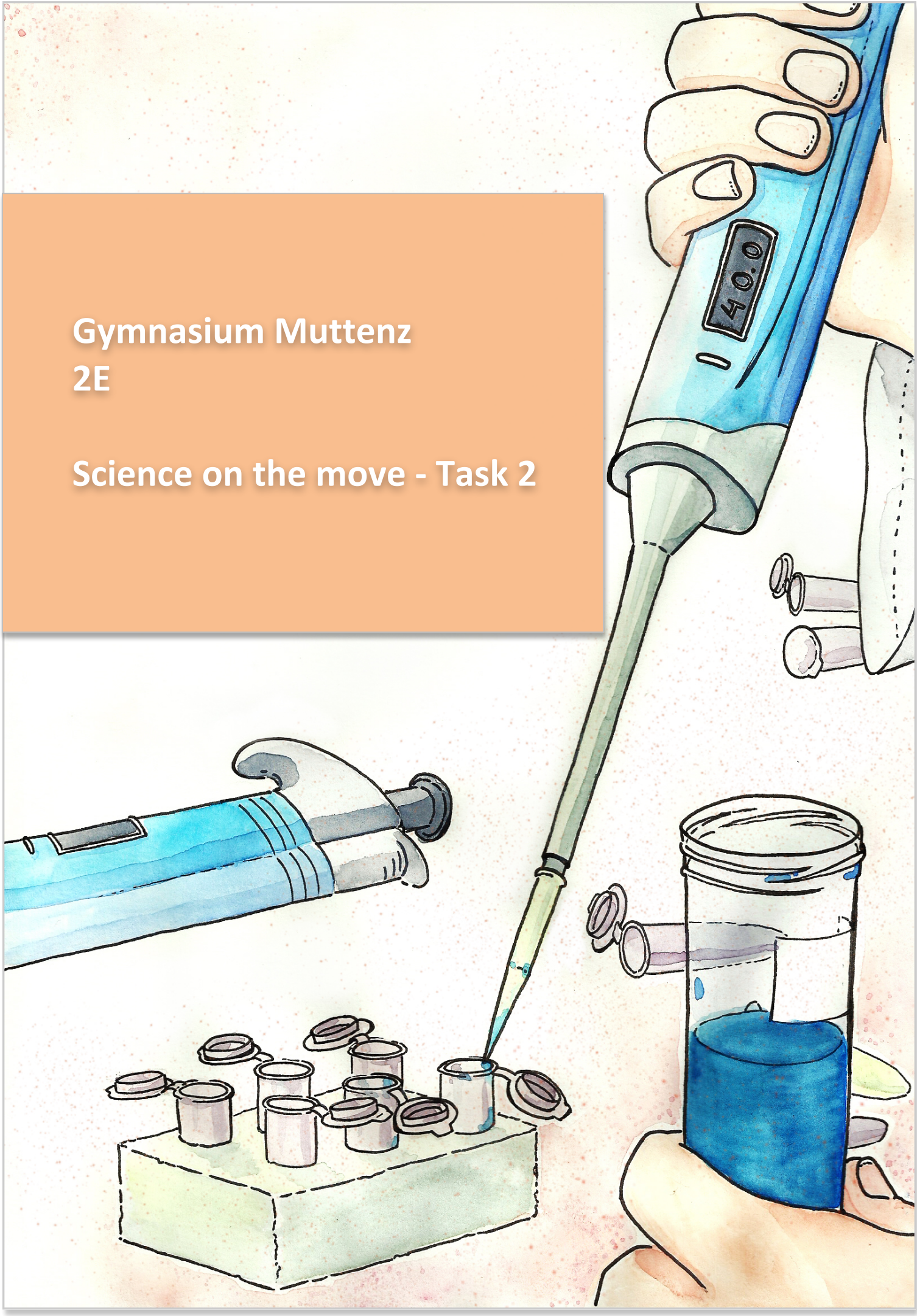
Gymnasium Muttenz
2E

Science on the move - Task 2



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Part 1 - Studying the literature

This task is about two things, which are very different from each other, but still linked together.
The first thing is regular baker`s yeast (lat. Saccharomyces cerevisiae), the other thing is phosphate (PO_4). Phosphates are extremely important for any kind of organism, since they take care of many important functions of the body. For instance, they are very important for the development and building of cell membranes and for higher vertebrates, also for the bone augmentation. But not only that, they are also important for the cell division, since parts of our DNA are made of phosphates, because every nucleotide "owns" a phosphate molecule. An interesting fact is though, that phosphate is also needed for the constant provision of energy[1]. Our body`s energy supplier is ATP, which is the shortening for adenosine triphosphate. As the name suggests, this molecule consists of three very energy-rich phosphate bonds[2]. As a result of this, an organism is constantly in need of enough phosphate to transform ADP into ATP. To ensure a preferably good ability of phosphate uptake, yeast cells have got different membrane proteins, which under several circumstances can uptake phosphate into the cell. Conditions that can influence these proteins are among others also the pH value, glucose concentration and also temperature. As an example for that, there are proteins, which are called transporters, that work best at a pH value of 4.5 and at the same time, there are others that work best at a pH value of 8[3]. This extreme specialization shows well, how much the yeast adjusted, so that it is provided with enough phosphate for the production of energy under many conditions as possible.
More about these factors see the results and discussions.
Yeast cells absorb phosphate through the transporters into their cells, where they get wrapped in vesicles and are carted off. When it is time to save the phosphate in the cell, the "storage vacuoles" take action, in which phosphates are stored in so-called polyphosphate bonds, until they get transported by vesicles to the mitochondria and get transformed into ATP. The phosphate uptake through microorganisms is because of many reasons important for our environment and our society. The most important ones though, are wastewater purification and the battle against eutrophication. Especially in the 80`s, microorganisms were used to filter phosphates out of waste waters to keep them away from lakes. Cause if there is too much phosphate in a lake, it can lead to an eutrophication[4]. An eutrophication is an extreme growth of algae, which can uptake phosphate incredibly fast and use it for their growth, that can lead to a destruction of whole ecosystems. As a consequence of this, a too large algae population can cause suffocations of fish, since algae use a lot of oxygen when they decay. A catastrophe like this can be prevented by microorganisms that function in lakes as natural phosphate regulators, that way not as much phosphate can reach the water. Yeast cells are also often used for research. Being eukaryotes, they actually are much closer to us than bacteria. For this reason we can learn a lot about humans and other eukaryotes by researching the yeast cells' membranes and abilities of uptake.

[1] http://themenpark-umwelt.badenwuerttemberg.de/servlet/is/15982/?viewMode=popupView&TB_iframe=true (2013/5/15)
[2] http://de.wikipedia.org/wiki/ATP (2013/5/15)
[3] RESEARCH ARTICLE: Characterization of the Pho89phosphate transporter by functional hyperexpression in Saccharomyces cerevisiae/ Renata A. Zvyagilskaya et al., first publication online July 2008
[4] http://www.wasser-wissen.de/abwasserlexikon/p/phosphate.htm (2013/5/15)

Part 2 - Calibration of the measuring system

Raw Data:

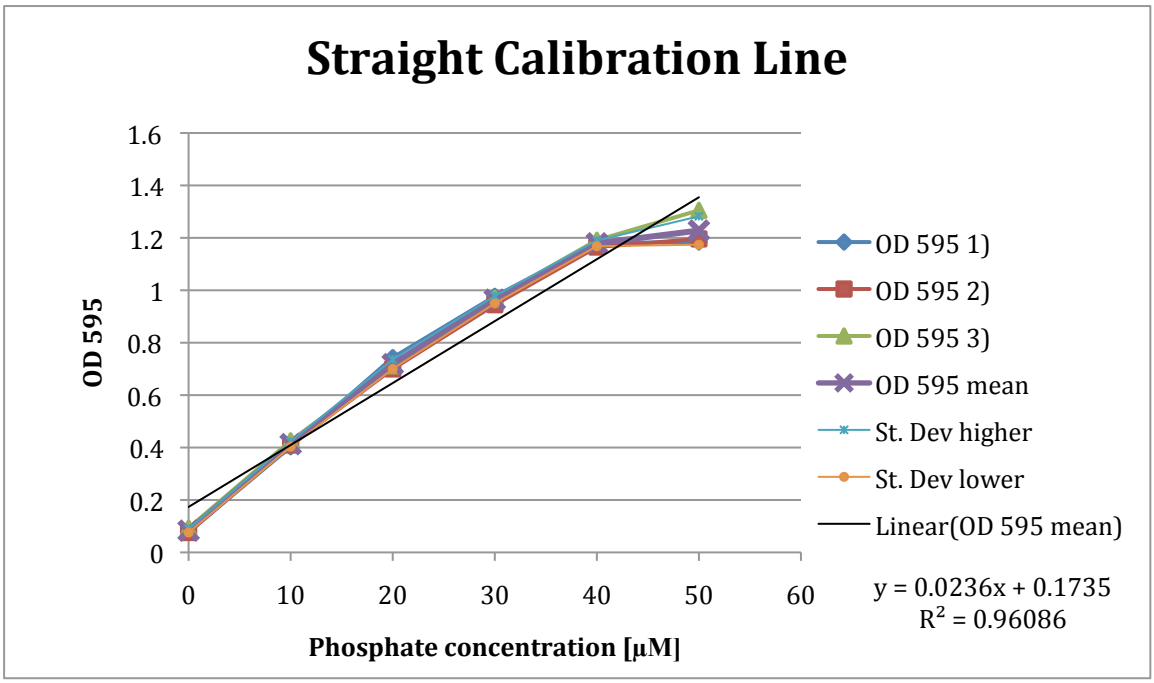
Phosphate concentration [µM]	OD 595
0	0.079
10	0.403
20	0.743
30	0.977
40	1.180
50	1.184

Phosphate concentration [µM]	OD 595
0	0.076
10	0.410
20	0.700
30	0.945
40	1.165
50	1.196

Phosphate concentration [µM]	OD 595
0	0.093
10	0.426
20	0.712
30	0.970
40	1.191
50	1.305

Processed Data:

Phosphate concentration [µM]	Mean OD 595	St.Dev. OD 595
0	0.083	0.007
10	0.413	0.010
20	0.718	0.018
30	0.964	0.014
40	1.179	0.011
50	1.228	0.054



Formula for the calculation of the phosphate concentration:

Phosphate concentration [µM] = (OD 595 - 0.1735) / 0.0236
r-value = 0.9803

Analysis:

There were certain problems we had to face: Inexact pipetting, doing several experiments at the same time, the sensitivity of the spectrometer and the collaboration of many people in one room. We solved our problems with the pipettes as well as the spectometer by doing not only the three required experiments, but the double amount. This was done in order to prevent false results, which can be caused through a little change in the handling of the cuvette, when putting it into the measuring machine. It proved to be necessary, since there was always a ricochet sneaking in.
The parallel working on the experiments increased the risk of mistakes, since one can easily mix up a pipette or an eppendorf-tube. Time forced us to do the experiments at the same time though. Having to coordinate with many other people, all in the same room, had the same effect as the parallel working and furthermore reduced the worker's concentration. We decided to minimize the involved people for the next experiments and to limit ourselves to the amount really needed .For example only one person who writes down the results, not three as in our experiments.
Looking at our graph we notice that the three experiments at the concentration of 0-40 µM are almost identical. However the results concerning the concentration of 50 µM deviate quite a lot. This could have originated from a lack of focusing on the researcher's behalf or because of the decrease of the spectrometer's accurateness. On the other hand experiment 3 could just be a ricochet.

Eppendorf-tubes

Part 3 - Measuring the phosphate uptake by yeast cells

Raw Data:

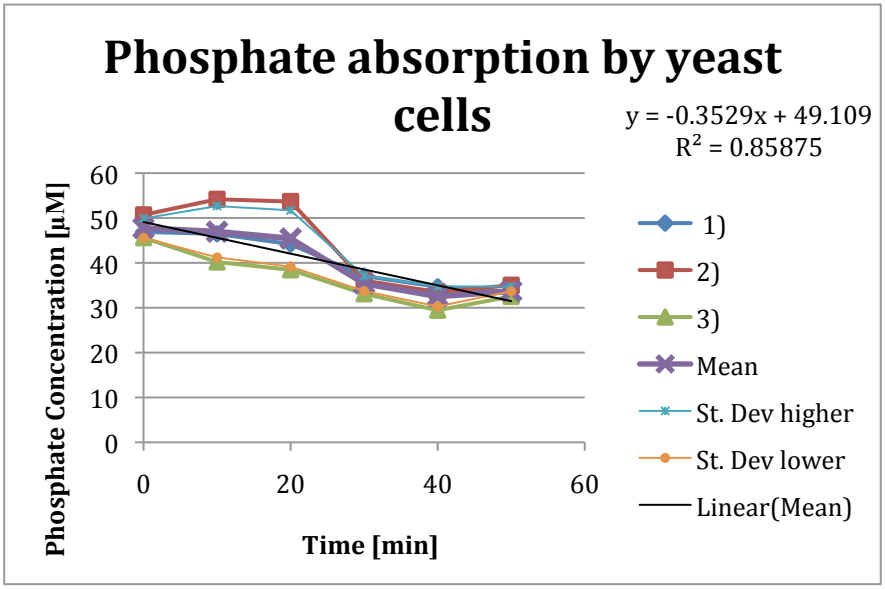
*: Calculated with the formula from part 2
P.C.= Phosphate concentration

Time [min]	OD 595	P.C. [µM]*
0	1.280	46.89
10	1.270	46.46
20	1.216	44.17
30	1.048	37.06
40	0.968	34.67
50	0.964	33.50

Time [min]	OD 595	P.C. [µM]*
0	1.370	50.70
10	1.452	54.17
20	1.440	53.67
30	1.024	36.04
40	0.964	33.50
50	1.000	35.02

Time [min]	Mean P.C. [µM]	St.Dev. P.C. [µM]
0	47.73	2.162
10	46.94	5.717
20	45.43	6.277
30	35.40	1.674
40	32.53	2.246
50	33.68	1.029

Analysis:
Time management was crucial in part 3. It took longer to prepare the stock solution than we've planned. This was because the students who were working on this hadn't realised that they couldn't work with the phosphate-buffer. They had to prepare another stock solution first in order to prepare the "real" stock solution. This was very time consuming.



r-value = 0.9267

Due to this we were only able to the experiment once with three people doing the experiment simultaneously. This is why there are only three results. Nonetheless, two of these three results are very similar as you can see in the graph above. The staining faded rather quickly and because of that we decided to measure it exactly one minute after the Malachite-green was added to the solution. We used the same method earlier in part 2.

At first sight you can see that the phosphate concentration drops after 20 minutes. This appears to be "the last act of uptake of phosphate" before an equilibrium is formed. It is also possible that the drop of the mean graph was caused by measurement errors which occurred in graph 2. Measurement errors are most likely the cause because the other two graphs are much flatter. Also, the majority of phosphate was absorbed by the yeast cells only seconds after it had been added (before 0min in the graph). The phosphate concentration was 0.5 mM and the highest value we've measured was only 54 µM.

Part 4 A - Temperature

Scientific question:
How much can the uptake of phosphate of yeast cells be improved by raising the temperature (*Saccharomyces cerevisiae*)?

Hypothesis:
The literature we have studied makes us assume that temperature only has a minor effect on the yeast cells' ability to take up phosphate. We figure the yeast cells absorb the most phosphate when they are in an environment which has a temperature of 30°C. 30°C is the optimal temperature for our experiment, we think, because it is just in between the optimal fermentation temperature (32°C) and the optimal reproduction temperature (28°C). Temperatures much higher or much lower will harm the cells' ability to take up phosphate and would eventually kill them.

Materials:
- Eppendorf-tubes
- Pipettes
- Cuvettes
- Malachitgreen-solution
- Molybdat-solution
- Sodium-Phosphate-Buffer
- 0.1M, pH 6.3, 40ml
- Distilled water
- Spectrometer

Variables :
The variables are:
amount of yeast cells, amount of water, temperature, light intensity, uptake of phosphate

The amount of yeast cells and the amount of water are the controlled variables.
The independent variable is the temperature.
The dependent variable is the uptake of phosphate.

Raw Data:

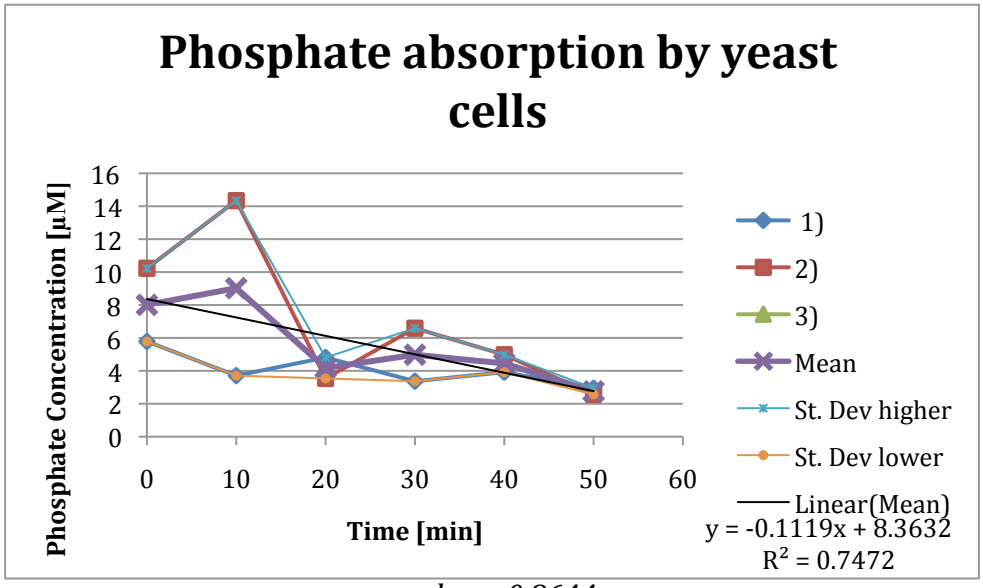
*: Calculated with the formula from part 2
P.C.= Phosphate concentration

Time [Min]	OD 595	P.C. [µM]*
0	0.310	5.784
10	0.261	3.708
20	0.287	4.809
30	0.253	3.369
40	0.266	3.919
50	0.243	2.945

Time [Min]	OD 595	P.C. [µM]*
0	0.415	10.23
10	0.512	14.34
20	0.257	3.538
30	0.329	6.589
40	0.291	4.979
50	0.234	2.564

Processed Data:

Time [min]	Mean P.C. [µM]	St.Dev. P.C. [µM]
0	8.007	2.223
10	9.024	5.316
20	4.1735	0.6355
30	4.979	1.61
40	4.449	0.53
50	2.7545	0.1905



r-value = 0.8644

Conclusion:
At first sight it doesn't look like it, but there clearly is a faster phosphate uptake, when working with higher temperatures than room temperatures. However, this happens already before the 0min-meassuring, namely in the first few seconds after adding the yeast. This happens because we first heated the solution on 30 degrees and then added the Yeast. We think the different values between the six measured times can be explained with the adjusting or already adjusted chemical balance between two different sorts of phosphates that can be more or less easy to uptake by yeast cells (Na_2HPO_4 and NaH_2PO_4). We are not sure whether or not our assumption about this is true. Sure is that we were able to improve the phosphate with help of the temperature.

Part 4 B - Glucose

Question:
How can the phosphate uptake of yeast cells be increased by enhancing the glucose concentration?

Hypothesis:
Before starting with our experiment, first of all we determined the pH value of our yeast solution and got a pH between about 4 and 4.5. During the Internet researches[i], we were able to figure out that the amount of open phosphate channels in the membrane depends on the glucose concentration and on the pH value of the medium. For our experiment we omitted to change the pH value as well because otherwise we wouldn't have been able to say whether the improvement of uptake is caused by the pH or by the higher glucose concentration. Through the internet research we figured out, that, for a pH-value of 4.5 , a concentration of 4% the minimum and 8% the maximum are to open absolutely all channel proteins, so we decided to go for the concentration of 6% glucose and assume now a significantly increase of phosphate uptake.

Materials:
- Eppendorf tubes
- Pipettes
- Cuvettes
- Malachite green – solution
- Sodium – Phosphate – Buffer 0.1M, pH 6.3, 40ml
- Distilled water
- Spectrometer
- Glucose

Controlled Variables:
- Temperature (room temperature)
- Amount of yeast cells (10g)
- Amount of stock solution (100ml)

Independent Variable:
- Glucose concentration 6% -> 6g in 100ml)

Dependent Variable:
- Phosphate uptake

Raw Data:

*: Calculated with the formula from part 2
P.C.= Phosphate concentration

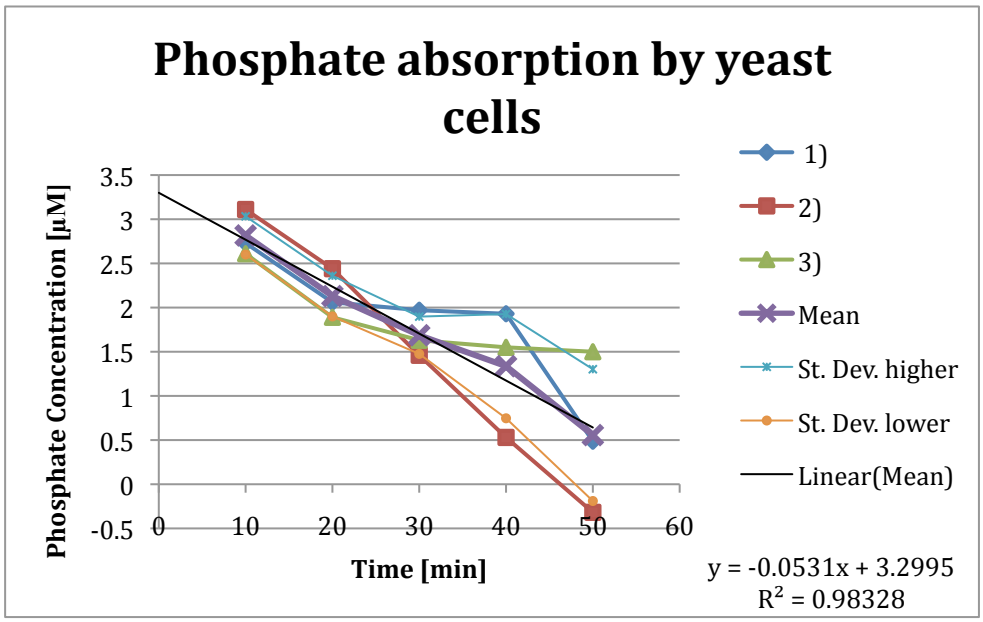
Time [min]	OD 595	P.C. [µM]*
0		
10	0.238	2.73
20	0.222	2.06
30	0.220	1.97
40	0.219	1.93
50	0.185	0.487

Time [min]	OD 595	P.C. [µM]*
0		
10	0.247	3.11
20	0.231	2.44
30	0.208	1.46
40	0.186	0.530
50	0.166	-0.318

Time [min]	OD 595	P.C. [µM]*
0		
10	0.235	2.61
20	0.218	1.89
30	0.212	1.63
40	0.210	1.55
50	0.209	1.50

Processed Data:

Time [min]	Mean P.C. [µM]	St.Dev. P.C. [µM]
0		
10	2.82	0.216
20	2.13	0.230
30	1.69	0.211
40	1.33	0.590
50	0.56	0.746



r-value: 0.9916

Conclusion:

During this experiment, recognizable is a clear downward trend, which however doesn`t turn out as strongly as the other one in Part 3 (-0.0531 in Part 4B, -0.3529 in Part 3). In return, the measured peak value of the phosphate concentration is only a fractional amount if you compare it with the peak value of Part 3.

This means, that especially during the first section (during the first few seconds, before the measuring with 0 minutes) was absorbed an extremely large amount of phosphate. That way also the increase of glucose concentration can be seen as a success. We assume that the reason why this uptake happened in the first few seconds is, that the yeast cells recognized, right after they were put into the solution, that they are under perfect glucose conditions and so open really absolute every transport protein, because of that they literally sucked up as much of their environment as possible.

A pretty confusing value exists though, looking at the result number 2) 50 min. It is below 0, even though the water can`t possibly have a negative P.C. This result may be explained by the equation from Part 2, which originates from our measurement data with the error in it. Anyhow, the result number 2) 50 min. is still above the average zero value, that was calculated for the Straight Calibration Curve (OD 595 = 0.083, P.C. calculated with our formula = -3.83 µM).

Evaluation:
Finally it worked with the time! In this third attempt (after Part 3 and 4A) for the first time we were able to carry out two experiment series (with three parallels) in a row, which pleased us with six results. These results though, were desperately needed, because three of the experiments were failed attempts. The other three results fitted perfectly. The only problem was that there was found a mistake during the measurement of the 0-value. The mistake was, that the pipettes were mixed up, which lead to distorted concentrations. Therefore, there are no 0-values presented in the conclusion above. The problem in fact was that the people weren't concentrated enough after the hard school day, after which the experiment had to be accomplished. Another reason could also be lack of refreshments between the experiment series.

Improvement:
The issue of the ability to concentrate is hard to fix. One option would be, having longer breaks, but this on the other hand would extend the working hours. This would mean that it could only be completed one single experiment series during the available time. Since the school day can`t be abolished, there is only one way to manage it with the ability to concentrate, which means leaving the working room and eat a quick snack, of course without forgetting to wash the hands.

Part 4 C - Shaking

Hypotheses:
Our Hypothesis is that we will be able to higher the phosphate uptake of yeast cells also by shaking the cells all the way through the experiment because this is used by all researchers that work with yeast cells. The reason why this works is because of the shaking more yeast cells come to the surface of the water and get access to oxygen and also more oxygen gets into the water for the other cells. Oxygen is very important for yeast cell's cell respiration, where glucose gets split up through oxidation to produce ATP.

Materials:
- Eppendorf-tubes
- Pipettes
- Cuvettes
- Malachitgreen-solution
- Molybdat-solution
- Sodium-Phosphate-Buffer 0.1M, pH 6.3, 40ml
- Distilled water
- Spectrometer
- Shaker

Variables :
The independent variable is the intensity of shaking
The dependent variable is the efficiency of the phosphate-uptake
The controlled variables is the concentration of the buffered solution and the temperature
We held the independent variable constant with a machine, in which we put the solution.

Raw Data:

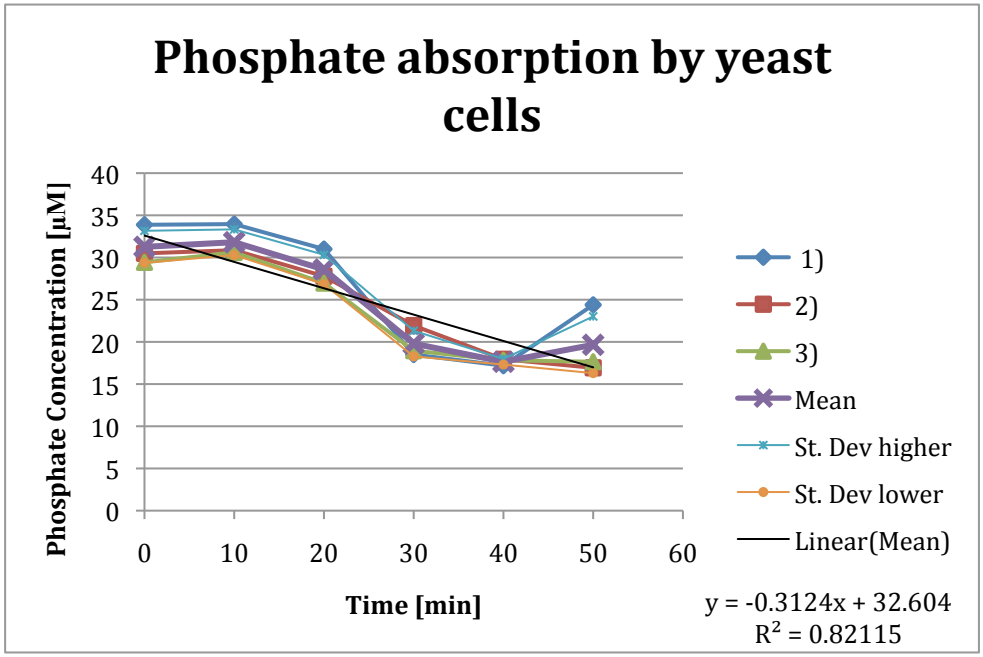
Time [min]	OD 595	P.C. [µM]*
0	0.973	33.88
10	0.975	33.96
20	0.905	31.00
30	0.610	18.50
40	0.579	17.18
50	0.749	24.39

Time [min]	OD 595	P.C. [µM]*
0	0.893	30.49
10	0.902	30.87
20	0.829	27.78
30	0.691	21.93
40	0.597	17.94
50	0.573	16.93

*: Calculated with the formula from part 2
P.C.= Phosphate concentration

Processed Data:

Time [min]	Mean P.C. [µM]	St.Dev. P.C. [µM]
0	31.26	1.898
10	31.81	1.522
20	28.58	1.739
30	19.80	1.520
40	17.65	0.334
50	19.66	3.359



R-value = 0.9061

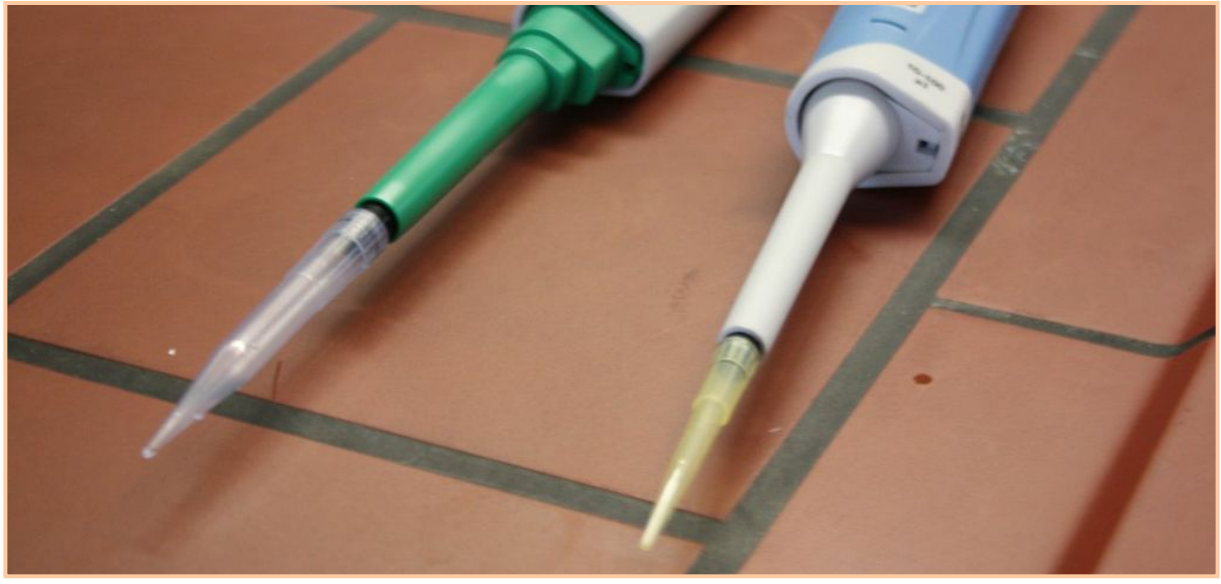
Conclusion:
The straight line looks as we've expected it to look like. It's very similar to the one from part 3 but it starts at a lower concentration. This means that shaking the solution has a positive effect on the uptake of phosphate of the yeast cells although the effect is smaller than the one achieved using the other two methods (part 4A and 4B). The variables which are usually changed are temperature, pH-value or concentration of the components but like said assumed above and proved in the experiment shaking the solution is still a good method for yeast cells because like that the yeast cells are more often on the surface and can therefore more easily access oxygen which is in the air. Due to the shaking there is also more oxygen in the water.

The concentration increases slightly at certain points in the experiment but this is caused by small measurement errors. An interesting aspect is also that the steep part of the curve is between 20 and 30 minutes, just like in part 3. Thus we conclude that we must not necessarily have made measurement errors during part 3 but that this is a real phenomenon. Our hypothesis is that the yeast cells need about 20 minutes to consume the phosphate and store it as polyphosphates in the storage vacuoles. After that they can take up more phosphate again. To prove this hypothesis we would have to do further measurements to see whether we were right or not.

Evaluation:
The ability to concentrate as a student is very important in an experiment like that. Unfortunately it is quite a challenge to remain concentrated at all times during the experiment if you had to be concentrated all day during lectures in school. Due to this we had to leave out two out of five results because there were made too many errors.
The planning of task 2 was remarkably better than the planning of task 1. This was necessary because task 2 was a lot more time consuming than task 1 (see activity list). Many of our conclusions are based on assumptions and some based on research. One weakness which still remained was that we simply didn't have enough time to get enough data to make a solid statistic to prove our hypothesis.

Improvement:
The only way to improve the ability to concentrate of the workers is to make them interested in the matter and to motivate them. They need to know what they are doing and why they are doing it. This will motivate them to work precisely and efficiently.
In order to gave more data we'd have to do more experiments simultaneously. To do this we'd need more people who are doing the experiments and also more rooms which we can work in.

Evaluation and Improvements for the future:
We think the whole team learned a lot from the task and we are sure we could do a lot more about this theme if we had more time. For instance we would like to repeat all the experiments to prove whether they really worked or not we also thought about some experiments we would like to do but that cost way too much time. An example would be that we want to change the pH-value to look whether we can higher the phosphate uptake by this but for that we would have to do way more control experiments to figure out how we can change the pH without destroy the right living conditions for the yeast cells and without distort the result measured by the spectrometer.

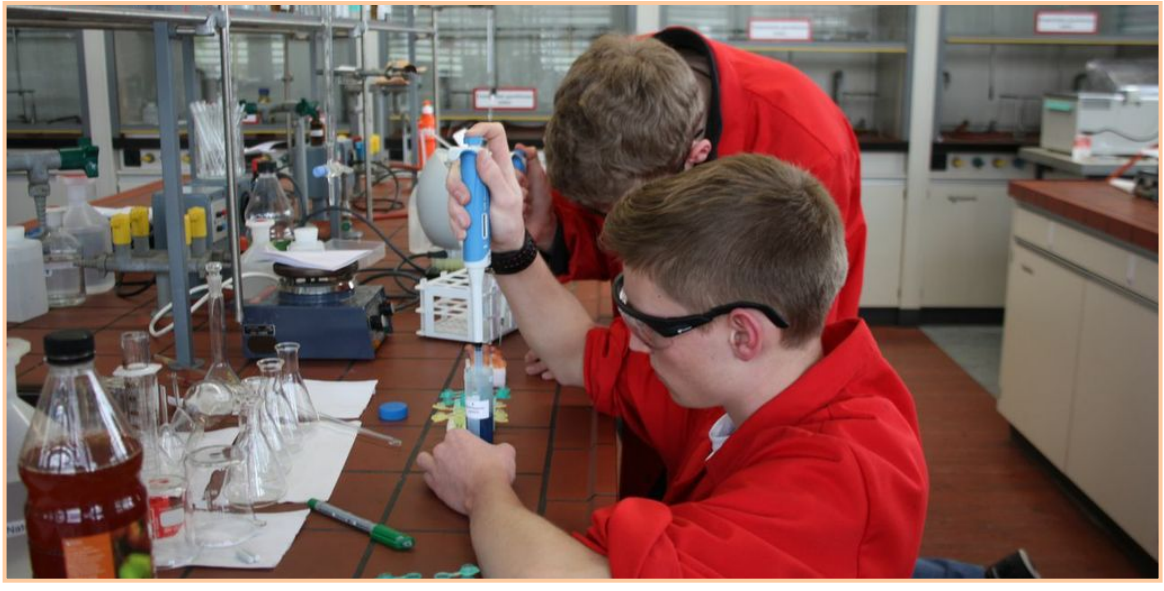


Pipettes

Reference List

1) http://themenpark-umwelt.badenwuerttemberg.de/servlet/is/15982/?viewMode=popupView&TB_iframe=true (2013/5/15)
2) http://de.wikipedia.org/wiki/ATP (2013/5/15)
3) RESEARCH ARTICLE: Characterization of the Pho89phosphate transporter by functional hyperexpression in Saccharomyces cerevisiae/ Renata A. Zvyagilskaya et al., first publication online July 2008
4) http://www.wasser-wissen.de/abwasserlexikon/p/phosphate.htm (2013/5/15)

Class 2E working



Activity list

Name	Work at school	Work at home	Total time
David **Project leader**	Assistant	Organisation Meetings with Mrs Bandi Evaluation of experiments Writer: Wrote Part 2, 3, 4A, 4B, 4C	35h
Mitchell **Research leader**	Pipette, Assistant	Research on experiment Meetings with Mrs Bandi Writer: Part 1 Text meeting Experiment design meeting	35h
Zoe **Layout leader**	Drawings, Assistant	Layout frontpage drawing	10h
Sabryna	Drawings	Layout	6.5h
Tobias	Pipette	Research on experiment	19h
Dennis	Pipette	-	15h
Dina	Pipette	-	10h
Roger	Spectrometer	Acitivity List	14h
Sandra	Spectrometer	-	11.5h
Barbara	Results	Layout	8h
Vanessa	Results, Pictures	-	9h
Andrea	Results, Pictures	Research on experiment	6h
Elisa	Results, Assistant	-	8h
Samuel	Assistant	-	6h
Raphael	Assistant	Research on experiment	4h
Michael	Assistant	Translator: Translated Part 3, 4B, 4C	11h
Christina	Assistant	Translator: Translated Part 2, 4A, 4B	8h
Imerio	Pictures	Research on experiment	4.5h
Rebecca	Pictures	Research on experiment	4.5h
Julia	Protocol	Research on experiment	5h
Jenny	Protocol	Translator: Translated Part 1	8h
Carmen	-	Research on experiment Translator: Translated Part 2	3h