Uptake of phosphate by yeast cells

Task 2

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Uptake of phosphate by yeast cells

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1 Studying the literature

1.1 Why is Phosphate important for all organisms?

Every metabolic action needs energy. This energy is provided by the ATPs (adenosine phosphates). The highly energized bonds of ATPs are derived from phosphate (Neligan, 2001). This is very important for actions such as muscle contractility, neuronal transmission or electrolyte transport. Phosphate also is one of the most important blocks for many essential intracellular compounds for example, enzymes or nucleoproteins. Phosphate also plays an important role as intracellular buffers for acids, particularly for volatile acid (University of Connecticut, 2006). But Phosphate is not only important for actions within the organisms it`s also the structural of bones and teeth as the hard dense enamel of mammalian teeth consists of a compound containing Phosphate (AZoM.com, 2001).

1.2 What is well known about the Phosphate uptake in yeast cells?

The cell membrane of the yeast cells consists of two protective layers. In these layers embedded carrier proteins provide transportation systems for active transport through the membrane (The Science of Yeast: Homebrew Science , 2002). The uptake of phosphates from outside the yeast cells is mediated by such transportation systems. One such system for example, has a high affinity for phosphate and is encoded by the *PHO84* gene. Other systems include a sodium/phosphate cotransporter and have a low affinity for phosphate (The Journal of biological Chemistry).

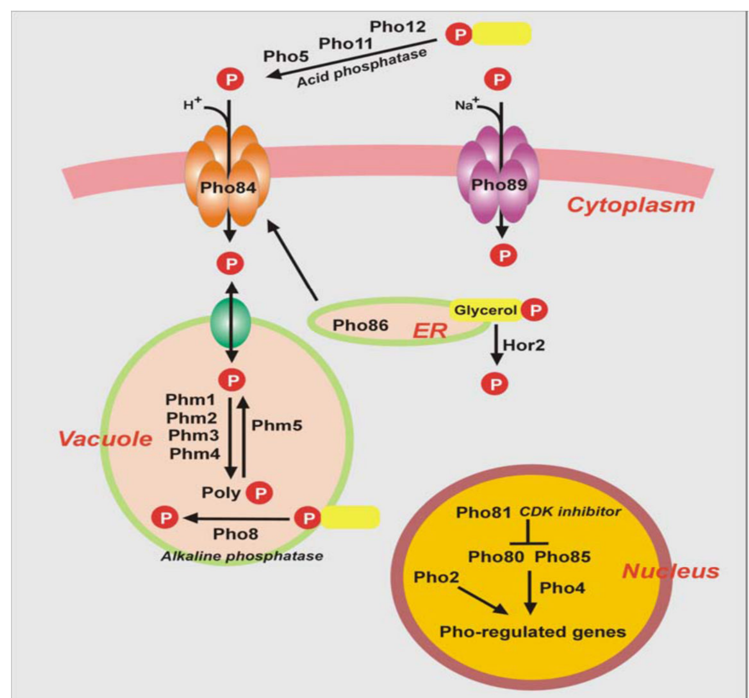

Fig. 1: Phosphate uptake and storage in yeast cells (Universität München).

1.3 How do yeast cells store Phosphate?

The vacuole of the yeast Saccharomyces cerevisiae (baker`s yeast) is the major storage compartment for phosphate. Therefore most phosphates are stored in the cell vacuole. There has also been measured phosphate transport through the vacuolar membrane, what proves this theory (Chemistry, 2013). In yeast cells the concentration of free phosphate is always kept very low, since most phosphates in yeast cells are bond in enzymes or nucleoproteins such as ATPs (Universität München).

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

Microorganisms are a very important part of the phosphorus cycle. Plants convert phosphorous to its organic form, and bacteria convert it back to the inorganic form through decomposition and make it available for plants again (Boundless). The process of making converting the phosphorus is called mineralization (Agriinfo, 2011). Without the phosphate uptake by microorganisms the phosphorus cycle would not work because the plants could not take up the phosphate. Neither could herbivore because they get their phosphate from the plants they eat. Since humans are also part of the cycle we couldn`t get the phosphates we need either and just as all the other organisms we would die.

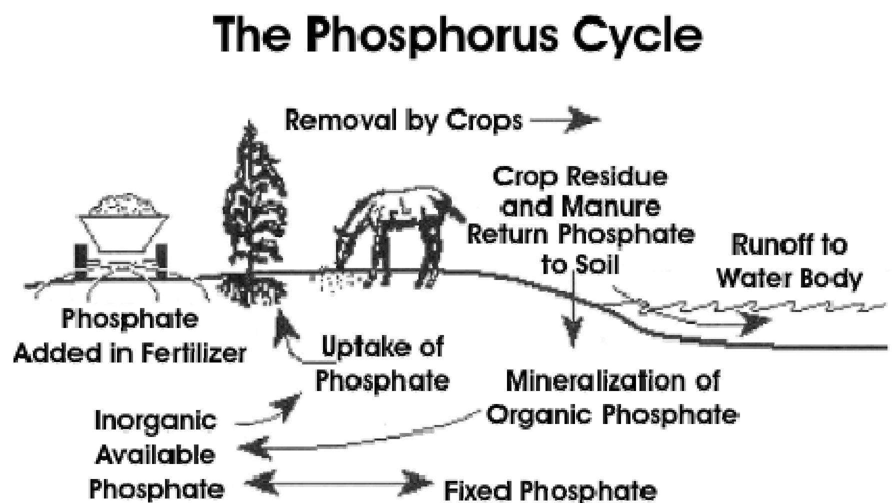

Fig. 2: Phosphorus cycle (U.S. Environmental Protection Agency, 2012)

2 Material and methods

2.1 Experiment

Our hypotheses are that the yeast cells will take up more phosphate, if we change de circumstances around the cells. So we changed the temperature (Q10), the Glucose concentration and the pH value and watched the solutions.

First we made two pretests in which we found out that one of the Molybdat-Solutions was polluted. Because of the pollution of the solution we didn't use it anymore in our experiment. After these pretests we made a straight calibration curve using a BMS SPEC5000 Spectrophotometer. We measured the absorbance and transmission of our phosphate solutions. We measured the absorbance because we would like to analyze the quantity. With the absorbance spectrum you see the proportionality of the concentration. But it is possible to convert the data from absorbance to absorption with the suitable formulas. (Beer-Lambert law)

We always measured at a wavelength of 595nm. We also prepared a medium solution (0.5nM Sodium-Phosphate-Buffer pH 6.3, 1% Glucose). After we made the medium solutions we prepared yeast solutions and measured the phosphate decline in them. To prove our hypotheses we changed the circumstances mentioned before and measured the phosphate uptake again as we did before.

2.2 Spectrophotometer

A Spectrophotometer is an instrument used to determine the intensity of the wavelengths transmitted by a solution. This provides a method for measuring the amount of phosphate in our solutions. The spectrophotometer we used had a wavelength range from 335-1000nm. As a light source the BMS SPEC5000 spectrophotometer had an inbuilt halogen Tungsten lamp. (20W/12V). The Photometric accuracy of this device is 1%. To measure the absorbance of a solution we first had to set the wavelength. Then we switched the Spectrophotometer to absorbance mode. Afterwards we had to adjust 0% (A) and set the sample for measuring.

Fig. 3: Spectrophotometer (Medicalexpo)

3 Pretest

We worked in two independent groups and each group made one run. After mixing the Molybdat-Solution with sulphuric acid, the complex from one group turned green and the other complex yellow. The reason, why this occurred must have been the contamination with phosphate in one system, according to the script. To prove our assumption, we exchanged our Molybdat-solution with the other group. After repeating the second part of the pretest, we observed again, that one solution turned green and the other yellow.

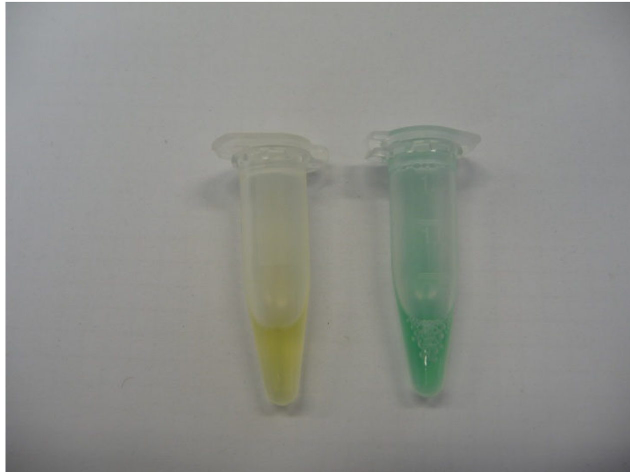

Fig. 4: Pure and contaminated Molybdat-Solution

Fig. 5: "Chem-Box"- equipment

Fig. 6:Lab-environment

4 Straight calibration

We had a lot of difficulties. First, we were very confused by the pretest because one of them was polluted with phosphate and we believed that it was our mistake. But when we made the test again and it showed us the same result. So we concluded that we got a polluted Molybdat-Solution. After doing this we started making the calibration curve. This task took us a lot of time because we had to do it more than three times. Every time there was phosphate in the samplings. It was also there where it should not have been (in the dest.-water).

After many tries we had clean distilled water and got some results which seemed logical and pretty similar. The figures show our three best results. We do not have the standard deviation because we did not know how it works and we had not enough time for understanding it.

The measured data we got over a concentration of 40 mikroMol was not in-line with the results of measurements at lower concentration. The straight calibration line decreased after the concentration limit of 40 mikroMol. The reason of this irregularity is that when the concentration is high, the gaps between the particles are very narrow and they interact. The Beer-Lambert law is not longer applicable then. (Otto, 2011)

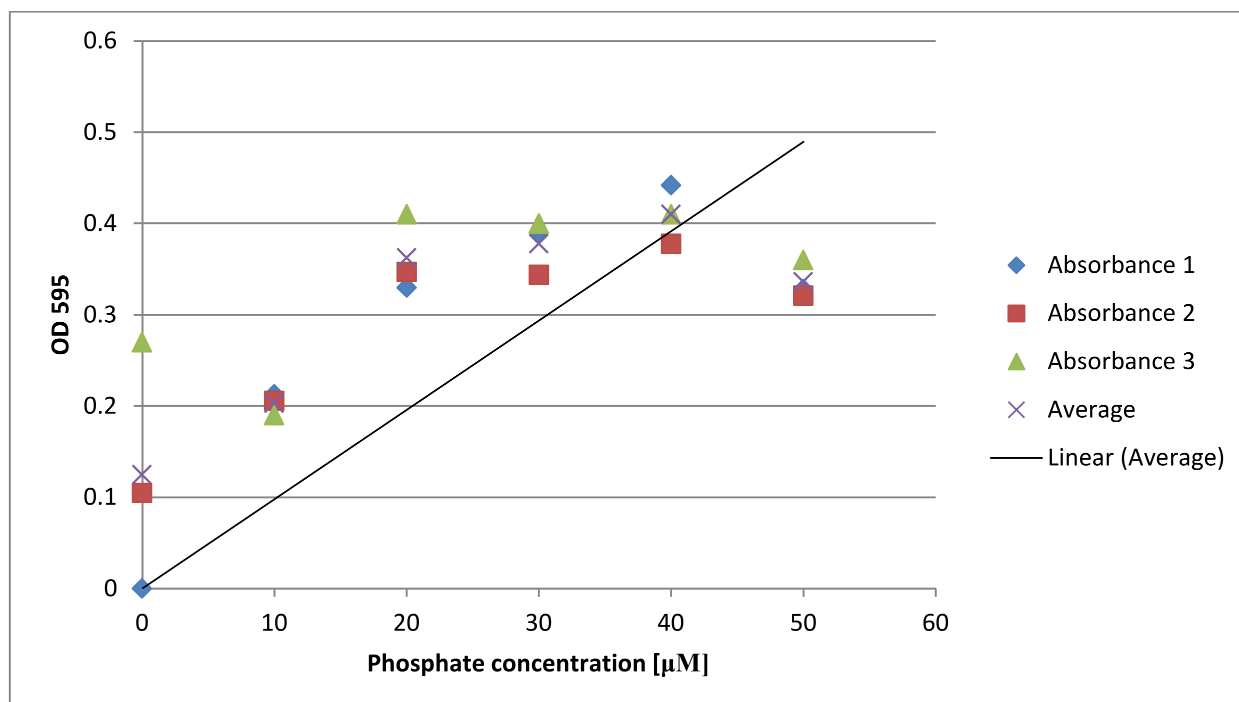

Fig. 7: Calibration curve (1, 2 and 3) with the average and the linear average

5 Measuring the phosphate uptake by yeast cells

5.1 3A Preparing the yeast solutions picking up phosphate over time

First we prepared the solution with 1% Glucose and with 0.5mM Sodium-Phosphate-Buffer. The solution was based on water. We made a mistake. We had not noticed that there is also phosphate in the distilled water of our school. Another problem was that everything we used for the experiment could also be contaminated by Phosphate. Because of the detergent you use to clean the laboratory objects. So this could affect the results. To avoid these problems we used deionised water, which doesn't contain Phosphate, to clean the laboratory objects. We dissolved 1g yeast in the solution. From now on we took 300µl out of the mixture every 10 minutes. We put these 300µl into an EP, which we centrifuged during 1 minute with max speed. We took exactly 40µl of the supernatant and transferred it into the corresponding EP labeled with 'S'. Now the probes were ready for the photometric measurement in 3B.

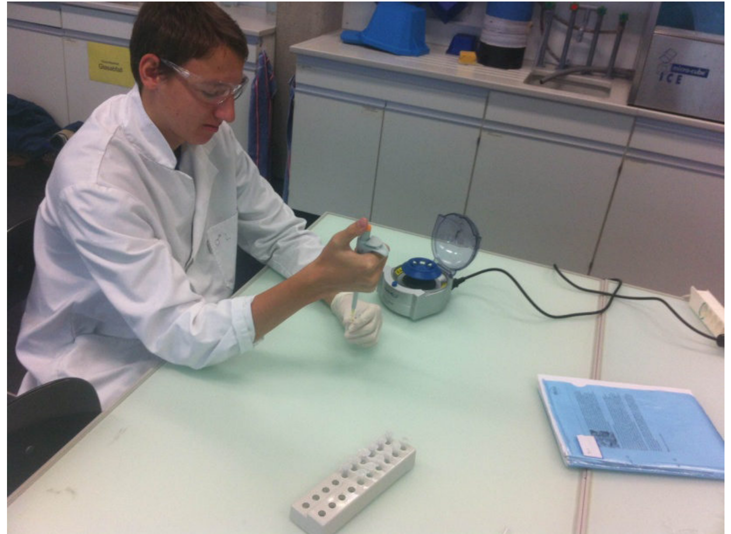

Fig. 8: 40µl of the supernatant

5.2 3B Measuring the decline of phosphate

We obeyed the steps strictly. We had to work in different groups so we could reach enough data material for 3 tables. But we only could use one spectrophotometer because otherwise we would get different results from the different spectrophotometers. We didn't want to lose too much time, so our approach was to work in 2 minutes rhythms. To explain it more detailed: In step 3, 4 and 5 we mixed a new sample every 2 minutes, simultaneously we measured the absorbance and transmission every 2 minutes. Later we decided to skip the transmission –measurements. We had a problem with our time-management, because each sample had to stand aside for five minutes before continuing the process. We attempted to mix and prepare the samples parallel, so the main difficulty was to observe the time exactly. The first time we did that, there was a problem with the solution, which our group (3B) got from the group of 3A. That means we had to repeat our measurements once more. In the end we got a curve and our data points were not in a line. At the end we had an average line that rises slightly with time. Which means that the yeast cells took up more phosphate.

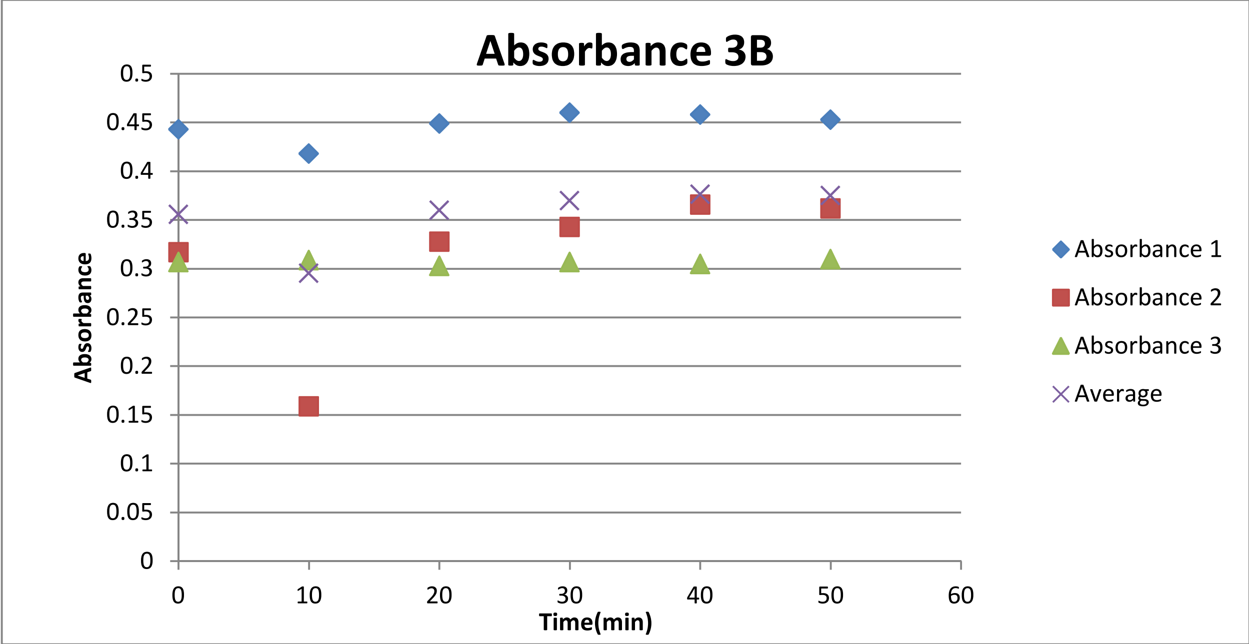

Fig. 9: Absorbance 3B

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

We think that the yeast cells will take up more phosphate, if we change the circumstances around the cells. We changed the temperature (Q10), the Glucose concentration and the pH-value and watched the solutions.

6.1 The absorbance with different glucose concentrations

How does the uptake of phosphate by yeast cells vary with different concentrations of glucose?

For this experiment we prepared two different mediums. One medium includes 0.5mM Sodium-Phosphate-Buffer, pH 6.3 and 1% glucose. We made this medium already in part 3A. The other medium includes 0.5mM Sodium-Phosphate-Buffer, pH 6.3 and 2% glucose. Then we took two 50ml tubes and weighed in each of them 1g fresh yeast. In tube 1 we filled 10ml of the medium with 1% glucose and in tube 2 we filled in 10ml of the medium with 2% glucose. After that we repeated part 3A and 3B with our tubes (1 and 2). We measured the uptake of phosphate in different points of time (at the beginning, after 10 minutes, 20 minutes, 30 minutes, 40 minutes and 50 minutes).

We expected higher values of absorbance when we put in more glucose. But as you can see in the figures below, the absorbance wasn't higher with more glucose (if you look at the average). Unfortunately we just were able to conduct one measurement with 2% glucose, because we had too little Molybdat-Solution.

So we can say that the yeast cells in these experiments didn't take up more phosphate when we raise the glucose concentration

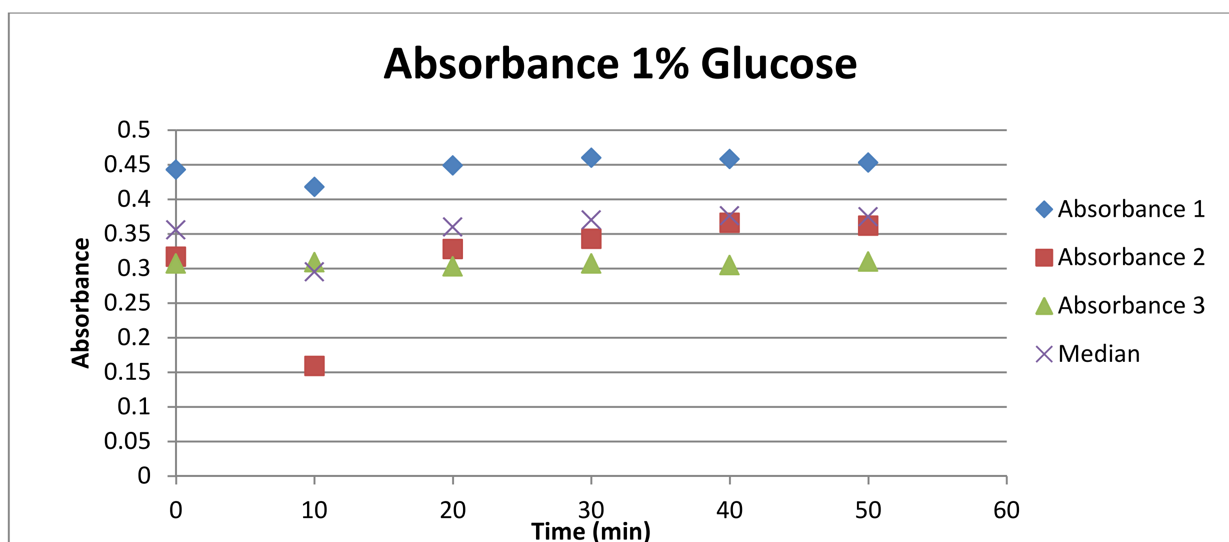

Fig. 10: Absorbance of 0.5mM Sodium-Phosphate-Buffer (pH 6.3), 1% Glucose

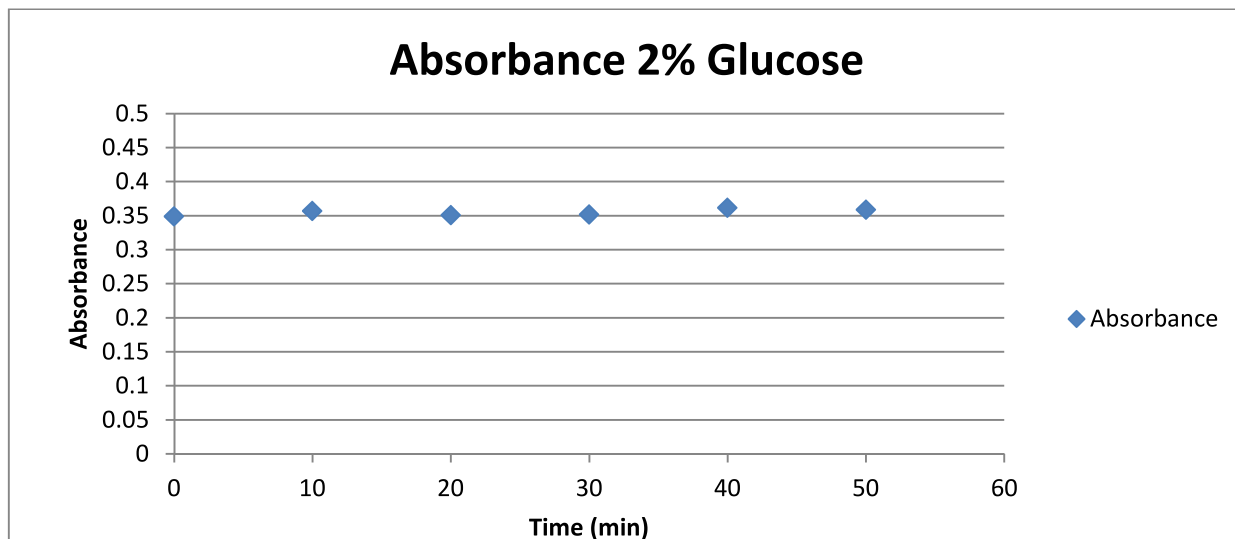

Fig. 11: Absorbance of 0.5mM Sodium-Phosphate-Buffer (pH 6.3), 2% Glucose

6.1.1 Discussion of the results of the glucose experiment

One problem was that the osmotic pressure rises if you put in more glucose. So the yeast cell loses more water because it wants to compensate the different glucose concentrations. As the cell loses water it is in the solution and so the concentration of the phosphate is also lower and we would think that the yeast cell had up taken more phosphate.

6.2 The absorbance with different pH-values

How does the uptake of phosphate by yeast cells vary with different pH-values?

We prepared two yeast solutions, to find out if the pH-value has any implication on the process. In one solution we added caustic soda (NaOH) and to the other hydrochloric acid (HCl). So we achieved for the alkaline solution a pH-value of 9.9 and for the acescent solution a pH-value of 4.1 (the neutral solution has a pH-value of 6.5). Then we repeat the test of 3B and measure the decline of phosphate.

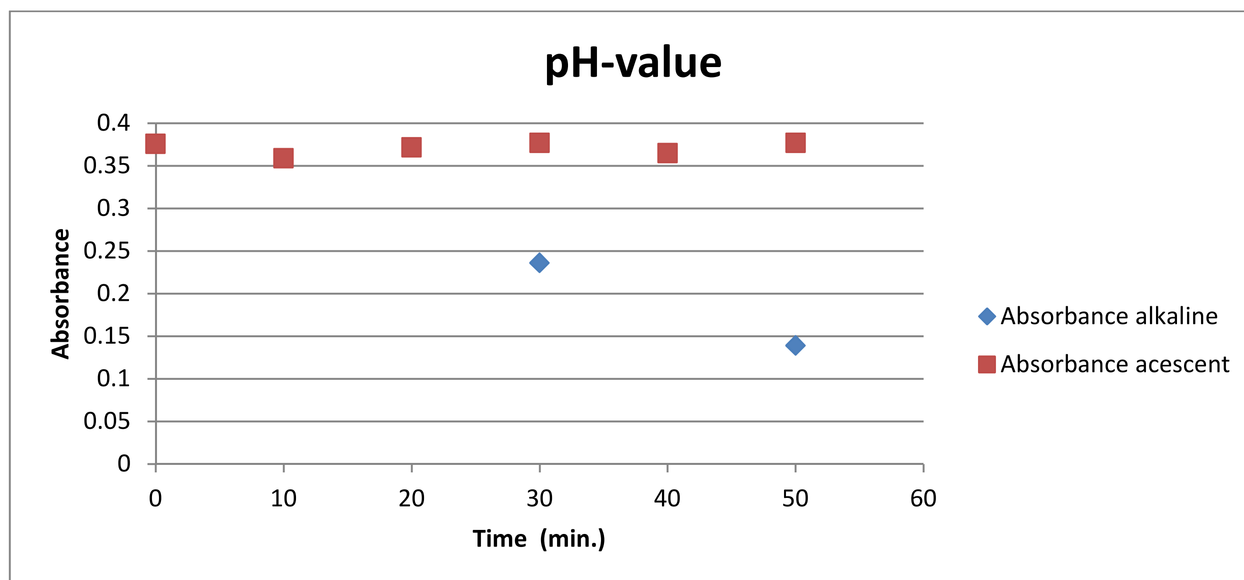

Fig. 12: Absorbance with different pH-values

6.2.1 Discussion of the results of the pH-value experiment

As you can see caustic soda has no implication on the phosphate decline. Unfortunately we hadn't enough Molybdat-Solution for each sample of the hydrochloric-containing yeast solution. You can see with hydrochloric the absorbance had weakened that means that the yeast cells had absorbed more phosphate. But we can't say why the yeast cells absorb more phosphate than before, because we haven't got any useful results out of test 3B. That means we can't compare the results with another result which isn't manipulated.

The low value of the absorbance of the basic solution could also depend on the discoloration of the Malachitgreen. It changes its colour clear, if it's blended with a basic solution. Our spectrometer can't measure the absorbance, because all the light goes through as only one specific wavelength (595nm) is measured.

Our second assumption is that the yeast cells take up more Phosphate in a basic solution because the absorbance is lower. We are not sure about it because we have only two values of the absorbance in the basic solution. (Wikipedia)

6.3 The absorbance with different temperatures

How does the uptake of phosphate by yeast cells vary with different temperatures?

For this experiment we did exactly the same as in parts 3A&B. We worked with the same mixture, but we varied the temperature. We worked with two Erlenmeyer flasks which we put into two different warm water baths. One was 30 degrees and the other 40 degrees warm. We only changed the temperature but no other circumstances to see how the temperature influences the results.

Uptake of phosphate by yeast cells

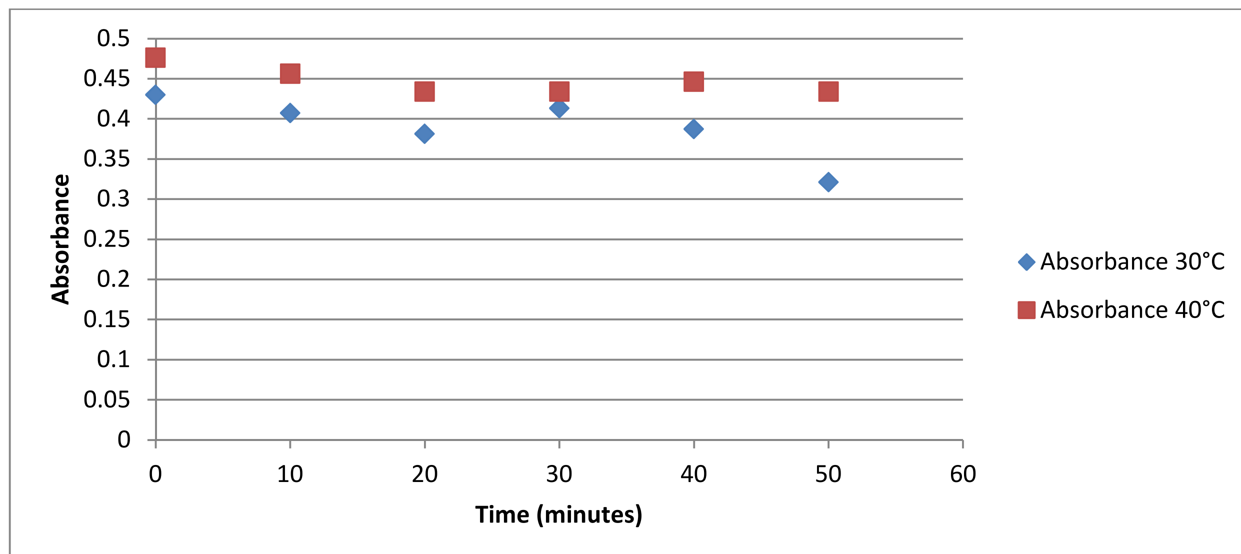

Fig. 13:Absorbance at 30°C and 40°C

6.3.1 Discussion of the results of the temperature experiment

The Absorbance at 40 degrees is higher than at 30 degrees. Obviously the absorbance at 30 degrees decreases, but starting at 40 degrees the value remains constant. If the value from the absorbance is higher the yeast cells didn't take up much phosphate. So if the value decreases you can see that the yeast cell worked well and took up much phosphate. You can also see that the value starting at 40 degrees stays virtually constant. That means that the yeast cells only took up a little amount of phosphate. At 30 degrees you can see the value of the absorbance is decreasing within the time. That means that at 30 degrees the cells take up more phosphate than at 40 degrees. The Q10 effect says that cells work better at higher temperature than at a low temperature. But at a certain point the temperature is too high and the cells work not as good as at lower temperatures. At about 35 degrees the temperature is too high.

7 Improving the investigation

We have experienced that there are many opportunities, how phosphate can get in our experiments. The first mistake in our findings was, that one of the Molybdat-Solution from the sent box was contaminated defiled with phosphate. Fortunately, during the pretest we found it out.

When the first EP in the experiment with the calibration curve, filled with Molybdat-Solution, Malachit-green-Solution and distilled water turned green we had a proof. There was some phosphate in the experiment. So we had to search the problem. With an easy test, we found out which distilled water was contaminated. Our distilled water from the school was contaminated with phosphate but the distilled water from the box was clean. But unfortunately we had too little distilled water from the box. So we had to distil our water to continue the experiment and to be sure, that we work without phosphate in the water. Another mistake was, that we had contaminated beaker. The beakers were contaminated because they were washed with dishwashing agent and in it is phosphate. So in every experiment in which we have used beaker, we had different and special results. To eliminate this error source we rinsed the beakers with distilled water before using it. The experiment was very sensitive for phosphate influence. For each pollution by us and for every dirty pipette, we got false results. So we had to make sure, that we had a clean experiment.

We found out that not all our spectrometers gave the same result for the same sample. So we worked with only one spectrometer during the whole project. To show the difference between the spectrometers we did one calibration curve with another one.

We had to repeat part 3 very often because we had problems with phosphate contamination. So we could measure the phosphate uptake by yeast cells with the changed temperature, glucose concentration and other pH-value only once. As we have found out before one measurement is no measurement and we would have liked to repeat them at least five times. But we had too little Molybdat-Solution which was not contaminated with phosphate.

Also in the part 3B we had problems with the time between pouring the mix into the supernatant and the measurement. We determined that we should wait exactly two minutes. But not everyone understood this from the beginning. So again we had to start anew. Next time we would give someone the task to help follow the timetable exactly.

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