Gymnasium Burgdorf

Class SPF BC 2d

Task 2

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Part 1

Phosphate is important, as it is necessary for every intracellular reaction and challenges the body's energy balance. Every metabolic process needs ATP, the high-energy bond results from phosphate. Many important intracellular compounds consist of phosphate: nucleic acids, phospholipids, enzymes and nucleoproteins. Phosphate is essential in the aerobic as well as in the anaerobic metabolism and is also indispensable in our body's buffer system: it's important to buffer carbon dioxide and could be called "the main source of intracellular buffer". Phosphate also takes part in the coagulation and immune systems. [1]

The optimal pH for the phosphate uptake in yeast cells (Saccharomyces cerevisiae) is 6.5. [2] The vacuole of the yeast stores a large amount of phosphate and there is transport measured vacuolar membrane. Isolated, they will absorb the added phosphate through counter flow exchange with the phosphate that was already in the vacuole before the isolation. [3]

Yeast cells win their energy with the use of carbohydrate. In order to do this, the yeast cells need nutrients like phosphate and amino acid. [4] There are a few plasma-membrane transport systems for yeast cells to absorb phosphate. Usually sodium cations enter the yeast cell with diffusion, so that the phosphate anions can enter the cell easier due to a higher electric potential inside the cell. [3]

Phosphate is one of the most important nutrients for all biota. Like oil, natural phosphate resources are being overused and will eventually disappear. Without phosphoric fertilizer, intensive agriculture as we have it today will not be possible anymore. World hunger is expected to increase again if we run out of phosphate. [5] Facing this scenario, scientists are trying to develop efficient methods of restoring phosphates from wastewater. This can be done using bacteria in purification plants. Under anaerobic circumstances they take up the phosphate and store it. Later, it can be extracted and reused. [6] Furthermore a high phosphate concentration in our water causes the removal of oxygen for other life and so it is another reason why the substance is being removed in purification plants. [5]

[1] Neligan, P. year unknown, *Phosphate why is ist important?,* retrieved May 7th, 2013 from www.ccmtutorials.com/

[2] Goodman, J. 1957, Article: *"The Active Transport of Phosphate Into the Yeast Cell",* retrieved May 6th, 2013 from www.ncbi.nlm.nih.gov/

[3] W. Booth, J. and Guidotti, G. 1997, *Phosphate Transport in Yeast Vacuoles*, retrieved May 5th, 2013 from www.jbc.org/

[4] Unknown, year unknown, *Backhefe*, retrieved May 3rd , 2013 fromm www.wikipedia.de

[5] Meier C. 2012, Spektrum – Article: „Bevor der Danger ausgeht". Retrieved May 3rd, 2013 from www.wissenschaft-online.de

[6] Sathasivan A. year unknown, *„Biological Phosphorus Removal Processes for Wastewater Treatment"*, Retrieved May 3rd, 2013 from www.eolss.net

Part 2

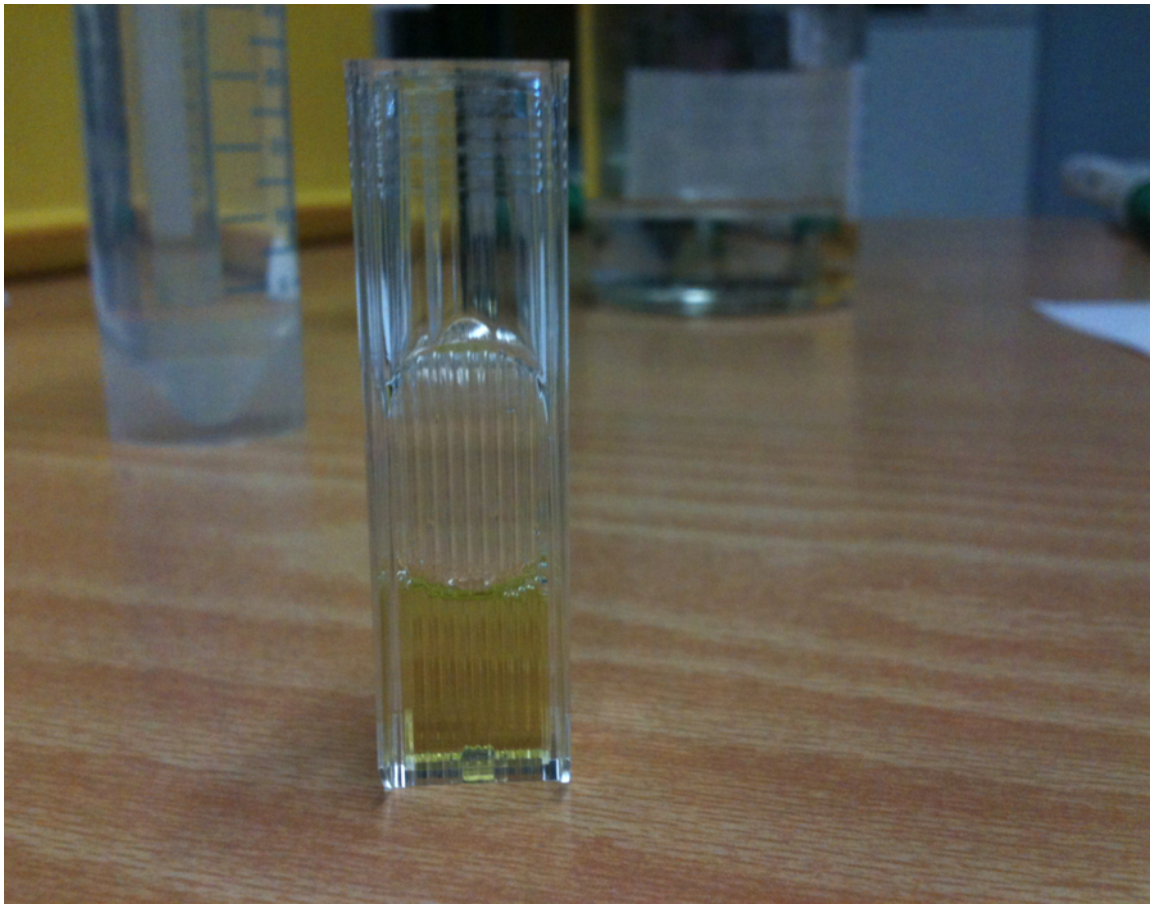

Fig. 1: *Result of our pretest*

Fig 2: *The chemistry lab of our school*

Table 1: Raw data of Part 2

Phosphate concentration	1st approach	2nd approach	3rd approach	Mean value	Standard deviation
0 µM	-0.086390363	-0.053157921	-0.044570241	-0.061372842	0.022087199
10 µM	0.241094887	0.251299983	0.24249316	0.244962677	0.00553262
20 µM	0.568501025	0.573191978	0.537484916	0.559725973	0.019403601
30 µM	0.890489563	0.862215478	0.846153968	0.866286336	0.022446384
40 µM	0.997278499	1.013423919	0.958826751	0.989843056	0.028047764
50 µM	1.037264708	1.042480834	1.048872771	1.042872771	0.005813948

Mean value of Standard deviation = 0.017221919

The r-value we reached was 0.94247, so our measurements are pretty precise.

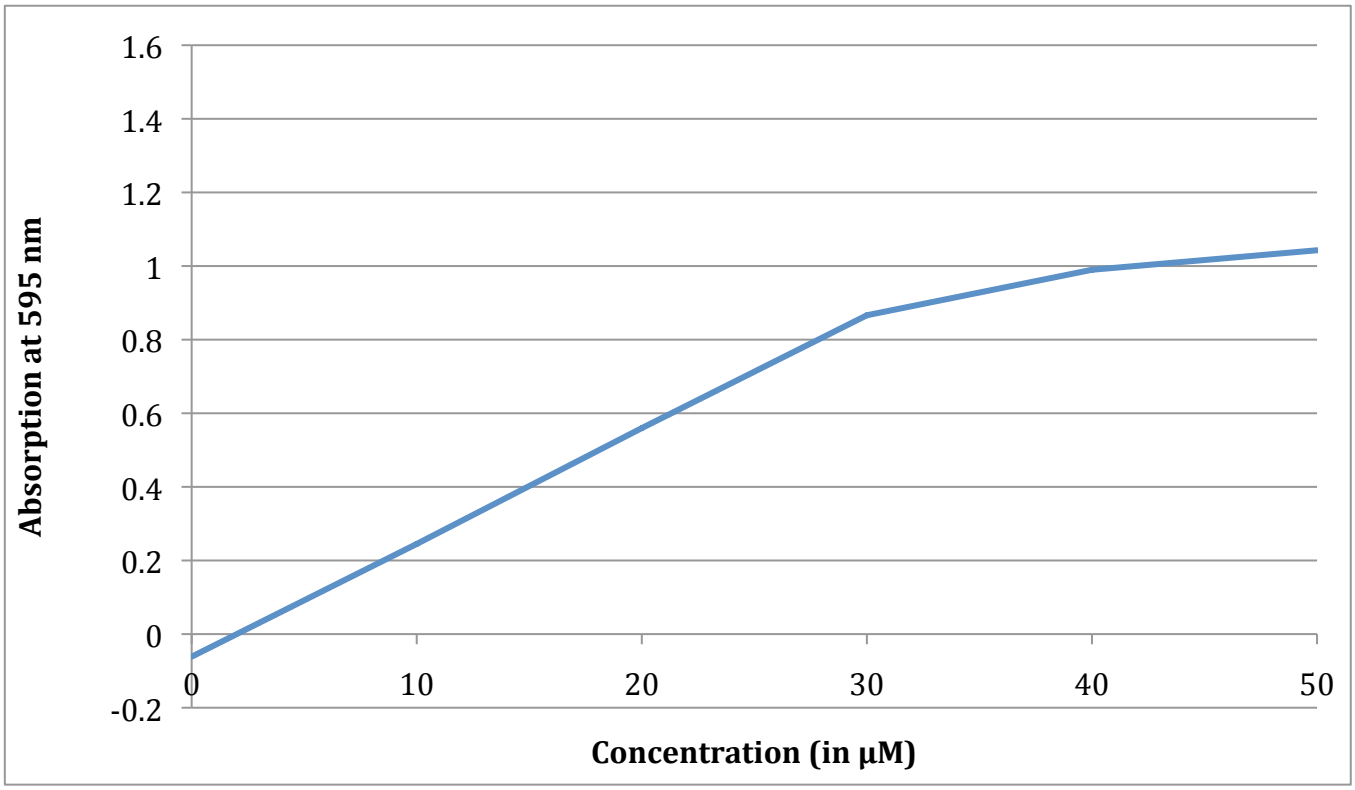

Fig. 3: *Chart with mean value of Part 2*

Difficulties

The biggest difficulty was to use the time as good as possible, because in this experiment we always had to wait a couple of minutes before we could go to the next step. As we did the first experiment-series we mixed the first solutions and waited until we had measured the absorption of this mix and then we started with mixing the second one. We realized that this uses too much time. We improved the time-management with mixing the next solutions while we were waiting the five minutes.

In the end everyone had his specific job that one had to do. So we could mix the solutions every two minutes and the others did their task after the exact time needed.

Another problem was that we didn't know that the download of the software for the photospectrometer would take so much time. That means that we first had to wait until the download was finished. We used this time for preparing all the EPs with the different concentrations of the phosphate.

Part 3

Table 2: Raw data and mean value of raw data from part 3

Time	1st attempt	2nd attempt	3rd attempt	Mean value	Standard deviation
0 min	0.985302707	0.947958523	0.811237766	0.914832999	0.091638563
10 min	0.873590687	0.985658616	0.872739179	0.910662828	0.064949653
20 min	0.326158416	0.438702831	0.053039855	0.272633701	0.198324618
30 min	-0.062816388	-0.062201864	-0.063519482	-0.062845911	0.000659305
40 min	-0.078033625	-0.060146262	-0.061934898	-0.066704929	0.009851616
50 min	-0.021311526	-0.085083583	-0.076390183	-0.06092843	0.034583495

Mean value of Standard deviation = 0.066667875

The r-value we reached was 0.80232, so it isn't as precise as our measurements in part 2.

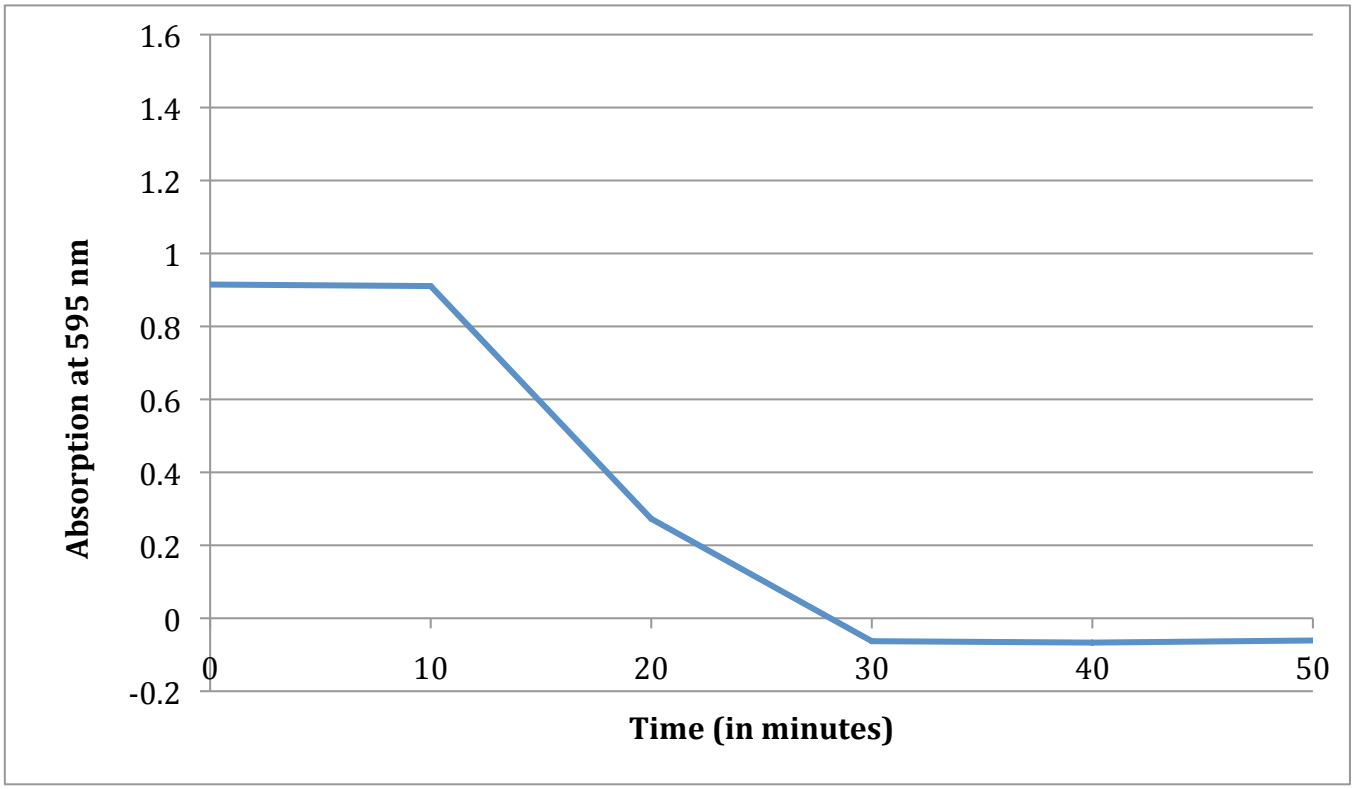

Fig. 4: *Chart with mean value of Part 3*

Difficulties

Once again, we struggled in using the time reasonable, because we had to wait always 50 minutes for having all the probes of one experiment-series. Because of that, we started three experiments parallel, but time-staggered. We had to wait first with the photometric measurement, because we were too few people for doing this while we were doing part 3A.

Another problem was, that the value of absorption in the photospectric measurement fluctuated after the calibration of the photospectrometer. Therefore we always calibrated the spectrometer before the measuring the absorption of the first solution of an experiment-series, so that at least for every experiment series the aberration will be the same.

Part 4

A 1st Approach

Design of experiment

We defined our specific research question as follows: „Does calcium chloride ($CaCl_2$) improve the phosphate uptake of yeast cells?“

We learned that with the help of the $CaCl_2$ the strength between the DNA and the cell membrane, both negatively charged, declines. There develop porosities[7], so theoretically the cell should be able to take up more phosphate. The independent variable in this experiment is the added amount of $CaCl_2$, which was chosen to be 1%, so it has the same percentage as the glucose. The dependent variable is the intensity of green color at each given point of time. Because the intensity stands in direct relation with the phosphate concentration, we could also say that the concentration is the dependent variable. Our constant variables are light intensity, pressure, temperature, added glucose and yeast and the time.

Light intensity, pressure and temperature could be held on the same level easily because we didn't change the anything in the room we were working. The time we controlled with little stop watches. The concentration of phosphate respectively the intensity of green light was measured with a photospectrometer.

We prepared three times the phosphate solution as in part 3, with the difference, that we added 0.1g $CaCl_2$ to the solution after adding the glucose. Then we continued with the experiment following the instructions given in the script. We started the three experiment-series parallel at three minutes intervals.

In order to get enough results, we decided to do three attempts. Three, because we are six people and so there are always two people responsible for one attempt.

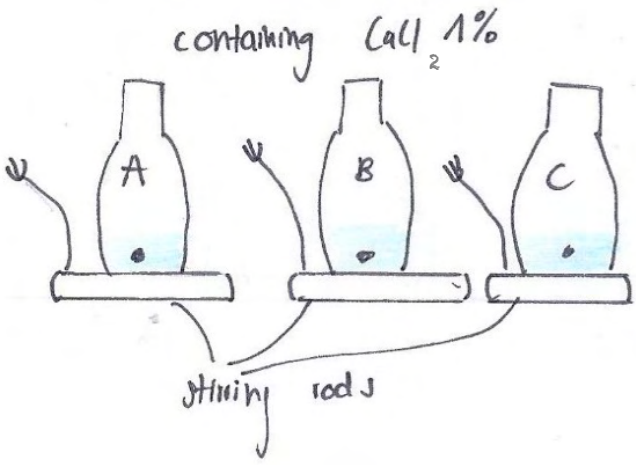

Fig. 5: *Experiment set-up of approach 1*

Data collection and processing

Table 3: Raw data and mean value of approach 1

Time	1st attempt	2nd attempt	3rd attempt	Mean value	Standard deviation
0 min	1.743469716	1.742706846	0.865453731	1.450543431	0.506702687
10 min	0.747661061	0.735337927	0.85822194	0.780406976	0.067670831
20 min	0.89409364	-0.02672178	0.27441838	0.380596747	0.469500419
30 min	-0.048517965	-0.010019802	-0.055277438	-0.037938402	0.024413291
40 min	-0.050385028	-0.010777219	-0.039930403	-0.03369755	0.020526349
50 min	0.105388091	-0.059234373	-0.04607285	2.69562E-05	0.091482419

Mean value of Standard deviation = 0.091482419

While measuring the absorption we were confronted with our first problem in this experiment: The photospectrometer fluctuated, even if we calibrated it after each attempt.

For processing our raw data we decided to calculate the mean value of all three experiment-series, because we think like this the results are most clear and to present it in a line chart together with the raw data, because with such a diagram the decline of the absorption can be seen very well and comparisons can easily be made.

[7] Transformation of plasmid DNA to competent *E. Coli* cells, Chenzhong Kiang, 2012 from www.wikipedia.com

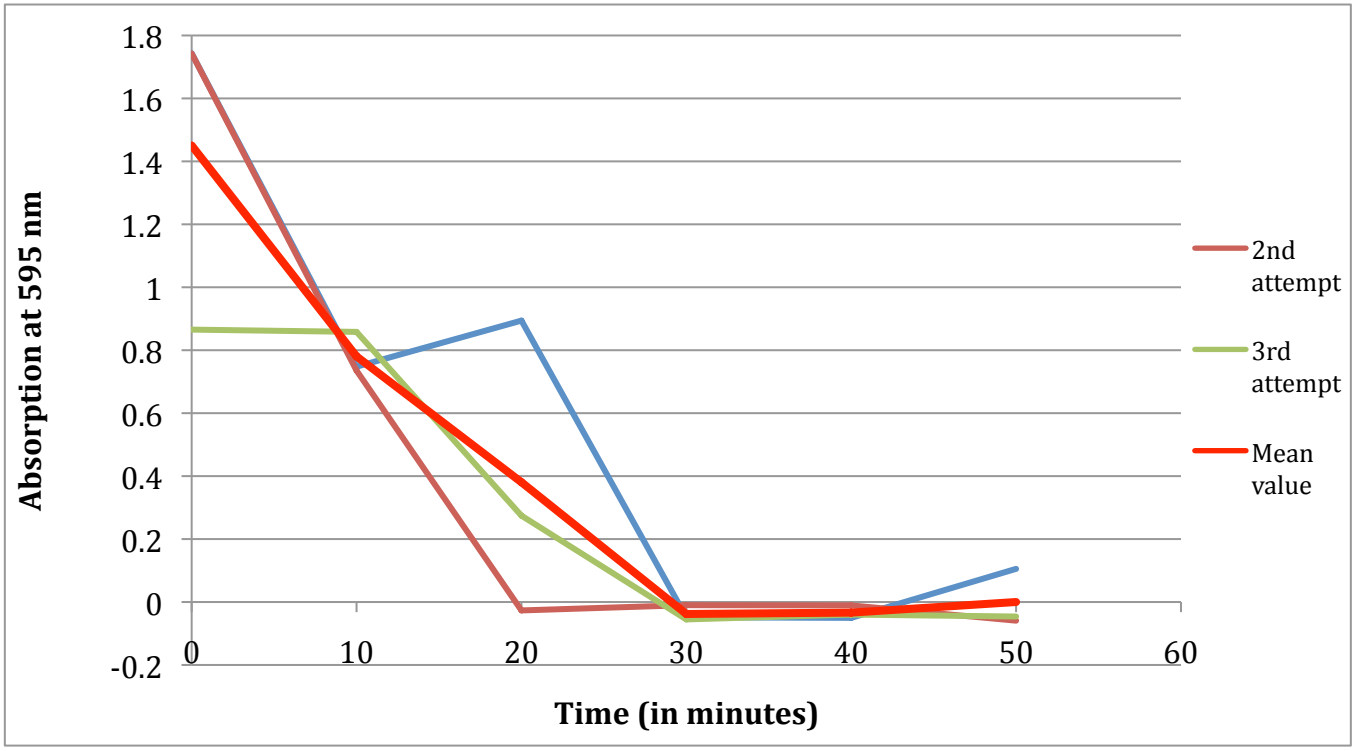

Fig. 6: *Chart of the raw data and mean value of approach 1*

Conclusion and evaluation

Concluding

The curves of this approach behave very strangely. The absorption at the beginning of attempt 3 and 4 is much higher compared to the results of part 3, which means that the phosphate concentration is probably higher as well. When we have a look on our chart from part 2 the concentration should be higher than 50 µM. An explanation for this strange fact could be that the $CaCl_2$ might have an intensifying effect on the coloration of the solution before putting it in the photospectrometer. After the first ten minutes the curve looks similar to the one in part 3, but we can see that between 20 and 30 minutes the curve is steeper, this would mean that the uptake of phosphate is faster than in the uninfluenced experiment. In the table we can see that the first two attempts are quite similar while the third looks more normal, compared to the result of part 3. So it is possible, that we made a mistake there, for example while diluting the phosphate-buffer. Out of our result it cannot yet be said, if calcium chloride has a positive effect on the phosphate uptake by yeast cells. It looks like a promising approach, but to be sure, it would be necessary to do some more attempts, but for this we unfortunately haven't enough time.

Evaluating procedures

The results we got from the three measurements show the same trends. But the values are not as similar to each other as they were in part 3. This inaccuracy may result from imprecise measurements of the different components, which were put in the EPs.

A weakness of this experiment may be that it was designed too easily. Meaning that the literature should have been studied more in order to have a more complex approach. This also could have helped to decide on the amount of $CaCl_2$ we added, because we haven't any idea, how much $CaCl_2$ is needed.

Another point to mention is, that we were already working on our SimplyScience-Project for five hours, so we weren't as concentrated as might be needed.

As already mentioned above, we had a problem with our photospectrometer, which could have a severe effect on our results.

Improving the investigation

For another time we should do in-depth research on this specific topic, this might help us with the design of the experiment so that we would know how much $CaCl_2$ we should add.

On top of that, this would give information about how to handle the $CaCl_2$, so that it can develop its effect. We could have dissolved it with the yeast first for example.

Furthermore different concentrations of the calcium chloride could be used as to be able to make a comparison from different sets of data later on.

We should also only do such experiments when we are "fresh" and fully concentrated.

B 2nd Approach

Design of experiment

The second experiment was made in a warm water bath with the temperature as the independent variable. We chose the heat, because we know from chemistry that chemical reactions run much faster at higher temperatures. By the gathering of information we found out that the metabolism of yeast cells works best if the temperature is around 32°. This was the reason why we decided to heat up the yeast in order to get better results with less phosphate in the solution. So our specific research question was as follows: „Does heat help the yeast cells to take up more phosphate?“

As already mentioned, the independent variable was the temperature. To get significant solutions one always has to have only one dependent variable and all the others have to stay constant. So the constant variables were again pressure, light intensity, added glucose and yeast, and the time. The dependent variable is, like in all approaches, the intensity of the green color at each given point of time.

To get good and significant results we had to look for a heating where we could set the temperature exactly to 32°C and where the origin of the temperature wasn't only one side. So we decided that a water heating system would fit best and we were very glad to find out that our school did have something like that. You can see the set-up of the experiment on the sketch.

Knowing that one attempt would not be significant enough we decided to make the experiment three times. Like we already did it in the first approach. We prepared the three solutions following the instructions given in the script.

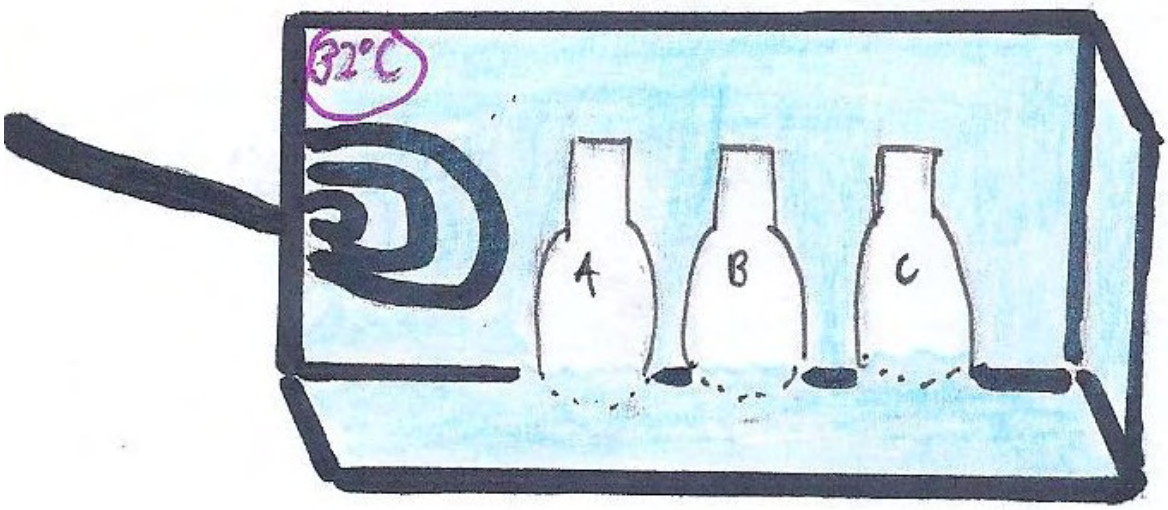

Fig. 7: *Experiment set-up of approach 2*

Data collection and processing

Table 4: Raw data and mean value of approach 2

Time	1st attempt	2nd attempt	3rd attempt	Mean value	Standard deviation
0 min	0.021264079	0.525097772	0.488441723	0.344934525	0.280905384
10 min	-0.070553815	0.497593181	0.499255456	0.308764941	0.32850073
20 min	-0.081244828	-0.017222699	-0.035676111	-0.044714546	0.032954184
30 min	-0.058723612	-0.069878943	-0.047996518	-0.058866357	0.010941911
40 min	-0.060574779	-0.074242896	-0.07076977	-0.068529148	0.007104199
50 min	-0.04343342	-0.084043729	-0.04914803	-0.058875059	0.02198319

Mean value of Standard deviation = 0.1137316

While collecting data of this approach we luckily hadn't the same problem with the photospectrometer as in the first approach.

For processing raw data we again decided to calculate the mean value of all three attempts and to put it together with the raw data into a line chart. So it is easy to compare the different results.

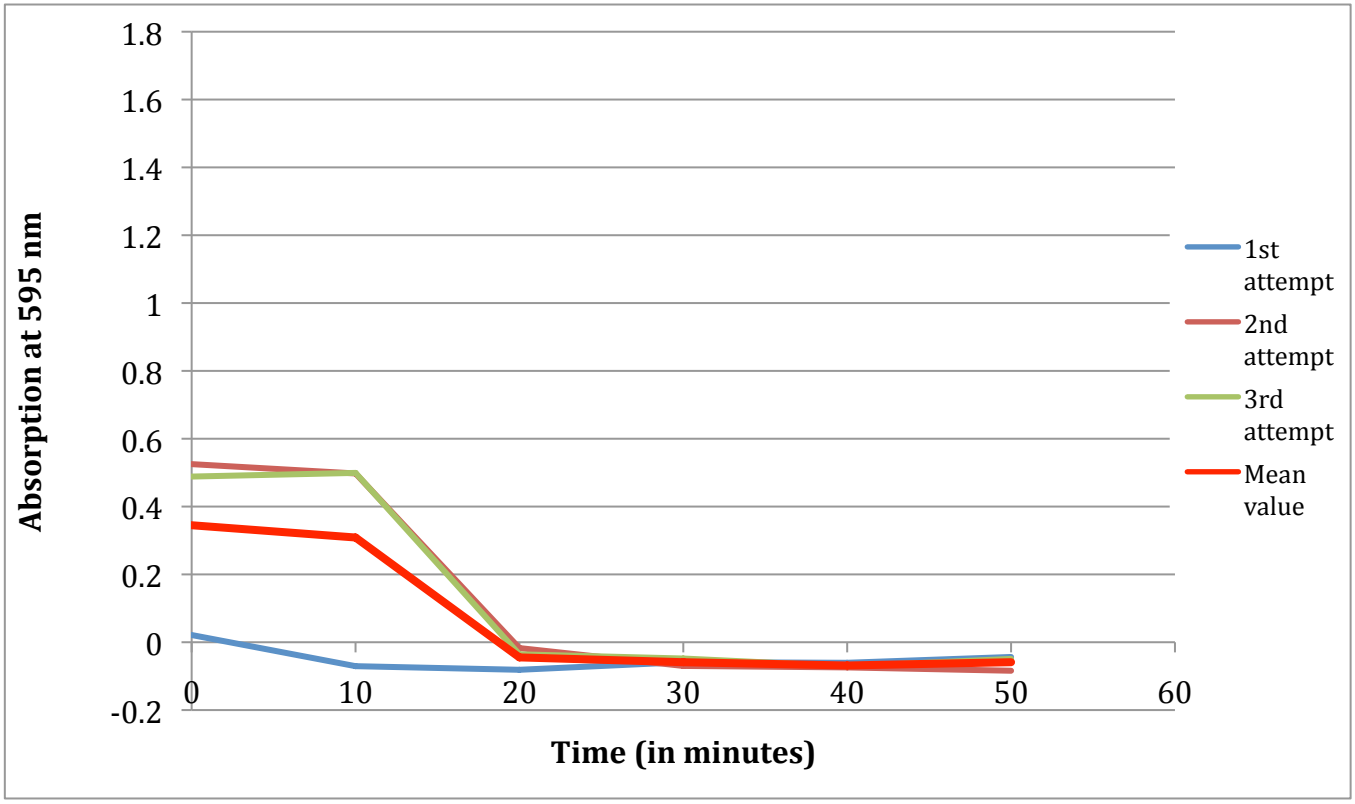

Fig. 8: *Chart of raw data and mean value of approach 2*

Conclusion and evaluation

Concluding

The highest concentration of Phosphate we got here was approximately 20 µM. The absorption at the very beginning is a bit less high compared to the curve in part 3. All three attempts reach negative values 10 minutes earlier than all other approaches and our comparison curve from part 3. This could be due to the lower start concentration or, as we predicted, due to the increased phosphate uptake by the yeast cells. So it seems as this approach has reached its goal. We were a bit irritated by the negative numbers as they occur in every attempt. The photospectrometer was calibrated with an empty cuvette, so with the intensity of green color in the air. Negative values would mean that the solution is less green than the air, but this is just non-sense, because all solutions were at least a bit green colored. So probably our photospectrometer has a serious problem. Also in this approach the mean value of the standard deviation is the highest, which means the results of the different experiment-series differ the most from each other compared to the other two approaches.

Evaluating procedures

Because of our experiment set-up, we could not stir the solutions all the time during the experiment, like we did it in approach 1. Due to this, less oxygen got into the solution, which could have affected the phosphate uptake by the yeast cells in a negative way.
The discrepancy of attempt 1 compared to attempt 2 and 3, could only be the result from imprecise pipeting, because all three attempts took place in the same water basin, so the temperature was the same for all as well and because the composition of all three solutions was equal.
As mentioned above we had a misfortune with our photospectrometer.

Improving the investigation

Instead of only choosing the temperature for optimal metabolism, several temperatures (for example 25°C, 28°C and 32°C) could have been chosen. The ideal temperature for the uptake of phosphate thus could have been found, because there were more available data.
Also should be designed a new experiment set-up, which would allow us to stir the solutions all the time during the whole experiment, so that the oxygen supply can be guaranteed.
Furthermore we should try to find out, what's going on with our photospectrometer and eventually use another one for the next time.

C 3rd Approach

Design of experiment

For the third experiment we took the concentration of oxygen in the solution as our independent variable. Yeast cells can make their metabolism either aerobic or anaerobic so the concentration of oxygen shouldn't have a big influence, but in the anaerobic metabolism the product is ethanol, which degrades the speed of the metabolic process. Our assumption was that the concentration of oxygen would then have an influence on the speed of the process. The controlled variables were again light intensity, pressure, temperature, added glucose and yeast, and the time. The dependent variable rests unchanged: the light intensity of green color at each given point of time.
In order to bring oxygen into the solution we needed a bottle of oxygen, which again wasn't a problem for us to get, because of the great equipment of our school. The oxygen went through a tube into a distribution system, which guaranteed us the same amount of oxygen in every Erlenmeyer tube. At the beginning we set the amount of added oxygen to 1.5 L/min, this means 0.5 L/min per Erlenmeyer tube, because they were all supplied from the same bottle of oxygen. Again you can see the set-up on the sketch.
As in the first two approaches we decided to do three attempts in order to get enough data. It wasn't possible to do more than three, because of a lack of time and a lack of people.

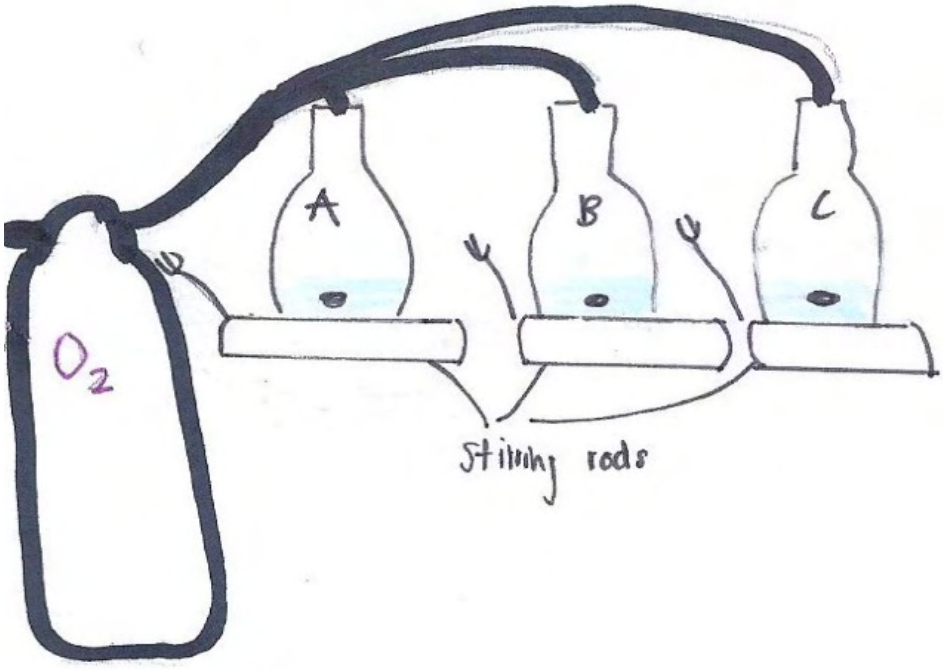

Fig. 9: *Experiment set-up of approach 3*

Data collection and processing

Table 5: Raw data and mean value of approach 3

Time	1st attempt	2nd attempt	3rd attempt	Mean value	Standard deviation
0 min	0.477032191	0.421227281	0.465754889	0.454671453	0.029507269
10 min	0.504093388	0.478647923	0.480181351	0.487640887	0.014268897
20 min	0.251474534	0.505475266	0.529909725	0.428953175	0.154185803
30 min	-0.042335977	-0.074180232	-0.069452907	-0.061989705	0.017183966
40 min	-0.048020053	-0.066535576	-0.071953828	-0.062169819	0.012549951
50 min	-0.015309971	-0.069146721	-0.079850948	-0.054769213	0.03458929

Mean value of Standard deviation = 0.043714196

For processing our raw data we decided to calculate again the mean value of all results and to put it together with the raw data of all three attempts into a line chart, because like this it is easy to compare the different results with each other.

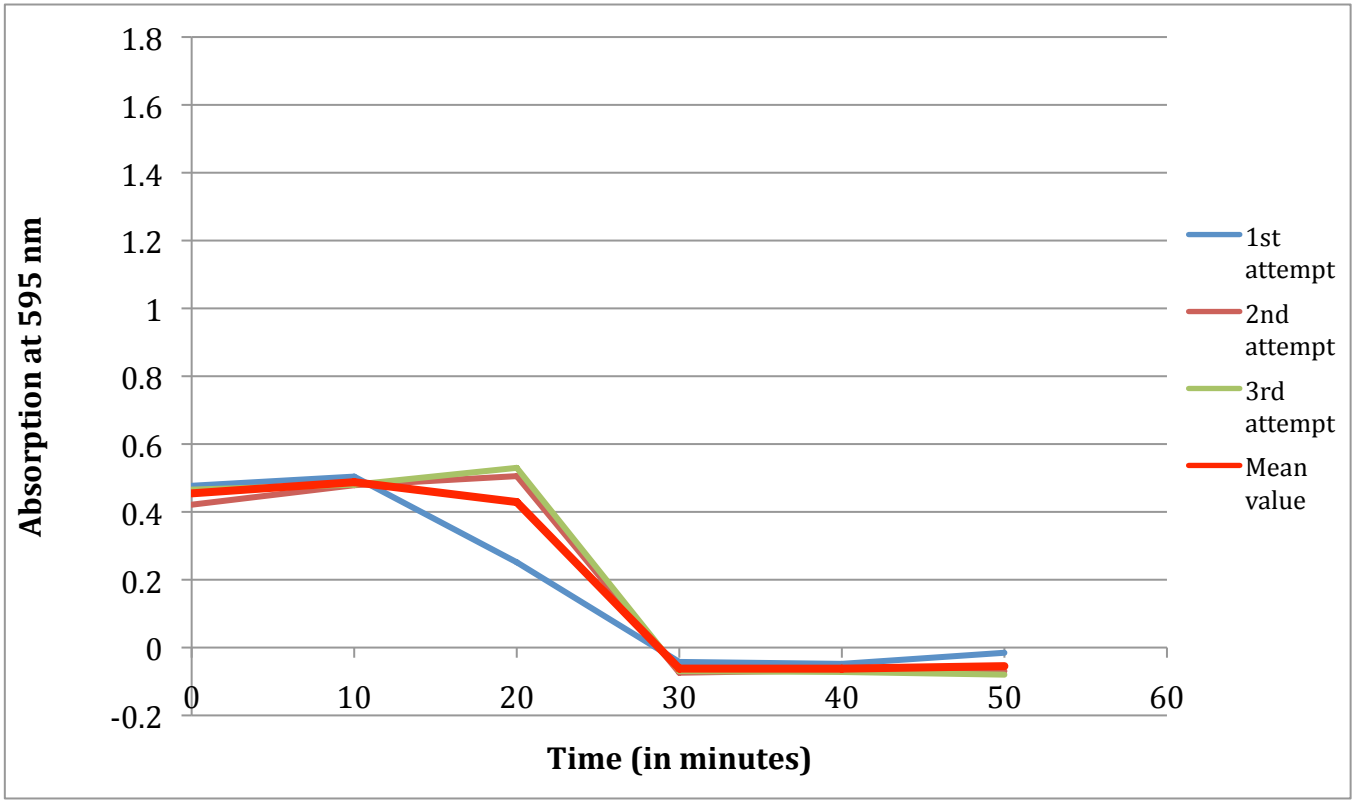

Fig. 10: *Chart of raw data and mean value of approach 3*

Conclusion and evaluation

Concluding

We can see in the graph that second and the third attempt are quite similar while the first behaves a bit different. But also with oxygen the start concentration of the phosphate seems to be a bit lower than in part 3. Perhaps this is due to the fact, that after the adding the yeast, the Erlenmeyer tubes were shaken by hand until the yeast has dissolved. But this time wasn't measured and not the same for every sample, because sometimes the yeast needed more time to dissolve. The decline in the intensity of the green color between 20 and 30 minutes from attempt 2 and 3 are the steepest ones of our whole project. This means the uptake of phosphate was much faster and so our assumption is approved. This attempt seems very promising to us and one should repeat the experiment after eliminating some errors, in order to get even more and better results.

Evaluating procedures

Although the experiment was started under identical conditions for all three suspensions, it is possible that the oxygen tubes have moved during the course of pipeting, which could have caused a different O_2 concentration in that receptacle. Neglecting this scenario, the equipment used was installed properly. The construction was built up as stable as possible to eliminate the risk of ripping out the oxygen tube or even overthrow the Erlenmeyer tube. Even though it took a while to figure out the ideal installation for this experiment, the time was used efficiently.

We didn't found out while studying the literature how much oxygen the yeast cells like, so the amount of 1.5L/min (0.5L/min) was chosen randomly. Another problem concerning the oxygen was that the pressure in of the bottle dropped, so there was less oxygen coming out of the bottle. The amount was around 1L/min for a short period of time (some minutes), because we didn't always survey the bottle.

Improving investigation

The oxygen supply should have been controlled better to eliminate the possibility that the concentration would be different in the three tubes and someone should always have an eye on the amount indicator of the oxygen bottle.

By using three oxygen cylinders, concentrations in the individual Erlenmeyer tubes could have been varied. Again this would have given us more data to work with and to make conclusions. So we could have found out which amount is the best to improve the phosphate uptake.

As mentioned above the time span between adding the yeast and taking the first probe wasn't always the same, this something that should be controlled another time.

Activitylist

Date	People involved/responsible person	Activity
29th April 2013	Julia, Ursina, Isabelle, Nicole, Christa, Lea	Studying the script, setting up a time plan, dividing up Part 1
30 th April 2013	Julia, Ursina, Isabelle, Nicole, Christa, Lea	Part 2 A&B
3rd May 2013	Ursina	Putting together part 1
7th May 2013	Julia, Ursina, Isabelle, Nicole, Christa, Lea	Conducting Part 3 A&B and Part 4 A We worked ca.7h
13th May 2013	Julia, Ursina, Isabelle, Nicole, Christa, Lea	Preparing solutions, EPs and Cuvettes for Part 4 B&C
13th May 2013	Lea	Making some pictures of class involvement
14th May 2013	Julia, Ursina, Isabelle, Nicole, Christa, Lea	Conducting 4 B&C
15th May 2013	Nicole	Difficulties of Part 1 and 2
15th May 2013	Christa	Part 4 A,B,C Step 1, Aspect 1
16th May 2013	Julia	Part 4 A,B,C Step 3 Apect 2 and 3
16th May 2013	Isabelle	Part 4 A,B,C Step 1 Aspect 1,2,3
16th/17th May 2013	Lea	Graphs of Part 1 and 2 4 A,B,C Step 2 and Step 3 Aspect 1
17th May 2013	Lea	Finishing the document

Fig. 11: *Ursina, Julia and Isabelle while conducting Part 4 A*

Fig. 12: *Nicole and Isabelle gazing at a Erlenmeyer tube filled with the yeast solution (Part 4A)*