Kantonsschule Trogen
Class Sf5 Bio-Chemie

THE UPTAKE OF PHOSPHATE BY YEAST CELLS

Fig. 1: The green solution with sodiumphosphate in comparison with the yellow test solution

TASK 2

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1. Some background information about Phosphate

1.1. Why is phosphate important for all organisms?

Phosphate is essential for all organisms because it is contained in the genetic information (DNA- and RNA-molecule). It is a very important component of ATP (Adenosintri**phosphate**), which delivers energy in the cells due to dock and undock. And they are also regulation in different metabolic processes.
In bones and teeth phosphate is included as calcium phosphate, which is responsible for the solidness.
Some minerals in the soil contain Phosphate. Phosphor is one of the most important nutrients of plants and it improves the soil structure. It boosts the water storage ability through its bindings. It enables many biological growth processes such as the root growth(1).

1.2. What is well known about phosphate uptake in yeast cells?

The uptake of phosphate in to yeast cells is an active transport by a number of plasma membrane transport systems and depends on the optimal production of energy of the yeast cells. There are two kinds of transport systems, one transporter with a high affinity for phosphates and another sodium/phosphate cotransporter and a constitutive transport system with a low affinity. The phosphate can only be transported into the cell and is strongly pH-dependent; this means that the transport activity increases with lower pH values. Phosphate can be transported to a concentration gradient of 100 to 1(2, 3).

1.3. How do yeast cells store phosphate?

Yeast cells (Saccharomyces cerevisiae) store phosphate in the vacuole in the form of polymeric orthophosphate(4). This consists of linear chains of polyphosphate(5).

1.4. Why is phosphate uptake by microorganisms an important issue in our society?

Phosphate is contained in aliment as molten salt or acidifier, in some fertilizers, or as softener in detergents(6). Because of our consumption the water contains too much phosphate, that's why we have to clean it. To precipitate phosphate they use chalk, iron chloride and aluminium sulphate(7). Without the phosphate uptake by microorganisms the waters would be charged by an eutrophication(6). Since 1986 phosphate isn't allowed in laundry detergents in Switzerland.
Microorganisms are used in the sewage-works to absorb phosphate and to store it as polyphosphate(8).

2. Calibration of the measuring system

2.1. Pretest

We did the pretest (Fig. 3) as it is described. Figure 2 shows our result. We would like to see, how the colour changes with phosphate. So we added two drops of sodiumphosphate into one of the yellow solutions. It changes suddenly to green (Fig. 1).

Fig. 1: The green solution with sodiumphosphate in comparison with the yellow test solution

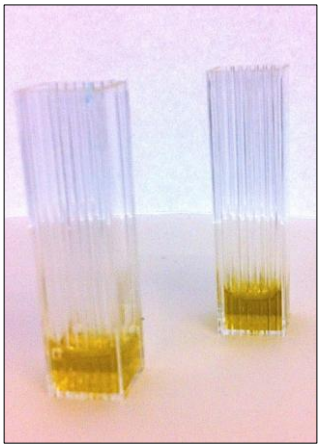

Fig. 2: Our test solution without phosphate

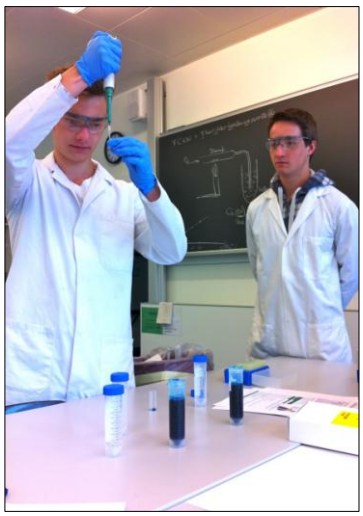

Fig. 3: Sammy and Markus carry out the pretest.

2.2. Establishing a straight calibration

Figure 4 shows the three best results of our attempts, the mean values and standard deviation. The r-values are close to 1.

phosphate concentration µM	measurements 1	measurements 2	measurements 3	mean-value	standard deviation
0	0.303	0.253	0.317	0.291	0.034
10	0.672	0.415	0.793	0.627	0.193
20	1.121	0.643	0.994	0.919	0.248
30	1.254	0.860	1.245	1.120	0.225
40	1.374	1.035	1.592	1.334	0.281
50	1.538	1.150	1.830	1.506	0.341
r-value	0.963	0.996	0.993	0.984	

Fig. 4: Phosphate measurement

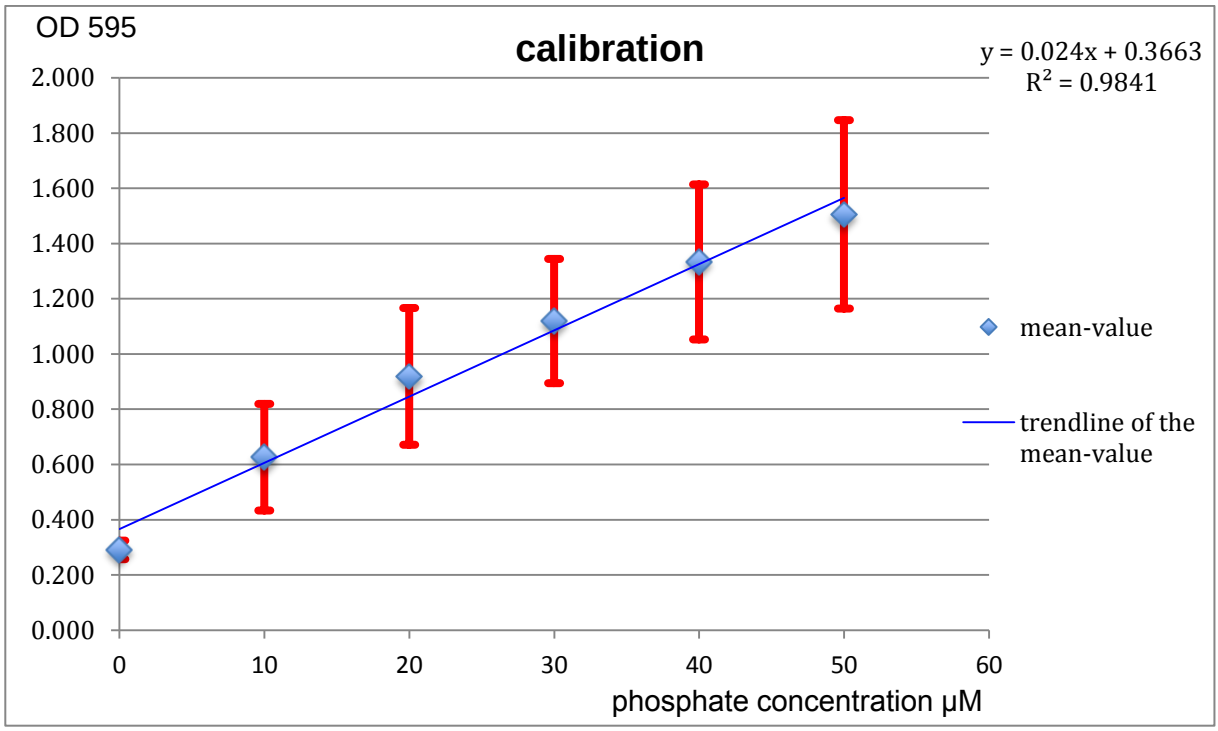

Fig.5: callibration line with acceptable standard deviation

Difficulties:
The first problem we had was that we flashed the dissolution in the eppendorf tubes together without micro pipets. So we had residues and the measure was incorrect.
The second problem was, in which time we should measure the dissolution so we tried it out and came to the conclusion, that we have to wait 20 minutes to get a solid straight calibration. These 20 minutes were also based on literature we found. (9)
The third problem we had was the handling with the micro pipets. We had to learn how we have to handle them to get correct solutions.
It could be that little backlogs are in the micropipets and so we couldn't pipet the correct volume.
Another reason for inexact results could be that our photometer didn't display the correct data, but we don't think that this is the reason because we have a really good photometer at our school. The values deviated even if we put the same cuvette into the photometer right after each other.

Design of the experiments part 3-4 (main design):
The time between adding the dye to the phosphate solution and the measurement in the photospectrometer is based on the 20 minutes used in the experiment for calibration (Part 2).

3. Phosphate uptake by yeast cells

3.1. Measuring the decline of phosphate in a well defined yeast solution

In Figure 6 you can see the decline of phosphate in the supernatant with the time. (Measurement every ten minutes)

time based measurements	measurements 1	measurements 2	measurements 3	mean-value
0	0.827	0.708	0.825	0.787
10	0.684	0.614	0.754	0.684
20	0.593	0.473	0.684	0.583
30	0.323	0.438	0.446	0.402
40	0.199	0.354	0.280	0.278
50	0.179	0.162	0.201	0.181

Fig.6: The three best attempts of the decline of phosphate

Figure 7 shows two graphs of the decline of phosphate in well defined yeast solutions. The red graph represents the data of Figure 6. The blue graph shows the mean values of the 9 measurements we did. They agree closely with each other.

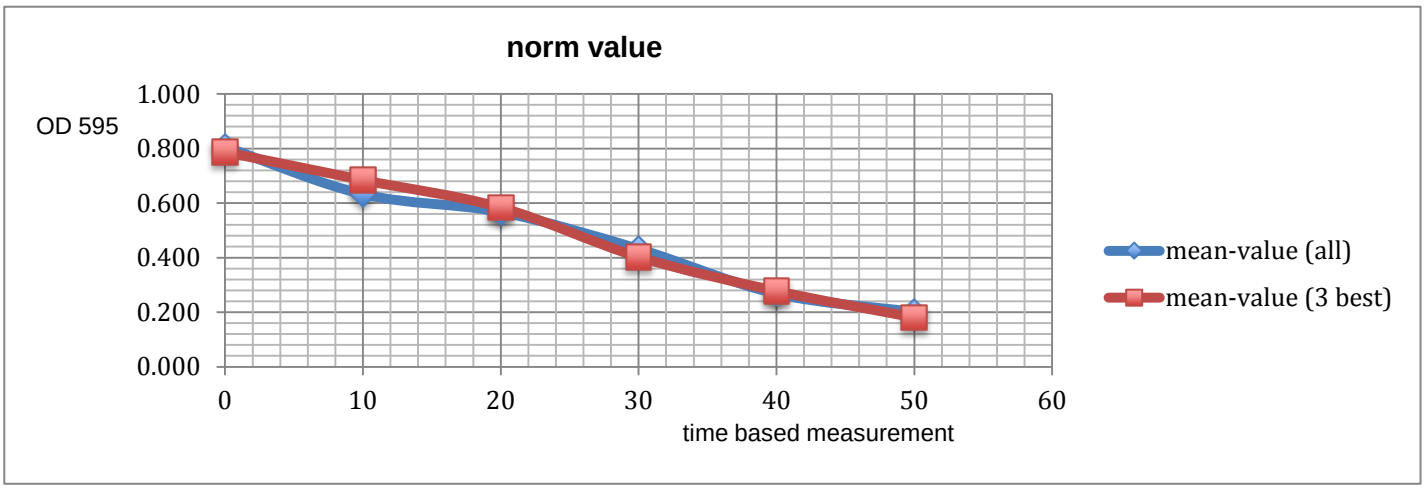

Fig.7: decline of the phosphate absorbance during the 50 minutes

We wanted to illustrate the decline of the phosphate concentration in another graph. Therefore we used just equation of the straight calibration, correlated the absorbance data in fig. 7 with the data of the straight calibration and calculated the phosphate concentration for the measure points S0 to S50. At measure point 10 and 20 much phosphate was uptaken by yeast cells and so the concentration of phosphate declined fast. When the phosphate concentration declines, the graph flattens. (after measure point 30). We can see that in the other two attempts and in the graph 3B too. We suppose that it takes more time for diffusion for the phosphate to reach the membrane transport proteins.

The problem was that we measured for the concentration of 50µM phosphate different absorbances. The absorptions in the straight calibrations were higher. As a result of this deviation the S50 phosphate concentration would have been negative. That is why we chose only the absorbance graph.

Difficulties:
All in all we did 9 measurements. The first three measurements we did in a group of three people. The problem of teamwork is that there can appear a lot of misunderstandings.
After that we worked alone but all at the same time. The problem here was that it was difficult to observe the time because sometimes we needed the same pipet at the same time.
The observation of the time is very important because of the very sensitive malachit solution.
Another problem was the stirring of the medium with the yeast. We suppose that if the stirring is too hard the yeast cells get damaged. Other problems were inaccuracy of the pipets (because we used pipets from our school, too) and imprecise samplings from the supernatant of the centrifuge. If there are still yeast cells they continue with the phosphate uptake, which might lead to further imprecisions. As we did the last five measurements we noticed that there is something wrong with the photometer.

4. Our experiment to improve phosphate uptake by yeast cells

4.1. First approach 4A: Temperature

Hypothesis:
The phosphate uptake of the yeast cells *(Saccharomyces cerevisae)* depends on the temperature, which, at an optimum of about 32°C accelerates the metabolism of the cells, whereby the phosphate uptake in a certain time is increased.

Does the phosphate uptake by yeast cells depend on the temperature?
We asumed for 32 °C as an optimum temperature for our experiment comparing to different sources from the internet and literature. We suppose that the metabolism of the yeast cells depends on the environmental temperature, because at a high enough temperature more energy in form of heat is available for the metabolism of the cells. Then the cells don't need energy to hold the metabolism on a certain level. And if the metabolism of the cells is accelerated, more phosphate will be taken up by the yeast cell. But at a too high temperature (45 – 50°C) the yeast cells will begin to die off. At a too low temperature the metabolism of the cells is almost inactive; it only degrades protein and carbohydrate reserves. This helps the cell to survive at low temperatures (2 – 8°C) for about ten/ twelve days.[8]

Experiment:
Independent variable: temperature
Dependent variable: phosphate uptake by the yeast cells
Controlled variables: preparation of yeast cells,

First it is to say that we didn't have enough malachitegreen solution for all experiments, which is the reason why we had to mix it on our own with the help of the recipe in part 2, page 3. We couldn't stir the solution several days, because we needed it immediately. Small particles in the solution could have influenced the measurement of part 4.1.
We put the yeast solutions in a water bath, which we broiled on a heating plate and tried to keep the temperature constant on 32°C. Once that was achieved, we measured the concentration of phosphate in the solution every 5 minutes, up to 40 min.
We started with the initial phosphate concentration and calculated the difference between the initial concentration and the concentration measured at a certain time (every 10 min).
In this way we figured out the phosphate decline, which is caused by the uptake of the yeast cells.

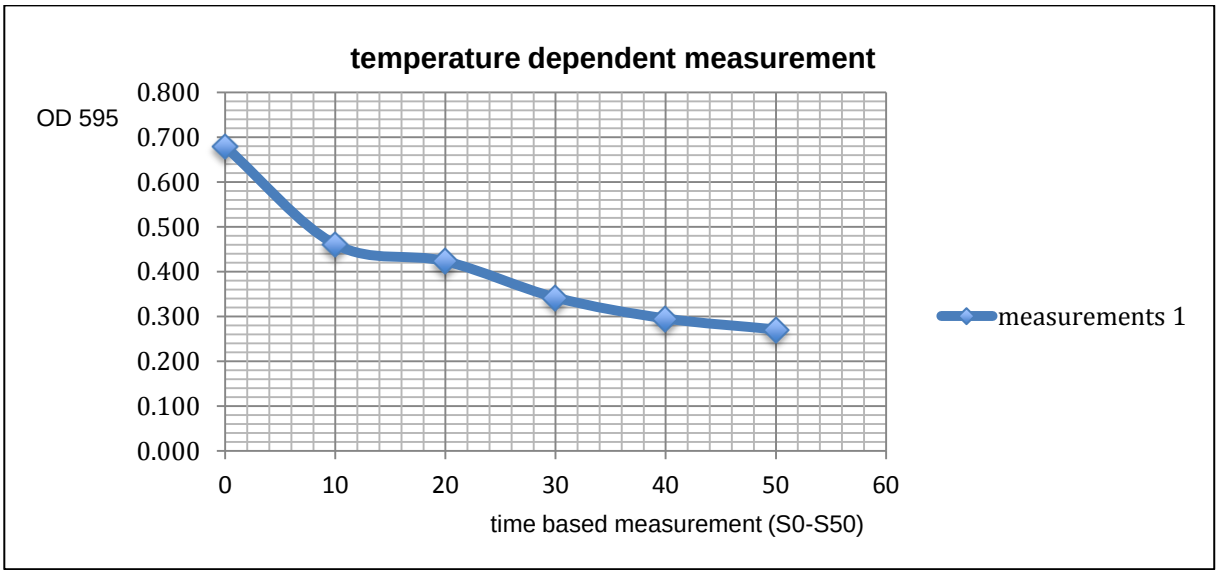

Fig 8: measurement 1 of the temperature measurement

Conclusion:

We were glad to see that our hypothesis approved. For our interpretation we pay attention to the shape of the curve and not to the starting level.

Our graph declined sharper than the graph of the norm-value (fig. 7). That means, that more phosphate got absorbed in our experiment that means again, that the yeast cells stored more phosphate.

We took the first measurement, because it was the best curve. Between S0 and S10 we observed, that the curve decline sharper than between S10 and S50. Because of the higher temperature (32°) the uptake of the phosphate in the yeast cells goes faster and is after the first 10 minutes mostly concluded.

4.2. Second approach 4B: pH value

Hypothesis:
The efficiency phosphate transport is dependent from the pH value. Phosphates can be transported into the yeast cells in a range of pH 2.5 – 8. The optimal value lays around pH 5.5 and decreases in both directions.[10, 11]

We want to prove that this is the optimal value for yeast cells.

Design of the experiment:
Does the phosphate uptake by yeast cells depend on the pH-value?
We think that the ideal pH value for phosphate transport in yeast cells is pH 5.5 according to literature we found in the internet. So we made a new buffer with pH 5.5[12]. But the rest of the experiment would be still same like the experiment explained in part 3.

Experiment:
Independent variable: pH-value
Dependent variable: phosphate uptake by the yeast cells
Controlled variables: preparation of yeast cells

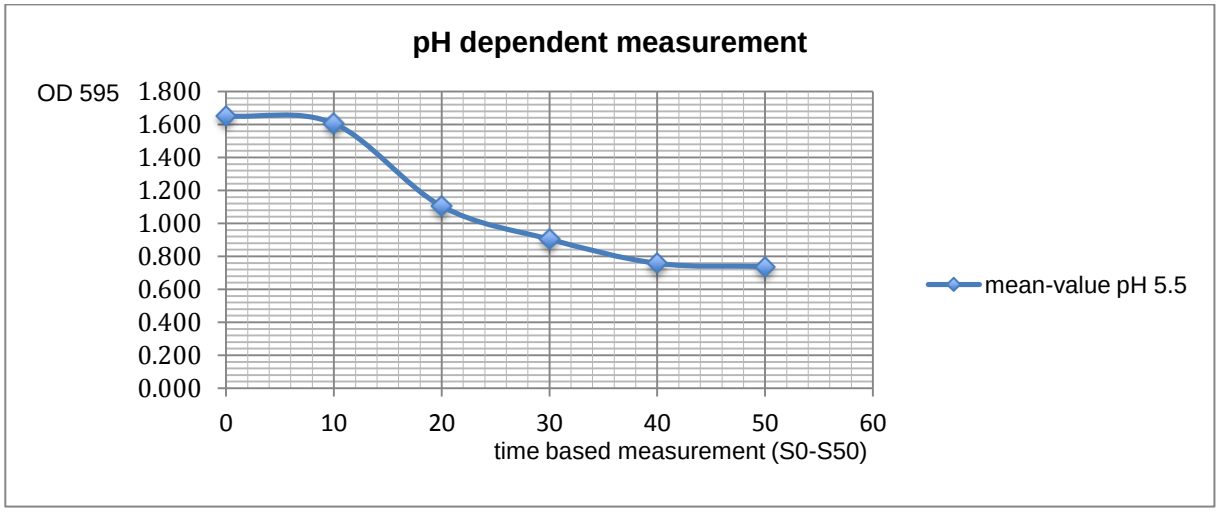

Fig 9: measurement pH 5.5

First we made a phosphate buffer with potassium dihydrogen phosphate and disodium hydrogen phosphate at a pH-value of 5.5[12] and mixed 0.5 ml of it with 1 g glucose and filled it up with destilled water until 100 ml (medium).
Then we proceeded with the experiment explained in part 3 except another medium with different pH-value.

Conclusion:
In fact we are pleased with the results, except for the part between S0 and S10, were it didn't reacted as we supposed to, because we thought that the curve instantly would decline. This could be due to mistakes made by us during the experiment or a wrong measurement.
We compared our curve to the curve of the norm-value (fig.7) and saw that our graph declined sharper. As a result of that, more phosphate was transported into the yeast cell and more phosphate was stored in them.
From S40 to S50 the curve flattens. This could be the reaction to the falling concentration of phosphate and this means that the transport would be slowed down.

4.3. Third approach 4C: glucose concentration 5%

Hypothesis:
The more sugar, the more energy. The more energy, the more yeast cells store phosphate.

The cell respiration functions with the supply of sugar ($C_6H_{12}O_6$) and oxygen (O_2), the product is carbon dioxide (CO_2) and water (H_2O). It converts biochemical energy into adenosine triphosphate (ATP). The energy stored in ATP can be used for the transportation of phosphate across the cell membrane and phosphate gets stored as polyphosphate.[13]

Experiment:
Independent variable: glucose content
Dependent variable: phosphate uptake by yeast cells
Controlled variable: preparation of yeast cells

Due to the graph on page 124[14] we calculated a concentration of glucose of 11 g in 100ml medium to get a maximum of phosphate transport/ storage. But briefly after this concentration the phosphate transport through the membrane will decelerate because the transport enzymes are overloaded. That is why we decided to perform our experiment with 5 g glucose.

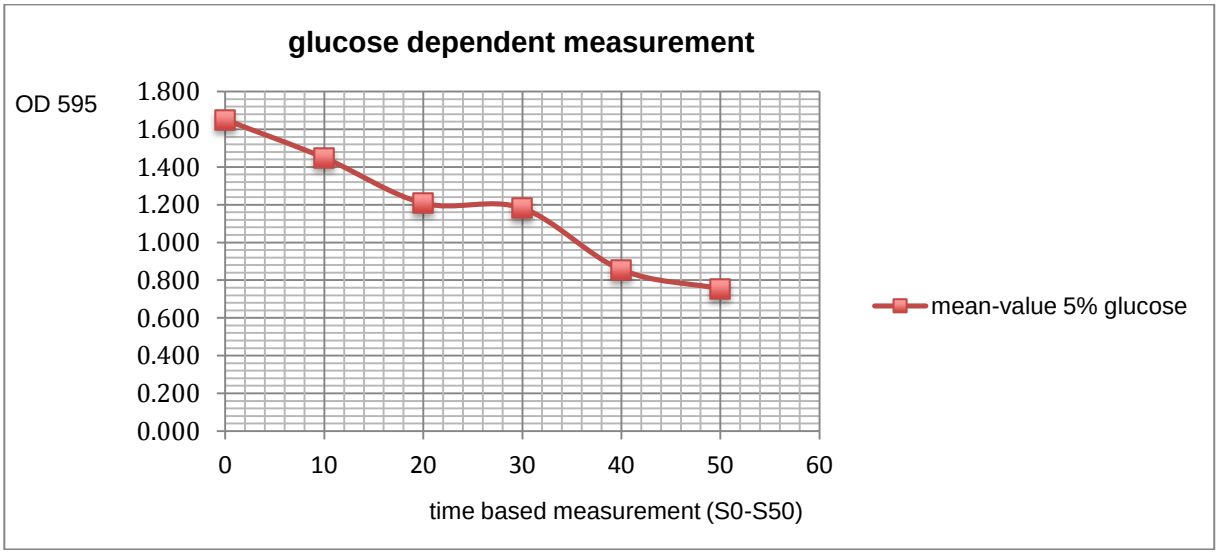

Fig: 10: mean-value of 5 % glucose

Conclusion:
We were glad to see that our hypothesis approved. In our interpretation we pay attention to the shape of the curve and not to the starting level.
Our graph declined stronger than the graph of the norm-value (fig. 7). That means, that more phosphate has been absorbed in our experiment. That means again, that the yeast cells stored more phosphate.
Between S40 and S50 we observed that the curves flatten with both glucose concentrations. Maybe because there is less phosphate that could be taken up, this could slow down the transport of the membrane enzymes even if there is enough glucose for cellular respiration.

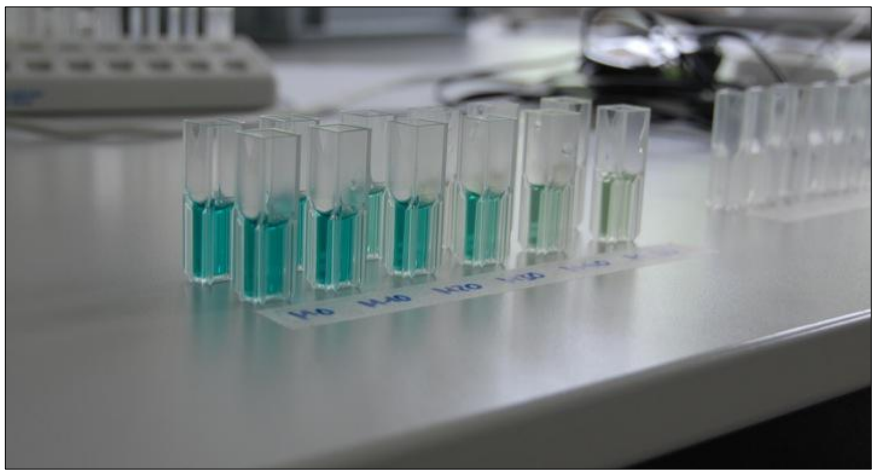

Fig 11: malachitgreen complex

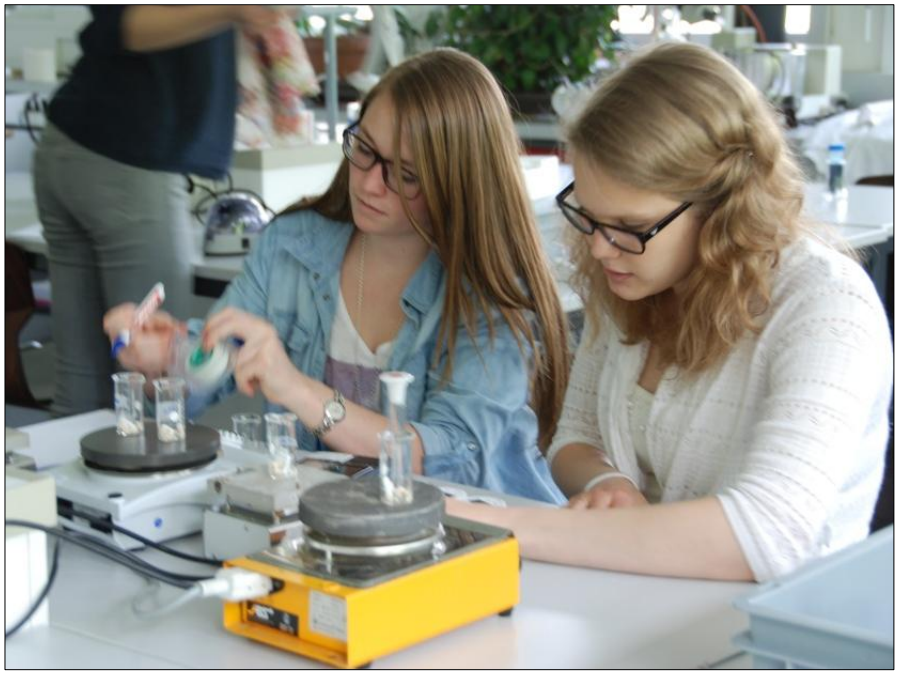

Fig 12: Jasmin and Mirjam preparing the experiment

Fig 13: Sf5 BioChemie at research

5. Reference list

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15. Fig. 1-13: all are self made

6. Activity list

Part 1 Studying the literature	Question 1	Mirjam Grögli Flavienne Landolt Johanna Reckhaus
	Question 2	Stephan Niederer Yannick Messmer Linda Beutler
	Question 3	Salome Germann Yannick Messmer Simona La Cioppa
	Question 4	Jasmin Maissen Sven Krähenbühl

Part 2 Calibration of the measuring system	labour	Jasmin Maissen Johanna Reckhaus Flavienne Landolt Markus Meier Carl Bauert Samuel Meili
	studying the hypotheses	Yannick Messmer Linda Beutler Salome Germann Simona La Cioppa Mirjam Grögli
	statistics	Sven Krähenbühl Donat Straessle Stephan Niederer

Part 3 Measuring the phosphate uptake by yeast cells	labour	Jasmin Maissen Mirjam Grögli Johanna Reckhaus

Part 4 How to improve the phosphate uptake by yeast cells	temperature	Yannick Messmer Donat Straessle Sven Krähenbühl Jasmin Maissen Johanna Reckhaus
	glucose content	Simona La Cioppa Salome Germann Markus Meier Samuel Meili
	pH	Stephan Niederer Linda Beutler Carl Bauert Flavienne Landolt

Reference List	All together
Activity List	Mirjam Grögli