SIMPLY science

SIMPLYSCIENCE.CH

Gymnasium Münchenstein

Class 2MRSWaWb

(Future Scientists)

# Task 2

**Table of contents:**

## Problems concerning the measurement

During the course of the first experiment, we soon noticed, that something had to be wrong.

Instead of the expected calibration line, we had very diversified results. Through all of the experiments there were variations, we had not expected. None of the experiments had results that were satisfying to us. First of all the pretest failed, also after the 3rd attempt. The calibration line was non-existent. Also the concentration-gradient of the yeast-uptake declined and there was a graph which was not understandable for us. . Therefore we searched the problem before starting our own experiments in part 4. Because we had spent a huge amount of time and repeated the experiments many times on different days and also with different people , we could be sure that we didn't have any technical problems ourselves. Also the photo spectrometer was well prepared and the software correctly installed. We controlled our spectrometer also in a control experiment with another pigment.

Because all of those experiments, of which not only one of them went wrong, we expected a basic problem.

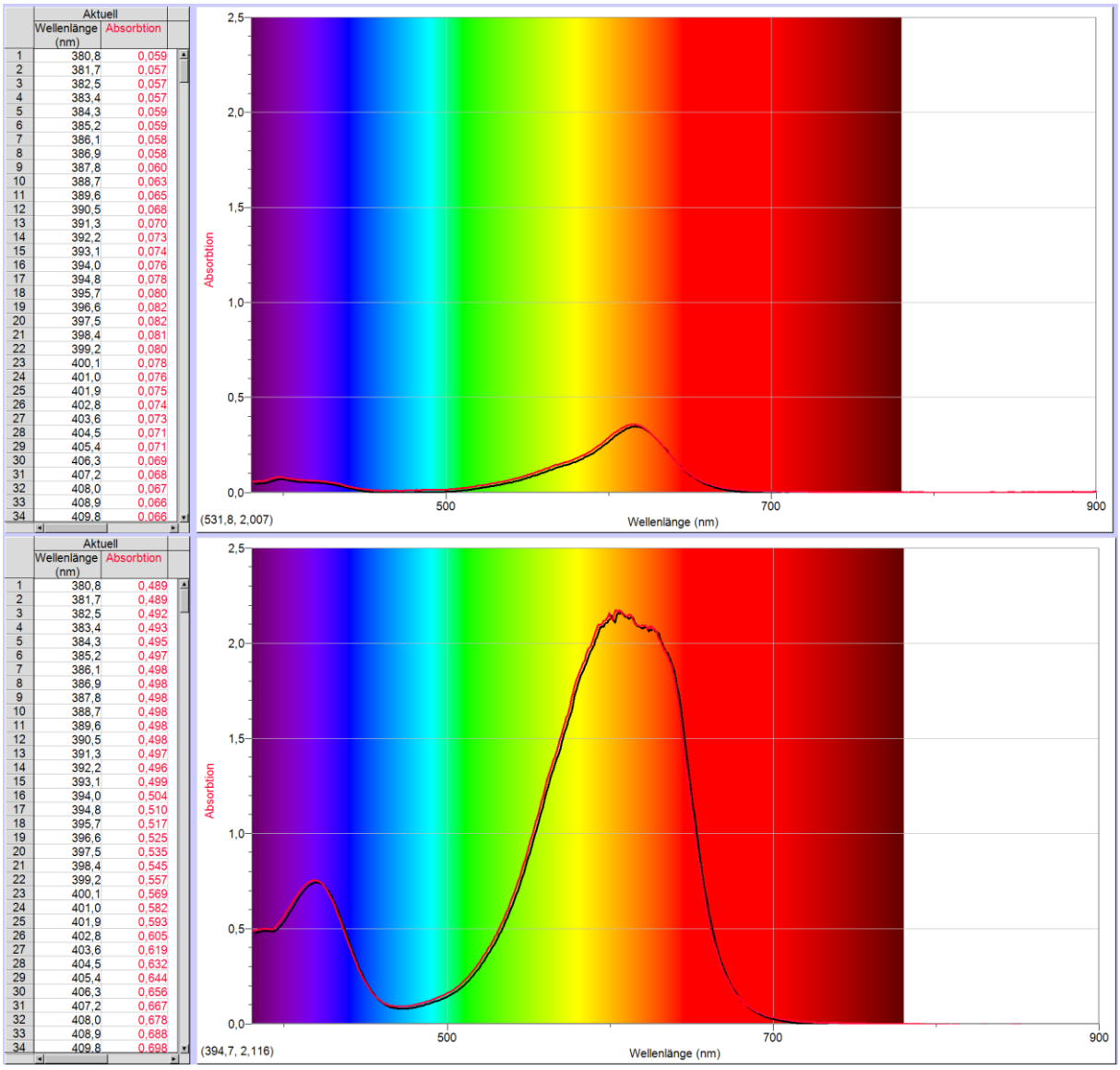
Another class of the Gymnasium Münchenstein is taking part in the competition and we compared our chemicals with theirs. Obviously our malachite -green solution wasn't as dark as theirs (see photo). That would also explain why in experiment 3 the curve wasn't fitting our hypothesis in the end. When our solution includes much less pigments than a "normal one", it is logical, that a phosphate uptake can't be shown later in the experiment. It could be that all pigments are uptaken through the reaction with the phosphate. That would explain why the graph from experiment 3 didn't rise in the end, or declined.
We understand the process which we investigate. The malachite -green shows us the concentration of phosphate in the solution and therefore shows also the phosphate uptake and variation in the solution with yeast. But when our malachite - green isn't representive for this whole process we fast realized that all the experiments have results which are wrong.
As a proof for this difference of our malachite -green solution we took pictures (Fig. 1) of our and the other's class solution and also checked both in the photo spectrometer:


Fig. 1

We didn’t dilute the solution (we would have needed a lot of water to dilute the malachite green so much). Another observation: There is a precipitation in the solution. Therefore we sent a message to simply science, and our teacher, Mister Dunant, spoke to one of the scientists of simply science. But producing a new solution takes a long time and so we had to do the experiments with this failing Malachtigreen.  All the following raw data and results are probably false or are estimated values.

Nevertheless we tried to write conclusions, not knowing what the results really are, for example in part 4.

For us that is a big problem. We could not find an own mistake and we spend a lot of time and energy doing these experiments and repeating them many times.



| | Aktuell | |
|---|---|---|
| | Wellenlänge (nm) | Absorbtion |
| 1 | 380,8 | 0,059 |
| 2 | 381,7 | 0,057 |
| 3 | 382,5 | 0,057 |
| 4 | 383,4 | 0,057 |
| 5 | 384,3 | 0,059 |
| 6 | 385,2 | 0,059 |
| 7 | 386,1 | 0,058 |
| 8 | 386,9 | 0,058 |
| 9 | 387,8 | 0,060 |
| 10 | 388,7 | 0,063 |
| 11 | 389,6 | 0,065 |
| 12 | 390,5 | 0,068 |
| 13 | 391,3 | 0,070 |
| 14 | 392,2 | 0,073 |
| 15 | 393,1 | 0,074 |
| 16 | 394,0 | 0,076 |
| 17 | 394,8 | 0,078 |
| 18 | 395,7 | 0,080 |
| 19 | 396,6 | 0,082 |
| 20 | 397,5 | 0,082 |
| 21 | 398,4 | 0,081 |
| 22 | 399,2 | 0,080 |
| 23 | 400,1 | 0,078 |
| 24 | 401,0 | 0,076 |
| 25 | 401,9 | 0,075 |
| 26 | 402,8 | 0,074 |
| 27 | 403,6 | 0,073 |
| 28 | 404,5 | 0,071 |
| 29 | 405,4 | 0,071 |
| 30 | 406,3 | 0,069 |
| 31 | 407,2 | 0,068 |
| 32 | 408,0 | 0,067 |
| 33 | 408,9 | 0,066 |
| 34 | 409,8 | 0,066 |



| | Aktuell | |
|---|---|---|
| | Wellenlänge (nm) | Absorbtion |
| 1 | 380,8 | 0,489 |
| 2 | 381,7 | 0,489 |
| 3 | 382,5 | 0,492 |
| 4 | 383,4 | 0,493 |
| 5 | 384,3 | 0,495 |
| 6 | 385,2 | 0,497 |
| 7 | 386,1 | 0,498 |
| 8 | 386,9 | 0,498 |
| 9 | 387,8 | 0,498 |
| 10 | 388,7 | 0,498 |
| 11 | 389,6 | 0,498 |
| 12 | 390,5 | 0,498 |
| 13 | 391,3 | 0,497 |
| 14 | 392,2 | 0,496 |
| 15 | 393,1 | 0,499 |
| 16 | 394,0 | 0,504 |
| 17 | 394,8 | 0,510 |
| 18 | 395,7 | 0,517 |
| 19 | 396,6 | 0,525 |
| 20 | 397,5 | 0,535 |
| 21 | 398,4 | 0,545 |
| 22 | 399,2 | 0,557 |
| 23 | 400,1 | 0,569 |
| 24 | 401,0 | 0,582 |
| 25 | 401,9 | 0,593 |
| 26 | 402,8 | 0,605 |
| 27 | 403,6 | 0,619 |
| 28 | 404,5 | 0,632 |
| 29 | 405,4 | 0,644 |
| 30 | 406,3 | 0,656 |
| 31 | 407,2 | 0,667 |
| 32 | 408,0 | 0,678 |
| 33 | 408,9 | 0,688 |
| 34 | 409,8 | 0,698 |

## Part 1: Studying the literature

Phosphates are salts of phosphoric acids. They are made of one phosphorus atom and four oxygen atoms [(1)].It is so important due to its variety of combinations. For example adenosine triphosphate (ATP) which is needed in the body for energy transport [(2)]. Phosphate is also used for cell division and as building block for bones [(3)].
It is an important nutrient for plants as well, that's why fertilizers contain phosphates [(4)].

We know about the uptake of phosphate by yeast cells that the optimum pH value is 6,5. The uptake takes place when there is a glucose assimilation, which is the reason why there is glucose in the medium needed [(5)].

Though with certain substances, it isn't absolutely necessary to have a sugary medium [(6)]. If yeast cells starve, their phosphate uptake capacity increases [(7)].

There is a difference of the uptake level under aerobic and anaerobic conditions [(8)].

Once Phosphate has been taken up by the yeast cell, it has to be stored somewhere. A major storage compartment is the vacuole of the yeast cell. Large amounts of phosphate and polyphosphate can be stored there [(9)]. Polyphosphate are linear polymers of phosphate in anhydrous linkage [(10)].The intracellular concentration of free phosphate is generally maintained at very low levels, except for when yeast cells switch from respiratory to fermentative metabolism [(11)].

Many microorganisms release some Enzymes into the ground, which hydrolyse the organic phosphorlipidester and release inorganic phosphate, which can be taken up and metabolised by the other organisms. These enzymes are called phosphatases [(9)].

The phosphate uptake by microorganisms can be quite problematic for our environment, because it leads to eutrophication of waters [(12)]. That means that if there is a too high amount of phosphate, there is an excessive growth of algae [(13)]. When the algae die and decompose, high levels of organic matter and the decomposing organisms deplete the water of available oxygen, causing the death of other organisms, such as fish [(14)]. The high amount of phosphate gets mostly through sewage water and manuring of the ground into the waters and is caused by our society [(15)].

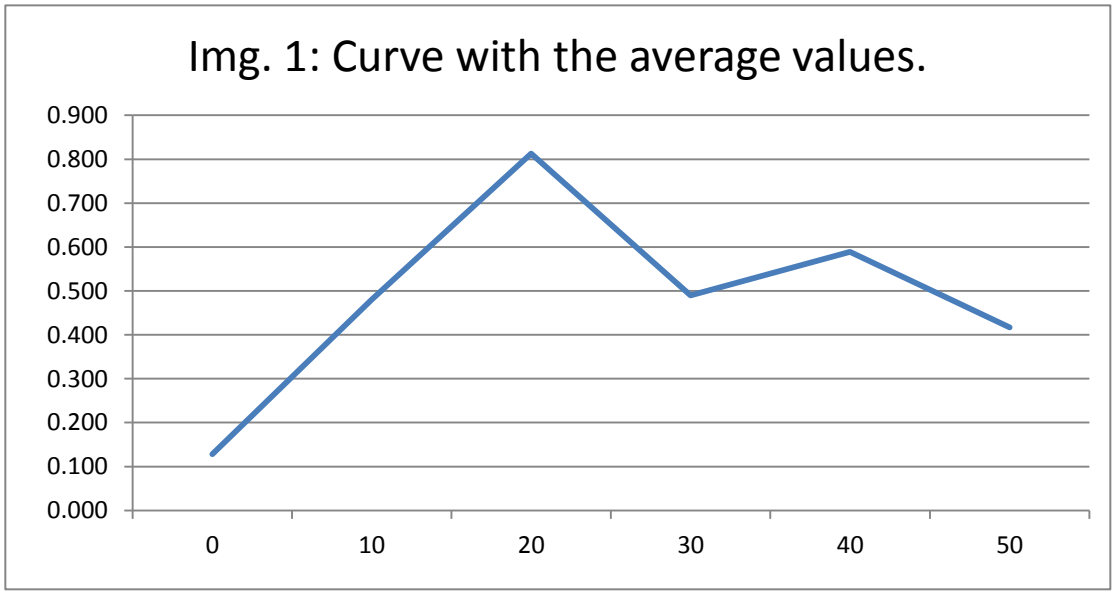
## Part 2: Calibration of the measuring system

Doing the pretest was easy, hence why it turned out well.
But when we tried to get a straight calibration line, we failed. We first thought that we didn't pipe well enough. Though even after giving it another shot, our attempts were in vain. As written in the problems concerning the measurement part, we did a carefully performed problem shooting and controlled everything. Additionally, we made sure the times between the measurements are always the same timed to a few seconds. But we couldn't figure out why it went wrong, until we finally found out that the problem is most likely connected to the chemicals. Therefore our results are now really strange.

Some Results:

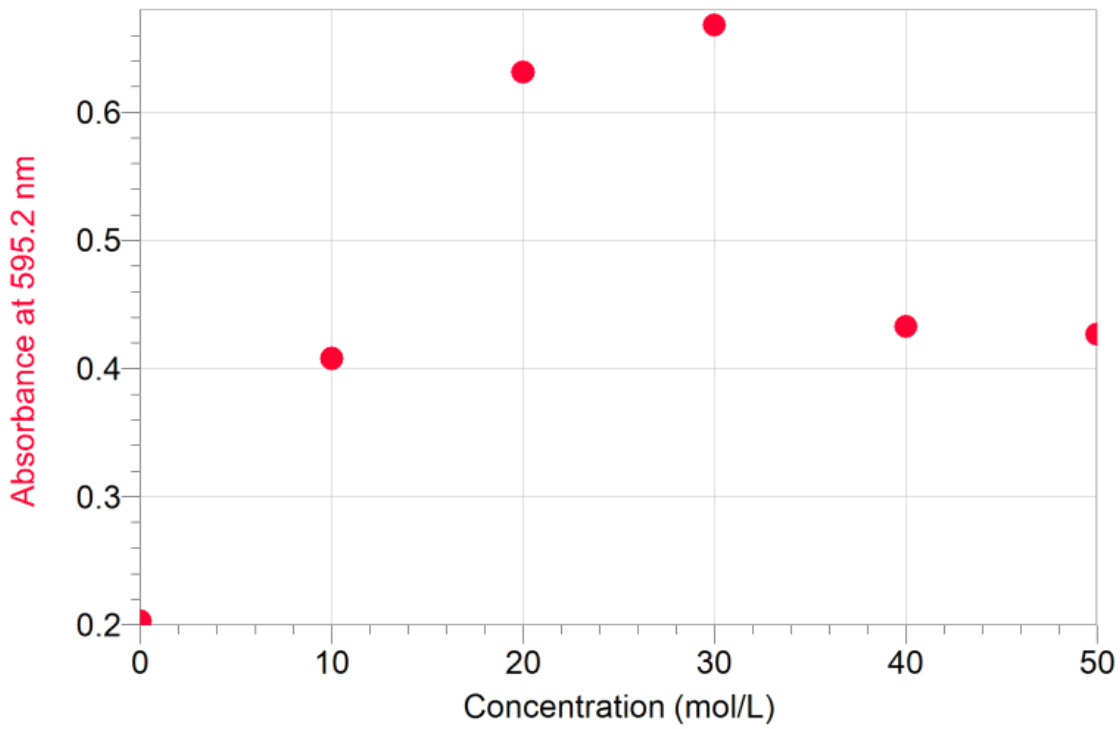
| Concentration | Trial #1 | Trial #2 | Trial #3 | Trial #4 | Trial #5 | Average |
|---|---|---|---|---|---|---|
| **0** | 2.075 | 0.138 | 0.203 | -0.006 | 0.178 | 0.128 |
| **10** | 2.231 | 0.423 | 0.408 | 0.693 | 0.395 | 0.480 |
| **20** | 2.095 | 0.611 | 0.631 | 0.351 | 1.659 | 0.813 |
| **30** | 2.108 | 0.609 | 0.668 | 0.012 | 0.671 | 0.490 |
| **40** | 2.080 | 0.549 | 0.433 | 0.082 | 1.291 | 0.589 |
| **50** | 2.111 | 0.475 | 0.427 | 0.082 | 0.686 | 0.417 |

The "best" measurements were achieved by trials #2, #3 and #5.
Trial #1 seems to be somehow corrupted (Maybe bad calibrating), but has a rather nice curve.
The "average column" excludes trial #1, because he is significantly higher than the rest.



The beginning of the curves always seems to be better than the rest. And it always looks similar. However, we suppose that the rest is rather wrong. The acclivity of the graph from point 0 - 20 is r= 0.04065.
Maybe the false chemical corrupted at the end. Another explanation is that the colour reaction, despite intensive shaking, wasn't finished at the last values. But then the cure should at least stay on its level and not go down. And it also wouldn't explain the strange up and down of some of the curves.

Although it was written that there can be exceptions where such a measurement doesn´t provide a straight line, we doubt that this is the case here. Since the part of the phosphate in the solution has always been increased by the same amount, we would also expect that the amount compounded, which arises during the reaction, is proportional as well. Nevertheless, our measurements could not confirm that. As said, our measurements are most likely false due to the corrupt chemicals.




**Img. 2: A typical measurement**

## Part 3: Measuring the phosphate uptake by yeast cells

### 3A Preparing the yeast solutions picking up phosphate over time

Material: See handout of “simply science task 2” on page 6

Methods: For the dilution of Sodium-Phosphate Buffer from 0.1 M to 0.5mM the following calculation was done:
0.5mM = 0.0005 mol/L -> the concentration at the beginning is 0.1 mol/L and for 0.5mM distilled water has to be given 0.1 M/0.0005 M = 200 that means that the solution has to be 1 to 200 diluted. For 1 ml Sodium-Phosphate Buffer 200 ml Water has to be given. But the solution has to be 10 ml. If for 1 ml -> 200ml then for 10ml -> 10/200 = 0.05 ml Sodium-Phosphate Buffer.
0.05 ml – 10 ml = the amount water to be given -> 9.5 ml distilled water.
For glucose 0.1 g was needed. Calculation 1g = 1ml, 10g accords the 100% of the whole solution and how much is 1%? 10/100 = 0.1
For our experiment we gave 0.005ml to the solution instead of 0.05ml.

### 3B Measuring the decline of phosphate in a well defined yeast solution

Methods: See handout of “simply science task 2” on page 7

Expectation: The data of the yeast uptake decreases.

Discussion: Because of some problems with the malachite- green solution in other experiments we are careful with our results in how far they are correct. If we look at the absorbance curve it seems the measurement point at 30 is wrong. Because without this point it would be possible to create more or less a curve and our expectation would be right. So it is most likely that this point is wrong.
At the beginning the yeast takes up a lot of phosphate. With the time there is less phosphate which means that the yeast takes constantly less phosphate up. In the graphic it is clearly visible that the curve decreases because there is less phosphate. The glucose which is mixed to the yeast helps that metabolism can continuously take place and ATP can be produced. The acid in the Malachtigreen-solution makes it much easier to detect the absorption of phosphate by yeast.

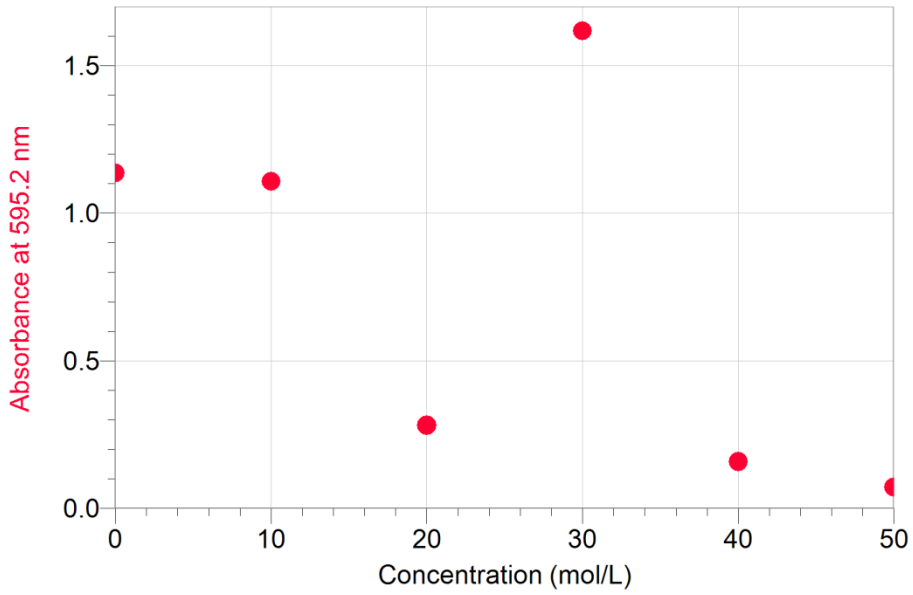



**Figure 2:**

**Absorbance curve**

## Part 4: How to improve the phosphate uptake by yeast cells

### 4A Increasing the phosphate by increasing the temperature

Step1: Design of Experiment

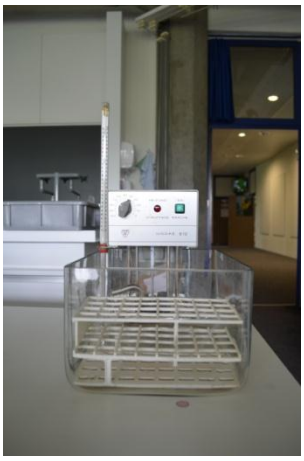
*Aspect 1:*

The goal of our experiment is to increase the phosphate uptake by increasing the temperature to 30°C and 40°C. We will measure the phosphate uptake increase in relation to the temperature increase.

*Aspect 2 & 3:*

Used materials

- Two water bathes
- 16 tubes
- Two Erlenmeyer flasks
- Fresh yeast
- Sugar=glucose
- Water
- Phosphate, c=1mol/L



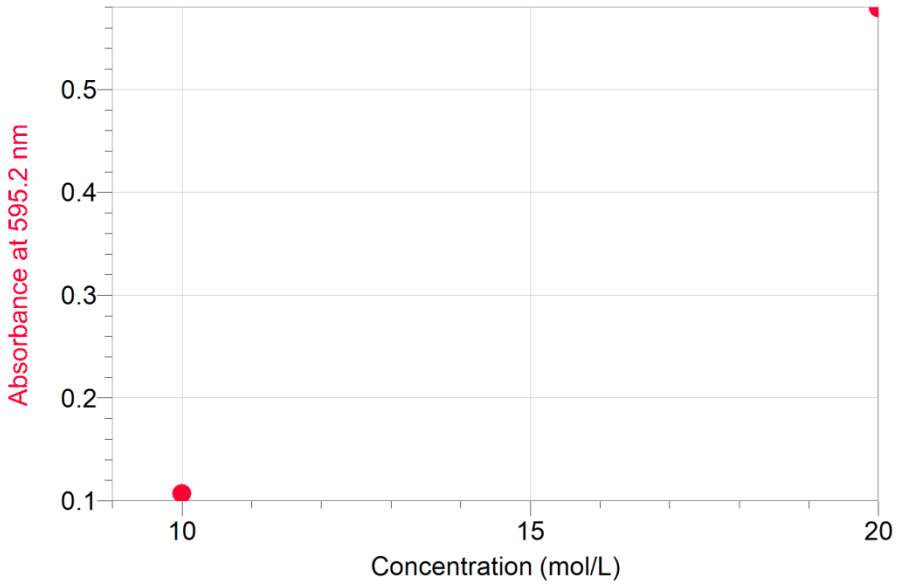
**Picture of our water bath Haake E12**

First, we had to install the two water bathes and start heating them up. During the waiting time we prepared our probes. We mixed 1g fresh yeast and 0.1g glucose with 9.995ml water in an Erlenmeyer flask. We repeated this procedure once and got two similar probes for the two different temperatures. We worked with the 30°C hot bath and the 40°C hot bath parallel. After heating up the water and adding the Erlenmeyer flask with the yeast-sugar-water solution, we waited another 10 minutes to make sure that our probes did adapt to the given temperature. After these 10 minutes we added 5µl liquid phosphate to each solution and reset the timer. Since we wanted to compare our results to the ones from our fellow colleagues, we did take the exact same amount of phosphate though it is instructed differently in the handout. We then proceeded to take two probes of each solution every 10 minutes. Sadly we had to end the experiment after 30 minutes due to a lack of time. Then we followed the exact instructions of the experiments 3A and 3B. Because we couldn't measure the phosphate uptake the given day, we froze all of our probes and measured the phosphate uptake a week later.

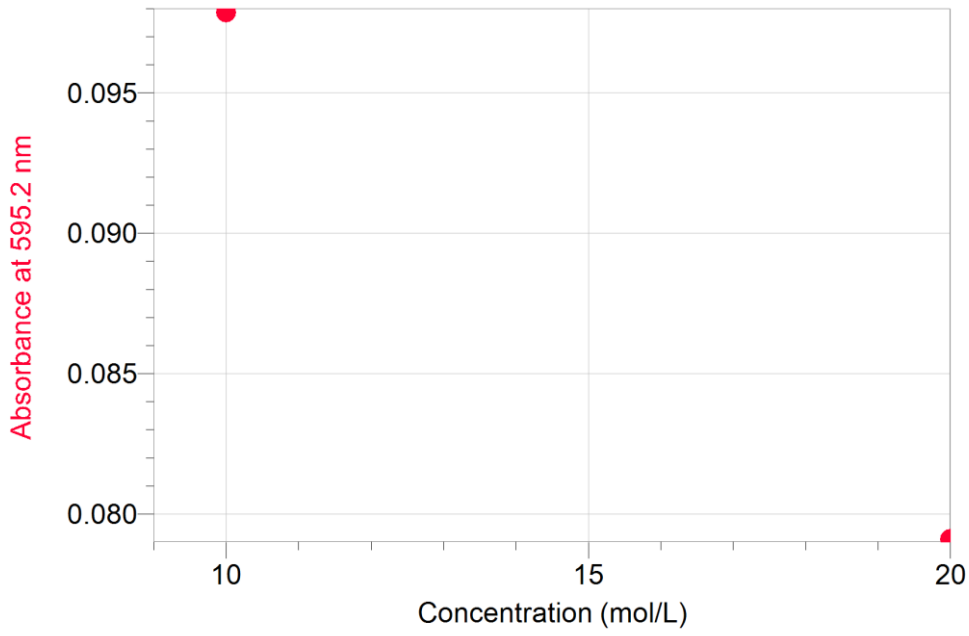
Step 2: Data collecting and processing

All of our results and data can be biased because, as we informed the board before, our Malachitgreen solution is of a lower concentration than needed.

30°C:




40°C:



Step 3: Conclusion and evaluation

*Expected results:*

Yeast is known to grow best in a temperature of 28°C and to be destroyed in a temperature of 45°C[1]. Therefore, we expected to get the highest value of phosphate uptake from the experiment with 30°C.

*Analysis:*

The reaction of yeast with phosphate is a biochemical reaction. Biochemical reactions get faster when increasing the temperature because the particles get more inner energy and therefore there will be more collisions in the same amount of time. If we compare our results with the results of experiment 3, you can clearly detect an increase of phosphate uptake when there is a higher temperature. This increase is highest at the point of 30°C. Despite our problems with correctly measuring the phosphate uptake we could get these results which prove our expectations.

**Heating up the solutions**

[1] German article in Wikipedia about baker's yeast: http://de.m.wikipedia.org/wiki/Backhefe, letzte Einsicht 14.5.13

**4B Increasing the phosphate by increasing oxygen supply**

Step 1: Design of experiment

*Aspect 1:*

Yeast cells have two ways to get energy. The first and more efficient method is the cellular respiration. There is glucose and oxygen needed. In the end, water and carbon dioxide are produced and there are 34 ATP.[2]

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2 + 34\ ATP$

The second method is happening when there's no oxygen available. There's just glucose present and the products are ethanol and carbon dioxide. Other than at the cellular (or aerobic) respiration here are only 2 ATP set free. This is called fermentation or also anaerobic respiration.[3]

$C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ C_2H_5OH + 2\ ATP$

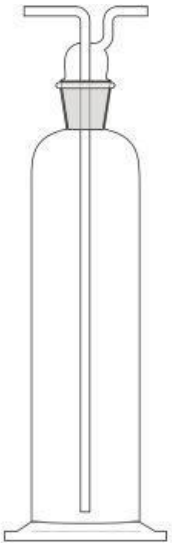
With oxygen the need for glucose is increased as it is needed for cellular respiration[4]. During this process the need for phosphate is increased. It is needed to synthesize the ATP (Adenosintriphosphate)[5], which is made up from ADP (Adenosindiphosphate).(2 There's a phosphate atom taken up by the ADP, this process is called oxidative phosphorylation.

Scientific question: How do aerobic or rather anaerobic conditions influence the phosphate uptake of yeast?

The independent variable is the yeast cell, the dependant variable the measured amount of phosphate (see chapter 3). A relevant controlled variable is the temperature.

*Aspect 2*

The experiment takes place under room temperature and is done as written in aspect 3.



Picture 1: Washing flask

Source: glossary.periodni.com

[2]Dunant, Patrick : School material about Fermentation and cellular respiration
[3]Dunant, Patrick : School material about Fermentation and cellular respiration
[4]Baltes, Werner / Matissek Reinhard : Lebensmittelchemie. 7. Auflage
[5]http://www.cornelsen.de/sites/medienelemente_cms/mel_xslt_gen/progs/medien/mels_stat/mel_809623.pdf (12.05.2013)

*Aspect* 3

First we prepared  the solution with yeast and glucose. Then we put this solution under aerobic / anaerobic conditons for about ten minutes. To create these conditions we used gas bottles with oxygen and carbodioxide and two washing flasks (see picture 1). We put the solution in this washing flask and through a flexible tube we were able to let the gas flow into the solution.  This ensured that there no more remaining carbondioxide or rather oxygen. After this ten minutes we added the phosphate. Sample of the solutions were taken every ten minutes. Totally there were 3  times examples taken. These examples should have been examined for their phosphate concentration.

Step 3: Conclusion and evaluation

We expected that the phosphate concentration in the solution with oxygen should be higher.

Unfortunately we could not examine our samples, because of the probles we had during the task. See: Problems concerning the measurement (page 3)

*Fig. 5: One student pipetting*

**4C Increasing the phosphate by starving the yeast cells**

Step 1: Design of experiment

*Aspect 1:*

As said in Part 1, the Phosphate uptake by yeast cells gets much quicker if the yeast cells are starving. The amount of the phosphate uptake is also much higher than in normal yeast cells.

If yeast cells starve, the reserve of useable substances such as phosphate will be used up. (1)

The cell is in nutrient deficiencies. The phosphate starvation means a huge change in the intracellular flux balance of the metabolic rate of phosphate. These imbalance causes, that some processes are blocked, but others, such as the phosphate uptake, are supported. (2)

So we wanted to find a way to starve the cell and like this increase the phosphate uptake. There are, based on literature, different ways to do this. We chose the one that was the easiest and quickest to achieve our goal:

Shaking the yeast for 18 hours in distilled water.(3)

Scientific question: How does the starvation of yeast cells enhance the efficiency of the phosphate uptake by these cells?

The independent variable is the staved yeast cell, the dependant variable the measured amount of phosphate (see chapter 3). Relevant controlled variables are the temperature and the preparation of the yeast cell starvation (amount of water, time, amount of yeast).

*Aspect 2:*

The Starvation takes place under room temperature and the samples are prepared as described in Aspect 3.

*Aspect 3:*

1g yeast is put in a test tube. The test tube is filled up with 100ml destilled water. Then it is put into a test tube shaker for 18h (overnight).

After that the proceeding is the same as in Part 3.

Step 2 & 3

Unfortunately we didn't do the experiment, because of the problems we had during the task (->see "problems concerning the measurements" (page

# List of references

## Part 1:


1. http://simple.wikipedia.org/wiki/Phosphate
2. http://www.ccmtutorials.com/misc/phosphate/page_03.htm
3. http://www.med4you.at/laborbefunde/lbef2/lbef_phosphat.htm
4. http://www.seilnacht.com/Lexikon/phosphat.html
5. http://jgp.rupress.org/content/40/6/915.abstract
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7. http://www.genetics.org/content/159/4/1491.full.pdf
8. http://books.google.ch/books?id=_34YAUed7xkC&pg=PA43&lpg=PA43&dq=aufnahme+phosphat+von+hefe+bedingungen&source=bl&ots=r7MMTr3yk9&sig=5v1xSUiM_T_EzwZ3CvVZ6-SO-FQ&hl=de&sa=X&ei=LvWBUc35AoSuOYeXgegF&ved=0CEwQ6AEwBQ#v=onepage&q=aufnahme%20phosphat%20von%20hefe%20bedingungen&f=false
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## Part 3:


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2. http://www.ncbi.nlm.nih.gov/pubmed/20971056
3. http://www.echelon-inc.com/index.php?module=Products&func=detail&id=259

## Activity List

| | |
|---|---|
| Problems concerning the measurement | Nicklas Müller |
| Part 1 | Lukas Strobel, Seline Schlotterbeck, Sina Buser |
| Part 2 | Pascal Kraft, Simon Streib |
| Part 3 | Carina Luchsinger, Olivia Widmer |
| Part 4A | Carola Sägesser, Isabelle Stebler |
| Part 4B | Lukas Strobel, Selina Schlotterbeck, Sina Buser |
| Part 4C | Sina Buser |
| Phosphate measurements | Pascal Kraft, Simon Streib |
| Layout | Tilman Kaim |

*2 Pictures showing the class involvement:*