Czech Technical University in Prague

Faculty of Electrical Engineering

Department of Circuit Theory

Master's Thesis

The Effect of Growth Phase and Illumination on the Antibacterial Effect of Photoactive Nanoparticles

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Study program: Medical Electronics and Bioinformatics
Specialization: Medical Instrumentation

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MASTER'S THESIS ASSIGNMENT

I. Personal and study details

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Study program: **Medical Electronics and Bioinformatics**

Specialisation: **Medical Instrumentation**

II. Master's thesis details

Master's thesis title in English:

The Effect of Growth Phase and Illumination on the Antibacterial Effect of Photoactive Nanoparticles

Master's thesis title in Czech:

Vliv růstové fáze a osvětlení na antibakteriální účinek fotoaktivních nanočástic

Guidelines:

This experiment-based project will attempt to investigate the influence of the bacteria growth stage on the antibacterial effect of photoactive nanoparticles (ZnO). Bacteria will be removed from different growth phases (i.e. lag, exponential and stationary) and adjusted to a standard cell concentration before adding to different concentrations of ZnO nanoparticles. ZnO will also be subjected to irradiation using white light to activate the surface and generate electron-hole pairs that results in the generation of reactive oxygen species (ROS) e.g., hydrogen peroxide. The antibacterial effect of ZnO will be monitored in real-time by measuring the change in optical density over incubation time and comparing growth kinetic profiles generated by the bacteria taken from different growth phases. e.g., lag phase duration, final optical density value, cell concentration etc. Upon completion of the project, the student will have gained experience in laboratory scientific research involving the antibacterial testing of photoactive nanomaterials. The student will also have expanded their knowledge in bacteria growth, material science and illumination.

Bibliography / sources:

[1] Cherchi C. & Gu A. Z. 2011 Effect of bacterial growth stage on resistance to chlorine disinfection. Water Sci. Technol. 64 (1), 7–13. https://doi.org/10.2166/wst.2011.536
[2] Makinoshima H, Aizawa S, Hayashi H, Miki T, Nishimura A, Ishihama A. 2003 Growth phase-coupled alterations in cell structure and function of Escherichia coli. J Bacteriol. 2003 Feb;185(4):1338-45. https://doi.org/10.1128/JB.185.4.1338-1345

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Assignment valid until: **21.09.2025**

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III. Assignment receipt

The student acknowledges that the master's thesis is an individual work. The student must produce her thesis without the assistance of others, with the exception of provided consultations. Within the master's thesis, the author must state the names of consultants and include a list of references.

Date of assignment receipt	Student's signature

Declaration

I declare that the presented work was developed independently and that I have listed all sources of information used within it in accordance with the methodical instructions for observing the ethical principles in the preparation of university theses.

Prague, date .....

.....
Author's signature

Acknowledgement

I would like to thank my supervisor David Rutherford, PhD., for his guidance and help with this thesis. I also really appreciate our countless discussions about the protocols or results, as well as his help with my English writing and proof reading.
I would like to thank my husband and my family for their great support throughout my studies and my work on this thesis.

Abstract: World Health Organization considers antimicrobial resistance as one of the top 10 global health threats. Therefore, it is important to study different antimicrobial treatments as well as potential resistance. Some nanoparticles, such as ZnO nanoparticles possess antibacterial properties and are studied and tested as a potential new antimicrobial treatment. The main aim of this thesis was to see if there was an effect of growth phase of *Escherichia coli* bacteria cells and continuous illumination on antibacterial effect of ZnO nanoparticles. Sub-lethal concentration of ZnO nanoparticles which was determined from Minimum inhibitory concentration test was used. Preparation protocols for different growth phase cells were developed for this experiment. Lag, log and stationary phase cells were cultivated in Mueller-Hinton broth or on Mueller-Hinton agar plates. The growth kinetics was monitored in real-time by measuring optical density throughout the 20 hours of the illumination experiment in bioreactors, and then cell concentration was measured by counting the number of colonies at the beginning and at the end of the illumination experiment by a series of dilutions and addition to agarose for growth.
I discovered statistically significant differences between the growth phases in their sensitivities towards the ZnO nanoparticles and ZnO nanoparticles combined with white illumination treatments. The least sensitive cells towards these treatments were the lag phase cells, while the most sensitive were the stationary phase cells.
The stationary phase cells also had the longest lag phase lengths, while the lag phase cells had the shortest lag phase length. These findings show that the growth phase of bacteria cells can influence the effect of sub-lethal concentrations of photoactive nanoparticles under illumination and should be taken into consideration when photoactive nanoparticles are to be used for antibacterial treatments in the future.

Key words: antibacterial effect, zinc oxide, nanoparticles, Escherichia coli, sub-lethal concentration, growth phases, lag phase, log phase, stationary phase, bacteria, illumination, white light

Abstrakt: Světová zdravotnická organizace považuje antimikrobiální rezistenci za jednu z deseti největších globálních zdravotních hrozeb. Proto je důležité studovat různé způsoby antimikrobiální léčby stejně jako potenciální rezistenci. Některé nanočástice, například nanočástice oxidu zinečnatého, mají antibakteriální vlastnosti a jsou studovány a testovány jako potenciální nová antibakteriální léčba.
Hlavním cílem této práce bylo zkoumat vliv růstové fáze buněk bakterie *Escherichia coli* a kontinuální osvětlení na antibakteriální účinek nanočástic oxidu zinečnatého.
Byla použita subletální koncentrace nanočástic oxidu zinečnatého, která byla stanovena na základě testu minimální inhibiční koncentrace. Pro tento experiment byly vypracovány protokoly pro přípravu buněk z různých růstových fázích. Buňky z lag, log a stacionární fáze byly kultivovány v Mueller-Hintonově bujónu nebo na petriho miskách s Mueller-Hintonovým agarem. Kinetika růstu byla sledována v reálném čase v bioreaktorech měřením optické hustoty po dobu 20 hodin, kdy osvětlovací experiment probíhal. A poté měřením koncentrace buněk pomocí množství kolonií na začátku a na konci experimentu s osvětlením pomocí série ředění a kultivací na petriho miskách s Mueller-Hintonovým agarem.
Zjistila jsem statisticky významné rozdíly mezi růstovými fázemi v jejich citlivosti vůči nanočásticím ZnO a nanočásticím ZnO v kombinaci s osvětlením bílým světlem. Nejméně citlivé vůči těmto ošetřením byly buňky z lag fáze, zatímco nejcitlivější byly buňky ze stacionární fáze.
Buňky stacionární fáze měly také nejdelší délku lag fáze zatímco nejkratší délku lag fáze měly buňky právě z lag fáze. Tato zjištění ukazují, že růstová fáze buněk bakterií může

ovlivnit účinek subletálních koncentrací fotoaktivních nanočástic při osvětlení a měla by být zohledněna při budoucím použití fotoaktivních nanočástic pro antibakteriální léčbu.

Klíčová slova: antibakteriální účinek, oxid zinečnatý, nanočástice, *Escherichia* coli, subletální koncentrace, růstové fáze, lag fáze, log fáze, stacionární fáze, bakterie, osvětlení, bílé světlo

List of abbreviations

$^{\bullet}HO$ – Hydroxyl radical
AMR – Antimicrobial resistance
ATP – Adenosine triphosphate
CB – Conduction band
CCM – Czech collection of microorganisms
CFU – Colony forming units
DNA – Deoxyribonucleic acid
E. coli – *Escherichia coli*
FDA – Food and Drug Administration
H_2O_2 – Hydrogen peroxide
HPLC – High Performance Liquid Chromatography
HUS – Hemolytic ureic syndrome
MDR – Multi-drug resistant
MHA – Muller-Hinton Agar
MHB – Muller-Hinton Broth
MIC – Minimum inhibitory concentration
MRSA – Methicillin-resistant *Staphylococcus aureus*
NCCLS – National Committee for Clinical Laboratory Standards
NPs – Nanoparticles
OD – Optical density
Plates – Petri dishes
RNA – Ribonucleic acid
ROS – Reactive oxygen species
RPM – Rotations per minute
Saline – Physiological Saline Solution (0.9% NaCl)
Stat – Stationary
STEC – Shiga-toxin producing *Escherichia coli*
UV – Utraviolet
VB – Valence band
Water+light – illuminated sample with water instead of zinc oxide nanoparticles
Water+no light – unilluminated sample with water instead of zinc oxide nanoparticles
WHO – World Health Organization
Zinc+light – illuminated sample with zinc oxide nanoparticles
Zinc+no light – unilluminated sample with zinc oxide nanoparticles
ZnO – Zinc oxide

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1. Introduction

Antibiotics were discovered more than 100 years ago. Penicillin-based antibiotics were discovered in 1928 and in the mid-1950s the discoveries of natural antibiotics had reached its peak.[1] Antibiotics have improved modern medicine and it is expected that they extended the average human lifespan by more than 20 years.[1,2]

However, antibiotic-resistant infections and bacteria are increasing at an alarming rate. Antimicrobial resistance (AMR) occurs when microorganisms become resistant to a treatment which was effective previously and has become ineffective.[3] This can be very dangerous since AMR makes infections harder to treat, and easier to spread. AMR not only affects humans, but it can also affect animals and plants reducing farm productivity and threatening food security.[4] AMR can also affect national economies, since it creates greater need for care and other treatments, which affects productivity of people and even agricultural productivity.[4] It is not only a health problem, but a global problem which threatens the whole world on several levels.

The main cause of the AMR is antibiotic overuse and misuse in humans, animals and plants.[2,4] It is expected that in 2019, AMR was associated with 4.95 million deaths.[5] And it is estimated that by 2050 around 10 million deaths annually will be caused by AMR.

Therefore, great emphasis is placed on discovery of new antibacterial treatment options as well as on studying AMR to these treatments prior to clinical use.

It is known that bacteria cells can show different sensitivity towards antibacterial treatments depending on the phase of growth there were harvested from.[6] Therefore, the efficiency of antibacterial treatments could be influenced by cell physiology. It is important to discover if bacteria from different growth phases become more resistant to the same treatments or if different growth phase cells can be more sensitive to the same treatment. It is not known if bacteria from different growth phases have different sensitivities towards nanoparticles with antibacterial properties.[6]

The plan for this study is to expose bacteria to sub-lethal concentrations of photoactive nanoparticles, which exhibit antibacterial effects, in addition to continuous illumination. The sub-lethal concentration will be determined from the Minimum inhibitory concentration (MIC) test.

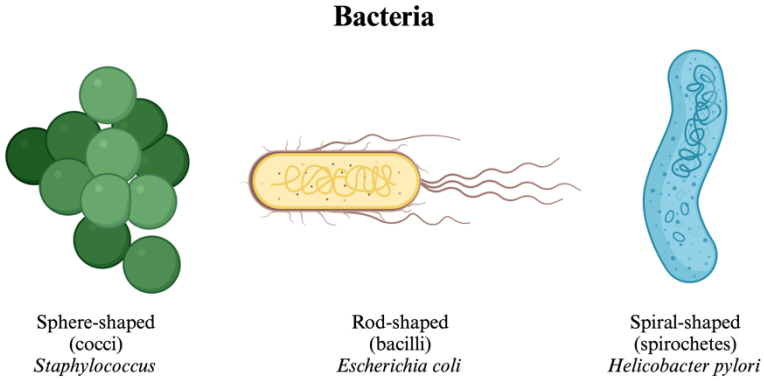
Stressing bacteria by exposure to sub-lethal concentrations of antibacterial nanoparticles under continuous illumination creates a non-ideal environment for growth and division. As mentioned previously, the nanoparticles used in this study are also photoactive, meaning they can have higher antibacterial effect when illuminated throughout the experiment. The bacteria from the different growth phases will be subjected to this treatment in order to discover if there are differences in the response that are dependent on the phase of growth the bacteria were harvested at.

2. Background

In this section, general information about bacteria is provided, along with specific details about the particular bacteria strain used in this study (*Escherichia coli*, *E. coli*), growth phases, antimicrobial resistance and nanoparticles.

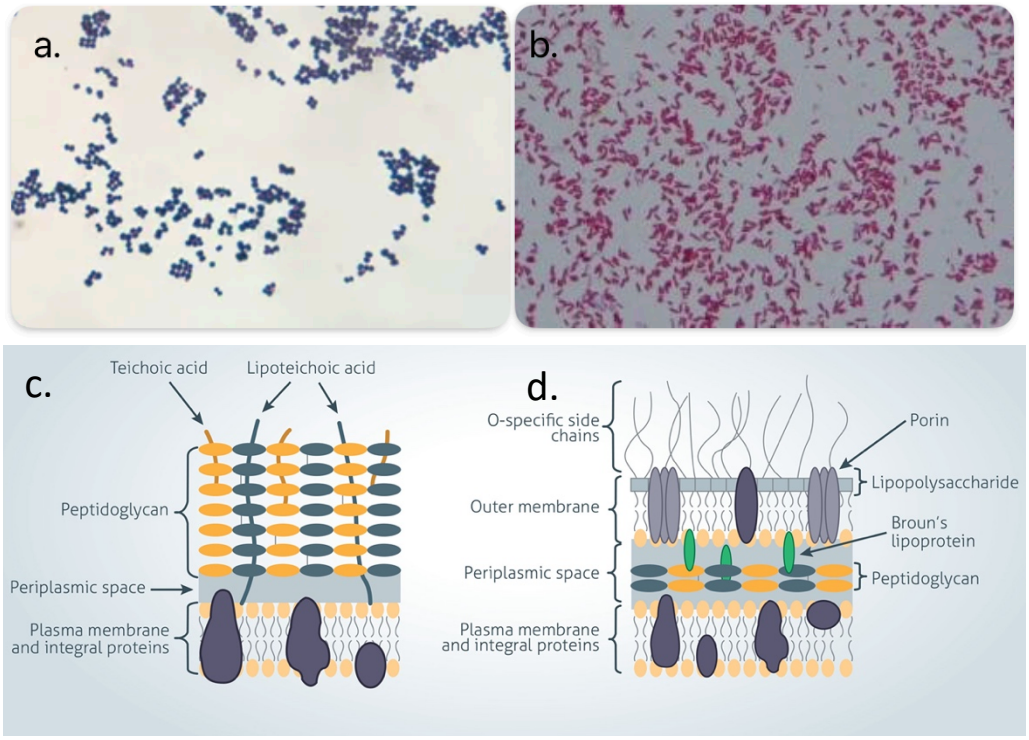
2.1 Bacteria

Bacteria are single-celled organisms.[7] Bacteria can be found almost everywhere on the planet and are essential to the planet's ecosystem.[8] The human body is estimated to contain more bacteria cells than human cells.[8] Some bacteria cells can also live under extreme conditions such as under extreme pressure or temperature.[8]
Since there are many types of bacteria cells, one way to classify bacteria is by their shape, *Picture 1*.



Picture 1 *Classification of bacteria by their shape. Created with Biorender.com.*

Sphere-shaped bacteria are called *cocci*, example of sphere-shaped bacteria is *Staphylococcus* or *Streptococcus*.[7,9]
Rod-shaped bacteria are called *bacilli*. Example of rod-shaped bacteria is *Escherichia coli* or *Salmonella*.[7,9]
Spiral-shaped bacteria are called *spirochetes*. Example of spiral-shaped bacteria is *Campylobacter jejuni* or *Helicobacter pylori*.[7,10]
Another way of classifying bacteria is by using optical microscopy to view bacteria after staining. The Gram-staining techniques creates two different groups. Gram-positive bacteria and Gram-negative bacteria[7]



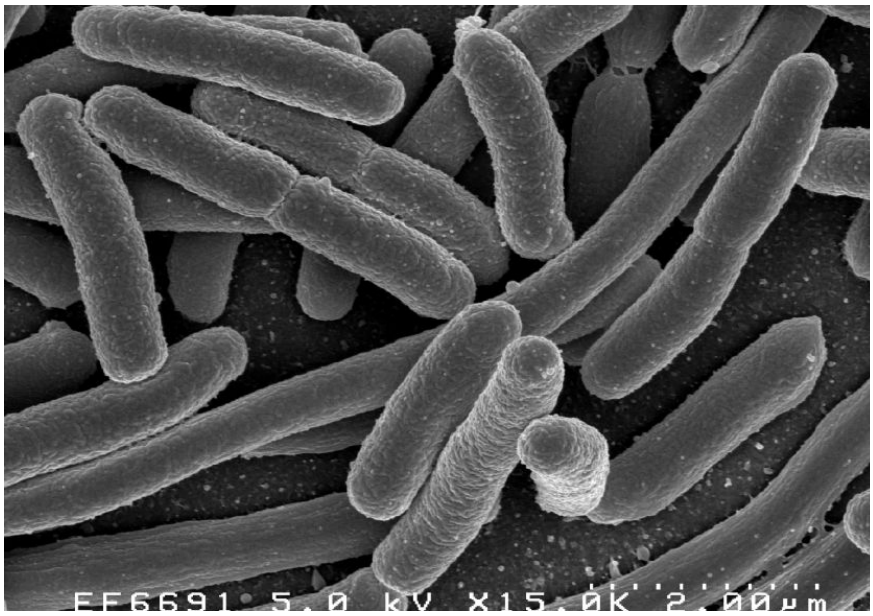
Picture 2 (*a) Gram-positive Staphylococcus aureus (b)Gram-negative Escherichia coli after Gram staining*[11]*, (c) schematic diagram of Gram positive and (d) Gram negative bacteria.*

In *Picture 2 (a)*, are examples of Gram-positive bacteria after Gram-staining, and *Picture 2 (b)*, shows Gram-negative bacteria.
Blue or purple stained bacteria after Gram-staining are called Gram-positive. Gram-positive bacteria is for example *Staphylococcus aureus*.[7,12]
Gram-positive bacteria have a thick layer of peptidoglycans, *Picture 2 (c)*, so they retain the colour after staining.[12] And they are more resistant to being killed as a result of physical damage like cell wall penetration.[12,13] For example, vancomycin-intermediate resistant *Staphylococcus aureus* strains developed thicker cell wall in order to protect themselves against the antibiotics.[14]
Gram-negative bacteria appear red or pink after Gram-staining.[7,12] This happens due to the Gram-negative bacteria having thinner peptidoglycan cell wall, but they have outer lipoprotein membrane[12,15], *Picture 2 (d)*, which can cause higher resistance to antibiotics.[16]
An example of Gram-negative bacteria is *Escherichia coli* or *Legionella*.[12]
The missing lipoprotein layer can also cause Gram-positive bacteria to absorb antibiotics more easily than Gram-negative bacteria.

2.1.1 Escherichia coli

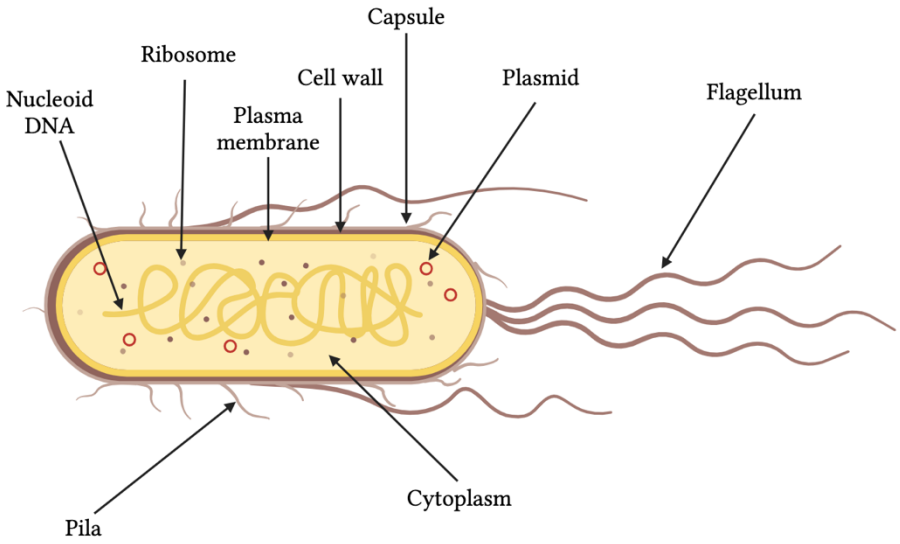
Escherichia coli (*E. coli*) is a rod-shaped, Gram-negative bacteria.[17,18] The bacteria cell has regular dimensions of 1-3 µm length, and 0.3-0.7 µm width.[19,20] It is normally found in human intestines and faeces.[21,22] Most *E. coli* strains are harmless. Although some can cause food poising or urinary infections and when *E. coli* is detected in water then it means that the water hasn't been decontaminated properly.[21] Multi-drug resistant (MDR) strains also exist making it important bacteria to study.[23] One of the dangerous strains of *E. coli* is called Shiga-toxin producing *Escherichia coli* (STEC), where infections due to this strain cause acute diarrhoea and sometimes life-threatening hemolytic uremic syndrome (HUS)[24], leading to kidney damage or failure and other serious health problems.[25]

E. coli is the most studied procaryotic microorganism and it is an important species in microbiological research.[19] Codons, were discovered thanks to E. coli. "By studying the viruses that infect *E. coli*, scientists have learned much about the form and function of viruses that infect humans, like the influenza virus or HIV."[26]
In *Picture 3* is an example of E. coli bacteria viewed at high magnification using electron microscopy.



Picture 3 *Hight magnification (15kx) scanning microscopy image of Escherichia coli bacteria*[27].

In *Picture 3* can be seen that some of the cells are longer and have a groove in the middle of the cell. These cells are in a phase of propagation. When the bacteria cells propagate, the cell first elongates to twice its length and then split in the middle, creating new bacteria cell.[28] Even though the two cells look the same, they somewhat differ in components.[29]
A schematic diagram of *E. coli* morphology is presented in *Picture 4*, detailing the intracellular components of bacteria that are not visible in *Picture 3*.

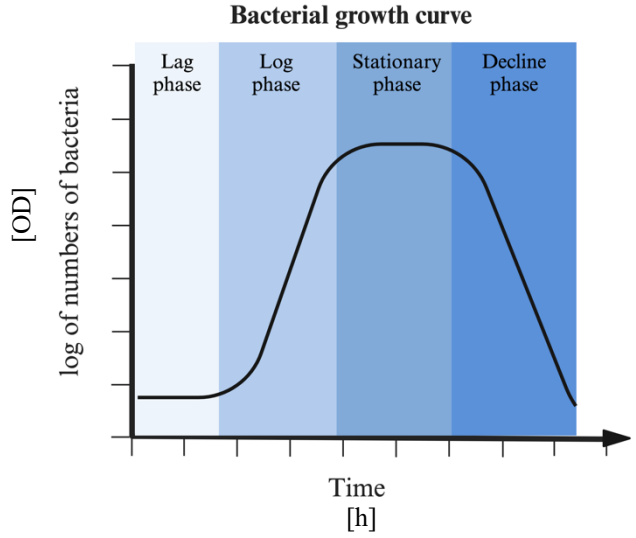


Picture 4 *Morphology of* E. coli *bacteria cell. Created with Biorender.com.*

The cell wall of E. coli cells is made of a thin layer of peptidoglycan and outer membrane around this peptidoglycan layer.[30] As discussed previously, bacteria can be grouped together depending on the cell wall composition (*Picture 2*). The capsule is another protective layer from the host immune system.[30] Flagella are long filamentous structures located outside the bacteria that are used for motility, and only few strains of E. coli are non-motile.[19] Pili are hair-like structures which help the cell stick to a surface.
The cytoplasm is a thicker intracellular solution mainly composed of water, salts and proteins. The nucleoid contains several proteins, ribonucleic acid (RNA) molecules and a single chromosome, where the deoxyribonucleic acid (DNA) is contained.[31,32] The plasma membrane is an inner semipermeable membrane which is responsible for transport and many other functions such as energy transduction.
Ribosomes are used for protein synthesis. Plasmids are usually circular DNA molecules, however, linear or RNA plasmids also exist.[33] "Bacteria can pick-up new plasmids from other bacterial cells (during conjugation) or from the environment."[32] Plasmids are able to copy themselves independently on the bacterial chromosome and therefore in one bacteria cell can be hundreds copies of a plasmid. The genes in the plasmids are usually not important for bacteria cell everyday survival, but they are used to overcome occasional stress. Some plasmids can make the bacteria cell resistant to antibiotics.[32] Virulence factors of bacteria cells are frequently encoded in plasmids.[34]

2.1.2 Growth curve

In closed system bacteria cells grow in a predictable pattern which is called bacterial growth curve.
The growth curve has four phases, lag phase, log phase, stationary phase and decline phase.[35] In *Picture 5* is an ordinary bacterial growth curve with all four stages clearly marked.



Picture 5 *General bacterial growth curve showing the change in number of bacteria cells in a closed system over time. Created with Biorender.com.*

Lag phase is the initial stage when the bacteria cells are getting used to the new environment and are adjusting to new conditions. Therefore, the curve is flat since the bacteria cells are not multiplying.[35,36]
After the lag phase the bacteria cells starts to divide by binary fission and are multiplying by two after every generation, the curve is exponential, and the phase is called log or exponential phase.[35,36]

When the bacteria cells have consumed almost all the nutrients and have run out of space, they are still multiplying but the rate has slowed down significantly, and the growth is balanced.[37] In stationary (Stat) phase, the number of bacteria cells is constant, the number of new bacteria is balanced by the same number of bacteria dying, and the line in the growth chart is flat.[35–37] The bacteria can multiply in the stat phase even when all the sources in the media have been used, because dying cells can lyse and provide some nutrients.
The last phase is called decline or death phase, it is a descending line in a growth chart. Due to the lack of nutrients and space and increase of waste products the bacteria cells are dying and the number of bacteria cells is decreasing. As well as growth, the decrease is also exponential.[35,36]
In Figure 1 is growth curve of *E. coli* with marked cell phases which were studied in the diploma thesis experiment.

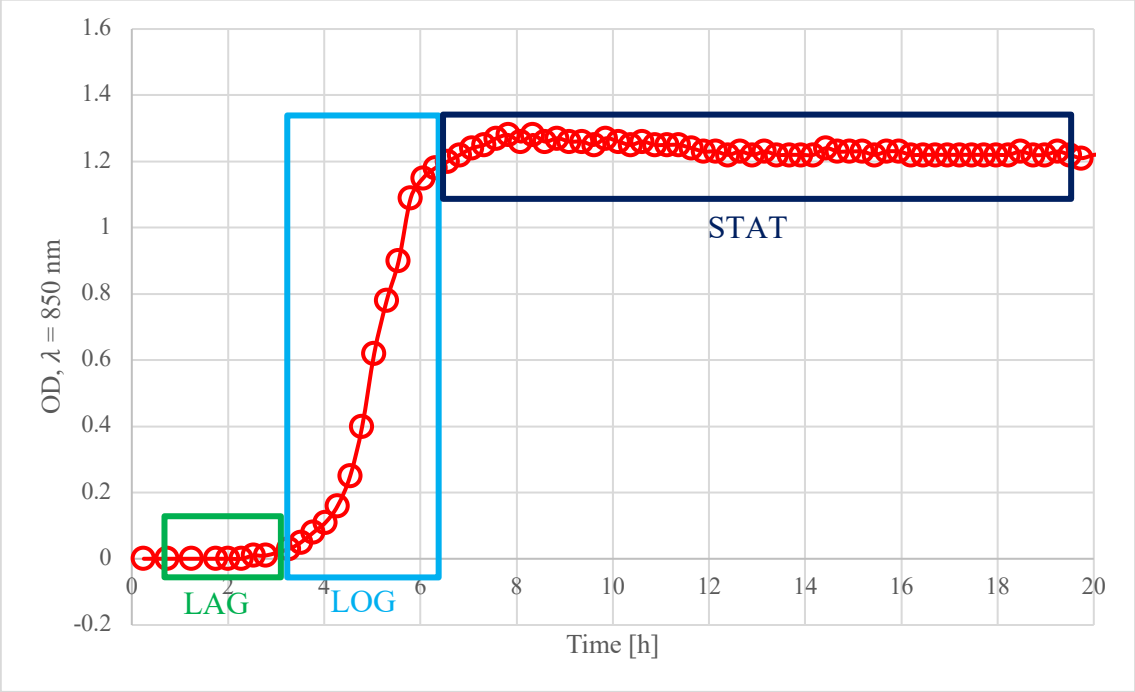


Figure 1 E. coli *growth curve showing the change in optical density over time with the separate growth phases marked.*

Bacteria cells have different physiological states depending on what phase of growth they are in. This can influence the cell sensitivity towards antibacterial treatments which is why different cell phases are studied in this diploma thesis.
The different growth phases have different phase-specific genes which were reported by many studies.[38–40]

a. Lag phase E. coli cells

When bacteria cells are added to new growth media, the first stage which they are in is the lag phase, the lag period refers to the time which is required for the bacteria cells to reach first cell division.[39] During the lag phase, the cells have to adjust to the new environment, hence they are not multiplying. The bacteria cells have to adapt physiologically to the culture conditions. To meet the requirements of the new environment, the adjusting time may include time for induction of a specific messenger RNA and protein synthesis.[37]
Even though the lag phase was documented more than century ago, the molecular structure and cellular events of the lag phase cells is not as documented as the other growth phases. This is due to technical problems which occur when small numbers of bacteria cells are studied.[39] The lag phase length is dependent on the inoculum concentration and type, as well

as media and the oxygen exchange as well. For higher inoculum concentration, the lag phase length will be shorter.[37]
Another factor that influences the lag phase length is the history of the inoculum, if the bacteria cells have been previously preadapted to pH, temperature or osmolarity extremes, they then have shorter lag phase lengths when encountered the same conditions.[39] Bacteria cells that were put under stress require more time to exit the lag phase. Individual cells however have different lag times, the variance is crucial for the bacteria survival.[39]
When cell that have grown previously to the stationary phase enter the lag phase, they have to change their transcriptome and proteome in order to be able to start the multiplication.[39]
Experiments on different bacteria cells, such as *Lactococcus lactis*, indicates that bacteria are producing new enzymes to digest food, build biomass and therefore prepare for the multiplication.[39]
Other research also shows that although the cells are not dividing in the lag phase, they are increasing in size.[38,39] During the lag phase the bacteria cells also have to reactivate ribosomes which were deactivated during the stat phase and as a primary source of glucose serves glycogen.[41] It was also shown that oxidative stress occurs during the lag phase, and reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or hydroxyl radical ($^{\bullet}HO$) are present. Associated with the oxidative stress are also higher concentrations of iron during the lag phase.

b. Log phase E. coli cells

After the lag phase, when bacteria cells start to multiply, they transition into the log phase. In the log phase, the cells have enough nutrients and they have adjusted to the new environment, therefore they have ideal conditions for growth.[37] Again, the log phase length is dependent on the media, inoculum concentration and oxygen exchange.
Log phase cells were reported to have the highest flagella count at late log stage. Therefore, the motility is lower at the beginning of the log phase and reaches its peak at late log.[40]
During the exponential phase approximately 1000 genes are which highly expressed are then shut off during the stat phase.[42]
During the log phase adenosine triphosphate (ATP), which is a chemical that that stores energy and releases it when it is needed[43], concentration increases about fifty times.[39] Also, electron transport chain activity is increased in intensity after the beginning of the log phase.[39] While esterase activity increases, membrane damage decreases.[39]
During the log phase, the glucose concentration from the media decreases as the bacteria cells are using it, and the glucose uptake reaches its peak at the late-log stage and the glycogen concentration slowly increases, as can be seen in *Figure 2*.

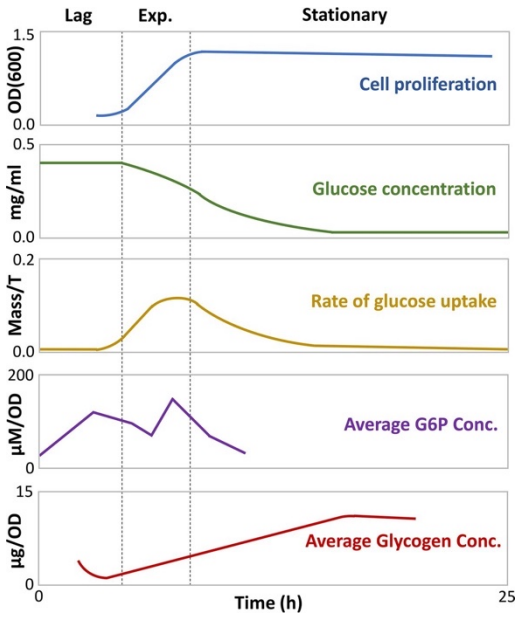
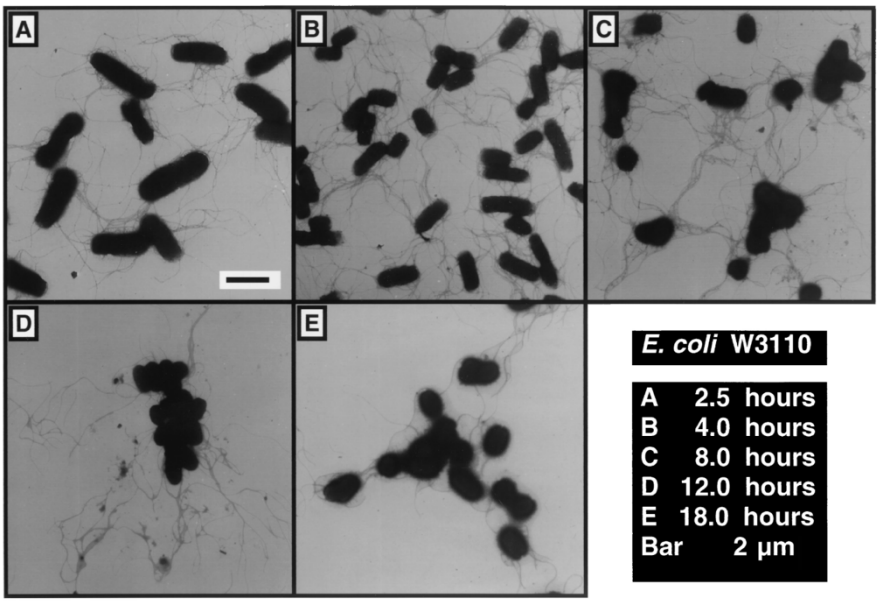


Figure 2 *Metabolic trends of* E. coli *cells in different growth phases, OD (600) means optical density at 600 nm, Exp. means exponential or log phase, and Conc. means concentration.*[39,41]

c. Stat phase E. coli cells

In nature bacteria cells usually stay in the stationary phase because the growth is limited.[42] Stat phase cells are widely studied, because they are easy to grow and obtain. From some research, it has also been reported that stat phase cells are smaller and more spherical.[42,44] During the stationary phase, the bacteria cells also condense their DNA, deactivate ribosomes to conserve energy or reduce their cytoplasmic volume.[39] During the stat phase, bacteria cells are also more prone to oxidative damage.[39]

Makinoshima et al.[40], studied the differences between log phase cells and stat phase cells of *E. coli*. They discovered in 2003 that stat phase cells have less flagella than the log phase cells[40], see *Picture 6.*

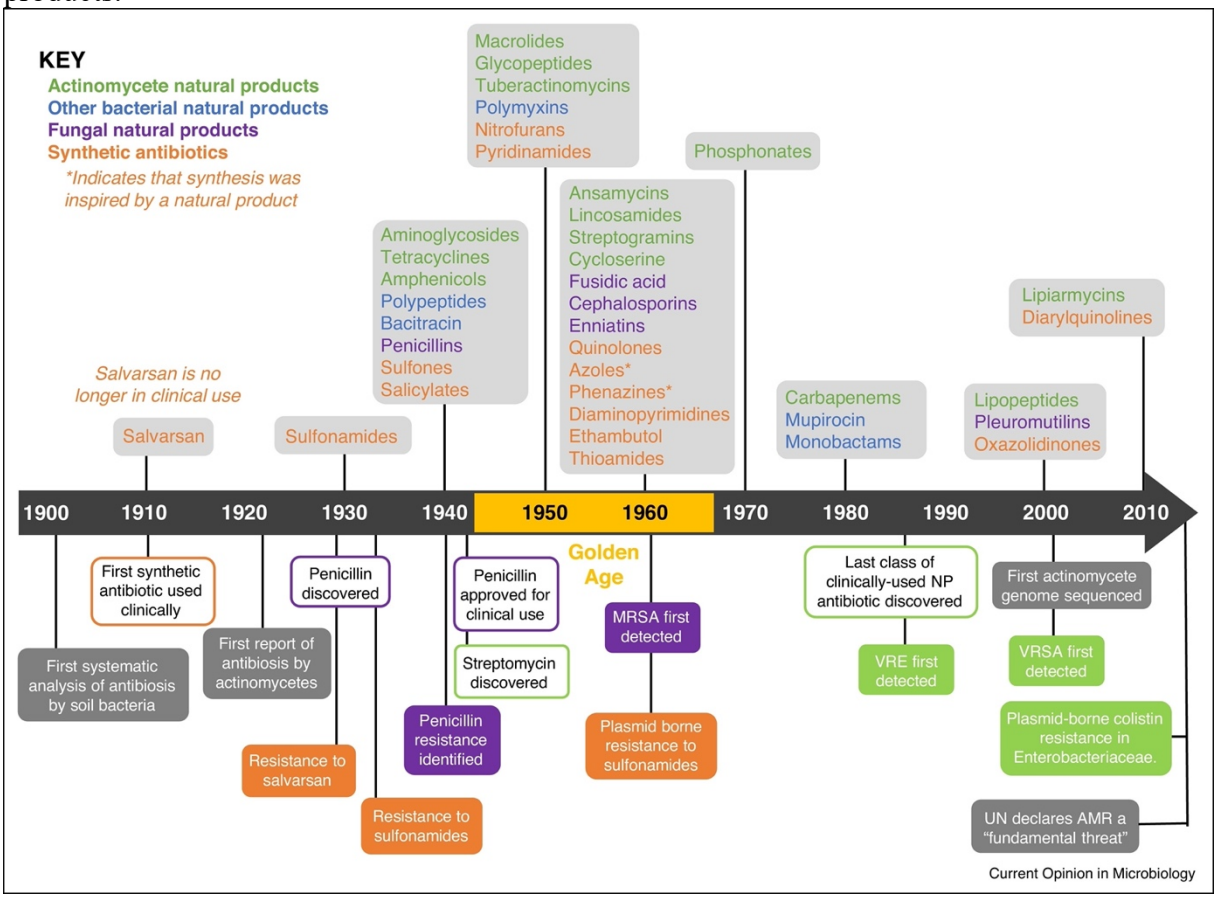


Picture 6 *Flagella counts during the exponential and stationary phase of E. coli bacteria, A to C, are log phase E. coli cells, and the decrease of flagella can be observed in D and E, where stat phase E. coli cells are.*[40]

Due to the lack of flagella, the stat phase cells also had lower motility than the log phase cells.[40]
The stat phase cells also had increased weight-to-volume ratio than the log phase cells, with accumulation of electron dense areas condensed in the cytoplasm was observed in electron microscopy.[39,40,42] This could have been caused by increase in glycogen.[40] During the stat phase, the average glycogen concentration reaches its peak, while glucose concentration decreases as well as glucose uptake.[39] Stat phase cells were also reported to have higher levels of storage materials such as trehalose, glycine betaine, glycogen and other[40] and to have around 100 stationary-phase-specific genes.[40,42]

2.2 Antibiotics and Antimicrobial resistance

Antibiotic clinical use can be considered the greatest medical breakthrough of the 20th century. Antibiotics made possible procedures impossible for that time, such as open-heart surgeries, organ transplants and even cancer treatments.[1] But, antibiotics were used for millennia, for example, the ancient Egyptians used mouldy bread to treat infected wounds.[1,45] Any substance that inhibits the bacterial growth can be called antibiotic, however the usual division of antibiotics is based on their source. There are actinomycetes-based, fungi-based, synthetic-based and other bacteria-based antibiotics. *Picture 7* shows timeline when which antibiotics reached the clinic. Most clinically relevant antibiotics are derived from natural products.[1]



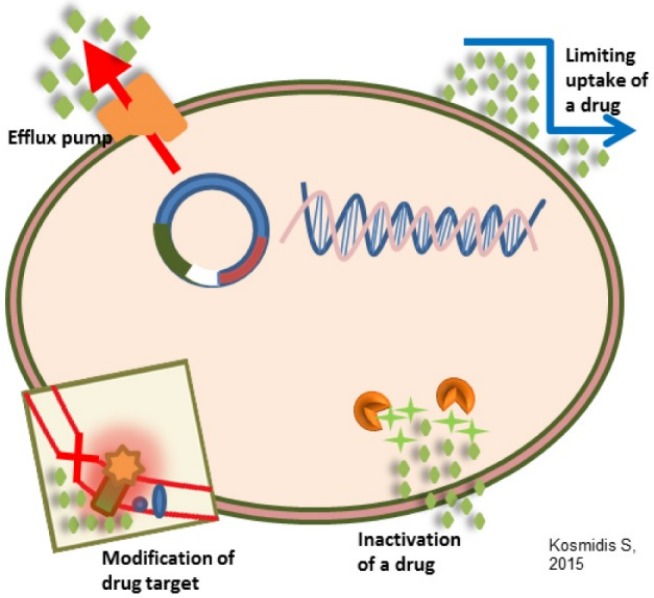
Picture 7 *Timeline showing when which antibiotics reached the clinic, colours are based on the source of the antibiotic, green-coloured are actinomycetes, blue-coloured are other bacteria, purple-coloured are fungi and orange-coloured are synthetic.*[1]

There are very specialised antibiotics which only work against specific bacteria, and then there are broad-spectrum antibiotics which attack even bacteria beneficial for human, e.g., in gut, where the microbiome helps with food digestion and nutrient absorption.[7,46] Antibiotics can either kill the bacteria or prevent the bacteria reproduction, by destroying the bacteria cell wall or DNA.[7,46]

First antibiotics were synthetic, arsenic-based, called salvarsan and were developed by Paul Ehrlich who used it to treat syphilis.[1,45] Although Ehrlich called it chemotherapy since he used chemical to treat the illness.[45] Penicillin, fungi-based antibiotics, was discovered by accident by Alexander Fleming in 1928 and got into clinical use in 1943.

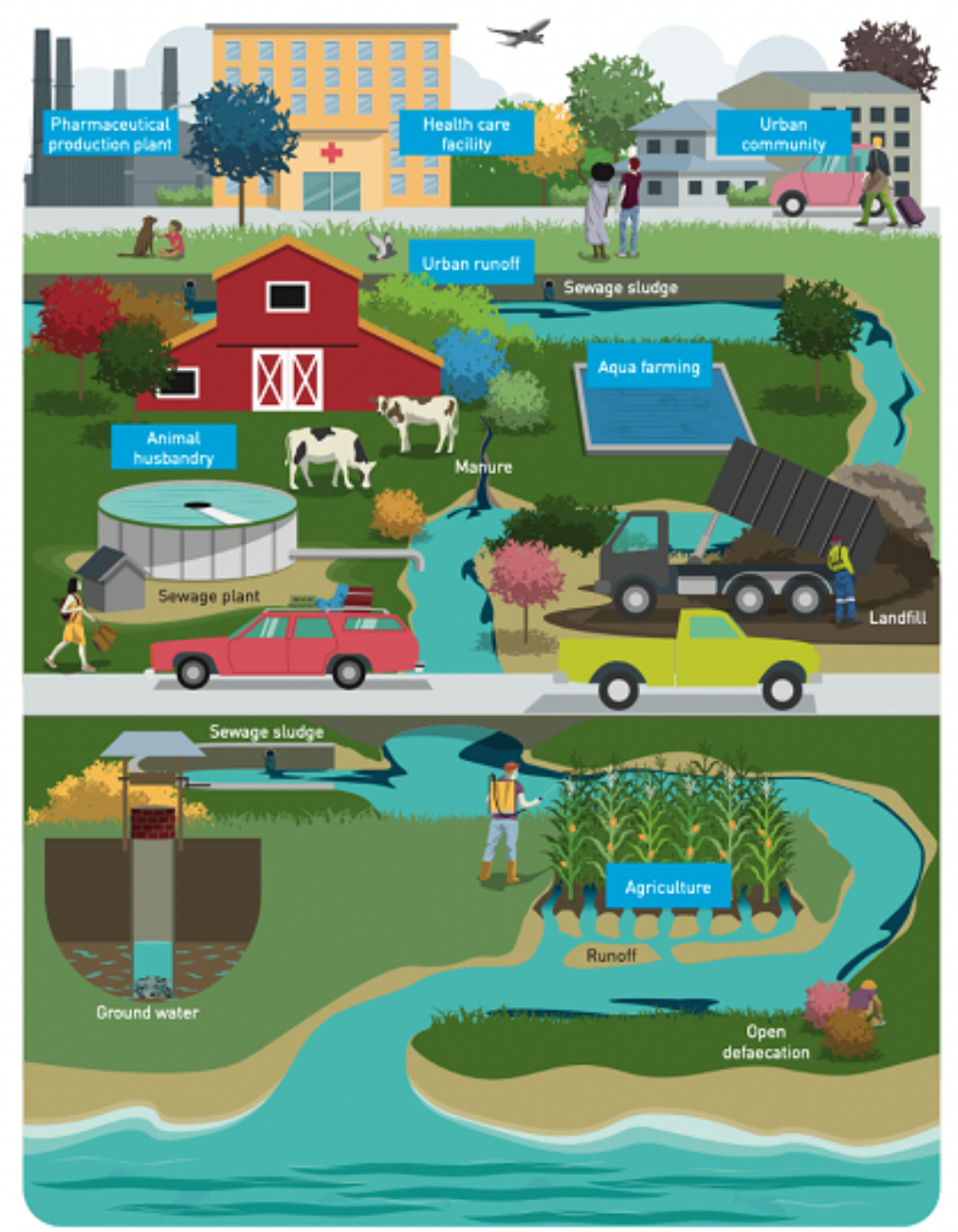
"Antimicrobial agents can be divided into groups based on the mechanism of antimicrobial activity. The main groups are: agents that inhibit cell wall synthesis, depolarize the cell

membrane, inhibit protein synthesis, inhibit nuclei acid synthesis and inhibit metabolic pathways in bacteria."[47]
Any successful use of a healing agent is compromised by the possibility of the target bacteria developing tolerance or resistance and becoming ineffective[48], e.g., methicillin-resistant *Staphylococcus aureus* (MRSA) was first discovered one year after the antibiotic methicillin being used.[49]
It is important to note, that there is a difference between persistence and resistance. If a bacteria cell is resistant to a certain antimicrobial agent, all the daughter cells will also be resistant as well, since daughter cells are genetic clones of parent cells. However, if the bacteria cells are persistent, the cells are not susceptible to the agent but do not have any resistance genes.[47] AMR can be either natural or acquired. Natural resistance can be either intrinsic, thus always expressed in the species, or induced, when the genes are naturally in the bacteria cells, but will only be expressed once the bacteria cells are exposed to the antibiotic.[47,50] Acquired resistance is when the bacteria cells acquire the genes that confers resistance from other nearby bacteria in the surrounding environment. This can happen through transformation, transposition or conjugations or the bacteria cells can experience mutation to their chromosomal DNA.[47,50]
The resistance mechanisms can be then divided into four main categories as shown in *Picture 8*: limiting uptake of a drug, modifying a drug target, inactivation of a drug and active drug reflux.[47] Gram-negative bacteria use all of these mechanisms.[47]



Picture 8 *Mechanisms of antimicrobial resistance.*[47]

Bacteria have many mechanisms how to fight against antibiotics and antimicrobial resistance has become a global threat. World Health Organization (WHO) lists AMR in the top 10 threats for global health.[3]
Society must understand that humans, animals and plants are interconnected and cannot be divided.[3,4] In *Picture 9* are different environmental sources of AMR pollution which shows the connection of different sectors. The approach to AMR and even to potential new treatments should be integrated and this connection between humans, animals and ecosystems needs to be considered.



Picture 9 *Environmental AMR pollution sources*.[3]

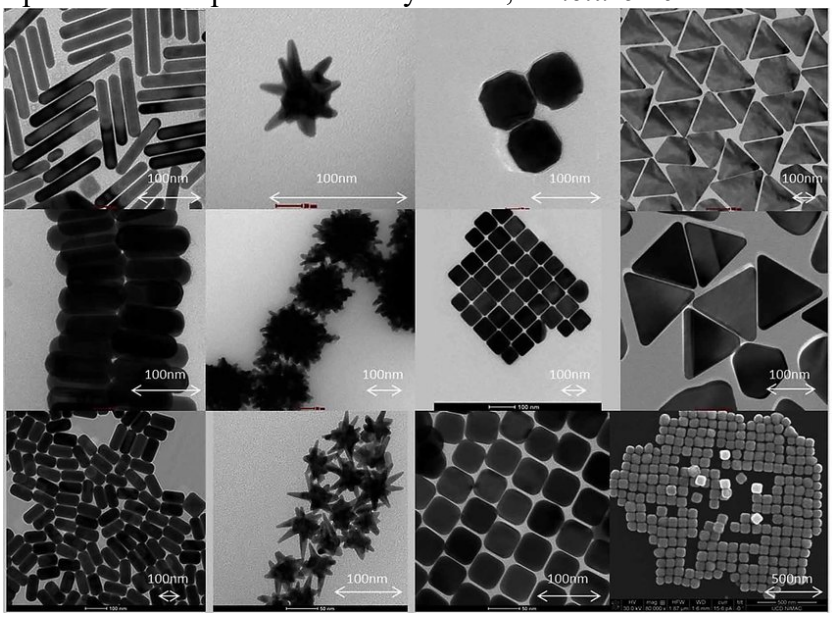
One of the potential antibacterial treatments is the use of nanoparticles since some nanoparticles have shown antibacterial properties and e.g., nanosilver particles have been widely used in a range of biomedical applications already.[51] However, when nanoparticles are considered as an antibacterial treatment, this connection of the environment and other sectors must be considered as well as AMR to nanoparticles should be considered and tested.

2.3 Nanoparticles (NPs)

Nanoparticles are substances with diameter of 1 nm to 100 nm.[52] Nanoparticles have unique properties which the same material in larger scale do not possess, because the unique properties are size-dependent.[53] The nanoparticles have these properties due to two main factors, one being surface effect, the second main factor are quantum effects.[53]
Dispersed nanoparticles have large surface area to volume ratio[53,54], and they have high particle number per unit of mass.[53] Also, the fraction of atoms at the surface in nanoparticles is increased and these atoms at the surface have fewer direct neighbours.[53]
For example, thanks to the lower number of direct neighbour atoms, the binding energy per atom for NPs is lower and this therefore change the melting temperature.[53] The higher surface area to volume ratio makes the NPs more reactive.[53] However, this reactivity can also have negative effects since it can lead to agglomeration of NPs. When the NPs are agglomerated, they lose their unique abilities because the surface area to volume becomes smaller, so it is important to keep the NPs dispersed.
Thanks to all these unique properties, nanoparticles are widely used in different sectors, such as medicine or pharma, electronics, agriculture, food industry[53] or for energy storage.[52]
Some nanoparticles were proven to have antibacterial properties such as silver NPs, which had shown antibacterial efficiency even against MDR strains.[55]
There are different ways how nanoparticles can be classified. They can be classified based on e.g.: their shape, their dimension, or their composition, or based on their physical or chemical properties.[56]

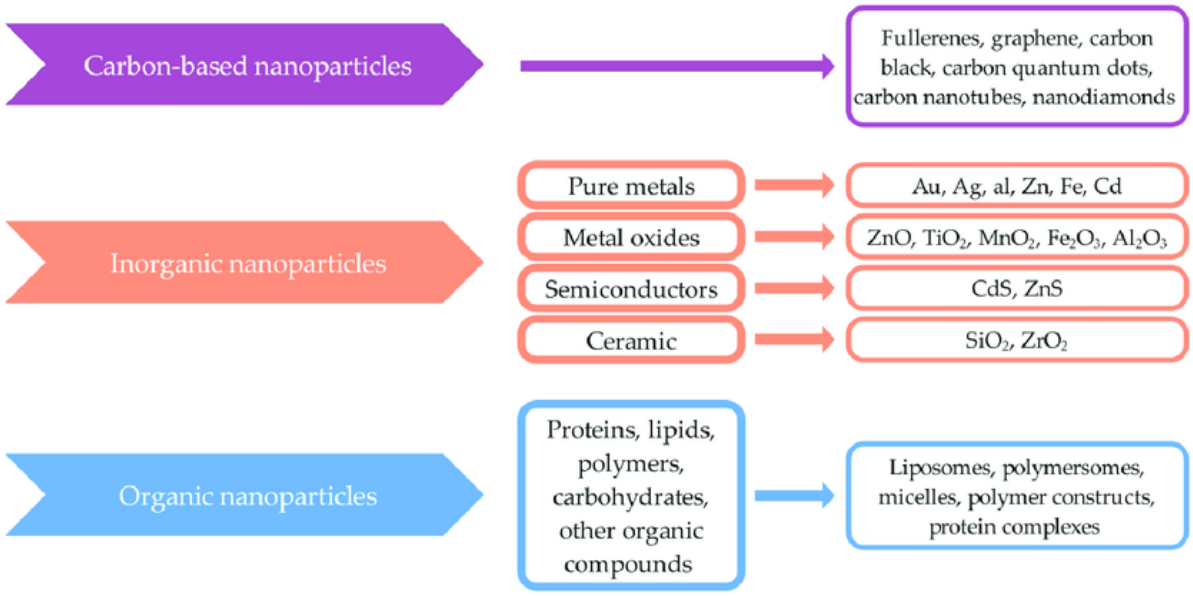
2.3.1 Types of nanoparticles

Based on the shape nanoparticles can be divided into many groups such as: nanotubes, nanorods, nanospheres or nanoplates and many others,[57] *Picture 10*.



Picture 10 *Different shapes of nanoparticles.*[57]

Based the composition, NPs are usually divided into three categories: organic NPs, carbon-based NPs and inorganic NPs, *Picture 11*.



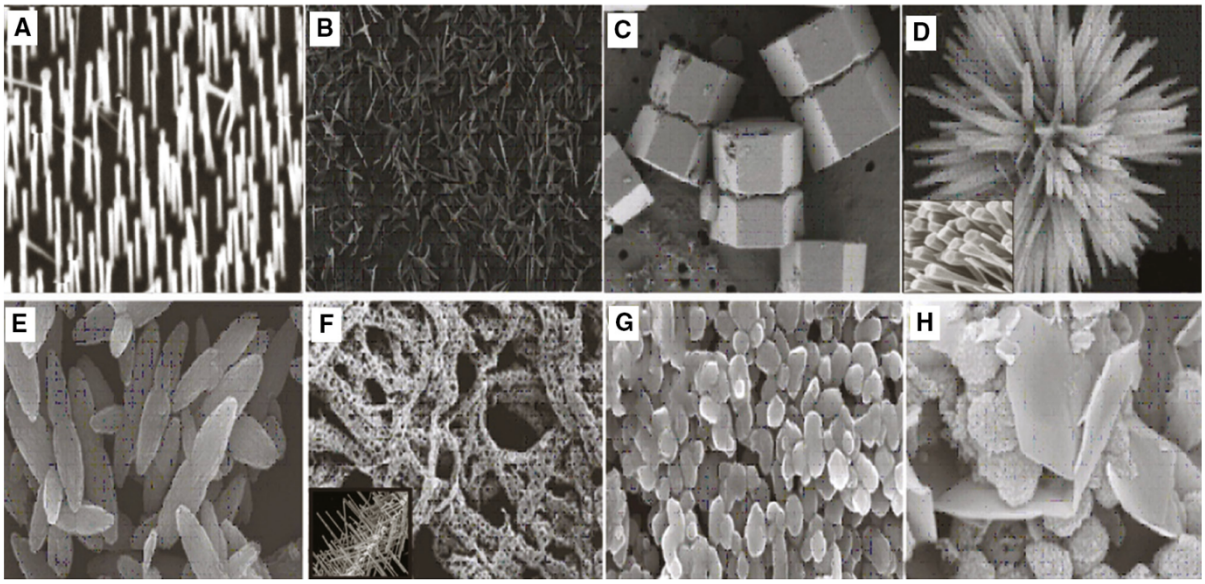
Picture 11 *Summarization of NP classification based on the composition.*[58]

Organic NPs are made of carbohydrates, proteins, lipids or other organic compounds and are primarily used in the biomedical field e.g., for cancer therapy.[53] Organic NPs are synthesized by milling ("Top-Down" Techniques) or by building up of NPs through physicochemical processes ("Bottom-Up" Techniques).[59]
Carbon-based NPs are split into carbon nanotubes and fullerenes, and they are primarily used for structural reinforcement since they are 100 times stronger than steel.[56] Some carbon-based NPs are also used in drug-delivery and tissue engineering application thanks to their low toxicity.[53] The carbon nanotubes are widely synthesized by deposition of carbon precursors especially the atomic carbons, vaporized from graphite by laser or by electric arc on to metal particles.[60]
In the inorganic group can be included metal NPs, ceramics NPs or semiconductor NPs.[53,56,60]
Metal NPs are purely prepared of metal precursors[60] and can be synthesized by chemical, photochemical or electrochemical methods.[56] Most common metal NPs are silver, gold, palladium, titanium, zinc or copper NPs. Metal NPs are used across several fields like in drug-delivery, or for imaging of biomololecules.[56]
Ceramics NPs are made of carbonates, carbides, phosphates and oxides of metals and metalloids like titanium or calcium, they are used in biomedical field thanks to their high stability.[53] Ceramic NPs are usually synthesized via heat and successive cooling.[53]
Semiconductor NPs are made of semiconductor materials with properties between metals and non-metals.[53,56] Semiconductor NPs are used in photocatalysis, electronics devices, nanophototonics and in many other fields.[56] They are synthesized e.g., by mechanochemical-thermal synthesis, or electrophoretic deposition[61], but there are many more ways how to synthesize semiconductor NPs and it very depends on the composition of the NPs. Semiconductor NPs include, among others, ZnO NPs, ZnS NPs, CdS NPs, GaN NPs or GaP NPs.[56]

2.3.2 ZnO NPs

ZnO NPs are inorganic, semiconductor NPs with a size range of 1-100 nm. ZnO NPs are a wide band gap semiconductor.[61]
ZnO NPs are one of the most studied inorganic nanoparticles. Some of their properties are high photostability, high chemical stability or high electrochemical coupling mechanisms.

ZnO NPs are widely used within many different industries such as additive products in ceramics, cement, plastics, glass, lubricants, adhesives but also in cosmetics or sunscreens.[62] ZnO NPs are biocompatible, highly selective[63], generally recognized as safe substances approved by Food and Drug Administration[64] and by many researchers recognized as safe for human cells.[65]
ZnO NPs have shown significant antibacterial properties.[62]
There are several ways how ZnO NPs can be prepared. Conventional methods include hydrothermal method, precipitation method and chemical/vapor method.[62] Biological or Green Synthesis methods include plant-mediated synthesis, microorganisms-mediated synthesis and algae-mediated synthesis.
Physical synthesis methods include arc plasma, physical vapor deposition, ultrasonic irradiation or laser ablation. And non-conventional methods include microfluidic reactor-based method.[62]
The ZnO NPs can then be modified to modify certain characteristics such as size, strength or morphology but many other properties of the NPs can be also modified.[62]
ZnO NPs exist in many different shapes such as nanospheres, nanoplates, nanorods or nanowires[66], *Picture 12*.

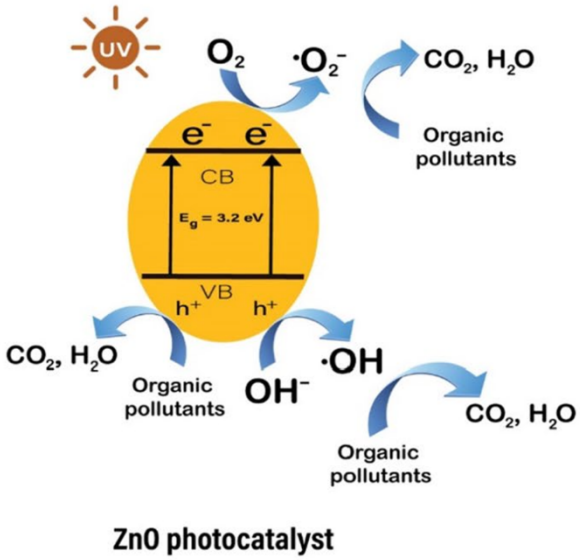


Picture 12 *ZnO NPs morphologies, A: needles, rods, wires, B: helixes, C: nanopellets, D: flower, snowflakes, dandelions, E: peanut-like, F: interwoven particle hierarchy, G: raspberry, nanosheet, H: nanospheres*[66]

Different shapes of ZnO NPs can influence the antibacterial effect of ZnO NPs.[65]

a. Band-gap theory

Since ZnO NPs are semiconductor nanoparticles, their electronic structure consists of conductive band (CB) and valence band (VB).[65] Wide band gap photocatalyst, such as ZnO NPs require ultraviolet (UV) light, wavelengths for UV light range from 100 nm to 400 nm[67], for excitation, so under solar spectrum with only 3-5% of UV light their practicability is restricted.[68] In *Picture 13*, is the graphical description of photocatalysis in ZnO NP.

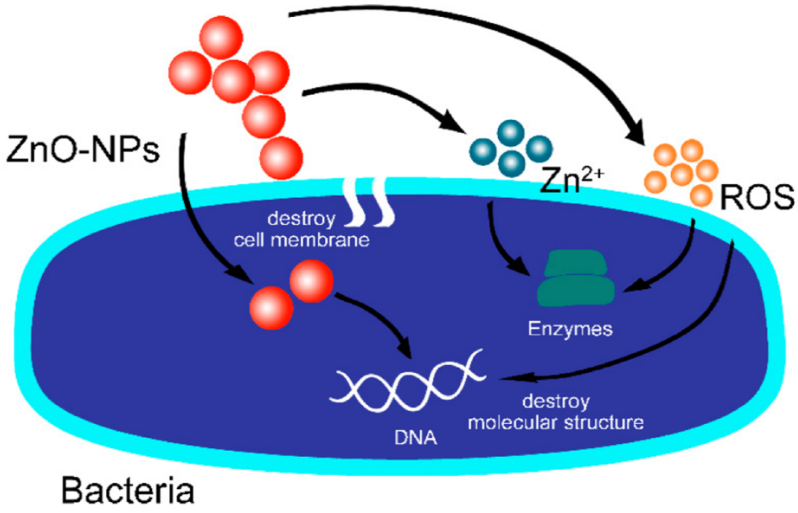


Picture 13 Graphic explanation of photocatalysis in ZnO NPs.[69]

Incident radiation of ZnO NPs with photons of energy higher than 3.3 eV is absorbed immediately and the electrons move from the valence band to the conductive band while creating positive holes in VB and free electrons in CB. The positive holes are direct oxidants and are essential for the creation of hydroxyl radical (˙HO), the most reactive ROS. The electrons in CB reduce oxygen, which is absorbed by the photocatalyst, or it can result in production of hydrogen peroxide (H_2O_2) which can penetrate the membrane causing fatal damage to the cell.[65]
Since ZnO NPs have a wide band gap, the photocatalytic effect can be enhanced by narrowing their band gap by doping the ZnO NPs with other semiconductor materials or metals. These doped ZnO NPs then showed photocatalytic effect even under visible light.[69]

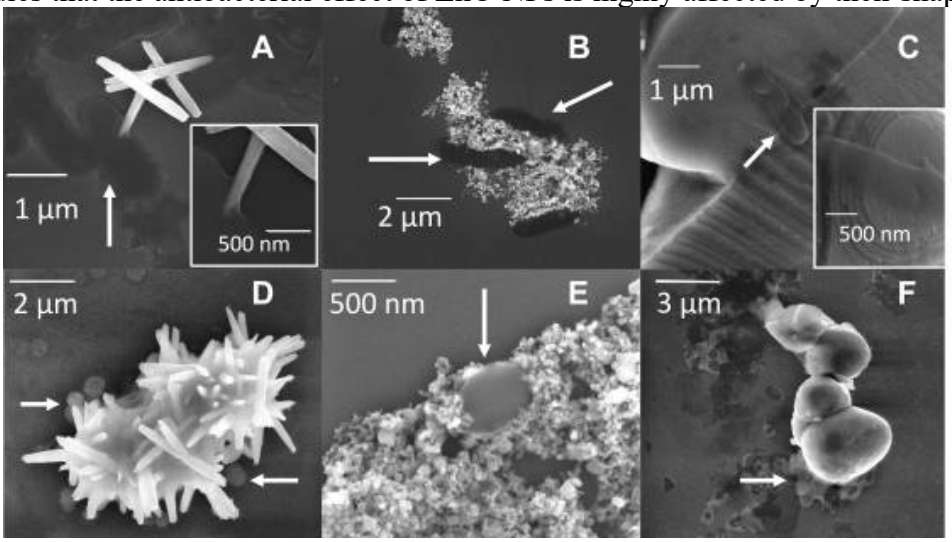
b. Antibacterial mechanisms

ZnO NPs have many mechanisms how to fight against bacteria cells, which are graphically indicated in *Picture 14*. These include the production of ROS, the loss of cellular integrity after contact with ZnO NPs, release of Zn^{2+} and ZnO NPs internalisation.[63]



Picture 14 *ZnO NPs antibacterial mechanisms*[62]

One of the antibacterial mechanisms is the UV illumination effect. Since one of ZnO NPs properties is high photocatalytic efficacy, ZnO NPs can highly absorb UV light. The photoconductivity persists long after the UV light was turned off. The photocatalytic effect results in improvement in conductivity which activates the interaction between ZnO NPs and bacteria cells and therefore results in higher antibacterial effect.[63]
ZnO NPs in watery solution under UV illumination have phototoxic effect that can produce ROS such as hydrogen peroxide (H_2O_2) and superoxide anions (O^{2-}). Some studies have shown that ROS can be created even without light[63], however UV light increases the production of ROS.
The ROS are capable of penetration into the cells and thus killing the cells. ROS can also cause damage inside the bacteria cell, they can damage structural proteins, organelles, DNA or enzymes which again leads to the death of the bacteria cell.[63]
Recent studies shown, that ZnO NPs can produce ROS even under dark conditions if the ZnO NPs had oxygen vacancy defects.[70]
The shape of ZnO NPs is determined by conditions during the synthesis and it was proven by many studies that the antibacterial effect of ZnO NPs is highly affected by their shape.[65]



*P*icture 15 *Growth inhibition of Gram-positive and Gram-negative bacteria study, scanning electron microscopy images, A-C: E. coli bacteria cells, D-E: S. aureus bacteria cells, A and D hedgehog ZnO NPs, B and E nanospheres ZnO NPs, C and F micrograins ZnO*[71]

For example, hedgehog ZnO NPs, Picture *15A* Picture *15D*, shown high antibacterial properties because they were able to pierce the outer cell structure of *E. coli* bacteria causing fatal damage to the cell[71], see *Picture 15A.*
Different shaped nanoparticles have different active facets, e.g., flower-shaped ZnO NPs showed higher antibacterial activity than nanospheres or nanorods. It has also been suggested that the higher number of polar surfaces have higher number of oxygen vacancies which increase the generation of ROS, and therefore have higher antibacterial effect.[65]
Concentration of ZnO NPs and their size are also very important factors of the antibacterial effect of the nanoparticles. The antibacterial effect of ZnO NPs directly correlates with their concentration which was proven by several studies: the higher the ZnO NPs concentration is, the higher the antibacterial effect is.[65]
Size of nanoparticles also plays an important role since smaller NPs can easily penetrate into bacteria cell membranes due to their large specific surface area.[65] It was reported that smaller NPs possess higher antibacterial activity.[65] Decrease in particle size will increase the antibacterial activity of NPs. Smaller sized NPs can accumulate in the bacteria cell until they reach the cytoplasmatic zone which can then lead to death of the bacteria cell.

The dissolution of Zn^{2+} ions was reported to be size dependent as well. When ZnO NPs are in solution, partial dissolution results in the release of Zn^{2+} ions, these ions have antimicrobial activity by decreasing amino acid metabolism and perturbing the enzymatic system.[63]
The dissolution of Zn^{2+} can only happen under acidic conditions e.g., in microorganism's lysosomes, the ions then bind to the biomolecules inside the bacteria cells and therefore inhibit its grow.[63]

3. Materials and Methods

3.1 Materials and Equipment

In this part the preparation of solutions including microbiological culture media which were used are described.

a. Muller-Hinton Broth (MHB) – Sigma

One of media used for bacteria cultivation is Muller-Hinton Broth (MHB). MHB is a liquid medium which is used for bacteria cultivation and also the specified media to use in many antimicrobial susceptibility methods including *Minimum Inhibitory concentration* (MIC) test.[72]

Mueller Hinton broth is a medium recommended by FDA, World Health Organization and NCCLS for testing most commonly encountered aerobic and facultative anaerobic bacteria such as *E. coli*.[72]

The medium show good reproducibility. MHB provides all the nutrients and vitamins necessary for bacterial growth, as well as starch, which is added to absorb any toxic metabolites which may be produced.[72]

Preparation:

1. Dissolve 2.1 g of MHB in 100 mL of distilled water.
2. Sterilize for 15 minutes in an autoclave at 121ºC.
3. Let it cool down.
4. Store in a fridge at 4ºC.

b. Muller-Hinton Agar (MHA) – Sigma

Muller-Hinton Agar (MHA) is a solid media used for bacterial growth in petri dishes. It contains the same ingredients as MHB with the addition of agarose which solidifies the mixture when cooled.

Mueller Hinton agar is a medium recommended by FDA, World Health Organization and NCCLS for testing most commonly encountered aerobic and facultative anaerobic bacteria such as *E. coli*.[73]

The medium shows good reproducibility. It provides all the nutrients and vitamins necessary for bacterial growth. And it can be used for *E.Coli* bacteria.[73]

Preparation:

1. Suspend 3.8 g of MHA in 100 mL of warm distilled water.
2. Dissolve completely.
3. Sterilize in an autoclave for 15 minutes at 121ºC.
4. Pour into 60 mm diameter petri dishes (plates), approximately 10 mL per petri dish and let it cool down.
5. Store in a fridge at 4ºC.

c. High Performance Liquid Chromatography (HPLC) water – P-lab

HPLC grade water is ultra-pure, filtered through membrane filter 0.02 µm.[74]

It was sterilized before use for 15 min at 121 ºC.

d. Physiological Saline Solution (Saline) – Penta

Physiological Saline Solution is used in medical field and other fields such as biology or chemistry when working with live bacteria cells. It is an isotonic solution which is used to

maintain cellular homeostasis and keeps the bacteria cells hydrated.[75] In this diploma thesis a standard concentration of 0.9% NaCl solution was used.

Preparation:

1. Dissolve 4.5 g of sodium chloride (NaCl) in 500 g (500 mL) of distilled water.
2. Sterilize in an autoclave at 121ºC.
3. Let it cool down to room temperature.
4. Can be stored in room temperature, protected from direct light.

e. Nanoparticles used in the illumination experiment

ZnO NPs with catalogue number: R.8278.1, by producer Carl Roth GmbH, delivered by P-lab a.s., were used for the illumination experiment.[76]
The ZnO NPs were grain-like shaped with 25nm diameter.[77]
Link to the safety sheet is provided in *Appendix 11.2.*

f. Bioreactor – RTS-1, BioSan

Bioreactors were used for optical density (OD) measurements and to graphically represent the growth curve. For the preliminary experiment only one bioreactor was needed. For the main diploma experiment, illumination experiment, four bioreactors were used for four bacterial samples.

Picture 16 *RTS-1 Bioreactor from BioSan*[78].

An example of bioreactor used for the experiment is shown in *Picture 16.*

g. *Automatic colony counter – SphereFlash*

Automatic colony counter from SphereFlash was used to count colonies on MHA plates after cultivation.

Picture 17 *Automatic colony counter from SphereFlash*[79].

Picture 17 shows how automatic colony counter from SphereFlash which was used for the experiment looks like.

h. *Light source – KL 2500 LED, Schott*

Light source was used for illuminating two samples during the illumination experiment. The illumination was used to examine the combination of ZnO NPs and illumination. Spectrum of the light source is in *Figure 3*.

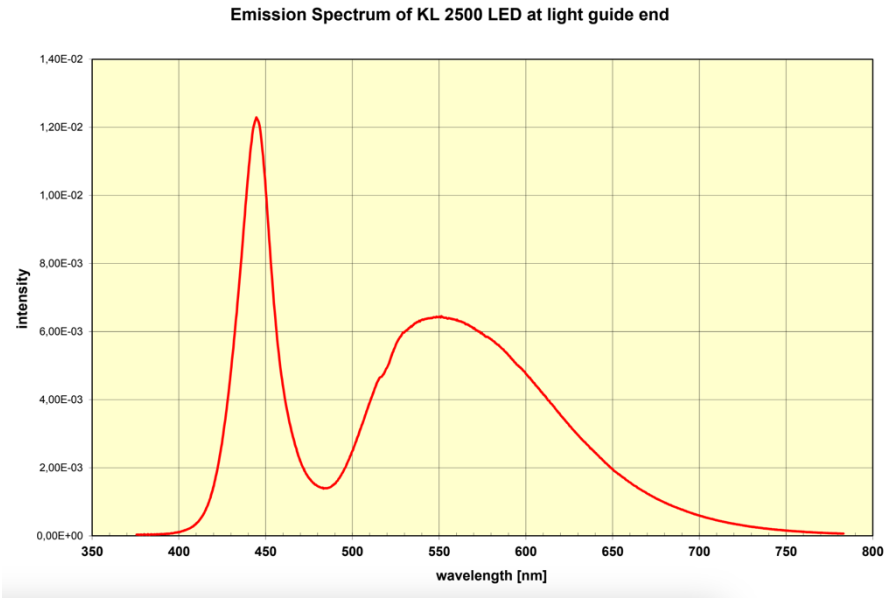
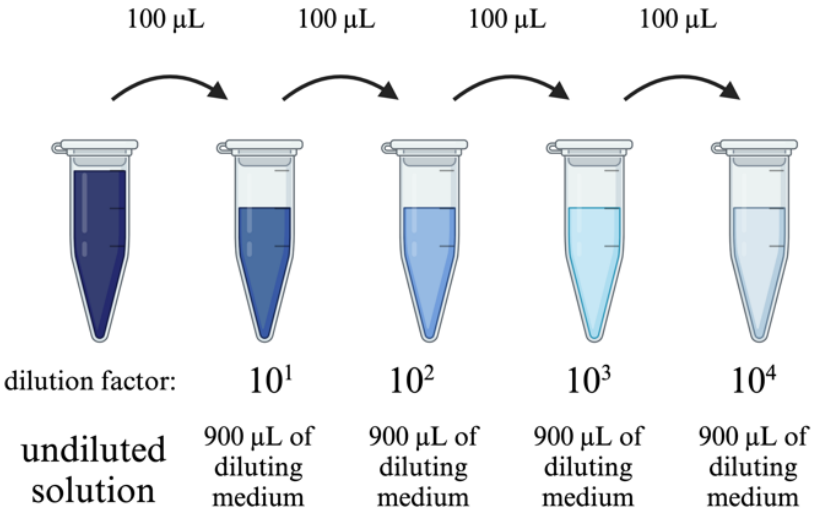


Figure 3 *Spectrum of the light source used in the illumination experiment.*

It is important to note that for the illumination experiment white light source was chosen, because the UV light alone would kill the bacteria cells and would not be suitable for the study that needs bacteria growth.

3.2 Dilution series

Dilution series was done many times throughout all the experiment using either 1.5 mL, 2 mL or 5 mL Eppendorf tubes or glass test tubes in case of larger volumes.
Based on the power of the dilution factor, the same amount of Eppendorf tubes or glass test tubes was used. For example, if wanted dilution factor was 10^{10}, then 10 Eppendorf tubes or glass test tubes were used and 1:10 dilution was performed.
For 1:10 dilution, 90% of the total volume was the diluting medium. Then 10% of the total volume is for the first Eppendorf tube the undiluted solution. Then for all other tubes 10% of the total volume is taken from the previous tube and then is pipetted to the next tube 'in the series'. Each tube is well mixed by hand or by using vortexer (Advanced Vortex Mixer ZX3, Velp Scientifica). An example of 1:10 dilution series is shown in *Picture 18.*



Picture 18 *1:10 dilution series with maximal dilution factor being 10^4 using Eppendorf tubes. Created with Biorender.com.*

In *Picture 18*, the maximal dilution factor of this series of dilutions is 10^4, so four Eppendorf tubes are used, and the total volume of the dilution is 1 mL (i.e., 1000 µL). 900 µL of the diluting medium is pipetted to all four Eppendorf tubes (i.e., 90% of 1000 µL). Then 100 µL is pipetted from the undiluted solution, which can be bacterial stock, ZnO nanoparticles solution etc, into the first Eppendorf tube. The first Eppendorf tube (10^1) is mixed well and then 100 µL from the first tube is pipetted to the second Eppendorf tube (10^2) and the process is repeated for all the tubes until the series of dilutions is complete.
For 1:1 dilution series, all tubes are pipetted with diluting medium of 50% of the total wanted volume. Then 50% of the total volume is the undiluted solution for the first Eppendorf tube, and for all the other tubes 50% of the wanted volume is taken from the previous tube and then is pipetted into the next tube in series.
Example: 1:1 dilution series diluted four times. 500 µL of the diluting medium is pipetted to all four tubes. Then 500 µL of the undiluted solution is pipetted to the first tube, and the solution is mixed well. Then again 500 µL from the fist tube is pipetted to the second tube and this process is again repeated for all four tubes in series.
Volumes can differ, the ratio needs to stay the same.

3.3 Methods

In this section the different methods which was used for the experiments are described, including statistical test that were used to compare the results statistically.

a. McFarland Standard (MF)

McFarland standard is an optical method which was used to standardize the approximate number of bacteria cells in media by measuring the turbidity of the suspension using a densitometer in order to ensure standardized microbial testing. The turbidity, also known as optical density, is approximately equivalent to the density, or concentration, of bacteria cells in media. The approximate bacteria concentration in a suspension with a value of MF 1.0 is $3.0x10^8$ cells.[80] Bacteria cells concentrations for other MF values are in *Table 1.*

For the McFarland standard measurements densitometer (Den1B, BioSan) was used.

Table 1 *McFarland standard*[81]

McFarland Standard	1% $BaCl_2$ (mL)	1% H_2SO_4 (mL)	Approximate Bacterial Suspension / mL
0.5	0.05	9.95	1.5×10^8
1.0	0.10	9.90	3.0×10^8
2.0	0.20	9.80	6.0×10^8
3.0	0.3	9.7	9.0×10^8
4.0	0.4	9.6	1.2×10^9
5.0	0.5	9.5	1.5×10^9
6.0	0.6	9.4	1.8×10^9
7.0	0.7	9.3	2.1×10^9
8.0	0.8	9.2	2.4×10^9
9.0	0.9	9.1	2.7×10^9
10.0	1.0	9.0	3.0×10^9

b. Colony forming units (cfu)

Colony forming unit is unit used in microbiology to determine the number of viable bacteria cells in a sample. To count the cfu, the sample usually has to be diluted, since the sample can be too concentrated and bacteria growth covers the entire surface instead of forming individual, countable, colonies[82,83]

The diluted sample is then added to the surface of MHA plates and cultivated in an incubator. The next day, it is possible to count the grown bacteria cells on the plate and then calculate the total colony forming units, usually per millilitre. For correct computation, dilution factor has to be considered.

For the colony count, automatic colony counter was used. The automatic colony counter counts the number of bacteria cells on the MHA plate and also the total cfu/mL. The settings for the automatic colony counter were sharp illumination method, 58 mm plate diameter for 0.5 mL plates and 85 mm plate diameter for 1 mL plates.

The sharp illumination method is used for colonies with sharp edges. E. coli can have different morphologies, some colonies can have sharp edges, then it is better to use the ‘sharp’ illumination method. Other colonies can have more blurry edges, then the ‘fuzzy’ illumination setting would be more suitable.

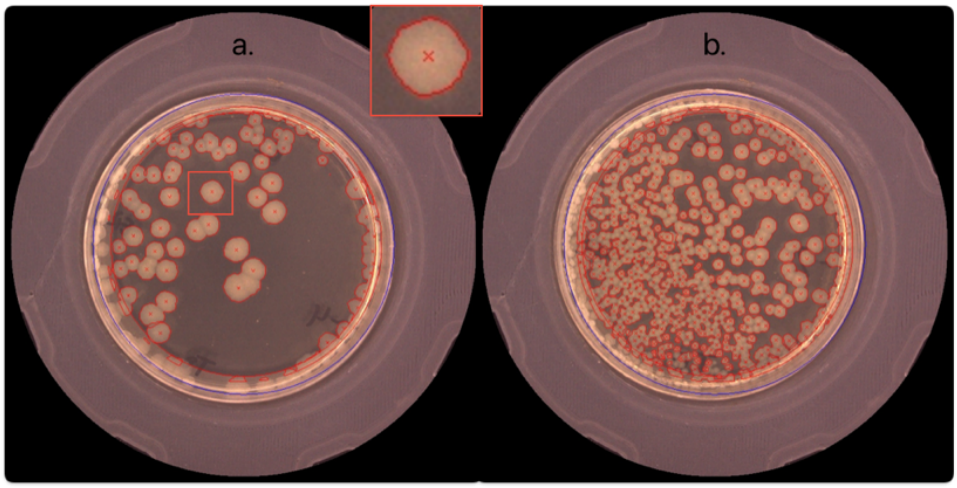
The MHA agar plates used within this experiment had a diameter of 60 mm, 0.5 mL volume plates, and diameter of 90 mm for 1 mL volume plates. But in the colony counter settings 58 mm diameter, or 85 mm, was used, because the edges of the plate can interfere with the image analysis and then the colony count can be incorrect. Also, near the plate's edges contamination is more likely to be present and so colonies near the edges are not considered for the total colony count.
If only 500 µL of bacteria solution was added to 0.5 mL MHA plates, the counts from the automatic colony counter had to be recalculated using equation [1], since values are reported as 'per millilitre' i.e. 1000 µL.

$$\frac{CFU}{mL} = \frac{colony\ count\ \times 2 \times dilution\ factor}{counted\ volume} \qquad [1]$$

In [1], colony count is the count of bacteria cells on the MHA plate, which is counted by the automatic colony counter. In the numerator is also the dilution factor, which describes how much the sample was diluted from the undiluted solution.
In denominator is the counted volume from the colony counter.

$$\frac{CFU}{mL} = \frac{colony\ count\ \times dilution\ factor}{counted\ volume} \qquad [2]$$

For 1 mL MHA plates the calculation was done according to [2].
The counted volume from the automatic colony counter and multiplying colony count by two corrects the final cfu/mL, so the cfu/mL data can be compared even when different volume plates were used.



Picture 19 *Processed pictures from the automatic colony counter, with magnified colony to show how the counter border the colony and create red cross in the middle.*
a. MHA plate with dilution factor 10^4, b. MHA plate with dilution factor 10^2.

In *Picture 19* are two pictures of MHA plates taken and processed by the automatic colony counter. The red circle line marks the area where the colonies are counted, the middle of the colony is circumscribed with red line and the middle is marked with a small x, which is then counted by the colony counter. It can be also observed, that for *Picture 19b.*, the dilution factor is too small, because the bacteria cell concentration on the MHA plate is too high and the colony counter is not able to count the number of colonies correctly, because colony growth overlaps onto adjacent colonies. In *Picture 19a.* the dilution factor is higher, and the colony counter is able count the colonies more accurately.

In *Table 2* is an example of data from the colony counter and recounted concentration.

Table 2 *Example of cfu/mL calculation from colony counter data*

Results Group Name	Plate Id	Counted Colonies	Counted Volume	Dilution Factor	Inoculated Volume	Counted concentration
Count Results	1e+02_EC_t2b	209	0.38471	1.00E+02	0.5	1.09E+05
Count Results	1e+03_EC_t2b	77	0.38471	1.00E+03	0.5	4.00E+05

Plate Id is a description of the MHA plate which is typed manually by the user and usually refers to the MHA plate's dilution factor, time when the dilution was done within the experiment, it can also include bacteria type, if the sample had nanoparticles or not and other features of the sample which are important for the user. Counted colonies is a number of colonies counted by the colony counter. Counted volume relates to the area that the software analyzes for counting colonies. It is automatically calculated by the software from the plate diameter, area of plate to be analyzed, and inoculated volume data. Dilution factor is entered manually by the user and refers to the dilution factor of the sample on the MHA plate and inoculated volume is a volume of sample which was injected on the MHA plate.
The counted concentration in cfu/mL is then the final sample concentration which is computed using equation [1], because 0.5 mL MHA plates were used.

c. Optical density (OD)

Bacteria growth was monitored using bioreactors that measured the change in optical density (OD) in real-time.[78] When the bacteria cells are multiplying the OD is increasing since the multiplying bacteria cells are making the MHB less translucent i.e. more optically dense.

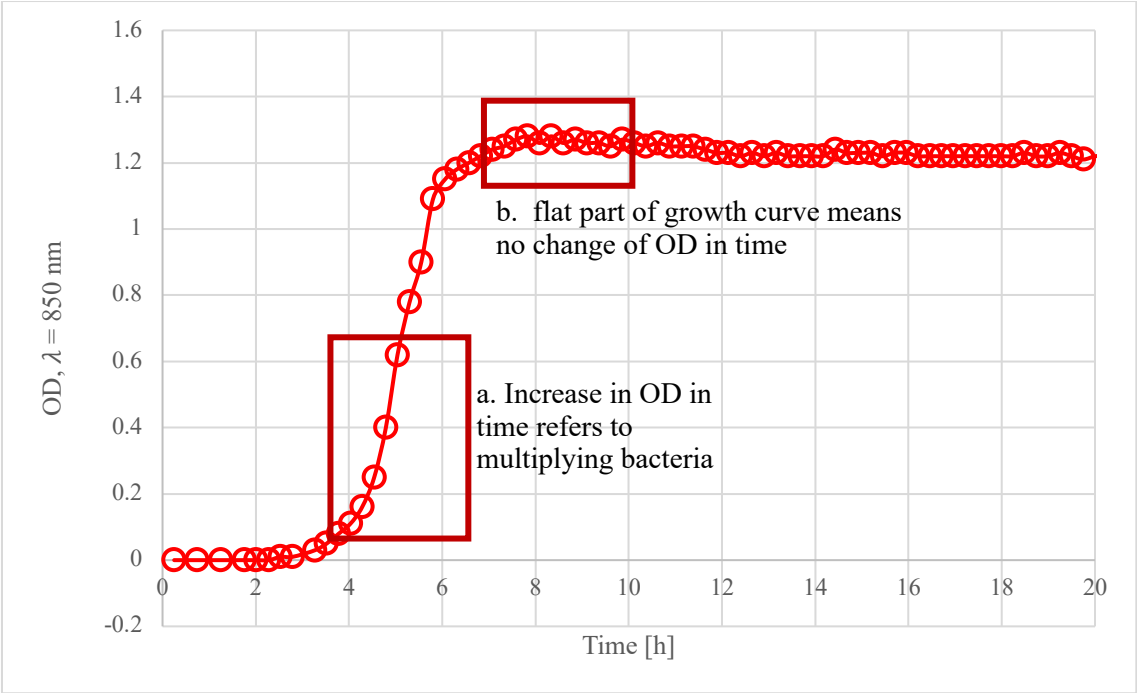


Figure 4 *Example of growing and flat part of growth curve of* E. coli*, which refers to the change of OD in time.*

Bacteria growth curve refers to the change in OD over time due to multiplying bacteria cells, shown in *Figure 4a.* flat OD curve means that the change of OD in time has the same value and so, the bacteria cells are not multiplying shown in *Figure 4b.*

The bioreactors were tested prior to the illumination experiment in order to make sure, that the used lighting does not interfere with the OD measurements by Ing. Bařinková within her diploma thesis experiment.[84] Also, the temperature of the broth was not influenced by continuous illumination and remained constant at the optimal growth temperature of 37 ºC. The OD data were corrected for the charts. The first measured value of OD, which corresponds to the optical density of the MHB, was subtracted from all the values so that the initial value of the OD measurement starts at zero i.e., blank corrected, and the OD values of the different samples can be compared.

d. Growth rate

Growth rate is another parameter which was measured in the bioreactors and used to monitor bacterial growth and development. Growth rate refers to speed at which the number of bacteria cells is increasing.[85] In the bioreactor, the growth rate can be calculated from the difference in OD values per unit of time and a steep rising curve means the bacteria cells are multiplying more quickly, as well as descending curve means that the speed of growth, bacteria cells multiplying, is slowing.

e. Statistics

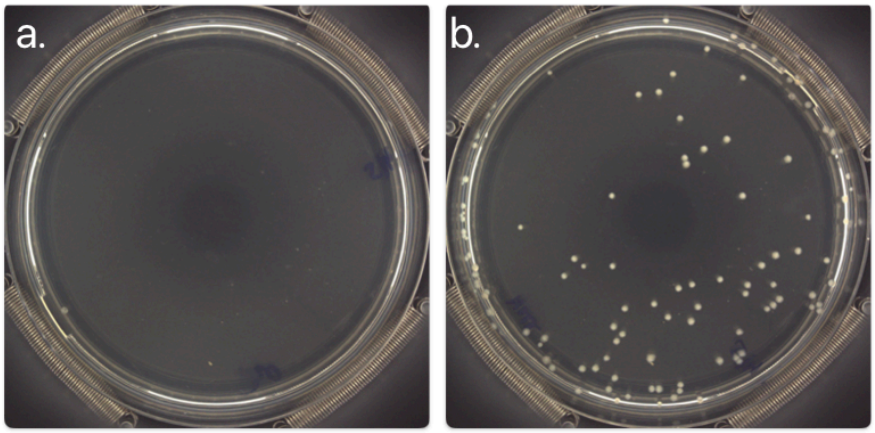
Statistical comparison of results was done in Python. The code is provided in *Appendix 11.5.*
The test which was used to compare the different samples through different weeks was either two-way Anova or three-way Anova, where the tested null hypothesis was that the samples are not different with 5% significance level alpha.
Anova test assumes that the samples are mutually independent, that the samples are normally distributed and also assumes homogeneity of variance.
In order to use the Anova test, all the cfu/mL results were first tested for normal distribution using Shapiro-Wilk test, null hypothesis being that the samples are from normal distribution with 5% significance level.
First, all samples were tested for normality together, and then all the different groups (weeks separately, all ZnO NPs samples, all water samples, all light samples, all no light samples) were tested for normality.
Anova is a robust method which can be used even when the samples are not from normal distribution but, the more groups are from non-normal distribution the higher chance to do type I statistical error (i.e. difference between the groups is found when there is actually no difference – false positive).
The less samples there are, the higher chance to do type II statistical error (i.e. difference between the groups is not found even though there is a difference between the groups – false negative).
All samples used for the statistics were independent.
To test the equal variance, Levene test was used. The Levene test tests null hypothesis that all samples have equal variances with 5% significance level.
The Anova test showed interactions and main effects (e.g. Zinc had p-value<0.05, then there was a main effect between zinc and no zinc samples, if Zinc:Light had p-value<0.05, then the interaction between zinc and light had the effect on the results).
When p-value for the interactions was bellow 0.05, below the level of significance, then post-hoc tests had to be performed, to figure out where the difference comes from. The post-hoc tests were t-tests, and all the different pairs where then tested separately.
For the t-tests the level of significance had to be modified using the Bonferroni correction [3]

$$Bonferroni\ correction = \frac{\alpha}{n} \qquad [3]$$

where α is the level of significance and n is the number of tests.
The statistical results from all the experiments are provided in *Appendix 11.7.*

f. Identification of contamination in microbiological experiments

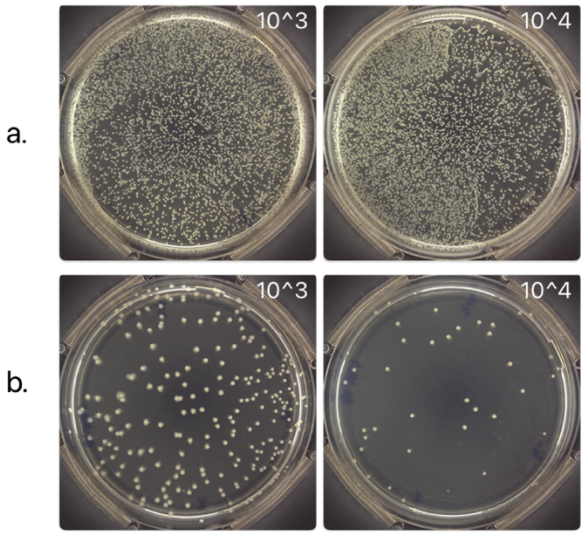
Contamination in microbiology means that there was bacterial growth observed which did not come from the initial inoculum but from some unspecified source. Contamination is quite common in microbial research which is why experimental controls are particularly important. Contamination can come from mistakes in the preparation of the experiment, from unsterilized or poorly sterilized equipment or media, like pipette tips or tubes but very often it is very difficult to locate the source of contamination in microbiological research.
The main problem with the contamination in microbiology is, that it can often be found only the next day. The contamination is usually not visible by human eye and can be found only after cultivating the media or MHA plates, so earliest the contamination would be found the next day.
In the experiments, experimental controls were done by cultivating used media after each use and the next day the media were checked for growth.
Contamination can be also observed on experimental data, for example on MHA plates, i.e. there are too many colonies on a plate, the colonies have different shapes or colours, or when bacterial growth curve is abnormal, i.e. if there is a contamination, the lag phase in the bacterial growth curve can be shorter due to higher concentration of bacteria.
In the Minimum inhibitory concentration (MIC) test, contamination is tested by negative control which do not contain any bacteria, just used media. If negative control in the MIC test grows, it means that there was contamination present in the media, the results should not be used, and the experiment should be repeated.
The same applies to experiments. If contamination is observed, the data should not be used, because the experiment could have been affected by the contamination and the source of the contamination is difficult to track. Therefore, the experiment should be repeated.
Different examples of contamination are described below.



Picture 20 *MHA plates inoculated with media used for experiment, a. MHA plates with 1 mL of HPLC water, b. MHA plate with 1 mL of saline.*

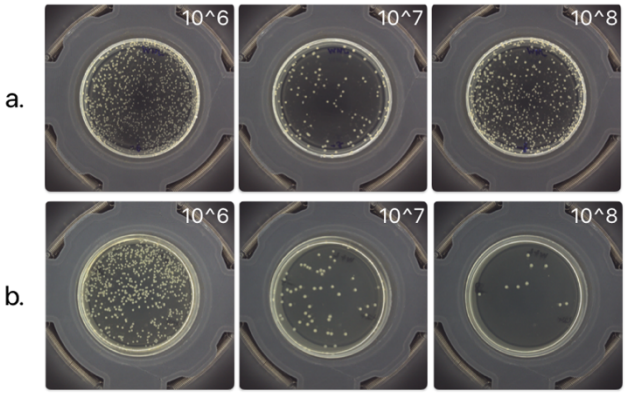
In *Picture 20* are two MHA plates inoculated with media used for experiment and then cultivated overnight. In *Picture 20a.* is MHA plate with 1 mL of HPLC water cultivated in incubator at 37 ºC overnight. There is no bacterial growth on this plate which means the HPLC water used for the experiment was not contaminated.
In *Picture 20b*. is MHA plate with 1 mL of saline cultivated in incubator at 37 ºC overnight. On this plate, bacteria colonies can be observed, so the saline which was used in the

experiment could have been contaminated. Even though growth on MHA plate with saline is observed, the contamination could have come from pipette tip used for the inoculation and therefore the contamination could have been only on this MHA plate. That is why even with proof of contaminated media it is still difficult to track the source of the contamination.
In *Picture 21* are two rows of MHA plates from the beginning of the illumination experiment.



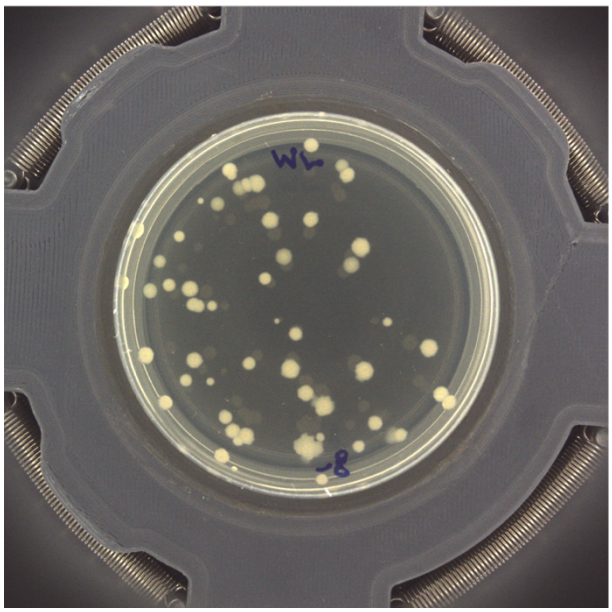
Picture 21 *MHA plates from the beginning of the illumination experiment with dilution factor in the right corner of each plate, a. contaminated samples, b. not contaminated samples.*

In *Picture 21b.* are reference MHA plates. These plates have normal growth for the beginning of the illumination experiment and there is a difference between number of colonies at MHA plate with dilution factor 10^3 when compared to MHA plate with 10^4 dilution factor. MHA plates with higher dilution factor should have less colonies than MHA plates with lower dilution factor.
In *Picture 21a.* are two MHA plates with different dilution factors, but the growth is very high for the beginning of the illumination experiment and also there is not any difference in the growth on the plates with different dilution factors. The fact that there was no reduction in the number of colonies on 10^4 leads to conclusion that MHA plates in *Picture 21a.* are contaminated.
In *Picture 22* are MHA plates from two different weeks of the illumination experiment.



Picture 22 MHA plates with water samples from two weeks of the illumination experiment, in the right corner of each plate is the dilution factor, a. contaminated samples, b. not contaminated samples.

In *Picture 22*a. are MHA plates with three different dilution factors, and the colony count is smaller for plates with higher dilution factor.
In *Picture 22*b. are MHA plates where on a plate with dilution factor 10^8 are more colonies than on MHA plate with dilution factor 10^7. The points again to contamination, as well as the different colony sizes, especially on MHA plate with 10^6 dilution factor. The size difference between colonies is easier to observe in *Picture 23.*



Picture 23 *Contaminated MHA plate from the illumination experiment, contamination is considered due to the different sized colonies.*

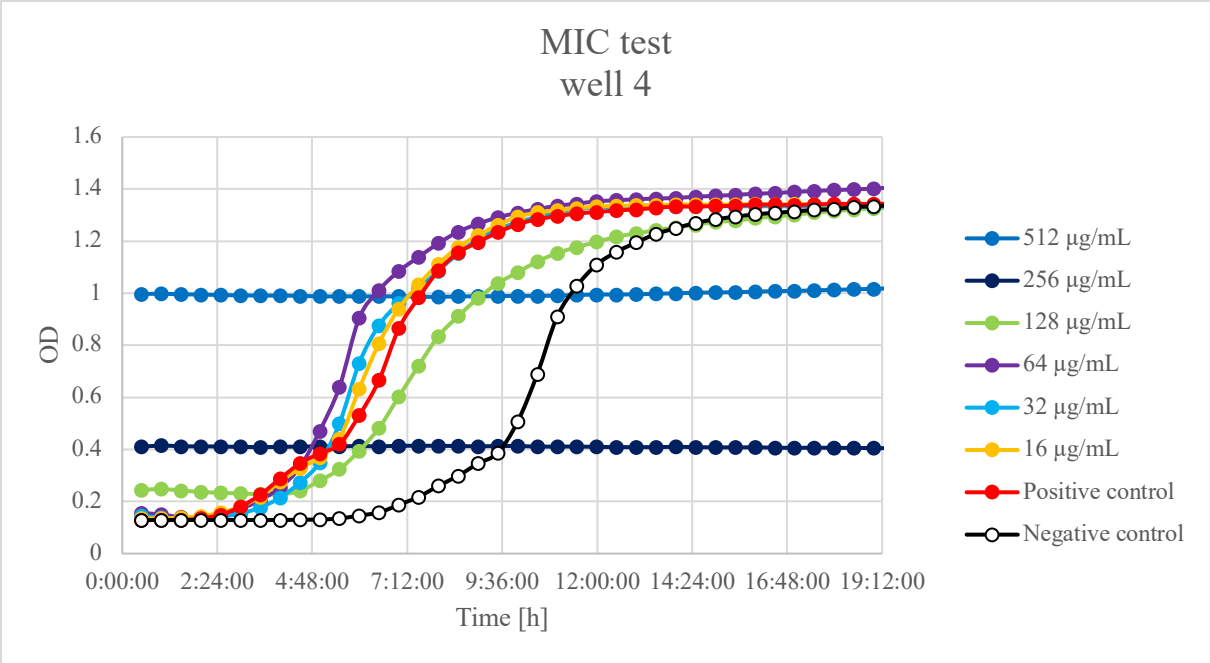


Figure 5 MIC test results from one row of wells where negative control grew, which points to contaminated negative control and therefore contaminated MIC test, names of the data series correspond with the final ZnO NPs concentration in the wells from 4th column.

In *Figure 5* are results from the MIC test, from wells number 4, where growth of negative control was observed which points to a contaminated negative control, contaminated media, and therefore the contaminated MIC test in these wells.

3.4 Bacteria preparation

Escherichia coli (*E. coli ATCC 25922)* were bought from the *Czech Collection of Microorganisms* (CCM, Brno) and used in the experiments. This particular strain of *E. coli* is one of the International standard reference strains for antibacterial susceptibility testing.[86] Main activities of CCM are deposition, preservation and distribution of bacteria cultures. The bacteria came as a lyophilised powder which is then added to 20 mL of MHB and then the bacteria are incubated at 37 ºC overnight on an orbital shaker (PSU-10i, BioSan), 150 rotations per minute (rpm). Then 2 mL of the bacteria in MHB solution is added to sterile brown glass tube containing 1 mL of glycerine to prevent damage from storage at -20 ºC. This frozen solution is then called bacterial stock and a single colony was needed for use in each experiment.

3.4.1 Single colony creation

1. One brown glass tube containing frozen *E. coli* bacterial stock was removed from freezer and let it thaw.
2. 1:10 dilution series with saline was performed, with 10^{10} being the maximal dilution factor in a final volume of 1.5 mL in each tube (i.e. 150 µL : 1350 µL).
3. 500 µL were inoculated from tubes no. 6, 7, 8, 9, 10 onto MHA plates. Therefore the dilution factors of these tubes were 10^6, 10^7, 10^8, 10^9 and 10^{10}.
4. The bacteria suspension were let dry before inverting and adding to an incubator overnight at 37 ºC. (n.b. the plates have to be upside down in the incubator, so the evaporated water does fall from the lid and interfere with the bacterial growth on MHA)
5. MHA plates were stored in the fridge at 4 ºC wrapped in parafilm, and placed bottom up, can be then used within the next 4 weeks.

3.4.2 Single colony cultivation

1. Single colony was removed from MHA plates using tip of a pipette and diluted in 5mL of sterile MHB in 15mL tube.
2. Bacteria were cultivated in the 15mL tube in the incubator (37 ºC) overnight placed on an orbital shaker (PSU-10i, BioSan), 150 rpm.

4. Preliminary Experiment

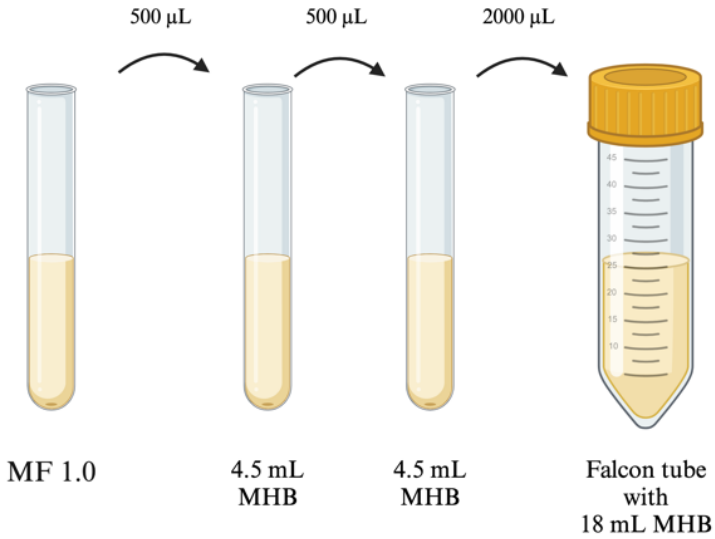
Prior to the main experiment, the preliminary experiment was done to find out the duration of *Lag phase* of *E. coli* in the bioreactor and to determine the concentration of *E. coli* during the *Lag phase*.

The concentration of *Lag phase* cells is impossible to determine by measuring optical density (OD), because the bacterial concentration is so low, that the change in OD is immeasurable in the densitometer and bioreactor. In order to determine the concentration of bacteria cells in the lag phase, it was necessary to perform a series of dilutions at regular time intervals from the start of the experiment (i.e. t = 0h, t0), and add to MHA for colony counting.

4.1 Preliminary experiment method

In this section, the step-by-step method that was followed for preliminary experiment will be provided.

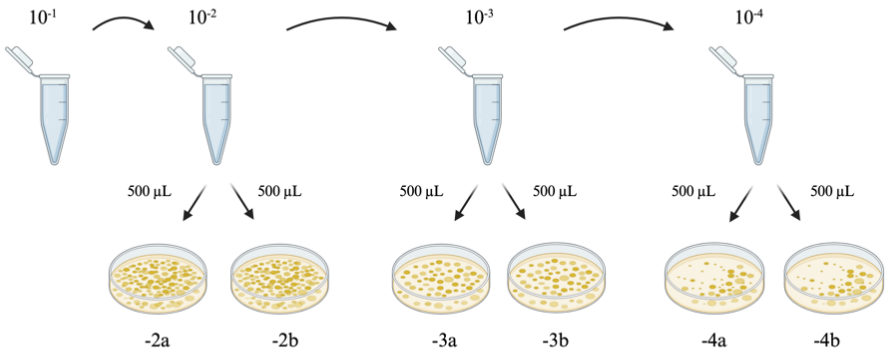
1. Single colony was created as described in *3.4.1.*
2. Single colony was cultivated as described in *3.4.2.*
3. The cultivated bacteria cells were removed from the incubator and then two 1.5mL Eppendorf tubes were filled with 1mL of the cultivated bacteria cells. The Eppendorf tubes were then placed in the centrifuge (MPW 150r) with 13 rpm for 10 minutes in order to separate the bacteria cells from the MHB.
4. The bacteria cells after spinning in the centrifuge should be concentrated at the bottom of the Eppendorf tube forming pellets. The MHB broth was removed from the Eppendorf tube using pipette.
5. The bacteria cells were re-suspended using fresh MHB in glass tube and then adjusted using MHB to MF 1.0.
6. Two 1:10 dilutions using MHB, from the MF 1.0 tube, were performed with the total dilution volume being 5mL, then one more 1:10 dilution with MHB as media was performed but with the total volume being 20 mL, so 50mL Falcon tube was used, represented in *Picture 24.*



Picture 24 *Dilution of MF 1.0 solution to Falcon tube. Created with Biorender.com.*

7. In order to get the initial bacterial concentration in the Falcon tube, also called t0 concentration, 1:10 dilution series with Saline as media was performed with 1500 µL being the total volume (150 µL : 1350 µL) with the maximal dilution factor being 10^4.

8. Then 500 µL from tubes 2, 3 and 4, dilution factors 10^2, 10^3, 10^4, were inoculated to MHA plates in duplicates. Shown in *Picture 25.*



Picture 25 *Dilution series with MHA plates inoculation. Created with Biorender.com.*

9. The Falcon tube was put to Bioreactor with settings: preset – water, 37 ºC, 2000 rpm, 15 minutes measurement interval, 1 second reverse spin, 20mL volume. And every hour steps 7 and 8 were repeated until increase in optical density (OD) was observed. All the MHA plates were marked with the hour when the sample was removed from the Falcon tube in order to then compare the concentrations at every hour and decide when the bacteria in the Falcon tube can be still consider Lag phase cells.
10. The following day, pictures of all MHA plates were taken using the automatic colony counter, in order to correctly count the cfu/mL, using settings and calculations described in *3.3b.*Colony forming units (cfu)

For week 2, week 3 and repeated week 3, only steps 2 to 10 were taken.
In repeated week 3, dilution series was done every half an hour in order to get concentration of the sample more often and only until hour 3 in the bioreactor.

4.2 Preliminary experiment results

The preliminary experiment as described in *4.1* was repeated three times and it took in total four weeks in a row. The third week had to be repeated due to contamination (red box, *Figure 6*). So, the whole preliminary experiment was performed four times.
Due to the contamination only data from week 1, week 2 and repeated week 3 were used. However, data from week 3 are shown as well even thought they were then not used for computations and the lag phase duration decision.

GANTT CHART - PRELIMINARY EXPERIMENT

	week 1					week 2					week 3					repeated week 3				
	day 1	day 2	day 3	day 4	day 5	day 1	day 2	day 3	day 4	day 5	day 1	day 2	day 3	day 4	day 5	day 1	day 2	day 3	day 4	day 5
Single colony creation	■																			
Single colony cultivation in MHB		■				■					■					■				
Pictures of plates				■				■					■					■		
Bioreactor experiment - lag phase			■				■					■					■			
Innoculation to MHA plates from dilution series			■				■					■					■			

Figure 6 *Gantt chart for Preliminary experiment, detailing the tasks that had to be performed on consecutive days, for a minimum of three weeks (i.e., 3 experiments) The red square marks a week were contamination occurred and therefore the data from this week were not used.*

Figure 6 shows Gantt chart which was created to show comprehensibly when which steps were taken.

4.2.1 Increase in OD

One of the measures taken to decide the length of the lag phase for *E. coli* was by measuring optical density in the bioreactor.
Unprocessed data from the bioreactor are provided in *Appendix 11.3.1-1*.
For all the weeks (week 1 to repeated week 3) increase in OD was observed between 2nd and 4th hour of measurement in the bioreactor.
In *Figure 7* are OD measurements for weeks 1 to 3 and for repeated week 3.

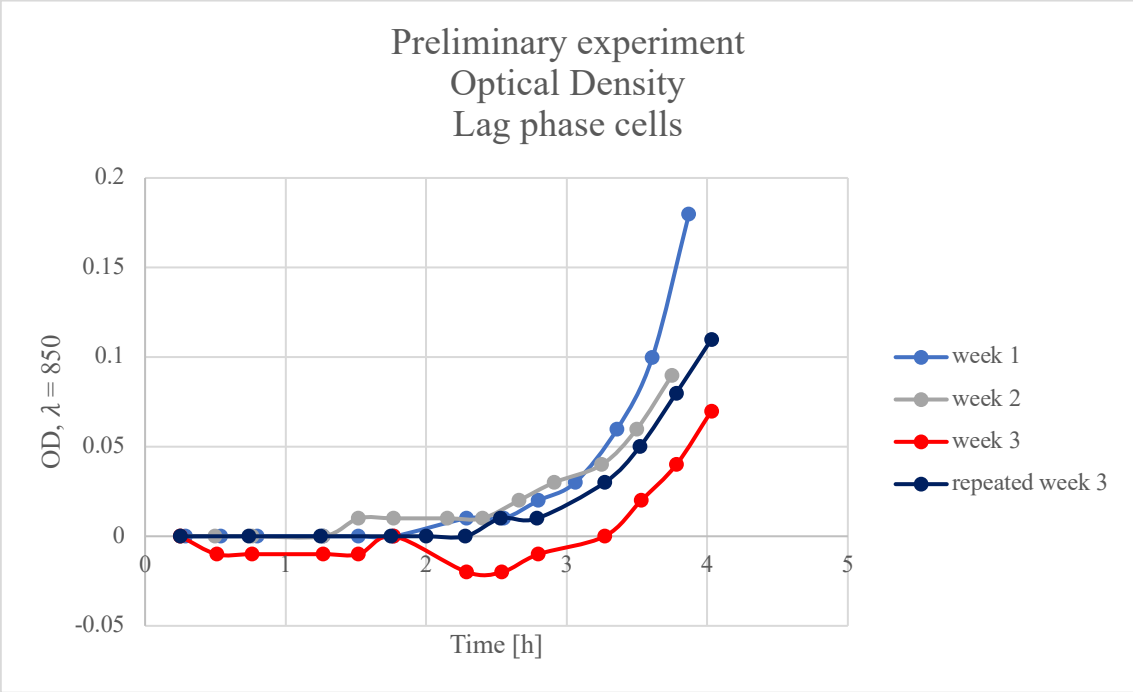


Figure 7 *Optical density measurements from the Preliminary experiment, data measured by the Bioreactor, increase in OD means transition from lag phase to log phase, red-marked is week 3, where contamination occurred and therefore the data from this week were not used.*

Week 3, where contamination was observed is marked with red color.
In the first week, increase in OD was observed in the first measurement after 2 hours and significant increase was observed a little before 4th hour.
Similar results were in week 2, even though the increase before 4th hour was not as significant, slight increase was still observed.
In week 3, around the 4th hour increase in OD can be observed, again increase seems to be not as significant as in week 1.
And in repeated week 3, increase in OD was observed around 3.5 hours, with more significant growth around 4th hour.
From only the OD measurement lag phase duration for E. coli in the bioreactor would be around 2 or 3 hours. After 3 hours in the bioreactor, increase in OD was observed indicating that the bacteria cells moved to log phase and had started to divide exponentially.

4.2.2 Growth rate

Figure 8 shows the growth rate for each week of the experiment. Week 3 is marked with red color because of the possible contamination.
Growth rate is counted by the bioreactor software and it is the change of OD per unit time.

Data from the bioreactor are provided in *Appendix 11.3.1-2.*

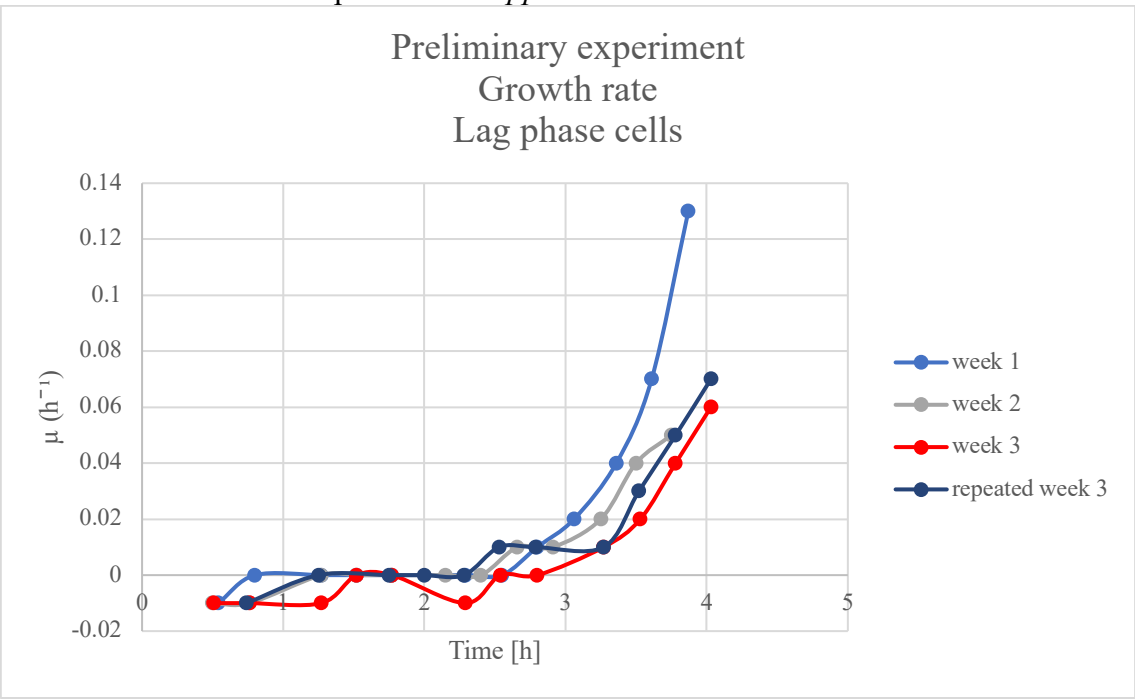


Figure 8 *Growth rate measurements from the Preliminary experiment, growth rate is the change in OD per unit time (i.e., ΔOD/Δt) and was calculated by the Bioreactor software, red-marked is week 3, where contamination occurred and therefore the data from this week were not used.*

For all weeks the growth rate curve is almost flat for the first hour, which indicates that the bacteria cells were not multiplying within the first hour and the speed of growth started to be more significant after 2nd hour. After three hours the growth rate is increasing significantly for all weeks, as shown by the sharp increase in line profiles in *Figure 8*. This indicates that after the 3rd hour the growth rate is rapidly increasing and therefore, the bacteria cells are moving from lag phase to the exponential, log phase.
The growth rate charts indicates that the log phase starts even earlier than from OD charts, where the growth is not as easy to observe. From the growth rate charts the lag phase seems to start between 2nd and 3rd hour.

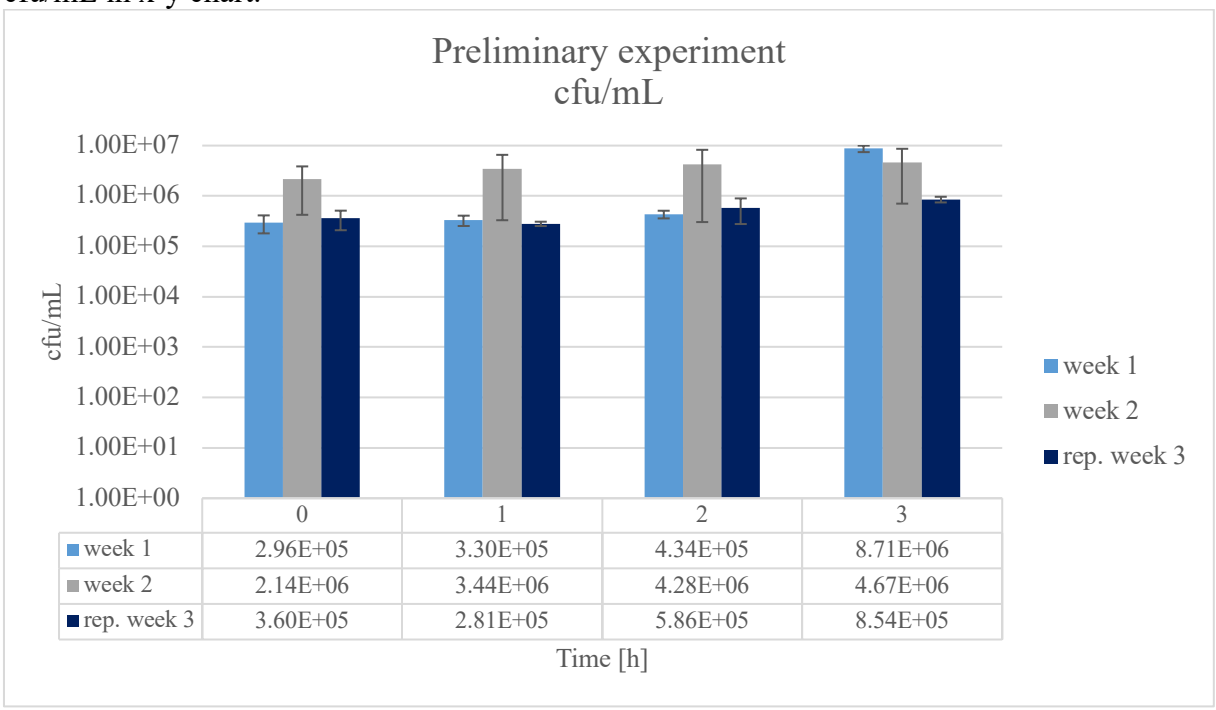
4.2.3 Colony forming units per mL

In this part results from the cfu/mL measurement are discussed. Cfu/mL values from week 1, week 2 and repeated week 3 are evaluated as well as pictures of MHA agar plates from all weeks. As mentioned previously, results from the experiment which had contamination were not included.
Cfu/mL values for week 1, week 2 and repeated week 3 are in *Table 3.*
Unprocessed data from the automatic colony counter are provided in *Appendix 11.3.2-1, Appendix 11.3.2-3, Appendix 11.3.2-2*

Table 3 *cfu/mL results for week 1, week 2 and repeated week 3 for t0, t1 and t2*

time (hours)	**T0**			**T1**			**T2**		
week	**week1**	**week2**	**rep. week3**	**week1**	**week2**	**rep. week3**	**week1**	**week2**	**rep. week3**
cfu/mL	2.34E+05	3.42E+06	5.72E+05	4.16E+05	5.95E+06	2.60E+05	4.00E+05	5.38E+06	5.20E+05
	2.24E+05	3.83E+06	3.64E+05	3.64E+05	6.31E+06	3.12E+05	3.48E+05	9.42E+06	1.04E+06
	4.68E+05	7.76E+05	2.65E+05	2.39E+05	6.78E+05	2.96E+05	5.20E+05	1.36E+06	3.69E+05
	2.60E+05	5.49E+05	2.39E+05	3.02E+05	8.23E+05	2.55E+05	4.68E+05	9.42E+05	4.16E+05
mean (cfu/mL)	**2.96E+05**	**2.14E+06**	**3.60E+05**	**3.30E+05**	**3.44E+06**	**2.81E+05**	**4.34E+05**	**4.28E+06**	**5.86E+05**

Even though the values at t0 differ between the weeks they are still within the range of MF 1.0 after three ten-fold dilutions, meaning the concentration at t0 should be around $3x10^5$ cells which corresponds with the cfu/mL values at t0. Also, in week 2, the concentration is slightly higher than in week 1 and in repeated week 3 but still within the range of one order of magnitude.
From the concentrations there was no growth between t0 and t1 in any of the weeks, but there was an increase in concentration measured from t1 to t2.
In week 1, the concentration increased from 3 $x10^5$ cfu/mL at t1 to 4 $x10^5$ cfu/mL at t2.
In week 2, the concentration increased from 3 $x10^6$ cfu/mL at t1 to 4 $x10^6$ cfu/mL at t2.
In repeated week 3, the concentration increased from $3x10^5$ cfu/mL at t1 to $6x10^5$ cfu/mL at t2.
The concentration growth was not marginal, but it seems that the bacteria cells can be multiplying between t1 and t2, but they are not multiplying or not multiplying significantly between t0 and t1.
Graphic representation of the mean of cfu/mL for week 1, week 2 and repeated week 3 with standard deviation bars is in *Figure 9*.
For better representation of the growth of cfu/mL in time, *Figure 10* shows the mean of cfu/mL in x-y chart.



	0	1	2	3
week 1	2.96E+05	3.30E+05	4.34E+05	8.71E+06
week 2	2.14E+06	3.44E+06	4.28E+06	4.67E+06
rep. week 3	3.60E+05	2.81E+05	5.86E+05	8.54E+05

Figure 9 *bar chart of mean colony forming units per millilitre (cfu/mL) measured from the preliminary experiment with error bars showing standard deviation of the mean (n = 4).*

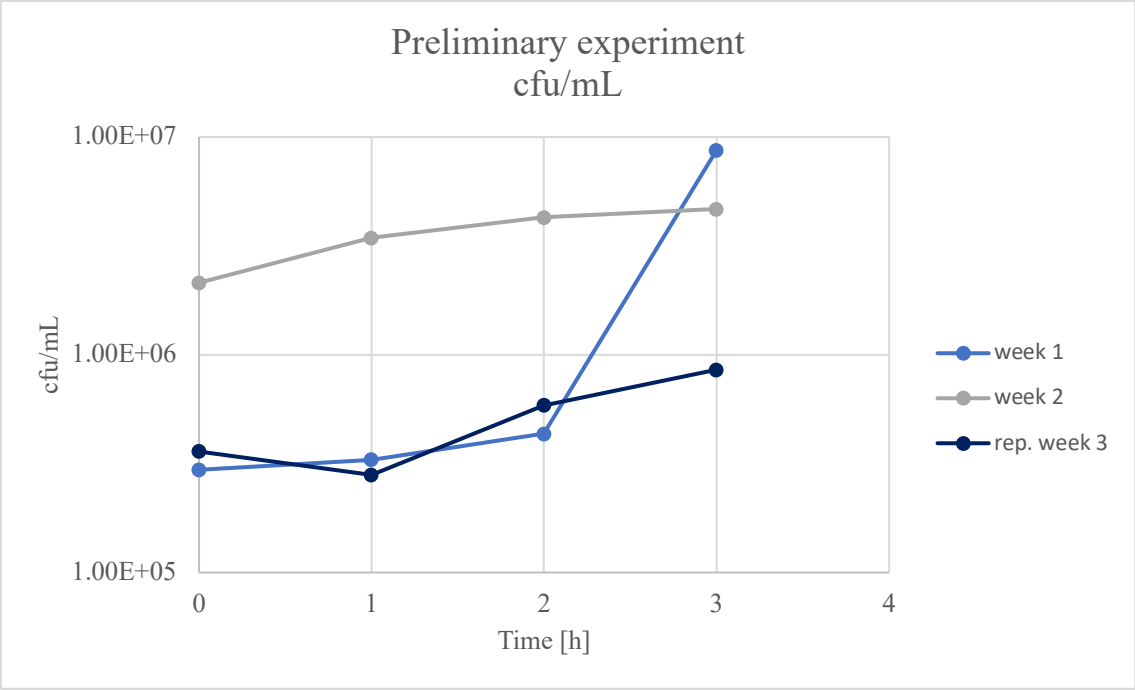
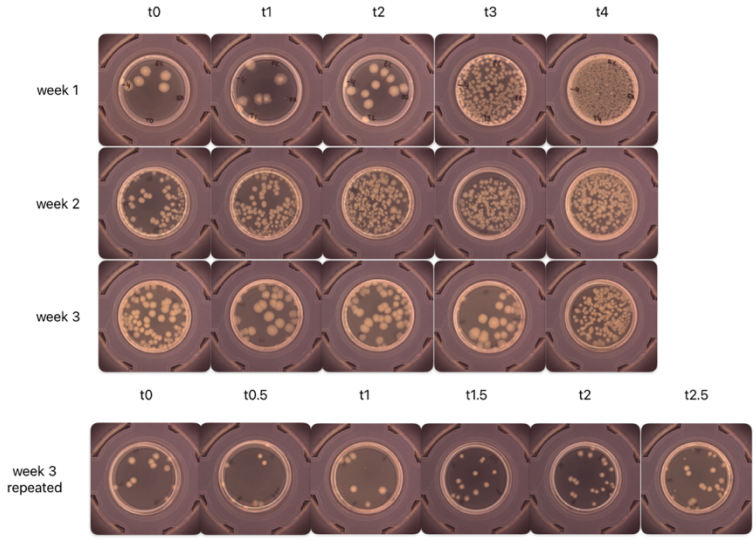


Figure 10 *x-y chart of mean colony forming units per millilitre (cfu/mL) measured from preliminary experiment, for weeks 1, 2 and repeated week 3.*

From these charts it can be observed that the log phase starts after the 3rd hour, since increase in cfu/mL can be observed.
As said before, in repeated week 3, the dilution series of the sample was done more often, every 30 minutes, to get more concentrations of the sample closer to each other time wise.
A composite image showing an example of bacteria growth as colonies on MHA plates from each timepoint and experiment is shown in *Picture 26.*



Picture 26 *MHA plates from preliminary experiment, weeks 1 to 3, with repeated week 3, all samples had the same dilution factor 10^4, t0 refers to the beginning of experiment, while t4 refers to sample taken after 4 hours in the bioreactor.*

For weeks 1 and 2, one can see that there is little difference in the number of colonies on t0 and t1 plates. There is a slight increase from t1 to t2, then a marked increase from t2 to t3.
Week 3 experiment suffered from unusual growth on MHA that was blamed on contamination

therefore the data from this week was not used but still shown in *Picture 26* for completeness. 'Week 3 repeated' used shorter time intervals than week 1 and 2 but follows a similar trend. there is little difference in the number of colonies on t0 and t1 plates. However, an increase in colony number was observed for t1.5.

4.3 Discussion and decision

From OD and growth rate it seemed that the bacteria cells started multiplying around 3rd hour in the bioreactor, so only from OD and growth rate, bacteria until 3rd hour would be considered lag phase cells bacteria, and after 3 hours they would be considered log phase cells bacteria.
But the cfu/mL results were slightly different. The growth was observed earlier in the cfu/mL measurements, earliest between 1st hour and 1.5 hours.
The statistics, two-way Anova test, showed that there was not any statistically significant difference between the cfu/mL values of t0, t1 and t2, the statistical results are provided in the *Appendix 11.7.1.*
However, since the bacteria cells are still growing when removed from the bioreactor and the preparation for the illumination experiment takes approximately 60 minutes, the bacteria cells could later move to the log phase if removed later, around 2 or 3 hours.
For all weeks, the cfu/mL results between t0 and t1 were stable so the bacteria at t1, after 1 hour in the bioreactor, can be considered lag phase cells, since there was not any bacterial growth observed.
This led to a decision to put the bacteria in the bioreactor for 1 hour in order to get lag phase cells of *E. coli* which were needed for the first part of the illumination experiment. The initial concentration of lag phase cells after 1 hour in the bioreactor is still close to the $3x10^5$ cells, which is a concentration of MF 1.0 suspension diluted 1:10 three times. This concentration is important because it will be the initial concentration for the illumination experiment for the log phase cells and the stationary phase cells.

5. Bacteria cells preparation

To study the effect of growth phase it was important to prepare the different growth phase cells using identical process and ensure same initial concentration of bacteria cells for the illumination experiment.

This section provides protocols for the preparation of the bacteria cells from different growth phases. Thus, prepared bacteria cells were then used for the MIC and for the illumination experiment.

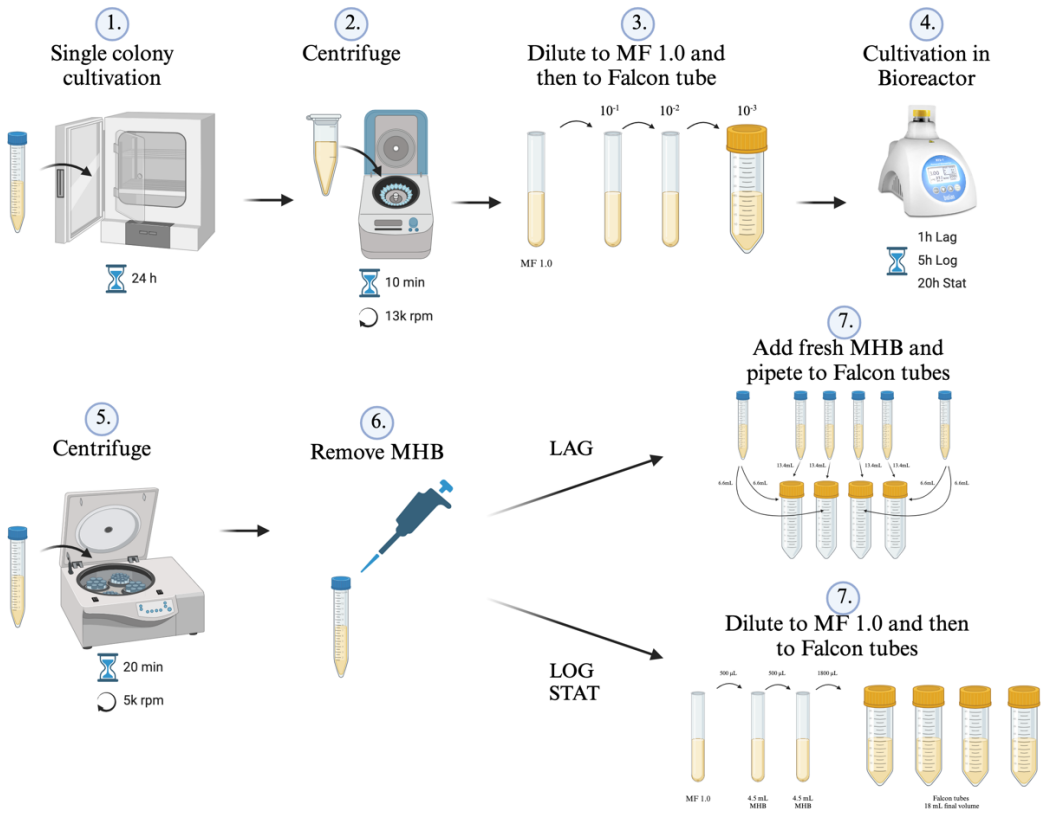
The initial concentration and the process of preparation was set accordingly to the results and methods from the preliminary experiment. The log phase cells, and the stat phase cells could have been diluted, so the initial concentration had to be set based on the initial concentration of the lag phase cells.

The process was first used on the lag phase cells and then repeated for all the other growth phases.

The initial concentration of bacteria for the illumination experiment at t0 was set to $3x10^5$ cells, based on the results from the preliminary experiment.

Each cell phase preparation is described separately with notes about cell phase preparation adjustments for the MIC test.

The summary of the preparation process is represented in *Picture 27.*



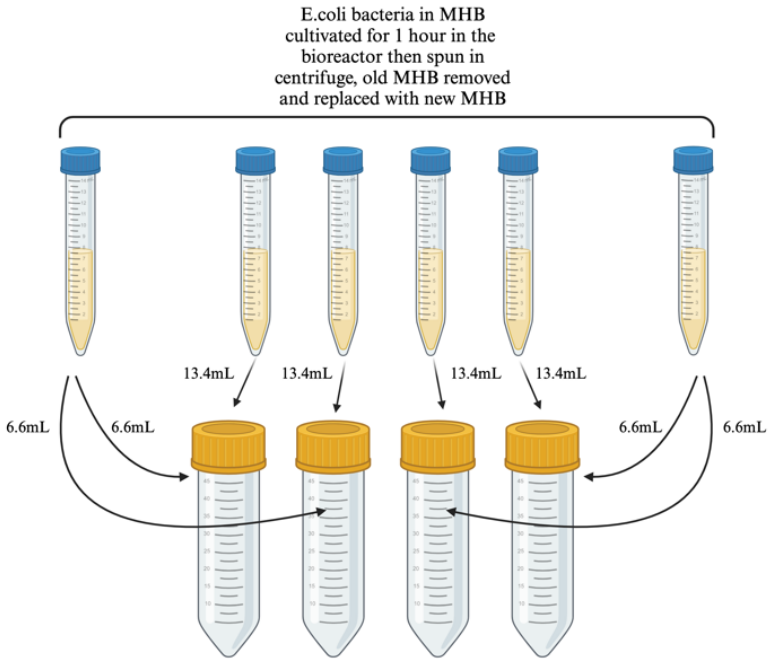
Picture 27 *Bacteria cell phase preparation process. Created with Biorender.com.*

5.1 LAG phase cells

In this part the process of lag phase bacteria preparation for the illumination experiment is described.

Lag cells preparation:

1. Single colony was created as described in *3.4.1* and the MHA agar plates with the colonies were then used for all the experiments with the lag phase cells.
2. Single colony was cultivated as described in *3.4.2*.
3. The cultivated bacteria cells were removed from the incubator and then two 1.5mL Eppendorf tubes were filled with 1mL of the cultivated solution. The Eppendorf tubes were then placed in the centrifuge (Tehtnica Centric 150r) with 13 rpm for 10 minutes in order to separate the bacteria cells from the MHB.
4. The bacteria cells after spinning in the centrifuge were concentrated at the bottom of the Eppendorf tube. The MHB broth was removed from the Eppendorf tube using pipette.
5. The bacteria cells were re-suspended using fresh MHB in glass tube and then adjusted using MHB to MF 1.0.
6. Two 1:10 dilutions using MHB as dilution media, from the MF 1.0 tube, were performed with the total dilution volume of the first dilution being 5mL, the second dilution had the total volume of 10mL.
7. Then four 50mL Falcon tubes were filled with 19.8mL of MHB and then to each Falcon tube 2.2mL from the second tube were added. So, in the end from the MF 1.0 solution, three continuous 1:10 dilutions were performed.
8. The Falcon tubes were then put in the Bioreactors with settings: preset – water, 37 ºC, 2000 rpm, 15 minutes measurement interval, 1 second reverse spin., 22mL volume. And let run for 1 hour.
9. Six 15mL tubes were used and 14mL from the Falcon tubes was pipetted into each of the 15mL tubes.
10. The 15mL tubes were then spun using centrifuge (Tehtnica Centric 150r) for 20 minutes, 5000 rpm.
11. Then 13mL were removed and fresh MHB broth was pipetted to the tubes instead. And the solution was mixed using vortexer. This corresponds to approximate bacteria cell concentration of MF 1.0 solutions diluted 1:10 three times.
12. 13.4mL of the solution from the four 15mL tubes were pipetted to four 50mL Falcon tubes. And then 6.6mL were pipetted to each Falcon tubes from the rest two 15mL tubes, so that in each Falcon tube was 20mL of the lag bacteria solution. The solutions in the Falcon tubes were mixed using vortexer. The distribution is graphically described in *Picture 28.*



Picture 28 *Lag phase cells preparation, distribution to Falcon tubes. Created with Biorender.com.*

13. Then 2mL were removed from each Falcon tube with the total volume in the tube being 18mL.

NOTE:
Preparation for the MIC test, lag phase cells.
For the MIC test, steps 1 to 11 were repeated, with small differences in the preparation process.
In step 6, second dilution had the total volume of 5mL.
In step 7 only one Falcon tube with the total volume of 20mL.
In step 9 only one 15mL tube was used and 10mL from the Falcon tube were pipetted to the 15mL tube.
In step 11, 9mL were removed from the 15mL tube and 9mL of fresh MHB were pipetted to the tube instead.

5.2 LOG phase cells

In this part the process of log phase bacteria cells preparation for the illumination experiment is described. The main difference from the lag phase preparation is in the length of time the bacteria cells has been in the bioreactor in order to get log phase cells. And also, the solution had to be diluted to the correct initial bacteria cell concentration before the illumination experiment, since the concentration of the lag bacteria cells in the solution was higher.
Log cells preparation:

1. Single colony was created as described in *3.4.1* and the MHA agar plates with the colonies were then used for all the experiments with the log phase cells.
2. Single colony was cultivated as described in *3.4.2*.
3. The cultivated bacteria cells were removed from the incubator and then two 1.5mL Eppendorf tubes were filled with 1mL of the cultivated solution. The Eppendorf tubes were then placed in the centrifuge (MPW 150r) with 13 rpm for 10 minutes in order to separate the bacteria cells from the MHB.

4. The bacteria cells after spinning in the centrifuge should be concentrated at the bottom of the Eppendorf tube, forming palates. The MHB broth was removed from the Eppendorf tube using pipette.
5. The bacteria cells were re-suspended using fresh MHB in glass tube and then adjusted using MHB to MF 1.0.
6. Two 1:10 dilutions using MHB as dilution media, from the MF 1.0 tube, were performed with the total dilution volume being 5mL.
7. Then one 50mL Falcon tube were filled with 18mL of MHB and then to each Falcon tube 2mL from the second tube were added. So, in the end from the MF 1.0 solution, three 1:10 dilutions were performed.
8. The Falcon tube was then put in the Bioreactor with settings: preset – water, 37 ºC, 2000 rpm, 15 minutes measurement interval, 1 second reverse spin, 20mL volume. And let run for 5 hours.
9. Two 15mL tubes were used and 9mL from the Falcon tube was pipetted into each of the 15mL tubes.
10. The 15mL tubes were then spun using centrifuge (Tehtnica Centric 150r) with 5000 rpm for 20 minutes.
11. Since the concentration of bacteria cells in the log phase solution was higher, the bacteria cells after spinning in the centrifuge were concentrated at the bottom of the tube. Therefore, the MHB was removed from the 15mL tubes and only the bacteria cells were left.
12. Then the bacteria cells from the 15mL tubes were pipetted into glass tube with fresh MHB and diluted using MHB to reach MF 1.0.
13. Then two 1:10 dilution with MHB were performed, first dilution had the total volume of 5mL the second dilution had the total volume of 10mL.
14. Clean four Falcon tubes were then filled with 16.2mL of fresh MHB broth and then 1.8mL from the second glass tube were pipetted into each Falcon tube being it the third 1:10 dilution. So, the initial concentration in the Falcon tubes of log phase cells should be then around $3x10^5$. It was important that the initial bacteria cells concentration was the same for all experiments. And the total volume in each of all four Falcon tubes was 18mL.

NOTE:
Preparation for the MIC test, log phase cells.
For the MIC test, steps 1 to 13 were repeated, with small differences in the preparation process.
In step 13, three 1:10 dilutions were performed using MHB.

5.3 STAT phase cells

In this part preparation of stat phase cells is described. The stat phase cells had to grow in the bioreactor even longer than log phase cells. The stat phase cells grew in the bioreactor overnight. As well as for the log phase cells, the stat phase cells had to be diluted before the illumination experiment, since the concentration was much higher than the lag phase cells.
Stat cells preparation:

1. Single colony was created as described in *3.4.1* and the MHA agar plates with the colonies were then used for all the experiments with the log phase cells.
2. Single colony was cultivated as described in *3.4.2*.
3. The cultivated bacteria cells were removed from the incubator and then two 1.5mL Eppendorf tubes were filled with 1mL of the cultivated solution. The Eppendorf tubes

were then placed in the centrifuge (MPW 150r) with 13 rpm for 10 minutes in order to separate the bacteria cells from the MHB.

4. The bacteria cells after spinning in the centrifuge should be concentrated at the bottom of the Eppendorf tube, forming palates. The MHB broth was removed from the Eppendorf tube using pipette.
5. The bacteria cells were re-suspended using fresh MHB in glass tube and then adjusted using MHB to MF 1.0.
6. Two 1:10 dilutions using MHB as dilution media, from the MF 1.0 tube, were performed with the total dilution volume being 5mL.
7. Then one 50mL Falcon tube were filled with 18mL of MHB and then to each Falcon tube 2mL from the second tube were added. So, in the end from the MF 1.0 solution, three 1:10 dilutions were performed.
8. The Falcon tube was then put in the Bioreactor with settings: preset – water, 37 ºC, 2000 rpm, 15 minutes measurement interval, 1 second reverse spin, 20mL volume. And let run overnight.
9. Two 15mL tubes were used and 9mL from the Falcon tube was pipetted into each of the 15mL tubes.
10. The 15mL tubes were then spun using centrifuge (Tehtnica Centric 150r) with 5000 rpm for 20 minutes.
11. Since the concentration of bacteria cells in the log phase solution was higher, the bacteria cells after spinning in the centrifuge were concentrated at the bottom of the tube. Therefore, the MHB was removed from the 15mL tubes and only the bacteria cells were left.
12. Then the bacteria cells from the 15mL tubes were pipetted into glass tube with fresh MHB and diluted using MHB to reach MF 1.0.
13. Then two 1:10 dilution with MHB were performed, first dilution had the total volume of 5mL the second dilution had the total volume of 10mL.
14. Clean four Falcon tubes were then filled with 16.2mL of fresh MHB broth and then 1.8mL from the second glass tube were pipetted into each Falcon tube being it the third 1:10 dilution. So, the initial concentration in the Falcon tubes of log phase cells should be then around $3x10^5$. It was important that the initial bacteria cells concentration was the same for all experiments. And the total volume in each of all four Falcon tubes was 18mL.

NOTE:
Preparation for the MIC test, stat phase cells.
For the MIC test, steps 1 to 13 were repeated, with small differences in the preparation process.
In step 13, three 1:10 dilutions were performed using MHB.

6. Minimum inhibitory concentration test

The MIC test was used to decide the concentration of ZnO NPs that would not be fatal for the bacteria cells but would still have antibacterial effect, the MIC test was done in order to find the sub-lethal concentration of ZnO NPs.
ZnO NPs have antibacterial effect in higher concentration so it was important to set the correct concentration of ZnO NPs for the illumination experiment. If the ZnO NPs concentration is too high the antibacterial effect is too dominant and therefore the bacteria will not grow, there will not be any increase in optical density of the solution and therefore there will be no bacterial growth curve to analyse. It is important to note that it was wanted to discover differences between bacteria recovered from different phases of growth to sub-lethal stress, therefore the optimal scenario is to use the highest concentration of ZnO NPs possible that still allows bacteria to grow.
The MIC test was done for all three bacteria cells phases – lag, log and stat.
The test was done for two different bacteria cells concentrations in order to match the initial concentration of the bacteria solution in the illumination experiment.
To also simulate similar conditions as there are in the illumination experiment the ratio between the ZnO NPs and the bacteria MHB solution was maintained. The ratio of MHB with bacteria solution to ZnO NPs solution was 10:1.
There are two control rows in the MIC test, positive control and negative control.
In this MIC test the positive control consisted of 90µL of the bacteria solution and 10µL of HPLC water. The positive control was then used as the base line, reference, for all the other sample which have the ZnO NPs solution added instead of the HPLC water.
The negative control in this MIC test consisted of 90µL of MHB and 10µL of HPLC water. The negative control ensures, that there was no contamination either in the MHB or the HPLC water used for the MIC test, therefore there should be no growth curve in the negative control.
For the MIC tests Microplate reader (Epoch2, BioTek) was used.

6.1 ZnO NPs preparation for MIC test

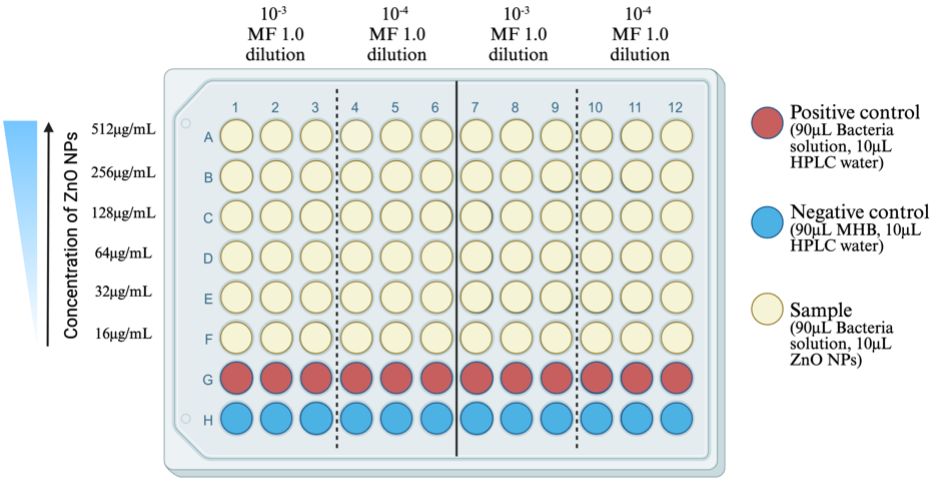
For the MIC experiment higher concentration of ZnO NPs was needed in order to then dilute it and get different ZnO NPs concentration for the MIC test.
In this part the preparation of the ZnO NPs for the MIC test is provided.
ZnO NPs preparation for MIC:

1. First 50 mg of ZnO NPs were weighed using scale (ABT 320-4M, Kern) into 5mL Eppendorf tube.
2. Then 2.5mL of HPLC water were added to the Eppendorf tube with the nanoparticles.
3. The Eppendorf tube was placed in the Ultrasound bath (Bandelin Sonorex Digitec), at 37kHz, 160 W for 5 minutes.
4. Then 2.5mL of HPLC water were added again to the Eppendorf tube.
5. The Eppendorf tube was placed again to the Ultrasound bath with the same setting as in step 3 for 5 minutes.
6. Then 512µL of the mixed ZnO NPs solution was pipetted to a 1.5mL Eppendorf and 488µL of HPLC water were added to the 1.5mL Eppendorf tube. The initial concentration of nanoparticles in this Eppendorf tube was then 5120µg/mL.
7. Then five one-fold dilutions were performed using HPLC water as dilution media with the total volume being 400µL. The concentration of NPs in these dilutions were then 2560µg/mL, 1280µg/mL, 640µg/mL, 320µg/mL and 160µg/mL.

6.2 MIC test protocol

Protocol for the MIC test is described in this section.

1. The bacteria cells were prepared as described in *5.1*, *5.2* and *5.3*, with the adjustments described in the note 'Preparation for the MIC test'.
2. The ZnO NPs were prepared as described in *6.1*.
3. Bacteria solution, which after the bacteria preparation had concentration around $3x10^5$ cfu/mL (MF 1.0 solution diluted 1:10 three times), was diluted 1:10 one more time using MHB with the total volume being 5mL, which then lead to a concentration around $3x10^4$ cfu/mL.
4. For the MIC test, sterile 96-well round bottomed microplate was used. For the negative control 90µL of MHB were pipetted into the first row with 10µL of HPLC water.
5. To all the other wells, 90µL of bacteria solution were pipetted. Two different bacteria cell concentrations were used. In half of the microplate the bacteria solution diluted to 10^{-3} from MF 1.0 solution was used (bacteria concentration around $3x10^5$ cfu/mL). In the second half of the microplate the bacteria solution diluted to 10^{-4} from MF 1.0 solution was used (bacteria concentration $3x10^4$ cfu/mL). This was repeated for the second half of the microplate as well, in order to get more data and more results.
6. Then 10µL of HPLC water was pipetted to each well in the second row, which was the positive control.
8. To the rest of the wells ZnO NPs solutions were pipetted, with the concentration of ZnO NPs increasing in each row. The ZnO NPs concentrations in the wells were then 512µg/mL, 256µg/mL, 128µg/mL, 64µg/mL, 32µg/mL and 16µg/mL. The layout of the wells, positive and negative control and the ZnO NPs concentrations are in *Picture 29*.



Picture 29 *Microplate used for the MIC, with the layout of bacteria and ZnO NPs concentrations. Created with Biorender.com.*

9. From the bacteria solution of 10^{-3} from MF 1.0 solution, dilution series was performed with the maximal volume being 1500µL with saline as the dilution media and the maximal dilution factor was 10^4. Then from 10^2, 10^3 and 10^4 Eppendorf tubes, 500µL were pipetted to MHA plates in duplicates. The MHA plates were then incubated overnight at 37 ºC and the next day pictures of the plates were taken using the automatic colony counter.
10. The 96-well microplate was then put to the microplate reader with settings: 37 ºC, OD measurements at 600nm every 30 min, continuous shaking This protocol was chosen

to simulate similar conditions to the conditions in the bioreactors during the illumination experiment, where the bacteria and the ZnO NPs are mixed throughout the whole experiment.

In *Figure 11* Gantt chart for the MIC test is provided for clearer understanding of the different steps, which were done for the different bacteria cell phases.

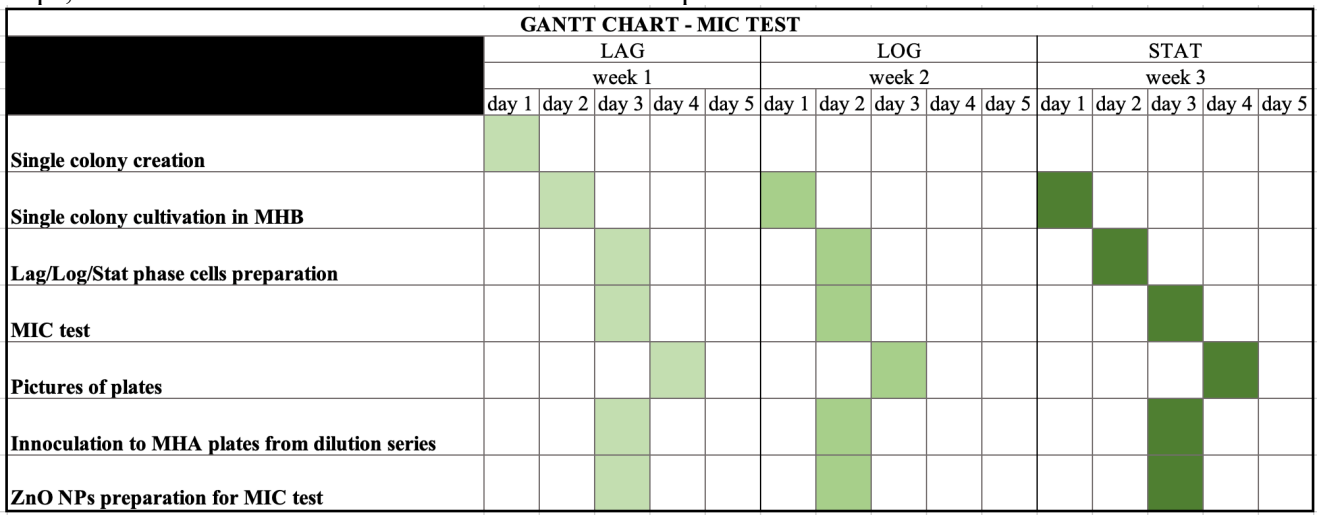


Figure 11 *Gantt chart for the MIC test which was performed for all the different bacteria cell phases (lag, log, stat). MIC test was divided into tasks which had to be performed on consecutive days.*

6.3 MIC test results

The MIC test was done to decide the right concentration of the ZnO NPs for the illumination experiment, which would be sub-lethal for the bacteria cells. The highest concentration which still allowed the bacteria cells to grow was chosen, since it was desired to observe and compare the growth curves of the different samples and different phases bacteria cells in the illumination experiment.

It was also important to treat all bacteria cell from different growth phases with the same concentration of ZnO NPs, to maintain the conditions the same for all the bacteria cells to then compare the treatments and the results as well as the bacteria cells from different phases.

In this section the results from the MIC test are provided with decision about the ZnO NPs concentration which was then used in the illumination experiment.

The data from the MIC test were blank-corrected meaning that the OD value of the negative control, which was just MHB and HPLC water, was subtracted from all the other samples, to show only the bacteria cells OD values and not the MHB OD value within the result curves.

Then, average value was calculated for every triplet of columns, which contained the same bacteria concentration. This average was calculated for every single ZnO NPs concentration as well as for the Positive control samples.

The average values in time were then plot, where x-axis was time in hours and y-axis were blank-corrected OD values.

The Positive control should portray the ideal growth curve since the bacteria cells in the Positive control are untreated and serve as a reference.

For the MIC result charts, wells 1-3 from the microplate were used. The bacteria cell concentration in these wells was around $3x10^5$ cfu/mL, which was the closest to the initial concentration of the illumination experiment. If the negative control grew the results could not be used since it points to the wells being contaminated. The MIC results which were used to draw the MIC charts are provided *Appendix 11.4.*

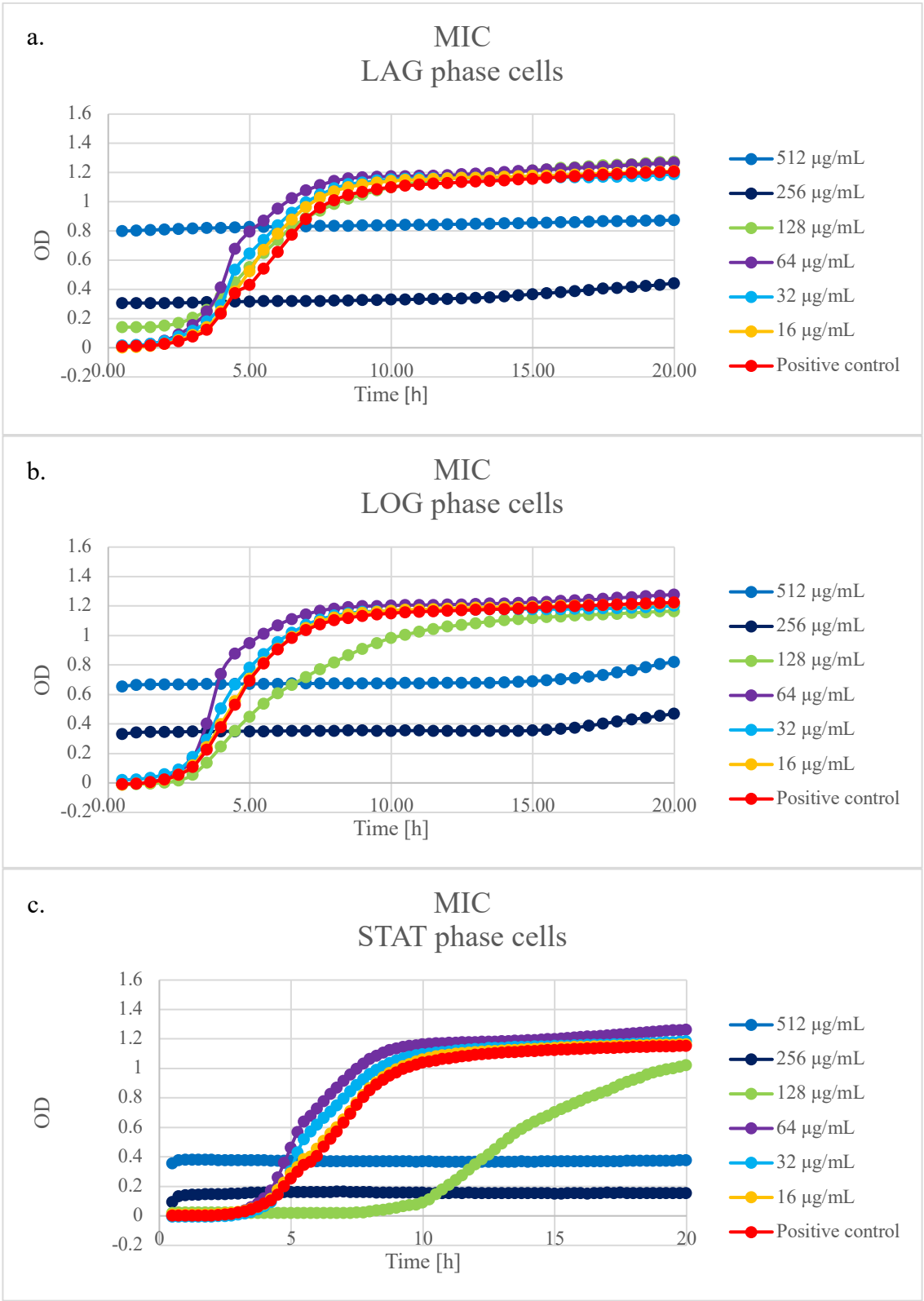


Figure 12 *MIC results growth curves, average values from wells 1-3, where initial bacteria concentration was around $3x10^5$. Marked red is a Positive control which shows reference growth curve, growth curve of untreated bacteria cells. All the other colours then portray growth curves of bacteria treated with different concentration of ZnO NPs. a. shows MIC results for lag phase cells, b. shows MIC results for log phase cells, c. shows MIC results for stat phase cells, for lag and log phase cells the measurements were taken every 30 minutes, for stat phase cells the measurements were taken every 15 minutes. The names of the data series correspond with the final ZnO NPs concentration in the wells.*

From *Figure 12a.*, the lethal concentration of ZnO NPs for the lag phase cells was 256 µg/mL. Lag phase bacteria cells treated with this or higher concentration did not grow. The curve for these highest ZnO NPs concentrations is higher placed because of the optical density of the ZnO NPs solution can be higher. The very concentrated ZnO NPs solutions tend to be white and optically dense because there are lots of nanoparticles diluted in a small volume of HPLC water. High concentrations of ZnO NPs result in increased particle agglomeration and these large agglomerates are heavy and fall out of suspension, leading to increased OD that was not caused by bacteria growth.
For all the other ZnO NPs concentrations treated lag phase bacteria, the growth curves looked very similar. It even seems that some of the lag phase bacteria cells treated with lower concentrations of ZnO NPs started to grow earlier than the reference, positive control, but the difference was very small. Also, the final OD values of lag phase cells treated with lower concentration of ZnO NPs are very similar to the reference final OD value. The sub-lethal concentration of ZnO NPs for the lag phase cells would be 128 µg/mL.
The growth curves from the MIC test of the log phase, *Figure 12b.*, cells are similar to the growth curves of the log phase cells. Also, the lethal concentrations of ZnO NPs for the log phase cells were 256 µg/mL and 512 µg/mL. The log phase cells treated 128 µg/mL ZnO NPs concentration had a little bit lower growth curve and it also seems that these cells started to grow later, but then the final OD value is very similar to the log phase cells treated with lower concentrations of ZnO NPs. For the log phase cells, the sub-lethal concentration of the ZnO NPs would be 128 µg/mL either.
However, from *Figure 12c.*, it seems that the stat phase cells are more sensitive to the ZnO NPs, since the shape of the growth curve of stat phase cells treated with 128 µg/mL concentration of ZnO NPs varied significantly from lower concentrations. Stat phase cells treated with lower concentrations of the ZnO NPs grew very similarly to the reference, but it can be observed that the stat phase cells had longer lag phase length than the lag and log phase cells for the same concentration of ZnO NPs. And even the log phase length for the stat phase cells seems to be longer than for lag and log cells for the same concentration of ZnO NPs.
Even the reference growth curve of the stat phase cells treated with 128 µg/mL had longer lag phase than the lag and log phase cells.
The sub-lethal concentration for the stat phase cells based on the results of the MIC test would be 128 µg/mL as well.
Based on the results from the MIC test it was decided to use the concentration of 100 µg/mL of ZnO NPs in the illumination experiment. This concentration would be sub-lethal for all the growth phases.
However, the stat phase cells grew differently than the lag and log phase cells and it seems that they were also more sensitive to the ZnO NPs treatment considering the MIC results.

7. Methods of the Illumination experiment

The illumination experiment was the main experiment of this diploma thesis. In the illumination experiment the effect of illumination and growth phase on the antibacterial effect of sub-lethal photoactive, ZnO nanoparticles, was studied.
This part provides the methods and process of the illumination experiment.

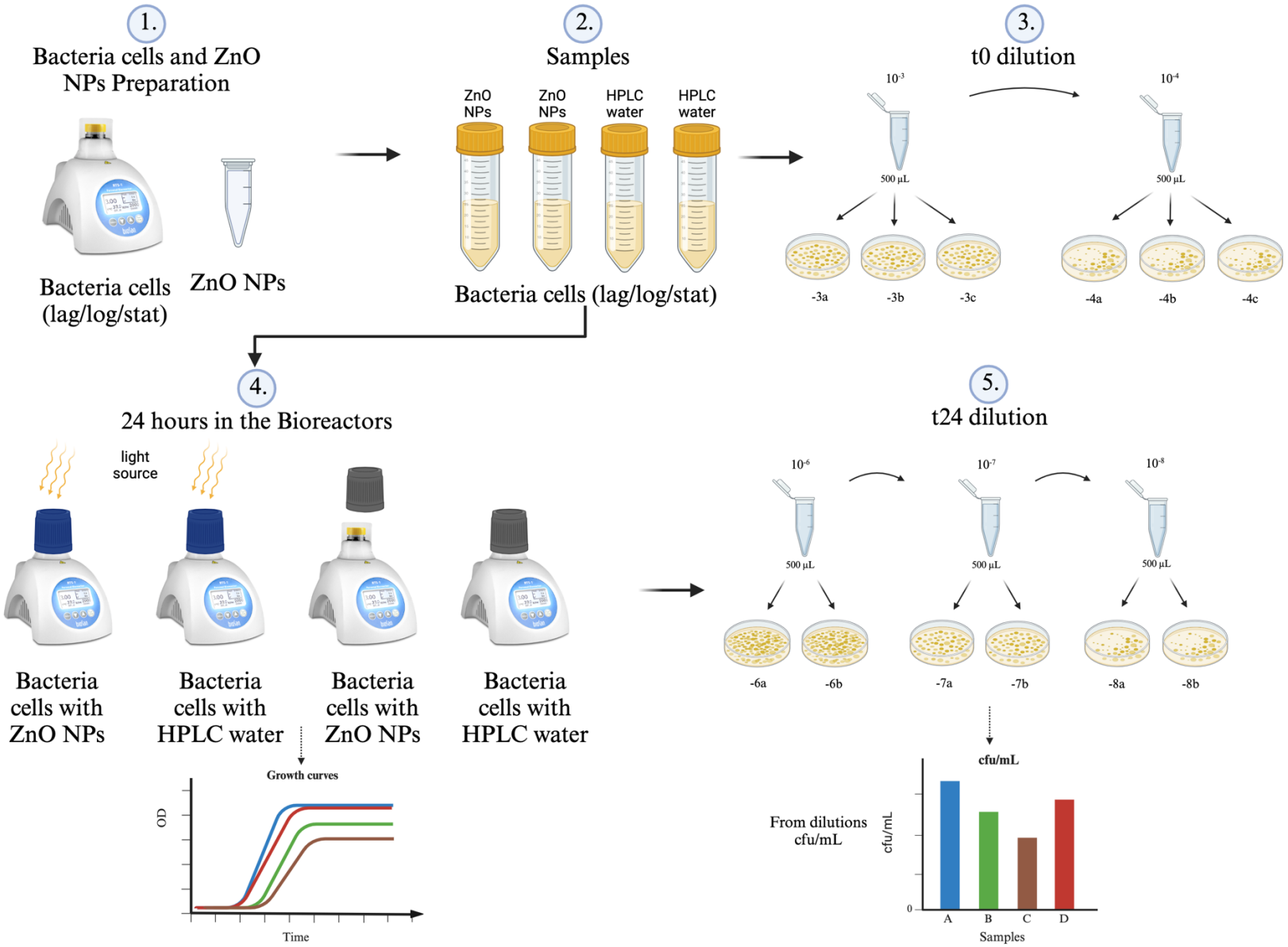
7.1 ZnO Nanoparticles preparation for the illumination experiment

The concentration on ZnO NPs was for the illumination experiment was set according to the results from the MIC experiment.
For the Bioreactor experiment only concentration of 1000µg/mL of the ZnO NPs was needed. Which then resulted in a concentration of 100µg/mL in the Falcon tube, as it was a 1:10 dilution with the MHB and bacteria cells.
ZnO NPs preparation for the illumination experiment:

1. First 5 mg were weighed into 5mL Eppendorf tube.
2. Then 2.5mL of HPLC water were added to the Eppendorf tube with the nanoparticles.
3. The Eppendorf tube was placed in the Ultrasound bath at 37kHz, 160 W for 5 minutes.
4. Then 2.5mL of HPLC water were added again to the Eppendorf tube.
5. The Eppendorf tube was placed again to the Ultrasound bath with the same setting as in step 3 for 5 minutes.
6. This led to a concentration of 1000µg/mL of ZnO NPs in the 5mL Eppendorf tube.

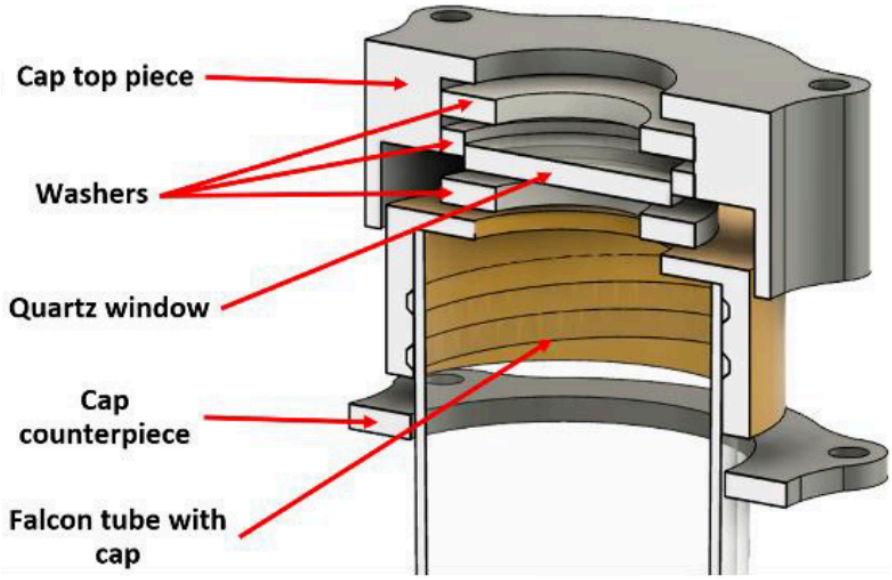
7.2 Illumination experiment protocol

The illumination experiment was the main experiment of this diploma thesis. In this part, the process of the illumination experiment is described.
For the illumination experiment four bioreactors were necessary. Two bacteria samples were mixed with ZnO NPs solution and the other two bacteria samples were mixed with HPLC water. The samples with water were reference samples.
Then one ZnO NPs sample and one water sample were illuminated using light source (KL LED 2500, Schott, 50%) the whole time for 20 hours. And one ZnO NPs sample and one water sample were kept in darkness as reference, in order to see if only the ZnO NPs have antibacterial effect alone, or if even the light alone has antibacterial effect or if the antibacterial effect is higher when the illumination and ZnO NPs are combined.
The process of one repetition of the illumination experiment is graphically described in *Picture 30*.



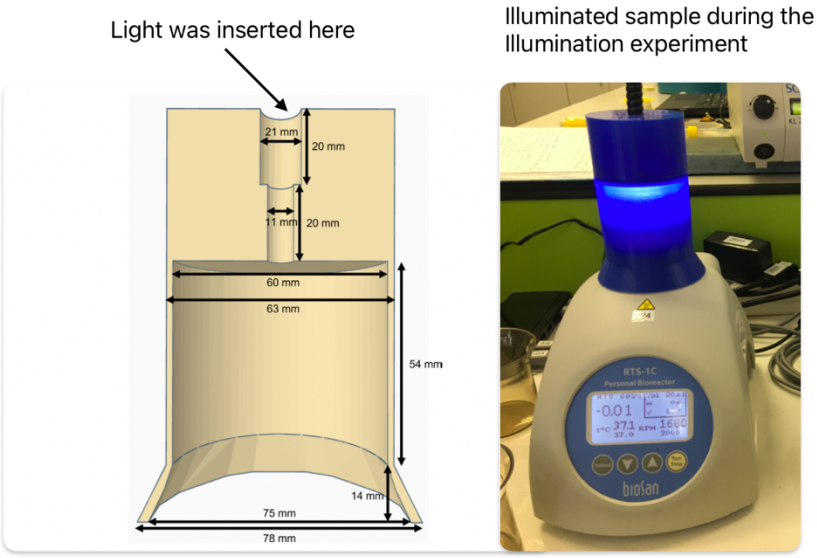
Picture 30 *Process of the illumination experiment graphically described. Created with Biorender.com.*

1. Bacteria cells were prepared as described in *5.1, 5.2, 5.3*.
2. ZnO NPs solution was prepared as described in *7.1*.
3. Then, 2mL of HPLC water were added to two bacteria samples in the Falcon tubes. These samples were marked ‘water’.
4. To the other two bacteria samples in Falcon tubes, 2mL of ZnO NPs solution were pipetted and these samples were marked ‘ZnO’.
5. One sample with water and one sample with ZnO NPs were then marked as ‘light’.
6. One sample with water and one sample with ZnO NPs were then marked as ‘no light’.
7. From one water sample and from one ZnO sample, 1:10 dilution series was performed, with saline as the dilution medium and with the maximal dilution factor of 10^4. The Eppendorf tubes had the maximal volume of 2000µL, and from tubes with dilution factors 10^3 and 10^4, 500µL were inoculated to MHA plates in triplicates. The MHA plates were marked with the dilution factor, with the sample title, either ‘W’ as for water or ‘Z’ as for ZnO NPs and with ‘t0’, as of beginning of the experiment, 0 hours in the bioreactor.
8. The MHA plates were then incubated overnight at 37 ºC. And the plates were captured, and the colonies counted the next day using the automatic colony counter. Then the concentrations, cfu/mL, were counted.
9. The two ‘light’ samples were placed to the bioreactors and special caps were used in order to ensure equality of the light source. The samples which were kept in dark had normal caps which come with the Falcon tubes. The special caps for illumination were designed and tested by Ing. Šlapal Bařinková, and the design is portrayed in *Picture 31*.



Picture 31 *Caps used for the illuminated samples, designed by Ing. Šlapal Bařinková*[87].

10. The ‘no light’ samples were also placed to the bioreactors, but were kept in the darkness using dark, 3D printed covers, which ensured that any light did not get in. In *Picture 32* is a model of a bioreactor cover which was designed by Ing. Šlapal Bařinková and which was used for the illumination samples.



Picture 32 *Model of bioreactor cover used for illuminated samples, designed by Ing. Šlapal Bařinková*[84], *picture adapted.*

11. The bioreactors were then started with settings: preset – water, 37 ºC, 2000 rpm, 15 minutes measurement interval, 1 second reverse spin, 20mL volume. And let run overnight, for approximately 20 hours, to ensure the samples after the experiment were all taken after spending similar time in the bioreactor.
12. To ensure that there was no contamination in the MHB used for the experiment, 1mL of the MHB was pipetted to 1.5mL Eppendorf tube and was put to the incubator overnight and checked for growth the next day.
13. After 24 hours in the bioreactors, from all samples 1:10 dilution series with MHA plates inoculation, where saline was used as the dilution medium, was performed.
14. The final volume in the Eppendorf tubes for the 1:10 dilutions series was 1500µL, the final dilution factor was estimated based on the OD values and the growth curve. Then from tubes the last three tubes in the dilution series, 500µL were inoculated to MHA plates in duplicates. All MHA plates were marked with an abbreviation of the sample (e.g. 'ZL' meaning ZnO NPs with light), dilution factor, and with 't20' to mark the duration the samples were in the bioreactor.
15. The MHA plates were then incubated overnight at 37 ºC.
16. Then, the MHA plates were captured, and the colonies were counted using the automatic colony counter. And the concentrations, cfu/mL, were counted.

To check that there was no contamination in the used media, 0.5mL of saline was pipetted to MHA plate and then cultivated overnight in the incubator at 37 ºC and checked for growth the next day every day of use. Same process of control was done for HPLC water. Contamination in MHB was checked by putting 1mL of MHB into Eppendorf tube and then cultivated overnight in the incubator at 37 ºC and checked for growth the next day.
The illumination experiment was repeated three to four times for each cells phase in order to get more accurate results.
On the next page, in *Figure 13*, Gantt chart for the illumination experiment is provided. In the Gantt chart there are all weeks of the illumination experiment for each bacteria cells phase marked with a schedule of what steps were done each day.

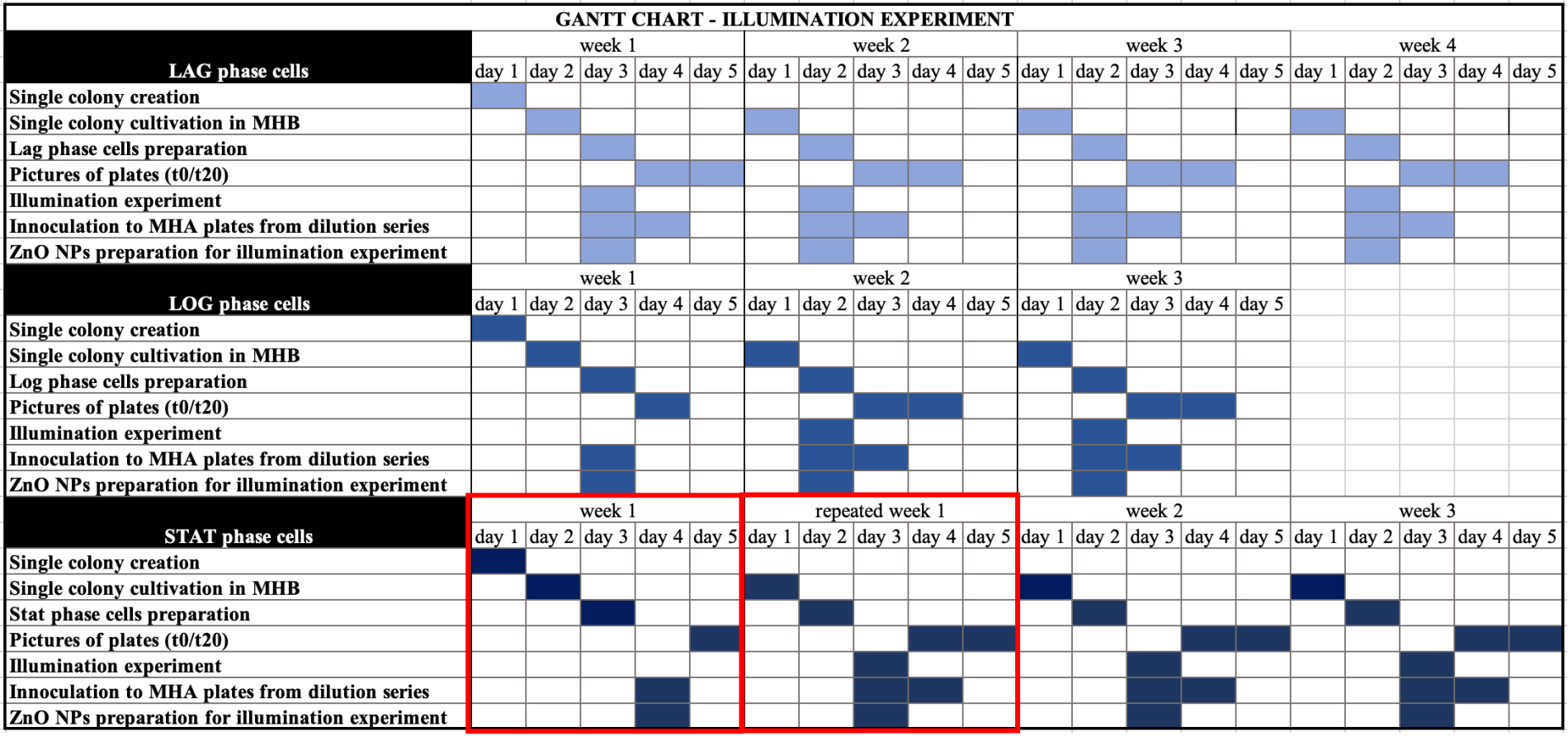


Figure 13 *Gantt chart of the illumination experiment, marked red are weeks where contamination occurred and results from these weeks were not used.*

8. Illumination Experiment Results

In this section, results from the illumination experiment are provided. The results are divided into four sections. First, the results are discussed for each bacteria phase cells separately and then the results are compared in a separate part.

In the charts provided in the sections below, abbreviations were used. *Zinc* samples means samples with ZnO NPs. *Water* samples means samples with HPLC water instead of ZnO NPs. *Zinc+light* means illuminated sample with ZnO NPs, *zinc+no light* means unilluminated sample with ZnO NPs. *Water+light* means illuminated sample with HPLC water instead of ZnO NPs and *water+no light* means unilluminated sample with HPLC water instead of ZnO NPs.

Another abbreviation which was used was for the time, t0 means time 0 hours of the illumination experiment, meaning that the dilutions were taken before the start of the illumination experiment, and t20 means that the dilutions were taken after 20 hours of the illumination experiment, after the samples spent 20 hours in the bioreactors.

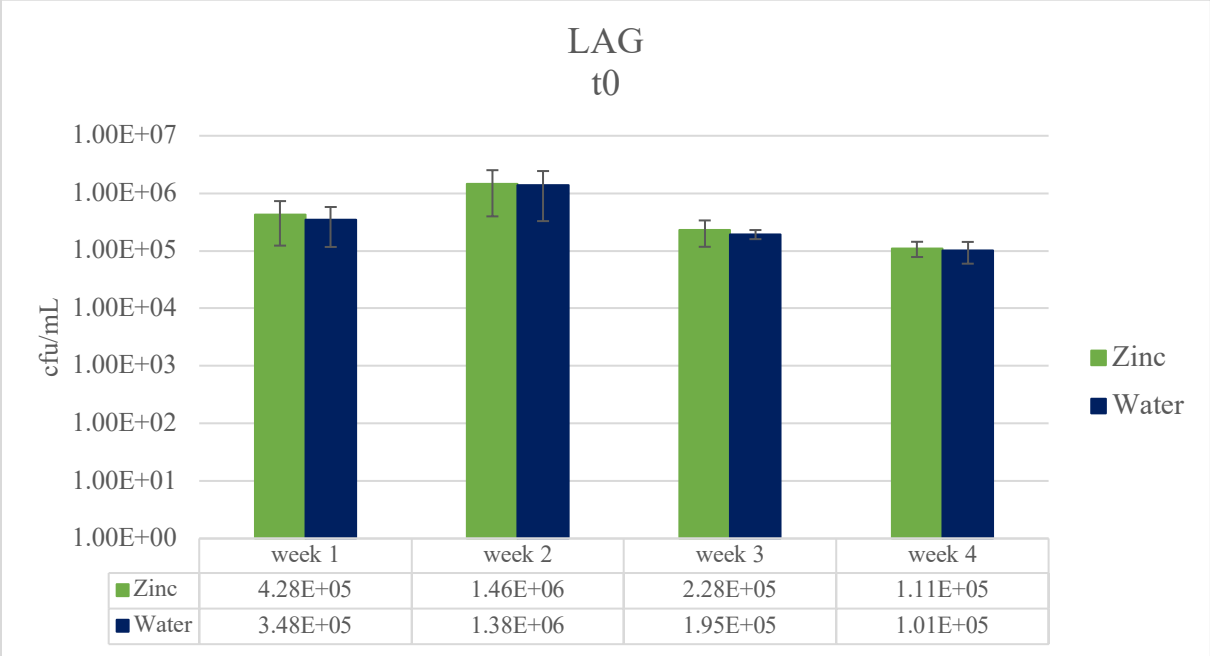
Statistical tests to check if the cfu/mL results for each bacteria cell phase fulfil the assumptions for Anova or t-test were performed. Levene test was used to test the groups for equal variances and Shapiro-wilk test was used to test the groups for normal distribution. The results from these assumptions tests are provided in *Appendix 11.7.2a.*

For all three cell phases, only 6 MHA plates were used for each sample to count the average cfu/mL at t0, and at t20 only 4 MHA plates were used to count cfu/mL, so it was more possible to do II type statistical error.

The four MHA plates at t20 were used because the dilution was estimated based on the final OD values, then 6 MHA plates for each sample were cultivated, duplicates from three dilution factors, but after cultivation usually only 4 MHA plates could have been used to count the cfu/mL results. One dilution factor, therefore two MHA plates, would be either too high or too low and then the results from the automatic colony counter could not been used to count correctly the concentration.

8.1 Lag phase cells

The illumination experiment was repeated four times for lag phase cells and showed good reproducibility. The only struggle with the lag phase cells was with the initial concentration. For the lag phase cells the initial concentration at t0 was fluctuating the most since it was not possible to use the McFarland standard densitometer to adjust and then dilute the bacteria solution. However, the differences were still within one order of magnitude. The concentration of the samples at t0, the initial concentration of the samples before the start of the illumination experiment, is in *Figure 14*.



	week 1	week 2	week 3	week 4
Zinc	4.28E+05	1.46E+06	2.28E+05	1.11E+05
Water	3.48E+05	1.38E+06	1.95E+05	1.01E+05

Figure 14 *bar chart of mean colony forming units per millilitre (cfu/mL) measured from the illumination experiment for lag phase cells at t0 with error bars showing standard deviation of the mean (n = 6).*

Levene test showed that there were not equal variances between weeks for t0 cfu/mL results, and Shapiro-Wilk test showed that a lot of groups did not have normal distribution, so it was more possible to do I type statistical error, for t0 cfu/mL results.
The cfu/mL results at t0 ranged from $1.0x10^5$ to $1.5x10^6$. Even though the concentrations were different between weeks, there was not a significant difference in concentrations between the samples in the same week and therefore the samples at t20 within the same week could have been compared.
This was also observed in the statistics. Since two-way Anova test for t0 cfu/mL results showed difference between weeks, t-tests were then performed, and they showed that only week 1 and week 3 had no difference between the values, but all the other weeks were statistically different at corrected level of significance alpha 0.8%.
All the statistics results are provided in *Appendix 11.7.2b* and *11.7.2c.*
In *Figure 15* are growth curves with blank-corrected data from the bioreactors. The blank-corrected data from the bioreactors are provided in *Appendix 11.5.1.*

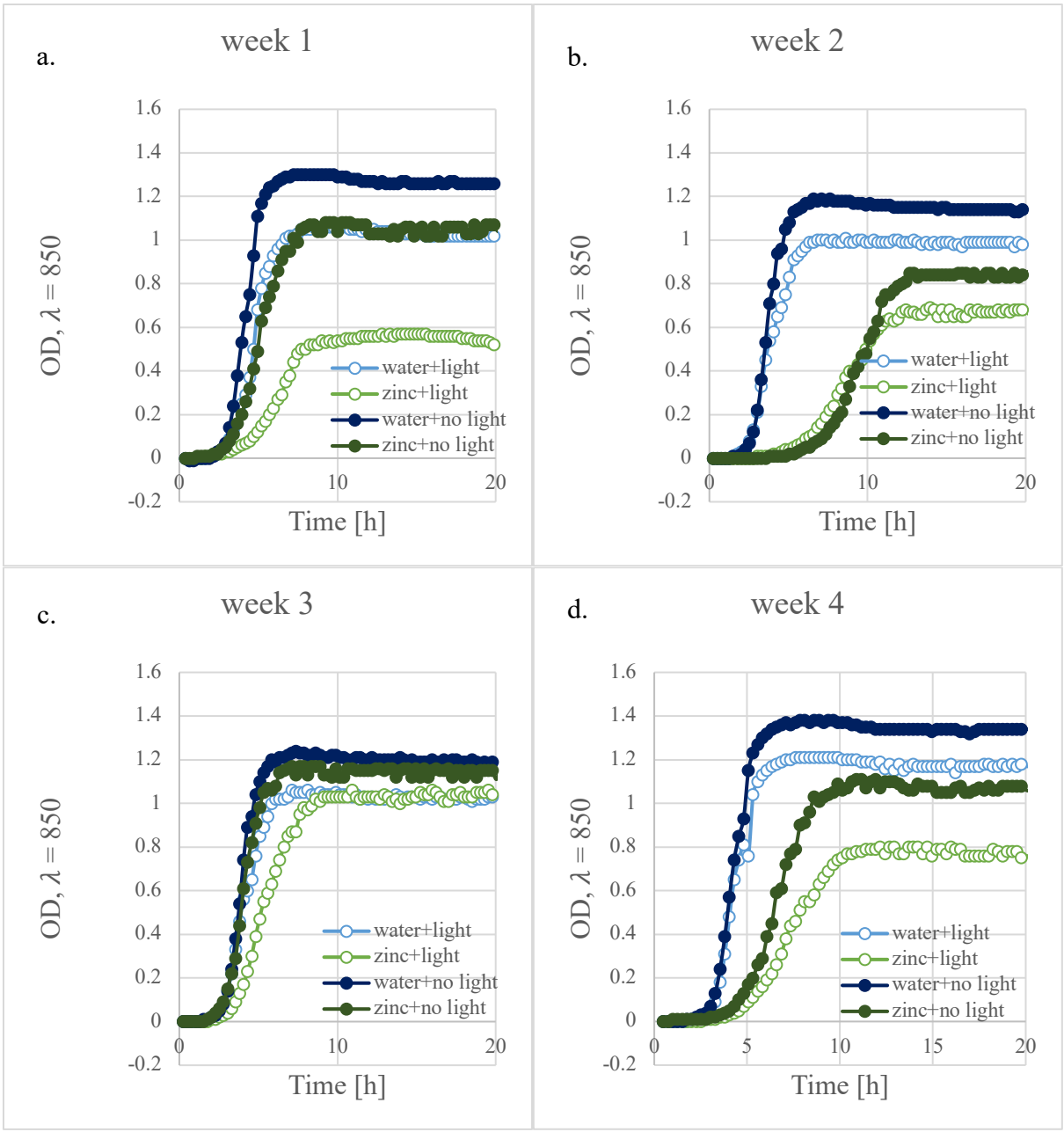
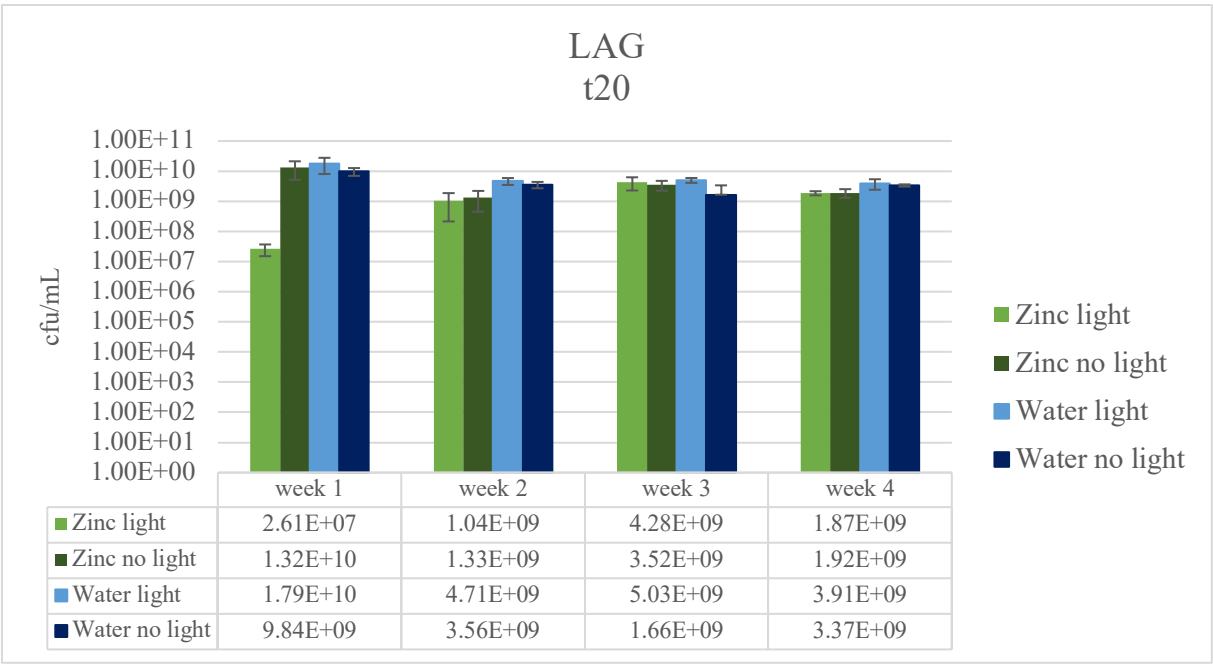


Figure 15 *OD charts from the illumination experiment for the lag phase cells, a. data from week 1, b. data from week 2, c. data from week 3, d. data from week 4. ZnO NPs samples are green and water samples are blue, with illuminated samples having white circle.*

The growth curves show that the *zinc* samples always had longer lag phase, which can be better observed on the *zinc-light* sample, where the longer lag-phase can be easily spotted in all the OD charts.
The cfu/mL results for lag phase cells at t20 are in *Figure 16*.



	week 1	week 2	week 3	week 4
Zinc light	2.61E+07	1.04E+09	4.28E+09	1.87E+09
Zinc no light	1.32E+10	1.33E+09	3.52E+09	1.92E+09
Water light	1.79E+10	4.71E+09	5.03E+09	3.91E+09
Water no light	9.84E+09	3.56E+09	1.66E+09	3.37E+09

Figure 16 *bar chart of mean colony forming units per millilitre (cfu/mL) measured from the illumination experiment for lag phase cells at t20 with error bars showing standard deviation of the mean (n = 4).*

Levene test showed that there were not equal variances between weeks for cfu/mL results at t20 and Shapiro-Wilk test showed that a lot of groups did not have normal distribution, so it was more possible to do I type statistical error, for t20 cfu/mL results. Therefore, statistical results, where the p-values were on the edges of the level of significance were considered carefully.

In all weeks, the *water-no light*, which was a reference sample, had the highest OD value at t20 which would be expected. The lowest OD value at t20 always had the *zinc-light* sample. The biggest differences between weeks had the *zinc-no light* sample, which, only based on OD value, was in week 1, *Figure 15a.*, and in week 3, *Figure 15c.*, very similar to the *water-light* sample. However, in week 2 and in week 4, *Figure 15b.* and *Figure 15d.*, the *zinc-no light* sample was in between the *zinc-light* sample and *water-no light* sample.

When compared to the cfu/mL at t0, it does not seem that the lower OD values for *zinc-no light* samples in weeks 2 and 4 were based on lower bacteria concentration for the *zinc* samples at t0, since in week 2 and in week 4, in *Figure 14*, the cfu/mL values were very different. Week 2 had the highest t0 bacteria concentration, while week 4 had the lowest bacteria concentration at t0 for both *zinc* and *water* samples.

Interestingly, the *water-light* samples always had higher cfu/mL values at t20, than the *water-no light* sample, even though the *water-no light* sample had higher final OD value for all weeks.

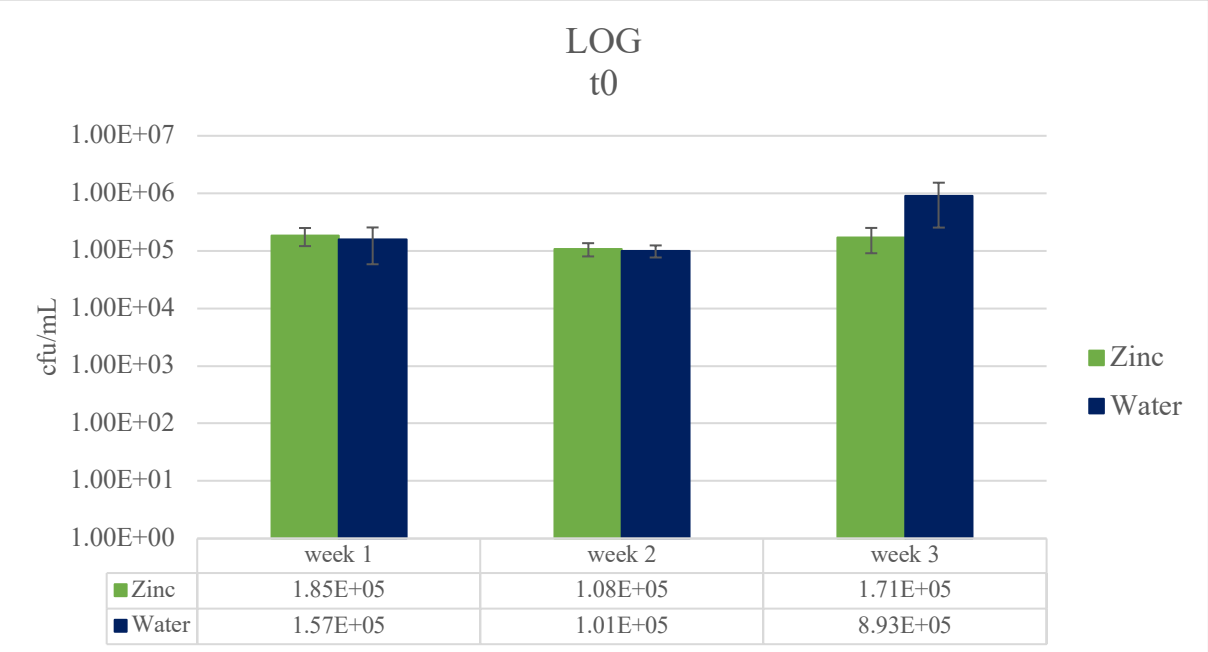
The same happened for *zinc-light* sample in week 2, which had lower final OD value at t20 than the *zinc-no light* sample, but then had a little higher cfu/mL value at t20 than *zinc-no light* sample. The difference was not very significant, the cfu/mL value at t20 for *zinc-light* sample was 4.28×10^9 and the final cfu/mL value for the *zinc-no light* sample at t20 was 3.52×10^9. Overall, the cfu/mL results at t20 ranged from 2.6×10^7 to 1.8×10^{10}. The lowest cfu/mL value had the *zinc-light* sample in week 1 and the highest cfu/mL value had the *water-light* sample in week 1.

The OD values and the cfu/mL results can lead to a conclusion that ZnO NPs and the combination of ZnO NPs and light had antibacterial effect on the bacteria cells. But the light alone did not show antibacterial effect.

This was proven by the statistics. Two-way Anova showed that ZnO NPs had a main effect on the cfu/mL results and also that the interaction between ZnO NPs and light had an effect on the cfu/mL results. The t-tests then showed that there was a difference between the the *zinc-light* samples and the *water-light* samples. So, the statistics proved that for the lag-phase cells the antibacterial effect was mostly concluded by the ZnO NPs in combination with illumination.

8.2 Log phase cells

For the log phase cells, the illumination experiment was repeated in total three times, so in this section results from three weeks of the log illumination experiment are provided. For the log phase cells the illumination experiment took 4 consecutive days in the lab for one growth curve and one series of t0 and t20 cfu/mL results.
The illumination experiment was easily repeatable for log phase cells, because the McFarland standard densitometer could have been used for the preparation of the cells for the illumination experiment. The only problem with the preparation of log phase cells was that it was necessary to start the cultivation of the log phase cells in the bioreactor, then wait 5 hours to let them cultivate and then prepare the cells for the illumination experiment and start the illumination experiment.
In *Figure 17*, are cfu/mL results for the log phase cells at t0.



	week 1	week 2	week 3
Zinc	1.85E+05	1.08E+05	1.71E+05
Water	1.57E+05	1.01E+05	8.93E+05

Figure 17 *bar chart of mean colony forming units per millilitre (cfu/mL) measured from the illumination experiment for log phase cells at t0 with error bars showing standard deviation of the mean (n = 6).*

Levene test showed that a lot of groups did not have equal variances for t0 samples. And Shapiro-Wilk test showed that only few groups had normal distribution, so it was more possible to do I type statistical error, for t0 cfu/mL results.
These were the initial concentrations of the samples before the start of the illumination experiment. The cfu/mL results at t0 ranged from $1.0x10^5$ to $8.9x10^5$. The *water* samples at t0 in week 3 had the highest cfu/mL values. Even though two-Anova showed statistically significant difference at t0 between weeks and *zinc* to *water* samples, there were not big differences between the cfu/mL values of different samples within the same week, so the

samples could have been compared at t20. This discrepancy between values and statistical analysis could be explained by low number of data that can be included for statistics (n=6, for t0), and the fact that small differences in colony counts can be magnified because of the dilution factor. Also, the difference in cfu/mL values at t0 was within one order of magnitude throughout all the weeks of the illumination experiment and t-tests showed only statistically significant difference between week 1 and week 2 at t0, at corrected level of significance alpha 1.6%.
In *Figure 18* are growth curves for log phase cells from the illumination experiment, already blank-corrected. The blank-corrected data from the bioreactors are provided in *Appendix 11.5.1.*

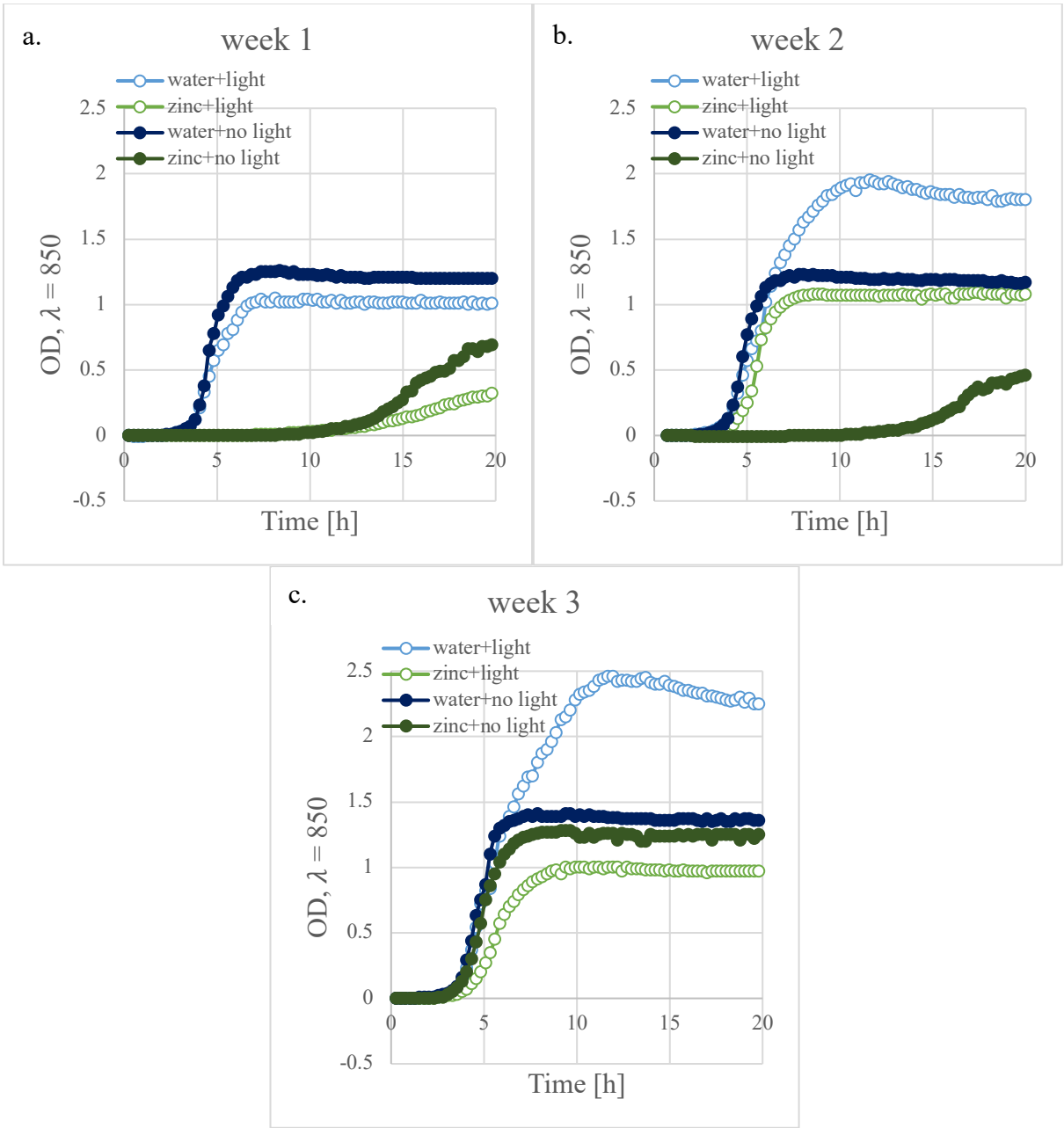
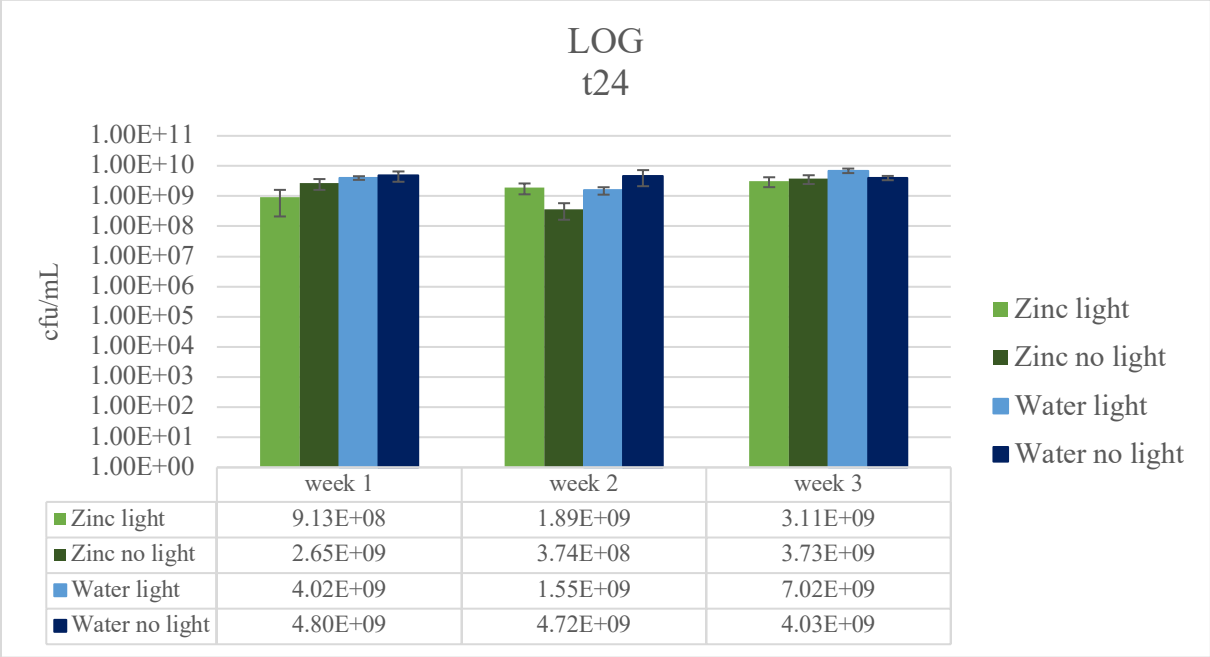


Figure 18 *OD charts from the illumination experiment for the log phase cells, a. data from week 1, b. data from week 2, c. data from week 3. ZnO NPs samples are green and water samples are blue, with illuminated samples having white circle.*



	week 1	week 2	week 3
Zinc light	9.13E+08	1.89E+09	3.11E+09
Zinc no light	2.65E+09	3.74E+08	3.73E+09
Water light	4.02E+09	1.55E+09	7.02E+09
Water no light	4.80E+09	4.72E+09	4.03E+09

Figure 19 *bar chart of mean colony forming units per millilitre (cfu/mL) measured from the illumination experiment for log phase cells at t20 with error bars showing standard deviation of the mean (n = 4).*

In *Figure 19* are cfu/mL at t20 for the log phase cells.
For the log phase cells for cfu/mL results, Levene test showed that at t20 samples had equal variances. Shapiro-Wilk test showed that only few groups had normal distribution, so it was more possible to do I type statistical error, for t20 cfu/mL results. Therefore, statistical results, where the p-values were on the edges of the level of significance were considered carefully.
In week 1, the *zinc-light* sample had the lowest final OD value, while the *water-no light* sample had the highest final OD value. The same results were for the cfu/mL results for week 1 at t20, *Figure 19*, where also *zinc-light* sample had the lowest cfu/mL value, 9.1×10^8, and the *water-no light* sample had the highest cfu/mL value, 4.8×10^9. The difference between the lowest and the highest cfu/mL at t20 value was not so high, not even one order of magnitude, even though based on the final OD values and the growth curves, the difference would be expected to be higher. The same happened in week 2, where *zinc-no light* sample did not have as low cfu/mL value at t20 as would be expected based on the final OD values and growth curves. This could have been caused by the bacteria cells in the *zinc* samples being smaller, and therefore the OD values were lower, but the cfu/mL results were higher than expected.
In week 2, problems with the caps and sealing occurred. The *zinc-light* sample had to be re-done, after few minutes of the experiment, because the falcon tube was leaking, and the *zinc-light* sample leaked out of the falcon tube. It can be also observed that the *water-light* sample had very high final OD value and the growth curve looked different than in week 1. This could have been caused again by sealing problem and extra oxygen entering into the falcon tube. Since the caps for the illuminated samples were 3D printed and cannot be bought anywhere, the washers sometimes could have been looser and therefore air could get inside, which then create better conditions for the bacteria growth
But even though the final OD value of the *water-light* sample was higher, the cfu/mL results at t20 showed that in week 2, the highest cfu/mL value had the reference, *water-no light* sample, 4.7×10^9.
In week 3, the *water-light* sample had the similar looking growth curve as in week 2. Again, this could have been caused by the imperfect sealing of the cap for the illuminated sample.

This time, the *water-light* sample had the highest cfu/mL value at t20, $7.0x10^9$, while the *water-no light* sample had cfu/mL value at t20 of $4.0x10^9$.
In all three weeks it seemed that the *zinc* samples had longer lag phase than the *water* samples and the *zinc-no light* sample had much longer lag phase than the *water* samples in the first two weeks.
Even though the difference between the *zinc* samples growth curves and the *water* samples growth curves seemed significant, the cfu/mL values did not show such a difference, and this was also proved by the statistics, where the two-way Anova test showed that there was only a statistically significant difference between the *zinc* and *water* samples at t20, but the *zinc-light* did not have statistically significant antibacterial effect on the cfu/mL results at t20. This could have been caused by low number of weeks, since the illumination experiment was only repeated three times and there was a big dispersion between the growth curves of the *zinc* samples, but also the *water-light* samples had the abnormal growth curves in two weeks out of three. And the *zinc-light* sample had higher final OD value than the *zinc-no light* sample in week 2.
For the log phase cells, the main antibacterial effect was caused by the ZnO NPs primarily, based on the cfu/mL results, OD values and the statistical results.

8.3 Stat phase cells

For the stat phase cells, the illumination experiment was repeated in total four times. Because the illumination experiment for the stat phase cells took 5 consecutive days in the lab in order to get one growth curve and one series of t0 and t20 cfu/mL results.
With the stat cells, contamination became an issue. The contamination occurred in the first weeks and repeated first week and therefore the results from these two weeks could not been used and are not provided in the results. This was due to the fact that the source of the contamination was not easily detectable and therefore the results could have been influenced by the contamination and show incorrect outcomes. Contamination of the sample with bacteria other than *E. coli* can affect both the OD and cfu/mL data and it is not possible to remove this artifact from the experimental results.
In the first week, there was higher bacterial growth on MHA plate with higher dilution factor, which lead to conclusion that there was a contamination present, because MHA plates with higher dilution factor should have lower colony count than the MHA plates with lower dilution factor. This higher growth was observed for two different samples at t20 and therefore not even the OD results could have been used, since the contamination could have been in the bioreactors throughout the illumination experiment and influence the growth curves.
The first week was then repeated but even in the repeated week 1, contamination occurred. The bacterial growths on MHA plates looked different, there were similar cell counts on all the plates and there was not any difference between the different dilution factors. Also, all the plates had much higher count of bacteria cells and smaller colonies even at t0, and then also on t20 MHA plates. Later, it was found that the saline used for the experiment was contaminated quite heavily, even though it was sterilized, and the initial source of the contamination was not found.
Beside the struggle with the contamination which really only occurred with the stat phase cells, the illumination experiment was easily repeatable for stat phase cells, because the McFarland standard densitometer could have been used to dilute the bacteria cells when preparing them for the illumination experiment.
The initial concentrations of samples, at t0, are in *Figure 20*.

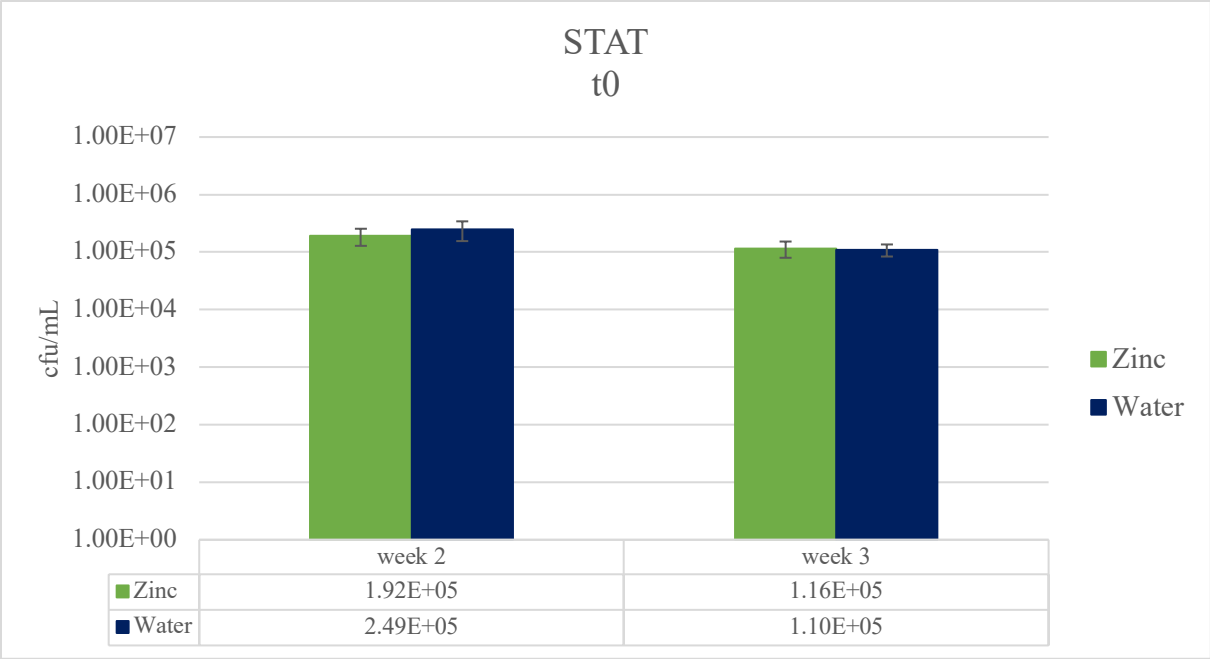


Figure 20 bar chart of mean colony forming units per millilitre (cfu/mL) measured from the illumination experiment for stat phase cells at t0 with error bars showing standard deviation of the mean (n = 6).

Levene test showed that *zinc* samples to samples without ZnO NPs had equal variances, but all the other groups did not for cfu/mL results at t0. And the Shapiro-Wilk test for the stat phase cells showed, that for t0 cfu/mL results many groups were from the normal distribution, with only few groups from not normal distribution.
The initial concentration of the samples ranged from $1.1x10^5$ to $2.5x10^5$. Even though, the two-way Anova showed difference between weeks, and also t-test showed that there was a statistically significant difference between week 2 and week 3 for cfu/mL values at t0, the difference was still within one order of magnitude. Also, there was not a big difference between the samples within the same weeks at t0, so the results could have been compared.
In *Figure 21*, are growth curves for stat phase cells from the illumination experiment, from week 2 and week 3, the growth curves are already blank-corrected. The blank-corrected data from the bioreactors are provided in *Appendix 11.5.1.*

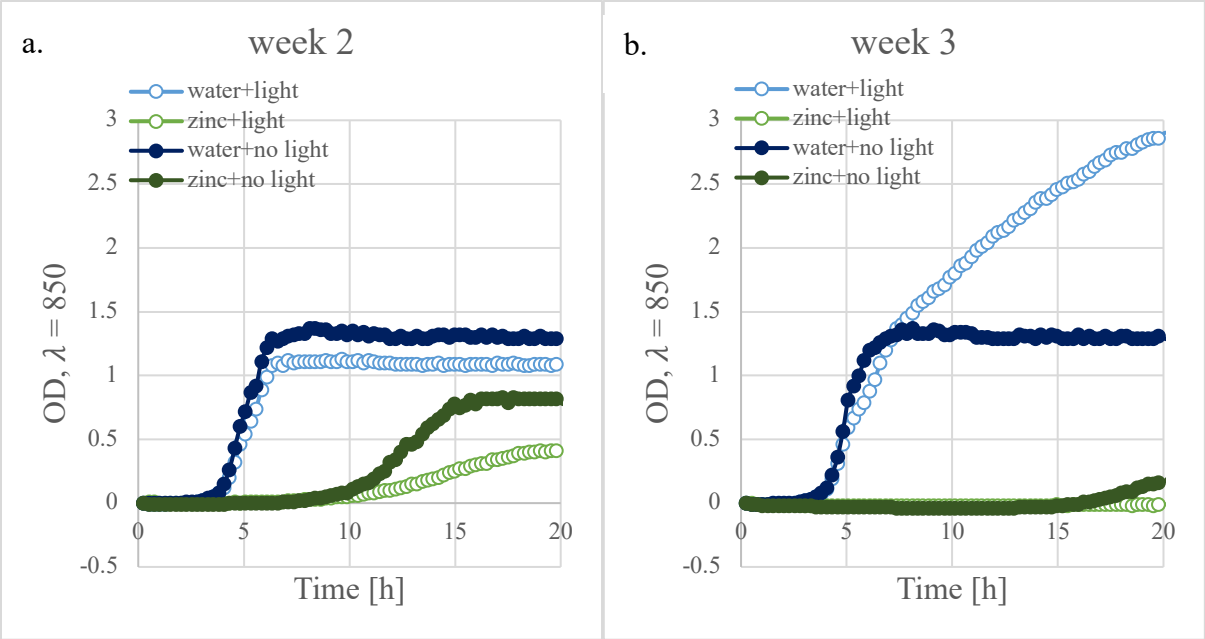
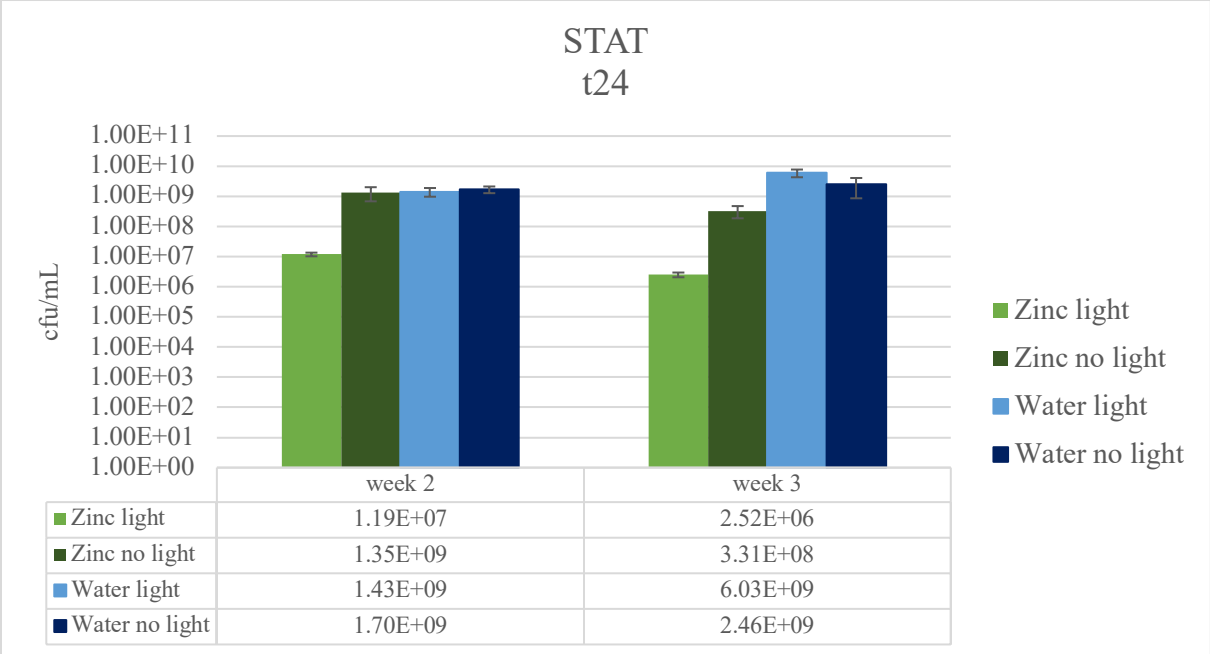


Figure 21 *OD charts from the illumination experiment for the stat phase cells, a. data from week 2, b. data from week 3. ZnO NPs samples are green and water samples are blue, with illuminated samples having white circle.*

In *Figure 22*, are cfu/mL results for the stat phase cells, at t20.



	week 2	week 3
Zinc light	1.19E+07	2.52E+06
Zinc no light	1.35E+09	3.31E+08
Water light	1.43E+09	6.03E+09
Water no light	1.70E+09	2.46E+09

Figure 22 bar chart of mean colony forming units per millilitre (cfu/mL) measured from the illumination experiment for stat phase cells at t20 with error bars showing standard deviation of the mean (n = 4), the no light samples in week 3 are showing cfu/mL results at t48, since the dilution series for these no-light samples had to be repeated and the pictures were taken again due to suspected contamination.

Levene test showed that *zinc* samples to samples without ZnO NPs had equal variances, but all the other groups did not for cfu/mL results at t20. And the Shapiro-Wilk test for the cfu/mL results at t20 showed that only one group was from normal distribution. Therefore, statistical results, where the p-values were on the edges of the level of significance were considered carefully.

In week 2, the *zinc* samples had much longer lag phase and then the final OD value was lower than for the *water* samples. In this week, the *water-no light* sample had the highest final OD value, while the *zinc-light* sample had the lowest final OD value. The same results can be observed in *Figure 22*, where the *zinc-light* sample had the lowest cfu/mL value at t20, which corresponds with the final OD value in week 2, and the highest cfu/mL value had the *water-no light* sample, but the cfu/mL value at t20 of *water-light* sample was very similar to *water-no light* sample.
In week 3, the growth curve for the *water-light* sample was similar to the log phase cells *water-light* samples in week 2 and week 3. Again, this could have been caused by problems with sealing of the cap used for the illuminated samples.
In week 3, *no-light* samples had abnormal MHA plates at t20, so for these samples the dilution series was repeated and the results in the *Figure 22* are cfu/mL results at t48 (dilution series was done after 48 hours from the beginning of the experiment and then MHA plates were cultivated in incubator at 37 ºC overnight and pictures were re-taken using the automatic colony counter the next day) for *zinc-no light* and *water-no light* samples. This approach was only done this one time for stat cells, in week 3. The samples were stored in fridge, so the growth was stopped and there should not be a difference between the MHA plates at t20 and at t48.
In week 3, the *zinc* samples had very long lag phase and only the *zinc-no light* sample grew, but only slightly. From the growth curve, it would be expected that the *zinc-light* sample would have the same concentration at t20 as it had at t0, but the *zinc-light* sample had $1.2x10^5$ cfu/mL at t0, and $2.5x10^6$ cfu/mL at t20. So, the *zinc-light* sample also grew a little, but it is not possible to observe that on the growth curve which is why also the cfu/mL measurements are important.
Interestingly, even though the growth curve showed that the final OD value for the *zinc-no light* sample was very low, the differences between the cfu/mL values at t20 between the *water-light* sample, *water-no light* sample and *zinc-no light* sample were not so different. The cfu/mL value at t20 of *zinc-no light* sample was $3.3x10^8$, while the *water-no light* sample had the cfu/mL value at t20 of $2.4x10^9$. The *water-light* had the highest cfu/mL value at t20, $6.0x10^9$, but not as high as it would be expected from the growth curve, since the final OD value in the growth curve was much higher than for the *zinc-no light sample* and *water-no light* sample. This shows that the OD values at t20 do not corresponds closely with the cfu/mL values at t20 for the illuminated and *zinc* samples. This could have been caused by the bacteria cells being smaller in the *zinc* samples, and therefore the growth could have been unnoticeable on OD values, but was then observed in cfu/mL results. This also shows that the dilutions had to be estimated based on the final OD values every week, but the estimated dilutions could have been incorrect because the final OD values were higher than the cfu/mL results. Unfortunately, the dilutions could not be repeated the next day, it was done only once in a special case, so there was only one chance to estimate the dilutions, and only the next day it could have been checked if the dilutions were correct.
From the results it seems that the stat phase cells are more sensitive to the ZnO NPs treatment than lag or log phase cells. It also seemed that the combination of ZnO NPs and illumination had higher antibacterial effect than ZnO NPs itself on the stat phase cells.
This was proved by the statistics, because the two-way Anova showed statistically significant difference for the main effect of ZnO NPs, but also for the interaction between ZnO NPs and light.
The t-tests then showed that the *zinc-light* samples were statistically significantly different than all the other samples, so for the stat phase cells the main antibacterial effect was caused by the combination of ZnO NPs and illumination.

8.4 Comparison

One of the goals of this thesis was to compare bacteria cells from different growth phases, to detect if the antibacterial effect of ZnO NPs and illumination differs based on the growth phase of the bacteria cells.
The comparison of the cells from different growth phases is provided in this section.
Based on the cfu/mL results at t0, see *Figure 14* for lag phase cells, *Figure 17* for log phase cells and *Figure 20* for stat phase cells, the highest range of the t0 cfu/mL values was for the lag phase cells, while the lowest range of cfu/mL values at t0 was for stat phase cells. This could have been caused either by the preparation of the cells, since for the log phase cells and stat phase cells, the McFarland standard densitometer could have been used to dilute the bacteria for the illumination experiment more precisely. This was not possible for the lag phase cells, because after cultivating one hour in the bioreactor to get the lag phase cells, the colonies did not create a visible pellet after spinning in the centrifuge and therefore the McFarland densitometer could not be used, because the concentration of the lag phase cells was too low to generate reading. Or this lowest range of cfu/mL values at t0 for stat phase cells could have been due to lower number of repetitions, since lag phase cells were repeated four times, log phase cells three times and stat phase cells four times as well, but only data from two weeks could have been used for stat phase cells due to contamination.
Lag phase cells always had similar growth curve, see *Figure* 15, to the reference sample, *water-no light*, in all weeks. Log phase cells had much lower final OD value for *zinc-no light* sample in weeks 1 and 2, and for *zinc-light* sample in week 1, see *Figure 18*, otherwise the *zinc* samples had similar growth curves to the reference, *water-no light*, sample. But the stat phase cells had very low final OD values in all weeks, and in week 3 *zinc-light* sample seemed to not grow at all, even though the cfu/mL results proved otherwise, and the *zinc-light* sample had slight increase in cfu/mL values from t0 to t20.
Based on the final OD values and the growth curves, the stat phase cells are more sensitive to the ZnO NPs treatment, and even to the combination of ZnO NPs and illumination. The higher sensitivity of stat phase cells to ZnO NPs was also proven in the MIC test, where even though the sub-lethal concentration for stat phase cells was also 128 µg/mL of ZnO NPs, the stat phase cells had longer lag phase length than lag and log phase cells, and the final OD value in the MIC test was lower for the same ZnO NPs concentration of 128 µg/mL.
This higher sensitivity can be also observed on the cfu/mL results at t20.
The cfu/mL results at t20 for lag phase cells for the *zinc-light* samples ranged from 2.7×10^7 to 4.2×10^9 and for *zinc-no light* samples ranged from 1.3×10^9 to 1.3×10^{10}.
The cfu/mL results at t20 for log phase cells for the *zinc-light* samples ranged from 9.1×10^8 to 3.1×10^9 and for *zinc-no light* samples ranged from 3.7×10^8 to 3.7×10^9.
The cfu/mL results at t20 for stat phase cells for the *zinc-light* samples ranged from 2.5×10^6 to 1.2×10^7 and for *zinc-no light* samples ranged from 3.3×10^8 to 1.3×10^9.
Therefore, there was not a big difference between the cfu/mL ranges for *zinc* samples for lag and log phase cells. But for stat phase cells, cfu/mL values at t20 differ by almost two orders of magnitude for *zinc-light* samples when compared to lag and log phase cells. The cfu/mL values at t20 for *zinc-no light* stat phase cells samples were more similar to *zinc-no light* cfu/mL values of lag and log phase cells.
The greater difference between the highest and lowest cfu/mL results for stat phase cells than either lag or log could indicate that the stat phase cells are more sensitive to the combination of ZnO NPs and illumination.
The average values of cfu/mL values at t20 for the different growth phase cells are in *Table 4*.

Table 4 *Average values of cfu/mL values at t20 for the different growth phase cells.*

LAG	
Mean cfu/mL at t20	
zinc+light	1.81E+09
zinc+no light	5.01E+09
water+light	7.90E+09
water+no light	4.61E+09
LOG	
Mean cfu/mL at t20	
zinc+light	1.97E+09
zinc+no light	2.25E+09
water+light	4.20E+09
water+no light	4.52E+09
STAT	
Mean cfu/mL at t20	
zinc+light	7.21E+06
zinc+no light	8.40E+08
water+light	3.73E+09
water+no light	2.08E+09

The average values of cfu/mL from *Table 4* shows that while there was not a big difference between the *water* samples for all the growth phases, the average cfu/mL values of the *zinc* samples vary for each growth phase. Average cfu/mL value at t20 for *zinc-light* sample was $1.8x10^9$ for lag phase cells, $2.0x10^9$ for log phase cells, but $7.2x10^6$ for stat phase cells. And average cfu/mL value at t20 for *zinc-no light* sample was $5.0x10^9$ for lag phase cells, $2.3x10^9$ for log phase cells and $8.4x10^8$.
The average of final OD values for the lag phase cells was 1.05 for *water-light*, 1.23 for *water-no light*, 0.75 for *zinc-light* and 1.02 for *zinc-no light*. The average of final OD values for the log phase cells was 1.00 for *water-light,* 1.24 for *water-no light*, 0.79 for *zinc-light* and 0.81 for *zinc-no light*. And the average of final OD values for the stat phase cells was 1.09 for *water-light*, 1.29 for *water-no light*, 0.21 for *zinc-light* and 0.48 for *zinc-no light*. To count the average of the final OD value, only normal looking growth curves were used, so the abnormal growth curves for *water-light* samples which occurred in two weeks for log phase cells and in one week for stat phase cells were not used for the calculation. The data are provided in *Appendix 11.5.6b.*
So, this evidence suggests that the ZnO NPs have higher antibacterial effect on the stat phase cells, and that the lag phase cells, and log phase cells are similar based on the cfu/mL values, growth curves and final OD values.
Even though the differences between the average values could have been caused by the lower number of repetitions for log and stat phase cells, the higher sensitivity of stat phase cells to ZnO NPs and the ZnO NPs treatment in combination with illumination was proven on different levels (final OD values, cfu/mL results, MIC test – only ZnO NPs treatment).
The lag phase length was also compared for the different growth phase cells. The threshold for the end of the lag phase was set to the time, when the sample reached blank-corrected OD value of 0.02, since after this value the exponential growth was always observed. The growth phase lengths are provided in *Appendix 11.5.6a.* The average lag phase length for lag phase

cells was 2.12 hours for *water-light*, 2.19 hours for *water-no light*, 3.07 hours for *zinc-light* and 3.10 hours for *zinc-no light*. The average lag phase length for log phase cells was 2.77 hours for water samples, 5.14 hours for *zinc-light* and 8.01 hours for *zinc-no light*. The average lag phase length for stat phase cells was 3.04 hours for water samples,13.55 hours for *zinc-light*, and 13.81 hours for *zinc-no light.*

Log phase cells had longer lag phase than lag phase cells, and stat phase cells had even longer lag phase than log phase cells, even for *water* samples, even though the difference for each growth phase was around one measurement (i.e. the OD measurements in the bioreactor were done every 15 minutes). But for the *zinc* samples the increased lag phase length was even more obvious.

Maximal growth rate and time when this maximum was reached was also compared for the different growth phases.

The data are provided in *Appendix 11.5.6c.* Even the abnormal growth curves for *water-light* samples could have been used because the growth at the end of the experiment did not interfere with the maximal growth rate value and when this maximum was reached.

For lag phase cells the average maximal growth rate was 0.36 h^{-1} at 4.34 hours for *water-light*, 0.50 h^{-1} at 4.66 hours for *water-no light*, 0.19 h^{-1} at 7.20 hours for *zinc-light* and 0.30 h^{-1} at 6.75 hours for *zinc-no light.*

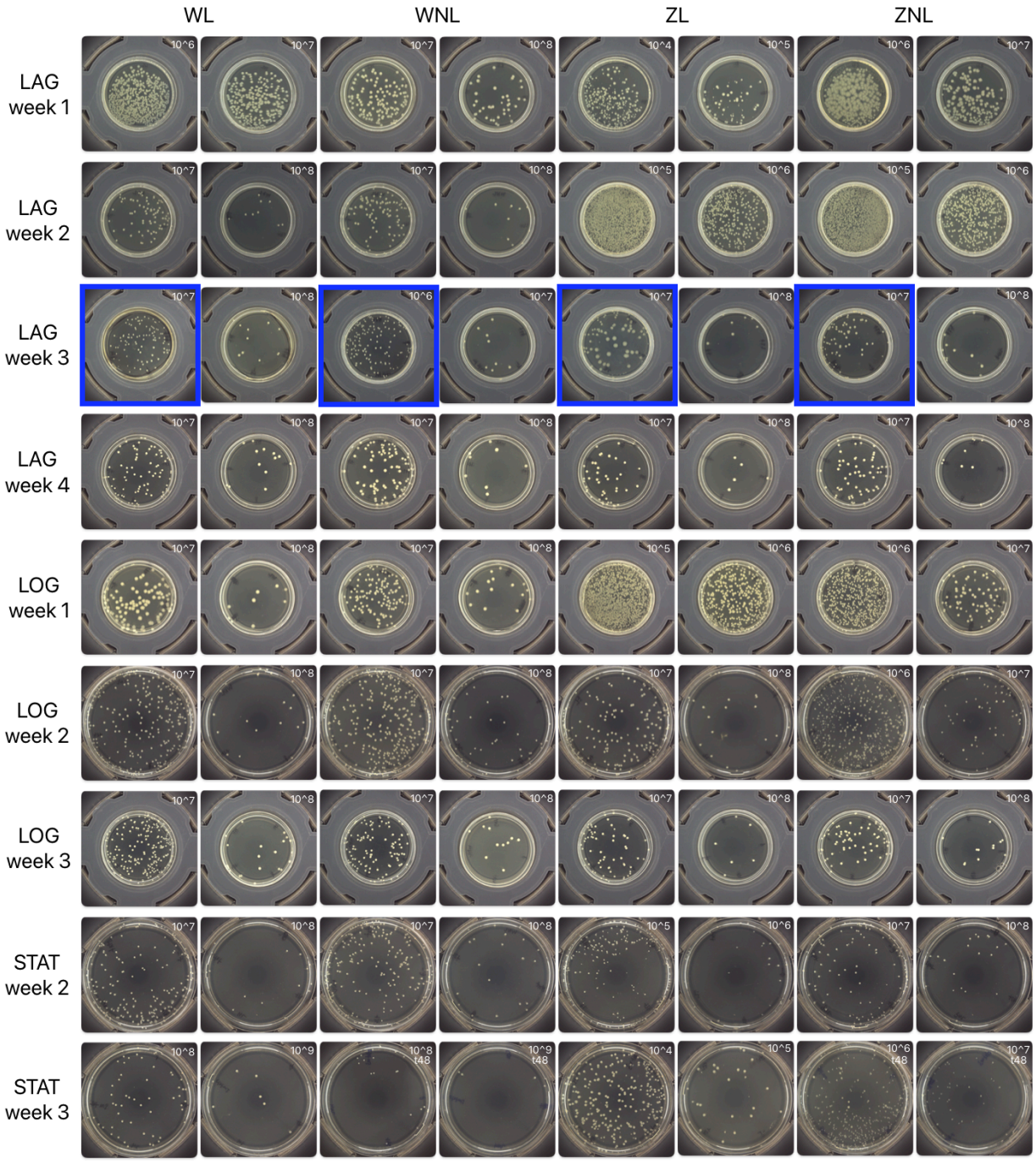
For log phase cells the average maximal growth rate was 0.45 h^{-1} at 5.64 for *water-light*, 0.52 h^{-1} at 5.14 hours for *water-no light,* 0.42 h^{-1} at 9.62 hours for *zinc-light*, and 0.23 h^{-1} at 12.66 hours for *zinc-no light*. For stat phase cells the average maximal growth rate was 0.39 h^{-1} at 6.21 hours for *water-light*, 0.50 h^{-1} at 5.58 hours for *water-no light,* 0.03 h^{-1} at 17.61 hours for *zinc-light* and 0.11 h^{-1} at 15.73 hours for *zinc-no light*.

Water-no light samples always had the highest average maximal growth rates. The *water-no light* sample usually reached the maximal growth rate between 4 and 5 hours of the experiment. Except from log phase cells, the lowest average maximal growth rate values had the *zinc* samples. Even for the *water* samples, the time when the maximal growth rate was reached was longer for log phase cells than for lag phase cells and for stat phase cells it was longer than for log phase cells in all weeks.

The main difference between the lag and log phase cells is in the lag phase length and in the maximal growth rate values and the time when the maximal growth rates were reached.

In the *Appendix 11.5.5* are provided pictures of the t0 MHA plates. There was not any difference in the colony size or shape for neither *zinc* nor *water* samples. The colonies on the t0 MHA plate always looked similar.

But on t20 MHA plates sometimes differences between the colony sizes and shapes were observed.



Picture 33 Selection of MHA plates pictures taken by the automatic colony counter, from the illumination experiment for all three growth phases, lag, log and stat. Aligned by the sample (WL - water+light, WNL - water+no light, ZL - zinc+light, ZNL - zinc+no light), with dilution factors marked in the right corner of each MHA plate picture. Blue-square marked pictures were processed with ImageJ software for comparison. In some weeks, larger petri dishes were used and therefore had around twice the number of colonies for the same dilution factor, this was then corrected using the corresponding equation [1] or [2] based on the petri dish size.

The differences were always only observed for the *zinc* samples. From *Picture 33.* it can be seen that the difference in shape was for lag phase cells in week 1 for both *zinc-light* and *zinc-no light* and then for lag phase cells in week 3 mostly *zinc-light* sample. The difference in colony sizes, i.e. on the same MHA plate where colonies with two or more different sizes were present, was observed for lag phase cells in week 3 for the *zinc-no light* sample, then for log phase cells in week 2 for *zinc-light* and *zinc-no light* samples and for stat phase cells in

week 3 for *zinc-light* and *zinc-no light* sample. From these results it seemed that the ZnO NPs used for the illumination experiment can also sometimes influence the size and shape of some colonies.
The MHA pictures marked with blue squares were processed using ImageJ software (https://imagej.net/ij/) to compare the pictures also analytically. The results from the ImageJ processing are in *Appendix 11.7.3*.
Summary with average values from the processing results from ImageJ are in *Table 5*.

Table 5 Mean values from the ImageJ processing results for MHA plates at t20 for lag phase cells from week 3

Mean values from the ImageJ proccessing results									
Sample	Colony Count	Colony Area [mm^2]	Mean Gray value	StdDev	Colony Perimeter [mm]	Colony Circularity	Aspect Ratio	Roundness	Solidity
WL	95	70.91	180.15	20.73	29.31	0.97	1.13	0.91	0.91
WNL	130	72.38	154.89	16.88	29.99	0.95	1.16	0.89	0.91
ZL	58	288.12	135.30	10.41	58.19	0.90	1.26	0.85	0.92
ZNL	70	101.19	147.38	14.34	33.39	0.93	1.32	0.86	0.92

Colony count refers to number of colonies on the MHA plate, which was processed. Area refers to a mean value of area of one colony on the MHA plate. Mean grey value refers to an average Gray value within the selection. StDev is a standard deviation of Gray values within the selection. Perimeter refers to an average value of one colony perimeter. Circularity is counted using [4].[88]

$$Circularity = \frac{4\pi \times area}{perimeter^2} \qquad [4]$$

Circularity of 1.0 means perfect circle and values close to 0.0 indicates more elongated shape of colony. Aspect ratio is a deviation of major axis to minor axis.[88] Roundness is counted using [5].[88]

$$Roundness = \frac{4 \times area}{\pi \times major\ axis^2} \qquad [5]$$

Solidity is the area divided by convex area.[88]
Based on the average values in *Table 5*, the *zinc* samples plates had larger colony area, especially the *zinc-light* sample, which can be also observed visually in *Picture 33*. The *zinc-light* sample had mean value of colony area 288 mm^2, the *zinc-no light* sample had mean value of colony area 101 mm^2, the *water-light* sample had mean value of colony area 71 mm^2 and the *water-no light* sample had mean value of colony area 72 mm^2. The *water-light* sample also had the most circular shaped colonies, the circularity was 0.97, while the *zinc-light* sample had the least circular shaped colonies, since the circularity was 0.90. However, mean circularity of 0.90 is still very close to perfect circle. The water samples had higher colony count than the *zinc* samples. The *water* samples had smaller perimeter, around 30 mm for *water* samples, 33 mm for *zinc-no light* sample and 58 mm for *zinc-light* sample. In general, the *zinc* treatment produced larger, less circular colonies than the *water*, suggesting that sub-lethal concentrations of ZnO NPs can affect size and shape of colonies.

9. Discussion

ZnO NPs possess antibacterial properties[66], which was also proved in the illumination experiment, since the samples with ZnO NPs had lower final OD values and lower cfu/mL results after 20 hours in the bioreactors for all growth phases in all weeks, and the effect of ZnO NPs was also proved in statistical tests.

Researches have shown that stat phase cells develop multi-stress resistance[89], they acquire ability to survive under extreme conditions and they have shown higher resistance to antimicrobials in comparison to lag or log phase cells.[6,90] However, the MIC test and the illumination experiment shown here suggests that the stationary phase cells were the most sensitive to the ZnO NPs treatment and also to the combined treatment of ZnO NPs and light. This could have been caused by the extra growth which the stat phase cells had to go through. The *E. coli* cells had to grow to stationary phase, meaning they had been cultivated overnight, in total 2 times for lag and log phase cells, before the illumination experiment or MIC test. But the stat phase cells had been cultivated overnight 3 times in total before the illumination experiment or MIC test, meaning they entered the lag phase three times before the illumination experiment, while lag and log phase cells only had to enter the lag phase two times before the illumination experiment.

When the cells enter the lag phase, after being in different growth phase, usually stat phase, they have to change their transcriptome and proteome, the cells must reorganize and restructure their metabolism, because the lag phase is an adaptive phase when the cells have to adjust their structure to be able to multiplicate and enter the log phase.[39]

This could be exhausting for the bacteria, since every growth phase have phase-specific genes which have to be turned on, while the previous-phase genes have to be turned off.[38–40]

This extra growth and therefore many repeated changes in the bacteria cell structure could have caused the higher sensitivity towards the ZnO NPs and ZnO NPs with illumination treatments.

Another explanation of the higher sensitivity for the stat phase cells towards the treatments could have been due to the lack of flagella. It was shown that stat phase cells have lower number of flagella when compared to log phase cells.[40] Flagella are used for movement and are helping bacteria to get away from stress and in turn get them to nutrients.[40] So, another explanation could be, that the stat phase cells were not able to get away from the stress, ZnO NPs in this case, and to nutrients as easily as log or lag phase cells and therefore could have been more sensitive towards it. That would also explain why the log phase cells had very diverse growth curves throughout the weeks, because it was shown that the late-log phase cells, had the highest flagella count, and therefore the highest motility.[40]

Prolonged lag phase is a defence mechanism for bacteria to tolerate stress.[39]

Therefore, longer lag phase means higher stress for bacteria and that the bacteria needed more time to adapt to the new environment. Since stat phase cells had the longest lag phase length, it seems that they were under the highest stress, when compared to lag or log phase cells, which is another proof that the stat phase cells had the highest sensitivity towards ZnO NPs.

Bacteria cells which were taken from log phase, with inoculum concentration of 10^6 cfu/mL and were put new growth media (Tryptic Soy Broth), did not have lag phase, but stationary phase cells had lag phase even for higher inoculum concentrations.[37]

However, in the illumination experiment the concentration of the log bacteria cells at t0 was around 10^5 cfu/mL, in all weeks lower than 10^6 cfu/mL, therefore the lag phase was expected for the growth phase cells and it was observed throughout all the weeks.

Due to the adaptation and restructuralization[39], it was expected that lag phase cells would have the shortest lag phase, when compared the log and stat phase cells, since they already started the adaptation and the solution was very similar to the solution in which they have already grown for 1 hour, only new MHB and the HPLC water or ZnO NPs.

Lag phase cells really had the shortest lag phase length and the stat phase cells had the longest lag phase length. However, the very long lag phase for stat phase cells could have been due to the higher sensitivity towards the treatments which was observed for the stat phase cells even in the MIC test.
In the MIC test for lag phase cells, some samples with ZnO NPs seemed to have shorter lag phase, than the positive control which was a reference sample. This was also observed by David Rutherford et al. in their study, where the sample with sub-lethal concentrations of ZnO nanospheres which had the shortest lag phase length.[71] In the illumination experiment and MIC test, grain-like shaped ZnO NPs were used which is similar shape to nanospheres. Different shapes and sizes at t20 MHA plates of ZnO NPs samples were also observed by Ing. Šlapal Bařinková in her Master's thesis experiment[84], even when using ZnO NPs with bigger diameter, 50 nm, and doped with aluminium.
Therefore, this effect of different sized colonies on MHA plates would be mainly caused by the ZnO NPs, not the doping nor the slightly bigger or smaller size of ZnO NPs.
She also observed the atypical growth curves for water-light samples.[84]
For wide band gap semiconductors such as ZnO NPs, UV light with wavelength of 100 nm to 400 nm is needed to cause the photocatalysis, therefore increase the production of ROS and increase the antimicrobial effect of the ZnO NPs.[68] Or doping ZnO NPs with other material such as metals is needed in order to increase the production of the ROS even under visible light.[69]
In the illumination experiment, higher antibacterial effect was observed for the combined treatment of ZnO NPs with white light even though the spectrum of the light source was not in the range of UV light with the light source intensity peak being at 440 nm. In the illumination experiment the antibacterial effect of ZnO NPs was proven to be higher with continuous illumination, which suggests that more ROS were produced even when the ZnO NPs were illuminated with white light, not UV light. Since higher production of ROS increases the antibacterial effect of ZnO NPs. But the illumination itself did not show any antibacterial effect.
Long-term *E. coli* survivors to antibiotic ciprofloxacin were from the log phase[91], it would be interesting to study if long-term survivors to ZnO NPs treatment or ZnO NPs combined with white light treatment would also be from log phase, since the illumination experiment have shown different results in every week.
Because of the different results for log phase cells and contamination problems with stat phase cells, it would be exciting to repeat the illumination experiment for all the growth phases, but especially for log and stat phase cells and to do more repetitions for each growth phase.
It would be also interesting to study resistance to the ZnO NPs treatment and ZnO NPs in combination with light treatment based on the growth phase, because stat phase cells are the ones which in many studies have shown the highest resistance to different treatments[6,90], and bacteria cells with longer lag phases have shown higher tolerance to antibiotics.[39]

10. Conclusion

In this thesis *Escherichia coli* bacteria cells from different growth stages were exposed to ZnO nanoparticles and to sub-lethal concentration of ZnO nanoparticles in combination with white light.

Specifically, lag, log and stationary phase *Escherichia coli* cells. The sub-lethal concentration of ZnO nanoparticles was determined from the Minimum inhibitory concentration test.

The initial bacteria concentration was determined from preliminary experiment, in which lag phase length for *E. coli* cells was studied.

Antimicrobial effect of ZnO nanoparticles and ZnO nanoparticles in combination with white light was studied. Four samples were tested each week, two samples with ZnO nanoparticles and two samples without the ZnO nanoparticles and with water instead. Then one water sample and one zinc sample were illuminated continuously for 20 hours while the other water and zinc sample were kept in the dark.

Two different methods to study the antibacterial effect were used. One of the methods was measuring optical density of the solutions in bioreactors, therefore studying the growth curves. And the other method was the measurement of colony forming units per millilitre, which were measured at the beginning and at the end of the illumination experiment. The results were compared and tested statistically, using two-way or three-way Anova.

The preparation protocols for different growth phases were developed for this thesis.

The lag phase cells were the least sensitive towards the ZnO NPs and combined ZnO NPs with illumination treatment, while stationary phase cells were the most sensitive and in one week the illuminated sample with ZnO NPs did not even grow for the stationary phase cells based on the optical density and growth curve results. However, this illuminated sample with ZnO NPs showed slight increase in cfu/mL results from t0 to t20. Therefore, the OD values did not fully correspond to cfu/mL values, which could have been caused by the bacteria cells in the ZnO NPs samples being smaller and therefore having smaller OD values while still growing.

Statistical tests have shown that the samples with ZnO nanoparticles were significantly different to the water samples at the end of the illumination experiment for all the growth phases. And for lag and stationary phase cells, the combined treatment of ZnO nanoparticles with continuous illumination with white light had statistically significant antibacterial effect.

The stationary phase cells had the longest lag phase length, even the reference samples with water instead of ZnO NPs had longer lag phase lengths for stationary phase cells than for lag phase cells. Samples with ZnO NPs in some weeks had different shaped and sized colonies on MHA plates, these colonies were usually bigger and less round than colonies on the MHA plates with water samples, which suggests that ZnO NPs can also influence the shape and size of bacteria colonies.

All the results concludes that the ZnO NPs had antibacterial effect on *E. coli* bacteria cells from all the studied growth phases. And for lag and stat phase cells, the main antibacterial effect was caused by the combined treatment of ZnO NPs with white light. Even though, white light should not cause photocatalysis in ZnO NPs, and therefore production of reactive oxygen species. The higher antibacterial effect of the combined treatment leads to a conclusion that reactive oxygen species were produced in ZnO NPs even with white illumination.

The results from this thesis show that the growth phase of bacteria cells can influence the effect of sub-lethal concentrations of photoactive ZnO NPs and the continuous illumination with white light even increased the antibacterial effect of the treatment, therefore it is important to consider these findings when photoactive nanoparticles are to be used for antibacterial treatment in the future.

11. Appendix

11.1 List of used Equipment

Advanced Vortex Mixer – ZX3, Velp Scientifica
Autoclave – Classic, Prestige Medical
Automatic Colony Counter – SphereFlash
Centrifuge 1 – MPW 150r, Med. Instruments
Centrifuge 2 – Tehtnica Centric 150
Densitometer – Den 1B, BioSan
Incubator – model 100-800, Memmert
Light source – KL 2500 LED, Schott
Microplate reader – Epoch2, BioTek
Orbital shaker – PSU-10i, BioSan
Personal Bioreactors – RTS-1, BioSan
Scale – ABT 320-4M, Kern
Ultrasound bath – Bandelin Sonorex Digitec

11.2 ZnO NPs used in the illumination experiment

Safety sheet:
https://onlinefolders.abra.eu/PLAB/BL_8278_20211126_OXID_ZINECNATY_nano_25_nm_VM5D300101.pdf

P-lab catalogue: https://www.p-lab.cz/nanomaterialy-2

Picture of the bottle with ZnO NPs used for the illumination experiment:

11.3 Preliminary Experiment data

11.3.1 Preliminary experiment data from the bioreactor e.g. OD and Growth rate data

Data: Preliminary experiment - OD							
week 1		**week 2**		**week 3**		**rep. week 3**	
Hours	**OD**	**Hours**	**OD**	**Hours**	**OD**	**Hours**	**OD**
0.29	-0.01	0.25	-0.06	0.25	-0.03	0.25	-0.04
0.54	-0.01	0.5	-0.06	0.51	-0.04	0.74	-0.04
0.8	-0.01	0.76	-0.06	0.76	-0.04	1.25	-0.04
1.27	-0.01	1.27	-0.06	1.27	-0.04	1.75	-0.04
1.52	-0.01	1.52	-0.05	1.52	-0.04	2	-0.04
1.77	-0.01	1.77	-0.05	1.77	-0.03	2.28	-0.04
2.29	0	2.15	-0.05	2.29	-0.05	2.53	-0.03
2.55	0	2.4	-0.05	2.54	-0.05	2.79	-0.03
2.8	0.01	2.66	-0.04	2.8	-0.04	3.27	-0.01
3.06	0.02	2.91	-0.03	3.27	-0.03	3.52	0.01
3.36	0.05	3.25	-0.02	3.53	-0.01	3.78	0.04
3.61	0.09	3.5	0	3.78	0.01	4.03	0.07
3.87	0.17	3.75	0.03	4.03	0.04		

Appendix 11.3.1-1 *OD values from the bioreactor, which were used to draw OD charts for all weeks of the preliminary experiment, including week 3, which had contamination.*

Data: Preliminary experiment - Growth rate							
week 1		**week 2**		**week 3**		**rep. week 3**	
Hours	**μ [h⁻¹]**	**Hours**	**μ [h⁻¹]**	**Hours**	**μ [h⁻¹]**	**Hours**	**μ [h⁻¹]**
0.54	-0.01	0.5	-0.01	0.51	-0.01	0.74	-0.01
0.8	0	0.76	-0.01	0.76	-0.01	1.25	0
1.27	0	1.27	0	1.27	-0.01	1.75	0
1.52	0	1.52	0	1.52	0	2	0
1.77	0	1.77	0	1.77	0	2.28	0
2.29	0	2.15	0	2.29	-0.01	2.53	0.01
2.55	0	2.4	0	2.54	0	2.79	0.01
2.8	0.01	2.66	0.01	2.8	0	3.27	0.01
3.06	0.02	2.91	0.01	3.27	0.01	3.52	0.03
3.36	0.04	3.25	0.02	3.53	0.02	3.78	0.05
3.61	0.07	3.5	0.04	3.78	0.04	4.03	0.07
3.87	0.13	3.75	0.05	4.03	0.06		

Appendix 11.3.1-2 *Growth rate values from the bioreactor, which were used to draw growth rate charts for all weeks of the preliminary experiment, including week 3, which had contamination.*

11.3.2 Preliminary experiment data from the automatic colony counter

Data: Preliminary experiment - week 1							
Results Group Name	Plate Id	Method	Counted Colonies	Counted Volume	Comments	Dilution Factor	Inoculated Volume	Inoculation mode
Count Results	1e+02_EC_t4b	TEMPLATE_Fuzzy	14	0.384709775	uncountable	100	0.5	Spread
Count Results	1e+02_EC_t4a	TEMPLATE_Fuzzy	14	0.384709775	uncountable	100	0.5	Spread
Count Results	1e+03_EC_t4b	TEMPLATE_Fuzzy	750	0.384709775	uncountable	1000	0.5	Spread
Count Results	1e+03_EC_t4a	TEMPLATE_Fuzzy	51	0.384709775	uncountable	1000	0.5	Spread
Count Results	1e+04_EC_t4b	TEMPLATE_Fuzzy	119	0.384709775	uncountable	10000	0.5	Spread
Count Results	1e+04_EC_t4a	TEMPLATE_Fuzzy	509	0.384709775	uncountable	10000	0.5	Spread
Count Results	1e+04_EC_t3b	TEMPLATE_Fuzzy	185	0.384709775		10000	0.5	Spread
Count Results	1e+04_EC_t3a	TEMPLATE_Fuzzy	150	0.384709775		10000	0.5	Spread
Count Results	1e+03_EC_t3b	TEMPLATE_Fuzzy	154	0.384709775	uncountable	1000	0.5	Spread
Count Results	1e+03_EC_t3a	TEMPLATE_Fuzzy	13	0.384709775	uncountable	1000	0.5	Spread
Count Results	1e+02_EC_t3b	TEMPLATE_Fuzzy	5	0.384709775	uncountable	100	0.5	Spread
Count Results	1e+02_EC_t3a	TEMPLATE_Fuzzy	0	0.384709775	uncountable	100	0.5	Spread
Count Results	1e+02_EC_t2b	TEMPLATE_Fuzzy	209	0.384709775		100	0.5	Spread
Count Results	1e+02_EC_t2a	TEMPLATE_Fuzzy	269	0.384709775		100	0.5	Spread
Count Results	1e+03_EC_t2b	TEMPLATE_Fuzzy	77	0.384709775		1000	0.5	Spread
Count Results	1e+03_EC_t2a	TEMPLATE_Fuzzy	67	0.384709775		1000	0.5	Spread
Count Results	1e+04_EC_t2b	TEMPLATE_Fuzzy	10	0.384709775		10000	0.5	Spread
Count Results	1e+04_EC_t2a	TEMPLATE_Fuzzy	9	0.384709775		10000	0.5	Spread
Count Results	1e+04_EC_t1b	TEMPLATE_Fuzzy	8	0.384709775		10000	0.5	Spread
Count Results	1e+04_EC_t1a	TEMPLATE_Fuzzy	13	0.384709775	7	10000	0.5	Spread
Count Results	1e+03_EC_t1a	TEMPLATE_Fuzzy	46	0.384709775		1000	0.5	Spread
Count Results	1e+03_EC_t1b	TEMPLATE_Fuzzy	58	0.384709775		1000	0.5	Spread
Count Results	1e+02_EC_t1b	TEMPLATE_Fuzzy	236	0.384709775		100	0.5	Spread
Count Results	1e+02_EC_t1a	TEMPLATE_Fuzzy	249	0.384709775		100	0.5	Spread
Count Results	1e+02_EC_t0b	TEMPLATE_Fuzzy	245	0.384709775		100	0.5	Spread
Count Results	1e+02_EC_t0a	TEMPLATE_Fuzzy	245	0.384709775		100	0.5	Spread
Count Results	1e+03_EC_t0b	TEMPLATE_Fuzzy	45	0.384709775		1000	0.5	Spread
Count Results	1e+03_EC_t0a	TEMPLATE_Fuzzy	43	0.384709775		1000	0.5	Spread
Count Results	1e+04_EC_t0b	TEMPLATE_Fuzzy	9	0.384709775		10000	0.5	Spread
Count Results	1e+04_EC_t0a	TEMPLATE_Fuzzy	11	0.384709775	5	10000	0.5	Spread

Appendix 11.3.2-1 *Unprocessed data from the automatic colony counter which were used for the calculation of the cfu/mL, data from week 1 of the preliminary experiment.*

Data: Preliminary experiment - week 2								
Results Group Name	**Plate Id**	**Method**	**Counted Colonies**	**Counted Volume**	**Comments**	**Dilution Factor**	**Inoculated Volume**	**Inoculation mode**
Count Results	1e+04_Ec_t4b	TEMPLATE_Fuzzy	167	0.38640213		10000	0.5	Spread
Count Results	1e+04_Ec_t4a	TEMPLATE_Fuzzy	201	0.38640213		1000	0.5	Spread
Count Results	1e+03_Ec_t4b	TEMPLATE_Fuzzy	3	0.38640213	uncountable	1000	0.5	Spread
Count Results	1e+03_Ec_t4a	TEMPLATE_Fuzzy	0	0.38640213	uncountable	1000	0.5	Spread
Count Results	1e+02_Ec_t4b	TEMPLATE_Fuzzy	2	0.38640213	uncountable	100	0.5	Spread
Count Results	1e+02_Ec_t4a	TEMPLATE_Fuzzy	12	0.38640213	uncountable	100	0.5	Spread
Count Results	1e+04_Ec_t3b	TEMPLATE_Fuzzy	150	0.38640213		10000	0.5	Spread
Count Results	1e+04_Ec_t3a	TEMPLATE_Fuzzy	163	0.38640213		10000	0.5	Spread
Count Results	1e+03_Ec_t3b	TEMPLATE_Fuzzy	279	0.38640213		1000	0.5	Spread
Count Results	1e+03_Ec_t3a	TEMPLATE_Fuzzy	203	0.38640213		1000	0.5	Spread
Count Results	1e+02_Ec_t3b	TEMPLATE_Fuzzy	122	0.38640213		100	0.5	Spread
Count Results	1e+02_Ec_t3a	TEMPLATE_Fuzzy	53	0.38640213		100	0.5	Spread
Count Results	1e+04_Ec_t2b	TEMPLATE_Fuzzy	104	0.38640213		10000	0.5	Spread
Count Results	1e+04_Ec_t2a	TEMPLATE_Fuzzy	182	0.38640213		10000	0.5	Spread
Count Results	1e+03_Ec_t2b	TEMPLATE_Fuzzy	262	0.38640213		1000	0.5	Spread
Count Results	1e+03_Ec_t2a	TEMPLATE_Fuzzy	182	0.38640213		1000	0.5	Spread
Count Results	1e+02_Ec_t2b	TEMPLATE_Fuzzy	162	0.38640213		100	0.5	Spread
Count Results	1e+02_Ec_t2a	TEMPLATE_Fuzzy	137	0.38640213		100	0.5	Spread
Count Results	1e+04_Ec_t1b	TEMPLATE_Fuzzy	72	0.38640213		10000	0.5	Spread
Count Results	1e+04_Ec_t1a	TEMPLATE_Fuzzy	111	0.38640213		10000	0.5	Spread
Count Results	1e+03_Ec_t1b	TEMPLATE_Fuzzy	131	0.38640213		1000	0.5	Spread
Count Results	1e+03_Ec_t1a	TEMPLATE_Fuzzy	159	0.38640213		1000	0.5	Spread
Count Results	1e+02_Ec_t1b	TEMPLATE_Fuzzy	233	0.38640213		100	0.5	Spread
Count Results	1e+02_Ec_t1a	TEMPLATE_Fuzzy	260	0.38640213		100	0.5	Spread
Count Results	1e+04_Ec_t0b	TEMPLATE_Fuzzy	62	0.38640213		10000	0.5	Spread
Count Results	1e+04_Ec_t0a	TEMPLATE_Fuzzy	67	0.38640213		10000	0.5	Spread
Count Results	1e+03_Ec_t0b	TEMPLATE_Fuzzy	123	0.38640213		1000	0.5	Spread
Count Results	1e+03_Ec_t0a	TEMPLATE_Fuzzy	87	0.38640213		1000	0.5	Spread
Count Results	1e+02_Ec_t0b	TEMPLATE_Fuzzy	318	0.38640213		100	0.5	Spread
Count Results	1e+02_Ec_t0a	TEMPLATE_Fuzzy	298	0.38640213		100	0.5	Spread

Appendix 11.3.2-2 *Unprocessed data from the automatic colony counter which were used for the calculation of the cfu/mL, data from week 2 of the preliminary experiment.*

Data: Preliminary experiment - repeated week 3								
Results Group Name	**Plate Id**	**Method**	**Counted Colonies**	**Counted Volume**	**Comments**	**Dilution Factor**	**Inoculated Volume**	**Inoculation mode**
Count Results	EC_ER_lag_week3_rep_t3_4b	TEMPLATE_Sharp	8	0.384709775	15	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t3_4a	TEMPLATE_Sharp	14	0.384709775	18	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t3_3b	TEMPLATE_Sharp	164	0.384709775	uncountable	1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t3_3b	TEMPLATE_Sharp	0	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t3_3a	TEMPLATE_Sharp	308	0.384709775	uncountable	1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t3_2b	TEMPLATE_Sharp	224	0.384709775		100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t3_2a	TEMPLATE_Sharp	318	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t2_4b	TEMPLATE_Sharp	7	0.384709775	10	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t2_4a	TEMPLATE_Sharp	20	0.384709775		10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t2_3b	TEMPLATE_Sharp	71	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t2_3a	TEMPLATE_Sharp	80	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t2_2b	TEMPLATE_Sharp	113	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t2_2a	TEMPLATE_Sharp	207	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1.5_4b	TEMPLATE_Sharp	7	0.384709775		10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1.5_4a	TEMPLATE_Sharp	13	0.384709775		10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1.5_3b	TEMPLATE_Sharp	69	0.384709775	74	1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1.5_3a	TEMPLATE_Sharp	74	0.384709775	64	1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1.5_2b	TEMPLATE_Sharp	159	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1.5_2a	TEMPLATE_Sharp	147	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1_4b	TEMPLATE_Sharp	19	0.384709775	5	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1_4a	TEMPLATE_Sharp	3	0.384709775	6	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1_3b	TEMPLATE_Sharp	34	0.384709775	57	1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1_3a	TEMPLATE_Sharp	44	0.384709775	49	1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1_2b	TEMPLATE_Sharp	187	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t1_2a	TEMPLATE_Sharp	92	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0.5_4b	TEMPLATE_Sharp	2	0.384709775	4	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0.5_4a	TEMPLATE_Sharp	5	0.384709775		10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0.5_3b	TEMPLATE_Sharp	49	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0.5_3a	TEMPLATE_Sharp	59	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0.5_2b	TEMPLATE_Sharp	165	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0.5_2a	TEMPLATE_Sharp	158	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0_4b	TEMPLATE_Sharp	6	0.384709775	11	10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0_4a	TEMPLATE_Sharp	7	0.384709775		10000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0_3b	TEMPLATE_Sharp	51	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0_3a	TEMPLATE_Sharp	46	0.384709775		1000	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0_2b	TEMPLATE_Sharp	220	0.384709775	uncountable	100	0.5	Spread
Count Results	EC_ER_lag_week3_rep_t0_2a	TEMPLATE_Sharp	279	0.384709775	uncountable	100	0.5	Spread

Appendix 11.3.2-3 Unprocessed data from the automatic colony counter which were used for the calculation of the cfu/mL, data from repeated week 3 of the preliminary experiment.

11.4 MIC test data

Blank-corrected data, negative control values subtracted from all the other values. In the columns are average values from wells 1-3.

MIC test blank-corrected data - lag phase cells							
Time [h]	OD values						
	512 µg	256 µg	128 µg	64 µg	32 µg	16 µg	Positive control
0.48	0.7980	0.3057	0.1407	0.0057	0.0137	0.0007	0.0083
0.98	0.8023	0.3063	0.1413	0.0120	0.0177	0.0033	0.0107
1.48	0.8060	0.3060	0.1420	0.0250	0.0267	0.0110	0.0153
1.98	0.8093	0.3063	0.1503	0.0490	0.0447	0.0260	0.0247
2.48	0.8127	0.3067	0.1680	0.0907	0.0750	0.0510	0.0440
2.98	0.8170	0.3093	0.2037	0.1530	0.1150	0.0883	0.0753
3.48	0.8190	0.3117	0.2587	0.2463	0.1807	0.1397	0.1220
3.98	0.8210	0.3133	0.3503	0.4117	0.2917	0.2470	0.2343
4.48	0.8230	0.3153	0.4493	0.6773	0.5333	0.3850	0.3727
4.98	0.8250	0.3163	0.5527	0.7930	0.6440	0.5230	0.4310
5.48	0.8273	0.3173	0.6470	0.8683	0.7383	0.6707	0.5403
5.98	0.8283	0.3183	0.7363	0.9523	0.8370	0.7787	0.6543
6.48	0.8310	0.3190	0.8257	1.0217	0.9250	0.8773	0.7723
6.98	0.8310	0.3193	0.8867	1.0760	0.9960	0.9643	0.8817
7.48	0.8323	0.3207	0.9393	1.1127	1.0470	1.0263	0.9590
7.98	0.8337	0.3213	0.9833	1.1420	1.0897	1.0710	1.0107
8.48	0.8357	0.3233	1.0183	1.1597	1.1190	1.0993	1.0457
8.98	0.8363	0.3247	1.0487	1.1657	1.1310	1.1167	1.0680
9.48	0.8370	0.3260	1.0753	1.1703	1.1433	1.1307	1.0857
9.98	0.8383	0.3283	1.0980	1.1730	1.1523	1.1413	1.0990
10.48	0.8397	0.3303	1.1163	1.1747	1.1563	1.1473	1.1090
10.98	0.8407	0.3317	1.1333	1.1760	1.1577	1.1503	1.1157
11.48	0.8427	0.3330	1.1467	1.1783	1.1600	1.1547	1.1227
11.98	0.8443	0.3350	1.1567	1.1810	1.1610	1.1587	1.1277
12.48	0.8457	0.3387	1.1677	1.1863	1.1613	1.1620	1.1327
12.98	0.8473	0.3410	1.1813	1.1917	1.1620	1.1643	1.1370
13.48	0.8490	0.3457	1.1917	1.1963	1.1633	1.1673	1.1417
13.98	0.8513	0.3497	1.1993	1.2027	1.1633	1.1707	1.1467
14.48	0.8527	0.3587	1.2067	1.2077	1.1647	1.1723	1.1523
14.98	0.8543	0.3663	1.2140	1.2133	1.1653	1.1760	1.1570
15.48	0.8567	0.3737	1.2207	1.2187	1.1657	1.1783	1.1633
15.98	0.8587	0.3800	1.2300	1.2237	1.1663	1.1823	1.1690
16.48	0.8607	0.3880	1.2360	1.2297	1.1673	1.1860	1.1740
16.98	0.8623	0.3957	1.2410	1.2350	1.1680	1.1897	1.1797
17.48	0.8647	0.4043	1.2470	1.2397	1.1693	1.1933	1.1837
17.98	0.8663	0.4097	1.2530	1.2450	1.1717	1.1967	1.1890
18.48	0.8673	0.4153	1.2570	1.2510	1.1737	1.2010	1.1937
18.98	0.8693	0.4223	1.2637	1.2570	1.1770	1.2033	1.1970
19.48	0.8713	0.4313	1.2690	1.2617	1.1823	1.2063	1.2010
19.98	0.8730	0.4420	1.2720	1.2663	1.1883	1.2087	1.2040
20.48	0.8753	0.4547	1.2747	1.2713	1.1927	1.2103	1.2080
20.98	0.8760	0.4690	1.2787	1.2787	1.1960	1.2123	1.2117

MIC test blank-corrected data - log phase cells							
Time [h]	OD values						
	512 µg	256 µg	128 µg	64 µg	32 µg	16 µg	Positive control
0.48	0.6550	0.3303	-0.0133	-0.0097	0.0203	-0.0107	-0.0077
0.98	0.6643	0.3420	-0.0090	-0.0050	0.0243	-0.0063	-0.0040
1.48	0.6677	0.3440	-0.0050	0.0063	0.0350	0.0027	0.0050
1.98	0.6687	0.3450	0.0020	0.0303	0.0570	0.0207	0.0227
2.48	0.6693	0.3463	0.0163	0.0737	0.0893	0.0537	0.0550
2.98	0.6693	0.3477	0.0540	0.1643	0.1740	0.1183	0.1097
3.48	0.6703	0.3483	0.1383	0.4030	0.2930	0.2427	0.2257
3.98	0.6710	0.3490	0.2453	0.7393	0.5040	0.3990	0.3783
4.48	0.6707	0.3507	0.3537	0.8777	0.6673	0.5530	0.5297
4.98	0.6713	0.3503	0.4500	0.9467	0.7797	0.7063	0.6927
5.48	0.6727	0.3507	0.5380	1.0127	0.8737	0.8167	0.8110
5.98	0.6727	0.3513	0.6077	1.0697	0.9563	0.9083	0.9053
6.48	0.6743	0.3530	0.6640	1.1123	1.0183	0.9890	0.9820
6.98	0.6740	0.3527	0.7193	1.1430	1.0707	1.0510	1.0360
7.48	0.6747	0.3540	0.7720	1.1673	1.1090	1.0940	1.0767
7.98	0.6750	0.3543	0.8183	1.1827	1.1340	1.1227	1.1020
8.48	0.6757	0.3550	0.8653	1.1910	1.1490	1.1417	1.1193
8.98	0.6760	0.3547	0.9107	1.1977	1.1610	1.1540	1.1333
9.48	0.6757	0.3543	0.9493	1.2010	1.1633	1.1617	1.1433
9.98	0.6767	0.3543	0.9817	1.2033	1.1670	1.1687	1.1507
10.48	0.6773	0.3547	1.0037	1.2057	1.1683	1.1733	1.1570
10.98	0.6767	0.3547	1.0267	1.2067	1.1703	1.1757	1.1610
11.48	0.6770	0.3540	1.0447	1.2077	1.1723	1.1793	1.1647
11.98	0.6777	0.3527	1.0600	1.2090	1.1730	1.1813	1.1673
12.48	0.6787	0.3540	1.0720	1.2117	1.1753	1.1850	1.1707
12.98	0.6783	0.3537	1.0840	1.2133	1.1763	1.1873	1.1733
13.48	0.6790	0.3527	1.0943	1.2160	1.1773	1.1897	1.1770
13.98	0.6823	0.3530	1.1023	1.2187	1.1783	1.1920	1.1797
14.48	0.6863	0.3530	1.1103	1.2223	1.1793	1.1937	1.1820
14.98	0.6907	0.3553	1.1167	1.2250	1.1793	1.1960	1.1880
15.48	0.6957	0.3607	1.1233	1.2290	1.1790	1.1980	1.1913
15.98	0.7027	0.3660	1.1297	1.2343	1.1793	1.2013	1.1947
16.48	0.7113	0.3740	1.1353	1.2383	1.1800	1.2033	1.1987
16.98	0.7223	0.3877	1.1397	1.2423	1.1830	1.2057	1.2030
17.48	0.7333	0.4020	1.1437	1.2473	1.1840	1.2063	1.2070
17.98	0.7483	0.4160	1.1480	1.2547	1.1847	1.2093	1.2103
18.48	0.7653	0.4293	1.1530	1.2603	1.1877	1.2107	1.2140
18.98	0.7837	0.4427	1.1573	1.2677	1.1907	1.2130	1.2170
19.48	0.8057	0.4557	1.1623	1.2720	1.1957	1.2177	1.2210
19.98	0.8220	0.4690	1.1643	1.2783	1.2013	1.2203	1.2237
20.48	0.8390	0.4817	1.1690	1.2847	1.2047	1.2250	1.2260
20.98	0.8547	0.4907	1.1717	1.2917	1.2087	1.2297	1.2280

MIC test blank-corrected data - stat cells							
	OD values						
Time [h]	512 µg	256 µg	128 µg	64 µg	32 µg	16 µg	Positive control
0.48	0.3563	0.0953	0.0190	0.0007	-0.0047	0.0080	0.0020
0.73	0.3783	0.1337	0.0233	0.0017	-0.0047	0.0087	0.0020
0.98	0.3807	0.1420	0.0247	0.0010	-0.0037	0.0090	0.0017
1.23	0.3810	0.1450	0.0240	0.0023	-0.0037	0.0093	0.0017
1.48	0.3810	0.1457	0.0237	0.0020	-0.0037	0.0097	0.0017
1.73	0.3800	0.1463	0.0240	0.0033	-0.0033	0.0103	0.0030
1.98	0.3800	0.1473	0.0227	0.0043	-0.0027	0.0113	0.0040
2.23	0.3793	0.1490	0.0233	0.0067	-0.0013	0.0133	0.0063
2.48	0.3790	0.1493	0.0227	0.0100	0.0007	0.0160	0.0097
2.73	0.3783	0.1500	0.0223	0.0167	0.0043	0.0207	0.0150
2.98	0.3787	0.1527	0.0220	0.0253	0.0100	0.0267	0.0223
3.23	0.3787	0.1550	0.0220	0.0393	0.0177	0.0360	0.0323
3.48	0.3777	0.1570	0.0220	0.0600	0.0290	0.0490	0.0457
3.73	0.3777	0.1580	0.0213	0.0853	0.0440	0.0627	0.0623
3.98	0.3773	0.1583	0.0213	0.1197	0.0647	0.0810	0.0830
4.23	0.3763	0.1597	0.0217	0.1743	0.0950	0.1163	0.1050
4.48	0.3753	0.1603	0.0210	0.2610	0.1517	0.1753	0.1443
4.73	0.3747	0.1607	0.0203	0.3587	0.2197	0.2267	0.2000
4.98	0.3737	0.1613	0.0200	0.4623	0.3033	0.2897	0.2543
5.23	0.3733	0.1617	0.0197	0.5687	0.4297	0.3337	0.3013
5.48	0.3730	0.1620	0.0197	0.6420	0.5210	0.3840	0.3480
5.73	0.3723	0.1637	0.0200	0.6777	0.5747	0.4043	0.3673
5.98	0.3720	0.1627	0.0197	0.7300	0.6187	0.4543	0.4060
6.23	0.3717	0.1633	0.0193	0.7790	0.6653	0.5107	0.4707
6.48	0.3720	0.1637	0.0197	0.8273	0.7067	0.5573	0.5230
6.73	0.3717	0.1640	0.0193	0.8713	0.7497	0.6027	0.5730
6.98	0.3717	0.1640	0.0207	0.9153	0.7973	0.6547	0.6320
7.23	0.3720	0.1630	0.0213	0.9593	0.8447	0.7103	0.6937
7.48	0.3720	0.1623	0.0223	0.9987	0.8877	0.7667	0.7540
7.73	0.3713	0.1607	0.0257	1.0347	0.9267	0.8197	0.8073
7.98	0.3713	0.1607	0.0317	1.0637	0.9620	0.8673	0.8527
8.23	0.3710	0.1600	0.0363	1.0880	0.9897	0.9067	0.8910
8.48	0.3707	0.1593	0.0423	1.1080	1.0150	0.9403	0.9227
8.73	0.3703	0.1593	0.0503	1.1243	1.0380	0.9687	0.9513
8.98	0.3700	0.1590	0.0567	1.1373	1.0550	0.9933	0.9750
9.23	0.3700	0.1583	0.0653	1.1473	1.0687	1.0147	0.9967
9.48	0.3693	0.1590	0.0720	1.1540	1.0790	1.0310	1.0120
9.73	0.3690	0.1573	0.0780	1.1590	1.0887	1.0473	1.0267
9.98	0.3693	0.1573	0.0920	1.1647	1.0960	1.0613	1.0403
10.23	0.3693	0.1563	0.1133	1.1670	1.1020	1.0727	1.0490
10.48	0.3683	0.1560	0.1467	1.1700	1.1077	1.0813	1.0563
10.73	0.3683	0.1567	0.1787	1.1727	1.1123	1.0890	1.0647
10.98	0.3687	0.1560	0.2103	1.1743	1.1167	1.0943	1.0707
11.23	0.3690	0.1577	0.2440	1.1760	1.1213	1.1003	1.0773

11.48	0.3687	0.1567	0.2747	1.1777	1.1243	1.1043	1.0827
11.73	0.3680	0.1570	0.3143	1.1790	1.1283	1.1090	1.0880
11.98	0.3683	0.1560	0.3507	1.1793	1.1320	1.1130	1.0930
12.23	0.3683	0.1550	0.3853	1.1807	1.1340	1.1150	1.0967
12.48	0.3687	0.1553	0.4177	1.1817	1.1380	1.1173	1.1007
12.73	0.3687	0.1547	0.4513	1.1833	1.1387	1.1200	1.1040
12.98	0.3687	0.1550	0.4917	1.1847	1.1413	1.1223	1.1077
13.23	0.3687	0.1557	0.5283	1.1870	1.1420	1.1240	1.1097
13.48	0.3687	0.1543	0.5637	1.1890	1.1457	1.1267	1.1127
13.73	0.3693	0.1540	0.5903	1.1907	1.1470	1.1287	1.1153
13.98	0.3687	0.1553	0.6147	1.1917	1.1483	1.1313	1.1180
14.23	0.3690	0.1557	0.6397	1.1940	1.1487	1.1333	1.1200
14.48	0.3693	0.1540	0.6613	1.1970	1.1510	1.1357	1.1230
14.73	0.3700	0.1537	0.6807	1.1990	1.1517	1.1377	1.1243
14.98	0.3700	0.1530	0.7040	1.2013	1.1533	1.1400	1.1267
15.23	0.3697	0.1527	0.7250	1.2040	1.1547	1.1420	1.1287
15.48	0.3707	0.1533	0.7440	1.2073	1.1553	1.1433	1.1307
15.73	0.3703	0.1540	0.7630	1.2103	1.1567	1.1453	1.1323
15.98	0.3713	0.1543	0.7823	1.2137	1.1577	1.1467	1.1340
16.23	0.3713	0.1527	0.8020	1.2163	1.1597	1.1483	1.1357
16.48	0.3713	0.1533	0.8187	1.2190	1.1603	1.1497	1.1370
16.73	0.3720	0.1543	0.8343	1.2227	1.1600	1.1507	1.1383
16.98	0.3723	0.1583	0.8510	1.2257	1.1613	1.1513	1.1397
17.23	0.3723	0.1547	0.8710	1.2293	1.1633	1.1527	1.1410
17.48	0.3727	0.1577	0.8907	1.2320	1.1653	1.1547	1.1430
17.73	0.3730	0.1550	0.9083	1.2353	1.1663	1.1557	1.1437
17.98	0.3737	0.1563	0.9237	1.2383	1.1680	1.1563	1.1453
18.23	0.3740	0.1567	0.9390	1.2417	1.1690	1.1580	1.1463
18.48	0.3747	0.1550	0.9600	1.2450	1.1710	1.1583	1.1470
18.73	0.3750	0.1563	0.9727	1.2487	1.1730	1.1597	1.1487
18.98	0.3747	0.1537	0.9823	1.2523	1.1750	1.1603	1.1500
19.23	0.3760	0.1537	0.9933	1.2557	1.1770	1.1610	1.1503
19.48	0.3763	0.1537	1.0033	1.2587	1.1797	1.1627	1.1517
19.73	0.3767	0.1540	1.0120	1.2617	1.1823	1.1640	1.1530
19.98	0.3770	0.1540	1.0213	1.2640	1.1847	1.1637	1.1540
20.23	0.3773	0.1530	1.0297	1.2670	1.1870	1.1647	1.1540

11.5 Illumination Experiment data

11.5.1 Blank-corrected OD data

LAG - week 1				
	OD, λ = 850			
Time [hours]	water+light	water+no light	zinc+light	zinc+no light
0.38	0	0	0	0
0.63	-0.01	-0.01	0	0
0.88	0	-0.01	0	0
1.14	0	0	0	0.01
1.39	0	0	0	0.01
1.64	0	0	0.01	0.01
1.9	0.01	0	0.01	0.01
2.15	0.02	0.01	0.02	0.02
2.4	0.02	0.03	0.02	0.02
2.66	0.04	0.04	0.02	0.04
2.91	0.06	0.07	0.03	0.05
3.16	0.09	0.14	0.03	0.08
3.42	0.13	0.24	0.04	0.11
3.67	0.19	0.38	0.05	0.16
3.93	0.25	0.53	0.06	0.2
4.18	0.29	0.65	0.07	0.26
4.43	0.37	0.75	0.08	0.32
4.69	0.5	0.93	0.1	0.41
4.94	0.68	1.11	0.12	0.49
5.19	0.78	1.17	0.15	0.63
5.45	0.85	1.21	0.17	0.69
5.7	0.88	1.24	0.2	0.74
5.95	0.93	1.25	0.23	0.79
6.21	0.96	1.27	0.27	0.86
6.46	0.98	1.28	0.29	0.91
6.72	1.01	1.29	0.35	0.95
6.97	1.02	1.29	0.38	0.95
7.22	1.02	1.3	0.44	1.01
7.48	1.03	1.3	0.48	0.99
7.73	1.05	1.3	0.5	1.05
7.98	1.05	1.3	0.5	1.06
8.24	1.05	1.3	0.52	1.07
8.49	1.06	1.3	0.52	1.07
8.74	1.05	1.3	0.52	1.04
9	1.06	1.3	0.54	1.04
9.25	1.06	1.3	0.53	1.08
9.51	1.06	1.3	0.54	1.08

9.76	1.06	1.3	0.53	1.08
10.01	1.05	1.29	0.54	1.04
10.27	1.06	1.29	0.54	1.08
10.52	1.06	1.29	0.55	1.08
10.77	1.05	1.28	0.55	1.08
11.03	1.05	1.28	0.55	1.07
11.28	1.05	1.28	0.55	1.07
11.53	1.04	1.27	0.56	1.07
11.79	1.05	1.27	0.56	1.07
12.04	1.05	1.27	0.56	1.03
12.29	1.05	1.27	0.56	1.03
12.55	1.04	1.26	0.56	1.03
12.8	1.04	1.27	0.57	1.03
13.06	1.04	1.26	0.56	1.03
13.31	1.04	1.26	0.57	1.02
13.56	1.04	1.26	0.56	1.05
13.82	1.03	1.26	0.57	1.02
14.07	1.04	1.27	0.57	1.05
14.32	1.03	1.27	0.57	1.06
14.58	1.03	1.26	0.57	1.06
14.83	1.03	1.26	0.57	1.02
15.08	1.03	1.26	0.57	1.05
15.34	1.03	1.26	0.57	1.06
15.59	1.02	1.26	0.57	1.02
15.85	1.02	1.26	0.56	1.02
16.1	1.02	1.26	0.56	1.06
16.35	1.03	1.27	0.56	1.02
16.61	1.03	1.26	0.56	1.02
16.86	1.02	1.26	0.56	1.06
17.11	1.02	1.27	0.56	1.06
17.37	1.02	1.27	0.56	1.06
17.62	1.02	1.26	0.56	1.06
17.87	1.02	1.26	0.55	1.06
18.13	1.02	1.26	0.55	1.03
18.38	1.02	1.26	0.55	1.03
18.63	1.02	1.26	0.54	1.07
18.89	1.02	1.26	0.54	1.03
19.14	1.02	1.26	0.54	1.04
19.4	1.02	1.26	0.54	1.07
19.65	1.02	1.26	0.53	1.07
19.9	1.02	1.26	0.52	1.07

LAG - week 2				
Time [hours]	OD, λ = 850			
	water+light	water+no light	zinc+light	zinc+no light
0.26	0	0	0	0
0.51	0	0	0	0
0.76	0	0	0	0
1.02	0	0	0	0
1.27	0	0	0	0
1.52	0.01	0.01	0	0
1.78	0.02	0.01	0	0
2.03	0.03	0.02	0	0
2.28	0.04	0.04	0	0
2.54	0.08	0.07	0	0
2.79	0.13	0.12	0	0
3.05	0.21	0.22	0.01	0
3.3	0.33	0.36	0.01	0
3.55	0.45	0.53	0.01	0
3.81	0.54	0.71	0.01	0.01
4.06	0.58	0.8	0.02	0.01
4.31	0.65	0.94	0.02	0.01
4.57	0.69	0.96	0.03	0.01
4.82	0.75	1.05	0.04	0.01
5.07	0.83	1.08	0.04	0.02
5.33	0.91	1.13	0.05	0.02
5.58	0.93	1.14	0.06	0.03
5.83	0.95	1.15	0.07	0.04
6.09	0.97	1.17	0.09	0.05
6.34	0.99	1.17	0.1	0.05
6.6	0.99	1.19	0.11	0.07
6.85	1	1.18	0.14	0.08
7.1	1	1.19	0.16	0.09
7.36	1	1.18	0.19	0.11
7.61	0.99	1.19	0.22	0.14
7.86	1	1.18	0.24	0.16
8.12	0.99	1.18	0.29	0.19
8.37	0.99	1.18	0.32	0.21
8.62	1.01	1.18	0.37	0.27
8.88	0.99	1.18	0.4	0.33
9.13	0.99	1.17	0.4	0.39
9.39	1	1.17	0.43	0.42
9.64	1	1.17	0.45	0.47

9.89	0.99	1.16	0.5	0.48
10.15	0.99	1.17	0.54	0.55
10.4	1	1.16	0.57	0.58
10.65	0.99	1.16	0.59	0.63
10.91	0.99	1.16	0.61	0.72
11.16	0.99	1.16	0.63	0.75
11.41	0.99	1.16	0.64	0.75
11.67	1	1.15	0.63	0.77
11.92	1	1.15	0.64	0.79
12.17	0.99	1.15	0.67	0.8
12.43	0.99	1.15	0.68	0.81
12.68	0.99	1.15	0.67	0.85
12.94	1	1.15	0.67	0.85
13.19	0.99	1.15	0.66	0.83
13.44	0.98	1.15	0.66	0.84
13.7	0.99	1.15	0.68	0.84
13.95	0.98	1.15	0.69	0.84
14.2	0.99	1.15	0.68	0.84
14.46	0.98	1.14	0.65	0.84
14.71	0.99	1.15	0.68	0.84
14.96	0.99	1.15	0.65	0.84
15.22	0.99	1.14	0.68	0.84
15.47	0.98	1.14	0.65	0.84
15.73	0.98	1.14	0.66	0.85
15.98	0.97	1.14	0.65	0.85
16.23	0.99	1.14	0.65	0.85
16.49	0.99	1.14	0.68	0.83
16.74	0.99	1.14	0.67	0.85
16.99	0.99	1.14	0.68	0.83
17.25	0.99	1.14	0.67	0.83
17.5	0.99	1.14	0.67	0.85
17.75	0.99	1.14	0.67	0.85
18.01	0.99	1.14	0.67	0.83
18.26	0.99	1.14	0.68	0.83
18.51	0.99	1.14	0.67	0.85
18.77	0.99	1.14	0.68	0.83
19.02	0.99	1.14	0.67	0.83
19.28	0.97	1.13	0.68	0.85
19.53	0.99	1.13	0.68	0.83
19.78	0.98	1.14	0.68	0.84
20.04	0.98	1.13	0.67	0.85

LAG - week 3				
	OD, λ = 850			
Time [hours]	water+light	water+no light	zinc+light	zinc+no light
0.26	0	0	0	0
0.51	0	0	0	0
0.76	0	0	0	0
1.02	0	0	0	0
1.27	0	0	0	0
1.52	0	0.01	0	0
1.78	0.01	0.01	0.01	0
2.03	0.02	0.02	0.02	0.01
2.28	0.03	0.03	0.04	0.01
2.54	0.05	0.05	0.06	0.02
2.79	0.08	0.08	0.09	0.03
3.04	0.14	0.14	0.15	0.04
3.3	0.22	0.24	0.22	0.06
3.55	0.33	0.38	0.29	0.09
3.81	0.46	0.54	0.44	0.13
4.06	0.56	0.74	0.61	0.17
4.31	0.6	0.89	0.73	0.23
4.57	0.65	0.94	0.82	0.3
4.82	0.76	1.04	0.91	0.39
5.07	0.85	1.1	0.98	0.47
5.33	0.89	1.14	1.05	0.55
5.58	0.94	1.17	1.07	0.59
5.83	1	1.2	1.06	0.63
6.09	1.02	1.19	1.08	0.69
6.34	1.02	1.21	1.14	0.74
6.6	1.02	1.21	1.15	0.8
6.85	1.03	1.22	1.16	0.85
7.1	1.06	1.23	1.17	0.87
7.36	1.05	1.24	1.13	0.87
7.61	1.05	1.23	1.17	0.95
7.86	1.05	1.23	1.17	0.98
8.12	1.06	1.22	1.13	0.97
8.37	1.04	1.22	1.17	0.99
8.62	1.04	1.23	1.17	1.02
8.88	1.05	1.22	1.17	1.01
9.13	1.04	1.21	1.13	1.03
9.38	1.04	1.21	1.13	1.03
9.64	1.04	1.22	1.12	1.03
9.89	1.05	1.22	1.16	1.03

10.15	1.04	1.21	1.12	1.03
10.4	1.04	1.21	1.12	1.03
10.65	1.04	1.2	1.16	1.03
10.91	1.03	1.2	1.16	1.06
11.16	1.04	1.21	1.15	1.03
11.41	1.02	1.21	1.15	1.02
11.67	1.03	1.2	1.15	1.03
11.92	1.02	1.2	1.15	1.03
12.17	1.03	1.21	1.15	1.03
12.43	1.02	1.2	1.16	1.03
12.68	1.03	1.2	1.16	1.02
12.94	1.02	1.2	1.15	1.04
13.19	1.03	1.2	1.16	1.03
13.44	1.02	1.2	1.16	1.01
13.7	1.04	1.2	1.12	1.02
13.95	1.03	1.21	1.15	1
14.2	1.02	1.2	1.16	1.02
14.46	1.03	1.19	1.16	1.02
14.71	1.03	1.2	1.12	1.03
14.96	1.03	1.19	1.16	1.03
15.22	1.02	1.19	1.12	1.05
15.47	1.03	1.19	1.16	1.04
15.73	1.03	1.19	1.12	1.04
15.98	1.02	1.2	1.15	1.06
16.23	1.03	1.19	1.16	1.05
16.49	1.03	1.19	1.15	1.03
16.74	1.02	1.2	1.15	1.01
16.99	1.02	1.19	1.16	1.01
17.25	1.02	1.2	1.12	1.04
17.5	1.02	1.2	1.15	1.04
17.75	1.03	1.19	1.15	1.05
18.01	1.03	1.19	1.12	1.03
18.26	1.02	1.19	1.15	1.03
18.51	1.01	1.2	1.15	1.03
18.77	1.02	1.19	1.12	1.05
19.02	1.02	1.18	1.15	1.05
19.28	1.02	1.19	1.15	1.05
19.53	1.03	1.18	1.15	1.06
19.78	1.03	1.19	1.15	1.04
20.04	1.03	1.18	1.11	1.04

LAG - week 4				
	OD, λ = 850			
Time [hours]	water+light	water+no light	zinc+light	zinc+no light
0.5	0	0	0	0
0.75	0	0	0	0
1.01	0	0	0	0.01
1.26	0	0	0	0.01
1.51	0	0	0	0.01
1.77	0	0.01	0	0.01
2.02	0.01	0.01	0	0.01
2.28	0.01	0.02	0	0.01
2.53	0.02	0.03	0	0.01
2.78	0.03	0.04	0.01	0.01
3.04	0.05	0.07	0.01	0.02
3.29	0.09	0.13	0.01	0.02
3.54	0.18	0.24	0.02	0.03
3.8	0.31	0.39	0.02	0.04
4.05	0.48	0.57	0.03	0.05
4.3	0.65	0.74	0.04	0.07
4.56	0.74	0.85	0.05	0.1
4.81	0.81	0.93	0.07	0.13
5.07	0.76	1.15	0.09	0.17
5.32	1.04	1.23	0.11	0.2
5.57	1.1	1.27	0.14	0.26
5.83	1.13	1.3	0.16	0.29
6.08	1.15	1.32	0.19	0.39
6.33	1.17	1.34	0.22	0.45
6.59	1.18	1.35	0.26	0.59
6.84	1.19	1.36	0.31	0.61
7.09	1.2	1.37	0.38	0.72
7.35	1.2	1.36	0.44	0.77
7.6	1.21	1.37	0.46	0.79
7.86	1.21	1.38	0.51	0.9
8.11	1.21	1.38	0.55	0.91
8.36	1.21	1.37	0.55	0.96
8.62	1.21	1.38	0.58	1.03
8.87	1.21	1.38	0.63	1.01
9.12	1.21	1.37	0.66	1.03
9.38	1.21	1.38	0.69	1.04
9.63	1.21	1.38	0.72	1.05
9.88	1.21	1.37	0.74	1.09
10.14	1.2	1.37	0.75	1.06

10.39	1.2	1.37	0.77	1.07
10.64	1.2	1.36	0.77	1.1
10.9	1.2	1.36	0.78	1.11
11.15	1.19	1.35	0.78	1.11
11.41	1.19	1.35	0.79	1.07
11.66	1.19	1.35	0.79	1.1
11.91	1.18	1.34	0.79	1.11
12.17	1.19	1.34	0.8	1.07
12.42	1.16	1.34	0.77	1.1
12.67	1.18	1.34	0.8	1.1
12.93	1.15	1.34	0.8	1.1
13.18	1.18	1.34	0.77	1.09
13.43	1.15	1.34	0.77	1.07
13.69	1.17	1.34	0.8	1.06
13.94	1.18	1.34	0.8	1.06
14.2	1.17	1.34	0.8	1.09
14.45	1.17	1.34	0.77	1.08
14.7	1.17	1.34	0.8	1.08
14.96	1.17	1.33	0.76	1.08
15.21	1.17	1.34	0.79	1.05
15.46	1.17	1.34	0.79	1.05
15.72	1.17	1.34	0.77	1.05
15.97	1.18	1.34	0.8	1.05
16.22	1.14	1.34	0.79	1.07
16.48	1.17	1.33	0.79	1.08
16.73	1.17	1.33	0.76	1.05
16.98	1.17	1.32	0.76	1.05
17.24	1.17	1.33	0.76	1.06
17.49	1.17	1.34	0.76	1.07
17.75	1.18	1.34	0.76	1.06
18	1.18	1.34	0.76	1.08
18.25	1.17	1.34	0.79	1.06
18.51	1.17	1.34	0.78	1.06
18.76	1.18	1.34	0.76	1.08
19.01	1.17	1.34	0.78	1.08
19.27	1.18	1.34	0.78	1.08
19.52	1.17	1.34	0.78	1.08
19.77	1.18	1.34	0.75	1.08
20.03	1.17	1.34	0.76	1.06

LOG - week 1				
	OD, λ = 850			
Time [hours]	water+light	water+no light	zinc+light	zinc+no light
0.25	0	0	0	0
0.51	-0.01	0	0	0
0.76	-0.01	0	0	0
1.01	-0.01	0	0	0
1.27	0	0	0	0
1.52	0	0	0	0
1.78	0	0	0	0
2.03	0	0	0	0
2.28	0	0.01	0	0
2.54	0.01	0.01	0	0
2.79	0.02	0.02	0	0
3.04	0.03	0.03	0	0
3.3	0.04	0.05	0	0
3.55	0.07	0.07	0	0
3.8	0.12	0.12	0	0
4.06	0.21	0.23	0	0
4.31	0.33	0.38	0	0
4.56	0.45	0.65	0	0
4.82	0.57	0.78	0	0
5.07	0.65	0.92	0	0
5.33	0.69	0.99	0	0
5.58	0.78	1.06	0	0
5.83	0.81	1.13	0	0
6.09	0.88	1.18	0	0
6.34	0.94	1.21	0	0
6.59	0.99	1.21	0	0
6.85	1.01	1.23	0	0
7.1	1.02	1.23	0.01	0
7.35	1.04	1.25	0.01	0
7.61	1.02	1.25	0.01	0
7.86	1.02	1.25	0.01	0
8.12	1.05	1.25	0.01	0
8.37	1.02	1.26	0.01	0.01
8.62	1.02	1.25	0.01	0.01
8.88	1.02	1.24	0.02	0.01
9.13	1.02	1.25	0.02	0.01
9.38	1.02	1.23	0.02	0.01
9.64	1.04	1.23	0.02	0.02
9.89	1.04	1.23	0.03	0.02

10.14	1.03	1.23	0.03	0.02
10.4	1.04	1.22	0.03	0.03
10.65	1.02	1.22	0.04	0.03
10.91	1.02	1.23	0.04	0.04
11.16	1.03	1.22	0.04	0.05
11.41	1.01	1.21	0.05	0.05
11.67	1.03	1.22	0.05	0.05
11.92	1.01	1.21	0.05	0.07
12.17	1.01	1.21	0.06	0.07
12.43	1.02	1.21	0.06	0.08
12.68	1.02	1.21	0.07	0.09
12.93	1	1.2	0.07	0.1
13.19	1.02	1.2	0.08	0.11
13.44	1.02	1.21	0.08	0.13
13.69	1.01	1.21	0.09	0.15
13.95	1.01	1.21	0.1	0.18
14.2	1.01	1.21	0.11	0.2
14.46	1.02	1.21	0.11	0.22
14.71	1.02	1.21	0.12	0.25
14.96	1.02	1.21	0.13	0.27
15.22	1.01	1.21	0.14	0.33
15.47	1.01	1.21	0.14	0.34
15.72	1.01	1.2	0.15	0.4
15.98	1.03	1.2	0.16	0.42
16.23	1.01	1.2	0.18	0.44
16.48	1.01	1.2	0.19	0.45
16.74	1.01	1.2	0.2	0.48
16.99	1.02	1.2	0.21	0.49
17.25	1.01	1.2	0.23	0.49
17.5	1.01	1.2	0.24	0.51
17.75	1.01	1.2	0.24	0.57
18.01	1.01	1.2	0.26	0.57
18.26	1.02	1.2	0.27	0.6
18.51	1	1.2	0.28	0.66
18.77	1.02	1.2	0.29	0.66
19.02	1	1.2	0.29	0.64
19.27	1.01	1.2	0.3	0.68
19.53	1	1.2	0.3	0.68
19.78	1.01	1.2	0.32	0.69
20.03	1	1.19	0.32	0.7

LOG - week 2				
Time [hours]	OD, λ = 850			
	water+light	water+no light	zinc+light	zinc+no light
0.7	0	0	0	0
0.95	0	0	0	0
1.2	0	0	0	0
1.46	0	0	0	0
1.71	0	0	0	0
1.97	0	0	0	0
2.22	0.01	0	0	-0.01
2.47	0.01	0.01	0	-0.01
2.73	0.02	0.01	0	-0.01
2.98	0.02	0.02	0.01	-0.01
3.23	0.04	0.03	0.01	-0.01
3.49	0.06	0.05	0.02	-0.01
3.74	0.08	0.08	0.03	-0.01
3.99	0.13	0.13	0.05	-0.01
4.25	0.21	0.23	0.08	-0.01
4.5	0.32	0.37	0.13	-0.01
4.76	0.46	0.6	0.19	-0.01
5.01	0.57	0.77	0.25	-0.01
5.26	0.66	0.89	0.34	-0.01
5.52	0.72	0.99	0.53	-0.01
5.77	0.8	1.06	0.73	-0.01
6.02	1.02	1.13	0.82	-0.01
6.28	1.14	1.16	0.89	-0.01
6.53	1.24	1.18	0.94	-0.01
6.78	1.32	1.18	0.98	-0.01
7.04	1.38	1.21	1.01	-0.01
7.29	1.45	1.22	1.03	0
7.55	1.5	1.21	1.05	0
7.8	1.57	1.23	1.06	0
8.05	1.63	1.23	1.07	0
8.31	1.67	1.22	1.07	0
8.56	1.71	1.23	1.08	0
8.81	1.76	1.22	1.08	0
9.07	1.79	1.22	1.08	0
9.32	1.83	1.22	1.07	0
9.57	1.84	1.22	1.07	0
9.83	1.87	1.21	1.07	0
10.08	1.89	1.21	1.07	0

10.33	1.91	1.21	1.07	0.01
10.59	1.92	1.21	1.07	0.01
10.84	1.87	1.2	1.07	0.01
11.1	1.93	1.2	1.07	0.01
11.35	1.93	1.2	1.07	0.02
11.6	1.95	1.19	1.07	0.02
11.86	1.94	1.19	1.07	0.02
12.11	1.92	1.19	1.06	0.03
12.36	1.92	1.2	1.07	0.03
12.62	1.94	1.19	1.07	0.04
12.87	1.92	1.19	1.07	0.04
13.12	1.91	1.2	1.07	0.04
13.38	1.89	1.19	1.08	0.05
13.63	1.9	1.19	1.07	0.06
13.89	1.88	1.19	1.07	0.06
14.14	1.88	1.18	1.07	0.07
14.39	1.86	1.18	1.04	0.09
14.65	1.85	1.19	1.07	0.1
14.9	1.86	1.19	1.08	0.11
15.15	1.85	1.19	1.07	0.13
15.41	1.84	1.18	1.08	0.14
15.66	1.84	1.19	1.08	0.17
15.91	1.84	1.19	1.05	0.19
16.17	1.82	1.19	1.05	0.21
16.42	1.84	1.18	1.08	0.22
16.67	1.82	1.18	1.08	0.27
16.93	1.82	1.18	1.08	0.31
17.18	1.81	1.18	1.09	0.34
17.44	1.82	1.18	1.09	0.37
17.69	1.82	1.18	1.08	0.35
17.94	1.8	1.17	1.09	0.36
18.2	1.83	1.17	1.08	0.4
18.45	1.79	1.17	1.08	0.4
18.7	1.79	1.18	1.08	0.41
18.96	1.8	1.17	1.05	0.39
19.21	1.81	1.17	1.08	0.43
19.46	1.8	1.16	1.08	0.44
19.72	1.8	1.16	1.07	0.45
19.97	1.8	1.17	1.08	0.46
20.23	1.79	1.17	1.08	0.46

LOG - week 3				
Time [hours]	OD, λ = 850			
	water+light	water+no light	zinc+light	zinc+no light
0.25	0	0	0	0
0.51	0	0	0	0
0.76	0	0	0	0
1.01	0	0	0	0
1.27	0	0	0	0
1.52	0	0.01	0	0
1.78	0	0.01	0	0
2.03	0	0.01	0	0
2.28	0.01	0.01	0	0
2.54	0.01	0.02	0.01	0.01
2.79	0.02	0.03	0.01	0.01
3.04	0.03	0.04	0.02	0.03
3.3	0.05	0.06	0.02	0.05
3.55	0.08	0.09	0.03	0.08
3.8	0.13	0.16	0.05	0.13
4.06	0.23	0.29	0.07	0.2
4.31	0.37	0.44	0.11	0.3
4.57	0.54	0.63	0.15	0.43
4.82	0.72	0.75	0.2	0.57
5.07	0.82	0.87	0.27	0.75
5.33	0.84	1.1	0.35	0.86
5.58	1.03	1.24	0.45	0.95
5.83	1.24	1.3	0.57	1.04
6.09	1.32	1.32	0.64	1.1
6.34	1.39	1.35	0.7	1.14
6.59	1.46	1.36	0.74	1.18
6.85	1.56	1.37	0.79	1.21
7.1	1.62	1.39	0.83	1.23
7.35	1.69	1.4	0.86	1.24
7.61	1.7	1.39	0.89	1.25
7.86	1.8	1.41	0.91	1.26
8.12	1.87	1.39	0.93	1.27
8.37	1.9	1.39	0.95	1.27
8.62	1.96	1.39	0.97	1.27
8.88	2.03	1.39	0.98	1.27
9.13	2.13	1.39	0.95	1.28
9.38	2.15	1.41	1	1.28
9.64	2.2	1.41	0.99	1.28

9.89	2.28	1.39	1	1.26
10.14	2.32	1.4	1	1.23
10.4	2.34	1.39	1	1.23
10.65	2.35	1.4	0.99	1.26
10.91	2.38	1.39	1	1.23
11.16	2.43	1.39	1	1.25
11.41	2.44	1.39	0.99	1.26
11.67	2.46	1.38	1	1.26
11.92	2.46	1.38	1	1.26
12.17	2.42	1.38	1	1.21
12.43	2.43	1.37	0.97	1.26
12.68	2.43	1.37	1	1.25
12.93	2.42	1.37	0.99	1.25
13.19	2.42	1.37	0.99	1.24
13.44	2.44	1.37	0.99	1.2
13.69	2.45	1.37	0.98	1.2
13.95	2.41	1.37	0.98	1.25
14.2	2.4	1.36	0.98	1.24
14.46	2.4	1.36	0.98	1.24
14.71	2.42	1.36	0.97	1.24
14.96	2.39	1.36	0.98	1.24
15.22	2.38	1.36	0.97	1.24
15.47	2.37	1.37	0.97	1.25
15.72	2.35	1.37	0.98	1.24
15.98	2.35	1.37	0.97	1.25
16.23	2.34	1.37	0.97	1.24
16.48	2.33	1.36	0.97	1.25
16.74	2.33	1.35	0.97	1.25
16.99	2.31	1.37	0.96	1.25
17.25	2.31	1.35	0.97	1.21
17.5	2.3	1.36	0.97	1.25
17.75	2.29	1.37	0.97	1.25
18.01	2.28	1.35	0.97	1.25
18.26	2.27	1.35	0.97	1.25
18.51	2.28	1.37	0.97	1.25
18.77	2.3	1.36	0.97	1.21
19.02	2.26	1.37	0.97	1.25
19.27	2.29	1.37	0.97	1.25
19.53	2.25	1.36	0.97	1.22
19.78	2.25	1.36	0.97	1.25
20.04	2.25	1.37	0.97	1.26

STAT - week 2				
Time [hours]	OD, λ = 850			
	water+light	water+no light	zinc+light	zinc+no light
0.25	0	0	0	0
0.51	-0.01	0	0.01	-0.01
0.76	-0.01	0	0.01	-0.01
1.01	-0.01	0	0	-0.01
1.27	-0.01	0	0	-0.01
1.52	0	0	0	-0.01
1.78	0	0	0	-0.01
2.03	0	0	0	-0.01
2.28	0	0.01	0	-0.01
2.54	0	0.01	0	-0.01
2.79	0.01	0.01	0	-0.01
3.04	0.02	0.02	0	-0.01
3.3	0.03	0.04	0	-0.01
3.55	0.05	0.05	0	-0.01
3.8	0.08	0.08	0	-0.01
4.06	0.12	0.15	0	-0.01
4.31	0.2	0.26	0	0
4.57	0.32	0.43	0.01	0
4.82	0.46	0.6	0	0
5.07	0.54	0.72	0.01	0
5.33	0.64	0.87	0.01	0
5.58	0.74	0.92	0.01	0
5.83	0.89	1.11	0.01	0
6.09	0.99	1.22	0.01	0
6.34	1.08	1.29	0.01	0
6.59	1.1	1.27	0.01	0
6.85	1.08	1.29	0.01	0.01
7.1	1.12	1.31	0.02	0.01
7.35	1.1	1.32	0.02	0.01
7.61	1.11	1.33	0.02	0.02
7.86	1.11	1.33	0.03	0.02
8.12	1.11	1.37	0.03	0.03
8.37	1.11	1.37	0.03	0.04
8.62	1.11	1.36	0.03	0.04
8.88	1.12	1.36	0.04	0.05
9.13	1.11	1.33	0.04	0.06
9.38	1.11	1.33	0.05	0.07
9.64	1.13	1.35	0.05	0.08

9.89	1.11	1.32	0.05	0.08
10.14	1.11	1.35	0.06	0.11
10.4	1.12	1.32	0.06	0.13
10.65	1.1	1.34	0.07	0.15
10.91	1.12	1.31	0.08	0.16
11.16	1.11	1.33	0.08	0.19
11.41	1.1	1.32	0.09	0.23
11.67	1.1	1.32	0.1	0.25
11.92	1.1	1.29	0.1	0.32
12.17	1.09	1.29	0.11	0.34
12.43	1.09	1.31	0.12	0.4
12.68	1.09	1.29	0.13	0.46
12.93	1.09	1.29	0.15	0.46
13.19	1.09	1.31	0.15	0.48
13.44	1.08	1.29	0.17	0.54
13.7	1.09	1.29	0.18	0.59
13.95	1.09	1.31	0.19	0.62
14.2	1.1	1.32	0.2	0.66
14.46	1.08	1.32	0.22	0.69
14.71	1.09	1.3	0.24	0.74
14.96	1.09	1.32	0.25	0.78
15.22	1.09	1.32	0.27	0.75
15.47	1.08	1.32	0.27	0.77
15.72	1.09	1.3	0.29	0.81
15.98	1.09	1.3	0.3	0.78
16.23	1.09	1.32	0.31	0.82
16.48	1.09	1.3	0.31	0.82
16.74	1.08	1.32	0.34	0.82
16.99	1.1	1.3	0.34	0.82
17.25	1.09	1.31	0.35	0.83
17.5	1.09	1.29	0.36	0.79
17.75	1.1	1.29	0.37	0.83
18.01	1.09	1.29	0.39	0.82
18.26	1.08	1.31	0.39	0.82
18.51	1.08	1.29	0.4	0.82
18.77	1.09	1.29	0.4	0.82
19.02	1.09	1.31	0.41	0.82
19.27	1.09	1.29	0.4	0.82
19.53	1.08	1.29	0.41	0.82
19.78	1.09	1.29	0.41	0.82
20.04	1.09	1.29	0.41	0.78

STAT - week 3				
Time [hours]	OD, λ = 850			
	water+light	water+no light	zinc+light	zinc+no light
0.25	0	0	0	0
0.51	-0.01	-0.01	0	0
0.76	-0.01	-0.01	0	0
1.01	-0.01	-0.01	0	0
1.27	-0.01	-0.01	0	0
1.52	0	0	0	0
1.78	0	0	0	0
2.03	0	0	0	0
2.28	0	0	0	0
2.54	0	0	0	0
2.79	0.01	0.01	0	0
3.04	0.02	0.02	0	0
3.3	0.03	0.03	0	0
3.55	0.05	0.05	0	0
3.8	0.07	0.08	0	0
4.06	0.11	0.12	0	0
4.31	0.19	0.22	0	0
4.57	0.31	0.36	0	0
4.82	0.46	0.56	0	0
5.07	0.59	0.81	0	0
5.33	0.67	0.92	0	0
5.58	0.74	1	0	0
5.83	0.79	1.12	0	0
6.09	0.88	1.2	0	0
6.34	0.97	1.22	0	0
6.59	1.1	1.26	0	0
6.85	1.2	1.29	0	0
7.1	1.28	1.31	0	0
7.36	1.37	1.32	0	0
7.61	1.4	1.36	0	0
7.86	1.45	1.33	0	0
8.12	1.49	1.37	0	0
8.37	1.55	1.33	0	0
8.62	1.58	1.33	0	0
8.88	1.61	1.33	0	0
9.13	1.66	1.36	0	0
9.38	1.68	1.35	0	0
9.64	1.71	1.32	0	0

9.89	1.77	1.32	0	0
10.14	1.8	1.34	0	0
10.4	1.86	1.34	0	0
10.65	1.88	1.34	0	0
10.91	1.93	1.33	0	0
11.16	1.98	1.3	0	0
11.41	2.01	1.3	0	0
11.67	2.04	1.3	0	0
11.92	2.09	1.29	0	0
12.17	2.12	1.29	0	0
12.43	2.14	1.29	0	0
12.68	2.17	1.29	0	0
12.93	2.22	1.29	0	0
13.19	2.24	1.32	0	0
13.44	2.28	1.31	0	0
13.7	2.31	1.31	0	0
13.95	2.36	1.29	0	0
14.2	2.39	1.32	0	0
14.46	2.39	1.3	0	0
14.71	2.42	1.32	0	0
14.96	2.46	1.31	0	0
15.22	2.48	1.29	0	0
15.47	2.51	1.29	0	0
15.72	2.52	1.29	0	0
15.98	2.54	1.32	0	0
16.23	2.58	1.29	0	0.01
16.48	2.6	1.31	0	0.01
16.74	2.64	1.29	0	0.02
16.99	2.67	1.29	0	0.03
17.25	2.69	1.31	0	0.04
17.5	2.73	1.29	0	0.05
17.75	2.75	1.29	0	0.06
18.01	2.75	1.31	0	0.08
18.26	2.78	1.31	0	0.09
18.51	2.78	1.29	0	0.09
18.77	2.81	1.29	0	0.12
19.02	2.83	1.29	0	0.13
19.27	2.85	1.29	0	0.15
19.53	2.86	1.29	0	0.15
19.78	2.86	1.31	0	0.16
20.04	2.9	1.29	0	0.17

11.5.2 Growth rate data

LAG - week 1				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.63	0	0	0	0
0.88	0	0	0	0
1.14	0	0	0	0
1.39	0	0	0	0
1.64	0.01	0	0.01	0.01
1.9	0.01	0.01	0.01	0.01
2.15	0.02	0.01	0.01	0.01
2.4	0.02	0.02	0.01	0.01
2.66	0.03	0.03	0.01	0.03
2.91	0.04	0.06	0.01	0.03
3.16	0.06	0.1	0.01	0.05
3.42	0.09	0.18	0.02	0.07
3.67	0.12	0.28	0.02	0.1
3.93	0.15	0.35	0.03	0.12
4.18	0.16	0.38	0.03	0.14
4.43	0.19	0.39	0.04	0.17
4.69	0.27	0.47	0.04	0.22
4.94	0.38	0.52	0.05	0.23
5.19	0.39	0.46	0.07	0.31
5.45	0.36	0.38	0.07	0.3
5.7	0.3	0.31	0.08	0.27
5.95	0.27	0.25	0.09	0.25
6.21	0.23	0.2	0.1	0.26
6.46	0.19	0.16	0.11	0.25
6.72	0.17	0.13	0.14	0.22
6.97	0.14	0.1	0.13	0.16
7.22	0.11	0.08	0.15	0.19
7.48	0.09	0.06	0.16	0.12
7.73	0.08	0.05	0.13	0.15
7.98	0.06	0.04	0.11	0.12
8.24	0.05	0.02	0.09	0.1
8.49	0.05	0.02	0.08	0.07
8.74	0.03	0.01	0.06	0.02
9	0.03	0.01	0.06	0.02
9.25	0.02	0.01	0.03	0.06
9.51	0.01	0	0.03	0.04
9.76	0.01	0	0.02	0.03

10.01	0	0	0.03	-0.01
10.27	0	0	0.02	0.03
10.52	0.01	-0.01	0.02	0.02
10.77	0	-0.01	0.02	0.01
11.03	0	-0.01	0.02	0.01
11.28	0	-0.01	0.01	0.01
11.53	-0.01	-0.01	0.01	0
11.79	0	-0.01	0.02	0
12.04	0	-0.01	0.01	-0.04
12.29	0	-0.01	0.01	-0.03
12.55	-0.01	-0.01	0.01	-0.03
12.8	-0.01	0	0.01	-0.02
13.06	-0.01	-0.01	0.01	-0.01
13.31	0	0	0.01	-0.01
13.56	0	0	0	0.02
13.82	-0.01	-0.01	0	-0.02
14.07	0	0	0	0.02
14.32	-0.01	0	0	0.02
14.58	-0.01	-0.01	0	0.01
14.83	-0.01	0	0	-0.03
15.08	0	0	0	0.01
15.34	-0.01	0	0	0.01
15.59	-0.01	0	0	-0.03
15.85	-0.01	0	0	-0.02
16.1	-0.01	0	0	0.02
16.35	0	0	0	-0.02
16.61	0	0	-0.01	-0.01
16.86	0	0	0	0.03
17.11	-0.01	0	-0.01	0.02
17.37	0	0	0	0.02
17.62	0	0	0	0.01
17.87	-0.01	0	-0.01	0.01
18.13	0	0	-0.01	-0.02
18.38	0	0	-0.01	-0.02
18.63	0	0	-0.01	0.02
18.89	0	0	-0.01	-0.02
19.14	0	0	-0.01	-0.01
19.4	0	0	-0.01	0.02
19.65	0	-0.01	-0.02	0.02
19.9	0	0	-0.02	0.02
20.16	0	0	-0.02	-0.02

LAG - week 2				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.26	0	0	-0.02	-0.01
0.51	0	0	-0.01	-0.01
0.76	0	0	-0.01	-0.01
1.02	0	0	-0.01	-0.01
1.27	0	0	0	0
1.52	0.01	0.01	0	0
1.78	0.01	0.01	0	0
2.03	0.02	0.02	0	0
2.28	0.03	0.03	0	0
2.54	0.06	0.05	0	0
2.79	0.09	0.09	0	0
3.05	0.15	0.16	0	0
3.3	0.23	0.26	0.01	0
3.55	0.29	0.36	0.01	0
3.81	0.31	0.45	0.01	0
4.06	0.27	0.43	0.01	0
4.31	0.27	0.46	0.01	0
4.57	0.25	0.36	0.01	0.01
4.82	0.24	0.36	0.02	0.01
5.07	0.26	0.3	0.02	0.01
5.33	0.27	0.28	0.02	0.01
5.58	0.22	0.21	0.03	0.02
5.83	0.19	0.18	0.03	0.02
6.09	0.16	0.14	0.04	0.02
6.34	0.14	0.12	0.04	0.03
6.6	0.1	0.1	0.04	0.03
6.85	0.09	0.07	0.06	0.04
7.1	0.07	0.06	0.06	0.04
7.36	0.05	0.04	0.08	0.05
7.61	0.03	0.03	0.08	0.07
7.86	0.03	0.02	0.08	0.06
8.12	0.02	0.02	0.12	0.08
8.37	0.01	0.01	0.12	0.09
8.62	0.02	0	0.14	0.12
8.88	0	0	0.13	0.15
9.13	0	0	0.1	0.17
9.39	0.01	0	0.11	0.15
9.64	0.01	0	0.1	0.17

9.89	-0.01	-0.01	0.12	0.14
10.15	0	-0.01	0.13	0.17
10.4	0.01	-0.01	0.13	0.16
10.65	0	-0.01	0.11	0.17
10.91	0	-0.01	0.11	0.21
11.16	-0.01	0	0.09	0.19
11.41	0	-0.01	0.08	0.14
11.67	0.01	-0.01	0.05	0.13
11.92	0	-0.01	0.05	0.12
12.17	0	-0.01	0.06	0.1
12.43	0	0	0.06	0.09
12.68	-0.01	0	0.04	0.1
12.94	0.01	0	0.03	0.07
13.19	0	0	0.01	0.04
13.44	-0.01	0	0	0.03
13.7	0	-0.01	0.03	0.02
13.95	-0.01	0	0.02	0.02
14.2	0	0	0.01	0.02
14.46	-0.01	0	-0.02	0.01
14.71	0	0	0.01	0.01
14.96	0.01	0	-0.02	0.01
15.22	0	0	0.02	0
15.47	-0.01	0	-0.01	0
15.73	-0.01	0	0	0.02
15.98	-0.02	0	-0.01	0.01
16.23	0	0	-0.01	0.01
16.49	0.01	0	0.02	-0.01
16.74	0.01	0	0.01	0
16.99	0	0	0.01	-0.01
17.25	0.01	0	0	-0.01
17.5	0.01	0	0	0.01
17.75	0	0	0	0.01
18.01	0	0	0	-0.01
18.26	0	0	0	-0.01
18.51	0	0	0	0.01
18.77	0	0	0.01	-0.01
19.02	0	0	0	0
19.28	-0.02	0	0	0.01
19.53	0	0	0.01	-0.01
19.78	-0.01	0	0	0
20.04	-0.01	0	0	0.02

LAG - week 3				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.26	-0.01	0	-0.01	0
0.51	-0.01	0	0	0
0.76	0	0	0	0
1.02	0	0	0	0
1.27	0	0	0	0
1.52	0	0.01	0	0.01
1.78	0.01	0.01	0	0.01
2.03	0.01	0.01	0	0.02
2.28	0.02	0.02	0.01	0.03
2.54	0.03	0.03	0.01	0.04
2.79	0.05	0.06	0.02	0.07
3.04	0.1	0.1	0.03	0.11
3.3	0.16	0.17	0.04	0.15
3.55	0.23	0.27	0.06	0.19
3.81	0.3	0.36	0.08	0.29
4.06	0.32	0.47	0.1	0.37
4.31	0.28	0.5	0.13	0.4
4.57	0.26	0.43	0.17	0.39
4.82	0.3	0.42	0.21	0.38
5.07	0.32	0.37	0.24	0.35
5.33	0.28	0.31	0.26	0.33
5.58	0.26	0.27	0.24	0.27
5.83	0.25	0.23	0.22	0.19
6.09	0.21	0.16	0.22	0.16
6.34	0.15	0.15	0.21	0.19
6.6	0.12	0.11	0.21	0.15
6.85	0.1	0.09	0.21	0.12
7.1	0.1	0.07	0.18	0.1
7.36	0.07	0.07	0.14	0.03
7.61	0.05	0.04	0.18	0.07
7.86	0.04	0.03	0.17	0.05
8.12	0.03	0.02	0.11	0
8.37	0.01	0.01	0.1	0.04
8.62	0	0.02	0.1	0.03
8.88	0.02	0	0.08	0.02
9.13	0	0	0.07	-0.03
9.38	0.01	0	0.06	-0.02
9.64	0.01	0.01	0.04	-0.02

9.89	0.01	0	0.04	0.02
10.15	0	-0.01	0.03	-0.02
10.4	0	-0.01	0.01	-0.02
10.65	0	-0.01	0.02	0.02
10.91	-0.01	-0.01	0.04	0.02
11.16	0	0	0	0.01
11.41	-0.01	0	-0.01	0
11.67	0	-0.01	0	0.01
11.92	-0.01	-0.01	0.01	0
12.17	-0.01	0	0.01	0
12.43	-0.01	-0.01	0	0
12.68	0	-0.01	-0.01	0
12.94	0	0	0.01	0
13.19	0.01	0	-0.01	0
13.44	-0.01	0	-0.02	0
13.7	0.01	0	0	-0.03
13.95	0	0.01	-0.03	0.01
14.2	-0.01	0	0	0.01
14.46	0	-0.01	0	0.01
14.71	0	0	0.01	-0.03
14.96	0	-0.01	0.01	0.01
15.22	-0.01	0	0.03	-0.03
15.47	0	-0.01	0.01	0.02
15.73	0	0	0	-0.03
15.98	-0.01	0	0.02	0.01
16.23	0	0	0.01	0.01
16.49	0	-0.01	-0.01	0.01
16.74	-0.01	0	-0.03	0.01
16.99	-0.01	-0.01	-0.03	0.01
17.25	0	0	0.01	-0.03
17.5	-0.01	0	0.01	0.01
17.75	0	-0.01	0.01	0.01
18.01	0	-0.01	0	-0.03
18.26	0	0	-0.01	0.01
18.51	-0.01	0.01	0	0.01
18.77	0	0	0.02	-0.03
19.02	0	-0.01	0.01	0.01
19.28	0	0	0.01	0.01
19.53	0.01	-0.01	0.01	0.01
19.78	0.01	0.01	0	0.01
20.04	0	-0.01	-0.01	-0.03

LAG - week 4				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.75	0	0	0	0
1.01	0	0	0	0
1.26	0	0	0	0
1.51	0	0	0	0
1.77	0	0.01	0	0
2.02	0.01	0.01	0	0
2.28	0.01	0.01	0	0
2.53	0.02	0.02	0	0
2.78	0.02	0.03	0	0.01
3.04	0.04	0.05	0.01	0.01
3.29	0.07	0.09	0.01	0.01
3.54	0.13	0.18	0.01	0.01
3.8	0.23	0.29	0.01	0.02
4.05	0.34	0.38	0.02	0.03
4.3	0.43	0.46	0.02	0.04
4.56	0.41	0.45	0.03	0.06
4.81	0.37	0.42	0.04	0.07
5.07	0.24	0.53	0.05	0.1
5.32	0.44	0.48	0.06	0.1
5.57	0.4	0.4	0.07	0.13
5.83	0.33	0.33	0.08	0.13
6.08	0.26	0.27	0.08	0.19
6.33	0.21	0.21	0.09	0.2
6.59	0.18	0.18	0.11	0.29
6.84	0.14	0.14	0.13	0.24
7.09	0.12	0.11	0.17	0.28
7.35	0.09	0.08	0.18	0.27
7.6	0.07	0.07	0.15	0.22
7.86	0.06	0.06	0.17	0.27
8.11	0.05	0.04	0.16	0.22
8.36	0.04	0.02	0.13	0.21
8.62	0.03	0.03	0.13	0.23
8.87	0.02	0.02	0.14	0.15
9.12	0.01	0.01	0.13	0.13
9.38	0.01	0.01	0.13	0.11
9.63	0.01	0.01	0.13	0.09
9.88	0	0	0.11	0.11
10.14	0	0	0.1	0.05

10.39	0	0	0.09	0.05
10.64	0	-0.01	0.07	0.07
10.9	-0.01	-0.01	0.06	0.06
11.15	-0.01	-0.01	0.05	0.04
11.41	-0.01	-0.01	0.04	0
11.66	-0.01	-0.01	0.03	0.03
11.91	-0.01	-0.01	0.03	0.02
12.17	-0.01	-0.01	0.03	-0.02
12.42	-0.03	-0.01	-0.01	0.01
12.67	0	-0.01	0.02	0.01
12.93	-0.03	-0.01	0.02	0
13.18	0.01	-0.01	-0.02	0
13.43	-0.03	-0.01	-0.01	-0.03
13.69	0	0	0.02	-0.02
13.94	0.01	0	0.01	-0.02
14.2	0	0	0.01	0.01
14.45	0	0	-0.02	0.01
14.7	0	0	0.01	0
14.96	0	0	-0.02	0
15.21	0	0	0.01	-0.03
15.46	0	0	0.01	-0.02
15.72	0	0	-0.02	-0.02
15.97	0.01	0	0.02	-0.01
16.22	-0.03	0	0.01	0.01
16.48	0.01	0	0.01	0.01
16.73	0	0	-0.03	-0.02
16.98	0	-0.01	-0.02	-0.01
17.24	0	0	-0.02	-0.01
17.49	0	0	-0.01	0.01
17.75	0.01	0	-0.01	-0.01
18	0	0	-0.01	0.02
18.25	0	0	0.02	-0.01
18.51	0	0	0.01	-0.01
18.76	0.01	0	-0.02	0.02
19.01	0	0	0.01	0.01
19.27	0	0	0.01	0.01
19.52	0	0	0.01	0.01
19.77	0.01	0	-0.02	0.01
20.03	0	0	-0.01	-0.01

LOG - week 1				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.51	0	0	-0.01	-0.01
0.76	0	0	-0.01	-0.01
1.01	0	0	0	0
1.27	0	0	0	0
1.52	0	0	0	0
1.78	0	0	0	0
2.03	0	0	0	0
2.28	0.01	0.01	0	0
2.54	0.01	0.01	0	0
2.79	0.02	0.01	0	0
3.04	0.02	0.02	0	0
3.3	0.03	0.03	0	0
3.55	0.05	0.05	0	0
3.8	0.08	0.09	0	0
4.06	0.15	0.17	0	0
4.31	0.24	0.27	0	0
4.56	0.29	0.47	0	0
4.82	0.34	0.48	0	0
5.07	0.33	0.5	0	0
5.33	0.29	0.45	0	0
5.58	0.31	0.41	0	0
5.83	0.26	0.37	0	0
6.09	0.27	0.32	0	0
6.34	0.25	0.28	0	0
6.59	0.25	0.21	0	0
6.85	0.2	0.18	0	0
7.1	0.16	0.14	0	0
7.35	0.13	0.11	0	0
7.61	0.08	0.09	0	0
7.86	0.06	0.07	0	0
8.12	0.07	0.05	0	0
8.37	0.03	0.04	0	0
8.62	0.02	0.03	0.01	0
8.88	0.02	0.01	0.01	0.01
9.13	0.02	0.01	0.01	0.01
9.38	0.01	0	0.01	0.01
9.64	0.02	0	0.01	0.01
9.89	0.02	0	0.01	0.01

10.14	0.01	-0.01	0.01	0.01
10.4	0.01	-0.01	0.01	0.01
10.65	-0.01	-0.01	0.01	0.01
10.91	-0.01	0	0.01	0.02
11.16	0.01	-0.01	0.01	0.02
11.41	-0.01	-0.01	0.01	0.02
11.67	0.01	-0.01	0.01	0.02
11.92	-0.01	-0.01	0.01	0.02
12.17	-0.01	-0.01	0.01	0.03
12.43	0.01	-0.01	0.02	0.03
12.68	0	-0.01	0.02	0.03
12.93	-0.01	-0.01	0.02	0.03
13.19	0.01	-0.01	0.02	0.03
13.44	0	0	0.02	0.05
13.69	-0.01	0	0.02	0.06
13.95	-0.01	0	0.02	0.06
14.2	0	0	0.02	0.07
14.46	0	0	0.03	0.08
14.71	0.01	0	0.03	0.09
14.96	0	0	0.03	0.08
15.22	0	0	0.03	0.12
15.47	-0.01	0	0.03	0.1
15.72	0	0	0.03	0.13
15.98	0.01	0	0.03	0.12
16.23	-0.01	0	0.04	0.11
16.48	0	0	0.04	0.1
16.74	-0.01	0	0.04	0.09
16.99	0.01	0	0.04	0.09
17.25	-0.01	0	0.05	0.06
17.5	-0.01	0	0.05	0.07
17.75	0	0	0.03	0.11
18.01	0	0	0.05	0.08
18.26	0.01	0	0.05	0.09
18.51	-0.01	0	0.04	0.12
18.77	0.01	0	0.04	0.1
19.02	-0.01	0	0.04	0.05
19.27	0	0	0.03	0.08
19.53	-0.01	0	0.03	0.06
19.78	0	0	0.03	0.05
20.03	-0.01	0	0.03	0.04

LOG - week 2				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.95	0	0	0	-0.01
1.2	0	0	0	0
1.46	0	0	0	0
1.71	0	0	0	0
1.97	0	0	0	0
2.22	0	0	0	0
2.47	0.01	0.01	0	0
2.73	0.01	0.01	0	0
2.98	0.02	0.01	0.01	0
3.23	0.03	0.02	0.01	0
3.49	0.04	0.04	0.01	0
3.74	0.06	0.05	0.02	0
3.99	0.08	0.09	0.04	0
4.25	0.14	0.17	0.06	0
4.5	0.22	0.27	0.09	0
4.76	0.31	0.43	0.13	0
5.01	0.33	0.49	0.15	0
5.26	0.34	0.48	0.21	0
5.52	0.32	0.46	0.34	0
5.77	0.31	0.41	0.45	0
6.02	0.45	0.38	0.42	0
6.28	0.46	0.32	0.39	0
6.53	0.44	0.25	0.35	0
6.78	0.4	0.2	0.29	0
7.04	0.36	0.17	0.25	0
7.29	0.35	0.13	0.21	0
7.55	0.31	0.1	0.17	0
7.8	0.3	0.09	0.14	0
8.05	0.28	0.07	0.11	0
8.31	0.25	0.04	0.09	0
8.56	0.23	0.04	0.07	0
8.81	0.22	0.02	0.06	0
9.07	0.19	0.02	0.04	0
9.32	0.19	0.01	0.03	0
9.57	0.15	0.01	0.02	0
9.83	0.14	0	0.02	0
10.08	0.12	0	0.01	0
10.33	0.12	0	0.01	0.01

10.59	0.09	0	0.01	0.01
10.84	0.02	-0.01	0	0.01
11.1	0.08	-0.01	0	0.01
11.35	0.06	-0.01	0	0.01
11.6	0.06	-0.02	0	0.01
11.86	0.04	-0.01	0	0.01
12.11	0.01	-0.01	-0.01	0.01
12.36	0	0	0	0.01
12.62	0.02	-0.01	0	0.02
12.87	0	0	0	0.01
13.12	-0.01	0	0	0.01
13.38	-0.02	-0.01	0.01	0.01
13.63	-0.01	-0.01	0	0.02
13.89	-0.03	0	0	0.02
14.14	-0.02	-0.01	0	0.03
14.39	-0.03	0	-0.03	0.03
14.65	-0.04	0	0.01	0.04
14.9	-0.01	0	0.02	0.04
15.15	-0.02	0	0	0.05
15.41	-0.02	-0.01	0.01	0.04
15.66	-0.02	0	0.01	0.06
15.91	-0.02	0	-0.02	0.07
16.17	-0.04	0	-0.02	0.07
16.42	0	-0.01	0.02	0.07
16.67	-0.02	-0.01	0.01	0.09
16.93	-0.02	0	0	0.11
17.18	-0.02	0	0.02	0.12
17.44	0	-0.01	0.01	0.11
17.69	-0.01	-0.01	0	0.06
17.94	-0.02	-0.01	0.01	0.06
18.2	0.02	-0.01	0	0.08
18.45	-0.03	-0.01	0	0.06
18.7	-0.02	0	0	0.06
18.96	-0.01	0	-0.03	0.03
19.21	0	0	0.01	0.06
19.46	-0.01	-0.01	0.01	0.05
19.72	0	-0.01	0	0.05
19.97	0	0	0	0.05

LOG - week 3				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.51	-0.01	0	-0.01	-0.01
0.76	0	0	-0.01	-0.01
1.01	0	0	-0.01	-0.01
1.27	0	0	0	-0.01
1.52	0	0	0	0
1.78	0	0	0	0
2.03	0	0	0	0
2.28	0.01	0.01	0	0
2.54	0.01	0.01	0	0.01
2.79	0.02	0.02	0.01	0.01
3.04	0.02	0.02	0.01	0.02
3.3	0.04	0.04	0.01	0.04
3.55	0.06	0.06	0.02	0.06
3.8	0.09	0.11	0.03	0.09
4.06	0.17	0.21	0.05	0.14
4.31	0.26	0.31	0.07	0.2
4.57	0.36	0.42	0.1	0.28
4.82	0.45	0.43	0.12	0.35
5.07	0.44	0.44	0.15	0.44
5.33	0.35	0.56	0.2	0.44
5.58	0.45	0.56	0.25	0.42
5.83	0.55	0.47	0.3	0.4
6.09	0.49	0.38	0.29	0.36
6.34	0.43	0.31	0.28	0.31
6.59	0.39	0.25	0.25	0.27
6.85	0.39	0.2	0.24	0.23
7.1	0.36	0.16	0.22	0.19
7.35	0.33	0.13	0.2	0.16
7.61	0.26	0.09	0.18	0.13
7.86	0.3	0.08	0.15	0.1
8.12	0.29	0.05	0.13	0.09
8.37	0.25	0.04	0.12	0.07
8.62	0.25	0.03	0.1	0.05
8.88	0.26	0.02	0.09	0.04
9.13	0.29	0.02	0.04	0.03
9.38	0.24	0.03	0.07	0.02
9.64	0.22	0.02	0.05	0.02
9.89	0.25	0	0.04	0

10.14	0.22	0.01	0.04	-0.03
10.4	0.19	0	0.02	-0.03
10.65	0.15	0	0.02	0.01
10.91	0.14	-0.01	0.01	-0.02
11.16	0.15	-0.01	0.02	0.01
11.41	0.13	-0.01	0	0.02
11.67	0.12	-0.01	0.01	0.01
11.92	0.09	-0.01	0.01	0.01
12.17	0.03	-0.01	0	-0.04
12.43	0.03	-0.02	-0.03	0.01
12.68	0.02	-0.01	0.01	0
12.93	0.01	-0.01	0	0
13.19	0	-0.01	0	-0.01
13.44	0.03	-0.01	0	-0.04
13.69	0.03	0	-0.01	-0.03
13.95	-0.02	0	-0.01	0.02
14.2	-0.02	-0.01	-0.01	0.01
14.46	-0.02	-0.01	0	0.01
14.71	0.01	-0.01	-0.01	0.01
14.96	-0.03	-0.01	0	0.01
15.22	-0.03	-0.01	-0.01	0
15.47	-0.04	0.01	-0.01	0
15.72	-0.04	0	0	0
15.98	-0.03	0	-0.01	0.01
16.23	-0.04	0	0	0
16.48	-0.03	-0.01	-0.01	0.01
16.74	-0.03	-0.01	0	0
16.99	-0.03	0.01	-0.01	0
17.25	-0.03	-0.01	0	-0.04
17.5	-0.03	0	0	0.01
17.75	-0.03	0.01	0	0.01
18.01	-0.03	-0.01	0	0.01
18.26	-0.03	0	0	0.01
18.51	-0.02	0.01	0	0
18.77	0	0	0	-0.03
19.02	-0.04	0.01	0	0.01
19.27	0	0.01	0	0.01
19.53	-0.03	-0.01	0	-0.03
19.78	-0.03	0	0	0.02
20.04	-0.02	0.01	0	0.02

STAT - week 2				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.51	-0.01	-0.01	0.02	-0.01
0.76	-0.01	0	0.02	-0.01
1.01	-0.01	0	0.01	-0.01
1.27	0	0	0	0
1.52	0	0	0	0
1.78	0	0	0	0
2.03	0	0	0	0
2.28	0	0	0	0
2.54	0.01	0.01	0	0
2.79	0.01	0.01	0	0
3.04	0.02	0.02	0	0
3.3	0.03	0.02	0	0
3.55	0.04	0.04	0	0
3.8	0.05	0.06	0	0
4.06	0.09	0.1	0	0
4.31	0.15	0.19	0	0
4.57	0.23	0.31	0	0
4.82	0.3	0.4	0	0
5.07	0.31	0.42	0	0
5.33	0.33	0.46	0	0
5.58	0.35	0.39	0	0
5.83	0.41	0.48	0	0
6.09	0.4	0.47	0	0
6.34	0.39	0.42	0	0
6.59	0.31	0.3	0	0.01
6.85	0.21	0.25	0	0.01
7.1	0.2	0.2	0.01	0.01
7.35	0.13	0.16	0.01	0.01
7.61	0.1	0.13	0.01	0.01
7.86	0.08	0.1	0.01	0.01
8.12	0.06	0.11	0.01	0.02
8.37	0.05	0.08	0.01	0.02
8.62	0.04	0.06	0.01	0.02
8.88	0.03	0.05	0.01	0.02
9.13	0.02	0	0.01	0.03
9.38	0.01	0	0.01	0.03
9.64	0.03	0.03	0.01	0.03
9.89	0	-0.01	0.02	0.03

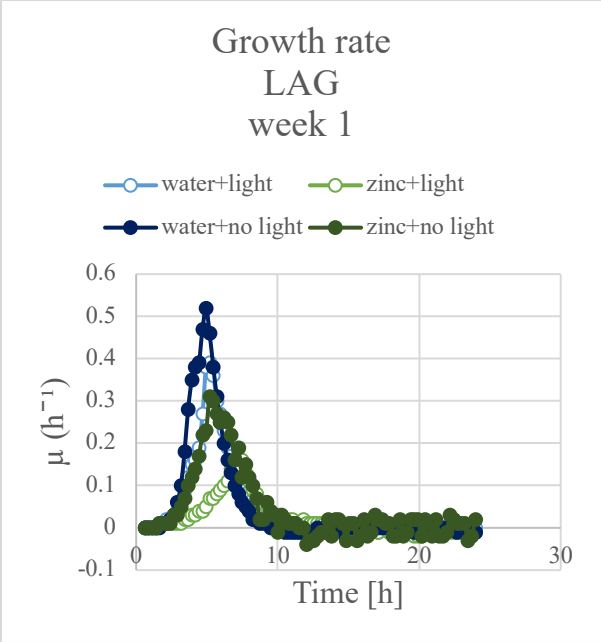
10.14	0	0.01	0.02	0.05
10.4	0.01	-0.02	0.02	0.05
10.65	-0.01	0.01	0.02	0.06
10.91	0	-0.02	0.02	0.06
11.16	0	0	0.02	0.07
11.41	-0.01	0	0.02	0.1
11.67	0	0	0.03	0.09
11.92	-0.01	-0.03	0.02	0.14
12.17	-0.01	-0.02	0.03	0.13
12.43	-0.01	0	0.03	0.15
12.68	-0.01	-0.02	0.03	0.17
12.93	-0.01	-0.01	0.04	0.12
13.19	0	0.01	0.04	0.12
13.44	-0.01	-0.01	0.04	0.15
13.7	0	-0.01	0.04	0.16
13.95	0	0.01	0.04	0.15
14.2	0	0.01	0.04	0.15
14.46	-0.01	0.01	0.05	0.15
14.71	0	-0.01	0.06	0.16
14.96	0	0.01	0.05	0.15
15.22	0	0.01	0.06	0.09
15.47	0	0.01	0.05	0.08
15.72	0	-0.02	0.05	0.1
15.98	0.01	-0.01	0.05	0.05
16.23	0	0.01	0.05	0.08
16.48	0	-0.01	0.04	0.06
16.74	-0.01	0.01	0.05	0.05
16.99	0.01	-0.02	0.05	0.03
17.25	0	0.01	0.04	0.03
17.5	-0.01	-0.01	0.04	-0.01
17.75	0.01	-0.01	0.04	0.02
18.01	0	-0.01	0.04	0.01
18.26	-0.01	0.01	0.03	0.01
18.51	-0.01	-0.01	0.04	0.01
18.77	0.01	-0.01	0.03	0
19.02	0	0.01	0.03	0
19.27	-0.01	-0.01	0.01	0
19.53	-0.01	-0.01	0.02	0.01
19.78	0.01	-0.01	0.01	0
20.04	0.01	-0.01	0.01	-0.03

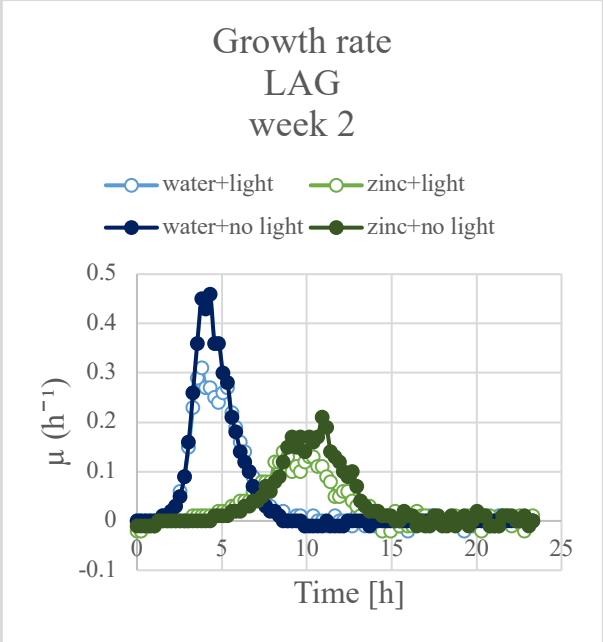
STAT - week 3				
Time [hours]	μ (h^{-1})			
	water+light	water+no light	zinc+light	zinc+no light
0.51	-0.02	-0.01	-0.02	-0.02
0.76	-0.01	0	-0.02	-0.02
1.01	-0.01	0	-0.01	-0.02
1.27	-0.01	0	-0.01	-0.01
1.52	0	0	-0.01	-0.01
1.78	0	0	-0.01	-0.01
2.03	0	0	-0.01	-0.01
2.28	0	0	-0.01	-0.01
2.54	0.01	0.01	-0.01	-0.01
2.79	0.01	0.01	0	-0.01
3.04	0.02	0.01	-0.01	-0.01
3.3	0.02	0.02	0	-0.01
3.55	0.03	0.04	0	0
3.8	0.05	0.06	-0.01	0
4.06	0.08	0.09	0	0
4.31	0.14	0.16	0	0
4.57	0.22	0.26	0	0
4.82	0.31	0.4	0	0
5.07	0.36	0.54	0	0
5.33	0.35	0.52	0	0
5.58	0.33	0.46	0	0
5.83	0.3	0.46	0	0
6.09	0.31	0.43	0	0
6.34	0.32	0.34	0	0
6.59	0.37	0.3	0	0
6.85	0.37	0.25	0	0
7.1	0.36	0.2	0	0
7.36	0.36	0.17	0	0
7.61	0.3	0.17	0	0
7.86	0.27	0.09	0	0
8.12	0.25	0.11	0	0
8.37	0.24	0.04	0	0
8.62	0.21	0.03	0	0
8.88	0.18	0.02	0	0
9.13	0.19	0.04	0	0
9.38	0.17	0.03	0	0
9.64	0.15	-0.01	0	0
9.89	0.18	-0.01	0	0

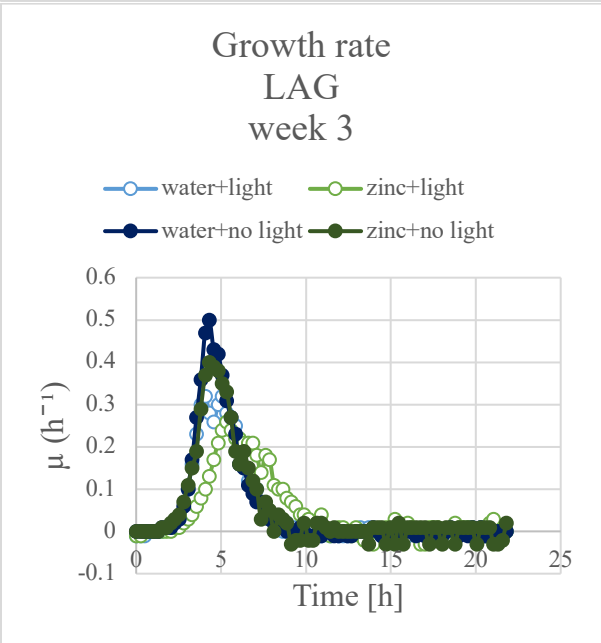
10.14	0.16	0.02	0	0
10.4	0.18	0.01	0	0
10.65	0.16	0.01	0	0
10.91	0.17	0	0	0
11.16	0.17	-0.03	0	0
11.41	0.16	-0.03	0	0
11.67	0.15	-0.02	0	0
11.92	0.16	-0.02	0	0
12.17	0.15	-0.02	0	0
12.43	0.14	-0.01	0	0
12.68	0.13	-0.01	0	0
12.93	0.14	-0.01	0	0
13.19	0.13	0.02	0	0
13.44	0.13	0.01	0	0
13.7	0.13	0.01	0	0
13.95	0.15	-0.01	0	0
14.2	0.14	0.01	0	0.01
14.46	0.11	-0.01	0	0.01
14.71	0.11	0.02	0	0.01
14.96	0.12	0.01	0	0.01
15.22	0.11	-0.01	0	0.01
15.47	0.11	-0.01	0	0.01
15.72	0.1	-0.01	0	0.01
15.98	0.09	0.02	0	0.01
16.23	0.11	-0.01	0	0.03
16.48	0.09	0.01	0	0.02
16.74	0.11	-0.01	0	0.02
16.99	0.11	-0.01	0	0.03
17.25	0.1	0.01	0	0.03
17.5	0.12	-0.01	0	0.04
17.75	0.11	-0.01	0	0.03
18.01	0.08	0.01	0	0.04
18.26	0.09	0.01	0	0.04
18.51	0.07	-0.01	0	0.04
18.77	0.08	-0.01	0	0.05
19.02	0.08	-0.01	0	0.05
19.27	0.08	-0.01	0	0.05
19.53	0.07	0	0	0.04
19.78	0.05	0.01	0	0.04
20.04	0.08	-0.01	0	0.05

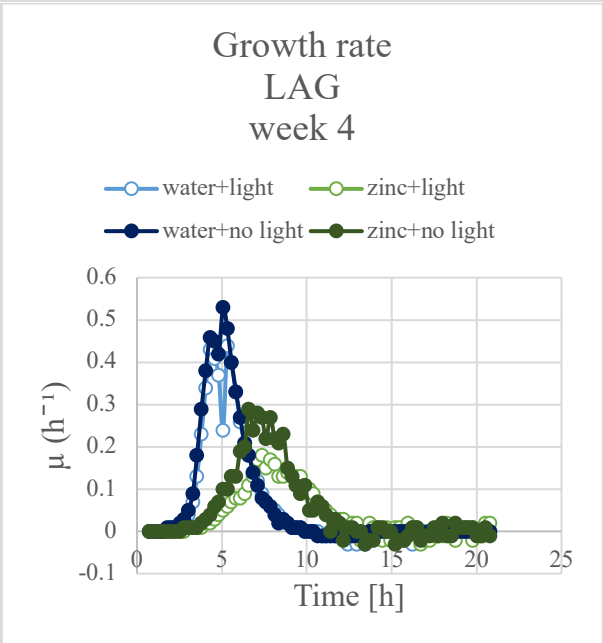
11.5.3 Growth rate charts

a. Lag phase cells

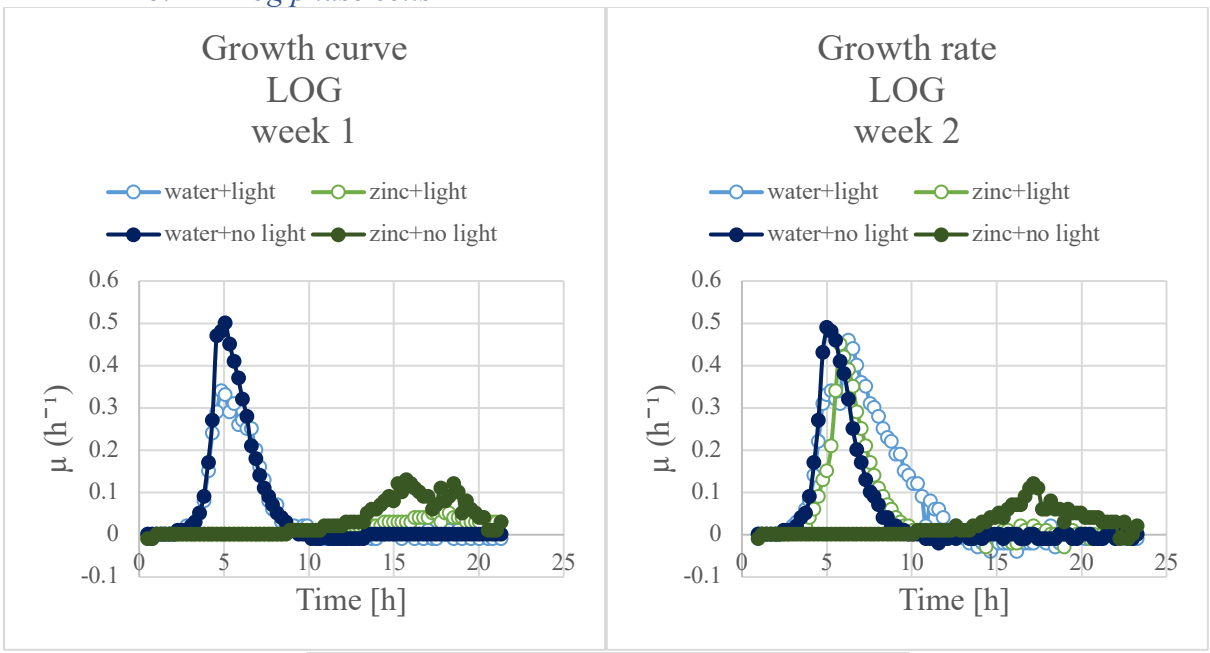


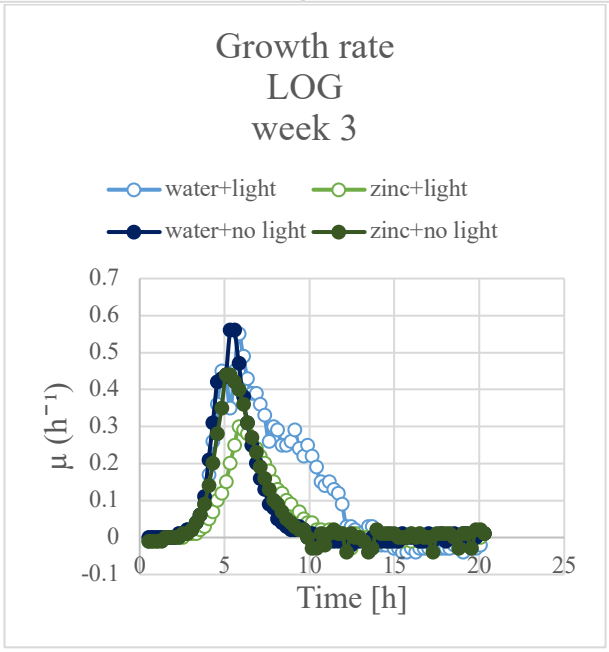




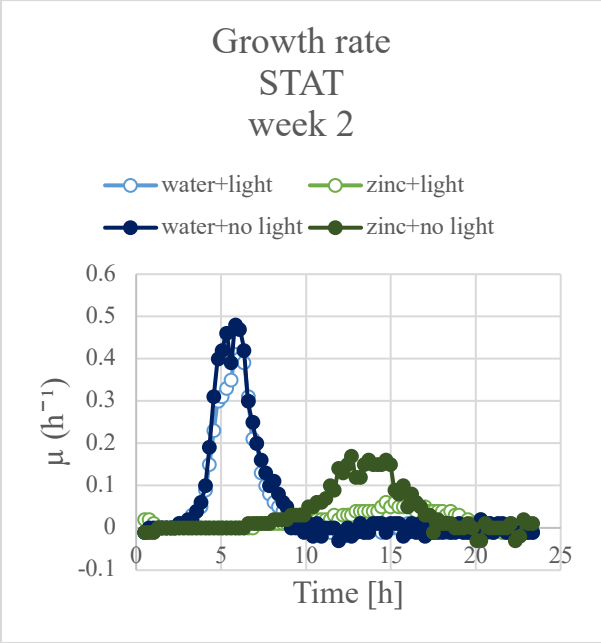


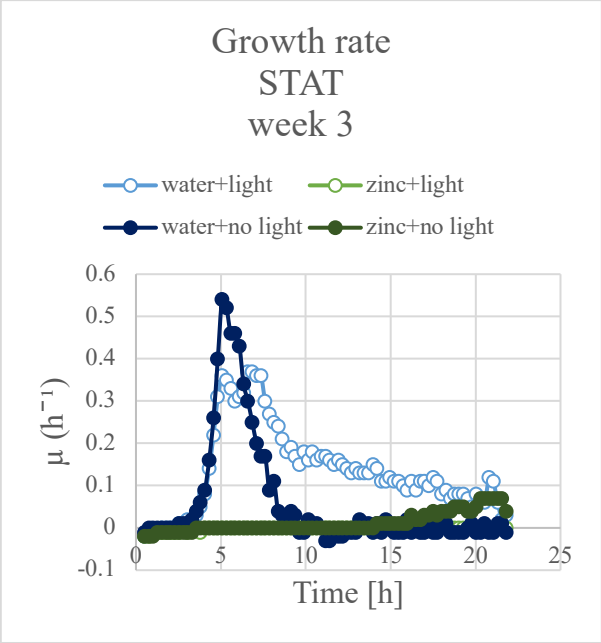
b. Log phase cells





c. Stat phase cells





11.5.4 Data from the automatic colony counter and counted cfu/mL

Appendix 11.5.4-1 *Data from the automatic colony counter with calculated cfu/mL for lag phase cells.*

Lag cells data from the automatic colony counter

Plate Id	Counted Colonies	Dilution Factor	Counted Volume [mL]	Counted Concentration [cfu/mL]	Zinc	Week	Time	Phase	Light
1e+04 Ec zinc lag t0_c	18	1.00E+04	0.39	9.31E+05	True	1	t0	lag	None
1e+04 Ec zinc lag t0_b	6	1.00E+04	0.39	3.10E+05	True	1	t0	lag	None
1e+04 Ec zinc lag t0_a	13	1.00E+04	0.39	6.73E+05	True	1	t0	lag	None
1e+03 Ec zinc lag t0_c	47	1.00E+03	0.39	2.43E+05	True	1	t0	lag	None
1e+03 Ec zinc lag t0_b	33	1.00E+03	0.39	1.71E+05	True	1	t0	lag	None
1e+03 Ec zinc lag t0_a	46	1.00E+03	0.39	2.38E+05	True	1	t0	lag	None
1e+04 Ec water lag t0_c	8	1.00E+04	0.39	4.14E+05	False	1	t0	lag	None
1e+04 Ec water lag t0_b	9	1.00E+04	0.39	4.66E+05	False	1	t0	lag	None
1e+04 Ec water lag t0_a	14	1.00E+04	0.39	7.24E+05	False	1	t0	lag	None
1e+03 Ec water lag t0_c	30	1.00E+03	0.39	1.55E+05	False	1	t0	lag	None
1e+03 Ec water lag t0_b	28	1.00E+03	0.39	1.45E+05	False	1	t0	lag	None
1e+03 Ec water lag t0_a	35	1.00E+03	0.39	1.81E+05	False	1	t0	lag	None
1e+08 Ec lag WD t20.b	25	1.00E+08	0.39	1.29E+10	False	1	t20	lag	False
1e+08 Ec lag WD t20.a	22	1.00E+08	0.39	1.14E+10	False	1	t20	lag	False
1e+07 Ec lag WD t20.b	164	1.00E+07	0.39	8.49E+09	False	1	t20	lag	False
1e+07 Ec lag WD t20.a	127	1.00E+07	0.39	6.57E+09	False	1	t20	lag	False
1e+08 Ec lag ZD t20.a	39	1.00E+08	0.39	2.02E+10	True	1	t20	lag	False
1e+08 Ec lag ZD t20.b	39	1.00E+08	0.39	2.02E+10	True	1	t20	lag	False
1e+07 Ec lag ZD t20.b	143	1.00E+07	0.39	7.40E+09	True	1	t20	lag	False
1e+07 Ec lag ZD t20.a	101	1.00E+07	0.39	5.23E+09	True	1	t20	lag	False

1e+08 Ec lag WL t20.b	52	1.00E+08	0.39	2.69E+10	False	1	t20	lag	True
1e+08 Ec lag WL t20.a	50	1.00E+08	0.39	2.59E+10	False	1	t20	lag	True
1e+07 Ec lag WL t20.b	214	1.00E+07	0.39	1.11E+10	False	1	t20	lag	True
1e+07 Ec lag WL t20.a	152	1.00E+07	0.39	7.87E+09	False	1	t20	lag	True
1e+06 Ec lag ZL t20.a1	3	1.00E+06	0.39	1.55E+07	True	1	t20	lag	True
1e+06 Ec lag ZL t20.a	8	1.00E+06	0.39	4.14E+07	True	1	t20	lag	True
1e+05 Ec lag ZL t20.b	49	1.00E+05	0.39	2.54E+07	True	1	t20	lag	True
1e+05 Ec lag ZL t20.a	43	1.00E+05	0.39	2.23E+07	True	1	t20	lag	True
EC_ER_lag_light_week2_t0_z_3c	91	1.00E+03	0.39	4.71E+05	True	2	t0	lag	None
EC_ER_lag_light_week2_t0_z_3b	92	1.00E+03	0.39	4.76E+05	True	2	t0	lag	None
EC_ER_lag_light_week2_t0_z_3a	121	1.00E+03	0.39	6.26E+05	True	2	t0	lag	None
EC_ER_lag_light_week2_t0_z_4c	42	1.00E+04	0.39	2.17E+06	True	2	t0	lag	None
EC_ER_lag_light_week2_t0_z_4b	41	1.00E+04	0.39	2.12E+06	True	2	t0	lag	None
EC_ER_lag_light_week2_t0_z_4a	56	1.00E+04	0.39	2.90E+06	True	2	t0	lag	None
EC_ER_lag_light_week2_t0_w_4c	41	1.00E+04	0.39	2.12E+06	False	2	t0	lag	None
EC_ER_lag_light_week2_t0_w_4b	52	1.00E+04	0.39	2.69E+06	False	2	t0	lag	None
EC_ER_lag_light_week2_t0_w_4a	42	1.00E+04	0.39	2.17E+06	False	2	t0	lag	None
EC_ER_lag_light_week2_t0_w_3c	79	1.00E+03	0.39	4.09E+05	False	2	t0	lag	None
EC_ER_lag_light_week2_t0_w_3b	91	1.00E+03	0.39	4.71E+05	False	2	t0	lag	None
EC_ER_lag_light_week2_t0_w_3a	84	1.00E+03	0.39	4.35E+05	False	2	t0	lag	None
EC_ER_lag_ZL_t20_1e+06_b	370	1.00E+06	0.39	1.91E+09	True	2	t20	lag	True
EC_ER_lag_ZL_t20_1e+06_a	306	1.00E+06	0.39	1.58E+09	True	2	t20	lag	True
EC_ER_lag_ZL_t20_1e+05_b	439	1.00E+05	0.39	2.27E+08	True	2	t20	lag	True
EC_ER_lag_ZL_t20_1e+05_a	876	1.00E+05	0.39	4.53E+08	True	2	t20	lag	True
EC_ER_lag_ZNL_t20_1e+06_b	407	1.00E+06	0.39	2.11E+09	True	2	t20	lag	False
EC_ER_lag_ZNL_t20_1e+06_a	393	1.00E+06	0.39	2.03E+09	True	2	t20	lag	False
EC_ER_lag_ZNL_t20_1e+05_b	588	1.00E+05	0.39	3.04E+08	True	2	t20	lag	False
EC_ER_lag_ZNL_t20_1e+05_a	1729	1.00E+05	0.39	8.95E+08	True	2	t20	lag	False
EC_ER_lag_WL_t20_1e+08_b	12	1.00E+08	0.39	6.21E+09	False	2	t20	lag	True
EC_ER_lag_WL_t20_1e+08_a	9	1.00E+08	0.39	4.66E+09	False	2	t20	lag	True
EC_ER_lag_WL_t20_1e+07_b	63	1.00E+07	0.39	3.26E+09	False	2	t20	lag	True
EC_ER_lag_WL_t20_1e+07_a	91	1.00E+07	0.39	4.71E+09	False	2	t20	lag	True
EC_ER_lag_WNL_t20_1e+08_b	5	1.00E+08	0.39	2.59E+09	False	2	t20	lag	False
EC_ER_lag_WNL_t20_1e+08_a	6	1.00E+08	0.39	3.10E+09	False	2	t20	lag	False
EC_ER_lag_WNL_t20_1e+07_b	84	1.00E+07	0.39	4.35E+09	False	2	t20	lag	False
EC_ER_lag_WNL_t20_1e+07_a	81	1.00E+07	0.39	4.19E+09	False	2	t20	lag	False
lag_week3_zinc_t0_-3c	60	1.00E+03	0.39	3.10E+05	True	3	t0	lag	None
lag_week3_zinc_t0_-3b	56	1.00E+03	0.39	2.90E+05	True	3	t0	lag	None
lag_week3_zinc_t0_-3a	38	1.00E+03	0.39	1.97E+05	True	3	t0	lag	None
lag_week3_zinc_t0_-4c	2	1.00E+04	0.39	1.03E+05	True	3	t0	lag	None
lag_week3_zinc_t0_-4b	2	1.00E+04	0.39	1.03E+05	True	3	t0	lag	None
lag_week3_zinc_t0_-4a	7	1.00E+04	0.39	3.62E+05	True	3	t0	lag	None

lag_week3_water_-3c	37	1.00E+03	0.39	1.91E+05	False	3	t0	lag	None
lag_week3_water_-3b	47	1.00E+03	0.39	2.43E+05	False	3	t0	lag	None
lag_week3_water_-3a	42	1.00E+03	0.39	2.17E+05	False	3	t0	lag	None
lag_week3_water_-4c	3	1.00E+04	0.39	1.55E+05	False	3	t0	lag	None
lag_week3_water_-4b	4	1.00E+04	0.39	2.07E+05	False	3	t0	lag	None
lag_week3_water_-4a	3	1.00E+04	0.39	1.55E+05	False	3	t0	lag	None
Lag_week3_t20_ZNL_7b	48	1.00E+07	0.39	2.48E+09	True	3	t20	lag	False
Lag_week3_t20_ZNL_7a	74	1.00E+07	0.39	3.83E+09	True	3	t20	lag	False
Lag_week3_t20_ZNL_8b	10	1.00E+08	0.39	5.17E+09	True	3	t20	lag	False
Lag_week3_t20_ZNL_8a	5	1.00E+08	0.39	2.59E+09	True	3	t20	lag	False
Lag_week3_t20_ZL_7b	58	1.00E+07	0.39	3.00E+09	True	3	t20	lag	True
Lag_week3_t20_ZL_7a	63	1.00E+07	0.39	3.26E+09	True	3	t20	lag	True
Lag_week3_t20_ZL_8b	14	1.00E+08	0.39	7.24E+09	True	3	t20	lag	True
Lag_week3_t20_ZL_8a	7	1.00E+08	0.39	3.62E+09	True	3	t20	lag	True
Lag_week3_t20_WNL_7b1	8	1.00E+07	0.39	4.14E+08	False	3	t20	lag	False
Lag_week3_t20_WNL_7b	10	1.00E+07	0.39	5.17E+08	False	3	t20	lag	False
Lag_week3_t20_WNL_8b	3	1.00E+08	0.39	1.55E+09	False	3	t20	lag	False
Lag_week3_t20_WNL_8a	8	1.00E+08	0.39	4.14E+09	False	3	t20	lag	False
Lag_week3_t20_WL_7b	92	1.00E+07	0.39	4.76E+09	False	3	t20	lag	True
Lag_week3_t20_WL_7a	77	1.00E+07	0.39	3.98E+09	False	3	t20	lag	True
Lag_week3_t20_WL_8b	12	1.00E+08	0.39	6.21E+09	False	3	t20	lag	True
Lag_week3_t20_WL_8a	10	1.00E+08	0.39	5.17E+09	False	3	t20	lag	True
lag_weeek4_t0_W_3c	22	1.00E+03	0.38	1.14E+05	False	4	t0	lag	None
lag_weeek4_t0_W_3b	24	1.00E+03	0.38	1.25E+05	False	4	t0	lag	None
lag_weeek4_t0_W_3a	21	1.00E+03	0.38	1.09E+05	False	4	t0	lag	None
lag_weeek4_t0_W_4c	1	1.00E+04	0.38	5.20E+04	False	4	t0	lag	None
lag_weeek4_t0_W_4b	3	1.00E+04	0.38	1.56E+05	False	4	t0	lag	None
lag_weeek4_t0_W_4a	1	1.00E+04	0.38	5.20E+04	False	4	t0	lag	None
lag_weeek4_t0_Z_3c	25	1.00E+03	0.38	1.30E+05	True	4	t0	lag	None
lag_weeek4_t0_Z_3b	27	1.00E+03	0.38	1.40E+05	True	4	t0	lag	None
lag_weeek4_t0_Z_3a	26	1.00E+03	0.38	1.35E+05	True	4	t0	lag	None
lag_weeek4_t0_Z_4c	2	1.00E+04	0.38	1.04E+05	True	4	t0	lag	None
lag_weeek4_t0_Z_4b	1	1.00E+04	0.38	5.20E+04	True	4	t0	lag	None
lag_weeek4_t0_Z_4a	2	1.00E+04	0.38	1.04E+05	True	4	t0	lag	None
lag_week4_t20_ZL_8b	4	1.00E+08	0.38	2.08E+09	True	4	t20	lag	True
lag_week4_t20_ZL_8a	4	1.00E+08	0.38	2.08E+09	True	4	t20	lag	True
lag_week4_t20_ZL_7b	28	1.00E+07	0.38	1.46E+09	True	4	t20	lag	True
lag_week4_t20_ZL_7a	36	1.00E+07	0.38	1.87E+09	True	4	t20	lag	True
lag_week4_t20_ZNL_8b	2	1.00E+08	0.38	1.04E+09	True	4	t20	lag	False
lag_week4_t20_ZNL_8a	4	1.00E+08	0.38	2.08E+09	True	4	t20	lag	False
lag_week4_t20_ZNL_7b	47	1.00E+07	0.38	2.44E+09	True	4	t20	lag	False
lag_week4_t20_ZNL_7a	41	1.00E+07	0.38	2.13E+09	True	4	t20	lag	False

lag_week4_t20_WL_7b	49	1.00E+07	0.38	2.55E+09	False	4	t20	lag	True
lag_week4_t20_WL_7a	52	1.00E+07	0.38	2.70E+09	False	4	t20	lag	True
lag_week4_t20_WL_8b	10	1.00E+08	0.38	5.20E+09	False	4	t20	lag	True
lag_week4_t20_WL_8a	10	1.00E+08	0.38	5.20E+09	False	4	t20	lag	True
lag_week4_t20_WNL_7b	69	1.00E+07	0.38	3.59E+09	False	4	t20	lag	False
lag_week4_t20_WNL_7	70	1.00E+07	0.38	3.64E+09	False	4	t20	lag	False
lag_week4_t20_WNL_8b	6	1.00E+08	0.38	3.12E+09	False	4	t20	lag	False
log_week4_t20_WNL_8a	6	1.00E+08	0.38	3.12E+09	False	4	t20	lag	False

Appendix 11.5.4-2 *Data from the automatic colony counter with calculated cfu/mL for log phase cells.*

Log cells data from the automatic colony counter									
Plate Id	**Counted Colonies**	**Dilution Factor**	**Counted Volume [mL]**	**Counted Concentration [cfu/mL]**	**Zinc**	**Week**	**Time**	**Phase**	**Light**
log_w1_t0_W_-3c	28	1.00E+03	0.39	1.45E+05	False	1	t0	log	None
log_w1_t0_W_-3b	35	1.00E+03	0.39	1.81E+05	False	1	t0	log	None
log_w1_t0_W_-3a	39	1.00E+03	0.39	2.02E+05	False	1	t0	log	None
log_w1_t0_W_-4c	6	1.00E+04	0.39	3.10E+05	False	1	t0	log	None
log_w1_t0_W_-4b	1	1.00E+04	0.39	5.17E+04	False	1	t0	log	None
log_w1_t0_W_-4a	1	1.00E+04	0.39	5.17E+04	False	1	t0	log	None
log_w1_t0_Z_-3c	25	1.00E+03	0.39	1.29E+05	True	1	t0	log	None
log_w1_t0_Z_-3b	46	1.00E+03	0.39	2.38E+05	True	1	t0	log	None
log_w1_t0_Z_-3a	44	1.00E+03	0.39	2.28E+05	True	1	t0	log	None
log_w1_t0_Z_-4c	2	1.00E+04	0.39	1.03E+05	True	1	t0	log	None
log_w1_t0_Z_-4b	3	1.00E+04	0.39	1.55E+05	True	1	t0	log	None
log_w1_t0_Z_-4a	5	1.00E+04	0.39	2.59E+05	True	1	t0	log	None
log_week1_t20_WNL_8b	6	1.00E+08	0.39	3.10E+09	False	1	t20	log	False
log_week1_t20_WNL_8a	14	1.00E+08	0.39	7.24E+09	False	1	t20	log	False
log_week1_t20_WNL_7b	96	1.00E+07	0.39	4.97E+09	False	1	t20	log	False
log_week1_t20_WNL_7a	75	1.00E+07	0.39	3.88E+09	False	1	t20	log	False
log_week1_t20_WL_8b	8	1.00E+08	0.39	4.14E+09	False	1	t20	log	True
log_week1_t20_WL_8a	7	1.00E+08	0.39	3.62E+09	False	1	t20	log	True
log_week1_t20_WL_7b	92	1.00E+07	0.39	4.76E+09	False	1	t20	log	True
log_week1_t20_WL_7a	69	1.00E+07	0.39	3.57E+09	False	1	t20	log	True
log_week1_t20_ZL_6b	312	1.00E+06	0.39	1.61E+09	True	1	t20	log	True
log_week1_t20_ZL_6a	274	1.00E+06	0.39	1.42E+09	True	1	t20	log	True
log_week1_t20_ZL_5b	607	1.00E+05	0.39	3.14E+08	True	1	t20	log	True
log_week1_t20_ZL_5a	590	1.00E+05	0.39	3.05E+08	True	1	t20	log	True
log_week1_t20_ZNL_7b	67	1.00E+07	0.39	3.47E+09	True	1	t20	log	False
log_week1_t20_ZNL_7a	70	1.00E+07	0.39	3.62E+09	True	1	t20	log	False
log_week1_t20_ZNL_6b	339	1.00E+06	0.39	1.75E+09	True	1	t20	log	False
log_week1_t20_ZNL_6a	339	1.00E+06	0.39	1.75E+09	True	1	t20	log	False

log_week2_t0_W_3c	107	1.00E+03	0.84	1.27E+05	False	2	t0	log	None
log_week2_t0_W_3b	83	1.00E+03	0.84	9.86E+04	False	2	t0	log	None
log_week2_t0_W_3a	78	1.00E+03	0.84	9.26E+04	False	2	t0	log	None
log_week2_t0_W_4b	11	1.00E+04	0.84	1.31E+05	False	2	t0	log	None
log_week2_t0_W_4a	6	1.00E+04	0.84	7.13E+04	False	2	t0	log	None
log_week2_t0_Z_3c	64	1.00E+03	0.84	7.60E+04	True	2	t0	log	None
log_week2_t0_Z_3b	76	1.00E+03	0.84	9.03E+04	True	2	t0	log	None
log_week2_t0_Z_3a	86	1.00E+03	0.84	1.02E+05	True	2	t0	log	None
log_week2_t0_Z_4b	8	1.00E+04	0.84	9.50E+04	True	2	t0	log	None
log_week2_t0_Z_4c	12	1.00E+04	0.84	1.43E+05	True	2	t0	log	None
log_week2_t0_W_4c	7	1.00E+04	0.84	8.31E+04	False	2	t0	log	None
log_week2_t0_Z_4a	12	1.00E+04	0.84	1.43E+05	True	2	t0	log	None
log_week2_t20_WL_7b	162	1.00E+07	0.84	1.92E+09	False	2	t20	log	True
log_week2_t20_WL_7a	149	1.00E+07	0.84	1.77E+09	False	2	t20	log	True
log_week2_t20_WL_8b	8	1.00E+08	0.84	9.50E+08	False	2	t20	log	True
log_week2_t20_WL_8a	13	1.00E+08	0.84	1.54E+09	False	2	t20	log	True
log_week2_t20_WNL_8b	20	1.00E+08	0.84	2.38E+09	False	2	t20	log	False
log_week2_t20_WNL_8a	29	1.00E+08	0.84	3.44E+09	False	2	t20	log	False
log_week2_t20_WNL_9b	4	1.00E+09	0.84	4.75E+09	False	2	t20	log	False
log_week2_t20_WNL_9a	7	1.00E+09	0.84	8.31E+09	False	2	t20	log	False
log_week2_t20_ZNL_6b	198	1.00E+06	0.84	2.35E+08	True	2	t20	log	False
log_week2_t20_ZNL_6a	141	1.00E+06	0.84	1.67E+08	True	2	t20	log	False
log_week2_t20_ZNL_7b	52	1.00E+07	0.84	6.18E+08	True	2	t20	log	False
log_week2_t20_ZNL_7a	40	1.00E+07	0.84	4.75E+08	True	2	t20	log	False
log_week2_t20_ZL_7b	112	1.00E+07	0.84	1.33E+09	True	2	t20	log	True
log_week2_t20_ZL_7a	125	1.00E+07	0.84	1.48E+09	True	2	t20	log	True
log_week2_t20_ZL_8b	25	1.00E+08	0.84	2.97E+09	True	2	t20	log	True
log_week2_t20_ZL_8a	15	1.00E+08	0.84	1.78E+09	True	2	t20	log	True
log_week3_t0_W_4c	28	1.00E+04	0.38	1.46E+06	False	3	t0	log	None
log_week3_t0_W_4b	30	1.00E+04	0.38	1.56E+06	False	3	t0	log	None
log_week3_t0_W_4a	27	1.00E+04	0.38	1.40E+06	False	3	t0	log	None
log_week3_t0_W_3c	50	1.00E+03	0.38	2.60E+05	False	3	t0	log	None
log_week3_t0_W_3b	63	1.00E+03	0.38	3.28E+05	False	3	t0	log	None
log_week3_t0_W_3a	67	1.00E+03	0.38	3.48E+05	False	3	t0	log	None
log_week3_t0_Z_4c	2	1.00E+04	0.38	1.04E+05	True	3	t0	log	None
log_week3_t0_Z_4b	4	1.00E+04	0.38	2.08E+05	True	3	t0	log	None
log_week3_t0_Z_4a	6	1.00E+04	0.38	3.12E+05	True	3	t0	log	None
log_week3_t0_Z_3c	28	1.00E+03	0.38	1.46E+05	True	3	t0	log	None
log_week3_t0_Z_3b	19	1.00E+03	0.38	9.88E+04	True	3	t0	log	None
log_week3_t0_Z_3a	30	1.00E+03	0.38	1.56E+05	True	3	t0	log	None
log_weeek3_t20_ZL_8b	9	1.00E+08	0.38	4.68E+09	True	3	t20	log	True
log_weeek3_t20_ZL_8a	6	1.00E+08	0.38	3.12E+09	True	3	t20	log	True

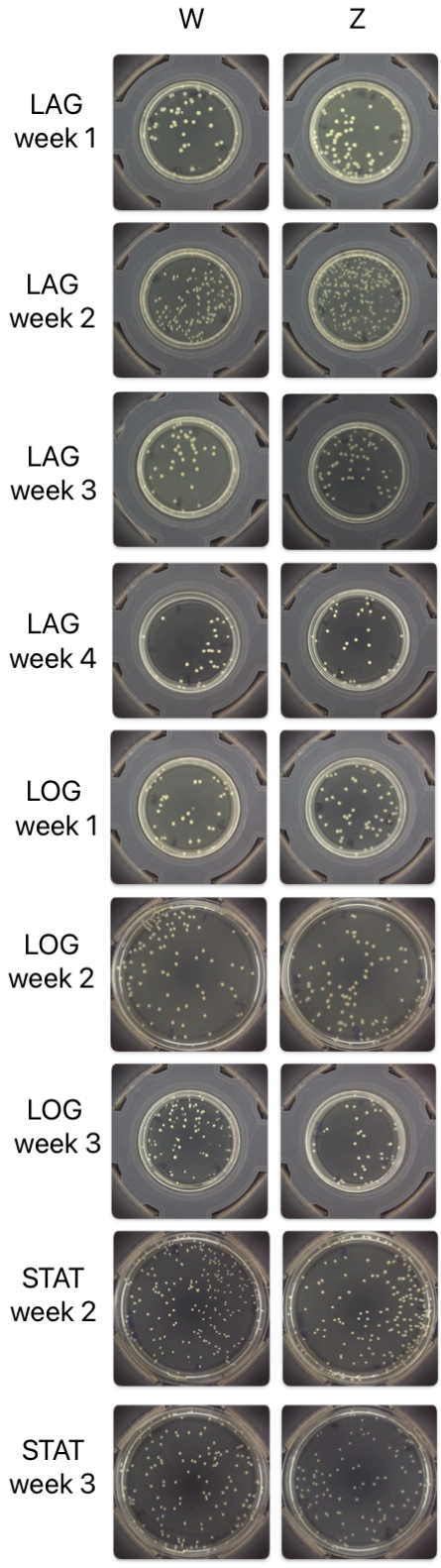
log_weeek3_t20_ZL_7b	42	1.00E+07	0.38	2.18E+09	True	3	t20	log	True
log_weeek3_t20_ZL_7a	47	1.00E+07	0.38	2.44E+09	True	3	t20	log	True
log_weeek3_t20_ZNL_7b	47	1.00E+07	0.38	2.44E+09	True	3	t20	log	False
log_weeek3_t20_ZNL_7a	80	1.00E+07	0.38	4.16E+09	True	3	t20	log	False
log_weeek3_t20_ZNL_8b	6	1.00E+08	0.38	3.12E+09	True	3	t20	log	False
log_weeek3_t20_ZNL_8a	10	1.00E+08	0.38	5.20E+09	True	3	t20	log	False
log_weeek3_t20_WNL_7b	80	1.00E+07	0.38	4.16E+09	False	3	t20	log	False
log_weeek3_t20_WNL_7a	60	1.00E+07	0.38	3.12E+09	False	3	t20	log	False
log_weeek3_t20_WNL_8b	9	1.00E+08	0.38	4.68E+09	False	3	t20	log	False
log_weeek3_t20_WNL_8a	8	1.00E+08	0.38	4.16E+09	False	3	t20	log	False
log_weeek3_t20_WL_7b	149	1.00E+07	0.38	7.75E+09	False	3	t20	log	True
log_weeek3_t20_WL_7a	101	1.00E+07	0.38	5.25E+09	False	3	t20	log	True
log_weeek3_t20_WL_8b	14	1.00E+08	0.38	7.28E+09	False	3	t20	log	True
log_weeek3_t20_WL_8a	15	1.00E+08	0.38	7.80E+09	False	3	t20	log	True

Appendix 11.5.4-3 *Data from the automatic colony counter with calculated cfu/mL for stat phase cells.*

Stat cells data from the automatic colony counter									
Plate Id	**Counted Colonies**	**Dilution Factor**	**Counted Volume [mL]**	**Counted Concentration [cfu/mL]**	**Zinc**	**Week**	**Time**	**Phase**	**Light**
stat_week2_t0_W_3c	204	1.00E+03	0.84	2.42E+05	False	2	t0	stat	None
stat_week2_t0_W_3b	88	1.00E+03	0.84	1.05E+05	False	2	t0	stat	None
stat_week2_t0_W_3a	176	1.00E+03	0.84	2.09E+05	False	2	t0	stat	None
stat_week2_t0_W_4c	31	1.00E+04	0.84	3.68E+05	False	2	t0	stat	None
stat_week2_t0_W_4b	28	1.00E+04	0.84	3.33E+05	False	2	t0	stat	None
stat_week2_t0_W_4a	20	1.00E+04	0.84	2.38E+05	False	2	t0	stat	None
stat_week2_t0_Z_3c	113	1.00E+03	0.84	1.34E+05	True	2	t0	stat	None
stat_week2_t0_Z_3b	122	1.00E+03	0.84	1.45E+05	True	2	t0	stat	None
stat_week2_t0_Z_3a	123	1.00E+03	0.84	1.46E+05	True	2	t0	stat	None
stat_week2_t0_Z_4c	19	1.00E+04	0.84	2.26E+05	True	2	t0	stat	None
stat_week2_t0_Z_4b	25	1.00E+04	0.84	2.97E+05	True	2	t0	stat	None
stat_week2_t0_Z_4a	17	1.00E+04	0.84	2.02E+05	True	2	t0	stat	None
stat_week2_t24_WNL_7b	164	1.00E+07	0.84	1.95E+09	False	2	t24	stat	False
stat_week2_t24_WNL_7a	129	1.00E+07	0.84	1.53E+09	False	2	t24	stat	False
stat_week2_t24_WNL_8b	18	1.00E+08	0.84	2.14E+09	False	2	t24	stat	False
stat_week2_t24_WNL_8a	10	1.00E+08	0.84	1.19E+09	False	2	t24	stat	False
stat_week2_t24_WL_7b	129	1.00E+07	0.84	1.53E+09	False	2	t24	stat	True
stat_week2_t24_WL_7a	103	1.00E+07	0.84	1.22E+09	False	2	t24	stat	True
stat_week2_t24_WL_8b	17	1.00E+08	0.84	2.02E+09	False	2	t24	stat	True
stat_week2_t24_WL_8a	8	1.00E+08	0.84	9.50E+08	False	2	t24	stat	True
stat_week2_t24_ZNL_7b	68	1.00E+07	0.84	8.08E+08	True	2	t24	stat	False
stat_week2_t24_ZNL_7a	76	1.00E+07	0.84	9.03E+08	True	2	t24	stat	False
stat_week2_t24_ZNL_8b	12	1.00E+08	0.84	1.43E+09	True	2	t24	stat	False

stat_week2_t24_ZNL_8a	19	1.00E+08	0.84	2.26E+09	True	2	t24	stat	False
stat_week2_t24_ZL_5b	89	1.00E+05	0.84	1.06E+07	True	2	t24	stat	True
stat_week2_t24_ZL_5a	92	1.00E+05	0.84	1.09E+07	True	2	t24	stat	True
stat_week2_t24_ZL_6b	10	1.00E+06	0.84	1.19E+07	True	2	t24	stat	True
stat_week2_t24_ZL_6a	12	1.00E+06	0.84	1.43E+07	True	2	t24	stat	True
stat_week3_t0_W_3c	102	1.00E+03	0.84	1.21E+05	False	3	t0	stat	None
stat_week3_t0_W_3b	109	1.00E+03	0.84	1.29E+05	False	3	t0	stat	None
stat_week3_t0_W_3a	84	1.00E+03	0.84	9.98E+04	False	3	t0	stat	None
stat_week3_t0_W_4c	8	1.00E+04	0.84	9.50E+04	False	3	t0	stat	None
stat_week3_t0_W_4b	6	1.00E+04	0.84	7.13E+04	False	3	t0	stat	None
stat_week3_t0_W_4a	12	1.00E+04	0.84	1.43E+05	False	3	t0	stat	None
stat_week3_t0_Z_3c	128	1.00E+03	0.84	1.52E+05	True	3	t0	stat	None
stat_week3_t0_Z_3b	97	1.00E+03	0.84	1.15E+05	True	3	t0	stat	None
stat_week3_t0_Z_3a	142	1.00E+03	0.84	1.69E+05	True	3	t0	stat	None
stat_week3_t0_Z_4c	7	1.00E+04	0.84	8.31E+04	True	3	t0	stat	None
stat_week3_t0_Z_4b	8	1.00E+04	0.84	9.50E+04	True	3	t0	stat	None
stat_week3_t0_Z_4a	7	1.00E+04	0.84	8.31E+04	True	3	t0	stat	None
stat_week3_t24_ZL_4b	193	1.00E+04	0.84	2.29E+06	True	3	t24	stat	True
stat_week3_t24_ZL_4a	255	1.00E+04	0.84	3.03E+06	True	3	t24	stat	True
stat_week3_t24_ZL_5b	23	1.00E+05	0.84	2.73E+06	True	3	t24	stat	True
stat_week3_t24_ZL_5a	17	1.00E+05	0.84	2.02E+06	True	3	t24	stat	True
stat_week3_t24_WL_8b	54	1.00E+08	0.84	6.41E+09	False	3	t24	stat	True
stat_week3_t24_WL_8a	39	1.00E+08	0.84	4.63E+09	False	3	t24	stat	True
stat_week3_t24_WL_9b	4	1.00E+09	0.84	4.75E+09	False	3	t24	stat	True
stat_week3_t24_WL_9a	7	1.00E+09	0.84	8.31E+09	False	3	t24	stat	True
stat_week3_t48_ZNL_6b	176	1.00E+06	0.84	2.09E+08	True	3	t24	stat	False
stat_week3_t48_ZNL_6a	179	1.00E+06	0.84	2.13E+08	True	3	t24	stat	False
stat_week3_t48_ZNL_7b	34	1.00E+07	0.84	4.04E+08	True	3	t24	stat	False
stat_week3_t48_ZNL_7a	42	1.00E+07	0.84	4.99E+08	True	3	t24	stat	False
stat_week3_t48_WNL_9b	4	1.00E+09	0.84	4.75E+09	False	3	t24	stat	False
stat_week3_t48_WNL_9a	1	1.00E+09	0.84	1.19E+09	False	3	t24	stat	False
stat_week3_t48_WNL_8b	20	1.00E+08	0.84	2.38E+09	False	3	t24	stat	False
stat_week3_t48_WNL_8a	13	1.00E+08	0.84	1.54E+09	False	3	t24	stat	False

11.5.5 Illumination experiment t0 plates pictures from the automatic colony counter



11.5.6 Illumination experiment lag phase length, final OD value, growth rate maximal value

a. Lag phase length

Lag phase length [h]					
LAG cells					
	week 1	**week 2**	**week 3**	**week 4**	**Mean [h]**
water+light	2.15	1.78	2.03	2.53	**2.12**
water+no light	2.40	2.03	2.03	2.28	**2.19**
zinc+light	2.15	4.06	2.54	3.54	**3.07**
zinc+no light	2.25	5.07	2.03	3.04	**3.10**
LOG cells					
	week 1	**week 2**	**week 3**	**week 4**	**Mean**
water+light	2.79	2.73	2.79	x	**2.77**
water+no light	2.79	2.98	2.54	x	**2.77**
zinc+light	8.88	3.49	3.04	x	**5.14**
zinc+no light	9.64	11.35	3.04	x	**8.01**
STAT cells					
	week 1	**week 2**	**week 3**	**week 4**	**Mean**
water+light	x	3.04	3.04	x	**3.04**
water+no light	x	3.04	3.04	x	**3.04**
zinc+light	x	7.10	20.00	x	**13.55**
zinc+no light	x	7.61	20.00	x	**13.81**

Appendix 11.5.6-1 *Table of lag phase length from the illumination experiment with calculated average lag phase length in hours for the different samples and cell phases. The end of lag phase was considered when OD value reached 0.02, since after this OD value, exponential growth was observed. Marked red are samples which did not grow over the 0.02 mark throughout the whole experiment and therefore the lag phase was considered to be the duration of the experiment.*

b. Final OD value

Final OD value (t20)					
LAG cells					
	week 1	**week 2**	**week 3**	**week 4**	**Mean**
water+light	1.02	0.98	1.03	1.17	**1.05**
water+no light	1.26	1.13	1.18	1.34	**1.23**
zinc+light	0.52	0.67	1.04	0.76	**0.75**
zinc+no light	1.07	0.85	1.11	1.06	**1.02**
LOG cells					
	week 1	**week 2**	**week 3**	**week 4**	**Mean**
water+light	1.00	1.80	2.25	x	**1.00**
water+no light	1.19	1.17	1.37	x	**1.24**
zinc+light	0.32	1.08	0.97	x	**0.79**
zinc+no light	0.70	0.46	1.26	x	**0.81**
STAT cells					
	week 1	**week 2**	**week 3**	**week 4**	**Mean**
water+light	x	1.09	2.90	x	**1.09**
water+no light	x	1.29	1.29	x	**1.29**
zinc+light	x	0.41	0.00	x	**0.21**
zinc+no light	x	0.78	0.17	x	**0.48**

Appendix 11.5.6-2 *Table of final OD values from the illumination experiment with calculated average OD value, for the different samples and cell phases. Marked red are samples which had oddly looking growth curve, which could have been caused by air getting inside the falcon tube. These values were not used for the calculation of the average.*

c. Maximal Growth rate

Maximal Growth rate										
LAG cells										
	week 1		**week 2**		**week 3**		**week 4**		**Mean**	
	max μ [h⁻¹]	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**
water+light	0.39	5.19	0.31	3.81	0.32	4.06	0.43	4.30	**0.36**	**4.34**
water+no light	0.52	4.94	0.46	4.31	0.50	4.31	0.53	5.07	**0.50**	**4.66**
zinc+light	0.16	7.48	0.14	8.62	0.26	5.33	0.18	7.35	**0.19**	**7.20**
zinc+no light	0.31	5.19	0.21	10.91	0.40	4.31	0.29	6.59	**0.30**	**6.75**
LOG cells										
	week 1		**week 2**		**week 3**		**week 4**		**Mean**	
	max μ [h⁻¹]	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**
water+light	0.34	4.82	0.46	6.28	0.55	5.83	x	x	**0.45**	**5.64**
water+no light	0.50	5.07	0.49	5.01	0.56	5.33	x	x	**0.52**	**5.14**
zinc+light	0.50	17.25	0.45	5.77	0.30	5.83	x	x	**0.42**	**9.62**
zinc+no light	0.13	15.72	0.12	17.18	0.44	5.07	x	x	**0.23**	**12.66**
STAT cells										
	week 1		**week 2**		**week 3**		**week 4**		**Mean**	
	max μ [h⁻¹]	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**	**max μ [h⁻¹]**	**time [h]**
water+light	x	x	0.41	5.83	0.37	6.59	x	x	**0.39**	**6.21**
water+no light	x	x	0.48	5.83	0.52	5.33	x	x	**0.50**	**5.58**
zinc+light	x	x	0.06	15.22	0.00	20.00	x	x	**0.03**	**17.61**
zinc+no light	x	x	0.17	12.68	0.05	18.77	x	x	**0.11**	**15.73**

Appendix 11.5.6-3 Table of maximal growth rate values [h⁻¹] with time in hours, when the maximal growth rate was reached, from the illumination experiment and shows for all the different samples and cell phases. The growth rate is automatically counted by the software in the bioreactor and it is the difference in OD values per unit of time. Marked red is a zinc light sample which did not grow. Therefore, the maximal growth rate was zero, and the time was the duration of the experiment.

11.6 Statistics code

In this section, code for the statistics tests, which were done in Python, is provided.

11.6.1 Preliminary experiment code

```
#Statistics Diploma thesis
#preliminary experiment
import numpy as np
import scipy.stats as stats
from scipy.stats import shapiro
import pandas as pd
import statsmodels.api as sm
from statsmodels.formula.api import ols

data =
pd.read_excel(r'/Users/evawohlgemuthova/Data/Preliminary_experiment_statistics.xlsx',
sheet_name="ALL DATA")
writer = pd.ExcelWriter("/Users/evawohlgemuthova/Data/preliminary.xlsx")

shapiro_test = pd.DataFrame(columns=('name', 'statistics', 'pvalue'))

d_t0 = data[data["Time"] == 0]
d_t1 = data[data["Time"] == 1]
d_t2 = data[data["Time"] == 2]

d_w1 = data[data["Week"] == 1]
d_w2 = data[data["Week"] == 2]
d_w3 = data[data["Week"] == 3]

#test of normality
shap = shapiro(d_t0["concentration"])
shapiro_test.loc[len(shapiro_test.index)] = ['t0', shap.statistic, shap.pvalue]
shap = shapiro(d_t1["concentration"])
shapiro_test.loc[len(shapiro_test.index)] = ['t1', shap.statistic, shap.pvalue]
shap = shapiro(d_t2["concentration"])
shapiro_test.loc[len(shapiro_test.index)] = ['t2', shap.statistic, shap.pvalue]
shap = shapiro(d_w1["concentration"])
shapiro_test.loc[len(shapiro_test.index)] = ['w1', shap.statistic, shap.pvalue]
shap = shapiro(d_w2["concentration"])
shapiro_test.loc[len(shapiro_test.index)] = ['w2', shap.statistic, shap.pvalue]
shap = shapiro(d_w3["concentration"])
shapiro_test.loc[len(shapiro_test.index)] = ['w3', shap.statistic, shap.pvalue]

shapiro_test.to_excel(writer, sheet_name="shapiro preliminary")
writer.save()

#Levene test to test equal variances
#equal variances t0
e_t0= np.array(d_t0["concentration"])
e_t1= np.array(d_t1["concentration"])
e_t2= np.array(d_t2["concentration"])
```

```
e_w1 = np.array(d_w1["concentration"])
e_w2 = np.array(d_w2["concentration"])
e_w3 = np.array(d_w3["concentration"])

res = stats.levene(e_w1, e_w2, e_w3)
print("preliminary weeks", res.statistic, res.pvalue)

res = stats.levene(e_t0, e_t1, e_t2)
print("preliminary time", res. statistic, res.pvalue)

mod_lm = ols('concentration ~ C(Time)+C(Week)+C(Time)*C(Week)', data=data).fit()
anova_pre = (sm.stats.anova_lm(mod_lm, typ=2))

anova_pre.to_excel(writer, sheet_name="anova preliminary")
writer.save()
writer.close()
```

11.6.2 Illumination experiment code

```
#Statistics Diploma thesis
#cells phase
import numpy as np
import scipy.stats as stats
from scipy.stats import shapiro
import pandas as pd
import statsmodels.api as sm
from statsmodels.formula.api import ols
import pingouin as pg
from statsmodels.iolib.summary2 import summary_col

writer = pd.ExcelWriter("/Users/evawohlgemuthova/Data/anova_lag.xlsx")
writer_test = pd.ExcelWriter("/Users/evawohlgemuthova/Data/shapiro_wilk.xlsx")

writer_t0 = pd.ExcelWriter("/Users/evawohlgemuthova/Data/t test t0 weeks.xlsx")
data = pd.read_excel(r'/Users/evawohlgemuthova/Data/Diploma_thesis_cfu_all data.xlsx',
sheet_name="sheet name")

d_t0 = data[data["Time"] == "t0"]
d_t20 = data[data["Time"] == "t20"]

test = pd.DataFrame(columns=('name', 'statistics', 'pvalue'))
t_test = pd.DataFrame(columns=('name', 'statistics', 'pvalue'))
t_test_t0 = pd.DataFrame(columns=('name', 'statistics', 'pvalue'))

#Shapiro-Wilk test used to test normality
#normality t0
#all data t0
t0 = d_t0[d_t0['Time'] == "t0"]
shap = (shapiro(t0["Counted Concentration"]))
test.loc[len(test.index)] = ['t0 all', shap.statistic, shap.pvalue]
```

```
#t0 zinc
t0_Z = d_t0[d_t0['Zinc'] == True]
shap = (shapiro(t0_Z["Counted Concentration"]))
test.loc[len(test.index)] = ['t0 Z', shap.statistic, shap.pvalue]

#t0 no zinc
t0_NZ = d_t0[d_t0['Zinc'] == False]
shap = shapiro(t0_NZ["Counted Concentration"])
test.loc[len(test.index)] = ['t0 NZ', shap.statistic, shap.pvalue

#t0 weeks
t0_w1 = d_t0[d_t0['Week'] == 1]
shap = shapiro(t0_w1["Counted Concentration"])
test.loc[len(test.index)] = ['t0 w1', shap.statistic, shap.pvalue]

t0_w2 = d_t0[d_t0['Week'] == 2]
shap = shapiro(t0_w2["Counted Concentration"])
test.loc[len(test.index)] = ['t0 w2', shap.statistic, shap.pvalue]

t0_w3 = d_t0[d_t0['Week'] == 3]
shap = shapiro(t0_w3["Counted Concentration"])
test.loc[len(test.index)] = ['t0 w3', shap.statistic, shap.pvalue]
#normality t20
#all data t20
t20 = d_t20[d_t20['Time'] == "t20"]
shap = shapiro(t20["Counted Concentration"])
test.loc[len(test.index)] = ['t20 all', shap.statistic, shap.pvalue]

#t20 zinc
t20_Z = d_t20[d_t20['Zinc'] == True]
shap = shapiro(t20_Z["Counted Concentration"])
test.loc[len(test.index)] = ['t20 Z', shap.statistic, shap.pvalue]

#t20 no zinc
t20_NZ = d_t20[d_t20['Zinc'] == False]
shap = shapiro(t20_NZ["Counted Concentration"])
test.loc[len(test.index)] = ['t20 NZ', shap.statistic, shap.pvalue]

#t20 light
t20_L = d_t20[d_t20['Light'] == True]
shap = shapiro(t20_L["Counted Concentration"])
test.loc[len(test.index)] = ['t20 L', shap.statistic, shap.pvalue]

t20_NL = d_t20[d_t20['Light'] == False]
shap = shapiro(t20_NL["Counted Concentration"])
test.loc[len(test.index)] = ['t20 NL', shap.statistic, shap.pvalue]

#t20 weeks
t20_w1 = d_t20[d_t20['Week'] == 1]
```

```
shap = shapiro(t0_w1["Counted Concentration"])
test.loc[len(test.index)] = ['t20 w1', shap.statistic, shap.pvalue]

t20_w2 = d_t20[d_t20['Week'] == 2]
shap = shapiro(t0_w2["Counted Concentration"])
test.loc[len(test.index)] = ['t20 w2', shap.statistic, shap.pvalue]

t20_w3 = d_t20[d_t20['Week'] == 3]
shap = shapiro(t0_w3["Counted Concentration"])
test.loc[len(test.index)] = ['t20 w3', shap.statistic, shap.pvalue]

#Levene test to test equal variances
#equal variances t0
e_t0_w1= np.array(t0_w1["Counted Concentration"])
e_t0_w2= np.array(t0_w2["Counted Concentration"])
e_t0_w3= np.array(t0_w3["Counted Concentration"])
e_t0_Z = np.array(t0_Z["Counted Concentration"])
e_t0_NZ = np.array(t0_NZ["Counted Concentration"])
res = stats.levene(e_t0_w1, e_t0_w2, e_t0_w3)
print("t0 weeks", res.statistic,  res.pvalue)
res = stats.levene(e_t0_Z, e_t0_NZ)
print("t0 zinc x nozinc",res.statistic, res.pvalue)

#equal variances t20
e_t20_w1= np.array(t20_w1["Counted Concentration"])
e_t20_w2= np.array(t20_w2["Counted Concentration"])
e_t20_w3= np.array(t20_w3["Counted Concentration"])
e_t20_Z = np.array(t20_Z["Counted Concentration"])
e_t20_NZ = np.array(t20_NZ["Counted Concentration"])
e_t20_L = np.array(t20_L["Counted Concentration"])
e_t20_NL = np.array(t20_NL["Counted Concentration"])

res = stats.levene(e_t20_w1, e_t20_w2, e_t20_w3)
print("t20 weeks", res. statistic, res.pvalue)

res = stats.levene(e_t20_Z, e_t20_NZ, e_t20_NL, e_t20_L)
print("t20 Z x NZ x L x NL", res.statistic, res.pvalue)

test.to_excel(writer_test, sheet_name="lag")
writer_test.save()
writer_test.close()

#t test for t0

t_stat, p_value = stats.ttest_ind(a=t0_w1['Counted Concentration'], b=t0_w2['Counted
Concentration'], equal_var=True)
t_test_t0.loc[len(t_test_t0.index)] = ['w1 x w2', t_stat, p_value]

t_stat, p_value = stats.ttest_ind(a=t0_w1['Counted Concentration'], b=t0_w3['Counted
Concentration'], equal_var=True)
```

```
t_test_t0.loc[len(t_test_t0.index)] = ['w1 x w3', t_stat, p_value]

t_stat, p_value = stats.ttest_ind(a=t0_w2['Counted Concentration'], b=t0_w3['Counted
Concentration'], equal_var=True)
t_test_t0.loc[len(t_test_t0.index)] = ['w2 x w3', t_stat, p_value]

t_test_t0.to_excel(writer_t0, sheet_name="lag")
writer_t0.save()
writer_t0.close()
#ANOVA

#t0 two way anova
datat0 = t0
datat0 = datat0.rename(columns={"Counted Concentration":"concentration"}) # make name
pythonic

mod_lm = ols('concentration ~ C(Zinc)+C(Week)+C(Zinc)*C(Week)', data=datat0).fit()
anova2_t0 = sm.stats.anova_lm(mod_lm, typ=2)
print("t0 anova\n", anova2_t0)# Type 2 ANOVA DataFrame
anova2_t0.to_excel(writer, sheet_name="t0_anova2")
writer.save()

#t20, two way anova
datat20 = t20

datat20 = datat20.rename(columns={"Counted Concentration":"concentration"})
mod_lm = ols('concentration ~ C(Zinc)+C(Light)+C(Zinc)*C(Light)', data=datat20).fit()
anova2_t20 = sm.stats.anova_lm(mod_lm, typ=2)
print("t20 two way anova\n", anova2_t20)# Type 2 ANOVA DataFrame
anova2_t20.to_excel(writer, sheet_name="t20_anova2")
writer.save()

#t20, three way anova include weeks
model = ols('concentration ~ C(Zinc) + C(Light) + C(Week) + C(Zinc):C(Light) +
C(Zinc):C(Week) + C(Light):C(Week) +C(Zinc):C(Light):C(Week)', data=datat20).fit()
anova3_t20 = (sm.stats.anova_lm(model, typ=2))
print("t20 three way anova\n",anova3_t20)
anova3_t20.to_excel(writer, sheet_name= "t20_anova3")
writer.save()

# non parametric t-test, if interactions are TRUE, then t-test was performed for the
interactions groups
# for t20 samples, Zinc and Light, 6 test, bonferroni correction = alpha/no.of tests, 0.05/6
z = d_t20[d_t20['Zinc'] == True]
w = d_t20[d_t20['Zinc'] == False]

zl = z[z['Light'] == True]
wl = w[w['Light'] == True]
znl = z[z['Light'] ==  False]
wnl = w[w['Light'] == False]
```

```
#zl_znl
print("t test\n")

t_stat, p_value = stats.ttest_ind(a=zl['Counted Concentration'], b=znl['Counted
Concentration'], equal_var=True)
t_test.loc[len(t_test.index)] = ['zl x znl', t_stat, p_value]


#zl_wl
t_stat, p_value = stats.ttest_ind(a=zl['Counted Concentration'], b=wl['Counted
Concentration'], equal_var=True)
t_test.loc[len(t_test.index)] = ['zl x wl', t_stat, p_value]

#zl_wnl
t_stat, p_value = stats.ttest_ind(a=zl['Counted Concentration'], b=wnl['Counted
Concentration'], equal_var=True)
t_test.loc[len(t_test.index)] = ['zl x wnl', t_stat, p_value]

#znl_wl
t_stat, p_value = stats.ttest_ind(a=znl['Counted Concentration'], b=wl['Counted
Concentration'], equal_var=True)
t_test.loc[len(t_test.index)] = ['znl x wl', t_stat, p_value]

#znl_wnl
t_stat, p_value = stats.ttest_ind(a=znl['Counted Concentration'], b=wnl['Counted
Concentration'], equal_var=True)
t_test.loc[len(t_test.index)] = ['znl x wnl', t_stat, p_value]

#wl_wnl
t_stat,p_value = stats.ttest_ind(a=wl['Counted Concentration'], b=wnl['Counted
Concentration'], equal_var=True)
t_test.loc[len(t_test.index)] = ['wl x wnl', t_stat, p_value]

t_test.to_excel(writer, sheet_name= "t_test_lag")
writer.save()
writer.close()
```

11.7 Statistics results

11.7.1 Preliminary experiment statistics results

a. Anova assumptions tests

Preliminary experiment - anova assumptions tests			
Shapiro-Wilk test			
name	**statistics**	**pvalue**	**result**
t0	0.589723	8.82E-05	Not Normal
t1	0.532213	3.08E-05	Not Normal
t2	0.573349	6.49E-05	Not Normal
w1	0.929481	3.75E-01	Normal
w2	0.856968	4.48E-02	Not Normal
w3	0.727091	1.56E-03	Not Normal
Levene test			
name	**statistics**	**pvalue**	**result**
Weeks	21.14807	1.23E-06	Not equal variances
Time	0.302065	7.41E-01	Equal variances

Appendix 11.7.1-1 *Anova assumptions tests results for the preliminary experiment, with 5% level of significance alpha.*

b. Anova results

Preliminary experiment - Anova test					
	sum_sq	**df**	**F**	**PR(>F)**	**result**
C(Time)	4.15E+12	2	0.654543	5.28E-01	No Difference
C(Week)	6.76E+13	2	10.64253	3.91E-04	Difference
C(Time):C(Week)	5.33E+12	4	0.419630	7.93E-01	No Difference

Appendix 11.7.1-2 *Anova results for the preliminary experiment, with 5% level of significance alpha.*

11.7.2 Illumination experiment statistics results

a. Anova assumptions tests

Shapiro-Wilk test			
LAG			
name	**statistics**	**pvalue**	**result**
t0 all	0.62	5.75E-10	Not Normal Distribution
t0 Z	0.64	1.83E-06	Not Normal Distribution
t0 NZ	0.59	5.11E-07	Not Normal Distribution
t0 w1	0.86	4.47E-02	Not Normal Distribution
t0 w2	0.79	7.32E-03	Not Normal Distribution
t0 w3	0.96	7.31E-01	Normal Distribution
t0 w4	0.88	8.26E-02	Normal Distribution
t20 all	0.69	2.66E-10	Not Normal Distribution
t20 Z	0.60	4.30E-08	Not Normal Distribution
t20 NZ	0.68	5.08E-07	Not Normal Distribution
t20 L	0.64	1.37E-07	Not Normal Distribution
t20 NL	0.73	2.55E-06	Not Normal Distribution
t20 w1	0.86	4.47E-02	Not Normal Distribution
t20 w2	0.79	7.32E-03	Not Normal Distribution
t20 w3	0.96	7.31E-01	Normal Distribution
t20 w4	0.88	8.26E-02	Normal Distribution
LOG			
name	**statistics**	**pvalue**	**result**
t0 all	0.51	7.26E-10	Not Normal Distribution
t0 Z	0.89	3.51E-02	Not Normal Distribution
t0 NZ	0.63	1.22E-05	Not Normal Distribution
t0 w1	0.97	9.26E-01	Normal Distribution
t0 w2	0.90	1.73E-01	Normal Distribution
t0 w3	0.69	7.32E-04	Not Normal Distribution
t20 all	0.93	3.99E-02	Not Normal Distribution
t20 Z	0.95	5.31E-01	Normal Distribution
t20 NZ	0.92	1.78E-01	Normal Distribution
t20 L	0.82	4.62E-03	Not Normal Distribution
t20 NL	0.93	2.06E-01	Normal Distribution
t20 w1	0.97	9.26E-01	Normal Distribution
t20 w2	0.90	1.73E-01	Normal Distribution
t20 w3	0.69	7.32E-04	Not Normal Distribution

STAT			
name	**statistics**	**pvalue**	**result**
t0 all	0.89	1.28E-02	Not Normal Distribution
t0 Z	0.91	2.37E-01	Normal Distribution
t0 NZ	0.88	9.05E-02	Normal Distribution
t0 w2	0.95	6.97E-01	Normal Distribution
t0 w3	0.95	6.83E-01	Normal Distribution
t20 all	0.78	1.46E-05	Not Normal Distribution
t20 Z	0.72	2.83E-04	Not Normal Distribution
t20 NZ	0.80	2.73E-03	Not Normal Distribution
t20 L	0.75	5.57E-04	Not Normal Distribution
t20 NL	0.86	1.89E-02	Not Normal Distribution
t20 w2	0.95	6.97E-01	Normal Distribution
t20 w3	0.95	6.83E-01	Normal Distribution

Appendix 11.7.2-1 *Anova assumption of normal distribution, Shapiro-Wilk test results, for the illumination experiment, with 5% level of significance alpha.*

Levene test			
LAG			
name	**statistics**	**p value**	**result**
t0 weeks	8.89E+01	1.05E-18	Not Equal Variances
t0 zinc x nozinc	3.85E-02	8.45E-01	Equal Variances
t20 weeks	1.35E+01	7.59E-07	Not Equal Variances
t20 Z x NZ x L x NL	2.78E-01	8.41E-01	Equal Variances
LOG			
name	**statistics**	**p value**	**result**
t0 weeks	5.94E+00	2.34E-02	Not Equal Variances
t0 zinc x nozinc	4.53E+00	4.05E-02	Not Equal Variances
t20 weeks	4.34E-01	6.52E-01	Equal Variances
t20 Z x NZ x L x NL	6.22E-01	6.03E-01	Equal Variances
STAT			
name	**statistics**	**p value**	**result**
t0 weeks	6.89E+00	1.55E-02	Not Equal Variances
t0 zinc x nozinc	1.63E+00	2.16E-01	Equal Variances
t20 weeks	6.30E+00	1.77E-02	Not Equal Variances
t20 Z x NZ x L x NL	2.88E+00	4.34E-02	Not Equal Variances

Appendix 11.7.2-2 *Anova assumption of equal variances, Levene test results, for the illumination experiment, with 5% level of significance alpha.*

b. Anova tests

Anova test t0					
LAG					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	3.00E+10	1	0.099908	7.54E-01	No Difference
C(Week)	1.32E+13	3	14.59908	1.43E-06	Difference
C(Zinc):C(Week)	1.08E+10	3	0.012005	9.98E-01	No Difference
LOG					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	4.71E+11	1	6.572741	1.56E-02	Difference
C(Week)	1.27E+12	2	8.858928	9.48E-04	Difference
C(Zinc):C(Week)	1.10E+12	2	7.652308	2.06E-03	Difference
STAT					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	3.91E+09	1	1.060226	3.15E-01	No Difference
C(Week)	6.91E+10	1	18.71619	3.28E-04	Difference
C(Zinc):C(Week)	6.09E+09	1	1.651471	2.13E-01	No Difference

Appendix 11.7.2-3 *Two-way Anova results for the t0 data from the illumination experiment, with 5% level of significance alpha.*

Two way Anova test t20					
LAG					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	1.29508E+20	1	4.666149	3.48E-02	Difference
C(Light)	3.23086E+16	1	0.001164	9.73E-01	No Difference
C(Zinc):C(Light)	1.68463E+20	1	6.069695	1.66E-02	Difference
LOG					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	3.25769E+19	1	7.622440	9.89E-03	Difference
C(Light)	4.83524E+17	1	0.113136	7.39E-01	No Difference
C(Zinc):C(Light)	3.25314E+17	1	0.076118	7.85E-01	No Difference
STAT					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	4.93199E+19	1	21.33710	7.85E-05	Difference
C(Light)	1.32572E+18	1	0.573541	4.55E-01	No Difference
C(Zinc):C(Light)	1.22905E+19	1	5.317199	2.87E-02	Difference

Appendix 11.7.2-4 *Two-way Anova results for the t20 data from the illumination experiment, with 5% level of significance alpha.*

Two-way Anova test - phases comparison					
name	**sum_sq**	**df**	**F**	**PR(>F)**	**result**
C(Zinc)	2.36829E+20	1	16.6623946	7.69E-05	Difference
C(Light)	3.78894E+15	1	0.00026657	9.87E-01	No Difference
C(Phase)	2.22872E+20	2	7.84019895	6.06E-04	Difference
C(Zinc):C(Light)	1.05398E+20	1	7.41538935	7.34E-03	Difference
C(Zinc):C(Phase)	2.53009E+18	2	0.08900377	9.15E-01	No Difference
C(Light):C(Phase)	2.43802E+18	2	0.08576482	9.18E-01	No Difference
C(Zinc):C(Light):C(Phase)	7.53602E+19	2	2.65102246	7.43E-02	No Difference

Appendix 11.7.2-5 *Three-way Anova results for the t20 data from the illumination experiment, the statistical comparison between cell phases, with 5% level of significance alpha.*

c. T-tests

T-test t0			
LAG			
Bonferroni correction			**0.0083**
name	**statistics**	**pvalue**	**result**
w1 x w2	-3.43338	0.0024	Difference
w1 x w3	2.239046	0.0356	No Difference
w2 x w3	4.137665	0.0004	Difference
w1 x w4	3.701658	0.0012	Difference
w2 x w4	4.507999	0.0002	Difference
w3 x w4	4.160040	0.0004	Difference
LOG			
Bonferroni correction			**0.0167**
name	**statistics**	**pvalue**	**result**
w1 x w2	2.741866	0.0119	Difference
w1 x w3	-2.15135	0.0427	No Difference
w2 x w3	-2.57286	0.0174	No Difference
STAT			
alpha			**0.05**
name	**statistics**	**pvalue**	**result**
w2 x w3	4.257898	0.0003	Difference

Appendix 11.7.2-6 *T-tests results for the t0 data from the illumination experiment, with 5% level of significance alpha, for lag phase cells and log phase cells Bonferroni correction was performed.*

T-test t20			
LAG			
Bonferroni correction			**0.0083**
name	**statistics**	**pvalue**	**result**
zl x znl	-1.97647	0.0574	No Difference
zl x wl	-3.14668	0.0037	Difference
zl x wnl	-2.77255	0.0095	No Difference
znl x wl	-1.18790	0.2442	No Difference
znl x wnl	0.223730	0.8245	No Difference
wl x wnl	1.583238	0.1239	No Difference
LOG			
Bonferroni correction			**0.0083**
name	**statistics**	**pvalue**	**result**
zl x znl	-0.46696	0.6451	No Difference
zl x wl	-2.81679	0.0100	No Difference
zl x wnl	-4.17753	0.0004	Difference
znl x wl	-2.26808	0.0335	No Difference
znl x wnl	-3.26130	0.0036	Difference
wl x wnl	-0.37079	0.7143	No Difference
STAT			
Bonferroni correction			**0.0083**
name	**statistics**	**pvalue**	**result**
zl x znl	-3.35200	0.0047	Difference
zl x wl	-3.86869	0.0017	Difference
zl x wnl	-5.06068	0.0002	Difference
znl x wl	-2.90829	0.0115	No Difference
znl x wnl	-2.59324	0.0213	No Difference
wl x wnl	1.574188	0.1378	No Difference

Appendix 11.7.2-7 *T-tests results for the t20 data from the illumination experiment, with 5% level of significance alpha and Bonferroni correction performed.*

11.7.3 ImageJ processing results for the illumination experiment

ImageJ software was used to process and analytically compare the MHA plates for lag phase cells from week 3.

ImageJ proccessing results - picture WL -7b								
Colony Number	Area [mm^2]	Mean Gray value	StdDev	Perimeter [mm]	Circularity	Aspect Ratio	Roundness	Solidity
1	68	180.221	20.603	29.213	1	1.057	0.946	0.907
2	73	184.014	23.662	30.385	0.994	1.089	0.918	0.93
3	52	176.981	16.197	25.556	1	1.076	0.929	0.92
4	64	185	22.147	28.971	0.958	1.004	0.996	0.914
5	52	169.327	14.104	24.971	1	1	1	0.929
6	67	187.567	23.395	28.971	1	1.047	0.955	0.931
7	60	177.733	21.583	27.799	0.976	1.053	0.949	0.902
8	75	180.333	21.935	30.627	1	1.05	0.952	0.909
9	62	179.468	21.073	29.213	0.913	1.093	0.914	0.861
10	58	181.741	19.736	27.556	0.96	1.154	0.867	0.921
11	44	174.477	14.353	24.385	0.93	1.088	0.919	0.871
12	50	171.98	17.312	25.556	0.962	1.057	0.946	0.893
13	59	186.424	20.899	26.971	1	1.09	0.917	0.929
14	80	183.912	23.273	31.799	0.994	1.179	0.848	0.93
15	65	181.415	21.411	29.213	0.957	1.086	0.921	0.884
16	71	186.085	23.27	29.799	1	1.06	0.943	0.916
17	53	172.302	16.087	25.556	1	1.041	0.961	0.93
18	64	181.438	21.995	28.385	0.998	1.034	0.968	0.921
19	60	172.117	19.006	27.799	0.976	1.034	0.967	0.902
20	66	189.955	24.337	29.799	0.934	1.055	0.948	0.892
21	25	175.52	18.469	20.142	0.774	2.123	0.471	0.847
22	60	174.367	17.411	26.971	1	1.111	0.9	0.938
23	60	176.433	19.177	27.799	0.976	1.032	0.969	0.902
24	69	184.275	24.916	29.213	1	1.039	0.963	0.914
25	55	173.655	18.561	26.971	0.95	1.042	0.96	0.894
26	57	176.772	18.793	26.385	1	1.031	0.97	0.912
27	57	178.263	19.316	26.971	0.985	1.044	0.958	0.912
28	87	174.897	17.354	34.971	0.894	1.455	0.687	0.916
29	85	177.494	19.59	32.971	0.983	1.018	0.982	0.944
30	61	178.443	19.271	26.971	1	1.103	0.907	0.938
31	67	175.104	19.587	28.971	1	1.037	0.965	0.937
32	60	186.75	20.999	27.314	1	1	1	0.968
33	74	187.986	24.212	30.385	1	1.069	0.935	0.925

34	55	185.091	20.756	27.799	0.894	1.201	0.832	0.873
35	68	185.103	22.467	29.799	0.962	1.012	0.988	0.907
36	67	185.209	21.643	28.971	1	1.035	0.966	0.931
37	68	184.206	21.578	29.556	0.978	1.055	0.948	0.932
38	84	187.238	25.575	34.042	0.911	1.023	0.978	0.875
39	69	183.304	21.737	29.213	1	1.054	0.949	0.914
40	70	185.029	26.328	29.799	0.991	1.06	0.943	0.921
41	58	186.224	20.363	28.385	0.905	1.138	0.878	0.879
42	76	185.697	26.416	30.385	1	1.069	0.936	0.938
43	117	186.111	21.344	42.042	0.832	1.707	0.586	0.886
44	66	186.985	23.424	28.971	0.988	1.029	0.972	0.923
45	105	168.933	13.725	39.799	0.833	1.597	0.626	0.886
46	70	182.871	22.16	29.556	1	1.041	0.961	0.946
47	74	184.662	22.56	30.971	0.969	1.066	0.938	0.925
48	74	185.365	21.242	31.213	0.954	1.102	0.907	0.897
49	78	189.603	25.709	31.799	0.969	1.016	0.984	0.918
50	91	187.747	24.952	34.627	0.954	1.042	0.96	0.91
51	89	187.652	23.99	33.799	0.979	1.053	0.949	0.922
52	68	183.103	21.47	28.971	1	1.028	0.973	0.938
53	69	181.768	23.132	29.799	0.976	1.033	0.968	0.902
54	76	174.487	18.471	31.213	0.98	1.056	0.947	0.905
55	75	188.693	26.714	30.627	1	1.05	0.952	0.909
56	44	180.25	18.103	23.314	1	1.043	0.959	0.946
57	85	182.906	23.205	32.627	1	1.051	0.952	0.919
58	87	190.862	27.071	33.213	0.991	1.067	0.937	0.921
59	73	183.63	25.38	30.385	0.994	1.017	0.984	0.918
60	73	183.822	24.162	30.971	0.956	1.054	0.949	0.918
61	80	187.925	20.847	31.213	1	1.042	0.959	0.93
62	10	153.3	13.442	10.485	1	1.551	0.645	0.909
63	101	197.743	27.133	35.799	0.99	1.036	0.965	0.944
64	127	199.567	28.239	40.042	0.995	1.021	0.98	0.937
65	63	176.905	16.066	28.385	0.983	1.036	0.966	0.913
66	63	176.111	18.601	28.385	0.983	1.057	0.946	0.913
67	112	185.402	23.879	38.627	0.943	1.043	0.959	0.914
68	93	196.946	28.851	34.385	0.988	1.06	0.944	0.935
69	66	184.015	25.148	29.799	0.934	1.06	0.943	0.892
70	112	181.277	22.199	38.042	0.973	1	1	0.918
71	4	147.25	9.845	7.071	1	2.031	0.492	0.8
72	107	196.327	28.336	36.627	1	1.067	0.937	0.943
73	126	186.722	23.468	40.87	0.948	1.011	0.989	0.91
74	90	184.289	16.235	36.385	0.854	1.378	0.725	0.914
75	49	169.061	15.853	24.971	0.988	1.076	0.93	0.907

76	144	195.958	27.316	43.698	0.948	1.038	0.964	0.914
77	53	182.057	23.068	26.385	0.957	1.065	0.939	0.891
78	2	137.5	2.121	5.657	0.785	2.646	0.378	0.667
79	53	156.755	11.059	27.213	0.899	1.665	0.601	0.869
80	135	189.993	24.413	42.042	0.96	1.054	0.949	0.934
81	83	188.952	23.485	32.627	0.98	1.02	0.98	0.917
82	32	162.562	14.346	19.314	1	1	1	0.941
83	133	191.586	25.075	42.042	0.946	1.005	0.995	0.927
84	94	189.17	26.3	35.213	0.953	1.035	0.966	0.917
85	125	192.816	26.468	40.042	0.98	1.02	0.98	0.929
86	1	135	0	2.828	1	1	1	1
87	101	187.059	27.143	37.456	0.905	1.067	0.937	0.902
88	4	140.75	6.946	5.657	1	1	1	1
89	35	171.229	17.917	20.142	1	1.129	0.885	0.921
90	110	181.618	18.195	38.627	0.926	1.356	0.738	0.924
91	66	184.409	25.788	29.213	0.972	1.06	0.943	0.904
92	98	194.99	29.11	35.213	0.993	1.063	0.94	0.933
93	1	147	0	2.828	1	1	1	1
94	117	177.735	18.769	39.456	0.944	1.034	0.968	0.911
95	57	185.596	26.437	26.971	0.985	1.031	0.97	0.912

ImageJ proccessing results - picture lag week 3 WNL -6a								
Colony Number	Area [mm^2]	Mean Gray value	StdDev	Perimeter [mm]	Circularity	Aspect Ratio	Roundness	Solidity
1	41	144.341	10.769	23.899	0.902	1.223	0.818	0.921
2	54	139.167	7.916	26.385	0.975	1.056	0.947	0.9
3	23	140.087	8.867	18.728	0.824	2.485	0.402	0.868
4	77	143.727	10.884	33.799	0.847	1.445	0.692	0.865
5	69	146.377	12.956	29.799	0.976	1.169	0.855	0.914
6	41	138.585	10.31	22.728	0.997	1.103	0.907	0.932
7	41	136	10.22	22.142	1	1.097	0.911	0.921
8	49	135.224	9.696	25.556	0.943	1.043	0.959	0.891
9	50	149.72	14.356	25.556	0.962	1.112	0.899	0.901
10	63	143.286	12.519	28.142	1	1.129	0.885	0.947
11	76	148.092	15.328	31.213	0.98	1.003	0.997	0.905
12	79	155.241	15.005	31.799	0.982	1.035	0.966	0.924
13	82	154.72	14.318	33.799	0.902	1.505	0.664	0.906
14	56	150.893	14.776	26.142	1	1.005	0.995	0.949
15	90	156.856	15.274	34.627	0.943	1.037	0.964	0.909
16	50	149.16	15.024	24.728	1	1.158	0.864	0.952

17	81	154.185	16.73	31.799	1	1.029	0.972	0.936
18	49	151.49	15.975	24.971	0.988	1.052	0.951	0.891
19	43	151.14	11.659	23.556	0.974	1.032	0.969	0.896
20	87	145.747	12.733	34.042	0.943	1.065	0.939	0.892
21	28	151.786	11.855	20.971	0.8	1.676	0.597	0.848
22	101	151.069	13.165	38.627	0.851	1.643	0.609	0.89
23	46	151.696	18.375	25.799	0.868	1.321	0.757	0.821
24	79	144.025	13.239	33.456	0.887	1.1	0.909	0.873
25	50	152.8	16.629	25.213	0.988	1.097	0.912	0.877
26	109	154.78	16.449	38.627	0.918	1.444	0.693	0.916
27	106	154.368	17.601	40.87	0.797	1.701	0.588	0.858
28	34	154.059	15.063	21.556	0.919	1.593	0.628	0.872
29	52	152.519	17.556	24.971	1	1.109	0.902	0.929
30	85	158.318	18.981	32.385	1	1.025	0.976	0.939
31	57	153.211	16.427	26.728	1	1.039	0.963	0.95
32	77	157.753	19.636	32.042	0.942	1.113	0.898	0.885
33	97	159.268	20.185	35.213	0.983	1.035	0.966	0.924
34	76	158.158	18.562	31.799	0.944	1.081	0.925	0.905
35	98	158.449	17.962	35.213	0.993	1.015	0.985	0.929
36	67	159.836	18.417	28.385	1	1.035	0.966	0.931
37	79	158.797	20.559	31.799	0.982	1.013	0.987	0.913
38	61	157.754	19.383	27.799	0.992	1.017	0.983	0.91
39	41	157.024	16.39	25.556	0.789	1.668	0.6	0.872
40	71	157.831	21.93	30.627	0.951	1.111	0.9	0.91
41	93	162.624	20.849	34.627	0.975	1.002	0.998	0.921
42	102	155.824	16.748	36.627	0.955	1.031	0.97	0.923
43	73	160.836	21.299	31.213	0.942	1.024	0.977	0.896
44	84	164.548	22.477	32.042	1	1.043	0.959	0.923
45	66	156.5	21.131	28.971	0.988	1.047	0.955	0.93
46	67	161.209	19.529	28.971	1	1.035	0.966	0.931
47	76	162.211	21.167	31.213	0.98	1.056	0.947	0.905
48	10	128	7.732	11.314	0.982	1.316	0.76	0.769
49	63	156.063	19.726	27.799	1	1.008	0.992	0.926
50	65	158.292	20.454	29.213	0.957	1.014	0.986	0.884
51	101	164.99	22.181	36.042	0.977	1.016	0.984	0.914
52	106	165.566	19.504	36.627	0.993	1.043	0.958	0.93
53	100	165.87	23.235	36.042	0.967	1.035	0.966	0.913
54	65	158.046	18.5	28.385	1	1.059	0.944	0.929
55	66	158.939	19.36	28.971	0.988	1.031	0.969	0.923
56	78	163.385	22.098	31.799	0.969	1.014	0.986	0.918
57	44	155.318	15.991	24.142	0.949	1.281	0.781	0.907
58	60	158.5	16.292	26.971	1	1.111	0.9	0.938

59	96	170.021	24.204	35.213	0.973	1.023	0.978	0.919
60	67	158.104	20.618	29.213	0.987	1.018	0.982	0.899
61	66	158.424	20.728	28.971	0.988	1.058	0.945	0.923
62	78	163.564	22.837	31.213	1	1.014	0.986	0.918
63	100	173.14	22.216	35.213	1	1.012	0.988	0.939
64	104	170.5	22.651	36.627	0.974	1.016	0.984	0.929
65	62	155.371	18.373	27.799	1	1.026	0.975	0.905
66	11	136	12.625	11.899	0.976	1.469	0.681	0.846
67	69	158	18.734	28.971	1	1	1	0.945
68	64	137.938	8.628	28.971	0.958	1.032	0.969	0.908
69	75	162.053	20.756	31.556	0.946	1.084	0.922	0.932
70	61	160.754	21.012	28.142	0.968	1.154	0.867	0.946
71	67	155.209	17.785	28.385	1	1.035	0.966	0.931
72	68	164.147	20.556	30.385	0.926	1.246	0.803	0.901
73	94	170.66	23.458	34.627	0.985	1.02	0.981	0.917
74	68	158.882	20.51	30.142	0.941	1.213	0.825	0.932
75	84	172.798	23.802	32.385	1	1.047	0.955	0.944
76	3	133.333	12.662	5.657	1	1.464	0.683	0.857
77	97	174.268	24.084	36.627	0.909	1.039	0.962	0.898
78	100	174.83	24.829	35.799	0.981	1.028	0.973	0.93
79	67	154.209	18.584	29.213	0.987	1.017	0.984	0.899
80	145	160.414	18.54	44.284	0.929	1.385	0.722	0.921
81	65	145.138	12.599	28.385	1	1.003	0.997	0.915
82	124	159.653	18.976	41.456	0.907	1.499	0.667	0.919
83	81	161.519	20.832	31.799	1	1.067	0.937	0.926
84	80	156.825	19.225	31.213	1	1.011	0.989	0.93
85	132	160.265	16.93	42.87	0.903	1.319	0.758	0.917
86	61	148.197	13.837	28.385	0.951	1.02	0.98	0.897
87	95	159.326	19.187	35.799	0.932	1.043	0.959	0.913
88	34	159.029	18.323	24.142	0.733	2.189	0.457	0.85
89	83	172.241	21.941	31.799	1	1.024	0.977	0.938
90	56	154.036	17.138	27.799	0.911	1.176	0.85	0.855
91	39	150.769	14.132	24.142	0.841	1.346	0.743	0.857
92	57	156.316	17.087	26.971	0.985	1.089	0.918	0.912
93	84	169.286	22.082	32.385	1	1.002	0.998	0.933
94	88	159.727	20.463	34.042	0.954	1.026	0.975	0.898
95	75	156.333	19.17	32.627	0.885	1.26	0.794	0.877
96	81	158.21	18.425	33.799	0.891	1.289	0.776	0.895
97	105	161.371	19.742	36.627	0.984	1.034	0.967	0.933
98	104	169.913	21.671	36.87	0.961	1.041	0.96	0.904
99	59	160.051	18.876	26.971	1	1.063	0.94	0.922
100	80	171.562	24.167	32.042	0.979	1.014	0.986	0.899

101	95	157.547	18.276	34.627	0.996	1.04	0.961	0.922
102	54	148.648	15.161	26.385	0.975	1.077	0.928	0.9
103	96	153.979	17.46	35.213	0.973	1.026	0.975	0.928
104	107	162.916	18.527	37.213	0.971	1.02	0.981	0.926
105	109	160.661	18.262	37.799	0.959	1.047	0.955	0.936
106	106	161.868	19.201	37.456	0.949	1.021	0.98	0.906
107	94	166.883	22.575	35.213	0.953	1.059	0.944	0.908
108	60	162.817	19.027	28.142	0.952	1.378	0.726	0.938
109	114	160.219	18.303	38.627	0.96	1.029	0.972	0.931
110	117	159.385	16.631	38.627	0.985	1.018	0.983	0.94
111	100	169.44	20.923	35.799	0.981	1.02	0.98	0.93
112	1	120	0	2.828	1	1	1	1
113	66	160.879	20.278	28.971	0.988	1.047	0.955	0.93
114	74	160.703	19.051	31.556	0.934	1.102	0.908	0.931
115	121	155.694	15.891	38.627	1	1.024	0.977	0.949
116	7	124.857	3.716	11.657	0.647	2.423	0.413	0.824
117	92	160.652	15.064	34.971	0.945	1.286	0.778	0.948
118	39	148.308	14.068	22.385	0.978	1.04	0.962	0.857
119	59	153.169	16.749	26.971	1	1.09	0.917	0.929
120	103	157.65	16.552	37.556	0.918	1.206	0.829	0.949
121	47	149.319	14.805	24.142	1	1.089	0.918	0.931
122	1	120	0	2.828	1	1	1	1
123	115	154.148	15.38	38.042	0.999	1.048	0.954	0.943
124	93	148.634	12.328	34.627	0.975	1.024	0.977	0.925
125	124	154.516	13.798	40.627	0.944	1.051	0.952	0.932
126	49	133.653	6.933	25.799	0.925	1.354	0.738	0.86
127	55	138.709	7.939	25.556	1	1.035	0.966	0.94
128	38	131.289	7.192	24.142	0.819	1.507	0.663	0.835
129	59	155.203	15.277	29.556	0.849	1.459	0.685	0.887
130	51	137.725	10.267	28.971	0.764	1.822	0.549	0.836

ImageJ proccessing results – picture lag week 3 ZL -7b								
Colony Number	Area [mm^2]	Mean Gray value	StdDev	Perimeter [mm]	Circularity	Aspect Ratio	Roundness	Solidity
1	138	134.543	10.945	42.284	0.97	1.104	0.906	0.926
2	175	134.109	10.759	62.77	0.558	2.267	0.441	0.839
3	174	134.178	11.35	49.698	0.885	1.064	0.94	0.923
4	219	122.639	6.812	54.284	0.934	1.062	0.941	0.944
5	101	139.356	11.772	36.627	0.946	1.216	0.822	0.931

6	312	123.349	7.214	70.184	0.796	1.465	0.683	0.908
7	144	140.542	11.81	44.284	0.923	1.052	0.951	0.914
8	179	140.469	13.107	50.527	0.881	1.258	0.795	0.918
9	386	136.067	10.203	75.497	0.851	1.4	0.714	0.914
10	366	138.344	11.245	69.841	0.943	1.047	0.955	0.947
11	251	134.458	10.105	59.698	0.885	1.034	0.968	0.923
12	307	139.775	11.586	65.598	0.897	1.088	0.919	0.937
13	443	144.115	13.005	78.669	0.9	1.009	0.991	0.942
14	278	138.906	10.095	61.355	0.928	1.101	0.909	0.936
15	135	136.97	10.487	43.698	0.888	1.089	0.918	0.903
16	257	141.012	10.672	63.941	0.79	1.688	0.592	0.905
17	141	138.213	11.764	44.284	0.904	1.035	0.966	0.904
18	354	141.489	12.11	72.083	0.856	1.128	0.887	0.919
19	437	137.744	11.325	77.841	0.906	1.102	0.907	0.939
20	277	144.48	12.501	62.77	0.883	1.288	0.777	0.933
21	253	137.439	12.171	59.598	0.895	1.001	0.999	0.918
22	149	140.98	12.623	46.042	0.883	1.112	0.899	0.92
23	329	143.343	12.666	67.012	0.921	1.035	0.966	0.941
24	209	137.349	9.677	55.355	0.857	1.311	0.763	0.915
25	161	140.13	12.327	46.627	0.931	1.009	0.991	0.936
26	239	140.205	12.28	56.77	0.932	1.102	0.907	0.928
27	152	137.026	11.859	44.87	0.949	1.02	0.98	0.924
28	333	136.33	11.147	67.598	0.916	1.122	0.892	0.931
29	628	146.833	12.934	93.154	0.909	1.025	0.975	0.949
30	531	142.36	11.792	85.255	0.918	1.034	0.967	0.952
31	224	140.268	12.302	54.284	0.955	1.052	0.951	0.945
32	34	132.824	16.236	20.728	0.994	1.164	0.859	0.919
33	678	144.712	12.405	96.326	0.918	1.038	0.964	0.952
34	661	143.082	11.709	97.154	0.88	1.065	0.939	0.94
35	9	125.778	12.901	10.485	1	1.817	0.55	0.857
36	150	138.46	11.478	46.284	0.88	1.257	0.796	0.917
37	301	139.412	11.33	62.77	0.96	1.026	0.975	0.953
38	525	140.046	11.493	85.012	0.913	1.052	0.95	0.96
39	491	145.56	12.595	81.841	0.921	1.037	0.964	0.95
40	489	145.027	13.552	82.083	0.912	1.102	0.908	0.946
41	414	133.812	10.226	76.669	0.885	1.014	0.987	0.94
42	220	139.573	11.124	54.77	0.922	1.035	0.966	0.924
43	639	140.119	11.225	92.669	0.935	1.022	0.978	0.96
44	761	144.011	12.547	105.154	0.865	1.31	0.763	0.946
45	573	136.827	9.42	88.083	0.928	1.035	0.966	0.952
46	3	113	1	5.657	1	1.464	0.683	0.857
47	378	128.19	10.09	74.326	0.86	1.064	0.94	0.929

48	520	135.358	9.404	84.326	0.919	1.112	0.899	0.945
49	6	115.667	5.785	8.485	1	1.809	0.553	0.8
50	425	133.188	9.52	78.184	0.874	1.155	0.866	0.938
51	5	116.8	3.493	7.071	1	1.553	0.644	0.909
52	435	132	9.106	77.255	0.916	1.006	0.994	0.945
53	442	134.328	9.624	77.841	0.917	1.085	0.922	0.944
54	245	135.576	9.665	61.012	0.827	1.36	0.736	0.914
55	6	124.833	9.827	8.485	1	1.291	0.775	0.8
56	9	117.556	3.358	11.899	0.799	2.406	0.416	0.783
57	5	113.6	2.702	9.071	0.764	2.61	0.383	0.769
58	5	115	1.581	9.071	0.764	2.61	0.383	0.769

ImageJ proccessing results - picture ZNL -7b								
Colony Number	Area [mm^2]	Mean Gray value	StdDev	Perimeter [mm]	Circularity	Aspect Ratio	Roundness	Solidity
1	1	116	0	2.828	1	1	1	1
2	1	114	0	2.828	1	1	1	1
3	52	156.538	21.991	30.971	0.681	2.241	0.446	0.86
4	124	148.476	15.108	40.87	0.933	1.049	0.954	0.905
5	1	114	0	2.828	1	1	1	1
6	1	118	0	2.828	1	1	1	1
7	141	148.277	13.067	42.87	0.964	1.02	0.98	0.925
8	16	134.812	11.996	18.728	0.573	3.394	0.295	0.78
9	138	154.007	16.228	44.627	0.871	1.358	0.737	0.905
10	175	158.183	17.493	47.698	0.967	1.014	0.986	0.933
11	13	132.538	11.042	16.142	0.627	3.162	0.316	0.765
12	160	155.544	16.861	47.213	0.902	1.153	0.867	0.941
13	185	155.568	16.241	49.113	0.964	1.036	0.965	0.934
14	147	154.993	18.533	44.284	0.942	1.063	0.941	0.919
15	89	152.854	16.392	34.284	0.952	1.207	0.829	0.904
16	100	147.79	14.705	36.042	0.967	1.018	0.982	0.909
17	156	153.385	19.798	44.87	0.974	1.031	0.969	0.937
18	148	156.649	19.591	43.698	0.974	1	1	0.925
19	203	157.877	19.142	51.941	0.946	1.021	0.979	0.927
20	86	145.395	12.182	34.042	0.933	1.171	0.854	0.905
21	120	157.792	16.826	38.627	1	1	1	0.952
22	96	149.427	14.215	34.627	1	1.037	0.964	0.928
23	172	155.61	17.409	47.698	0.95	1.062	0.942	0.927
24	138	148.971	13.023	48.184	0.747	1.858	0.538	0.849
25	130	156.977	18.343	40.87	0.978	1.018	0.982	0.925

26	90	152.467	15.848	34.042	0.976	1.227	0.815	0.928
27	134	157.007	18.804	42.284	0.942	1.017	0.983	0.918
28	350	154.691	16.511	71.012	0.872	1.132	0.884	0.93
29	148	156.203	19.513	43.456	0.985	1.044	0.958	0.94
30	136	149.456	13.769	42.042	0.967	1.04	0.962	0.932
31	200	160.9	17.955	51.355	0.953	1.011	0.989	0.926
32	156	155.936	18.244	45.698	0.939	1.022	0.979	0.92
33	10	123.8	8.6	11.314	0.982	1.327	0.754	0.8
34	107	154.159	15.111	38.042	0.929	1.185	0.844	0.918
35	169	161.083	18.551	46.87	0.967	1.041	0.961	0.939
36	8	139	16.895	10.485	0.914	1.939	0.516	0.842
37	125	160.008	19.357	40.042	0.98	1.042	0.96	0.933
38	116	152.5	17.936	38.627	0.977	1.035	0.966	0.928
39	82	152.549	15.931	31.799	1	1.073	0.932	0.932
40	157	153.49	15.699	48.042	0.855	1.636	0.611	0.913
41	132	159.167	20.548	40.87	0.993	1.023	0.978	0.933
42	138	167.855	26.245	42.042	0.981	1.008	0.992	0.936
43	160	169.306	24.985	45.456	0.973	1.023	0.977	0.938
44	100	152.21	17.502	35.213	1	1.021	0.98	0.939
45	177	160.04	17.723	47.698	0.978	1	1	0.937
46	119	158.697	18.148	41.213	0.88	1.325	0.755	0.905
47	164	160.963	17.533	45.698	0.987	1.022	0.978	0.937
48	124	159.774	17.257	40.385	0.955	1.185	0.844	0.95
49	3	132.333	6.028	5.657	1	1.464	0.683	0.857
50	68	148.868	15.166	29.799	0.962	1.002	0.998	0.907
51	172	169.36	23.703	47.113	0.974	1.031	0.97	0.932
52	3	118.333	0.577	6.828	0.809	3	0.333	1
53	127	157.354	19.419	40.627	0.967	1.035	0.966	0.934
54	110	151.718	17.39	37.213	0.998	1.05	0.952	0.94
55	166	166.048	22.636	46.042	0.984	1.009	0.991	0.949
56	43	149.093	16.246	23.314	0.994	1.033	0.968	0.935
57	69	148.826	12.027	28.971	1	1	1	0.945
58	140	161.65	19.147	42.627	0.968	1.014	0.986	0.943
59	53	151.792	18.076	25.799	1	1.125	0.889	0.891
60	30	133.633	11.346	30.971	0.393	4.746	0.211	0.682
61	1	115	0	2.828	1	1	1	1
62	42	142.429	12.488	23.799	0.932	1.431	0.699	0.857
63	75	142.133	11.225	30.971	0.983	1.057	0.947	0.932
64	71	132.817	10.767	30.627	0.951	1.111	0.9	0.882
65	1	124	0	2.828	1	1	1	1
66	7	126.571	4.315	9.657	0.943	1.936	0.517	0.933
67	72	132.056	8.528	30.627	0.965	1.123	0.891	0.894

68	1	121	0	2.828	1	1	1	1
69	122	142.943	14.159	39.698	0.973	1.017	0.984	0.914
70	12	133.667	12.01	15.899	0.597	3.7	0.27	0.75

11.8 List of Figures

11.9 List of Pictures

11.10 List of Tables

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