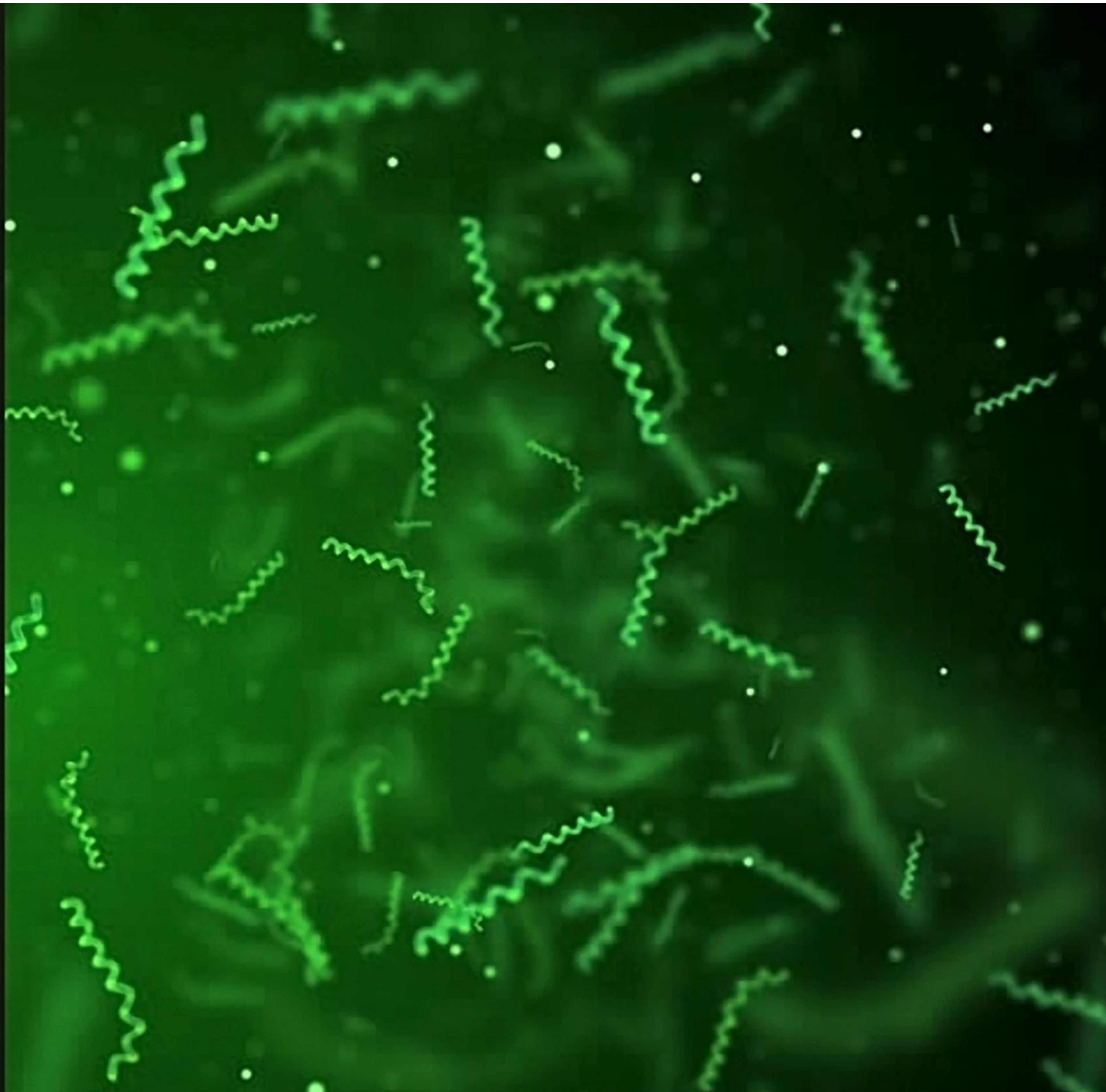
ALGAECULTURE



ALGAECULTURE

Microalgae as an aid for a better agriculture

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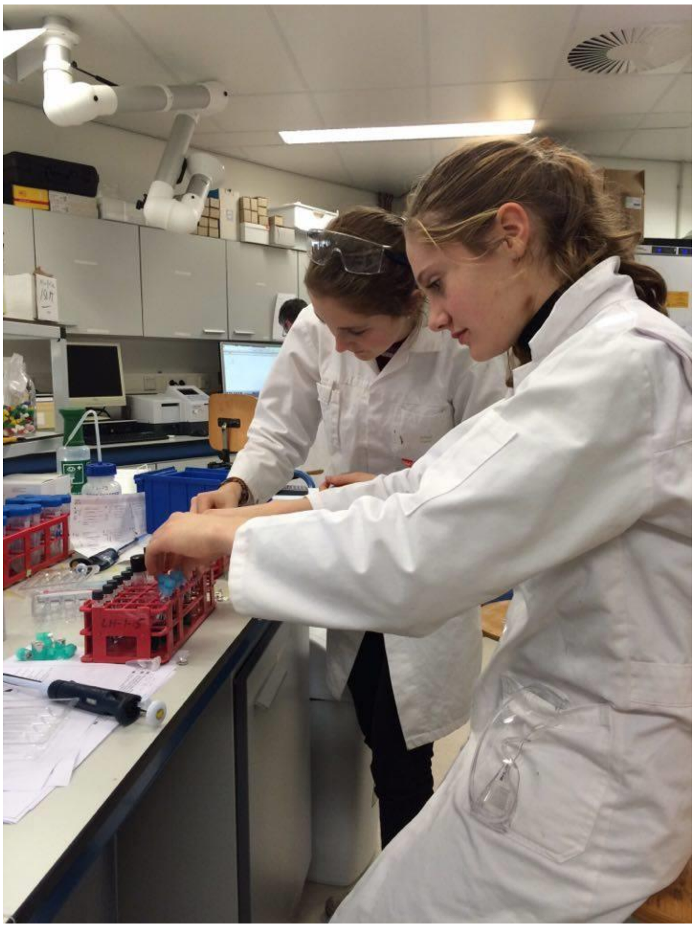
Preface

When we had to choose the topic of our research, we had a lot of different ideas, because of the broad interest we both have. However, one thing was certain: we wanted to investigate a topic that is currently under research, yet still offers enough to be discovered.
After having orientated and gained more information about multiple contemporary topics, we realised that we both started to kindle an interest in the microalgae technology. As the scientific research on microalgae is currently still at an early stage, we were very curious to find out what the possibilities of this new technology might be. We firstly wanted to carry out our research at school, but we soon discovered that there was more material required than available there. When we heard about 'Imagine Life Sciences', we immediately felt that this was a fantastic opportunity for us. Not only because one of the topics in question was on microalgae, but also because the combination of scientific, economic and societal aspects really appealed to us. Due to our cooperation with the scientists Ben van den Broek and Dorinde Kleinegris, we had the possibility to conduct our own research at the University of Wageningen with the necessary equipment and help. We therefore want to especially thank them for all their feedback and cooperation.

Needless to say, we encountered some difficulties during the process of conceptualising our ideas and starting up our own management organisation. At school, our Biology and Geography teachers gave us valuable advice when we needed help or feedback. Furthermore, before handing in our report, our English teacher checked our work for mistakes. We also want to thank these teachers for their helpful contribution to our project .



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Management summary

The aim of this project is to use the technology of microalgae in order to improve agricultural produce in Bangladesh. Bangladesh suffers from extreme poverty and is also one of the most densely populated countries in the world. For most Bangladeshis, agriculture is their main source of income. Both farmers and consumers depend on mostly rice as an agricultural product. Because of the demographic pressure, many farmers have started using high yielding varieties. These crops have an increased growth rate, but also require a higher level of agricultural care, which cannot be fully realised in Bangladesh, since it is one of the poorest countries in the world. Therefore, the varieties do not grow efficiently and cannot meet the large demand. Furthermore, farmers use polluted water from the local rivers to irrigate their crops. This makes the agricultural products less healthy and causes even more pollution of the irrigation systems. By introducing the usage of microalgae, this situation will be improved.

Microalgae are unicellular organisms that can convert water and carbon dioxide in the presence of sunlight to biomass and oxygen. Algae need different nutrients such as nitrates and phosphates for their cultivation. Specific species, like *Chlorella*, can also absorb heavy metals and pesticides. Therefore, polluted water, also called ‘black water’, appears to be an ideal nutrient medium for algae species like *Chlorella*. Algae contain lipids, proteins, carbohydrates, various pigments and vitamins and can therefore be used for many applications, dependent on the nutritious substance. *Chlorella*, for instance, cannot only be used to produce oil and colouring agent, but also as a food supplement. *Spirulina* is especially suitable for the production of chemical fertiliser, due to the high absorption of nitrates and phosphates.

To improve the current situation in Bangladesh, two open plastic tanks will be allocated in between the river and the acre of a farmer. The polluted water of the river will flow into the first tank, which is filled with *Chlorella*. *Chlorella* will absorb the pesticides, heavy metals and the extensive nitrates and phosphates out of the black water, which hereby will be roughly cleaned. The water has to stay in the tank until all the heavy metals and pesticides are absorbed, which then will be transferred to the second tank, filled with *Spirulina* or *Chlorella*, depending on the farmers demand. If a farmer wants to produce a bio-fertiliser and plant protection product, the second tank has to be filled with *Spirulina. Chlorella* is suitable as a food supplement for forage, which can be chosen by cattle farmers.

The *Chlorella* in the first tank will be cultivated with the nutrients of the black water and will therefore not be suitable for the production of nourishment. The *Chlorella* can be sold to other companies, which can produce colouring agent or oil. However, the algae in the second tank grow on the filtered water without the heavy metals and pesticides. Therefore, it is possible to use *Spirulina* as chemical fertiliser for farmers that work in agriculture and *Chlorella* as a food supplement for forage.

By placing the two open tanks with *Chlorella* and *Spirulina*, the problems in the local agriculture and cattle farms in Bangladesh can be partly solved. Polluted water from the river will be cleaned and used for irrigation of the acres. This will improve the quality of the crops and counteract the downward spiral of water pollution. Furthermore, the *Spirulina* can be used as a chemical fertiliser to increase the profit of the high yielding varieties and the *Chlorella* as a food supplement in forage. These products can then be sold or used by the farmers themselves. Therefore, this project has many benefits which will improve the agricultural productivity in Bangladesh.

Introduction

The aim of our project, 'AlgaeCulture', is to improve aspects of agriculture in Bangladesh on a small scale with the aid of the microalgae technology. Of the many agricultural aspects of cultivation we will focus on improving water quality, providing bio-fertilisers, plant protection products and nutritious supplements for forage. We chose to focus on these aspects, when we became familiar with all the possibilities that microalgae offer, ranging from a source for fuel to nutritional use. The fact that microalgae are able to purify polluted water, can be used as fertiliser and added to forage nutrition, brought us to combine these three qualities and focus on agriculture. As Bangladesh suffers from food shortage, we thought that applying these qualities of microalgae will solve part of this major issue that Bangladeshis deals with. Thus, our main question for this project is:

How can we improve local agriculture in Bangladesh with the help of microalgae to increase the produce of the farmers?

The aim of this business plan is forming a clear answer to this question by providing general information about Bangladesh, explaining the technique of the microalgae and discussing our experiment.

A polluted river in Bangladesh

Global overview of Bangladesh

The People's Republic of Bangladesh -the country referred to as Bangladesh- has only become an independent nation in 1971, during the liberation war against Pakistan. Before being a sovereign state, the present territory belonged to Pakistan and was known as East-Pakistan. This southern Asian country is part of the historic region of Bengal and situated between Burma and India. Bangladesh is a low lying country formed by the biggest delta of the world: the Ganges-Brahmaputra river system. With a population of 160 million people, Bangladesh is not only the eighth most populous country worldwide, but also among the most densely populated countries, as the landmass only covers 147500 square kilometers. Unfortunately, the population of this country still suffers from floods and persistent food insecurity. Despite the economic development of the past decades, many inhabitants still live below the poverty line[1].

Economy

Although Bangladesh is still amongst the poorest countries, the economic situation has significantly improved over the past decade. Its economy has grown steadily over the past few years and the estimated 45% of the population that was living below the poverty line in 2004 has been reduced to 30%. With a rate of 7,1%, the economy is the second fastest growing major economy of 2016, according to the IMF. Despite the fact that about 50% of the GDP[2] comes from the service sector, approximately two thirds of the labour force is employed in agriculture. This sector contributes about 20% of the GDP. The other 30% of the GDP comes from the industrial sector. The export earnings of Bangladesh mainly come from the garment industry. The partial dependence of the economy on the agriculture sector makes the economy very vulnerable for droughts, floods and cyclones. Being defined as a low-income country, Bangladesh aspires to be a middle income country[3] by 2021. It is estimated by The Labour Force Survey that approximately 90% of the jobs in the labour market are part of informal employment. Informal employment occurs more often in rural areas and is more likely applied to women.

Agriculture

Despite that only 20% of the GDP comes from the agriculture, this sector is the major source of employment in Bangladesh. The agricultural sector employs more than half of the labour force in Bangladesh and over 60% of the land of Bangladesh is cultivated. Many macro-economic aspects such as employment generation, food security and poverty alleviation are strongly influenced by this sector. The primary products of agriculture are rice, wheat and jute. Of these products, rice can be harvested three times a year. The land is constantly in use for the cultivation of products. Furthermore, cultivated land is overcrowded and mostly dominated by subsistence farmers. Agricultural food production has increased in the past few years, due to better irrigation, more effective use of fertilisers and improved distribution. High yielding varieties have been introduced by the government of Bangladesh and international aid programs to meet the challenges of food shortages. However, high yielding varieties require a higher level of agricultural care, such as intensive disease control, more chemical fertiliser and controlled water supply. Unfortunately, this is not fully possible in Bangladesh, since it is one of the poorest countries in the world. Overall, there is still a pressure on productive capacity due to population pressure, which requires an even more efficient agricultural sector.

[1] The World Bank determined the international poverty line to US$1.90 a day.

[2] Gross Domestic Product

[3] Middle-income countries (MICs) are classified by the World Bank as countries with a GNI per capita between 1036 and 12615 dollar. Middle income economies are referred to as developing countries just like low income countries.

Climate
The climate of Bangladesh is characterised by its seasonal variations. Bangladesh has a tropical monsoon climate with three distinct seasons. The rainy season is from May to September when 80% of the annual rain falls. The winter lasts from October to March, which is a cool and dry period. The summer season is from March till May. During this period the temperature quickly rises, the air becomes very humid and the rainfall increases. Tropical cyclones occur from April until June and October or November. In summer the maximum temperatures can range between 30 and 40°C. The minimum temperature in the winter is 10°C. Most parts of the country receive over 2000 mm rainfall a year, which mostly falls during the monsoon season.

Language and communication
The language that is spoken in Bangladesh is Bengali, also known by its endonym Bangla. This language has many dialects in different regions of Bangladesh. Besides Bengali, English, Indi and Hurdu are spoken by a part of the population. English is spoken by 18% of the population. Approximately 40% of the population is illiterate. It is of importance to bear this in mind, while contacting Bangladeshis. Overall, Bangladeshis are rather indirect communicators. Direct communication styles may be seen as rude, which is important to be aware of while communicating with Bangladeshis. When Bangladeshis communicate with someone of the same gender, they tend to stand rather close to each other and touch is common. However, when men speak to women there is a bigger distance between both. When men meet each other it is common to shake hands, however when a man meets a woman, he should wait to see if the woman extends her hand first.

Religion and culture
The main religion of Bangladesh is Islam. The Muslim population constitutes approximately 90% of the total population, which makes Bangladesh the world's fourth largest Muslim-majority country. Other religions in Bangladesh include Hinduism, Buddhism and Christianity. In Bangladesh, however, there exist no boundaries between the people of various religions. People are respected for their age and position and therefore this country is a hierarchical society. Older people are granted more respect as they are naturally viewed as wise. Only when people are of the same age they call each other by their first names.

Current situation and how 'AlgaeCulture' can help

Despite the fact that Bangladesh has developed increasingly in the past decades, the country still has many issues to deal with. One of these problems is the high demand of food production that is not able to be reached, which is caused by the fact that Bangladesh is highly populated. As a result, extremely high pressure is put on the agriculture sector. Furthermore, local farmers do not make enough profit, as a very large part of their produce stays within the family to avoid malnutrition. Moreover, the introduced high yielding varieties do not grow efficiently. Due to polluted water, which is used for irrigation, the low quality of the grown crops can cause health issues on a small scale. By cleaning the polluted irrigation water and providing algae that can serve as fertiliser, plant protection product and supplement to forage, the current situation for farmers can be improved and more produce for the population can be provided. The tropical monsoon climate, with its high temperatures, many hours of sunshine and rainfall, is very beneficial for the cultivation of algae. Besides, as a major part of the population is employed in the agriculture sector, 'AlgaeCulture' will benefit many inhabitants. Bangladesh is therefore a very suitable country for the implementation of 'AlgaeCulture'.

The microalgae technology

Microalgae are primary producers, found in either freshwater, marine systems or wastewater. Algae are a natural component of most aquatic ecosystems. These microscopic algae can convert water and carbon dioxide to oxygen and biomass, which can be used for various purposes. The photosynthesis counteracts the greenhouse effect and microalgae can be a replacement for fossil fuels. These microorganisms have many benefits and are a source for a promising new technology, which can benefit agriculture in Bangladesh. Firstly, we will introduce the different algae species and their characteristics, then we will explain how and where the cultivation takes place. Furthermore, the wide range of possibilities will be illustrated and lastly, our experiment will be discussed.

Algae Species

Over the last few years, an increasing number of algal species has been investigated. The number of algae species is estimated from 30.000 to 1 million. Only ten thousand species of this enormous amount have been defined and processed in the Algae Base[4]. These species can be classified into different groups. The main groups of algae are Diatoms, Chlorophyta (green algae), Euglenophyta, Dinoflagellata, Chrysophyta (golden algae), Phaeophyta (brown algae), Rhodophyta (red algae) and Cyanobacteria (blue-green algae)[5].

As we will only be using the *Chlorella* and *Spirulina* for our project, we will mainly focus on these two species. *Chlorella* is one of the most well-known algae species and is part of the single-celled Chlorophyta-group. When dried, *Chlorella* consists of 45% protein, 20% fat, 20% carbohydrate, 5% fibre and 10% minerals and vitamins. Due to the high content of proteins and other essential nutrients, this algae specie is very useful as replenishment in alimentation and forage. Besides, *Chlorella* can have a purifying function, since it can absorb carbon dioxide and various heavy metals. *Spirulina* is a genus of the single-celled Cyanobacteria. This algae specie has an impressive nutrition factor, consisting mainly of protein for 50 to 70%. *Spirulina* also contains 7% fat and embodies a number of essential minerals as well as vitamins. Besides, cyanobacteria can be used as bio-fertiliser and plant protection product, due to their role as diazotrophs[6], their ability to compete with native flora and fauna and their function as an ameliorant, which is a chemical that is applied to improve the quality of the soil and thereby improve plant growth. This algae specie is therefore very effective to be used as a bio-fertiliser.

Cultivation

Algae can reproduce very rapidly and only need light, water, carbon dioxide and the right nutrients to grow. Subsequently, for cultivation, the algae have to be placed in CO_2-rich water, with the required nutrients and the right amount of sunlight. The number of times a day that they can multiply depends on the algae specie and the circumstances. *Chlorella* needs a temperature of approximately 37 °C and an optimum pH of 6.7 to obtain the best cultivation. *Spirulina* reproduces the fastest at a temperature of 35-37 °C and a pH of 9. From the four different cultivation systems[7] that are used to grow algae in, we chose to use small open pond systems, as this is the most suitable system for our project in Bangladesh. This system is the cheapest and the process to place and install the open ponds is not very complex, which will keep the investment costs low. Furthermore, ingredients can be added very easily, which will be convenient for the farmers.

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[4] The Algae Base is a Global Species Database of information on all groups of algae.
[5] Further explanation in appendix A
[6] Bacteria that can fix atmospheric nitrogen gas into a more usable form such as ammonia.
[7] Further explanation in appendix B

Experiment

With our experiment[8], we wanted to find out if and how quickly *Chlorella* absorbs heavy metals and nutrients, such as nitrate and phosphate. It was crucial for our experiment that we imitated the circumstances in Bangladesh as accurately as possible, as our project will be realised there. Therefore, we used polluted water from a ditch, which we transfused into two open tanks, in which the algae were grown. To examine the effect of a biofilm on algae cultivation, we added a dishcloth to one of the tanks as a biofilm. The added nutrients were ammonium, nitrate and phosphate. We also added the heavy metals copper and zinc. We focussed mainly on the absorption of copper, which can be found in polluted rivers, ammonium and phosphate. We measured the reduction of these components every three hours in the course of four days, and compared the two tanks, one *with* and one *without* the biofilm. To determine the different concentrations, we used various measuring methods and materials, such as spectrophotometry and ion detecting kits. The *Chlorella* seems to absorb the heavy metals, phosphate and ammonium out of the medium, as expected. This indicates that additional nutrients have to be added to the medium with *Chlorella* or *Spirulina* in the second tank in order to make the algae grow properly. Also the copper concentration reduced, which indicates the quick absorption of heavy metals by *Chlorella*. Furthermore, we examined how to make the products out of the *Chlorella* and *Spirulina*. In our experiment we dried the *Chlorella* to make powder out of it. Finally, we examined the structures of the algae species under a microscope.

Economic aspects

Customer segments and value propositions

Our direct customers will be the local farmers in Bangladesh. They will have to invest in the product we offer, which is indispensable for the setup of their own self-sufficient 'AlgaeCulture'. They can pay this pack[9] in monthly instalments. Which pack we will deliver, is dependent on which products the farmers value most.

Most farmers in Bangladesh suffer from extreme poverty, which makes them unable to apply more chemical fertiliser to their crops. Furthermore, clean water for irrigation is not at disposal. Cattle farmers cannot afford good quality forage for their livestock. This causes a reduction in profit and therefore income. By offering our product to local farmers, we can help to solve these problems. The technology of using algae not only creates the possibility of producing their own bio-fertiliser and food supplement, but farmers also have clean irrigation water at disposal. The microalgae fertiliser is organic and does not do damage to the environment. Besides, *Spirulina* also works as a plant protection product, which helps to increase the harvest. The *Chlorella* can be dried and added to the forage as a food supplement.

The farmers only have to make one rather big investment, which they can pay back over time. However, the nutrients for the algae in the second tank can run out and there is a chance that the missing nutrients have to be bought multiple times, which will be their only annual payment.

Key resources

The products of the first investment contain two plastic tanks, a mirror, two biofilms, additional nutrients and a baseline microalgae count. It also contains extra nutrients (nitrate and phosphate), that can be added to the second tank with one of the two offered algae

[8] Further explanation in appendix C
[9] Further explanation in 'key resources'

species, which are *Chlorella* and *Spirulina*. *Chlorella* can easily be delivered, since this specie can be found in almost every part of Bangladesh. The *Spirulina* will firstly be cultivated from a baseline count, which will be delivered in Dhaka by *CSIR*[10]. These cultivated algae will then be delivered to the local farmers, together with the other materials. The farmers do not have to buy complementary algae, as they can constantly use their own cultivated algae as a baseline count.

Obviously, the farmers have to be given explanations about the cultivation of algae. Therefore, we want to hire an instructed local as an intermediary to approach the farmers, share their knowledge about algae and illustrate how the cultivation and the processing works. We intended to inform our intermediary by offering a instructing package, containing explanative booklets and videos.

Furthermore, we will provide a promotion video which can be shared by the Dutch embassy, one of our key partners, among local farmers and companies to motivate them to participate in our project. We will make two versions of this promoting video, one for motivating the farmers and one for the companies to provide products and become a sponsor of our project. In this video, the project and its procedures will be explained, alongside the emphasized benefits of participating in this project. The video for the farmers will be more simplified and will mainly outline the benefits for the farmers themselves, such as an increase in their profit. To motivate interested companies, we will be focusing on primarily publicity of their participation in our project, such as advertisement posts on our website. In addition we will create a 'paper version' of this video, a promotion booklet. In the case that the video cannot be shared due to lack of facilities, these booklets can still be spread among the farmers.

Key partners

In Bangladesh, most of the river water is under control of a water management organisation. The Dutch embassy in Bangladesh leads this project, called IPSWAM. The IPSWAM-project dominates the introduction of integrated and sustainable water management in local communities in Bangladesh. For this purpose, close collaboration with the local population is required, including local farmers that make use of the river water. Because of the existing cooperation between the Dutch embassy and the local farmers in Bangladesh, our key partner will be the Dutch embassy. They can easily contact local farmers and communities to sell our products to. By sharing the promotion video or booklets, they can attract farmers to participate in the project. Furthermore, the embassy will recruit a local who will function as an intermediary for our project, who should be literate and who masters, besides Bengali, the basics of the English language. However, we ourselves will instruct the local throughout our project. The embassy merely contacts the farmers and intermediaries, as we do not have any connections yet in Bangladesh, apart from the embassy.

To deliver and produce our products, other collaborations with companies or partners are required. Firstly, the plastic tanks have to be produced by a company in Bangladesh, just like the biofilms and the mirrors. Besides, all these products, including the algae and the nutrients, have to be delivered to the local farmers, which requires a truck and a driver.

Channels

It is crucial for the setup of our business management to consider the ways of communication with the customers. Through which channels do we want to reach our customers? Can we integrate them with customer routines? Firstly, our stakeholder, the Dutch embassy, can easily reach the local farmers, due to the already existing cooperation of the IPSWAM-project. This moderates the communication with our customers and helps us to integrate our channels in their routines. By spreading the promotion video or booklet the

[10] http://www.csiro.au/en/Research/Collections/ANACC/Australian-National-Algae-Supply-service

Dutch embassy can recruit farmers for our project. They can recruit an intermediary by placing ads in the neighbourhood, which we will provide. We will stay in contact with the embassy by Skype. Whenever this is not possible, we will contact them by phone.

Besides, we will have regular contact with the intermediary for our project. We will start this contact by an in-person meeting in Bangladesh. We will then hand out an instructive package, containing all the information needed for his/her tasks and provide a cell-phone for this person. We will regularly stay in contact via email or cell-phone, whenever contact via Skype is not possible, to stay updated over the project and to answer all of his/her questions. The intermediary will communicate orally with the farmers, our customers.

Furthermore, we want to use the internet to contact our potential partners by sending emails and ads, which is a very cost-efficient method. To acquire more publicity abroad, we will set up an accessible website, that can be visited by both our (potential) partners and our customers. Therefore, the website has to mention the plan itself, the available options for different farmers, the costs, the locations, the collaborative companies and our contact details. In this way, we cannot only inform the farmers and local companies about 'AlgaeCulture', but the website is also an easy way to reach sponsors and contributors.

Phases

- Phase 1: Publicity (2017)

Before our business management can take off, we firstly have to approach and inform our most important key partners, which will be the Dutch embassy in Bangladesh, and local companies in Bangladesh that produce the plastic tanks, mirrors and biofilms. Besides, we have to instruct our intermediaries and teach them the basic knowledge about microalgae and their cultivation. For this purpose, we will have an in-person meeting to explain and practise this technique. In this stage, our main focus will be on gaining more publicity amongst the customers and interested companies, by using our promoting video and booklets. The embassy plays an important role in this stage, as they can easily reach our prospective customers.

- Phase 2: Funding (2018-2019)

When the first stage is completed, the materials and equipment for the beginner's kit have to be acquired. Our product can be delivered to the customers and the local contact person can explain how to use it. At the beginning of this stage, we need to make certain investments. To help start up our project, we will need a baseline amount of funding. We think that, due to our connection to the Dutch embassy, our business management has good chances to be considered for government funding. We also hope that our website will attract contributors and sponsors. Our project also can obtain the needed support for the following reasons:

1. This project contains a very new and innovative science technique
2. 'AlgaeCulture' improves the profit of the agricultural sector and thus increases the incomes and living standards of the farmers in Bangladesh.
3. Our project creates new jobs and provides a higher produce.

If this does not function at will, we will organise a crowdfunding to raise money for the setup of our project.

- Phase 3: Expanding (from 2019 onward)

After three years of advertising and gaining more customers, we can finally begin to expand our project and hopefully involve more companies and farmers. In this way, we hope to improve the agricultural situation in an increasing number of communities throughout Bangladesh. This project has many possibilities for farmers, such as creating a self-sufficient ecological system. It also contributes to higher living standards and more sources of income. Moreover, new and more challenging job possibilities are created for the local population, for example our local intermediary.

Revenue streams[11]

As the costs of 'AlgaeCulture' mostly depend on the number of farmers that participate in the project and on the phase our business is in, we have calculated the revenue streams per ten farmers and one intermediary, to guide this group of farmers. We have distinguished a one-off investment and repetitive costs to represent the first phase of the project. The required equipment, algae and the nutrients have to be paid back by the farmers in monthly terms, but to create a complete overview of the first phase of our business, we have included these costs as investments. We have not only calculated equipment and ingredient prices, but also labour, transport, advertising and communication costs.

per 10 farmers	Investment (€)	Annual costs (€)
Equipment	**290,00**	
20 plastic tanks	200,00	
20 biofilms	20,00	
10 mirrors	35,00	
Harvest materials	35,00	
Ingredients	**213,90**	**53,00**
Chlorella	0,00	
Spirulina	213,90	
Nutrient package		53,00
Intermediary costs		**320,00**
Transport costs		**48,60**
Vehicles		30,00
Fuel		15,60
Bus driver		3,00
Communication costs	**21,75**	**30,00**
Telephone costs	10,80	30,00
Instruction package	10,95	
Promotion costs		**84,00**
Website costs		84,00
Promotion video	0,00	
Unexpected costs (10%)	**52,57**	**53,56**
Total costs	**578,22**	**589,16**

Revenue incomes[7]

As the farmers will pay back the equipment and ingredients in monthly instalments, this will eventually be an income for our project. We hope to attract as many companies and sponsors as possible for 'AlgaeCulture', as they can provide discount on the required equipment and support us financially. However, we assume that this will not be the case in the first phase of our project. When 'AlgaeCulture' will enter the expanding phase, there will be more sponsoring and funding to cover the costs. Therefore, we have calculated the revenue incomes *without* the help of the recruited companies and sponsors to represent the first phase of the project.

[11] Further explanation in Appendix D

Per 10 farmers	Investment incomes(€)	Yearly incomes(€)
Sponsors and crowdfunding	Unknown	unknown
Government funding	1000,00	
Farmers	494,90	53,00
Total incomes	1494,90	53,00

Conclusion

The aim of this project is to use the technology of microalgae in order to partly solve the current problems in the agriculture of Bangladesh and therefore increase the produce of local farmers. For this purpose, we have set up a potential sustainable business management in the form of this business plan. Throughout this business plan, we have attempted to create a clear answer to our main question '*How can we improve the local agriculture in Bangladesh with the help of microalgae to increase the produce of the farmers?"*. Therefore, we started with asking ourselves how we could improve this situation with the help of the microalgae technology. After having conceptualised our ideas, we collected the required information, which we could use for our business plan. By describing the possibilities of the microalgae technology and providing general information about Bangladesh and the problems of the agricultural sector, we illustrated that Bangladesh is a very suitable country for the implementation of 'AlgaeCulture'. Furthermore, by elaborating our concept, we demonstrated that 'AlgaeCulture' is enforceable in reality and in fact cost-effective.

Although we have endeavoured to set up our business plan as accurately as possible, we encountered some difficulties while estimating the exact circumstances which can influence the progress of the implementation of 'AlgaeCulture'. We especially had difficulty forming an overview of the exact revenue streams and incomes. Due to the fact that we have not yet been able to test the algae cultivation in Bangladesh under the present circumstances, we cannot state with certainty that the algae will grow as well as expected. Despite the fact that it will inevitably require more improvements in the future, we believe that 'AlgaeCulture' will be a helpful contribution to the Bangladesh farming community.

We hope to contribute to the well-being of many farmers in Bangladesh

Postface

When we chose to participate in Imagine, we were very excited about where the project would take us and curious about its result. We have learned a lot during the months we worked on this project. First of all, due to the cooperation with scientists and the carrying out of our experiment, we have increased our knowledge about microalgae and their applications. We found the tour through the algae park at Wageningen University very informative and we saw our experiment in the laboratory in the bio-based food and research department as a unique experience. Furthermore, during the workshop of sustainable business management, we gained knowledge of a field that we were both unfamiliar with. This was an inspirational experience, which gave us new insights in possibilities for our project.

Needless to say, we came across some obstacles during our project. The first difficulty we encountered in one of the earliest stages of our project was the fact that we wanted to use as many applications of the algae as possible, but found it difficult to bring these applications together in one coherent concept. The possibilities of using microalgae are in fact endless and we found it hard to exclude some of these applications. Later we realised that it is of great importance to focus on one particular problem or sector, such as agriculture, before conceptualising multiple diverging ideas. As soon as we chose to only focus on the agricultural sector, everything seemed to fit together and we started to make progress. Furthermore, we struggled with expressing our ideas in English instead of Dutch. We found it especially difficult to discuss our experiment in English, as we were not familiar with the English terms is this field. However, this became less difficult the more we made progress.

We already knew before participating in this project that we would have to work hard, especially because we are a group of only two. However, we only figured out how much work it really was, when we were working out our idea in the form of a business plan. We kept on realising that we had not taken everything into account and that aspects of our project had to be edited and improved. The fact that we enjoyed working on this project – all the while becoming enthusiastic for 'AlgaeCulture'- and worked very efficiently as a team, helped us to overcome the workload and other challenges. Now, after having finished our business plan, we can look back at 'Imagine' as a journey, because that is exactly how we can describe it: an intense but inspirational journey which has brought us different experiences, has widened our knowledge in different fields and constantly challenged us.

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Appendices

A. Algae species

‘Algae’ is a term for a large, diverse group of photosynthetic organisms. These organisms are not necessarily closely related. Algae species mutually vary considerably in size, shape, and growth form. Based on these qualities, a classification of different algae species is made. First of all, algae can be single celled eukaryotic organisms, which are referred to as protozoa, or multicellular eukaryotic organisms, which are referred to as metazoans. The most important single-celled algae species are Diatoms, Chlorophyta (green algae) and the Cyanobacteria. Furthermore, the Chrysophyta, Phaeophyta and Rhodophyta are consistent multicellular algae.

Diatoms

Diatoms form a major group of algae, consisting of over 100.000 species. They are among the most common types of phytoplankton and are producers within the food chain. Most diatoms exist singly, although some join together to form colonies. They are usually yellowish or brownish and can be found in either fresh- and saltwater. An interesting feature of diatom cells is that they are enclosed by a cell wall made of silica, hydrated silicon dioxide, called a frustule. This frustule consists of two valves, of which one is slightly larger. This causes a wide diversity in form.

Chlorophyta (green algae)

Chlorophyta is a division of green algae and includes about 7.000 species. This group contains mostly unicellular species, although some multicellular species can also be found within this group. The various species can be coenocytic, containing more than one nucleus in a cell, or can form colonies. They can be found in freshwater and marine water and even a few types can be found on moist soil, on the trunks of trees, on moist rocks and in snow banks.

Cyanobacteria (blue-green algae)

The cyanobacteria are the least closely related to other algae groups. These cells can form colonies. Cyanobacteria contain the green pigment chlorophyll and are thus primary producers. Cyanobacteria have no habitat, as they can be found almost everywhere on earth.

Chrysophyta (golden algae)

Chrysophyta mostly live in freshwater and includes about 1000 species. Their cell walls are composed of cellulose, which also contains silica. These ‘golden algae’ contain the photosynthetic pigments chlorophyll a and c and beta-carotene, which give them a gold colour.

Phaeophyta (brown algae)

Many of these multicellular algae are marine and form the familiar seaweeds. These algae can join together to form slime layers on wood and stone. They contain brownish carotenoid pigments, which give them their characteristic brown colour.

Rhodophyta (red algae)

Members of this multicellular division have a characteristic clear red or purplish colour, which is caused by pigments called phycobilins. Most of the world's seaweeds belong to this group and these algae can be found in oceans where there is a tropical climate.

B. Cultivation systems

Nowadays, four different algae cultivation systems are used. Which system brings the highest produce depends on circumstances, such as temperature, pH, amount of sunshine, rainfall, landscape and the presence of other microorganisms.

Open pond system

The open pond system is the most common system of algae cultivation and consists of an open, shallow pond in which the algae are exposed to sunlight. The benefits of these ponds are their simple design and thus the low instalment costs. However, these systems are more difficult to monitor, as they are very sensitive to surrounding circumstances. For instance, water can evaporate easily and the system can be intruded by various infectors. Furthermore, the percentage of light that algae convert into biomass is approximately 1.5% in open pond systems, while in theory algae can convert about 10% of the light into biomass. The amount of light at the surface of the pond is rather high, while algae in a lower part of the tank receive hardly any light. This aspect makes the open pond a rather inefficient cultivation system. The most common pond system within the open ponds is the raceway system. This pond consists of shallow annular channels. The raceway system contains paddles wheels to make the water flow around the circuit continuously. However, we will not use the raceway system for our project as it is a more complex system and requires more equipment.

A raceway system

Horizontal tube reactors

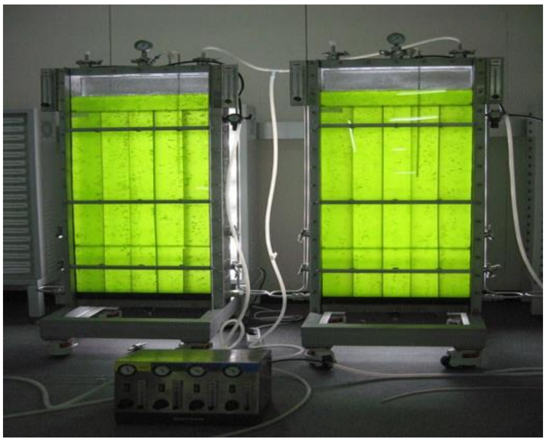
The horizontal tube reactors system is one of the closed systems for growing algae. The main advantage of this system is the possibility to control various circumstances such as temperature. Compared to the open ponds system, this system is a more productive one. Furthermore, the tubes of this system can be easily extended, which facilitates the upscaling process. One of the drawbacks of this system is the too high intensity of light, which keeps the algae from growing optimally. Another drawback is the fact that O_2 accumulates easily in the tubes, which can limit the algae growth as O_2 is toxic in a high concentration for the algae. Besides, the construction costs of such a system are relatively high. The energy costs are also higher as the algae have to be continuously circulated through the system.

A horizontal tube reactor

Three-dimensional tube reactors
The multiple layers of this system, which are vertically placed on top of each other, form virtual vertical panels. Some of the drawbacks of this system are similar to those of the horizontal tube reactors, such as the high costs and the accumulation of O_2.This system does not however have to cope with the problem of a too high light intensity, as the tubes are placed in each other's shadows. This system is one of the most productive systems, as more tubes can be placed on a terrain, which increases the production per square meter.


Three-dimensional tube reactors

Flat plate reactors
Probably the most efficient cultivation system is the one with flat plate reactors. This system consists of multiple flat parallel plates. The main advantage of this system is that accumulation of O_2 does not occur and that the right intensity of light can be reached. However, a high amount of electricity is required to keep the nutrients composite and to avoid the algae from settling. It is also difficult to add extra CO_2 into this system. Furthermore, upscaling this system will cause more difficulty, than upscaling other systems.


Flat plate reactors

C. Experiment

In our experiment, we chose to focus on the absorption of heavy metals and nutrients of *Chlorella*. The aim of our experiment was to find out if and how quickly the absorption elapses and what the effect of growing algae on biofilm compared to growing algae in an open tank would be. As preparation for our experiment in Wageningen, we conducted the first part of our experiment at school. In the undermentioned overview, we listed the different experiments and their aspects that will be discussed.

1. Experiment at school
   - Heavy metals and nutrients
   - Equipment used
   - Method
   - Observations
2. Experiment at Wageningen University & Research
   - Preparations
   - Microscope examination
   - From grown algae to powder
   - Absorption measurements
     - Copper sulphate
     - Phosphate
     - Ammonium
3. Overview experiment

Experiment at school

Nutrients and heavy metals

As we wanted to simulate the situation in Bangladesh as accurately as possible, we used polluted water from a ditch. We added the *Chlorella* to two Liter of this polluted water and added 2.0 gram zinc nitrate, 1.18 gram ammonium, 1.5 gram phosphate and 1.53 gram copper sulphate. These quantities give the following concentrations:

Zinc nitrate	1.000 gram/L
Ammonium	0.905 gram/L
phosphate	0.750 gram/L
Copper sulphate	0.705 gram/L

We chose to add these quantities to the medium, based on the minimum quantity of nutrients that *Chlorella* needs in order to grow. We chose these heavy metals, as they can be found in polluted rivers in Bangladesh and because we had the possibility of using these metals.

Equipment used

We divided the two Liter medium into two separate small tanks, each tank containing one Liter. We placed a piece of washcloth, as biofilm, on one of the tanks. We further used a big lamp, to provide enough light for the algae to grow. As the temperature in the room, where our algae setting was based, was not as high as the temperature in Bangladesh, we used an electric heater. We further used a thermometer connected to a laptop to measure the temperature and to make sure the temperature did not vary too much, which might influence our results.

Method

As we wanted to measure the concentrations during the growth of the algae, we took a sample, of approximately 3 mL, out of the medium of both tanks, four times a day. The times at which we took samples were 8.00 am, 11.00 am, 2.00 pm and 5.00 pm. We started the experiment on Tuesday 8.00 pm and we took our last sample on Friday 2.00 pm. We also took a sample of the water without the algae in it, which we could use as a control test. After carrying out this experiment for four days, we had thirty test tubes filled with a compound of algae and the medium. For more reliable results, we ruled out the effects of light by covering the test tubes to prevent the algae from growing further and absorb more nutrients. Besides this, we preserved the cultivated algae in order to use them for our further experiment.

Observations

During our experiment at school, we noticed that the amount of water in both tanks had decreased over time. This occurred especially in the tank with the biofilm due to the absorption of water in the dishcloth. As the dimensions of the washcloth exceeded those of the surface of the tank, a part of the water left the tank through the outskirts of the biofilm. Despite the larger reduction of water in the tank with the biofilm, it seemed that the *Chlorella* grew quicker while attaching to the biofilm. Unfortunately, we could not harvest the algae grown on the biofilm, as the dishcloth and the algae were dried out, due to lack of water available in the tank. However, we are quite certain that it would be most efficient to harvest the algae from the biofilm, as we observed that all the grown algae attached to this biofilm. By raking the algae from the biofilm, all the grown algae would be collected at once.

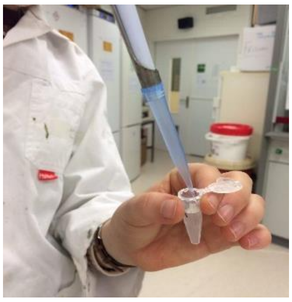
Experiment at Wageningen University & Research

Introduction experiment at the university of Wageningen

As we did not have the right measurement equipment at our disposal at school, we took the test tubes to Wageningen University for the continuation of our experiment. In Wageningen, we had access to the laboratory and the available equipment. We firstly examined our own cultivated algae, *Chlorella*, and *Spirulina* under the microscope. Secondly, we fabricated the grown *Chlorella* to powder. Moreover, we measured the concentrations of copper, phosphate and ammonium.

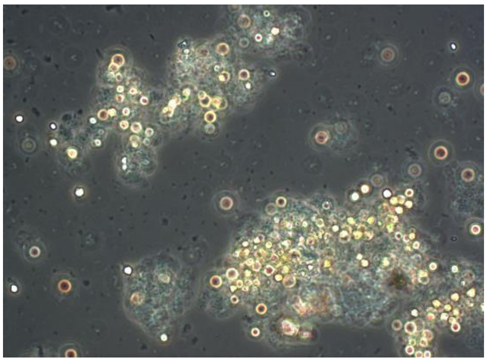
Preparations

Before we could start with our experiment, we had to be aware of safety measures. We were required to wear safety goggles and laboratory jackets. It also was explained what to do in case of fire and other calamities. To prepare for the multiple measurement experiments, we firstly organised our test tubes neatly and transferred the liquid out of the test tubes using a pipette to the centrifuge tubes. After this, we centrifuged the tubes, in order to separate the algae biomass from the liquid compound.



Microscope examination

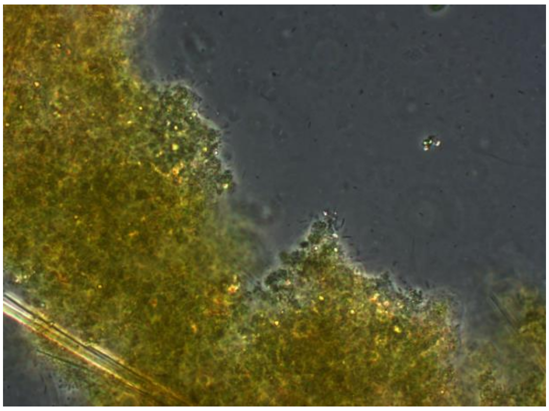
To examine the structure, shape and the condition of our grown *Chlorella*, we observed multiple microscope slide preparations with drops of the microalgae medium under a microscope. *Chlorella* is an algae specie with a round structure and shape of a small globule. This structure was also clearly visible under the microscope. The grown algae cells were heaped together in different chunks. The *Chlorella* was in a good condition and there were not many bacteria present.



Chlorella under the microscope

Moreover, we looked at *Spirulina*, which we received from the University of Wageningen as well, but which we did not cultivate at school. We noticed that the *Spirulina*, which we had preserved for multiple weeks, had been infected by bacteria. Although we did not open the test tube with *Spirulina*, there were most likely some bacteria in the medium from the start. Because we covered the test tubes and ruled out the effects of light, the *Spirulina* had no possibility of growing. However, various bacteria are not dependent on light, which meant that the bacteria started to multiply in the algae medium. It was very interesting to see how the bacteria lived on the algae biomass and adhered to the microalgae cells.

The structure of *Spirulina*[12] differs a lot from the roundly shaped *Chlorella*. The *Spirulina* is spiroid and its main photosynthetic pigment is phycocyanin, which is blue in colour. Under the microscope, there was also agglomeration of the *Spirulina*. Unfortunately, this made it more difficult to distinguish this characteristic structure.

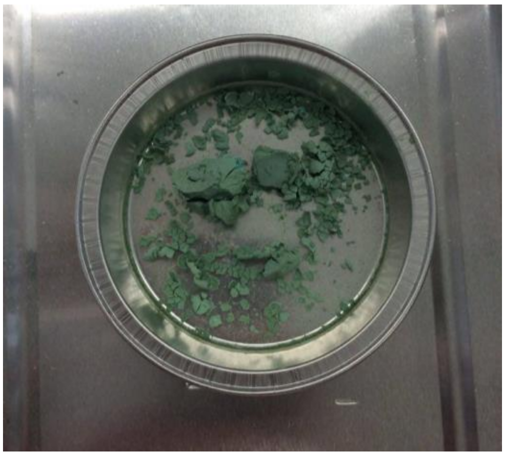


Spirulina under the microscope

From grown algae to powder

We brought a portion of the grown *Chlorella* to Wageningen to fabricate it to powder. We firstly centrifuged the algae compound. Afterwards we heated the compound of solely algae in an oven at the temperature of 90 °C. After two and a half hours, we went back to check the algae and only dry material was left. We made this into powder by crushing it.

In Bangladesh, using an oven would be out of question, due to the high purchase and electricity prices. Instead of an oven, we would therefore use mirrors to heat the algae compound. When the mirrors are placed in the right angle under the right circumstances, a high temperature of 50 °C can be reached[13]. As the algae dried very quickly in an oven (at 90 °C), we assume that this also would go rapid using mirrors. We predicted that drying the algae using a mirror would take around four hours.



What struck us, was that the powder had a slightly blue colour, while a powder of *Chlorella* is supposed to be intense green. From this we can conclude that the copper has not been completely absorbed by the grown *Chlorella*. We think that the main reason for the copper not being fully absorbed, is that the copper sulphate was not mixed well enough with the algae compound. We already noticed this while we were growing the *Chlorella*. We saw little lumps of blue powder sticking together.

Dried Chlorella

[12] https://microbewiki.kenyon.edu/index.php/Spirulina
[13] https://newscientist.nl/blogs/adembenemend-mooie-energiecentrale/

Absorption measurements

Copper Sulphate

Method

To measure the reduction of the copper sulphate concentration, we used the blueish colour of copper as an indicator. We used a spectrophotometric method[14] to analyse how the concentration of copper sulphate elapsed. Firstly, we prepared five samples for the analysis of the absorption, containing 0,00 M (water), 0,02 M, 0,04 M, 0,06 M, 0,08 M and 0,10 M of copper sulphate. We used 1 cm cuvettes and a wavelength of 635 nm. The absorbance of each of the standard solutions was then determined. Based on the results, we constructed a calibration line, in which the absorbances of the standard solutions were directly proportional to their concentrations. Subsequently, we prepared the samples with the liquid of the *Chlorella* test tubes in 1 cm cuvettes, beginning with sample no. 1 *with* biofilm and sample no. 1 *without* biofilm. We did not calibrate all the cuvettes, because the range between the samples was very small. We examined the following cuvettes, indicated in the tables.

Results and discussion

Sample ID-standard solutions	Absorption rate
water	-0,001
0.02M	0,064
0.04M	0,107
0.06M	0,142
0.08M	0,173
0.10M	0,224

Sample ID	Absorption rate
blanco	0,015
sample 1 without biofilm	0,015
sample 1 with biofilm	0,016
sample 7 with biofilm	0,025
sample 8 with biofilm	0,096
sample 11 with biofilm	0,02
sample 13 with biofilm	0,022
sample 14 without biofilm	0,008
sample 15 with biofilm	0,026

While processing this data, we did not see a clear relation between the two variables. The independent variable is the time, which is linked to the number of the cuvettes. The dependent variable is the reduction in the copper sulphate concentration (M). The absorbance and thus the concentration gradually became higher. We tried to explain this phenomenon by using the following information. When we cultivated the *Chlorella*, we took samples of approximately 3 mL out of the water. Moreover, due to the warmth of the electric heater, there was evaporation. This obviously caused a higher concentration of nutrients and heavy metals. Moreover, *Chlorella* makes the pigment chlorophyll, when absorbing nutrients or heavy metals. This pigment has the same greenish/blueish colour as copper and thus influences the extent of absorption with a wavelength of 635 nm. However, the differences between the measurements were not significant enough and thus we can unfortunately not come to a conclusion based on this experiment. Despite not having proved the decrease of copper sulphate by using spectrophotometry, we clearly observed the reduction of the

[14] Further explanation in Appendix E

blueish colour of the compound as the time elapsed during our experiment at school. The colour of the medium had already turned from turquoise to green after the first day of examination. At the end of our experiment the blue colour had totally disappeared.

The difference in colour between the first and last tube

Phosphate measurement

Method

For the measurement of the phosphate concentration, we used the 'LCK 349'-kit. The principle on which this method is based is the reaction of phosphate ions molybdate and antimony ions in an acidic solution. As a result of this reaction an Antimonyl phosphomolybdate complex is formed, which is reduced by ascorbic to phosphomolybdenum blue. We firstly added 2.0 mL of the first, sixth, eleventh and fifteenth sample (with and without biofilm), each in a separate tube, by using pipettes. Then we added 0.2 mL of the reagent LCK 349 B and inverted the tubes a few times. After waiting for ten minutes, for the reaction to elapse, we analysed the various shades of blue of the different tubes. We used a spectrophotometric method to measure the light absorbance of the solution in the different tubes.

Results

After having carried out the instructions of the kit, we measured the phosphate concentrations by using spectrophotometry. We firstly analysed five tubes containing the medium of the algae grown *without* biofilm. The measurements are noted in the table below.

Test tube without biofilm	Concentration
1 (Tuesday 8.00 am)	0,186 mg/L
6 (Wednesday 11.00 am)	0,666 mg/L
11 (Thursday 3.00 pm)	0,585 mg/L
15 (Friday 2.00 pm)	0,818 mg/L

Test tubes of the phosphate measurement

Subsequently, we analysed five tubes containing the medium of the algae grown *with* biofilm. However, the concentration of the second tube (Wednesday 11.00 am) was already too high to be measured by the spectrophotometry.

Conclusion and discussion

From the results we can conclude that during our experiment the concentrations have increased instead of decreased. This can be explained by the evaporation that took place in both tanks, which we had already observed during the first step of our experiment (growing the algae) and that not all the added nutrients and heavy metals were completely absorbed. That the concentration increased faster in the tank with the biofilm, can be explained by the fact that the biofilm absorbed an amount of water, which even left the tank in a liquid form. This phenomenon can explain why the light absorbance and thus the concentration, measured by the spectrophotometry, has increased.

Ammonium measurement

Method

For the measurement of ammonium, we used the ‘LCK 305 Ammonium-Nitrogen’-kit. The principle on which this method is based is the reaction between ammonium ions with hypochlorite ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue. These ions react at a pH of 12.6 and a temperature of 20 °C. We firstly added the required amounts of water and liquid out of the centrifuge tubes, by using pipettes, in order to let the reaction elapse correctly. We did this for the first, third, seventh, eleventh and fourteenth sample *with* and *without* biofilm. Then, we removed the foil from the caps of the test tubes, reversed them and screwed the caps back on tightly. We shook the tubes firmly and waited for 15 minutes. The compounds all turned into a different shade of green, which we could therefore analyse with a spectrophotometric method. The compound only remained stable for a further 15 minutes, so we analysed the samples directly after the first 15 minutes had passed. We used a spectrophotometric method to measure the light absorbance of the solution in the test tubes. As we had to measure a green colour, we used a wavelength of 694 nm.

Test tubes of the ammonium measurement

Results

After we had analysed the test tubes of the ammonium kit with a spectrophotometric method, we noted the following measurements of the light absorption.

Test tube with biofilm	Absorption rate
1 (Tuesday, 8:00 am)	4,450
3 (Tuesday, 2:00 pm)	3,003
7 (Wednesday, 2:00 pm)	4,273
11 (Thursday, 3:00 pm)	4,299
14 (Friday, 11:00 am)	3,957

Test tube without biofilm	Absorption rate
1 (Tuesday, 8:00 am)	4,450
3 (Tuesday, 2:00 pm)	4,100
7 (Wednesday, 2:00 pm)	4,371
11 (Thursday, 3:00 pm)	4,267
14 (Friday, 11:00 am)	3,920

Conclusion and discussion

Alike the copper sulphate determination, a higher absorbance level and thus a higher ammonium-concentration is measured in most of the test tubes. This is the result of evaporation in the open cultivation tanks and the constant removal of 3 mL of liquid out of the tanks by taking samples. However, the range between the measurements is rather diminutive, which indicates a very small increase in concentration. Due to evaporation, the concentrations of all nutrients should become higher than before. As evaporation occurred in both tanks and there was only a minimal increase and even a decrease at the end in concentration, we can conclude that the *Chlorella* has absorbed the ammonium. Besides, a decrease is visible in the last measurements of test tubes 14. The ammonium-concentration has therefore decreased over the respective period of time.

Overview of the experiment

Overall conclusion

Several other scientific experiments have proved the ability of *Chlorella* to absorb heavy metals and nutrients such as ammonium and phosphate. With our conducted research, we were not able to draw clear conclusions based on our measurements, due to encountered difficulties. However, with this experiment we have obtained useful information for our business plan and ideas for further research. We now know that choosing a suitable biofilm with corresponding dimensions is important to avoid significant decrease of water. Using a biofilm, however, facilitates the algae to grow and especially to harvest them efficiently. Moreover, we have come to the conclusion that a right amount of various nutrients is vital for the algae to grow at a proper speed. We can conclude this by the fact that we only added a limited variety of nutrients in our first experiment and the fact that the *Chlorella* did, by far, not grow to its full potential. Furthermore, we noticed that the algae dried very quickly in the oven and were easy to granulate. Therefore, we can conclude that drying the algae by using mirrors will be a convenient way for processing the algae to powder, as a high temperature can be reached by the reflexion of the sun by mirrors.

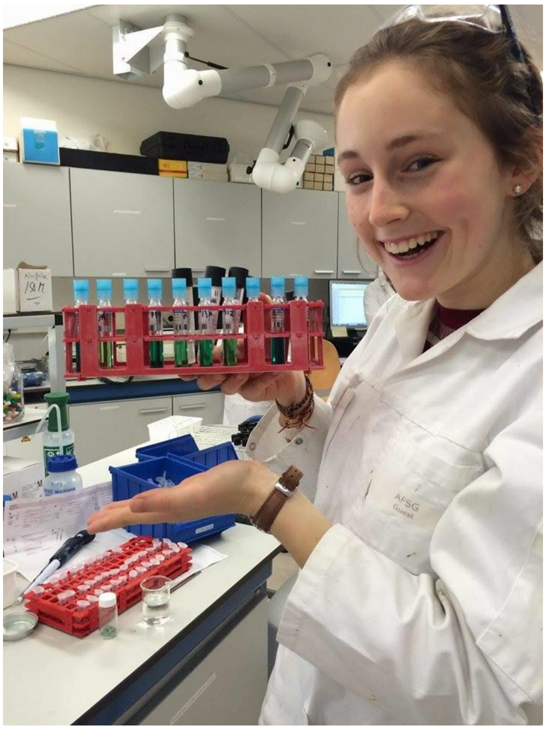
Overall discussion

During our experiment in Wageningen, we measured the elapse in concentration of the added heavy metals and nutrients. However, while carrying out our examination we encountered some difficulties. Now, after the completion of our experiment, we have become aware of the flaws in our method, which need to be taken into account for further research.

Firstly, the biofilm absorbed water, which left the tank through the outside parts of the washcloth, which we used as biofilm. Also, evaporation occurred of the water out of the tanks. This caused an increase in the concentrations of the nutrients. Some of the nutrients were not mixed well enough in the compound, which caused the fact that not all the required nutrients were available for the *Chlorella*. Furthermore, we did not have access to all the required nutrients for *Chlorella* to grow, which might have caused limited algae growth and thus a limitation of absorption of the heavy metals and nutrients. Overall, we found it challenging to simulate the circumstances of Bangladesh, as we did not exactly know the concentrations of the heavy metals and other contaminations in the polluted water used for irrigation. In addition, we did not have access to the equipment which we would use for the project in Bangladesh.

Further research

If we were able to carry out our experiment again, we would make sure that the nutrients and heavy metals would be mixed well enough in the compound, as absorption could take place more easily. Furthermore, we would experiment with different materials as a biofilm and choose the material that absorbs the least water. We would also make sure that no part of the biofilm would be outside the tank, to minimize the flooded water. In general we would do an experiment to determine the minimum required nutrients for *Chlorella* to grow. In this experiment we would also include CO_2 which we have not done in our experiment which probably was a restrictive factor for our *Chlorella* to grow well. This experiment would be useful, as we want to provide as little as possible nutrients, as it will save costs, but also provide a sufficient amount for the algae to grow well.



We enjoyed our experiment in Wageningen

D. Further explanation of revenue streams and incomes

Equipment costs

Plastic tanks
A tank in Europe costs approximately €10,00. Although these costs will most likely be lower in Bangladesh, we have calculated the total costs with this price. However, we assume that these costs will definitely not be higher. The total price of €200,00 is based on the fact that every farmer will receive two tanks and that we have calculated the costs per ten farmers.

Biofilms
A piece of cotton of 1,5 by 1,0 m, which is enough to cover two tanks, costs €2,00 in Bangladesh. Subsequently, the total costs per ten farmers will amount €20,00.

Mirrors
The price of the mirrors is based on the fact that one mirror approximately costs €3,50 and that every farmer needs one mirror.

Harvest materials
For the harvest of the algae, multiple materials are required. The material package consists of a bucket; aluminum tanks, in which the farmers can dry the *Chlorella;* and a spatula which is required to scrape the algae compound off the biofilm. The bucket costs €2,00 per piece, the aluminum tank costs €0,50 per piece and the price of one spatula is €1,00. This amounts to a total of €3,50 per farmer.

Ingredients

Chlorella
As *Chlorella* can be found in Bangladesh, no costs will have to be made for this algae specie.

Spirulina
The algae will be bought from the company ANACC[15], which sells *Spirulina* at a price of $230 per 250 mL tissue-culture flask. We will start off with a baseline count of 250 mL, which equals an amount of €213,90. After having grown these algae in Bangladesh, the *Spirulina* will be divided between the ten farmers.

Nutrient package
We will use nutrient packages, named KAS[16], which cost €26,50 per 100 kg. We estimated that the farmers will approximately need 20 kg of this package per year. As the baseline count of algae will first be cultivated in order to provide enough for ten farmers,1 kg of the total amount of nutrients will be used in advance, because it is only possible to buy the nutrients in packages of 100 kg. In total this will cost €53,00 a year, as two times an amount of 100 kg has to be bought.

Intermediary costs
We assume that the intermediary has to work 8 hours a day and 200 days a year. We will pay the intermediary twice the minimum wage of Bangladesh[17]. As the minimum wage is €0,10 per hour, they will be paid €0,20 per hour. The yearly salary will thus be €320,00.

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[15] http://www.csiro.au/en/Research/Collections/ANACC/Australian-National-Algae-Supply-service
[16] http://www.boerderij.nl/Home/Achtergrond/2016/4/Kunstmest-KAS-prijzen-flink-omlaag-2790598W/
[17] https://en.wikipedia.org/wiki/List_of_minimum_wages_by_country

Transport costs

We have calculated the transport costs for a further stage, bearing in mind that the project will expand over the years and more groups of ten farmers will participate. More intermediaries from different areas can then be recruited.

Vehicle costs

In Bangladesh, a bus can be rented for 2500,00 Taka a day[18], which equals €30,00[19]. The equipment and the ingredients have to be delivered at once to all of the ten farmers.

Fuel costs

The bus will be rented in the capital, Dhaka, and from there the equipment and ingredients will be delivered. The ten farmers have to live all in the same area, due to practical reasons. We assume these farmers will not live any further than 150 kilometers from Dhaka. Thus the bus-driver has to drive a maximum distance of 300 kilometers. With one Liter of Diesel, 15 km can be driven. For this delivery 20 Liter is required, which cost 65,00 Tk/Liter[20]. This amounts 1300,00 Taka, equaling €15,60.

Bus-driver costs

We will pay the bus driver €3,00 for the delivery of the products to the farmers. As the delivery will take a maximum of 10 hours, the bus-driver has to be paid for 10 hours. We will pay him three times the minimum wage, based on the salary of a bus-driver[21].

Communication costs

Telephone costs

We assume that we will be calling the Dutch embassy, especially during the first stage of our project. We will preferably contact our key partners and the intermediary through Skype. In the case that either we or our key partners or the intermediary do not have access to the internet, we will contact each other by phone. We estimated that one conversation will take 10 minutes and that we will have to call once every two months in the first stage of our project. Calling to Bangladesh from the Netherlands costs €0,50 per minute. Therefore our yearly telephone costs will be €30,00. Furthermore, we will provide a mobile phone for the intermediary, which costs €10,80.[22]

Instruction package

To produce an instruction package, the costs of the printing of the booklets will have to be paid, which amounts €10,95[23].

Promotion costs

Promotion videos

We will film and edit the promotion video ourselves. Therefore, no costs will be involved for these videos.

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[18] http://www.xe.com/currency/bdt-bangladeshi-taka

[19] http://bikroy.com/en/ad/comfort-car-rent-a-car-for-sale-dhaka-3

[20] http://www.bpc.gov.bd/contactus.php?id=39

[21] http://siteresources.worldbank.org/EXTNWDR2013/Resources/8258024-1320950747192/8260293-1320956712276/8261091-1348683883703/WDR2013_bp_Occupational_Wages.pdf

[22] https://www.daraz.com.bd/mobile-phones/?sort=Price%3A%20Low%20to%20High&dir=asc

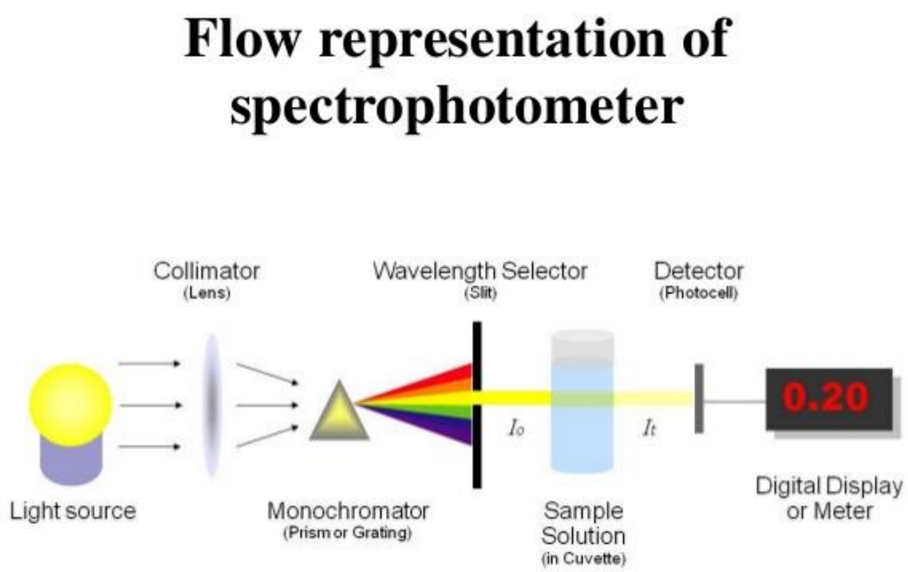
[23] https://www.drukzo.nl/boekjes-a5-8-90gsmsilk-135coversilk-250#turnaround

Website costs
We discovered that a suitable website will cost €7 per month[24] and thus €84 per year.

Revenue incomes
The revenue incomes of the investment for our clients, the farmers, will fully cover the investment costs that will have to be made for them. The remaining €1000,00, which is the minimum amount that can be received as a subsidy from the Dutch government[25] when we start our project, will enable us to cover the remaining investment cost as well as the annual costs of the first and partly the second year. We assume that by the second year our project will have gained enough publicity and thus enough sponsors to finance our project over the following years.

E. Spectrophotometry

Spectrophotometry is an effective method to analyse the absorbance and the concentration of different solutions, by using wavelengths. This method is based on the relationship between the absorbance and concentration of coloured solutions. The wavelengths of light that are absorbed are determined by the electrons in a compound. They can absorb energy, the wavelength of the light, and then become aroused. This depends on the type of atoms found in the compound. Because the colour of a species is due to its ability to absorb light, the colour should become darker or more intense as the concentration increases. An increase in concentration leads to more electrons and thus more absorption of light. This relationship is most efficiently determined using wavelengths that correspond with the colour of the compound. Therefore this method only uses visible light (390-700 nm), near-ultraviolet (above 700 nm) and near-infrared (smaller than 390 nm). Firstly, a calibration line is created to show the relationship between the absorbance of the standard solutions and their concentrations, that are known beforehand. Subsequently, the absorbance and the concentration of unknown compounds can be read from this calibration line.



A systematic overview of spectrophotometry

[24] https://www.hostnet.nl/website/website-maker
[25] http://www.ondernemersplein.nl/ondernemen/geldzaken/subsidies/horizon-2020-leiderschap/

F. Fabrication of products

From Chlorella to nourishment product

For the cattle farmers, who choose to fill the second tank with *Chlorella,* the *Chlorella* has to be dried in order to produce a powder compound. This powder can then be added to the forage as a nourishment supplement. *Chlorella* is very suitable as a nourishment product, as it contains many proteins, omega-3 fatty acids, beta-carotene, antioxidants, and vitamin A, B2 and B3. Its rich green colour comes from a high concentration of chlorophyll, which contributes to good health. Moreover, *Chlorella* has a detoxifying effect as it absorbs heavy metals and toxins. Due to these beneficial qualities, *Chlorella* is nowadays seen as one of the healthiest superfoods and food supplements. It is not only added to human nutrition, but also to forage. The algae firstly have to be harvested from the biofilm (this can be done using a spatula) and transferred to small aluminium buckets, which are heat resistant. Then the *Chlorella* has to be dried, using the heat of sunlight and the reflection of mirrors. As the average temperature in Bangladesh is approximately 34°C and there are enough hours of sunshine[26], using mirrors for heating the algae would be a very effective method. During our experiment, we examined how long this process would take in an oven and then predicted how long it would take using mirrors.

As the *Chlorella* out of the first tank will be cultivated with the nutrients of the polluted water, it will not be suitable for the production of nourishment or bio-fertiliser. Although the farmers have to harvest the grown *Chlorella*, they cannot use it on their own farms. However, in order to still increase their profit, the algae can be sold to local companies, which can produce rough oil or colouring agent from the *Chlorella.* The intermediary can collect the harvested *Chlorella* and transfer them to interested companies. The incomes will go to the farmer.

From Spirulina to bio-fertiliser

Spirulina is part of the cyanobacterial group, which contains a large amount of phosphate and nitrate. This specie could be served as a useful nitrogen source to increase crop productivity. Moreover, it is a sustainable alternative for the protection of agricultural crops. To produce the bio-fertiliser, no further fabrication process is required. The harvested algae can be spread out over the acres in its wet condition[27].

[26] approximately 8 hours from November to May and 3 from June to September
http://www.klimaatinfo.nl/bangladesh/dhaka.htm

[27] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4789255/

G. Schematic survey of the Business plan