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BIOTECHNICAL SOLUTIONS TO WATER SCARCITY

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We would first like to thank our promotors Marjan De Mey and Wim Van Criekinge. They were always willing to help us and they definitely encouraged us to think outside the box. Thanks as well to the students who were involved in the iGEM-team 2020, as they discussed possible ideas for a project together with us. We received a lot of interest and input of PhD students, some of them were also involved in the project of 2016 namely ’The Dewpal’. Finally we would like to thank Snomax, KMI, Dr. Thomas Chubb of Snowy Hydro and prof. Steven Siems of the Monash University who all gave us more advice, especially about the practical aspects of our project.

Despite the measures concerning the COVID-19 pandemic, we did not have any additional problems like cancelled experiments in the lab. Our tests were already planned in August and September. We received all the information around our topic or about the thesis through mail, video-call or just the internet. This is obviously different than face-to-face contact, but everyone was very helpful nevertheless.
The only thing that we found a little bit more difficult, was meetings with all of the team members.

This preamble was drawn up in consultation between the student and the supervisor and approved by both.

CONTENTS

ACRONYMS

aa amino acids.

AgI silver iodide.

CNN cloud condensation nuclei.

DNA deoxyribonucleic acid.

FAO The Food and Agriculture Organization.

iGEM International Genetically Engineered Machine.

IN ice nuclei.

INPs ice-nucleating proteins.

MEMS microelectromechanical systems.

MIT Massachusetts Institute of Technology.

MOFs metal organic frameworks.

S serine.

SBC Synthetic Biology Competition.

SDG Sustainable Development Goals.

T threonine.

UGent University of Ghent.

UN United Nations.

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 What is the iGEM competition

The competition International Genetically Engineered Machine, or better known as iGEM, started as an independent study course at the Massachusetts Institute of Technology (MIT, Boston, US) in January 2003. It was meant for students to develop biological devices to popularize the subject of synthetic biology according to their website (iGEM Foundation, 2020). Soon after, in 2004, it became a summer competition with 5 teams. They initially called it the Synthetic Biology Competition (SBC). The next year there were already 13 teams participating and in 2019 it expanded to 353 teams representing more than 40 countries as shown in Figure 1.1.

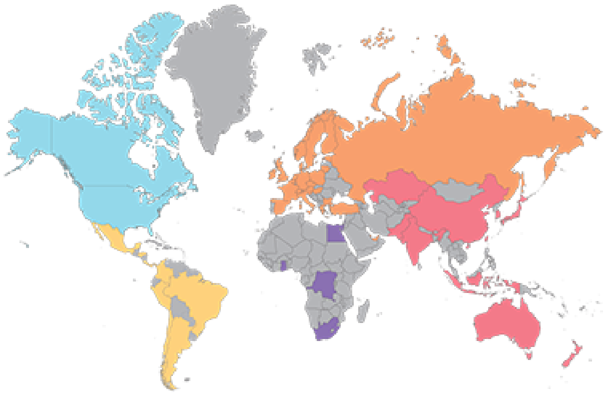

Figure 1.1: World map of countries participating in the competition International Genetically Engineered Machine (iGEM) (iGEM Foundation, 2020). Different colours indicate countries from different parts of the world put in groups according to iGEM, countries in grey-colour not included. Blue = North America, Yellow = South America, Purple = Africa, Orange = Europe + Russia, Pink = Asia + Oceania.

The iGEM Competition gives students the opportunity to find solutions for everyday issues facing the world by applying synthetic biology. Originally the competition consisted of teams of undergraduate college students, but now graduate and high school students are included as well. The multidisciplinary teams work together to design, build, test and measure a system of their own design using interchangeable biological parts and standard molecular biology techniques. There are a lot of skills that are developed and put into practice in iGEM, for example project planning, administration, fundraising, problem based knowledge, safe lab work and project design.

Finally, the students have to present their project for an international jury at the iGEM Jamboree. In the beginning, there existed the 'Regional Jamborees', with the highest scoring teams from each region proceeding on to the World Championships in Boston. Eventually in 2014 the first 'Giant Jamboree' was organised at the Hynes Convention Center in Boston so the whole iGEM community could be united at one single event.

The iGEM competition is divided in different tracks so the teams can focus more on their project (see Figure 1.2). The subject areas enclosed by the tracks aim to solve crucial global challenges. There are 10 standard tracks:

- Diagnostics, e.g. detecting illness and disease
- Energy, e.g. own transportation fuel, irrespective of available natural resources
- Environment, e.g. biosensors
- Food & Nutrition, e.g. calories from sustainable fishing practices
- Foundational Advance, e.g. advancing high-throughput quality control
- High School, teams of high school students
- Information Processing, e.g. building elements of a biological computer
- Manufacturing, e.g. micro-scale production of drugs, therapeutics or other high-value molecules
- New Application, novel, forward-thinking projects and innovative ideas that don't fit into conventional paradigms
- Therapeutics, e.g. treating medical conditions

There are also 2 special tracks:

- Open, synthetic biology project, but no lab work using DNA parts
- Software, using software tools

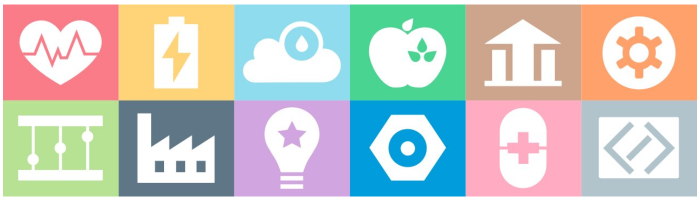

Figure 1.2: Icons of the different iGEM tracks (iGEM Foundation, 2020).

Each team has to make a Wiki (an informative website which can be extended pretty fast by everyone who has excess to it), a poster and a presentation. It is not only about figuring out a relative new synthetic biology concept and design it in the lab.

The goal is to convince companies, fellow participants and judges, not only in order to obtain funding, but also to have a chance of winning medals at the iGEM competition. The students really need to work together in order to convert their idea into an actual concept, get fundraising, plan their excursion to Boston and of course let the general public know what can be achieved using synthetic biology.

1.2 Problems that fit iGEM

According to Panel et al. (2011), by 2030 almost half of the world's population will suffer severe water stress without altering current levels of water consumption and pollution. This is still an actual problem and scientists are therefore actively searching for low-cost solutions especially for developing countries.

Although some solutions have been found, individually these do not solve the problem of water scarcity. AQUASTAT (2014), FAO's Global Information System on Water and Agriculture, states that water scarcity is either due to physical shortage or scarcity due to the inability to access water caused by the failure of institutions to ensure a regular supply or a lack of adequate infrastructure. At this moment there is no global water scarcity as such, but individual countries and regions need to urgently tackle the critical problems presented by water stress (see Figure 1.3).

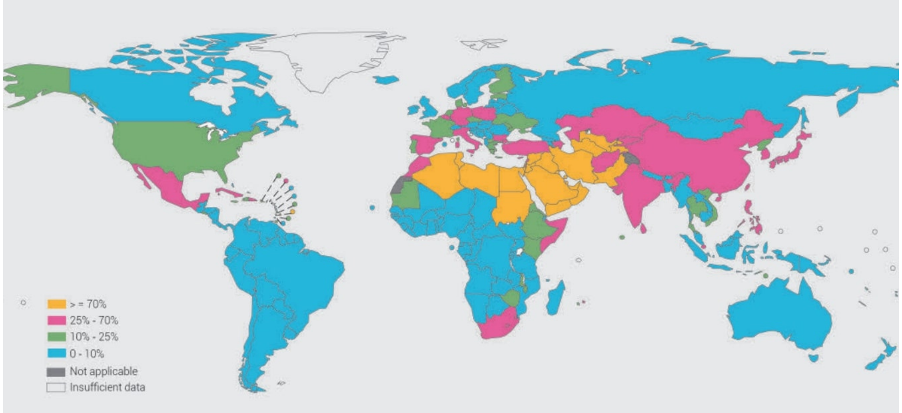

Figure 1.3: World map showing the levels of water stress worldwide (AQUASTAT, 2014).

In a paper of 1998, they stated already that safe drinking water remains inaccessible for about 1.1 billion people in the world, and the hourly toll from biological contamination of drinking water is 400 deaths of children below the age of 5 (Gadgil, 1998). Ashraf (2003) wrote in his article that each person needs a minimum of 5 L of drinking water per day. The reality for people living in 40 of the world's most water-famished countries is that they must survive on 5-7 L per day for all their water needs. According to the FAO (2020) 2 L of water is often sufficient for daily drinking purposes, but it takes about 3,000 L to produce the daily food needs of one person. People do not only need water to drink or to produce food, but also for their toilet, shower, washing machine and more. This does not have to be potable water. Farmers cannot grow anything without water and because of the growing population and the ongoing climate change, water scarcity is becoming a big problem. The main source of water for farmers, or in fact their crops, comes from the rain. So more rainfall in the right amount would not only solve the problem of water shortage, but also of crop damage.

Finding biotechnological solutions to solve water scarcity would perfectly fit in the environment track of iGEM and can easily be related to Belgium since 44% of the land area is agricultural acreage (Statbel, 2019). On top of that, Belgium is ranked 23rd out of 164 countries in water scarcity, the third highest in Europe, according to Aqueduct (2019). Belgium falls into the high-risk category, the second highest of the five categories. Dry summers are increasingly common in Flanders, with the summer of 2018 severe enough to be formally declared a farming disaster for the region.
Furthermore, our idea not only fit in one of the tracks of iGEM, but it also is in line with the Sustainable Development Goals (SDG) for 2030 from the UN (see Figure 1.4). Particularly to SDG 6: ’ensure availability and sustainable management of water and sanitation for all’.

Figure 1.4: The 17 Sustainable Development Goals from the UN (UN, 2015).

Since an UGent-team participated in 2016 with their project called ‘Dewpal’, a structure to collect water droplets from the air, we thought it was a good idea to improve upon this project. Through the 2016 team was not able to demonstrate that their device could effectively capture a decent amount of water, we tried to search for solutions that would actually work.

Nowadays rain is induced by using chemicals such as silver iodide (AgI) and this technique is called ’cloud seeding’. These chemicals could be potentially harmful to the natural environment and come in contact with the plants that depend on the contaminated rain to grow. There has not been enough research to know what the long-term effects on animals and plants will be, according to Fajardo et al. (2016).

CHAPTER 2

STATE OF THE ART

2.1 Collection of atmospheric water

2.1.1 Water scarcity, too little for too many

As stated before, water scarcity is already a big problem in large parts of the world and the problem will most likely increase with climate change and the growth of the human population. Safe drinking water is a basic human right and one of the SDG, yet very hard to get in some places like deserts or regions with a contaminated water supply.

The largest causes for drinking water scarcity today are water pollution, agriculture and flawed infrastructure (UN, 2015). Still, places like the Sahara or Atacama Desert will not see much more water if these problems are solved. Therefore, large scale innovation of potable water collection is necessary.

2.1.2 Biomimetic materials for water collection

It would be foolish not to look in nature when looking for answers to any (engineering) problem, especially involving water capture. The reason being that most organisms and mechanisms have been through thousands if not millions of years of trial-and-error or evolution. As a result, many organisms living in arid regions are equipped with special materials or pathways in order to collect water and stay alive. Biomimetic materials are materials inspired by natural designs such as honeycomb structure of the beehive for improved structural strength or shark-skin-inspired bathing suits to decrease friction and thus increasing athletes performance. Some of these materials have been studied and can be used by humans in order to provide clean water.

Organisms like tree frogs (e.g. *Litoria caerulea*), toads, tortoises, lizards, snakes, elephants and sandgrouse, but also spiders, desert beetles and cacti can get access to different types of water that include rain, dew, water from thermally facilitated condensation on the skin, fog or moisture from a damp substrate (Comanns, 2018). They use their integument to capture water using one or more of the 6 basic mechanisms:

1. Increased surface wettability
2. Increased spreading area
3. Transport of water over relatively large distances
4. Accumulation and storage of collected water
5. Condensation
6. Utilization of gravity

Of the 6 mechanisms, condensation is the most interesting for our objective, i.e., improving the dewpal device, and will therefore be discussed more thoroughly. One of the implementations of condensation is the hydrophilic/hydrophobic mosaic inspired by the fogstand beetle (*Stenocara gracilipes*) used in the design of the 2016 Dewpal project. The fogstand beetle lives in the Namib desert where it cannot rely on rain or puddles for the water it needs to survive. In order to occupy this niche the fogstand beetle found a way to collect water from humid air. It tilts its back towards the humid breeze in order to capture small droplets of water on its wings. The wings display a hydrophilic/hydrophobic mosaic. The hydrophilic patches bind small water droplets and the hydrophobic patches repel water causing the droplets to be channeled towards the beetle's head (UGent Belgium iGEM 2016, 2016).

The tree frog *Litoria caerulea* (Figure 2.1) causes condensation on its skin by secreting a hygroscopic substance on its slightly granular skin in combination with a temperature gradient the frog creates by cooling down its body to a temperature lower than the surrounding air temperature. It can achieve this by cooling down in the open and entering a warm and humid tree hole, where the temperature difference causes condensation on the frog's skin. The tree frog is able to change its core temperature to the surrounding temperature, because it is an ectotherm (Comanns, 2018).

Figure 2.1: Picture of the the tree frog *Litoria caerulea* (The Australian Museum, 2018).

It has been found that uniformly hydrophilic surfaces (like on tree frogs) have higher rate of water condensation than a mosaic pattern of hydrophilic/ hydrophobic surfaces (like on fogstand beetles) (Comanns, 2018). Therefore, it might be better to have a full hydrophobic surface similar to the surface of the lotus flower (Figure 2.2) which is ultra-hydrophobic (Mozumder et al., 2019).

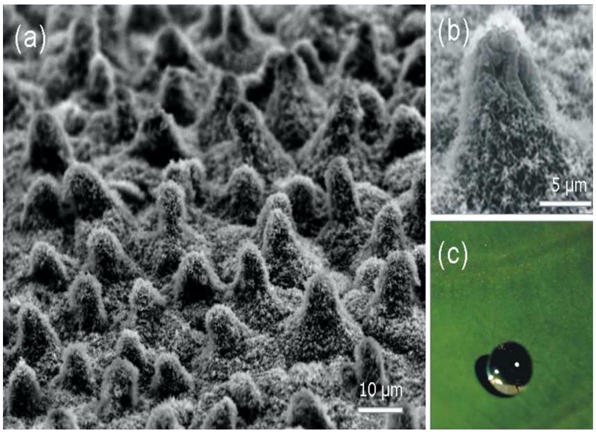

Figure 2.2: The lotus leaf (Mozumder et al., 2019). **(A)** Visualisation of the structure of a lotus leaf under a scanning electron microscope. **(B)** Higher magnification image of the lotus leaf surface. **(C)** A water droplet on the surface of the lotus leaf.

2.1.3 Nanorods

Nanorods are a form of nanoscale objects, that are often synthesized from metals or semiconducting materials. Nanorods are mostly used for display technologies and microelectromechanical systems (MEMS), but when attempting to make magnetic nanowires, carbon-rich nanorods were discovered that have the ability to capture water from the humidity in the air. These nanorods can absorb water in conditions with low humidity and then release the water as vapor at a high humidity level. The material behaves similar to a sponge as it absorbs water and wrings itself before it is fully saturated with water. Almost 50 % of the water can be expelled and on top of that the water expulsion process is reversible (Nune et al., 2016).

The reversibility of this process can be attributed to the interfacial forces between the confined rod surfaces. When the rods are widely spaced apart, a monolayer of water can form on the surface of the rods. This subsequently leads to the condensation of the water in the confined spaces between adjacent carbon-rich nanorods. At increasing relative humidity, adjacent nanorods are drawn closer together via capillary forces. When a critical relative humidity is reached and 2 intersecting nanorods reach a distance of about 1.5 nm apart, the size of the spaces between the nanorods have become so small that a process called solvent cavitation or surface-induced evaporation takes place and the water that had condensed inside the confined area is released as water vapour as seen in Figure 2.3 (Nune et al., 2016).

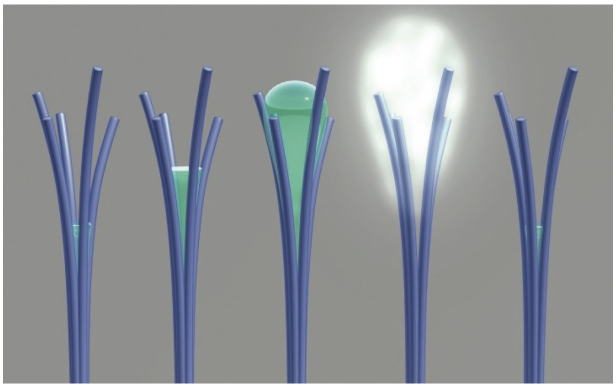

Figure 2.3: Illustration of the water expulsion mechanism (Nune et al., 2016). The nanorods are being pulled together by the surface tension created by the water. When adjacent nanorods are drawn closer together, the size of the spaces between the nanorods have become so small that the water, that had condensed inside the confined area, is released as water vapour.

Unlike biomimetic materials, Choo et al. (2015) found that a mosaic pattern of hydrophilic/hydrophobic nanorod surfaces collect more water than either a superhydrophilic or superhydrophobic nanorod surface on its own.

This technology has the potential to be used for low-energy water harvesting and purification in deserts or developing regions, since it can capture water during the day when the relative humidity is low and set it free during the night when the relative humidity is high. This water expulsion behaviour at high relative humidity was not known to be present in any class of known inorganic materials like metal organic frameworks (MOFs) (Nune et al., 2016).

Although it was not certain that the same water expulsion phenomenon would be present at larger scale at the time of the article, there now is a Canadian company called ’AWN Nanotech’ that has a product on the market to produce cheap and clean drinking water using the carbon-rich nanorod technology (Awn Nanotech Inc., 2018). The AWN setup consumes less than 0.01 kWh/L of fresh water, which is 20 to 70 times less than the other devices on the market. There has also been more research into carbon-rich nanorod application such as the cactus stem-inspired water harvesting system which is estimated to collect about 50 L of water/unit area of 1 m^2/day given that the water harvester experiences wind of 70 cm/s created by a humidifier. Unlike the iron based nanorods Satish et al. discovered, the nanorods or nanoneedles used by Sang et al. are based on copper-composites (Nune et al., 2016; Lee et al., 2019).

Similar technologies could not only be used for remote, arid and developing regions, but also on a larger scale to produce clean drinking water in cities. Places with a contaminated water supply could also incorporate the technology to purify water.

2.1.4 Metal organic frameworks (MOFs)

MOFs are compounds consisting of metal-ions or -clusters that are arranged together with organic ligands to form modular structures. This results in a large diversity of structures that allow a chemical and geometrical optimization needed to obtain certain qualities. These structures have a wide range of properties including high porosity and being able to adsorb gases like hydrogen and CO_2 which makes them quite interesting in applications for gas purification and separation, catalysis and off course water capture (Kalmutzki et al., 2018).

In a recent study, Kim et al. (2017) has found MOFs that have a maximal yield for water capture and consume minimal energy. These new types of MOFs display an isoterm with a steep increase in water uptake within a narrow range of relative humidity. This quality enables maximal regeneration with minimal temperature increase, which minimizes the energy necessary to capture water from the air.

MOF-801 and UiO-66 are 2 MOFs created to adsorb water (Trapani et al., 2016). In regions with a relative humidity of just 20 % like in North Africa, MOF-801 has the ability to be used and UiO-66 is best suited for regions with a relative humidity of 40 % like northern India. Even though the adsorption capacity of UiO-66 is lower than some zeolites, zeolites have to be heated up to at least 60°C in order to release water. This means that although the newly discovered MOFs have great potential, there is not just one type of MOF to suit all different types of conditions (Kim et al., 2018).

The setup in Figure 2.4 (Kim et al., 2017) can capture water from the atmosphere at ambient conditions using low grade heat with no additional energy required. This heat is originating from the sun at a flux of less than 1 sun. This is equal to 1 kilowatt/m^2. Per kilogram of MOF the setup can harvest 2.8 L/day at a humidity level of 20 %.

These yields are obtained by letting the MOFs absorb water at night by opening the device, shown in Figure 2.4, and then closing the box during the day so the sun can heat up the MOFs which in turn release the water that is then finally condensed and stored. The water condenses at ambient temperatures created by the passive heat sink, the condenser. This requires no additional energy.

Even though this relatively small yet effective device is still not cheap, as zirconium (used in MOF-801) costs €140 per kilogram, this device or improvements could provide clean drinking water to homes in the most arid places on earth. Zirconium could possibly be replaced by aluminum which is about 100 times cheaper than zirconium (Kim et al., 2017). Together with carbon-rich nanorods MOFs could be quite the improvement on existing products on the market. Existing technologies are often very energy intensive or have a low yield. If MOFs and nanorods were to be used simultaneously in 1 installation it could give rise to a setup that needs very little energy and captures water day and night with a relative high yield. On top of that, both the MOFs and nanorods can still be improved individual. Since atmospheric water accounts for a volume estimated to be as much as all the freshwater in our planet's rivers, swamps and marshes, this is a large source of freshwater that could be used for drinking water (Shiklomanov, 1991).

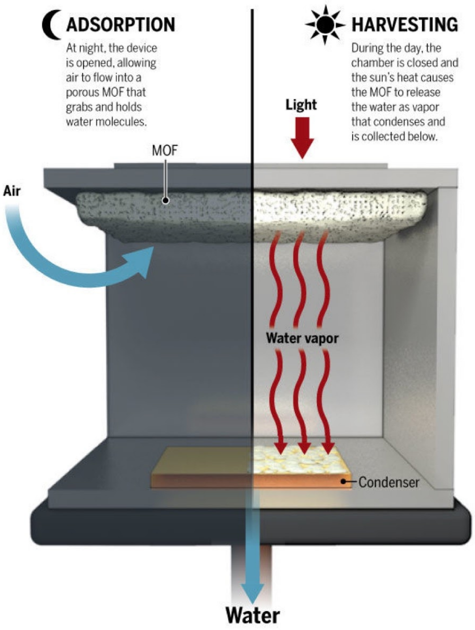

Figure 2.4: Device based on metal organic frameworks (MOFs) that pulls water from the air (Service, 2017). At night the device soaks up water vapor from the air and uses heat from the sun to release it as liquid water during the day.

2.1.5 The Dewpal 2016-project

In 2016 a project called 'Dewpal' was created by the iGEM team from UGent. Dewpal is a 3D-printed shape, optimized for the condensation and collection of atmospheric water. It was inspired by the Fogstand beetle *Stenocara gracilipes* which uses fog to capture large droplets on its back (UGent Belgium iGEM 2016, 2016). The beetle's back is a bumpy surface consisting of alternating hydrophobic, wax-coated and hydrophilic regions (Parker and Lawrence, 2001). In an attempt to enhance the Dewpal's functionality, a filament containing biotin was added in order to bind functional proteins. Ice-nucleating proteins (INPs) of *Pseudomonas syringae*, also found in clouds, were used. These INPs improve the nucleation of ice at temperatures higher than what is normally observed. Since nucleation is a very important step in condensation, the INPs were used to increase condensation and therefore also water collection.

The IPNs where then expressed as a fusion protein with streptavidin to bind it to the biotin - as illustrated in the Figure 2.5 - or produced as membrane-bound proteins in order to bind the whole organism to the biotin.

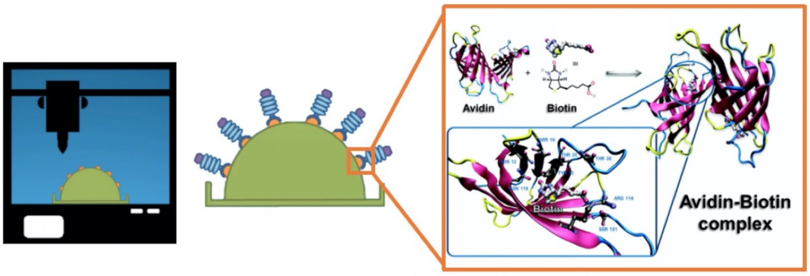

Figure 2.5: Representation of the avidin-biotin complex on top of 3D printed shape (UGent Belgium iGEM 2016, 2016).

In the first part of our case study we worked on a Dewpal 2.0, to find out what technologies would be best to produce drinking water in desert areas, places with a contaminated water supply and even areas that have seasonal droughts. Since the 2016 Dewpal project was unable to produce large amounts of water, we hypothesised that adding a temperature gradient to the setup would improve condensation. Similarly to the tree frog *Litoria caerulea*, the Dewpal 2.0 could use an underground piping to create a temperature gradient as the ground temperature at certain depths is lower than the air temperature. In addition, the existing concept could be improved upon using other technologies like nanorods, different types of biomimetic materials and MOFs. The feasibility of the project will be discussed further in the case study.

2.2 Potable water via cloud seeding

2.2.1 Formation of rain

Precipitation is defined as water in a liquid or solid state falling down from clouds onto the Earth’s surface, e.g. in the form of rain or snow. The main process enabling precipitation is condensation, leading to the formation of water droplets high in the sky which will eventually aggregate into clouds.

The process providing the water vapor needed for condensation is called evaporation and can be divided into two categories: one being evaporation itself, meaning liquid water is turned into vapor by solar radiation, and another one being transpiration, leading to the release of water vapor by plants in order to maintain their metabolism. Transpiration on its own accounts for 39 % of terrestrial precipitation and evapotranspiration for 61 % globally (Schlesinger and Jasechko, 2014).

Condensation, evaporation and transpiration are part of the hydrological cycle, which describes the movement of water throughout the atmosphere, the soil and the environment in general. This cycle is the driving force behind the formation of precipitation and can be altered by using cloud seeding.

2.2.2 The concept of cloud seeding

The term ‘cloud seeding’ is sometimes used in a general sense to describe the release of any material designed to modify cloud properties, thus not only to create precipitation (Dennis, 1980). However, in the context of our bachelor thesis, a more narrow definition is applied, stating that cloud seeding or cloud modification is a technique used to create precipitation in an artificial way using cloud condensation nuclei (CNN) or ice nuclei (IN), which alter the microphysical processes within the cloud.

CCN are specifically used to create rain; if the goal is to form snow or ice, IN should be used. Both techniques are based upon the principle of heterogeneous nucleation, meaning cloud droplets form in the atmosphere by condensation on already existing particles, rather than water molecules from the vapor state uniting to form pure water droplets (homogeneous nucleation). An example is shown in figure 2.6. Important to notice is that the underlying principle of heterogeneous nucleation is exactly the same as the one that occurs during the natural formation of clouds: a regular cloud also contains particles called ‘condensation nuclei’ upon which the water vapor initially condenses into droplets. Common condensation nuclei are for example dust, pollen and smoke particles or even sand or sea salt.

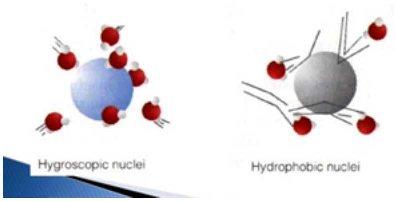

Figure 2.6: Heterogeneous nucleation (Beasley, 2009), left: hygroscopic nuclei, right: hydrophobic nuclei. Water molecules are displayed in red and white.

The role of both CNN and IN is of major impact: in the absence of catalysts for freezing, water can remain in a metastable liquid state at temperatures well below 0°C, referred to as supercooled water. Spontaneous freezing of supercooled water occurs below a temperature of -39°C without nuclei and at around -5°C with nuclei. Hence, freezing catalysts are clearly essential for natural freezing processes outside of polar regions and extreme winter seasons on Earth and in much of the corresponding troposphere (Morris et al., 2004).

Based upon these understandings, many ways have been developed to enable the modification of clouds using CNN or IN. The initial start of the modern era of weather modification began with the discoveries of Schafer in 1946 and Vonnegut in 1947, who discovered that supercooled liquid water could be converted into ice crystals using either dry ice or silver iodide. Both materials enhance the ice crystal concentrations in clouds by either nucleating new crystals or freezing cloud droplets. In accordance to all these discoveries, the following cloud seeding methods can be distinguished (McDonald, 1958; Bruintjes, 1999):

1. Static cloud seeding
2. Dynamic cloud seeding
3. Hygroscopic cloud seeding

All three methods differ based on their ice-nucleating capabilities. The effect of static and dynamic cloud seeding concepts has already been investigated numerous times, while hygroscopic cloud seeding is a newer approach to the subject of weather modification.

Static cloud seeding involves spreading a chemical like previously mentioned AgI into clouds. The AgI provides a crystal around which moisture can condense. The moisture is already present in the clouds, but AgI essentially makes rain clouds more effective at dispensing their water. Important to know is that static seeding per definition only has a significant effect when used on a supercooled cloud, a.k.a. a ‘cold-based cloud’ or ‘cold cloud’, which is also preferably continental and convective, meaning it was formed by convection above a landmass. A cloud type that seems to match all of these requirements consists of cumulus clouds, low-level clouds which are generally less than 2,000 m in altitude. Nonetheless, even this claim is doubted due to the large natural variability in temperature and moisture ratio of the clouds and an incomplete understanding of the physical processes considering convection (Bruintjes, 1999).

The second concept, commonly called dynamic-mode seeding or seeding for dynamic effects, is based on the postulate that ‘the seeding-induced conversion of supercooled rain drops into ice particles will result in the production of more rain and stronger downdrafts from the seeded cells which, in turn, will enable the cloud system to grow larger, process more water vapor, and yield even more precipitation’ (Silverman, 2001). In other words, by adding CNN or IN to a cloud system during static cloud seeding, the cloud system will automatically grow larger and produce even more rain or ice by itself, entering dynamic cloud seeding.

Identical to static cloud seeding, the validity of this technique is still doubted. According to Silverman (2001), ‘based on a rigorous examination of the accumulated results of the numerous experimental tests of the static-mode and dynamic-mode seeding concepts conducted over the past four decades, it has been found that they have not yet provided either the statistical or physical evidence required to establish their scientific validity’. More recent sources confirm this as well (Pelley, 2016). However, the former author states that cloud seeding as a whole remains promising, unproven and worth pursuing, for example by further investigation of hygroscopic cloud seeding.

Hygroscopic cloud seeding is aimed at accelerating autoconversion in warm clouds, meaning the conversion of cloud water to precipitation, using flares or explosives of salt in the lower portions of clouds. The salts grow in size as they absorb water, creating larger droplets more quickly. The issue with hygroscopic cloud seeding is that, according to research by Rosenfeld et al. (2010), the technique appears to be far more complex than envisioned originally. Using models, they found that similarly to static and dynamic cloud seeding, the seeding outcome for individual clouds and for groups of convective clouds is strongly dependent on the sizes and amounts of the dispersed nucleant and on the time the seeding action is taken. Initiating precipitation too early in the convective cycle can sometimes result in less precipitation than if no seeding were undertaken at all.

In conclusion, all three techniques have proven to be functional on a certain level, but taking into consideration the immense variability in temperature, moisture ratio, nature of the used CNN or IN, location and many more, none of these methods have generated both sufficient statistical and physical evidence. Of course this does not mean that the concept of cloud seeding is invalid: the extreme diversity and dependence on external circumstances just makes it difficult to fully prove the usefulness of the technique.

Furthermore, in the scientific community, cloud and weather modification in general is still viewed as a somewhat controversial topic due to a few different factors, such as proceeding with an inadequate scientific knowledge base and differing views between funding agencies and project scientists (Bruintjes, 1999). Most of these items are focused on the financial side of the project, although there exist many more controversies considering the opinion of the general public and the ethical side of cloud modification (Dennis, 1980).

Another major issue is that the chemicals used in cloud seeding can potentially damage the environment, especially the plants on the fields that they intend to protect using cloud seeding. It has been found to be highly toxic to fish, livestock and humans as well (Malik et al., 2018). Even today, there is no substantial study done on the implications of AgI on the environment, but it is suspected to cause ‘iodism’, a type of iodine poisoning where the patient exhibits running nose, headache, skin rash, anemia, and diarrhea among others. Taking into consideration that AgI is, as previously mentioned, still one of the most used chemicals in cloud seeding, this is most definitely a noticeable problem related to the subject.

2.2.3 Ice nucleation proteins

What are ice nucleation proteins?

Besides inorganic particles and chemicals, there also exist bacteria that produce certain protein. These proteins are called INPs. INPs are a family of proteins that are produced by several species of Gram-negative bacteria, including *Pseudomonas syringae*, *Erwinia herbicola*, *Pseudomonas viridiflora*, *Pseudomonas borealis* and *Xanthomonas campestris pathovar translucens* (Gurian-Sherman and Lindow, 1993; Newby, 2017). *Pseudomonas syringae* generally is the model organism for studies involving INPs. InaZ, the INP produced by these species therefore is also a model INP (Han et al., 2017). The proteins can be found on the outer membrane of the bacteria and they act as a nucleus for the formation of ice crystals. This can happen at a higher temperature than normally possible when non-biological particles such as dirt for example act as nuclei. This is due to their specific protein structure that forms a surface that can attract water molecules and aligns them in such a way that they resemble an ice lattice, promoting crystal growth.

The ice nucleation activity of INP is possible due to its specific protein structure and protein sequence. It has been shown that the sequence of INP consists of 3 large regions: a C-terminal region on one end of the protein, a N-terminal region on the other end and in between a highly repetitive central domain. These regions respectively represent around 4 %, 15 % and 81 % of the total sequence (Li et al., 2012). The 2 terminal regions are less important for the ice nucleation activity of INP. They are also very similar in terms of sequence between versions of INPs of different bacterial species. The N-terminal region makes it possible to couple the protein to certain groups found in the outer membrane (Li et al., 2012) of bacteria. Therefore this part is important for the fixation of INP on the membrane, but the N-terminal region also is important for the transportation of intracellular produced INP towards the membrane. The function of the C-terminal region is not exactly known, but is thought to have a stabilizing function (Kajava and Lindow, 1993). The most important region, the central domain, consists of a number of repeats of series of 16 amino acids (aa) with a few aa that are not always the same. The consensus sequence of this repeat is AGYGSTxTAxxxSxLx (Kumaki et al., 2008). The x's can represent any aa. INPs are generally made out of 50-80 of these repeats. The 16 residues long repeat can also be divided into repeats of 8 aa. Between these repeats there is less similarity than between the repeats of 16 residues. This means that a repeat of 8 residues does have a lower similarity with the one just after it, but a higher similarity with the one after that. Furthermore, the repeats of 16 residues can be seen as a part of a 48 aa long repeat. This repeat again has a higher similarity than that of 3 consecutive repeats of 16 residues.

Although there still is a lot of uncertainty about the exact cause of the ice nucleation activity, it is thought that the combination of the different aa form regions in the strand that are alternatingly hydrophilic and hydrophobic, or in other words attract and repel water. Because of this the protein attracts and orients the water molecules in such a way that it resembles the beginning of an ice lattice. Starting from this structure, the protein can easily attract more water and the ice lattice can grow further. The exact tertiary structures and the 3D-shape of INP are not known. This is due to their large size (around 150 kD) (Govindarajan and Lindow, 1988), their membrane association and their tendency to form aggregates with each other, which make it difficult to analyse it. However, several attempts have been made to create models of INP that could explain the functionality of INP, most of which came to similar conclusions (Graether and Jia, 2001; Kajava and Lindow, 1993).

An example of such a model is the one proposed by Garnham et al. (2011). For their model they studied the INP produced by the bacteria *Pseudomonas borealis*. By looking at proteins that can also bind water molecules, they proposed that the structure of INP could be a beta-helix. A beta-helix is a protein strand that is wound up in a helix formation and that is stabilized through bonding between the functional groups on the aa of the protein. The difference between a beta helix and an alpha helix is that the sequence in a beta helix has repeats of certain sequences. The helix structure is wound up in such a manner that these sequences are always oriented towards the same side of the helix.

Also the specific aa responsible for the activity of INP are not known. By looking at catalytic centres of other water binding proteins, similarly as with the structure, it is proposed that the motif TxT could be responsible. This motif is found within each repeat and is therefore represented on one side of the helix. Also another water binding motif is found in each repeat and is represented on the other side of the helix. In the specific case of *Pseudomonas borealis*, these 2 motifs are TQTA and SLTA. The protein contains 2 nucleation sites, formed by the 2 motifs, with each site positioned on another side of the protein. Both nucleation sites are supposed to be relatively flat surfaces. A visual representation of the proposed structure is seen in Figure 2.7 (Garnham et al., 2011).

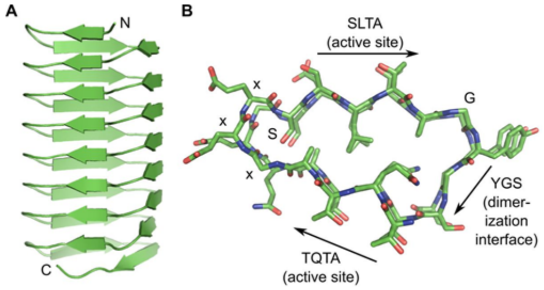

Figure 2.7: Beta helix structure (Garnham et al., 2011). **(A)**: visual representation of the beta helix structure, the arrows represent the tandem repeats. **(B)**: a top view of subfigure (A), SLTA and TQTA are the nucleation sites, YGS is site where a dimer can be formed, the green parts represents carbon, the blue parts nitrogen and the red parts oxygen.

So how do the 2 nucleation sites achieve the water bonding? The surfaces of the protein are relatively hydrophobic, but there also are functional groups that can form hydrogen bonds with the water molecules approaching the surface. These groups are the hydroxyl groups of threonine (T) and serine (S) in the motifs. Besides these groups, T also has a hydrophobic methyl group, which cannot form hydrogen bonds. When a water molecule binds on the surface through the hydroxyl groups, it is also repelled by the methyl groups. This traps them in a clathrate-like structure, with a clathrate being a compound that can trap molecules. The water molecules in their trapped state have a distance in between them equal to the distance of water molecules in an ice lattice. When the ice lattice is formed, it becomes thermodynamically more interesting for water to bind to the INP (see Figure 2.8) (Davies et al., 2002). This results in a much more efficient way to start ice nucleation than non-biological particles.

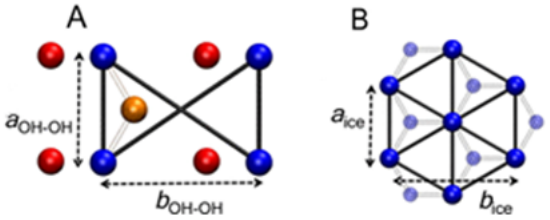

Figure 2.8: Water trapped in INP (Davies et al., 2002). **(A)**: a visual representation of a water molecule (orange dot) trapped between the TxT motifs of the INP. **(B)**: the distances between the water molecules in an ice lattice are the same as the ones in between the hydroxyl groups in subfigure (A).

Factors determining ice nucleation activity

The structure of INP makes sure that it has ice nucleation activity, but besides this, there are 2 big factors that determine how strong this activity is. The first factor is the amount of ice-binding surface that the protein has. The bigger the surface area, the more water molecules can bind to it. This means that the INP will be more effective and the ice nucleation will occur at higher temperatures. An increased surface can be achieved by three things: a higher number of repeats in the central domain, aggregation of several individual INPs to form oligomers according to Ling et al. (2018) and a higher expression resulting in more INPs on the membrane. Expression will be discussed later in this section.

The second factor affecting the efficiency of INP, is the membrane association. For the activity of INP to be optimal, it has to be located on the membrane of the bacteria (Schmid et al., 1997). The monomers forming the aggregates on the membrane have to be positioned with a certain distance from each other, otherwise the formed ice lattices will have additional stress and deformities in their lattices. These distances are assured by the positioning of the monomers on the membrane (Qiu et al., 2019).

A higher expression of INP in the bacteria results in more INPs on the membrane surface, increasing aggregation and therefore more active surface. This results in a higher activity of the protein and consequently a higher temperature where nucleation is possible. Three types of bacteria capable of forming ice nuclei can be defined, each with a different level of INP expression: type I nuclei have a nucleation activity at temperatures between -5°C and -2°C, type II between -5°C and -7°C, and type III between -7°C and -10°C (Yankofsky et al., 1981). Since a more polymerized INP has a higher nucleating temperature, type I nuclei are the cells that have the most aggregated structures of INP.

Not all of the types are expressed equally in a population. The bigger the aggregates get, the harder it becomes for the cell membrane to hold them together (Morris et al., 2004). This results in higher frequency of the smaller type II and type III nuclei, and a lower frequency of the type I bacteria in a normal bacterial population. However, there can be shift towards the type I of INP under certain conditions. A combination of lower temperatures and nutrient starvation can induce the production of type I nuclei. Nemecek-Marshall et al. (1993) showed that the expression of type I nuclei can be increased from less than 1 per 107 cells, to 1 per cell with a medium depleted of nutrients and a temperature shift from 32°C to around 14-18°C. With increasing temperatures the type I nuclei are degraded. Lindow (1983) proposed that this was due to the fact that membranes become more liquid when heated, and that because of this the expression of type I nuclei is decreased.

For the increased expression the cells have to be in the stationary phase, the phase after the growth phase where the bacterial population does not increase anymore. Increased ice nucleation activity can be seen in a medium that contained just enough nitrogen for the cells to complete their growth phase, and therefore the medium contained no more nitrogen in the stationary phase. Also phosphates proved to have a similar effect, this effect was even notable when the bacteria were in the growth phase. Further optimization could be done with the addition of Mn_2^+ ions or sugars, such as inositol, mannose and glucosamine to the medium. These are suggested to be components needed for the aggregation of the INP and their linkage to the membrane (Kozloff et al., 1991).

Function in nature

INPs have 2 major functions in nature. The best studied and known function is that they help the bacteria on which they are expressed to become more pathogenic to certain plants. A much less proven function is aiding in natural rain formation. These 2 functions are heavily linked with each other. The pathogenic effect of INPs is visible in plants during periods with temperatures below 0°C. More specifically, the proteins play a part in frost injuries in plants (Gurian-Sherman and Lindow, 1993). In most plant tissue, water can be supercooled to a certain extent. In normal conditions, if no INP is present in the tissue, water will not freeze above a temperature of -5°C. This is because, as mentioned before, water only freezes at temperatures below 0°C if certain ice nuclei are present. There are ice nuclei in the plant tissue, but most nuclei are active at temperatures of -10°C or lower. Ice nuclei that are active at -5°C or higher exist, but are very rare in plant tissue. However, if the plant is colonized with INP producing bacteria, ice nucleation will take place at a higher temperature.

This means that ice nucleation above -5°C becomes possible. The exact effect of this phenomenon depends on the extent of frost-sensitivity of the plant.

First, plants that are frost-sensitive will experience serious disadvantages from an infection with the bacteria. Since they are harmful to these plant species, INP producing bacteria are plant pathogens. At temperatures below 0°C but above -5°C, frost-sensitive plants are able to survive since there are not any active ice nuclei at these temperatures. In case of an infection, freezing does become possible. The probability of frost damage consequently increases. The cells of these plants cannot handle the ice formation since the formed ice crystals penetrate them and destroy the cells. For the pathogenic bacteria, this means that they can take advantage of all the nutrients that are released when the plant cells break (Buttner and Amy, 1989).

Secondly, there is also an effect on non frost-sensitive plants, though positive in this case. INP can help to tolerate ice formation in these plants. This is because supercooling in frost-hardy plants is disadvantageous to them. If freezing happens at relatively high temperatures, approximately between 0°C and -5°C, ice formation will occur more slowly. This allows a controlled propagation of ice formation in the apoplast, which is the space in between the cells. Because of this, water is drawn from the cells by a water potential gradient. This results in an increased solute concentration inside of the cells. A liquid with a higher concentration of solutes has a lower freezing point, which means that freezing in the cells will occur at a temperature lower than 0°C. On the other hand, if freezing happens at lower temperatures, the tissues will freeze too fast and cell dehydration will not occur. This results in intracellular ice formation and the ice will penetrate the cells and cause the cell death, just like in frost-sensitive plants (Baertlein et al., 1992).

The pathogenicity of these bacteria is probably the reason for the increase in INP expression due to nutrient starvation, as mentioned in the previous part. If the bacteria experiences low nutrient levels when they have infected a plant, they can increase the quantity of INP on their membranes. Because of the resulting ice formation, the plant cells break and the nutrients inside of these cells are released, which the bacteria can use for their growth (Nemecek-Marshall et al., 1993).

Other than their aid in pathogenic processes, INPs also play a role in the enhancement of precipitation in nature. Metagenomic studies of cloud water have shown that several species expressing INPs are present in this water, including *P. syringae*, *Xanthomonas* spp. and *Pseudoxanthomonas* sp. Therefore it is thought that INP plays a significant role in rainfall through a process called bioprecipitation.

The hypothesis explaining this process suggests that bacteria may be present in clouds as part of an evolved process of dispersal. The cycle starts with pathogenic bacteria using their INPs to gather nutrients from the plants. Thanks to this nutrients the bacterial colonies grow and eventually there are so much bacteria that there are not enough nutrients left. By then the number of bacteria is so big that get blown in the sky. Eventually these bacteria get in the clouds which is also an environment with very little nutrient availability. This is where the bacteria use their INPs again, here to help induce rainfall. Along with the rain, the bacteria get back to the surface and on new plants that they can colonise. Additionally, because of the rain the plants can grow better, thus producing more nutrients for the bacteria to use. As the bacterial population grows in the plant and its surface, the chances of bacteria being blown in the sky by the wind increase as well, and the whole cycle starts again. This hypothesis proves that INP has a part in creating rain naturally, though only to a limited extent, as it is merely a way for these bacteria to spread, in much the same way as plants rely on wind blown pollen grains (Morris et al., 2004).

Applications

Besides their function in nature, INPs also have several applications in the food industry, agriculture, entertainment and for scientific purposes. An example of such an application is the making of artificial snow with snow cannons. These are used in ski resorts when there is not enough natural snow for skiing. Artificial snow making does not necessarily need INP to work, but they can make the process much more efficient and make more and denser snow (Snomax, 2015).

Another application of INP is the use in frozen food products. Because they make freezing at higher temperatures possible, less energy is needed for cooling. Additionally the quality of the frozen food is better. This is because using INP results in bigger crystals and sizable crystals are beneficial for the quality of frozen food (Zhang et al., 2010).

Also in agriculture can INPs be beneficial. As described earlier INP can cause serious frost damage in plants, but if used in a right way, crops can benefit from inoculation with bacteria that can express INP. One specific application is to inoculate the crops with INP expressing bacteria and use the freezing effect to make sure that insect eggs cannot make it through the winter, thus controlling insect pests in the plants. Another use of the bacteria is applying them on the plants, but without expressing INP. Since these bacteria already colonised the plant, bacteria that do express INP, do not have any room left on the plant, and are therefore unable to colonise it (Cid et al., 2016).

Finally, INPs also have their possible uses in scientific applications. They have been proven useful as a basis for reporter gene systems. With these systems, it is possible to see if a certain gene is being expressed by checking if the ice nucleation gene it is combined with, is also expressed. It can be concluded that INPs are present if water freezes at certain temperatures (Gurian-Sherman and Lindow, 1993).

Ice-nucleation proteins for cloud seeding

Similarly to the chemicals mentioned in §2.2.2, INP can be used as a heterogeneous nucleus upon which water vapor condenses into droplets. The microorganisms that produce INP efficiently catalyse ice formation at temperatures much higher than most organic or inorganic substances, meaning INP can be used to seed many different types of clouds whereas static or dynamic seeding techniques only seem to have an effect on heavily supercooled clouds, as stated before. In other words, INP operates the same as previously mentioned chemicals, but with a far greater range of possibilities while being more friendly for the environment, nontoxic and biodegradable. Another interesting feature of bacterial ice nucleation is its quantitative and qualitative variability. The use of INPs to induce precipitation is further investigated in the second part of the case study.

CHAPTER 3

CASE STUDY

3.1 Dewpal 2.0

As the 2020 iGEM team at UGent we wanted to find a way to bring potable water to arid and remote regions with the help of synthetic biology. We were intrigued by the idea of the 2016 team to capture atmospheric water using a 3D structure with a hydrophobic/hydrophilic mosaic and INP. As the 2016 team did not succeed in producing large amounts of water, our first idea was to add a temperature gradient to the setup based upon a survival mechanism of the tree frog *Litoria caerulea*. The frog can cool itself down in the open air and then enter a warmer humid place like a tree hole in order to create condensation on its skin.

It is known that in most places, a constant temperature is present at certain depths underground. The idea was to install piping which starts from the surface, goes down to a region with a more or less constant temperature and then goes back up again. The hot desert air would then be sent through the piping, causing the air to gradually cool down as it got deeper underground. This would be facilitated by a fan powered by solar energy from solar panels. The cooler air would then have a higher humidity percentage, facilitating condensation. On top of that the INP might have a larger effect on the overall water capturing process, since it normally works at negative or in some cases slightly positive temperatures. Besides using the underground piping to cool the air, we wanted to maximize the condensation area inside the piping. We thought this might be possible by introducing nanorods that, instead of vaporizing the captured water, would divert the captured water to somewhere else, where it could be stored and ultimately be pumped up to the surface for consumption.

In order to explore the feasibility of the project some rough calculations were done (See appendix A). The energy that had to be dissipated and the amount of water that could be retained, using approximations of temperatures and humidity for the Sahara Desert, were calculated (see Figure 3.1). Using the rough estimates, we concluded that for the setup we would be able to collect sufficient amounts of water, but the main problems were the large amount of energy that had to be dissipated underground, the warming of the ground around the setup and the depth the pipes would need to reach.

The energy from the heat of the air has to be dissipated through the ground. The dissipated energy subsequently warms up the ground and taking into consideration that sand has a relatively low heat capacity, the surrounding ground would warm quickly. The rising temperature of the sand would diminish the temperature gradient between the ground and air temperature and thus rendering the whole point of the setup useless. On top of that, the average ground temperatures in deserts are slightly higher than those used in the calculations and the piping would need to reach a depth of at least 6 meters to have a temperature that is somewhat constant disregarding the air temperature. The fact that we would have to dig this deep makes the setup not portable and more expensive.

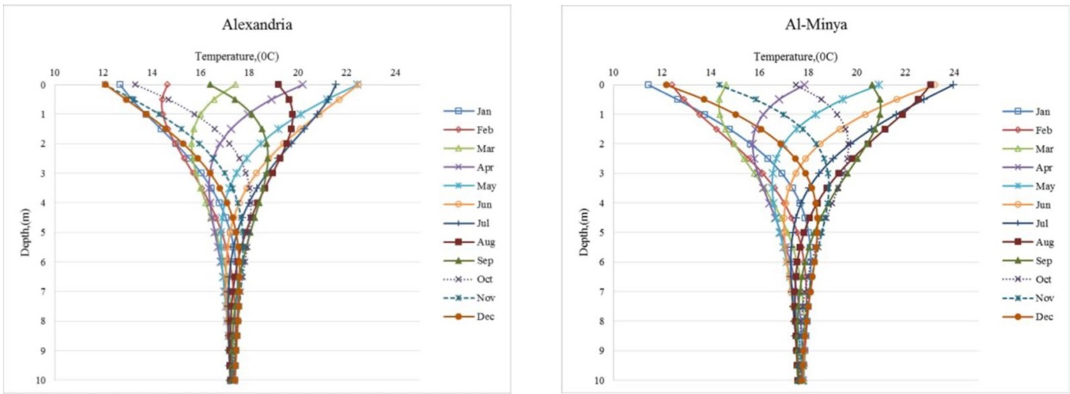

Figure 3.1: The monthly average soil temperature profiles in Alexandria (left) and Al-Minya (right) in Egypt (Serageldin et al., 2015).

Although some problems might be solved by incorporating nanorods, MOFs or other technologies, we decided to abandon the idea because it was getting too complex and it would have little added value over existing setups like the setup from the University of California Berkeley (Service, 2017).

Furthermore, the MOF setup from the University of California Berkeley is quite compact, portable and scalable, whereas the Dewpal 2.0 would be large and would have to remain where it was initially installed. We decided to redirect our efforts into creating a way to increase the natural phenomenon of rain instead of trying to capture atmospheric water. Nonetheless, there is still room for improvement with atmospheric water capture using MOFs and nanorods. Our main concern was that the idea would add almost no value to the subject and that it would steer us away from further improvement to the Dewpal 2.0 through biotechnological and synthetic biological solutions, which are obligatory requirements for the iGEM competition.

3.2 Cloud seeding

Since the Dewpal 2.0 did not prove to be a viable project for the iGEM competition, other possible projects were considered. Eventually we decided to build the project around the concept of cloud seeding. We decided to use bacterial INPs to achieve this. The general idea is to let certain bacteria produce extracellular vesicles that express INPs on their surface and use these vesicles as a cloud seeding agent. Since the vesicles alter the water cycle, the name ‘Vsycle’ was chosen for the project, combining the words vesicle and cycle. The inspiration for this idea came from the iGEM project of the UGent team of 2016, that also used INPs, more specifically the INP of *P. syringae*, to help condensate water. As mentioned in §2.2.1, INP can lower the temperature at which freezing of supercooled water becomes possible, and possibly plays a role in precipitation cycles. Therefore, it seemed a good idea to use these proteins to create rain in an artificial way. This could have numerous possible applications, including solutions for droughts, floods, forest fires, but also for making sure it does not rain during big outdoor events. This rain could also be used to water crops that are in need for precipitation or to protect them from storms and hail. As mentioned in §2.2.2, cloud seeding is something that is already being used, but is currently done with potentially harmful chemical agents like AgI. With this idea cloud seeding could be made more friendly to the environment, using biological, nontoxic and biodegradable components. Additionally, bacteria expressing INP have shown to be more efficient in freezing supercooled water than their chemical counterparts (Levin et al., 1987).

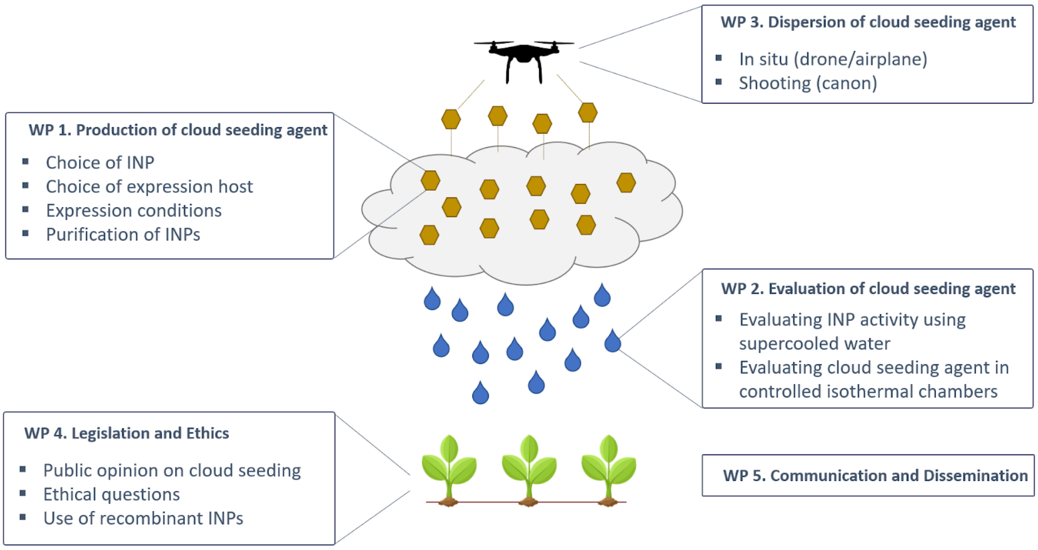

Figure 3.2: The PERT Chart of the Vsycle project with its five work packages. WP = work packages, INP = ice nucleating protein.

The Pert chart of the Vsycle project is given in Figure 3.2. More specifically, the project comprises five highly interconnected work packages (WPs). In WP1, the genetic part of Vsycle and the production and purification of a cloud seeding agent will be researched. In WP2 these constructs will be tested on their efficiency and capability of ice nucleation by performing various tests. WP3 will look at the dispersion of the produced agent into clouds. WP4 focuses on the legislation and ethics of the project. Finally, WP5 will be about communication and dissemination of the project. The first four WPs will be discussed further below. The risks regarding the different aspects of the Vsycle project and their mitigations are discussed in Table 3.1.

Table 3.1: The risks regarding the Vsycle project, and their possible mitigations.

Work package (WP)	Risk	Mitigation
WP1	Pathway for vesicle formation is not functional in *E. coli*	Fine-tuning of the pathway OR use of bacterial ghosts (Kassmannhuber et al., 2017), which are bacterial cells that have a kill switch that causes it to expel all cytoplasmatic content
WP1	Candidate INPs not displayed on vesicle	Evaluation of other membrane tags OR use of scaffold proteins
WP1	Vesicles cannot be separated using centrifugation, filtration or phase separation	Adding tag sequences on the vesicles
WP2	It is not possible or very hard to make a controlled chamber	Looking for institutes that already have such chambers and asking if these could be used
WP2	No physical evidence that cloud seeding actually works in clouds	Contact with institutes that do tests in real clouds (Siems, 2020)

3.2.1 Production of a cloud seeding agent (WP1)

In WP1 the optimal method for producing a biological cloud seeding agent containing INP is investigated. First, the most promising INP candidate will be selected. Next, an easy and efficient expression system to functionally express INPs through vesicle display will be developed and optimised. Finally, several purifications methods to recover the vesicles with membrane integrated INPs will be evaluated.

The INP of *P. syringae*, InaZ (Han et al., 2017), is the most investigated INP. Besides, this INP is already successfully expressed both attached to the *E. coli* outer cell membrane as well as to a soluble protein at the Centre of Synthetic Biology from UGent, by the iGEM UGent 2016 team. For these reason, InaZ from *Pseudomonas syringae* is chosen as a reference INP for inducing precipitation. In addition, literature and metagenomic data from cloud samples will be investigated for candidate INPs. Other than *P. syringae*, potential INP activities can be found in *Xanthomonas* spp. and *Pseudoxanthomonas* sp., as these were found in clouds (Joly et al., 2013).

Next, a novel expression system to display INPs on a vesicle will be developed. Such a system has the advantage that INPs are membrane bound, which is previously reported in §2.2.3, to enhance functionality of INPs in comparison to soluble INPs. Furthermore, recovery of these vesicles carrying INPs from cell cultures, will allow to avoid the use of GMOs and the accompanying risks, legislation and negative public perception. To this end, the natural vesicle system of *Erwinia herbicola* will be investigated (Phelps et al., 1986). Here, we opt to use *E. coli* as a production host as *E. herbicola* is a plant pathogen and few molecular techniques are described. More specifically, first the pathway for forming vesicles from *E. herbicola* will be introduced in *E. coli* first. Next, the candidate INPs will be targeted to the outer membrane of the vesicles by fusing them to the N-terminal transmembrane anchoring domain of the INP from *E. herbicola*. In addition, the optimal conditions (media, temperature induction time/level, etc.) will be investigated and optimised to increase type I nuclei expression in view of more efficient cloud seeding induction.

Finally, a downstream process to recover the vesicles from the *E. coli* culture will be developed and optimised. As these vesicles differ from the *E. coli* cell size, (ultra)centrifugation and filtration steps will be applicated. Additionally, based on the hydrophobic character of the vesicles, purification through phase separation will be used.

3.2.2 Evaluation of cloud seeding agent (WP2)

WP2 of the Vsycle project will search for methods to test the produced vesicles on their efficiency and capability to induce rainfall. To do this, first the ice nucleation activity will be tested on supercooled water. After this, the vesicles will be evaluated by testing them in artificial clouds in controlled chambers.

The ice nucleation activity efficiency of INPs is generally tested by looking at the lowest sub-zero temperature at which supercooled water starts to freeze. Practically, this is done through a droplet-freezing assay (Ling et al., 2018). In this assay ice nuclei are added to a number of water droplets and by repeating the test with a series of temperatures and looking at which temperature most droplets freeze, the freezing temperature of the droplets with ice nuclei is determined. Since this method is well described in literature and easy to perform, it will be used to test the efficiency of the ice nucleation activity of the vesicles. Other than different temperatures, alternating concentrations of vesicles will further be tested to see if this has an effect. As a positive control for these tests, nuclei that are known to increase the freezing temperature of supercooled water will be used. Options include known cloud seeding agents such as AgI, but also whole *P. syringae* cells expressing INPs, as both options have been proven to be as effective as ice nuclei (Han et al., 2017; Marcolli et al., 2016). For the negative control, *E. coli* cells without the gene for INPs or a gene with a stop codon, causing incompletes INPs, will be used as these are not fit as ice nuclei.

In a later stage the goal is to test the effect of the vesicles as cloud seeding agents. In order to explore this, a cloud will be made in a controlled isothermal chamber and the effects of adding the agents will be observed. Such chambers have already been used successfully in the past to test *P. syringae* cells on their effect of forming ice nuclei in clouds (Ward and DeMott, 1989). This was done by the company Snomax, that uses this bacteria as ice nuclei to make snow for snow machines. One reason for doing these tests in chambers instead of real clouds, is that it is not allowed to release a untested substance into the environment. Another reason is that it is very difficult to prove that a cloud seeding agent actually induces rain, as the process is dependant on a multiple of different parameters, including temperature, humidity and the type of cloud. By choosing to test in a controlled chamber, these parameters can be chosen freely and make the results much more informative.

Snowy Hydro, a company in Australia, uses chest freezers (see Figure 3.3) for their cloud seeding program (Chubb, 2020). A supercooled cloud can be made by blowing gently into a hole in the cover, and this is seeded using dry ice (CO_2) fragments dropped into the ’cloud’. The dry ice is so cold that it freezes water droplets as it falls through the cloud, and these are clearly visible thanks to the back-lighting.

Figure 3.3: Chest freezer from Snowy Hydro (Chubb, 2020)

3.2.3 Dispersion of cloud seeding agent (WP3)

WP3 will focus on how the produced cloud seeding agent will be dispersed in actual clouds. Options to do this are the use of drones or airplanes, as these are dispersion methods that are being used nowadays in dispersing chemical cloud seeding agents. Also cannons that shoot the agent in the clouds are an option. Further research is needed to find out if these methods can be used for biological particles and eventual alternatives as well.

3.2.4 Legislation and ethics (WP4)

In WP4 the impact of the novel cloud seeding agent and how they would be perceived by the general public will be evaluated. The legal aspects will be reviewed as well.

A question that could be asked for example, is whether the novel cloud seeding agent and cloud seeding in general will have a meaningful impact on the problems it could theoretically solve. Another issue could be that when you induce rain in a certain place, it will not rain in another place whereas under normal conditions, this would have happened. Therefore, farmers could ‘steal’ each other’s rain. This also raises the question whether cloud seeding could be ‘weaponised’ to let it rain in specific places where it could possibly have disastrous consequences. To find the answers to these questions and to gather solutions, we will contact Massimiliano Simons, postdoc at the department of philosophy and moral sciences at UGent. He will be able to help us with selecting and setting up the right methods to find these solutions. Finally, a survey regarding the public opinion around cloud seeding will be held. Additional information about cloud seeding is planned to be given to the public through media such as the news and newspapers, this to get rid of the negative connotations of cloud seeding.

CHAPTER 4

CONCLUSION

In the first part of our bachelor dissertation, we evaluated possible improvements on the Dewpal for water capture from the atmosphere. The idea of using an underground pipeline was feasible to collect sufficient amount of water, but the calculations proved that it needed large amounts of energy. The fact that the pipe had to reach a depth of at least 6 meters, made the setup more expensive and not portable. Even if some problems were solved, using already existing techniques like nanorods or MOFs, the Dewpal 2.0 would add little to no added value to the already existing devices.

All of these solutions or devices do not directly solve the global water shortage problems, because for example farmers need a lot of water to produce food, but it will definitely be an improvement for the people in countries where there is a lack of potable water. If the techniques are made on a portable device, citizens close to a desert area can take these with them if they have to travel through these regions for instance. An interesting research would be to use all of the positive aspects of the existing techniques and bring them into one efficient device.

Secondly, we investigated the collection of water through precipitation induced by biotechnical solutions. Inducing rain does not mean that there is more rainfall in general, but it leads to rain at the right moment so no atmospheric water gets lost. There are several countries like India and China that are using cloud seeding not only to protect their crop fields, but also to collect atmospheric water because it is easier to purificate than salt sea water or contaminated water. Cloud seeding can even help to prevent rainfall during important events when rain is not desired. To this end, we have developed a project proposal called Vsycle.

Scientists still need to do a lot of research on the actual positive effects of cloud seeding, but nevertheless the biodegradable inducers are an improvement on the chemicals that are used nowadays. The concept of making vesicles with the desired proteins could be used in other scientific fields to by-pass the legislation of GMOs especially in Europe.

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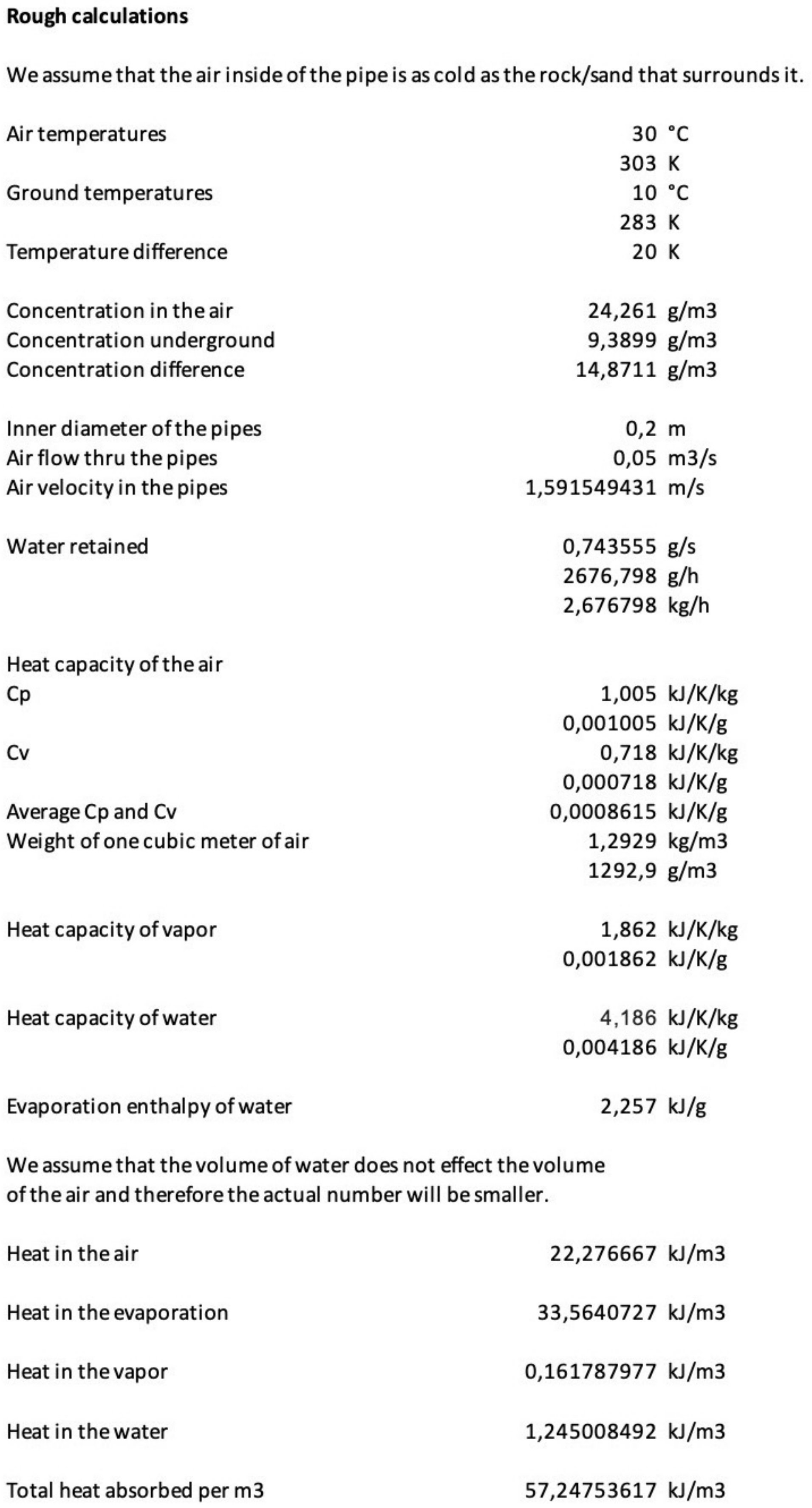
APPENDIX A

CALCULATIONS DEWPAL 2.0



Rough calculations

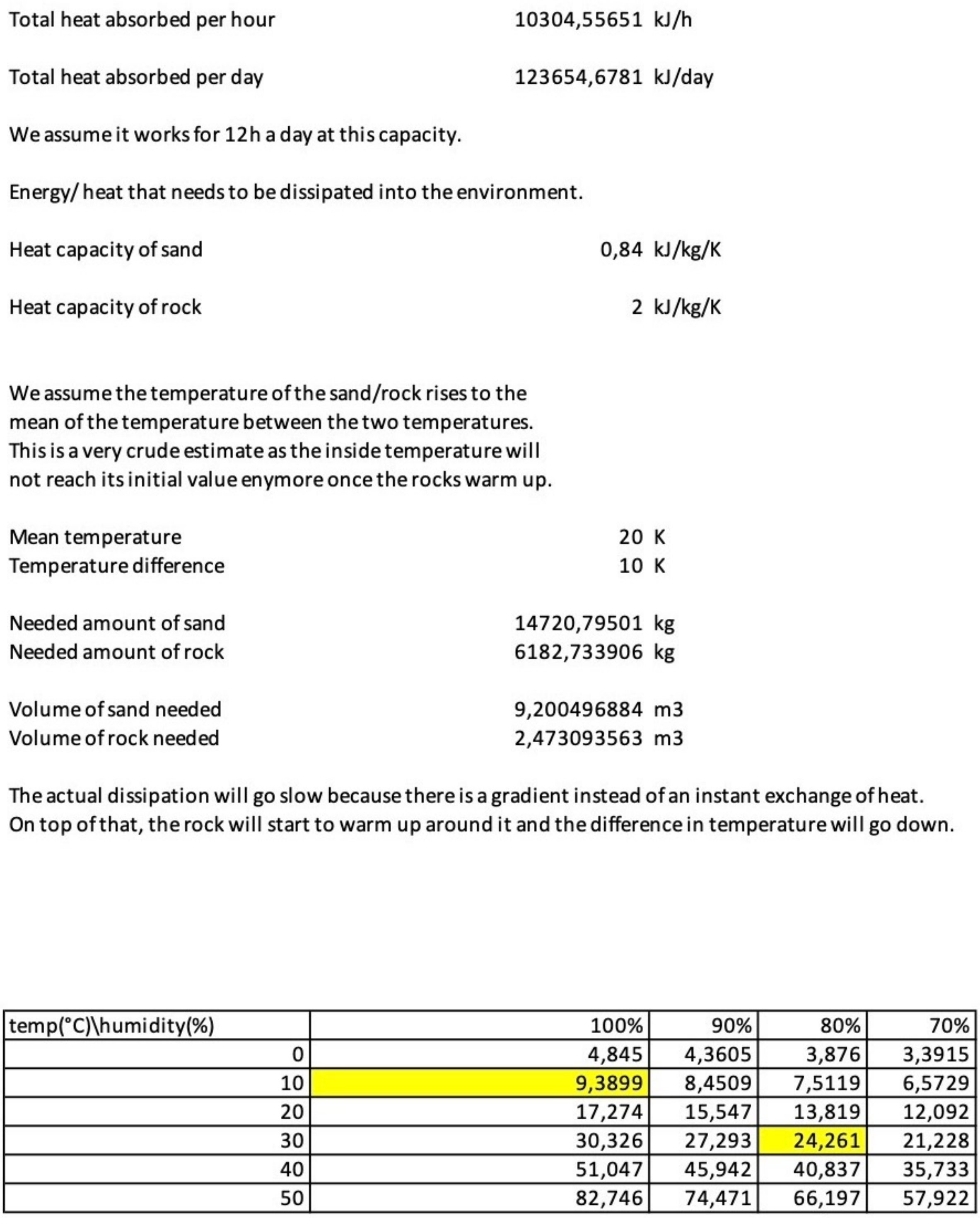
We assume that the air inside of the pipe is as cold as the rock/sand that surrounds it.

Air temperatures	30	°C
	303	K
Ground temperatures	10	°C
	283	K
Temperature difference	20	K
Concentration in the air	24,261	g/m3
Concentration underground	9,3899	g/m3
Concentration difference	14,8711	g/m3
Inner diameter of the pipes	0,2	m
Air flow thru the pipes	0,05	m3/s
Air velocity in the pipes	1,591549431	m/s
Water retained	0,743555	g/s
	2676,798	g/h
	2,676798	kg/h
Heat capacity of the air		
Cp	1,005	kJ/K/kg
	0,001005	kJ/K/g
Cv	0,718	kJ/K/kg
	0,000718	kJ/K/g
Average Cp and Cv	0,0008615	kJ/K/g
Weight of one cubic meter of air	1,2929	kg/m3
	1292,9	g/m3
Heat capacity of vapor	1,862	kJ/K/kg
	0,001862	kJ/K/g
Heat capacity of water	4,186	kJ/K/kg
	0,004186	kJ/K/g
Evaporation enthalpy of water	2,257	kJ/g

We assume that the volume of water does not effect the volume
of the air and therefore the actual number will be smaller.

Heat in the air	22,276667	kJ/m3
Heat in the evaporation	33,5640727	kJ/m3
Heat in the vapor	0,161787977	kJ/m3
Heat in the water	1,245008492	kJ/m3
Total heat absorbed per m3	57,24753617	kJ/m3

Figure A.1: Rough calculations Dewpal 2.0



Total heat absorbed per hour	10304,55651	kJ/h
Total heat absorbed per day	123654,6781	kJ/day

We assume it works for 12h a day at this capacity.

Energy/ heat that needs to be dissipated into the environment.

Heat capacity of sand	0,84	kJ/kg/K
Heat capacity of rock	2	kJ/kg/K

We assume the temperature of the sand/rock rises to the
mean of the temperature between the two temperatures.
This is a very crude estimate as the inside temperature will
not reach its initial value enymore once the rocks warm up.

Mean temperature	20	K
Temperature difference	10	K
Needed amount of sand	14720,79501	kg
Needed amount of rock	6182,733906	kg
Volume of sand needed	9,200496884	m3
Volume of rock needed	2,473093563	m3

The actual dissipation will go slow because there is a gradient instead of an instant exchange of heat.
On top of that, the rock will start to warm up around it and the difference in temperature will go down.

temp(°C)\humidity(%)	100%	90%	80%	70%
0	4,845	4,3605	3,876	3,3915
10	9,3899	8,4509	7,5119	6,5729
20	17,274	15,547	13,819	12,092
30	30,326	27,293	24,261	21,228
40	51,047	45,942	40,837	35,733
50	82,746	74,471	66,197	57,922

Figure A.1: Rough calculations Dewpal 2.0 (continued)