Bündner Kantonsschule Chur

# 5 SPF Group Manzanell

Task 2

# Table of Contents

# 1 – Background information

The group of phosphates contains all molecules with a phosphate ester as well as inorganic phosphates. A typical property of phosphates is that they easily yield protons, which is called an acid reaction.[1]

1. Why is phosphate important for all organisms?
   Many molecules important for the metabolism of an organism are phosphates. For example ATP, cAMP, creatine phosphate and phospholipids. More important than that, DNA and RNA contain a phosphodiester sugar backbone.[2]

2. What is well known about phosphate uptake in yeast cells (*Saccharomyces cerevisiae*)?
   The yeast cells take up the phosphate with the help of phosphate transport molecules located in the membrane. The active uptake of phosphate needs energy, which is provided by the energy metabolism of the cell, e.g. by respiration and by degradation of glycogen. (The cell accumulates glycogen when there is an abundance of nutrients in its surroundings.) [3]

3. How do yeast cells store phosphate?
   The cells store the phosphate internally in the form of so-called polyphosphate, which can be used during periods of phosphate limitations.[4]
   Depending on its surroundings, yeast contains a varying amount of so-called metaphosphate, which is built up during certain energy providing processes. The energy won by these processes is stored by the metaphosphate.[5]

4. Why is phosphate uptake by microorganisms an important issue in our society/environment?
   The problem lies in the so-called Eutrophication. This refers to the accumulation of nutrients in a water ecosystem, which can cause needless and even damaging growth of plants and algae. Phosphates from sewage and intensely manured fields reach rivers and lakes where they boost the growth of micro-algae and phytoplankton. This biomass sinks to the ground and is being decomposed by bacteria. More material to decompose means more oxygen is needed. When the concentration of oxygen declines in a waterbody, phosphate is being released from the sediments into the water. The ecological balance is disturbed. The phosphate can be removed from the sewage by biological elimination, e.g. with certain plants, or chemical precipitation processes. The phosphate containing sludge can be disposed of.
   Another issue is that the phosphate resources on earth are limited and without it, no plant growth is possible. Even though there are ways to regain the phosphate from the sludge, they are not being used, as of yet, since they are expensive but may be necessary in the future.[6 7]

# 2A – Pretest

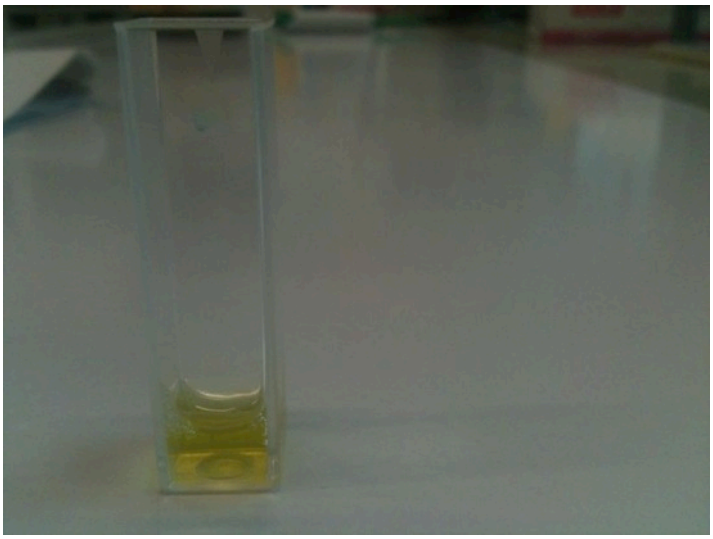


*Figure 1*

*Figure 2*

*Figure 3*

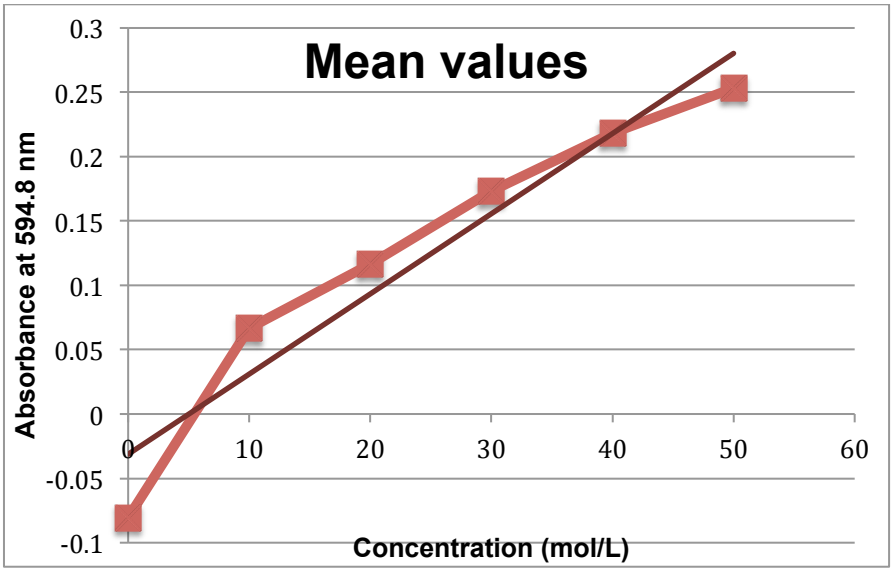
Luckily, our pretest was successful on the first try.

*Fig.1 – the result of our pretest*
*Fig.2 – the work environment*
*Fig.3 – the equipment and solutions used for the test*

# 2B – Establishing a straight calibration

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| Time | Absorbance | Time | Absorbance | Time | Absorbance |
| 0 | -0.053 | 0 | -0.040 | 0 | -0.080 |
| 10 | 0.043 | 10 | 0.101 | 10 | 0.186 |
| 20 | 0.094 | 20 | 0.171 | 20 | 0.412 |
| 30 | 0.119 | 30 | 0.198 | 30 | 0.573 |
| 40 | 0.124 | 40 | 0.236 | 40 | 0.598 |
| 50 | 0.143 | 50 | 0.240 | 50 | 0.613 |
| Deviation: 0.913 | | Deviation: 0.919 | | Deviation: 0.963 | |

| Mean value | |
|---|---|
| Time | Absorbance |
| 0 | -0.058 |
| 10 | 0.070 |
| 20 | 0.127 |
| 30 | 0.163 |
| 40 | 0.193 |
| 50 | 0.212 |
| Deviation: 0.939 | |




*Graph 1: mean values of the calibration experiments*

Trying to establish a straight calibration, the main problem we encountered was working with the photo spectrometer. After our first try, our results were not even close to being on one straight line but rather all over the place. With the help of our teacher though, we managed to find out that this was not our fault but much rather an inadequacy in the spectrometer. Since we had so little liquid in our cuvettes, it would sometimes just measure the air above it which would obviously lead to incorrect results. So what we did was place the cuvettes on a little piece of cork and then measure them like that – not the most-scientific solution but it worked. Thankfully, most of us had worked with this kind of pipets before so we didn't have any problems there.

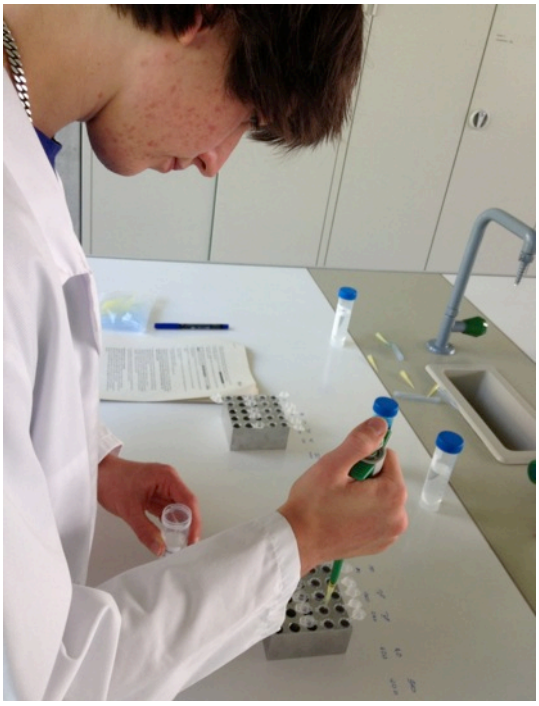


*Figure 4*

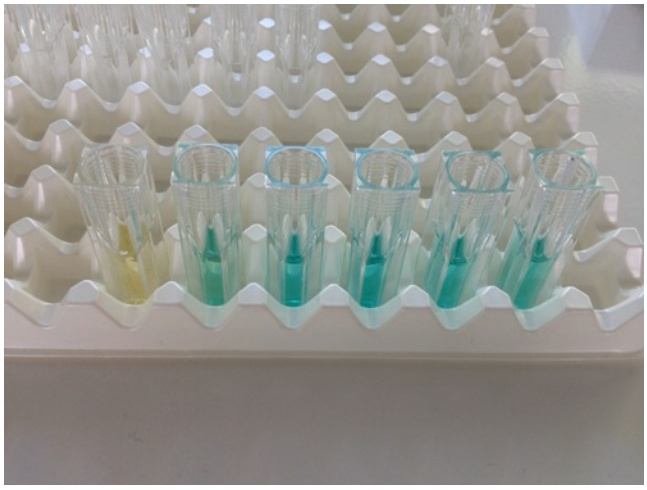


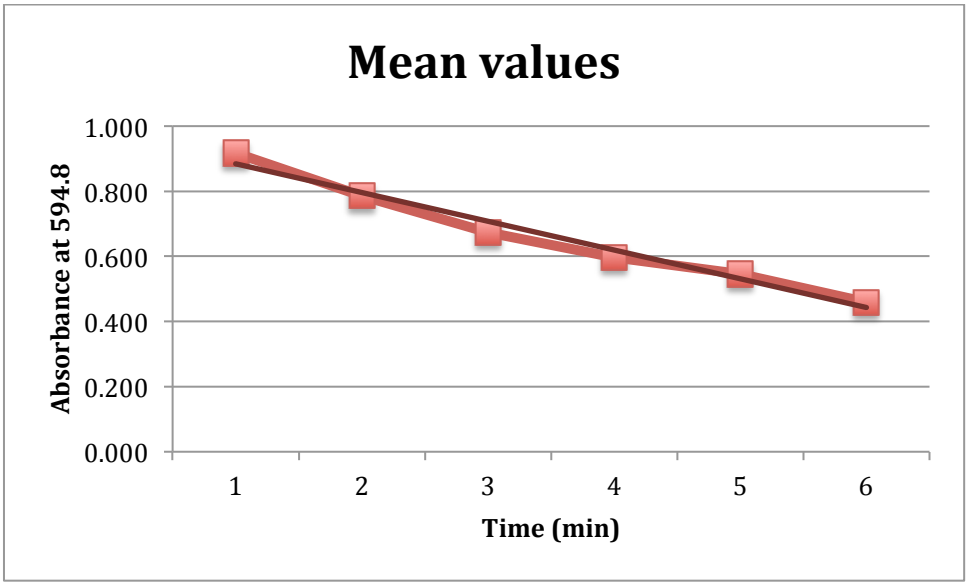
*Figure 5*

*Fig. 4 – Noël is preparing the solution*
*Fig. 5 – The result of the calibration*

# 3 – Measuring the phosphate uptake by yeast cells

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| Time (min) | Absorbance | Time (min) | Absorbance | Time (min) | Absorbance |
| 0 | 0.713 | 0 | 1.078 | 0 | 0.965 |
| 10 | 0.579 | 10 | 0.903 | 10 | 0.876 |
| 20 | 0.496 | 20 | 0.833 | 20 | 0.693 |
| 30 | 0.514 | 30 | 0.650 | 30 | 0.629 |
| 40 | 0.445 | 40 | 0.688 | 40 | 0.506 |
| 50 | 0.382 | 50 | 0.512 | 50 | 0.484 |
| Deviation: -0.948 | | Deviation: -0.969 | | Deviation: -0.981 | |

| Mean value | |
|---|---|
| Time | Absorbance |
| 0 | 0.918 |
| 10 | 0.786 |
| 20 | 0.674 |
| 30 | 0.598 |
| 40 | 0.546 |
| 50 | 0.460 |
| Deviation: -0.988 | |




*Graph 2: mean values of the phosphate uptake experiments*

For this part, the main difficulty was the fact that the solution fades quickly after the Malachitgreen is added. Again, the results didn't seem to make much sense after our first try because we forgot to consider this factor.
We also noticed that it is important to be exact with the amount of Malachitgreen- and Molybdat-solution since the probes don't have a very big color distinction to begin with.
Lastly, it took us quite a while how to prepare the 0.5 mM Sodium-Phosphate-Buffer with 1% glucose.

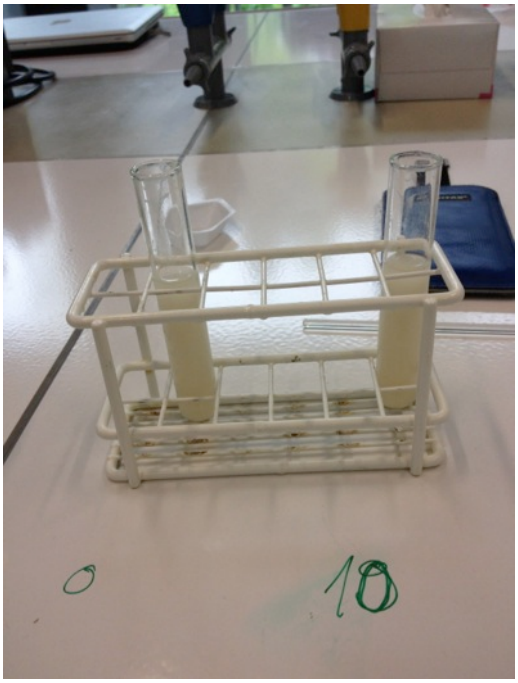



*Figure 6*

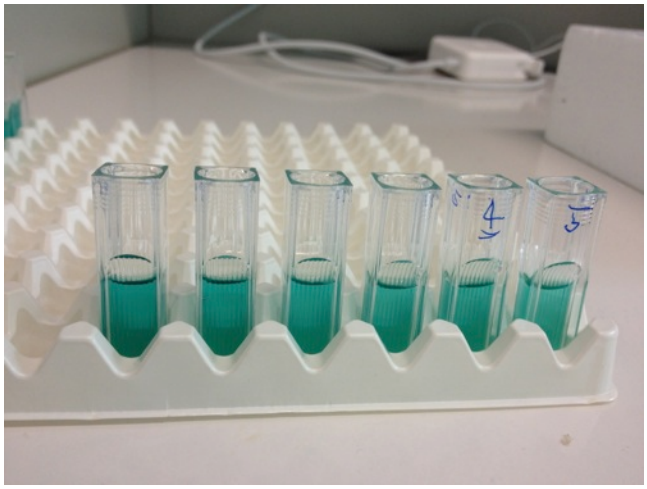


*Figure 7*

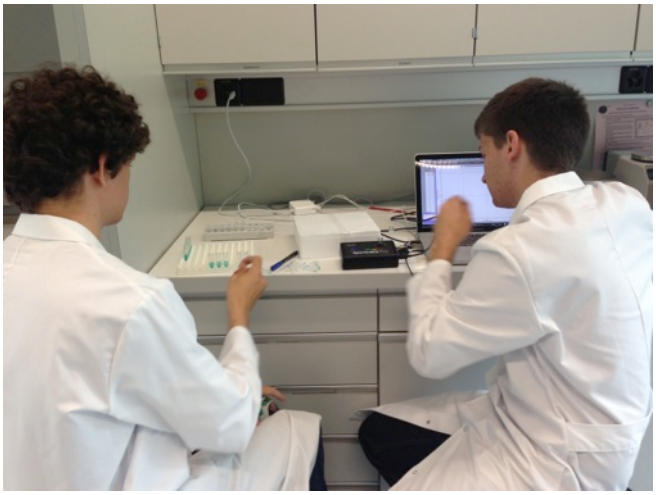


*Figure 8*

*Fig. 6 – the prepared yeast solutions*
*Fig. 7 – the probes after the photospectrometic measurement*
*Fig. 8 – Andrea and Pascal measuring the probes*

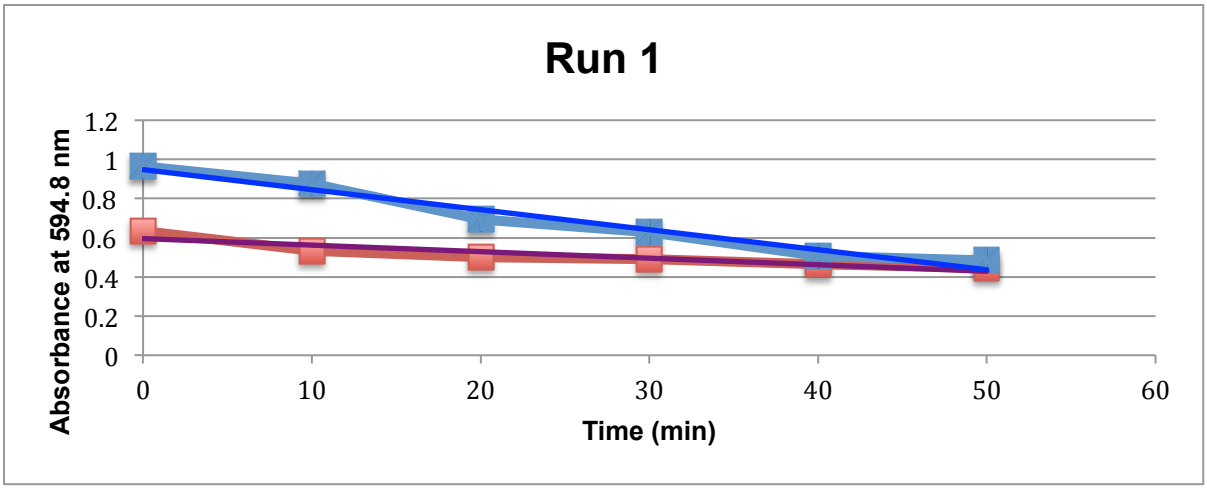
# 4A – How does light affect the phosphate uptake of yeast cells?

## Design of the experiment - Method and materials

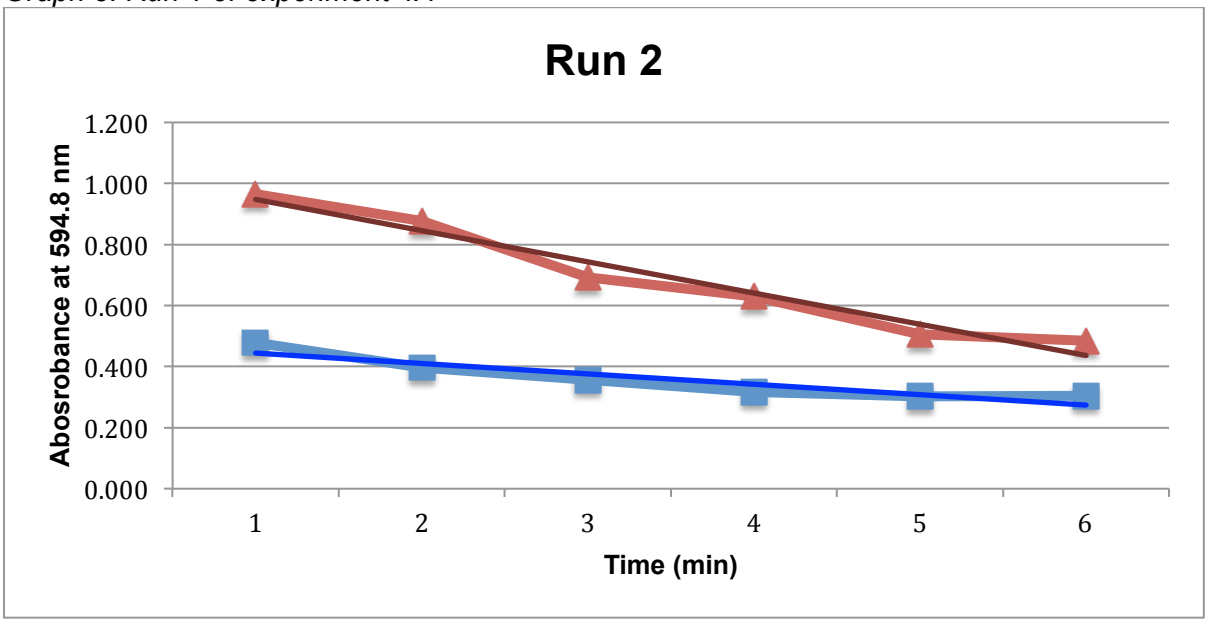
In this first experiment, we wanted to test the effect light has on the phosphate uptake of yeast cells.
We ran two tests two, one (Compare test) at daylight (496 lux) and 21.1°C and the other (Test) at 0-1 lux and 21.1°C.
We used the methods we had adapted to in part 3A and 3B to prepare the solution and measure its phosphate uptake.

## Data collection and processing

| | | | | Test: | | | Compare test: | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | light intesity: 0-1 lux | | | light intensity: 496 lux | |
| | | | | temperature: 21,1°C | | | temperature: 21.3°C | |
| | | | | | | | | |
| Run 1: | | Time (min) | | Absorbance at 594.8 nm | | | Absorbance at 594.8 nm | |
| | | | | | | | | |
| | | 0 | | 0.635 | | | 0.965 | |
| | | 10 | | 0.535 | | | 0.876 | |
| | | 20 | | 0.502 | | | 0.693 | |
| | | 30 | | 0.491 | | | 0.629 | |
| | | 40 | | 0.465 | | | 0.506 | |
| | | 50 | | 0.446 | | | 0.484 | |
| | | | | | | | | |
| | | | | | | | | |
| Run 2: | | Time (min) | | Absorbance at 594.8 nm | | | Absorbance at 594.8 nm | |
| | | | | | | | | |
| | | 0 | | 0.421 | | | 1.078 | |
| | | 10 | | 0.295 | | | 0.903 | |
| | | 20 | | 0.285 | | | 0.833 | |
| | | 30 | | 0.290 | | | 0.650 | |
| | | 40 | | 0.242 | | | 0.575 | |
| | | 50 | | 0.233 | | | 0.512 | |




*Graph 3: Run 1 of experiment 4A*




*Graph 4: Run 2 of experiment 4A*

## Conclusion and evaluation

From the results we can see that the phosphate uptake of the yeast cells is faster in the darkness than in daylight.
One possible explanation is that the UV-radiation reduces the activity of yeast. It is also possible that the daylight activates other functions of yeast, like enzyme or vitamin production and that the yeast invests more energy in this procedure than in the phosphate uptake when left in the light.
We had considered the fact that most mushrooms live in a place with high humidity and without direct sunlight exposure. Although humidity is obviously an important factor in the life of fungus, there is plenty of evidence, that light is influencing growth and metabolism of various fungus species in various ways, e.g. positive phototropism in Pilobolus sp. [8 9]

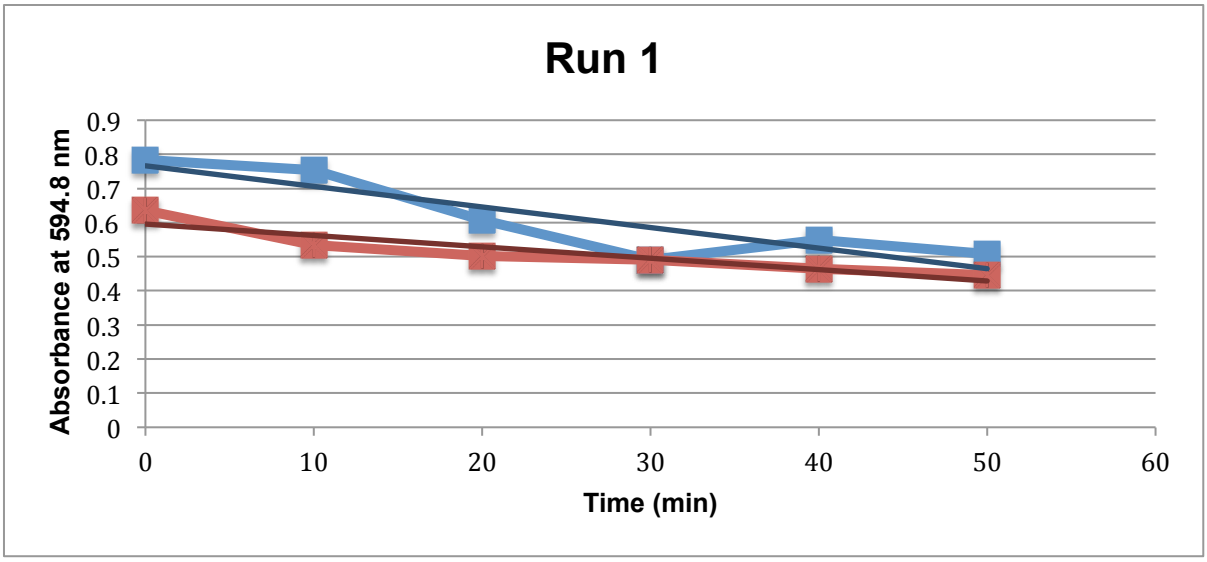
# 4B – How does temperature affect the phosphate uptake of yeast cells?

## Design of the experiment – Methods and materials

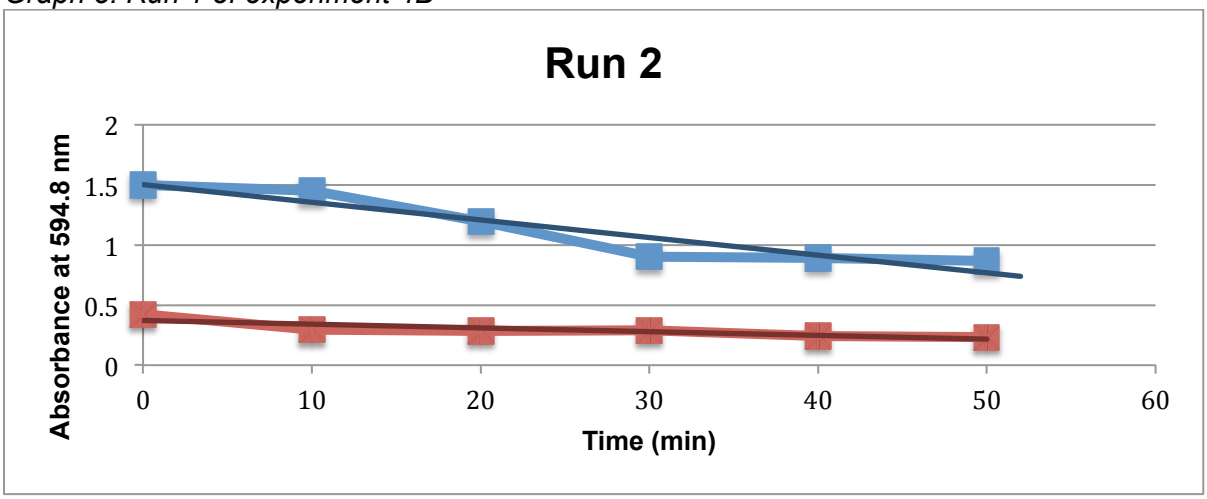
For this experiment we wanted to know if and how the surrounding temperature affects the phosphate uptake of the yeast cells.
In order to find this out, we ran one test at a temperature of 21.1°C and a light-intensity of 0-1 lux. For the second test, we increased the temperature to 28°C but let the light intensity stay at 0-1 lux in order to only have one independent variable. This second test was placed in a heated cubicle, which provided perfect condition for our experiment.
We used the methods we had adapted to in part 3A and 3B to prepare the solution and measure its phosphate uptake.

## Data collection and processing

| | | | | Test: | | | Compare test: | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | light intesity: 0-1 lux | | | light intensity: 0-1 lux | |
| | | | | temperature: 28,6°C | | | temperature: 21.1°C | |
| | | | | | | | | |
| | | | | | | | | |
| Run 1: | | Time (min) | | Absorbance at 594.8 nm | | | Absorbance at 594.8 nm | |
| | | | | | | | | |
| | | 0 | | 0.784 | | | 0.635 | |
| | | 10 | | 0.754 | | | 0.535 | |
| | | 20 | | 0.606 | | | 0.502 | |
| | | 30 | | 0.491 | | | 0.491 | |
| | | 40 | | 0.549 | | | 0.465 | |
| | | 50 | | 0.508 | | | 0.446 | |
| | | | | | | | | |
| | | | | | | | | |
| Run 2: | | Time (min) | | Absorbance at 594.8 nm | | | Absorbance at 594.8 nm | |
| | | | | | | | | |
| | | 0 | | 1.501 | | | 0.421 | |
| | | 10 | | 1.454 | | | 0.295 | |
| | | 20 | | 1.194 | | | 0.285 | |
| | | 30 | | 0.903 | | | 0.290 | |
| | | 40 | | 0.894 | | | 0.242 | |
| | | 50 | | 0.867 | | | 0.233 | |
| | | | | | | | | |




*Graph 5: Run 1 of experiment 4B*




*Graph 6: Run 2 of experiment 4B*

## Conclusion and evaluation

From the results we can see that the phosphate uptake is faster at a temperature of 28°C than at 21°C. The higher the temperature is, the faster is the diffusion. That means that molecules are faster and that reactions happen faster. If the temperature rises to high though, the enzymes of the yeast crumble and become ineffective. 28°C is actually the optimum temperature for yeast to reproduce itself, which explains our results.[5]

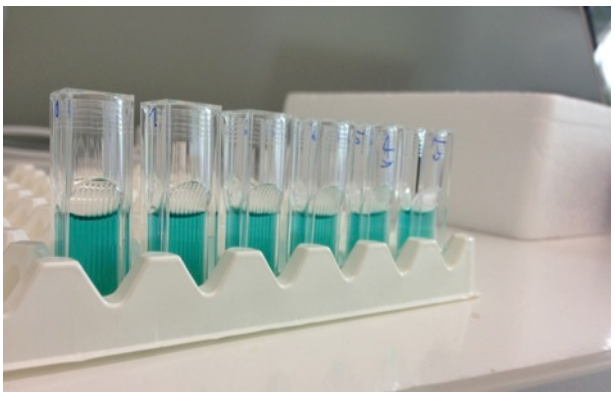


*Figure 10: the results of experiment 4B, run 2*

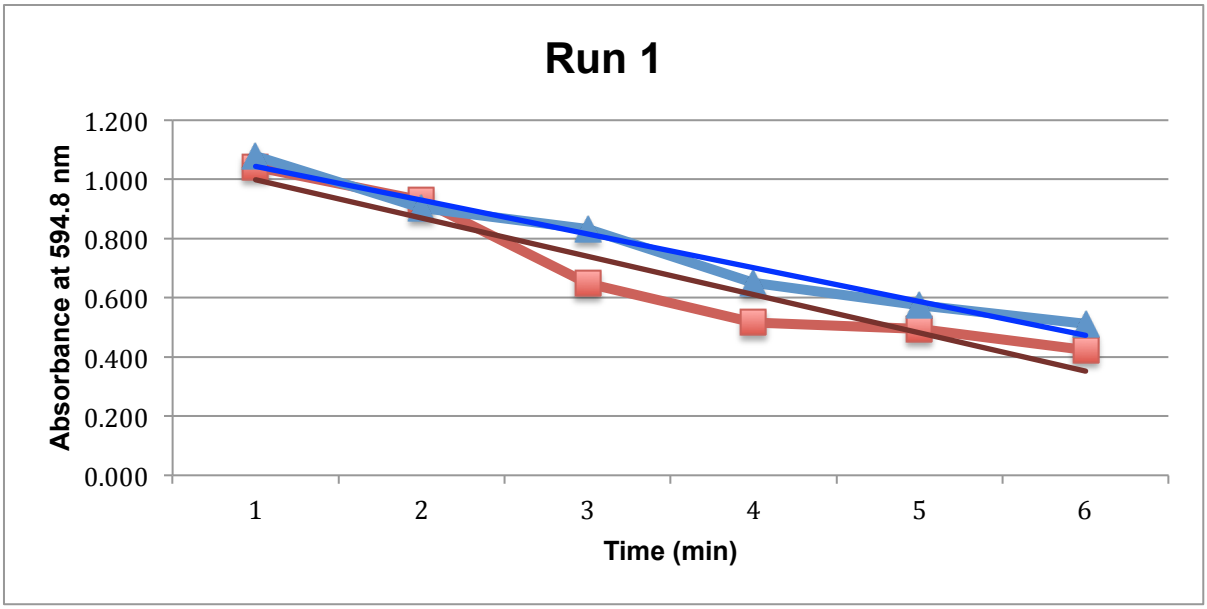
# 4C – How does electricity affect the phosphate uptake of yeast cells?

## Design of our experiment – Method and materials

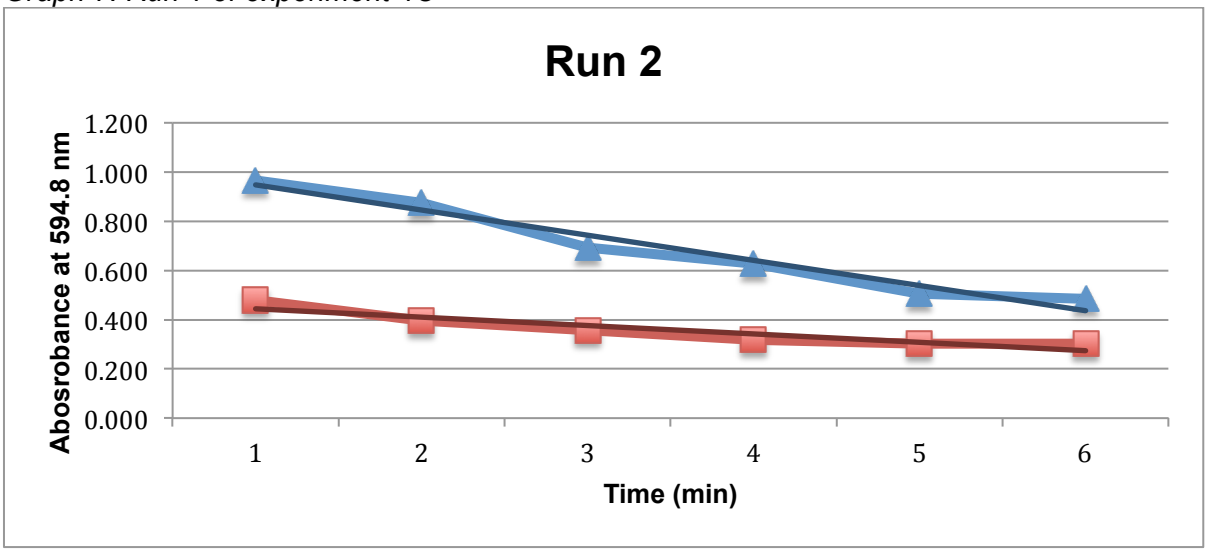
For this last experiment, we wanted to see if and how electricity affects the phosphate uptake.
We ran two tests in which we put a cathode and an anode into the tubes with the yeast solution. We then put a voltage of 4.5 V on the electrodes during the 50 min of the experiment.
We used the methods we had adapted to in part 3A and 3B to prepare the solution and measure its phosphate uptake.

## Data collection and processing

| | | Test: | Compare test: |
|---|---|---|---|
| | | Voltage: 4.5 V | Voltage: 0 V |
| Run 1: | Time (min) | Absorbance at 594.8 nm | Absorbance at 594.8 nm |
| | 0 | 1.041 | 1.078 |
| | 10 | 0.929 | 0.903 |
| | 20 | 0.649 | 0.833 |
| | 30 | 0.516 | 0.650 |
| | 40 | 0.494 | 0.575 |
| | 50 | 0.423 | 0.512 |
| Run 2: | Time (min) | Absorbance at 594.8 nm | Absorbance at 594.8 nm |
| | 0 | 0.478 | 0.965 |
| | 10 | 0.396 | 0.876 |
| | 20 | 0.357 | 0.693 |
| | 30 | 0.318 | 0.629 |
| | 40 | 0.303 | 0.506 |
| | 50 | 0.304 | 0.484 |




*Graph 7: Run 1 of experiment 4C*




*Graph 8: Run 2 of experiment 4C*

Conclusion and evaluation

The yeast cells under a voltage of 4.5 V tend to have a larger phosphate uptake than the ones under normal circumstances.
An interpretation of this result could be, that the positive voltage of the solution around the yeast cells interferes the transport of $K^+$ ions from within the cell to the surrounding solution. This might possibly motivate the yeast cell to absorb more $PO_4^{3-}$ ions to keep the membrane potential at a constant level.

# Reference list

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# Activity list

| | |
|---|---|
| 1.1/1.2 Why is phosphate important for all organisms?; What is well known about phosphate uptake in yeast cells (Saccharomyces cerevisiae) | Juno Kim, Gian-Marco Camenisch |
| 1.3/1.4 How do yeast cells (Saccharomyces cerevisiae) store phosphate?; Why is phosphate uptake by microorganisms an important issue in our society/environment? | Marco Zablonier, Livio Colombi |
| 2A Pretest (+ 2 pictures: lab environment and result) | Noël Heinz, Michael Heinz, Gian-Marco Camenisch |
| 2B calibration – experiment *(repeat at least 3 times !)* | Andrea Brütsch, Pascal Arpagaus, Gabriel Zala |
| 2B calibration – creating a table for results (excel) | Marco Zablonier |
| 2B analysis – 5-10 sentences (struggle and consequences) | Daria Ryffel, Rike Teuber |
| 3A preparing yeast solutions | Livio Colombi, Jeffrey Hunger, Nadja Keller |
| 3B measuring decline of phosphate – experiment *(repeat at least 3 times!)* | Ruben Meier, Semira Margreth, Juno Kim |
| 3B noting results (excel) and illustrating them in a graph | Marco Caduff |
| 3B analysis – 5-10 sentences (struggle and consequences) | Daria Ryffel, Rike Teuber |
| 4A first approach (design, data collection&processing, conclusion&evaluation) | Andrea Brütsch, Pascal Arpagaus, Noël Heinz, Rike Teuber, Gian-Marco Camenisch |
| 4B second approach (design, data collection&processing, conclusion&evaluation) | Ruben Meier, Marco Caduff, Nicolas Walker, Gabriel Zala, Semira Margreth, Marco Zablonier |
| 4C third approach (design, data collection&processing, conclusion&evaluation) | Daria Ryffel, Jeffrey Hunger, Michael Heinz, Nadja Keller, Juno Kim, Livio Colombi |