*Collège Saint-Michel*

Class 2. D1

# Task 2

17 May 2013

Collège St-Michel

SIMPLY SCIENCE

# Content

# 1 Part 1 – Studying the literature

## 1.1 Why is phosphate important for all organisms?

Phosphate is utterly important for the production and storage of energy in organisms. Cells benefit from the energy for cell division. Furthermore phosphate is a building brick for cell membranes and bones.

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

Phosphate is an essential nutrient for the synthesis of nucleic acids and phospholipids for cell membranes[1]. Cells use phosphate transporters which are strongly dependent on pH values in order to concentrate phosphate inside the cell membrane [2].
Five subunits of those phosphate transporters are responsible for the uptake of phosphate and the growth of yeast in high phosphate media [3].
A big amount of phosphate is absorbed by counter current exchange in the vacuole[4]. The vacuole serves as phosphate storage, but does not possess any active transport system like the plasma membrane[5]. The absorption of free phosphate from outside of the cell is transmitted by a series of transport systems by the plasma membrane [5].

## 1.3 How do yeast cells (Saccharomyces cerevisiae) store phosphate?

Protozoons produce high energy molecules in order to store excessive energy.
The so obtained sugar und the fats (polysaccharides and lipids) are stored in a reservoir within the cell.
Fungi are like animal cells able to synthesize complex polymers of glucose. A cell can immediately activate those particles in order to receive energy. This causes extended glucose reservoirs.
The energy is stored in the form of fat which is produced out of glucose. One gram of fat contains approximately six times more energy than glucose and is easier to access.
It is stored as droplets in the cytoplasm. Animal fat cells are specialized for this type of storage, because of the exceptionally big fat droplets [6].

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

Usually, stagnant waters (i.e. lakes and ponds) contain only a small concentration of phosphate, but without it, there would not be any life possible. The supply of soluble phosphate has a positive effect on the growth of the microorganisms, but this can have a negative effect on the eco system. Just a small increase of phosphate concentration increases the growth of algae[7] and leads to an overgrowth also called an eutrophication [8]. Due to the excessive supply of nutrition for the zooplankton, all organism of the zooplankton proliferate.
If the zooplankton proliferates, the crabs will have more nutrition and begin to reciprocate. Because of the eutrophication, all organisms of water begin to proliferate, but a huge amount dies too. Bacteria proliferate and use more oxygen, because of the overgrowth of algae, no light reaches the ground, and phytoplankton cannot make any photosynthesis. This causes living creatures like fish, crabs and snails to die because of the lack of oxygen [9].
The key source of phosphate in Germany (1998-2000, in tons $PO_4^{2-}$ per year) are erosions (26.8%, 8167 tons * $a^{-1}$) and communal wastewater (24.6%, 8898 tons * $a^{-1}$).
Phosphates were used in washing agent and washers too, but are forbidden today to prevent the phenomenon above.

# 2 Part 2: Calibration of the measuring system

## 2A Establishing a straight calibration (theory and introduction)

The aim of this first experiment was to get familiar with the measuring technique and to establish a nice straight calibration curve.
The calibration curve should enable us to determine the phosphate concentration directly from the measured absorption.
The theory behind is described by the law of Beer-Lambert:

$$A = c \, l \, \varepsilon$$

A: Absorption
c: concentration [mol/L]
l: length of the cuvette [cm]
ε: extinction coefficient

According to this linear relationship between the Absorption A and the concentration c we expected to get a straight calibration line which would enable us to determine the ε-value for our colored substance at 595 nm.

**Pretest:**
We made a first pretest, adding a mixture of 128 µl Malachitgreen-Solution and 172 µl molybdat solution to 200 µl D-water. The solution we obtained was yellow, indicating that no phosphate was present.
In the presence of phosphate, the mixture of Molybdate and Malachitegreen would give a blue solution.

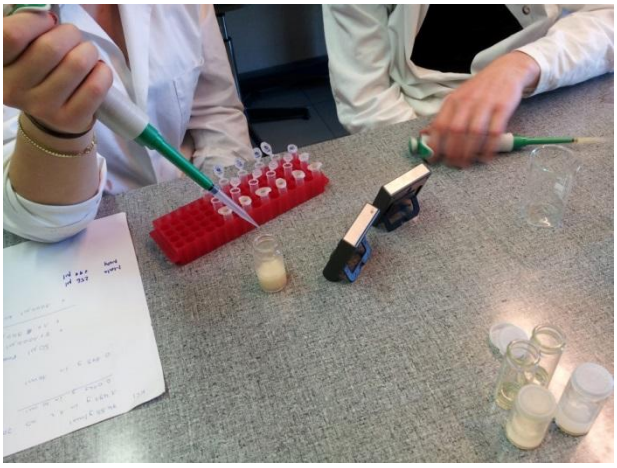
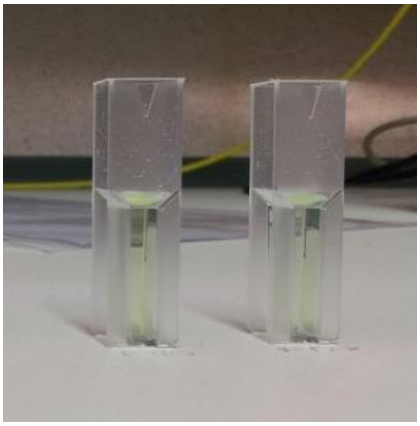


**Image 1**

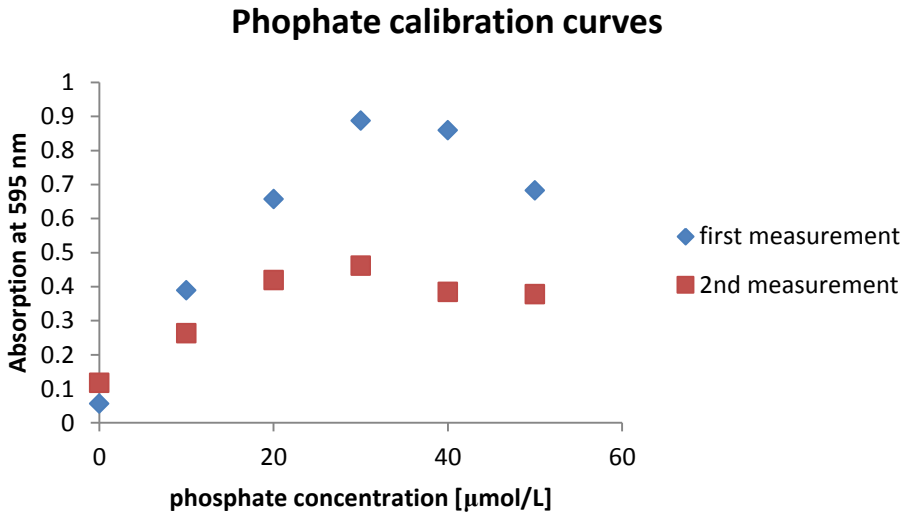
**Image 2**

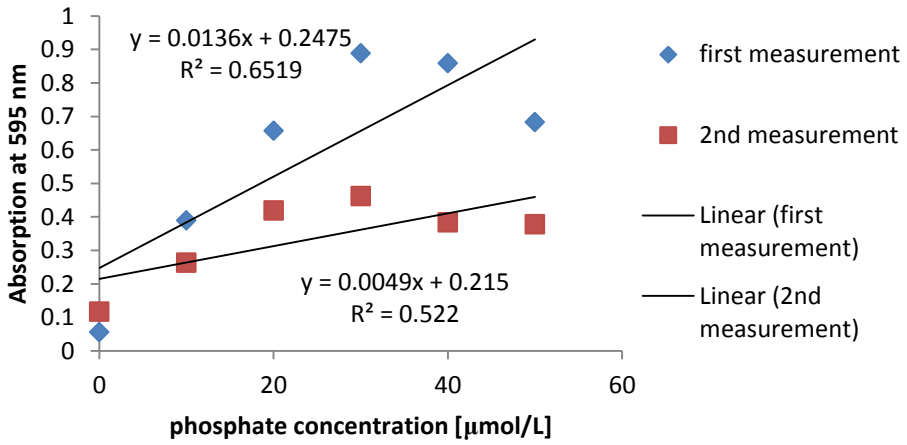


## 2B Establishing a straight calibration

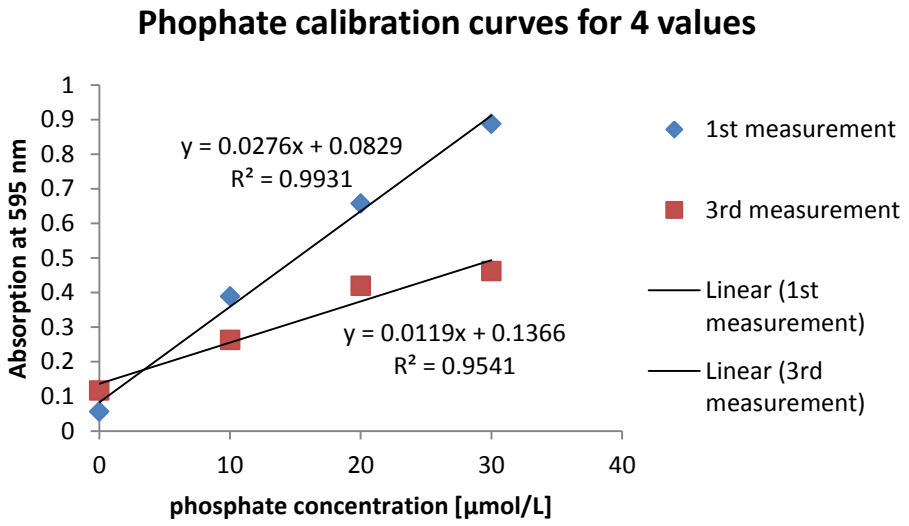
**Measurements:**
By making a linear regression for the absorptions at a given wavelength (here 595 nm) at different concentrations, we should be able to determine the extinction coefficient ε for our colored substance at that wavelength.

| 1st measurement | | 2nd measurement | |
|---|---|---|---|
| **Time (min)** | **A at 595 nm** | **time (min)** | **A at 595 nm** |
| 0 | 0.056 | 0 | 0.117 |
| 10 | 0.389 | 10 | 0.263 |
| 20 | 0.657 | 20 | 0.419 |
| 30 | 0.888 | 30 | 0.462 |
| 40 | 0.859 | 40 | 0.384 |
| 50 | 0.683 | 50 | 0.378 |



**Problems**: Our calibration curve cannot be fitted by a straight line. During the first measurement too many mistakes were made, the results could not be used.
However, it would be quite ok for the first 4 values. In our second measurement we got a straight line for the first four values, the last two values lay unfortunately not on the straight line.




**Other problem**: The linearity is good for the first 4 values ($R^2$ values of 0.9931 and 0.9541 respectively). Unfortunately the two curves are very different for the two measurements so we suppose that the error is very big. We made a whole series of measurements but in the other measurements we made mistakes at some points so the values cannot be trusted. However, with our results for the calibration, we cannot be sure about the extinction coefficient, so we cannot use these calibration curves for the determination of the phosphate concentration for a given absorption.

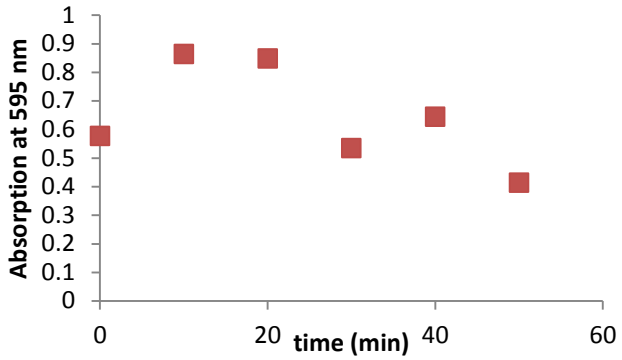
One big problem for us was the fact that the color of the solutions change with time. We found out, that the color increases during around 6 minutes, before bleaching. As different groups were carrying out the measurements, everyone waited for another time period before measuring the absorption spectra. Some groups measured immediately after mixing the solutions, some waited for 2 or 3 min.

**Proposed solution**: For the further measurements with the yeast solutions, we will not determine the absolute value for the concentrations, but rather concentrate on the changes. It is anyhow more important for us to see how the phosphate concentration changes over time than to determine absolute concentrations. We will therefore use relative concentrations instead of absolute values for the phosphate concentrations.

# 3 Measuring the phosphate uptake by yeast cells under standard conditions

## 3.1 Data

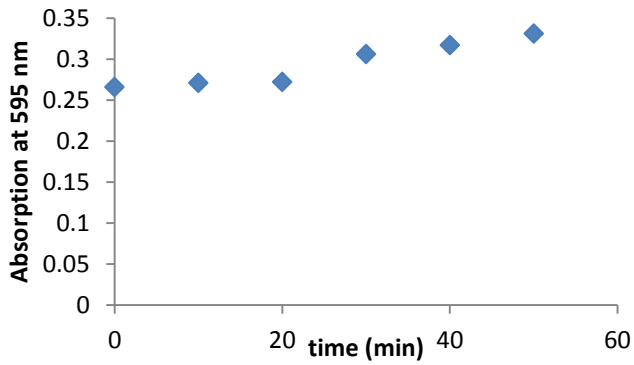
### 1st measurement (standard conditions)




| time (min) | Absorption at 595 nm |
|---|---|
| 0 | 0.576 |
| 10 | 0.863 |
| 20 | 0.847 |
| 30 | 0.534 |
| 40 | 0.644 |
| 50 | 0.413 |

In the first measurement, no clear trend can be seen observed. We will have made too many mistakes.

### 2nd measurement (standard conditions)




| time (min) | Absorption at 595 nm |
|---|---|
| 0 | 0.266 |
| 10 | 0.271 |
| 20 | 0.272 |
| 30 | 0.306 |
| 40 | 0.317 |
| 50 | 0.331 |

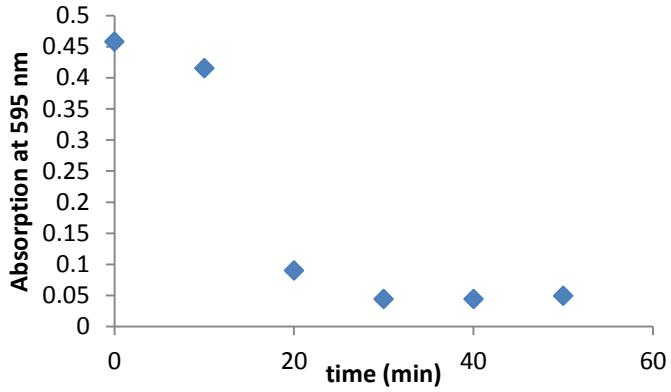
In the second measurement, the phosphate concentration seems to increase with time. Also this result is not what we expect to see.

### 3rd measurement

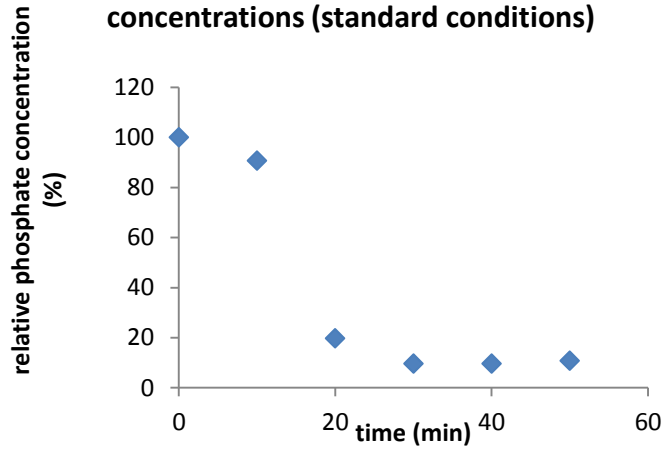
| time (min) | Absorption at 595 nm | Relative phosphate concentration |
|---|---|---|
| 0 | 0.458 | 100 % |
| 1 | 0.415 | 90.6113537 % |
| 2 | 0.090 | 19.650655 % |
| 3 | 0.044 | 9.6069869 % |
| 4 | 0.044 | 9.6069869 % |
| 5 | 0.049 | 10.69869 % |

The results of the third measurement seem to make sense. We will take this result for comparison with the other conditions.
In the beginning, we have 100% phosphate concentration. This concentration decreases over time. In this way, we can compare different measurements without knowing the real concentrations.

**3rd measurement (standard conditions)**




**3rd measurement with relative phosphate concentrations (standard conditions)**



# 4 Part 4 – How to improve the phosphate uptake by yeast cells

## 4A Does the temperature influence the absorption of phosphate in yeast cells?

**Short introduction**

At higher temperatures, yeast cells reproduce themselves quicker and with a higher number of yeast cells, they absorb more phosphate[10]. We increased the temperature up to 35°C which is 11°C above room temperature. We chose this temperature, because a lower temperature would not be significant enough and yeast cells die at temperatures above 45°C.

**Determination of the variables**

How can the absorption of phosphate be increased? In this experiment, we try to achieve this by increasing the temperature from 24°C to 35°C. In order to stabilize the temperature level we put the prepared yeast cells in a warmed water bath. We put the yeast cells back in there for 10 minutes after each measurement.
So the independent variable here is the temperature and the dependent variable is the speed of the phosphate absorption. The remaining, controlled variables which could affect the result are preparation and number of yeast cells, water quality and the glucose and phosphate concentration in the medium.

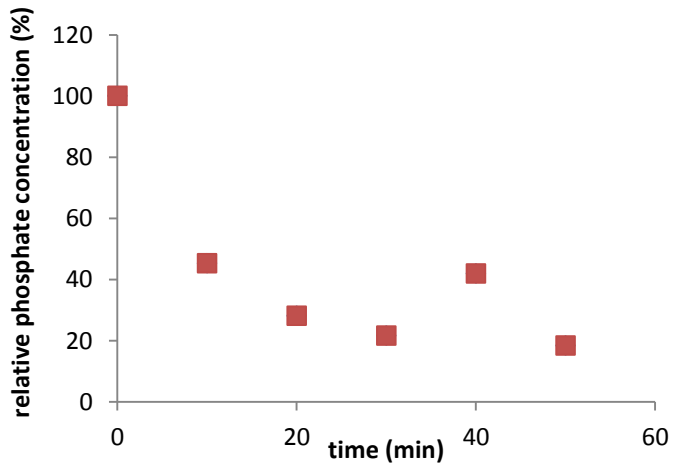
**Controlled variables**

We changed just one variable from the original experiment (standard conditions) for the measurement. The rest stayed the same.

**35°C**
(1 g yeast in 9950 µl H2O / 50 µl phosphate buffer 0.1 M / 0.1 g glucose at **35°C**)

| time (min) | Absorption at 595 nm | Relative phosphate concentration |
|---|---|---|
| 0 | 1.214 | 100 % |
| 10 | 0.55 | 45.3047776 % |
| 20 | 0.342 | 28.1713344 % |
| 30 | 0.263 | 21.6639209 % |
| 40 | 0.51 | 42.0098847 % |
| 50 | 0.223 | 18.369028 % |

**at 35°C**



**Problems**

- Time pressure (missed classes, problems with teachers) → We did not have the occasion to execute the experiment more than once and received strange results. We could neither confirm, establish nor improve them.
- We don't know where the problem in the value after 40 min lies. Apparently, we did not work precisely enough with the pipette or we put the cuvette the wrong way in.

## 4B Second Approach: Does the addition of $K^+$ ions increase the phosphate absorption?

**Short introduction**

Yeast cells possess different techniques to absorb phosphates. One is the ion pump. It is a complex, inserted into the cell membrane, which is permeable for charged particles like ions. Yeast cells possess those pumps to achieve the ion exchange. Phosphates can stick themselves to those ions and invade the cell with them. Thereby, the phosphate absorption is increased indirectly. Phosphate can stick to $K^+$ ions the best and are therefore the most suitable ions.
Our experiment and approach are based on a report from 1957, which observes, in match to our project, the active transportation of phosphate in yeast cells. The team back then used the method with the addition of $K^+$ ions to the solution in order to increase the absorption. Their success cheered us on to try the same method with our project.

**Determination of the variables**

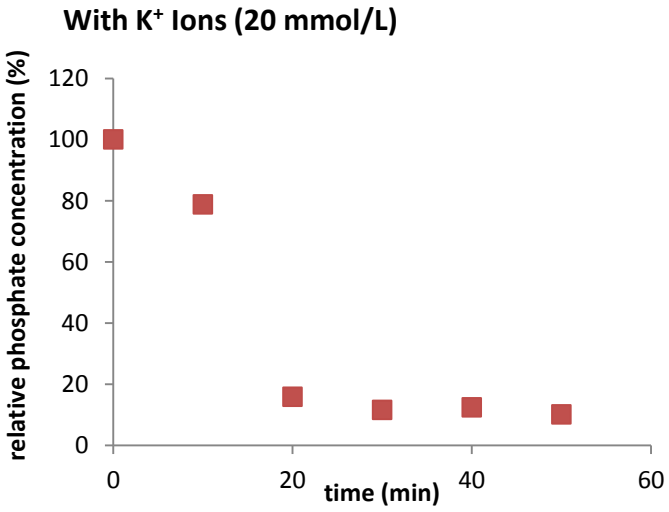
How can the absorption of phosphate be increased? In this experiment, we try to achieve this with the addition of $K^+$ ions. Thereby, we vary the K+ ion concentration in the solution. We try to investigate if the addition of $K^+$ ions causes an increase in speed of the phosphate absorption. The $K^+$ ion concentration is an independent variable; the dependent variable the speed of phosphate absorption. The remaining, controlled variables which could affect the result are temperature, preparation and number of yeast cells, water quality and the glucose and the phosphate concentration of the medium.

**Controlled variables**

In this experiment we respected the conditions and quantities described in the protocol for a well-defined yeast solution (standard conditions). The only exception is that we added KCl (20 mmol/L) to the medium.

**With addition of $K^+$-Ions (20 mmol/L)**
(1 g yeast in 9950 µl H2O / 50 µl phosphate buffer 0.1 M / 0.1 g glucose at 23°C/**20 mmol/L KCl**)

| time (min) | Absorption at 595 nm | Relative phosphate concentration |
|---|---|---|
| 0 | 0.353 | 100 % |
| 10 | 0.278 | 78.7535411 % |
| 20 | 0.056 | 15.8640227 % |
| 30 | 0.041 | 11.6147309 % |
| 40 | 0.044 | 12.4645892 % |
| 50 | 0.036 | 10.1983003 % |




**Problems**

- No big problems during the experiment
- Time pressure (missed classes, problems with teachers) → We did not have the occasion to execute the experiment more than once.

## 4C Third Approach: Does the addition of more glucose increase the phosphate absorption?

**Short introduction**The yeast cells grow more quickly and become bigger, if the medium contains more glucose. We prepared a medium which now contains 5% glucose instead of just 1%. The method was the same as in the standard solution.

**Determination of the variables**

The independent variable is now the glucose concentration in the medium. The dependent variable is the phosphate absorption. The remaining, controlled variables which could affect the result are preparation and size of the yeast cells, water quality, the addition of glucose and the phosphate concentration of the medium.

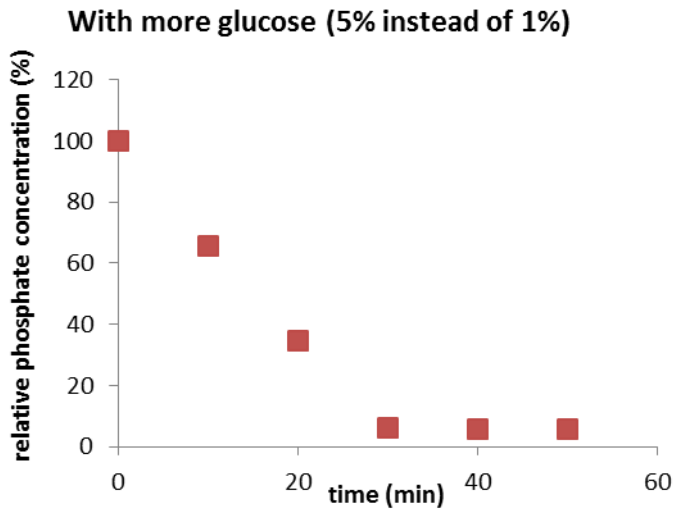
**Controlled variables**

In this experiment we changed only the glucose concentration in comparison to the original experiment (standard conditions). The rest stayed the same.

**With more glucose (5% instead of 1%)**

(1 g yeast in 9950 µl H2O / 50 µl phosphate buffer 0.1 M / **0.5 g glucose** at 23°C)

| time (min) | Absorption at 595 nm | Relative phosphate concentration |
|---|---|---|
| 0 | 0.807 | 100 % |
| 10 | 0.529 | 65.551425 % |
| 20 | 0.28 | 34.6964064 % |
| 30 | 0.05 | 6.19578686 % |
| 40 | 0.045 | 5.57620818 % |
| 50 | 0.045 | 5.57620818 % |




**Problems**

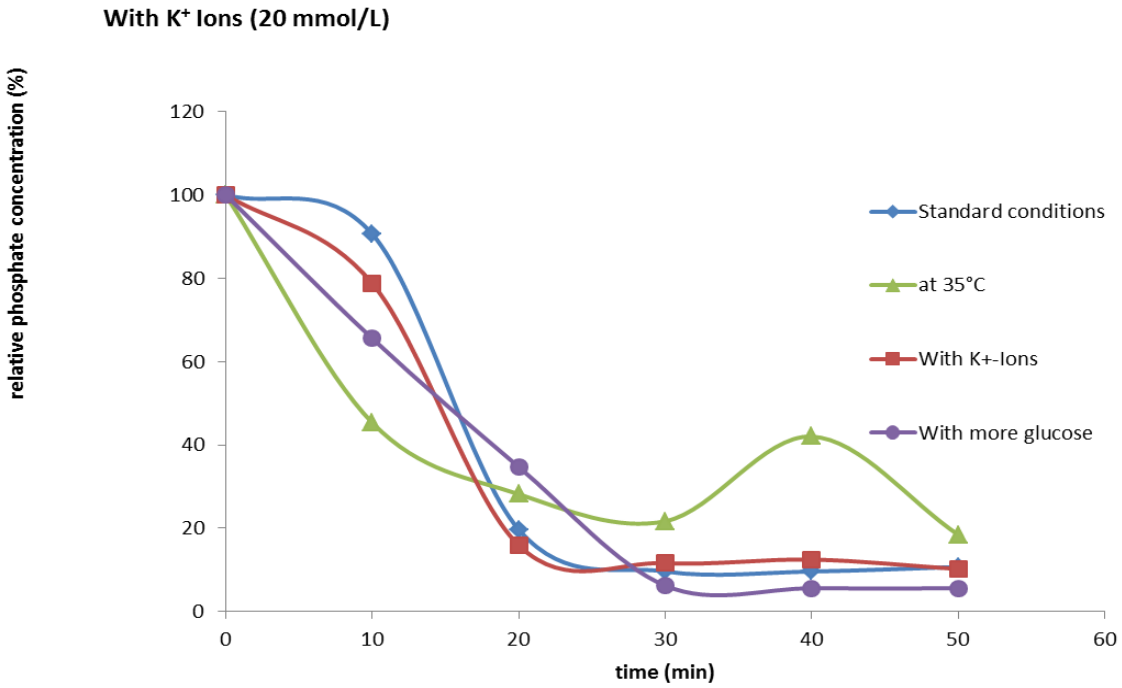
- We received way too much solution. Explanation: The pipette was set to a wrong value. • Time pressure (missed classes, problems with teachers) → We did not have the occasion to execute the experiment more than once.

# 4 Step 3: Conclusion and Evaluation

## Aspect 1: Concluding

You cannot see a clear trend. It is conspicuous that the changed variables did not lead to higher phosphate absorptions.

In the first 10 minutes, the method with the increased temperature seems to be most reliable. However, if we have a look at the other values obtained at 35°C, we can see that the value after 40 min




differs and will have to be excluded. It seems like we did some kind of a mistake there. Eventually, this method performs worst.

Furthermore, you can see that the addition of $K^+$ ions increases the absorption of phosphate at first (after 10 min), but eventually does not differ much from the standard conditions.

The concentration of phosphate decreases linear with more glucose at first, but performs worst after 20 minutes. But the concentration continues to decrease linear and is stable after 30 minutes. Finally, the method with adding more glucose was the best method we came up with which increased the absorption of phosphate in comparison the standard solution the most. However the differences between the four conditions are very small after 30 min already and after 50 min, the differences are insignificant.

## Aspect 2: Evaluating procedures

Possible mistakes we made:

- Pipet mistakes (bubbles in the pipette, remainders in the pipette)
- You could not set the pipette to 344 µl respectively 256 µl. We tried different method: Combinations with different pipettes, rounding, mixing the whole solution for all 6 solutions in one jar and put then 600 µl in each solution  less rounding mistakes.
- Waiting too long (i.e. after the preparation before putting them into the centrifuge)
- Bad time measurement (not long enough in the centrifuge)
- Time pressure

One big problem for us was the fact that the color of the solutions change with time. We found out, that the color increases during around 6 minutes, before bleaching. As different groups were carrying out the measurements, everyone waited for another time period before measuring the absorption spectra. Some groups measured immediately after mixing the solutions, some waited for 2 or 3 min. This is an additional reason why it seemed intelligent to use relative concentrations instead of using absolute concentration values for the phosphate concentrations.

### Aspect 3: Suggestions for improvement

It would be much better to repeat the experiments more often

It would be very interesting to carry out the experiments at several different temperatures, with several different glucose concentrations and with different K+-ion concentrations

Suggestions for improvement for experiment #1:
- Pipet more precisely, train some specialists for pipetting
- Accurate time measurement
- Work in smaller groups
- 28°C instead of 35°C (optimum for reproduction)
- Yeast cells are pressure-sensitive, maybe the water pressure changed too much?

Suggestions for improvement for experiment #2:
- Add more $K^+$ ions
- Execute the experiment for a longer period of time in order to see if the concentration decreases more (start with bigger concentrations)
- Take more time
- Work in smaller groups, specialists for pipetting

Suggestions for improvement for experiment #3:
- Execute the experiment for a longer period of time in order to see if the concentration stays stable
- Increase the phosphate concentration in the beginning
- Add more glucose
- Work in smaller groups, specialists for pipetting

Problems with the standard solution:
- The cooperation was difficult because someone was always putting the cuvette the wrong way in. Therefore, one time it was in the right way, one time not.
- We received 3 completely different graphs, but we don't have any good explanation at all.

In general, we just did not have enough time. We could execute each experiment only once or twice.

# 5 Reference list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10. “Backhefe”. www.de.wikipedia.org. Retrieved May 10, 2013

Image 1: Two student pipeting and preparing the experiment

Image 2: Results of our pretest

# 6 Activity list

| | |
|---|---|
| Berisha Shpetim | theory and experiment |
| Bielmann Mel | experiment |
| Chantaraviwat Tanatip | curve measure |
| Cotting Marina | experiments and evaluation |
| Dang My-Hanh | experiment assistant |
| Diesbach Jonas | activity list |
| Dutly Nicolas | theory |
| Fiore Silas | measure of the calibration curve |
| Göttel Dominique | theory |
| Haberditz Xenia | curve measure and experiment |
| Häring Tom | template |
| Kaeser David | graphs |
| Krattiger Nicolas | layout and experiment assistant |
| Liechti Zoel | theory and experiment |
| Lomazzi Rémy | pictures |
| Marti Denis | theory, experiment and evaluation |
| Pütter Kim | template |
| Schori Julia | experiment and problems |
| Schweizer Yannick | experiment |
| Tatli Mevlüt | graphs |
| Weber Dominic | translation |
| Wider Simon | graphs |