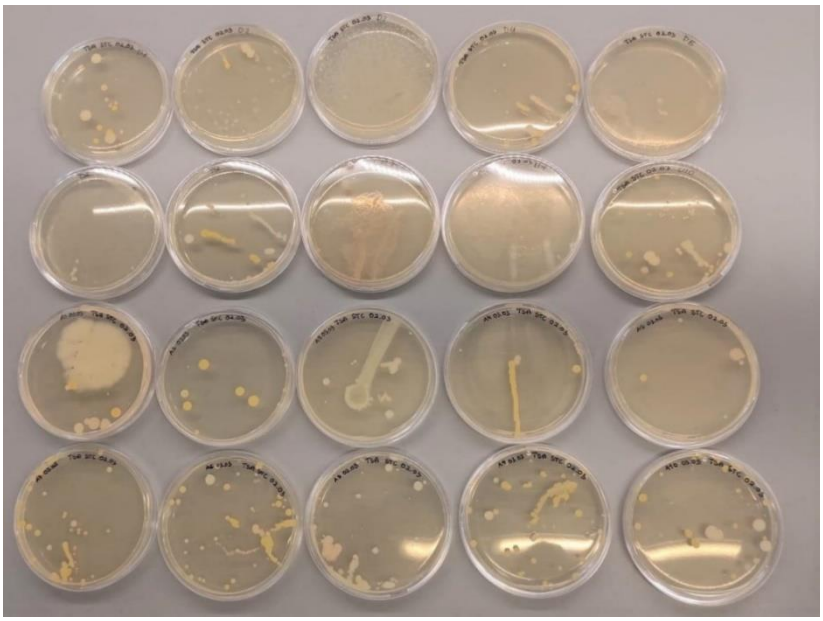

Figure 18. The agar plates of the first week two days after inoculation. Samples from the top left corner to the right: D1-D10 and A1-A10.

The agar plates from area A contained several bacterial colonies in the first week of testing. The A1 sample from the first week contained one fungal colony, and several plates of the first week contained bundled colonies near the edges of the plates. In the tests from the second week, the A2 sample is excluded from the averages and standard deviations, and this also the case for the A4 sample from the third week. The A7 sample from the third week did not contain bacterial growth. The number of isolated and bundled colonies on each agar plate from area A samples is shown in table 3.

Table 3. The number of isolated and bundled colonies on agar plates inoculated with the samples from area A, with averages and standard deviations.

	Week 1		Week 2		Week 3		Week 4	
Sample	**Isolated**	**Bundled**	**Isolated**	**Bundled**	**Isolated**	**Bundled**	**Isolated**	**Bundled**
A1	11	0	38	0	64	0	10	0
A2	12	1	240	6	40	4	26	0
A3	12	3	33	2	38	5	40	0
A4	46	2	10	3	250	3	20	1
A5	7	2	9	1	29	4	8	0
A6	33	3	21	4	43	2	7	1
A7	47	6	8	1	-	-	6	2
A8	25	5	10	2	29	0	15	0
A9	42	2	17	0	59	0	13	1
A10	28	1	8	4	26	3	30	1
Average	**26,3**	**2,5**	**17,1**	**1,9**	**41,0**	**2,3**	**17,5**	**0,6**
Std. Dev.	**15,4**	**1,8**	**11,4**	**1,5**	**14,0**	**2,1**	**11,3**	**0,7**

On the agar plates that contained samples from area D there were also observed many bacterial colonies. Two of the plates (D3 and D9) in the first week did not contain any form of bacterial growth at all. The same applies for the D8 sample of the second week and D3 from the third week. The colony count of each sample, including isolated and bundled colonies, from area D are shown in table 4. In many of the agar plates from each week, fungal growth was also observed.

Table 4. The number of isolated and bundled colonies on agar plates inoculated with the samples from area D, with averages and standard deviations.

	Week 1		Week 2		Week 3		Week 4	
Sample	**Isolated**	**Bundled**	**Isolated**	**Bundled**	**Isolated**	**Bundled**	**Isolated**	**Bundled**
D1	15	0	5	0	33	1	20	0
D2	29	3	7	1	25	5	10	2
D3	-	-	19	3	-	-	25	2
D4	13	3	8	2	8	0	16	0
D5	3	2	14	4	13	3	40	1
D6	7	2	2	1	13	0	6	1
D7	16	6	13	2	0	3	10	1
D8	7	1	-	-	10	1	5	1
D9	-	-	9	9	10	1	10	2
D10	33	3	7	7	6	0	10	0
Average	**15,4**	**2,5**	**9,3**	**3,2**	**13,1**	**1,6**	**15,2**	**1,0**
Std. Dev.	**10,7**	**1,8**	**5,2**	**3,0**	**10,0**	**1,7**	**10,7**	**0,8**

4.2.2. Absorbance of bacterial samples

The absorbances of the samples from area A are shown in table 5. Sample A5 was left out of the calculation of average absorption and standard deviation and is marked in red in table 5.

Table 5. Absorbances of each sample from area A, with averages and standard deviations for every week of testing.

Sample	**Week 1**	**Week 2**	**Week 3**	**Week 4**
A1	0,065	0,034	0,050	0,034
A2	0,044	0,031	0,053	0,032
A3	0,040	0,035	0,042	0,028
A4	0,050	0,019	0,041	0,026
A5	0,050	0,030	0,029	0,017
A6	0,057	0,031	0,058	0,025
A7	0,057	0,019	0,072	0,031
A8	0,052	0,034	0,050	0,036
A9	0,038	0,020	0,034	0,032
A10	0,052	0,029	0,046	0,030
Average	**0,051**	**0,028**	**0,048**	**0,030**
Std. Dev.	**0,008**	**0,006**	**0,012**	**0,004**

Table 6 shows the absorbances of every sample from area D. Sample D7 is marked in red as it was left out of the calculation of average absorption and standard deviation.

Table 6. Absorbances of each sample from area D, with averages and standard deviations for every week of testing.

Sample	**Week 1**	**Week 2**	**Week 3**	**Week 4**
D1	0,049	0,044	0,040	0,034
D2	0,063	0,045	0,041	0,036
D3	0,063	0,043	0,033	0,029
D4	0,048	0,030	0,036	0,028
D5	0,052	0,034	0,030	0,041
D6	0,064	0,036	0,029	0,039
D7	0,049	0,041	0,035	0,013
D8	0,054	0,040	0,048	0,031
D9	0,048	0,033	0,039	0,031
D10	0,040	0,027	0,032	0,035
Average	**0,053**	**0,037**	**0,036**	**0,034**
Std. Dev.	**0,008**	**0,006**	**0,006**	**0,004**

4.2.3. Use and maintenance of the field during the test period

A monitor registered the average number of people in Flatåshallen every whole hour of the day, and these numbers were obtained from the hall managers through contact person Bjørn Aas. The activity is presented in the dark blue plot in figure 19, where the total number of people is plotted for each day from February 22nd to March 27th. The yellow points represent the days on which samples were collected. There was no data available from March 8th to March 21st regarding hall activity, and these days are marked red in figure 19. Maintenance of the field was performed once every fortnight, and these days are marked as a green point in the figure. The CFU and absorbance (x1000) values are also plotted in the figure.

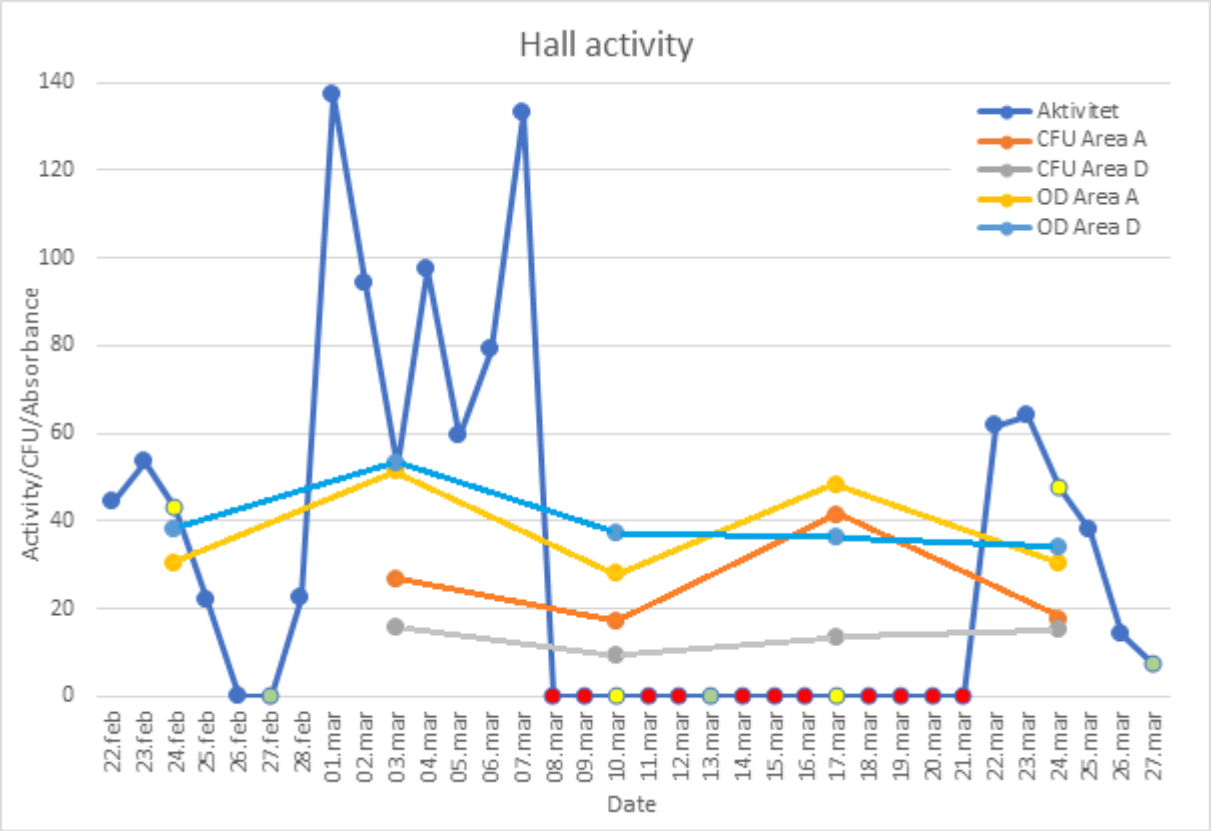

Figure 19. The activity in Flatåshallen during the testing period, based on the average number of people in the hall every hour. In Yellow points represent the testing days, green points represent days where maintenance was performed, and red points represent days with no data. CFUs and absorbance values from area A and D are also plotted in the figure.

4.3. Testing of disinfection methods in vitro

In the first try of disinfection, fungal growth dominated the plates from both before and after disinfection, and bacterial growth was not common on these plates. Some representative plates of the different levels of fungal growth are shown in figure 20.

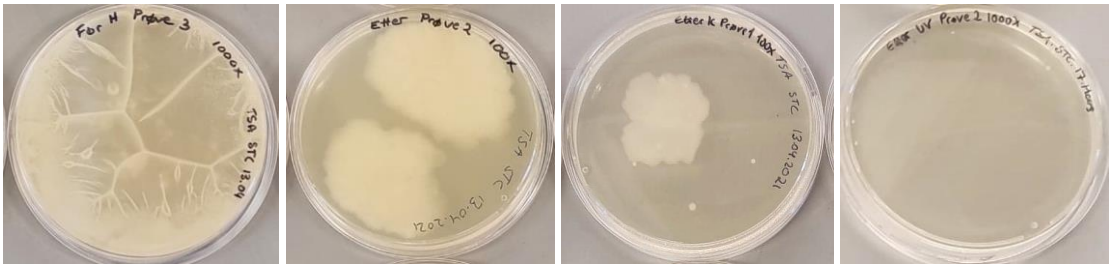

Figure 20. Representations of each degree of fungal growth. From the left: High degree, medium degree, low degree and no growth.

The results from all plates are shown in table 7 and 8, each one analysed by the different levels of growth on the plates both before and after disinfection, and with both a 100-fold and 1000-fold dilution.

Table 7. The degree of growth on the agar plates before and after disinfection with citric acid and hydrogen peroxide.

	Citric Acid				**Hydrogen peroxide**			
Dilution	*100x dilution*		*1000x dilution*		*100x dilution*		*1000x dilution*	
Time	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Sample 1	Medium	Low	High	None	High	High	None	None
Sample 2	High	None	Low	None	High	Medium	High	None
Sample 3	None	High	None	None	High	High	High	None

Table 8. The degree of growth on the agar plates before and after disinfection with sodium hypochlorite and UV.

	Sodium hypochlorite				**UV**			
Dilution	*100x dilution*		*1000x dilution*		*100x dilution*		*1000x dilution*	
Time	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Sample 1	High	Low	Medium	None	None	None	High	None
Sample 2	None	High	None	None	High	None	None	None
Sample 3	High	High	Medium	Medium	High	Low	None	None

The results from the try of disinfecting the grass offcuts with the samples diluted with a factor of a hundred are presented in table 9-12, including the calculated average numbers of isolated and bundled CFUs and the bacterial reduction both ten minutes and one day after disinfection. The numbers marked with a star in the table are estimated.

Table 9. The individual and average number of isolated and bundled CFUs on the agar plates from the 100-fold dilution and the bacterial reduction ten minutes and one day after disinfection with citric acid.

Citric Acid

	Pre		**10 min**		**1 day**	
	Isolated	*Bundled*	*Isolated*	*Bundled*	*Isolated*	*Bundled*
Sample 1	40	0	1	1	5	0
Sample 2	150*	2	10	1	3	0
Sample 3	13	2	5	2	36	1
Average	67,7	1,3	5,3	1,3	14,7	0,3
Reduction (%)			**92,1 %**	**0,0 %**	**78,3 %**	**75,0 %**

Table 10. The individual and average number of isolated and bundled CFUs on the agar plates from the 100-fold dilution and the bacterial reduction ten minutes and one day after disinfection with hydrogen peroxide.

Hydrogen peroxide

	Pre		**10 min**		**1 day**	
	Isolated	*Bundled*	*Isolated*	*Bundled*	*Isolated*	*Bundled*
Sample 1	13	1	0	0	2	0
Sample 2	21	0	1	0	4	0
Sample 3	90*	1	3	1	4	1
Average	41,3	0,7	1,3	0,3	3,3	0,3
Reduction (%)			**96,8 %**	**50,0 %**	**91,9 %**	**50,0 %**

Table 11. The individual and average number of isolated and bundled CFUs on the agar plates from the 100-fold dilution and the bacterial reduction ten minutes and one day after disinfection with sodium hypochlorite.

Sodium hypochlorite

	Pre		**10 min**		**1 day**	
	Isolated	*Bundled*	*Isolated*	*Bundled*	*Isolated*	*Bundled*
Sample 1	2	1	0	0	9	0
Sample 2	1	0	2	0	1	1
Sample 3	2	3	0	1	2	0
Average	1,7	1,3	0,7	0,3	4,0	0,3
Reduction (%)			**60,0 %**	**75,0 %**	**-140,0 %**	**75,0 %**

Table 12. The individual and average number of isolated and bundled CFUs on the agar plates from the 100-fold dilution and the bacterial reduction ten minutes after disinfection with UV-radiation.

UV-radiation

	Pre		**10 min**	
	Isolated	*Bundled*	*Isolated*	*Bundled*
Sample 1	5	0	100*	0
Sample 2	2	0	5	1
Sample 3	11	1	2	0
Average	6,0	0,3	35,7	0,3
Reduction (%)			**-494,4 %**	**0,0 %**

The results from the try of disinfecting the grass offcuts with the samples diluted with a factor of a thousand are presented in table 13-16, including the calculated average numbers of isolated and bundled CFUs and the bacterial reduction both ten minutes and one day after disinfection. Sample 3 from ten minutes after disinfection with hydrogen peroxide contained fungal growth and is not included in the calculations.

Table 13. The individual and average number of isolated and bundled CFUs on the agar plates from the 1000-fold dilution and the bacterial reduction ten minutes and one day after disinfection with citric acid.

Citric Acid

	Pre		**10 min**		**1 day**	
	Isolated	*Bundled*	*Isolated*	*Bundled*	*Isolated*	*Bundled*
Sample 1	1	0	0	0	7	1
Sample 2	11	1	0	0	1	0
Sample 3	100*	1	200*	1	2	0
Average	37,3	0,7	66,7	0,3	3,3	0,3
Reduction (%)			**-78,6 %**	**50,0 %**	**91,1 %**	**50,0 %**

Table 14. The individual and average number of isolated and bundled CFUs on the agar plates from the 1000-fold dilution and the bacterial reduction ten minutes and one day after disinfection with hydrogen peroxide.

Hydrogen peroxide

	Pre		**10 min**		**1 day**	
	Isolated	*Bundled*	*Isolated*	*Bundled*	*Isolated*	*Bundled*
Sample 1	0	1	4	0	3	1
Sample 2	3	0	7	0	3	1
Sample 3	0	2	-	-	45	0
Average	1,0	1,0	5,5	0,0	17,0	0,7
Reduction (%)			**-450,0 %**	**100,0 %**	**-1600,0 %**	**33,3 %**

Table 15. The individual and average number of isolated and bundled CFUs on the agar plates from the 1000-fold dilution and the bacterial reduction ten minutes and one day after disinfection with sodium hypochlorite.

Sodium hypochlorite

	Pre		**10 min**		**1 day**	
	Isolated	*Bundled*	*Isolated*	*Bundled*	*Isolated*	*Bundled*
Sample 1	0	1	0	0	3	0
Sample 2	13	3	2	0	3	1
Sample 3	200*	1	8	0	3	0
Average	71,0	1,7	3,3	0,0	3,0	0,3
Reduction (%)			**95,3 %**	**100,0 %**	**95,8 %**	**80,0 %**

Table 16. The individual and average number of isolated and bundled CFUs on the agar plates from the 1000-fold dilution and the bacterial reduction ten minutes after disinfection with UV-radiation.

UV-radiation

	Pre		**10 min**	
	Isolated	*Bundled*	*Isolated*	*Bundled*
Sample 1	3	0	1	0
Sample 2	1	0	5	1
Sample 3	4	1	5	1
Average	2,7	0,3	3,7	0,7
Reduction (%)			**-37,5 %**	**-100,0 %**

5. Discussion

The experimental work was split into three main stages. The initial testing, where the goal was to evaluate the methods and test different areas of the field. The second part was the main testing which was done to examine the degree of microbial contamination and how the microbial presence in the artificial grass evolved during a month. The last part of the experimental work was the testing of disinfection methods to find out if some methods are more suitable than others in disinfecting the grass. All these results are therefore evaluated in addition to the methods used.

5.1. Experiences from initial testing

The objective of the initial testing was to determine faults and limitations of the methods to be able to apply changes before the main testing. The three methods of analysis that were trialed were cell counting with a hemocytometer, counting CFUs on agar plates and a spectrophotometric analysis. The other objective was to figure out which areas was the most relevant for further testing. The two areas with the highest estimated contamination from these tests were analyzed further, because these areas would likely be the areas with the most activity.

5.1.1. Optical density method and choice of field positions

The optical density method had the advantage of not being time consuming and not requiring a lot of preparation. It only required vortex to loosen the sample as well as dilution to fill the volume of the cuvettes. The initial testing results showed a low absorption which indicated that further dilution was unnecessary.

The method's main disadvantage is that it measures all matter in the samples, such as bacteria, dust, dead cells, fungi, EPS and other irrelevant matter (67, 71). Therefore, it needed to be combined with another method to gain enough information about the sample as it measured more than just the bacteria. Each bacterium and fungi species will absorb and scatter light differently, and since the samples composition of the samples was unknown it was difficult to use standards such as the McFarland standards (68, 70). This resulted in none of the samples being able to be quantified in terms of bacteria. Although the samples could still be an aid to assume the level of contamination by comparing the absorbance. High absorbance likely meant a high degree of contamination.

The use of cuvettes was also a plausible source of error. In all tests there were used different cuvettes for each set of samples. Small differences in the transparency of each cuvette, either in form of barely visible marks or scratches, could have affected the absorbance (95). Precautions were taken by cleaning each cuvette with lens paper before adding the samples, but variations may have occurred.

The results indicated that area A and D had the highest absorption as shown in table 2, and by the aid of the CFU method as well, were chosen as the foundation for further testing. This test did not give a definitive answer to which areas contained the most bacteria, as other factors (fungi, EPS) might have affected measurements, but the method still gives a good indication of the overall contamination of the turf (67, 71).

A Q-test was performed for the second sample of area A, marked red in table 2, as this had a considerably lower absorption than the other parallels. The Q-value was higher than the critical Q-value of an analysis of five parallels with a confidence of 95%, and the sample was therefore considered a plausible outlier and was not used in the calculation of average absorption and standard deviation.

5.1.2. Experiences with the CFU method

The CFU method was tested during the analysis of the initial samples to find out how to use this method as effectively as possible. The results of this testing showed that some improvements had to be made for further testing. The inoculated volume of bacteria suspension was changed from 0,5 milliliters in the initial testing to 0,1 milliliters in the main testing. This was due to overfilling of the agar plates, and a lower volume would settle on the plates instead of dripping down when turned upside down. Although some plates contained many bacterial colonies, the dilution was usable for further testing, considering that the volume of suspension was to be lowered considerably for further tests. Since many of the plates contained an uncountable number of bacteria, the bacterial quantities of each plate were not noted and is therefore not presented in the results.

Furthermore, the initial testing also resulted in growth of fungi on some of the agar plates. This was in itself important to establish, as fungi also is a form of contamination on the artificial turf and can have an health impact for the athletes (15). The decision was therefore to note fungal growth in the main tests as well, as this also would contribute to a fuller picture of the total contamination in the artificial turf.

5.1.3. Problems with hemocytometer counts

The challenges of using a hemocytometer for quantification were quickly recognised when it was first used. The importance of finding the right dilution factor was apparent as the initial tries of counting the cells were difficult due to a too large number of bacteria and extracellular matter in each square of the Bürker-chamber and because the different matter was difficult to differentiate. Several dilution factors were tested to get a countable number that still was large enough to be representative for the sample (64, 96). In the initial tests the original bacteria sample was dilutions with a factor of ten, a hundred and a thousand were tested, but the difficulty of distinguishing bacteria cells from other matter in the sample prevailed. Even with the use of a phase-contrast device attached to the microscope, it was hard to separate the bacteria from other matter, which made this method unreliable for quantification. This was also a very time-consuming method to perform, and the hemocytometer was not used in the testing at all after the initial testing (66).

5.2. Bacterial growth during the test period

The main testing was performed over a period of four weeks with the intention of investigating the change of the quantity of bacteria. In this process the CFU method and optical density method were used to quantify the bacteria, but there were several sources of error connected to both the collection of samples and the methods used (61, 62, 68).

5.2.1. Faults and limitations with sample collection and preparation

The collection of samples was done by two different people. Generally, one person collected the samples from area A and another from area D, but in week 2 of testing the three first samples from area D were collected by the person responsible for area A. When the samples then were analysed with the optical density method the results showed a significant drop in absorbance when the other person started collecting samples (D4-D10). This is likely due to the amount of pressure applied when collecting samples, as the first person (D1-D3) probably applied more pressure than the other which led to more pollution and microbes being picked up. The rough surface of the artificial grass could have resulted in variations in the amount of matter picked up by the swab. Studies have shown that use of a moist swab will leave a bit of liquid on the surfaces and therefore not pick up all microbes (97). Additional factors affecting this could be the way the grass fibres were facing in relation to the movement of the swab, how deep into the grass the swab got and the pressure applied by the sampler.

It is unlikely that the spread of bacteria was completely equal all over the field, even within the specific areas chosen for collecting the samples. It is also possible that biofilms were encountered at random within the sample collection, and these usually contain many bacteria (34). This would probably enable variations within each sample set. Samples were taken right next to each other, but variations could have occurred because of factors such as the activity level on a particular spot, spill from drinking bottles and blood or spit from the users (13). This would mean that from one week to another a lot of factors could affect the results, and this could be one of the causes for some of the samples that had a significantly lower or higher absorbance or CFU value than the other samples within a sample set. These factors make the quantification of bacteria uncertain, something the variety in the results of the spectrophotometric analysis and CFU count show. The main problems were the lack of a suitable sample method that could take samples with a low variety and the unequal spread of bacteria on the turf.

5.2.2. Bacterial growth based on CFUs

Over the four weeks of testing no increase was observed, which makes it difficult to draw any conclusions on the bacterial growth in the artificial grass of Flatåshallen by itself. The results from area A shows that the number of bacteria seemed to increase every other week, before decreasing the next week. In area D however, this was not the case as the number of bacteria appeared to stay relatively constant throughout the testing period, except for the first week in which the number of bacteria was higher than in the other weeks. Although the number of isolated bacteria was lower in week two than in the two last weeks, there were more bundled colonies this week which possibly could even the balance. As the number variates over time, it is likely to assume that they do not have very good terms for long term growth on the surface in form of optimum temperature and nutrition, and that other factors therefore play a part in the presence. The temperature in the grass is about 10-15 °C and bacteria usually prefers higher temperatures than this (10, 89).

The standard deviation is noteworthy in every week in both areas, which makes the average number uncertain. This shows that there was great variation in the number of bacteria from sample to sample.

Another factor contributing to this uncertainty is that two of the samples (A2 from week two and A4 from week three) had a considerably higher number of colonies than almost all other samples. There were considered outliers as their Q-value was higher than the critical Q-value of an analysis of ten parallels with a confidence of 95%. These samples could have been outliers either because the samples were collected in an area where the activity had been particularly high, biofilm was encountered or an unknown factor had contaminated the area (13, 34). There were also other sources of error connected to the growth on the agar plates. One factor which made it difficult to count the colonies was that some of them overlapped and therefore could not be counted individually. These bundled colonies could contain everything from a few to tens of CFUs, and therefore the average number of colonies would have been significantly higher if these bundled colonies had been countable (98). The bundled colonies did not have any defined lines between each single colony and the size of the bundled colonies differed too much to compare them to each other making an estimation of CFUs difficult. This could be due to the water dilution making it difficult for the bacteria to survive planktonically when diluting the samples before applying them to agar plates (30).

Some plates contained growth of fungi which might have slightly affected the number of bacterial colonies. Nystatin was added to the medium to inhibit fungal growth but the antibiotic did not have any effect (99). This was assumed to be a result of the nystatin decomposing in the medium because of the high temperate and consequently not having an effect when the samples were inoculated. Later nystatin was added both in cold medium and on top of agar plates, but this did not work. It is likely to assume that the nystatin simply did not have an effect potentially due to deterioration after exposure to air, heat or light or that it had expired (100). Some of the plates had notable and dominating fungal growth that interfered with the bacterial growth. These agar plates were excluded from the results among with the plates that did not contain any growth at all.

An important fault with the method was that the samples was diluted with sterilized distilled water instead of diluents such as media or saline water. These liquids contain nutrients and growth factors the bacteria needed to grow (89). Distilled water does not contain enough of these, which can lead to hypotonic conditions and consequently cell swelling and potentially cell lysis (101). Since the testing within half an hour of dilution in every stage the water might not have had a significant effect, but the microbial quantity may still have been somewhat reduced.

It was apparent in this part of the analysis that the field contained several bacterial and fungal microbes throughout the process, and even though it proved difficult to estimate the level of bacterial growth it is still clear that the microbial activity does not die out when the field is in active use. There was a significant quantity of bacteria present in all weeks of testing, ranging from 4 650 to 20 500 CFU per dm^2. This number is likely much higher, mainly because the bundled colonies were not a part of the calculation and because the swabs did not manage to pick up every single bacterium in the section of the grass (97, 98). This number does also not include the number of fungal colonies present in the grass, which are also a significant part of the overall contamination (15).

Compared to other surfaces in former projects this is a high number. In the study from 2009 at Ullevål University Hospital, the number of bacteria on hospital floors before mopping was between 300 and 500 CFU per dm^2 which is significantly lower than the findings in Flatåshallen (41). The other mentioned study performed in Belgium from 2005 showed a bacterial quantity on surfaces in office buildings ranged from 4 to 4000 CFU per dm^2 which is also lower than the quantity in Flatåshallen (42). Surfaces in office buildings and hospitals are regularly cleaned in contrast to the artificial grass in Flatåshallen, which might be some of the explanation of the difference between these surfaces.

5.2.3. Artificial grass contamination based on optical density

The absorbance in area A seemed to increase one week and decrease the next, a pattern consistent with the results from the CFU method. The results of area D were also consistent with the CFU method, but did not contain a pattern as clear as area A. The samples from week four in area D was the exception from the pattern that appeared in area. In this week, the absorbance and CFU increased in relation to week two in area A, but stayed the same as the former week in area D. The A5 and the D7 sample of the fourth week were considered outliers as their Q-value was higher than the critical Q-value of an analysis of ten parallels with a confidence of 95%.

In most weeks there were a few samples that clearly deviated from the rest of the samples from that area. This suggests that other factors such variations within each area and the activity levels between each week might have a bigger impact than the sampling method. The composition of bacteria and fungi seemed to affect the spectrophotometer analysis, something a comparison of the results from the spectrophotometer and CFU methods indicated (68). In general, area A contained more bacteria than area D but less fungi according to the CFU method but gave lower absorbances. This indicates that fungi affect the absorbance of a sample more than bacteria.

One source of error was posed by the blanks used for analysing the samples. The blanks were unused transport media, that was diluted and prepared the same way as the samples. A new blank was used every week, and both the preparation of the sample and the cuvette used could have influenced the absorbance. The alternative to this would have been to make one blank the first week and store it for future tests. The reason this was not done was because bacteria could have grown in the blank over time if the blank had been slightly contaminated, which would have affected the absorbance (68).

5.2.4. Microbial presence in relation to activity and maintenance

The results from the spectrophotometer analysis and the CFU method gave similar results. Including the results from the first week gives a pattern of higher and lower values every other week. In area A the initial tests and the tests from the second and fourth week of the main tests showed similar microbial presence, while the tests from the first and third week showed higher microbial presence. This pattern was also somewhat apparent in area D, apart from week three where the results were as low as in the week of initial testing and the second and fourth of testing.

These results indicate that there are factors affecting the presence. Therefore, it is of interest to compare the activity and maintenance work in the hall with the results from the testing period. Therefore, the absorbance (x1000), CFUs and activity results were plotted in figure 19 and compared to get an insight into how these factors potentially could have affected the microbial presence. The hall activity is only available for the initial testing, and the first and last week of the main test, and only these weeks can therefore be compared. In the days prior to the initial testing, the activity was moderate (seen in relation to the minimum and maximum of the period), and this was one of the weeks with lower microbial presence according to the tests. However, in the first week of the main testing, a week with some of the highest microbial presence measured, the activity in the two days prior to the testing was at a very high level. For the last week of testing however, the activity in the days prior to testing was only slightly higher than before the initial testing and had a similar level of microbial presence. After this comparison, it seems that the activity may be linked to the presence of microbes as the week with high results in testing, was also the week with the highest activity level prior to the sample collection whereas the two other weeks had lower activity prior testing and lower microbial presence. The main source of the microbes on indoor artificial fields is likely the users of the pitch (13, 38). Hence, it would be likely that higher activity results in more microbes on the field.

When comparing the microbial presence with the maintenance, it seems that this might have affected the results. The maintenance was performed every other Saturday in the testing period, and testing days closer to the maintenance gave higher results than the other testing days. On the Saturdays, four days before the first and third week of the main testing, maintenance was done, and these weeks were the weeks with the highest presence of bacteria, especially notable in area A. The other weeks of testing had lower results, and had maintenance performed just under two weeks prior to the tests. If the maintenance increases the microbial presence on the surface of the pitch, this poses a question of how this happens. In an experiment by Green Cleaner AS, measurements found that the microbial content in the air was higher after maintenance, likely as a result of the microbes being stirred up during maintenance (27). This might also explain the connection between maintenance and the results of this project. One of the limitations of the sampling in this project is that only microbes near the top of the grass can be collected as the swab does not go deep into the grass. If microbes in the grass are stirred up during maintenance, this might result in more of the microbes deep in the grass moving up to the surface or that the microorganisms stirred up into the air ending up closer to the top of the grass surface when they come down. Consequently, more microbes might have been able to attach to the swab in the testing days closest after maintenance. That means that disinfection can be more effective after maintenance.

5.3. Testing of disinfection methods

The testing of the three chemical disinfectants and UV-radiation was supposed give an idea of the efficiency of each method and use these results to suggest good alternatives for disinfection of artificial grass. This was supposed to focus on bacterial reduction after disinfection, but fungal growth made the first try unsuccessful and it had to be done over again with focus on not contaminating the grass with fungi. The results of the first try are still interesting, as it shows the difficulties of fungal disinfection. There were quite a few limitations and sources of error in this experiment, which are important to review.

5.3.1. Efficiency of disinfection methods

In the first trial set fungi seemed to dominate the agar plates and therefore bacterial colonies were impossible to quantify. The fungi were prevalent both before and after disinfection as shown in table 7 and 8. Although there generally was less fungal growth on plates after disinfection, there were still many plates with notable fungal growth, which might indicate that the methods used were not very effective against fungi. It is also likely that the disinfectants did not reach the lower layers of the artificial grass where moisture from the microbial application probably was located, and therefore did not have a good effect on the fungi (3, 81). However, as the samples were collected from areas near the surface, this might not have influenced the tests. To remove the fungi before the second try, the artificial grass offcuts had to be soaked in ethanol and sodium hypochlorite solutions, which was massaged into the grass. It was difficult to remove fungi with simple disinfection, but this process was successful.

The results presented in table 9-16 generally portray reduction of CFUs both ten minutes and one day after disinfection with the chemical substances. The exceptions are largely a result of either very few bacterial colonies before disinfection or one plate with a high number of CFUs after disinfection. Examples of the former are the 1000-fold dilution of hydrogen peroxide and the 100-fold dilution of sodium hypochlorite. These had an average number of 1,0 and 1,7 CFUs respectively. On the other hand, the 1000-fold dilution of citric acid has two plates with zero colonies after disinfection but one

with about two hundred, which on its own raises the average to a higher number than before the disinfection. Because of these uncertainties it is difficult to compare the efficiencies of each disinfectant, and more parallels should have been taken to get a fuller picture of their effect. The UV-disinfection did show an increase of bacterial colonies after disinfection in both the 100-fold and the 1000-fold disinfection, which may indicate that this is not an effective method of disinfection on artificial turf. This may be because the UV-light likely does not reach all areas of the turf because of microshadows and protective layers in form of infill (81). This means that only places that the light can reach are disinfected while most of the grass is not being affected.

It is difficult to evaluate the efficiency of each disinfectant based on these results, but it is evident that the disinfection generally is not as effective as what is desirable. Most samples contained a significant presence of fungi in the first try and bacteria in the second try after the disinfection. This might be because of the complex structure of the artificial grass which possibly makes it difficult for the disinfectants to reach all areas in the grass or that the execution of the disinfection was not as effective as the disinfection potentially could have been with this method (2, 81). It is not certain if the disinfectants or the apparatus used is the liming factor for the disinfection. The problem could be that too low concentrations of the disinfectants were used or that the method does not reach all areas of the grass even with the small drops produced by the prototype from Elergy AS. The apparatus has many variables related to the collision nozzle, such as the design chosen, the distance from the apparatus to the grass and angle of the nozzle in relation to the grass. These could affect the efficiency of the disinfection and needs to be optimized for both this and similar products.

5.3.2. Limitations of the disinfection testing

There were several challenges with the testing of the disinfectants. The first problem was in the first stage of the process, when preparing the artificial grass pieces. It was difficult to spread out the sand and olive stones evenly, especially near the edges of the offcuts. This resulted in more of the infill being located near the middle which might have affected the testing as microbes got different conditions on different locations on the grass. This was adjusted before the second try as the infill was spread out better during the disinfection between the two tries, but this may still have been a source of error on the second try.

As the grass offcuts were disinfected between the first and second try by soaking them in ethanol and sodium hypochlorite, parts of these disinfectants might have remained on the grass when the testing was performed, although they were placed in fume cupboards for a few days for the disinfectants to dry and evaporate. Another factor that could have impacted the results was that the grass offcuts used in the testing were not sterile when delivered. The disinfection before the experiment was not fully effective, which meant that bacteria that are generally not found in artificial grass could have been present during testing. It might also have contributed to the uneven bacterial presence in the grass and therefore led to variation between parallels. This could have affected some of the bacteria when it was added to the grass if the remaining disinfectants still had an effect. The application of bacteria on the grass may have affected the bacterial distribution on the grass offcuts. This was done by soaking the offcuts in bacterial suspension to get it as evenly distributed as possible. Although the bacteria in theory would be evenly concentrated in the liquid and therefore be evenly concentrated on the grass, this was likely not the case in practise as many aspects could have affected this. This includes aspects such as how the grass was lowered into the suspension, the survivability of bacteria in the water suspension, how the infill was distributed and the angles of the grass fibres. It is apparent that these factors were very prevalent by the variation of CFUs in the results.

The collection of the samples and the CFU method might have affected the results. These include the uncertainty of how much of the bacteria the swab picks up and the bundled colonies which were difficult to quantify (97, 98). On many plates there very also many small colonies which were difficult to count and therefore human error also played a part in the counting. One factor which was not prevalent in the second try was fungal growth, as the disinfection before the second try removed fungi. Since the samples inoculated on the grass offcuts only contained bacteria, fungi did not affect the results in this part.

Another problem during the disinfection was replicating how the process would work in full-scale. The exposure time that the disinfectants would have had in practise would likely have been shorter in full-scale use, especially for the UV-disinfection, but replicating the pace and movement of relevant UV-devices was difficult manually and with a handheld device that used different light bulb than these devices (102).

5.4. Further research

As the results of this project show, more testing should be done in terms which disinfectants to use and how to use them. To optimize the effect of the apparatus that was used for disinfection, all the alternative nozzles should be compared, as well as the distance from nozzle to grass and the angle between them. It would be advantageous to do the disinfection tests in a larger scale to get more parallels of each stage of the disinfection process, as three parallels seemed to be too few to get conclusive results. This is clear in the results shown in table 9-16, where there is a significant variation in some of the sample series. Another aspect to research further would be testing of the fungal cultures in the artificial grass since they proved to be very hard to irradicate and very prominent in the grass. Identification of the species in the grass would be important to find out which health risks may be associated with them (15). In addition to general studies of the fungi, thorough studies of the disinfectant's efficiency on these species as well as on the bacteria would also be important.

There is a need to develop better methods to get optimal results when studying microbial growth on artificial grass, especially in terms of a method of collecting samples as the swabs account for a considerable uncertainty in collecting microbes on a surface as complex as artificial grass (2, 97). The quantification should also have been performed with more advanced instruments than what was available in this case. Flow cytometry or PCR could have made the quantification in both the bacterial growth part and the disinfection part easier and more accurate, as human error might affect the counting and bundled colonies on agar plates contains an uncertain number of CFUs (58, 72, 98). It is also necessary to perform full-scale studies and use instruments that are built for this purpose to replicate real conditions and exposure time.

6. Conclusion

To conclude, the microbial presence was significant throughout the entire testing period. The average number of bacteria in the tested areas was between 4 650 and 20 500 CFUs per dm^2, which is a higher number than similar studies on surfaces such as hospital floors and office buildings have found. These numbers are likely underestimated too, as the test method had limitations in terms of collecting all bacteria in the selected area and these numbers does not take the bundled colonies on the agar plates into account. These numbers do also not include the fungal contamination in the grass, which was substantial in all weeks of testing. The degree of bacterial contamination seemed be increase or decrease in correlation with the activity in the hall and to increase after maintenance was performed, both according to the spectrophotometric and plating analyses. These results are slightly unsure due to uncertainty connected to the testing methods, but still indicate that cleaning measures should be taken on the artificial grass, especially in periods with high activity.

In the disinfection part citric acid, hydrogen peroxide and sodium hypochlorite appeared to have a positive but unsatisfying effect on both fungi and bacteria. UV-radiation did not seem to be effective, likely because of the complex structure of the grass, which provides cover for many microorganisms from the UV-rays (2, 81).

Due to the uncertainty posed by the sampling method and quantification methods that were used, further research should focus on finding a more reliable method of collecting samples and use more advanced methods such as PCR or flow cytometry to quantify the bacteria in the artificial grass. In the disinfection testing, the effect of the disinfectants, especially in terms of fungi removal was substandard, and it would therefore be of interest to thoroughly study which chemical disinfectants are most effective and how to effectively apply them to the grass.

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List of attachments

Attachment 1 – Popular science article

Mikrodråpene som vil kunne forhindre smitte mellom fotballspillere

Kjemiingeniør-studenter ved NTNU har foretatt tester av kunstgress for å kunne se om bakterier akkumulerer over tid, samt hvilke rengjøringsmidler som har størst effekt på bakteriene i gresset.

Figur 1: Nærkontakt og taklinger kan føre til skrubbsår som bakterier kan trenge inni (1). Foto: Kieran Cleeves (Pa Photos)

Når fotballspillere får sår fra taklinger eller fall på gresset gjør det at huden, den ytre barrieren i immunforsvaret, blir brutt. Dette kan føre til spredning av sykdommer og virus mellom fotballspillere. Det er da viktig at sårene er rene for å forhindre sykdommer og farlige infeksjoner (2,3). Ettersom det blir etterlatt spytt, blod, svette og tårer på gresset, vil dette kunne gi gode vilkår for sopp og bakterier å blomstre opp. Ofte er fotballspillere såpass unge og friske at immunforsvaret deres tar knekken på det meste, men det er fortsatt viktig å forhindre krisetilfeller. Infeksjoner kan føre til ulike sykdommer, noen til og med livstruende (4).

Mikrodråper

Derfor kommer mikrodråper til utsetning! De ser ikke så store ut, faktisk kan du ikke se dem ikke i det hele tatt. Tanken med disse dråpene er at de skal kunne trenge seg inn i alle kriker og kroker i kunstgresset for å tilintetgjøre trusselen. Disse blir laget inni en dyse produsert av Elergy AS (5). En dyse er et traktformet rør som kontrollerer hvordan væske under trykk skal presses ut (6). En kollisjonsdyse vil skape innvendige væskekollisjoner som fører til at dråpene deler seg opp. Verdien av dette er at væsken da vil kunne dekke store overflater og trenge godt nedi gresset, samtidig som det reduserer væskeforbruk (7).

Rengjøringsresultater

I studentenes forsøk var det hydrogenperoksid som hadde størst effekt. Dette er samme middel som blir brukt i hår og tann-bleking. Molekylet består av et oksygenatom mer enn vann, altså H_2O_2 istedenfor H_2O. I forsøket førte middelet til en reduksjon av bakterier med 97% innen 10 minutter. Det vil si at den kvittet seg med ca. 24 av 25 bakterier. I tillegg ble det testet ut sitronsyre, klor og UV-stråling (8). Som navnet tilsier, finnes sitronsyren i sitrusfrukter og har en desinfiserende effekt (9). Klor har også en desinfiserende effekt og blir ofte brukt i svømmehaller (10). Både sitronsyre og klor hadde en god effekt, men ikke like god som hydrogenperoksid. Desinfeksjonen med UV-lys hadde tilnærmet ingen effekt. Dette kan være på grunn av at lyset hadde for kort virkningstid eller på grunn av at lyset ikke greier å trenge seg langt lok ned i gresset (8).

Vedlikehold og vekst

Noe som trenger seg lenger ned i gresset er børstene som blir brukt ved vedlikehold (11). I kunstgresshallen som ble testet blir det foretatt vedlikehold annenhver uke (12). Dette er for å ivareta spillegenskapene i gresset, samt fjerne større forurensninger som snus og tyggegummi (11). Samtidig ble det også målt antall bakterier over en 4-ukers periode hvor det viste seg at antall bakterier i gresset økte de ukene det hadde vært foretatt vedlikehold. Tilfeldighet? Det er lite sannsynlig. Noe som er mer sannsynlig er at børsten virvler opp bakterier som allerede er dypt i gresset (8). Da havner nok mange av disse på overflaten av gresset. Det vil si at rengjøring av gresset kan være mest aktuelt rett etter vedlikehold (8,13).

Renholdsmiddel langt over middels!

Ettersom naturlig innfyll ikke inneholder stoffer som virker bakteriehemmende, slik som innfyllet som har blitt brukt til nå, gjør dette at bakteriene kan kose seg uforstyrret (14,15). Derfor er det enda større nødvendighet med renhold i dag kontra tidligere. Renholdsmiddelet som skal brukes har en lang ønskeliste å oppfylle. Det skal ikke skumme, være helseskadelig, etterlate seg fukt og det skal trenge inni alle kriker og kroker i gresset (16). Samtidig skal selvfølgelig desinfiseringen være god nok. Som nevnt kom hydrogenperoksid ut på topp i testen (8). Etter bruk vil stoffet kunne fordampe i form av hydrogen og oksygen- gass, noe som allerede er naturlig forekommende i vanlig luft (17). I tillegg vil kollisjonsdysen sørge for at det blir brukt en lav mengde stoff, helt ned mot 3 liter på en hel kunstgresshall (6)! Det vil da etterlate seg svært lite fukt i gresset. De små dråpene vil også gjøre at stoffet trenges dypt ned, samtidig som det blir godt fordelt. Dette vil kunne være bakterienes mareritt.

Veien videre

Bakterier er ikke det eneste problemet når det kommer til kunstgress. Det var også funnet sopp. Sopp stortrives i miljø som er varme, fuktige og litt sure (18). Akkurat som besteforeldre på ferietur i Gran Canaria. I motsetning til glade besteforeldre er ikke sopp ønskelig. Det er nettopp derfor det er viktig å bruke små mengder flytende stoff på gresset, ettersom det kan ta tid før gresset får drenert seg. Sopp produserer små sporer som kan forurense luften og kan muligens skape problemer for spillere med for eksempel astma (19). Hvordan å effektivt fjerne sopp er noe som kan være en spennende problemstilling ved videre eksperimenter (8). Annet enn å undersøke soppvekst i kunstgress er det også relevant å finne ut mer om effekten hydrogenperoksid i større skala, med mer avansert utstyr. I tillegg kan det være nyttig å vite hvor store mengder som skal til for å oppnå en ønsket effekt av desinfeksjon.

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