Kantonsschule Im Lee
Class 3dg
Science on the Move – Task 2

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Abstract

Phosphate is a vital resource, which is, among other things, used as a building block for DNA and the energy vector ATP and is a basic requirement for all life on earth. In this paper the influence of the elements potassium, calcium and magnesium on the rate of uptake of phosphate in yeast cells was analyzed along with how the pH-value affected this uptake. Each of these three elements is supposed to accelerate the growth and reproduction of yeast cells, which in turn directly influences the culture's uptake of phosphate. To test the magnitude of this influence, different salts were introduced to the testing environments, each with one of these elements as a cation. The added salts were KCl, $MgCl_2$ and $CaCl_2$, which were dissolved respectively in a 0.4M solution. The experiments were each carried out with three different pH-values (4.8, 5.65, 6.5). The results showed that two of the tested compounds, namely calcium and potassium, lead to the concentration of phosphate decreasing more rapidly than in a control medium without the added compounds. In the experiment with magnesium the rate of uptake was actually lower than in the control medium. In addition, all of the experiments demonstrated that a pH-value of 6.5 yielded the greatest rates of uptake.

1 Introduction

Without phosphorous, life on Earth would not be possible. Most often it is taken up by organisms as phosphate and used by the same for different purposes.

1.1 Why is phosphate important for all organisms?

As phosphate is a part of DNA, it is a vital material that has to be present in every organism. On the one hand, phosphate forms the backbone of the DNA double helix and therefore grants it its structure. It serves as the connection between the glucose molecules and constitutes a part of the DNA strand. On the other hand, phosphate is an important part of ADP and ATP, the energy units of the body. During cell respiration a phosphate is connected to ADP, using the energy provided by sugar, to create ATP. This universally applicable energy vector is necessary for the cell during catabolism.
During this process phosphates are once again released from ATP, which creates a small amount of energy that can be used for metabolism. [1]

1.2 What is well known about phosphate uptake in yeast cells?

Phosphate is taken up by yeast cells via the membrane and stored in vacuoles. [1] There are two different ways to for the yeast cell to utilize phosphate. It is used for the processes mentioned under point 1.1. Temperature, pressure, vitamins, PH-value and oxygen concentration can, along with several other factors, have an effect on the rate of intake of phosphate into the yeast cells. [1]

1.3 How do yeast cells store phosphate?

During the intake of phosphate through cellular respiration phosphate is stored in the cell's vacuoles, so that during the next cellular division there is enough phosphate present to create new DNA. The cell's own phosphate is also used during reparation processes. When the yeast cell practices fermentation, the phosphate will be embedded in Glucose-6-phosphate, which then is used as a precursor for further metabolic reactions. [2]

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

Phosphate, which can be found in fertilizers amongst other things, often acts as the limiting factor in over-fertilized bodies of water and can even endanger a whole ecosystem. Excess phosphate effectuates an explosive growth of algae. [3] Phosphate can also have a negative effect in the area of agriculture. For example; phosphate over-fertilization can cause poor harvests and discolor leaves. [4] Therefore it is of great importance to society to develop an efficient and ecological way to dispose of this excess phosphate in different ecosystems. Microorganisms such as yeast cells can assume this task.

1.5 Research questions and hypothesis

Experiments have already been carried out to analyze the increase of phosphate uptake in yeast and therefore many factors like temperature, pressure or oxygen content have already been tested frequently to optimize their values. Temperatures between 30 C° and 38 C° for example maximize the activity of yeast, because of the high kinetic energy [5, 6, 7]. Due to the high numbers of sources that came to same conclusions, these findings were taken as given

facts and so it was decided to focus on other, less-known factors with the ability to increase the uptake of phosphate in yeast. There seem to be discordances about the optimum pH as well as about the interaction of certain cations and the correlating amount of yeast cells, which corresponds to the phosphate uptake. Roomans and Borst-Pauwels (1978) tested the interaction between cations and the phosphate uptake rate at a pH of 7.2 [8] whereas other findings came to conclusion that a neutral or slightly acidic pH are the most preferable condition for the phosphate uptake of yeast [7, 9, 10]. Goodman and Rothstein (1957) could show, the addition of potassium at the concentration 0.2 mol/l increases the uptake of phosphate up to 700 percent at a pH of 4.8 compared to an optimum pH of 6.5 without. [11] And finally; Olson and Johnson (1948), stated that the addition of potassium, nitrogen or magnesium accelerates the reproduction of yeast, and thus indirectly the increase of up taken phosphate. [12]

This contradiction concerning the pH as well as the not yet fully analyzed functions of different cations raised interest and it was decided to test the impact on the uptake rate of phosphate of the three following substances at three different pH.

1. How does the addition of potassium at three different pH influence the uptake rate of phosphate into yeast cells?

Every living cell requires potassium, without it, no life would exist. While many animals use potassium-sodium pumps to obtain the necessary phosphate, taking the required energy from ATPase, plants and fungi, including yeast, have developed other mechanisms to ensure the transport of K^+ into the cell. One mechanism is the proton pumping via H^+-ATPase, where a transporter, Trk1, with high affinity for K+ is responsible for the major uptake. Buch-Pedersen et al. (2006) from the Royal Veterinary and Agricultural University in Copenhagen, Denmark, states that this transport mechanism is characterized by a phosphorylated intermediate as part of their catalytic cycle. However, the direct influence of potassium on this phosporylation remains yet to be researched. [13]

In a yeast cell, potassium takes up prominent roles in many intracellular processes like regulation of cell volume, intracellular pH, protein synthesis, activation of enzymes and regulation of plasma membrane potential. [15] All these major intracellular processes that include potassium are essential for the cell's growth and reproduction. Since potassium is vital for yeast, it must be able to enter the cells even against a strong concentration and charge gradient. Ramos et al. (1993) stated that the growth of yeast increases constantly with the concentration of potassium inside the cell, as long the concentration does not exceed the natural limit of 2.1M of K^+. [14] The natural question therefore, is; does an additional amount of potassium in the surroundings of yeast and therefore a relatively high amount of potassium inside the cell accelerate the reproduction and therefore also the corresponding uptake of phosphate?

The mechanism that is responsible for the uptake of K^+ ensures that the limit is not exceeded, and therefore no harm is done. This means the addition of phosphate into the surrounding of yeast will not have a negative impact on its uptake on phosphate; it can only stay the same or increase. As potassium only has positive effects on yeast cell, there is a good chance that the cell will only profit from the increased amount of potassium in it. Besides the transport by Trk1, the anion bicarbonate is also important for the potassium accumulation. Bicarbonate is produced in a series of steps, from decarboxylation to carbonic anhydrase. The bicarbonate anions, due to their high permeability, cannot be extruded via Pma1, a mechanism inside cell that extrudes ions to prevent an imbalance of charge. The negative charge of bicarbonate creates an imbalance of charges, which is in turn balanced by the accumulation of K^+. [15] Since positively charged ions can be attracted to negatively charged ions into the cell, it was took as read that a surplus of accumulated potassium, being positively charged, can attract the negatively charged phosphate ($PO4^{3-}$), which finally would lead to a faster uptake rate. Originally

it was also discussed to test sodium. Due to chemical similarities between the two elements in the group of alkaline metals, potassium and sodium, it was believed that sodium also had an impact on the uptake rate of phosphate. However, since the provided sodium-phosphate-buffer, as already contains Na^+ ions, it was decided to eliminate sodium.

Hypothesis 1: The addition of potassium will increase the uptake of phosphate in yeast cells, firstly because potassium helps the growth of yeast and secondly acts as a stimulator in the uptake of phosphate. It will also attract the negatively charged phosphate-ions.

2. How does the addition of calcium at three different pH influence the uptake rate of phosphate into yeast cells?

According to Olson and Johnson (1948) the growth of yeast cells can increase up to seven times by adding calcium at a pH of 5.0 [12] By speeding up the reproduction rate of yeast cells, they indirectly also increase the amount of phosphate accumulated inside the cells.

According to Demaurex and Poburko (2009), calcium enhances cellular respiration within cells. The mitochondria, which convert energy from glucose to ATP, code and decode cytosolic calcium signals. These signals augment the cellular respiration by enzyme-regulation.[16]

Since every process carried out in a cell requires energy, an increased energy production leads to a faster division of the cells, which results in an enhancement of cell reproduction and therefor a faster phosphate uptake.

Hypothesis: The addition of calcium to the surrounding of a cell will indirectly increase the amount of phosphate uptake, due to an enhancement of the respiration oft the cell, which will lead to an improved growth rate.

3. How does the addition of magnesium at three different pH influence the uptake rate of phosphate into yeast cells?

Magnesium is an important ion in the biological system of a cell. It is a necessary mineral nutrient for life as it occurs in every cell type in every organism. Moreover, it plays an important role in gaining energy, which again is necessary for the reproduction. As the main source of energy, ATP must be bound to Magnesium in order to be biologically active. [17] In like manner, Magnesium contributes to the stability of all phosphate compounds within the cell, including those associated with DNA- and RNA synthesis. [18]

Magnesium is essential for a proper cellular function. Lack in nutrients results in diseases in the affected organism. Especially in single-celled organisms such as yeast a lack of magnesium can lead to a greatly reduced growth rate.

Hypothesis: The addition of magnesium will increase the uptake of phosphate into the yeast cell. This is explained with the fact that magnesium is of vital importance for the stability of all phosphate compounds within a cell.

2 Methodology

As all three hypotheses possess a very similar experimental setup and as such they do not have to be explained individually, but can be described collectively.

2.1 Culture

Normal backing yeast, which can be purchased in any convenience store, was used for the working process in the laboratory.

2.2 Contents of the analysis medium

For the fabrication of the buffer solutions, a 0.1M Na_3PO_4 and a 0.1M H_3PO_4 solution were used. Thereby, three pH-values for the buffer solution were created for each hypothesis. The pH differences were achieved by titrating the 0.1M H_3PO_4 solution with the 0.1M Na_3PO_4 solution until the desired pH-values were reached. This way, it was assured that the concentration of the phosphate species was at a constant of 0.1M. The pH-values were selected to be 4.8, 5.65 and 6.5.
The pH-value of 4.8 represented the optimum value for the phosphate uptake in the presence of potassium. [7] Without potassium, the optimum lay at 6.5. [7] The mean of those two pH-values was selected as the third value to be tested.
In the three experimental series, the influence of potassium, calcium and magnesium on the rate of division of yeast cells and therefore their uptake of phosphate was to be tested. Depending on the hypothesis, a salt was used that contained the to-be-examined cation. Thereby, a salt bond that contained chloride was always selected, so that the anion did not have any influence on the result (Used salts: KCl, $MgCl_2$, $CaCl_2$).

0.25 ml of the buffer solution were, with the corresponding pH-value and 5ml 0.4M of the saline solution (KCl, $MgCl_2$ bzw. $CaCl_2$) inserted into a 50ml volumetric flask and filled up to a total of 50ml with demineralized water. Thus, the buffer concentration in the finished solution was 0.5mM (the same as in part 3) and the concentration of the salt was 0.04M.
Goodman et al. used a 0.02M potassium concentration for a yeast concentration of 0.5g/10ml.[7]

The yeast concentration in this experiment was 1g/10ml and therefore a proportional potassium concentration was used that, as a result, was twice as high (0.04M).

2.3 Experiment

During the experiment, 0.1g of glucose ($C_6H_{12}O_6$) and 10ml of the before produced solution were added to an Erlenmeyer flask and mixed. After that, 1g of backing yeast was added and dissolved. From this moment onwards, in regular spaces of ten minutes, a probe of the solution was extracted for measuring. The probe was centrifuged so that the yeast was no longer spread out through the whole solution, but collected at the bottom. 40ml of the supernatant were extracted and used for a photospectrometric measurement. These measurements were executed and evaluated according to the instructions in Task 2.
The experiments for all hypotheses were performed at the same time, so that all of them were set in conditions that were as similar as possible to each other concerning external influences. The experiment was carried out in a room with a constant temperature of 21.9°C.

3 Results

3.1 Calibration Results

The first difficulty was a problem commonly faced when handling new software, in this case the one that was necessary for the spectrophotometer. This was however only a minor concern that was resolved eventually and did not hinder the first part of the experimental process.
The second difficulty laid in understanding the instructions correctly, which was solved by careful rereading of the documents over and over again.
The straight calibration presented several problems during its execution, which was attempted several times in order to obtain the correct results.
At first the wrong cuvettes were used to measure the absorption. This mistake was discovered and corrected in time and did not influence the results.
The next difficulty appeared at step 7 of the straight calibration, concerning the lapse of time between step 6 and the measurement of absorption.
The first resulting absorption graph did not appear as expected, and therefore it was thought that during the experimental process there had been some sort of mistake. The process was repeated several times, whereby it was carried out by two different people to eliminate the potential human error. However, some of the resulting graphs were unsatisfactory, though the experiment was carried out meticulously.

Concentration	Dilution series 1 – Measurement 1	Dilution series 1 – Measurement 2	Dilution series 1 – Measurement 3	Average	Standard Deviation
0	0.023	0.025	0.028	0.025	0.003
10	0.349	0.348	0.343	0.347	0.003
20	0.705	0.698	0.709	0.704	0.006
30	1.053	1.048	1.054	1.052	0.003
40	1.348	1.333	1.346	1.342	0.008
50	1.611	1.585	1.589	1.595	0.014

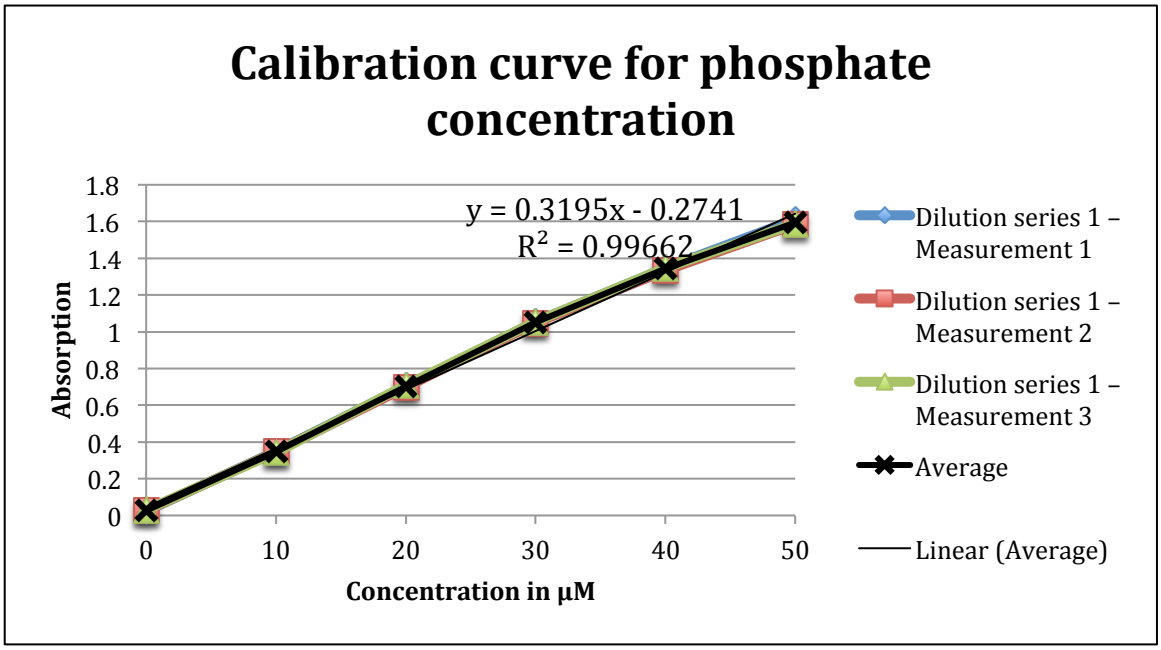

Figure 1: All results of the serial dilution in comparison to the average N. Da Mutten, 2013

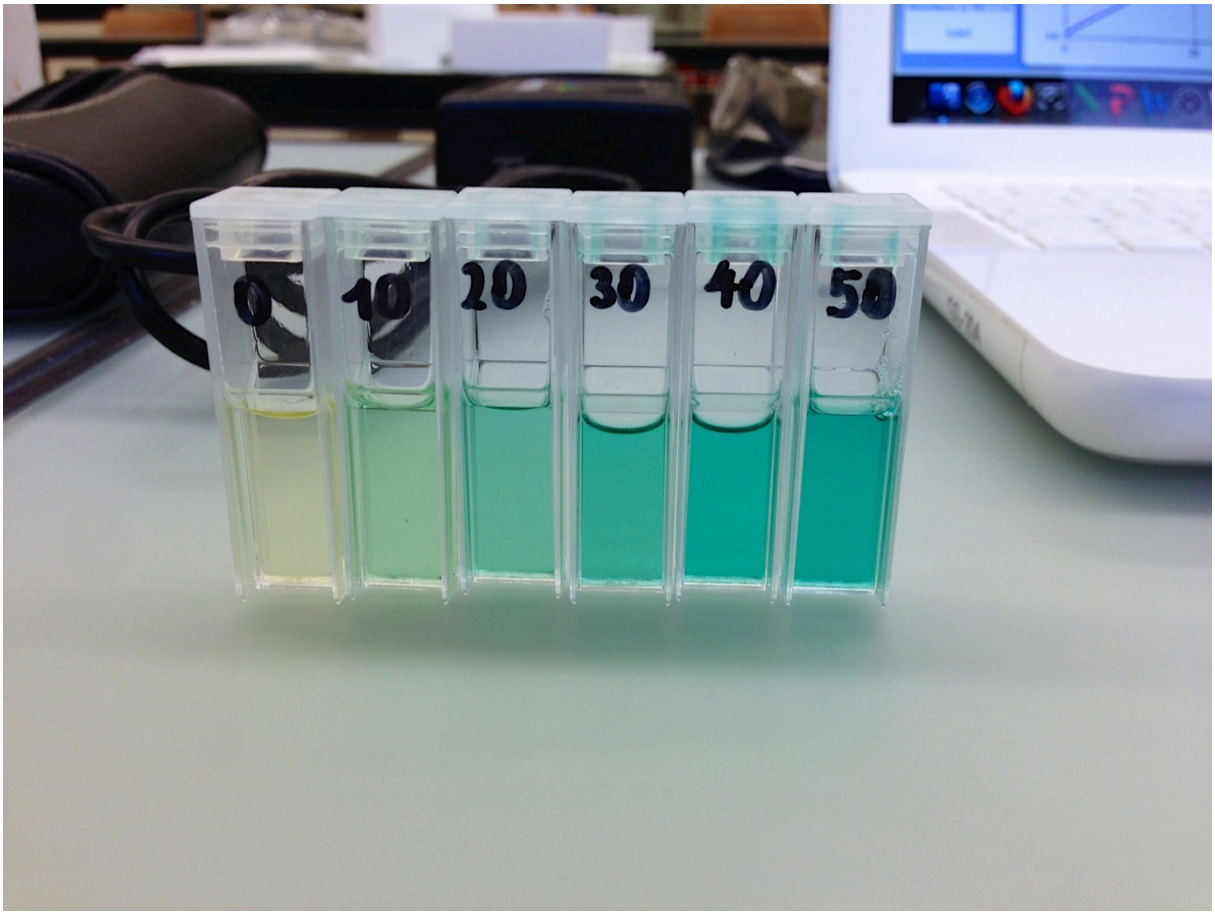

Figure 2: The calibration solutions. N. Da Mutten, 2013

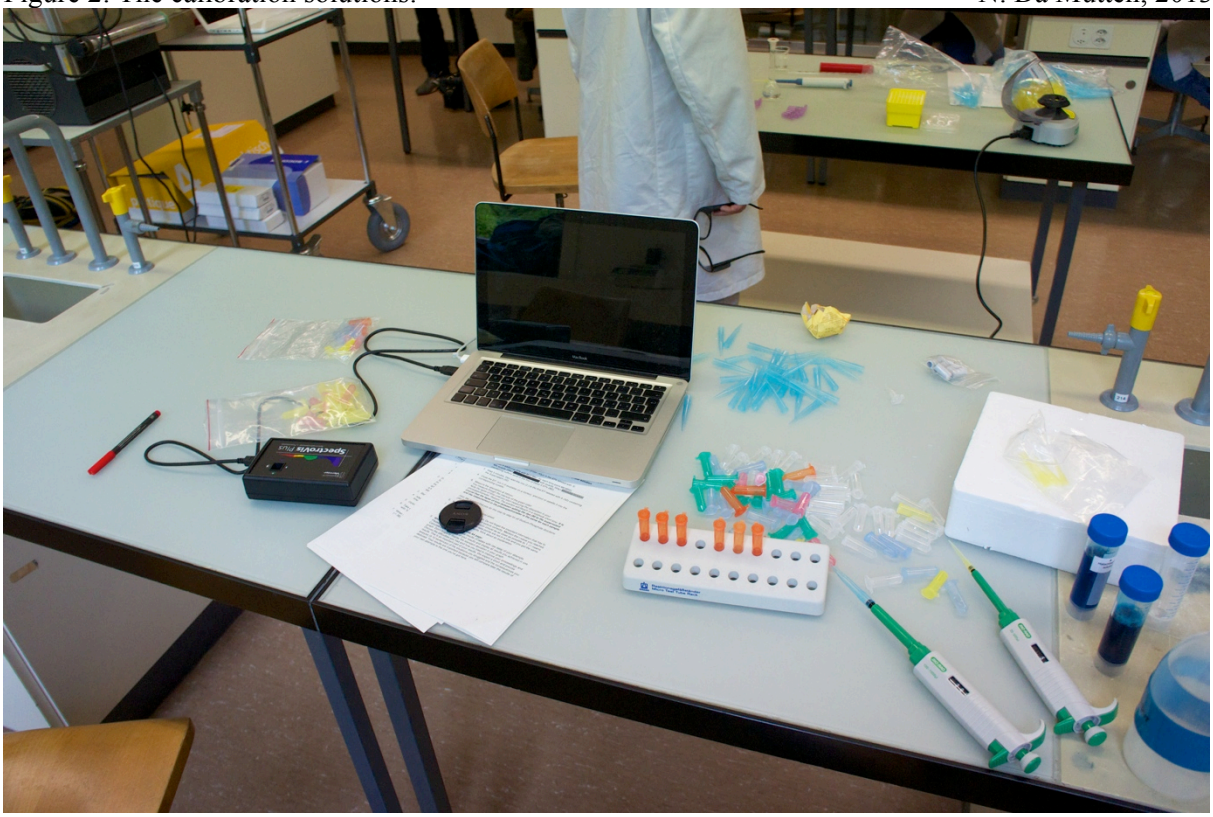

Figure 3: Our lab environment. N. Da Mutten, 2013

3.2 Control group results

Since it was not known how the preparation of the yeast solution corresponded to the measurements, multiple tries were required until the preparations had been executed properly. Due to this, instead of the supernatant, the yeast was pipetted during the first experiment, which naturally did not lead to the desired results.
After the hypotheses had been formed, the problem that the control series was not meaningful suddenly sprung up, as it was decided that the measurements were to be carried out with varying pH-values, and therefore an individual result was needed for every pH-value.
Hence, the whole experiment was carried out anew for each value.

Time	ph = 4.8	ph = 5.65	ph = 6.5
0	4.066260438	4.080417168	3.984284705
10	3.745530382	3.997442913	4.015728461
20	3.418013949	3.852384805	4.016216803
30	1.755568409	3.822928183	2.037266318
40	2.020964965	1.187633998	1.062488014
50	1.321263434	1.033490384	1.104931774

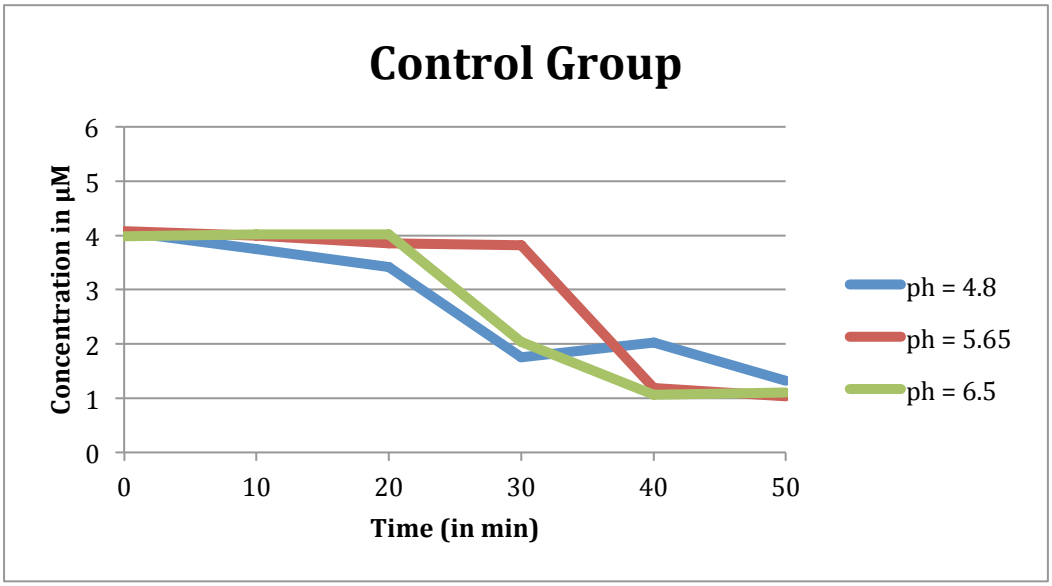

Figure 4: Phosphate concentrations of the control group solutions, in absence of any additional salts. N. Da Mutten, 2013

3.3 Experiment results

Pertaining the duration of the experiment, the series were terminated after five measurements, or a to total of fifty minutes. This was due to the fact that professor Rothstein et al. (1957) demonstrated that the phosphate uptake was greatest in the first thirty minutes and after that the rate of decline of phosphate decreased rapidly. [15] Therefore it was decided to compare the results after thirty minutes. The extra twenty minutes served as a buffer zone to clarify the result.

The results showed that in all the experimental series the phosphate was ultimately depleted.

The measurements at different pH-values differed clearly from each other. The highest pH-value displayed the most rapid uptake of phosphate in all the experimental series except for the control group.
In addition it is notable that all salts had an increase of the decline of phosphate as a consequence.

The extremes after 30 minutes were the rates of uptake of potassium with a pH of 6.5, which exhibited a value of 85% and the solution without salts and a pH of 5.65 and a value of 13%.

An error occurred during the experimental series of potassium and magnesium, as the phosphate concentration increases inexplicably. In addition there is a mistake in the control series with a pH of 4.8 at the benchmark of 30 minutes, but this is the only deviation (see discussion).

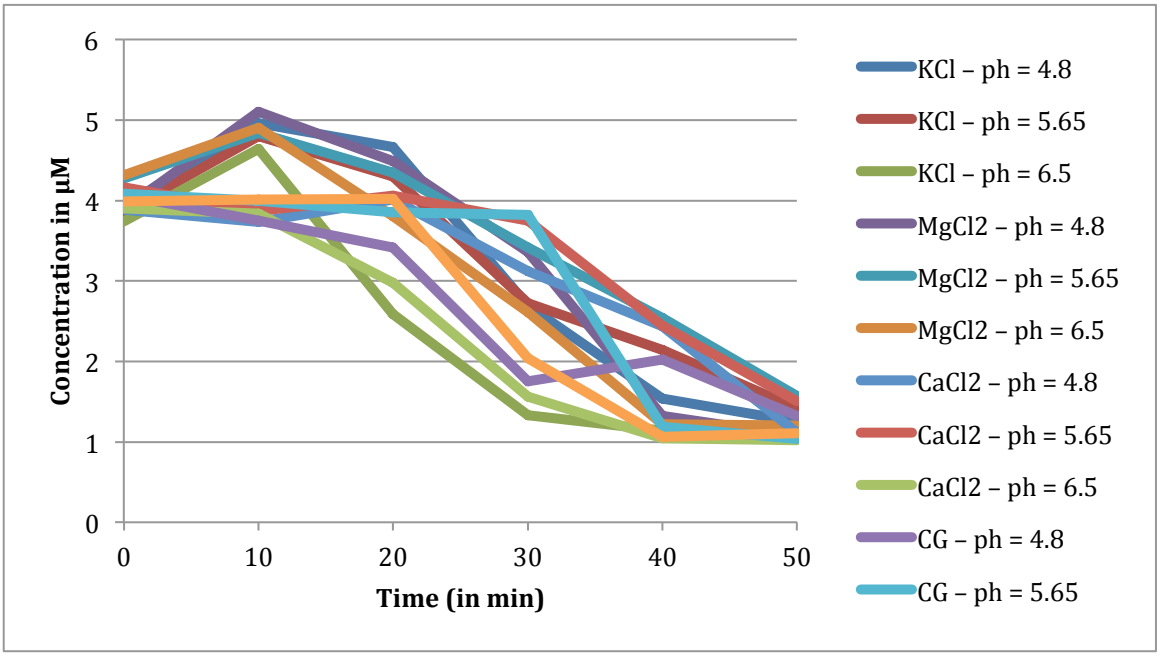

Figure 5: Phosphate concentration of all solutions oft he experiment. N. Da Mutten, 2013

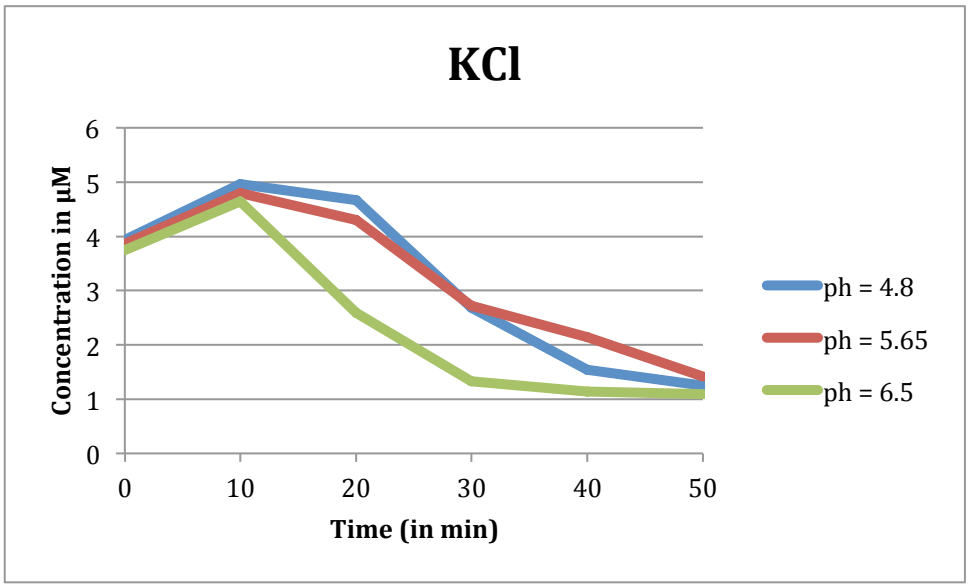

Figure 6: Phosphate concentration with the influence of potassium.
N. Da Mutten, 2013

The highest pH-value (6.5) causes the most rapid reduction in phosphate. In this way, 85% oft he phosphate was already taken up while with the other two pH-values the amount was only at 38%.

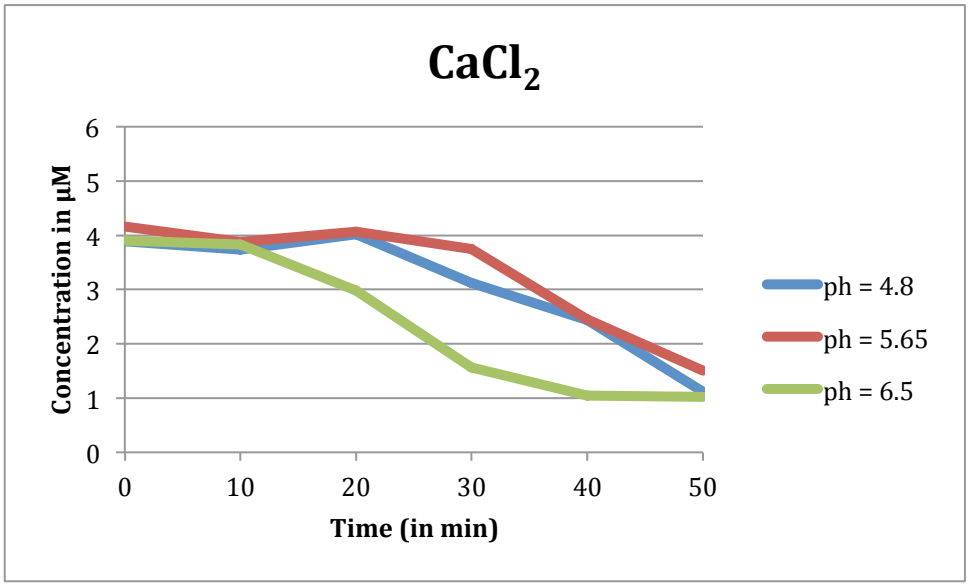

Figure 7: Phosphate concentration with the influence of calcium.
N. Da Mutten, 2013

The experiment indicated that the yeast cells with calcium and the pH-value of 6.5. 80% oft he phosphate was taken up during the first 30 minutes.
The values of the buffer with pH 4.8 are a lot lower (20% uptake) and the pH 5.65 (5% uptake)

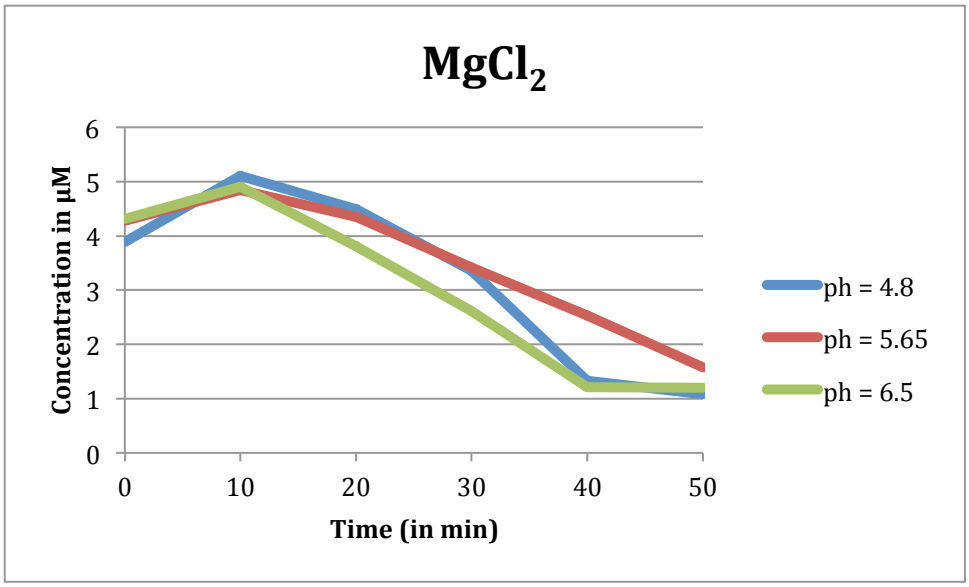

Figure 8: Phosphate concentration with the influence of magnesium
N. Da Mutten, 2013

What is noticeable with magnesium is that the phosphate concentrations lie close together than with other salts, though the concentration declines at the highest pH value (6.5) at the fastest rate. The uptake is at pH 6.5 is 75%, at pH 4.8 and pH 5.65 it is 60%

The decline of phosphate in the yeast solution without any salt at all was the lowest (Figure 4). The rate of uptake 72% was achieved at pH 4.8, 62% at pH 6.5 and 13% at pH 5.65. This

exception, that the rate of uptake of the pH 4.8 is higher than the one at pH 6.5, can be traced tot he deviant result, as the following values show a more expedient course.

4 Discussion

4.1 Potassium

The results show that potassium had the predicted effect on the uptake of phosphate in yeast. Next to the important roles potassium has in various cell functions [20], potassium is needed to protect the cells from the ammonium toxicity [21]. Mary Hoff conducted an experiment in 2006, looking at the influence of different levels of ammonium and potassium in the reproduction of yeast. The findings showed that the number of yeast, which had been potassium-deprived, in an environment with high levels of ammoniums decreased drastically. This suggests that "ammonium was toxic to yeast when potassium was limited". [21]

Another positive effect of potassium in yeast is the enhanced utilization of the present glucose in yeast, as the John Muntz described [22]. Glucose is the main source of energy for yeast, therefore an increased amount of glucose that is used by the yeast, means more energy, which means faster reproduction and finally leads to an increased uptake of phosphate.

On the other hand, there are things that do not correspond to our hypothesis. The potassium curve of pH 4.8 and 5.65 did not show mentionable changes, as with the pH6.5, even though Rothstein and Goodman took 4.8 to be the optimum pH with the addition of potassium. This can be explained as follows:

The transport of potassium is not a one-way process. Potassium can be taken up or extruded, depending on the intracellular conditions. The overall mechanism for flux of potassium into and out of the cell has developed a way to regulate the amount of potassium that gets transported both ways. While Trk1 is mainly responsible for the influx, the anti-porter Nha is the main extruder of K^+ [20]. One condition where Nha1 is more active is under acidic environmental conditions [23]. This is precisely what the results show: under a low pH the positive effect of the added potassium is limited because it could not be taken up by cell and therefore not support the uptake of phosphate. The values of the experiment with pH 4.8 and 5.65 show little distinction from the values of the control-group-experiment at the same acidic conditions.

In their natural habitat, the occurrence of potassium is usually quite low. Due to the high demand for it, its anti-porter Nha1 is not activated. In the conducted experiment, the habitat founded for the yeast does not correspond with the natural composition of ponds and lakes found outdoors, since the concentration of K^+ was deliberately enhanced to a level not present in natural waters; the exact condition at which Nha1 has been shown to be involved in K^+ efflux through the plasma membrane. [24]

Moreover the flux of potassium is a never resting process. "About two-thirds of the K^+ taken up is returned to the external medium through the K^+ efflux system(s), suggesting that efficient processes regulate K^+ homeostasis" [24]. Following this, Banuelos et al. (2001) investigated the influence of Nha1p on the in- AND efflux of potassium and found that it had not only the function of extruding potassium but also of the overall regulation. It "improved the growth of S. cerevisiae cells (especially) at low external K^+ concentrations and pH" [24], the exact opposite of the conditions in our experiment. From this we can conclude, that although yeast cells require potassium for fast cell processes, the addition of potassium in the surrounding can also have negative impacts. To optimize the uptake of phosphate, the potassium would have to be inserted directly into the cell, without the effect of an unnatural environment.

4.2 Calcium

The hypothesis, which stated that through the addition of calcium the phosphate uptake will be increased could be validated by the experiment. The results showed that after thirty minutes the group with the salt containing calcium (~1.56 µM) contained less phosphate than the control group (~2.04 µM). Due to imprecision during the measurement process the difference (pH 4.8: ~3.12 und pH 5.65: ~3.75 also 0.62) between the pH-values 4.8 and 5.65 can be neglected. Only the measurement with a pH of 6.5 (~1.56µM) showed a distinct difference from the others. As this pH-value lied closest to the neutral value, it is evident that the biological processes in the yeast cells and therefore also the uptake of phosphate function best in this situation.
The assumption of Olson and Johnson, [12] that a significant growth of the phosphate taken up could be achieved through the increase of the calcium concentration could not be affirmed by the experiments. In comparison to the control group more phosphate was taken up during the calcium experiment with pH 6.5 than with other investigated pH-values after the benchmark of thirty minutes. The results could however not demonstrate that there was a distinct increase in the uptake of phosphate. This can be traced back to different possible causes. In their work, different concentrations were tested, but this experiment was only to determine the best possible pH-value.

4.3 Magnesium

It is true that the uptake of phosphate is increased by the addition of magnesium-ions. However, yeast cells take up magnesium only in certain conditions. This might be the reason why magnesium did not enhance the uptake of phosphate compared to the control group.

It is known that 28 degrees are the ideal temperature for the magnesium uptake by yeast cells1, yet the experiment took place at room temperature. Also, the concentration of magnesium influences the increase in uptake. The optimal magnesium concentration is said to be between 0.24- 0.48 g/L. [25] The magnesium concentration in the experiment was 0.97 g/L.

Additionally, phosphate-saturated yeast cells are not affected by the divalent cation Mg^{2+}, whereas the cells, which are phosphate starved, are stimulated by Mg^{2+} and the amount of phosphate intake increases. [26] It was assumed that the yeast cells used in the experiment were already saturated.

As shown in the graph, the more neutral the pH-value was, the more favorable was the magnesium uptake and consequently the phosphate uptake. This might had been caused by the easier formation of connection between free electron pairs of ligands of yeast cell wall and Mg^{2+} ions with a lower concentration of H^{+} ions. [25]

4.4 Evaluation

During the evaluation of the elements potassium and magnesium, an increase in the phosphate that was present in the solution from the first to the second measurements can be identified. This, however, was not possible, as the yeast cells were not capable of producing phosphate.
As the results of the second measurements were prepared at the same time for the photospectrometric evaluation, it is most probably that a mistake was introduced at this point in the process.
For example, it could have been possible, that during the mixing in the centrifuge the solution, which still contained yeast, a mistake occurred involving the timing, or that the centrifuge was shaken in such a way that the yeast once again mixed with the supernatant. This would have

led to an error during the measurement of the translucence of the solution on the basis of a turbidity caused by the yeast.
All other deviations however could not be properly analyzed, as the complex work process offered a lot of room for such errors.
It might also be possible that as the different substances were pipetted after being mixed in the centrifuge a part of the yeast was sucked up and that the same mistake described above occurred again, but in this case merely for a singular measurement. An other explanation could be that a single pipette was adjusted wrongly and a result was therefore falsified.

4.5 Improvements

The most effective way to increase the significance of the obtained results is certainly by repeating the experiment multiple times. Subsequently, enough data would be accumulated in order to be able to mathematically determine the significance of the results. Additionally, the range of pH could be enlarged and the difference between selected pH could be narrowed. A variation of the concentration of the added cations could also be tested.

A higher precision could be reached by periodically checking the pipettes to confirm their settings. Moreover, one could perform the experiments in an environment where all optimal conditions can be reached. Individual optimizations in the experiments can also be achieved, for example potassium could be inserted directly into the cell, to avoid the effect of an environment supersaturated with potassium.

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Activity List

Canora, Konstantin:	preparing yeast solution for measurement and experiment, leading experiment
Da Mutten, Nicolas:	photographer, layout, measuring, straight calibration
Galm, Roman:	research, hypothese caltium
Hasler, Lukas:	research, hypothese magnesium
Krüger, Constantin:	research, hypothese caltium, hypothese magnesium, introduction
Lüthi, Stephanie:	research, hypothese potassium, language, introduction
Marti, Dominic:	research, measuring
Moser, Dave:	research, methodology, measuring
Müller, Livia:	research, hypothese potassium, introduction
Sriram, Ravina:	research, hypothese magnesium, introduction
Ungricht, Christoph:	research, hypothese magnesium
Watkins, Dylan:	straight calibration, language
Wick, Cyrill:	preparing yeast solution for measurement and experiment, leading experiment

Every student contributed a part of the written text.

Figure 9: The class working on the experiments. N. Da Mutten, 2013

Figure 10: One of the students. N. Da Mutten, 2013

Figure 11: Another student. N. Da Mutten, 2013