

# iGEM Vcycle – Business plan

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iGEM TEAM UGENT 2019-2020

Vcycle

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# 1 INTRODUCTION

WATER. The basis for all life on earth. A vital resource without which nothing can survive, provided all around the globe by rivers, lakes and rain. But not always provided in sufficient amounts. Water scarcity is an increasingly important problem everywhere on our planet. Because of the large impact water has on human lives and the increasing urgency for solutions, scientists have been actively searching for low-cost solutions both for developing countries and the western world. Although some solutions have been found, individually none can solve this immense problem on its own. According to Panel et al. (2011), by 2030 almost half of the world's population will suffer severe water scarcity if no action is taken. 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.

In a paper of 1998, it was stated 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. Farmers cannot grow anything without water and both the growing population and the ongoing climate change contribute to water scarcity. The main source of water for farmers, or in fact their crops, comes from the rain. More rainfall in the right amount could help to solve this problem. Besides this, control over where and how much it rains could be advantageous to solving numerous other climate change caused problems such as preventing crop damage due to storms and hail, extinguishing of forest fires and preventing floods.

Methods to do this already exist, known as cloud seeding. This is a technique where certain particles are dispersed into clouds and induce rainfall. This works because for rainfall to be possible, large aggregates of water molecules are needed, ice crystals being an example of this. These ice crystals, once they are heavy enough, fall out of the clouds and melt as the air gets warmer going to the ground. This results in rain droplets. Therefore, the formation of ice is an essential phenomenon for rain formation. However, ice crystals are not formed easily out of pure water, as this would happen at temperatures of  $-40^{\circ}\text{C}$ . For ice crystals to form at relatively high temperatures, the water molecules need attachment points, the so-called ice nuclei. The particles used in cloud seeding act as such ice nuclei. Today the most used cloud seeding agent is silver iodide (AgI). AgI has a crystal structure very similar to that of an ice lattice which makes it very easy for it to attract more water molecules. Even though AgI is able to induce rain, silver compounds are as toxic as lead both for the environment and for humans. Using AgI as a cloud seeding agent would be a serious risk for the environments where cloud seeding is used if it were used more regularly and intensively. This prevents the use of cloud seeding on a large scale, both in residential and rural regions.

If a non-toxic alternative were discovered, cloud seeding could be used by farmers to prevent heavy hail and rain storms from destroying their crops. Governments and private entities could protect buildings, cars and machinery from the same risks and avoid billions of euros of damage combined. Next to hail suppression, cloud seeding can be used to enhance or reduce rain. This could be useful when farmers need some rain in a dry season or when infrastructure has to be protected from superfluous rainfall. On top of that, fog and low clouds can be dispersed in an environmentally friendly way. This is extremely useful at airports where improved visibility is vital on foggy days as there are so many in places like the UK and Belgium. Last, but most definitely not least, the amount of natural snowfall can be increased to supplement and sustain fresh water reserves or create the ideal conditions for winter sports. Because of climate change, many rivers and canals in Europe and the US contain less and less water especially in the dry seasons, which are becoming more and more ubiquitous. Unfortunately, many of these rivers are vital for industry and trade. Last year the water levels in the Rhine, Germany's most important river for in- and export, got so low in some areas that ships could not pass and transport was blocked. This did not just cost a lot of money for many businesses, but caused more road traffic, increasing emissions and worsening climate change even more. The largest cause of the lack of water is the shrinking glacier at the source of the Rhine. This could have been prevented if cloud seeding was used to create more snowfall at the source of these types of rivers to ensure transport. The largest most important upside a non-toxic cloud seeding agent would have, would be the scale and variety of problems it could solve.

Our team at the UGhent university decided to tackle the problem and meanwhile participate in the iGEM (International Genetically Engineered Machine) competition, a competition that challenges teams from all over the world to find solutions for all kinds of problems with the help of synthetic biology. Eventually, each team has to present their project for an international jury at the iGEM Jamboree, uniting all the teams in one giant convention. iGEM is divided in multiple tracks, a kind of classification to categorise the different projects into multiple subjects. One of these is the environmental track. Finding a biotechnological solution to solve water scarcity and more specifically making cloud seeding more sustainable, perfectly fits in this track.

The idea for our project, named Vcycle (see logo in Figure 1), is to design alternative ice nuclei that are biological, nontoxic and biodegradable. This with the help of proteins expressed on the outer membrane of several bacterial species, ice nucleation proteins (INPs). The proteins make sure these bacteria can act as nuclei for the formation of ice crystals. This can happen at a higher temperature and therefore with a higher efficiency, than normally possible when non-biological particles such as dirt for example act as nuclei. These bacteria use this as a way to acquire nutrients from plant cells by damaging them through the formation of ice crystals. Besides that, they also use them as a dispersal method to reach new hosts by inducing rain when they get in clouds (Morris et al., 2004). The INP produced by *Pseudomonas syringae* (InaZ), the most efficient and studied one (Han et al., 2017), was also used by our predecessors of the UGent university in 2016. The goal of their project, the Dewpal, was to design an optimized 3D shape for condensing water, using a fusion protein of INP and streptavidin to increase water collection. In their experiments, they were able to prove the ice nucleation efficiency of INP both as a soluble protein and bound to the outer membrane of *E. coli* cells. For these reasons, the Vcycle agent also uses this specific INP.



*Figure 1: Logo of project Vcycle.*

To transform bacteria expressing INPs on their membrane into an actual cloud seeding product, the idea is to make *E. coli* cells express the INPs of *Pseudomonas syringae* on their membrane. This instead of pure protein because it has been proven that INPs bound to membranes can achieve better results as ice nuclei (Schmid et al., 1997). The INPs are targeted to the outer membrane of the bacteria by the N-terminal transmembrane anchoring domain of INP.

However, as these cells would be living GMOs containing synthetically engineered DNA and would therefore suffer from the negative perception and legislation of GMOs, the principle of bacterial ghosts (BG) will be used on them. BG are bacteria containing a plasmid coding for a gene that makes the cells lysate themselves, causing them to expel all of their cytoplasmic content. The result is an empty bacterial membrane, still containing the proteins bound to it (Kassmannhuber et al., 2017). In this specific case, this means the cloud seeding agent contains empty *E. coli* cells with the INPs still on their surface. To obtain the final product, these cells go through a separation process and a lyophilisation. This because the used dispersion mechanism, pneumatic flares, requires a powder. We use this specific mechanism instead of the conventionally used heat-based mechanisms, as these would destroy both the cells and the proteins.

## **2 WHO ARE WE: THE TEAM & ITS ORGANISATION**

### **2.1 Structure of the team**

Project Vcycle consists of six members, all with a biological engineering and biotechnology background. The chairwoman, Laure Craeye, led the way for the entire group. The team was divided into different modules to work in: module engineering, cloud, business and ethics, dispersion and legislation. Due to the COVID-19 crisis, all the work and communication was performed via online meetings and tools. Weekly meetings to discuss the progress in every module were planned. Moreover, the people who worked on a certain module, arranged small meetings amongst themselves. Every two weeks, meetings with the other iGEM UGent team and the supervisors Marjan De Mey and Wim Van Criekinge as well as some PhD students, were scheduled to receive feedback and suggestions for the project.

## 2.2 Future organisation

The market in which Vcycle would position itself is very new. Therefore, it is quite unclear how the use of the product could be implemented. To gain insight in how to do this, we sent questionnaires to select groups of people. As the ethical questions in these questionnaires are also about in-depth scientifically discussions such as geo-engineering, we decided to not give the survey to just everyone. It is important to take prior knowledge to a subject before an objective statement can be made. Therefore, the surveys with the most in-depth ethical questions were only sent to experts such as professors at the university, philosophers, civil servants, climate experts, etc. To a lesser extent, we asked farmers about the ethics of the use of the cloud seeding technology.

Since like every scientific technology cloud seeding can be abused. One of the questions in the survey was: "who should manage the access of the technology?" We concluded that the farmers would not make cloud seeding a government-controlled activity: 50% of farmers would rather give control over the technology to meteorological experts, while the other ones would let cloud seeding performing companies, possibly together with experts, manage the accessibility. However, experts preferred the technology to be regulated by the government or an agency that is controlled by the government.

A question 100% of experts and farmers positively agreed on was if every act of cloud seeding should be registered. This not only to control the use of the technique or to prevent abuse, but also because this can be a way to evaluate the effect of using cloud seeding on the weather. Making registration obligatory could be a way to prevent political abuse of cloud seeding and other techniques (war, blackmail, new form of power, etc.). This abuse is something we as a team but also the experts and farmers fear for. Especially if the ability to perform cloud seeding would become a new form of power, it could lead to a new form of inequality as well. This is a common concern, as the surveys show. The use of such techniques can lead to more inequality amongst countries. However, the experts and our team are convinced that global regulation as well as global laws could reduce this problem to a certain extent.

Using these opinions and insights, we came up with a way to propose an organisation structure in case this technique becomes available in the future. The production and sale of the cloud seeding agent should be regulated by a private company. This private company should in its turn be controlled by an international committee, where all countries can be part of. If an individual or another company wants to use cloud seeding, they should file in an application form. This form should be evaluated by a group of national experts, who are chosen and paid by the government. These experts should be looking into the actual situation regarding weather conditions and/or damage caused by extreme weather conditions.

When one country is in conflict with another, it is possible that the cloud seeding technology can be abused to dry out each other. Since the use of cloud seeding for political purposes is something we surely want to avoid, we propose to have an international group of experts as well, that are under surveillance of higher authorities like the above proposed international committee, the EU and the UN. This group will evaluate the request of a nation to undertake a cloud seeding action. Ideally groups of experts should consist of people like experts in geo-politics, economics, climate experts

and meteorologists. We feel that a democratic approach and a frequent re-election of those experts would be the way to go.

If the application form is accepted by the committee, a certification will be given to the customer nation which will be required to order the needed amount of cloud seeding agent by the private company. If a country will perform cloud seeding activities, its experts have to inform the government which will in its turn inform the people by releasing press messages.

Also it would be obligatory to have an insurance that covers any damage a cloud seeding activity may inflict on third parties. Another proposition is that all users of the cloud seeding technology follow a mandatory course that learns them about the possible consequences and dangers.

### **3 MISSION & VISION**

#### **3.1 Mission**

Water scarcity is one of the many consequences of climate change. In addition, water scarcity itself is a cause for many other drought related problems: failed harvests, starvation, diseases, etc. On the other hand, climate change results in more extreme weather conditions and floods which are destroying a lot of people's lives. In the past decennia, more and more climate organisations rose up and wanted their voice to be heard. They asked the politicians to do something before it is too late. It does not seem like something is changing. If there is the scientific knowledge to undertake action, it should be used.

Project Vcycle wants to work on a solution. By making biological particles to induce rain, the project wants to anticipate on any other problems due to drought in the future. As mentioned before, the process of inducing rain is called cloud seeding. This is already done the past by the use of chemicals such as the well-known silver iodide. By providing a biological, nontoxic and biodegradable alternative for these chemicals, Vcycle wants to work on a sustainable future and planet. The project wants to help people by helping the planet. The Vcycle product and the cloud seeding technology aim to protect nature, biodiversity and every single organism from any drought or water scarcity issues.

#### **3.2 Vision**

Climate change has an enormous impact on the water supplies (Wang et al., 2018). That is problematic since every single organism, plant or animal needs water to survive. Water scarcity is becoming a bigger problem that already affects a lot of countries and thus affects a lot of humans, animals and other organisms. With this project, that might change. By stimulating the water cycle and the formation of rain, water resources can be provided. Vcycle wants to create a world, where every organism has the chance to grow and live. This project also wants to guarantee the possibility for the current and future generations to live. This by controlling the weather to protect people from extreme weather conditions. As such, we are taking a step in the right direction.

This means water for everyone, for every plant, for every animal and for every human being. Just by using our product, Vcycle, which can provide eco rain on demand.

### **3.3 Values**

Respect and taking care for the beautiful planet we have, is the baseline for this project. Climate change, which is a consequence of human activities, is threatening us more than ever before. Therefore, we want to help in making our planet better by controlling the weather. And as we want our product to be ecological, we made a biological, non-toxic and biodegradable product that can be used as cloud seeding agent. This to replace harmful toxic chemicals that are used these days. As every scientific project, the cloud seeding technique could be abused for political reasons. Since respect for our fellow human beings is of prior importance, we propose a structural approach on as both the national and international level in case this technique actually becomes available in the future.



## 4 BUSINESS MODEL CANVAS (BMC)

<p><b>Key Partners</b></p> <ul style="list-style-type: none"> <li>• Sequencing companies</li> <li>• Transport firms</li> </ul>	<p><b>Key Activities</b></p> <ul style="list-style-type: none"> <li>• Research and development</li> <li>• Fermentation</li> <li>• Quality control</li> <li>• Packaging</li> </ul>	<p><b>Value Propositions</b></p> <ul style="list-style-type: none"> <li>• Possible solution for long-term droughts</li> <li>• Minimising storm damage</li> <li>• Non-toxic alternative</li> <li>• Non-GMO</li> </ul>	<p><b>Customer Relationships</b></p> <ul style="list-style-type: none"> <li>• Information through social media</li> <li>• Education about GMOs and the product</li> <li>• Public cooperation with weather and climate experts</li> </ul>	<p><b>Customer Segments</b></p> <ul style="list-style-type: none"> <li>• Farmers</li> <li>• All individuals living in the region where the cloud seeding is performed</li> </ul>
	<p><b>Key Resources</b></p> <ul style="list-style-type: none"> <li>• Human <ul style="list-style-type: none"> <li>○ Team of experts for assessing damage</li> <li>○ Company employees</li> </ul> </li> <li>• Financial <ul style="list-style-type: none"> <li>○ Taxes</li> </ul> </li> <li>• Physical <ul style="list-style-type: none"> <li>○ Buildings and equipment</li> </ul> </li> </ul>		<p><b>Channels</b></p> <ul style="list-style-type: none"> <li>• Social media</li> <li>• Website</li> <li>• Evaluation by team of experts</li> <li>• Agreements in paper format</li> </ul>	
<p><b>Cost Structure</b></p> <ul style="list-style-type: none"> <li>• Estimation of all products and materials that would contribute to the total variable costs: nucleid acid sequences, enzymes, testkits, cell cultures, chemicals, lab materials &amp; packaging material</li> <li>• Estimation of all aspects that would contribute to the fixed costs: formation expenses, infrastructure (establishment, laboratory equipment), assurance and manpower costs</li> </ul>			<p><b>Revenue Streams</b></p> <ul style="list-style-type: none"> <li>• Revenue from selling the product to government of a country</li> </ul>	

## 4.1 Creating Value

### 4.1.1 Value propositions

Climate change is an actual problem, a problem that strikes each of us. But climate change as such is one thing, the extreme weather conditions as a consequence of it are another. Every day, there are newspapers and -releases telling us how much damage another storm or another period of drought is causing. Even here in Belgium, summers are getting hotter and dryer while snow is remaining out during winter. The temperatures are rising, it is clear.

The extreme weather conditions as a consequence of climate change and drought in particular, are the focus of this project. Cloud seeding is a method that can control precipitation type and precipitation levels. This means by influencing weather, more extreme conditions can be avoided. In fact, '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 this project, a narrower 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 (CCN) 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).

IN's are thus essential in the formation of rain. The concept of cloud seeding therefore exists of bringing more IN's in clouds to enhance rain formation. Cloud seeding is already done by the use of chemicals such as the toxic silver iodide. To date, cloud seeding is not a commonly used technique, but if it would be, the use of chemicals on a larger scale could be harmful to the environment. With the prospective that cloud seeding could be a possible solution to long-term droughts, the use of chemicals on a larger scale should be avoided. Therefore, an alternative is needed and the design of this biological agent was the aim of project Vcycle.

Ice nucleation proteins (INPs) of *Pseudomonas syringae* are well known and the bacteria is the most studied INP producing organism. These proteins are responsible for the formation of ice crystals at higher temperatures in comparison to dirt particles acting as nuclei for instance. Therefore, this protein forms a good basic line to produce a new cloud seeding agent. Expressing this protein on the membrane of *E. coli* cells, followed by induced leaking of the cells results in the Vcycle agent. Making the cells to leak, which is according to the bacterial ghost principle, is a way to make sure no GMO material is used. This is done to anticipate on legislation issues regarding GMOs in countries and to make sure this product is really available for every country regardless from contemporary laws. As a team and as inventors of this product, it must be guaranteed that the technique is available for everyone and that it will only be used for scientific and ethical correct purposes. This project aims to help people in countries suffering from drought and water scarcity

on one hand and to protect people who are affected by floods or heavy hailstorms on the other hand.

#### **4.1.2 Customer segments**

The aim of project Vcycle is to help people in countries suffering from extreme droughts or extreme storms. Although the goal of this project is to help the whole world, a main category of stakeholders is identified. This category includes people working in the agricultural sector. Drought, water scarcity and extreme climate conditions have disadvantages for everyone. But it is certainly a problem that particularly affects the farmers, who are also responsible for our food supply. During prolonged droughts with no precipitation, crops often need extra irrigation. On the one hand this means that the harvest is threatened by the drought and if it is irrigated, it also means that extra water is extracted from rivers and canals, which further contributes to the drought. On the other hand, there are the more extreme hail storms or floods that cause crop damage. These problems have direct effects for farmers and their harvests, but also indirectly for us. This would not mean that every farmer can “order” rain if his or her harvest is suffering from drought or hailstorms. The technique should only be used on a larger scale.

#### **4.1.3 Customer relationships**

Using scientific knowledge and expertise to solve world problems through synthetic biology is a nice concept. However, accomplishing such a project is not easy if it is not supported by the general public. The discussions about whether or not GMOs are ethically acceptable and the use of synthetic biology in daily life are highly topical. Scientists, politicians, companies, climate activists as well as private individuals take part in these discussions.

If a company is making a product, one has to make sure there are customers who actually need that product and want to buy it. Even though the Vcycle agent is not a standard product that will be sold in a store, knowing what our customers think and want is important. What may seem like a good solution for drought and water scarcity to us, may rather raise questions to others.

Therefore, it is very important to let people know who we are and what this project is about. Besides that, they have to be informed very well about this product and for what purposes it can be used. To do so, social media is nowadays one of the most important channels to reach customers. Social media is not a common channel used by companies. Although, it is better to do so especially for this type of product. Influencing the weather is something that has consequences for everyone which means that even if the product will not be accessible for every human, it is important that all individuals are informed regarding what is done to help avoid extreme weather conditions. It is important to give access to product and technique information for everyone. Another way to provide this information is by organizing lectures, webinars, school lessons, etc. where information regarding the use of this product, the influence on the weather and possible consequences is explained.

As mentioned before, the use of GMOs is not widely accepted. To anticipate on this and to have a bigger chance to win confidence and interest in the product, the production of the agent is based on the bacterial ghost principle which means no GMOs are used. Besides that, customers do have to trust the product and have confidence in the inventors who made and designed the agent. From the company's side, it is important to give that trust to the customers. This is done by setting up a cooperation with a team of experts in weather and climate and by showing this cooperation to the outside world. Gaining trust from private individuals is not done by making this product commercial, but by ensuring that this actually works, that it is safe to use and that it will be used only if necessary, i.e. when countries are suffering from extreme weather conditions.

It is clear that there is only potential for this product to be used in future if every party from scientists, politicians, industrial companies to private individuals are informed well. The relationship between the responsible company and other parties is the most essential thing for the product to come alive.

## **4.2 Delivering value**

### **4.2.1 Channels**

Trust in the product and the responsible company is the most important requirement to make product use realistic and possible. As mentioned before, all classes of people have to be informed about the product: what it is, how it works and when it will be used. This can be done by just using channels which are rapidly available: social media. In addition, a website which gives a detailed overview regarding product properties and reasons for use, is recommended.

For the company who produces the Vcycle agent and exports it, it is important to be accessible to countries across the world as this project has to be accessible to everyone. In fact, this means that government of countries who are planning to use cloud seeding, should assign a group of experts who can be in direct contact with the responsible company. Communication can be done by mail. As the production and use of this product has immediate consequences for all private individuals, decisions cannot be made just by phone. A team of experts of a certain country has to decide how necessary the use of this technique is. This means that it is important to make an evaluation regarding the state of the country: how hard is the country suffering from extreme weather conditions due to climate change the past weeks or months? If the decision is taken that the use of cloud seeding will be used, this has to be discussed first with the international expert team. Then, they have to give permission whether or not a country or individual gets a certificate, which is needed to order the cloud seeding agent. It is important that every decision and agreement is captured in paper format and no agreements are just made by phone. After giving the permission, the experts have to inform the national government who in turn has to inform the nation by press releases.

## **4.2.2 Key resources**

### **4.2.2.1 Human resources**

As mentioned before, it is important to have the access to the use of the technique strictly regulated. Therefore, a team of experts per country is required. Their responsibility includes assessing the stress from which a country is suffering and possible damage that can occur as a result of a prolonged stress situation. The results from this assessment have to be written down in a report. Besides that, every national team has to be evaluated and certificated by an international team. This international team has to reassess the report made by any national team. If a report makes clear that cloud seeding could definitely be a solution to stop suffering from extreme weather conditions and all criteria are met to be allowed to use the technique, the international experts have to assign an official certificate to the countries. Only with this certificate, countries are able to order cloud seeding agent and to perform the cloud seeding technique.

Moreover, human resources includes the people who are working in the company itself. This means a management team, employees, lab researchers – and workers, fermentation specialists as well as plant workers.

### **4.2.2.2 Financial resources**

As the frequency of use of this product is estimated low (can only be performed when really necessary), one company should be responsible for the production and export of this Vcycle product. This company should have two establishments which can be built up by using the start-up capital whereof every country, that is a member of the earlier mentioned committee, has to pay its part. If a country will perform the cloud seeding technique after it obtained its certificate, this country has to pay the ordered product.

### **4.2.2.3 Physical resources**

As mentioned above, with the start-up capital, two establishments can be set up. These establishments will be provided with all the necessary equipment to produce the Vcycle product. This includes labs to design and improve the production organism, fermentation reactors, -media, lyophilization equipment and control equipment as pH gauges, spectrometers and plating materials as well as antibiotics and packaging materials. Moreover, offices for company employees have to be provided as well.

## **4.2.3 Key Activities**

As it is almost impossible and cost inefficient for a company to perform all tasks, a company has to focus on one or more specific activities. For a Vcycle producing company, the key activity should be the design and production of lyophilized, ice nucleating bacterial ghosts. With this project, the design of the Vcycles is already provided. Nevertheless, improving the production organism can often lead to higher

production rates or less byproducts. Therefore, the company has to have an own specialized lab where research is done regarding the improvement of the efficiency of the production and downstream processing.

To produce the actual cloud seeding agent, fermentations have to be performed. To make sure the bacteria are growing well and high production rates are obtained, a lot of parameters have to be controlled continuously during fermentation: temperature, pH, dissolved oxygen, stirring rate and aeration level. Besides that, optical density (OD) as well as colony forming units (CFU) have to be followed up to control the bacterial growth within the fermentation vessel. It is obvious that these actions are also done by the producing company. Moreover, the lab can also be responsible to check the E-lysis and ice nucleation efficiency on a regular basis. Finally, packaging of the product is also required and should be done by the company itself.

#### **4.2.4 Key Partnerships**

However, it is important that the condition of the production organism is checked on a regular basis as mutations can occur. Therefore, it is recommended to sequence the bacterial construct frequently. This can be done by external companies which have specialized high-throughput equipment for this. As such, the company does not have to provide this equipment and can therefore completely focus on the production and optimization of the Vcycle product.

After designing, producing and packaging the product, it has to be exported to different countries as only two establishments will be set up at the beginning. For this, the company can collaborate with external transport firms. There are no special requirements to transport the product as the bacterial cells will be lyophilized to form a dry powder, which make transport and export easy.

## 4.3 Valorising value

### 4.3.1 Cost structure

As mentioned above, two establishments should be set up at the start of this business. These companies are supervised by an international committee, whereof all countries can be a member. The countries representing this committee each pay a contribution to form the start-up capital. The start-up capital is used to finance the founding of the company.

A distinction can also be made between **fixed costs** and **variable costs**. **Variable costs** include costs such as the purchase of chemicals and substances to create media or buffers, enzymes, genes, primers, test kits, cell cultures and small materials to evaluate growth. Packaging material also belongs to this category of costs. These costs vary over time as they are tailored to the demand for the product. **The fixed costs** include infrastructure costs such as office and establishment costs. In addition, insurance costs (industrial accident insurance, fire insurance, etc.) as well as personnel costs belong to the fixed costs. A detailed overview is given in Table 2 in Appendix. As this project was a theoretical project, estimation of the real cost was not feasible. With this cost structure, the prior goal is to give future companies an idea which costs a start-up includes.

### 4.3.2 Revenue streams

The revenues for the Vcycle producing company will be gained from selling the agent. Due to the COVID-19 pandemic, we were not allowed to perform any lab work. This means that calculations of the actual costs and thus a determination of a possible price were not feasible. However, based on the experience of the iGEM UGent team in 2016, an estimation of the lab cost can be made. This includes the design, lab scale fermentation and lyophilisation cost of the cloud seeding agent and is estimated to be 5-15 €/kg. This indirectly gives an idea about what a possible price could be. If a quick comparison is made towards the price per kilogram for silver iodide, a price of 4440 €/kg can be found on the website from SigmaAldrich. This comparison shows that the purchase price of our cloud seeding agent can be advantageous to that of silver iodide. However, on an industrial scale it is possible that the cost can be much higher depending on the production volume. There is a company, Snomax, which produces a similar product that can act as ice nuclei that consists of lyophilised *Pseudomonas syringae* cells with ice nucleation proteins. One kilogram of this product costs 322,5 euros. It seems that our product would be cheaper. One reason why the price of our product can be lower is because of the rapid growth rate of *E. coli* cells in comparison to *Pseudomonas syringae* cells. This can lead to a higher productivity. In any case, it is important to keep in mind that this price is just an estimation and exact calculations have to be conducted to have a reliable view.

## 5 COMPETITOR ANALYSIS

### 5.1 SWOT

#### 5.1.1 Strengths

- S1. Not toxic and biodegradable
- S2. It is a new alternative that has not been used before in cloud seeding
- S3. *E. coli* is a fast-growing bacterium which leads to higher productivity
- S4. Bacteria are dead
- S5. Non-GMOs

#### 5.1.2 Weaknesses

- W1. There is no experience with INPs as a cloud seeding agent
- W2. Not much experience with cloud seeding
- W3. Difficult to prove the efficiency of cloud seeding
- W4. Usage is limited by the regional climate
- W5. High start-up cost because the production of the products is new

#### 5.1.3 Opportunities

- O1. Possible solution for water scarcity
- O2. Possible solution to prevent extreme weather conditions such as hailstorms, floods, etc.
- O3. Can be used internationally
- O4. Potentially more efficient in ice nucleation than AgI
- O5. Boost for the use of biotechnological applications

#### 5.1.4 Threats

- T1. AgI is the standard cloud seeding agent for the moment, might be difficult to replace it
- T2. Cloud seeding could be used for political purposes
- T3. People might not like the idea of 'throwing bacteria in the clouds'
- T4. People might think we are disturbing the natural ecosystem
- T5. Legislation around cloud seeding might be a limiting factor in many countries



## **5.2 SOR**

### **5.2.1 Orientation towards strengths**

Agl can be toxic for many organisms in high concentrations. Intensive use of cloud seeding could let Agl accumulate in the environment, this with disastrous consequences. This would be the case if cloud seeding would be used more frequently to help solving drought and other climate change related problems. The Vcycle product provides a non-toxic, biodegradable alternative for Agl by using INPs. INPs have not been used in cloud seeding before, making our product a new and unique addition to the cloud seeding market.

An advantage of the product in comparison to using *Pseudomonas syringae* as a carrier for INPs, is that *E. coli* can be grown much faster. This enables the product to be produced much faster thus leading to a higher productivity. In addition, by using the bacterial ghost principle there are no live bacteria or bacterial DNA present in the product, which ensures that no reproduction of the bacteria is possible. This also means that the resulting membranes are no longer perceived as GMOs, avoiding GMO related legislation issues.

### **5.2.2 Orientation towards weaknesses**

A problem with the use of the Vcycle cloud seeding agent is that there is no experience with using INP based agents. The results on rain formation are therefore difficult to predict. This is also an issue with cloud seeding in general, as it is often hard to proof that additional precipitation is due to the performed cloud seeding event. Experience in cloud seeding is also limited.

Limitations due to regional climates can be another disadvantage. This because cloud seeding methods using ice inducing particles, such as INP and Agl, are most efficient in environments with cold clouds. This makes using our agent unsuitable in regions with warm clouds.

Since this product is entirely new, a new production system would have to be set up. This would result in high start-up costs to make the product in the quantities needed for the use for cloud seeding.

### **5.2.3 Orientation towards opportunities**

Cloud seeding can be used as a potential remedy for several climate change related problems. Inducing rain in regions struck by prolonged droughts are one of the options. But by altering the type of precipitation and redirecting where and when the precipitation falls, natural disasters such as heavy hailstorms and floods could also be prevented. The technique could help to solve such problems all over the world. Our agent specifically could help even more in mediating these problems, since INPs are more efficient in the formation of ice crystals than their chemical counterparts.

The designing of the cloud seeding agent is possible through the use synthetic biology. By using the product for helping to solve an important world problem, we boost the reputation of the biotechnological sector.

#### **5.2.4 Orientation towards threats**

An important threat for the usage of an INP based cloud seeding agent, is the fact that AgI is the current product of choice. Additionally, the commonly used heat-based flares would need to be exchanged to an alternative suited for the dispersion of biological particles. Companies who practise cloud seeding might not be willing to change their products and dispersion devices, as this requires additional investments. These include new equipment such as fermentation tanks, freeze-drying machines, etc. and new flares.

The public opinion around cloud seeding can be another threat. Some people might not like the idea of cloud seeding as they think that it would disturb the natural ecosystem. Also the possibility to 'weaponize' cloud seeding to influence rainfall in ways that hinder other people, might cause some people to frown at its use. Additionally, bacteria often have a negative connotation with people who are not familiar with them. This might cause those people to believe that dispersing bacteria in clouds, even though they are dead, is a bad thing.

Finally, cloud seeding is not allowed in every country. This legislation might make it difficult to use it to its full potential.

#### **5.2.5 SOR strategy**

Based on the identified strengths, weaknesses, opportunities and threats in the SWOT analysis, a SOR strategy is made to evaluate the strategy the Vcycle producing companies should implement. See Appendix Table 1 for the SOR matrix.

#### **5.2.6 Marketingmix**

In the SOR analysis, the attack-strategy received the highest score. This means a company producing the Vcycle product should focus on using its strengths in order to succeed in taking its opportunities. In our marketing, it is important to focus on the fact that our product is an ecological, more environmentally friendly way of doing conventional cloud seeding. We should also stress that our product contains no GMOs or live bacteria. This to get trust and confidence from our customers as well as all the other people.

##### **5.2.6.1 Price**

Estimations of prices are difficult since this concept is not tested in the lab due to the COVID-19 pandemic. Table 1 in Appendix gives an overview of all estimated equipment that would contribute to the variable and fixed costs. Based on lab experience from the iGEM UGent 2016 team, a lab production cost of 5-15 €/kg is estimated. However, to have a reliable view on the cost, more precise calculations have to be conducted.

#### **5.2.6.2 Place**

As mentioned before, the start-up company should have two establishments. These companies will produce, optimize and package the Vcycle agent. Distribution of the product will be conducted by external firms.

#### **5.2.6.3 Product**

The Vcycle cloud seeding agent consists of *E. coli* cells with INPs bound on their outer membrane. These proteins assure that the cells are good ice nuclei and therefore are able to promote ice formation in the clouds they are dispersed in. This results in more rain formation in those clouds.

To ensure that the cells do not contain any recombinant DNA and do not have the ability to replicate themselves in the environment, the bacteria are designed to produce protein E. This protein makes pores in the membrane of the bacteria, through which the contents of the cells can be expelled, resulting in empty membranes with INPs attached to them.

To disperse the bacteria in clouds, pneumatic flares are used. This instead of the commonly used heat-based flares and ejection mechanisms because the heat from those would destroy the cells and proteins. The pneumatic flares are electronically ignited, but no burning of the active compound takes place: a pre-prepared powder is ejected by a gas and its chemical composition does not change. Since a powder is required, the empty cells are freeze dried.

#### **5.2.6.4 Promotion**

To promote our product, several channels can be used. Firstly, social media can be used to inform a wide range of possible clients of the possibilities of the Vcycle cloud seeding agents. Secondly, since farmers are an important group of clients, special announcements from a central organisation that controls agricultural practices should be sent out to farmers. Through this way farmers are informed about how cloud seeding with our biological agent can benefit the growth of their crops. Further advertising can also be done through governmental advertising campaigns. This also provides opportunities to reach a very wide range of people.

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## 7 APPENDIX

**Table 1:** SOR matrix

	Opportunities					Treats					Row sum
	O1	O2	O3	O4	O5	T1	T2	T3	T4	T5	
<b>Strengths</b>											
S1	1	1	2	0	2	2	0	1	0	1	10
S2	0	0	0	3	2	0	0	0	0	0	5
S3	1	1	0	2	3	3	0	0	0	0	9
S4	1	1	2	1	2	1	0	3	0	1	10
OS5	1	1	3	0	2	1	0	2	0	1	11
<b>Weaknesses</b>											
W1	1	1	0	1	0	2	0	1	0	0	6
W2	2	2	0	0	0	0	0	0	3	0	7
W3	2	2	0	0	0	0	0	0	3	0	7
W4	2	2	3	0	0	0	0	0	0	0	7
W5	1	1	2	1	1	3	0	0	0	0	9
Colom sum	12	12	12	8	12	12	0	7	6	3	
Attack	31										
Defence	16										
Clean ship	24										
Crisis	12										

**Table 2:** Overview of estimated variable and fixed costs.

<b>Variabele costs</b>	
<b>Nucleic acid sequences</b>	Ice nucleation gene Green fluorescent protein gene Bacterial ghost gene PCR primers with overhangs for CPEC PCR primers for colony PCR 1_BBa_K584000 backbone Molecular ladder for gel electrophoresis dNTPs Q5 DNA-polymerase
<b>Enzymes</b>	Restriction enzymes: EcoRI, XbaI, SpeI Ligation enzyme High fidelity DNA polymerase: PrimeSTAR HS, PrimeSTAR GXL (TaKaRa Bio) or Q5 (New England Biolabs) Taq DNA polymerase
<b>Testkits</b>	PCR purification kit
<b>Cell cultures</b>	<i>Escherichia coli</i> cells
<b>Chemicals</b>	Chloroamphenicol Agarose Tryptone Yeast extract NaCl Q5 buffer DMSO
<b>Lab materials</b>	Eppendorf tubes Petri dishes Drigalski scoops Ent needle Cuvets PCR tubes Pipettes Pipette tips
<b>Packaging material</b>	

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**Fixed costs**

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**Formation expenses****Infrastructure****Laboratory equipment**

Vortex  
Droplet freezer  
Centrifuge  
Electroporation device  
Gel electrophoresis device  
PCR machine  
Light microscopes  
Spectrofotometer + cuvetts  
Nanodrop device  
Warmwater baths  
Diafilters  
Tangential flow filter system  
Freezer  
-80°C Freezer  
Autoclave  
Laminar flows  
Fermentor  
pH gauges  
Thermometer  
Freeze dryer  
Packaging equipment

**Establishment**

Buildings  
Office supplies  
Telephone subscription  
Internet subscription  
Maintenance costs

**Assurance costs**

Fire insurance  
Accident at work (staff)

**Manpower costs**

Salaries  
PPE (Personal protective equipment): lab coats,  
safety goggles, gloves