Science on the Move Competition 2013

Task 2:
Phosphate uptake by yeast

Gymnasium Bäumlihof 4C

Table of contents

1. About Phosphate

1.1 Phosphate - essential for all organisms

Phosphate is common in nature and found as different minerals like apatite, pyromorphite and vivianite and it is essential as a nutrient for every living organism - animals, plants, fungi and bacteria[1]. 85% of the phosphates in the human body are found in bones and teeth as hydroxylapatite ($Ca_5(PO_4)_3OH$) - this is what makes them strong and resistant. The remaining 15% are found in cells as phosphate or polyphosphate in different molecules as listed below:

- Phosphate is the main building block of compounds rich in energy like ATP and GTP and is required in the process of releasing energy into the body. Phosphate groups from these compounds are transferred to other substrates, transferring energy[2].
- Phosphate is important for signal transduction: enzymes called kinases and phosphatases add, respectively, remove phosphate groups on different target proteins and hence create different cellular responses [3].
- Phosphate is an important element of the cell membrane as it is part of the phospholipids, which are the main building block of the membrane bilayer.
- Phosphate is also found in the backbone of DNA and RNA, both of which store and transfer genetic information.
- Phosphate can be used in buffer solutions to help keep the pH at a constant value[2].

In humans a lack of phosphate causes problems like loss of appetite, muscle weakness and bone softening. A greater lack of phosphate causes hemolysis[2]. Consequently, in all organisms a lack of phosphate causes disease.

Eventually, phosphate is involved in molecules essential for genetic information, energy transfer, communication during signal transduction, physiological functions and use as building block[2].

1.2 Phosphate uptake - an important issue for our society and for the environment

Liebig's Law of the Minimum indicates that the fewest available nutrient always defines the growth of organisms and usually this is nitrate and/or phosphate[8]. This minimal factor can be expanded with dung[8]. Fertilizing is therefore extremely important to grow enough food to feed humanity. The nutrition pyramid shows us how the different nutritive levels depend on each other. For instance phosphate influences them in water ecosystems, for example plankton or the lower and the higher fishes. Until 2003 it was a big problem that through sewage water too much phosphate got into lakes and rivers. This Phosphate is absorbed by algae. Too large quantities of phosphate in water can lead to eutrophication of aquatic ecosystems: the increase in phosphates, which can often be put down to agricultural waste, fertilisers and detergent in sewage, results in shortage of oxygen due to mushrooming growth of microorganisms especially phytoplankton[7]. Nowadays, phosphate is forbidden in laundry detergent and microorganisms which take up phosphate are used in purification plants to degrade phosphate and thus stabilise the phosphate level of water that is drained off to open water bodies[7]. The yeast experiments in this report can therefore be studied as a model system to understand phosphate uptake by organisms[7].

1.3 Phosphate uptake and storage in the yeast cell Saccharomyces cerevisiae

Uptake: During the metabolism of sugars, phosphate is transported into yeast cells involving glycolysis reactions[6]. It seems like phosphate can only enter into cells which have metabolic processes at the cell surface. "Uptake of free phosphate from outside the cell is mediated by a number of plasma membrane transport systems. One has a high affinity for phosphate and is

encoded by the *PHO84* gene, whose expression is derepressed under conditions of phosphate starvation. Others include a sodium/phosphate cotransporter and a low affinity, constitutive transport system"[4].

Storage: Yeast stores phosphate mostly in vacuoles[4]. The phosphates often occur as organic linkages or in form of polyphosphates. The concentration of free phosphate within the cell is normally maintained very low[6].

2. Calibration of the measuring system

We followed the instructions for part 2. The calibration curve is shown in Figure 1 and the measurement values are given in Table 1. The standard deviation increases at higher phosphate concentrations, indicating uncertainty about these values. Measurement 1 (M1, Tab. 1) shows a nearly straight line with a correlation coefficient R^2 of 0.982.

For our first experiments, the lapse of time between mixing the sodium-phosphate-buffer with the molybdat-malachitgreen-mixture (step 6 of part 2) was set at 1 minute. After problems with discolouration of cuvettes and a too low fill level of the cuvettes, which resulted in anomalous results, the original cuvettes were replaced by more narrow cuvettes, also the lapse of time between mixing the sodium-phosphate-buffer with the molybdat-malachitgreen-mixture (step 6 of part 2) was increased from 1 minute to 10 minutes, a completely new cuvette was used for each measurement and in order to clarify any uncertainties about precipitating substances in molybdat-solution and the stability of molybdat-solution, we sought advice from *„simplyscience"* but we were later reassured that there were no inconveniences to expect.

Figure 2 shows the result of the pretest, the result of the pretest, the molybdat-malachitgren-mixture without phosphate as a negative control. In all experiments (part 2 to 4) the photospectrometer was calibrated to zero using such a negative control.

Figure 3 shows our lab environment. A series of cuvettes used for one measurement as well as the school photospectrometer are shown at the front page.

$c[PO_4^{3-}]$	M 1	M 2	M 3	Mean	SD
0	0	0	0	0	0
10	0.57	0.395	0.313	0.426	0.107
20	0.834	0.763	0.779	0.792	0.03
30	1.033	0.88	0.896	0.936	0.069
40	1.564	1.314	1.222	1.367	0.145
50	1.883	1.332	1.278	1.498	0.273
R^2	0.982	0.957	0.954	0.977	
y =	0.036x+0.081	0.272x-0.173	0.264x-0.175	0.299x-0.209	

Tab. 1: Measurements used to establish the calibration curve. $c[PO_4^{3-}]$: concentration of phosphate in µM. M1-3: measurement 1-3. Mean: arithmetic mean of M1-3. SD: Standard deviation. R^2: r-value (correlation coefficient). y=: linear equation
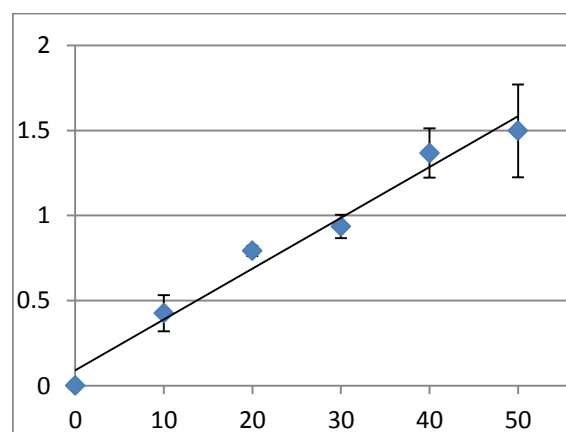

Fig. 1: Linear Regression of calibration values from table 1. OD_{595} versus phosphate concentration in µM.
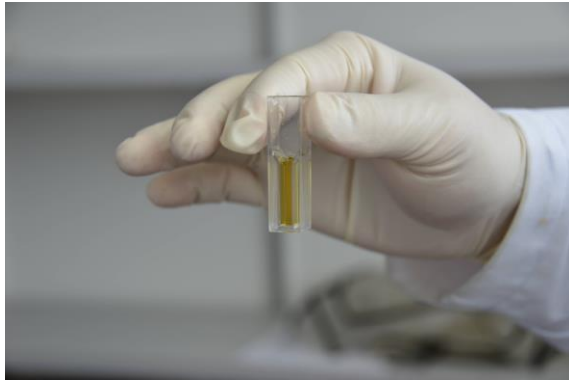
Fig. 2: Negative control

Fig. 3: Lab equipment

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

In the standard experiment the phosphate uptake of yeast was tested at room temperature (21°C), untreated yeast (Coop) and the phosphate-buffer sent by „*simplyscience*" (200x diluted with the addition of Glucose: final concentration: 1% glucose, 0.5mM, pH6.3). While doing the standard experiment we already started to conduct experiments to test accelerating factors for phosphate uptake, as shown in part 4. Most promising experiments were repeated to gain statistical values.

In table 2 and figure 4 the five measurements of phosphate decrease under standard conditions are illustrated with the mean and standard deviation. A clear decrease of phosphate concentration is visible. Problems arrived at the standard experiment as well as in part 4 mostly because of the indicator solution (malachitgreen + molybdat), in which the light absorption changed over time and the experiment had to be repeated multiple times. We solved this problem through a constant keep of the same period of time between the preparing of the indicator solution and the metering of the light absorption in the photospectrometer: the indicator solution (256 µL malachitgreen-solution + 344 µL molybdat-solution) was added to the probe exactly 5 minutes after mixing and of those the light absorbance was measured 10 minutes afterwards. Instead of only showing the best 3 results of the experiments, 5 results were used in order to achieve better statistical data. This was as well done because it cannot simply be chosen the *"best"* data in statistics: This would be subjective to an individual interpretation of the data. It would be necessary to cancel out the two extreme values on both sides of the collected data: This would lead to elimination of measurement 3 (Tab. 2) which seems to be the best measurement, as there is a straight linear decrease in phosphate.

Time	M1	M2	M3	M4	M5	Mean	SD
0	1.46	1.438	1.318	1.303	1.479	1.4	0.074
10	1.415	1.471	1.286	1.037	1.443	1.33	0.16
20	1.275	1.269	1.159	1.065	1.155	1.185	0.079
30	1.108	1.064	0.934	0.99	0.825	0.984	0.1
40	0.886	0.96	0.463	0.858	0.712	0.776	0.176
50	0.912	0.886	0.176	0.78	0.694	0.69	0.268

Tab. 2: Absorption at OD_{595} under standard conditions, time in minutes.
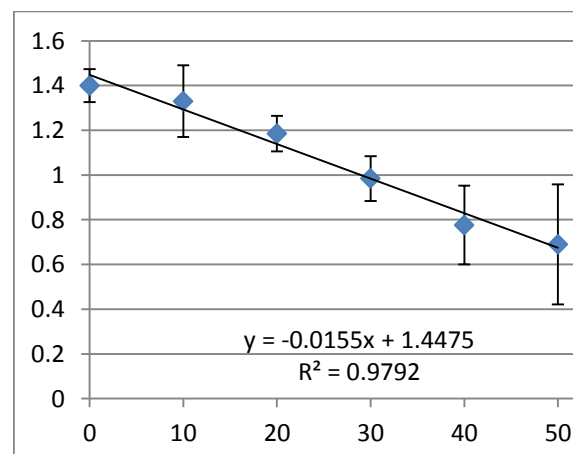

$$y = -0.0155x + 1.4475$$
$$R^2 = 0.9792$$

Fig. 4: Phosphate uptake by untreated yeast cells. OD_{595} versus time in minutes.

4. How to improve the phosphate uptake by yeast cells

Different approaches were taken in order to increase phosphate uptake by yeast cells:

Our first idea was to raise the temperature (4.1). This seemed reasonable because we had learned about the Van't Hoff's rule in class which tells us that chemical reactions proceed two times faster if the temperature is raised by 10 degrees. We tested two different temperatues: 28°C because this is the natural growth optimum of yeast[9] and 37°C because this temperature is often used for incubations in our biology experiments done with bacterial cells.

In the literature we found the information, that the yeast phosphate uptake depends on the pH: A lower pH should increase the efficiency of the yeast phosphate uptake[4]. Therefore we tested yeast cells after adding 100 µl of 0.1 M HCl or 100 µl of 0.1 M NaOH to the essay (4.2).

The third aspect we investigated, was to starve the yeast cells by keeping them in a phosphate poor environment for one night prior to the essay (4.3)[10]. It seems obvious that cells depleted of phosphate will take up phosphate faster as soon as they are again in a phosphate-rich medium and the gene of the high affinity phosphate transporter PHO84 gets derepressed under conditions of phosphate starvation[4].

As a fourth approach we considered increasing the concentration of yeast or phosphate because we assumed that phosphate uptake would increase the more cells there are that take up phosphate and the more phosphate there is to take up. In addition, an increase of phosphate in the medium would be in accordance with the law of Le Chatelier, which says that the least available resource defines the amount of the chemical reaction. We didn't try increasing the amount of yeast cells after considering that this wouldn't change anything about the uptake per cell and only tried increasing phosphate concentration (4.4).

The last method we tried was adding potassium and magnesium to the yeast cells that were taking up the phosphate since we found in the literature the information that this might improve the uptake (4.4)[5].

At the end of all experiments we plotted a linear regressions through the mean values. Even though we cannot say that the reactions definitely occur linearly, this makes it easier to compare the phosphate decrease with the decrease observed in the standard experiment (part 3).

4.1 Temperature

At 37°C the yeast took phosphate up more slowly compared to the standard temperature (Fig. 5, Tab. 3). At 28°C however the phosphate uptake was heightened significantly (faster and more) in comparison to the standard (Fig. 6, Tab. 4). This is due to the fact, that phosphate uptake is not solely based on the Van't Hoff rule because yeast is a living organism with a growth optimum, which is at 28°C[9]. Hence yeast has the fastest and biggest uptake of phosphate at this temperature. 37°C is the growth optimum for some bacteria but is already too high for yeast and thus phosphate uptake is already reduced. We have made a linear regression at both of these temperatures, but we don't know if these are correct as after approximately 20 minutes the uptake declines strongly to eventually even out completely. This might be put down to signs of saturation the yeast is exhibiting after an intense short-term uptake. This would mean that the decrease in phosphate was not linear and consequently the linear regression was incorrect.

In further experiments it would be necessary to measure every degree Celsius between 28°C and 37°C. Out of this the exact optimum for the temperature during phosphate uptake would result. Because of the consideration above we estimate that the optimum would be close to 28°C.

Time	M1	M2	M3	Mean	SD
0	1.277			1.277	0
10	1.137		1.094	1.116	0.022
20	0.51	0.67	0.667	0.616	0.075
30	0.693	0.7	0.68	0.691	0.008
40	0.838	0.753	0.819	0.803	0.036
50	0.928	0.757	0.738	0.808	0.085

Tab. 3: Absorption at OD$_{595}$. The experiment was performed at 37°C.
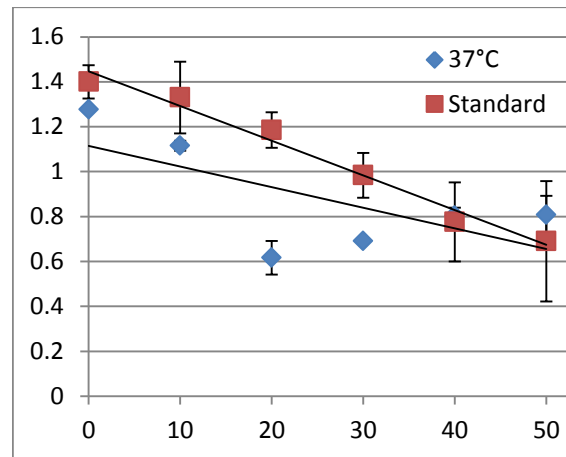
Fig. 5: Graphic display of the values at 37°C compared to the standard. OD$_{595}$ versus time in minutes.

Time	M1	M2	M3	Mean	SD
0	1.499		1.109	1.304	0.195
10	1.469		0.991	1.23	0.239
20	0.686	0.541	0.55	0.592	0.066
30	0.272	0.246	0.364	0.294	0.051
40	0.201	0.211	0.32	0.244	0.054
50	0.196	0.228	0.311	0.245	0.048

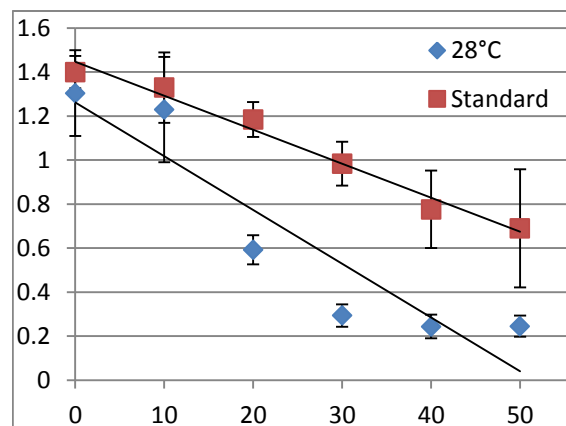**Tab. 4:** Absorption at OD$_{595}$. The experiment was performed at 28°C.

Fig. 6: Graphic display of the values at 28°C compared to the standard. OD$_{595}$ versus time in minutes.

4.2 pH

At the addition of HCl (100 µl 0.1 M) (Fig. 7, Tab. 5), as well as at the addition of NaOH (100 µl, 0.1 M) (Fig. 8, Tab. 6) the phosphate uptake increases with significant amounts. Both of the curves are below the standard curve. It is to conclude that phosphate at a changed pH is taken in faster. Though one can not find a significant difference between the addition of H_3O^+ and the addition of NaOH. The phosphate is taken in at the same speed with both of the additions.

In references it is stated that phosphate uptake in a low pH is faster, we did not found any references for a high pH[4].

We added 100 µl of 0.1 M HCl or 0.1 M NaOH respectively to 10 ml of yeast solution. This would give a calculated pH of 3 and 11 if diluted in water. Since we added it to the yeast suspension in phosphate-buffer the change of pH was less than expected and not gaugeable with pH paper. Hence it is questionable if we changed the pH strong enough.

In more experiments the same measurements with different pH buffers would have to be done to obtain more extensive data at an exactly defined pH value from pH 3 to pH 11 in steps of pH 0.5.

Time	M1	M2	M3	Mean	SD
0	1.177	2.018	1.103	1.433	0.415
10	1.009	1.07	1.074	1.051	0.03
20	1.108	1.061	0.824	0.998	0.124
30	0.833	0.752	0.411	0.665	0.183
40	0.419	0.483	0.203	0.368	0.12
50	0.181	0.348	0.135	0.221	0.092

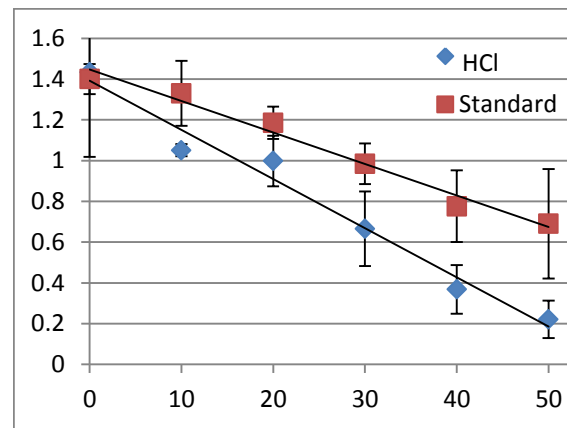**Tab. 5:** Absorption at OD_{595}. The experiment was performed by adding HCl.

Fig. 7: Graphic display of the values by adding HCl compared with the standard. OD_{595} versus time in minutes.

Time	M1	M2	M3	Mean	SD
0	1.363	1.044	1.379	1.262	0.154
10	1.34	0.551	1.124	1.005	0.333
20	0.869	0.902	0.682	0.818	0.097
30	0.513	0.41	0.168	0.364	0.145
40	0.36	0.334	0.191	0.295	0.074
50	0.155	0.187	0.173	0.172	0.013

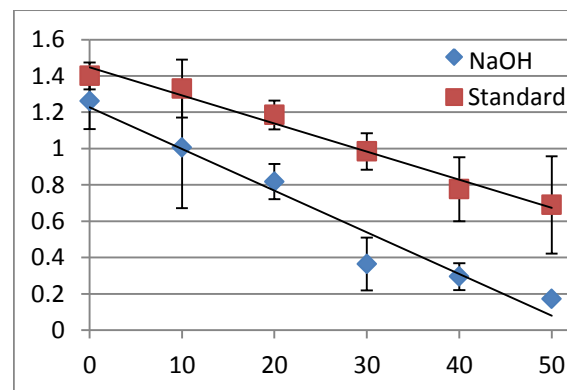**Tab. 6:** Absorption at OD_{595}. The experiment was performed by adding NaOH**.**

Fig. 8: Graphic display of the values by adding NaOH compared to the standard. OD_{595} versus time in minutes.

4.3 Starvation of yeast cells prior to the essay

The yeast was kept overnight in a starch-solution of 1% starch because we assumed that in these conditions yeast were not able to metabolise this and would be famished in the next day. Against all odds, the phosphate uptake was reduced after the starvation in comparison to the standard experiment (Fig. 9, Tab. 7) and we have no explanation for these results, which contradict our hypothesis. Especially as starvation should lead to the upregulation of genes increasing phosphate uptake[4].

In order to conduct these experiments better two parallel experimental preparations would need to be conducted simultaneously and the yeast kept in the same nutrient solution with and without phosphate overnight. Then the yeast could be used for the experiments and be compared exactly to each, giving an accurate result if the starvation of the yeast led to an increase in phosphate uptake. In addition, another approach would be to vary the duration of the starvation of the yeast.

Time	M1	M2	Mean	SD
0	1.399	1.394	1.397	0.003
10	1.143	1.347	1.245	0.102
20	1.266	1.273	1.27	0.003
30	1.213	1.14	1.177	0.037
40	1.147	1.133	1.14	0.007
50	1.166	1.14	1.153	0.013

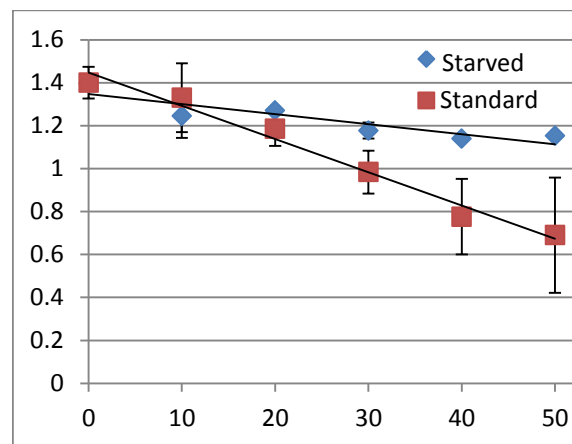**Tab. 7:** Experiment with starved yeast.

Fig. 9: Experiment with starved yeast. OD_{595} versus time in minutes.

4.4 Change of phosphate concentration and addition of potassium or magnesium ions

An increase of the phosphate concentration to 10 µM led hardly to a change (Fig. 10, Tab. 8). This is in retrospect explicable since the decrease is similar but on a higher level, therefore the concentration in the medium stays high. This experiment was only done once.

We tried to increase phosphate uptake by yeast cells by adding a solution of 5 µM potassium chloride in one essay and 5 µM magnesium chloride in another essay.

The phosphate uptake by yeast cells is increased with 5 µM KCl (Fig. 11, Tab. 9) but shows no difference by the addition of 5 µM $MgCl_2$ (Fig. 12, Tab. 10). As we were running out of staining solution we decided to repeat the experiment with KCl, but performed the experiment with MgCl only once. It would be necessary to repeat both experiments to receive statistically significant results.

Additionally, it is stated in literature that the amount of taken up phosphate only increases and does not accelerate, when for example potassium or magnesium is present[5]. Nonetheless, the uptake of phosphate in the presence of potassium was accelerated.

Time	M1
0	1.401
10	1.341
20	1.292
30	1.345
40	1.282
50	1.294

Tab. 8: Absorption at OD_{595}. The experiment was performed by increasing the phosphate concentration.

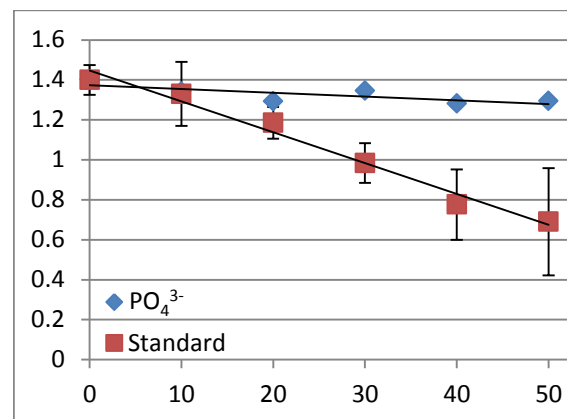**Fig. 10:** Graphic display of the values by increasing phosphate concentration with the standard. OD_{595} versus time in minutes.

Time	M1
0	1.461
10	1.558
20	1.085
30	0.941
40	0.776
50	0.592

Tab. 9: Absorption at OD_{595}. The experiment was performed by adding 5 µM $MgCl_2$.
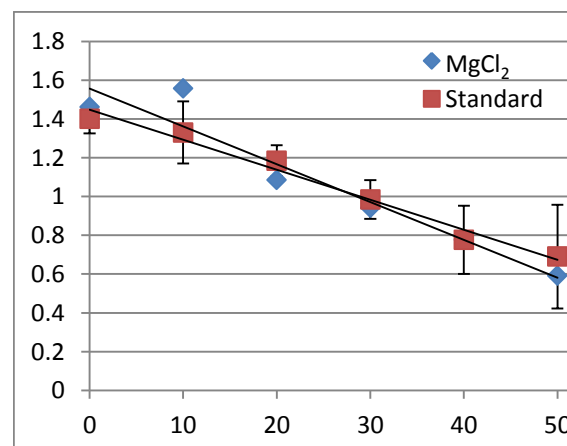
Fig. 11: Graphic display of the values by adding 5mM $MgCl_2$. OD_{595} versus time in minutes.

Time	M1	M2	Mean
0	1.22	1.4	1.31
10	1.373	1.243	1.308
20	0.971	0.978	0.975
30	0.565	0.421	0.493
40	0.189	0.14	0.165
50	0.194	0.194	0.194

Tab. 10: Absorption at OD_{595}. The experiment was performed by adding 5 µM KCl.
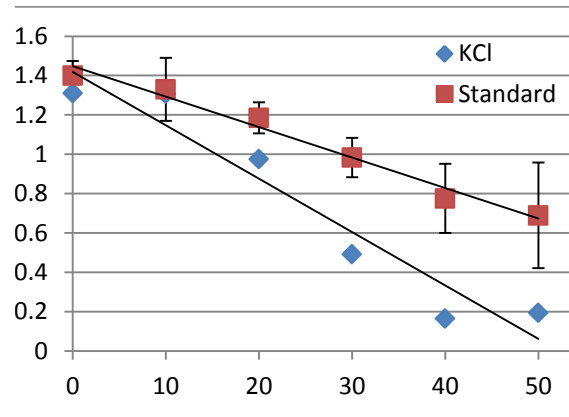

Fig. 12: Graphic display of the values by adding 5 µM KCl. OD_{595} versus time in minutes.

4.5 Summary

We achieved an increase in phosphate uptake by yeast by increasing temperature to 28°C and by changing pH. An increase could as well be observed by adding potassium ions, but regarding this experiment the data set is low. Starvation did not lead to an increase.

It would be interesting to combine different experimental set-ups to ascertain whether a synergistic effect is obtainable.

5. Reference list

1. Seilnacht, T. 2012. *SEILNACHT* – Article: "Phosphate". Retrieved April 18, 2013 from www.seilnacht.com.

2. Anonymous. 2013. *MedizInfo* – Article: "Phosphate". Retrieved April 18, 2013 from www.medizinfo.de.

3. Kössel, H. et al. 1986. *Lexikon der Biologie*. pp 370-371. Verlag Herder, Freiburg.

4. Booth, J.W. et al. 1997. *JBC* – Article: "Phosphate Transport in Yeast Vacuoles". Retrieved April 18, 2013 from http://www.jbc.org/content/272/33/20408.long.

5. Goodman, J. et al. 1957. *University of* Rochester – Article: "The active transport of phosphate into the yeast cells". Retrieved April 19, 2013 from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2147585/.

6. Feldmann, H. 2005. *Uni-München* – Article: "Yeast Molecular Biology". Retrieved April 18, 2013 from http://biochemie.web.med.uni-muenchen.de/Yeast_Biol/.

7. Anonymous. No date. *World Resources Institute* – Article: "Eutrophication and Hypoxia". Retrieved April 19, 2013 from http://www.wri.org/.

8. Baur, W. 1980. *Gewässergüte bestimmen und beurteilen*. 3rd edition. Verlag Paul Parey, Hamburg/Berlin.

9. Renz, K. No date. *Uni Düsseldorf* – Article: „Wachstumskurve einer Hefepopulation". Retrieved May 7, 2013 from http://www.uni-duesseldorf.de/MathNat/Biologie/Didaktik/Hefe/ .

10. Markham, et al. 1968. "Uptake, storage and utilization of phosphate by yeast III. The behaviour of phosphate-starved yeast." *Journal of the Institute of Brewing*, vol. 74, issue 4. July-August 1968. pp 374–378.

6. Activity List

The entire class was fully involved with the experiments involving the uptake of phosphate by yeast cells, as well as writing the texts.

The scientific texts were written by Sara, Bárbara, Michal, Lena, Noemi, Sabine and Reto. The phosphate measurements were conducted by Dany, Francesco, Tim, Michael, Diego, Emmanuel, Floriana, Giulia, Rebecca, Fabian, Damian, Ibrahim, Ehi, Mathias, Dominique and Ruben.

Fig. 13: Some of the boys at the pipetting training

We obtained the pictures of the working scenes using Dany's Camera. He was also the main photographer of our experiments.

Fig. 14: Concentrated pipetting by Floriana