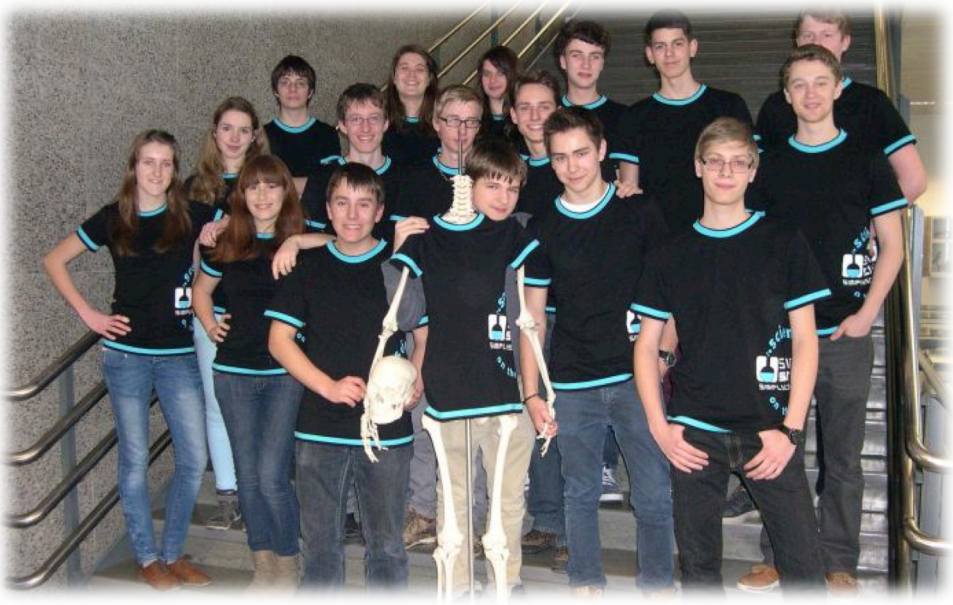
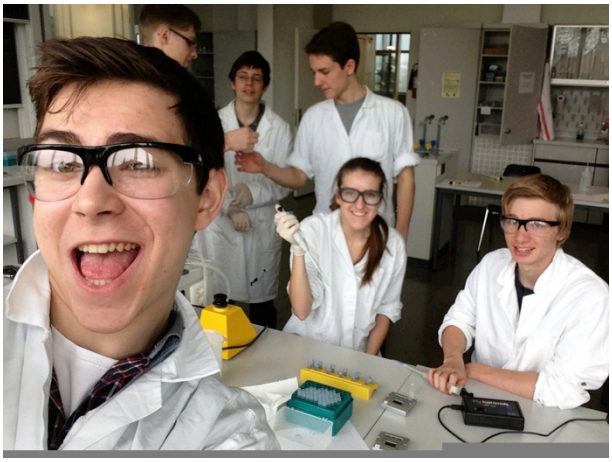
Phosphate uptake by yeast cells

Science on the move - task 2



Alte Kantonsschule Aarau

Class G2D



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Part 1

Why is phosphate important for all organisms?

Phosphates are very essential for life. Billions of phosphate molecules help us to do daily activities. Phosphates provide adenosine triphosphate, or short ATP. ATP is the energy carrier of the cells in each organism. Phosphates establish chains with sugar, protein and fats. The brain and the nerves cable with phosphates because they contain a lot of phosphor-fat-links. Our liver stores a lot of sugar. This energy will transfer to the fuel triphosphate.
Furthermore the genetic constitution has the form of a double helix whose skeletal structure is a ladder of sugar and phosphate. The muscles contain billions of Myosin. These are molecular motors which jerk frequently with the aid of phosphate. Vesicles and organelles will be carried with these molecular motors. Our hormones control the metabolism and transfer the signals with phosphate and the bones and tooth consist of apatite, which is a bond of calcium and phosphate. [1,2]

What is well known about phosphate uptake in yeast cells?

It is well known, that the phosphate in many plants is taken up by their roots.
So it would be a possibility that the yeast also uptakes the phosphate by a similar mechanism.
Therefore, plants must have specialized transporters at the root/soil interface for extraction of phosphate, as well as other mechanisms for transporting phosphate across membranes.
If you observe the pH-value during the uptake of the phosphate you can see one thing, which was also found out in many studies: The uptake rates of phosphate in higher plants are most decorated between pH 5.0 and 6.0. Interesting is, that $H_2PO_4^-$ dominates in this area. Moreover, this fact let us suggest that phosphate is taken up as the monovalent form.
We also assume, that the yeast cells mainly take up phosphate by diffusion. This would be the easiest way for them. [3-6]

How do yeast cells store phosphate?

We assume that the yeast cells are able to store the phosphate in their vacuoles in the structure of so-called polyphosphategranula. [7,8]

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

Some microorganisms have the ability to uptake phosphate out of the water because they need it for their anabolism and energy metabolism. This phenomenon is used for cleaning the polluted water since the 1980's. With the help of these microorganisms it's possible to eliminate a huge part of the phosphate content in a clarification

plant. Without this cleaning the rivers and the lakes would be sated with phosphate. This would lead to a fast growth of the algae populations. The reduction of the dead algae would need a lot of oxygen and so many other individuals wouldn't have enough to survive. An example of this terrible act is the "Hallwilersee" in Switzerland. [9]

Part 2

Creating this calibration curve was our first experience in a science lab. Before this we had to work in smaller groups when we had to deal with chemicals and complicated electronical measure systems. It was really not easy to get a proper and more or less accurate calibration curve. First there were just six people in a science lab, equipped with chemicals and a photometric measurement system. On the first day there was quite a mess in our lab. We did our best, all ended up in a chaos just because we didn`t make a time table with the exact process of the experiment.

On this first day, we used the photspectrometers we received from simply science. This emerged as a fatal mistake. We got terrible results for our calibration curve, the results were scattered all over the table, and there was no visible straight calibration curve. Maybe these terrible results are due to the inaccurate pipetting we did, but we assume it was also because of the low quality photospectrometers we received from simply science.

On the second day we tried to make things better. For the beginning, we created a time table with the accurate process of the experiment. With this table, everyone knew what to do, everything was controlled and planned. This time we used the photospectrometers from our school, which are very accurate. Our pipetting was also improved a lot. We did it really careful and slow, so no chemical was spilt and could falsify our results. With these improvements we got a really good calibration curve with accurate measurement. We were really satisfied with this day.

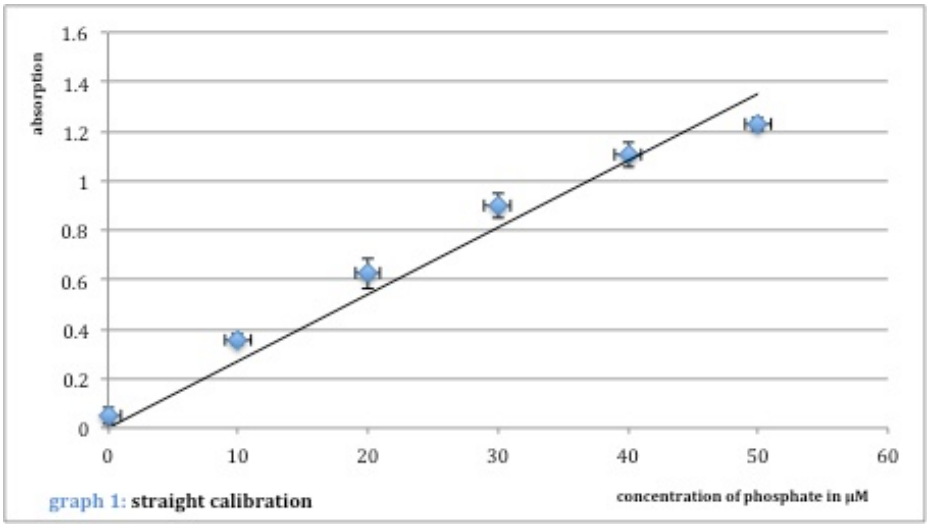
Time (in min)	Person 1	Person 2	Person 3
0	Adding Molybdat to Malachitgreen (1)		
1	"		
2	"		
3	"		
4	"		
5	"	0s: Add the phosphate solution to (1)	0s:
		10s: the sample, 5s in the Vortex	10s:
		30s:	30s: Put the solution in a cuvette
		35s:	35s: measure the absorption
6	"	"	"

Tbl 1: Time table for analysing the sample

This is the data we collected:

Concentration	Absorption A	Absorption B	Absoprtion C	Average	Standart Deviation
0	0.056	0.047	0.04	0.0477	0.03403
10	0.395	0.33	0.345	0.357	0.01992
20	0.649	0.615	0.614	0.626	0.06253
30	0.96	0.901	0.835	0.8987	0.05050
40	1.155	1.054	1.105	1.1047	0.05248
50	1.216	1.182	1.285	1.2277	0.02702
R^2	0.9295	0.9538	0.9835	0.9606	

Tbl 2: Raw data of the straight calibration



graph 1: straight calibration

As you can see the R^2-values of the three experiments are very near at 1, of the average it's

$$R^2 = 0{,}961$$

So the experiment was very successful. We were able to handle well and exactly with the pipettes, the photospetrometers worked also well and we got a good calibration curve.

Part 3

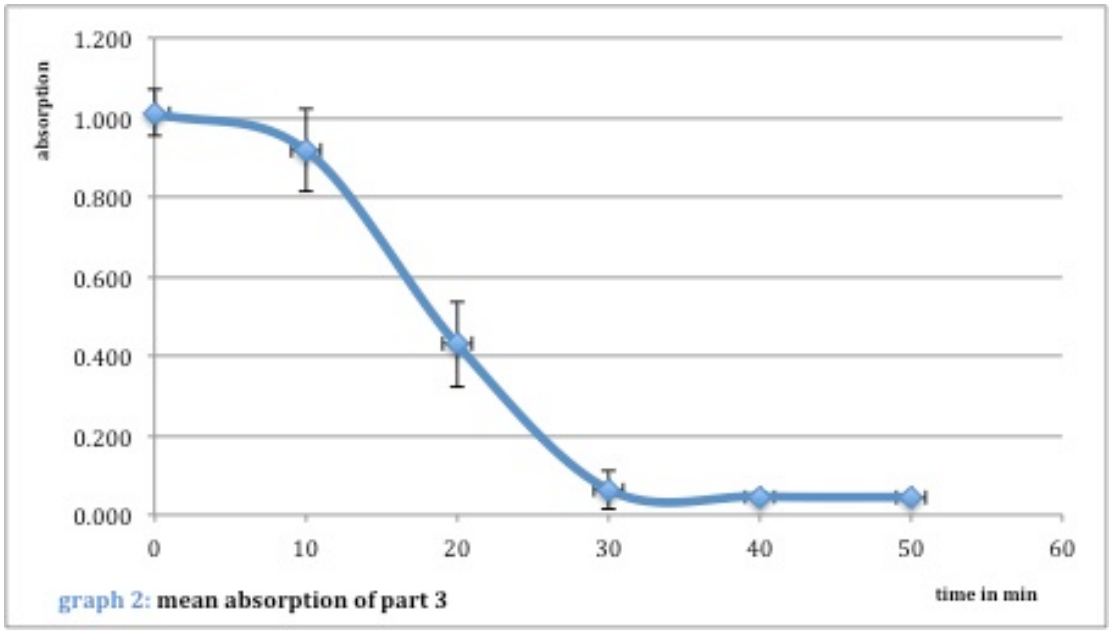
To receive useful results it was especially important, to work very precisely in all aspects. Inaccurate pipetting would have led to imprecice absorption in the photospectrometer, which means terrible wrong results. Another problem was that we spilt some samples due to our very stressful situation.

We learned a lot in the experiments we had done to achieve the regression line in part 2. But nevertheless we made a big mistake in this part. We took the specified amount of yeast to measure the phosphate uptake. This turned out as an error when we noticed that all the phosphate was taken up in just ten minutes. We couldn't measure fast enough to collect enough good results in this short period of time. We decided to halve the amount of yeast. This allowed us to do measurements over a longer time.

We had followed the same time table to analyse the samples as in Part 2.

Time	Part 3 attempt 1	Part 3 attempt 2	Part 3 attempt 3	Average	Standard Deviation
0	0.969	1.077	0.986	1.011	0.0581
10	0.988	0.963	0.799	0.917	0.1027
20	0.317	0.530	0.440	0.429	0.1069
30	0.035	0.120	0.036	0.064	0.0488
40	0.043	0.054	0.042	0.046	0.0067
50	0.046	0.042	0.045	0.044	0.0021

Tbl 3: Raw data of part 3



graph 2: mean absorption of part 3

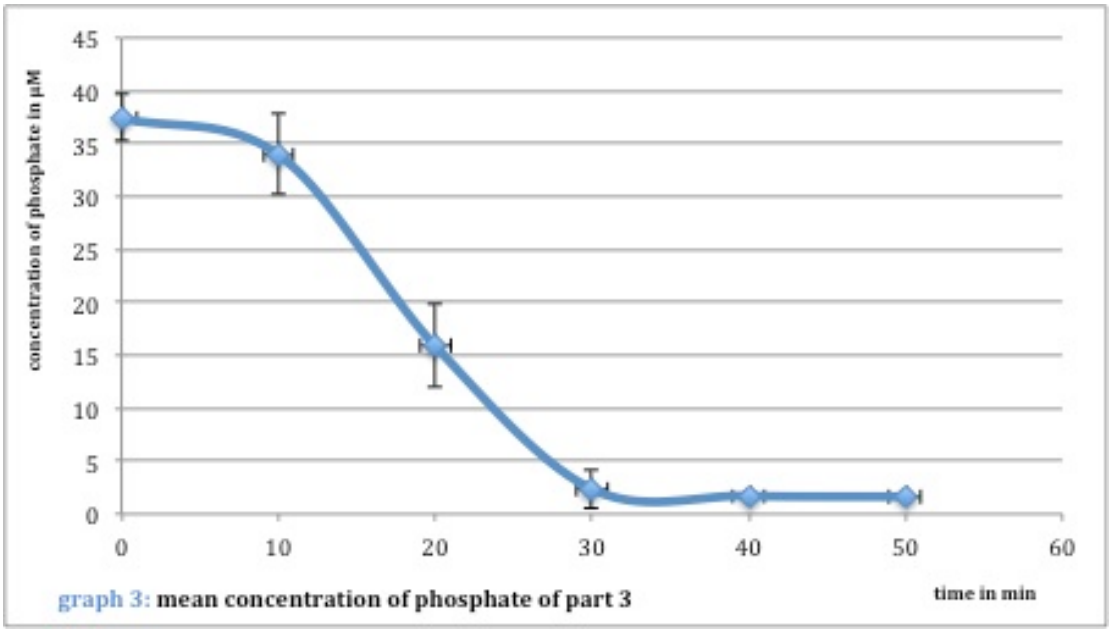
Now we could calculate these data with the linear equation of the straight calibration curve:

$$y = 0.027*x$$

“Y” is the absorption and “x” the concentration. So, when we would calculate the concentration at each point of time, we just had to compute like this:

$$x = y/0.027$$

If we do that at every point of time we receive a diagram like graph 3. It’s mostly the same, but the data on the y-axis are definitely in another range



graph 3: **mean concentration of phosphate of part 3**

Part 4

Step 1

Now we like to set up three approaches concerning the experiment we have brought through. We came up with three attempts to enhance the efficiency of the phosphate uptake by yeast cells.

Approach 1: pH value

Aspect 1

Based on our knowledge, we quickly came onto the idea that the pH value might be a solution concerning our experiment. Based on part one, question 2, we liked to give some time to the pH value. And this topic is probably connected with the diffusion. You have to consider that there exist three different sorts of phosphate: the primary phosphates, the secondary phosphates and the tertiary phosphates. It has probably an effect on the experiment. And it could also have an effect on the diffusion, respective on the speed of the uptake of the phosphate. Altogether, you can set up one central question:
How does the speed change itself by other pH values?

Independent variables:	pH value
Dependent variable:	speed of the uptake
Controlled variables:	temperature of the water
	Amount of the water
	Amount of the yeast cells
	Room temperature

Aspect 2

The independent variable was reached by a specific pH meter, which controlled the pH value constantly. It would also have been possible to use a special pH indicator. This would have been a pH indicator paper. The function of this paper is the following one: if you give a tiny water drop onto this paper with the help of a pipette it will change its colour. And each colour stands for a specific pH value.
The dependent variable, namely the speed of the uptake, was measured by the students of our class.
Further to the controlled variables. The temperature of the water was hold by the room temperature. We also controlled it constantly by the help of a thermometer.
The amount of the water was set up before we made the experiment. And we didn't change it during the whole experiment.
It was probably the same concept concerning the amount of the yeast cells: We set it up before the experiment and we didn't change it during the experiment.

Aspect 3

To collect the data we had to measure the pH value correctly and the speed of the uptake. We could identify the pH value with the help of the concept which we explained in the passage “Aspect 2”.
We collected the speed of the uptake the same way as we did it in part 2 and mainly part 3. You can put those two factors against each other in a diagram. So you can see the data trend.

Approach two: water temperature

Aspect one:

Based on our first approach we set up a second one. We had the idea that the yeast cells might take up more phosphate by a higher water temperature. And it might take up the most amount of phosphate by 32°C. We came onto this assumption because of our knowledge. Based on our biology class, we know that the yeast cells take up the most phosphate at 32°C. This leads us to our second question: How does the uptake rate of phosphate changes by other water temperatures?

Independent variables:	water temperature
Dependent variable:	speed of the uptake
Controlled variables:	amount of the yeast cells
	Amount of the water
	Room temperature
	pH value

Aspect two

The independent variable was controlled by our measures. We controlled it with the help of a temperature control device. The speed of the uptake was measured by the students of our class with some stopwatches.
Further to the controlled variables. The amount of the water was set up before we made the experiment. And we didn’t change it during the whole experiment.
The amount of the yeast cells was also set up before the experiment. And we didn’t change it during the whole experiment, too.
The room temperature was held by the heater in the room, so it couldn’t have been changed.
We took the water from the tap. The pH value in this water is 7. So, we had a constant value during the experiment.

Aspect three

To collect the data we had to measure the temperature of the water correctly and the speed of the uptake. We could measure the temperature of the water by a thermometer. The speed of the uptake was measured in the same way as we did it in the approach number one. We collected it the same way as we did it in part 2 and mainly

part 3. We could put those two factors against each other in a diagram. So we could see the data trend.

Approach 3: glucose

Aspect one

We spent a lot of time on setting up the last approach. We finally thought about the glucose share. We talked about it in the first year of the Kantonsschule. Glucose is a simple monosaccharide and it can easily and directly be taken up by the human being. You might know glucose as “Traubenzucker”. And this is well known as a great energetic substance. We assume that it has the same effect on yeast cells. This would mean that they have more energy and can take up more phosphate.
That leads us to our hypothesis:
We thought that through more glucose share in the water, the yeast cells can take up more phosphate.

Independent variables:	amount of glucose in the water
Dependent variable:	speed of the uptake
Controlled variables:	amount of the yeast cells
	Amount of the water
	Room temperature
	pH value
	Temperature of the water

Aspect two

The independent variable was set up before the experiment. To define a specific amount of glucose we used glucose powder. And this specific amount was set up before the experiment and we didn’t change it during the experiment.
The speed of the uptake was measured by the students of our class with the help of stopwatches in the same way as in the approaches one and two.
Further to the controlled variables. The amount of the water was set up before we made the experiment. And we didn’t change it during the whole experiment.
It was probably the same concept concerning the amount of the yeast cells. We also determined the amount before the experiment and we didn’t change it, too.
The room temperature was held by the heater in the room. So, the students couldn't change it.
The water was taken from the tap. The pH value in this water was 7. So, we had a constant value during the whole experiment.
And last but not least, the temperature of the water. This controlled variable was defined by the room temperature and it also couldn’t be changed by the students.

Aspect three

Now we had to collect the data of the speed of the uptake in the same way as we did it in the approaches number one and number two.
Now, we compared it with the speed of the uptake under normal conditions which means that there was the original content of glucose. We could put those two data against each other. For this, we constituted the data of the uptake rates under normal

conditions in a diagram and also the data of the experiment we just brought through. We could probably see if the uptake rate was higher under normal conditions or by the changing of the glucose share.

Step 2

Time	Part 4 PH attempt 1	Part 4 PH attempt 2	Part 4 PH attempt 3	Average	Standard Deviation
0	0.820	1.431	0.901	1.051	0.3319
10	0.057	0.158	0.128	0.114	0.0519
20	0.047	0.162	0.117	0.109	0.0580
30	0.090	0.031	0.109	0.077	0.0407
40	0.096	0.099	0.136	0.110	0.0223
50	0.125	0.079	0.145	0.116	0.0338

Tbl 4: Raw data of approach 1

Time	Part 4 temp attempt 1	Part 4 temp attempt 2	Part 4 temp attempt 3	Average	Standard Deviation
0	1.550	1.400	1.588	1.513	0.0994
10	0.400	0.500	0.400	0.433	0.0577
20	0.089	0.095	0.090	0.091	0.0032
30	0.092	0.097	0.107	0.099	0.0076
40	0.097	0.094	0.086	0.092	0.0057
50	0.076	0.096	0.085	0.086	0.0100

Tbl 5: Raw data of approach 2

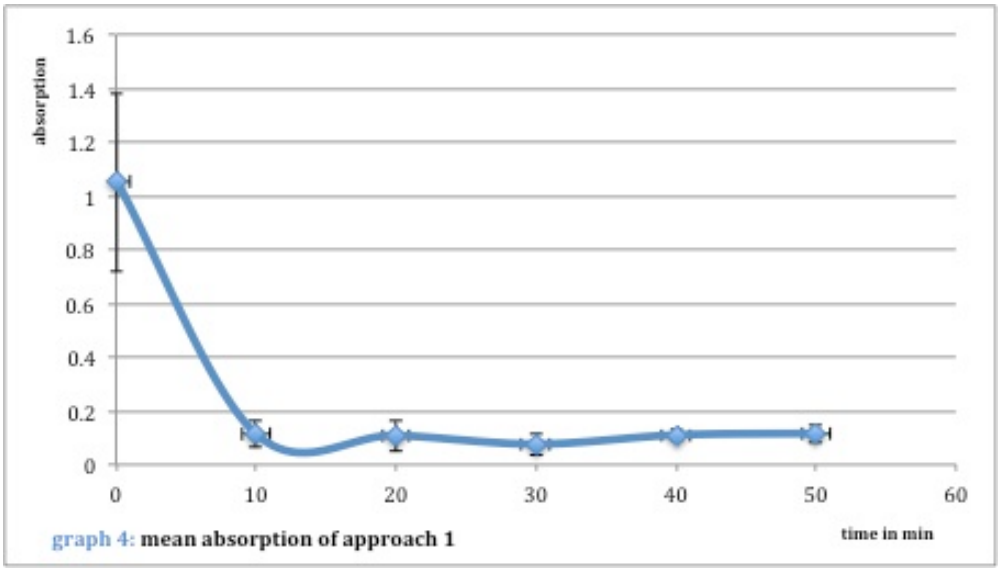
Time	Part 4 gluc attempt 1	Part 4 gluc attempt 2	Part 4 gluc attempt 3	Average	Standard Deviation
0	1.122	1.126	1.459	1.236	0.1934
10	0.968	0.930	0.833	0.910	0.0696
20	0.268	0.087	0.098	0.151	0.1015
30	0.114	0.080	0.077	0.090	0.0206
40	0.110	0.095	0.083	0.096	0.0135
50	0.108	0.104	0.100	0.104	0.0040

Tbl 6: Raw data of approach 3

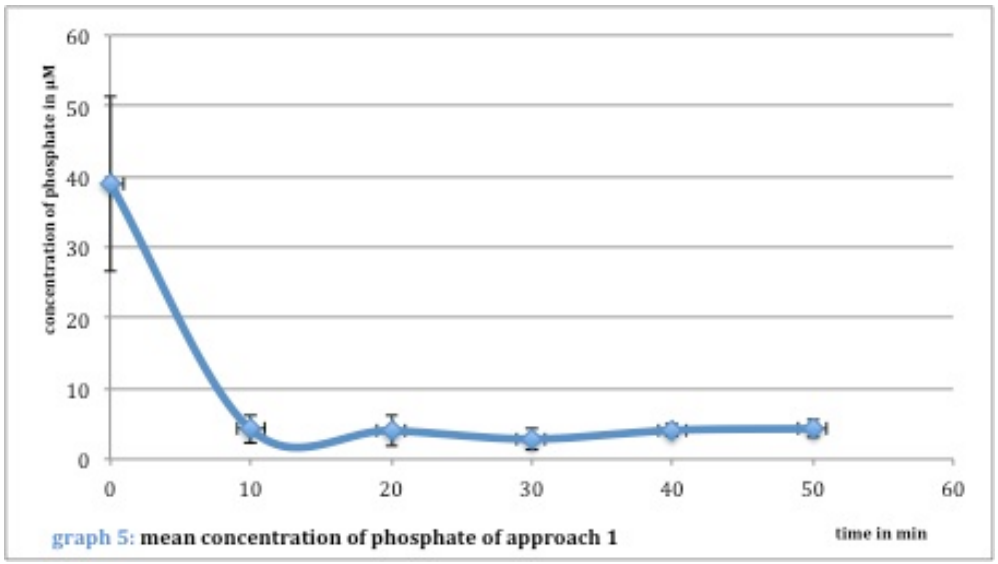
In this part we calculate the concentration the same way like in part 3

Step 3

Approach 1 – pH-value



graph 4: mean absorption of approach 1



graph 5: mean concentration of phosphate of approach 1

Aspect 1

This graph of the modified pH value (graph 5) shows how the phosphate concentration is changing during a time period of 50 minutes under the influence of the pH value. The massive reduction of the concentration during the first ten minutes is very remarkable. After that the concentration stagnates among five µMol. In difference to the graph of the modified pH value, the graph under normal conditions doesn't stagnate among five µMol. The phosphate concentration drops to nearly one and a half µMol.

Aspect2

To lower the pH value we added tot he phosphate bufer 40 µl HCl so the pH value reached 5.5.

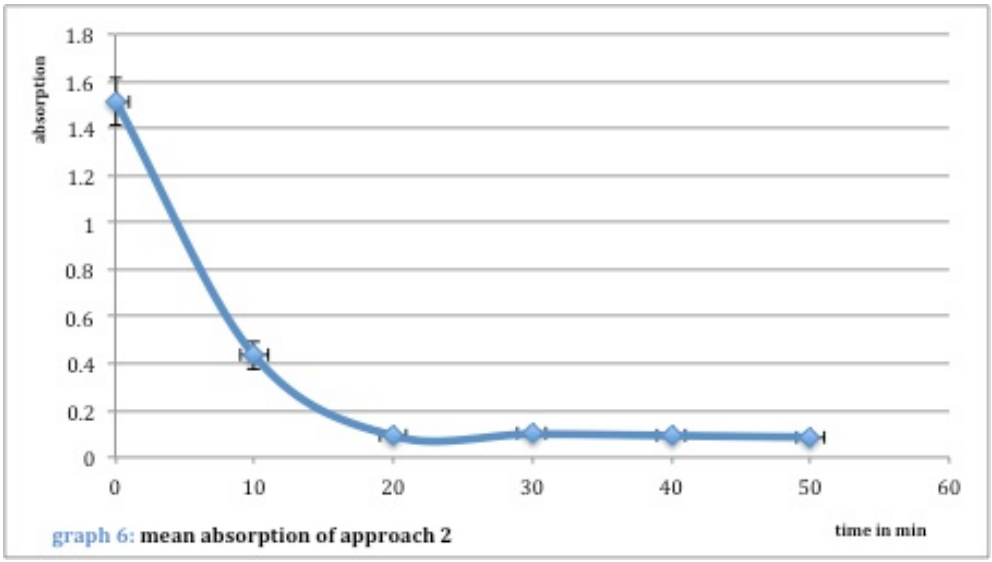
In our experiment we have the following weaknesses. Our devices to measure the concentration have a limited exactitude and we can't repeat the experiment more than three times to take the average afterwards. Because of these weaknesses our measured data have some inaccuracies but they aren't that big and the reduction of the phosphate concentration in the first ten minutes is as important as the graphs (graph 5) show.

Aspect 3

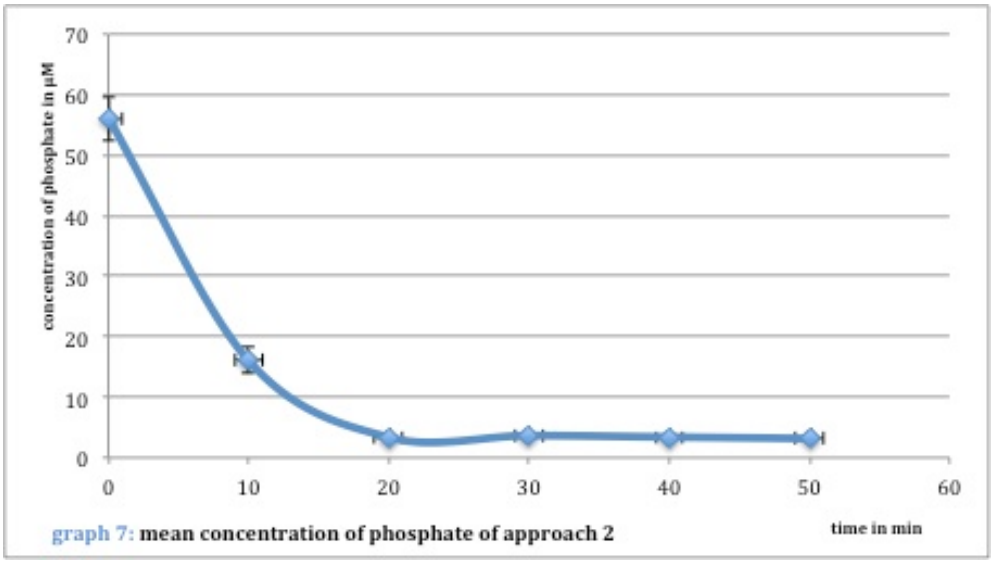
To improve the results of the experiment with the modified pH value, there are some approaches to solve the problem. Then we could measure the absorption more frequently. With this change we would have a better look over the process of the phosphate uptake and we could make clearer statements about the phosphate concentration.

Approach 2 – Watertemperatur

In the following graphics it abounds that with the rising of the water temperature the values of absorption rise.



graph 6: **mean absorption of approach 2**



graph 7: mean concentration of phosphate of approach 2

Aspect 1: Concluding

It's remarkable that the graphs of the beginning conditions are similar to the graphs of approach 2. The only bigger difference is that the curve is much steeper in approach 2. That's because of the yeast cells absorb the phosphate much faster. The curve isn't just steeper the reaction also speeds up much faster. In the beginning conditions the yeast cells need some time to begin with the uptake. The reason of this is the rule of Jacobus Henricus van't Hoff. It says that with the elevation of the temperature of 10°C of a biochemical reaction, the speed of reaction doubles. [10]

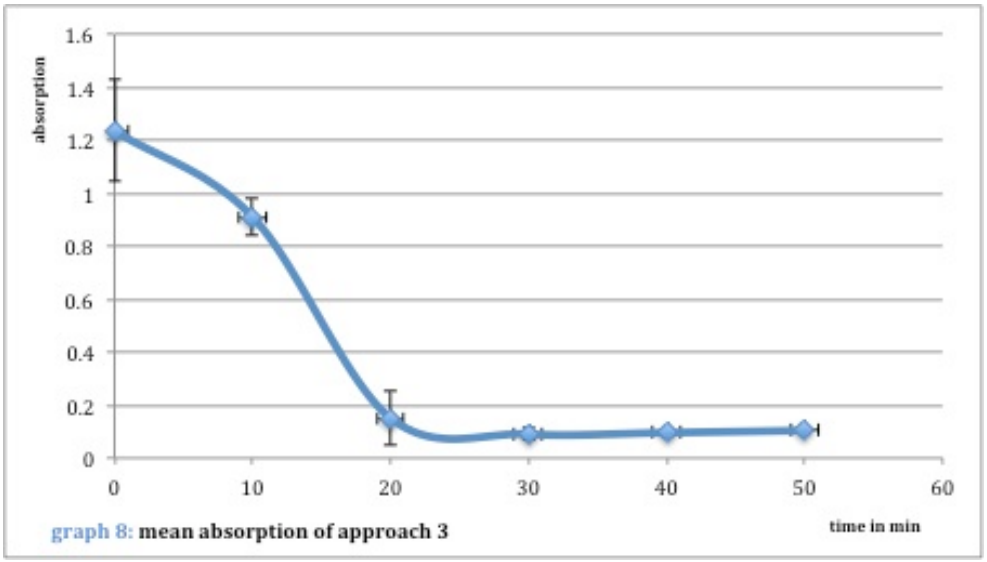
Aspect 2: Evaluating procedures

We changed the water temperature by putting the Erlenmeyer tubes in water, which we warmed up later on. We used a thermometer to check the water temperature so that it reached 32 degrees. This method wasn't very useful because we couldn't stir the salvation anymore. This might have changed the outcome a bit but not very much. Some variations of the data can be explained with mistakes of pipette, which can't be avoided totally. The whole experiment was really stressful because three rounds of this approach were made simultaneously and had to be measured by a single person. When we evaluated the probes with the timetable above in part 2 we once came in a great stress because some probes haven't been thinned down and we had to do it while measuring the others.

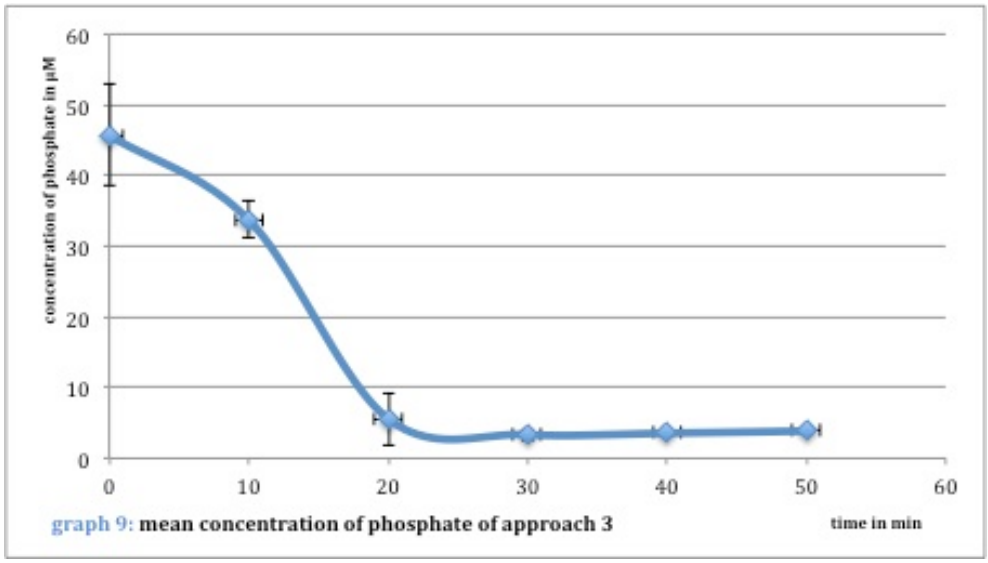
Aspect 3: Improving the investigations

We could have improved our method by using magnetic heating stirrer, then we could have warmed up the probes and simultaneously stir them. It would have also been more precise. The first time we made this experiment we had a more imprecise heater. We even think that some yeast cells died because the water was too hot.

Approach 3 – Glucose



graph 8: mean absorption of approach 3



graph 9: mean concentration of phosphate of approach 3

Aspect 1: Concluding

We now would like to compare the two graphics we made. The first graphic shows the uptake rates of phosphate under normal conditions.

You can see that the starting valuation is always located around one. Then it diminishes pretty quickly. After 30 minutes, all graphics are nearly at the zero valuation. And there they rest. But altogether, you can say that the uptake of phosphate decreases quickly and then rests at a low uptake level.

If you have a look at the graphic under changed conditions, you swiftly see that the starting valuation is located at a higher level. The valuations decrease quicker and the rest at a very low level until the end.

If you compare the both graphics, you can see that the uptake rate is higher in the beginning. But under changed conditions, they do not stagger.

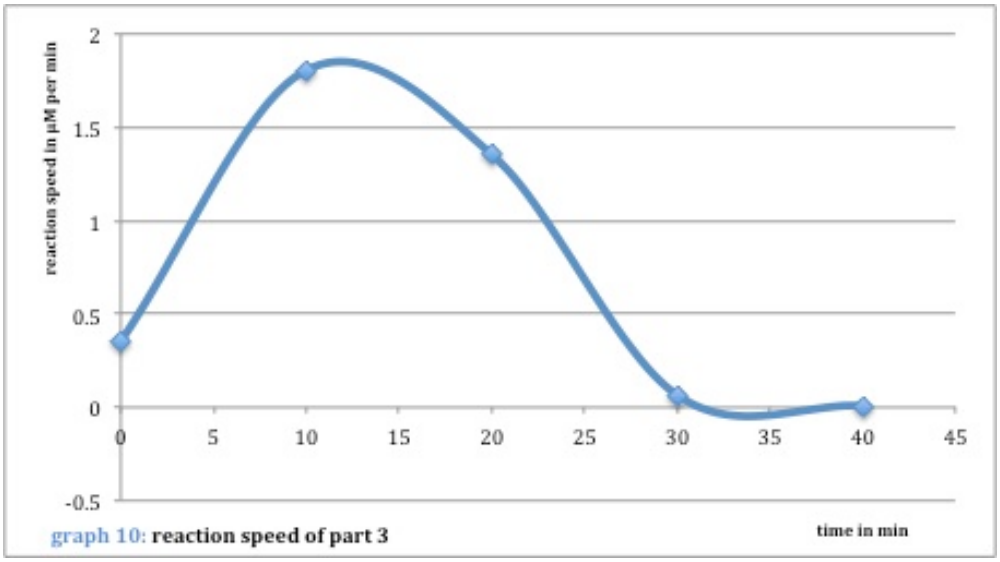
Aspect 2: evaluating procedures

To win the best datas we could, we brought through the experiment two times. To be honest, we made it three times. The first lasted about six hours and we made it on a day off. But we didn't gained useful datas. This was because the first photometer didn't worked well. So, we had to make the experiment two more times.
We were very careful and used the best measuring instruments of our school. We used a special and very expensive photometer of our chemistry teacher. To manage the time, we utilized stop watches. Based on the fact, that we made the experiment for several times and that we used measuring instruments of very high quality, we can say that the quality of the data is good. The measuring failures are tiny and they do not have a great influence on the results. Under the conditions of our school, this was the best quality of the measuring which was possible.

Aspect 3: Improving the investigation

First, it is pretty obvious that more precise equipment should be used. But this is not the only suggestion. Further, you should be more careful to important solutions, which you haven't already analysed. We say that, because one of our students (Michael Vogel =)) spilt up almost all of one very important solution. But fortunately we could analyse it anyway. Moreover, we had problems with the pressure of time. We were very nervous and a few students collected a number of data at the same time. So, we frequently had to help each other. Another struggle was, not to get sidetracked from other students or birds which fly past our windows. The last improvement would be, to gauge the different liquids exactly. You generally have to work as careful as it is possible. In our first experiment we didn't work very careful. And because of this and the not well working photometer, we collected unusable data.

Adding



graph 10: **reaction speed of part 3**

With our collected data we were also able to calculate the speed of the biochemical reactions at a certain point in time of the whole reaction. The reaction speed was calculated by dividing the difference between to concentrations with the time passed between them.
In the shown graph 10 you can see the developing of the speed during the raction . In other words: the height of graph ten symbolizes the gradient of the graph 3 in part 3 at a certain point of the curve. It's remarkable how the beautiful curve shows how the reaction first needs some time to reach full speed and the slowly gets slower. Because it would have used too much space we decided not to do this procedure for the other results in part 3 and 4, but we hope, that this short adding demonstrates how the developing of a reaction speed can be illustrated.

Reference List

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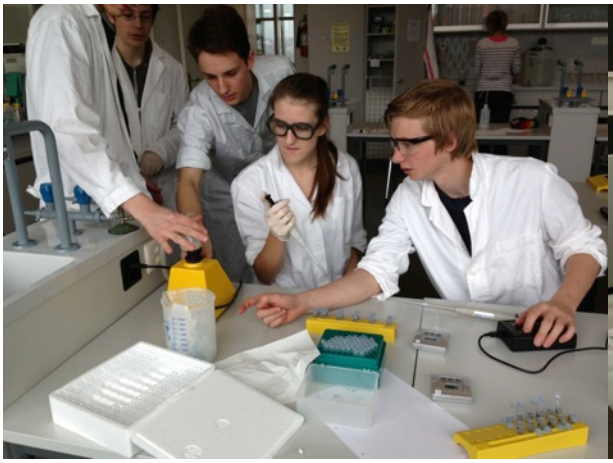
[9] Dr. –Ing. G. Kayser, Gewässerschutz/Abwasserreinigung, www.ihi-zittau.de/de/dnl/gewaesserschutz.657.pdf, visited: 28.4.2013

[10] RGT – Regel, http://www.u-helmich.de/bio/stw/enz/enzym/rgt.htm, visited: 10.5.2013

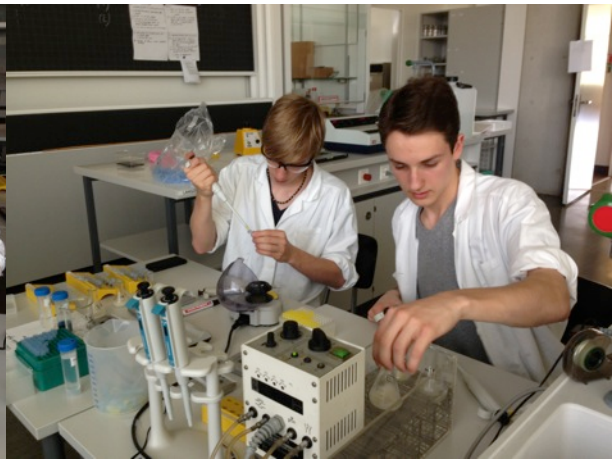
Activity List

Who?	What?
Timo, Michael, Benjamin, Christoph, Tina, Lucien	All the experiments
Sarah, Sevde, Toni	Part 4: Step 1, Step 3 – Glucose, Part 1: Question 4
Marilena, Severin, Ivan	Part 4: Step 3 – Watertemperatur, Part 1: Question 1/3
Leon, Nathanael	Part 2
Marc, Salome, Nicola	Part 4: Step 3 – Ph-value, Part 1: Question 2
Rinor, Christoph	Part 3
Jamina	Controlling our english
Lucien	Put everything together

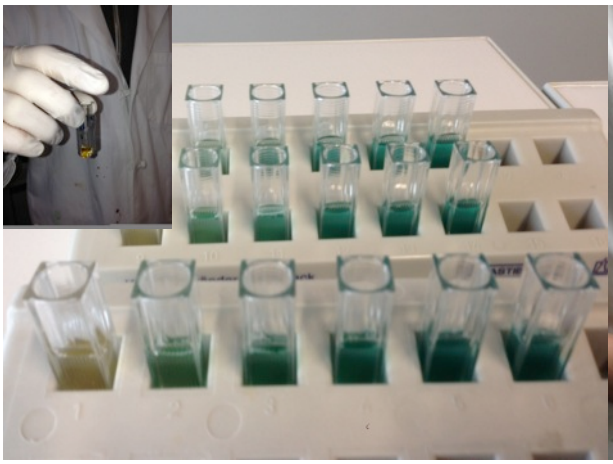
Photos



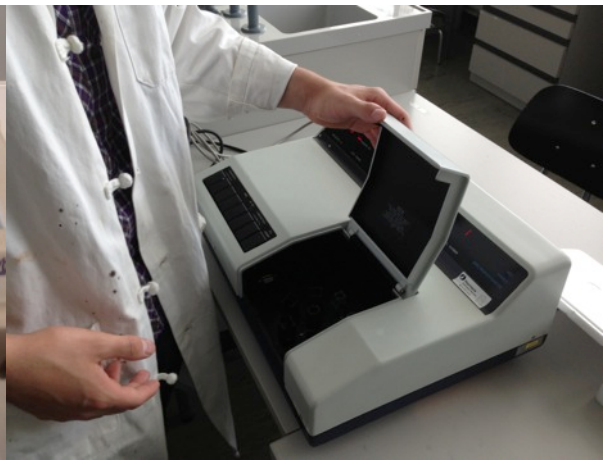
Pic 1: try to hand the vortex



Pic 2: Part 4 Approach 2 and 3



Pic 3: the samples of the straight calibration curve and the pretest (left upper corner)



Pic 4: the photospectrometer from our school