Supporting Information

New Procedure To Readily Investigate Lactase Enzymatic Activity Using Fehling's Reagent

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Contents

S1: List of chemicals and materials for the experiment

Reagents: lactose monohydrate, copper(II) sulfate, potassium sodium tartrate, sodium carbonate, sodium hydroxide, methylene blue and sodium acetate. They were bought from the Sigma Aldrich or PanReac Corporations and they were used without any other process of purification. Distilled water was used in the preparation of aqueous solutions.

Compound	CAS Number	Catalog	Amount	Price (July 2017)
lactose monohydrate	[10039-26-6]	CA0880.1000 PanReac AppliChem	1Kg	26.54€
copper(II) sulfate pentahydrate	[7758-99-8]	C8027-500G Sigma-Aldrich	0.5Kg	75.70€
potassium sodium tartrate	[6381-59-5]	S2377-500G Sigma-Aldrich	0.5Kg	36.70€
sodium carbonate	[497-19-8]	CA3900.0500 PanReac AppliChem	0.5Kg	26.68€
sodium hydroxide	[1310-73-2]	30620-1KG-M Sigma-Aldrich	1Kg	28.30€
methylene blue hydrate	[28514-100G]	28514-100G Sigma-Aldrich	100g	47.40€
sodium acetate	[127-09-3]	S7545-500G Sigma-Aldrich	0.5Kg	58.80€
Lactase	[9031-11-2] E.C.3.2.1.23	SilactFast® 4500 International Unit (UI) per pill	30 pills	8.95€

The glassware used was cleaned and dried just before the execution of the experiments. All the lab glassware used was class A equipment and it is claimed to have the following relative error:

Pipette (5 ml): ± 0.015 ml

Pipette (10 ml): ± 0.02 ml

Pipette (20 ml): ± 0.03 ml

Pipette (50 ml): ± 0.05 ml

Volumetric flask (100 ml): ± 0.1 ml

Burette: Range 50 ml, sensitivity 0.05 ml

For the weighting operation an analytical balance with a sensitivity of 0.0001 g was used.

For the heating of solution during the titrations induction heating plants were used.

For the heating of enzymatic kinetic solutions a thermoset water bath (± 1 °C) was used.

S2. Hazards

The experiment uses dilute solutions, which can be prepared in advance by laboratory assistants to minimize the risk represented by pure compounds and concentrated solutions to students' health. Labeled containers for wastes must be available. Gloves, lab coat, and safety glasses must be worn throughout the experiment. All laboratory activities must be carried out under the supervision of trained and qualified personnel. Copper (II) solution (Fehling A) may cause eye and skin irritations; it may be harmful if it is absorbed through skin, if it is swallowed or inhaled. Sodium hydroxide (Fehling B) is corrosive: it may cause eye and skin burns as well as severe damage to the digestive tract if ingested. The methylene blue hydrate is toxic if it is swallowed. The diluted acetate buffer and potassium sodium tartrate solutions used for the preparation of the reactive Fehling B have no known hazards. The heating plates (set at the temperature of 180 °C) may cause burns if accidentally touched. In order to move the Erlenmeyer flasks necessary for the titration, it is necessary to use a pair of metal pliers. The obtained solutions containing lactose, glucose and galactose could be dangerous only if ingested in high quantity.

S3. Detailed procedure of the experiment for the students:

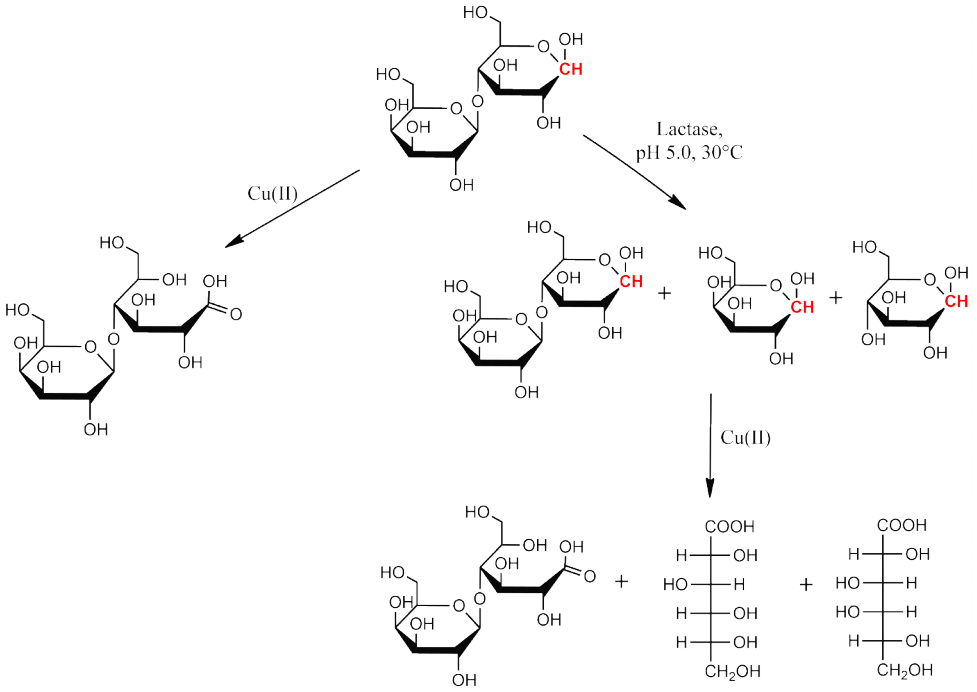
Title: Determination of K_M and V_{MAX} of lactase enzyme through redox titrations

Introduction

Lactase (E.C.3.2.1.23) is a β-galactosidase enzyme which catalyzes the hydrolysis of lactose into galactose and glucose.

This experiment is based on the observation that the number of reductive groups increases progressively during hydrolysis. Lactose presents only one reducing group, in fact only glucose within the molecule of lactose is in equilibrium with the aldehydic open-chain form. The concentration of reducing groups can be determined by titration using a basic solution of Cu(II)-ions complexed with tartrate ions with the subsequent formation of the corresponding gluconic acid and copper(I) oxide (Fehling's method). A video describing the Fehling's test can be found at https://www.youtube.com/watch?v=nDglRuHBTkw.

The number of the reducing groups in the solution changes in a different way if there is the enzyme or not (SH1). In fact the hydrolysis causes the release of glucose and galactose, giving throughout two reducing groups.



SH1: Oxidation of sugars in absence (left) and in presence (right) of lactase (reducing groups are written in red).

Endpoint of the titration is signaled because all Copper (II)-Ions are consumed and the sugars reduce the indicator giving leucomethylene blue (SH 2b, colorless) so Copper (I) oxide (red) will be visible. To consider the titration a reliable source of information, it has to occur in less than 3 minutes and the indicator is added just before the endpoint. These expedients are necessary to limit the oxygen interference and to minimize the evaporation of Fehling's solution.

SH2: Reduction of methylene blue: oxidized form (a), reduced form (b).

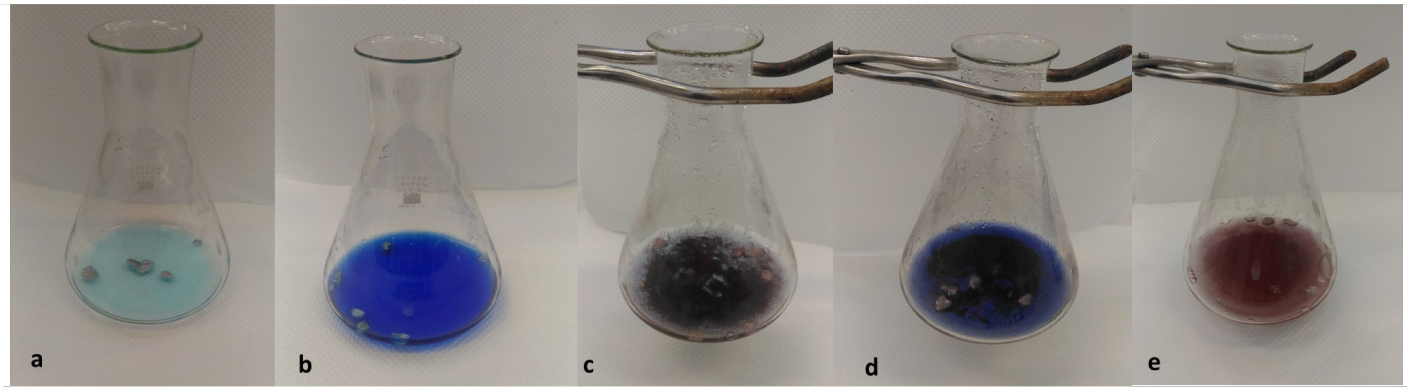
First series of titrations

The class should be divided into pairs. Each pair has to do only two titrations that will be used to build the final graph. Through the Lineweaver-Burk plot you can obtain the values of K_M and V_{MAX} of lactose. Every group has to work with a different concentration of lactose.

- In a 100 ml volumetric flask, you have to add 5.00 ml of Acetate buffer 0.50 M at pH = 5.00, the needed quantity of a lactose solution 35.0 g/l (0.102 M) and water till 100 ml. You have to put the solution in a water bath at 30°C for 15 min.
- Stop each reaction by adding 5.00 ml of a solution of Na_2CO_3 1.00 M. You have to pour this solution in a burette and to use it for titrating 20.00 ml of Fehling's solution.

General procedure for the titration

You have to use the following procedure to carry out the titrations: 10.0 ml of solution of Fehling A (6.93 g $CuSO_4 \cdot 5\ H_2O$ dissolved in distilled water and brought the volume to 1 l) and 10.0 ml of solution of Fehling B (352 g of potassium sodium tartrate (Rochelle Salt) and 154 g of sodium hydroxide in distilled water and made the solution up to 1 l) are poured in a 250 ml Erlenmeyer flask with also some pieces of pumice stone, necessary to prevent spurts during the boiling. Cu(II)-Ions are complexed by tartrate and the solution from light blue becomes deep blue. Now you have to place the Erlenmeyer flask on the heating plates (set at the temperature of 180 °C or to the "med-high" level) until the solution boils. This solution is titrated with the solution of reducing sugar (while it is boiling) until the solution turns pale red. At this point you have to add two drops of methylene blue hydrate 1% in water: that reagent is used as a redox indicator. Finally, it is necessary to titrate until the turning from blue to red (figure SH3). To consider the titration a reliable source of information, it must occur in less than 3 minutes. This titration is necessary to observe the change of color of the solution and to verify that without enzyme the hydrolysis of substrate doesn't happen.



SH3: Steps of the titration: (a) Fehling A, (b) Fehling A + B, (c) Before indicator addition, (d) After indicator addition, (e) Endpoint.

Preparation of the enzyme solution

Prepare the solution of enzyme in the following way: by grinding in a mortar 1 pill of Beta-galactosidase (from Aspergillus Oryzae, SilactFast®, 4500 International Unit (UI) per pill). Then the dust is dispersed in 20 ml of distilled water and it is centrifuged at 4000 rpm for 10 minutes. The clear solution was pipetted out and brought to a volume of 50.0 ml. It has to be kept in a refrigerator at the temperature of 10 °C.

Second series of titrations

Conduct the same experiment after the addition of 2.00 ml of the final enzyme solution to the lactose buffered solution just before making to the mark.

Waste disposal and clean-up

After the titration solutions are cold, dispose them in the properly labeled container (solutions containing metal ions). At the end of experiment, you have to wash lab glassware with distilled water.

Theoretical background

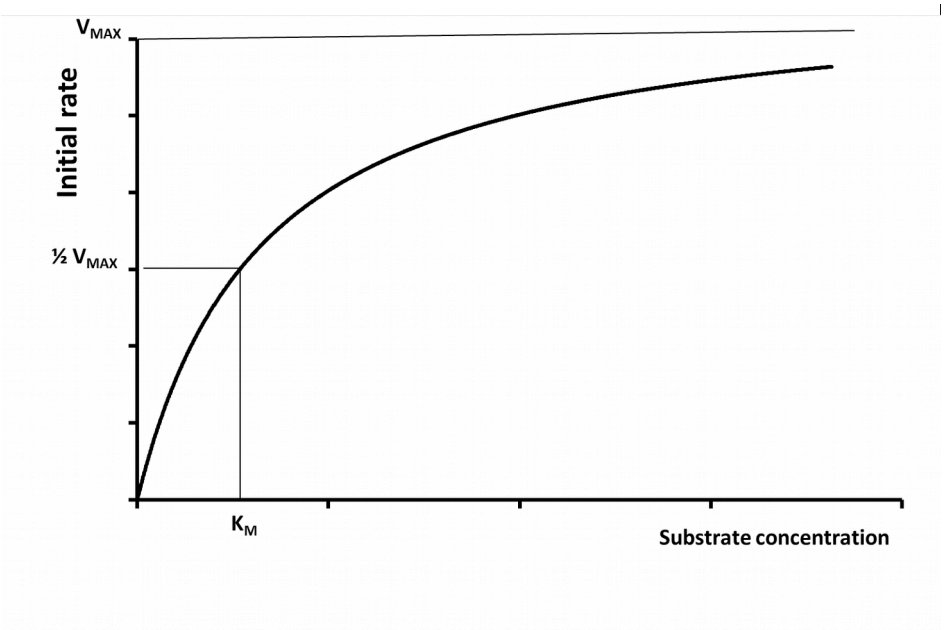
The theory of Michaelis and Menten refers to the reaction between enzyme (E) and substrate (S) to give the enzyme-substrate complex (ES) which dissociates into enzyme (E) and product (P):

$$S + E \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} ES \underset{K_{-2}}{\overset{K_{+2}}{\rightleftharpoons}} P + E \qquad (1)$$

Under steady-state conditions and saturation of the enzyme with substrate, the equation with initial rate v_o leads to

$$v_0 = \frac{V_{MAX}\,[S]}{K_M + [S]} \quad (2)$$

where V_{MAX} is the maximal initial rate that enzyme can achieve and K_M is the Michaelis constant.



It represents the initial rate correlated with the concentration of substrate in correspondence of $V_{MAX}/2$. Lineweaver and Burk suggested the double-reciprocal form:

$$\frac{1}{v_0} = \frac{K_M + [S]}{V_{MAX}\,[S]} = \frac{K_M}{V_{MAX}}\,\frac{1}{[S]} + \frac{1}{V_{MAX}} \quad (3)$$

The graph of v_o^{-1} against $[S]^{-1}$ is the Lineweaver-Burk plot. The x-axis intercept gives $-1/K_M$ and

the y-axis intercept gives the value of $1/V_{MAX}$. There are two methods in order to determine the concentration of the product [P] to obtain v_o: continuous and discontinuous. A typical discontinuous method, we also used in this experiment, is the end-point assay where [P] is measured after a fixed time.

Data Analysis

In order to verify that the reaction doesn't happen without enzyme, you need to compare the molarity of the prepared solution with the molarity obtained through following equation:

$$M_{lact} = \frac{3.94 \times 10^{-4}}{ml_{titration\ 1}} \times 1000 \times 1.05$$

This equation is obtained considering that 20.0 ml of Fehling's solution reacts with 3.94×10^{-4} mol of lactose. The correcting factor 1.05 is due to the 5.0 ml of the Na_2CO_3 solution added to quench the reaction.

After all groups have done the second titration, you have to write the obtained volumes in the fourth and fifth column of the following table.

Group	Concentration of lactose [M]	Volume of Fehling's solution [ml]	Volume of the first titration (without enzyme) [ml]	Volume of the second titration (with enzyme) [ml]	Initial rate (V_0) [mM min^{-1}]
1	0,0102	20.00			
2	0.0117	20.00			
3	0.0146	20.00			
4	0.0175	20.00			
5	0.0190	20.00			
6	0.0204	20.00			
7	0.0256	20.00			
8	0.0402	20.00			
9	0.0584	20.00			

In order to obtain the values of the initial rates, you have to calculate them through the

following equation (use the spreadsheet or a calculator):

$$V_0 = \frac{1000}{time}\left(\frac{1.24}{ml_{\ second\ titration}} - 3.00 \times M_{lactose\ sol.}\right)$$

Time: min V_0: mM min^{-1}

Through the spreadsheet, you can obtain the Lineweaver-Burk plot and the values of K_M and V_{MAX}. If you are interested in knowing how the equations have been obtained, you can read the following section.

Data analysis (depth discussion)

The quantity of lactose hydrolyzed in each titration was determined through the following system of equation:

	Lactose	→	Galactose	+	Glucose
t_0	z		0		0
t_f	y		x		x

z is the initial number of moles of lactose in the titration volume, and it is equal to:

$$z = \frac{mL_{\ second\ titration}}{1000 \times 1.05} M_{lactose\ sol.} \quad (4)$$

It is also possible to set up the following proportions and matrix in order to determine the number of moles of lactose reacted and glucose and galactose obtained:

$$3.94 \times 10^{-4} : 20ml = y : a_{ml} \quad (5a)$$

$$[(5.72 \times 10^{-4} + 6.10 \times 10^{-4}) \times 0.5\,] : 20ml = 2x : b_{ml} \qquad (5b)$$

Where a_{mL} and b_{mL} are respectively the volumes of Fehling's solution consumed by a y quantity of lactose and by a x quantity of glucose and galactose. The first member of equation 5b represents the medium value between glucose and galactose. Formula 5b is applicable because the amount of glucose and galactose in the solution is the same. This equivalence is confirmed by the stoichiometry of the reaction.

$$\begin{cases} a_{ml} = \dfrac{20y}{3.94 * 10^{-4}} = 50760\ y \\ b_{ml} = \dfrac{40x}{5.90 * 10^{-4}} = 67682x \\ a_{ml} + b_{ml} = 20 \\ z = y + x \end{cases} \qquad (6)$$

With some rearrangements it is possible to find that in the following equations:

$x = 1.18 \times 10^{-3} - 3.00\ z$ (7)

$y = -\ 1.18 \times 10^{-3} + 4.00\ z$ (8)

Replacing the equation (4) in equation (7), we have:

$$x = 1.18 \times 10^{-3} - 3.00\left(\frac{mL_{\,second\ titration}}{1000 \times 1.05}\ M_{lactose\ sol.}\right) \qquad (9)$$

The initial rate is $\Delta x/(\text{Vol} \cdot \text{time})$:

$$V_0 = x\ \frac{1000 * 1.05}{ml_{\,second\ titration} \times time} \qquad (10)$$

Replacing the equation (9) in equation (10) and simplifying we obtain this final equation:

$$V_0 = \frac{1000}{time}\left(\frac{1.24}{ml_{\,second\ titration}} - 3.00 \times M_{lactose\ sol.}\right) \qquad (11)$$

V_0: mM min^{-1}

Final questions

1) What is the K_M meaning? And V_{MAX} one?
2) Calculate how long it takes a person who suffers from lactose intolerance and who has taken a lactase pill to digest a glass of milk (230 ml, 4.7g of lactose/100 ml). Consider the enzyme in condition of saturation (K_M = 29 mM, V_{MAX} = 0.57 mM min^{-1}).
3) A kinetic enzymatic experiment was conducted at 30°C and pH 5: these are the optimal values for that enzyme. If you use a different pH, what happens to the enzyme structure? And with a different temperature?
4) To stop an enzymatic kinetic a sodium carbonate solution was added? Why?
5) Why are the values of the volumes of the second titration lower than the first one?
6) Compare the value of K_M e V_{MAX} you have obtained with the value you can find in the online database.
7) People who suffer from lactose intolerance take lactase before meals. Compare the value of K_M obtained during this experiment with the concentration of lactose in milk.
8) What is the role of the Fehling B solution? What happens if there isn't?
9) Methylene blue is the redox indicator we used. You have to search the structure of its reduced form. Why is it colorless?
10) You have to search for information about Fehling's method and about its use in the laboratory.

Instructor Information

New Procedure To Readily Investigate Lactase Enzymatic Activity Using Fehling's Reagent

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II 1. Data analysis

In the determination of the quantity of lactose hydrolyzed it is important to keep in mind that 20 ml of Fehling's reagent (10 ml A + 10 ml B) reacts with 3.94×10^{-4} mol of lactose, 5.72×10^{-4} mol of glucose and 6.10×10^{-4} mol of galactose.[1-3]

The quantity of lactose hydrolyzed in each titration was determined through the following system of equation:

	Lactose	→	Galactose	+	Glucose
t_0	z		0		0
t_f	y		x		x

z is the initial number of moles of lactose in the titration volume, and it is equal to:

$$z = \frac{mL_{\ second\ titration}}{1000 \times 1.05} M_{lactose\ sol.} \qquad (1)$$

The correcting factor 1.05 is due to the 5.0 ml of the Na_2CO_3 solution added to quench the reaction.

It is also possible to set up the following proportions and matrix in order to determine the number of moles of lactose reacted and glucose/galactose obtained:

$$3.94 \times 10^{-4} : 20\ ml = y : a_{ml} \qquad (2a)$$

$$[(5.72 \times 10^{-4} + 6.10 \times 10^{-4}) \times 0.5] : 20\ ml = 2x : b_{ml} \qquad (2b)$$

Where a_{mL} and b_{mL} are respectively the volumes of Fehling's solution consumed by a y quantity of lactose and by a x quantity of glucose and galactose. The first member of equation 2b represents the medium value between glucose and galactose. Formula 2b is applicable because the amount of glucose and galactose in the solution is the same. This equivalence is confirmed by the stoichiometry of the reaction.

$$\begin{cases} a_{ml} = \dfrac{20y}{3.94 \times 10^{-4}} = 50760\ y \\ b_{ml} = \dfrac{40x}{5.91 \times 10^{-4}} = 67680\ x \\ a_{ml} + b_{ml} = 20 \\ z = y + x \end{cases} \qquad (3)$$

With some rearrangements, it is possible to find the following equations:

$x = 1.18 \times 10^{-3} - 3.00\ z$ (4)

$y = - 1.18 \times 10^{-3} + 4.00\ z$ (5)

Replacing the equation (1) in equation (4), we have:

$$x = 1.18 \times 10^{-3} - 3.00\left(\frac{mL_{\ second\ titration}}{1000 \times 1.05} M_{lactose\ sol.}\right) \qquad (6)$$

The initial rate is Δx/(Vol · time):

$$V_0 = \mathrm{x}\frac{1000 \times 1.05}{ml_{\ second\ titration} \times time} \qquad (7)$$

Replacing the equation (6) in equation (7) and simplifying we obtain these final equations:

$$V_0 = \frac{1000}{time}\left(\frac{1.24}{ml_{\,second\ titration}} - 3.00 \times M_{lactose\ sol.}\right) \qquad (8)$$

$$V_0 = \frac{1000}{15}\left(\frac{1.24}{ml_{\,second\ titration}} - 3.00 \times M_{lactose\ sol.}\right) \qquad (9)$$

This formula can be pre-included in a spreadsheet or used with a calculator and it provides the initial rate in mM min^{-1}.

To point out the trend of expected data, the experiment has been simulated with a 0.02 M substrate concentration (Figure II1).

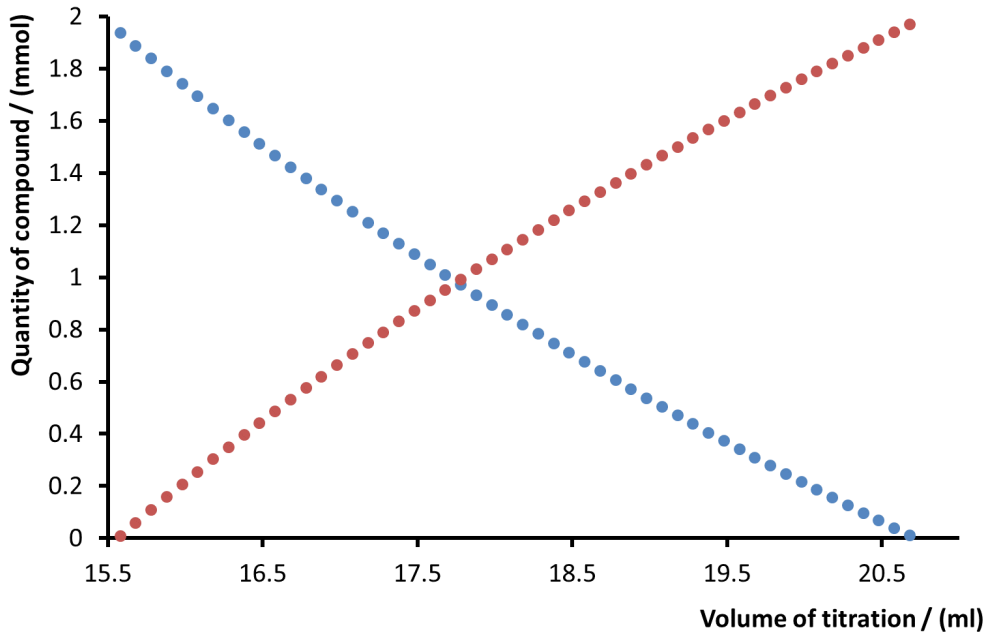

Figure II1: Simulation, using the previous equations, of the trend of the quantity of lactose (red points) and of glucose (blue points) with the volume used in the titration ($M_{lactose}$: 0.02 M).

In figure II2 it is possible to visualize the expected difference between the volumes of the two titrations. It is very easy to observe that the higher the lactose concentrations are, the lower the differences between the volumes will be. Nevertheless, also when the used concentration is at the maximum value (0.051 M), the error is little respect to the difference between the volumes of the two measures (See error bars in the insert of Figure II2).

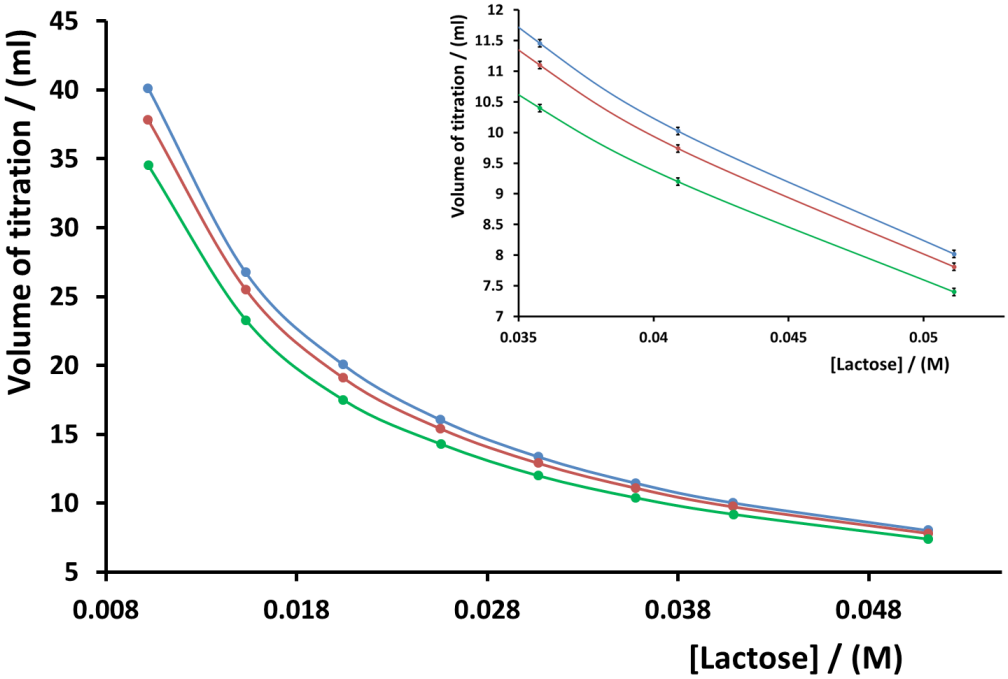

Figure II2: Expected values of titrations without enzyme (blue points) and with enzyme (3 pills green points, 1 pill red points) in function of lactose concentration.

II 2. Concentrations and students' results

The data obtained through the students' experiments, used for the rate of lactose hydrolysis and Lineweaver–Burk plot, are reported in this table:

Class 1:

Group	Concentration of lactose [M] (±0.0001)	Volume of Fehling's solution [ml] (±0.03)	Volume of the first titration (without enzyme) [ml] (±0.05)	Volume of the second titration (with enzyme) [ml] (±0.05)	Initial rate [mM min^{-1}]
1	0,0102	20.00	40.50	37.80	0.15 ± 0.01
2	0.0153	20.00	27.05	25.50	0.18 ± 0.01
3	0.0180	20.00	23.00	21.70	0.21 ± 0.02
4	0.0205	20.00	20.25	19.10	0.23 ± 0.03
5	0.0256	20.00	16.20	15.40	0.25 ± 0,02

6	0.0307	20.00	13.50	12.90	0.27 ± 0.03
7	0.0358	20.00	11.60	11.10	0.29 ± 0.04
8	0.0409	20.00	10.15	9.74	0.31 ± 0.05
9	0.0511	20.00	8.10	7.81	0.37 ± 0.06

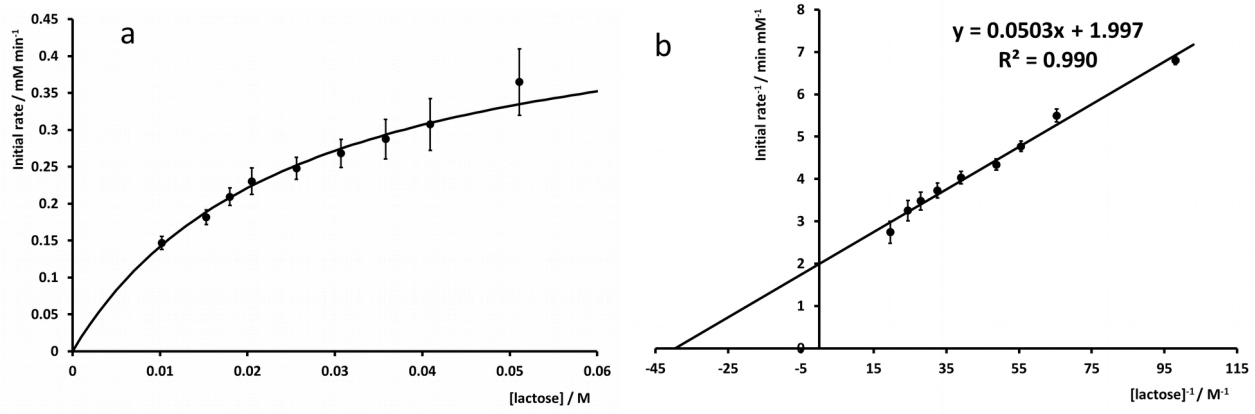

Figure II3: Rate of lactose hydrolysis (a) and Lineweaver–Burk plot (b) (T = 30°C, pH 5.0).

In this experiment the kinetic parameters obtained were respectively K_M = 25 ± 3 mM and V_{MAX} = 0.50 ± 0.05 mM min^{-1}.

II 3. Other considerations

- After the addition of the basic solution of Sodium Carbonate, the final pH measured was of 10.95. At this value of pH the activity of the enyzme is almost negligible.[3]

- If the solution that contains sugars is put too quickly in the burette it forms little bubbles. Therefore, to solve this problem and to facilitate the reading, it is necessary to remove them before starting the titration. It is easy to do it using a Pasteur pipette.

- Prepared Fehling A and B solutions are also commercially available. In this case, the experiment will be a little more expensive.

- The titration with Fehling's reagent is very accurate and precise but it is necessay a good manual skill. It's commonly used in the analytical laboratory for determining reducing sugars contained in fruits and derivates.

- The best range of lactose concentration is about 0.01 to 0.08 M. A lower concentration causes a higher volume titration that cannot exceed 50 ml. If the concentration is greater, we observe a saturation of the solutions.

- Another additional experiment that may be done by students is following the behavior of the complete kinetic course. In order to obtain this goal it is necessary to stop the reaction at different times.

- Tablets contain also cellulose, sorbitol, silica and magnesium stearate. Through centrifugation, insoluble silica and cellulose are separated. Sorbitol and magnesium stearate do not interfere with the titration because they are not reducing.

- The procedure was thought to give a lactose conversion of 10 % with the aim of maximizing the difference between the volumes of the titrations. It was obtained an averaged conversion of 9 %. For decreasing the error and increasing the gap between the titrations it is necessary to augment the incubation time or the amount of enzyme. It was demonstrated through an experiment with 3 pills instead of one. In this case, the averaged conversion was of 17% and the kinetic parameters were similar to the values previously obtained (see II 6). We suggest this experiment for obtaining a better correlated points.

- There are several procedures to conduct Fehling's titration. In the majority of cases, the indicator is added just before the endpoint and the titration has to be completed in less than 3 minutes. These expedients are necessary to limit the oxygen interference and to minimize the evaporation of Fehling's solution.

II 4: Students' assessment

Student's improvement was evaluated observing them during the activity and through a conclusive text that concerns enzymatic kinetic. It was noticed that in the second titration the students' skills were augmented, mostly in the use of pipettes and burettes. The final test results (questions 1,2,3,4 of the Supporting Information) were compared with the results of other two classes that did not carry out the experiment: the average of the marks raised from

6.1 to 6.7 in a “1 to 10” range (Figure II4).

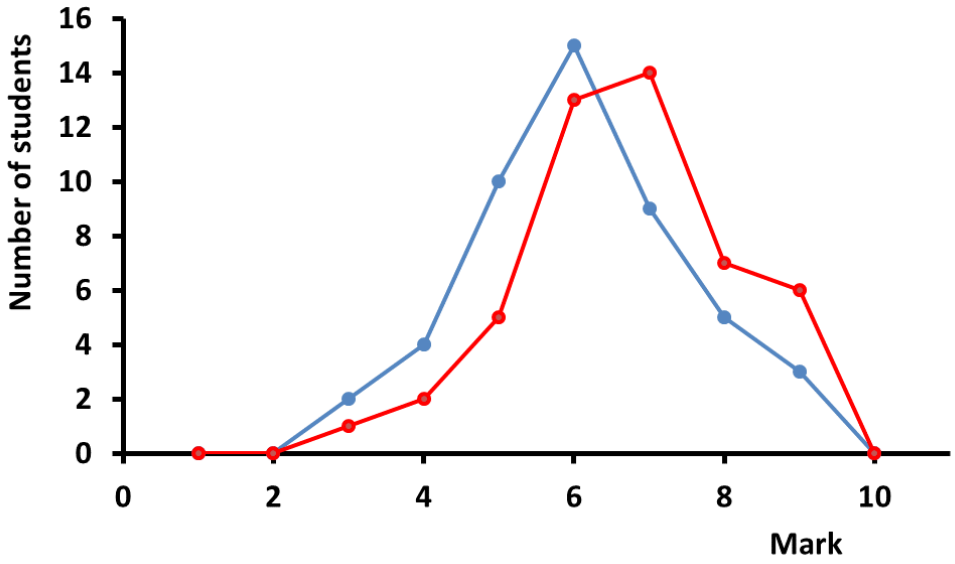

Figure II4: Results of the students’ final test. With (red) and without (blue) carried out the experiment.

In figure II5 the results of the other questions are reported. Obviously these were obtained by the class that had carried out the experiment.

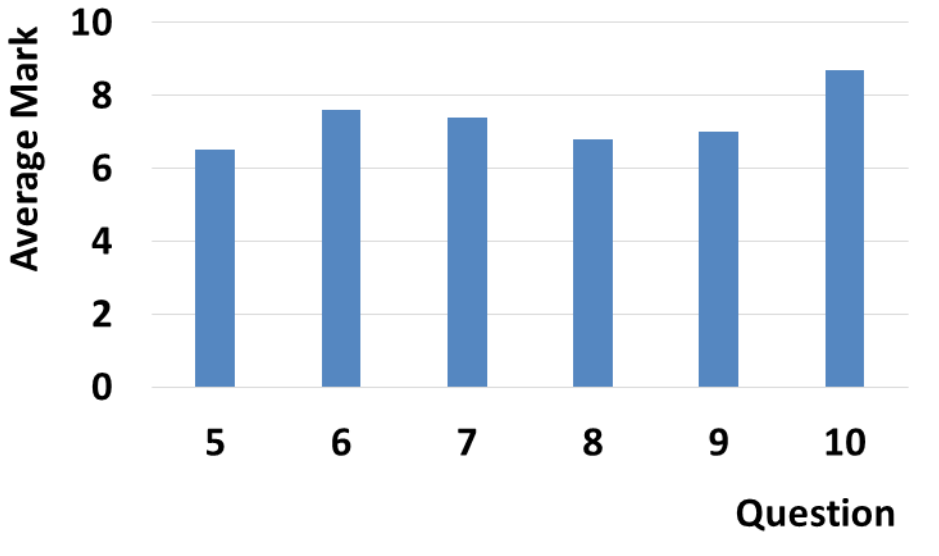

Figure II5: Average results of the students’ final test (questions 5-10).

II 5: Results with more enzyme (3 pills)

Class 2:

Group	Concentration of lactose [M] (±0.0001)	Volume of Fehling's solution [ml] (±0.03)	Volume of the first titration (without enzyme) [ml] (±0.05)	Volume of the second titration (with enzyme) [ml] (±0.05)	Initial rate [mM min^{-1}]
1	0,0102	20.00	40.50	34.52	0.35 ± 0.02
2	0.0153	20.00	27.05	23.30	0.49 ± 0.03
3	0.0205	20.00	20.25	17.50	0.62 ± 0.04
4	0.0256	20.00	16.20	14.30	0.66 ± 0.04
5	0.0307	20.00	13.50	12.00	0.75 ± 0,05
6	0.0358	20.00	11.60	10.40	0.79 ± 0.05
7	0.0409	20.00	10.15	9.20	0.81 ± 0.06
8	0.0511	20.00	8.10	7.40	0.95 ± 0.07
9	0.0614	20.00	6.75	6.20	1.1 ± 0.1

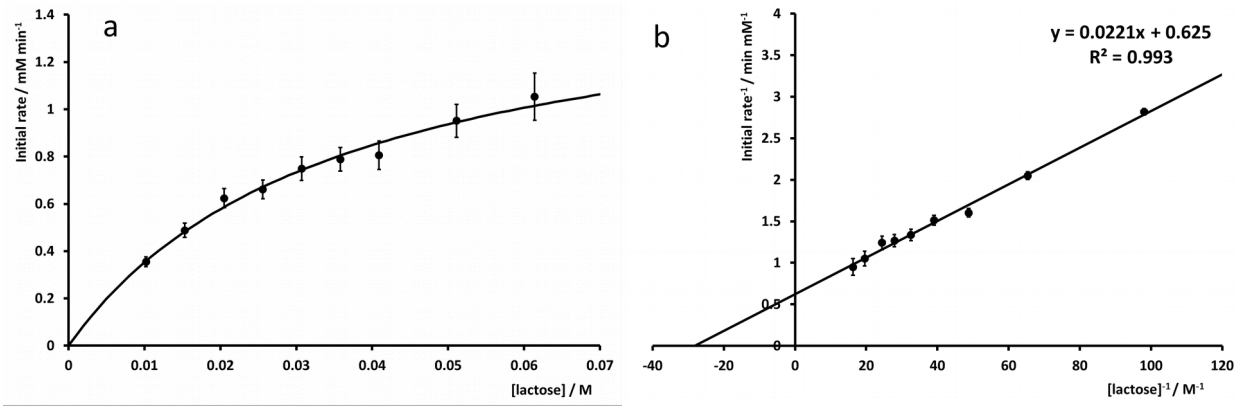

Figure II6: Rate of lactose hydrolysis (a) and Lineweaver–Burk plot (b) (T = 30°C, pH 5.0).

In this experiment the kinetic parameters obtained were respectively K_M = 35 ± 3 mM and V_{MAX} = 1.6 ± 0.2 mM min^{-1}.

Class 3:

Group	Concentration of lactose	Volume of Fehling's	Volume of the first titration	Volume of the second titration	Initial rate [mM min^{-1}]

	[M] (±0.0001)	solution [ml] (±0.03)	(without enzyme) [ml] (±0.05)	(with enzyme) [ml] (±0.05)	
1	0,0102	20.00	40.50	35.53	0.29 ± 0.02
2	0.0153	20.00	27.05	23.80	0.41 ± 0.03
3	0.0205	20.00	20.25	18.09	0.47 ± 0.04
4	0.0256	20.00	16.20	14.55	0.56 ± 0.05
5	0.0307	20.00	13.50	12.23	0.62 ± 0,05
6	0.0358	20.00	11.60	10.55	0.68 ± 0.06
7	0.0409	20.00	10.15	9.28	0.73 ± 0.07
8	0.0511	20.00	8.10	7.45	0.88 ± 0.07
9	0.0614	20.00	6.75	6.25	0.95 ± 0.08

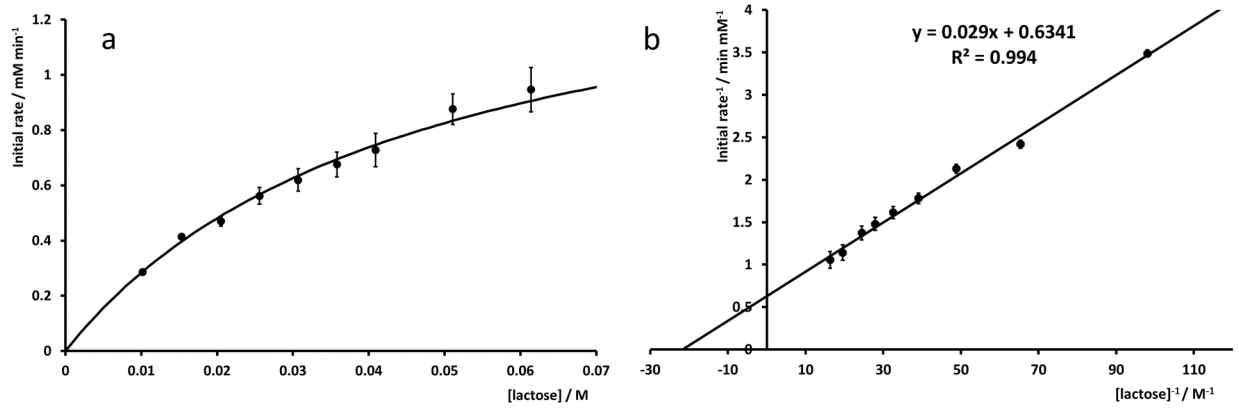

Figure II7: Rate of lactose hydrolysis (a) and Lineweaver–Burk plot (b) (T = 30°C, pH 5.0).

In this experiment the kinetic parameters obtained were respectively K_M = 46 ± 3 mM and V_{MAX} = 1.6 ± 0.2 mM min^{-1}.

II 6. Evaluation grid for the experiment

Evaluated skills **Competence in:**	**Observed behavior**	**Score**

Working in team	• Listening to classmates • Discussing in a quiet way	1	
Following the instructions	• Reading the procedure • Carrying out the procedure without “skips”	1	
Assembling the glassware	• Recognizing the glassware • Assembling the glassware in the right way	1	
Making measurements	• Choosing the right unit of measurements • Writing correctly the result	2	
Making calculations	• Not making mistakes • Rounding up correctly • Indicating the right number of significant digits	3	
Constructing graphs	• Collecting data neatly • Opportunely choosing independent and dependent variables • Showing the unit of measurements • Representing data in the right way	2	
		10	

II 7. References

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4. Tanaka, Y.; Kagamiishi, A.; Kiuchi, A.; Horiuchi, H. Purification and Properties of β-Galactosidase from *Aspergillus oryzae*. *J. Biochem.* **1975**, *77*, 241-247.