Neue Kantonsschule Aarau G3BEF Task 2

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Part 1

Text

The first term that comes to mind when thinking of genetics is DNA and RNA. This is because our DNA are the building blocks of who we are and what we look like. Phosphorous is a key structural component in the backbone of DNA and RNA. Our DNA is made up of infinite amounts of nucleotide structures, which are made up of a sugar (ribose or deoxyribose), a base, and a phosphate molecule. So, all living organisms need phosphorous to build these structures to transmit genetic material and synthesise proteins. Additionally, our energy on a cell level is stored in molecules called ATP(Adenosine **triphosphate**). Shown by the 'tri', each ATP molecule contains three phosphates, and breaking off one of these phosphates, which contain phosphorous releases relatively large amounts of energy in a controlled and orderly fashion. Every time you flex your muscles, or when your cells reproduce, this process occurs. None of this could talk place without phosphorous. Furthermore, every cell in our body and in every other living organism is surrounded by a cell membrane. Cell membranes are made by a combination of phospholipids, a type of fat that can form a bilayer, and proteins.²

The vacuole of the yeast *Saccharomyces cerevisiae* is a major storage compartment for phosphate. Infact, the phosphate metabolism in yeast has been extensively studied. It helps us to understand how cells make a coordinated response to environment changes. Since phosphate is often only available in low amounts, the yeast has involved as evolved complex mechanisms to deal with changes in phosphate availability.³

Phosphate is transported across the yeast cells vacuolar membrane and most of it is stored in the vacuole. The cells already contain a certain amount of phosphate and together with the phosphate from the environment a bidirectional phosphate transport is built up. Depending on the environment changes the cells take up more or less phosphate.

Phosphate is very important for all organisms, but it also has a bad side. Algae for example really like phosphate and if there is too much of it in a lake, algae start growing and within a short time the whole surface could be covered with green leave. There is also not enough oxygen on the bottom of the pond where decomposition should take place. This leads to bacteria starting to decompose the biomass and produce methane and other toxic compounds. As a result most of the organisms living in that lake or pond die. Also other plants can die because of the competition. All these events would happen if there weren't microorganisms that are able to remove the excess.⁴

See References for Bibliography

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¹ "Phosphorous".Wikipedia. May 2, 2013. Accessed May 2, 2013.

² "Why is phosphourus important to living things?". Yahoo Answers. 2008. Accessed May 2,2013. http://answers.yahoo.com/question/index?qid=20080306002322AA4IQzh

³ Phosphate Transport in Yeast Vacuoles". Jbc. Accessed May 2, 2013. http://www.jbc.org/content/272/33/20408.long

⁴ "Phosphor (P) und Wasser". Lenntech. Accessed May 5, 2013. http://www.lenntech.de/element-und-wasser/phosphor-und-wasser.htm

D, DCP and CE: Simply Science Task 2

Part 2

Raw Data (Quantitative)

These are the raw results of part 1 of Task 2. Measured here was the absorption of different concentrations of phosphate. The goal of this step was not data collection, but just a test to see how accurate our class could pipet. Optimally, the absorption should be linearly proportional to the concentration of the phosphate. (See processed data of part 2)

Concentration of Phosphate (µmol)	Set 1 (Abs)±0.0005	Set 2(Abs)±0.0005	Set 3(Abs)±0.0005
0	0.0994	0.0930	0.1048
10	0.1203	0.2954	0.2470
20	0.3876	0.3928	0.3862
30	0.5219	0.4839	0.5302
40	0.6245	0.5714	0.6179
50	0.6892	0.6223	0.6849

Table 1: Three data sets measured in absorption for each concentration of phosphate.

Processed Data (Quantitative)

The first step is to calulate the average absorption in each set for every concentration. All the averages are as follows.

Concentration of Phosphate (µmol)	Average ±0.0015
0	0.0991
10	0.2209
20	0.3889
30	0.5120
40	0.6046
50	0.6655

Table 2: The average of all 3 data sets per concentration

As stated before, if the process was done without mistake, absorption would be linear to the concentration. We can see this if the data is set in a diagram.

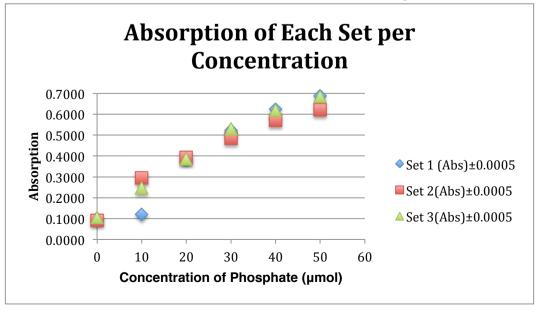


Diagram 1: Absorption of Each Set per Concentration

In the diagram it is already clear that the points aren't completely linear. For set 1 we see that there was some type of mistake, however, the other sets are relatively exact. Now we will make one line for the average and then make a trendline to compare to the equation of the line.

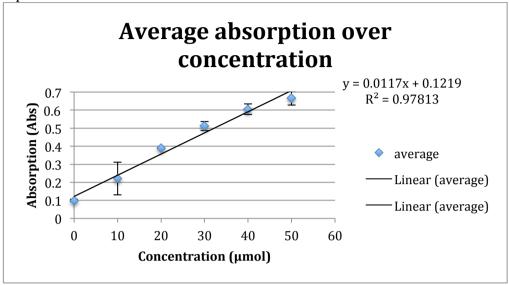


Diagram 2: The average absorption per concentration with the equation and regression of the line.

In the diagram above, it is clear that the line is not linear, but it also isn't terribly far away. This basically means that our pipetting technique wasn't terribly inaccurate, but it wasn't perfect. The equation to figure out the phosphate concentration is as follows: $Absorbance = Molar \ Absorptivity \ x \ Path \ Length \ x \ Concentration$

Difficulties

The biggest problem at the beginning was the time management since we tried to stagger the runs at step 4 by starting with a new sample when the prior sample was not yet finished. We underestimated the time we needed to measure the absorption resulting in a delay of all the following samples. Since the time gaps between mixing and measuring increased with each sample the color faded out and the measuring results are not significant. To prevent lack of time in the following repeats we adjusted the time gap between each sample to 2.5 min. Another problem arose with the use of cuvettes, which were too big for 1ml of solution since the light beam did not hit the solution properly. We exchanged them with smaller cuvettes, which resulted in significant results. Additionally we realized that the sides of the cuvettes were not clean but showed fingerprints so we cleaned them and made sure that the cuvettes were not touched at the smooth side.





Part 3

Raw Data (Quantitative Data)

time (min)	e (min) Set 1±0.0005 Set 2±0.0005 (Abs)		Set 3±0.0005 (Abs)
0	0.6399	0.7853	0.5149
10	0.7267	0.7458	0.4883
20	0.7771	0.7272	0.3123
30	0.6403	0.5797	0.0509
40	0.2828	0.3356	0.0465
50	0.2841	0.2849	0.1566

Table 3: The raw data for part 3. The 3 best sets were taken.

Processed Data (Quantitative Data)

time (min)	Average (Abs)	e±0.0005	standard deviation	
	0	0.647		0.1353
	10	0.654		0.1435
	20	0.606		0.2552
3	30	0.424		0.3242
4	40	0.222		0.1540
!	50	0.242		0.0738

Table 4: Processed data of experiment 3 which shows the average and the standard deviation

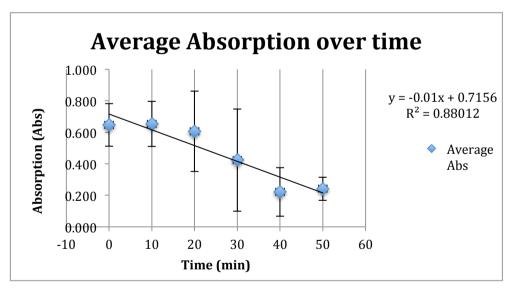


Diagram 3: The average absorption over the time. Here the equation of the line an the regression is also shown. If the regression is close to 1, the points are all close to the line.

Difficulties

It was quite difficult to pipet accurately enough. We tried it four times, because one set was very unaccurate. We think that this was the effect of touching the cuvettes on the wrong side. In later runs, we could eliminate this error. Time management was also difficult. And we tried to work as fast as possible without destroying the experiment. There was one set, where we prepared the first solution one day before. Maybe the yeast took up more phosphate during this time, so there is a tiny error too. All the other solution we prepared and used them right away.

Part 4A-Phosphate Concentration and Phosphate Uptake

Design

Phosphate Concentration and Phosphate Uptake

How does the uptake of phosphate by yeast cells change when the concentration of phosphate in the yeast-phosphate solution is changed?

Independent variable: Phosphate concentration (M) \rightarrow 0.3, 0.4, 0.5, 0.6, 0.7 mM

Dependent variable: Phosphate uptake, measured by the absorption of the coloured solution with

remaining phosphate (abs)

Controlled variables: Glucose concentration, time yeast cells can uptake phosphate, temperature

Hypothesis: The phosphate uptake will be higher at a higher concentration, but at some point a limit will be reached so that a higher concentration will not make a big difference in uptake anymore because of a saturation or because the yeast cells cannot take up the phosphate up any faster. We therefore expect a curve that goes up slowly in the beginning, showing the little phosphate that is still present in the solution, but whose slope increases at higher concentrations as relatively much phosphate remains in the solution.

Material

- Glass beakers, Erlenmeyer flasks and glass rods
- 5 ml glucose solution (2%)
- 5 ml sodium phosphate buffer (1M)
- Distilled water
- 5 x 0.2 g yeast
- Glass rod
- 45 Eppendorf tubes (EP)
- EP rack
- Timer
- Centrifuge
- Malachitgreen solution
- Molvbdate solution
- Vortex
- Spectrometer

Method

- 1. For the 0.3 mM run, add 994 ml distilled water to 6 ml 0.1 M phosphate solution.
- 2. Add 1 ml of the phosphate solution to 1 ml of the glucose solution.
- 3. Dissolve 0.2 g yeast in the solution and stir it with the glass rod.
- 4. Fill the yeast solution in 3 EPs (300 ml each) as soon as the yeast is completely dissolved.
- 5. After 10 minutes, centrifuge 1 min at max speed.
- 6. Pipette 40 ml of the supernatant in each tube into a new EP and dissolve each sample with 360 ml distilled water.
- 7. Mix 344 ml molybdate solution with 256 ml malachitgreen solution in a new EP.
- 8. After five minutes, transfer this mix into the EP with the phosphate solution and mix it on the vortex
- 9. Transfer the content of the EP into a cuvette and measure the absorption at 595 nm exactly one minute after mixing the colouring solution with the phosphate solution.
- 10. Repeat steps 7-9 for each sample.
- 11. For the 0.4 mM run, add 992 ml distilled water to 8 ml 1 M phosphate solution and repeat steps 2-10.
- 12. For the 0.5 mM run, add 990 ml distilled water to 10 ml 1 M phosphate solution and repeat steps 2-10
- 13. For the 0.6 mM run, add 988 ml distilled water to 12 ml 1 M phosphate solution and repeat steps 2-10.
- 14. For the 0.7 mM run, add 986 ml distilled water to 14 ml 1 M phosphate solution and repeat steps 2-10.

Raw Data (Quantitative Data)

The raw data for experiment 4C is as follows:

phosphate concentration(mM)	Absorption (Abs) ±0.0005		
	Set 1	Set 2	Set 3
0.3	0.4131	0.4865	0.4456
0.4	0.5969	0.5225	0.5341
0.5	0.5944	0.6610	0.6134
0.6	0.6431	0.6593	0.6293
0.7	0.6593	0.6541	0.6278

Table 5: A table of the 3 sets measured for 5 different concentrations

In a graphical form it appears like this: In an optimal situation, there should be a logarithmic curve.

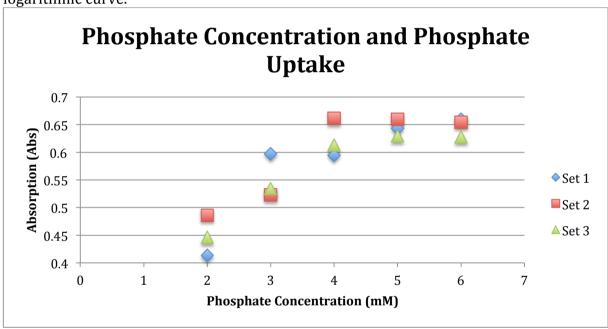


Diagram 3: The raw data shown in the table above in a graph

Processed Data (Quantitative Data)

Now we have the processed data. As always, the average was calculated from the data of the three sets, as well as the standard deviation to help us see how significant the data is. Our processed data looks like this:

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phosphate	Average	Standard		
concentration(mM)	Absorption	Deviation		
	$(Abs)\pm0.0015$			
0.3	0.4484	0.03678		
0.4	0.5512	0.04003		
0.5	0.6229	0.03431		
0.6	0.6439	0.01502		
0.7	0.6471	0.01689		

Table 6: Processed Data table with the average and standard deviation

Here we have put the processed data in a graph. This graph is very important because we are able to see if the data are coherent or not. From the data we can also do a

regression analysis which tells us if the data are regressive. The closer the regression line is to 1, the more precise the data is.

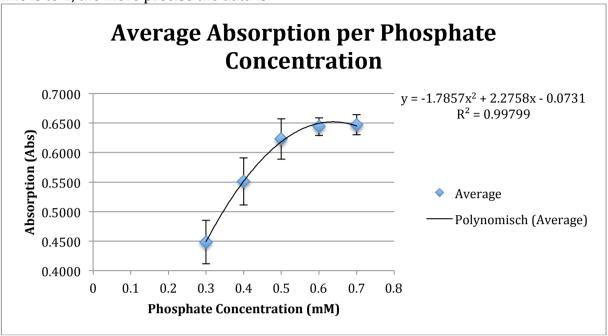


Diagram 4: The graph of the table 4

Conclusion and Evaluation

As we predicted, the absorption increased over increasing phosphate concentration, but the slope of the curve decreased. Our hypothesis was therefore verified. However, this does not yet give us any information on what an optimum concentration would be. The relative absorption which also has to be taken into consideration as well. It can be calculated by dividing the absorption of the starting solution by the absorption of the final solution and find the percentage still present. Most probably we would then get the result that the relative absorption was highest at the lowest concentration which means that there the effectiveness of the yeast is highest. Still, this is not necessarily the optimum phosphate concentration because a much higher absolute amount of phosphate was absorbed at higher concentrations. This means that probably the optimum concentration lies somewhere between the two values.

A weakness of our experiment is that it is not possible to find a perfect optimum, we can only say between which values it probably lies, and it is therefore not the most relevant experiment. Also, because we had to pipette a lot, the errors of measurement accumulated and the results are maybe not that accurate. However, we could only have changed this if we had already had the required phosphate solutions.

Part 4B- Glucose Concentration and Phosphate Uptake

Design

Glucose Concentration and Phosphate Uptake

How does the uptake of phosphate by yeast cells change when the concentration of glucose in the yeast-phosphate solution is changed?

Independent variable: Glucose concentration (%) \rightarrow 0.1, 0.5, 1, 5, 10%

Dependent variable: Phosphate uptake, measured by the absorption of the coloured solution with

remaining phosphate (abs)

Controlled variables: Phosphate concentration, time yeast cells can uptake phosphate, temperature

Hypothesis: Because phosphate can only be taken up by the yeast as long as enough glucose is present to allow the mitochondria to form ATP, we expect the phosphate uptake to be lower at lower concentrations of glucose where the yeast does not have enough energy. Therefore the curve should decrease with increasing glucose concentration as there more phosphate will still be present in the solution we colour. We are not sure about the slope of the curve. At very high glucose concentrations, the phosphate will also not be taken up because the osmotic pressure will cause a hypertonicity in the yeast cells and they will not be able to take up the phosphate at all. So probably at 10% there will be almost all phosphate left and there will be a very high absorbtion.

Material

- Glass beakers, Erlenmeyer flasks and glass rods
- 5 ml glucose solution (10%)
- Distilled water
- 0.5 ml Sodium phosphate buffer (0.1M)
- 5 x 0.2 g yeast
- 45 Eppendorf tubes (EP)
- EP rack
- Timer
- Centrifuge
- Malachitgreen solution
- Molybdate solution
- Vortex
- Spectrometer

Method

- 1. For the 10% run, use the given glucose solution.
- 2. Add 1990 ml of the glucose solution to 10 ml sodium phosphate solution.
- 3. Dissolve 0.2 g yeast in the solution and stir it with a glass rod.
- 4. Fill the yeast solution in 3 EPs (300 ml each) as soon as the yeast is completely dissolved.
- 5. After 10 minutes, centrifuge 1 min at max speed.
- 6. Pipette 40 ml of the supernatant in each tube into a new EP and dissolve each sample with 360 ml distilled water.
- 7. Mix 344 ml molybdate solution with 256 ml malachitgreen solution in a new EP.
- 8. After five minutes, transfer this mix into the EP with the phosphate solution and mix it on the vortex.
- 9. Transfer the content of the EP into a cuvette and measure the absorption at 595 nm exactly one minute after mixing the colouring solution with the phosphate solution.
- 10. Repeat steps 6-8 for each sample.
- 11. For the 5% run, dissolve 1000 ml 10% glucose solution in 1000 ml distilled water and repeat steps 2-10.
- 12. For the 1% run, dissolve 200 ml 10% solution in 1800 ml distilled water and repeat steps 2-10.
- 13. For the 0.5% run, dissolve 100 ml 10% solution in 1900 ml distilled water and repeat steps 2-10. For the 0.1% run, dissolve 20 ml 10% solution in 1980 ml distilled water and repeat steps 2-10.

Raw Data (Qualitative Data)

The raw data for experiment 4B is as follows:

	Absorption (Abs) ±0.0005		
Glucose Concentration (%)	Set 1	Set 2	Set 3
0.1	0.6115	0.6060	0.6371
0.5	0.6134	0.4387	0.6107
1	0.6243	0.6085	0.6049
5	0.5859	0.6093	0.5912
10	0.6001	0.6138	0.5933

Table 7: Raw Data for experiment 4B. The absorbance was measured for different glucose concentrations each 3 different times

The graph of the raw data looks like this. It seems to be a slopeless line with 1 piece of data from set 2 out of order.

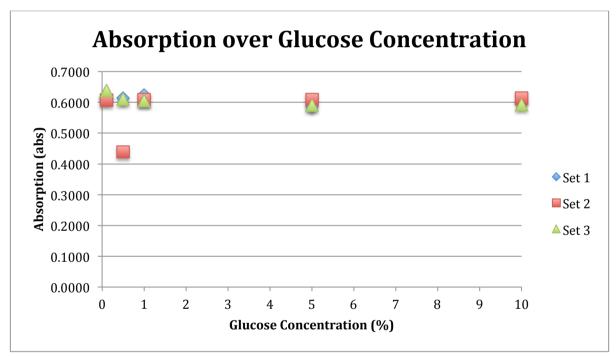
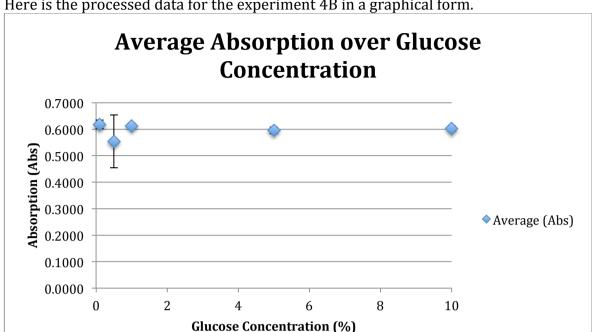


Diagram 5: The raw data shown in a graphical form. The trend seems to be one straight line.

Processed Data (Quantitative Data)

Glucose	Average	Standard
Concentration (%)	(Abs)	Deviation
	±0.0015	
0.1	0.6182	0.0166
0.5	0.5543	0.1001
1	0.6126	0.0103
5	0.5955	0.0123
10	0.6024	0.0104

Table 8: The average and standard deviation of table 5



Here is the processed data for the experiment 4B in a graphical form.

Diagram 6: The average absorption put in a graph. There doesnt seem to be anything conclusive.

Conclusion and Evaluation

The first part of our hypothesis was that the phosphate uptake increases with the increasing glucose concentration. However we can't be sure whether this is true or not, because it only worked in one of three sets. In the results from set 1 and 2 you can't recognize a pattern, because the results go up and down. However, in set 3 the absorption decreases with a higher glucose concentration (table 5). The graph of the absorption over the glucose concentration is more or less a straight line (diagram 5 and 6). But if you compare our results with the control value, you can see that there is a decrease in the absorption, so glucose can enhance the phosphate uptake. This is because the yeast needs energy to take up phosphate. The energy for this process is extracted from glucose. The second part of our hypothesis was that at a very high glucose concentration, less phosphate is taken up. In set 1 and 2 this was true, but it might just be a coincidence. To prove our hypothesis, we should have done some more tests. However this wasn't possible because of a lack of time. Furthermore the spectrometer couldn't give an exact value, so the absorption differs in ±0.0005. Another measuring error could have occurred in weighing the yeast, because the balance we used, only gives two decimals, so the error would be ±0.005g. This could be prevented by using a more accurate balance. Another problem was, that the steps between the different glucose concentrations were rather small, especially in the first few experiments where the difference was only 0.4 and 0.5%. Instead we should have used bigger differences. We also should have waited longer before we centrifuged the solution, so the yeast would have more time to take up the phosphate.

Part 4C-Temperature and Phosphate Uptake

Design

Temperature and Phosphate Uptake

How does the uptake of phosphate by yeast cells change when the temperature of the yeast-phosphate solution is changed?

Independent variable: Temperature (°C) \rightarrow 20, 25, 30, 35, 40 °C

Dependent variable: Phosphate uptake, measured by the absorption of the coloured solution with

remaining phosphate (abs)

Controlled variables: Phosphate concentration, time yeast cells are left to uptake phosphate, glucose

concentration

Hypothesis: We expect an optimum temperature of around 30 °C because this is what we found in the literature about at which temperature yeast performs best. Probably the phosphate uptake will increase slowly at increasing temperature because the kinetic energy of all particles will increase. The drop after the optimum temperature will be more sudden and steep because some structures are deformed at high temperatures and some processes will thus not work properly anymore. As we are measuring the remaining phosphate in the solution, we expect the shape of the curve to look similar to a U.

Material

- Glass beakers, Erlenmeyer flasks and glass rods
- 5 x 0.2 g yeast
- 10 ml glucose solution (1%)
- 0.5 ml Sodium phosphate buffer (0.1M)
- Glass rod
- 45 Eppendorf tubes (EP)
- 5 waterbaths (20, 30, 40, 50 and 60°C) with EP racks
- Timer
- Centrifuge
- Distilled water
- Malachitgreen solution
- Molybdate solution
- Vortex
- Spectrometer

Method

- 1. Add 9950 ml glucose solution to 50 ml sodium phosphate buffer.
- 2. For the 20°C run, dissolve the first 0.2 g yeast in 2 ml solution and stir it with a glass rod.
- 3. As soon as the yeast is completely dissolved, incubate for 10 minutes at 20 °C.
- 4. Fill the yeast solution in 3 EPs (300 ml each).
- 5. Centrifuge 1 min at max speed.
- 6. Pipette 40 ml of the supernatant in each tube into a new EP and dissolve each sample with 360 ml distilled water.
- 7. Mix 344 ml molybdate solution with 256 ml malachitegreen solution in a new EP.
- 8. After five minutes, transfer this mix into the EP with the phosphate solution and mix it on the vortex.
- 9. Transfer the content of the EP into a cuvette and measure the absorption at 595 nm exactly one minute after mixing the colouring solution with the phosphate solution.
- 10. Repeat steps 6-8 for each sample.

Repeat steps 1-9 for all temperatures (25, 30, 35 and 40 $^{\circ}$ C).

Raw Data (Qualitative Data)

Temperature(°C)	Se	et 1 (Abs)±0.0005	Set 2(Abs)±0.0005	Set 3(Abs)±0.0005
	20	0.5997	0.5901	0.6553
	25	0.5887	0.5927	0.6553
	30	0.5835	0.6274	0.6467
	35	0.5616	0.6096	0.6027
	40	0.5875	0.6211	0.6781

Table 9: The Raw data for experiment 4c. The temperature is the independent variable here

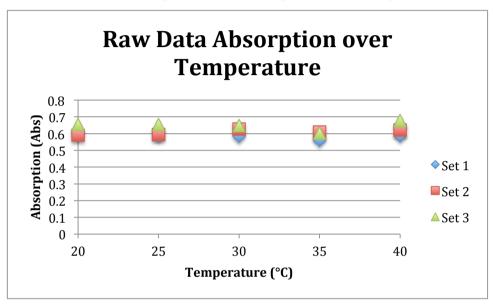


Diagram 7: The Raw data of 4c put into a diagram form

Processed Data (Quantitative Data)

Temperature(°C)±1°		Average±0.0015	Standard Deviation
	20	0.6150	0.0352
	25	0.6122	0.0374
	30	0.6192	0.0324
	35	0.5913	0.0260
	40	0.6289	0.0458

Table 10: processed data of 4c showing the average of the sets as well as the standard deviation

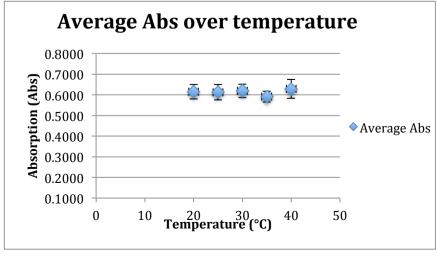


Diagram 8: Processed Data of 4c. The average is shown with the standad deviation.

Conclusion and Evaluation

The data shows a slightly decreased amount of phosphate at 35° C, thus the yeast cells have their highest phosphate uptake capacity at around 35° C. Phosphate uptake decreases at a temperature above and below 35° C, resulting in a U shaped graph. Therefore our hypothesis can be partly confirmed since the phosphate uptake corresponds to our expectations but the optimum temperature lies at ca. 35° C and not at 30°C as expected.

Our results can be explained by the energy the temperature supplies. The higher the temperature the higher the kinetic energy of the particles, thus the yeasts cells have more energy to perform the reaction and the reaction happens faster. When the temperature exceeds 35° C phosphate uptake is slowed down since the high temperature leads to a denaturation of proteins in the yeast cells.

This experiment contains several limitations:

The results only show small differences in absorption between the different temperatures that makes it hard to interpret the results since it is not clear whether the differences are random or not resulting in low significance of the data. As an improvement the time span in which the yeast and the phosphate solution are mixed can be extended so that the yeast has more time to take up phosphate and the differences in phosphate uptake become more clear between the different temperatures. We only made 3 samples per temperature thus we had little data which sometimes differed a lot. In a set of only 3 samples a high distribution of data leads to insignificant results since one outlier can change the average of the whole set considerably thus our results are not significant. A higher number of samples (at least 6 samples) would lead to more significant results and should be considered in further experiments. Our data does not show a high difference in absorbance between the different temperatures and the data provides an almost straight line. This may also be due to the small overall temperature range of only 20° C. However this does not limit the significance of our results heavily since we found a slight curve. To make our curve more clearer the temperature range could be exceeded up to 0° C and 100° C.

References

Only used for Part 1

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- 3. "Phosphate Transport in Yeast Vacuoles". Jbc. Accessed May 2, 2013. http://www.jbc.org/content/272/33/20408.long
- 4. "Phosphor (P) und Wasser". Lenntech. Accessed May 5, 2013. http://www.lenntech.de/element-und-wasser/phosphor-und-wasser.htm

Activity List

Ailin Lüthi	Participated in experiments for part 2,3 and 4, and wrote the
	discussion for part 2 and conclusion and evaulation for part 4c.
Anita Streit	Wrote part of the text for part 1 as well as taking photos and
	participation in part 3 experiment.
Katharina	Participated in all experiments and wrote the conclusion and
Lüscher	evaluation for part 4a.
Allen Grau	Participated in the part 2 experimentation and did the entire data
	collection and processing for all experiments. He also wrote part of the
	text for part 1. Created the activity list as well.
Julia Lienhard	Participated in experiment for part 3 and 2. She also took photos and
	wrote the conclusion and evaluation for part 4c.
Anna Löpfe	Participated in experiment 3 and 2 and took photos.
Arta Arifaj	Participated in experiment 3 and 2
Morena Sager	Participated in experiment part 2.



