Polymers from Microalgae Bioreactor

Raffaël Marth

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Director: Nicolas Aschman

Je déclare sur honneur avoir développé et rédigé ce mémoire sans l'aide abusive d'autrui.

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2. Introduction

Plastic has become one of the most important materials of our time. However, the conventional plastic production is based on mineral oil, which is not a sustainable resource. Biobased plastic is an alternative to this. Research is conducted to use different plants or waste products to produce plastic in the future. This project aims to provide an alternative to conventional plastic production by using microalgae as resource for plastic production.

2.1. Use of and dependency on plastic material

Plastic has become an elemental part of our modern world. More than 350 million tonnes of plastic have been produced in 2018[1]. These relatively cheap materials, offering so many variations and possibilities to be adapted to their specific use is omnipresent. Most of it is used for packaging (39,9%), other uses are construction (19,8%) or applications in appliances, mechanical engineering, furniture, textiles or the medical sector. It is further used in cars, electrical devices, as varnish, glue and many more things are made of plastic nowadays.[2]

The most common type of plastic is polyethene (PE), making up almost 30% of the plastic demand in the EU. Its primary use is packaging. It is followed by polypropene (PP) (19%), which is also mainly used for packaging. Besides these two polymers constituting about half of the plastic demand, other common types are polyvinyl chloride (PVC), polyurethane (PUR), polyethene terephthalate (PET) and polystyrene (PS).[3]

While it may not be necessary or reasonable for all of these products to be made of plastic, it certainly plays a key role in our everyday life and we will resort to this fantastic material many times in the future too. While a world with less plastic, and most importantly less single use plastic and plastic pollution is definitely a good idea, a world completely without plastic is unimaginable.

2.2. Problems of conventional polymer production

The majority of the polymers we produce are based on petroleum. The use of petroleum however leads to several ecological, but also political problems.

2.2.1. Limitation of fossil resources

Firstly, oil is a fossil resource and therefore limited. Although the world oil reserves still consist of over 200 trillion litres, at the current exploitation rate, they will drain in about 50 years[4]. In truth, they will likely last longer, as the word *reserves* refers to economically exploitable oil, so new technologies or rising oil prices would increase the reserves and some new reserves might also be discovered. Nonetheless petroleum is a limited resource of which we will run out in the near or distant future.

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[1]PlasticsEurope, Plastics – the Facts 2019, from: plasticseurope.org, URL: https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts2019_14102019.pdf (consulted 03.05.2020), p. 14

[2]Beckman, Eric, The world's plastic problem in numbers, from: weforum.org, URL: https://www.weforum.org/agenda/2018/08/the-world-of-plastics-in-numbers (consulted: 01.05.2020)
Wikipedia, Kunststoff, URL: https://de.wikipedia.org/wiki/Kunststoff (consulted 01.05.2020)
Percentages, referring to EU, Norway and Switzerland, according to:
PlasticsEurope, Plastics – the Facts 2019, from: plasticseurope.org, URL: https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts2019_14102019.pdf (consulted 03.05.2020), p. 20

[3] Ibid. p. 22

[4] Calculation based on OPEC Annual Statistical Bulletin, 54th edition, Vienna 2019, p. 26&31

2.2.2. Carbon footprint

Secondly, petroleum has stored carbon for millions of years. Extracted from the soil this carbon is now susceptible to be released into the atmosphere as carbon dioxide. Although this is rather a problem when the oil is burned, for example as fuel, the polymers might also be burned or they will eventually decompose, thereby releasing carbon dioxide. This carbon dioxide will then further contribute to the greenhouse effect and enhance global warming.

2.2.3. Geopolitical considerations

Furthermore, the exploitation of oil is dependent on oil deposits. These are not evenly distributed around the world, making countries without oil resources dependent on supply from others. This becomes even more problematic when large oil reserves are located in politically unstable regions or when interests in oil become part of war objectives.

The resulting long transport routes further increase environmental damage caused by oil usage. The vehicles or ships used for transport consume energy and the construction of oil pipelines leads to further logistical and ecological problems.

2.2.4. Ecological footprint

Also, the accidental liberation of crude oil into the environment causes massive damage to ecosystems. Oil contains toxic components which may lead to intoxication of animals ingesting it. Also smothering of feathers and fur poses problems to animals as it reduces its isolating capacities. Seabirds are especially vulnerable as their buoyancy is also reduced. Furthermore, some fragile ecosystems may also suffer by losing a key species. For example, coral reefs are highly dependent on the microorganisms building the corals, if those die the whole ecosystem is altered extremely. Another example are mangroves which depend on salt water resistant trees, which also suffer from oil pollution.[5]

2.3. Advantages of biobased polymer production

The use of alternative sources for polymer production can solve these problems. The main alternatives are the use of waste products, crops and algae. This project will focus on the use of microalgae.

One of the key advantages of these alternative resources is that they are renewable. In contrast to oil, we will never run out of plants, as long as environmental conditions allow for their growth and cultivation. Thereby, humanity will have access to polymers (as well as other now petrochemical products) in the future too.

Another advantage of biobased polymers is that their production fixes carbon. While the plants grow, they absorb carbon dioxide from the atmosphere. As they build up their biomolecules, they bind the carbon obtained from the carbon dioxide therein. As long as the polymers obtained from those plants exist, the carbon is bound. So not only do they not contribute to the greenhouse effect, they can even help prevent excessive greenhouse effect by removing excess

[5] Water pollution, Oil Pollution in Water, from: .water-pollution.org.uk, URL: https://www.water-pollution.org.uk/oil-pollution-in-water/ (consulted: 02.04.2020)
Wikipedia, Oil spill, URL: https://en.wikipedia.org/wiki/Oil_spill (consulted: 02.04.2020)
Ramseur, Jonathan L., Oil Spills: Background and Governance, from fas.org, URL: https://fas.org/sgp/crs/misc/RL33705.pdf (consulted 02.04.2020)
Wikipedia, Ölverschmutzung, URL: https://de.wikipedia.org/wiki/Ölverschmutzung (consulted 02.04.2020)
ITOPF Ltd, Effects of oil pollution on the marine environment, from: itopf.org, URL: https://www.itopf.org/fileadmin/data/Documents/TIPS%20TAPS/TIP_13_Effects_of_Oil_Pollution_on_the_Marine_Environment.pdf (consulted 02.04.2020)

carbon dioxide from the atmosphere. If eventually they get burned or decompose, the carbon is again released into the atmosphere, however it only releases carbon dioxide that was previously bound, opposed to oil which adds carbon dioxide to the carbon cycle which was not part of it for millions of years.

Then, plants, especially microalgae have the advantage that they are not so resource dependant as oil. Plants can be grown in many places of the world. The cultivation of microalgae needs only two primary resources, carbon dioxide which is present in the air on the whole planet and water which is also quite abundant on earth. In contrast to land plants, they do not even need soil, which makes it possible to cultivate them in even more places, where other plants couldn't grow.

This is also a further advantage of microalgae over land-based plants for polymer production. They do not compete with the production of food and they do not rely on the presence of fertile soils. Also, they can be grown in immediate proximity of the manufacturing facilities that use them, thereby enormously reducing transport routes.

To conclude, the use of biobased resources for polymer production solves several of the problems resulting from the use of oil, such as the limited availability of fossil resources, the release of additional carbon dioxide into the atmosphere and the dependency on resources. Microalgae furthermore offer the advantage over land plants of not being dependent on fertile soil.

2.4. Biology of microalgae

Microalgae are a polyphyletic group of unicellular photosynthetic eukaryotic organisms. They are phototrophic, meaning they get their energy by using light for photosynthesis thereby converting inorganic matter into organic matter. They lack typical features of land plants such as roots, stems or leaves[6]. They offer huge biodiversity with 200 000 to 800 000 species[7]. They play a very important role for the marine ecosystems and the whole biosphere, by being responsible for the production of about half of the atmospheric oxygen and by serving as food for various organisms such as brine shrimps, copepods, rotifers as well as larvae of crustacean, fish and molluscs, which are in turn eaten by larger organisms.[8]

As unicellular organisms they reproduce by dividing. This leads to exponential growth of algae cultures. In general, an algae culture goes through five phases.

Lag phase: At the beginning the growth of the culture is low as the cells are still adapting to their new environment and preparing for division.

Exponential phase: Once the cells have adapted to the environment and have prepared for division, the exponential phase starts. During this the algae reproduce exponentially

Linear phase: Later scarcity of nutriments slows the culture's growth to being linear.

[6] The Bio2 Solution, What Are Microalgae?, from: bio2solution.com, URL: www.bio2solution.com/how-why-it-works/what-are-microalgae (consulted: 04.05.20209)

[7] Wikipedia, Microalgae, URL: https://en.wikipedia.org/wiki/Microalgae (consulted: 04.05.2020)

[8] Adarme-Vega, T Catalina & Lim, David & Timmins, Matthew & Vernen, Felicitas & Li, Yan & Schenk, Peer. (2012). Microalgal biofactories: A promising approach towards sustainable omega-3 fatty acid production. Microbial cell factories. 11. 96. 10.1186/1475-2859-11-96

Stationary phase: At one point the culture will reach the maximum cell number the medium can hold. This does not mean that no divisions happen, but rather that the death rate and growth rate are equal.

Death phase: Eventually the death rate will exceed the growth rate and the number of cells will decrease.[9]

2.5. Current use of microalgae

2.5.1. Nutrition

Microalgae have applications as food supplements. They are considered a source of protein, omega-3 fatty acids and vitamins. Another use includes the use of algal pigments such as carotenoids or chlorophyll as natural colorants. Products derived from microalgae are thus used either as ingredients for food production or microalgae powder is sold as dietary supplement. They may also be a solution to guarantee sufficient protein supply in the future for a growing world population[10]

2.5.2. Biofuel

Microalgae are seen as an alternative to oil-based fuel. They can be used to produce oil which can then be manufactured into biofuel. It is possible to obtain fatty acid methyl esters trough transesterification of the fatty acids contained therein, which can be used as biodiesel.[11]

2.5.3. Bioplastic

Another highly oil dependant area is the production of plastic. There are several possible applications of microalgae in this sector that are subject to research and a few that have been commercialized. One approach is to blend algal biomass with other already available plastic types (petroleum-based or biobased). Another possible application is the synthesis of polyhydroxybutyrate (PHB), a natural polyester that some bacteria and microalgae produce under certain conditions. Genetical engineering can also be part of the plastic production process from microalgae, either by using genetically modified algae to produce polymers or by using algal biomass as feedstock for other recombinant organisms leading to plastic production. Finally, there is the biorefinery approach, similar to the current treatment of oil, using different refinery treatments to produce a variety of chemicals, some of which can then be used for polymer production.[12]

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[9] Bassiri, Eby, BACTERIAL GROWTH CURVE, from. sas.upenn.edu, URL: https://www.sas.upenn.edu/LabManuals/biol275/Table_of_Contents_files/6-BacterialGrowthCurve.pdf (consulted01.05.2020)
Fernandez Sevilla ,Jose M. , Microalgal Growth Kinetics, from: w3.ual.es, URL: https://w3.ual.es/~jfernand/MBio70411204/Lesson2/L2.1.html (consulted 01.05.2020)

[10] Becker, W. (n.d.). Microalgae in Human and Animal Nutrition. Handbook of Microalgal Culture, 312–351
Robert A. Kay & Larry L. Barton (1991): Microalgae as food and supplement, Critical Reviews in Food Science and Nutrition, 30:6, 555-573

[11] Pienkos, Philip T. et al., Making Biofuel from Microalgae, from: americanscientist.org, URL: https://www.americanscientist.org/article/making-biofuel-from-microalgae (consulted 01.05.2020)
Wikipedia, Biodiesel, URL: https://de.wikipedia.org/wiki/Biodiesel (consulted 01.05.2020)
Contact: Bioenergy Education Initiative, from: agsci.oregonstate.edu, URL: https://agsci.oregonstate.edu/sites/agscid7/files/bioenergy/education/algae_final_interactive.pdf (consulted: 01.05.2020)

[12] Rahman, A., & Miller, C. D. (2017). Microalgae as a Source of Bioplastics. Algal Green Chemistry, 121–138
Abdo, S.M., Ali, G.H. Analysis of polyhydroxybutrate and bioplastic production from microalgae. Bull Natl Res Cent 43, 97 (2019)

2.5.4. Other uses

Other applications of microalgae include the incorporation in cosmetic products or medicine. Furthermore, microalgae can be used to gain energy, for example by using algal biomass in a biogas plant. Another usage is wastewater treatment, the microalgae are then used to fixate phosphor and nitrogen compounds. The latter can be combined with the other applications, using wastewater as culture medium, thereby cleaning it and in addition producing biomass.[13]

2.6. Overview of polymer production

Polymers are long chain-like molecules. Typically, they are produces from repeating subunits, the monomers. There are three principal chemical reactions used for this purpose: chain-growth polymerization, polycondensation and polyaddition. The first two shall be briefly presented here as they might be of use for the project.

2.6.1. Chain-growth polymerization

Classical chain-growth polymerization requires monomers with double bonds. Those can be broken, enabling the monomers to bond with other monomers. The polymer chain-growth reaction needs one initiator which initiates the reaction and then the reaction continues whilst one monomer after the next gets added to the chain until the reaction eventually stops.

The process shall be explained by the example of the production of polyethene from ethene, the most basic organic molecule with a double bond. There are several possibilities to start the reaction, including the use of cations or radicals. The latter will be used as example: radicals are atoms or molecules with unpaired electrons, which makes the extremely reactive as unpaired electrons are not a stable configuration.

In the first step of the reaction, the radical is added to the ethene. Only a few molecules of radical are needed as the reaction later continues in a chain reaction. The radical attaches itself to one ethene molecule, breaking the double bond. One of the two carbon atoms originally constituting the ethene molecule is now bound to the former radical, the other one however now lacks a bond and has therefore an unpaired electron. The new molecule is again a radical. This radical will now again bond with an ethene molecule, breaking its double bond. The chain will

"Science for Environment Policy": European Commission DG Environment News Alert Service, edited by SCU, The University of the West of England, Bristol, URL: https://ec.europa.eu/environment/integration/research/newsalert/pdf/273na2_en.pdf (consulted: 01.05.2020)

Diaz, Jesus, The Creators Of This Algae Plastic Want To Start A Maker Revolution, from: fastcompany.com, URL: https://www.fastcompany.com/90154210/the-creators-of-this-algae-plastic-want-to-start-a-maker-revolution (consulted: 01.05.2020)

[13] Abdel-Raouf, N., Al-Homaidan, A. A., & Ibraheem, I. B. M. (2012). Microalgae and wastewater treatment. Saudi Journal of Biological Sciences, 19(3), 257–275Contact: Bioenergy Education Initiative, from: agsci.oregonstate.edu, URL: https://agsci.oregonstate.edu/sites/agscid7/files/bioenergy/education/algae_final_interactive.pdf (consulted: 01.05.2020)

Minhat, Z.B.T. & RAHAMAN, MUHAMMAD & Takriff, Mohd & Kofli, Noorhisham. (2016). Differentiation of biomass composition between isolated and commercial strains of microalgae. Journal of Engineering Science and Technology. 11. 737-744.

grow adding more and more ethene molecules until eventually two radicals react, pairing up their unbonded electrons and thus stopping the reaction.

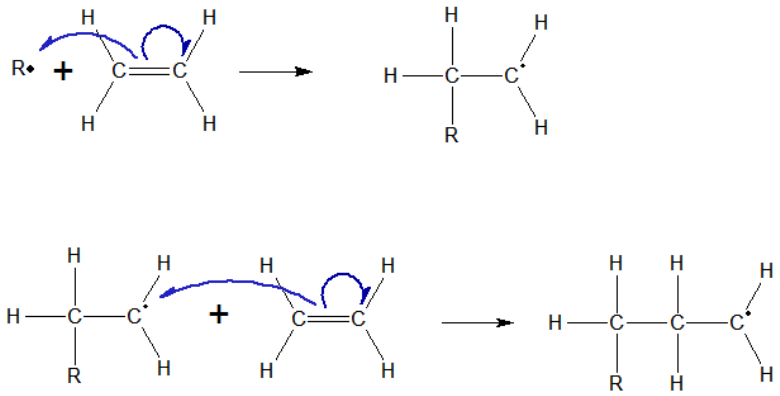


Image 1: Reaction mechanism of chain-growth polymerization

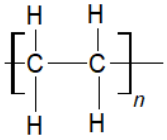


Image 2: Polyethene

2.6.2. Polycondensation

A condensation reaction[14] is a chemical reaction, where two molecules bind by liberating a small molecule, often water. During polycondensation, this happens multiple times and leads to the formation of polymers. In opposition to the polymer chain-growth reaction, monomers are not added one after the other to a growing chain, but rather many reaction between only two monomers take place, building dimers which then again react to build bigger molecules which can react again. The growth of the chains is therefore not continuous but happens at many places at the same time and suddenly polymers emerge.

To allow polycondensation, the monomers need functional groups that can react together in a condensation reaction. The molecules need to have at least two functional groups, with only one they could just combine with a single other monomer, which would make it impossible to obtain chains.

One example of a polycondensation is the formation of polyesters from alcohols and carboxylic acids. This ether requires monomers containing an alcohol group and an acid group or the se of two different molecules, one containing two acid groups and the other containing two alcohol groups. Lactic acid contains both these groups and can therefore undergo polycondensation, leading to polylactide (PLA). The formation of polyesters requires an energy input, which can be given by heating and strong acids can be used as catalysts.

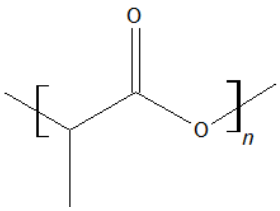


Image 3: PLA

[14] Not to be confused with the physical process of condensation, meaning the liquefaction of a gas

During the reaction, the acid group of one molecule reacts with the alcohol group of another one. This leads to the formation of an ester bond. The newly formed ester still has another available alcohol group and another available acid group. These can react again and after many such reactions a polymer is obtained.

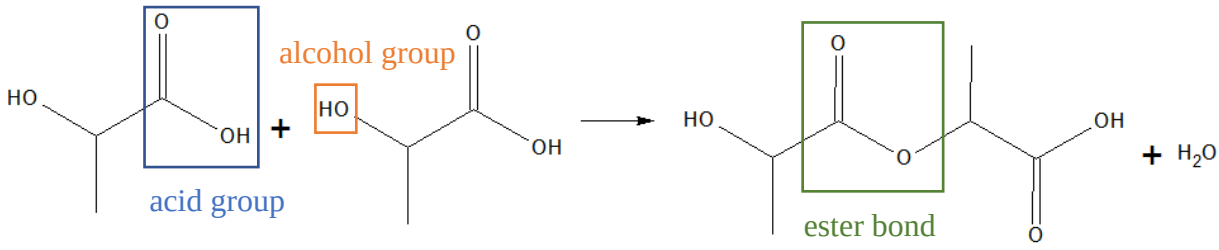


Image 4: polycondensation reaction mechanism

3. Objectives

The goal of this project is to create plastic from microalgae. This shall be achieved by the following steps:

First it is necessary to grow the algae and therefore build a reactor, produce growth medium and measure the algae's growth kinetics in order to maintain the cultures properly.

Once algae can be cultivated successfully, the produced biomass needs further processing: After harvesting the microalgae the different components will be extracted from them and those suitable for polymer production need to be isolated.

Carbohydrates may be used as starting point to produce monomers. They ca be fermented to ethanol or lactic acid. From ethanol one can obtain ethene by dehydration. From this ethene polyethene can then be produced. This route from ethanol to polyethene offers the advantage that it is already used on industrial scale and leads to a common polymer with wide applications. Another use for the carbohydrates might be the fermentation to lactic acid. This can then be used to produce polylactide.

Another major constituent of microalgae is fat. The triglycerides could be split into glycerol and fatty acids by saponification. The obtained glycerol could then be used as part of a copolymer with another monomer or maybe it might be further transformed to obtain a monomer suitable for plastic production. The fatty acids are more difficult to use for polymer production, as they only contain one functional group. One idea regarding their use could be to try to apply cracking to them. This is a chemical process that breaks down long molecules to shorter ones, which will contain double bonds and can therefore be used for polymer production.

4. Bioreactor design and construction

4.1. Materials

- Plexiglas tubes
- Support material
- Rubber plug
- PVC-Pipes
- Taps
- Plexiglass caps
- Hoses
- Distributor
- Air stone bubblers
- Aluminium foil

4.2. Designs

4.2.1. First Ideas

For the algae to be able to grow, they essentially need water, carbon dioxide and light. The bioreactor must provide them with these. In addition to this, they also need lesser quantities of nitrogen and some minerals, which are provided by the culture medium

There are many ways to set up an algae bioreactor. It is possible to grow algae in large open ponds, which is seen as low cost option and easy to operate having the drawback of being exposed to the environment, being therefore subject to many varying factors, leading to a variety of different treats to the culture as well as poor control over it. The other possibility is to grow the algae in a closed system. These make it possible to grow algae in controlled lab conditions and therefore it was opted for this possibility in this project. Furthermore, closed reactors can save space by vertical design and may be used as parts of buildings in the future.

The main material for the reactor is plexiglass. It is permeable for light of the visible spectrum, which the algae need. It lets through 92% of the incoming light. Additionally, it is rather robust and affordable. The light input also depends upon the shape and size of the reactor. Tubular reactors for example allow light to enter from every direction at the same conditions. The diameter of the tubes can also be of interest, if the tubes are too thick, denser cultures will not allow the light to penetrate the whole depth of the reactor. [15]It was opted for a reactor build of multiple plexiglass tubes, each having a volume of about 1 L. The total reactor volume with 10 tubes would then be about 10 L. Using multiple tubes makes it possible to start with a smaller culture and gradually expand the reactor.

Another element of the reactor is an air pump. As the reactor is closed, it is necessary to supply the algae with carbon dioxide, this is done by pumping air into the culture. One could also use pure carbon dioxide, however using air was simpler. Besides supplying the algae with carbon

[15] Fernandez Sevilla ,Jose M. , Microalgal Growth Kinetics, from: w3.ual.es, URL: https://w3.ual.es/~jfernand/MBio70411204/Lesson1/L1.4.html (consulted 01.05.2020)

dioxide, using a pump also brings the benefits of creating some flow inside the reactor and preventing the algae from sedimentation.

As air naturally moves upwards inside water, the easiest setup is to pump air into the reactor at the bottom and let it flow through the culture to the top of the reactor. This however leads to two possible designs: One possibility is to place the tubes vertically next to each other. This though makes it necessary to use multiple pumps or split up the airflow of one pump in order to connect every tube to the aeration. Thus, another design came into consideration. One could also put the tubes (almost) horizontally on top of each other. This way, a single air input at the bottom of the first tube would be enough to supply the whole reactor, as the air would then flow from this point upwards through the whole bioreactor.

4.2.2. First provisional setup

Whilst trying to implement the first idea, it got clear that the solution with horizontal tubes is not as easy to build as assumed. It would be complicated to connect the tubes, as the connection would need to have an angle of a bit less than 180, fit waterproof on the tubes and ideally be transparent, as otherwise major parts of the culture would not receive light.

Therefore, the actual bioreactor got build with the vertical setup. Three tubes where connected by a PVC-tube which would also allow to add a tap through which the whole reactor could be harvested at once. Air was provided by hoses entering the reactor from the top and releasing the air at the bottom between the tubes in order to provide all the tubes with air. The tubes were closed off at the top with aluminium foil.

However, this design proved unsuitable. As a culture was put into it, after about a week, the reactor turned almost transparent. The algae were not multiplying as they should, but rather the concentration of algae cells seemed to approach zero.

Image 5: First reactor setup

The problem turned out to be that the algae sedimented and as the bottom part of the reactor was not transparent, they did not get enough light anymore.

4.2.3. Last provisional setup

As it proved unpractical to have an opaque reactor part, the design was readjusted. In the new design, the tubes were not connected anymore and just closed with a rubber plug. This way, even if the algae sedimented they would still get light. This made aeration more complicated as every tube would need its own air supply and it would also not be possible to empty the whole reactor an once, however this also provides the opportunity for future experiments to run several algae cultures in different tubes at different conditions. Again, the tubes were closed at the top with aluminium foil.

In order to supply every tube with air a distributor was added.

Another adjustment to prevent sedimentation, was the addition of air stone bubblers. In the first setup, the air just formed a sequence of big bubbles that were not effective to prevent sedimentation. Having a continuous stream of small bubbles turned out to be more effective to prevent sedimentation.

The reactor was first set up with 3 tubes, 2 more have been added consecutively later.

Whilst the intensity of the green colour of the cultures visually augmented, in some of the tubes, the aeration ceased after some time (as this happened during holidays, it is not known when it happened exactly). It turned out that the majority of the air was flowing into one single tube. To distribute the air more evenly, those hoses where pinched off with paperclips. This reduced the airflow in these hoses and increased it in the other ones.

Another problem with this reactor design was that the water in the tubes evaporated. In a final design, the tubes should therefore be properly closed, so that no water can leave them. Also, it was difficult to harvest the algae from this reactor as it was not possible to open the tubes at the bottom in a controlled manner. Therefore, it was only possible to pipette from the top or to flip the whole tube. In the final design, taps for every tube are therefore necessary.

Image 6: Reactor directly after building it

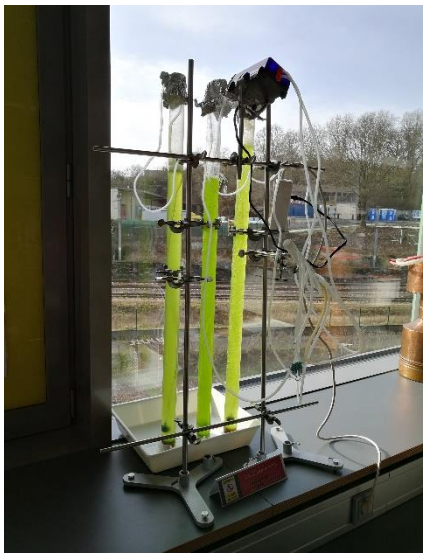


Image 7: Reactor after 3 weeks

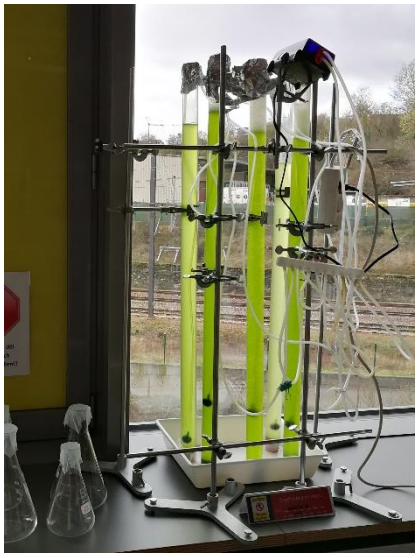


Image 8: Complete reactor on the 13th March

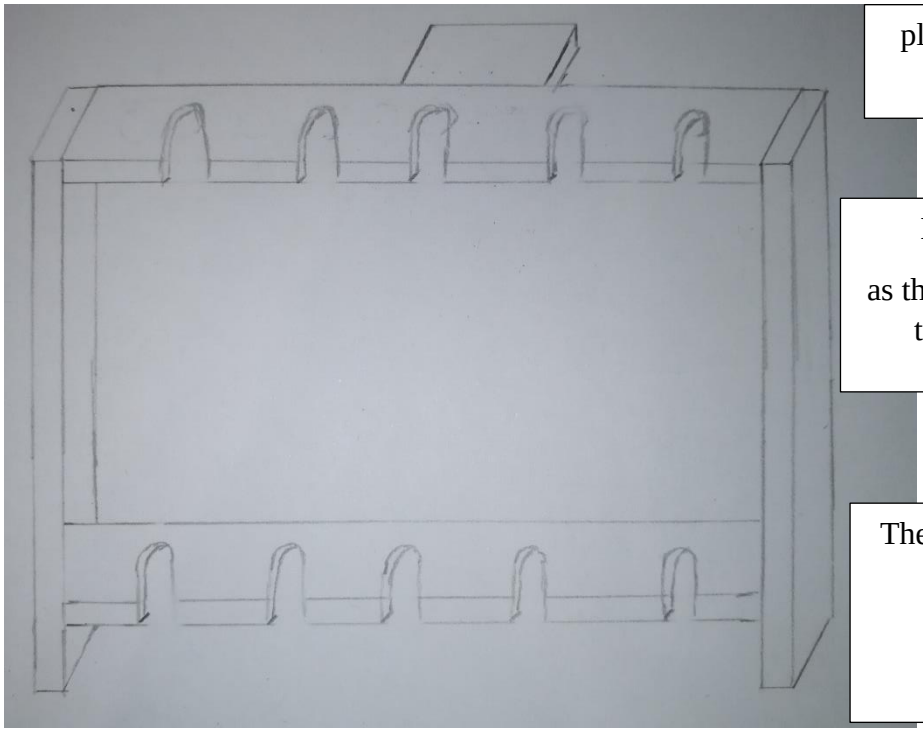
4.2.4. Final design

The preceding setups made clear, which elements are important for proper reactor function: The air supply must come in little bubbles to prevent sedimentation and every form of opaque parts should be avoided. Furthermore, it is necessary to close the top of the tubes properly to eliminate evaporation. To allow an easy harvest, it is necessary to have a tap at every tube. In addition to this I would also like to add a sterile filter to the air supply. This was already an idea for the previous setup, however the filter did not fit properly on the houses and I have not yet found an adequate solution.

For the final setup, the tubes shall be closed with affixed plexiglass caps, with a tap in the bottom one. Furthermore, is shall be possible to tilt the whole reactor in different angles to adapt it to the current angle of the sun at every moment for optimal light absorption.

It is intended to have a wooden frame built for the final reactor. The construction of it could not be started due to the outbreak of the COVID-19 pandemic and here are only the first sketches:

This is the basic component of the reactor. It is a frame to hold the tubes. The tubes lie inside the holes, which will also have some sort of fastener on the front side to prevent the tubes from falling out of the frame:



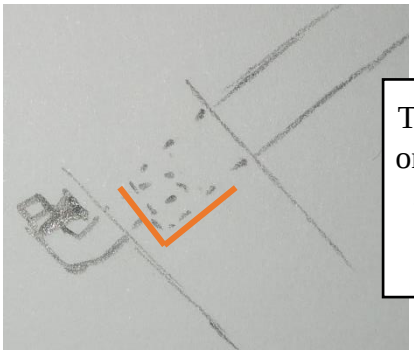
platform for air pump

Holes fort the tubes,

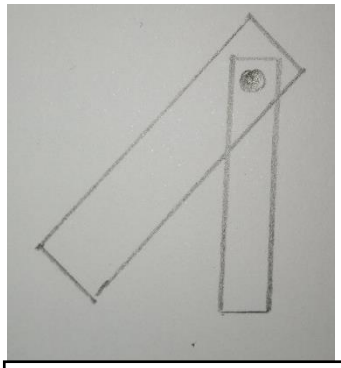
as the reactor is inclined, the tubes will lie in these

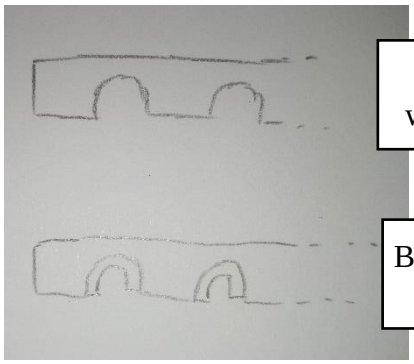
The bottom holes will have a rim inside (not shown on sketch) to prevent the tubes from falling through

The bottom holes will differ from the top ones by a rim inside, which holds the tubes in place and allows the tap to pass through . The whole frame shall be fixed to another part which makes it possible to incline the reactor at different angles.



The region highlighted in orange will be the contact area of the tubes inside the frame part





Top part of the frame with holes for the tubes

Bottom part with inner rim to hold the tubes vertically

Frame shown from the side, with second part to control inclination

5. Culture management

5.1. Sterilization

To prevent contamination of the algae cultures sterilizing the material and working under sterile conditions was important.

The medium as well as smaller containers for cultures were sterilized by autoclavation. Larger flasks and the reactor tubes were treated with oxidizing agent (hydrogen peroxide, Chemipro OXI) The hoses for the air supply were decocted in boiling water and fragile parts as the air stone bubblers have been rinsed with distilled water. All the operations with the cultures have been performed under a Bunsen burner. (This was not possible with the reactor anymore, as it was impractical to get a Bunsen burner over it, however the cultures were also larger at that moment and therefore less vulnerable)

5.2. Cultures

A start culture of *Chlorella vulgaris* algae was purchased from Sordalab.

In order to maintain cultures over prolonged periods and to ensure maximal biomass production it is important to dilute the cultures at the right moment, thus adding nutriments and reducing cell concentration. Thereby the culture can be kept in the exponential phase.

Basically, cultures were started by 10x diluting 1 ml of the purchased algae and later maintained by 10x diluting either to larger volumes or by discarding the rest. See the following explanations for details about the different cultures.

A first test culture (named FTC) was obtained on the 19.11.2019 by diluting 1 ml of the original culture with 9 ml of medium. The culture was placed in a reactor tube.

On the 5th December, 5 new cultures have been started by diluting 1 ml of the original culture in 9 ml of medium. These cultures where labelled C1-C5.

On the 27th November from C1 respectively 1 ml was taken and diluted to 10 ml to obtain 10 cultures labelled C1.1-C1.10. C4 and C5 were diluted by discarding 9 ml and replacing them by 9 ml of fresh medium.

On the 2nd December C4 and C1.1 were added to 100 ml medium in an Erlenmeyer flask respectively. The same was done with C2 on the 5th December.

To prevent sedimentation, the cultures were placed on a shaker on the 16th December. From that day on, all cultures that were still used were placed on the shaker.

Image 9: Cultures on shaker

On the 20th December C2 was moved into a larger flask containing 1 L of medium. C3 was diluted to as much as fitted into the Erlenmeyer flask which should be about 300ml. A new culture labelled CB was started.

Image 10: Diluted cultures on the 20th Decmber, showing the three stages of a growing culture

On the 6th January respectively 90 ml of C1.1 and C4 were put into an Erlenmeyer flask labelled EXP. This was used in to conduct experiments without fear of contamination of a culture. The cultures were refilled with 90 ml of fresh medium.

C2 was transferred into the reactor on the 10th January, 3 L of medium were added.

On the 17th January C3 was transferred to a larger flask with 1 L medium.

CB was transferred to an Erlenmeyer flask containing 100 ml of medium in the 24th January.

On the 2nd January about half of C3 was added to the reactor because it was nearly transparent, the rest of the culture was refilled with fresh medium.

On the 7th February, the new reactor setup was finished and started to run with algae from C3.

The reactor was expanded with a 4th tube on the 2nd March, by taking 25 ml of culture from every of the three other tubes and filling the 4th tube up with fresh medium. The same way a 5th tube was added o the 6th March.

On the 13th March, most of the culture contained in the reactor was moved into Erlenmeyer flasks which got put into the fridge. The reactor got diluted again. This early harvest was necessary, because the schools were about to close for 2 weeks due to the COVID-19 pandemic. As later became clear that the confinement would last much longer, all that was left into the reactor got put into the fridge.

Image 11: Cultures before being put into the fridge

Initially it was intended to dilute the cultures according to the established growth kinetics. As this failed to be achieved (see 6.1 and 6.2), it was judged by colour when solutions would be diluted. Thereby, the cultures were usually diluted in intervals of about 2 weeks. Often cultures were diluted right before a holiday, so that they would not enter death phase during the holiday and in order to somehow use this time where no other experiments could be done to a least multiply the algae.below

5.3. Medium

The microalgae need to grow in some sort of medium. This must supply them with nitrogen and the minerals they need to grow

A culture medium was prepared by adapting the following procedure from "Das grosse Kosmos-Buch der Mikroskopie"[16].

The stock solutions were all prepared on the 14th November 2019 and diluted, when needed.

5.3.1. Substances

Sodium nitrate ($NaNO_3$), calcium chloride ($CaCl_2$) magnesium sulphate ($MgSO_4 \cdot 7\ H_2O$), dipotassium phosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4), natrium chloride ($NaCl$)

5.3.2. Method

1. Dissolve in respectively 400 ml H_2O the given amount of the following nutrient salts: 10 g $NaNO_3$, 1 g $CaCl_2$, 1 g $MgSO_4 \cdot 7\ H_2O$, 3 g K_2HPO_4, 7 g KH_2PO_4, 1g $NaCl$

2. Of each of these stock solutions add 10 ml to 940 ml distilled water and add one drop of a 1% aqueous solution of iron chloride ($FeCl_3$)

5.3.3. Adaptations

Some of the substances were not present in the lab and thus replaced:

The dry calcium chloride was replaced by 1,46 g of $CaCl_2 \cdot 2\ H_2O$.

K_2HPO_4 was not available, instead the same quantity of g KH_2PO_4 was used.

As no $NaNO_3$ was available, it was prepared from ammonium nitrate and sodium hydroxide:

$NH_4NO_3 + NaOH \rightarrow NaNO_3 + NH_4OH$

Therefore 7,8 g of NH_4NO_3 and 3,7 g of NaOH were dissolved in distilled water respectively. Then the two solutions were mixed. As NH_4OH degrades into ammonia and water, the solution was put under the fume hood overnight. The obtained solution was then diluted to 400 ml with distilled water.

[16] Kremer, Bruno P. Das grosse Kosmos-Buch der Mikroskopie, 3th edition, Franckh Kosmos Verlag, 2015

6. Experimental Procedures

6.1. Measurement of growth 1

In order to operate the reactor properly, it is important to know the growth kinetics of the algae. These help to determine when it is necessary to dilute the cultures or harvest them. It is also of interest to know how fast the algae actually reproduce.

One possibility to determine how fast the algae reproduce and when their number doesn't increase anymore, is to count the algae cells in a given volume. This count can be performed under a microscope with a Neubauer chamber (see 9.7. Neubauer chamber) and directly indicates the concentration of cell in the culture. This concentration can then be used to establish growth kinetics.

6.1.1. Method

For cell counting using a Neubauer chamber, 100 µL of culture are diluted with 900 µL medium in an Eppendorf tube in order to obtain a 10x diluted solution. A drop of methylene blue is added and the solution is mixed. One drop of solution is then placed in the Neubauer chamber for counting. The cells are counted in the middle square of the Neubauer chamber. Cells on the inside of the square and such touching the left or upper lines are counted. Except where indicated, counting was performed 4 times with every sample. As the Neubauer contains 2 chambers, it is rinsed with water and isopropanol after every 2 counts. A clicker is used to keep track of the counting.

6.1.2. Observations

date	culture	dilution	count 1 (cells)	count 2 (cells)	count 3 (cells)	count 4 (cells)	average (cells)	original concentration (cells/ml)
19.11.2019	Purchased culture	10	280	139	82	486	247	2,47E-06
20.11.2019	FTC	10	42	142			92	9,20E-06
21.11.2019	FTC	10	278	254	108	277	229	2,29E-05
22.11.2019	FTC	10	182	473	207	258	280	2,80E-05
25.11.2019	FTC	10	304	188	195	165	213	2,13E-05

date	culture	dilution	count 1 (cells)	count 2 (cells)	count 3 (cells)	count 4 (cells)	average (cells)

29.11.2019	C4	10	63	91	75	86	79
29.11.2019	C5	10	107	100	73	86	92
29.11.2019	C2	10	171	252	258	216	224
02.12.2019	C4	10	1206	1083			1145
03.12.2019	C1.7	10	376	252	236	209	268
04.12.2019	C2	10	109	111	153	226	150
05.12.2019	C2	10	231	251	267	201	238
05.12.2019	C1.7	10	287	264	302	276	282
05.12.2019	C1.1	10	219	148	32*	51*	113
05.12.2019	C2**	10	20	57			39

*On the 05th December was observed, that even if the count was performed on a blank Neubauer slide, there were still many structures that looked like cells. Therefore, the slide was rinsed multiple times after the counting with water and isopropanol. It was also observed, that by focusing differently, different cells, seemingly on different levels appeared, thereby leading to different counts. The marked counts were performed after this rinsing procedure

** This count was performed on C2 after diluting the culture from 10 ml to 100 ml.

6.1.3. Interpretation

The different counts do not seem coherent. The measurement of C4 on the 29th November shows a multiplication of cell number by roughly 15. This is much more than the expected growth. Also the measurements of the 5th December show irregularities: C1.7 should actually be 10 times more diluted than C2, leading to an expectation of a cell count 10 times lower. However, this is not the case, the cell number in C1.7 is even higher than the one in C2. Additionally to this, C2 also optically looked greener, suggesting it to be denser, as it should actually be. This lead to suspicions regarding the reliability of the method. Also, the second count on C2 was performed after diluting the culture, which should result in a 10 times lower cell count, which is not the case. The facts that even on blank slides where counted cells and that cells seemed to be on different levels further invalidates the measurements. The later could be due to the algae not sinking onto the ground of the chamber and instead floating inside it.

6.1.4. Conclusion

The irregular and unexplainable measurements as well as the other problems encountered show that the used method is not reliable.

6.2. Measurement of growth 2

As counting the cells in the Neubauer chamber turned out to be unreliable, a different method for measuring cell growth was adopted. The density of the cultures was measured through spectroscopy. Cells absorb light at a wavelength of 600 nm. The absorption rate is proportional to the density of cell in the culture. As this method does not allow for absolute cell count, growth rate can nevertheless be measured.

6.2.1. Method

Cell growth is determined by measuring the optical density of cultures at a wavelength of 600 nm (OD_{600}) using a visible-light photospectrometer. First the spectrometer is zeroed with medium for absorption at a wavelength of 600 nm. Then 2 mL of culture are placed in a cuvette. Absorption at 600 nm is measured. Afterwards, 1mL is removed from the cuvette and 1 mL of medium was added. The solution is mixed and measured again. This step is repeated in order to get a total of 3 measurements, at 1x, 2x and 4x dilution respectively.

6.2.2. Observations

		OD600	
date	dilution	C2	C3
10.12.2019	1x	0,128	0,082
	2x	0,074	0,048
	4x	0,024	0,026
12.12.2019	1x	0,141	0,090
	2x	0,075	0,049
	4x	0,040	0,026
13.12.2019	1x	0,152	0,111
	2x	0,101	0,067
	4x	0,083	0,042
16.12.2019	1x	0,217	0,122
	2x	0,144	0,085
	4x	0,072	0,065
	1x		0,118
	2x		0,065
	4x		0,036
17.12.2019	1x	0,223	0,146

	2x	0,115	0,081
	4x	0,054	0,093

The experiment could not be continued after this date because the lab ran out of cuvettes.

6.2.3. Interpretation

Except for the last measurement of the 17th December, the results look rather reasonable. Due to the limited amount of data it is not possible to establish a proper growth curve, all that can be said is that the daily growth rate for both cultures is about 10% during the observed period.

6.2.4. Conclusion

The experiment should be repeated over a prolonged period in order to establish a proper growth curve. The used method seems reliable.

6.3. Estimation of dry mass

To know what the yield of an eventual algae harvest would be, respectively to know how much resources could be gained from the reactor, it is important to know, how much biomass it contains. To determine this, samples of algal solution can be dried by evaporation, leaving behind the algal biomass which can then be weighed. To add a second measurement, one can then also remove the biomass from the beaker after drying and by measuring the difference of the beakers mass before and after, calculate how much mass was removed from the beaker, this being equal to the mass it previously contained.

6.3.1. Method

A beaker has been weighed before and after adding the culture EXP (see p. 17).

The sample is then heated to boiling until all liquid has evaporated.

After cooling down to room temperature the beaker is weighed again.

The beaker is then placed in the oven at 120°C for a least 24 hours and weighed again.

The experiment was repeated twice, the second time the beaker was additionally cleaned after drying in the oven, by scraping the sides. It was weighed again. Finally, it got rinsed with water, put in the oven and afterwards weighed another time.

6.3.2. Observations

The first time the experiment was performed, 10 ml of culture EXP were used.

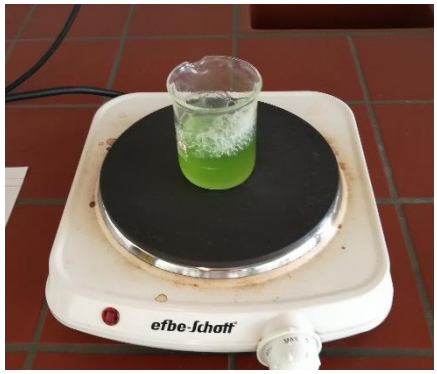


Image 12: Algae during heating

Weight of beaker: 45,417 g

Weight of beaker with algae culture: 55,200 g

Weight of beaker after cooling down: 45,409 g

Weight of beaker after drying 4 days: 45,424 g

The experiment was repeated with 100 ml of dense algae culture from C3.

Weight of beaker: 82,349 g

Weight of beaker with algae culture: 180,609 g

Weight of beaker after cooling down: 82,387 g

Weight of beaker after drying 1 hour: 82,365 g

Weight of beaker after cleaning: 82,345 g

Weight of beaker after 3 days in oven: 82,349

6.3.3. Interpretation

As the beaker was lighter after the water evaporated than at the initial weighting, this would imply negative mass for the algae, since this does not make sense, the measurement is invalid. Also the gain of mass after drying of 15 mg does not make sense. The quantities seem to be too small to get accurate results.

The second experiment seems to have a better accuracy. The gain of mass is of 16 mg after drying. The removed algal biomass from the beaker had a mass of 20 mg, however the beaker was again lighter at that moment than at the initial measurement and it regained 4 g of mass after drying again. This would again indicate the presence of 16 mg algal biomass in 100 ml solution.

6.3.4. Conclusion

As there is still some error, a reasonable estimate for the concentration of algal biomass in the algae solution would be a concentration of 0,1 g/L – 0,2 g/L. However, the procedure needs to be repeated with larger amounts in order to validate the protocol.

6.4. Cell lysis 1

Numerous methods exist for cell lysis. One possibility is freeze and thaw; first the cells are frozen and then they are thawed again. As water expands when freezing, this leads to the disruption of the cell walls.

6.4.1. Method

1)10 ml of culture EXP are put into Eppendorf tubes (1 ml per tube). The sample is placed in the freezer for about 2 hours. Afterwards the tubes where put in a bain-marie at 80 °C until they were completely thawed.

The probes are observed under the microscope and compared with non-treated cells.

2)The probes are again placed in the freezer for 24 hours, then thawed again and observed anew.

6.4.2. Observations

1) The cells had sedimented in the tubes. Under the microscope, the treated cell showed no visible difference to untreated cells.

2) Again, no visible difference could be established under the microscope

Image 13: Probes in bain-marie

6.4.3. Interpretation

1) The process has not affected the cells, they are still intact.

2) The process has not affected the cells, they are still intact.

6.4.4. Conclusion

1) The process has not worked. Maybe the freezing was too short, the experiment should be repeated with a longer freezing period. This led to conduction of 2).

2) Even with the prolonged freezing period, freeze and thaw is no suitable method for the lysis of this particular algae.

6.5. Cell lysis 2

According to a paper from Ángel Darío González-Delgado et al., acid treatment can be an effective pre-treatment for microalgae. Not only does this process lead to cell lysis, but furthermore, by filtering the obtained biomass, one can immediately separate the solid lipids from hydrophilic substances such as monosaccharides.[17]

For this experiment, primarily the capacity of the process for cell lysis should be observed in a first step.

6.5.1. Method

10 ml have been taken from culture EXP. Respectively 1 ml has been put into 10 Eppendorf tubes for centrifugation. They were centrifuged for 2 minutes at 14 rpm. The supernatant was removed with a micropipette. A few drops of distilled water were added to the pellets in order to transfer them into a single Eppendorf tube. The obtained sample was centrifuged again for 4 minutes.

The supernatant was removed. To the sample were added 60 µL of 0,5 molar hydrochloric acid. The sample was put on the shaker for 24 hours.

The sample was observed under the microscope.

6.5.2. Observations

The sample had changed colour. It was not green anymore, but rather brown or brown-green. Under the microscope many of the cells have irregular forms. The individual cells also look greener under the microscope, usually individual cells look transparent under the microscope.

Image 14: Probe after treatment with hydrochloric acid

6.5.3. Interpretation

The irregular form of the cell is a sign of disruption. The colour change could be explained by action of acid onto chlorophyll, which leads to the production of pheophytin[18], which indeed has a brown colour. As the acid could penetrate into the cell, the cell wall and membrane must be damaged.

[17] Gonzalez-Delgado, A. D., Martinez, J. B. G., & Peralta-Ruiz, Y. Y. (2017). Cell disruption and lipid extraction from microalgae Amphiprora sp. using acid hydrolysis-solvent extraction route. Contemporary Engineering Sciences, 10, 841–849

[18] Koca, N., Karadeniz, F., & Burdurlu, H. S. (2007). Effect of pH on chlorophyll degradation and colour loss in blanched green peas. Food Chemistry, 100(2), 609–615

Amanda, Pigments, pH, and Beautiful Food Colors, from: decodingdelicious.com, URL: www.decodingdelicious.com/pigments-ph/ (consulted: 04.05.2020)

Wikipedia, Pheophytin, URL: https://en.wikipedia.org/wiki/Pheophytin (consulted: 04.05.2020)

6.5.4. Conclusion

The treatment with hydrochloric acid is an effective method for cell lysis for the present algae. Further treatment of the biomass, such as the separation of the solid phase and the liquid phase must be established in further experiments.

7. Further experiments

Not all the planned experiments could be carried out due to school closure. I will nevertheless outline the plans for the continuation of this project.

7.1. Obtaining ethanol

After filtering the pre-treated biomass, the samples need to be neutralized with sodium hydroxide. The samples are then subjected to alcoholic fermentation by adding yeast. Finally, the obtained product is distilled to isolate ethanol.

7.2. Ethanol dehydration 1

Ethene can be obtained from ethanol through dehydration. This reaction can be catalysed with aluminium oxide (Al_2O_3).

7.2.1. Method

The ethanol is heated in a flask. The vapour passes through a heated tube containing the aluminium oxide. The obtained gases are then conducted through a glass tube that lies in water and ends in a flask put in the water upside down. [19]

7.2.2. Expectation

Ethene should collect in the top of the flask in the water.

7.3. Ethanol dehydration 2

Another method for dehydration of ethanol consists in using a strong acid as catalyst. Suitable acids include sulfuric acid and phosphoric acid.

7.3.1. Method

In this process, ethanol is heated with an excess of concentrated sulfuric acid at a temperature of 170°C. The gases produced are passed through a sodium hydroxide solution to remove the carbon dioxide and sulfur dioxide produced from side reactions. The ethene is collected over water.[20]

7.3.2. Expectation

Ethene will collect in the tube.

7.4. Polyethene production

Polyethene can be produced from ethene in a number of ways. Once the lab is open again, it shall be figured out with the people in charge there, which off the following methods fits best with the available chemicals, infrastructure and safety restrictions:

Polyethene can be formed under high temperatures of over 200 °C and high pressure of over 2000 atmospheres. Small oxygen impurities act as initiator in this method.[21]

Another possibility is to use a catalyst instead of radical polymerization. These require lower temperature and pressure. One such catalyst is a Ziegler-Natta catalyst. This type of catalyst

[19] Clark, James, Dehydrating Alcohols to Make Alkenes, from: chem.libretexts.org, URL: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_%28Organic_Chemistry%29/Alcohols/Reactivity_of_Alcohols/Dehydrating_Alcohols_to_Make_Alkenes (consulted 18.04.2020)

[20] Ibid.

[21] Clark, Jim, THE POLYMERISATION OF ALKENES, from: chemguide.uk, URL: http://www.chemguide.uk/organicprops/alkenes/polymerisation.html (consulted: 03.05.2020)

usually is a mixture of two compounds, one possibility is titanium(IV) chloride ($TiCl_4$) with triethylaluminium ($Al(C_2H_5)_3$).[22]

7.5. Obtaining lactic acid

After filtering the pre-treated biomass, the sample is neutralized with sodium hydroxide. The sample is subjected to lactic acid fermentation by addition of lactobacillus culture. Purify the lactic acid by repeated distillation and crystallization.[23]

7.6. PLA production

7.6.1. Method

A small quantity of sulfuric acid is added to a lactic acid solution.

The solution is heated over a Bunsen burner until the viscosity or the colour changes. This step may need to be repeated several times with different samples for different periods of time to determine after which time a polymer is actually formed.

7.6.2. Expectation

As longer molecules form, the viscosity of the sample should increase, probably the colour will also change. If the sample is not heated long enough, this will result in a solution of oligomers rather than polymers. Heating the sample for to long time will lead to degradation of the formed polymers. After having figured out the correct time for heating, it should be possible to produce intact PLA.

7.7. Fat treatment

After the acid pre-treatment of the algae, the remaining solid part should contain fat. For further processing, the triglycerides constituting the fat should be split into fatty acids and glycerol. As they are bound by ester bonds, this can be achieved by saponification using a base as catalyst.

7.8. Fatty acids cracking

Cracking is a procedure which breaks long molecules into smaller ones. Those smaller molecules contain double bonds. Cracking requires heat as well as pressure or a catalyst.

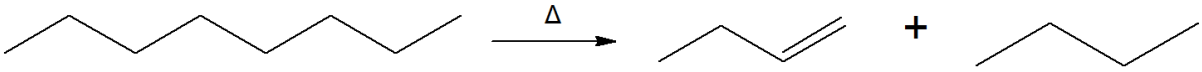


Image 15: Example for cracking of octane, producing butane and butene

7.8.1. Method

For this method, broken pumice stone is used as catalyst. Other silica compounds might be suitable to.

In the bottom of a test tube is placed some mineral wool. A solution containing molecules to be cracked is filled into to the tube, so that it is soaked up by wool. The tube is hold almost vertically and the catalyst is inserted.

[22] Clark, Jim, THE POLYMERISATION OF ALKENES, from: chemguide.uk, URL: http://www.chemguide.uk/organicprops/alkenes/polymerisation.html (consulted: 03.05.2020)
Polymerdatabase, Coordination polymerization and Ziegler-Natta Catalyst, from: polymerdatabase.com, URL: https://polymerdatabase.com/polymer%20chemistry/Ziegler-Natta.html (consulted: 03.05.2020)

[23] Researchgate, How to purify lactic acid?, from: researchgate.net, URL: https://www.researchgate.net/post/How_to_purify_lactic_acid (consulted 03.05.2020)

The tube is heated in almost position over a Bunsen burner. First heat the catalyst until it is hot, then alternate between heating the catalyst for 10 second and heating the mineral wool for 1 second.

The forming gases are collected over water in test tubes.

To show that light molecules containing double bonds have been formed, one can check whether the obtained gases can be ignited and if they decolorate bromine water or acidifed potassium permanganate.[24]

7.8.2. Expectation

The first few filled test tubes should only contain air. The following one should contain small, gaseous alkenes, obtained from cracking. They should burn and be able to decolour the bromine water and acidified permanganate. The latter two reactions are tests for the presence of carbon double bonds. If all these tests are positive, this means that small alkenes have been produced which are suitable for the production of polymers by chain-growth polymerization.

[24] Nuffield Foundation and the Royal Society of Chemistry, Cracking hydrocarbons in liquid paraffin with a catalyst, from: edu.rsc.org, URL: https://edu.rsc.org/resources/cracking-hydrocarbons/681.article (consulted: 04.05.2020)

8. Discussion

Of the set objectives, building the bioreactor is the one with the most progress. It has been figured out, by going through several setups and continuously improving them, what elements and considerations are important for algae bioreactor building. This allowed for a design for a final reactor which has yet to be implemented. While running a bioreactor is thereby possible, one limitation persists, which is the supply with sterilized medium. The autoclave in the lab only permits for the sterilization of 1 L medium at the time, making it hard to produce large quantities of medium. Solutions for mass sterilization of medium must be examined in the future course of this project.

Proper maintenance of the cultures was not done extensively enough. Establishing the algae's growth kinetics failed. The First approach using a Neubauer chamber to count the cells proved to be unreliable and the second approach using spectrometry is promising, but it was interrupted and never repeated. Therefore, the culture maintenance was guided by estimates based on the culture's colour by the naked eye. Furthermore, other important parameters of the medium, such as pH, temperature and nutriment content, were not tracked at all.

On the processing of biomass and actual polymer production, almost no experiments could be conducted. A suitable method for cell lysis by acid treatment was established. This should be followed by extraction and purification procedures of the different compounds, followed by the production of polymers from them. All this was not done.

In general, it can be said that some parts of the project were undertaken quite hastily, especially those about algal growth, and that many of the results lack precision. This is due to several factors, such as availability of only small quantities of probes, lack of repetition of the experiments and a general urge to move quickly to the next step in order get to the final result of polymers quickly.

One major limitation to the project was the availability of time. The whole project turned out to be much more time consuming as I had expected, one could have made an entire project about any single objective of it. Especially the experimental parts require a lot of time, which is complicated by the fact that I only had limited time to spend in the lab. This got further intensified by the closure of schools due to the Covid-19 pandemic, which interrupted all the practical work.

I intend to continue the project in the future and in the following school year. The first, rather biology linked, half of the project, which consisted in setting up a bioreactor as well as cultivating and harvesting algae has been completed to a degree which, although not being optimized and lacking scientific accuracy, allows nevertheless for maintaining cultures and producing biomass for further use. Therefore, future work on the project shall first focus on the second, rather chemistry linked part, which consists in the synthesis of polymers from the obtained biomass. This part was only presented in theory, but no practical results have been achieved. Furthermore, the reactor maintenance shall be optimised and general precision shall be increased, by repeating the experiments on growth kinetics and estimation of biomass over a prolonged period and with bigger quantities. A more intense surveillance of the algae cultures and the medium properties shall also be taken into account.

9. Glossary

9.1. Polymer and plastic

The material commonly referred to as plastic is composed of organic polymers. The name polymer originates from the ancient Greek “poly” – many and “meros” – part and that is essentially what they are: long chains composed of many atoms. In the case of organic polymers, those chains are in majority composed of carbon and hydrogen, including also other elements amongst which oxygen and nitrogen are the most common.

More precisely, polymers are composed by a repetition of a small subunit, called monomer (mono, Greek – single). This may be a repetition of a single monomer or several monomers. One polymer molecule can contain several hundreds or thousands of monomers.

There are natural polymers and artificial ones. Starch for example is a natural polymer composed of many glucose molecules. An example for an artificial polymer is polyethene (PE), the most common plastic, which is obtained from the connection of many ethene molecules.

The terms “plastic” and “polymer” are used here almost as synonyms, although there are of course natural polymers which are not considered plastic, such as starch or proteins.

9.2. Monomer

A monomer is a small molecule that is able to form a polymer.

9.3. Microalgae

Microalgae are single-celled aquatic plants. Like all plants, they live and grow by performing photosynthesis. They reproduce like other unicellular organisms by simply dividing into two new cells once they are big enough. As they live on photosynthesis, they essentially only need light, carbon dioxide and water to live. In addition, they also require some other nutriments in form of diverse salts.

9.4. Chlorella

Chlorella is a microalgae genus. It is an immobile alga with a shape of 2 to 10 µm.[25]

9.5. Autoclave

An autoclave is a device used for sterilization. It sterilizes material as well as liquids by exposure to hot water vapour. Therefor it heats water up to 119 °C in a pressure chamber.

9.6. Centrifuge

A centrifuge is a device used for separating substances. It acts by rotating the probes, thereby exerting centrifugal force. Upon the action of the centrifugal force, heavier particles move to the outside (usually downwards in the tubes). In cell cultures for example, the cells end up at the bottom of the centrifuge tubes, whereas the medium floats above them.

[25] Wikipedia, Chlorella, URL: https://en.wikipedia.org/wiki/Chlorella (consulted: 03.05.2020)

9.7. Neubauer chamber

A Neubauer chamber is a special microscope slide designed for cell count. One part of fit is lower than the rest, creating a chamber, which can be filled through capillary action. In the chamber is engraved a grid with known size. By counting the cells inside several squares of the grid, one can then calculate the number and concentration of cell in the probe, as the volume of the chamber is known. [26]

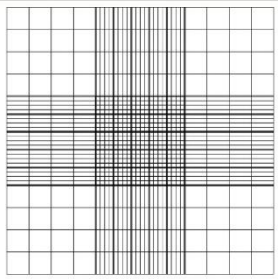


Image 16: Neubauer grid

9.8. Organic (chemistry)

The terms organic chemistry and organic compounds go back to the historical belief, that only living beings could create certain compounds. However, this was overthrown by Friedrich Wöhler who managed to synthetize urea, an organic compound from non-organic compounds. Nonetheless the distinction still exists, nowadays carbon-based compounds, with the exception of pure carbon compounds and carbon dioxide, are usually classified as organic. Therefore, the cells of organisms are composed of organic compounds, but mineral oil which is definitely not living is also organic, as it is composed of hydrocarbons.

9.9. Photosynthesis

Photosynthesis is a reaction converting water and carbon dioxide into glucose and oxygen, using the energy of light. It is catalysed by the green compound chlorophyll. Photosynthesis happen in all photoautotroph organisms, such as plants and cyanobacteria. The glucose thereby obtained can then be used to gain energy of further used by the organism to build other organic molecules.

[26] Image 15: from microbehunter.com, URL: www.microbehunter.com/the-hemocytometer-counting-chamber/ (consulted 04.05.2020)

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