Bündner Kantonsschule Chur

Class SPF 4Gbde

Task 2

16.05.13

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Part 1

1. Phosphate is important for all organisms; it is used to build nucleotides. *Nucleotides* are biological molecules that form the building blocks of DNA and RNA. They are formed from a sugar molecule, a base and a phosphate. The most important energy molecule in our body, ATP (adenosine triphosphate), contains three phosphates, which activate essential cell processes in every organism [(1)]. Phosphate is also very important for our teeth and bones - where we find 85% of the phosphate content in the human body [(2)].

2. There are different ways to supply phosphate to a yeast cell. They all function by means of a transport-system of enzymes from the outside of the yeast cell through the cell membrane into the yeast cell. They can pass through the membrane with a sodium-co-transporter or, also, with a lithium or potassium [(3)].

3. In the vacuoles of yeast cells, black particles appear which actively move around and then disappear within the time space of 10 minutes to several hours. It is generally accepted that the main component of these particles is sodium polyphosphate. This would mean that three phosphate-ions have crystallized with five sodium-ions and are stored in the vacuole in form of sodium polyphosphate [(4)].

4. Generally, there is too much phosphate in our natural water systems. If microorganisms did not absorb the phosphate, there would be too much growth of algae [(5)]. Humans therefore need the phosphate uptake by microorganisms in plants to clean the water. And phosphate, on the other hand, is very important for the growth of plants.

Part 2

After our first trials we noticed that the measurements of our spectrophotometer were very inaccurate and inconsistent.
We discovered that the light beam of the device was positioned too high, hit the top surface of the test-liquid and therefore gave unreliable results. To solve the problem we inserted a cut piece of cork under the cuvettes so that the light beam passed through the test-liquid properly.

Fig. 1: Lab environment

We also had to mix a new molybdat-solution with sulphuric acid because we ran out of our first batch. The new solution, however, had a lower standard curve than the first. We suspect that the higher temperature at the moment of measurement was the reason.

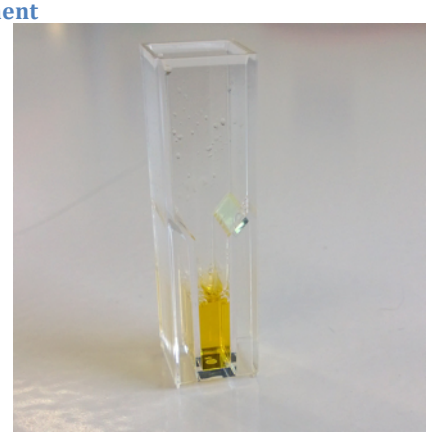

Fig. 2: Pretest

Time (min.)	Trial 1	Trial 2	Trial 3	Mean vlaue	StDev
0	0.13	0.02	0.06	**0.07**	0.057
10	0.44	0.32	0.31	**0.36**	0.072
20	0.74	0.62	0.56	**0.64**	0.092
30	1.07	0.81	0.79	**0.89**	0.156
40	1.31	0.91	1.07	**1.10**	0.201
50	1.51	1.38	1.45	**1.45**	0.065
R^2	0.997	0.971	0.992	0.987	

Tab. 1: Raw data Part 2

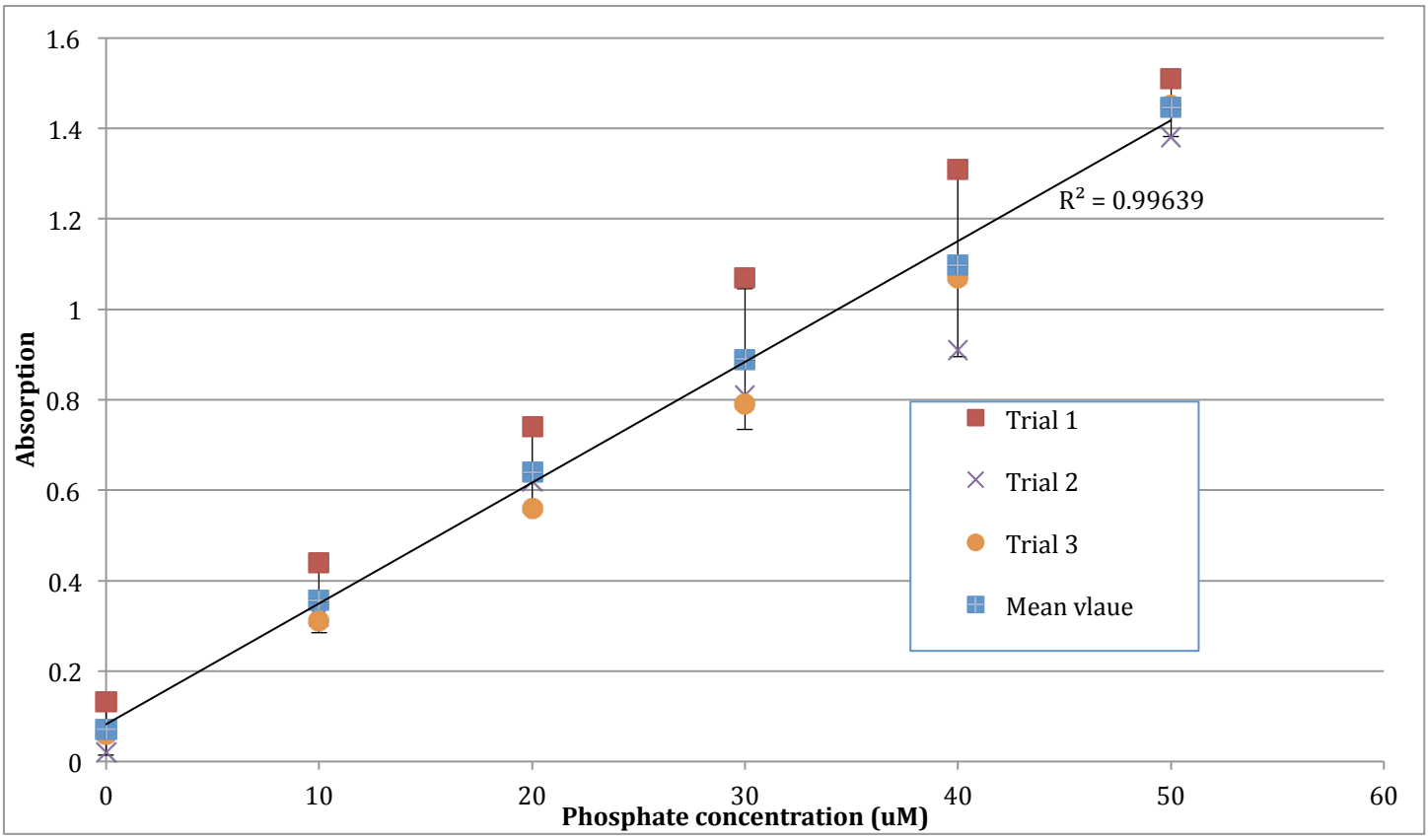

Fig. 3: Raw data with standard deviation

Part 3

In this trial we calculated how much phosphate a yeast culture can absorb over time. We prepared a yeast solution with 1 gram of yeast and 0.1 gram of sugar and 10 ml of water. To this solution we added the phosphate and started timing. Every ten minutes we took a sample and put it in the centrifuge to separate the yeast from the liquid, then mixed the liquid with di-water. We then put this sample into the prepared mixture of malachitgreen and molybdat-solution. As a last step, we measured the absorption of phosphate in the spectrophotometer. We had some difficulties managing the time and coordinating the right sequence of adding the substances. Due to problems of managing the spectrophotometer, we had to re-do the trials several times. To improve the procedure, we selected a few students, who were only responsible for concentrating on this part of the experiment. These students read the instructions precisely and discussed how to handle it before starting. Thanks to favourable collaboration between all of these students who did this trial, we could solve all the problems we had had in the beginning.

Time (min)	Trial 1	Trial 2	Trial 4	Mean value	StDev
0	2.188	1.326	1.314	1.609	0.762125974
10	1.374	1.190	1.181	1.248	5.089055937
20	1.153	1.033	1.003	1.063	10.95927308
30	0.718	0.914	0.901	0.844	16.79656396
40	0.017	0.818	0.810	0.548	22.624048
50	0.01	0.802	0.792	0.535	28.40736573
R^2	0.953	0.956	0.948	0.952	

Tab. 2: Raw data Part 3

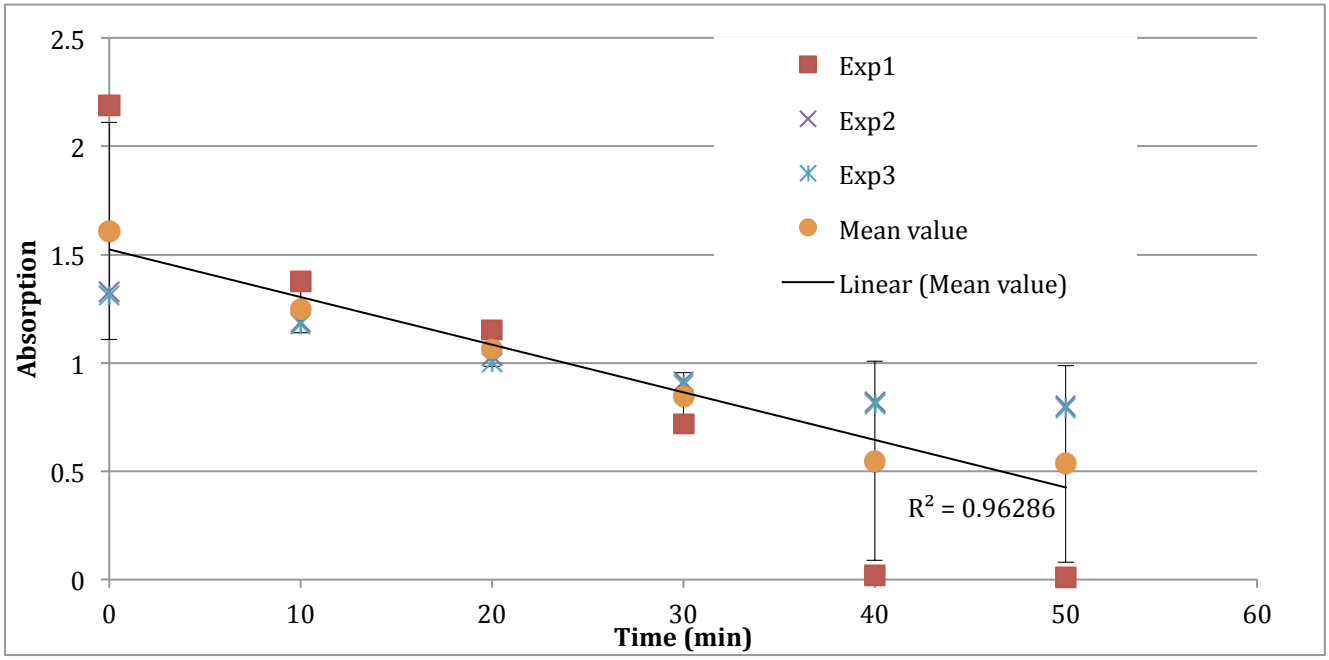

Fig. 4: Raw data with standard deviation

Part 4

Part 4 A: Heat

Can increasing the temperature stimulate the absorption of phosphate?

According to the Q10 (temperature coefficient)[6], the biochemical processes of the yeast cells should run faster when the temperature is raised. We did this trial to see if yeast can process phosphate more quickly with increasing temperatures.

We mixed the same yeast-solution as used in Part 3. In order to improve the experiment we had conducted previously, we heated the yeast-solution in a water bath to 30°C. once the desired temperature had been reached, we added the phosphates to the solution and conducted the same experiment as described in Part 3.

Unfortunately, our results were not as they should have been. There was no absorption of any phosphate (Tab. 3 and Fig. 5). The measurements were not viable, so we have no conclusions.

Time (min)	Absorption
0	0.604
10	0.592
20	0.637
30	0.632
40	0.621
50	0.616

Tab. 3: Raw data heat

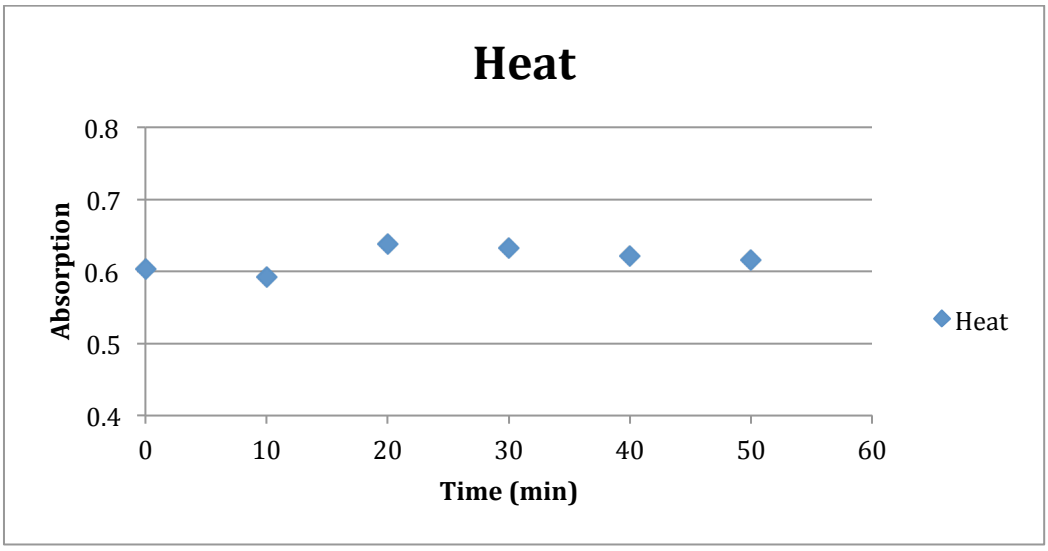

Fig. 5: Raw data heat

Part 4 B: Sodium chloride

Can adding sodium chloride boost the absorption of phosphate?

We prepared the same yeast solution as in Part 3, but we added sodium chloride. To achieve the correct concentration we first prepared a 5mM stock solution. We added the phosphate and the stock solution at the same time as we started timing this trial. The rest of the trial took place in the same way as in Part 3.
The idea was that the yeast cell would absorb both the phosphate and sodium chloride at the same time, so that the absorption rate of the phosphate would be heightened [(7)].
The absorption stayed constant (Tab. 4 and Fig. 6). We do not know why this was the result. A possible explanation is that the yeast was too old. We did this part of the trial independently from Part A and C but these two parts also had this result (constant absorptions rates) so we suppose that the error was not made by us.

Time (min)	Absorption
0	1.812
10	1.050
20	1.278
30	1.116
40	1.018
50	1.382

Tab. 4: Raw data NaCl

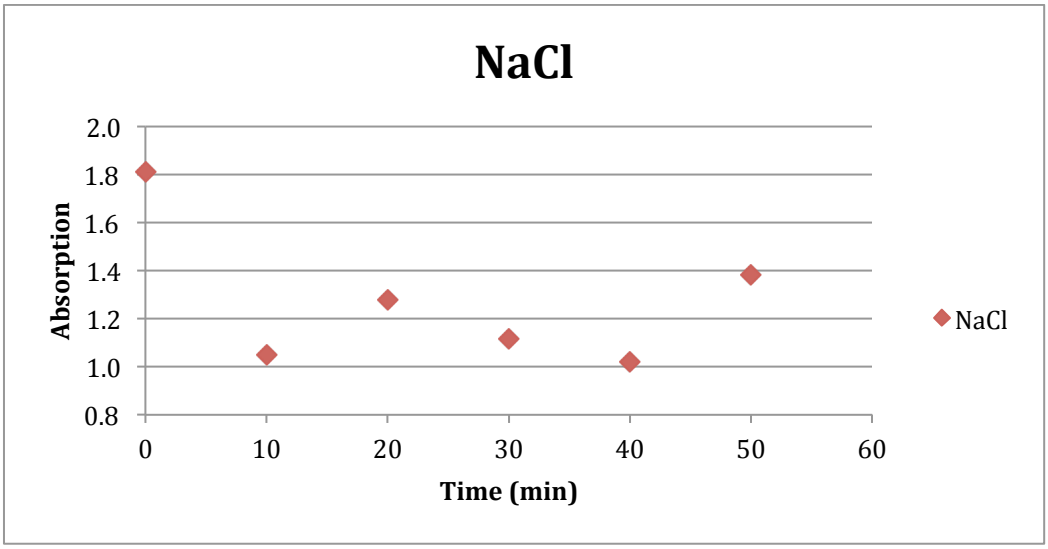

Fig. 6: Raw data NaCl

Part 4 C: Potassium nitrate

Can adding potassium nitrate stimulate the absorption of phosphate?

We proceeded with our third self-made trial in the exact same manner as in Part 3, with the notable exception that we additionally added 5 microliters of a solution of potassium nitrate and water alongside with the normal sodium phosphate. The potassium nitrate solution was composed of 100 microliters KNO_3 and 900 microliters di-H_2O.
The rest of the experiment was conducted in the same manner as described in Part 3, including the measurements of the different absorptions. We received none of the results that we had expected (almost no phosphate was absorbed) (Tab. 5 and Fig. 7).
In Part 4 C as in Part 4 A and B, we also came to the same result. Although we had mixed all the solutions in separate work teams, all the results remained constant (Tab. 3, 4, 5) although a change in phosphate absorption was expected. We really do not know what could have caused this. One possibility might be that the yeast was too old.

Time (min)	Absorption
0	0.714
10	0.712
20	0.640
30	0.650
40	0.674
50	0.675

Tab. 5: raw data KNO_3

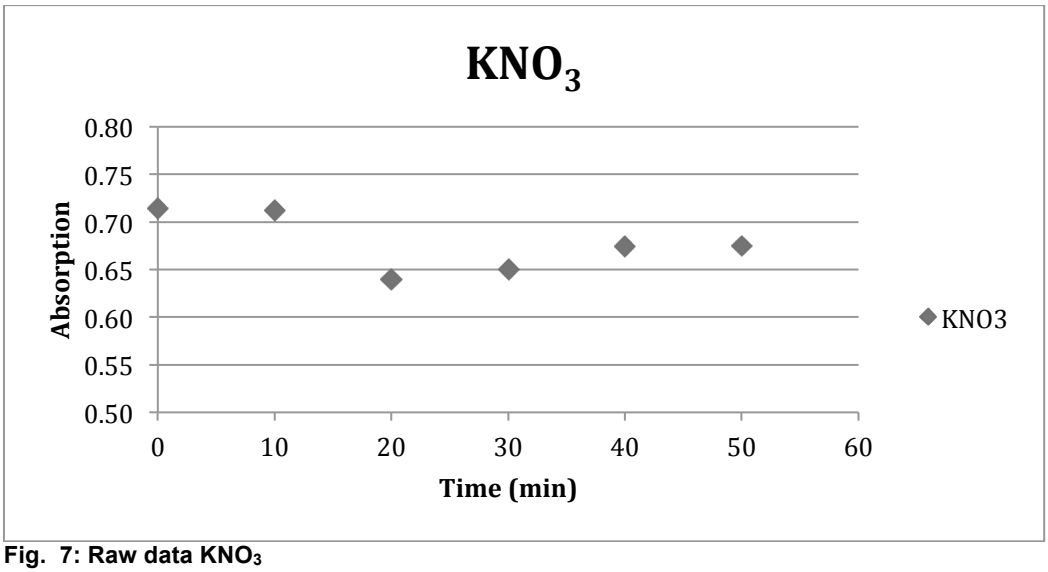

Fig. 7: Raw data KNO_3

Reference List

(1) Anonymous: „Nukleotid“. Retrieved April 26, 2013 from http://www.simplyscience.ch/definitionen/articles/nukleotid/selection/N.html

(2) Anonymous: „Phosphat“. Retrieved April 26, 2013 from http://www.medizinfo.de/labormedizin/elektrolyte/phosphat.shtml

(3) Roomans, G.M. et al. 1977. ”Biochimica e Biophysica Acta (BBA) – Biomembrans”, Retrieved April 26, 2013 from http://www.sciencedirect.com/science/article/pii/0005273677902425

(4) „Vacuole“. Retrieved April 26, 2013 from http://www.newworldencyclopedia.org/entry/vacuole

(5) Dannewitz, P. „Klärwerk-online.de“, Retrieved April 26, 2013 from http://www.crinum.de/abwasser/mikroorganismen.html

(6) 1. Anonymous: “Q10 (temperature coefficient)”. Retrieved May 16, 2013 from http://en.wikipedia.org/wiki/Q10_%28temperature_coefficient%29

(7) Dr. F. Jungnickel. 2007 (online; Retrieved May 16, 2013). „Untersuchungen zur phosphatinduzierten Aufnahme und Exkretion von Kalium bei Phosphatmangelhefe“. *Zeitschrift für allgemeine Mikrobiologie,* vol. 10, issue 3. 1970. pp 197-207

Activity List

Domeni, Seline	Photography/ Experiments Part 1, 2, 3 and 4
Geissberger, Stefan	Measuring the data
Hautle, Noel	Measuring the data/Writing
Menghini, Arianna	Experiments Part 3 and Part 4
Oswald, Jan	Experiments Part 3 and Part 4/Translation
Räschle, Gianna	Team Leader/Experiments Part 3 and Part 4
Rizzi, Marina	Part 3 and Part 4
Scalisi, Nadia	Experiments Part 3 and Part 4
Schindler, Anna	Experiments Part 2 and Part 3/Background information Part 4
Schmid, Cinzia	Experiment Part 1 and 2/Writing
Thomann, Gianin	Assistant/Experiment Part 1, 2 and 3/Graphs
Willi, Tonia	Experiments Part 2 and Part 4
Zulauf, Lukas	Activity List/Measuring the data