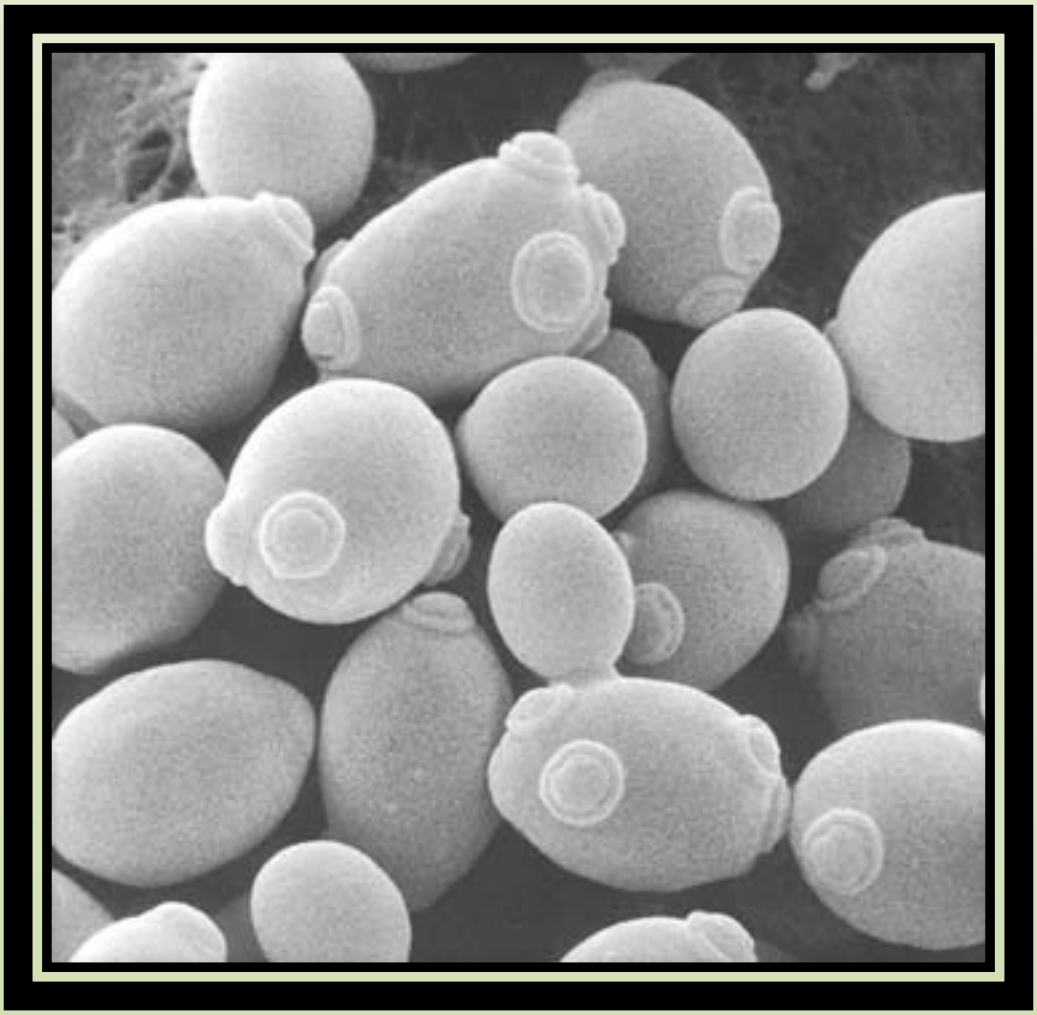
Task 2 - Phosphate uptake in yeast cells

Gymnase d'Yverdon

Class 1M03



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Part 1 – Studying the literature

The phosphate is an essential component for the life of all organisms. It enter in the composition of two of the most important organic macromolecules: the DNA and the ATP. Phosphate is present in the structure of the DNA, where it allows the liaison of sugar deoxyribose wich compose the frame of the molecule. Otherwise, the ATP provides the energy for the cell. It is three phosphates bonded and if a phosphate breaks off, it releases lots of energy that a cell can use to make movement, chemical reactions and other cells activities.[1]

The yeast cell is an unicellular organism, so it uptake the phosphate it needs directly in its environment. The phosphate enters by endocytosis in the yeast cell.[2]

Once the phosphate has been take up by the yeast cell, the vesicule formed is transported to the nucleus where the phosphate is used to build DNA. [2] The vesicule can also bring the phosphate in a structure called vacuole, wich is the site of storage of large amounts of phosphate in a polymer form. These vacuolar pools are either augmented or depleted depending on changes in phosphate availability.[3]

The phosphate ions are taken from the soil by plants. When herbivores eat plants or carnivores eat plants or herbivores, then the phosphates shift from plants to animals. Then carnivores give back the phosphate ions to the soil through their urines and feces, or through their decompositions after their deaths.[4]

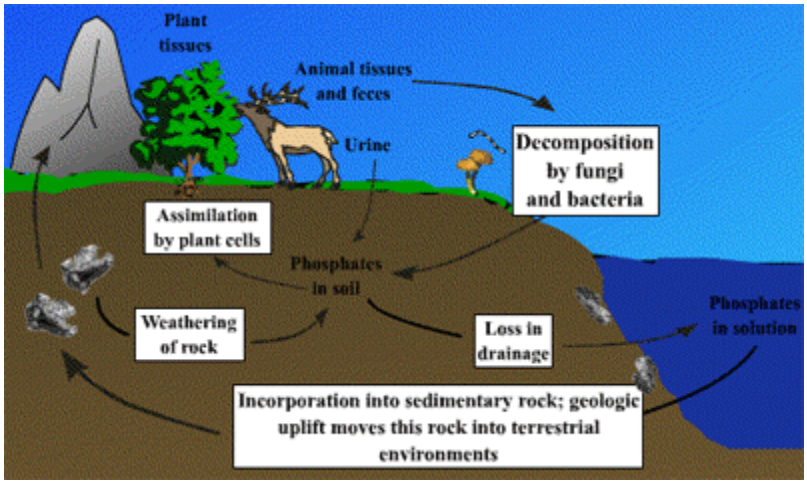

Figure 1. The cycle of phosphate. Source: www.enviroliteracy.org

As we can see on figure 1, the phosphate uptake by microorganisms like fungi (taxonomic group in wich are the yeasts) and bacteria is very important to return phosphates from urines, feces and death organisms in soil in a form that can be assimilated by other living organisms.

It's important to mention here that in natural environment, plants have difficulty to obtain phosphates. Phosphate is actually a limiting nutrient in many ecosystems. When humans add extra phosphates to water, they cause the eutrophication of it. The adding of phosphate leads to the rapid growth in the plant population. "With more living plants comes more dead plants needing decomposition. The bacteria that decompose the dead plants use oxygen, and eventually burn up so much that not enough remains to support [other livings organisms of the aquatic ecosystem].[1]

Part 2 – Calibration of the measuring system

Before calibrating our measuring system, we did a pre-test to confirm the absence of contamination by phosphate in our solutions. It was negative as we can see on figure 2 below.

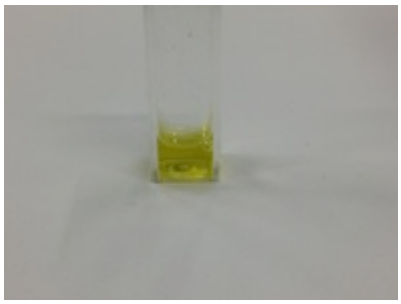

Figure 2. Pre-test result showing that there was no contamination in our test-solution.

With a spectrophotometer, we did several measure of the optical density at 595nm of different concentration in phosphate. We took the three best curve and calculated a mean value of the O.D. related to phosphate concentration.

Figure 3. Our lab and a part of the crew.

Table 1. Raw data of the three best results obtained in the calibration.

Data 1		Data 2		Data 3	
PO4 [µM]	OD [595 nM]	PO4 [µM]	OD [595nM]	PO4 [µM]	OD [595nM]
0	0	0	0	0	0
10	0.096	10	0.084	10	0.088
20	0.165	20	0.188	20	0.168
30	0.231	30	0.212	30	0.220
40	0.286	40	0.261	40	0.251
50	0.296	50	0.282	50	0.288

Table 2. Mean value and standard deviation.

Mean value		Standard deviation
PO4 [µM]	OD [595nM]	
0	0	0
10	0.089	0.006
20	0.174	0.013
30	0.221	0.010
40	0.266	0.018
50	0.289	0.007

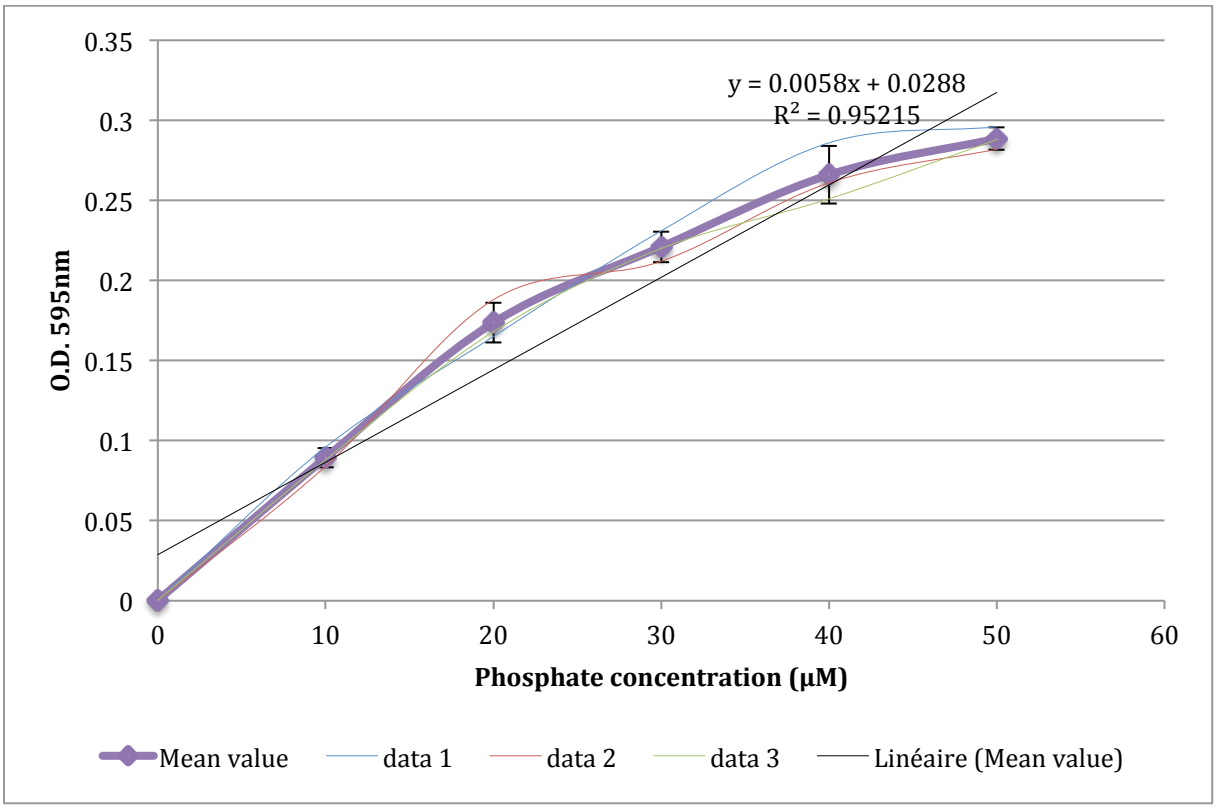

Figure 4. Calibration curve of the O.D. $_{595}$ related to phosphate concentration.

While working on part two, we encountered different difficulties. The first try, we realized that the quantities of reagents provided by the protocol amounted to a volume wich was too small to be read by our spectrophotometer. We diluted the volume with 2 mL of additional water. It should be noted that we had to add 2 mL also for points 3 and 4, to avoid calculating each time the concentrations of the standard curve. Subsequently, we noticed some contamination issues during our pre-test. At first we thought our water was contaminated with phosphate because our solution became green when we added 2 mL of water when it should have been yellow. We tested it with deionized water and then with distilled water, but the solution still had a tendency to become green. We calibrated the zero with this solution, it was a reference for each time so it's not a problem for the experiment.

Part 3 – Measuring the phosphate uptake by yeast cells

For parts 3 and 4, to measure the speed of phosphate absorption by the yeast cells, we measured the absorbance of the supernatant of the solution with the photospectrometer. This absorbance was put in connection with the calibration curve done in the part 2. The variation of the concentration of phosphate in the environment in function of the time corresponds at the speed of absorption.

For this third step, we first saw that the phosphate uptake by yeasts was very quick with the magnetic stirring rod. Actually, all phosphate was uptaken after 10 or 20 minutes. It was probably because the agitation with the stirring rod added oxygen in the medium and also increased movement of molecules. Those two conditions probably contributed to increase phosphate uptake by yeast cells. It has given us the idea to increase agitation as a condition to test in the part 4. Also, to improve our results, we made the choice to shake our yeast culture by hand but on a regular basis. The uptake of phosphate by yeast cells was then slower and that allowed us an easier collection of the data. We noticed that the first time we did the experience some errors were done with the photo spectrometer too. For example, the time between the different steps wasn't always the same, so we decided to attribute one specific job to each student. We needed some time to find a good work's method, but when we had found it, we were efficient. The table 3 shows the three best results we collected (raw data). Table 4 shows the same data converted in concentration of phosphate, using the equation given by the calibration curve obtained in part 2 (note: we adjusted the equation in order to consider the 1:10 dilution of the supernatant implied by the protocol we used).[5]

Table 3. Time-course of the optic density at 595nm of the supernatant of a yeast culture.

	O.D. [595 nm]		
Time [min]	**Experiment 1**	**Experiment 2**	**Experiment 3**
0	0.357	0.342	0.337
10	0.306	0.298	0.285
20	0.269	0.287	0.242
30	0.045	0.044	0.064
40	-0.036	-0.037	-0.047
50	-0.048	-0.041	-0.051

Table 4. Time-course of phosphate concentration in the supernatant of a yeast culture.

	Phosphate concentration [mM]		
Time [min]	**Experiment 1**	**Experiment 2**	**Experiment 3**
0	0.566	0.540	0.531
10	0.478	0.464	0.442
20	0.414	0.445	0.368
30	0.028	0.026	0.061
40	-0.112	-0.113	-0.131

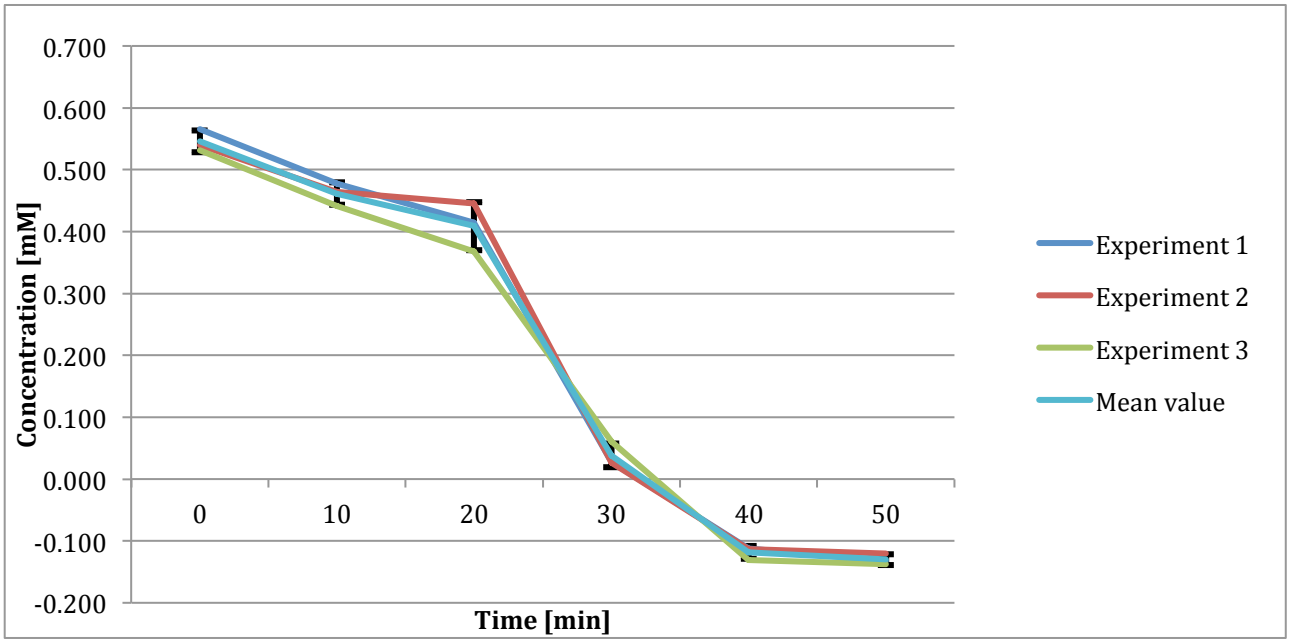

Figure 5. Variation of the phosphate concentration in a yeast culture in time.

The mean speed of uptake was calculated by dividing the mean variation of phosphate concentration by 50 minutes: 0.0135 mM/min.

Part 4 – How to improve the phosphate uptake by yeast cells

A. Enhancing the efficiency of the phosphate uptake by yeast cells by increasing glucose concentration

Step 1: Design the experiment

Aspect 1: Defining the problem and selecting variables

During our researches, we found that the glucose could have an effect on the absorption of the phosphate by the yeast cells[6]. We then decided to find the answer to the following question: does a higher glucose concentration could increase the speed of phosphate absorption by the yeast cells. According to the literature, we supposed that higher would be the concentration of glucose in the solution, faster would be the phosphate absorption by the yeast cells.

The independent variable in our experience was the percentage of glucose in the medium. We have tested three different concentration of glucose.

The dependent variable was the speed of the phosphate uptake by the yeast cells.

Controlled variables were the temperature and the agitation of the solution. During the experience we also discovered that we had to use yeast from the same lot to avoid too much variation in our results.

Aspect 2: Controlling variables

We decided to do the experience under three different concentrations of glucose: 0 %, 2 % and 4 %.

To minimize the variations in the measuring method, the same person measured the absorbance of solutions at the photospectrometer.

According to the instructions, we let the same lapse of time between the adding of the dye to the supernatant and the measurement in the photospectrometer for each sample.

We made the experience in temperature room.

Aspect 3: Developing a method for collection of data

To collect our data, we did the experience in the way indicated in the protocol provided by the Science On The Move team once. We used different concentrations of glucose from 0% to 4%.

Step 2: Data collection and processing

Aspect 1: Recording raw data

Table 5. Time-course of the optical density of supernatant of yeasts cultures at different glucose concentrations.

Time (min)	Glucose concentration		
	0%	2%	4%
0	0.248	0.257	0.189
10	0.210	0.242	0.235
20	0.247	0.210	0.198
30	0.256	0.123	0.120
40	0.196	0.051	0.044
50	0.196	0.041	0.048

Aspect 2: Processing raw data

The results we collected were converted from optical density to phosphate concentration with the calibration curve we did in part 2. Our standard curve gave us an equation to find the phosphate concentration with the optic density.

Phosphate concentration = (((OD-0.0288)/0.0058)*10)/1000

As mentioned above, the factor « 10 » was added in the equation because we harvested 40 µl of supernatant that we had into 360 µl of D-water so the solution has been dissolved 10 times. We divided the result by 1000 to convert the concentration unit from µM to mM.

To know the speed of uptake we have to do this following formula:

$$([PO_4^{3-}]_{T0}-[PO_4^{3}]_{T50})/50 = x \text{ mM/min.}$$

We can't include a treatment of uncertainties and errors with your processed because we hadn't done many experiences.

Table 6. Time-course of phosphate concentration (mM) in supernatant of yeasts cultures at different glucose concentration

Time (min)	Glucose concentration		
	0%	2%	4%
0	0.38	0.39	0.28
10	0.31	0.37	0.36
20	0.38	0.31	0.29
30	0.39	0.16	0.16
40	0.29	0.04	0.03
50	0.29	0.02	0.03

Aspect 3: Presenting processed data

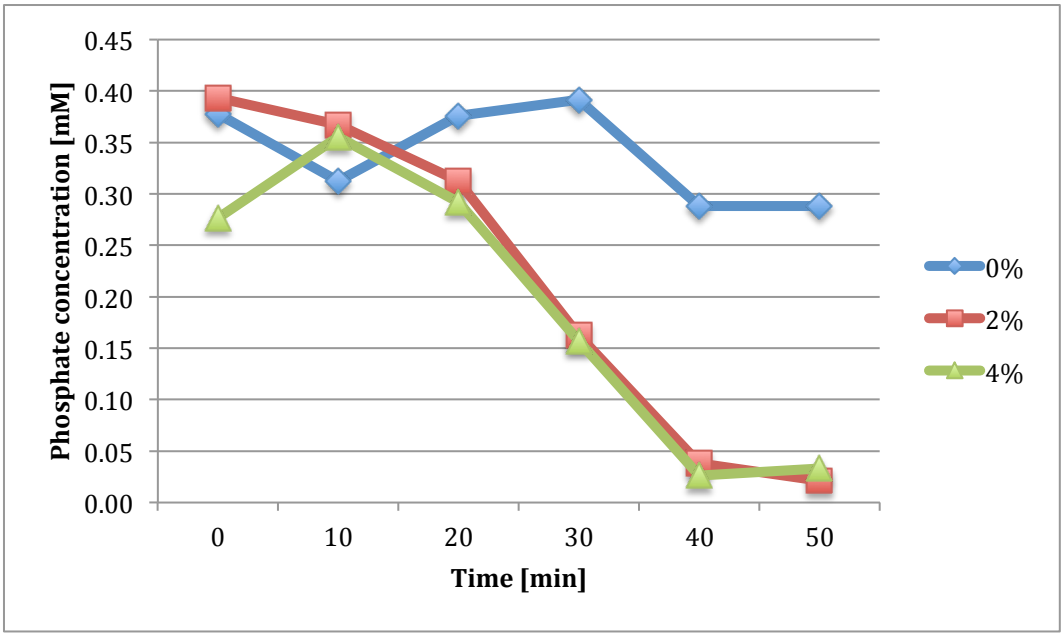

Figure 6. Variation of the phosphate concentration in a yeast culture in time according to the glucose concentration.

Table 7. Mean speed of phosphate uptake according to the glucose concentration.

Glucose concentration [%]	Mean speed of phosphate uptake [mM/min]
0%	0.0018
2%	0.0074
4%	0.0050

Step 3: Conclusion and evaluation

Aspect 1: Concluding

Regarding the results of our first trial, where we test 0%, 2% and 4%, we can observe some remarkable aspects. At 2% and 4%, except for the first value at 0 minute, the phosphate concentration is very similar during all the experiment. The main speeds of phosphate uptake with these two concentrations are in the same order. However, to 0% of glucose the speed of phosphate uptake is slower, but the variation is very unregular.

To 2% and 4% of glucose, the phosphate absorption seems faster than for 0%.

Aspect 2 : Evaluating procedures

During the experiment, we just experiment three different concentrations of glucose. Conclusions we can make about effect of glucose concentration on phosphate absorption by yeast cells are then limited and it could be one of our weaknesses.

We also tested 6% and 8% of glucose. Unfortunately, we arrived at the end of our yeast pack. We bought another pack but the results in the first control test were pretty different from the ones we had with the first batch of yeast, so we decided to compare only the results obtained in the first experiment.

Aspect 3 : Improving the investigation

Our main weakness here is the insufficient range of concentrations of glucose tested. With more different concentrations, maybe we would be been able to better characterize the effect of glucose concentration on phosphate uptake.

B. Enhancing the efficiency of the phosphate uptake by yeast cells by increasing the temperature

Step 1: Design the experiment

Aspect 1: defining the problem and selecting variables

In our researches, we found that the absorption of the phosphate by the cells of yeast is blocked at low temperature.[6] We then decided to try to answer to the following question: does the temperature in which the yeast cells are growing during influence the speed of absorption of phosphate by the yeast cells?

We supposed that the speed of absorption of phosphate would increase with the temperature.

The independent variable in our experience was the temperature variation. Four solutions of yeast and phosphate has been maintain in different temperatures. We kept these temperatures as stable as possible during the experience.

The dependent variable was the speed of the absorption of phosphate by the yeast cells.

Controlled variable was the glucose concentration and the agitation of the solution.

Aspect 2: controlling variables

We decided to do the experience (of the part 3) under four different temperatures: 5-10 degrees, 20 degrees, 35 degrees and 50 degrees. To keep our solutions in these various temperatures, we used water bath in witch temperatures remained constant. We also used thermometers to make sure that the temperature in which the solutions were placed did not vary too much.

For each temperature, we placed a test tube there containing 10 ml of the solution (phosphate and yeast). The absorption in phosphate was measured by the photospectrometer and by the same person.

For time, as it was said in the instruction, we watched that the same lapse of time between adding the dye to the phosphate solution and the measurement in the photospectrometer is the same example.

Aspect 3: Developing a method for collection of data

To collect our data, we did the experience in the way indicated in the protocol provided by the SOTM team.

Step 2: Data collection and processing

Aspect 1: Recording raw data

Table 8. Optic density in yeast culture placed at different temperatures.

Time (min)	Temperatures			
	0-10°C	20°C	35°C	50°C
0	0.301	0.335	0.325	0.271
10	0.322	0.315	0.266	0.311
20	0.335	0.23	0.151	0.323
30	0.295	0.222	0.113	0.302
40	0.306	0.163	0.15	0.317
50	0.272	0.142	0.159	0.269

Aspect 2: Processing raw data

Ref. p. 9 – A. Step 2 Aspect 2.

Aspect 3: Presenting processed data

Table 9. Phosphate concentration (mM) in a yeast culture placed at different temperatures.

Time (min)	Temperature			
	0-10°C	20°C	35°C	50°C
0	0.47	0.53	0.51	0.42
10	0.51	0.49	0.41	0.49
20	0.53	0.35	0.21	0.51
30	0.46	0.33	0.15	0.47
40	0.48	0.23	0.21	0.50
50	0.42	0.20	0.22	0.41

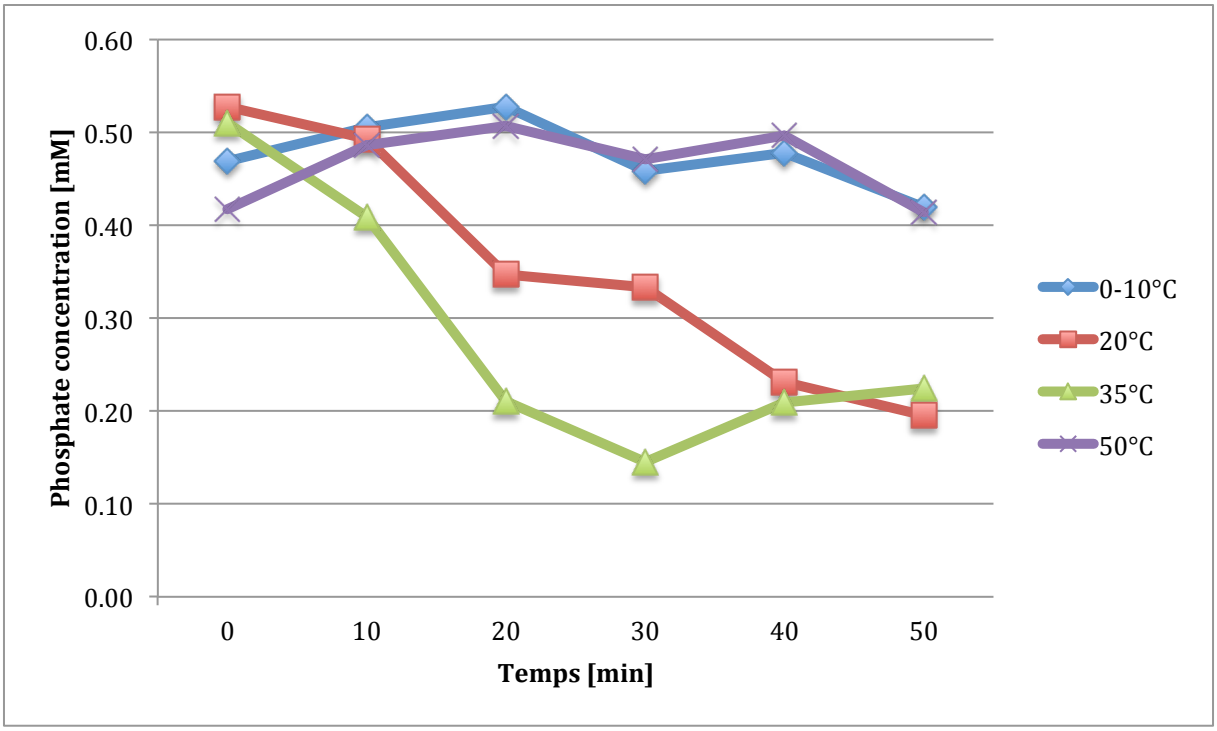

Figure 7. Variation of the phosphate concentration in a yeast culture in time according to the temperature.

Table 10. Mean speed of phosphate uptake according to the temperature.

Temperatures (°C)	Main speed of uptake (mM/min)
0-10°C	0.0010
20°C	0.0066
35°C	0.0051
50°C	0.0002

Step 3: Conclusion and evaluation

Aspect 1: Concluding

To 10°C and to 50°C, the phosphate absorption is very similar. We can also see that the yeast uptake the phosphate faster to 20°C and to 35°C. At 40 minutes and 50 minutes, the two curves come closer to each other and almost show the same results. The faster absorption of phosphate in the 30 first minutes is observed to 35°C. According to the kinetic theory, at 10°C, the move slowly and since the yeast cells are less in contact with the molecules of phosphate, their absorption is slowed down too. To 50°C, the temperature is too high and the cells of yeasts probably died or had their proteins denatured, so the phosphate absorption is about zero.

Aspect 2 : Evaluating procedures

During the experiment, we have used the same type of water bath (with different temperature) except for the 10°C one for which we used cold water with ice. For this one, the temperature was not stable and was varying from 0°C to 10°C.
We could not do the experiment several times and only did it twice which doesn't allow us to get conclusive results.

Aspect 3 : Improving the investigation

We shall experiment some other temperatures between which we test to see how the absorption. We also need to find a way to maintain the temperature 0-10°C more stable.

C. Enhancing the efficiency of the phosphate uptake by yeast cells by increasing the agitation in the medium of culture

Step 1: Design the experiment

Aspect 1: defining the problem and selecting variables

Our research question for the 4C section is about the role of the agitation of the medium in the phosphate uptake by yeast cells. During our previous experiments, we noticed a difference in our results following our technique to mix the solution. We expected that the yeast absorb the phosphate better with oxygen. We found that *Saccharomyces cerevisiae* is aerobic but able to survive without oxygen. *Saccharomyces cerevisiae* makes more energy as ATP in aerobic condition than in anaerobic condition. However, more ATP means more phosphate uptake since there are three phosphates by ATP molecule. Consequently, we expect that there will be an increase of phosphate uptake when we add oxygen in the medium with agitation.

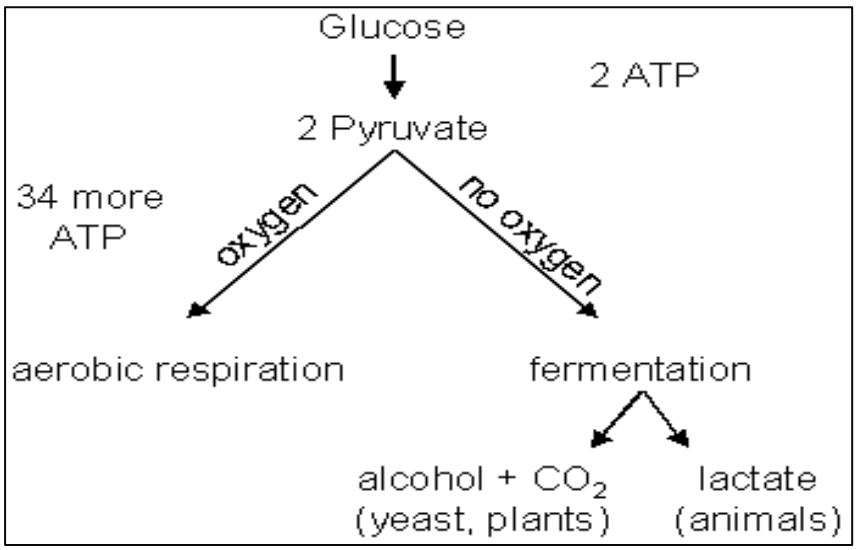

Figure 8. ATP production in aerobic and in anaerobic condition.
Source: http://faculty.clintoncc.suny.edu/

If we increase the agitation in the medium, there will be more oxygen. A significant mixing of the water increases the contact of it with air and therefore increases the level of dissolved oxygen.[7] More we mix, more there will be oxygen and it has less oxygen if we do not mix. From there, we understood that more we mix our solution, faster the absorption should be. The question is: how does the speed of the absorption of the yeast cells vary with a bigger agitation?

The independent variable was the agitation speed. We controlled the degree of the agitation to see the absorption by the yeast cells with three different kinds of agitation.

The dependent variable will be the speed of the absorption of phosphate by the yeast cells.

Controlled variables were the temperature and the glucose concentration of the solution.

Aspect 2: Controlling variables

We have been experimenting with three different kinds of agitation. We put the yeast and the phosphate in the three Erlenmeyer and placed the magnetic stirring rod inside the two others. We mixed the first one with a light and regular handshaking just to homogenize the medium. The two others yeast cultures was mixed with the magnetic stirring rod. We don't know exactly the speed of the agitation on the magnetics plates, but one had a light agitation and the other a high agitation. The agitation was continual during all the experiment. Three persons controlled each agitation and harvested the solution.

Our technique of measure is the same as the Part 4 A and B: To measure the speed of absorption of the phosphate by the cells of yeasts, we have measured the absorbance of the supernatant of the solution with the photospectrometer. This absorbance is put in connection with the calibration curve done in part 2. To control the agitation intensity, we used two similar magnetic plate, one set at the middle position and the other set at the maximal intensity. The variation of the concentration of phosphate in the environment according to time corresponds at the speed of absorption.

Aspect 3: Developing a method for collection of data

To collect our data, we did the experience in the way indicated in the protocol provided by the SOTM team. We did only once the experiment so we have only one set of data. We should have taken two others sets of data. We got the expected results, which confirms our hypothesis. If we had more time, we would have enjoyed doing the experiment again.

Step 2: Data collection and processing

Aspect 1: Recording raw data

Table 11. Optic density in yeast culture placed at different level of agitation.

Time (min)	O.D. 595 nm		
	no agitation	light agitation	max agitation
0	0.219	0.263	0.26
10	0.238	0.251	0.228
20	0.248	0.16	0.081
30	0.208	0	0
40	0.176	0.006	-0.008
50	0.15	0.003	0.005

Aspect 2: Processing raw data

Ref. p. 9 – A. Step 2 Aspect 2.

Aspect 3: Presenting processed data

Table 12. Concentration of phosphate in yeast culture at various agitation

Time (min)	No agitation	Light agitation	Max agitation
0	0.33	0.40	0.40
10	0.36	0.38	0.34
20	0.38	0.23	0.09
30	0.31	-0.05	-0.05
40	0.25	-0.04	-0.06
50	0.21	-0.04	-0.04

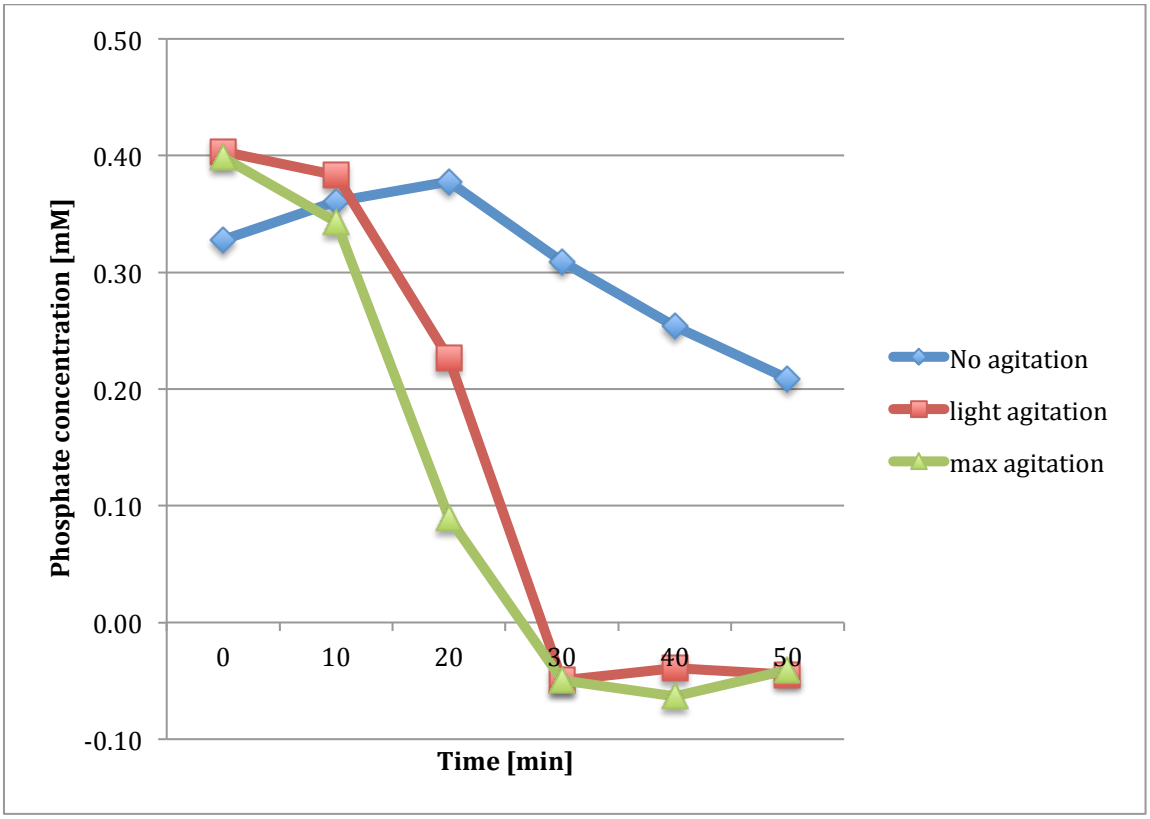

Figure 9. Variation of the phosphate concentration in a yeast culture in time according to the agitation

Table 13. Main speed of the phosphate uptake according to the agitation

Agitation	Speed of uptake (mM/min)
No	0.0024
Light	0.0088
Max	0.0088

Step 3: Conclusion and evaluation

Aspect 1 : Concluding

As we can see in the graph with no agitation there is almost any phosphate uptake in the yeast cells and there is a huge difference between the phosphate uptake with agitation and without agitation. The results show also that the rate of the phosphate-uptake between the light agitation and the high agitation is very similar , almost identical. However we can note that in the first 30 minutes the high agitation absorb slightly more phosphate than the light agitation until both trends intersect at 30 minutes. According to our hypothesis the yeast cells absorb more phosphate when there is an increase of the agitation probably due to the movements of the molecules and perhaps to the increase of the oxygen level or maybe a combination of the two factors.

Aspect 2 : Evaluating procedures

During our experiment we used two magnetic plates to do the light and low agitation , the one that did not use the magnet though was a bit agitated by hand regulary at each photometric measurement just to homogenize the mix. Our idea and method was actually pretty effective despite the risk of deviate the magnet placed in the centre of the erlenmeyer during the taking of the yeast cells (300 µl). The factors present in the experiment is the magnet plate and the magnetic stalk used to homogenize the mix . As we said earlier the two plates and the magnetic stalks which were used simultaneously were also identical. Otherwise the experiment is basically just like the one in Part 3. Unfortunately we could not do other experiments to make sure that our results are satisfaying due to the lack of time we had and because only three of us did this experiment. But we are very pleased with our data , it shows us that our hypothesis was not wrong.

Aspect 3 : Improving the investigation

Obviously if we had had more time , we could have done more experiments and we would have thought perhaps of a better way to do our approach. Also we did not know the speed of our agitation , during the experiment we only ajust our magnetic plates to agitated at the highest speed it could go and at a lower one. It would have been useful if we had measured the speed and the oxygen uptake with an oxygen sensor.

Activity list – task 2

Date	Names	Activity
April, 18th	All the group	Meeting - planning of the experiments - formation of small workgroups - shedule of the experiments - Part 1 (at home)
April, 22th	Talyssa Justine Laurane Aymen Ludovic Myriam Laurie Agathe	Part 2 - testing some dilution to have a correct volume in the cuvette
April, 23th	Ludovic Myriam Laurie Juliane Nina Camille Talyssa Laurane Mélanie	Part 2 - resolving problems of “contamination” of the water - distillation of water - No calibration curve obtained
April, 24th	Lucien Léonard Augustin Justine	Part 2 - 3 calibration curves
April, 25th	All the group	Meeting - briefing about results and planning the rest of the experiments
April, 25th	Camille Nina Mélanie Juliane Soren Yves Ilan Léonard Jean Magjun	Part 2 - 5 calibration curves
April, 30th	Camille Nina Mélanie Juliane	Part 3 - make 100 mL of medium - write part 2

	Laurane Talyssa	
May, 1st	Agathe Justine Ornella Léonard Lucien Magjun	Part 3 - the medium with yeast was agitated vigourously and all the phosphate disapeared after 30 minutes
May, 2nd	All the group	Meeting - briefing about results and planning the rest of the experiments
May, 2nd	Simon Ilan Jean Ludovic Laurie Myriam	Part 3 - test the experiment in new conditions: 1. without agitation of the yeast (little handshake at every 5 minutes instead of magnetic stiring rod 2. take 80µl of supernatant
May, 3th	Diana Myriam Laurie Lucien Augustin Ornella Talyssa Laurane Justine Aymen Agathe	Part 3 - experiment 4X
May, 6th	Simon Juliane Soren Mélanie Léonard Jean Maeva Magjun Nina Ilan Camille Yves	Part 3 - experiment 4X
May, 7th	Camille Nina Mélanie Juliane Laurane Talyssa	Part 4 - Make 100 ml of medium 1% glucose 0.5 mM phosphate for part 4B and 4C - Make 100 ml of a solution 10% glucose for part 4A

May, 9th	Laurie Laurane Juliane Talyssa Jean Myriam Agathe Diana	Part 4B - Test 2X each following temperature (°C): 1. 0-10 2. 20 3. 35 4. 50
May, 9th	Magjun Justine	Part 4C - Test 3 conditions: 1. No agitation 2. Light agitation 3. Maximal agitation
May 13th	Simon Juliane Soren Mélanie Léonard Jean Maeva Magjun Nina Ilan Camille Yves	Part 4A - test 3 concentration of glucose 1. 0% 2. 2% 3. 4%
May, 14th	All the group	Part 4A - test four concentrations of glucose 1. 0% 2. 1% 3. 6% 4. 8% But the experiment was made with new yeast , diferent result Redaction of the parts 3 and 4
May, 16th	Myriam Laurie Laurane Justine Agathe Juliane Magjun	Redaction of parts 3 and 4

Figure 10. All the group, working at the last part of the experiment or redacting parts 3 and 4, on May, 14th .

Figure 11. D-day minus one: still working on the texts, until very late! Pizzas and a picture of the Golden Gate help us to keep going on with motivation…

Reference list

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[5] Simply Science. *Science on the move. Task 2 document*. 2013.

[6] Hevesy, G., Linderström-Lang, K., and Neilsen, N., ''Phosphorus Echange in Yeast'', nature, 1937, 140, 725

[7] Group of education and eco monitoring of the water : http://www.g3e-ewag.ca/programmes/adopte/accueil/physico.html