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# Task 2

## Phosphate uptake by yeast cells



Class 14gG

Gymnasium Thun Schadau

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## 2 Abstract

The second task of the science on the move contest is about the efficiency of the uptake of phosphate by yeast cells. At the beginning, very little knowledge about this topic was there. The goal of this report is to figure out the “normal” rate of phosphate-uptake by yeast cells and then to develop three methods to improve the efficiency. This report investigates how the addition of glucose, the addition of sodium, as well as the increase/decrease in pH influences the yeast cells. The final solutions are measured by a photo spectrometer, whose output shows the amount of phosphate remaining in the solution.

This creation of the solutions is done by adding different amounts of the investigated variable to the phosphate buffer. In order to increase the reliability of the data collected, the experiments were conducted twice.

Unfortunately, the data collected showed errors, due to the problems we had during the procedure of the experiments. Yet still, a conclusion was able to be done: none of the investigated variables illustrated an optimization of the uptake of phosphate efficiency. The hypothesis was proven to be wrong.

### 3 Basic Information about phosphate uptake

#### 3.1 Why is phosphate important for all organisms?

Phosphate is an essential substance for living organisms. It is used as a particle in deoxyribonucleic acid (DNA) and in adenosine triphosphate (ATP) as well. The DNA is a macromolecule on which the genetic code is written. A phosphate ester bridge is responsible for combining the double helix of the DNA.<sup>(1)</sup>

The ATP molecule is used as an energy storage. When needed, it breaks off one of its three phosphate and releases lots of energy a cell can use for specific function, such as moving or starting a chemical reaction.<sup>(2)</sup>

#### 3.2 What is well known about phosphate uptake in yeast cells?

There are two different transport systems responsible for the uptake of phosphate into the yeast cells. In one of them, Sodium ( $\text{Na}^+$ ) regulates the phosphate uptake, while the other mechanism is  $\text{Na}^+$  independent. At a pH 7.2 the  $\text{Na}^+$ -dependent mechanism is stimulated and the affinity for the transport of phosphate increases, while the affinity for the  $\text{Na}^+$ -Ions decreases.<sup>(3)</sup>

In the  $\text{Na}^+$ -independent transport system, two positive hydrogen atoms ( $\text{H}^+$ ) flow into the cell, while one potassium atom ( $\text{K}^+$ ) flows out for each phosphate ion assimilated. The  $\text{Na}^+$ -independent transport mechanism is stimulated at a pH 4.5 and inhibited above pH 7.2. Therefore, it is blocked when the  $\text{Na}^+$ -dependent is stimulated.<sup>(4)</sup>

Aside from that, the phosphate uptake can also be stimulated by a concentration under 100mmol/L of 2-deoxy-D-glucose (2-dGlc), but at a concentration over 100mmol/L the uptake is inhibited.<sup>(5)</sup>

#### 3.3 How do yeast cells store phosphate?

A yeast cell is able to store phosphate and also polyphosphate in its inside. A major storage for phosphate is the vacuole of a yeast cell. The phosphate is stored in this vacuole in so called vacuolar pools, which are either enlarged or used up, depends on how much phosphate is available for the cell in the moment. Like this the cell is able to regulate the amount of phosphate.<sup>(6)</sup>

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

Microorganisms are very important for the soil phosphorus cycle as they make phosphorus available to plants. There is often a shortage of phosphorus in weathered and tropical soils, the prices for phosphorus fertilizers are rising, and plants cannot use phosphorus from soil and fertilizer sources very efficiently even though many soils contain a relatively large amount of total phosphorus. But unfortunately these are only poorly available to plants, which makes it very important to find out more about the effects of microorganisms on plant phosphorus nutrition and to discover opportunities for manipulating specific microorganisms to increase the availability of phosphorus to plants. Moreover, there are no never-ending deposits of high-quality phosphate. It is an attractive

subject for research to find and manipulate microorganisms in order to make them capable of making phosphorus available to plants and therefore develop a more sustainable agriculture.<sup>(7)</sup>

### 3.5 Literaturverzeichnis

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## 4 Calibration of the measuring system

The calibration of the measuring system was done following exactly the steps described in the Science on the Move task sheet.

Conducting the calibration, many difficulties had to be faced. In the first trial, one solution settled and therefore adulterated the absorption spectra. In the second trial, the five minutes waiting were not maintained and once again the absorption spectra showed meanderings of the expected one. After a third trial, in which every assignation was observed, the data of the absorption spectra was much higher than expected. Therefore a fourth trial had to be done. The solutions for the calibration line were diluted at the rate 1:1 with distilled water. Now the indicated absorption line was almost super imposable with the expected calibration line.



Figure 1: The students in the lab conducting an experiment.



Figure 2: The result of the pretest.

#### 4.1 Measuring the phosphate uptake by yeast cells

At first, one test run was done to see what issues might occur and how the measuring technique worked. As a result of this test series, it became clear that the values measured by the spectrometer were too high to still be accurate. The cause of this was found fast: the wrong, unfinished Molybdatsolution was used instead of the finished one. Some further tests were done, checking whether or not the solution measured should be further diluted or not. Because the absorption was still in a range where it could be measured exactly, the solution wasn't further diluted in the second series. Having already some practice and knowing of potential problems, the actual experiment went without problems. The three best runs and their average are represented in the table below.

Time	Run 1	Run 3	Run 4	Average
0	1.450	1.294	1.238	1.327
10	1.662	1.560	1.435	1.552
20	1.427	1.478	1.474	1.460
30	1.095	1.210	1.651	1.319
40	1.157	1.300	1.243	1.233
50	1.179	1.279	1.141	1.200
Standard Dev	0.220	0.135	0.190	0.135
Average	1.457	1.354	1.364	1.391

A graphical representation of the data is found in figure 3. For each point in time, an average of the three runs considered was calculated. This resulted in another graph, shown in figure 4. A best fit line was drawn, indicating an overall decreasing slope. This suggests that the phosphate in the

supernatant had decreased over time. The rather low  $R^2$  value suggests non-linear data that doesn't always follow the overall trend. In this example, phosphate tends to have left the yeast in the first ten minutes. This may be due to the new, liquid environment the yeast cell was put into for the experiment, such that phosphate has left the yeast cell instead of being uptaken.

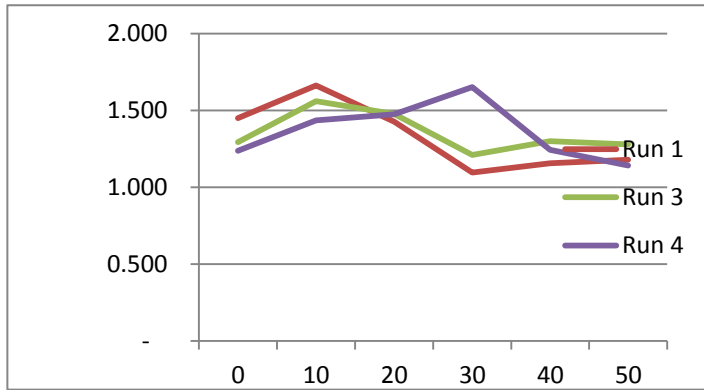


Figure 3: The diagram is showing the results of the change of phosphate in the solution over time.

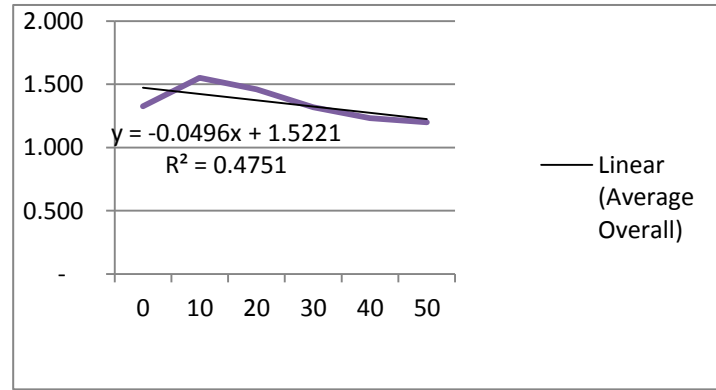


Figure 4: The diagram is showing the decrease of phosphate supported with a line of best fit.



## 5 How to improve the phosphate uptake by yeast cells

The goal was to enhance the efficiency of the phosphate-uptake by yeast cells. As described in Chapter 3.1, Sodium, glucose and the variation of the pH-value influence, among a fair amount of other factors, the phosphate uptake by yeast cells. To find out how exactly these factors influence the phosphate uptake as well as to find where the optimum lies, was the goal of each of the experiments described in this chapter.

### 5.1 Sodium

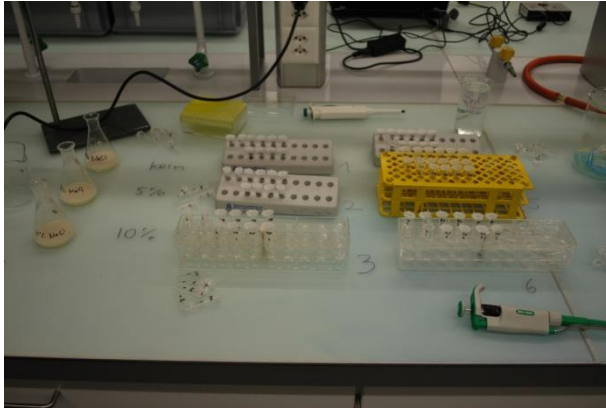


Figure 5: The design of the experiment conducted.

#### 5.1.1 Design of the experiment

##### 5.1.1.1 Defining the problem and selecting variables

The goal of this set of experiments is to figure out whether the amount of sodium in the yeast solution has an influence on the phosphate uptake of yeast cells.

The independent variable in this experiment was the concentration of sodium as it was manipulated and led to the measurement of the dependent variable, which in this case was the uptake of phosphate. The controlled variables included keeping the time intervals, temperature and the preparation of the samples.

##### 5.1.1.2 Controlling variables

A new buffer had to be produced, because the buffer we had gotten already contained sodium. Therefore, a 0.1M  $\text{KH}_2\text{PO}_4$  solution was mixed with a 0.1M  $\text{K}_2\text{HPO}_4$  solution, whereas much more acid was used than base to reach a pH-value of 6.3, like the sodium-phosphate buffer received.

To three solutions, each of which containing 20ml of our buffer, no NaCl, 1g and 2g of NaCl was added. After mixing the particular yeast solutions with the specific amount of NaCl, the yeast solutions were established as in Part 3 but with the new buffer instead of the Sodium-Phosphate-Buffer. Expected was a higher phosphate uptake by the yeast cells.

Strictly following the same procedure as used for the experiment described in Chapter 5 and making our own plan how to proceed, the preparation of the samples was completed as in Part 3 and the keeping of the time intervals could be achieved. Because the entire experiment was conducted in the same room and during a short time period, there were no major temperature changes or other important environmental factors which could have had an influence on the experiment.

### 5.1.1.3 Developing a method for collection of data

After having finished the experiment as it is written in Part 3, the cuvettes were placed in a photo spectrometer at the given time. At the first trial, the absorption values were much higher than they should be according to our knowledge. Therefore, the experiment was repeated whereas this time two series were enforced parallel to ensure the reliability of the data. The solution was diluted with distilled water at the rate of 1:4 (220  $\mu$ l of the solution and 880  $\mu$ l of distilled water). The results had then the values which were expected and the phosphate uptake by the yeast cells with additional sodium could be visualized by creating a graph of the results.

## 5.1.2 Data collection and processing

### 5.1.2.1 Recording raw data

Table 1: The results of the series one of the investigation of the sodium variable are shown in this table.

Series 1	Phosphate		
	no NaCl	1 g NaCl	2 g NaCl
0 min	0.246	0.184	0.149
10 min	0.222	0.211	0.203
20 min	0.360	0.275	0.231
30 min	0.300	0.315	0.288
40 min	0.251	0.340	0.336
50 min	0.181	0.339	0.336

Table 2: The results of the second series conducted about the sodium influence.

Series 2	Phosphate		
	no NaCl	1 g NaCl	2 g NaCl
0 min	0.270	0.405	0.156
10 min	0.308	0.310	0.276
20 min	0.299	0.300	0.270
30 min	0.272	0.350	0.320
40 min	0.210	0.343	0.288
50 min	0.259	0.321	0.274

### 5.1.2.2 Processing raw data

Table 3: The average of series one and two are illustrated in this table. Also the standard deviation is calculated.

Average	Phosphate		
	no NaCl	1 g NaCl	2 g NaCl
0 min	0.258	0.295	0.153
10 min	0.265	0.261	0.240
20 min	0.330	0.288	0.251
30 min	0.286	0.333	0.304
40 min	0.231	0.342	0.312
50 min	0.220	0.330	0.305
Standard deviation	0.040	0.032	0.061

### 5.1.2.3 Presenting processed data

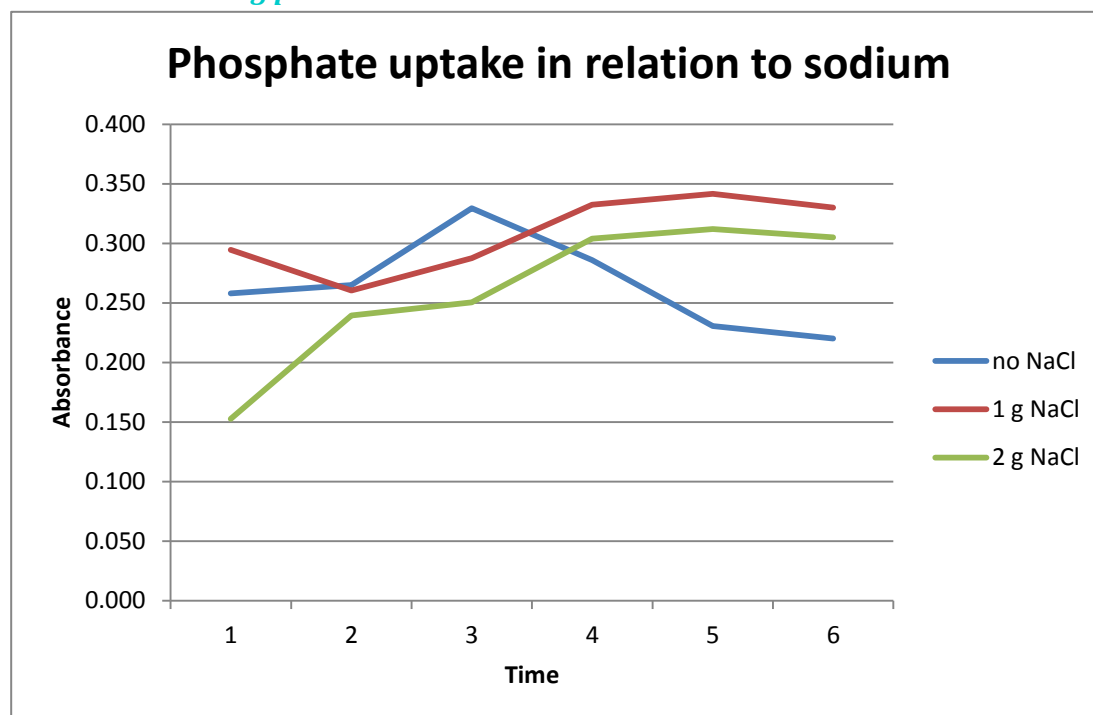


Figure 6: The diagram is showing the amount of phosphate within the measured solutions.

## 5.1.3 Conclusion and evaluation

### 5.1.3.1 Concluding

Considering the curves of phosphate uptake in relation to sodium, there are two mainly ascending trends and one primarily descending trend. It is conspicuous that the phosphate uptake in the solution with 1g and 2g sodium is worse than without additional sodium. In fact, phosphate had left the yeast cells over time, suggesting that the phosphate uptake by yeast cells works best without any additional sodium. After a particular period of time, a decrease of the upwards trend is visible in the curves with 1g and 2g of sodium in the 200ml solution.

One possible explanation for the decrease of phosphate in the yeast cells is that it left the cells due to the osmotic imbalance created by the addition of NaCl. After some time, the balance slowly had been restored, resulting in a flattening of the curves with 1g and 2g of sodium. Another explanation of this could be that the yeast cells start to take up again some phosphate, and that the curve will

start to go down again. It might be of interest whether the curve goes down again, and if it goes down faster than before.

### **5.1.3.2 Evaluating procedures**

The decision to make the experiment by adding additional sodium was based on internet and literature research. Since this self-created experiment was conducted the same way as the experiment in Chapter 5, some practice was already there and maintaining the time target was not a problem.

Thanks to a created plan of procedures assembled before the experiments, the whole process blended nicely.

A weakness of this experiment was that there was only one photo spectrometer available but three groups needed it at the same time. Therefore, the absorption rate could not be measured right after the mixing of the solutions. As a result of that, the absorption values may be somewhat imprecise. The significance of this weakness is apparent in a few big jumps of the absorption listed in **chapter 3**. This experiment was conducted two times, because little faults disturbed the whole process. First the dissolving of the mixed solutions in the cuvettes was forgotten, but the third trial was planned beforehand limiting potential mistakes. Overall, this trial went well.

### **5.1.3.3 Improving the investigation**

A definitive improvement would be, if there were more than one photo spectrometer so that the adulteration because of the time could be avoided. It might also be of interest how the curves behave after the 50 minutes of an experiment. What is also important is to always use fresh yeast to ensure the output data will be accurate. Always keep the abridgment, otherwise it could happen that the wrong solutions are mixed together or that one part of a mixture will be forgotten to add. Moreover, having more time to conduct this project would have definitely had more accurate results as a consequence.

## 5.2 pH-Value

### 5.2.1 Design of the experiment

#### 5.2.1.1 Defining the problem and selecting the variables

Is there a variation of the results of the uptake of phosphate by yeast cells if the pH-value is changed?

The independent variable is the change in pH and the dependent variable is again the amount of phosphate taken up by the yeast cells. The controlled variables are the same as mentioned in Part 5.1.1.1

#### 5.2.1.2 Controlling variables

Two solutions had to be mixed: 10ml 0.5 mM  $\text{Na}_2\text{HPO}_4$  (Base) and 10 ml 0.5mM  $\text{NaH}_2\text{PO}_4$  (Acid). To get the six solutions with the different pH-values we mixed this to solutions to get the buffer. The more acid is in, the lower the pH, the more base is in, the higher the pH. The acidity was measured with a pH meter. Chosen pH-values were 5, 5.5, 6, 6.5, 7 and 8.

After mixing the new buffers, the solution was mixed with yeast and glucose as it is described in Part 3.

The further process of the experiment was exactly the same as in Part 3. The circumstances like temperature were held constant as in Part 5.1.

#### 5.2.1.3 Developing a method for collecting data

To make sure that all possibly influencing pH-values are taken, steps of half a pH were made between the pH-values that had the biggest difference in respect to the uptake of phosphate. Only one series was made, due to the lack of Molybdat-Solution and Malachitgreen-Solution. The time needed to do one series played a big role such that we decided to do six different pH-values without control series.

The results when measuring them with the photo spectrometer were too high, so a dilution was made. But only with a rate of 1:3 and not 1:4.

### 5.2.2 Data collection and processing

#### 5.2.2.1 Recording raw data

Table 4: The raw data of the first series is represented by this table.

Series 1	Phosphate		
	pH 6	pH 7	pH 8
0 min	0.539	1.68	2.012
10 min	2.395	1.97	1.989
20 min	0.485	0.521	0.572
30 min	0.625	0.52	0.55
40 min	0.565	0.435	0.48
50 min	0.462	0.515	0.456

The results of the first series are presented in table 4.

Table 5: The measurements of the second series about the influence of the pH is listed in this table

Series 1	Phosphate		
	pH 5	pH 5.5	pH 6.5
0 min	0.55	0.35	0.586
10 min	0.638	0.479	0.692
20 min	0.43	0.455	0.521
30 min	0.62	0.481	0.655
40 min	0.565	0.504	0.586
50 min	0.632	0.406	0.613

The results of the first series are presented in table 5.

### 5.2.2.2 Processing raw data

Table 6: This table shows the average between the values of series one and two. It also shows the standard deviation and the median of the different runs conducted.

Average	Phosphate					
	pH 5	pH 5.5	pH 6	pH 6.5	pH 7	pH 8
0 min	0.55	0.35	0.539	0.586	1.68	2.012
10 min	0.638	0.479	2.395	0.692	1.97	1.989
20 min	0.43	0.455	0.485	0.521	0.521	0.572
30 min	0.62	0.481	0.625	0.655	0.52	0.55
40 min	0.565	0.504	0.565	0.586	0.435	0.48
50 min	0.632	0.406	0.462	0.613	0.515	0.456
Standard Deviation	0.07182792	0.05255923	0.69512886	0.05450204	0.63194288	0.70162963
Median	0.5925	0.467	0.552	0.5995	0.5205	0.561

To show the differences between the pH-values, the median (average) was taken. The standard deviation was taken as well. The results are shown in table 6.

### 5.2.2.3 Presenting processed data

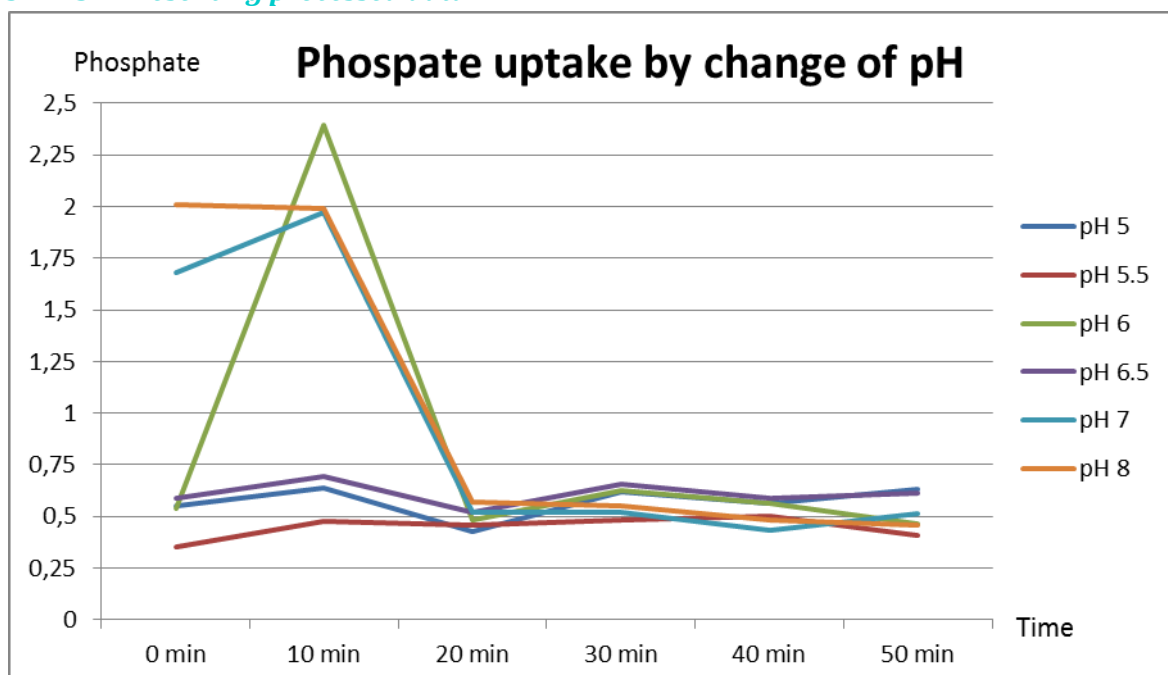


Figure 7: This diagram presents the change in concentration of phosphate in relation to the time.

The line graph was chosen because it shows best that the differences are minimal, seen in figure 7.

Unfortunately, there are a couple of errors. The values of phosphate uptake should steadily decrease, whereas the values of the two series once decline and then again increase. The increase of phosphate is not possible. The phosphate should be taken up by the yeast. As a possible reason the yeast may have given off some phosphate or there were simply mistakes made during the procedure. The high values marked red are a lot higher than they should be. This may be because of a mistake of the diluting proportions.

### 5.2.3 Conclusion and evaluation

#### 5.2.3.1 Concluding

Series 1 with the incredible high values of pH 6, 7 and 8 on the beginning cannot directly be compared with series 2, where there are none of these high values. The standard deviations of pH 6, 7 and 8 are close to each other and the same with the standard deviations of pH 5, 5.5 and 6.5. If there was made a control series, the differences would have been crossed out because they were generated by a mistake during the dilution. Without these high values, the standard deviations would be about the same.

In the estimated experiments it is best with a pH about 5.5 and the difference between pH 5 and 5.5 is there. Even though some errors it can be said that there is a tendency that the uptake of phosphate by yeast cells is affected by the pH of the surrounding area. But the differences are quite small. The standard deviation supports this. The hypothesis that the uptake of phosphate by yeast cells was not proven to be wrong or right. The tendency could be proven as right by repeating the experiments.

#### 5.2.3.2 Evaluating procedures

One of the major problems with the developed technique for the experiments was the lack of time. To save some time 3 experiments were made at the same time, which led to a stressful operation and therefore mistakes. Two students worked together, trying to optimize the time-result proportion. Because there were three developed experiments (Na-influence, pH-influence and glucose-influence) the series had to be done one after another due to a lack of students and equipment (mostly pipets), which cost time.

#### 5.2.3.3 Improving the investigation

The just mentioned problems could be solved by having more students included, more equipment and especially more time. With this the experiments could be repeated until the results look always the same and errors could be minimized. This would lead to a better conclusion due to clearer results.

## 5.3 Glucose

### 5.3.1 Design of the experiment

#### 5.3.1.1 Defining the problem and selecting variables

Another approach of how to improve the phosphate uptake of yeast cells is established. As written in Part 1, the phosphate uptake of yeast cells can be controlled by the amount of glucose in the environment. So for this experiment, the independent variable is the glucose contained in the buffer. The dependent variable as always is the amount of phosphate taken up by the yeast cells. The controlled variables are the temperature, which in this case is room temperature, the time between the different measurements, as well as the amount of phosphate and yeast put into reaction.

#### 5.3.1.2 Controlling variables

For this experiment, three experiments have been launched at the same time. One buffer is containing 5%, so 0.5g, of glucose instead of just 1% as it was done in Part 3. The second experiment contained 10% (1g) and the third one was conducted containing 20% (2g). Everything else was done exactly the same way as described in Part 3. The amount of glucose was purposely chosen not to exceed a concentration above 100mmol/l, since glucose starts to inhibit further uptake from there.

The dependent variable is again measured with the photo spectrometer. The solution had to be attenuated, because the photo spectrometer showed a value of 3.000, which is the maximum value it can measure. It has been attenuated with distilled water on a scale 1:3 whereby the 1 represents the phosphate-yeast solution. The resulting values were directly put into an excel table.

The variable temperature is controlled by the experiment never leaving the room. So in other words, all the experiments were conducted inside the same laboratory. The variable time was controlled by the students and an alarm clock, which told them when to start pipetting the solutions. This also counts for the amount of yeast and phosphate. The students always took exact measurements with the help of scales and pipettes.

#### 5.3.1.3 Developing a method for collecting data

In order to ensure the reliability of the data collected in the first series, a second one has been conducted. A third one was planned, the time played against the experiment though. So in the end only two series have been done.



## 5.3.2 Data collection and processing

### 5.3.2.1 Recording raw data

Table 7: The results of the first series of the investigation of the glucose variable are listed in this table.

Series 1	Phosphate		
	0.5 g Glucose	1 g Glucose	2 g Glucose
0 min	0.418	0.403	0.387
10 min	0.404	0.401	0.327
20 min	0.379	0.309	0.416
30 min	0.303	0.298	0.316
40 min	0.270	0.297	0.295
50 min	0.216	0.203	0.268

The results of the first series are presented in table 7.

Table 8: The results of series 2 of the glucose variable is illustrated with this table.

Series 2	Phosphate		
	0.5 g Glucose	1 g Glucose	2 g Glucose
0 min	0.413	0.457	0.406
10 min	0.399	0.358	0.372
20 min	0.359	0.344	0.377
30 min	0.367	0.357	0.370
40 min	0.322	0.320	0.336
50 min	0.249	0.278	0.306

The results of the second conducted series are presented in table 8.

### 5.3.2.2 Processing raw data

Table 9: The average was taken of the series one and two of the glucose experiment and listen in this table. There's also the calculation of the standard deviation.

Average	Phosphate		
	0.5 g Glucose	1 g Glucose	2 g Glucose
0 min	0.416	0.430	0.397
10 min	0.402	0.380	0.350
20 min	0.369	0.327	0.397
30 min	0.335	0.328	0.343
40 min	0.296	0.309	0.316
50 min	0.233	0.241	0.287
Standard Deviation	0.069	0.064	0.044

In order to make this clearly arranged as well as to cross out any imprecision, the average between the data of the series 1 and series 2 was taken. The standard deviation is calculated as well. These results are shown in table 9.

### 5.3.2.3 Presenting processed data

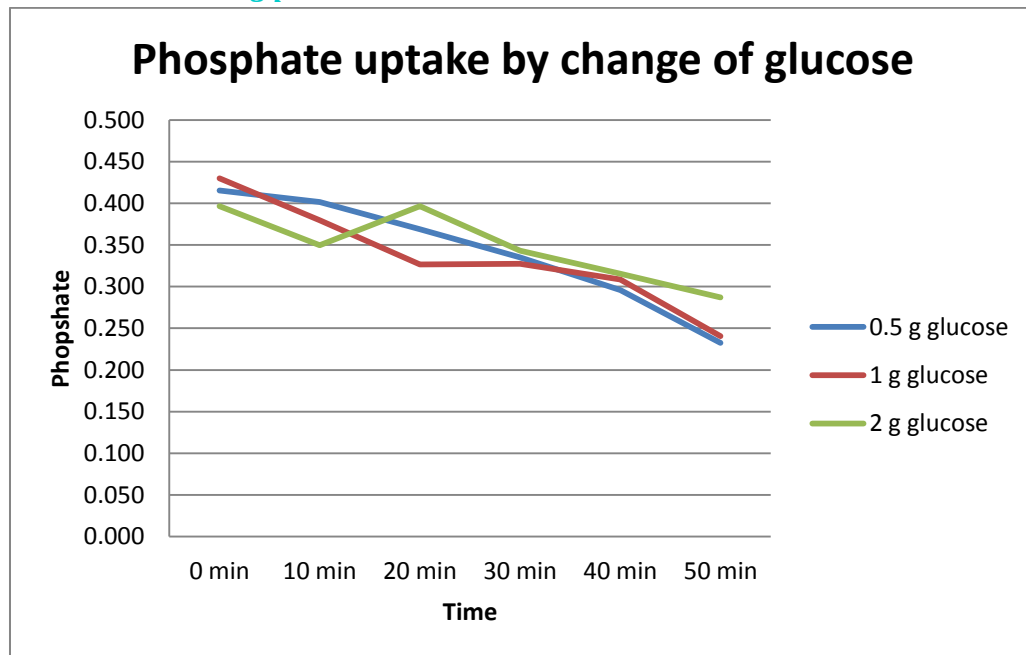


Figure 8: The diagram shows the decline of the concentration of phosphate in the treated solutions.

The line graph was chosen because it perfectly illustrates the decline of the phosphate inside the solution. It directly compares the different experiments with each other.

There are though, unfortunately, a couple of errors within the collected data. The increase of phosphate over time, as it happens in the red marked places in table 7 and table 8, is simply impossible. It has to be assumed that mistakes with the controlled variables were made.

### 5.3.3 Conclusion and evaluating

#### 5.3.3.1 Concluding

Even though there are a couple of imprecisions, one can still make the observation that the data from the different experiments do not differ a lot from each other. This observation is supported by the values of the standard deviation in table 9. In other words, the hypothesis, which said that the adding of glucose to the phosphate increases the uptake of the latter by yeast cells, is proved to be wrong.

#### 5.3.3.2 Evaluating procedures

The major problem with the developed technique for the experiments was the lack of time. Usually, two students worked at one experiment. For example, they started the glucose experiment with all three runs (0.5g, 1g and 2g glucose) and five minutes later they launched the second series with three runs. That was to make up for the lacking time. It was no rarity that this ended in a stressful situation due to time delay, which caused uncertainties and mistakes. Another rather significant source of errors was the pipetting. It occurred that the pipettes were pulled out of the liquid too early, causing the pipette to soak air into the tip. This decreases the amount of liquid the pipette is supposed to contain, which, obviously, falsifies the controlled variables.

### ***5.3.3.3 Improving the investigation***

All the in the upper Aspect declared problems can be solved by allowing extra time. This includes launching the experiments separately. This would then take way more time, but the results would definitely be more accurate and a better conclusion could be made.

## 6 Activity list

Who?	When?	How long?	What?
Rebekka, Cyril, Ramona, Nadja, Matthias, Manuel, Marcel	4/23/13	90min	Research to part 1
Rilana, Shalin, Luca, Romano	4/23/13	90min	Finished Molybdat-Solution, Pretest, created the calibration line (unsuccessfully)
Rilana, Shalin, Romano, Luca	4/24/13	180min	Created the calibration line (successfully)
Nadja, Ramona, Cyril	4/24/13	180min	Measured the phosphate uptake by yeast cells (unsuccessfully)
Manuel, Matthias, Marcel	4/24/13	90min	Research to part 1
Nadja, Ramona, Cyril, Matthias, Marcel, Rebekka	4/26/13	90min	Measured the phosphate uptake by yeast cells (successfully)
Rilana, Shalin	4/26/13	90min	Research to create our own experiments, writing parts of part 2
Entire class	5/08/13	180min	Conduct part 4 (unsuccessfully)
Matthias, Shalin, Ramona	5/09/13	180min	Part 4 – Sodium (successfully)
Luca, Marcel	5/09/13	180min	Part 4 – Glucose (successfully)
Rilana, Nadja	5/09/13	300min	Part 4 – pH-value (successfully)
Romano	5/09/13	180min	Assisted with the photo spectrometer
Entire class	5/15/13	180min	Writing all the parts of the report
Shalin, Ramona, Marcel	5/16/13	150min	Continuing writing the different parts
Shalin, Ramona	5/16/13	120min	Put report together
Matthias, Ramona	5/16/13	60min	Read through report and correct it
Matthias	5/16/13	180min	Formatting report



Figure 9: The class 14gG conducting the experiments.



Figure 10: the class 14gG conducting another experiment.