Task 2

Class 2E at Liceo Lugano 2

May 17, 2013

Contents

1 Studying the literature

All cells of every organism require phosphate (PO_4^{3-}) to allow the various biological systems to carry out (or motivate) some fundamental processes such as cellular respiration, mitosis, meiosis, etc. and this makes the ion in question an indispensable resource for life on our planet.

The molecule ATP (adenosine triphosphate), for example, the means by which the cells recognize and store bio-available energy, is formed, in addition to a nitrogenous and ribose (a sugar) base, from three phosphate groups. The energy left from the cellular respiration (ATP + H_2O + H^+internal = ADP + phosphate + H^+external) is used to bind a phosphate (Pi inorganic phosphate) to ADP (adenosine diphosphate). When this energy will be necessary for the cell, the Pi can be detached from ATP making the energy available. The maximum peak of consumption of phosphate is formed because when the organism grows, or the amount of the individuals increases, in other words, when new cells require new ATP molecules.[1]

In addition to this the ion phosphate is involved in the duplication of DNA and therefore an important consequence for meiosis and mitosis. In DNA and RNA the phosphate group is found in the "backbone" (in other words, the external part of the double helix) and has a function of support: a PO_4^{3-} converts to a sugar creating the so-called sugar-phosphate backbone. To keep in mind, since all organisms require both from ATP and from the duplication of DNA to grow and reproduce (in spite of requiring only a discrete quantity) and thus the explanation for the connection between life and the ion.[1]

Having verified the absolute dependence of the organisms on phosphate, the question that arises spontaneously is how the cells are capable of storing within themselves. The absorption takes place through precise anionic carriers, transporting proteins which take advantage of the possibility of changing conformation in order to penetrate the other part of the cell and release, once inside, the ion. In reference to Saccharomyces cerevisiae, a unicellular fungus and pivot of all following experiments, one observes how in the course of evolution this has improved the system of external phosphate absorption, that provides the cell with a sufficient quantity of this essential inorganic molecule. The topic of numerous research, the absorption of PO_4^{3-} by yeast (common name of *Saccharomyces cerevisiae*) depends essentially on two different transporting proteins, one (called Pho84p) which moves the "bi proton phosphate" and another (called Pho89p) that transfers the "sodium phosphate". These last two directly depend from the pH, this proves that the first is more active in acidic conditions and the second in alkaline conditions instead, however, the reasons for this reaction are still unclear.[2] In the growing yeast the

absorption of the Pi requires the presence of sugar and is blocked at low temperatures and from metabolic inhibitors. The total absorption depends on the presence of cations such as potassium, sodium, magnesium or the proton (H^+), which are also eventually absorbed.[3] Concerning the storage in itself, the cell often makes use of the vacuole, a cellular compartment that has different functions depending on the type of cell, and on various occasions is a valid reserve of nutrients. In the case of Saccharomyces cerevisiae, this is the compartment where phosphate and poly-phosphate (a linear polymer of phosphate) are principally stored, which besides containing a high capacity as a buffer, are also the principal anions. These vacuole pools are increased or emptied according to the availability of the ion. Also for the transport inside the vacuole there are precise bidirectional transporters, that facilitate the entry and exit of phosphate, so as to capture it when there is an abundance and make it available in case of scarcity. In contrast to the case of carriers on the cellular membrane the transporters on the vacuole membrane have yet to be identified, even though for some cations the vacuole transport systems are known. However it is known that these transporting proteins depend, as that of the membrane, on pH: in particular, they have increased activity at a low pH.[4]

There's need of yeast cells even for ecologic stuff. In fact, aside from its use in cooking and oenology because of its fermentation, it's often used to eliminate eutrophication (because of its membership in the Effective Microorganisms [EM], which are very important for their skills about absorbing toxic substances). This phenomenon, which is nothing but an excess of nutritional substances (like nitrogen, phosphorus and sulfur) from anthropic sources, generates important failures at the ecologic level. This happens because of the increase of the population of plants (especially algae) which imply a degradation of the ecosystem: they aren't disposed by primary cunsomers, so they imply an increased bacterial activity which decreases the amount of available oxygen, and after some time the animal population (fishes are particularly sensitive) decreases.

2 Calibration of the measuring system

2.1 Data collection and processing

Since we had to estabilish a straight calibration line and we had a strict procedure to follow, we managed to obtain these three tables quite easily:

Experiments:

Concentration (µmol/L)	Absorbance	Concentration (µmol/L)	Absorbance	Concentration (µmol/L)	Absorbance
0.000	0.010	0.000	0.037	0.000	0.000
10.000	0.310	0.100	0.361	0.100	0.333
20.000	0.695	0.200	0.687	0.200	0.717
30.000	0.998	0.300	1.021	0.300	1.021
40.000	1.278	0.400	1.287	0.400	1.297
50.000	1.427	0.500	1.565	0.500	1.569

Figure 1: The data we collected to obtain a calibration line

It's been much easier to combine these data and obtain a unique calibration line:

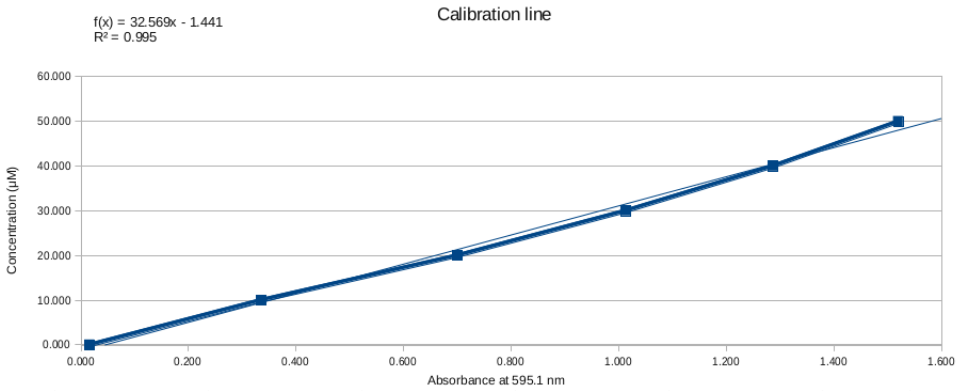

Figure 2: This is what we obtained as a calibration line

It's easy to see that our results are precise thanks to the R^2 factor. The equation we obtained has also been used later to estabilish the concentration of phosphate in our future solutions.

2.2 Problems and solutions

Although it wasn't a very hard job, we encountered a very hard to solve problem. In fact, in the very beginning we always obtained logarithmic graphs. This was because we didn't let the solution of molybdate, malachitegreen

and phosphate react well. In fact, we waited only one minute after its preparation to get the data. We were able to solve this problem simply by waiting and observe when the measured absorbance became stable. This happened at 30 minutes from the creation of the solution.

3 Phosphate absorption by yeast

3.1 Data collection and analysis

To ensure the correctness of our data we had to perform three successful experiments and present them here. However, we soon discovered that we hadn't enough molybdate solution to perform every experiment, so we couldn't repeat any of these works. These are our three tables:

		Experiments			
Time	Absorbance	Time	Absorbance	Time	Absorbance
0.000	1.261	0.000	2.059	0.000	1.569
10.000	1.180	10.000	1.181	10.000	1.396
20.000	0.569	20.000	0.401	20.000	1.326
30.000	0.015	30.000	0.174	30.000	1.087
40.000	0.039	40.000	0.064	40.000	1.003
50.000	0.000	50.000	0.066	50.000	0.913

Figure 3: These are the results of the three experiments

It's very easy to notice that the third table is completely different from the other two and from all of the other experiments we performed. We think that the yeast partially died and that there wasn't enough to use all the phosphate we put in the solution. To analyse the data we prefered to leave that experiment out, because he would change everything. So, this is what we obtained:

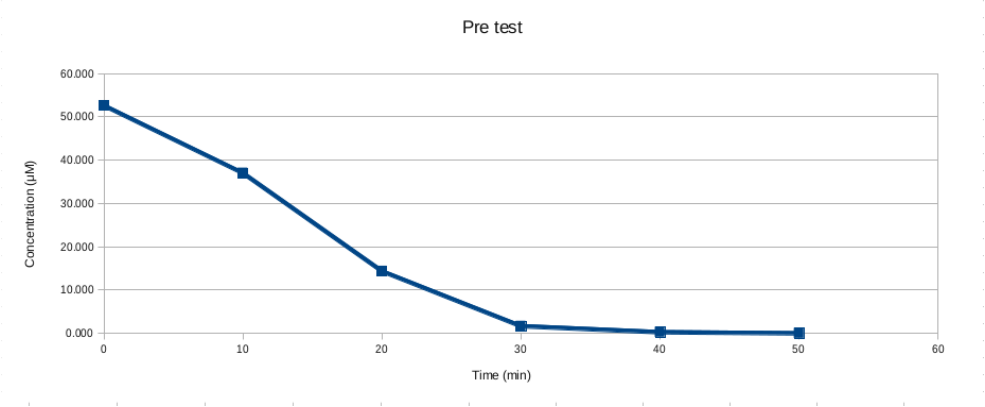

Figure 4: This is the line we obtained from our experiment. The most important thing we discovered is that this line always reaches the bottom, which means that the yeast consumed all the phosphate in the solution.

We have discovered another thing: the starting concentration of the solution varies a lot between two different experiments. We do not really know why, but we think it's a simple human error. However, to prevent stupid comparisons we also decided to calculate the slope of the line while it's descending. This way we're going to compare our data to discover wether we obtained what we wanted, which is an increase of the phosphate uptake. To calculate the slope we calculate the difference of hieght during the decreasing of phosphate and divide it for the time it took to approach 0. In this example, the line approaches 0 as 30 minutes are passed. The slope is then calculated with $(52.624 - 1.653)/30 = 1.754$.

3.2 Problems and solutions

We discussed about all the problems we found while commenting the data, but there's still something to say. The shortage of solutions we had forced us to calculate how many experiments we could make. The other important issue is the differences of starting concentrations. Fortunately, a simple slope can tell us much more than raw data, so this problem also became a minor problem, though we wanted to discover why does these differences appear.

4 How to improve the phosphate uptake by yeast cells

4.1 Introduction

After some careful reflexions and an analysis of biographical sources, we designed three experiments which would have allowed us to modify (and possibly increase) the phosphate uptake by yeast. The variables we decided to vary are: glucose concentration, pH and temperature. The advantage of these experiments is that all the data can be related to the ones we obtained during the tests in normal conditions. This way we achieved a number of experiments which is, in reality, one more than the experiments we actually performed.

We also had to think about how many experiments to do. In the very beginning, we wanted to vary every variable 4 times, and do 5 repetitions for each experiment. Unfortunately, we soon discovered that the quantity of molybdate we had wasn't simply enough to do so many experiments. The number of variations has then been decreased to 2 and the repetitions also to 2.

As we were working on these experiments, we soon discovered that the molybdate solution wasn't our only real problem: both of our sodium phosphate buffers were also finished, and we still had to prepare a solution at a concentration of 0.5 mM. We first tried to recreate the solution by using the salt Na_3PO_4. We managed to recreate the original concentration, but there was another important problem: pH. In fact, being Na_3PO_4 a base, the solution had a pH of 12. So we had to create a buffer that would have allowed us to obtain a value of 6.3. Fortunately, there are two simple salts which containt phosphate and of which one is an acid, the other a base. These are Na_2HPO_4 (base) and NaH_2PO_4 (acid). It wasn't hard this time to obtain a solution which was very similar to the one we had before.

To facilitate ourselves during these experiments, we mantained the procedure used during the third part of this project. We choose to do this for two main reasons: we could compare the data we obtained in that part; we had already done some experiments and we were somehow familiar with that way of working.

4.2 4A - Glucose concentration

4.2.1 Design of the experiment

This has surely been the easiest experiment to perform. In fact, it's very easy to change the concentration of the glucose in a concentration: it's enough to add it and it's done. The advantage of the glucose is that it isn't (obviously) a salt, so it doesn't change the concentration of any ion we already had in the solution. It also isn't able to change the pH of the solution, as we saw after a measurement with the pH meter. As said during the introduction, we choose two different concentrations of glucose: 3% and 6% (concentrations are expressed as mass over mass). We choose these two concentration to have somehow a significative gap between this experiment and the ones of the third part, and because 6 is the double of two. We wanted to verify wether by doubling the concentration, doubles the phosphate uptake too. Before starting to talk about the real experiment, there's still one consideration to do. 6% is a high concentration for a solid, so we were afraid that it could produce some unwanted osmotic effects that would have killed the yeast without obviously improving its phsophate uptake.

4.2.2 Data collection and processing

After having done all the work, all we had were tables of raw data which represented the absorbance of the solution in function of time.

time (min)	absorbance
0	1.397
10	1.368
20	0.630
30	0.003
40	0.003
50	0.008

Figure 5: An example of raw data we obtained while working with a 3% concentrated solution. Other ones are very similar.

Of course this kind of table is completely useless, because we want to know about concentrations of phosphate. According to the equation we obtained while working on the calibration line, we managed to calculate the concentration in our solution and these graphs represent so:

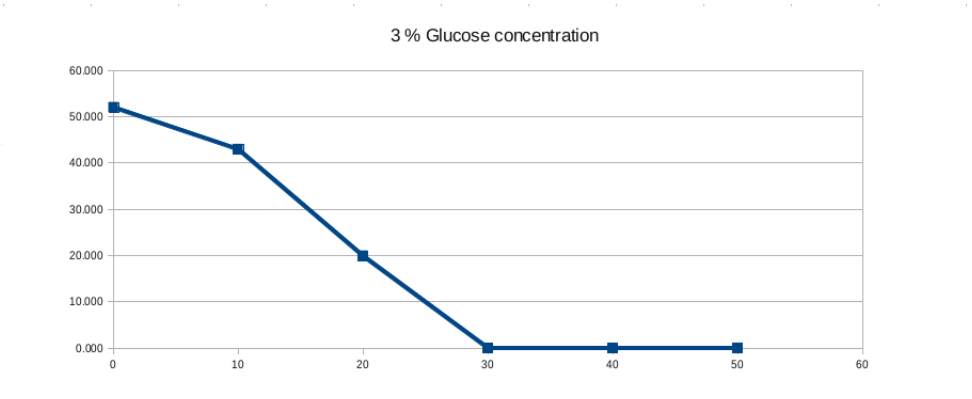

Figure 6: This is what we obtained during our experiments at 3% glucose concentration

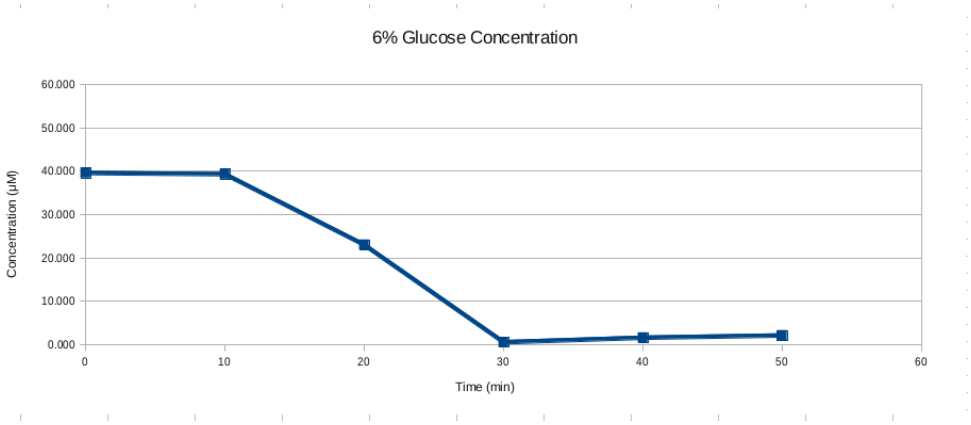

Figure 7: This is what we obtained during our experiments at 6% glucose concentration

There are two significative differences for this two graphs: the first is the starting point, the second is the concentration at the beginning. In fact, there's a strange difference of $10\mu M$, which is probably due to an human error. The starting point is also a strange thing. In fact, as the concentration increases, the yeast seems to be shocked for a while, and just after that it begins to react and uptake phosphate. As said before, it's useful to compare the slope of the descending line. In this case, the slope of the 3% graph is 1.732, and the one of the 6% (as it really starts, at 10 minutes) is 1.935.

4.2.3 Conclusion and evaluation

According to the analysed data (and especially to the slopes of our lines), we can say that, as the concentration of glucose increases, the phosphate uptake also increases. Arrived at a certain point, the yeast seems to be shocked for a while before starting the cellular respiration. This is possibily due to some osmotic processes, and we think that at higher concentrations the yeast would die without even beginning the absorption. To improve the result, we would use even higher glucose concentrations, to find what's the limit of yeast cells. Another improvement would also be the quantity of repetitions for every experiment. Unfortunately we have done only two, but with more repetitions we would have obtained more precise data and would have found better results. Since this experiment was the first we performed after the third part, it took us some time to manage all the experiments together (to gain some time), but in the end we realised a performing system that allowed us to finish all the experiments.

4.3 4B - Temperature

4.3.1 Design of the experiment

The second experiment we performed was about changing the temperature of the solution to try to increase the phosphate uptake. Since it's known that increasing the temperature also increases the metabolic activity of lots of organisms,[1] we thouht that this could have also worked for yeast cells. To perform this experiment, we used a cooker and a thermometer to control the temperature. We decided to mantain two temperatures: at 25℃ and at 29℃, since the third part has been executed at a standard temperature of 21℃.

4.3.2 Data collection and processing

The method we obtained and analyse data is the same we used for the glucose concentration. So here it is a simple table of raw data:

time (min)	absorbance
0	1.364
10	1.717
20	0.510
30	0.099
40	0.600
50	0.070

Figure 8: An example of raw data we obtained while working with a 25℃ temperature

Again, this tables don't mean nothing. So here there are our final graphs of the concentration in function of time:

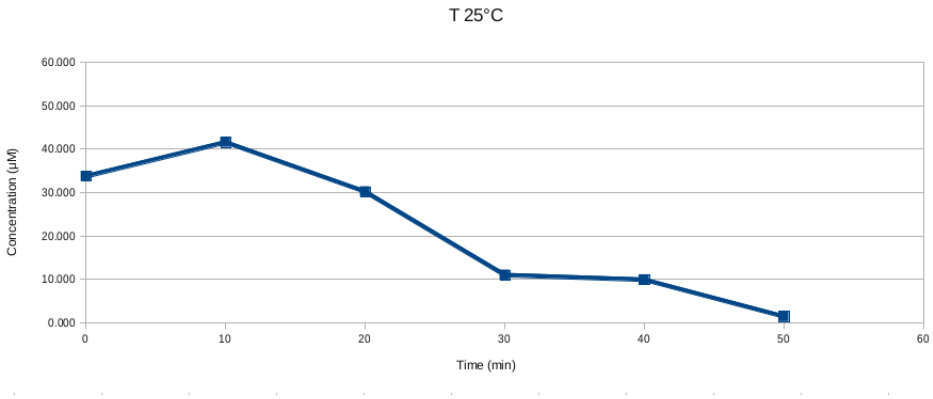

Figure 9: This is what we obtained during our experiments at 25℃

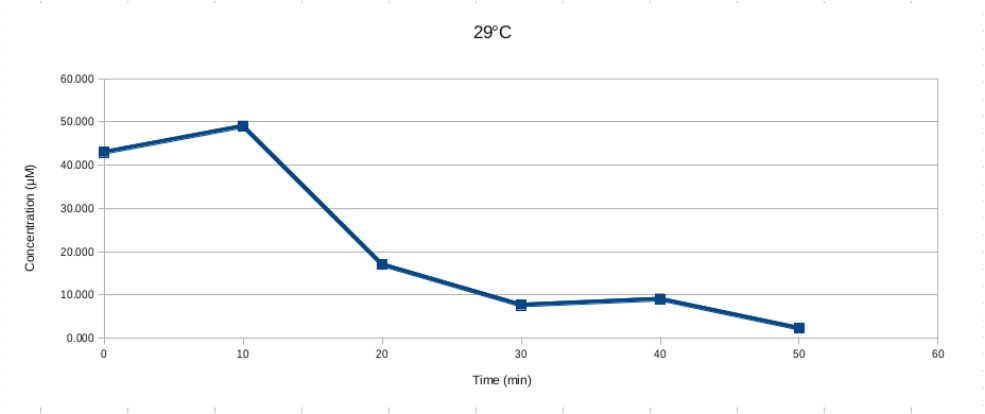

Figure 10: This is what we obtained during our experiments at 29°C

Again, the starting concentration is different for the two graphs, so we calculated the slope again, starting at 10 minutes and ending at 30 minutes for the experiment at 25°C and ending at 20 minutes for the one at 29°C. We obtained a slope of 1.526 for the first experiment and 1.599 for the second one.

4.3.3 Conclusion

It's somehow depressing that there wasn't any significant change of phosphate uptake at different temperatures. The other unexpected thing is that this slope values are minor than the ones obtained during the part 3. This means that increasing temperature decreased the phosphate uptake by yeast. We can think that yeast doesn't endure high temperatures. This would also explain the data obtained at 10 minutes, which is higher than the concentration at 0 minutes. This would mean that yeast cells didn't produce enough ATP to cover its consumption. It's also possible that yeast cells tend to die at high temperatures, and maybe the low phosphate uptake is due by this point.

4.4 4C - pH

4.4.1 Design of the experiment

This has absolutely been the hardest experiment we performed. Changing the pH isn't easy at all, and we had to think carefully before starting the work. Of course we found in the literature that a change of pH do changes the absorption of phosphate.[2] It was also obvious that a too much acid or base solution would have completely killed all of our yeast, so we decided to perform our experiments at values of 5.3 and 7.3, to also see the difference with a base and an acid. Unfortunately, the concentration of the solution used during the tests isn't high enough to change the pH by varying the concentrations of salts in the buffer. So we had to use hydrochloric acid HCl and sodium hydroxide $NaOH$. After some trials we obtained the solutions we wanted. Of course we had to think about one important thing: the sodium ions producted by sodium hydroxide could have significantly changed the starting one. Fortunately, a very minimal part of this salt was enough to produce the pH variation we needed, so we haven't took any precautions about this problem.

4.4.2 Data collection and processing

The prcedure has been the same this time too, so here it is one of our data table:

time (min)	absorbance
0	0.922
10	0.584
20	0.000
30	0.000
40	0.019
50	0.015

Figure 11: An example of raw data we obtained while working with a solution at a pH of 7.3

And now, the graphs:

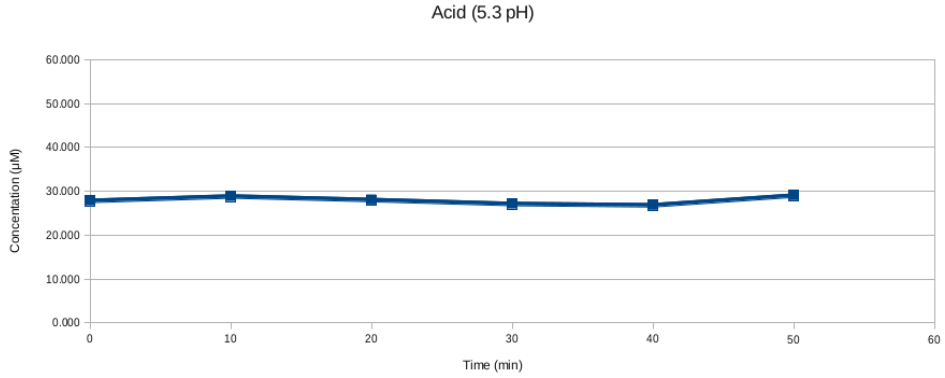

Figure 12: This is what we obtained during our experiments at a pH of 5.3

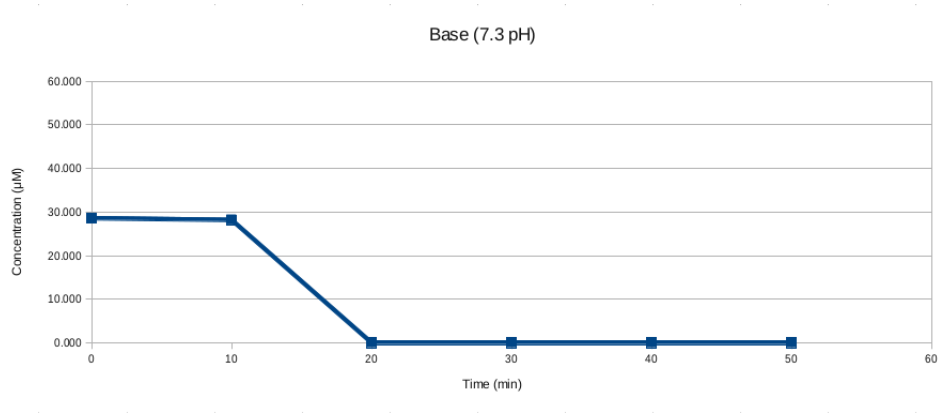

Figure 13: This is what we obtained during our experiments at a pH of 7.3

This time the starting concentration was the same, but the strange thing is the graph of the acid solution: there hasn't been any phosphate uptake by yeast cells during these experiments. Here are the slopes for our graphs: 2.820 for the basic solution and 0 for the acid one.

4.4.3 Conclusion

We were actually expecting an increase of the phosphate uptake in the base solution, beacuse we found in the literature that these solutions work better when there is also an acceptable quantity of sodium in the solution.[2] There strange thing is actually the results from the acid solution. We knew that there shouldn't have been any significant increase in phosphate uptake, but it was strange that there wasn't any uptake at all. Probably yeast cells don't

endure acid solutions and die easily, while thrive in a basic solution. However, when confronted with a change of their conditions, yeast cells seem to take a while to perform the right way in the new ambient, as seens in the basic solution.

4.5 Conclusions

Arrived at this point, we had to collect all the data we had to see which experiment increased the phosphate uptake the most. So here is our table collecting all the calculated slopes:

Base	2.820
6% glucose	1.935
Standard	1.754
3% glucose	1.732
29°C	1.6
25°C	1.526
Acid	0

Figure 14: It's easy to see which experiment performed the most when compared to the standard one

According to this table, the only successful experiments were the ones performes with a basic solution and one with a glucose concentration of 6%. However, this can be considered as a success because we were actually able to increase the phopshate uptake by yeast cells. We also discovered one another important thing: when yeast cells are put in a ambient different from the one they're used to live, they take a while to adapt themselves and react to this change. This time can be approximated to 10 minutes.

The problems we encountered were all solved but one: the shortage of given solutions. We had to drastically drop the number of experiments, and this could have caused some imprecision in our results. To improve our results, we would have liked to vary even more variables, like the concentration of sodium ions (which are used during the transport of phosphate into the yeast cell), the oxygen concentration (which is unfortunately quite hard to control), the phosphate concentration, ...

List of Figures

References

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[8] D. C. H. D. H. Jennings and A. Rothstein, "The participation of phosphate in the formation of a 'carrier' for the transport of mg^{++} and mn^{++} ions in yeast cells,"

A Activity List

	Design the experiment	Experiment	Text Writing	Photographs	Layout	
Davide Apolloni	-	-	-		-	-
Kevin Barbieri		-		-		
Noa Bernasconi	-	-	-	-		
Elena Bertolini		-				
Gaia Bianchi		-				
Manuela Bizzozero		-		-		
Clarissa Bonzanigo		-				
Cristina Broggini		-				
Lapo Carnevali		-				
Anna Citterio		-				
Mara Giovanelli		-	-	-		
Christopher Magnoli		-				
Francesca Nicoli		-				
Chiara Ortelli		-				
Sara Pellegrino		-				
Sofia Talarico	-	-		-		
Leonardo Tognola	-	-	-			

B Photos

Figure 15: Our class at work

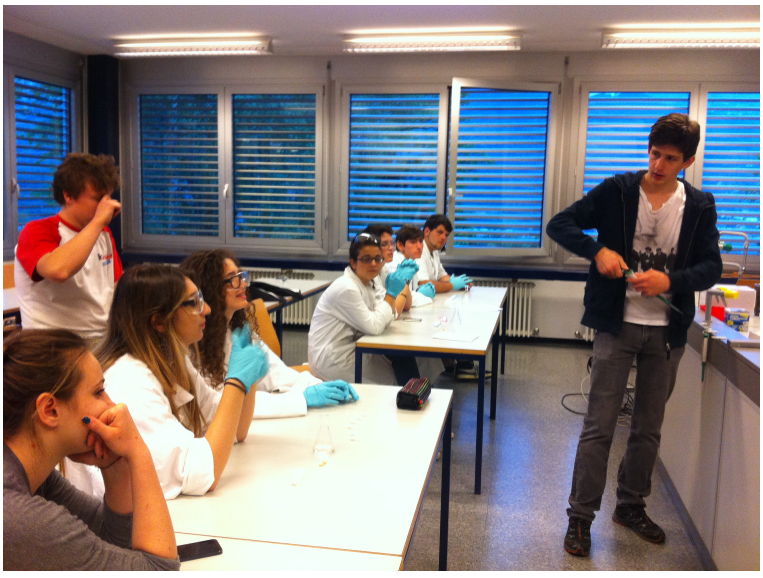

Figure 16: Our class at work