Kantonsschule Beromünster, class 4D
Science on the Move

Uptake of phosphate by yeast cells

Table of contents

1. Researches

Why is phosphate important for all organism?

All creatures in this world need Phosphate, humans, animals and plants. There are two important constituent in the organism which would not work without phosphate(1). Frist, cell respiration: in cell respiration the phosphates are important for compilation energy for the cells. Secondly, cell division: Phosphate is included in the cell membrane. Because of this it is needed for the cell division. Besides, it's in the genetic constitution and it's important for the DNA(1).

What is well known about phosphate uptake in yeast cells?

The yeast cells start growing if phosphate gets in the cell and they work a lot faster, because of the phosphate (2). We also found, that if there's a high osmotic concentration on the outside of the cell there's less phosphate on the inside (3). This is what is called "hypertonic". The other case is also possible: if there's a higher concentration inside of the cell, then the cell absorbs more phosphate than before (3). If this is the case, you call it "hypotonic".
Metaphosphate, which is the product of condensation of normal phosphate, can't be a reservoir of phosphate (3).
"In growing yeast, the uptake of P32-labelled phosphate requires the presence of sugar and is blocked at low temperature, and by metabolic inhibitors." (Goodman and Rothstein, 1957)
"Cellular metabolism depends on the appropriate concentration of intracellular inorganic phosphate, (...) the similarity of Pho84p, a high-affinity phosphate transporter in Saccharomyces cerevisiae (...)."(Wykoff and O'Shea, 2001)

How do yeast cells store phosphate?

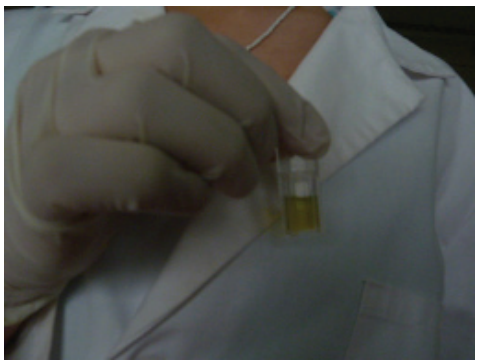
In the human body, phosphate is found in the DNA and the phospholipids in the cell membrane (1). We follow the hypothesis that this is the same in yeast cells. In another paper, we found the information, that many things about phosphate in yeast cells aren't exactly investigated and well known (9). Definitely sure is that yeast is in nearly every part of a yeast cell and their compartments, especially in the vacuole(9).
Yeast cells take up phosphate as ATP and diphosphoglycerate (8). Then they convert it into polymerized forms of phosphate (8) which are often found in the mitochondria of these cells (7). The mitochondria cells are very important to our energy balance(7). It is also interesting that chlamydomonas, some type of green algae, store the polyphosphates in their cell walls (10). Probably the yeast cells do something similar.

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

There are two aspects.
The first one is over-fertilisation. In excrements there are a lot of Phosphate recesses. Clarification plants clean the sewage. However after the clarification, the Phosphate is still in the mood. If too much Phosphate is put into water, microorganisms like algae or bacteria are growing fast. Phosphate works like a fertiliser(4). Therefore, farmers use slurry to fertilise their fields. With the rain phosphate, which is bound to ton minerals as well reach waters. In fact of this the oxygen austerity develops. In order to prevent a population turnover, ventilations are installed in the concerned waters, for example in Lake Hallwyl or Lake Sempach.
Secondly, scientists are worried that Phosphate sources will be running low like mineral oil. However it's much harder to find an alternative to Phosphate than in the case of mineral oil. People need 0.7 gram Phosphate per day, otherwise deficiency symptoms occur(5). Another problem will be that 80% of the Phosphate sources are located in only four countries, Morocco, China, South Africa and Jordan. So Europe is dependent on 90% imports. For this reason, new technologies are checked to use the Phosphate which accumulates in the clarification plants(6).

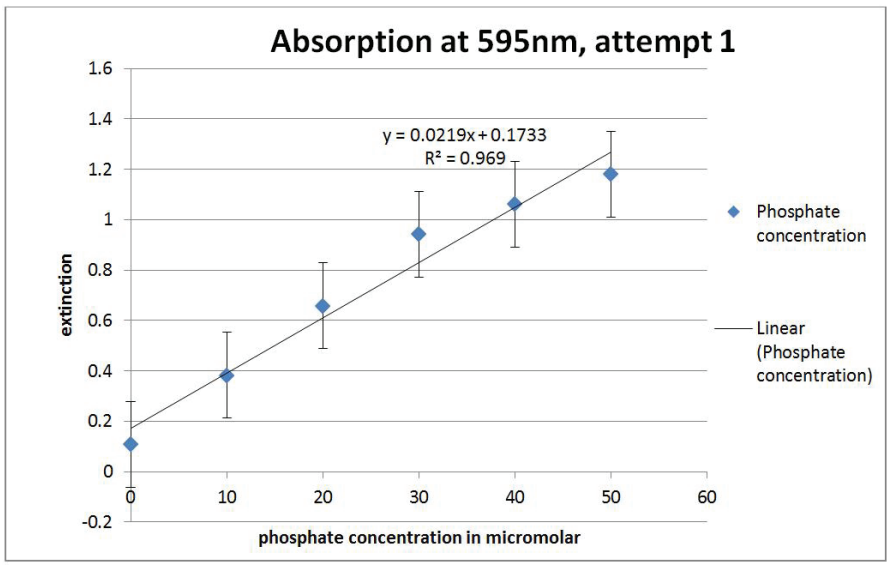
2. Calibration

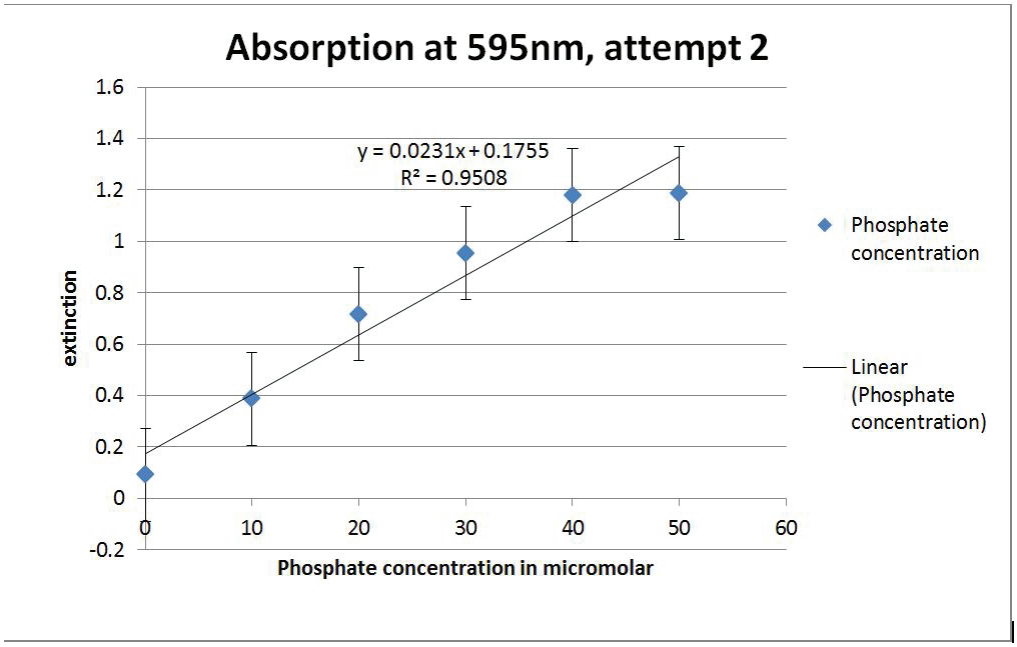
Part 2

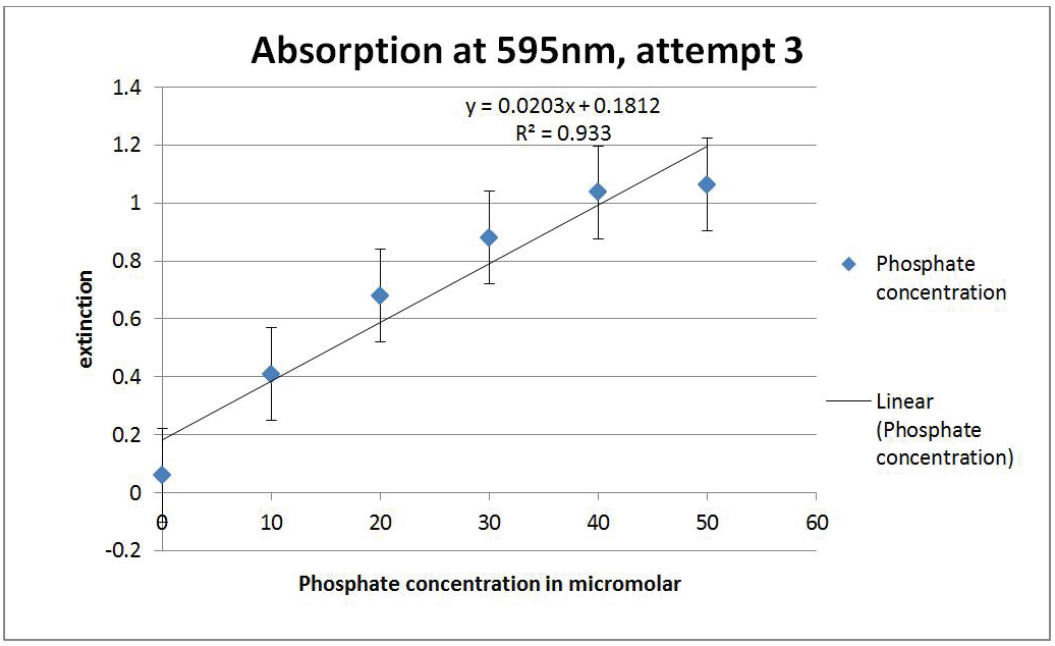


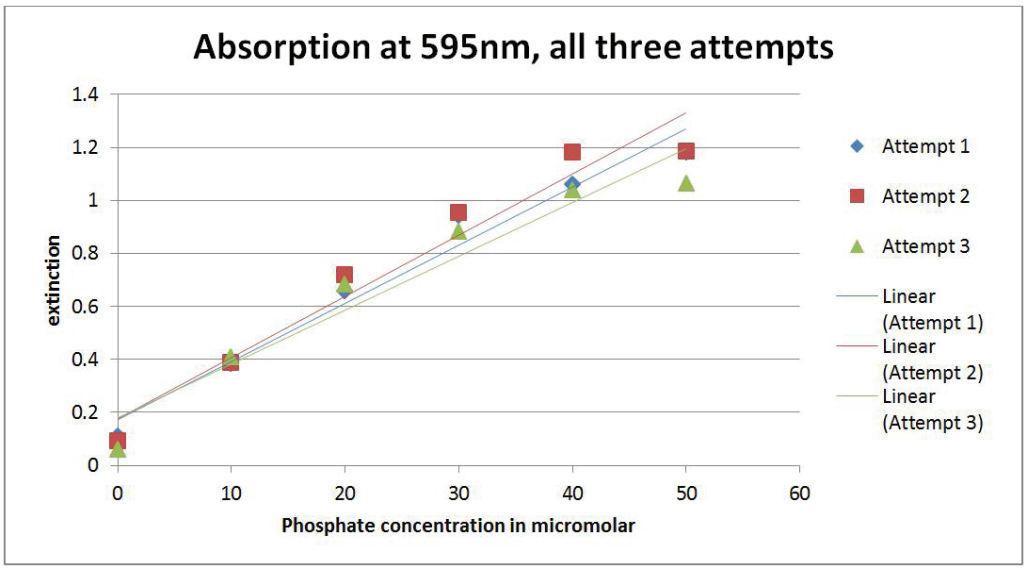
Picture 1 result of the pretest

Picture 2 lab environment







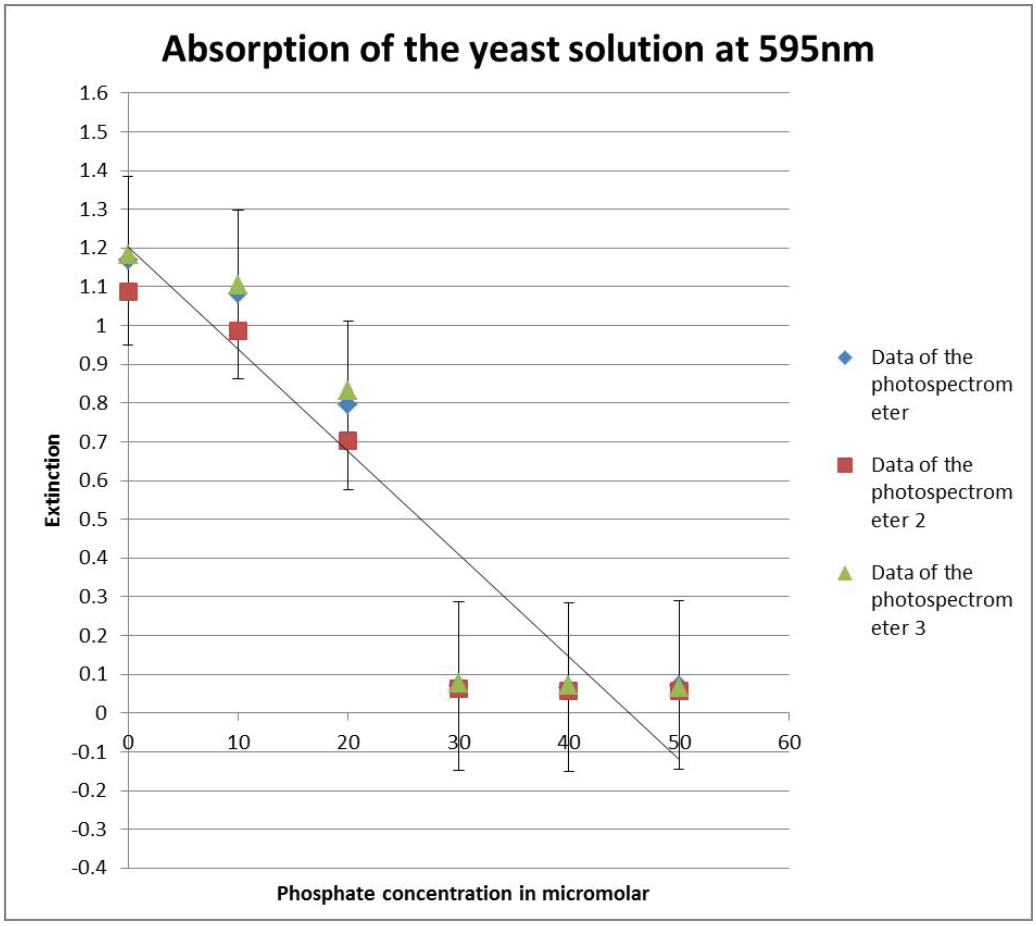


At first we needed time for arranging ourselves with the theme and our task. Before that we had never worked in a lab environment. Besides, we had never operated with these materials. However, the class became active and understood fast how everything works step by step and that we only have to follow the instructions. Finally, the difficulty consisted of adding the exact amount of the substance in the Eppendorf-Tubes. We had to decant the solutions a few times so that we lost a part of our liquids. The first attempt took a long time. After that we learned and worked more optimistically for the other tests. In the end we possessed our results and had to present our data in an excel table. If our results are not exact, the fault is down to the decanting.

3. Measurings with yeast cells

Part 3

First we mixed the liquids we had received in the right concentration. For this we needed 0.1g Glucose and 50 microliter Sodium-Phosphate-Buffers. Our problem was that we got the phosphate value 6.54 instead of the expected 6.3. We have two reasons for this: either the PH value wasn't calibrated correctly or we made a mistake in the mixing process. In other respects the mixing causes no problems. There were some problems with the interpretation of the Phosphate results, because we have been waiting too long. Finally, it worked after a lot of training.



4. Our own experiments

Part 4a
Step 1
Variables

First we considered in which parts of a yeast cell there is phosphate. We found out that phosphate especially exists in the DNA and in the lipid bilayer of a cell membrane (in the hydrophilic parts of the phospholipids). Out of this, we concluded that if a cell accretes, it also produces more DNA and it forms more cell membrane, the phosphate-concentration is growing up and probably phosphate is absorbed from outside. We also found that if a cell makes cellular respiration or fermentation, ADP converts into ATP. The more the cell makes fermentation/cellular respiration, the more it absorbs phosphate when yeast grows, it also makes more cellular respiration/fermentation and it absorbs more phosphate, too.
Furthermore, our question for the first approach was under which condition the yeast-cell accretes at most. The quantity of yeast, the light and the quantity of water in which the yeast is, are the controlled variables. The substance which you add to watch when the yeast grows at most is the dependent variable. The quantify of sugar, salt, ammonium nitrate and the temperature are these in our example. The increase of the yeast is the missing variable.

Control of the variables

For the first pre-test we had to consider which substances we want to add to the yeast, so that it grows faster. We found that every cell needs energy for the growth, so we decided to use sugar. The second substance we chose is ammonium nitrate, because it often is used for dunging. The last substance is salt. We did also a test without any additives, only with the yeast. We did this to have a comparison. For 60 minutes, we kept these four tests in three respective temperatures:3.8°C, 20°C and 30°C. Now we still needed an as much as good method to prove the increase of the yeast. We decided to take a bottle in which we dissolve the yeast in water, and then we added the additives. At last we attached a balloon to the bottle. In the fermentation ethanol is formed. More ethanol is accrued when there are more yeast-cells, which are doing fermentation. After we waited 60 minutes, we corded the balloon and took it away. To compare the results, we focused on the volume of the balloons.

Material: 12 1.5l bottles, per bottle 500ml water (to dissolve the yeast in it), one yeast (42g).
Additives: 100g sugar, 50g salt, 3g ammonium nitrate.

Data

In order that we didn't make any mistakes, we chalked the bottles up exactly, we created a timetable and we did same experiment twice. The second attempt caused the same results. This allowed us to make sure we did not make any mistakes.

Step 2
Raw data

Room temperature (20°C)

Normal	Less inflated
sugar	Less inflated
salt	Nothing
Ammonium nitrate	nothing

Fridge (3.8°C)

Normal	Balloon was absorbed
sugar	Less inflated
salt	Balloon was absorbed
Ammonium nitrate	Balloon was absorbed

Oven (30°C)

Normal	middle inflated
sugar	strongly inflated
salt	Less inflated
Ammonium nitrate	Less inflated

Step 3
Conclusion

It was easy to assert that all balloons with the additive sugar at every of the three temperatures at most were inflated. In addition you were able to see well, that the balloon inflated in the oven. In the fridge some balloons were even absorbed. Through this experiment we asserted that salt and ammonium nitrate don't do many for the increase of the yeast. It could even be that they prevent the growth of the yeast, because some balloons grew more in the normal-condition (without any additives) than with salt or ammonium nitrate. In our second approach, we won't use these two additives anymore and we will especially concentrate on sugar and the different temperatures. Our result of the first pre-test is also that yeast quickly accretes at high temperatures and with sugar.

Evaluation of the method

The drawback of our method is that we haven't got any numerical value as result and it isn't very exact in that respect. Yet, to us it wasn't primarily important to have exact results, because we saw the large differences well and we were able to draw conclusions without any problems. However, if accurate measurings are made, this method isn't recommendable.
In the oven, all balloons were inflated, but that could also be because particles are moving faster if it's warmer and because of this, the balloon is inflated. To diminish this effect, we let the detached balloon rest for 10 minutes. For our first approach this experiment was exactly the right one to follow. It roughly showed us on which substances we should concentrate in our second approach and precisely this was our purpose.

Suggestions for improvement

If you wanted to have precise result, you could measure the volume of the balloons and then compare theses volumes. Yet, this is very complicated and probably it isn't exact, either.

Part 4b
Step 1

After the experiment at part 4A we established the assumption that yeast grows the most with sugar (glucose) as adding and so also needs the most phosphate. Now we wanted to find some measurable results which we purposed with our new experiment and a new research question.
Research question: With which percentage of glucose yeast takes up the most phosphate?

Variables

Our independent variable was the concentration of the glucose. We worked with the amounts of 0g/l, 100g/l, 200g/l, 300g/l, 400g/l und 500g/l. Depending on this variable was the increase aggrandizement and growth of the yeast cells, as well as the value of phosphate in our liquids.
We tried to control as many variables as possible, so that our results could be distorted by as few factors as possible. In order to achieve this, we always added the same amount of yeast and also worked with the same percentage of water and phosphate-buffer like we did in part 3.

The lotions were shaken in the vortex for 10 minutes and then sedated for 30 minutes. The location they were being placed in was dark. We also controlled the factor of time as well as possible. The temperature was also controlled; it was always around 19 degrees.

Control of the variables

We always produced our lotion the following way:

- 9950µl distillated water
- 50µl Phosphat-buffer
- 1g yeast
- Je 0g, 1g, 2g, 3g, 4g oder 5g glucose.

The increase or the growth of the cells weren't measured or controlled, because it didn't matter for us. Instead, we measured the value of the phosphate with the phosphate spectrometer. In order that the lotion was in a constantly dark place (this didn't matter in the experiments 4A and C), we stored our samples in a closed cupboard. The time was measured with a stopwatch. Our samples were being shaken the same time each time.

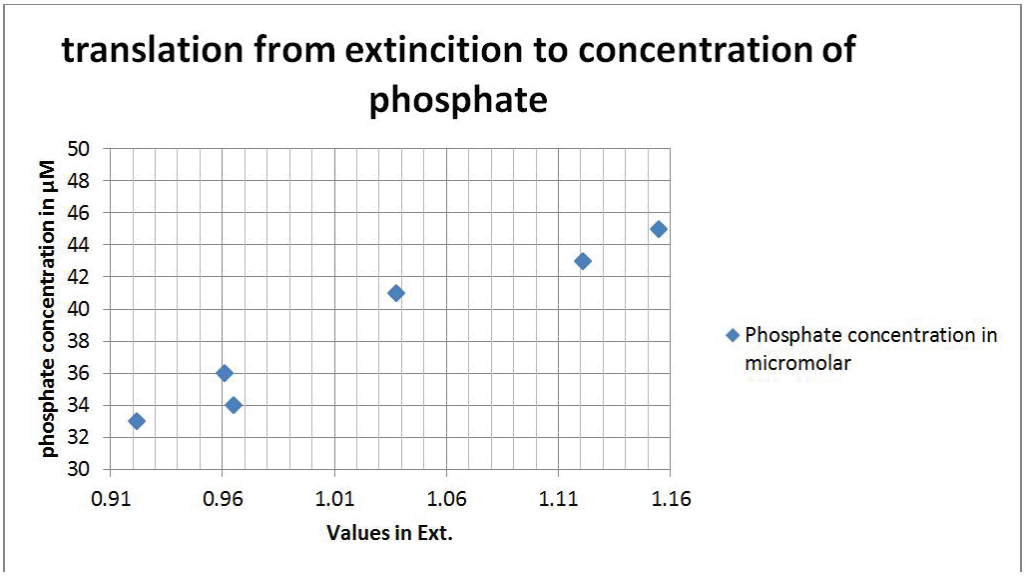
Data

In the end, we got six different values of phosphate for the glucose experiment, i.e. for every concentration one result. We were able to use the results of Part 2, 3 and 4 A/C to compare our acquired results.

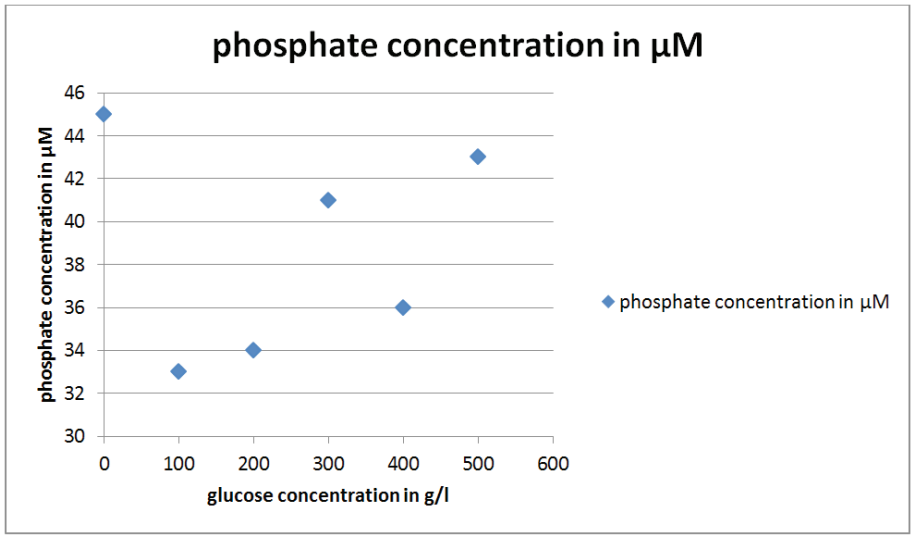
Step 2
Our raw data

Glucose concentration	Value in extinction
0g/l	1,155
100g/l	0,922
200g/l	0,965
300g/l	1.038
400g/l	0,961
500g/l	1,121

These values in extinction didn't really help us, but fortunately we'd already got the values form part, which helped us to establish the values of the phosphate.



Accordingly, it seemed to be more reasonable for us to associate the values of phosphate with those of the glucose.



Step 3
Conclusions

In our data, there weren't any visible trends and there wasn't a clear pattern. From this we followed that the glucose content of the environment doesn't play a big role at the phosphate absorption, On the contrary, if you use too much glucose, it impacts badly on the absorption. Probably it's just important for the development, that there is some glucose, because one time we put 0g in it and then the yeast recorded almost no phosphate.

Evaluation of the method

In our opinion we worked accurately at many points of the experiment. For example, we paid much attention on the time, such as how long we left the yeast behind or how long it was shaken in the Vortex. At the same time there was also inaccuracy. We produced the 6 samples at the same time, which means that we first put the yeast and afterwards the glucose in the prepared solution. In this process, the mixture was sometimes standing in the room and the yeast had different time to develop. Later we couldn't centrifuge every solvent or measure the phosphate content, either. So there some inaccuracies might have happened. Yet, overall we worked very clean and precisely. We worked with gloves and we used new pipette tips for every compound. Personally, we think we probably need more data for meaningful results, but we didn't have enough time. Beyond, there could also have happened some mistakes at the measure with the photo spectrometer, because only Noelle knew exactly how to use the instrument. At the measuring Noelle wasn't there and neither our chemic teacher. After some time we found out how to handle the instrument but nevertheless there could be some mistakes. Because of the fact that we needed more distillate water at part 3 than scheduled, we had to take the disposal water from the chemistry laboratory. It is possible that in this water there was less phosphate which also distorts the results. Our teacher also noted that a linear calibration straight probably isn't the optimal evaluation method. A logarithm would probably have fit better. But we didn't enter into this because we had a lack of knowledge. In certain circumstances we worked also with wrong glucose values. Perhaps values of 0 to 100g/l would have been more useful.

Suggestions for improvement

At a repetition there should always be a competent person at the meters. Either this person is always around to ask or a couple of people have to experience how the instruments work. Further, we have to be more exact with the time. Every single solvent would have to be prepared individually and with determined times. The best would be if first everything is prepared except for the yeast, so that they can't multiply without any control. Another point would be that we have to collect more data, which means that we can work more efficiently and with less difficult methods and maybe even less time at the yeast development. If we'd do another experiment, we should also have enough distillate water. If this weren't there again we could also measure the phosphate content of the water and then we could include it in our account. Maybe there is also another possibility to free the water of the phosphate. Instead of a linear best-fit line, we could use a logarithmic function. But for this we'd have to study this function and we would also have to make a more exact test. If we wanted to learn if the glucose content at a lower value plays a role, we'd have to repeat the experiment with the corresponding values.

Part 4c
Step 1

Also from part 4A we conclused that the temperature also plays a role at the uptake of phosphate by yeast cells. For measuring this we worked with similar methods like at part 4B.
Research question: At which temperature yeast takes up the most phosphate?

Variables

The independent variable was the temperature this time. We embed our patterns for 30min at 3,8°C, 19°C, 30°C and 40°C.
Dependent variables were again the growing and the increase of yeast and the percentage of phosphate. Farther we again controlled the variables yeast-, water- and phosphate-buffer-percentage, the times and the light intensity. Before keeping quiet the patterns had been shook on the vortex for 10min again. This time the glucose percentage also belonged to the controlled variables, we always added 0.1g.

Control of the variables

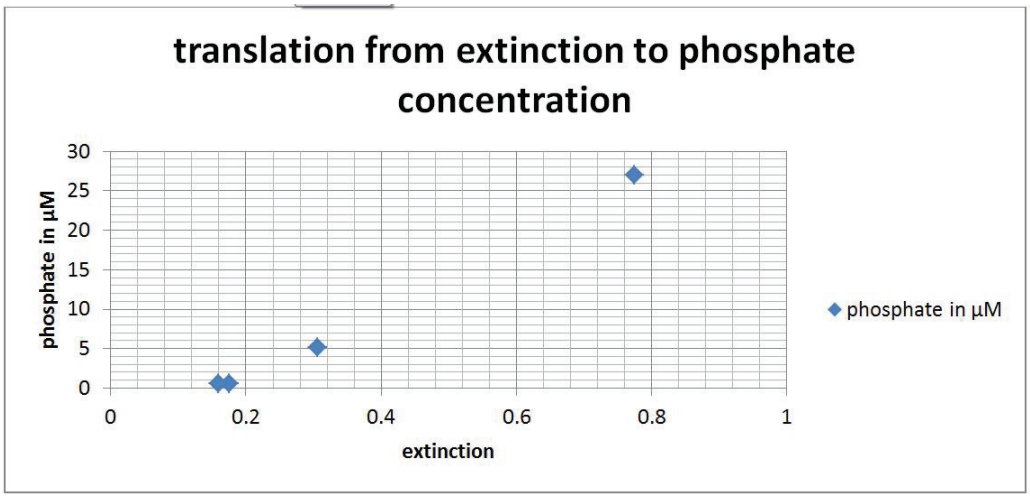
Our solution looked this time like this:

- 9950µl distilled water
- 50µl phosphate buffer
- 1g yeast
- 0.1g glucose

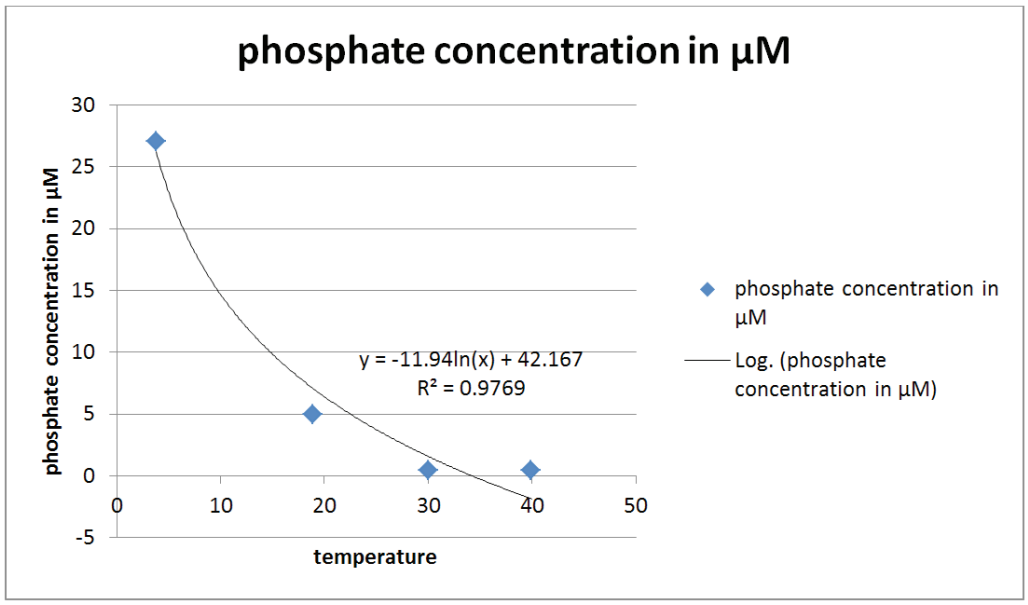
For controlling the temperature we embed a pattern in a fridge, one by room temperature and two more in two ovens with 30 degrees and 40 degrees. In the fridge and in the ovens it was dark so we put the patterns by room temperature in a cupboard so that there was the same light intensity everywhere. Again we measured the time with a stopwatch and all patterns had been shaken on a Vortex for 10min.
Data: for the temperature we got four results which we compared with the results from part 2 und 3 as well as part 4A and B.

Step 2
Raw data

Temperature	Value in extinction
3,8°C	0,775
19°C	0,307
30°C	0,176
40°C	0,161



For getting useful informations, we tried to link the phosphate concentration to the corresponding temperature.



Step 3
Conclusion

From explanations we learned how a logarithmic function looks like and we utilized it here for once. It really works with the values we got, the standard deviation lays really close to 1. Here you can clearly see the trend that the phosphate concentration sinks with the increase of phosphate. We conclude from this, that yeast takes up the most phosphate at a high temperature. Probably the values would be even better, we though have the purpose that it would increase after a certain degree.

Evaluation of the procedures

At part 4C we worked very similar to part 4B, nearly with the same methods. This time there were less impreciseness with the time because we were able to use the same solution for every four patterns. There were only minimal time differences, per example at the measuring or at the centrifuge. We again worked with gloves and new pipette-tops all the time. We got even less results than at part 4B, tough these were much more responsible. Even more high temperature could have been very interesting (like already noticed). This time we had an expert person at our measuring equipment, so there couldn't be any faults. Unfortunately we had to use again distilled water from the chemistry lab, which is eventually phosphatic. The translation from the values in extinction to the phosphate concentration would probably also been more exact with a logarhythmus.

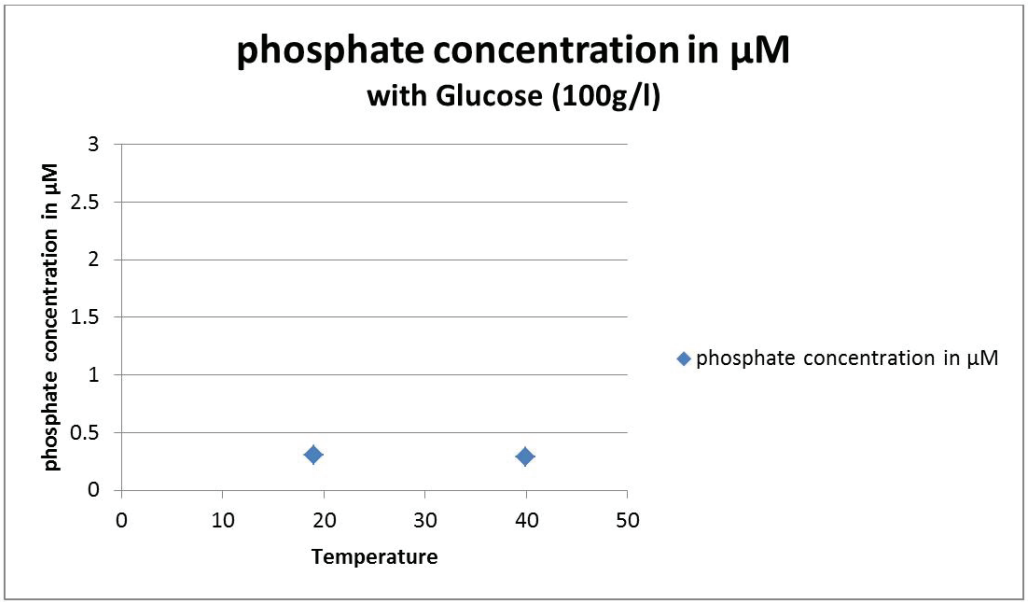
Improving suggestions

For getting even more results, we could have taken several patterns per temperature. New temperatures weren't possible because we didn't have enough fridges or ovens in our chemistry lab, but probably it would have been really interesting with temperatures like over 100 degrees. Like already said we should have had more distilled water, or we had to measure the phosphate in our distilled water and remove it or add it to our calculations.

Part 4D

After we did the two experiments with the glucose and the temperature, we asked ourselves, what the combinated result would have been like. For this we embed a pattern with 100g glucose per liter 30min at 40°C as well as a pattern at 19°C.

Our results already translated and processed



The result at room temperature really surprised us, because there were never these good results at room temperature in the investigations before. Eventually here happened measuring faults. Though we conclude from our experiment that the combination of warmth and enough nourishment in form of glucose are really important for the uptake of phosphate and supposably also for the increase and the growth of yeast cells.

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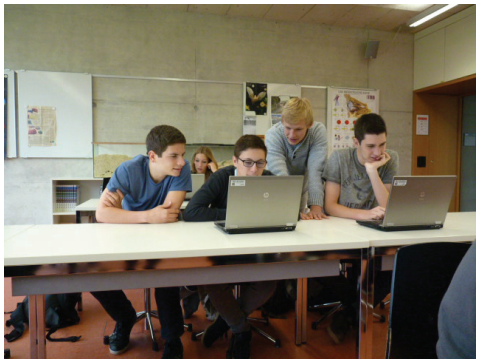
7. Activity List

Raphael	the list for the activities, some researches, wrote part 1, investigation of phosphate
Ramona	groups, researches, develop experiments, catching the planarians, wrote the part 5, and built the planarian trap, investigation of the pH-value
Noelle	groups, researches, develop experiments, catching the planarians, wrote the part 3, and built the planarian trap, investigation of nitrate
Nives:	groups, researches, catching the planarians, wrote the part 3 in English, investigation of calcium and temper
Lea	groups, researches, develop experiments, develop the layout, investigation of calcium and temper
Joel	develop experiments, develop and complete the layout, investigation of phosphate
Roger	develop experiments, wrote part 1, investigation of calcium and temper
Elio	experiments, wrote part 1, investigation of nitrate
Sabrina	wrote part 2, helped with the part 1, investigation of phosphate
Meret	groups, catching the planarians, controlled our English texts, brought water of the river, built the planarian trap, investigation of the pH-value
Lelia	develop experiments, catching the planarians, wrote part 4 in English, brought water of the river, investigation of iron
Daniel	wrote part 4 in German, investigation of iron
Maria-Laura	helped catching the planarians, develop experiments, investigation of nitrate
Baris	wrote part 1, investigation of the pH-value
Seline	investigated the water, investigated the experiments, investigation of iron



Picture 3 class working

Picture 4 daniel in the lab