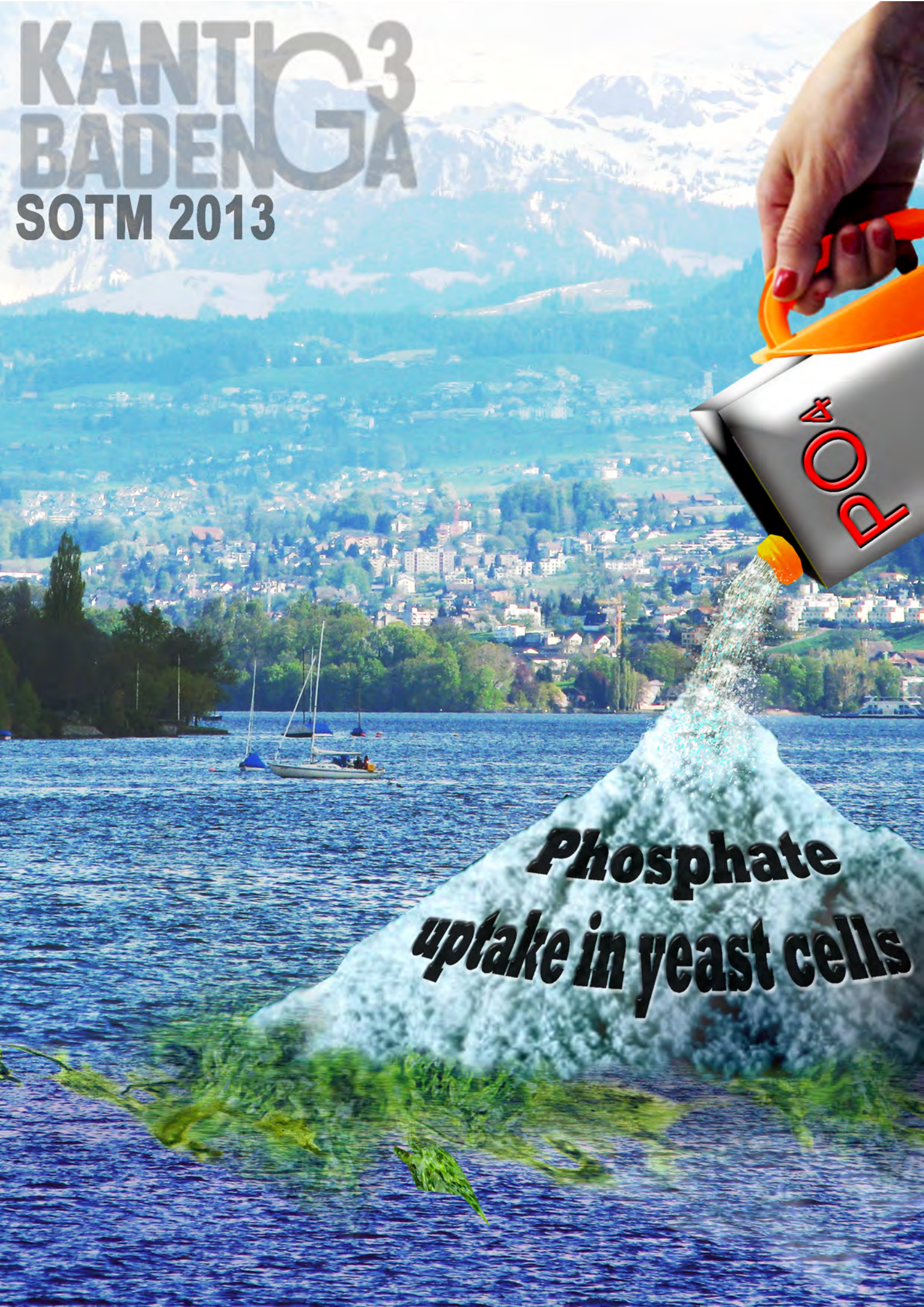
KANTI BADEN G3

SOTM 2013



Contents

Part 1

Information about phosphate

Phosphate is a negative ion ($PO_4$3-)[1], it is mined from minerals such as Apatit Ca[(PO_4)$_3$(OH,F,Cl)][2]. It is often found in North Africa (Morocco, Western Sahara), Jordan, United States (Florida), Russia (Kola Peninsula), South Africa and China[2] and often occurs in connection with heavy metals[2]. However, the occurrence of phosphate is limited and will be years[3].

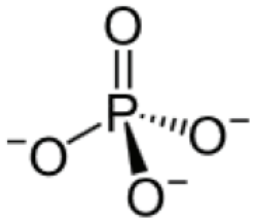


Fig. 1 Phosphate

Humans and animals absorb phosphate through their food[4] and plants take it up through the soil[3]. Phosphate is essential for all organisms[2]. Phosphate and alternating sugars (deoxyribose) form the DNA double helix, one of the three major macromolecules[2]. Phosphate is also involved in the energy metabolism, where in the shape of ATP, it sets energy free and conserves it[1]. Phosphate with potassium ion form the molecule calcium apatite which strengthens the bones and teeth[5]. In addition, hormones transmit their signals with the help of phosphate[3]. The brain and the nerves need phosphate to function properly[3] and the muscles thicken with the help of phosphate[3].

Phosphate is an important nutrient to the cell. It goes across the membrane of the vacuole into the vacuole and it is able to take large amounts of phosphate in. This is done by means of counter-current exchange. The exchange is highly dependent on the pH value, where a lower value will enhance the activity [6].

In the vacuole, the phosphates are also stored. The phosphates and polyphosphates form polymers, anhydrous compounds, which are also called vacuolar pools. Depending on the actual occurrence of phosphate they multiply or disappear[6].

Due to the increasing population, more and more biological substances are needed . Phosphate is used as fertilizer to multiply these organical substances[7]. In the shape of a fertilizer it reaches various water sources[8], ,where it is responsible for the eutrophication[8]. We use treatment plants to filter the phosphate out before it reaches streams and lakes[8]. Microorganisms play an important role in this process[8]. In the biological phosphorelimination microorganisms are set under stress in an anaerobic pool[7]. In this situation the organisms emit the phosphate which they had stored to produce the necessary energy to survive without oxygen[8]. After this stress situation the microorgansims reach an aerobic pool. There they take up their phosphate again and some additional ones too[8]. Hence the total content of phosphate in the water falls[8].

1 Author unknown, 2013. Apothekenumschau – Article: „Phosphat: Wichtig für viele Stoffwechselprozesse“. Retrieved April 17,2013 from www.apotheken-umschau.de.

2 Wikipedia. 2013. Article: “Phosphate”. Retrieved April 17, 2013 from www.wikipedia.org.

3 Hans Schuh, 2005. Zeit – Article: “Ressourcen: Ohne Phosphat läuft nichts“. Retrieved April 17, 2012 from www.zeit.de.

4 Univ.Doz.Dr.med. Wolfgang Hübl, 2003. Med4you – Article: „Phosphat (bzw. „Phosphor“) – Übersicht“. Retrieved April 17, 2013 from www.med4you.at.

5 Author unknown, 2013. BDI – Article: “Blutbild – Erklärung Phosphat”. Retrieved April 17. 2013 from www.internisten-im-netz.de.

6 James W. Booth and Guido Guidotti, 1997. The Journal of Biological Chemistry.

Part 2

Difficulties and Improvements

During our measurements, we were confronted with several difficulties, which we had to master. The first difficulty we met was how to use the photospectrometer. We had to learn everything about the measurement methods by trial and error because nobody really understood how to operate the machine and we did not have a manual. However, after some test runs we finally managed to get used to the machine.
The next problem was that the cuvettes we got from our school laboratory had already been used before and as a result they were contaminated with phosphate from cleaning products. We noticed this while adding the first "zero solutions" which did not contain any phosphate, only distilled water and the Molybdite-Malachite green-Indicator, into the cuvettes. Before filling the solution in the cuvettes, it was yellow, while after we filled it into the cuvette, it suddenly turned green. This meant that the cuvettes were contaminated with phosphate. So we asked our school laboratory for cuvettes which had never been used before.
With this knowledge, we accomplished our first series of measurements. Although after this series, we ran out of Sodium-Phosphate-Buffer (50µM), so we had to reproduce it with the 0.1M. We tried to calculate the dilution factor. First, we unfortunately calculated a wrong dilution factor of 1:20. We did not notice this mistake until we saw that every solution to which we added the Sodium-Phosphate-Buffer coagulated. This was an effect of the too high concentration of phosphate. After that we recalculated the dilution factor and eventually got the right factor of 1:2000.
Following this, we mastered all remaining series of measurements with success and received quite good results.

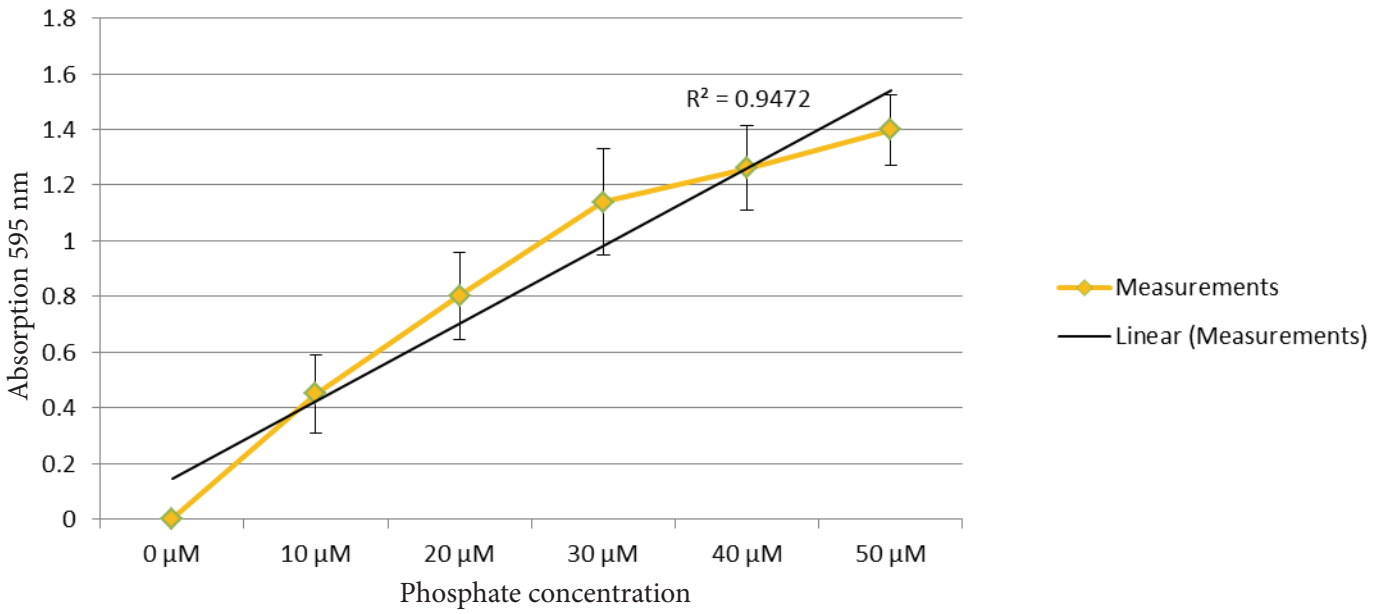
	Measurement 1				
	Mean average	Standard deviation			
0 µM	0.005966667	0.000416333	0.0063	0.0061	0.0055
10 µM	0.1747	0.003504283	0.1711	0.1749	0.1781
20 µM	0.497366667	0.000503322	0.4969	0.4973	0.4979
30 µM	0.764233333	0.001101514	0.7631	0.7643	0.7653
40 µM	0.967033333	0.001650253	0.9654	0.967	0.9687
50 µM	1.264366667	0.012502133	1.252	1.2641	1.277

	Measurement 2				
	Mean average	Standard deviation			
0 µM	0.010666667	0.00057735	0.011	0.01	0.011
10 µM	0.3613	1E-04	0.3612	0.3613	0.3614
20 µM	0.713333333	0.00141892	0.7118	0.7136	0.7146
30 µM	0.985333333	0.002023199	0.983	0.9864	0.9866
40 µM	1.1648	0.004550824	1.1603	1.1647	1.1694
50 µM	1.5193	0.007208329	1.5123	1.5189	1.5267

	Measurement 3				
	Mean average	Standard deviation			
0 µM	0.002666667	0.00057735	0.003	0.003	0.002
10 µM	0.4506	0.001705872	0.4492	0.4501	0.4525
20 µM	0.8025	0.004881598	0.7984	0.8012	0.8079
30 µM	1.14	0.010901376	1.1292	1.1398	1.151
40 µM	1.261766667	0.013361262	1.2481	1.2624	1.2748
50 µM	1.399066667	0.015257894	1.3841	1.3985	1.4146

Part 2

The Average of all three measurements is displayed in the graph below.



	Measurement 1	Measurement 2	Measurement 3	Mean average:	Standard deviation:
0 µM	0.005966667	0.01066667	0.00266667	0.00643333	0.00402036
10 µM	0.1747	0.3613	0.4506	0.32886667	0.14078048
20 µM	0.497366667	0.71333333	0.8025	0.67106667	0.15689628
30 µM	0.764233333	0.98533333	1.14	0.96318889	0.18885955
40 µM	0.967033333	1.1648	1.26176667	1.1312	0.15021203
50 µM	1.264366667	1.5193	1.39906667	1.39424444	0.12753506

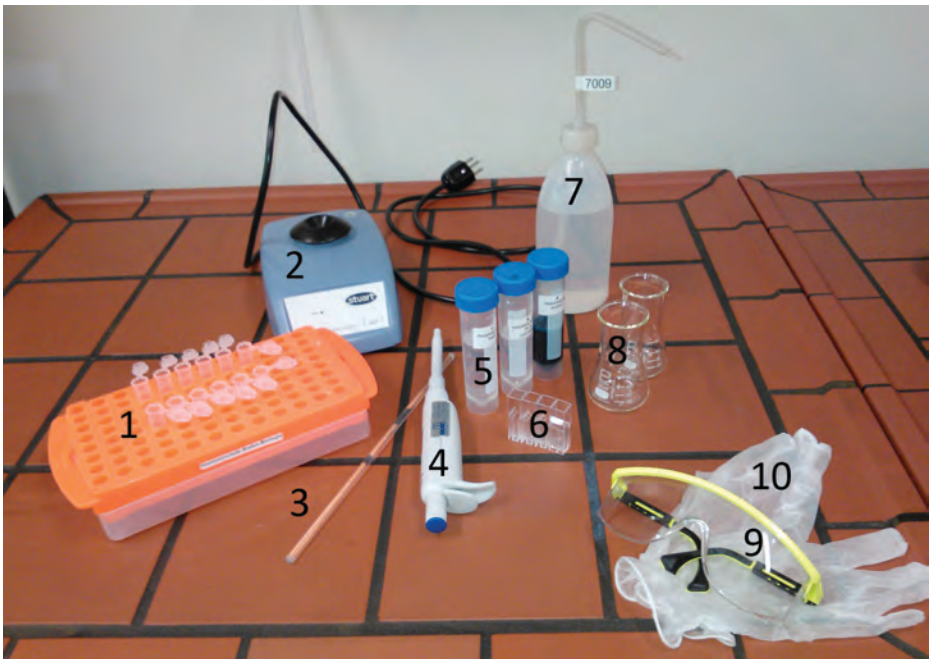


Fig. 2 Laboratory material
1. Eppendorf-Tubes
2. Vortex
3. Stirring rod
4. Pipet
5. Solutions
6. Cuvettes
7. Distillated water
8. Pots
9. Protective glasses
10. Protective gloves

Part 2

Calculations

To calculate the concentration out of the absorption, we can use the Beer-Lambert-equation.

This says: $A = c^* \varepsilon^* d$

A stands for our measured absorption, c for the concentration and d for the distance, the light has to travel through the cuvette. A, c and d are given from our measurements and ε is a constant which varies from substance to substance. We had to calculate ε by adding all given variables and solving for ε.

$$\varepsilon = \frac{A}{d * c} = \frac{0{,}3288}{1cm * 10\mu M} = 328800$$

Now we can calculate the concentration of our measurements. We simply add our calculated constant to the Beer-Lambert-equation:

$$A = c * 328800 * d$$

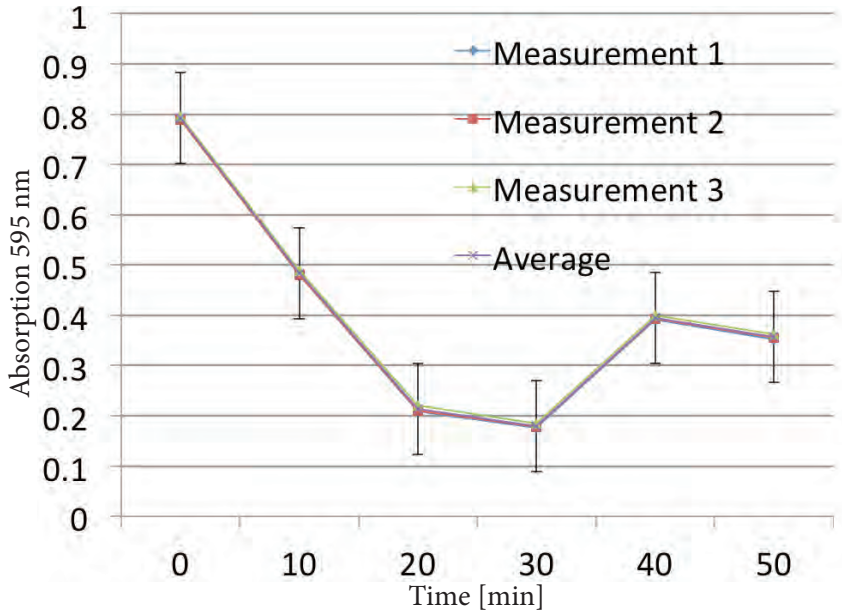
$$c = \frac{A}{10\mu M * 328800}$$

Part 3

Raw Data

Time [min]	Measurement 1	Measurement 2	Measurement 3
0	0.7900	0.7897	0.7965
10	0.4806	0.4807	0.488
20	0.2079	0.2109	0.2206
30	0.1756	0.1782	0.1782
40	0.3911	0.3934	0.3934
50	0.3525	0.3554	0.3554

Time [min]	Average
0	0.79206667
10	0.4831
20	0.21313333
30	0.1795
40	0.39486667
50	0.35663333



Expectation and Explanation

We were somewhat surprised about the results of this experiment. We expected to see a curve that is constantly (exponentially) falling. This would mean that the concentration of phosphates in the solution decreases constantly. We came to this conclusion because we thought the yeast cells would uptake the phosphates at a regular rate. What we measured though was different. As you can see in the curve above we have a considerable increase after 40 minutes in all of our three measurements. Due to that we excluded the possibility of an error concerning our measurements. So either our method of measuring was wrong or it is a phenomenon which we did not include in our considerations. We discussed several possible explanations for this occurrence.

Part 3

A very logical, biology-based explanation would be osmosis. After 40 minutes, the phosphate concentration inside the yeast cells is much higher than outside, causing the cell to start rejecting the surplus phosphate ions. However, a study of the yeast cell revealed that since the phosphate is stored in vacuoles, the concentration in the cell plasma does not rise sufficiently for this explanation to be viable.

Another explanation could be that somehow our solution contained a kind of bacteria which disposed a substance, therefore forming a coloured complex that absorbs at exactly 595 nm.

Further we considered the possibility of bursting yeast cells due to the changing osmolarity caused by increasing concentration of phosphates in the cells. However, yeast cells have a very strong outer membrane with a special structure difficult to damage, which makes this explanation highly unlikely.

Problems and Solutions

In order to get useful data out of our experiment we had to overcome various problems. First of all we had to fight for permission to work on a photometer. When we finally got access to the machine it took us a considerable amount of time to get it running. The problem was that there was a group of chemistry teachers who could not decide on how to use the photometer correctly in order to fulfil the task.

This was also the cause of our next problem. Since we used a great deal of solutions we received from simply science without getting any useful results we were now running out of material for our real experiments. We tried to produce the solutions ourselves which did not work because the solution was contaminated with phosphates. We believe that the first sulphuric acid at Kanti Baden contained phosphates.

Also we had to use all new material (vessels, etc.) for our experiment because all material that had been used before was contaminated with phosphor which probably came from the process of washing.

Fig. 3 Working on Part 3

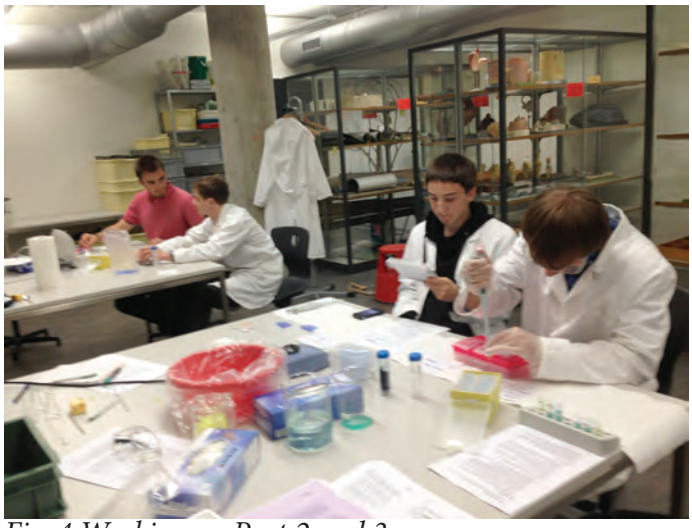


Fig. 4 Working on Part 2 and 3

Part 4 A

First approach: fermentation

Improvement of the phosphate uptake of the yeast cells through anaerobic cellular respiration (fermentation).

Conclusion

The hypothesis of this experiment was to improve the phosphate uptake of yeast cells using anaerobic fermentation. The explanation to this theory lies in the following chemical reaction:

Aerobic cellular respiration: $C_2H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 30\ ATP$
Fermentation: $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP$

Due to the missing oxygen the cells start to consume more glucose. The cells are now in a state of anaerobic cellular respiration, hence fermentation. As a result of this higher consumation of glucose and uptake of phosphate the following experiment design was created.

The Experiment

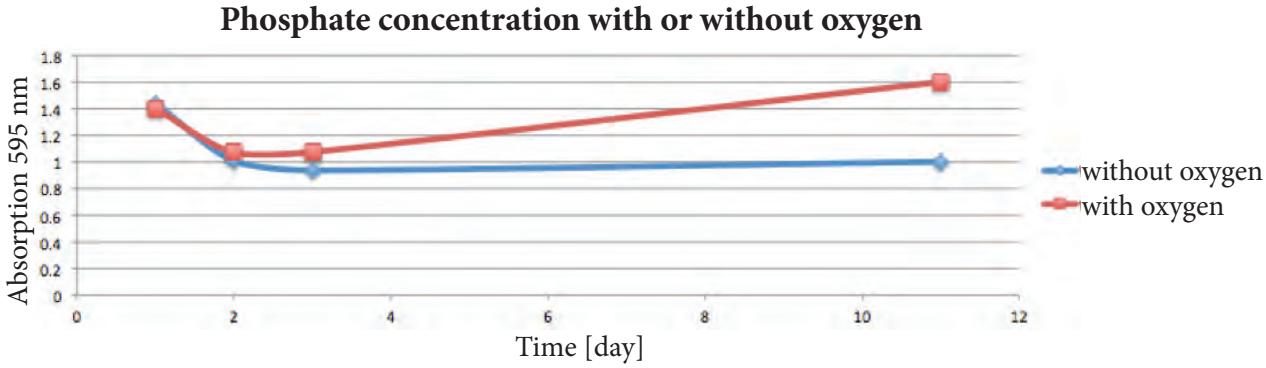
Controlled variables:
- yeast cell solution

Independent variables:
- aerobic / anaerobic state
- time

The basis of the experiment was the 60cl yeast cell solution: 99% of this was the phosphate buffer (0.1 M), 1% a glucose solution (1%), as in task 2. Six grams of yeast was added. We put this solution into 30 Eppendorfs (à 2cl). Then 15 of them were closed and sealed off, while the other 15 were left open to control and measure the difference. Under the assumption that the oxygen in the Eppendorfs would be consumed after a day, the measurements started.
From this point on the measurements were supposed to be performed daily, to have as much data as possible. After one series the Malachitgreen and Molybat solutions had been consumed. This complicated everything in that we had to wait for these to be delivered, and this was a highly time consuming progress.
New Eppendorfs had to be prepared, because the original ones were too old to produce useful information. This time, only a 30cl yeast cell solution was used, hence producing only 12 Eppendorfs. Due to the data that had been collected from the original series, the first measurement was after two days.

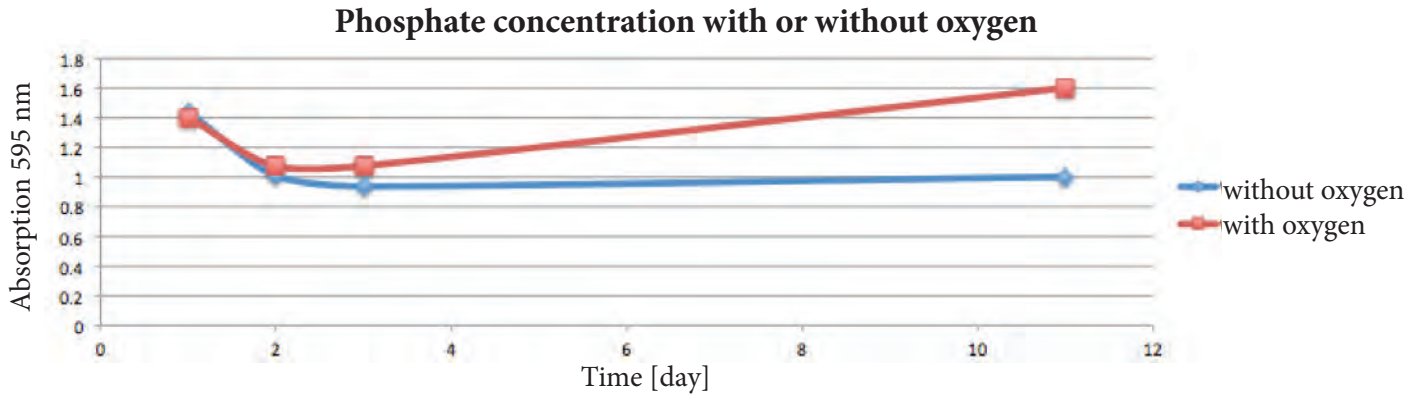
Evaluation

These are our graphs of the experiment:

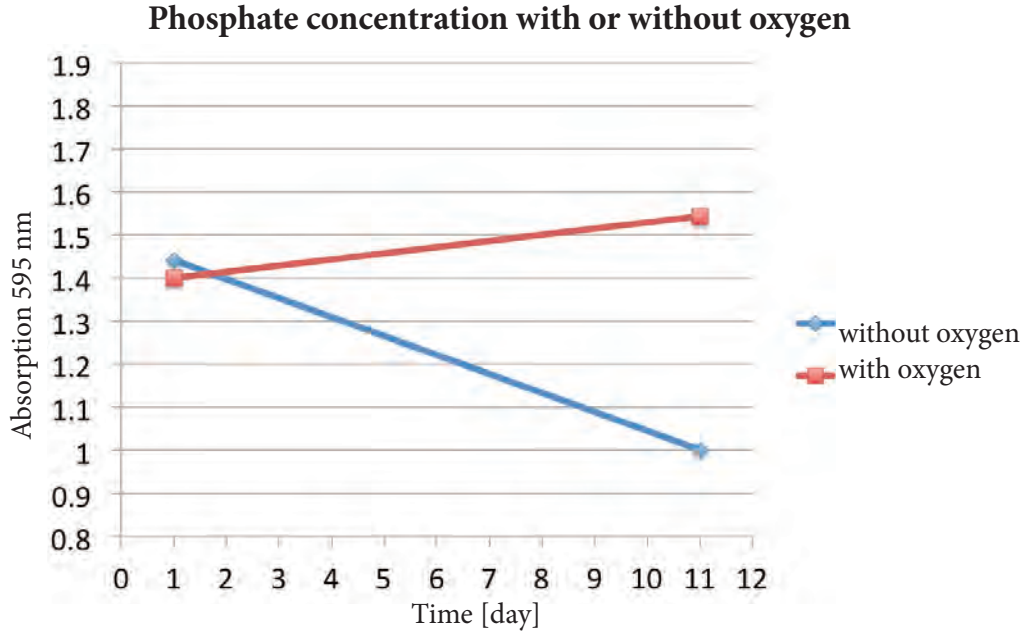


Part 4 A

Here you can see the results we had on the first, second, third and eleventh day. We then interpolated these results.



Here are the results of the second and third day.



This is the interpolation between the first and eleventh day.

This is our theory about the outcome of the measurements:
(1) The results do not verify our hypothesis at all. It could be that the concentration of the phosphate in the solution increased due to condensation. This would be a plausible explanation for the increasing concentration. This seems likely, because we could observe how the amount of the solution decreased daily.

Investigation improvement
(1) Similar to the rest, this experiment was complicated by the missing solutions. It had to be restarted and was very time consuming. Because of this, the quantity and quality of the data suffered severely. It was planned to measure as much and as regularly as possible to keep the systematic errors as small as possible.
(2) Something that we noticed was the rapid sedimentation of the yeast in the Eppendorfs. We believe this is a problem for the amount of the phosphate uptake. If they could be left in a constant vibrating machine, the solution would be better mixed and would possibly guarantee a higher uptake.
(3) It is not guaranteed that the used utensils and recipients are phosphate-free.
(4) We did not expect the strong condensation that was observed. To avoid this falsification, it would be better to have a way to fill the Eppendorfs with distilled water.

Part 4 B

Second approach: Potassium solution

Would a higher potassium concentration improve yeast cell's phosphate uptake?

Thesis

Yeast's phosphate absorption levels can be improved by increasing the potassium molarity in the solution. Phosphate has a negative charge, and as more of it flows into the cell, the inside turns too negative and starts repelling further phosphate ions. However, potassium cations could serve as equalizers by balancing the inner charge back to neutral so as to avoid this repelling effect, thereby making it easier for the cell to absorb more phosphate.

Variables

Controlled:

- Yeast solution
- Phosphate Buffer
- Photospectrometer

Independent:

- Potassium molarity
- Time

Experiment procedure

The day before the experiment, the potassium solution was prepared with different molarities ranging from 0.0 M to 0.2M in 0.05M steps (and one with 0.5M in addition, as an extremum of sorts), to test their efficiency.

Once the potassium solutions were ready, the same procedure as in Part 3 was followed with a modification at the last step, with the addition of 10 ml of every potassium solution variation right after inserting the yeast into the phosphate solution. For every molarity of the potassium solution, a sample was taken every ten minutes (five samples per molarity in total). Each sample was centrifuged and had its light absorption measured and recorded. This is proportional to the phosphate concentration, so a higher light absorption signifies lower phosphate absorption.

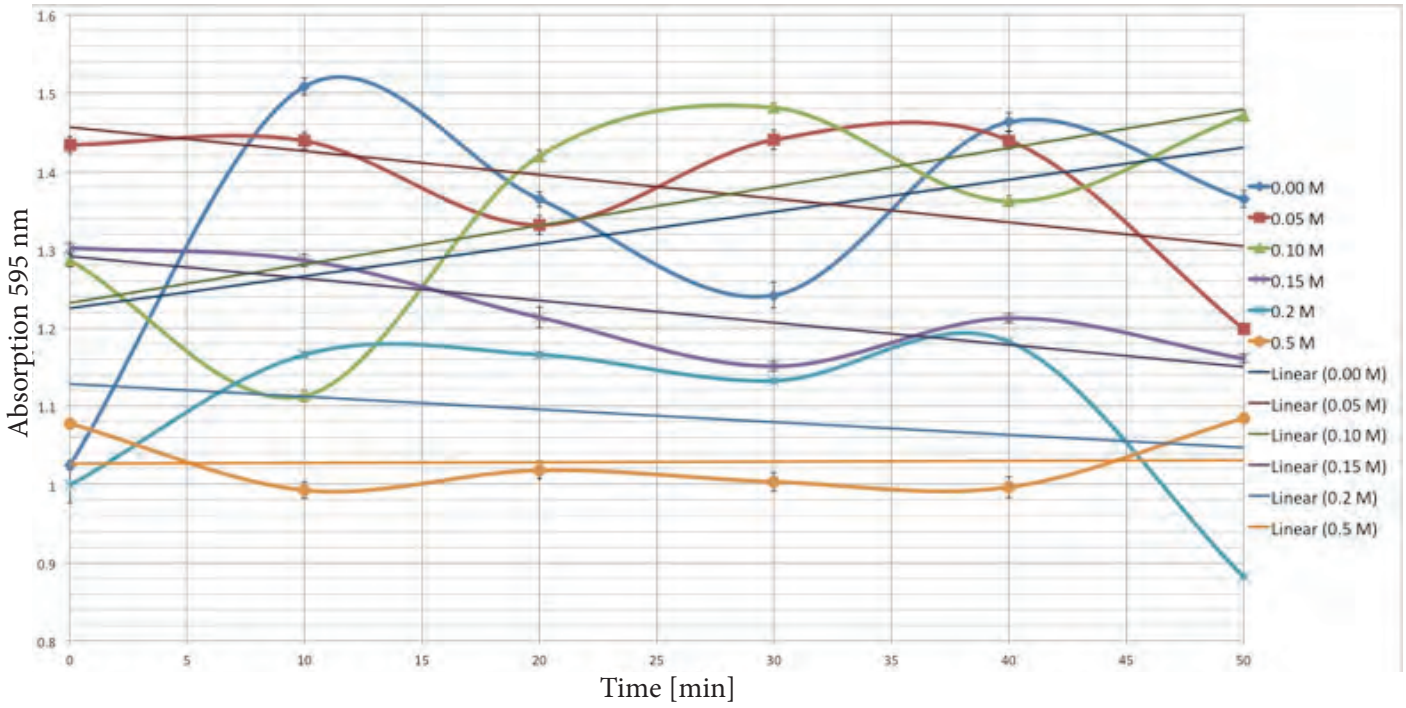
Difficulties

The first experiment was ruined because we used the wrong Sodium-Phosphate-Buffer (due to misreading and a wrong calculation of the dilution factor), leading to a lack of malachite green solution and molybdite solution for a sufficient amount of experiments. We were only able to conduct a single experiment for each variable, which was then measured three times to eliminate any deviation of the photospectrometer, which already has a low deviation to begin with.

Since we only had enough solution left for a single independent measurement, the error in measurement could not be correctly reduced. In addition, there were many problems due to phosphate contaminated cuvettes – something we did not find out about until it was too late to do anything about it. Because of all these problems, our results were not as reliable as we would have hoped, and our data did not show as clear a trend as expected.

Part 4 B

Interpretation of the graph



Despite the inexact values, the data seems to prove our thesis. The lower concentrations are mostly on the upper part of the graph (i.e.: lower phosphate absorption), while the higher concentrations are generally lower. The linear regression graph clearly shows how the phosphate absorption increases with higher potassium molarities.

There is a sudden increase in light absorption between t=30 and t=40 in all experiments conducted, which must mean that not only has the cell stopped absorbing phosphate, it has begun to emit phosphate back out. Either that or another substance is being formed, which absorbs light at approximately 595 nm, that is, a substance with a similar colour. We have come up with several theories that could explain this strange phenomenon. An incorrect measurement is doubtful and improvable given that the same phosphate concentration increase occurs at the 30-minute-mark in different independently conducted experiments.

If the measured substance is, in fact, only phosphate and not something else, the phosphate release may be due to the yeast cells getting too full and bursting, or simply expelling some of the phosphate ions out (this could be because the potassium can freely go out and the charge would eventually turn negative again) or it could be related to the on-going cell division process, but this is unlikely, since all cells are going through different phases at the same time, so there should not be any sudden changes.

It is difficult to say with certainty what happened, because of the many variables that could not be easily controlled, such as the bacterium contamination or the yeast cell count.

Part 4 C

Third approach: UV-radiation

How does the phosphate uptake of yeast cells vary under exposure of UV-radiation?

Thesis

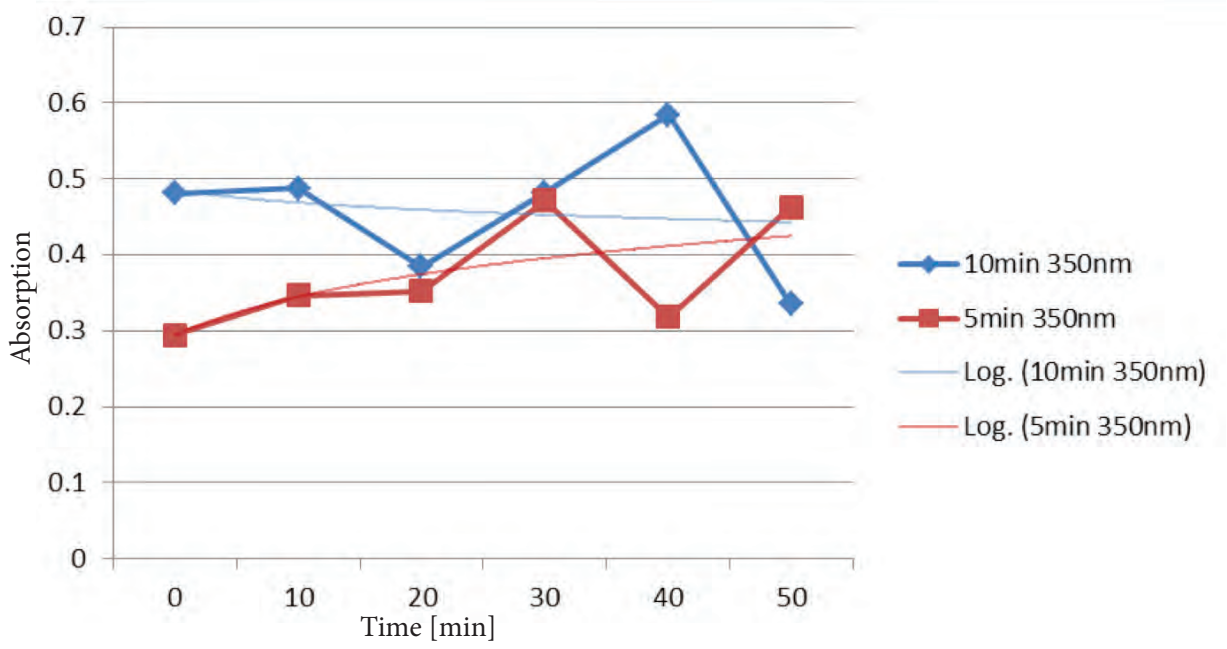
As we were collecting information we stumbled upon a research paper which suggested that UV-radiation could increase the phosphate uptake in yeast cells . This seemingly occurs through the change of phospholipid content in the membrane caused by the UV-radiation, allowing the membrane to take more phosphate in. But the fact is that the UV-radiation is able to significantly increase the probability of mutations.

We used two different wavelengths (350 nm and 254 nm). For each of the two wavelengths we did a measure series in which the yeast cells were under exposure for either 5 or 10 minutes. We decided to use these time spans because we thought that the yeast cells might mutate or die if they are exposed to UV-radiation for too long.

So that we were able to compare our results with the original experiment we decided to change as little as possible and came up with the following variables: The independent variable is the wavelength of the UV-radiation and the time the yeast cells are exposed to it. The dependent variable is the phosphate uptake by the yeast cells. The variables we had to control were the following: temperature, sample size, preparation of Elodea cells and the solutions used, measuring in 10 minute intervals.

Results for 350nm

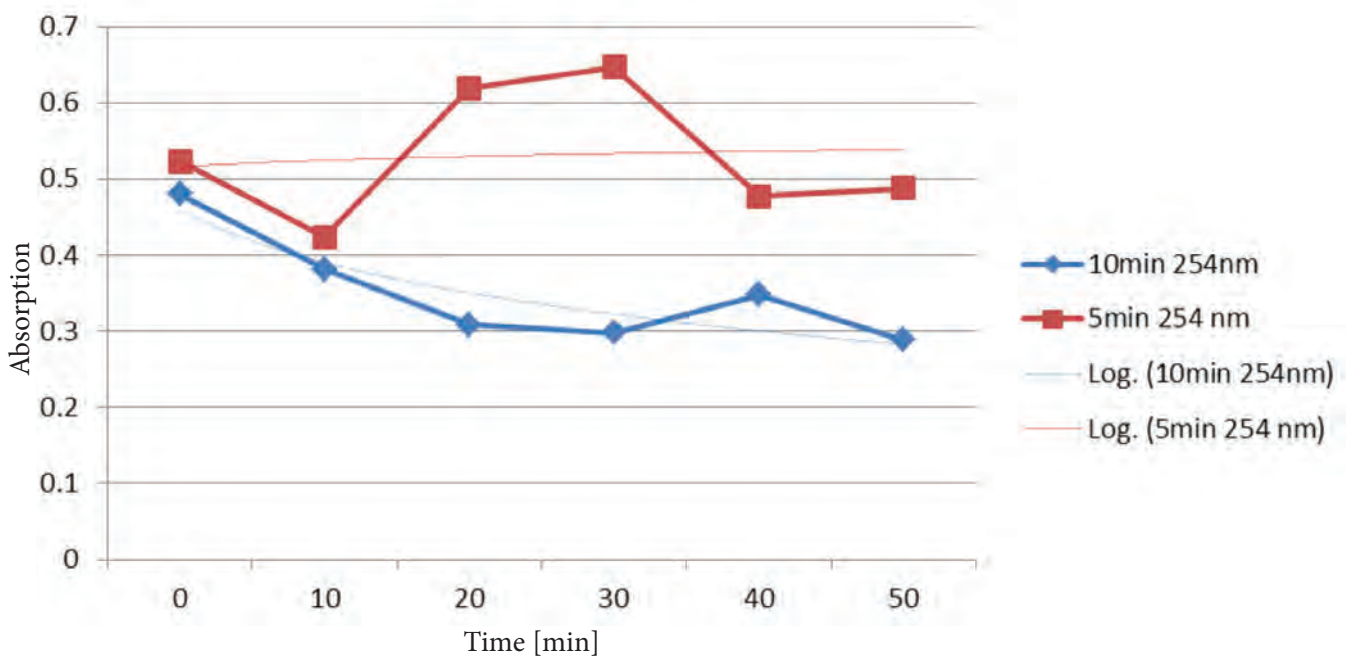
Time in Minutes	Absorption 10 min	Absorption 5 min
0	0.4814	0.2944
10	0.4879	0.3464
20	0.3847	0.3524
30	0.4824	0.4715
40	0.5847	0.3184
50	0.3352	0.4629



Part 4 C

Results for 254nm

Time in Minutes	Absorption 10 min	Absorption 5 min
0	0.4804	0.5232
10	0.3817	0.4236
20	0.308	0.619
30	0.2975	0.647
40	0.3477	0.4771
50	0.288	0.4879



Interpretation

Our thesis seems to be correct in the measurement series with 254nm because the 10 min. exposed measurement series has a bigger phosphate uptake than the 5 min. measurement series. We do not know why the measurements at 20 and 30 min. are very high. But the two measurement series with 350nm contradict our thesis because the 5 min. series has absorbed more phosphate than the 10 min. series. Since there is too little data we are not able to generalize our observations.

Feedback

We found the second task to be a really interesting challenge and we enjoyed the opportunity to work together with the whole class towards a common goal. There were difficulties, of course, and we did not always get along well, but we were able to overcome every obstacle and improve our teamwork as a result. It was also great being able to acquire some experience working in a laboratory, and getting to know the different useful devices that are a normal part of a scientists work-life, giving us some insight into what being a scientist actually is, day to day. Taking part in this competition was a very nice experience and we all feel that we have learned a lot in the last couple of weeks.

Activity List

Part 1

Sheila Peterhans
Nadine Thierer
Therese Albert
26 - 28 April 2013

Part 2

Yannick Merkli
Raphael Kuhn
30 April, 1. May 2013

Part 3

Florent Aziri
Fabian Schnyder
Simon Meyer
Oliver Tejero
30 April, 1 May 2013

Part 4 A

Paco Canales
Fabian Schnyder
Florent Aziri
Stefan Carlen
Fabian Näf
Therese Albert
Nadine Thierer
5 May, 10 May 2013

Fig. 5 Study the instructions

Part 4 B

Andrea Keller
Raphael Kuhn
Yannick Merkli
Adrian Strupler
Thomas Benz
Sheila Peterhans
Felix Rüssli
5 May, 9 May 2013

Part 4 C

Ajith Kumaran
Simon Meyer
Oliver Tejero
Silvan Renggli
Aleks Jovanovic
Sara Bär
Sarah Busslinger
5 May, 9 May, 10 May 2013

Pictures

Sarah Busslinger
Seline Frei

Organisation & Layout

Seline Frei
Team leader

Fig. 6 Working on Part 2 and 3

Fig. 7 Photospectrometer