

IGEMAmsterdam 2020 x
ARTIS MICROPIA

**WORKSHOP
SUSTAINABLE
MICRO-FACTORIES**

WORKSHOP:

SUSTAINABLE MICRO-FACTORIES

BY IGEM AMSTERDAM 2020

INTRODUCTION

Thank you for attending the workshop that we developed together with Micropia. During this workshop we will guide you through our iGEM project in which we use microbes, in particular cyanobacteria, to make substances for us. Just like Micropia, we find it most important to show that microbes can contribute to a better world!

WHO ARE WE?

We are a group of enthusiastic students from various backgrounds, from engineering to forensics to life sciences. Together we want to make biotechnology accessible to you. In this way we hope to contribute to a world that wants to and can become more sustainable.

WHAT DO WE DO?

In November this year we participate in a competition called iGEM (international Genetically Engineered Machine). iGEM is an annual competition in which teams come up with creative solutions to current problems through genetic modification.

WHAT IS GENETIC MODIFICATION?

According to the scientific committee of the European Union (EU), genetic modification is defined as: “the application of science, technology & engineering to enable / accelerate the design, manufacture & modification of (genetic) material in living microbes”. Genetic modification is therefore a way to change microbes in a very targeted manner.

CYANOBACTERIA: WHAT ARE THEY

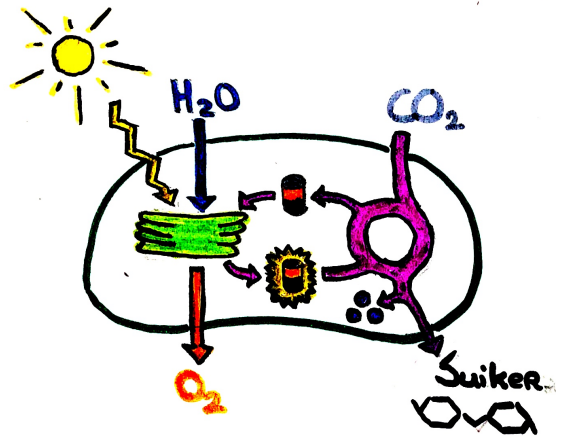
Cyanobacteria are among the oldest life forms on our planet. Fossil evidence suggests they date back to some 2.7 billion years ago. In comparison, we, humans, have existed for about 125,000 years. Basically cyanobacteria are nothing more than bacteria that can carry out photosynthesis. This allows them to live on light, carbon dioxide and nutrients. Normally cyanobacteria are unicellular but when they multiply they can form colonies, this is a group of cells together. If enough nutrients are present, colonies can grow back into large branched threads. We know cyanobacteria as blue-green algae that can ruin our swimming pleasure in large puddles and lakes in the summer. If we ingest blue-green algae, this can contribute to all kinds of health complaints such as stomach and intestinal complaints. But they can also help us....



Algae growth found in the water near the John Frostburg in Arnhem
©Municipality of Arnhem



Cyanobacteria
Synechococcus
aeruginosus
 © Protist Information
 Server



Schematic drawing of photosynthesis linked
 to metabolism in a cyanobacterium
 © iGEM Amsterdam

PHOTOSYNTHESIS

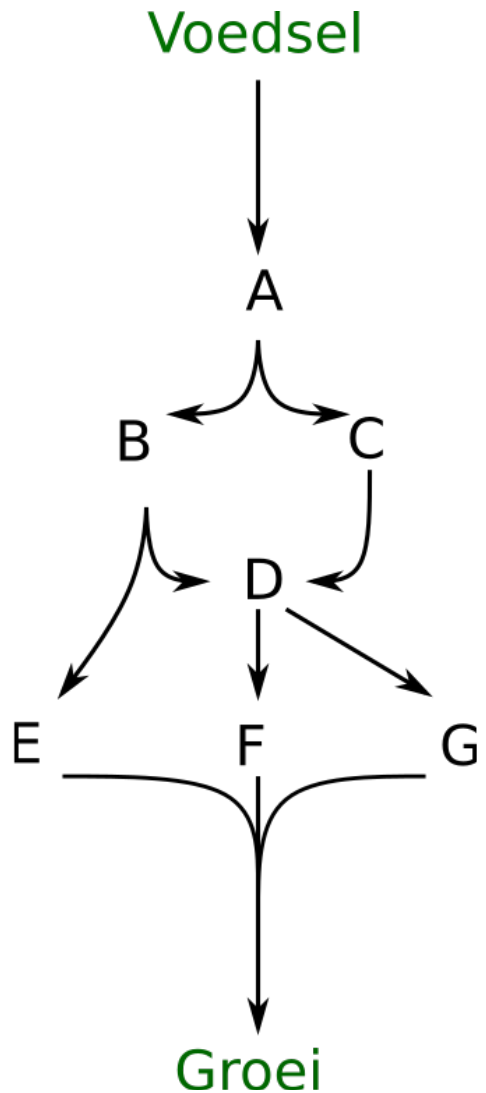
As with plants, cyanobacteria therefore make use of photosynthesis. Photosynthesis is a process that converts water, carbon dioxide and sunlight into sugars. This process may be due to chloroplasts in cyanobacteria. Chloroplasts convert light into energy. During this conversion water is consumed and oxygen is released into the environment.

METABOLISM

The energy created by photosynthesis is then used for processes to keep the cyanobacterium alive. We call all these processes together the metabolism. The metabolism consists of all kinds of different routes that keep the cell alive, an example of this is the production of building blocks for the cell. A route consists of a substance that is gradually converted into another substance. These conversions can take place through energy from photosynthesis.

NETWORKS

A microbe is very complex, you can see it as a small factory. Many substances, also called metabolites, are in contact with each other through certain reactions that are accelerated by proteins. Just like in a factory, we also have a map this response: a network. An example of a network that you have probably seen is the map of a metro. Below you can see a simplified example of a network in a cell. We can adapt this network so that we can link the production of a particular substance to growth. Can you find all possible routes to growth in the image on the right?



Schematic example of a microbe network. The arrows indicate that a certain substance (substance A) is converted into another substance (substance B).

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PRODUCTION OF COMPOUNDS BY CYANOBACTERIA



With our project we have one goal: to make useful fabrics in a sustainable way. To do this, we make a computer program that tells us what to do to turn microbes into factories. Our “pets” for testing our program are cyanobacteria. Because of their photosynthesis, cyanobacteria are great and sustainable factories! Read on and learn how we do this.

BIOTECHNOLOGY

The goal of biotechnology is to make products from raw materials with the help of microbes. These can be plants, but also bacteria, animal cells or yeast (known from bread and beer). One application of biotechnology that you probably don't even consider is the production of cheese and yoghurt. But parts made of plastic and biofuel can also be made in this way.

SUSTAINABILITY

But why do we want to use biotechnology for substances that we can also make in other ways? Using biotechnology, we can make products in a more sustainable way. Fossil fuels are used in a factory that generate carbon dioxide emissions. Microbes and plants use the carbon dioxide that is released for food. This creates a closed circle. Cyanobacteria are particularly promising, as they are easier to adapt than plants.



A schematic representation of a closed ecosystem (circulation).
In this, products are always reused.
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LARGE-SCALE APPLICATIONS

Why is biotechnology not yet applied everywhere? Unfortunately, many of the processes are not yet efficient enough to be applied on a large scale. In short, they are often too expensive and not fast enough. We, iGEM Amsterdam, want to enable large-scale production by microbes. A microbe really only has one purpose in life: to grow and reproduce as quickly as possible. Because a modified microbe uses energy to make products for us, less energy is left for its own growth. Over time, cells will form that do not produce and grow faster. Due to the faster growing microbes, our adapted microbes that make substances disappear. As a result, we are back to square one.

PRODUCTION COUPLED TO GROWTH

We want to use the ability of a microbe to grow and reproduce to enable large-scale production. How then you might ask? By linking the making of a substance to the

growth and reproduction of the microbe, we prevent faster growing microbes from developing. But how do we link growth and reproduction to the production of substances? For this we write a computer program that calculates this for us. We give our program the blueprint of a microbe (the “network”) and the substance we want to make. The program then calculates the best way to link the growth and reproduction of the microbe to making the substance. The result: a good microbial factory!

WORKSHOP EXPERIMENTS

INTRODUCTION

In the experiments you will be conducting, we will take you along in our daily research. We take you through the steps we take to make microbial factories. In the first experiment, you will learn how to see what your microbe needs to be happy. This is important because happy microbes are better factories! Then you will discover how you can see if your microbial factory is working: by means of smelling you can find out what makes the microbe. Finally, we show you that the substances that our microbial factories make are actually used in everyday products. Just like a real scientist, you have to plan your experiments well. This means that you don't always follow the logical order when you run your experiments. Since we have to wait in experiment 3 to see the result, we recommend that you start with this experiment.

VEILIGHEID

Your safety is our top priority, so please read the text below and pay close attention when we show and explain something to you.

EXPERIMENTS

For the different experiments we will briefly demonstrate how you should perform certain actions. We'll show you in advance how to smell and mix fabrics together. The bottles will always be closed and only used by us. You can only smell when the bottle is open and we indicate it.

COVID-19

In connection with the new corona measures, we wear mouth masks during the workshops and we ensure that the materials are regularly disinfected so that everyone can safely participate in the experiments during the experiments.

WORKSHOP EXPERIMENTS

1. GROWTH OF CYANOBACTERIA

Cyanobacteria want to grow and reproduce naturally to form an ever larger group of cells. We call growing and reproducing "cultivation" and the large group of cells that arise in the bottle we call a "culture". To keep and cultivate this culture of cyanobacteria, we have to keep them under the best conditions and provide the right nutrients.

RESEARCH QUESTION

Which cyanobacterial culture has been grown under the best conditions and the right nutrients? And why do you think so? Can you think of what we need to make them grow as well as possible?

SETUP

Materials

- * One bottle of optimally grown cyanobacterial culture
- * One bottle of non-optimally grown cyanobacterial culture

Method

Using what you see and your knowledge of cyanobacteria, find out which culture has been cultivated under the best conditions. What circumstances are these? What would you add to help these microbes grow in the best way possible?

WORKSHOP EXPERIMENTS

2. PRODUCTION OF SMELLY COMPOUNDS

Cyanobacteria can therefore make substances for us in an environmentally friendly way because they absorb carbon dioxide. For this we have to adjust their metabolism in some way so that they make what we want. There are already a number of substances that can make cyanobacteria, but can we identify the same substances by smelling?

RESEARCH QUESTION

Can you smell what substances our modified cyanobacteria make for us?

SETUP

Materials

- * 3 vials with different cyanobacterial cultures
- * Your own nose

Methods

Here you will receive three bottles (“Erlenmeyers”) with different cultures of cyanobacteria. Each of these cultures produces a different substance, but we don't remember which one? On the basis of the smell of the substances you should be able to find out which substance is in which culture. Using the smell table, you can find out the substances.

WORKSHOP EXPERIMENTS



3. ASPIRIN MADE BY CYANOBACTERIA

For the next part of the experiments, we want to show you that cyanobacteria make substances that are actually found in everyday products. One of the substances that we want our cyanobacteria to make is salicylic acid. With salicylic acid we can make acetyl-salicylic acid. Acetylsalicylic acid is used in Aspirin®.



Aspirine® of Bayer with a schematic drawing of a cyanobacteria

What's cool is that acetone can also be made by cyanobacteria. Acetone can react with acetylsalicylic acid and thus detect the presence thereof. So in theory we could mix a culture of cyanobacteria that makes acetylsalicylic acid and a culture that makes acetone together to detect acetylsalicylic acid. Normally we would first purify both substances from the culture, but this can take quite a long time. That is why we now use aspirin & acetone (nail polish remover).

RESEARCH QUESTION

Can we purify an acetylsalicylic acid from aspirin using acetone?

SETUP

We can extract acetylsalicylic acid from aspirin tablets using acetone, which is a well-known nail polish remover component.

Materials

- * Aspirin tablets
- * Nail polish remover with acetone
- * Mortar / Grinder
- * Glass measuring cup with stirrer
- * Coffee filter
- * Petri dish

Method

- 1) Crush one aspirin tablet with a mortar / grinder.
- 2) Mix the aspirin powder with 10 milliliters of acetone for 5 minutes.
- 3) Filter the mixture with a coffee filter and collect the liquid in a Petri dish.
- 4) Let the liquid dry in the Petri dish, this will take about 10 min.

FINAL WORD

We are very happy that Micropia has given us the opportunity to share our project with you. We hope you enjoyed the experiments and learned a little about how to make everyday products in a more sustainable way. We would like to thank you as a visitor for participating in the experiments. Have you become enthusiastic? You can follow us on Instagram, Facebook and LinkedIn via iGEM Amsterdam 2020. Through these channels we share our developments in science and our collaboration with others. For more detailed explanation of our project, please visit our English website (<https://2020.igem.org/Team:Amsterdam>). In addition, various webinars about genetic modification can be found on our youtube channel.

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