
NKSA, G2B Task 2

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PART 1: STUDYING THE LITERATURE

Phosphate is a major constituent of nucleic acids such as DNA and RNA and of phospholipids. Furthermore, it is important for ATP and other energy-storing molecules. As a mineral, phosphate is also important for bones and teeth[1], and is therefore necessary for all living organisms.
Yeast also requires phosphate and absorbs it actively, the cells taking up free phosphate with the help of a number of plasma membrane systems.[2]
Many experiments with yeast have been conducted in the past. We analysed a report found on the Internet and derived our hypothesis from this analysis. Our hypothesis is as follows:
Potassium +ions increase the rate of phosphate uptake by yeast cells.[3]
After the uptake of phosphate, large amounts are stored in the vacuoles. [4]
Phosphate is a limiting factor for all organisms, as plants are directly dependent on phosphate, whereas herbivores eat plants, and carnivores come next in the food chain, hence they are also indirectly dependent on phosphate.[5] Because microorganisms stand first in the food chain, they are the first to be effected by phosphate variability.
The natural phosphate cycle is stable, but the interference from humans has resulted in over-fertilization in some parts of the world, as intensive modern agriculture aims for constant crop production, without allowing time for natural regeneration. Therefore, large quantities of artificial fertilizer are used, and these contain phosphate.[6] One of the main problems caused by the use of fertilizer is that it can seep into groundwater or watercourses, and this can result in accelerated growth of organisms, especially microorganisms and algae, and especially in small lakes. This leads to a big problem, as when algae die, oxygen in the water is used in the decomposing process, and this causes a shortage of oxygen for all organisms in the lake. Consequently, the organisms that require oxygen to survive die. [7]

[1] Campbell, Reece; *Biology*. Pearson, 2008, p.1233
[2] "Phosphate Transport in Yeast Vacuoles", http://www.jbc.org/content/272/33/20408.long, 14.05.13
[3] "The Effect of Potassium Ions on the Absorption of Orthophosphate and the Formation of Metaphosphate by Bakers Yeast", http://www.jbc.org/content/178/2/733.full.pdf, 15.05.13
[4] "Phosphate Transport in Yeast Vacuoles", http://www.jbc.org/content/272/33/20408.long, 14.05.13
[5] Michael Kent; *Advanced Biology.* Oxford, 2000, p.527
[6] "Fertilizer", http://en.wikipedia.org/wiki/Fertilizer, 15.05.13
[7] „5.6 Phosphorus", http://water.epa.gov/type/rsl/monitoring/vms56.cfm, 15.05.13

PART 2: CALIBRATION OF THE MEASURING SYSTEM

Picture 1: Result of the pre-test

Picture 2: Lab environment

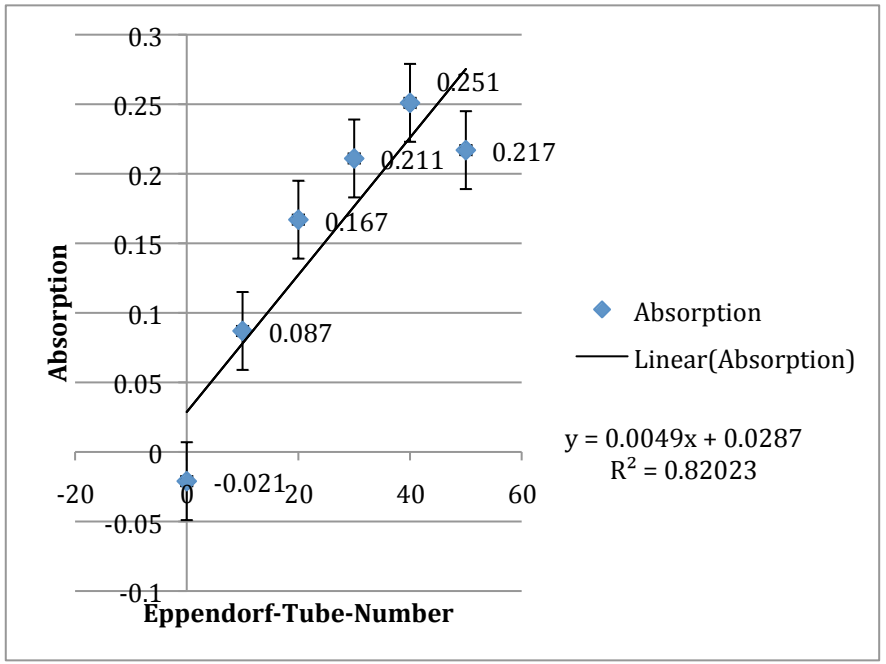

Diagram 1: Absorption at 595 nm

	Relative Uncertainty	Values	Values	Values	Values	Values	Values
Eppendorf-Tube Number	-	0	10	20	30	40	50
Sodium-Phosphate-Buffer (50µM) in µl	+/- 0.5 (µl)	0	80	160	240	320	400
Distilled water in µl	+/- 0.5 (µl)	400	320	240	160	80	0
Time lapse between adding dye and measurement in photospectroeter	+/- 0.5 (s)	60	60	60	60	60	60
absorption at 595 nm first attempt	+/- 0.0005	-0.022	0.077	0.212	0.288	0.291	0.264
absorption at 595 nm second attempt	+/- 0.0005	-0.013	0.097	0.141	0.177	0.252	0.205
absorption at 595 nm third attempt	+/- 0.0005	-0.027	0.087	0.149	0.167	0.211	0.183
maximal absorption	-	-0.013	0.097	0.212	0.288	0.291	0.264
minimal absorption	-	-0.027	0.077	0.141	0.167	0.211	0.183
mean absorption	-	-0.021	0.087	0.167	0.211	0.251	0.217
median absorption	-	-0.022	0.087	0.149	0.177	0.252	0.205
standard deviation	-	0.006	0.008	0.032	0.055	0.033	0.034
absolute uncertainty	-	0.007	0.01	0.0355	0.0605	0.04	0.0405

Table 1: Results of the three best attempts, mean values, standard deviation

Discussion

No difficulties were experienced when conduction the pre-test and a very yellow solution was produced. Establishing a straight calibration proved to be more difficult as we had to conduct the experiment twice. The first time it was not possible to produce graphs with a visible trend. The problem during the first attempt could have been that the time lapse of 1min 45s was too great. An improvement was made by using a newer photospectrometer that could be connected to the computer and then use “Logger Pro” software. The older photospectrometer had constantly changing values, which made it difficult to assess the correct values. Another problem was our organization as a class. During the first attempt, nobody knew exactly what to do and there were probably too many students in the laboratory. However, during the second attempt we were well organized and split the class into groups which each concentrated on different parts of the experiment. This allowed us to improve our accuracy whilst working as we could focus on a small part of the experiment.

PART 3: MEASURING THE PHOSPHATE UPTAKE BY YEAST CELLS

	Relative Uncertainty	Values	Values	Values	Values	Values	Values
Eppendorf-Tube Number	-	0	10	20	30	40	50
Sodium-Phosphate-Buffer (50µM) in µl	+/- 0.5 (µl)	0	80	160	240	320	400
Distilled water in µl	+/- 0.5 (µl)	400	320	240	160	80	0
Time lapse between adding dye and measurement in photospectroeter	+/- 0.5 (s)	60	60	60	60	60	60
absorption at 595 nm first attempt	+/- 0.0005	0.600	0.693	0.624	0.63	0.622	0.619
absorption at 595 nm second attempt	+/- 0.0005	0.132	0.114	0.083	-0.059	-0.043	-0.028
absorption at 595 nm third attempt	+/- 0.0005	0.133	0.119	0.1	-0.058	-0.057	-0.058
maximal absorption	-	0.600	0.693	0.624	0.630	0.622	0.619
minimal absorption	-	0.132	0.114	0.083	-0.059	-0.057	-0.058
mean absorption	-	0.288	0.309	0.269	0.171	0.174	0.178
median absorption	-	0.133	0.119	0.100	-0.058	-0.043	-0.028
standard deviation	-	0.220	0.272	0.251	0.325	0.317	0.312
absolute uncertainty	-	0.234	0.2895	0.2705	0.3445	0.3395	0.3385

Table 2: Results of the three best attempts of the phosphate uptake experiment, mean values, standard deviation

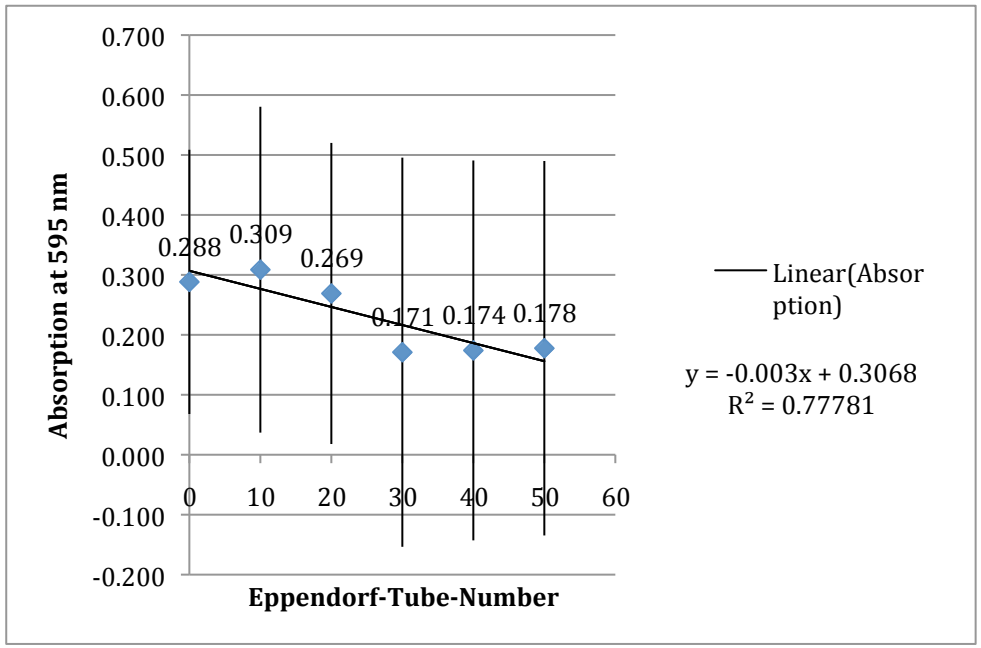

Diagram 2: Absorption at 595 nm of the phophate uptake experiment

Discussion

Totally wrong values were obtained in the first attempt of the third part because of a mistake made whilst conducting the experiment, although we could not establish exactly what the mistake was, it may have been due to false handling of the pipettes. After the mistake was noticed, we made sure all the participants knew how to handle the pipettes correctly and after this our results improved significantly. This error was obviously quite serious because the amounts needed to be measured were so small that any error can have a large effect. We also realized during the experiment that it is of great importance to regularly re-dissolve the yeast in the solution as false results may be obtained if this is not done to prevent the yeast settlings on the bottom of the container. A further reason that our first measurement was not successful could have been that we had only just taken the yeast out of the fridge and the low temperature could have affected the reaction time and our results.

PART 4: HOW TO IMPROVE THE PHOSPHATE UPTAKE BY YEAST CELLS

4A: First Approach: Glucose

Design

How does the amount of glucose added to the solution vary the rate of the phosphate uptake by yeast cells?
Yeast cells will uptake more phosphate if the amount of glucose gets increased, because glucose stimulates the yeast activities.

Independent variable: Amount of glucose added; 1g and 0.5g
Dependent variable: Amount of phosphate, that remains in the solution after 0 min, 10min, 20 min, 30 min, 40 min and 50 min.
Controlled variable: lapse of time between adding the solution into the cuvette and measure it, room temperature, air pressure.

Method

1. Make a homogenous solution out of 50 µl 0.1M Sodium-Phosphate-Buffer, pH 6.3 and 9950 µl H_20 and dissolve 1g, respectively 0.5 glucose and 1 g yeast in it until you get a homogenous solution.
2. Take an aliquot of 300µl at each defined time point and transfer it into an EP.
3. Centrifuge during 1min with max high speed
4. Take exactly 40µl of the supernatant and transfer it into another EP and add 360µl D-Water to each probe.
5. When you collected all the probes you may start the photometric measurement.
6. Label 6 additional EPs with M (=”Mix”, for each time point M0-M50).
7. Mix exactly 256µl Malachite green solution with exactly 344µl Molybdat-Solution in the first of these 6 EPs (M0).
8. Wait 5 minutes, then add this mix (7.) to the first of the collected probes containing the Supernatant.
9. Close the EP, mix it, and pour or pipette it into the corresponding cuvette.
10. Wait for 1 Minute.
11. Measure the absorption at 595nm.
12. Do this step by step for all the collected probes containing the Supernatant.

Data collection and processing

absorp. at 595 nm 1. att.	+/- 0.0005	0.082	0.133	0.172	0.067	-0.032	-0.073
absorp. at 595 nm 2. att.	+/- 0.0005	0.062	0.128	0.083	-0.059	-0.051	-0.062
absorp. at 595 nm 3. att.	+/- 0.0005	0.156	0.204	0.149	-0.04	-0.037	0
EP's		0	10	20	30	40	50
mean absorp.	-	0.100	0.155	0.135	-0.011	-0.040	-0.045
median absorp.	-	0.082	0.133	0.149	-0.040	-0.037	-0.062
standard deviation	-	0.013	0.016	0.010	0.021	0.002	0.012
absolute uncertainty	-	0.047	0.038	0.0445	0.063	0.0095	0.0365

Table 3: Results of three best attempts, glucose experiment, 1 g glucose

absorp. at 595 nm 1. att.		+/- 0.0005	0.165	0.157	0.161	0.011	-0.046	-0.035
absorp. at 595 nm 2. att.		+/- 0.0005	0.187	0.192	0.157	-0.016	-0.061	-0.04
absorp. at 595 nm 3. att.		+/- 0.0005	0.203	0.159	0.104	0.02	-0.06	-0.052
EP's			0	10	20	30	40	50
mean absorp.	-		0.185	0.169	0.141	0.005	-0.056	-0.042
median absorp.	-		0.187	0.159	0.157	0.011	-0.060	-0.040
standard deviation	-		0.001	0.007	0.012	0.004	0.003	0.002
absolute uncertainty	-		0.019	0.0175	0.0285	0.018	0.0075	0.0085

Table 4: Results of three best attempts, glucose experiment, 0.5 g glucose

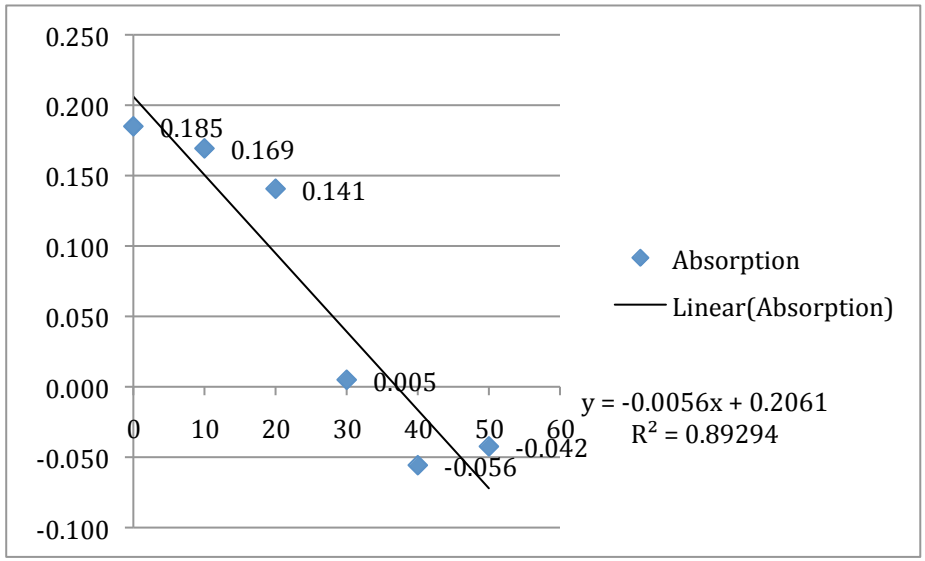

Diagram 4: Absorption at 595 nm of the means of the glucose experiment, 0.5 g glucose

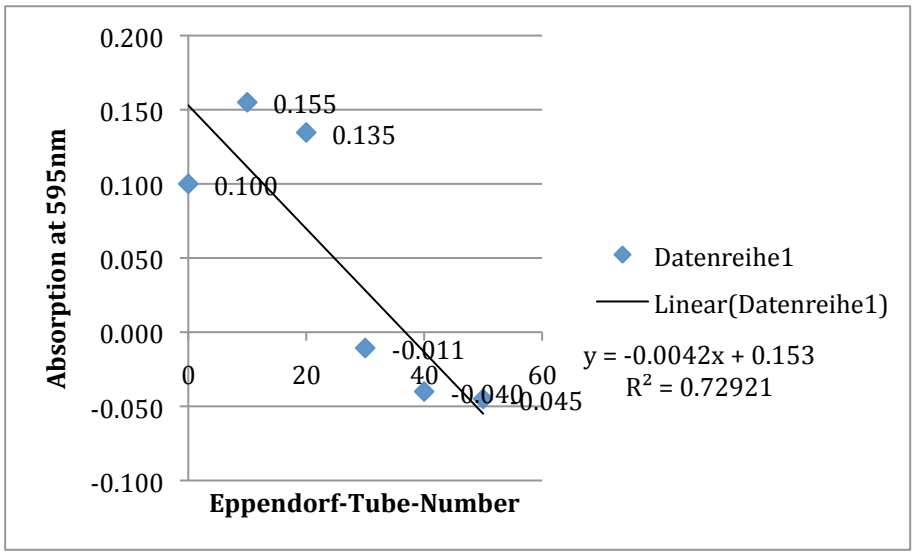

Diagram 3: Absorption at 595 nm of the means of the glucose experiment, 1 g glucose

Conclusion and evaluation

The mean values in diagram 4 are much higher than the values in diagram 3. However, a similar tendency can be seen in both diagrams. In diagram 4 the range of the values is much bigger than in diagram 3. The range of the values in diagram 4 goes from 0.185 to -0.056 whereas the range of the values of the diagram 3 reaches from 0.155 to -0.040.

It was relatively difficult to handle the pipettes accurately, and although the handling improved with time, some big mistakes could have been made here.

As can be seen in the tables, the values became less precise the longer the solution is left. This could be due to the fact that the photospectrometer does not have the capacity to measure such small values.

Another reason could be the factor of temperature. The yeast had been removed from the fridge right at the start of the experiment so it was rather cold in the beginning, and warmed up during the experiment.

We would certainly improve the controlling of the temperature of the yeast, so that there is no temperature factor to distort the results.

4B Second approach: Temperature

Design

How does a variation of the temperature influence the rate of phosphate uptake in yeast cells?

Yeast cells will uptake more phosphate if the temperature is higher because higher temperature stimulates the yeast activities.

Independent variable: Temperature of the solution

Dependent variable: Amount of phosphate, that remains in the solution after 0 min, 10 min, 20 min, 30 min, 40 min and 50 min.

Controlled variable: lapse of time between adding the solution into the cuvette and measure it, temperature of the environment, air pressure.

Method

1. Make a homogenous solution out of 50 µl 0.1M Sodium-Phosphate-Buffer, pH 6.3, 9950 µl H_20 0.1 g gluciose and 1 g yeast..
2. Maintain this solution during the whole experiment by 25°C, respectively by 35°C
3. Take an aliquot of 300µl at each defined time point and transfer it into an EP.
4. Centrifuge during 1min with max high speed.
5. Take exactly 40µl of the supernatant, transfer it into another EP an add 360µl D-Water.
6. 7.When you collected all the probes you may start the photometric measurement.
7. Label 6 additional EPs with M (=”Mix”, for each time point M0-M50).
8. Mix exactly 256µl Malachite green solution with exactly 344µl Molybdat-Solution in the first of these 6 EPs (M0).
9. Wait 5 minutes, and then add this mix (8.) to the first of the collected probes containing the Supernatant.
10. Close the EP, mix it, and pour or pipette it into the corresponding cuvette.
11. Wait for 1 minute.
12. Measure the absorption at 595nm.
13. Do this step by step for all the collected probes containing the Supernatant.

Data collection and processing

	Units	Rel. Unc.	P0	P10	P20	P30	P40	P50
absorp. at 595 nm 1. att.		+/- 0.0005	0.418	0.21	0.013	0.006	-0.052	-0.043
absorp. at 595 nm 2. att.		+/- 0.0005	0.383	0.027	0.023	-0.025	-0.025	-0.03
absorp. at 595 nm 3. att.		+/- 0.0005	0.392	0.226	0.033	0.023	-0.047	-0.024
maximal absorption		-	0.418	0.226	0.033	0.023	-0.025	-0.024
minimal absorption		-	0.383	0.027	0.013	-0.025	-0.052	-0.043
mean absorption		-	0.398	0.154	0.023	0.001	-0.041	-0.032
median absorption		-	0.392	0.210	0.023	0.006	-0.047	-0.030
standard deviation		-	0.018	0.111	0.010	0.024	0.014	0.010
absolute uncertainty		-	0.0175	0.0995	0.01	0.024	0.0135	0.0095

Table 5: Results of three best attempts, temperature experiment, 25°C

		Rel. Unc.	P0	P10	P20	P30	P40	P50
absorp. at 595 nm 1. att.		+/- 0.0005	0.404	0.059	-0.023	-0.017	-0.021	0.013
absorp. at 595 nm 2. att.		+/- 0.0005	-0.032	-0.032	-0.057	-0.048	-0.03	-0.041
absorp. at 595 nm 3. att.		+/- 0.0005	0.4	0.048	-0.016	0.002	-0.042	-0.032
maximal absorption		-	0.404	0.059	-0.016	0.002	-0.021	0.013
minimal absorption		-	-0.032	-0.032	-0.057	-0.048	-0.042	-0.041
mean absorption		-	0.257	0.025	-0.032	-0.021	-0.031	-0.020
median absorption		-	0.400	0.048	-0.023	-0.017	-0.030	-0.032
standard deviation		-	0.251	0.050	0.022	0.025	0.011	0.029
absolute uncertainty		-	0.218	0.0455	0.0205	0.025	0.0105	0.027

Table 6: Results of three best attempts, temperature experiment, 25°C

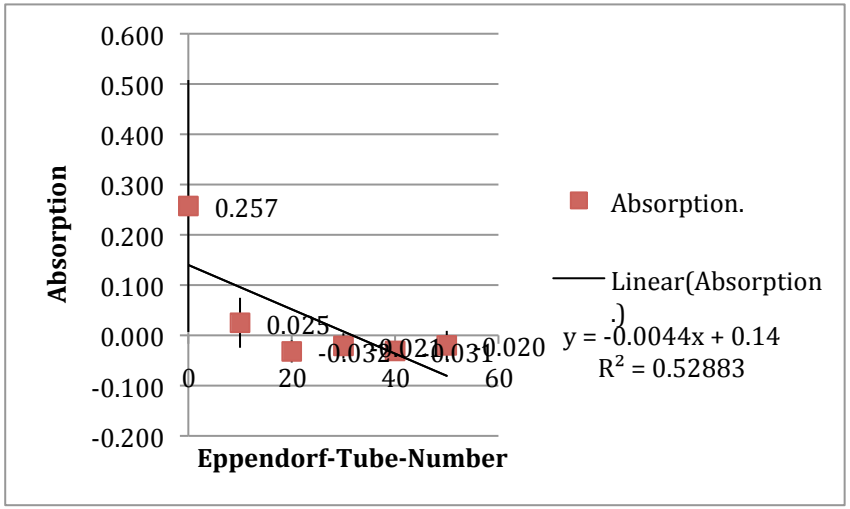

Diagram 6: Absorption at 595 nm of the mean values of the temperature experiment, 35° C

Conclusion and evaluation

The trend shown on all the graphs indicates that the absorption decreases quite linearly with time. This means that the phosphate uptake increases the longer the solution is left. The graphs also show that increasing the temperature makes the absorption decrease. This trend is especially visible at time 0, 10, 20 and 30; although both data points at time 0 are visibly too high and this means that they are most probably outliers. This could be due to the fact that it is rather difficult to take measurements at time 0.

Our procedure and investigation could have been improved quite significantly if we had taken measurements at more different temperatures. This weakness is very severe as seriously affects data evaluation.

Apart from these errors we consider our procedure of raising the temperature as successful as we had no difficulties achieving the temperatures, especially after we had found a more effective way of keeping our solutions in the water bath without someone having to hold them down.

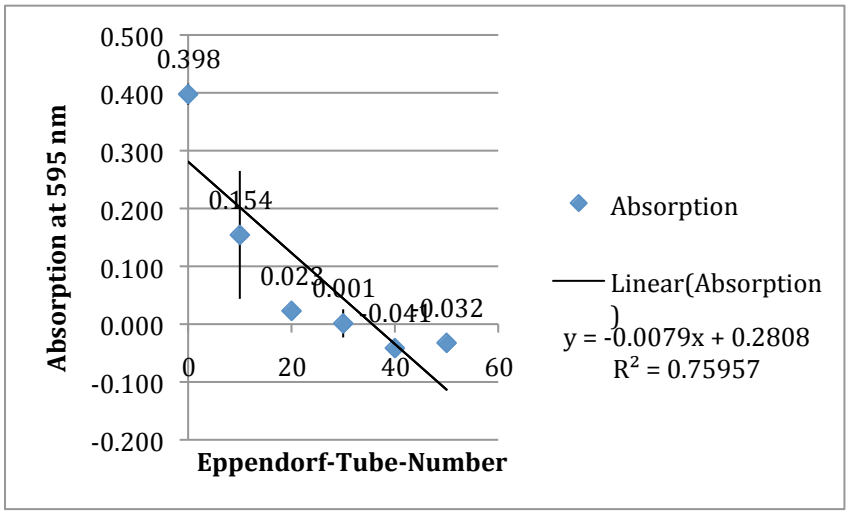

Diagram 5: Absorption at 595 nm of the mean values of the temperature experiment, 25°C

4C Third approach: Potassium Chloride

Design

How does adding Potassium Chloride (KCL) to the solution influence the rate of the phosphate uptake by yeast cells?

Yeast cells will uptake more phosphate if KCL gets added to the solution, because KCL stimulates the yeast activities.

Independent variable: Amount of KCL added to the solution ; 0.007456g and 0.01912g

Dependent variable: Amount of phosphate, that remains in the solution after 0 min, 10min, 20 min, 30 min, 40 min and 50 min.

Controlled variable: lapse of time between adding the solution into the cuvette and measure it, room temperature, air pressure.

Method

1. Make a homogenous solution out of 50 µl 0.1M Sodium-Phosphate-Buffer, pH 6.3, 9950 µl H_20, 0.1 g glucose, 0.007456g respectively 0.01912 g KCl and 1 g freh yeast.
2. Take an aliquot of 300µl at each defined time point and transfer it into an EP.
3. Centrifuge during 1min with max high speed.
4. Take exactly 40µl of the supernatant, transfer it into the corresponding another EP and add 360µl D-Water to it.
5. When you collected all the probes you may start the photometric measurement.
6. Label 6 additional EPs with M (=”Mix”, for each time point M0-M50).
7. Mix exactly 256µl Malachite green solution with exactly 344µl Molybdat-Solution in the first of these 6 EPs (M0).
8. Wait 5 minutes, then add this mix (7.) to the first of the collected probes containing the Supernatant.
9. Close the EP, mix it, and pour or pipette it into the corresponding cuvette.
10. Wait for 1 minute.
11. Measure the absorption at 595nm.
12. Do this step by step for all the collected probes containing the Supernatant.

Data collection and processing

absorp. at 595 nm 1. att.	+/- 0.0005	0.125	0.07	0.013	0.013	0.007	0.016
absorp. at 595 nm 2. att.	+/- 0.0005	0.055	0.034	0.031	0.01	-0.001	0.156
absorp. at 595 nm 3. att.	+/- 0.0005	0.92	0.056	0.013	0.021	-0.001	0.011
	EP's	0	10	20	30	40	50
maximal absorption	-	0.92	0.07	0.031	0.021	0.007	0.156
minimal absorption	-	0.055	0.034	0.013	0.01	-0.001	0.011
mean absorption	-	0.3667	0.0533	0.0190	0.0147	0.0017	0.0610
median absorption	-	0.125	0.087	0.149	0.177	0.252	0.205
standard deviation	-	0.48048	0.01815	0.01039	0.00569	0.00462	0.08231
uncertainty	-	0.4325	0.018	0.009	0.0055	0.004	0.0725

Table 7: Results of three best attempts of mean values of potassium experiment, 0.007456 g KCl

absorp. at 595 nm 1. att.	+/- 0.0005	0.256	0.056	0.018	0.025	0.018	-0.013
absorp. at 595 nm 2. att.	+/- 0.0005	0.072	0.014	0.018	0.029	0.024	-0.008
absorp. at 595 nm 3. att.	+/- 0.0005	0.069	0.076	0.028	0.039	0.017	0.012
	EP's	0	10	20	30	40	50
maximal absorption	-	0.256	0.076	0.028	0.039	0.024	0.012
minimal absorption	-	0.069	0.014	0.018	0.025	0.017	-0.013
mean absorption	-	0.1323	0.0487	0.0213	0.0310	0.0197	-0.0030
median absorption	-	0.072	0.087	0.149	0.177	0.252	0.205
standard deviation	-	0.10711	0.03164	0.00577	0.00721	0.00379	0.01323
absolute uncertainty	-	0.0935	0.031	0.005	0.007	0.0035	0.0125

Table 8: Results of three best attempts of mean values of potassium experiment, 0.01912 g KCl

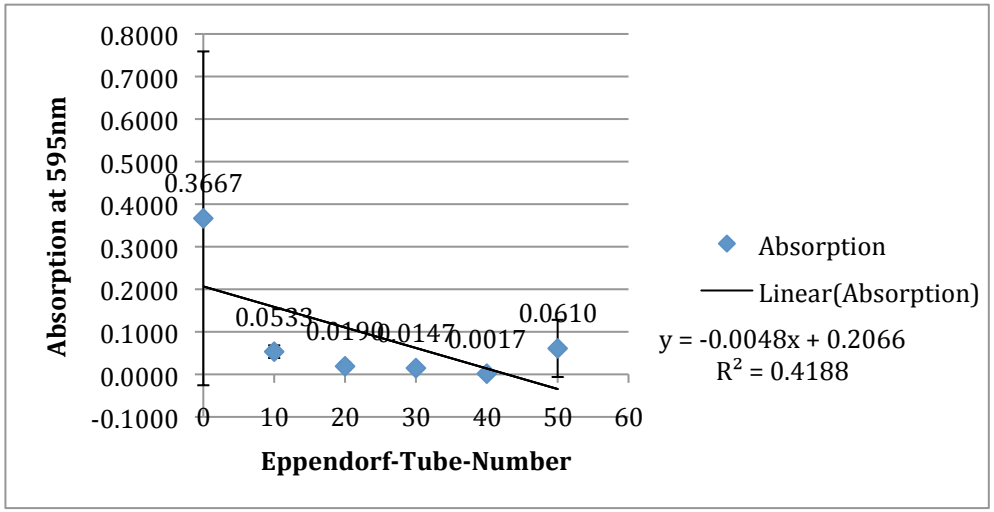

Diagram 6: Absorption at 595 nm of the mean values of the potassium experiment, 0.007456g KCl

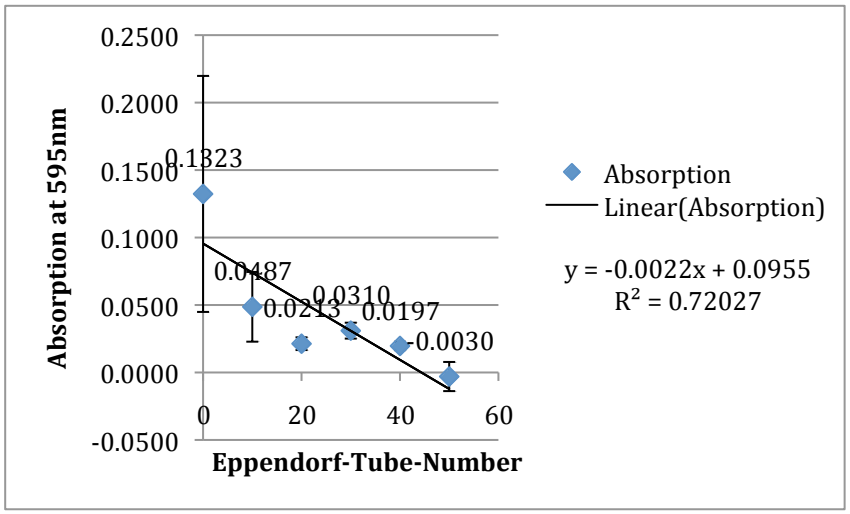

Diagram 7: Absorption at 595 nm of the mean values of the potassium experiment, 0.01912 g KCl

Conclusion and evaluation

Both the graphs of the experiment with 0.007456g and 0.01912g of Potassium chloride show a clearly decreasing trend. The graph of 0.007456g starts with a higher value but then decreases rapidly in contrary the graph of 0.01912 starts at lower value and decreases slower.

Unfortunately, our thesis hasn't been verified, as the higher concentration of Potassium chloride didn't have a positive effect on the Phosphate uptake of yeast cells.

Taking more measurements could have given clearer results.

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Campbell, Reece 2008 Biology. 8th edition. Pp. 65/1226. Pearson International Edition
Phosphate Transport in Yeast Vacuoles", http://www.jbc.org/content/272/33/20408.long, 14.05.13

Oral informations:
Isabelle Zumsteg, Biology Teacher at Neue Kantonsschule Aarau
Patrik Hunziker, Physics Teacher at Neue Kantonsschule Aarau
Graham Carver, Chemistry Teacher at Neue Kantonsschule Aarau

ACTIVITY LIST

Name	*Activity*
Jennifer Seiz	Making pictures; Part 3
Christina Pallikudiyil	Part 1 Searching literature, background information; Part 4 data collecting
Sirijana Grob	Part 1 Searching literature, background information; Part 3
Stefania Plüss	Part 1 Searching literature, background information
Silvio Müller	Part 3 & 4 data collecting
Léa Pistorio	Part 3 & 4 data collecting
Christina Dössegger	Part 1 Searching literature, background information; Part 2; Part 3; Part 4; Activity list
Samuel Cook	Part 1 Searching literature, background information; Part 2; Part 3; Part 4
Jeannine Hersche	Part 1 Searching literature, background information; Part 2; Part 3; Part 4
Attila Hirschi	Part 2; Part 3; Part 4
Alexandra Siebert	Part 3 data collecting; Layout
Elena Siegrist	Part 2; Part 3; Part 4

Picture 3: Léa, Samuel and Silvio collecting data

Picture 4: Jeannine and Christina conducting the experiment