

Experiment

Growing Spirulina-algae



by



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Algae III The cultivation of Spirulina

Introduction

For every living creature our planet, the earth, is the place to live and to provide them with food. For people our planet earth also provides a lot of energy. As a result of the growing world population and the growing prosperity, the demand for food and energy has increased rapidly in recent decades. The supplies of fossil fuels (like natural gas and crude oil) are slowly decreasing and more and more complex techniques are needed to make the exploitation of oil and gas possible. And more complex techniques will lead to higher prices for oil and gas. On the other side the demand for energy is getting bigger every day so people are constantly looking/searching for other energy sources as an alternative for the fossil fuels.

Solar and wind energy are well known, but also biofuels such as biodiesel and bioethanol are rising in popularity. But the production of biofuels in most cases need that corn or palm trees are specially grown for this purpose. But if farmers make their choice to use precious cultivated land for the production of biomass for energy, this means that this land will be used at the expense of food production. This may eventually lead to food shortages. And as a result the prices for food prices will be continuously rising. In many cases big pieces of rainforest are destroyed and prepared for growing palm trees for their oil. This brings really a lot of damage to the environment and the beauty of the earth with all its wonderful animals and plants.



Despite of all critical remarks, energy from biomass is one of the leading energy providers of the future. For this reason energy made out of 'green' residuals (often called waste) is a good option. Residuals to be used as biomass. But the question remains if this can provide in the total global energy needs.

On the other side, 'green waste' should not always be labelled as waste. If the green residuals contain a reasonable amount of nutrients, minerals and/or ingredients for medicines, then it might be better to consider the waste as useful raw material (feedstock). If the extraction of nutrients or other useful products from the biomass can be easily performed, companies can make more profit out biomass. Normally people and/or companies have to pay if they want to get rid of their 'green' residuals. So by investigating the possibilities and contents of 'green waste' it may turn out that waste is not waste anymore, and people can even make profit out of waste! Burning (and making energy out of) biomass should always be seen as the last option. The ratio of the value of the products that can be compared to the amount of product recovered from a certain amount of biomass can be displayed with a so-called "eco-pyramid" (see Figure 1).

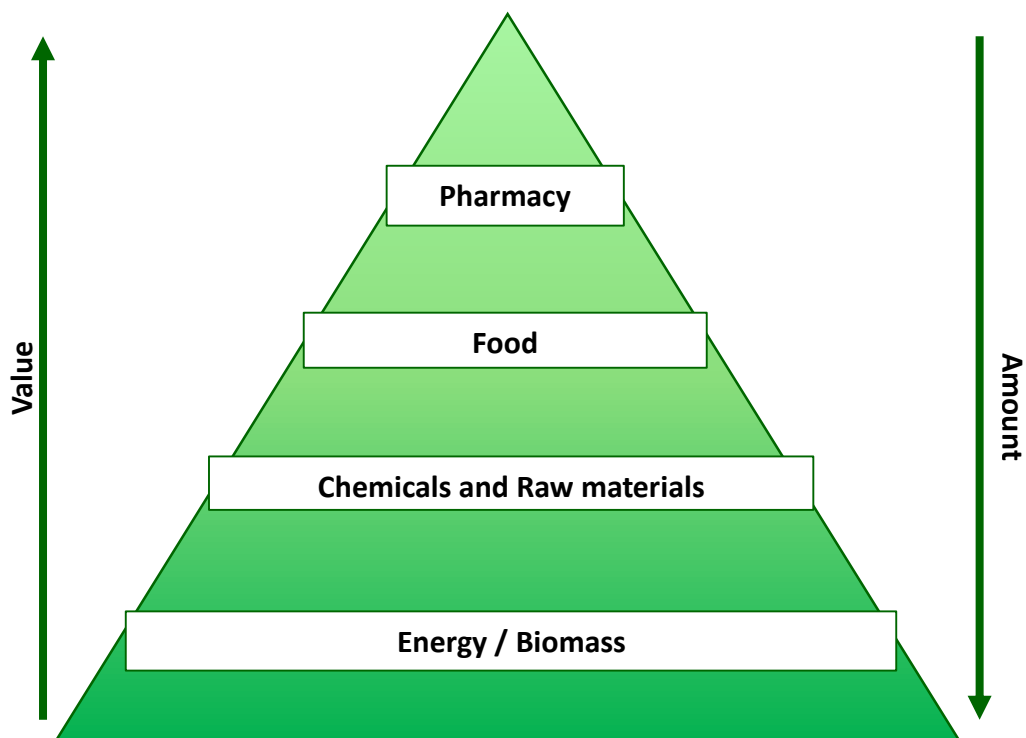


Figure 1: 'Eco-pyramid'

Nowadays a lot of research is done to find alternatives to create biomass in a relatively easy way without disturbing or compromising food production from agriculture. So how to create biomass in a cheap and efficient way?

In the world we have two kinds of algae-cultivation. The cultivation of big seaweeds and the cultivation of micro-algae. In some part of the world seaweeds are just only harvested for food, agar or to abstract other compounds. The seaweeds can be very big, more than 5 meter long and have big leaves. However microalgae are only harvested in some alkaline lakes in semi-arid areas like in Tjaad and Mexico. There are more than 100.00 species of micro-algae. Some of them are not really algae but cyanobacteria's. In the world there are some commercial Spirulina-farms (Hawaii, France and Greece).

In places where cultivated land is scarce and plenty of (fresh) water is available, biomass can be grown in water. For instance seaweed or algae easily grow in water if the conditions are

optimal. The positive thing about this approach is that the farmers can keep using their cultivated land for agriculture, so for the cultivation of food.

Especially certain species of algae are suitable to be grown for food, pharmacy or biomass. They easily can be grown in large reservoirs. But this may sound easier than it is. For a good yield the conditions for growing/multiplying of the algae should be optimal! Factors such as the concentration of nutrients in the water, temperature, amount of light and aeration play a major role. When one or more factors are not optimal, the yield will be low or minimal and the algae may even die.

By varying different conditions, this experiment will guide students step by step to investigate what will have a positive or negative effect on the yield of algae. The main goal should be to have the best yield of algae, but for a reasonable price **AND** with minimal environmental impact!

If needed, the students should be able to finetune or troubleshoot on the process. If something goes wrong, what should be done to minimize the damage?

The algae used for this experiment are so-called Spirulina-algae (see Figure 2).



Figure 2: Spirulina algae in water

Spirulina-algae grow in a salty aqueous solution with a relatively high pH (9-10). The positive thing about this is that it makes the growth medium reasonably resistant to contamination from the outside, such as:

- Bacteria from bird droppings
- Traces of other species of algae coming from (rain)water and/or out of the air
- Dead insects that ended up in the water

A positive thing for this experiment is that good results can be obtained in a relatively short time. Algae grow rapidly and do provide the first visible results within a few days by change of the color of the solution. And from setting up a new culture of algae to harvesting, it takes about 4 weeks! Even if some parts of the experiments go wrong there is still enough time for troubleshooting and 'repair'.

Moreover, Spirulina algae is a very interesting product because it contains a reasonable amount of nutrients, antioxidants and minerals. The diagram as displayed on the next page shows the composition of dried Spirulina (see Figure 3):

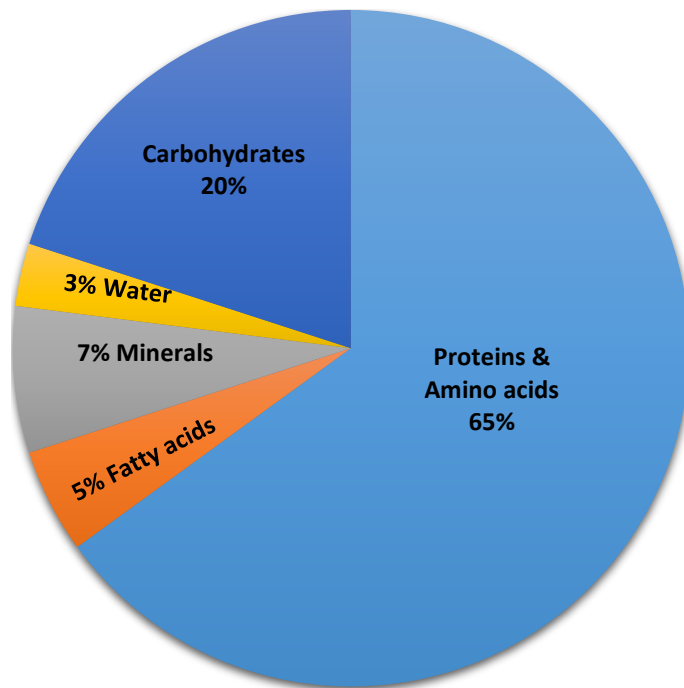


Figure 3: Composition of (dried) Spirulina-algae

Spirulina is for this reason more than just biomass. It is known as a dietary supplement, highly nutritious due to the high content of proteins and minerals. In many shops where so-called 'reform-products' are sold, Spirulina-based dietary supplements are one of the main products. In addition, this alga contains a blue pigment which may be suitable to be used in the food industry. A big company in the food-industry like Unilever is investigating if the blue (consumable) pigment can be isolated from the algae in an easy and cheap way. Beside from this the Spirulina-algae produce, if treated under certain cultivation conditions, a relatively high content of oil. This "bio-oil" might be used as biofuel or bio-lubricant. As a last resort algae can be used as biomass for the production of energy.

So growing algae for food or energy is far more interesting than most people think. Seen as a raw material, (dried) algae may lead to a number of valuable bio-based products. For optimization of growing the algae students need to use their knowledge of chemistry (fertilizers, salts, pH), physics (light, movement) and biology (plant growth), or learn about it.

Experiment: Growing and harvesting of Spirulina-algae.

Introduction

This experiment will guide you step by step to obtain from a small amount of algae (start-concentration) a big amount (10 liters or more) of Spirulina-algae in water in a relatively high concentration. After a couple of weeks this algae-concentrate can/should be used to perform experiments in the classroom and let every student grow his/her own algae under specified conditions.

So the obtained algae-concentrate can be used for:

- The small-scale cultivation of algae by students (liter bottles)
- Demonstrating the harvesting of the algae
- A new starting point to grow a new algae 'mother culture' in a new freshly made medium.

In this experiment, the mother culture grown in the classroom will be used primarily to provide for every student a small amount of (living) algae concentrate for their own experiments.

As a student you should select/determine your own 'optimal' conditions to obtain a maximum amount of algae. After a couple of weeks the results will be compared will become clear what are the best conditions for getting the highest yield in algae.

For a good, successful cultivation of the algae you will have to deal with some very important parameters:

- **The exposure of the algae to light**
- **The temperature of the growth medium**
- **The agitation of the algae in the growth medium**
- **The aeration of the growth medium**

What equipment and other tools are required?

- Concentrate of spirulina algae
- Fertilizer in powder form (so-called. NaK mixture)
- Liquid concentrate of trace elements (Chelal CaFeMg concentrate)
- Cylindrical vase (capacity approximately 12 L and smaller vases for individual experiments)
- Aeration pump device, tubing material and brushstone
- Sufficient amount of demineralized water
- pH meter



- Thermometer (conventional is sufficient)
- Analytical weighing balance

How to prepare HM5 growth medium:

- Clean the vase(s) thoroughly with warm water and a small amount of detergent.
- Rinse the vase after extensive cleaning with clean water.
- After rinsing with water make sure that the vase is completely dry. Eventually use a piece of clean tissue paper to remove the remaining droplets. (Remaining traces of water in the vase may contain traces of (non Spirulina) algae as well)
- Fill the large vase with 10 liters of distilled water (at ambient temperature).
- Use the HM5-calculationtool (excel-file) to calculate the required amount of NaK mixture

HM5 medium volume	15	L
NaK mixture	236,3	g
Chelal-CaFeMg concentrate	150	mL
HM5 total ' <i>solids</i> '	241,1	g

NaK mixture		
Potassium nitrate	44,3	g
Potassium dihydrogen phosphate	7,5	g
Baking soda (NaHCO ₃)	157,5	g
Sodiumchloride	15,0	g
Sodiumsulphate	12,0	g
NaK total ' <i>solids</i> '	236,3	g

***Example calculated amounts of required chemicals
for the preparation of HM5-medium***

- Weigh and create according to this recipe the required amount of NaK-mixture and Chelal-Flor solution.
- Dissolve the white NaK-powder while stirring the demineralized water. (The NaK mixture dissolves easily in water)
- Continue stirring until a clear liquid is obtained **without** solid particles visible at the bottom of the vase!
- Add the calculated amount (milliliters) of Chelal CaFeMg concentrate by using the calculation tool) to the solution.
- Once more stir the solution thoroughly.

Starting to grow algae:

- Now the vase should be placed in a room with sufficient light. Straight sunlight shining on the solution with algae will normally give very good results, so near the window (south side) is probably a good position.
- Connect a brushstone to a piece of plastic/silicone tubing and put it into the solution. Make sure that the brushstone reaches the bottom of the vase. Connect the other end of the tubing to the air pump and switch it on.

Basically the aeration has two functions:

- It ensures a good exchange of oxygen and carbon dioxide (CO₂) between growth medium and air.
 - It ensures a continuous movement/flow in the vase to prevent that algae will sink to the bottom of the vase.
-
- Adjust the airflow to have a reasonable amount of air bubbles to keep the solution 'in motion'. On the other side, too much airbubbles may lead to foaming (but will **not** have a negative effect on the growth of the algae). So make sure to adjust a steady stream of air bubbles.
 - By using the pH-meter or 'pH-paper', measure the pH of the solution. The measured pH value should be at least **pH = 8**.
 - Pour a small amount of with 'algae concentrate' in the medium. A good startingpoint is a concentration of about 5-10 % (v/v).
 - Measure the pH again (this is expected to be pH = 9 – 9,5).
 - Now all conditions are fine to grow Spirulina algae. It just takes time and optimal conditions to grow!
 - From this moment please check every day the condition of solution. It should have a fresh green color and the smell of the solution should be normal smell (a nasty smell is an indication that algae are dying).
 - Measure and record daily the pH of the growth medium.

Table: Registration measurement of pH

Day #	pH	Remark(s):	Day #	pH	Remark(s):
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

- Measure and record daily the temperature of the growth medium

Table: Registration measurement of temperature

Day #	Temperature (°C)	Weather conditions (sunny/cloudy . .)	Day #	Temperature (°C)	Weather conditions (sunny/cloudy . .)
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

- Check daily if the aeration is still OK. Do you see bubbles?
- If you notice a very thin layer of sediment on the bottom of the vase after 1 to 2 days, this is just normal and not alarming. If a thicker, dark colored layer of deposits on the bottom arises this may indicate that the algae have died.
- Within a few days, the first results of multiplying of the algae should be visible. The color of the solution should change from (semi-transparent) bright green to a (non-transparent) dark green colored solution.

- If all conditions are chosen correctly, after about two weeks the concentration of algae has increased to such a level that the algae medium is now ready for the first time harvesting.

Some additional notes:

- If the vase is filled with 10-11 liters of HM5-solution it might be possible that the brushstone does not completely sink to the bottom. Eventually you can make the brushstone heavier by connecting an extra well-cleaned pebble or other stone with a tie wrap to the tubing.
- The temperature is an important parameter for the growth of algae. When the growth medium is too cold ($<15^{\circ}\text{C}$), the growth is stagnating. At room temperature, the algae will grow well. A temperature of approximately 30°C is optimal, but will also cause extra evaporation. When the temperature is chosen too high ($> 35^{\circ}\text{C}$), the algae will die. When the vase is put in a place at ambient temperature and sufficient light it is fine. But when there is a lot of sunlight shining directly on the vase and the color of the medium is already quite dark in color, you will notice an increase in the temperature of the growth medium during the day. This may lead to a temperature that exceeds the maximum temperature of 35°C . In this case move the vase to a bright spot with less direct solar radiation.

Harvesting the algae:

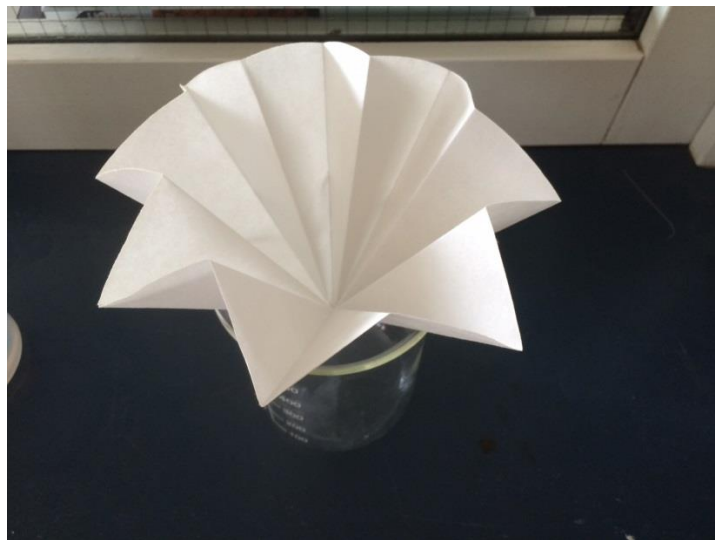
Introduction:

How much algae can be obtained after 4 weeks?

The easiest way to determine this is to use a conventional coffee filter :



or by a folded filter paper:





If the conditions for growing the algae were selected quite optimal, normally after **two weeks** the the color of the solution has turned from almost clear faint green to dark green:



So if this is the case, you may start harvesting the algae for the first time. Take about 95% of the solution for harvesting and use the remaining 5% to grow a new algae-concentrate in another two weeks. This volume of 5% of algae-concentrate is added to the filtrate (that remains after harvesting), and allowed to grow for two weeks.

Determine the yield of Spirulina-algae:

- Take a new filter paper or a coffee filter.
- Determine the weight of it on a analytical weighing balance. Write it down:

Weight of the filter paper: g (= W1)

- Put the filter in a stove at 70°C for one hour.
- Take the filter paper out of the stove and weigh it again.

Weight of the filter paper after drying: g (= W2)

- Now select a specified volume of your algae-concentrate by using a measuring glass. Suggested volume: 500 – 1000 mL (take a reasonable amount!)

Selected volume: mL (= V)

- Pour this volume of algae-concentrate slowly in the filter paper until the whole solution passed the filter.



- The Spirulina-algae will now be collected in the filter paper and the color of filtrate should be between clear colorless and clear faint green.
- Carefully take the wet filter paper out of the coffee filter holder or funnel and put it in a stove, set at 70°C, for several hours.
- If the filter paper and the algae are completely dry, determine the weight of it for the last time.

Weight of the filter paper PLUS dried algae: g (= W3)

- Calculate the yield of Spirulina-algae by using the following formula:

$$\text{Yield of Spirulina-algae (g/L)} = \frac{(W3 - W2)}{(V:1000)}$$

W3 = Total weight of dried filter paper PLUS algae (g)

W2 = Weight of a clean, dried filter paper (g)

V = selected volume of algae-concentrate that has been filtrated (mL)

- Depending on how many times this harvesting procedure was performed during a complete growth-cycle of four weeks, the total yield can be calculated as the sum of the different yields:

$$\text{Total yield (g/L)} = \text{yield1} + \text{yield2} + \dots$$

And the winner is

Who has chosen the best conditions for growing algae? Compare the yield of the different groups or students and find out what was the key for better results!



Teacher's guide: Theoretical background:

Biology.

Most of the algae have a size of between 3 and 50 microns (μ , pronounced mu, = micrometer, = 1/1000 mm), some algae, bear the name of algae (e.g., blue-green algae), but they are not true algae, they belong to the bacteria. They have no nucleus, no cell walls and no chloroplasts, something the real algae have. Real algae have the structure of a plant cell.

Growth, glucose, cellulose and protein.

Unicellular algae multiply by division. They take basic substances from the water and grow to a maximum size and then they divide into two. The two halves grow back out to their maximum cell size. Because they grow exponentially they grow faster than normal plants. Only their number is growing exponentially, the growth rate per cell therefore does not increase. (See section growth rate and growth factor)

All algae do photosynthesis, this they do with sunlight. They make their own food as glucose molecules in which they capture the solar energy. The algae produces these molecules of glucose from two basic molecules, carbon dioxide and water. Organisms that do this are called autotrophic. Only green plants are also autotrophic. The glucose molecules are later used both as fuel and as construction material. Molecules like starch and cellulose can be built directly from glucoses. A cell wall consists for a major part of cellulose. Furthermore, glucose can be converted to fat or oil, often as a reserve fuel. Some fat-like structures serve as building blocks for cell membranes. Furthermore, glucose can be converted together with nitrate into amino acids. These amino acids are linked in chains to form proteins later. Proteins are the major building blocks for all living organisms.

Primary production of plants.

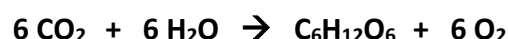
Only plants can make glucose (photosynthesis).

Together with some other bacteria, they are the only organisms that can produce glucoses, amino acids and proteins.

All other organisms steal these substances from plants or bacteria.

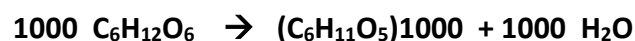
1. Photosynthesis:

Carbon dioxide and water are converted to glucose and oxygen:



2. Production poly sugars:

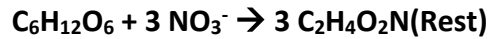
Thousands of glucose molecules are string together in a chain of cellulose or starch



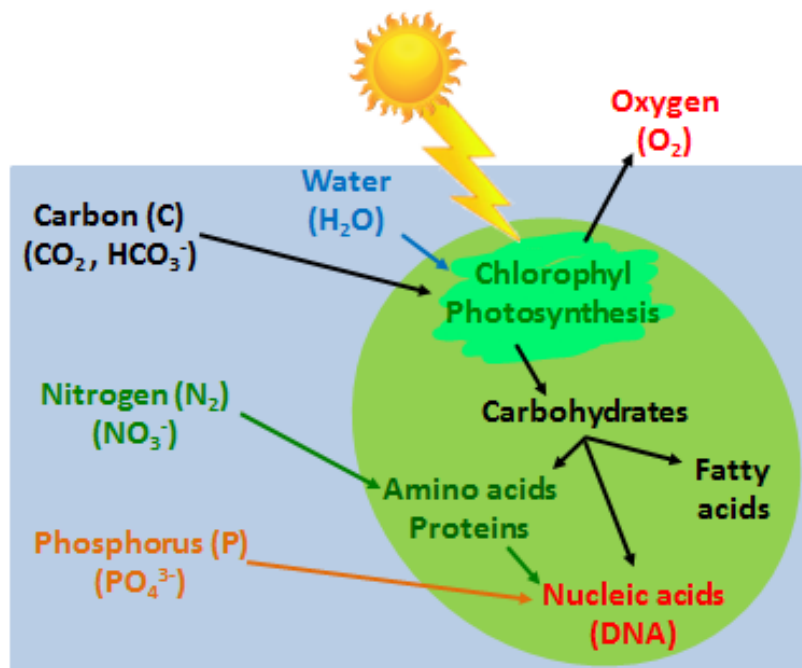
3. **Formation of fatty acids and glycerol from glucose:**
Glucose is eventually converted into fat.



4. **Glucose and nitrate (containing N) can be converted into amino acids.**
Then thousands of amino acids are string together into protein chains



5. **Later on the algae can produce other metabolites like pigments and DNA- and RNA-acids**



Photosynthesis.

Photosynthetic algae should take in carbon dioxide and water. Ofcourse the take in of water is usually not a problem because these algae live in the water. The supply of carbon dioxide can be more difficult because there in not much carbon dioxide (CO₂) in the water and it can run out. From the air carbon dioxide can come into the water again because it dissolves easy in water. But when it is cultivated in glass you have to give carbon in the hydrogen carbonate form (HCO₃). This is a given by a adding a salt. Also this salt can run out. By nature, there is also not always enough carbon dioxide in the water in order to guarantee proper growth expired. Thus, we can go aerating CO₂ active to get more carbon dioxide into the water. Because in air is only 0.04% CO₂ we can add additional CO₂ mixid with air, mostly not more than 1% CO₂.

Primary production, energy and growth.

Plants and algae produce the first to glucose. This glucose can the algae use for fuel or as construction material. When converting glucose into building materials such as cellulose, glucose and amino acids it will demand glucose as raw building material and this process will demand gluceses as energy supplier. An important part of glucose is burned during this construction.

Also the formation from glucose to fat as reserve fuel ask a portion of the glucose burned down in order to deliver energy during formation of fat.

Amino acids and proteins are important nutrients for growth.

The residual energy can be converted into fats. Fats (oils) stock 3 times more energy in a same volume as starch. Moreover fats are extremely flexible and can absorb vitamins as A,D, E and K.

The production and storage of oil costs the plant relatively much more energy than the production and storage of starch.

The glucose content in the algal cells then drops sharply when fats are formed and they consume than more oxygen.

Minerals, salts or nutrients

Algae not only take in carbon dioxide and water they also need quite a number of minerals, often known as salts. Salts consist of positive and negative ions (charged particles) and divide into their separated ions when dissolving in water.

Important salts are the macronutrients such as nitrate (NO_3^-) phosphate (PO_4^{3-}) potassium K^+ .

Other macro-nutrients are sulphate (SO_4^{2-}), calcium (Ca^{2+}) and magnesium (Mg^{2+})

Some salts the algae need in small quantities named micronutrients.

Examples for micronutrients are Iron (Fe^{2+}), copper (Cu^{2+}) and manganese (Mn^{2+}), Borium (B)

Salts

In dietetics salts are often called minerals. Salts always exist of positive and negative ions.

If you would to dissolve any of the ions above it is impossible that you only can dissolve positive ions (eg only K^+) without dissolving negative ions.

E.g. If you want to bring potassium ions in solution, you can choose for example: from KCl , KNO_3 from, or K_2SO_4 .

You must have the necessary chemical knowledge for making a good, efficient choice of salts which you can use the best.

Some salts should be careful regarding their toxicity. For example, for iron and copper.

Overdoses of these nutrients are not only harmful to the algae but especially for the eaters of these algae. The content of salts can rough measure with an EC meter.

However, an EC-meter measures all dissolved salts. Some ions (Al^{3+}) indicate a higher conductivity on the meter than others (Na^+). Also, you know anything about the types of

ions dissolved. Also, other dissolved ions can be measured, such as acids or bases. The EC-measurement therefore is not more than an indication-measurement.

Technical prescriptions for growing *Spirulina* algae

Measuring the temperature of the solution

The temperature is preferably measured with a common 'glass' alcohol thermometer.

Measuring the acidity, the pH

The pH is determined by using a calibrated pH-meter with a glass electrode.



- If a forced air-flow (bubbles) is used in the solution, switch it off during measuring the pH.
- Slowly move the electrode in the 'algaesoup' and wait for stability of the measured value on the display of the pH-meter.
- It might take up to 30 seconds before stability is obtained.
- Write down the measured value of the pH with an accuracy of 1 decimal.
- Once finished measuring, switch the device off. Be sure to store the sensitive electrode in a solution of 3M KCl in demineralized water (3M = 3 mol/L).

Determination of the dry matter content of the algaesoup

A known volume (for example 500 mL) of the algaesoup is filtered by pouring it in a Büchner funnel with filter paper. This filter paper should first be weighed on an analytical balance. After collecting the algae and dried in an oven this filter paper should be weighed again. The filter paper plus the algae should be dried for 3 hours at 30°C.

$$\text{Dry matter content (g/L)} = \frac{\text{mass of dried filterpaper with algae (g)} - \text{mass of filter before measuring (g)}}{\text{Volume of the algaesoup (L)}}$$