CLASS 4LD

Task 2: Phosphate uptake in yeast cells

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1. Part 1

1.1. Why is phosphate important for all organisms?

A phosphate is a salt of phosphoric acid[(1)]. Organic phosphates are essential for all living beings in order to produce two of the most important organic macro-molecules: deoxyribonucleic acid (DNA) and adenosine triphosphate (ATP)[(2)]. In addition, phosphate is an important nutrient for plants. It helps them to activate root development and flowering while protecting them from diseases and stress[(3)]. Therefore many phosphates are used in fertilizers.

1.2. What is well known about phosphate uptake in yeast cells *(Saccharomyces cerevisiae)*?

The yeast secretes phosphatases to free phosphate in the extracellular space. This free phosphate is mediated by "a number of plasma membrane transport systems"[(4)]. The activity of transport is increased at a lower pH-value[(4)]. The energy for the transport is primarily provided by glycolysis[(5)].

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

Phosphate is primarily stored in the vacuole of yeast cells. Large amounts of phosphate are absorbed by the vacuole via counter flow exchange, an exchange of two liquids or gases in different compartments in opposite directions[(4)]. 3-5 % of the dry weight of yeast cells consists of phosphate [(6)].

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

In aquatic ecosystems phosphate uptake by plankton enhances their growth, but only unto a certain level where too high concentrations of phosphate lead to eutrophication, a depletion of oxygen in waters resulting in disturbance of the "equilibrium" of the ecosystem[(2)]. However, since the introduction of sewage treatment and the prohibition of phosphate containing detergents the concentration of phosphate has dropped below the limit of detection in some of Switzerland's lakes. This has led to fish becoming smaller and fewer over the past few decades because phosphate is at the beginning of the food chain in aquatic ecosystems and as a consequence it is impossible for today's fishermen to live of their profession in most of Switzerland[(7)].

2. Part 2

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Phosphate concentration [µM]	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00000000
	10	0.2734	0.2732	0.2738	0.2731	0.2736	0.2734	0.00028636
	20	0.3356	0.3361	0.3361	0.3361	0.3363	0.3360	0.00026077
	30	0.5413	0.5411	0.5420	0.5428	0.5435	0.5421	0.00101143
	40	0.6603	0.6623	0.6618	0.6631	0.6637	0.6622	0.00130690
	50	0.5859	0.5880	0.5902	0.5916	0.5939	0.5899	0.00310596

Table 1: The measured observed densities at 595 nm light of solutions containing different concentrations of phosphate. The vial containing no phosphate was used as reference.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Phosphate concentration [µM]	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00000000
	10	0.2915	0.2902	0.2885	0.2864	0.2852	0.2884	0.00260250
	20	0.4950	0.4944	0.4927	0.4936	0.4931	0.4938	0.00093968
	30	0.6511	0.6536	0.6520	0.6530	0.6537	0.6527	0.00111221
	40	0.9119	0.9111	0.9123	0.9135	0.9143	0.9126	0.00127750
	50	1.1540	1.1566	1.1542	1.1533	1.1535	1.1543	0.00132552

Table 2: The measured observed densities at 595 nm light of solutions containing different concentrations of phosphate. The vial containing no phosphate was used as reference.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Phosphate concentration [µM]	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00000000
	10	0.3482	0.3476	0.3458	0.3451	0.3446	0.3463	0.00157099
	20	0.5337	0.5342	0.5323	0.5299	0.5287	0.5318	0.00238914
	30	0.9421	0.9449	0.9416	0.9426	0.9426	0.9428	0.00126610
	40	1.2215	1.2191	1.2234	1.2223	1.2219	1.2218	0.00158682
	50	1.5662	1.5682	1.5671	1.5665	1.5634	1.5663	0.00178241

Table 3: The measured observed densities at 595 nm light of solutions containing different concentrations of phosphate. The same reference as for the second set of samples was used.

		Average	SD
Phosphate concentration [µM]	0	0.0000	0.00000000
	10	0.3027	0.03255701
	20	0.4539	0.08772379
	30	0.7125	0.17487109
	40	0.9322	0.23682572
	50	1.1035	0.41426531

Table 4: The averaged measured observed densities at 595 nm light for the above three sets of samples.

Function for determination of the phosphate concentration:

$$y \approx 0.0186x + 0.0805 \quad [1]$$
$$y - 0.0805 \approx 0.0186x \quad [2]$$
$$x \approx (y - 0.0805)/0.0186 \quad [3]$$

Equations 1-3: Equation 1 shows the function equation of the trend line depicted in figure 1. The third equation gives the formula to approximate the phosphate concentration x in a sample if the observed density is known.

Changes made to the procedure:

Due to difficulties in obtaining coherent results in part 3 of the task, a few alterations to the guidelines were made.

Because of the adhesive properties of the malachite green solution, this was diluted with distilled water (H_2O) by the factor of two.

In order to eliminate measuring inaccuracies by the photo spectrometer, five measurements of the same vial were taken at intervals of around two seconds.

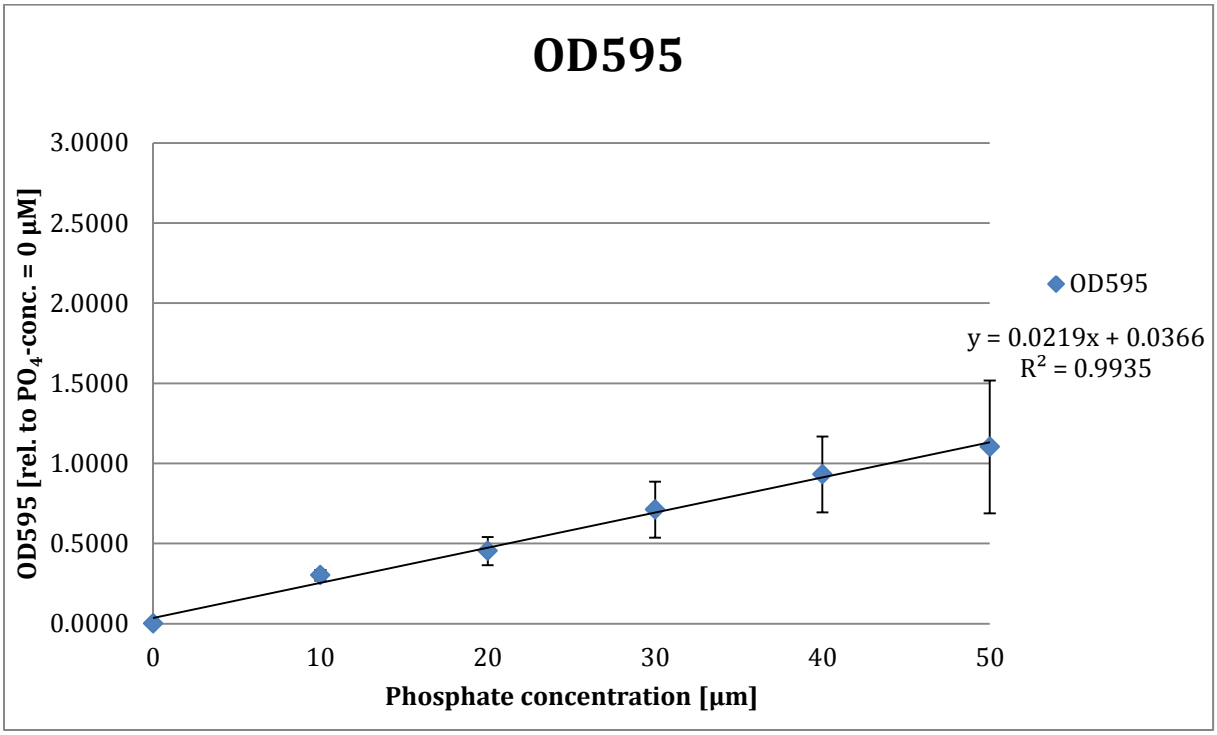

Figure 1: Depicted are the average measured observed densities at 595 nm light fitted with a trend line and standard deviation error bars. The variable y in the given formula denotes the observed density, x the phosphate concentration.

Figure 2: Centrifugation of the yeast suspension during experiment 4B. The rack in the foreground holds the EPs containing the malachite green solution and those filled with the supernatant.

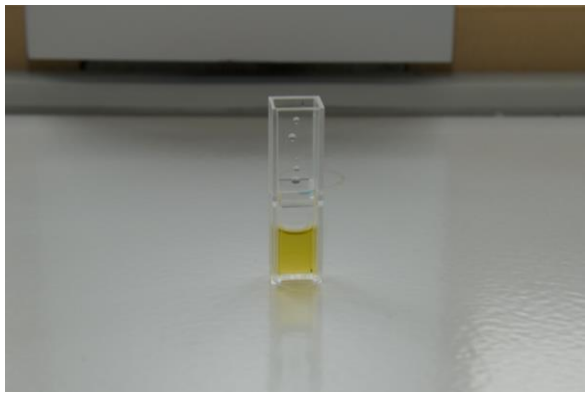

Figure 3: The yellow pretest showing that no phosphate contamination of the reagents existed.

3. Part 3

		Culture 1	Culture 2
		Value 1	Value 1
Time passed	0 min	1.7209	1.7511
	10 min	1.9316	1.8100
	20 min	1.8026	1.8424
	30 min	1.7410	-
	40 min	1.5868	1.6731
	50 min	1.4126	1.5519

Table 5: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time. These measurements were taken before the implementation of the "five measurements per sample" policy. The OD for the second culture at the 30 min mark was lost during data processing.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	1.7082	1.7128	1.6958	1.6949	1.7068	1.7037	0.00794544
	10 min	1.1388	1.1740	1.1930	1.2112	1.2189	1.1872	0.03213770
	20 min	1.5479	1.5486	1.5489	1.5544	1.5947	1.5589	0.02018031
	30 min	1.4958	1.5009	1.4904	1.4894	1.4895	1.4932	0.00505025
	40 min	1.1216	1.1212	1.1144	1.1175	1.1148	1.1179	0.00341321
	50 min	0.8663	0.8649	0.8636	0.8633	0.8635	0.8643	0.00127358

Table 6: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time. The measurements leading to the values in the cells marked beige were taken with 688 µl if molybdate solution instead of the usual 344 µl.

		Average	SD
Time passed	0 min	1.7252	0.02399528
	10 min	1.6429	0.39934372
	20 min	1.7346	0.15348506
	30 min	1.6171	0.17522106
	40 min	1.4593	0.29876466
	50 min	1.2763	0.36349730

Table 7: The average measured observed densities at 595 nm light for the above three sets of samples. The values in the cells marked beige were influenced by the incorrect amount of molybdate solution present in the measurements shown in table 6.

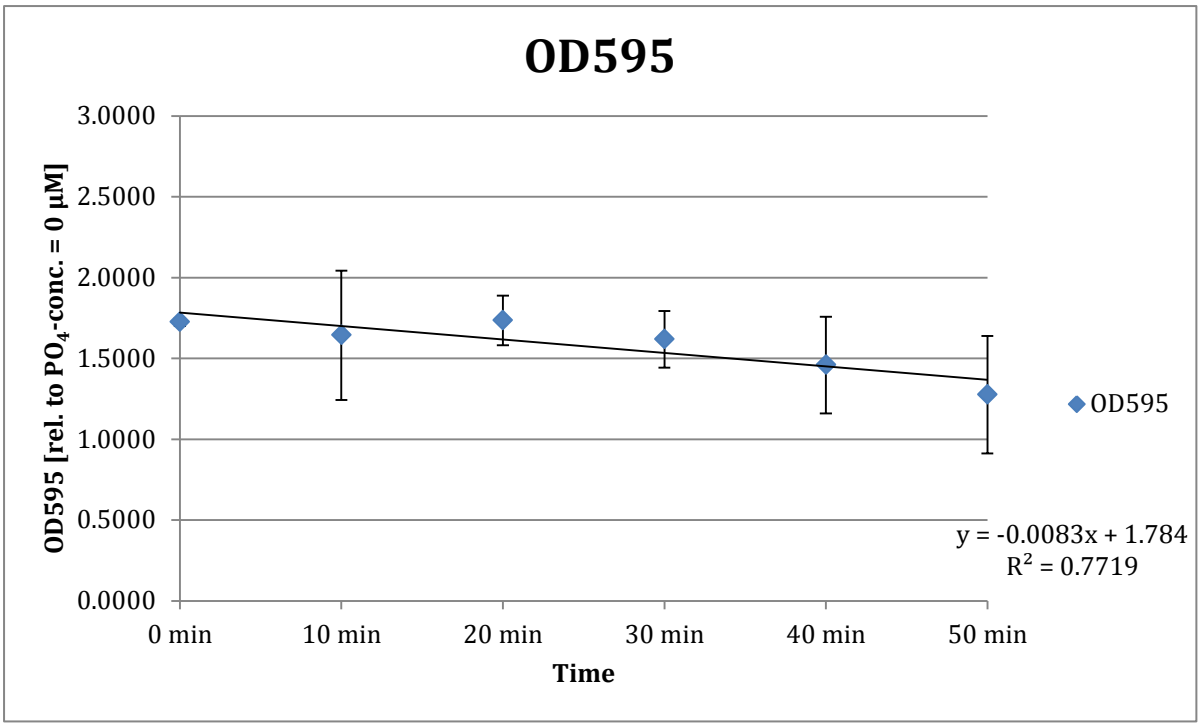

Figure 2: The average measured observed densities at 595 nm light fitted with a trend line and standard deviation error bars.

At the beginning of the experiment low R^2 values were obtained. To ameliorate the results, several changes in the experimental procedure were made. It was observed that a source for the seemingly arbitrarily oscillating results might be the adhesion of drops of the different solutions to the Eppendorf tubes and the pipetting tips during transfer to other containers. For this purpose, the contents of the tube containing the supernatant were pipetted into the tube holding the phosphate indicator. This still didn't yield satisfying results, so it was decided to dilute the malachite green solution by a factor of two with distilled water (H_2O) to further reduce adhesion to the pipetting tips.
To eliminate possible fluctuations caused by false measurements by the photo spectrometer five measurements of each sample were taken in direct succession.
To assess the remaining disturbing influence, one experiment was conducted with yeast that previously had been boiled for several minutes in order to kill it. It yielded a very low R^2 value (0.0137), however with a low average standard deviation of around 0.00732654.
Unfortunately, we did not have the time and capacity, limited by our schedule and the availability of only one centrifuge, to realize other ideas such as the pipetting of smaller volumes than the total volume of the EP to eliminate the influence of incomplete pipetting.

4. Part 4A

4.1. Step 1

4.1.1. Aspect 1

In research conducted in the 1950s a group of researchers surrounding John Goodman and Aser Rothstein[5] found that adding potassium chloride (KCl) to the medium containing the yeast cells increased the speed of the phosphate uptake.
This can be attributed to the fact that unequal electrical charges in a system balance out over time. When the K^+ ions of the KCl compound are transported into the yeast cells, a flow of phosphate into the cell is induced and the uptake of the negatively charged phosphate is increased.
For this reason, adding KCl to the medium was chosen as an approach for increasing phosphate uptake.
The variables needed to be defined for conducting the experiment are listed below:

- Independent variables: K^+ concentration (appr. 0.02 M), pH-value (appr. 4.8)
- Dependent variable: phosphate uptake speed
- Controlled variables: medium temperature (appr. 21 °C), origin of yeast used (MIGROS), medium composition, amount of yeast used (appr. 1 g), phosphate indicator composition, time lapse between mixing of indicator with phosphate and measurement (appr. 45 sec.)

4.1.2. Aspect 2

<u>K^+ concentration</u>
According to equations 4-6 it was calculated that about 746 mg of potassium chloride (KCl) needed to be added to the parent medium solution to reach a concentration of roughly 0.02 M of K^+ ions in the medium (see "medium solution" below). This amount was weighed and then tipped into the concerning beaker.

Molar mass (KCl) · 0.02 M = x	[4]
74.55 g/Mol · 0.02 M = 1.491 g [KCl / litre H_2O]	[5]
=> 0.07455 g KCl / 50 ml H_2O	[6]

<u>pH-value</u>
To reduce the pH-value of the medium hydrochloric acid (HCl, 0.1 M) was diluted in distilled water (H_2O) by a factor of 20 and then added drip by drip to the parent medium solution until a pH-value of around 4.8 had been achieved.

<u>Medium composition</u>
The solution containing the phosphate to be processed by the yeast cells was prepared using a parent solution containing 50 ml of distilled water (H_2O) and 250 µl of the 0.1 M sodium-phosphate-buffer. The amount of water was measured with a volumetric pipette and filled into a beaker, then the buffer was added using a volume-handled pipette.
Approximately 9.9 g of this parent solution were filled into a separate beaker before finishing the medium by adding 0.1 g of glucose ($C_6H_{12}O_6$).

Medium temperature
All the liquids used to prepare the medium were stored in the lab at room temperature, thus had adopted a temperature of around 21 °C beforehand.

Amount of yeast used
The yeast used for the experiment was weighed with a precision of 0.01 g before beginning with the experiment.

Phosphate indicator composition
In order to mix the indicator of phosphate employed for the photometric measurement, the malachite green solution provided was diluted with distilled water (H_2O) with a ratio of 1 : 2, meaning that double the amount of water was added to the amount of malachite green solution provided. 768 µl of this parent solution were added to the corresponding Eppendorf tubes.
To these tubes, 344 µl of the molybdate solution provided were added and the concerning tube vortexed.
After 5 minutes the supernatant mixed with 360 µl of distilled water (H_2O) was pipetted into the tube containing the indicator solution and vortexed.

Time lapse between mixing of the indicator with the supernatant and the measurement
The interval between adding the supernatant to the phosphate indicator and conducting the photometric measurement was kept in close proximity to 45 seconds.

4.1.3. Aspect 3

For each sample it was decided to take five directly subsequent measurements in order to eliminate the danger of missing potential errors of measurement. Overall four measurement series were conducted.

4.2. Step 2

4.2.1. Aspect 1

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.2693	0.2695	0.2693	0.2698	0.2694	0.2695	0.000207364
	10 min	0.312	0.3119	0.3125	0.3127	0.3125	0.3123	0.000349285
	20 min	0.2859	0.2853	0.2839	0.2846	0.2848	0.2849	0.000751665
	30 min	0.1888	0.1888	0.1882	0.1891	0.1893	0.1888	0.000415933
	40 min	0.1046	0.1054	0.1065	0.1074	0.1079	0.1064	0.001368576
	50 min	0.1115	0.1123	0.1126	0.1139	0.1146	0.1130	0.001251799

Table 8: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.2807	0.2813	0.2817	0.2818	0.2817	0.2814	0.00045607
	10 min	0.2665	0.2667	0.2662	0.2671	0.2675	0.2668	0.000509902
	20 min	0.2731	0.2727	0.2741	0.2746	0.2743	0.2738	0.000817313
	30 min	0.2037	0.2045	0.2045	0.2054	0.2052	0.2047	0.000673053
	40 min	0.1204	0.121	0.1215	0.1222	0.1253	0.1221	0.001917551
	50 min	0.1206	0.1216	0.1219	0.1223	0.1243	0.1221	0.001361249

Table 9: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.2943	0.2936	0.2929	0.2931	0.2962	0.2940	0.001333042
	10 min	0.3196	0.3198	0.3228	0.3227	0.3206	0.3211	0.001552417
	20 min	0.3063	0.3072	0.3083	0.3094	0.3086	0.3080	0.001217785
	30 min	0.2471	0.2475	0.2505	0.2508	0.2488	0.2489	0.00168612
	40 min	0.1535	0.1544	0.1557	0.1556	0.158	0.1554	0.001694993
	50 min	0.0691	0.07	0.0711	0.0726	0.0743	0.0714	0.002072921

Table 10: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

4.2.2. Aspect 2

		Average	SD
Time passed	0 min	0.2816	0.02399528
	10 min	0.3000	0.39934372
	20 min	0.2888	0.15348506
	30 min	0.2141	0.17522106
	40 min	0.1279	0.29876466
	50 min	0.1021	0.36349730

Table 11: The average measured observed densities at 595 nm light for the above three sets of samples.

4.2.3. Aspect 3

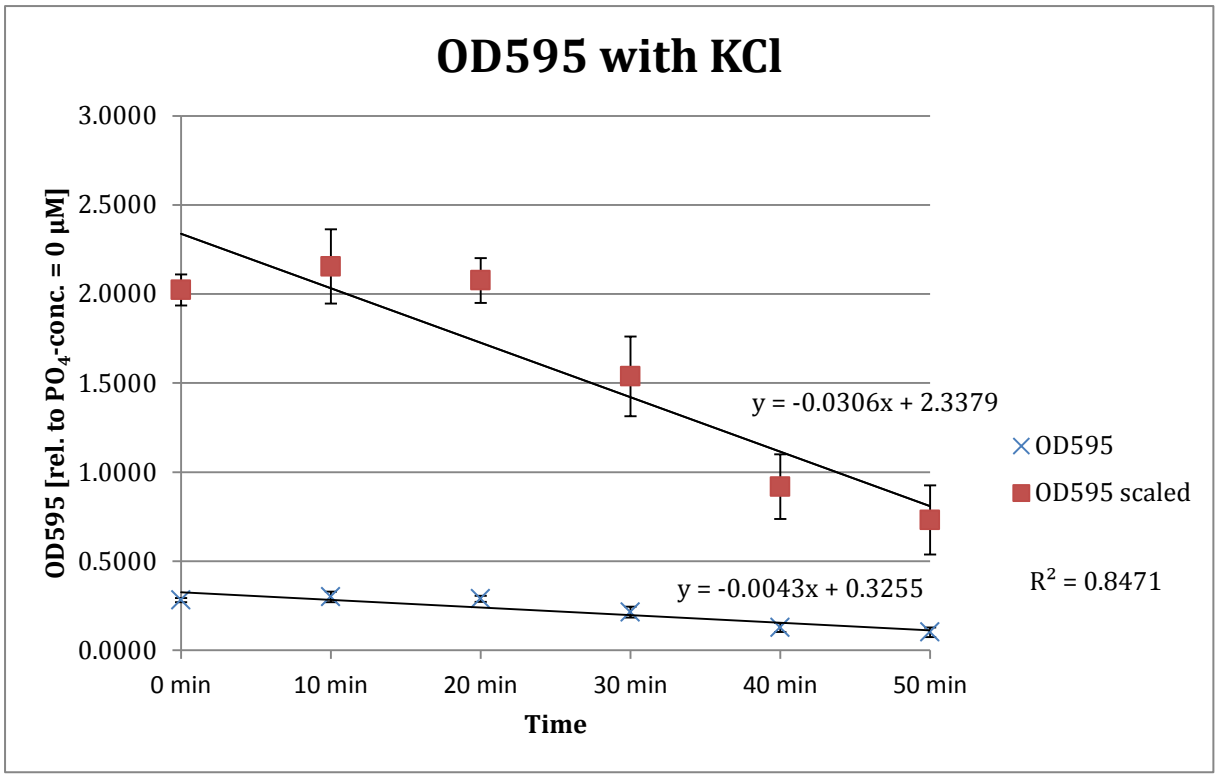

Figure 3: The average observed densities at 595 nm wavelength light fitted with trend lines and standard deviation error bars. The scale factor of the unscaled data was chosen as the factor between the mean measurement at 0 min with KCl and the mean measurement at 0 min from part 3.

4.3. Step 3

4.3.1. Aspect 1

As visible from figure 3, a slight decrease of the amount of phosphate contained in the medium was recorded in the unscaled data with a slope of -0.0043.
After scaling, a larger slope of -0.0306 becomes apparent.
The decrease in phosphate for the unscaled data progresses slower with respect to the decrease under normal conditions by a factor of approximately 2 whereas the decrease in the scaled data turns out relatively rapid, faster with respect to that under normal conditions by a factor of around 3.7.

4.3.2. Aspect 2

As can be seen in tables 8-12 and figure 3 above, the standard deviation of the unscaled data turned out extremely small. This stands in stark contrast to the standard deviations obtained for the other experiments. Additionally, the initial phosphate concentration lies much lower with respect to the phosphate levels measured in the other approaches.
Possibly, sodium phosphate exhibits an affinity for one or both ions of the KCl compound, which could lead to ion binding and thus hinder the phosphate indicator from reacting with the phosphate apparent in the medium, although no references to such a bond could be found in the literature. Such a bond, however, would not explain why the uptake of phosphate proceeded at a comparatively slow pace, when compared to the other experiments, because in their research, John Goodman and Aser Rothstein [5] state, that the presence of K^+ ions in the medium induces a stimulation of the yeast cells between pH-values of 3 and 6.
For this reason, the data was scaled. This led to a standard deviation closer to that obtained from the other investigations and a more significant slope in the graph. This data was

considered potentially meaningful because another source of error might be due to a proportional decrease in activity of the indicator due to the quantity of KCl in the medium. Because the results of the three measurement series carried out exhibit extreme similarities with respect to the value of the data gained, errors of measurement seem highly unlikely. Nevertheless, it would have been advisable to conduct the same investigation a few times more, but this was rendered impossible due the times of laboratory availability and the rapidly dwindling supply of chemicals needed for the experiment.

4.3.3. Aspect 3

As addressed above, an additional run of the experiment with a separate parent solution and a new block of yeast would have been preferable in order to verify the significance of the results obtained.

5. Part 4B

5.1. Step 1

5.1.1. Aspect 1

The goal of the experiment was to optimize the phosphate uptake of yeast cells by manipulating the temperature. The optimal temperature for fermentation is situated at approximately 32 °C and for reproduction at 28 °C. [8]
No literature treating the connection between phosphate uptake and temperature was found, so the temperature for the experiment was chosen around 30 °C because it was supposed that the optimum for reproduction would be similar to the optimum for the metabolism, and the glycolysis, being a central part of the metabolism and the main provider of energy for the phosphate uptake [5], would also work well at that temperature and thus improve the phosphate uptake.
The independent variable is the temperature, which was set at 30 ± 2 °C. Controlled and dependent variables were already treated in part 4.1.1. The only difference is that no KCl was added to the parent solution for this experiment.

Figure 4: The apparatus used for controlling the temperature of the medium.

5.1.2. Aspect 2

The control of the independent variable was achieved by employing a magnetic stirrer with a hot plate: The beakers containing the medium were placed inside a pan containing a multivalent alcohol. With a temperature sensor immersed in the heating fluid and connected to the magnetic stirrer, the temperature was set to 30 °C, while two magnetic stir bars provided a good distribution of the warmth inside the pan. A thermometer was placed inside one of the beakers to ensure that the medium had reached the targeted temperature. The controlled variables were kept the same as in part 3.

5.1.3. Aspect 3

The process of data collection remains the same as described in part 4.1.3.

5.2. Step 2

5.2.1. Aspect 1

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.6850	0.6860	0.6861	0.6860	0.6845	0.6855	0.00072595
	10 min	0.5460	0.5421	0.5427	0.5437	0.5431	0.5435	0.00150399
	20 min	0.4988	0.4990	0.4989	0.4990	0.4994	0.4990	0.00022804
	30 min	0.1556	0.1553	0.1556	0.1558	0.1562	0.1557	0.00033166
	40 min	0.1219	0.1218	0.1210	0.1203	0.1198	0.1210	0.00091815
	50 min	0.2694	0.2703	0.2691	0.2670	0.2643	0.2680	0.00240562

Table 12: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.8568	0.8595	0.8604	0.8615	0.8616	0.8600	0.00196545
	10 min	0.6129	0.6130	0.6136	0.6142	0.6140	0.6135	0.00058138
	20 min	0.6763	0.6762	0.6734	0.6685	0.6637	0.6716	0.00544307
	30 min	0.3907	0.3909	0.3911	0.3918	0.3912	0.3911	0.00041593
	40 min	0.6330	0.6343	0.6341	0.6338	0.6348	0.6340	0.00066708
	50 min	0.0150	0.0148	0.0149	0.0147	0.0145	0.0148	0.00019235

Table 13: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.9031	0.9015	0.9055	0.9023	0.9031	0.9031	0.00149666
	10 min	0.6975	0.6979	0.6976	0.6971	0.6967	0.6974	0.00046690
	20 min	0.5750	0.5751	0.5754	0.5750	0.5754	0.5752	0.00020494
	30 min	0.2921	0.2928	0.2930	0.2929	0.2927	0.2927	0.00035355
	40 min	0.3877	0.3873	0.3873	0.3866	0.3872	0.3872	0.00039623
	50 min	0.4483	0.4462	0.4416	0.4363	0.4318	0.4408	0.00684273

Table 14: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

5.2.2. Aspect 2

		Average	SD
Time passed	0 min	0.8162	0.115203754
	10 min	0.6181	0.077023090
	20 min	0.5819	0.086498342
	30 min	0.2798	0.118245100
	40 min	0.3807	0.256581630
	50 min	0.2412	0.214291225

Table 15: The average measured observed densities at 595 nm light for the above three sets of samples.

5.2.3. Aspect 3

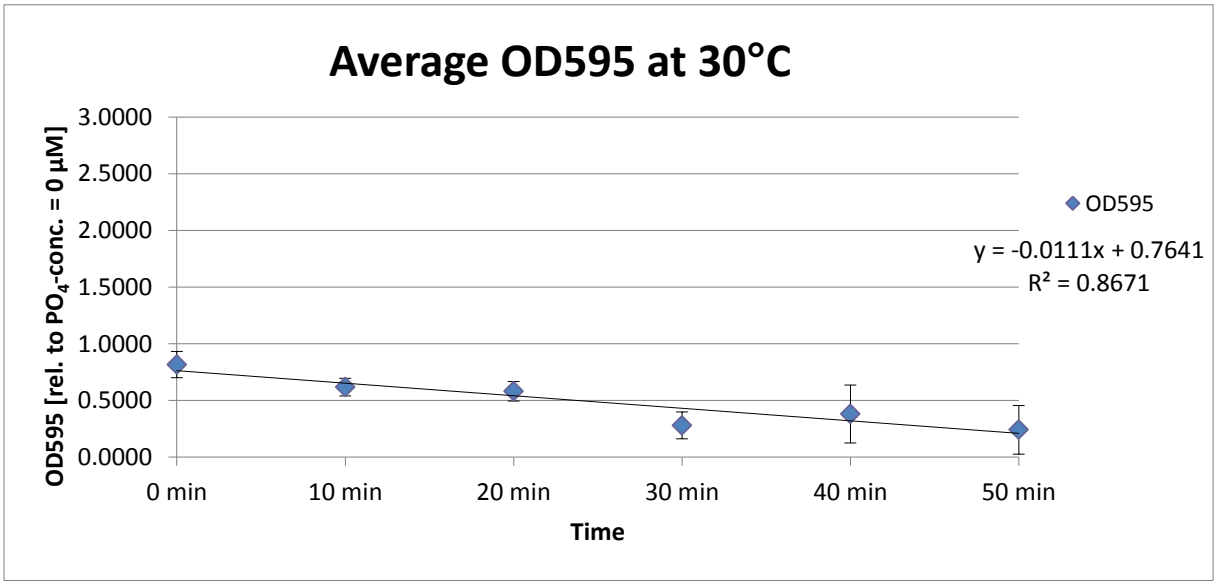

Figure 5: The average observed densities at 595 nm wavelength light fitted with trend lines and standard deviation error bars.

5.3. Step 3

5.3.1. Aspect 1

The slopes of the best-fit line of the experiment, -0.0111 resp. -0.0389 show a 1.3 resp. 4.7 times quicker decrease of the phosphate concentration than in part 3. This indicates that a temperature of 30 °C indeed is better for the phosphate uptake by yeast cells in comparison to about 21 °C which was the temperature during the conducting of the experiments for part 3.

5.3.2. Aspect 2

In general, the measurements are as precise as in part 3 since no controlled variables were changed. What was not taken into consideration is the increased evaporation, which might account for marginally higher concentrations of phosphate especially for samples taken towards the end of the experiment. It remains unclear why the values were relatively low in comparison to the values obtained in part 3.

5.3.3. Aspect 3

The impact of increased evaporation could either have been lessened by covering the beakers with a watch glass, or taken into account with a reference experiment without yeast. To gain more precise results, some ideas for improvement already addressed in part 3 should have been realized, but could not because of limited time and materials. Overall, more experiments should have been conducted.

6. Part 4C

6.1. Step 1

6.1.1. Aspect 1

In his 1961 piece of work "Entry of phosphate into yeast cell" J. E. Leggett[9] finds that yeast cells process phosphate quicker under anaerobic conditions than under aerobic conditions. So it was decided to attempt an increase of the speed of phosphate uptake by pumping nitrogen (N_2) into the medium containing the yeast cells during the experiment in order to decrease the oxygen level in the solution.

The independent variable is the concentration of nitrogen respectively oxygen solved in water. For the controlled and dependent variables see part 5.1.1.

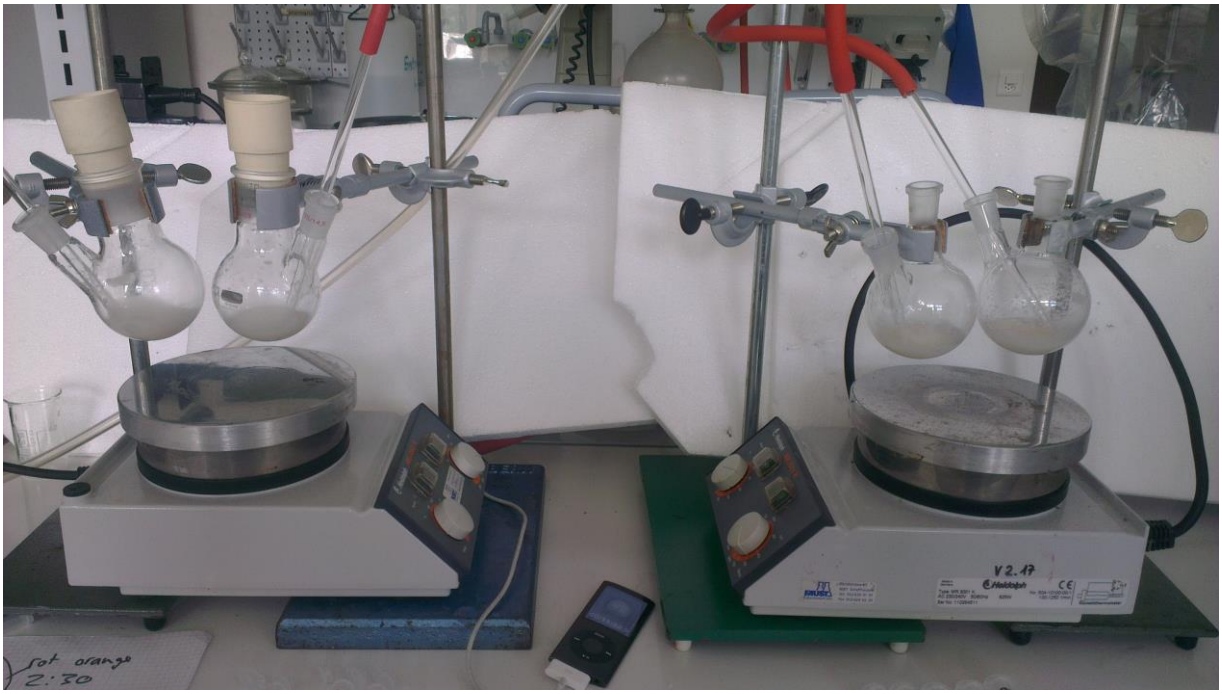

Figure 6: The four separate yeast cultures in the medium being treated with nitrogen.

6.1.2. Aspect 2

By pumping nitrogen into the medium, a low concentration of oxygen was ensured. Furthermore, plugs respectively plastic film were used to limit the gas exchange through the opening of the double-neck flasks. Nitrogenation of the medium was commenced a quarter of an hour before the addition of the yeast.
Since stirring the solution by gently shaking the flasks wasn't possible with the apparatus involved, magnetic stir bars were used, hence the usage of a magnetic stirrer.

6.1.3. Aspect 3

The method of data collection remains the same as in part 4.1.3.

6.2. Step 2

6.2.1. Aspect 1

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.4298	0.4308	0.4302	0.4309	0.4315	0.4306	0.00065803
	10 min	0.4258	0.4246	0.4256	0.4250	0.4258	0.4254	0.00053666
	20 min	0.6670	0.6676	0.6668	0.6678	0.6701	0.6679	0.00131833
	30 min	0.6807	0.6797	0.6819	0.6821	0.6810	0.6811	0.00097057
	40 min	0.7262	0.7261	0.7270	0.7263	0.7283	0.7268	0.00092033
	50 min	0.5323	0.5328	0.5337	0.5328	0.5344	0.5332	0.00083964

Table 16: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.8227	0.8223	0.8240	0.8257	0.8283	0.8246	0.00245764
	10 min	0.6835	0.6838	0.6837	0.6855	0.6854	0.6844	0.00098336
	20 min	0.7246	0.7233	0.7252	0.7262	0.7282	0.7255	0.00183848
	30 min	0.4272	0.4274	0.4267	0.4274	0.4270	0.4271	0.00029665
	40 min	0.9928	0.9921	0.9937	0.9982	0.9982	0.9950	0.00297574
	50 min	0.7116	0.7129	0.7132	0.7152	0.7144	0.7135	0.00139212

Table 17: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

		Value 1	Value 2	Value 3	Value 4	Value 5	Average	SD
Time passed	0 min	0.4416	0.4423	0.4425	0.4427	0.4429	0.4424	0.00050000
	10 min	0.7316	0.7324	0.7331	0.7337	0.7335	0.7329	0.00086197
	20 min	0.4511	0.4520	0.4517	0.4526	0.4522	0.4519	0.00056303
	30 min	0.5535	0.5534	0.5531	0.5536	0.5539	0.5535	0.00029155
	40 min	0.6357	0.6355	0.6352	0.6359	0.6370	0.6359	0.00068775
	50 min	0.5809	0.5813	0.5822	0.5824	0.5841	0.5822	0.00123976

Table 18: The measured observed densities at 595 nm light for samples taken from the medium containing yeast at different points in time.

6.2.2. Aspect 2

		Average	SD
Time passed	0 min	0.5659	0.22413523
	10 min	0.6142	0.16532691
	20 min	0.6151	0.14422117
	30 min	0.5539	0.12697049
	40 min	0.7859	0.18672170
	50 min	0.6096	0.09320868

Table 19: The average measured observed densities at 595 nm light for the above three sets of samples.

6.2.3. Aspect 3

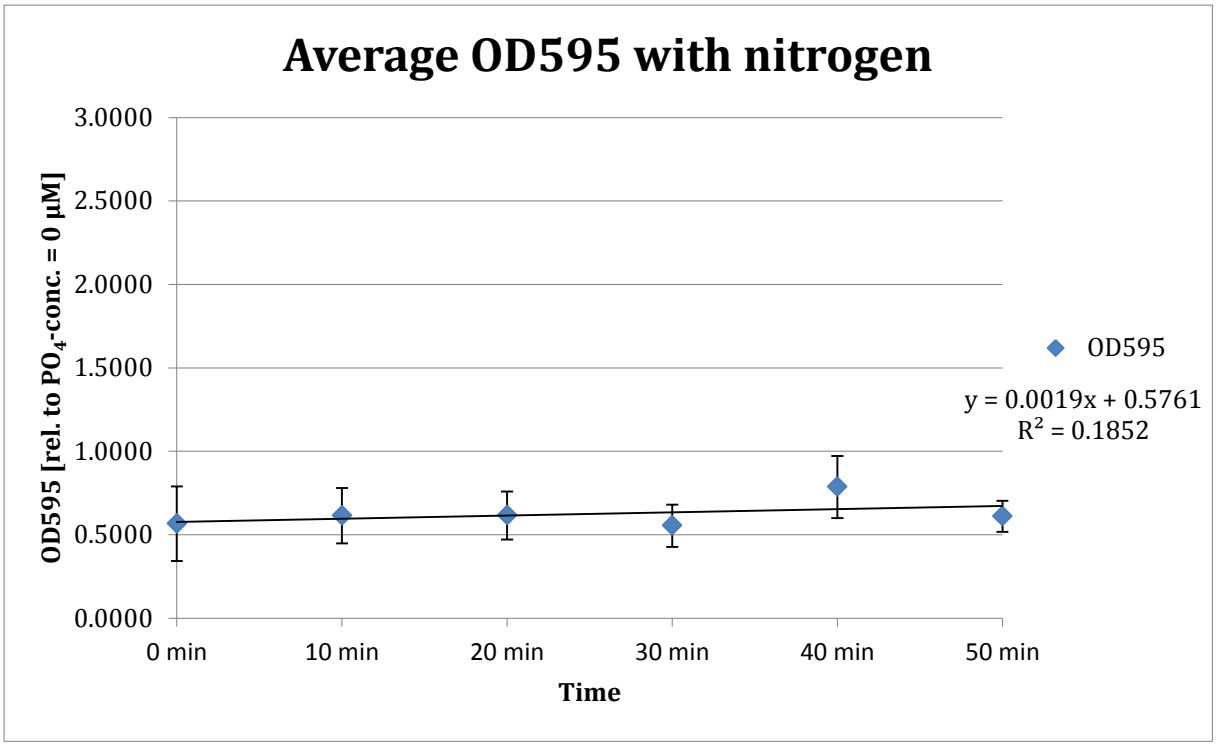

Figure 7: The average observed densities at 595 nm wavelength light fitted with trend lines and standard deviation error bars.

6.3. Step 3

6.3.1. Aspect 1

A slight increase in the phosphate concentration was observed, but the extremely low R^2-value makes the significance of this interpretation questionable.

6.3.2. Aspect 2

The design of the experiment might have caused the death of the yeast, but other possibilities, such as the yeast having been dead beforehand, remain.
The placement of magnetic stir bars within the solution containing the yeast wasn't consistent with the procedure of the previously conducted experiments, so the additional agitation could have induced different behaviour of the yeast cells when compared to that of the cells in the other assays or caused their death.

6.3.3. Aspect 3

The viability of the yeast cells before beginning with the assay could have been investigated by an experiment under normal conditions using yeast from the same block. If this had turned out successful, it would have led to the conclusion that the yeast had died during the conduction of the experiment and alterations to the setup would have had to be implemented. This could have included removing the stir rods from the double-neck flasks or reducing the influx of gas into the medium.
If the yeast had been proven viable, the assay could have been repeated with the same experimental setup in order to strengthen the significance of the obtained results.

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9. Leggett, J. E. 1961. “Plant Physiology”, p.284.

8. Activity list

Date	Description	Involved people
17.04.13	Part 1 Question 1	Erik, Livia, Till, Sarina
	Part 1 Question 2	Hyun-Min, Lucien, Marimo, Jonny
	Part 1 Question 3	Kim, Patrick, Fabio, Andrea
	Part 1 Question 4	Loraine, Virginia, Joel, Murielle
	Take pictures	Jan, Kim
	Part 2 experiment	Tamara, Timothy, Mara, Sonja, Livia S., Anja
	XY scatter gram graphs of part 2B	Hyun-Min, Sarina
	Research about the properties of yeast cells	Jonny, Till, Marimo
	Preparing the activity list	Joel
22.04.13	Take pictures	Jan
	Part 1 Question 3	Fabio, Andrea, Patrick, Kim
	Research for part 4	Tamara, Lucien
	Organizing part 3	Mara
24.04.13	Research about the properties of yeast cells	Jonny, Till, Marimo, Murielle, Livia Z., Fabio

	Part 1 question 4	Joel, Loraine, Murielle, Virginia
	Research for part 4	Sonja, Tamara, Lucien
	Research about electroporation	Marimo, Joel
	Research about the properties of phosphate	Livia Z., Murielle, Till, Jonny
	Research about phosphorylation	Virginia, Loraine, Anja
	Part 1 question 3	Andrea, Fabio, Kim, Patrick
	Research about storage and uptake of phosphate in yeast cells	Fabio, Till
	Part 3 experiment	Tim, Mara
	Take pictures	Jan
29.04.13	Take pictures	Jan
	Part 3	Tim, Livia, Lucien
	Formatting of data for part 2	Hyun-Min, Erik, Mara, Tamara
01.05.13	Part 1 Question 1	Erik Häller
	Part 4 experiment	Lucien, Tim, Livia, Virginia
	Part 2	Hyun-Min, Mara, Lucien
	Research about storage and uptake of phosphate in yeast cells	Andrea, Patrick, Jonny, Mara, Jan, Tamara, Sonja, Till, Marimo
	Creative ideas	Kim, Murielle, Livia Z.
	Part 3	Hyun-Min
06.05.13	Part 2 experiment	Tim, Livia S., Lucien
07.05.13	Activity list	Joel
	Part 4 experiment	Lucien, Tim, Livia S.
08.05.13	Formatting and correcting part 1	Virginia, Livia Z., Anja
	Part 4 experiment	Tim, Livia S., Lucien
	Text writing for part 2 and 3	Patrick, Jonny, Fabio, Till, Sonja, Andrea, Mara, Tim, Lucien
	Research about electroporation	Marimo, Joel, Erik
13.05.13	Part 4 experiment	Lucien, Tim, Livia S.
15.05.13	Formatting, proof-reading, text writing part 1 to 4	Jan, Fabio, Sonja, Till, Tim, Hyun-Min, Jonny, Joel, Tamara, Erik, Marimo, Sarina, Loraine, Mara, Murielle, Kim, Lucien, Virginia, Livia Z., Anja, Andrea, Patrick, Livia S.
16.05.13	Writing of part 4, assembling of all documents	Tim, Virginia, Lucien
17.05.13	Assembling of all documents, proof reading	Tim, Virginia, Lucien, Livia S.

Table 20: Activity list

Figure 9: Murielle and Jonny searching the literature for information on the properties of yeast cells.

Figure 8: Class searching the web during research for part 1.