Phosphate Uptake in Yeast Cells - Task 2

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1

Studying the literature

„The hugest amounts of Phosphate are found in Russia, Marucco, Florida, Tennesse, Utah and Idaho.“

Why is phosphate important for all organisms?

Without phosphate no organism could grow, move, reproduce or even exist. All organisms need phosphate. Every cell needs to keep alive at least one cell nucleus to stay alive.[1] A cell nucleus has a membrane and contains the DNA. Membranes consist of Phospholipids. Phospholipids contain phosphate. Membranes separate cells and organelles and at the same time they connect them and hold them together. DNA also contains phosphate. They are needed as Phosphatedeoxiribosebackbone, which is responsible for their stability. DNA is the container of the genetic information and is needed for reproduction.

In order that organisms can perform active transport of molecules, cellular movements, like muscle contraction and synthesis to biomolecules from easier substances, they need to store energy in a way in wich it can be transported to the places where the energy is needed. ATP (adenosine triphosphate) is used for this purpose. By splitting off a phosphate group, they can set free energy.[2] Phosphate also plays a main role in the process of building up bones and teeth.

What is well known about phosphate uptake in yeast cells (Saccharomyces cerevisiae)?

CNS, the centralized nervous system, is a key organ for understanding the evolutionary divergence of metazoans. All bilateral animals posess a centralized nervous system, but it was suggested that the CNS of deuterostomes has arisen from different origins than the one of protostomes. But now support for the opposite conclusion has been provided. Planaria are a good organism to answer the question whether both deuterostomes and protostomes are derived from a common ancestor because they possess a CNS with simple, primitive morphology similar to the vertebrate CNS.[3] There is also no doubt that Planaria are descendants of early bilateral animals because in their phylogenic tree they are positioned near the root of the Lophotrochozoa, one of the three groups of bilateral animals.

How do yeast cells store phosphate?

An important part of the phosphate storage in yeast cells plays the polyphosphates. They are one of the most important ways to store energy in yeast cells and other microorganisms. The trombocytes contain structures which store polyphosphate; the so called dense granules.[4] Another major storage compartment of the yeast cell is the vacuole. There is a huge transport of phosphate across the vacuolar membrane by counterflow exchange with with the phosphate present in the vacuoles. The carrier which transports the phosphate is highly selective for phosphate and doesn't let any other substrates through.[5]

Why is phosphate uptake by microorganisms an important issue in our society/environment?

Microorganisms can be used for lowering the phosphate content in water, which is very important for our environment. Phosphate is often used as a compound of fertilizers, in washing powder for softening the water or also in food to preserve it.[6] The high rate of phosphate in these products has an effect on the rate of phosphate in the ground water. Too much phosphate also causes a rapid growth of seaweeds in aquatic systems and therefore a total change of the ecosystem.

In the process called biological phosphorus removal in purification in effluents, yeast cells are brought into an anaerobe environment. If microorganisms don't have any oxygen for surviving they automatically use phosphate to produce energy, which they use instead of oxygen. To support this process, easily degradable substratum has to be made available. When microorganisms reach an aerobe area of life again they absorbe the phosphate used before with further more phosphate present in the water.[7] Therefore the concentration of phosphate in there lowers.

2
Calibration of the measuring system

The results of our three best measured experiments are shown in this table followed by a graph of the raw data and the mean value of our measuring. All the measuring was done with the spectrometer received which was set to 595 nm.

The standard deviation is calculated with the following formula:

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2}$$

1. Result of our pretest.

2. Our lab environment.

Phosphate concentrati-on	Experiment 1	Experiment 2	Experiment 3	Mean value	Standard deviation
10	0.247	0.259	0.248	0.251	0.007
20	0.555	0.576	0.572	0.568	0.011
30	0.770	0.830	0.815	0.805	0.031
40	0.865	0.944	0.907	0.905	0.040
50	0.904	0.981	0.958	0.948	0.039

3. Pretest results.

The precedent results are quite acceptable and useful for part 3, where the uptake of phosphate by yeast cells is measured. But first we had some problems with the spectrometer; our measuring was totally wrong and without any regularity. So we tried to find out the weak points of the experiment from different points of view.
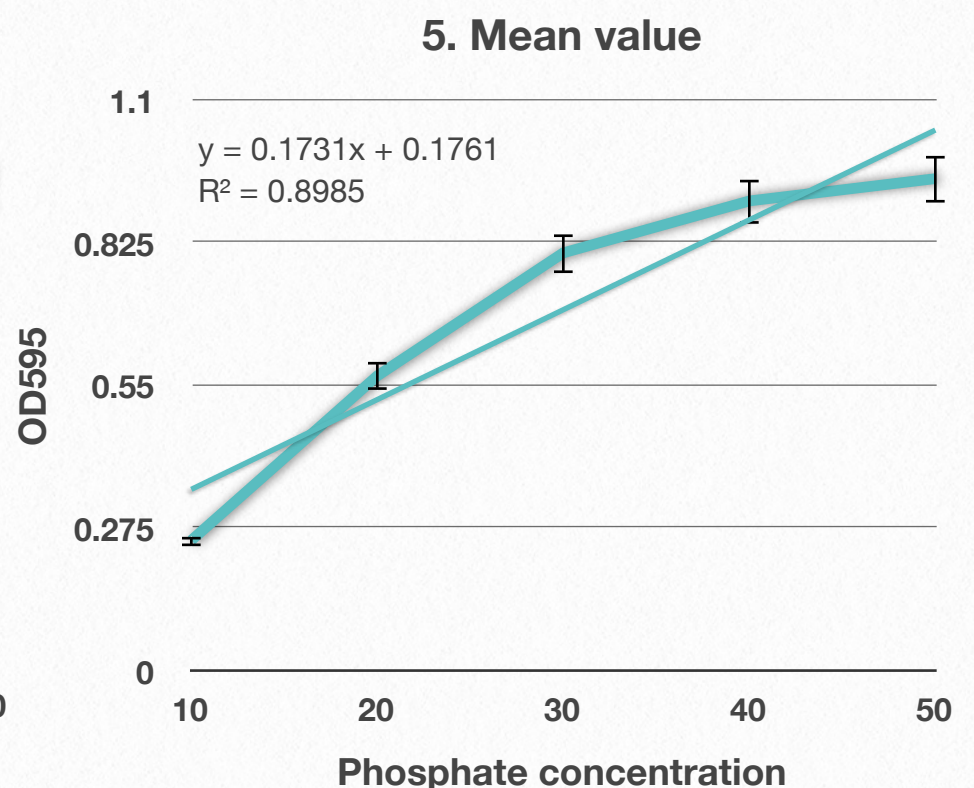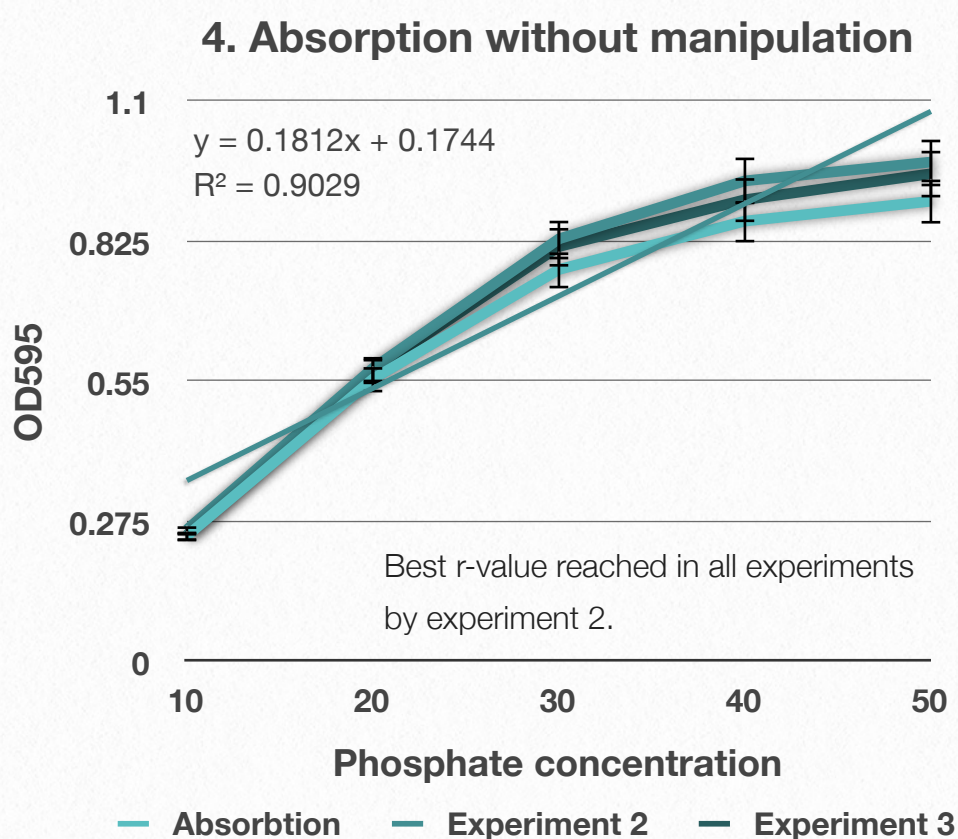
Our observations were that:

- The measuring inaccuracy is +/- 0.01 maximal and therefore not very exact.

- The cuvettes of the Simply Science Chem-Box are eventually a bit less accurate than the new cuvettes we bought (so we used our own cuvettes for the next experiments).

- The measuring does not dependent on whether we measure with the exact wavelength of 595 nm or with a wavelength of 585 nm or 605 nm.

After some experimenting we found out that the measuring becomes more exact and regular when the lapse of time from the preparation of the solution until the start of the measurement is longer (30 minutes minimum). Frederike Schmid has written in her dissertation work that a time span between 20 and 30 minutes would be enough to reach exact results.[8] With the acquired knowledge we measured all the data again and reached the results described beforehand.

Another obstacle in this task was that it took much time to understand the computer programme for the spectrometer. Finally our male classmates managed to get all the important parts of the programme and lead us through the task.

All these things were among other little things the hardest difficulties in this task, but still we managed to obtain results with acceptable fluctuation.

4. Absorption without manipulation

$y = 0.1812x + 0.1744$
$R^2 = 0.9029$

Best r-value reached in all experiments by experiment 2.

Phosphate concentration

— Absorbtion   — Experiment 2   — Experiment 3

5. Mean value

$y = 0.1731x + 0.1761$
$R^2 = 0.8985$

Phosphate concentration

3

Measuring the phosphate uptake by yeast cells

„The original production process of phosphate was the extraction from urine.“
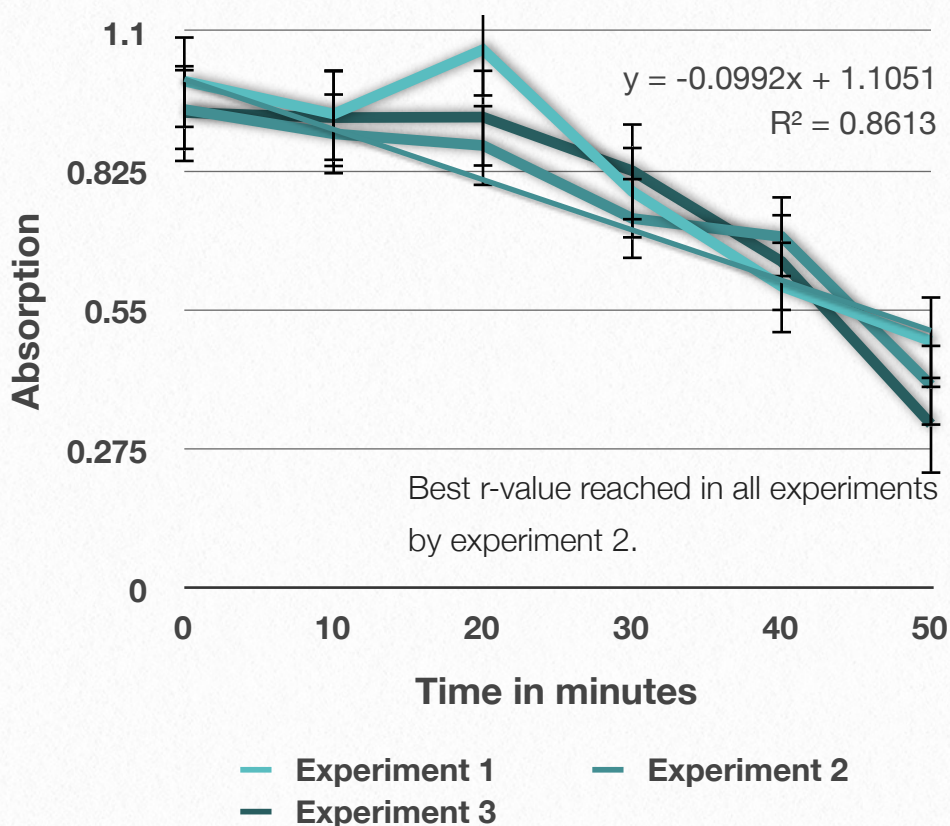
Discuss the difficulties

In these experiments we did not have many difficulties because in part 2 we have already improved our working with the spectrometer by far. To prepare the solutions we used the pipettes accurately just like we learnt it in part 2. So we worked as precisely as possible and tried to avoid any mistakes. When we worked with the centrifuge we centrifuged the EP's exactly 1 minute and we always used a counter piece in order to balance it out. When we took 40 µl of the supernatant we were always careful so we did not rai-

se the pellet. We think we may say that we worked precisely because our graphs turned out pretty good.
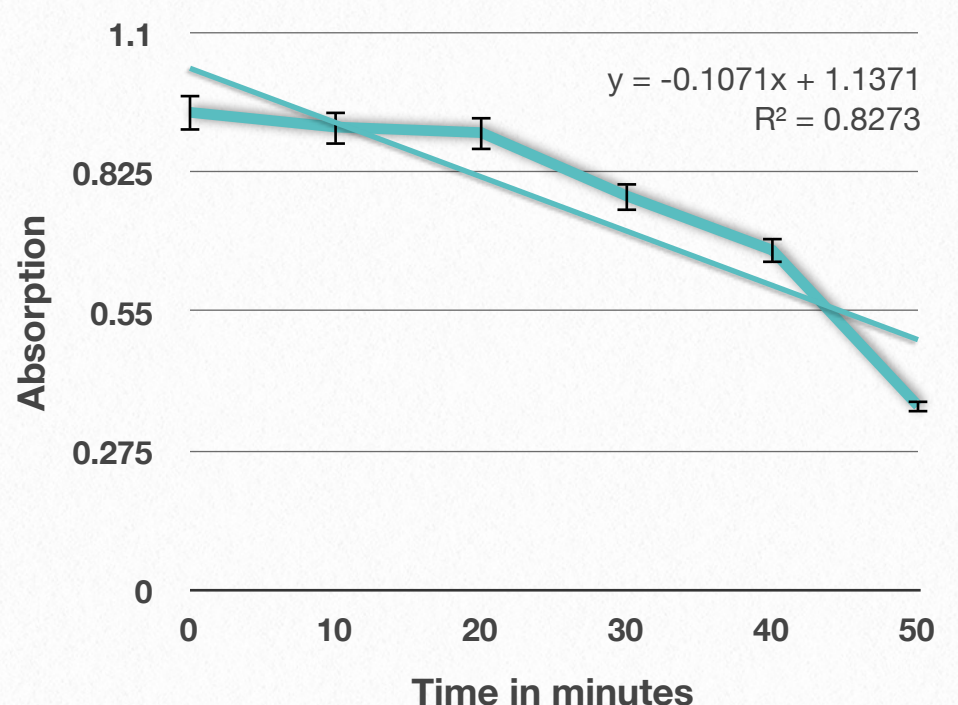
Time in minutes	Experiment 1	Experiment 2	Experiment 3	Mean value	Standard deviation
0	1.002	0.946	0.940	0.943	0.034
10	0.937	0.898	0.930	0.914	0.021
20	1.064	0.875	0.931	0.903	0.097
30	0.784	0.731	0.825	0.778	0.047
40	0.597	0.695	0.646	0.671	0.049
50	0.489	0.402	0.325	0.364	0.082

6. Absorption of phosphate by yeast cells without changing variables during three experiments.

7. Absorbtion of phosphate by yest cells

$y = -0.0992x + 1.1051$
$R^2 = 0.8613$

Best r-value reached in all experiments by experiment 2.

Absorption / Time in minutes

8. Mean value without manipulation

$y = -0.1071x + 1.1371$
$R^2 = 0.8273$

Absorption / Time in minutes

— Experiment 1   — Experiment 2
— Experiment 3

4

Improving of the phosphate uptake by yeast cells

4A: pH-value

Step 1

Aspect 1

We know from our biology classes that the phosphate uptake by cells is through membranes and that there is an active transport system in the membranes with the help of carriers, they can also perform a co-transport with two substrats, and we have read that there is a co-transport with sodium and phosphate through the membrans in the yeast cells and that with this transport system is accompanied with 2 H^+. We think that is because phosphate is only absorbed when it's instead of three times negative charged only one time negative charged. So the H^+ regulates that and because of that we have figured out that the pH-value could influence the phosphate uptake. So how does the phosphate uptake of yeast cells vary with an acrid pH-value outside the cell? We've decided to make the experiment with a 4.5 pH-value because it is not too acrid and we've read that it's the best pH-value for phosphate uptake of yeast cells.

Dependent variable: phosphate uptake

Independent variable: pH-value outside the cell

Controlled variable: temparature (25 °C), light quality, preparation of the yeast cell are the same as in the other experiment (Part 2), sample size (100 ml), time
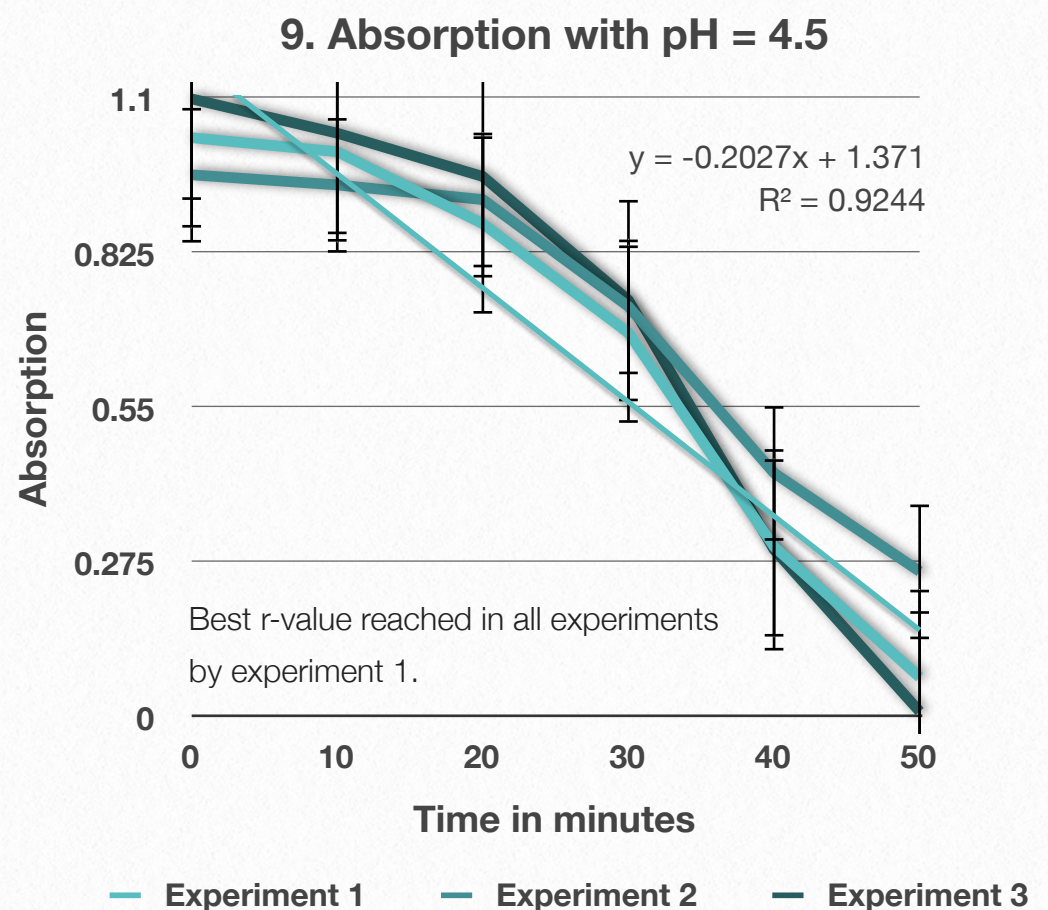
Aspect 2

We have always made the experiments at the same place so the temperature and the light quality was always the same. We decided to use HCl as acid, to control the pH-value we used a pH-meter. With the eppendorf-pipette only a few HCl was added so we could exactly stimulalte the pH-value. When there was a ph-value of 4.51, we stopped adding HCl and controlled the pH-value again with a pH-stick and it was again 4.5.
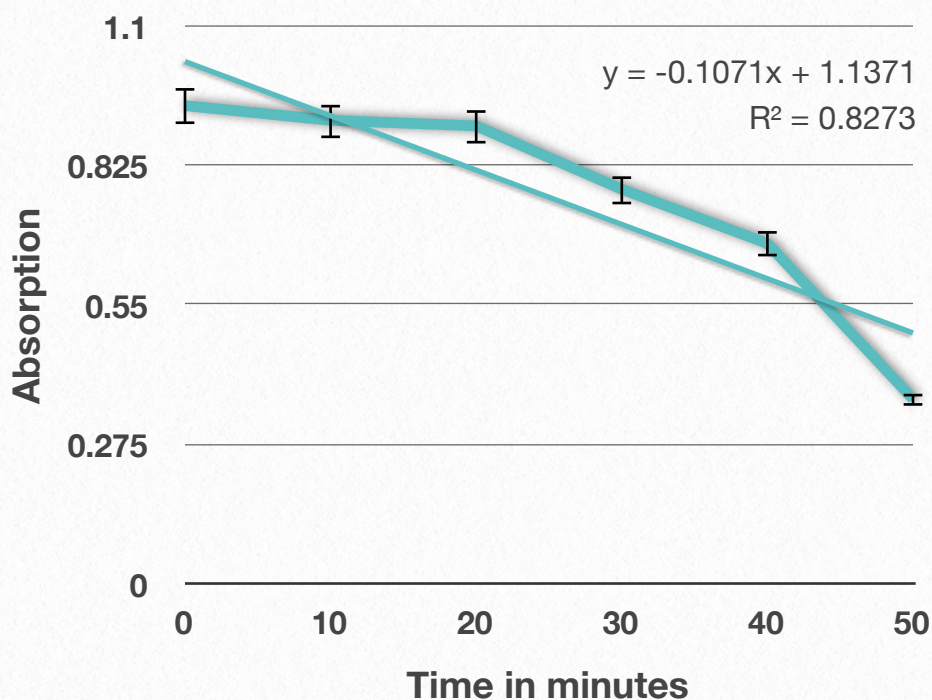
Aspect 3

To get enough data we made five attempts and took the best three ones. We would have liked to make more attempts but we were running out of molybdate because we used a lot of it in part 2 and we liked to have enough molybdate left for part 2 and 3.

Step 2

9. Absorption with pH = 4.5

$$y = -0.2027x + 1.371$$
$$R^2 = 0.9244$$

Best r-value reached in all experiments by experiment 1.

Absorption (y-axis): 0, 0.275, 0.55, 0.825, 1.1

Time in minutes (x-axis): 0, 10, 20, 30, 40, 50

— Experiment 1   — Experiment 2   — Experiment 3

10. Mean value without manipulation

$y = -0.1071x + 1.1371$
$R^2 = 0.8273$

11. Mean value with pH = 4.5

$y = -0.1929x + 1.3608$
$R^2 = 0.9117$
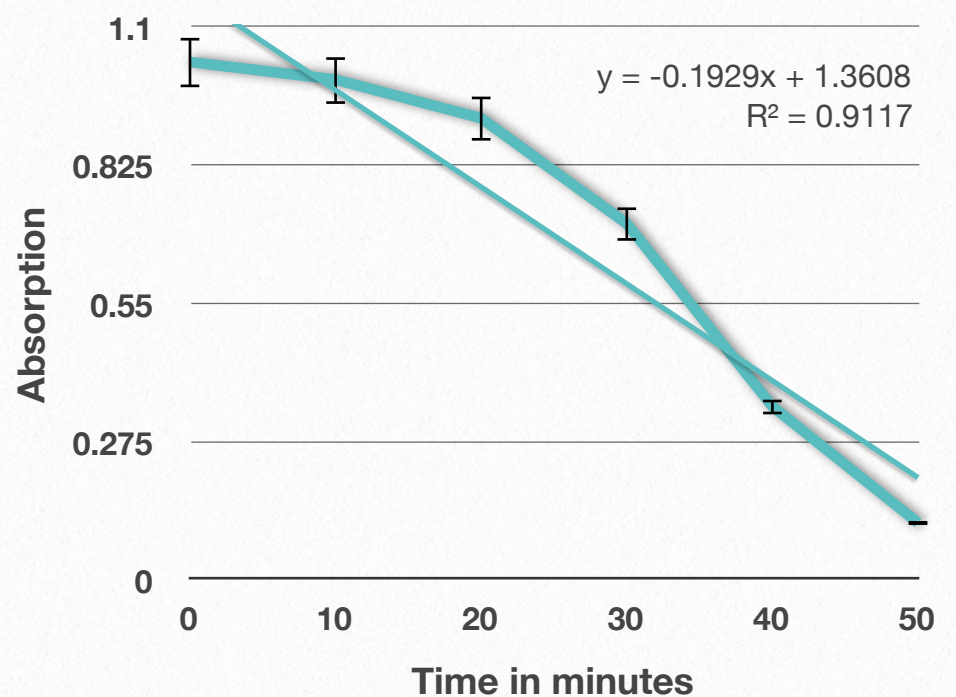
Step 3

The vertical axes represent the absorption at a wavelength of 595 nm. The horizontal axes represent the time in minutes.

In general, the yeast cells take up more phosphate at a pH-value of 4.5 because less light is absorbed already after the first 20min after adding the yeast solution to the phosphate solution. The graph and the bends of experiment one and three are very similar to eachother. Experiment three is the most successful one: in the end it was zero, so all phosphate was absorbed. Experiment two was still better than without the pH 4.5, but the worst one and it has a buckle.

Time in minutes	Experiment 1	Experiment 2	Experiment 3	Mean value	Standard deviation
0	1.030	0.964	1.099	1.031	0.067
10	1.005	0.946	1.038	0.996	0.047
20	0.877	0.920	0.961	0.919	0.042
30	0.683	0.730	0.714	0.709	0.024
40	0.303	0.434	0.298	0.345	0.077
50	0.071	0.259	0.010	0.113	0.130

12. Absorption of phosphate by yeast cells with changed pH-value.

Aspect 2

A weakness of our experiment was the pH-meter because the value it showed varied and did not stay the same altough no more HCl was added. But the variations were not significant because the value did not change for more than 0.1 and we double checked it with a pH-stick. It was not that important that the pH-value is exatctly 4.5 because we only wanted to see how the phosphate uptake changes in an acrid environment.
The acid was added after the solution was prepared so it changed the phosphate concentration because is changed the volume of the solution, but the volume of the acid was so small (0.17 ml) in comparison to the whole solution (100 ml)

so we could neglect it..
Due to the lack of time and molybdate we could only repeat it five times. Therefore we used the three best results. It would have been better to have made more attempts to compare but all three results were similar so we think they are not by chance.
Perhaps the yeast cell was a bit desiccated because they were prepared some time before they were mixed up with the phosphat solution.

Aspect 3

If the pH-value would be adjusted with conentrated HCl and more phosphate(i.e. creating 1 l of phosphate solution with the pH-value 4.5 instead of just 0.1 l) solution the phosphate concentration would not be changed as much as with the applied method. In future experiments we would repeat the experiment more often. And also the yeast cells shouldn't have been prepared and dried on by the air before the solution with the HCl and phosophate was finished.

4B: UV-light

Step 1

Aspect 1

Does treating the yeast cells with UV-light improve their uptake of phosphate?

Our considerations are that the UV-irradiances destroy some of the phospholipids in the cell membrane. Therefore phosphate is taken up by the cells so that new phospholipids can be produced.

Dependent variable: phosphate uptake
Independent variable: UV-light intensity
Controlled variable: temperature (25 °C), pH (6.3), preparation of the yeast cell are the same as in the other experiments (part 2), sample size (100 ml), time

Aspect 2

With the UV-cabinet of our school (Camag UV-cabinet II) the cuvettes are irradiated for 0, 10, 20, 30, 40 and 50 minutes at a wave length of 254 nm. The temperature and the preparation of the solutions are the same as in the other experiments.

Aspect 3

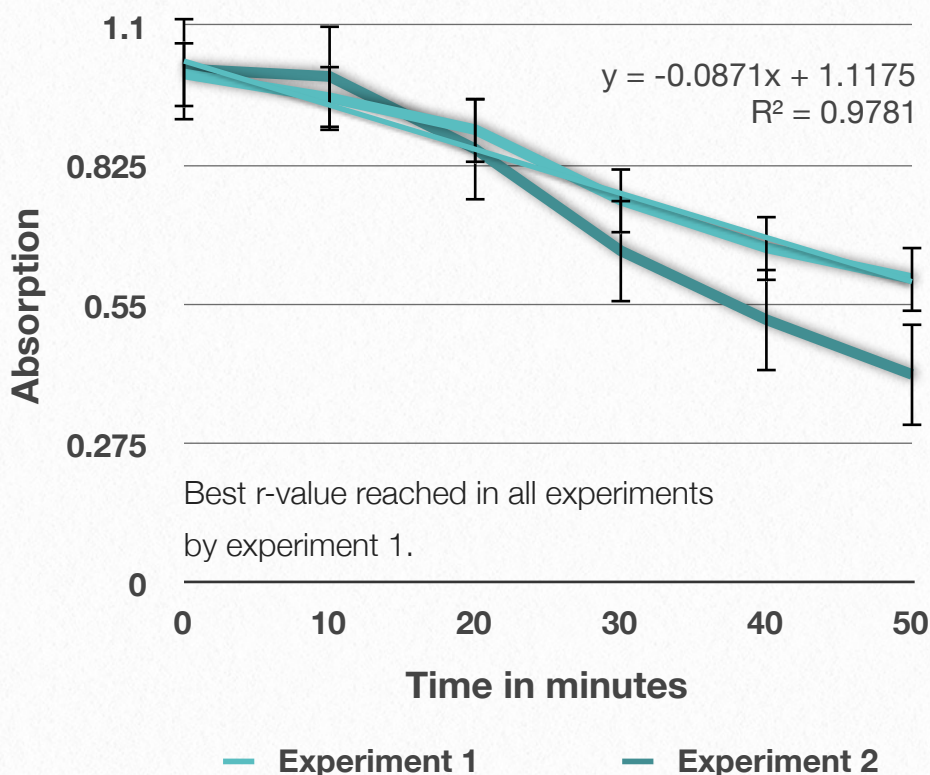We were only able to make the experiment two times, because we ran out of molybdat. As a consequense of this, the concentration of molybdat in the cuvettes in the third experiment was not equal because air had been in the EP's, too.

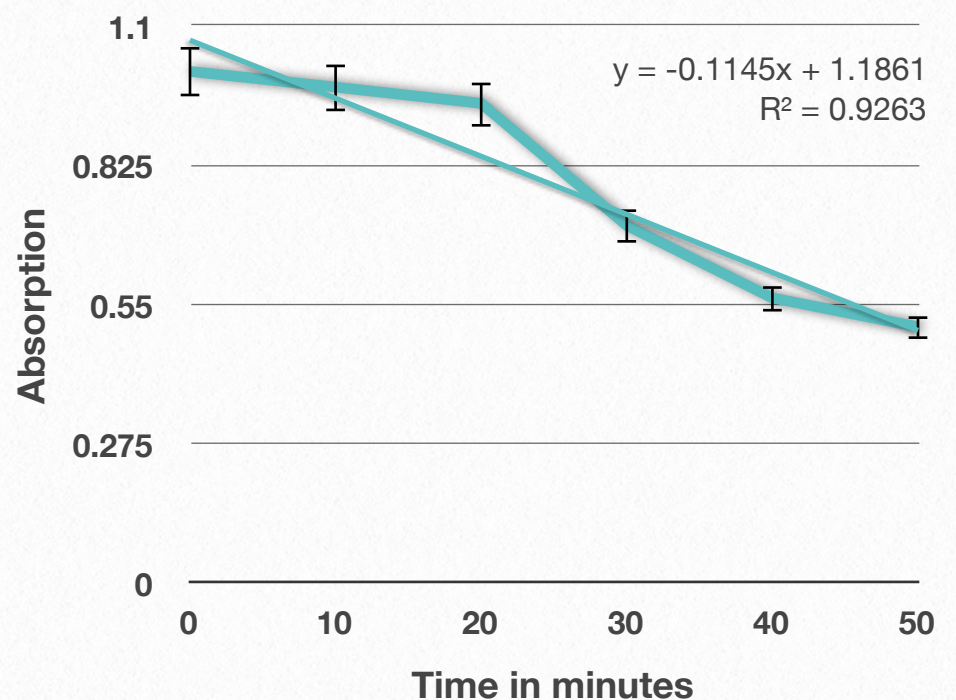### Step 2

Time in minutes	Experiment 1	Experiment 2	Mean value	Standard deviation
0	1.005	1.015	1.010	0.007
10	0.958	1.000	0.979	0.030
20	0.895	0.857	0.876	0.027
30	0.756	0.656	0.706	0.071
40	0.662	0.520	0.591	0.100
50	0.601	0.412	0.507	0.134

13. Absorption of phosphate by yeast cells with UV-light.
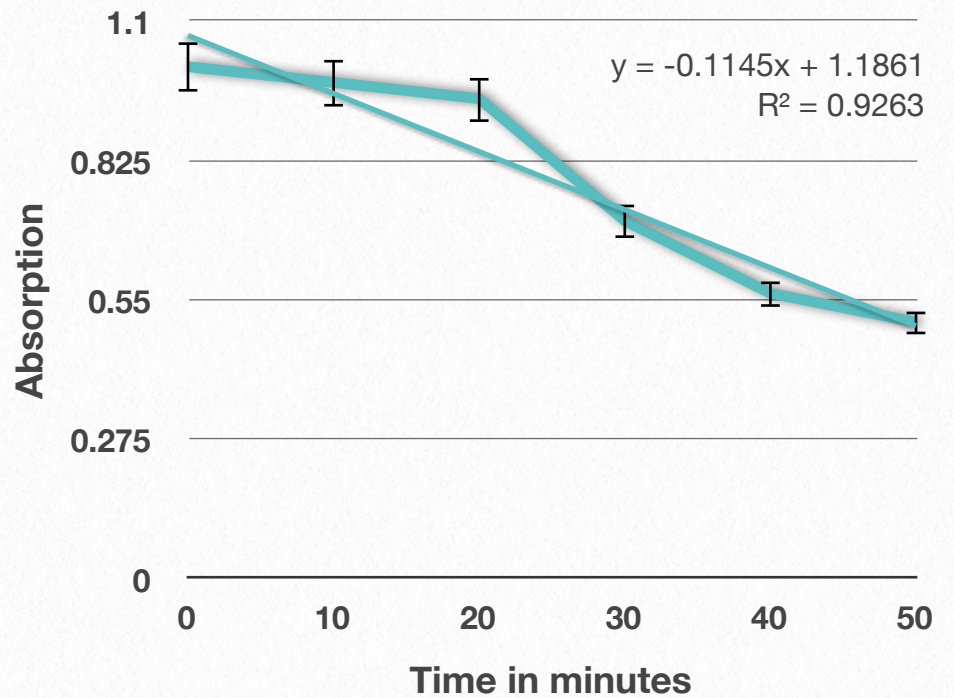

14. Absorption with UV-light

$y = -0.0871x + 1.1175$
$R^2 = 0.9781$

Best r-value reached in all experiments by experiment 1.

Experiment 1   Experiment 2

15. Mean value with UV-light

$y = -0.1145x + 1.1861$
$R^2 = 0.9263$

16. Mean value without manipulation

$y = -0.1071x + 1.1371$
$R^2 = 0.8273$

Absorption (y-axis): 1.1, 0.825, 0.55, 0.275, 0
Time in minutes (x-axis): 0, 10, 20, 30, 40, 50

17. Mean value with UV-light

$y = -0.1145x + 1.1861$
$R^2 = 0.9263$

Absorption (y-axis): 1.1, 0.825, 0.55, 0.275, 0
Time in minutes (x-axis): 0, 10, 20, 30, 40, 50
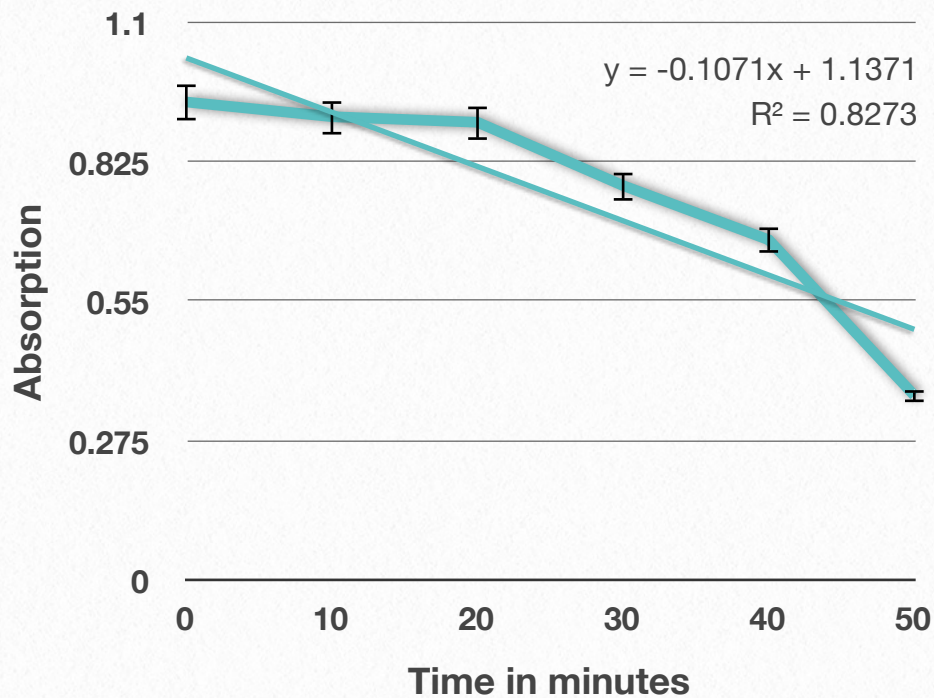
Step 3

Aspect 1

In Experiment 1 the absorption was worse than in part 3. In Experiment 2 the absorption was increased a little bit. Between the last points of the two graphs there is a difference of 0.2 what is quite huge. The solution of experiment 1 was exposed more direct to the UV-irradiances. Maybe the influence of UV-irradiances helps the yeast to take up phosphate but if it is exposed to directly to the UV-irradiances, the yeast cells are destroyed by it like the human skin (sunburn) or anything that has DNA.

Aspect 2

The equipment to make this experiment was professional (see Step 1, Aspect 2). The experiment was executed three times using a beaker and after 0, 10, 20, 30, 40 and 50 minutes a little dose was taken out and the absorption at 595 nm was measured. We paid a lot of attention to work exactly but because of the problems in Part 2 we had a lack of molybdate, which is the reason that in the last experiment two data are missing and we had not enough time to repeat it so we only made it at one of the two probable UV-wave lengths (254 nm).

Aspect 3

If we would have had more molybdate, we could have done more experiments and maybe with the other wave length. Also we could have made the experiments with the second wave length of the UV-cabinet (366 nm).

Part 4C: Mg²⁺-Concentration

Step 1

Aspect 1

We know from literature that bivalent cations, for example Ca^{2+} and Mg^{2+}, have a great influence on phosphate uptake of yeast cells. They increase the affinity of phosphate and decrease the affinity for Na^+, which is used as a co-transporter with phosphate (see part 4A Step 1 Aspect 1). They stimulate the Na^+ independent transport system as well.

Our consideration is that for this reason the adding of Mg^{2+} will increase the amount of phosphate taken up by the yeast cells. We decided to use Mg^{2+} because we can easily add it as $MgCl_2$. So how does phosphate uptake of yeast cells depend on the addition of bivalent cations?

Dependent variable: phosphate uptake
Independent variable: Mg^{2+} concentration
Controlled variable: temperature (25 °C), light quality, preparation of yeast cells the same as in every experiment, time, natural pH-value of the solution (6.3).

Aspect 2

We made all experiments at the same place so the temperature and the light intensity stayed the same as in the other experiments.

We used the pH value, which was used in the experiments in part 2 and 3, so that we only changed one variable by adding the $MgCl_2$. Because we added nothing that could have changed the pH-value, it stayed the same and we controlled this with a pH-stick similar to those we used in Part 4A.

The liquid was prepared with an Mg^{2+} concentration of 0.1mol /l. As 50 microlitres of phosphate solution (0.1 mol/l) were added to the yeast cells we decided to add the same amount of magnesium. That is why we created 100 ml of magnesium chloride solution (0.1 mol/l). To create this solution we weighed 2.033g of magnesium chloride ($MgCl_2 * 6H_2O$) which is 0.01mol. We then added so-

me distilled water and waited until all of the magnesium chloride was dissolved and then added more distilled water up to the 100 ml mark of the graduated flask. To ponder the $MgCl_2$, we used a scale which is precise to the amount of 0.001 g. Apart from that we did not use any new equipment.
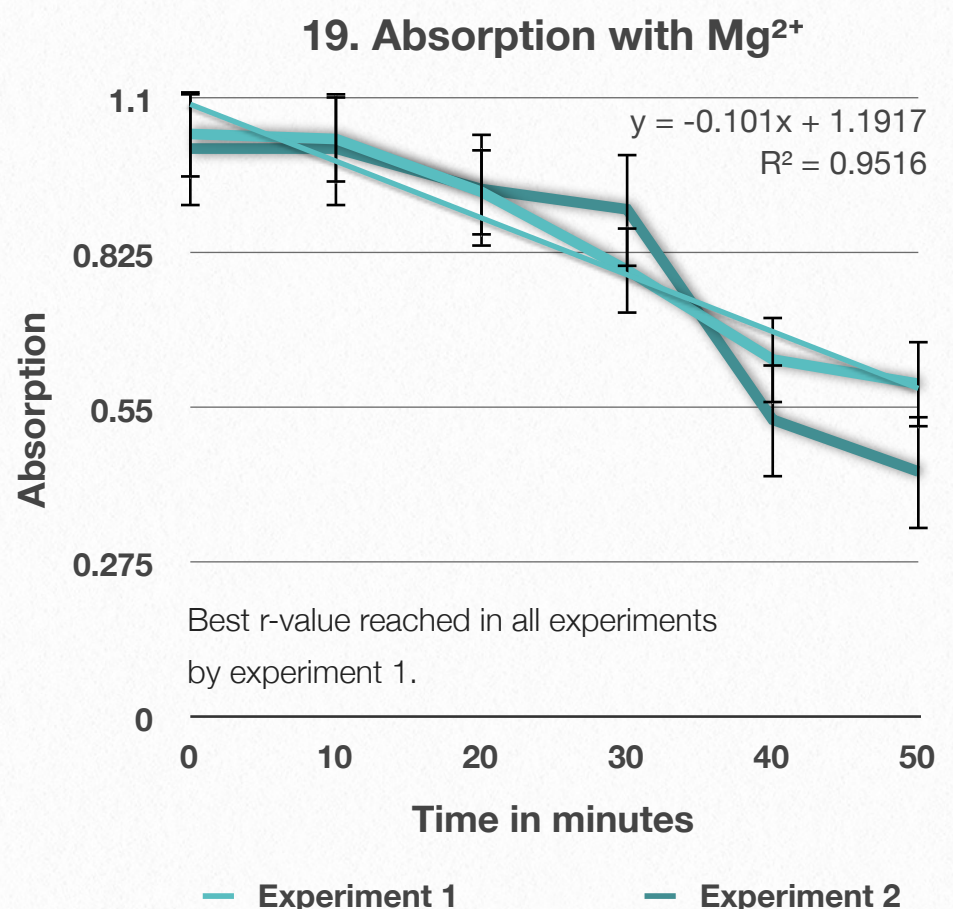
Aspect 3

We were only able to make the experiment three times, because we ran out of molybdate and time.

Step 2

Time in minutes	Experiment 1	Experiment 2	Mean value	Standard deviation
0	1.038	1.012	1.025	0.018
10	1.029	1.012	1.021	0.012
20	0.935	0.940	0.938	0.004
30	0.796	0.904	0.850	0.076
40	0.637	0.530	0.584	0.076
50	0.594	0.438	0.516	0.110

18. Absorption of phosphate by yeast cells with changed Mg^{2+}-concentration.

19. Absorption with Mg²⁺

$y = -0.101x + 1.1917$
$R^2 = 0.9516$

Best r-value reached in all experiments by experiment 1.

Absorption

Time in minutes

— Experiment 1   — Experiment 2

Step 3

Aspect 1

Experiment 2 seems to be the best one because the graph is exponential diminishing and the absorption of phosphate is the highest and it is better than without the adding of the $MgCl_2$, but not as much as we expected. In experiment 1 the graph looks quite similar to the graph of the mean value, especially from its bend, so this graph it quite the same as without the addition, so there was no improvement. And in experiment 3 the absorption was even worse because the graph shows that the absorption was in the end worse than without the addition. The three graphs have a similar bend and start at the same value but do not end up at the same value so they are quite different.

Aspect 2

In literature we read that Mg^{2+} stimulates the phosphate uptake of the Na^+ dependent transport system with a pH value at 7.2 and that the Na+ independent transport system is stimulated with a pH value at 4.5. It was also written that the uptake of phosphate with the Na^+ dependent transport system is inhibited at pH 7.2.

It might be that the phosphate uptake wasn't as good as expected in this experiment, because the yeast cells took up the phosphate partly with the Na^+ dependent transport system and that the phosphate uptake was already inhibited at pH 6.3.

Because we can't control which transport system is used for phosphate uptake, we decided to repeat the experiment with a pH value of 4.5 so we could see whether our consideration that the experiment failed because of the pH-value were right or wrong. This experiment we performed twice because the molybdate then was depleted. The only variable we changed newly was the pH-value so we used the solution we have also used in Part 4A. Our considerations were right because one can see in the new graphs that the absorption was extremely good compared to the mean value and also compared to the graph without the pH-value of 4.5. So the main problem with this experiment was that we made it with the natural pH-value which was too high, but in the instructions we were only allowed to change one variable of the phosphate concentration because of the adding of $MgCl_2$ but the volume was extremely small (0.05 ml) compared with the volume of the whole solution so this did not have an influence.
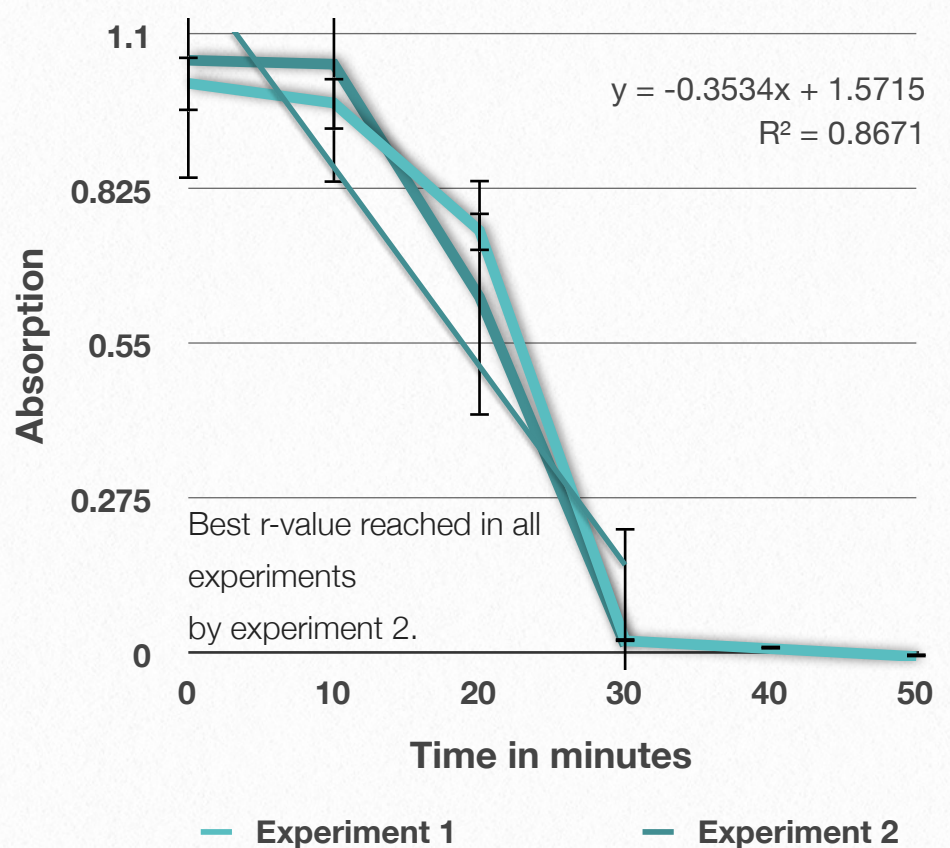
And the yeast cells were already a bit desiccated and old so perhaps it did not work as well as if they had been fresh ones. We can't say how much this weakness influenced the results but the experiment failed anyway because of the pH-value, which had been proved. The rigidity of the yeast cells was probably the reason why the results were worse than under normal conditions.

Aspect 3

In order to improve this experiment it is obvious to use a solution with a lower pH-value and to utilize only fresh yeast cells just as we did in our experiment with a pH-value of 4.5.

Due to our lack of Molybdate-solution we were only able to finish the first experiment. The graph is seen below.

20. Absorption with Mg^{2+} and changed pH

$$y = -0.3534x + 1.5715$$
$$R^2 = 0.8671$$

Best r-value reached in all experiments by experiment 2.

Axis label: Absorption

X-axis: Time in minutes

Legend: — Experiment 1   — Experiment 2

Time in minutes	Experiment 1	Experiment 2	Mean value	Standard deviation
0	1.014	1.055	1.035	0.029
10	0.979	1.048	1.014	0.049
20	0.752	0.634	0.693	0.083
30	0.023	0.015	0.019	0.006
40	0.009			
50	-0.005			

21. Absorption of phosphate by yeast cells with changed Mg^{2+}-concentration and pH.

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    NAADP.

Activity List

„Red phosphorus is used in Striker surface of safety matches.“

Responsibilities

Part 1	Maja Wolleb, Pauline Meier
Part 2	Maja Wolleb Sara Gehrig
Part 3	Maja Wolleb Sara Gehrig
Part 4	Pascaline Jermann Eric Jutzi Manon Stich Patrick Schwyzer Pauline Meier
Experiments	Jeannette Eckert Stefan Kohler Sara Gehrig Sabine Tschirren Pascaline Jermann Maja Wolleb Pauline Meier Patrick Schwyzer
Layout	Patrick Schwyzer
Teachers	Dr. Peter Sandmeier Karoline Knapinski Monika Fluri

22. Sara Gehrig working with sulfric acid.

23. Sara Gehrig and Sabine Tschirren preparing yeast solutions.