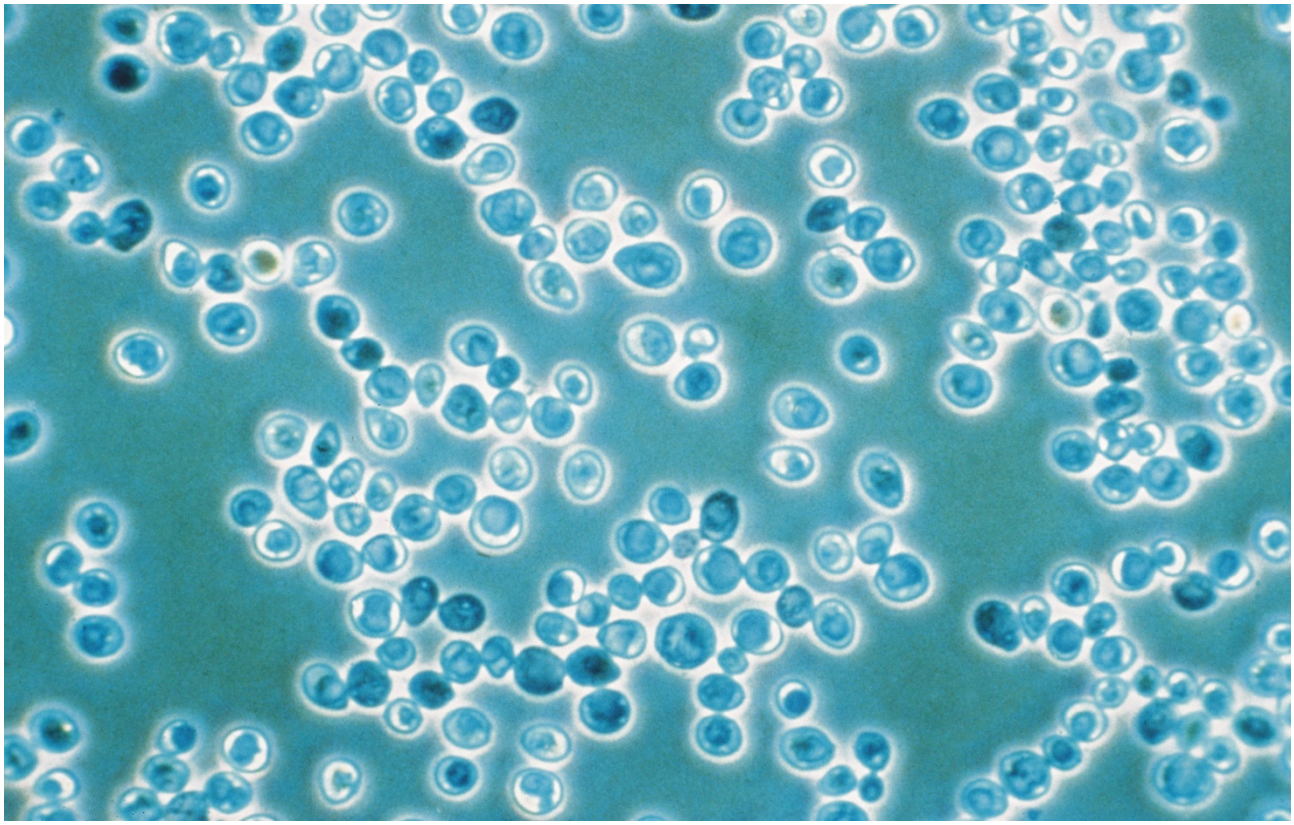
Task 2

Phosphate uptake by yeast cells

Gymnasium Köniz-Lerbermatt, Class 2cdh

13.05.2013



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Part 1: Background Information

Why is phosphate important for all organisms?

Phophate is important for all organisms because it is used to make two of the most important organic macro-molecules : deoxyribonucleic acid (DNA) and adenosine triphosphate (ATP) [(1)]. Furthermore the organisms require Phosphate for the biosynthesis of other cellular components including proteins, lipids and sugar. Therefore phospate is essential for organisms to develope regulatory mechanisms for acquisition, storage and release of this molecule [(2)]. In humans and animals phosphate is also an important component of bones and teeth [(3)]. The diverse importances of phosphates make them an essential nutrient.

What is well known about phosphate uptake in yeast cells (Saccharomyces cerevisiae)?

Although phosphate uptake is one of the vital functions to survive we know little about how exactly yeast cells take up phosphate. A yeast cell contains cytosol, which also have vacuoles [(4)] which are important organelles in the phosphate uptake and use of the yeast cell. There are different phosphate transporters in the yeast cells which all function slightly differently. Briefly said, yeast cells take up phosphate through specific channels with help of transporter molecules who work differently. Later on we will take a closer look at two such transporters and their dependencies on ions.

There are also a number of transporters who are vital for transfer of phosphate in the interior of the cell. They have a major role in the budding of the yeast cell and in regulation of phosphate household. The most important is Pho91, which attends as a vacuole phosphate transporter. It executes phosphate from the vacuole lumen so that it`s in the cytosol. The concentration in the vacuole is regulated by Pho4 [(5)].

How do yeast cells store Phosphate?

The phosphate concentration in yeast cells is about 3-5% of their dry weight. The major part is stored as Orthophosphate ($H_2PO_4^-$), an inorganic phosphate, mostly in the vacuole and to a small part in the cytoplasm. [(6)]

There are several genes that regulate the concentration of phosphate in the yeast cell. These genes depend on the phosphate transporters which in turn depend on the phosphate condition. When the phosphate condition is on a low level, a phosphate transporter activates the transcription of phosphate-regulating genes. [(5)]

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

Yeast cells have two important functions which affect phosphor. As we know the phosphate is essential for all organisms for survival. We all depend on the phosphor cycle. The function of the yeast cells in the ecosystem is decomposing the waste of other organisms. This is vital to the phosphor cycle because otherwise the used phosphor wouldn't be returned to the cycle. The other important function of the yeast cell in our environment is its role as phosphate regulator in water ecosystems. When there is a large amount of phosphate there are automatically a very large number of algae who grow in the water. If there are too many algae's there is lack oxygen because if the algae are decomposed all the oxygen is used. The yeast cells prevent this by controlling the phosphate amount. So we see that yeast cells play an important role in our ecosystem.

Part 2: Pre-test

The main difficulty was to work with extreme precision. We were continuously under time pressure. Throughout the tests multi-tasking skills were required. From the moment after adding the sodium-phosphate-buffer to the tube containing malachitgreen-solution and molybdat-solution we had to proceed fast.

Picture 1: **Working in the laboratory**

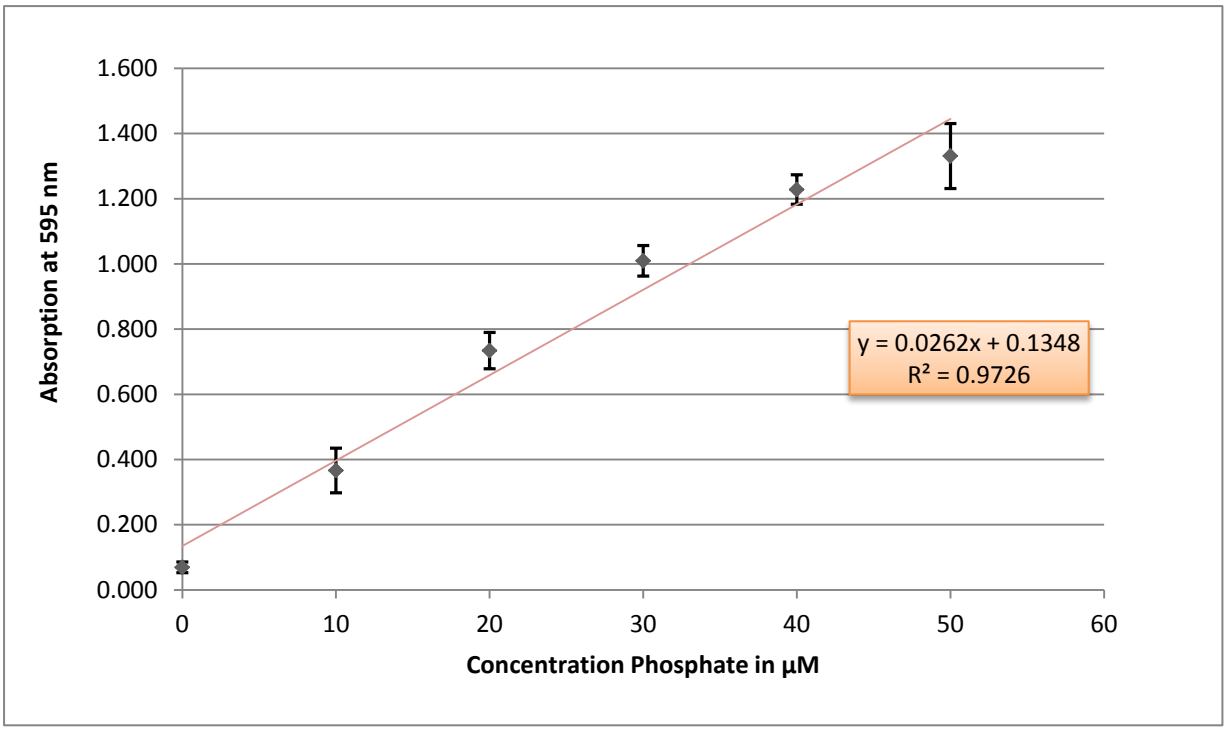
In addition, one had to change the pipette tips after every use. This was because of the high probability that substances could mix and contaminate the other substances.
Another problem with the pipette tips was that they were made of plastic to which some substances like malachitgreen adsorbed.

To optimize the results of this experiment one could apply following improvements. For one thing we really have to work with precise lapses of time. For example wait exactly 5 minutes after pouring the malachitgreen-solution in the molybdat-solution, then add this mix to the sodium-phosphate-buffer and mix it exactly 30 seconds and finally wait exactly 1 minute to measure the absorption.

Picture 2: **Measuring with the photospectrometer**

Concentration phosphate [µM]	Absorption [-]	Absorption [-]	Absorption [-]	Mean value [-]	Standard deviation [-]
0	0.086	0.053	0.069	0.069	0.0165
10	0.414	0.288	0.397	0.366	0.0684
20	0.760	0.670	0.772	0.734	0.0557
30	1.059	0.966	1.004	1.010	0.0468
40	1.279	1.213	1.193	1.228	0.0450
50	1.364	1.219	1.410	1.331	0.0997

Table 1 **The three best phosphate concentrations we found**



What we can see is that the more phosphate in the solution, the higher the absorption rate is. This is so because the phosphate absorbs light. The slope is practically constant. The last measurement is below the slope so it is possible that after a certain phosphate concentration the absorption stay constant.

Part 3: Measuring the phosphate uptake by yeast cells

Time	Extinction 1	Pi-conc. 1	Extinction 2	Pi-conc. 2	Extinction 3	Pi-conc. 3	Mean value	Standard Deviation
0	1.279	48.68	1.249	47.54	1.79	68.19	54.80	11.60520855
10	0.418	15.82	1.218	46.35	1.81	68.95	43.71	26.66355866
20	0.655	24.87	0.928	35.29	0.821	31.20	30.45	5.250323375
30	0.132	4.90	0.702	26.66	0.249	9.37	13.64	11.4905783
40	0.177	6.62	0.454	17.19	0.127	4.71	9.51	6.723012763
50	0.108	3.99	0.714	27.12	0.108	3.99	11.70	13.35397951

Table 2 **This table shows the extinction we measured and the phosphate concentration we calculated.**

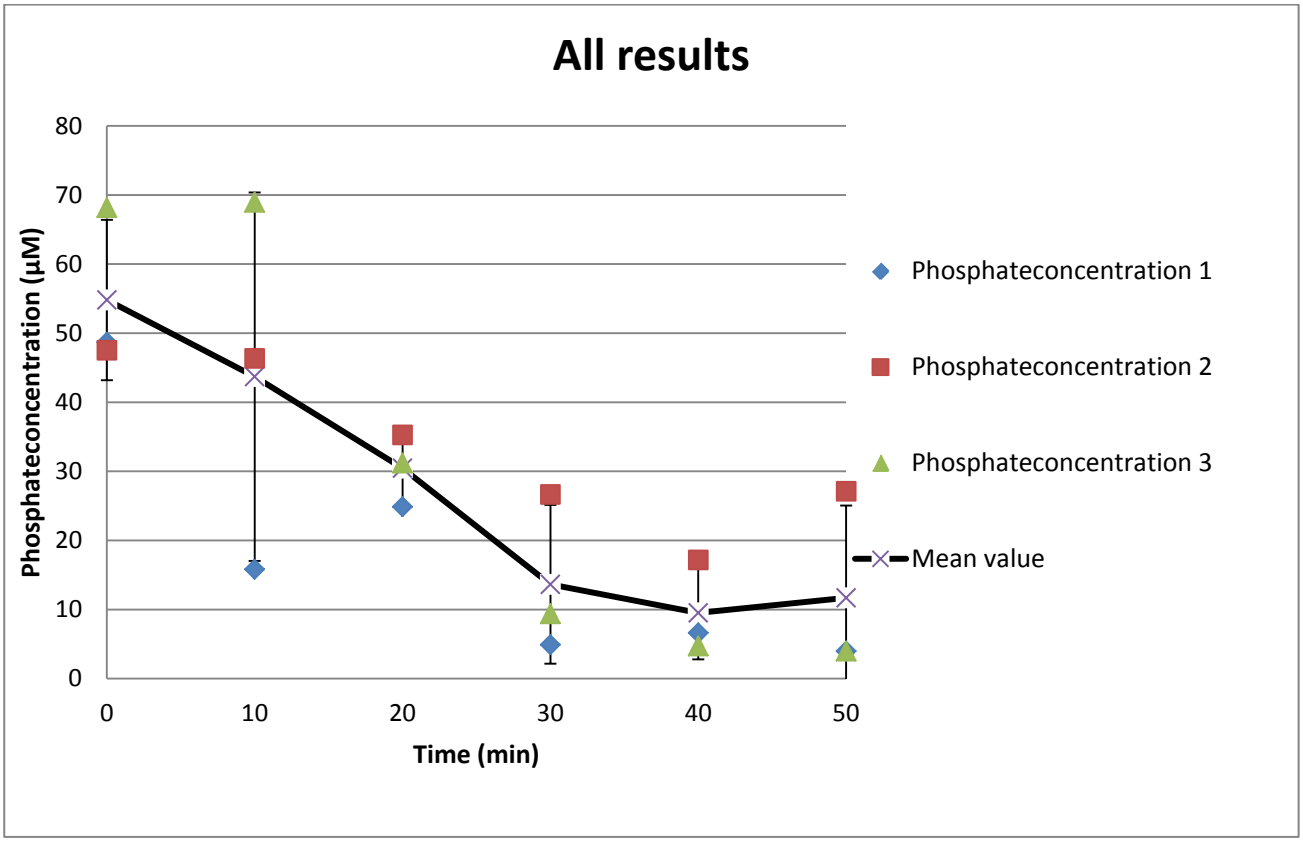


Figure 2 **On the figure above you see the different Extinctions per time. The black lines show the standard deviation on each point of time.**

We had several problems during our experiment. A minor problem at the beginning was the handling with the pipettes. To maintain the amount of the different dilutions we had to use more than one pipette. For example for a dilution of 344µl we had to use one pipette of 300µl and another one with 44µl. To improve this difficulty we chose the simplest way to reach the amount which was twice 172µl.
Another problem was to be on time. We had to stop the time of many different dilutions and it was difficult not to lose the overview. We started working in groups of 2 and more persons so we could share the work.

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

Approach 1: Addition of O_2 and CO_2

Hypothesis:

If yeast cells have enough oxygen they do cell respiration, on the other side they're able to do fermentation if they haven't got enough oxygen. When doing cell respiration yeast cells produce much more ATP in comparison to fermentation. Because of that fact we assume that the oxygen concentration might have an influence on the phosphate uptake.

Implementation:

We did the experiment like in part 3 two more times. After we finished the first medium, we added Oxygen. We used a gas bottle and a pipette. To make sure the oxygen doesn't escape, we immediately covered the medium. In intervals of ten minutes we added more oxygen to compensate for the loss. We repeated this procedure, but instead of oxygen we took CO_2.

Picture 3: Gas bottles with CO_2/O_2

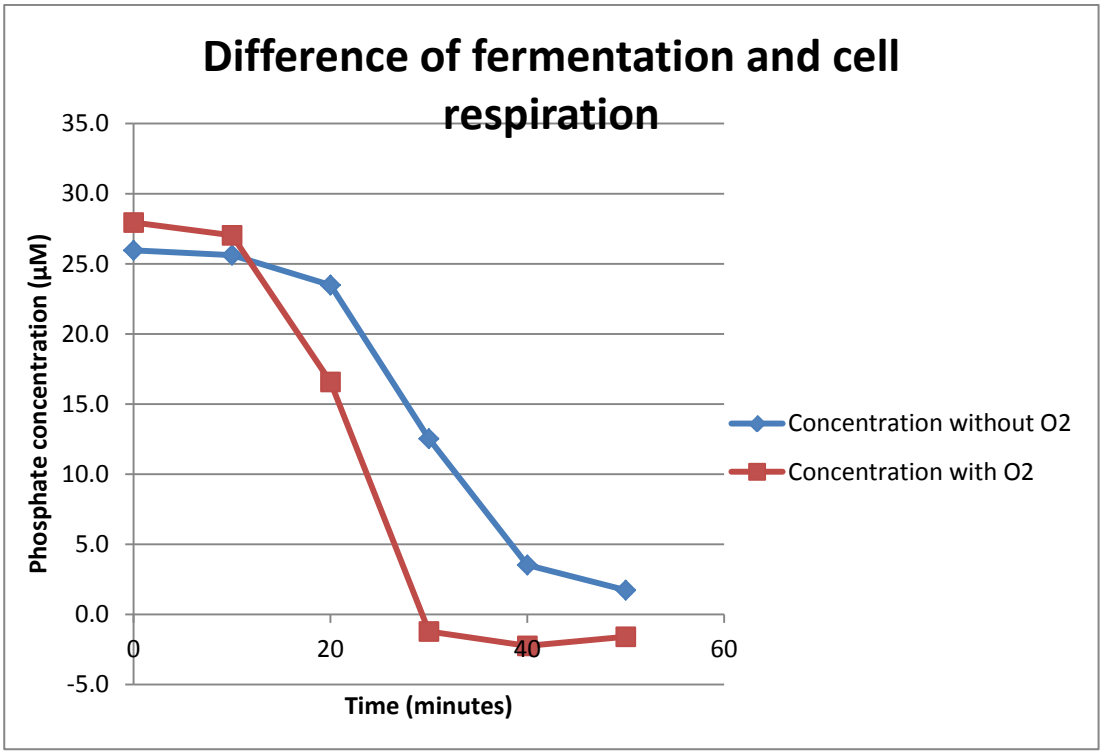


Figure 3 **Phosphate concentration measured**

The first ten minutes the phosphate concentration of both experiments was approximately the same. But in the next twenty minutes the concentration of the phosphate in the O_2medium decreased a lot faster than the CO_2medium.

Interpretation and conclusion:

From the results we conclude that oxygen has a positive effect on the phosphate uptake. But it's difficult to interpret something, because we worked with colourless gas, so we can't surely say if the medium was full with the gas and if something escaped during the extraction of the medium. Every time we opened the medium, we refilled the glass but it is possible, that there was still normal air in the medium. If we compare our measure with Part 3, we have to admit that our way to optimize the uptake of phosphate wasn't as successful as expected. But we can say that the yeast cells are more effective in taking up phosphate when the cells do cell respiration than when they do fermentation.

With our experiment we can give a direction, how we should do next experiments so we could really optimize the process of taking up phosphate in the yeast cells.

Approach 2: Glucose concentration

Hypothesis:

In this approach we tried to change the phosphate uptake by changing the concentration of Glucose. We thought that if the cells would have more glucose they'd have more energy because with a higher amount of glucose more ATP is produced and as such more phosphate is used.

Implementation:

We changed the concentration in the Sodium-Phosphat-Buffer, which contains Glucose. So we produced three times this buffer, where we changed as already said the Glucose concentration, so we made one buffer with 1%, one with 4% and the third with 8% Glucose. Then we put in every buffer, containing 10ml of this liquid, exactly 1 gram of fresh yeast cells. Then we took every 10 minutes 300µl of the suspension. Then we centrifuged it and took 40µl of the supernatant and mixed it with 360µl distilled water. After this we mixed it with 256µl Malachitgreen-Solution and 344µl Molybdat-Solution. Then we measured the absorption in the photometer.

Results:

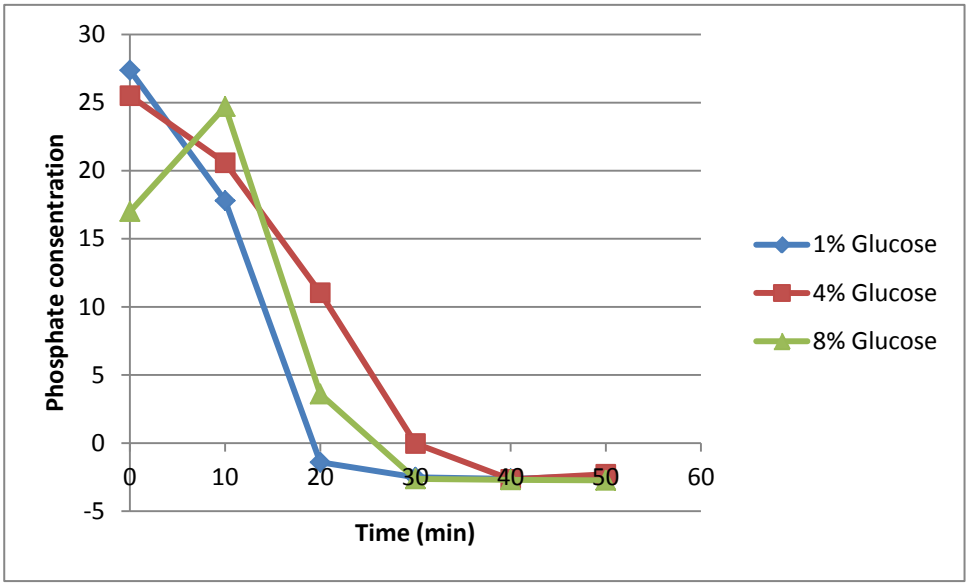


Figure 4 Phosphate concentration against the time and influence of glucose concentration

Time	1% Glucose	4% Glucose	8% Glucose
0	0.852	0.803	0.58
10	0.601	0.674	0.782
20	0.098	0.424	0.229
30	0.069	0.134	0.066
40	0.066	0.065	0.064
50	0.072	0.075	0.063

Table 3 : Absorbtion at different glucose-concentrations

Time	1% Glucose	4% Glucose	8% Glucose
0	27.374	25.504	16.992
10	17.794	20.580	24.702
20	-1.405	11.038	3.595
30	-2.511	-0.031	-2.626
40	-2.626	-2.664	-2.702
50	-2.397	-2.282	-2.740

Table 4: Phosphate-concentration calculated with (absorbtion-0,1348):0,0262 = phosphate-concentration

In our measurements we recognized that the curve with 1% Glucose takes up the most phosphate at the beginning and but then the phosphate concentration decreases fast and stays in the end constant. The 4%-curve decreases as well, but doesn`t decrease as fast as the 1%-curve. The 8%-curve increases at the beginning but decreases after 10 minutes really fast.

Conclusion and evaluation:

In our approach we made some mistakes. First we forgot to take a measurement with 0% Glucose so we can`t say if yeast cells take up more or less phosphate with Glucose. Then our yeast cell concentration in the Sodium-Phosphate-buffer is much too high. It is 100g/l, so our Glucose-concentration is much too low for a significant measurement.

Because of those mistakes, we can`t analyze the graph by reason that the difference of the phosphate-concentration of the different glucose-concentrations isn`t really high. So it doesn`t express a significant statement.

To improve our investigation we should make at the beginning a 0%-Glucose measurement, that we can compare the data. Then we should decrease the yeast cell-concentration in the buffer, and on the other side increase the glucose-concentration.

Approach 3: Temperature

Hypothesis:

We think that because of the Q10 temperature rule the yeast cell might take up more phosphate. This is so because the chemical reactions as a whole happen twice as fast when the temperature is risen by 10˚C. So the reactions in the cell interior would also happen faster meaning that also more ATP is used at the same time. This requires a higher rate of phosphate uptake to maintain the needed amount. The temperature mustn’t be too high otherwise the cell would die.

Implementation:

We executed the experiment as described in the instruction. We only changed the conditions for the suspension by putting it in a 32 °C warm water bath. The experiment was repeated and we calculated the mean value. We also did the experiment again without the water bath to compare the results by room temperature and by 32 °C.

Results

We measured the phosphate concentration by 32°C twice and by room temperature, which is around 22°C, once more. The mean value of the two measurements by 32°C is shown in the graph (Fig.1). The values are lower than the values for the room temperature. The starting concentration of phosphate in the water when measuring the first time was about 24µM/L. After ten minutes the concentration dropped to 7.15 µM/L. Ten minutes later the concentration drops even to a negative number. Up to 40 minutes after starting the measurements, it increases slightly. The last ten minutes it decreases again. The second measurement follows a similar pattern, but the starting concentration was much lower. In order to have more accurate data, we calculated the mean value. The measurement under standard condition (22°C) doesn`t follow that pattern. The phosphate concentration drops slower and does not reach the zero point in 50 minutes.

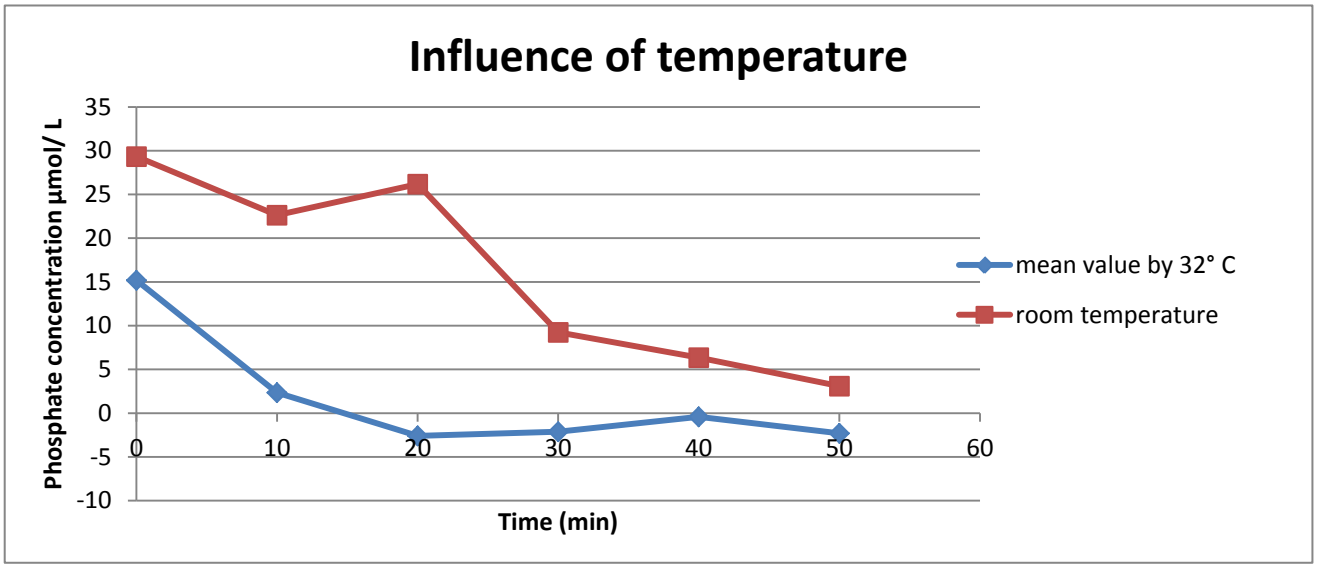


Figure 5 **The influence of higher temperature in the phosphate uptake of yeast cells**

Time (min)	1st time 32°C	2nd time 32°C	Mean value	Room temp.
0	23.900	6.458	15.179	29.321
10	7.145	-2.435	2.355	22.641
20	-2.893	-2.282	-2.588	26.191
30	-2.282	-1.939	-2.111	9.244
40	0.733	-1.557	-0.412	6.344
50	-2.511	-2.092	-2.302	3.099

Table 5 **The different Phosphate concentrations at different points in time and different temperatures**

Interpretation

The most important thing we could see was that in 32°C water, the phosphate uptake occurs faster. This is the proof that the q10 temperature coefficient also works for this experiment. The uptake of Phosphate goes much faster when the temperature is increasing. This difference of 10 °C between room temperature and the 32°C in the water bath has a big effect on the metabolism of the yeast cells. That is the reason why the uptake occurs much faster.
In the graph of the measurements with 32°C there is a peak, where the concentration of phosphate rises suddenly. We can't explain it exactly, but we have a few hypothesis:

- Trough metabolism, Phosphate might get released as a waste product.
- Errors could have happened because of inaccurate pipetting.
- Yeast cells release phosphate when they die.

A similar pattern appears in the graph for the room temperature

Conclusion

This approach is a good method to improve the phosphate uptake. The temperature should not be higher than 45°C, because yeast cells die when is gets warmer. It also does not help to get away of the optimum for metabolism in yeast cells, which is around 32°C.[8]

Improving the measurement

The only thing we could do is to do the experiment more than twice, so that we can be sure that we have more or less the same starting concentrations of phosphate in the yeast. We also weren`t use to the pipetting, so this was also an effort we could make.

Approach 4: Influence of Na^+ on the phosphate uptake:

Hypothesis:

As we know yeast cells depend on the uptake of phosphate. But how exactly do they transport the phosphate into the interior of the cell? As we explained earlier the yeast cells have specific channels that transport the phosphate inside the cell. But what we didn't mention until now is that there are specific mechanisms which transport the phosphate ions. The mechanisms are made out of rather complicated molecules but from what we know there are two different types. The first mechanism is Na^+ dependent and the other one is Na^+ independent. The Na^+ dependent mechanism has a double affinity to Na^+ and a single affinity to phosphate. This means that two Na^+ ions and one phosphate ion can be transported with this mechanism. To keep things simple we can imagine that the Na^+ independent mechanism transports only phosphate into the cell.[7] Considering these mechanisms we developed our hypothesis. Our idea was that if we would increase the Na^+ concentration the phosphate uptake would be more effective and faster because this would influence both mechanisms which transport the phosphate.

Implementation:

We made a NaCl solution with a concentration of 1mol. After that we prepared 3 different mediums with yeast cells as described in the instructions. In the first medium we added 25µl of our NaCl solution. In the second we added 50µl and in the third we put 100µl. We also adapted the amount of water that was added to each medium considering the amount of NaCl solution we put into them. The amount of NaCl that was added in the thirst medium through this way correlated the half of the phosphate concentration. In the second medium it was the same amount as phosphate and in the last medium the NaCl concentration was the double of the phosphate concentration.

Results:

Time (minutes)	25µl 0.1 Mol/l NaCl	50µl 0.1 Mol/l Nacl	100µl 0.1 Mol/l NaCl
0	22.4	25.2	28.7
10	21.7	24.7	26.6
20	10.1	11.1	5.0
30	-1.6	-1.3	-1.6
40	-1.3	-2.2	-1.6
50	-1.6	-2.1	-1.5

Table 7: Concentration of phosphate in the solution

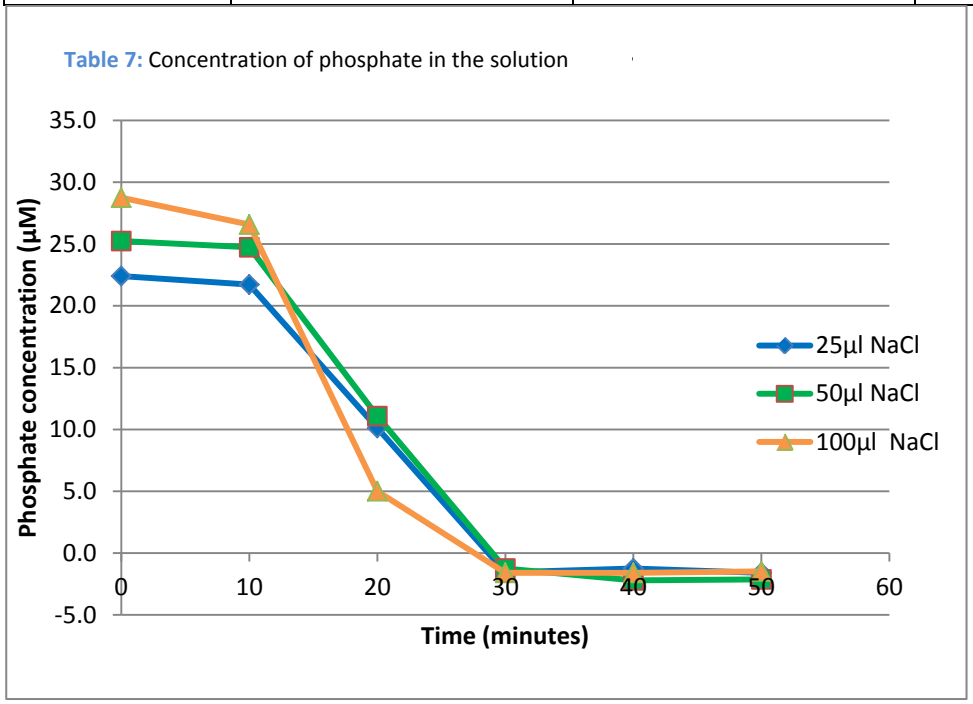


Figure 6 **Influence of Na on Phosphate uptake**

We can see that if Na^+ is added the concentration of phosphate drops rapidly after the ca. 10 minutes. The solutions with 25µl and 50µl drop at about the same rate while the solution with 100µl NaCl drops a bit faster. After 30 minutes the values stay constant and the phosphate uptake in the solution doesn't drop any further. The graphic shows that if we have a higher concentration of NaCl we also have a higher concentration of phosphate at the beginning. This may be correlating or just a coincidence.

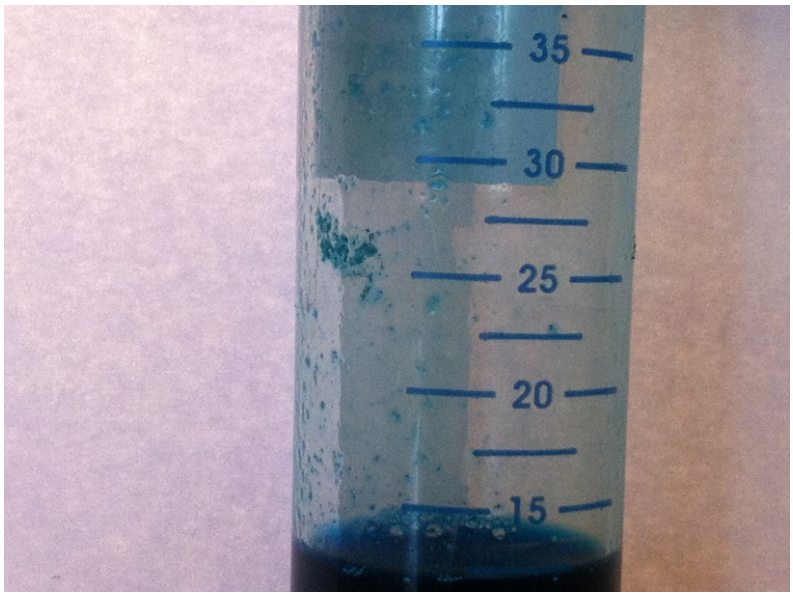
Interpretation and conclusion:

According to our results one could say that Na^+ has an influence on the phosphate uptake of yeast cells. Especially when looking at the curve of the solution of 100µl NaCl. We know that there are two different mechanisms which transport phosphate into the yeast cell. We explained that the Na^+ dependent mechanism has a double affinity for Na^+ and one for phosphate. However this doesn't mean that all these mechanisms automatically transport two Na^+ ions but that maybe only one or two spots for Na^+ ions are left unoccupied because the Na^+ concentration is too low. Now if we raise the Na^+ concentration in the solution all Na^+ dependent mechanisms carry two Na^+ ions. This means that much more Na^+ is transported into the yeast cell. This results in a positive charging of the cell interior. But this state is contradictory to the charging balance and as such the yeast cell tries to restore its neutral state. To achieve that it opens all channels for the Na^+ independent mechanism and with so let's in phosphate that neutralizes the positive charge of the yeast cell.

In the first ten minutes we see that there is only a slow dropping of phosphate concentration independent of the amount of NaCl. This can be explained through the primary uptake of Na^+ through the dependent mechanism. After those 10 minutes the yeast cells are strongly positively charged and we see a rapid drop in the phosphate concentration in the solution because of the Na^+ independent uptake. As a result the yeast cell has taken up the maximum amount of phosphate it can implement and the phosphate concentration in the surrounding solution doesn't drop any further.
We can't say that our theory is 100% correct because we couldn't make enough tests concerning these mechanisms to fully prove our theory. But we can say that a higher amount of Na^+ results in a much faster and with that effective phosphate uptake.

Conclusion and evaluation

It is clear that our three best approaches are number 1, 3 and 4. It may be possible, that if we would combine a great concentration of O_2, the temperature on approximately 32°C and 100µl of a 1mol NaCl solution we would see an even more effective phosphate uptake but we can only assume that.
What we can see in all graphs is that some values are below zero. This is because we measured the straight calibration curve on the 1st of May and we did the approaches a week after that. So we think the concentration of the malachitgreen changed because it partly crystallized.



Picture 4: **Malachitgreen with crystallization**

During Task 2 we always had the same problems throughout all parts. What was really important and difficult was to work with extreme precision while using the pipettes and having a good time management. We can see the problems surfacing in our results because they are not always accurate. We also didn't have sufficient time to collect enough data so that we didn't obtain fully accurate results. For example if just one result is imprecise it falsifies the mean value and it also negatively influences the graph. Also due to the little data it was tricky to make a proper observation from the graphs for a fully correct interpretation.
Another weakness was the realization of our ideas for the approaches. We had a lot of ideas but we didn't know how to realize all of them because some of them were rather complex and required further knowledge about specific structures and mechanisms of the yeast cell. For some of them we didn't have all the equipment we needed.
We would need a lot more time to receive better and more precise results. With the time we had it was impossible to collect sufficient data. For example if we had more time we would have tested if a combination of all our approaches would have resulted in an even better phosphate uptake.

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8) Wikipedia.org, *Backhefe*, retrieved 15.05.2013, from http://de.wikipedia.org/wiki/Backhefe#Stoffwechsel

Activity list

Part	Responsible Member(s)
Part 1	
Why is phosphate important for all organisms?	Rahel
What is well known about phosphate uptake in yeast cells (Saccharomyces cerevisiae)	Thuy, Stephanie, James, Nicolas
How do yeast cells store phosphate?	Doris, Edda
Why is phosphate uptake by microorganisms an important issue in our environment/society?	Dominik, Nicolas
Part 2	
Pretest	Kai, Tim, Alexander, Léa, James, Dario, Roman, Jonas, Giulia, Lauranne
Processing of raw data and presentation	Tim, Lauranne, James
Part 3	
Measuring the phosphate uptake by yeast cells	Léa, Kai, Alexander, James, Dario, Roman, Jonas, Giulia, Rahel
Processing of raw data and presentation	Doris, Kai, Edda
Part 4	
1. Approach: fermentation vs respiration	Giulia, Alex
2. Approach: Glucose	Dominik, Edda, Kai, James
3. Approach: Temperature	Roman, Dario, Edda
4. Approach:	Nicolas, Tim, Jonas, Léa, James
Conclusion and evaluation	Edda, Lauranne, Nicolas
Layout	Jonas, Léa
Reference List	Jonas, Thuy, Stephanie
Corrections	James, Nicolas
Activity List	Edda, Léa