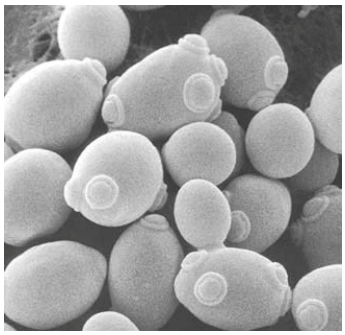
Task 2

Introduction



Phosphate is often a limiting factor in water ecosystems. Too much phosphate in a lake can cause an algal bloom and in the last consequence, it can finally lead to a disaster (Hallwylersee, Greifensee). One consequence may be that a whole fish-population might die. Lots of microorganisms like algae, fungi and microorganisms developed strategies to uptake the often rare phosphate quickly and efficient. To eliminate phosphate in sewage purification plant by microorganisms, constant efforts have to be done to find the most effective techniques to get rid of the phosphate.

The goal of this experiment

In this experiment you will prove first that you can handle pipets accurately and follow precisely a protocol to measure the decline of phosphate. In the beginning, you will learn to work with yeast cell cultures. Instead of a mixture of microorganisms like in a sewage purification plant you will work with baker's yeast (*Saccharomyces cerevisiae*) cell cultures. As soon as you can handle these cells, the pipetting and the technical equipment to measure precisely the decline of phosphate in this system, **you will establish a method to improve the uptake of phosphate by yeast cells**. The goal is to manipulate the circumstances to get a maximized uptake speed of phosphate by the yeast cells. It is up to you to test different approaches after having studied the literature.

You will be asked to establish **three different hypotheses** how the uptake of phosphate in this system could be optimized. Explain the biochemical context of your assumptions. Design the appropriate experiments to test your hypotheses. Carefully collect and analyze your data. Discuss the results, bringing your biochemical knowledge and your research of the literature into consideration.

What you have to do…

Part 1
Studying the literature

First find out some basic information about phosphate uptake in yeast cells. To get your first points you will have to write down a **short** text (1/2 - 1 A4 page) including the discussion of the following questions. Don't forget to create a reference list as described below:

1. Why is phosphate important for all organisms?
2. What is well known about phosphate uptake in yeast cells (*Saccharomyces cerevisiae*) (Not too many details. Try to find out the most important basic facts that could be helpful for your own hypothesis).
3. How do yeast cells (*Saccharomyces cerevisiae*) store phosphate?
4. Why is phosphate uptake by microorganisms an important issue in our society/environment?

An important aspect will be the citation of your sources and the establishing of a bibliography (reference list) at the end of your paper. Please read the following instructions how to cite carefully and use the added examples as model for your own list:

„In-text" citations: Superscripted numerical markers

To let the reader know where you have used a piece of information in your work, please use the superscripted numerical marker. Here is an example:

"Monkey prefer ripe bananas to unripe bananas[(1)]. This is due to the extra sugars present in ripe bananas[(2)], and scientists think that monkeys may have a similar range of tastes to humans[(3)]. It has yet been unproven whether or not monkeys find it funny when someone slips and falls on a discarded banana skin[(1)].

Bibliography (reference list)

Supply complete details of the source you have used – so that the reader could find them easily to check them or learn more. You can add the bibliography at the end of Part 1. You must list your sources in the order in which they are used in your piece of work. The very first source you cite in your text listed in position 1 in your bibliography, the second in position two and so on. If you use a source again later on, cite it in the text with the same number as the first time you used it. You don't need to write the same source twice in your bibliography. Here an example referring to the "in-text" citations above:

1. Taylor, S. 2006. *Monkey Nutrition Handbook*. 2nd edition. pp 198-199. Primate Press, Bandung.

2. Triandafillou, A. 2011. *Livestrong* – Article: "Nutritional difference in ripe bananas". Retrieved February 22, 2013 from www.livestrong.com.

3. Murphy, R. et al. 2005. "A Study into the taste pallet of primates". *Monkey Journal*, vol. 2, issue 12. Dec.2005. pp 12-15.

Part 2
Calibration of the measuring system

Before you start to investigate the phosphate uptake by yeast cells you must get familiar with the measuring technique. You will learn to pipet a serial dilution of different phosphate concentrations. Afterwards you will establish a linear trend line by comparing the absorption of the stained phosphate solution with the corresponding phosphate concentration. To detect the intensity of the color reaction you first must learn how to handle a photospectrometer.

2A Establishing a straight calibration (theory and introduction)

- According to the law of Beer Lambert you can measure the absorption of light by a particular solution according to the concentration of the substance in the solution. If you know the concentration you can measure the absorbance and finally get a calibration curve. Within certain limits this is very often a straight calibration line.

- If a substance itself is not absorbing light in a region which is selective and measurable, you will have to add another solution to get a reaction product which can be detected and measured.

- During your investigation you will measure phosphate concentrations with a very sensitive dye. This means, the result of your first assignment during this task will visualize how accurate you can pipet. You will get familiar with the technical equipment so that you easily can handle all technical challenges.

- As a result of your first efforts you will get the calibration line you need to determine the decline of phosphate in your further investigations.

We provide you with most of the reactants you need to measure phosphate.

In your “Chem-Box” (you received from us) you find following liquids:

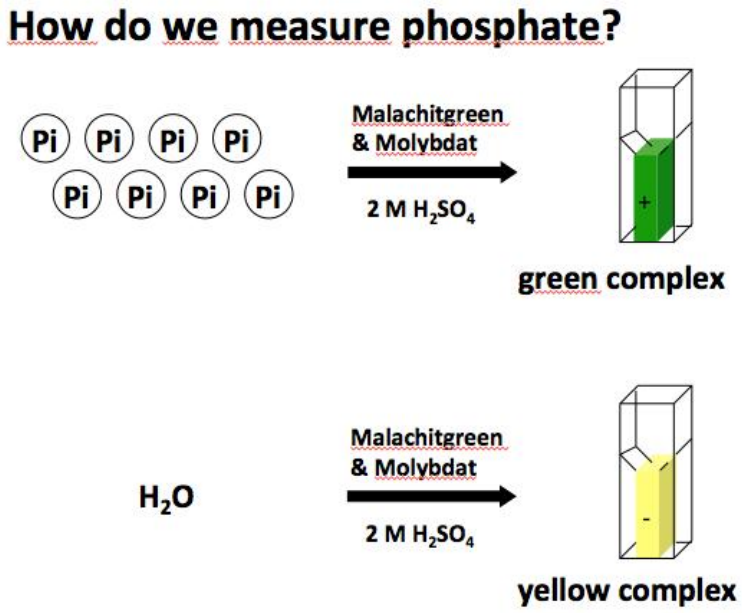
- **A**: **Malachitgreen-Solution** (2x40ml). We already prepared this solution for you. (12.7mg Malachitgreen, 140mg Polyvinylalcohol, filled-up to an end volume of 40ml with water, stirred over several days and finally filtered.)
- **B:** The **Molybdat-Solution** (2x30ml) we prepared for you. This solution has to be finished by yourself, adding concentrated sulfuric acid (4.48ml) to each tube. Attention! Wear safety gear! (see box below) and fill up each tube with 5.52ml D-water (included in your box) to an end volume of 40ml
- **C: Sodium-Phosphate-Buffer** 50µM, pH 6.3, 40ml
- **D: Sodium-Phosphate-Buffer** 0.1M, pH 6.3, 40ml
- **E: D-Water** (distilled water), 45ml

Attention! You have to wear (as usual during chemical experiments) safety glasses, gloves and a lab coat. We strongly suggest that you teacher supports you during this step and you protect your eyes, your skin and your cloths to exclude any harm.
Please work fully concentrated especially during this step “working with sulfuric acid”.

Pretest

- Add as described before 4.48ml sulfuric acid conc. to the Molybdat-Solution (30ml, in your “Chem-Box”) and fill-up with D-Water (5.52ml) to an end volume of 40ml.
- Make now a pretest (see below) to eliminate a contamination with phosphate in your test-solution:

- Take a cuvette and add 128µl Malachitgreen-Solution to 200µl D-Water. - Add now 172µl of the Molybdat-Solution (containing sulfuric acid). - *Pipet the solutions in sequence as described.* The result should be a yellow solution. If your solution is green, there is a contamination with phosphate in your system you should eliminate first.



Please take two pictures for your documentation: one of the results of your pretest and one of your lab environment.

2B Establishing a straight calibration (instructions)

Become familiar with the pipettes and the process of pipetting. Read the instructions of your photospectrometer of your school or of the one you find in your “Chem-box”. Ask a teacher for support.

The teacher may help you to handle all technical problems concerning your photospectrometer. *She or he is not permitted to support you during your measurements.*

Hint: To test the photospectrometer, use a diluted colored solution. Create a table in your lab-book in which you can easily note your results later during the measurements.

Now it becomes serious…

1. Label 6 cuvettes and 6 Eppendorf-Tubes (EPs) with 0; 10; 20; 30; 40; 50. The numbers stand for the concentration in micromolar (µM) phosphate.

2. Add exactly 0; 80; 160; 240; 320; 400µl of the Sodium-Phosphate-Buffer (50µM) into the corresponding EPs.

3. Add D-Water (distilled water) to an end volume of 400µl (400; 320; 240; 160; 80; 0µl D-Water) to each EP.

4. Add 6 EP's labeled with 'M' for Mix (M0-M50) next to each phosphate concentration. Mix in the first one exactly 256µl Malachitgreen-Solution with exactly 344µl Molybdat-Solution (containing sulfuric acid).

5. Wait 5 minutes, and then add this mix (4.) to the EP with the corresponding Sodium-Phosphate-Buffer.

6. Close the EP, mix it (if available on a Vortex), and pour it into the corresponding cuvette.

   Hint: We suggest that you do this step by step for all Sodium-Phosphate-Solutions (0-50). Why? Check 7.!

7. Measure the absorption at 595nm.
You measure though the intensity of the green color.

   *Note the lapse of time (right after performing step 6.) until you start measuring the absorption in your photospectrometer. Reason: The solution will bleach in a relatively short time**. It is crucial that the lapse of time between adding the dye (M) to the phosphate solution and the measurement in the photospectrometer is the same for each sample.***

8. Create a table with your results. Be careful not to loose any important data or any other information about the measuring process. Gather all raw-data carefully and label the columns of your table.
   Insert your data in an excel sheet. Create an XY scatter gram (Y OD595, X phosphate concentration) with a regression line (best straight line) and read out the corresponding linear equation. Excel or 'Calc' provides you this opportunity. Mention the r-value you reached. The r-value of your regression line should be as close as possible to 1. (1 means all points are on the line).

9. You might repeat this measurements several times until you are satisfied with the results. Keep the results of the different attempts in your lab book.

10. **What we expect to see on 1-2 A4 pages:**
    Please present the **three** best results (3 tables with raw data) of your attempts. Calculate the mean value of your results and display all results you achieved in one graph. Don't forget to display the standard deviation in your graph!
    Discuss the difficulties you had and how you tried to improve your proceedings and results. This is an important part of your results. We expect a short and precise analysis (5-10 sentences) of the situations you struggled and the consequences you deduced.

Part 3
Measuring the phosphate uptake by yeast cells

Since you finalized part 2, you know how to measure the phosphate concentration of a solution. During part 3 you learn how to handle the yeast cells and to measure the decline of phosphate in the solution, caused by the excessive uptake by these unicellular creatures.

3A Preparing the yeast solutions picking up phosphate over time

What you have to wear: **safety glasses, gloves, lab coat! It's a MUST!**

1. Prepare and label 2 EPs for each point in time. One with P for pellet and one with S for supernatant (12 EPs all in all).

   P0; P10; P20; P30; P40; P50min and
   S0; S10; S20; S30; S40; S50min

2. Dissolve 1g fresh yeast (you find in COOP or MIGROS) in 10ml medium (**0.5mM** Sodium-Phosphate-Buffer, pH 6.3, 1% Glucose) in an Erlenmeyer tube. To prepare this solution you can use the concentrated Sodium-Phosphate-Buffer we sent you in the box (0.1M Sodium-Phosphate-Buffer, pH 6.3). Think about the dilution you must achieve! Please do not forget to add glucose! Take a stirring rod to get the yeast into a homogenous suspension. Start the timing right after the yeast-bloc is dissolved allover.
3. Blend the cultures regularly during the whole experiment.
4. After shaking the yeast solution once again, take an aliquot of 300µl at each defined time point and transfer it into the appropriate EP labeled with 'P'.
5. Centrifuge during 1min with max high speed.
6. Take exactly 40µl of the supernatant and transfer it into the corresponding EP labeled with 'S'.
7. When you collected all the probes you may start the photometric measurement (-> 3B).

3B Measuring the decline of phosphate in a well defined yeast solution

1. Add 360µl D-Water to each probe (40µl) in the EPs labeled with ‘S’.
2. Label 6 additional EPs with M (=”Mix”, for each time point M0-M50).
   Mix exactly 256µl Malachitgreen-Solution with exactly 344µl Molybdat-Solution (containing sulfuric acid) in the first of these 6 EPs (M0).
3. Wait 5 minutes, then add this mix (2.) to the first EP labeled with S (S0) containing the Supernatant (S0).
4. Close the EP, mix it (if available on a Vortex), and pour or pipette it into the corresponding cuvette.
5. Measure the absorption at 595nm.
   You measure though the intensity of the green color.
   *Note the lapse of time until you start measuring the absorption in your photospectrometer. Reason: The solution will bleach in a relatively short time****. It is crucial that the lapse of time between adding the dye (M) to the supernatant and the measurement in the photospectrometer is the same for each sample.***

   Hint: We suggest that you do this step by step for all Sodium-Phosphate-Solutions (S0-S50).
6. Note all the results in an Excel-sheet.
7. Illustrate your results in a graph. Do not forget the important information that has to be part of your graph. Label your picture correctly. Mention the r-value you reached. The r-value of your linear regression should be as close as possible to 1. (1 means all points are on the line). If your data points are not in a line and you get the same results after several measurements, think about an explanation.
8. **What we expect to see on one A4 page:**
   Please present the **three** best results (3 tables with raw data) of your attempts. Calculate the mean value of your results and display all results you achieved in one graph. Don’t forget to display the standard deviation in your graph.
   Discuss the difficulties you had and how you tried to improve your proceedings and results. This is an important part of your results. We expect a short and precise analysis (5-10 sentences) of the situations you struggled and the consequences you deduced. Your results are important because you will compare later the results of your own attempts to the one you’ve got here.

Part 4
How to improve the phosphate uptake by yeast cells

After being familiar with the photospectrometer, the pipets and the whole setup of the experiment, the most important part starts now. Your knowledge in biology and biochemistry and your phantasy will be important. The question is how to change the circumstances so the yeast cells will pick up the phosphate quicker then under normal conditions. In other words: How can you enhance the process of transferring the phosphate out of the nutrient-solution into the yeast cells? Think about different transportation systems through membranes. Search the literature for helpful hints and take all your knowledge about biochemical processes into account.

4 A-C Enhancing the efficiency of the phosphate-uptake by yeast cells

What we expect to see:
Establish and present **three approaches** (First, second and third approach, 4 A-C) to reach that goal. From the first task of this competition you know already the roster (Design, Data Collection and Processing, Conclusion and Evaluation). The scoring list on page 11 and the summary below will help you to structure your experiments.

Each approach should be presented on 1-2 A4 pages. Use a new page for each approach.

Thinking about the corrections of your work, it is crucial to follow exactly the given structure you find in the scoring list. Please do not miss one aspect in part 4A - 4C, we will judge all of them.

Please follow the three steps carefully:

Step 1: Design of your experiment

Aspect 1: Defining the problem and selecting variables

- **You identify a specific research question**. In a short introduction you describe your considerations based on your knowledge in biology and biochemistry and the literature.

- You define the **variables**. Variables are factors that can be measured and/or controlled.
  Independent variables are those that are manipulated, and the result of this manipulation leads to the measurement of the dependent variable.
  A controlled variable is one that should be held constant so as not to obscure the effects of the independent variable on the dependent variable.

- Example: 'How does the speed of movement of chloroplasts in *Elodea* cells vary with light intensity?' The independent variable is the light intensity; the dependent variable is the speed of movement. Relevant controlled variables would include temperature, preparation of *Elodea* cells, sample size and light quality.

Aspect 2: Controlling variables

- You describe carefully how the control of the variables is achieved.

- If a standard measurement technique is used, it should be referenced.

- Example: While planning an investigation to study the effect of light wavelength on the rate of photosynthesis in *Cabomba*, you may have adapted method to measure the rate of photosynthesis taken from a textbook. A standard reference would then be expected as a reference, for example, “Freeland, PW (1985) *Problems in Practical Advanced Level Biology*, Hodder and Stoughton”.

Aspect 3: Developing a method for collection of data

- The planned investigation should anticipate the collection of sufficient data so that the aim of research question can be suitably addressed and an evaluation oft he reliability of the data can be made.
- Be sure to collect enough data, so an error analysis involving the calculation of a mean value and a standard deviation is possible.

Step 2: Data collection and processing

Aspect 1: Recording raw data

- Raw data is the actual data measured.
- Within tables of quantitative data, columns should be clearly annotated with a heading, units and an indication of the uncertainty of measurement. The number of significant digits should reflect the precision of the measurement.

Aspect 2: Processing raw data

- Data processing involves, for example, combining and manipulating raw data to determine the value of a physical quantity (such as adding, subtracting, squaring, dividing), and taking the average of several measurements and transforming data into a form suitable for graphical representation. It might be that the data is already in a form suitable for graphical presentation. If the raw data is represented in this way and a best-fit line graph is drawn, the raw data has been processed. Plotting raw data (without a graph line) does not constitute processing data.

Aspect 3: Presenting processed data

- You are expected to decide upon a suitable presentation format yourself (for example spreadsheet, table, graph, chart, flow diagram and so on). There should be clear, unambiguous headings for calculations, tables or graphs. Graphs need to have appropriate scales, labeled axes with units, and accurately plotted data point with a suitable best-fit line or curve (not a scatter graph with data-point to data-point connecting lines. You should present the data so that all the stages to the final result can be followed. The way how you present your data is not only a matter of design. It should be meaningful and prove that you didn’t choose the type of diagram by chance.
- You should include a treatment of uncertainties and errors with your processed data, where relevant.

Step 3: Conclusion and evaluation

Aspect 1: Concluding

- Analysis may include comparisons of different graphs or descriptions of trends shown in graphs. The explanations should contain observations, trends or patterns revealed by the data.

Aspect 2: Evaluating procedures

- The design and method of the investigation must be commented upon as well as the quality of the data. You must not only list the weaknesses but must also appreciate how significant the weaknesses are. Comments about the precision and accuracy of the measurements are relevant here. When evaluating the procedure used, you should specially look at the processes, use of equipment and management of time.

Aspect 3: Improving the investigation

- Suggestions for improvements should be based on the weaknesses and limitations identified in aspect 2. Modifications to the experimental techniques and the data range can be addressed here. The modifications proposed should be realistic and clearly specified. It is not sufficient to state generally that more precise equipment should be used.

Scoring List

Part	Subject	Score (%)
1	Searching the literature, background information about phosphate uptake by yeast cells (Summary). (For your short text and the correct references to the literature you can get 10% of the total score).	10
2A & 2B	Establishing a nice straight calibration curve (If you show 3 tables with all your results (raw data), 1 straight calibration curve based on the mean value of the raw data (including the standard deviation) with equations to calculate the phosphate concentration out of the absorptions measured and two pictures, you can make up to 10% of the total score).	10
3A & 3B	Measuring the decline of phosphate under normal conditions (If you are able to follow the instructions correctly and getting nice results (3 tables with raw data) and one corresponding graph (see 2A & 2B), you can make up to 15% of the total score).	15
4A	First approach: Design, Data Collection and Processing, Conclusion and evaluation (If you fulfill all 3 aspects for each category perfectly you can get 15% of the total score.).	15
4B	Second approach: Design, Data Collection and Processing, Conclusion and evaluation (If you fulfill all 3 aspects for each category perfectly you can get 15% of the total score.).	15
4C	Third approach: Design, Data Collection and Processing, Conclusion and evaluation (If you fulfill all 3 aspects for each category perfectly you can get 15% of the total score.).	15
Layout	Layout (If all pictures, graphics and tables are labeled and numbered correctly and the quality of your charts and diagrams is convincing, if your layout is pleasant and consistent, you will get 10% of the total score).	10
Extra Points	Extra-points for successful approaches (You will get these extra-points (10% of the total score) *only* if you present us outstanding, unique and fanciful approaches with convincing results and explanations during part 4A-4C).	10
Total		100

Don’t forget…

Expected documentation and further information
Create **one single** PDF file (the size of the PDF file must not exceed **6 MB**!) containing all your answers, solutions, pictures, other documenting material and the activity list. Use page numbers and use a new page at the beginning of each part:

Front page: must include name of school, name of class and number of task
Page 1: Table of Contents
Page 2: Part 1
Page 3-4: Part 2
Page 5: Part 3
Page 6-11: Part 4 (A-C)
Page 12: Reference List
Page 13: “Activity List” including two pictures

Use font size 11-12.
You are free to choose the font, but it should be easily readable.

Name the file following strictly these conventions:

Name of School

Name of Class (same as on application form or homepage)

Number of Task

Date (year/month/day)

→ Please use underlines instead of spaces!

Here is an example: **Kantonsschule_Muster_Class3b_Task_20120308.pdf**

Reference List
Do not forget to **list** your **references** at the end of your documentation according to the guidelines explained in Part 1.

Activity List
Do not forget to add the **activity list** to your documentation! Each class needs to report which member was or is responsible for which portion or aspect of the work. Each person in the class must have participated at least once (during the entire competition) in the experimental portion.
Therefore, **take 2 digital photos showing the class involvement.** Place them next to the activity list in your documentation file at the very end.

Closing Date of Task 1: Friday, 17.05.2013, 13:00