IB BIOLOGY

Internal Assessment

For the International Baccalaureate Diploma

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TABLE OF CONTENTS

PART I: THE BIOLOGY IA GUIDE.....9

1. GENERAL INTRODUCTION.....10

2. FINDING A GREAT TOPIC.....12

3. PERSONAL ENGAGEMENT.....14

4. EXPLORATION.....14

5. ANALYSIS.....19

6. EVALUATION.....29

7. COMMUNICATION.....31

PART II: SEVEN EXAMPLES OF EXCELLENT INTERNAL ASSESSMENT.....33

1. THE EFFECT OF VARYING INITIAL CONCENTRATIONS (M) OF COPPER (II) SULFATE SOLUTION ON THE CHANGE IN CONCENTRATION OF THE FINAL SOLUTION.....35

2. THE EFFECT OF DIFFERENT ELECTRICAL VOLTAGES ON THE GROWTH OF VIGNA RADIATA.....49

3. CHANGING LIGHT INTENSITIES AND THE EFFECT ON THE PHOTOSYNTHETIC RATE MEASURED USING A COLORIMETER IN ABSORBANCE UNITS (AU) USING ALGAE BALLS IN SEA LETTUCE.....63

4. THE EFFECT OF ETHANOL CONCENTRATION ON THE HEART RATE OF DAPHNIA MAGNA.....77

5. THE EFFECT OF E-CIGARETTE USE ON THE HUMAN.....91

SALIVARY A-AMYLASE ACTIVITY MEASURED THROUGH THE SPECTROPHOTOMETRIC ABSORBANCE OF MALTOSE.....91

6. TO WHAT EXTENT DOES THE SAP OF THE HERACLEUM MANTEGAZZIANUM CAUSE BURN MARKS (CM2) ON THE LEAVES OF FIVE DIFFERENT PLANT TYPES.....105

7. WHAT IS THE EFFECT OF PH CONCENTRATIONS ON THE EFFICIENCY OF IMMOBILIZED LACTASE? 119

PART I

THE BIOLOGY IA GUIDE

1. GENERAL INTRODUCTION

The Internal Assessment in Biology is a personal research that comprises the 21st century skills in Science; research skills, thinking and communication skills, analytical skills as well as self-management and time-management. As a component, it weighs 20% of the total grade, it is externally graded but it is, initially, moderated internally by the teacher.

Biology is a Life Science, which allows you to be a risk taker and inquisitive. Hence, you can carry out any type of investigation, even beyond the syllabus, given does not challenge ethical or environmental issues.

Tip: Going beyond the syllabus shows serendipity and courage but you must be careful not to choose a topic that demands skills you have not acquired or a method that is too complex to be carried out in a school setting. IB allows students to carry out an investigation in an external lab such as university lab, as long as there is validation and proof.

1.1 The Criteria of Internal Assessment

There are 5 criteria that are tabulated below. The approach in overall marking is the best-fit approach, which means that the moderators will mark positively to what you have achieved overall. The total number of marks is 24.

Criterion and marks	What is marked?
Personal Engagement (PE) – 2 marks	Your curiosity, your input and insight in the topic, your creativity or serendipity. It is marked by the whole investigation, not just the introduction
Exploration (Ex) – 6 marks	The background theory, the research question, the method, the variables and their manipulation.
Analysis (A) – 6 marks	The presentation of raw data, the manipulation of numbers and significant figures, dealing with uncertainty, graphical

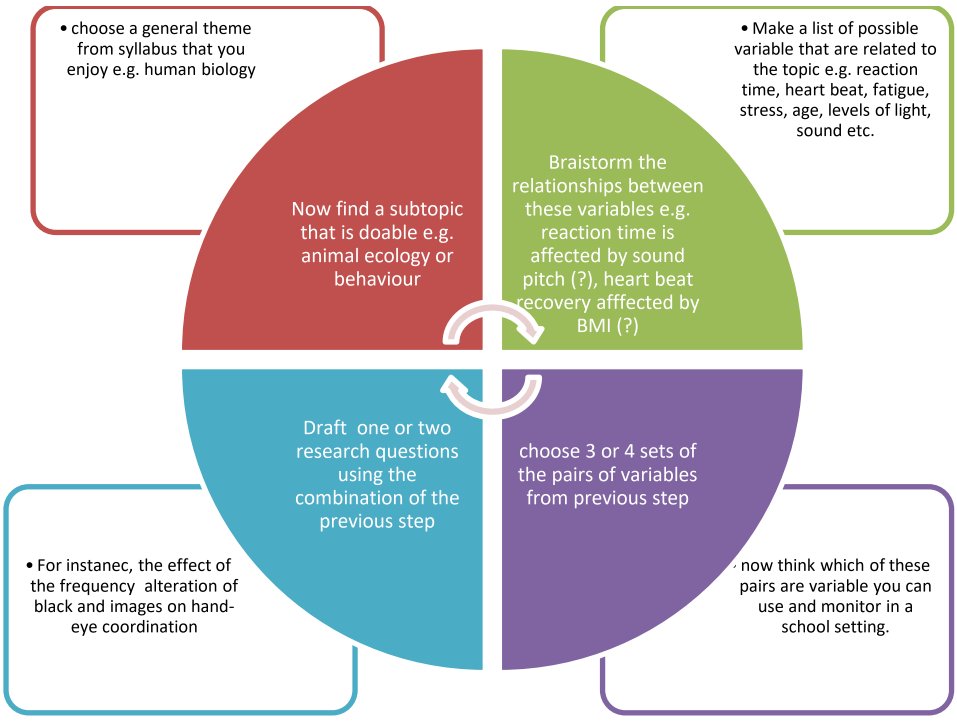
	analysis, data processing, statistical processing, interpretation of data.
Evaluation (Ev) – 6 marks	This criterion comprises the Conclusion, addressing strengths and limitations, suggesting viable improvements and future extensions.
Communication (C) – 4 marks	The general flow and coherence of your report, the clarity and presentation of the graphs and tables, the use of scientific terminology, proper referencing etc.

1.2 The layout of the IA report

The IA report should be written in either 11 or 12 font size. Make sure you keep the spacing between 1.15 and 2.0. Avoid making it colorful as this is not graded, and it may be not likeable by the moderator. The report should not exceed the 12 pages, although 13 pages may be acceptable if page 13 contains part of the bibliography. Generally, avoid, too densely written text. As for the Appendix, this part is never checked or graded by the moderator, so it is not a good idea to put important data there. However, if you have lengthy raw data tables, you may keep some sample tables in the main body and add the rest in an appendix; in this case the teacher must point out in the comment of your report, prior to submission, that they have checked your appendix.

2. FINDING A GREAT TOPIC

This is probably the most exciting part of your investigation. However, it can be overwhelming and lead to either a very complex or a very simplistic research question. So, here are the steps you are advised to take to find a topic that excites you and will lead to a meaningful research question:



TIP: If your investigation involves human subjects, you must obtain a consent form. If your investigation involves animals (including arthropods), then make sure your method abides by the protocol of ethical treatment of animals as laid out by IB.

2.1 Choosing the Source Of Raw Data

A vital factor that may affect the choice of topic and research question is how you are planning to collect raw data. There are two types of data collection: primary data collection and secondary data collection.

i. Primary data collection

Data is collected with an actual, hands-on experiment in the lab or a field, or from a simulation. Here are great resources:

https://www.wolfram.com/system-modeler/libraries/high-school-biology/?src=google&458&gclid=Cj0KCQiA1KiBBhCcARIsAPWqoSqVc-pvfE7oElp_v7N0G_PXGp2ypiVh6IHPEdOJQKxCdtSNYlqrKlQaAvVzEALw_wcB

https://phet.colorado.edu/en/simulations/filter?subjects=biology&type=html

https://i-biology.net/ict-in-ib-biology/modeling-simulation/

https://www.sciencecourseware.org/FlyLabJS/

ii. Secondary data collection

Data is collected from a data base. It is advisable you receive feedback from your teacher as to which data base will provide sufficient data for a focused research question:

DNA Data Bank of Japan

UniGene

Explore worm Biology

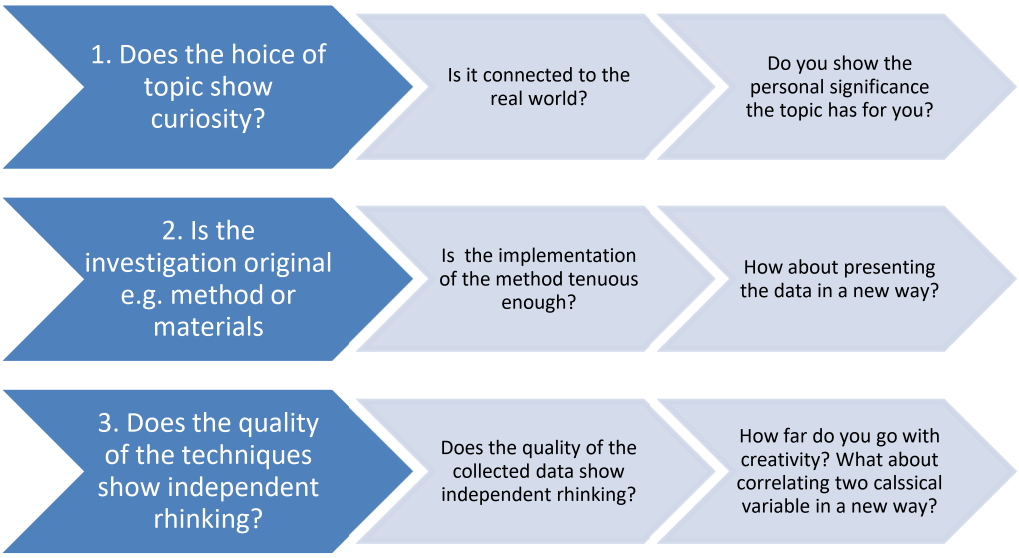
Genomes, Genome features and maps

Protein Data Bank

http://www.rcsb.org

3. PERSONAL ENGAGEMENT

The criterion of PE applies holistically to the whole body of the lab report. The areas that are assessed are seen below:



Tip: It is advisable that you write a clear statement of the purpose of the investigation. For instance:

The concerning accumulation of painkillers in waste water, such as ibuprofenoic acid, inspired me to investigate the effect of this drug on the growth of Phaseolus vulgaris.

4. EXPLORATION

The criterion of exploration consists of the following components: the background theory, the research question, the variables, the risk assessment and acknowledgment of limitations.

4.1 The background theory

The background information provided can enhance significantly your report as long as it is focused on the research question, it has plenty of references and it is not too general or simplistic. Normally, a well written background theory should take up 1.5 to 2 pages.

2. THE EFFECT OF DIFFERENT ELECTRICAL VOLTAGES ON THE GROWTH OF VIGNA RADIATA

Author: Bui Philong
Moderated Mark: 22/24

The effect of different electrical voltages on the growth of *Vigna radiata*

Background

According to Collins dictionary (n.d.), electro culture is the practice of using electricity in agriculture in order to stimulate plant growth. Plants do not have a nervous system for electricity to pass through; however, electricity affect its growth and biological properties (Nytimes.com, 1985). There have been studies showing how manipulating electric current around plants, could boost their growth. This could help in agriculture preferences, as an alternative to using fertilizers or when a growing season is limited, to boost the growth of crops. Electrical charges are used as transport of materials in and out of cells, and they further regulate metabolism in cells (Jeanty, n.d.). The increase in metabolism would increase the ion pumping transport of hydrogen ions inside the cells increasing the nutrition value of the plant, additionally, the electricity would also affect and increase the water uptake of plants (What-when-how, n.d.). The flow of electrical surges passing through the plant has the potential of manipulating and stimulating the increase in the nutritional uptake for plants, and thus, affecting the growth of plants. This experiment would expose the plant to different electrical voltages.

Statement of purpose

I was intrigued to do this experiment after I saw my dad leaving a charger by accident in a pot of plants. I was curious how the electrical stimulation could have affected the plants. It is quite common for outdoor wires to be damaged exposing their bare-naked electrical cables to the ground. Furthermore, the bare wires could send electrical surges to the soil affecting the plants around it. As the world is developing with technological advances and increasing electrical wires filling the planet, this research could convey on how bare electrical wires in the industrial world would affect the plants or in the practice of electro culture to help in agriculture growth.

Figure 1. Complex apparatus

A complex apparatus had to be made for the experiment, as seen in Figure 1, involving a parallel circuit around each group of plants with two electrodes inserted into the soil of each pot. Direct current (DC), an electrical charge would flow from one rode into the soil and plant and then back into the second rode. This would allow the soil to get electrically charged and potentially allow the plants to be exposed to electrical surges with different voltages. For the experiment, *Vigna radiata* (also known as mung bean), from the family of Leguminosae (bean plants family) has been selected due to the fast-growing natural properties of the plant. The experiment would use pre-germinated seeds, which ensures that all the plant is alive by imbibing the seeds in water, to allow absorption of liquid and trigger the seeds germination before the experiment.

Additionally, ammonium sulfate, $(NH_4)_2SO_4$, would be added to the soil, which will act as a fertilizer, but mainly used to allow electricity to conduct through the soil. As growth is described as the increase in size, the height and mass would be measured. Finally, as it is unsure whether the electrical stimulation would make plants grow by cell division through more nutrition or just an increase in water uptake; the wet mass, and dry mass would be measured to check the water content of the plants.

Aim

This biology experiment aims to investigate the effects of different electrical voltages in soil on pregerminated seeds of *V. radiata* by measuring its growth through the height and the final mass of the plant.

Research Question

How does different electrical voltage (3V, 6V, 9V, 12V, 16V) affect the growth of pre-germinated seeds of *Vigna radiata* measured through their average height (mm), dry mass (mg) and wet mass (mg) over 20-day period experiment?

Hypothesis

Electrical voltage will successfully help boost the growth of *V. radiata* up to an optimum level. It will help boost the plants uptake of water and increase the metabolism allowing the plants to grow at a faster rate through their height and increase in mass (Jeanty, n.d.).

Prediction
It is predicted that up to an electrical voltage, the growth of the plant would be the highest with the highest average height and mass (higher than control group), however, higher than the optimum voltage level, the vitality of the plant will decrease as the heat from the electricity would kill and stunt the plant growth decreasing its growth.

Variables

Table 1: Groups for the experiment

Experimental Groups	3V, 6V, 9V, 12V, 16V	Each group with 18 seeds would have the same conditions, but only the voltage of the electrical surge would be manipulated. There is an increase of 3V per each group, to see the effect of increasing voltage per plant.
Control Groups	0V	Group 0V would have the same conditions as the experimental group, except it will not be exposed to any electrical surge. This would be the control group for the experiment.

Table 2: Independent and Dependent Variables

Independent variable	Different level of voltages passing through the soil: 3V, 6V, 9V, 12V, and 16V	This was controlled with a power supply by turning the voltage switch. Model "MCP-m10-sp303e-DC" and "Frederiksen-DC" power supply was used. Each pot in each group was ensured the same voltage due to the parallel circuit and nails attached to the pots.
Dependent variable	The growth of *V. radiata* measured through: -Height every 2 days (mm) -Wet mass (mg) measured on day 20 -Dry mass (mg) measured on day 20	The height was measured using the same ruler for the whole experiment with a ± 5mm uncertainty. The mass was weight with the same digital scale all the time with a ±100mg uncertainty.

Table 3: Controlled variables and method of control

	Reason for it to be controlled	**Method of control**
Soil	Soil provides adequate nutrients and foundation base for the plants to grow in optimal conditions.	All the pots received the same amount of soil of 130g (weight on a digital scale) from the same company which provided 100% natural organic soil for growing crops.
Water content during watering [ml]	To make sure that all the pots receive an equal amount of water, as water it one of the factors which affect plant growth	With a measuring beaker, 20ml of water was measured and poured in every pot
Temperature [°C]	Temperature is a factor affecting enzyme activity. It is also a limiting factor, as after an optimum level, it decreases the rate of photosynthesis of *V. Radiata*	All the plants were arranged and placed in the same closed room with a air-conditioning which regulated the temperature so they were all exposed to the same temperature of 23-25°C. It was monitored using the air conditioning.
Humidity	Water vapor affects the transpiration and respiration of plants, by opening the stomata (Polygon, n.d.)	All the plants were arranged in the same enclosed room with the air-condition regulating and measuring the same humidity rate of 40% RH. This ensured that all the plants would be surrounded with the same humidity rate equally.

Pre-germinated seeds	With the seeds already pre-germinated in the experiment, this would ensure that the seeds are in a vital state and growing before being exposed to the treatment.	In order to make all the seeds germinated, they were imbibed in a bowl of water. After that, visually selecting the best seeds with a similar growing radicle ensured that the seeds are alive and starting to grow at the same time period.
Sunlight [lux]	Sunlight intensity affects the Carbon dioxide uptake, and thus the rate of photosynthesis	The plants were positioned in an enclosed balcony, where the walls and roof were made of transparent glass. This allowed the sunlight to spread equally among the plants without shadows caused by concrete walls. This was monitored with a smartphone app "light meter" which ensured that all pots received the same sunlight, measured in Lux
Ammonium Sulfate [g]	The salt concentration of $(NH_4)_2SO_4$ is a fertilizer for plants. They would support the components of the plant to help them grow (Mosaic Crop Nutrition, 2018).	In order to ensure all the plants received the same amount of fertilizer, the water-salt mixture was poured from one bucket containing 36g of Ammonium Sulfate allowing equal spread and then mixed. It was later poured to every pot equally.

Apparatus

Table 4: Materials needed for the experiment

Materials			
Equipment	**Amount**		**Amount**
Bucket 5L	1x	Pots 10cm diameter	36
Forceps	1x	Trays for pots	7
Scissors	1x	Nails 7cm	60x
Digital Scale [± 0.01g]	1x	Wires with crocodile endings	60x
Digital Multimeter (200m)	1x	Power Supply (Frederiksen-DC) [± 0.1V]	1x
Ruler [±5mm]	1x	Power Supply (MCP-m10-sp303e-DC) [± 0.1V]	1x
Transparent bowl	1x	Beaker 50ml [± 0.1ml]	1x
Paper towel	1x	Spatula	1x
Oven	1x	Permanent Marker	1x
Baking paper	1x	Table Spoon 15ml	1x
Substances			
	Amount		**Amount**
Ammonium Sulfate Pure (Chempur)	30g	Tap Water	18.85L
Universal Soil (COMPO BIO)	5kg	Mung Beans (PNOS)	300x

Figure 2.1 Apparatus set-up

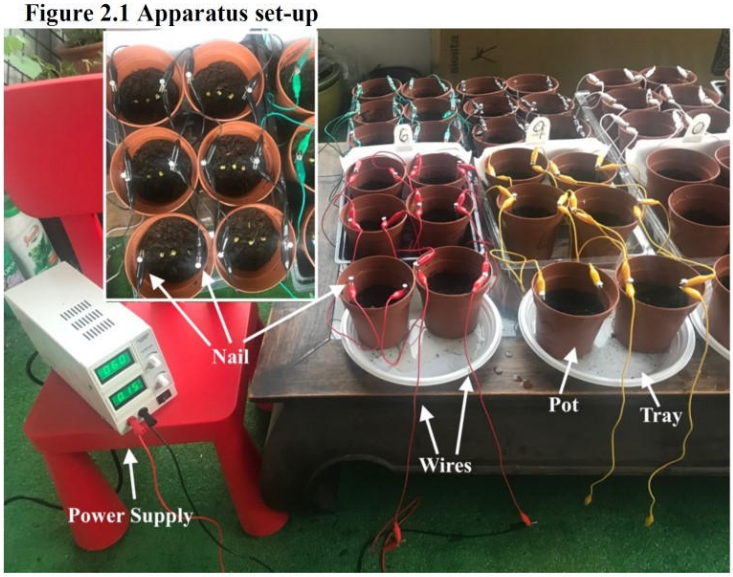
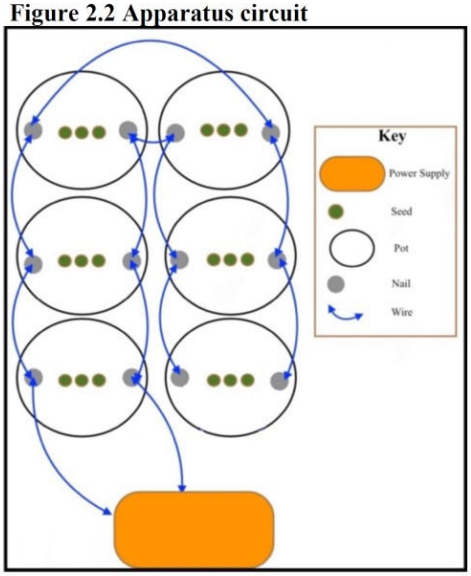


Figure 2.2 Apparatus circuit



Creating the apparatus

1) 6 pots were arranged on a tray. The tray would collect excess water. 130 grams of soil was added to each pot to fill in 2/3 of the pot. The soil was weight on a digital scale to ensure that all pots have the same amount of soil. Draw a straight horizontal line with a marker on the inside of all the pots where the soil reaches. This would be the starting measuring point. It is advised not to measure from the soil, as the soil could condense with water over time (Measuring Plant Growth, n.d.).
2) 2 nails were pressed inside the soil, to act as conducting electro rod, 8cm apart from each other in each pot.
3) The nail would be 4cm deep inside the soil.
4) Wires with alligator clips were clipped into each nail of the consecutive pot to form a parallel circuit as seen in Figure 2.2. Every nail would have two wires connecting into it, except the last two nails of the last pot. Position the wires so they do not cover the top of the seed. The parallel circuit will allow electricity to continue to flow through the rest of the pots in case one pot fails to conduct the electricity.
5) The first pot would have wires connected from its nail, into the power supply, to allow electricity to flow into the closed circuit. Make sure the power supply is switched off when preparing the apparatus to not get shocked.
6) 3 pregerminated seeds are placed in each pot (with the radicle faced downwards), on top of the soil, arranged in a straight line parallel to the nails. 2cm apart from the next seed and the rode. The arrangement would allow electricity to pass through the seeds when the current is passing from one nail to the other.
7) Sprinkle extra soil in each pot to cover the seeds about 1 cm from the top.
8) With a permanent marker, the apparatus is labeled with the voltage number which would be used on the group.
9) The apparatus is repeated 5 more times for each group

- For control groups of 0V, the apparatus is the same, except that all wires, nails, and power supply were excluded, as electricity is not passing through that group.

Method and Procedure

Preliminary experiment

With a digital multimeter, it was possible to check if there was electricity flowing through the soil, by plugging in 2 test probes in the soil. A test was made to check the conductivity of the soil, by preparing the same apparatus but manipulating the soil. Current was not detected in the soil when it was dry or after being watered. By creating a water-salt mixture and then watering the soil, currents were detected. Water-salt mixture successfully increases conductivity through the soil due to the salts ions, that are charged particles with high mobility to carry charge (Brubaker, 2018). However, the salt water does not allow osmosis through the plant's tissues, causing dehydration in plants and harming them (Lacoma, 2018). An alternative solution with salt was ammonium sulfate $(NH_4)_2SO_4$ which is a salt fertilizer for plants. Furthermore, adding ammonium sulfate to the soil, conducted electricity. It was decided that 1 gram of fertilizer would be added to every pot at the beginning of the experiment, to allow conductivity throughout 3 weeks experiment.

Sample description

For each treatment, 18 seeds were used. 3 seeds placed in each pot, to allow electricity to flow through the plants. With 5 groups exposed to the treatment and 1 control groups, a total of 108 seeds and 36 pots were used to give highly reliable and viable data.

Pre-germinating the seeds

1) The whole package of mung beans (300 seeds) have been dispersed and evenly spread in a bowl.
2) 750ml of warm tap water is added to the bowl and is placed in a window sill to obtain sunlight.
3) Allow the soaked seeds to imbibe in water for 8 hours until a 0.5cm radicle is seen breaking through the seed coating.
4) Select 108 seeds in which the radicle is the same in length and with forceps, carefully place the seed in a towel to dry.
5) Gently add each pre-germinated seed in the soil, to the designated group's apparatus, with forceps, making sure that the radicle does not break.

Preparing the Salt fertilizer

1) With a spatula, measure and weight 36 grams of ammonium sulfate.
2) Place the ammonium sulfate in a bucket and add 3.6 liters of room temperature tap water.
3) Mix the solution thoroughly with a tablespoon until all the ammonium sulfate has dissolved.

7. WHAT IS THE EFFECT OF PH CONCENTRATIONS ON THE EFFICIENCY OF IMMOBILIZED LACTASE

Author: Fiona Erskine
Moderated Mark: 23/24

Research Question

What is the effect of (*4, 5, 6, 7, 8*) pH concentrations on the efficiency of immobilised lactase by measuring the amount of glucose in the solution using a blood glucometer after 24 hours of exposure of lactase to lactose?

Gathered Information

Lactose intolerance is a chronic medical condition resulting from the inability of a certain percentage of the population to digest the lactose sugar, most commonly found in dairy products. Symptoms include bloating, cramping, and diarrhoea, which can lead to severe dehydration. “Approximately 65 percent of the human population has a reduced ability to digest lactose after infancy. Lactose intolerance in adulthood is most prevalent in people of East Asian descent, affecting more than 90 percent of adults in some of these communities.” (Lactose Intolerance, 1). From this we can see that around 65% of the population suffers from some form lactose intolerance, the highest concentrations of which are found in Asia, Southern Africa, and South America. It is important to mention that these are also the regions with the highest incidence of poverty, unfortunately making lactose-free products an unaffordable luxury. By finding more efficient and exact methods of catabolising the lactose substrate, firms may be able to increase their productivity and sell products for cheaper prices without decreasing their revenue. This would make their products more accessible and affordable to people of lower socioeconomic status. I think this is a valuable lab to pursue because lactose intolerance is an incredibly uncomfortable and even dangerous ailment to deal with, especially if you don’t have the resources to handle it properly. I think there should absolutely be more research on lactose intolerance and action taken to make products available to people in less than ideal situations.

Immobilised enzymes are enzyme units that have been adhesively attached to an insoluble material and cannot move freely about the substance, but are still able to carry out their function. Advantages to using immobilised enzymes include making the enzyme less sensitive to changes in temperature, giving the enzyme a better reaction rate, and increasing the stability of the enzyme. This is due to the binding of an enzyme to a surface, which allows the protein to become more stable and less likely to denature (Twadell, 1). In addition, immobilised enzymes can give greater yields of the product than free-moving enzymes, which is particularly interesting to the economics perspective of this lab (Markoglou, 215). Immobilised enzymes can also be easily separated from the product and even reused several times which saves both time and money for the producer of the enzyme-affected product (Zhang, 106). In this lab, the immobilised lactase enzyme will be used to break down lactose. There are many different methods of immobilising an enzyme, but here I will be using a process called crosslinking. This involves mixing sodium alginate and lactase enzyme solution with another solution of calcium chloride. The enzyme binds the insoluble $CaCl_2$ and forms beads. These beads can be taken from the solution and then introduced to the lactose sugar for metabolism (Amato, 1).

Lactase is used to break down lactose, a disaccharide sugar with the chemical formula $C_{12}H_{22}O_{11}$ into the universal monosaccharides of glucose $C_6H_{12}O_6$ and galactose

$C_6H_{12}O_6$ through a process called catabolism. Once broken down, the organism can use these monomers like building blocks to assemble the molecules necessary through a process called anabolism, or use them as resources for cellular respiration to obtain energy. Catabolism of lactose is done by the lactase enzyme. When lactose bonds to the active site of the enzyme, the enzyme's orientation allows it to split the molecule into glucose and galactose, which then separate from the lactase and enter the bloodstream (White, 1).Once in the bloodstream, several enzymes work to take the molecules through the different stages of cellular respiration: glycolysis, the citric acid cycle, and the electron transport chain. This aerobic process releases 36 ATP (Beck, What is). ATP is an organic chemical that supplies energy for cells to do work and maintain homeostasis. Most glucose that is broken down goes towards the making of ATP for cell energy. Lactose is a source of necessary sugar for many organisms, especially mammals in infancy. People who suffer from lactose intolerance may get their sugars from other foods as well, but not having the ability to eat any dairy products could seriously cut down on the glucose-intake of the organism. Research on lactose-free products, such as milk that has already been broken down into the monomers of glucose and galactose, allows lactose-intolerant patients maintain their necessary sugar levels.

All enzymes have a range of optimum pH and temperature, this is the pH or temperature where the enzyme works most effectively. Enzymes that are placed in temperatures lower than their optimum experience a decrease in their ability as the decrease in kinetic energy reduces the amount of collisions between the enzyme and the substrate. Without these collisions the enzyme cannot carry out its function as well, reducing the output. Temperatures that are higher than the enzyme's optimum can denature, or change the shape of the enzyme, making it ineffective. Lactase is efficient over a pretty wide range of temperatures but was found to function best at a temperature of 37ºC (Hermida, 4836), around human body temperature. Optimum pH for lactase enzyme is known to be around 5.8 to 6.0 (Skovbjerg, 653), but the enzyme is still at least partially effective from a pH of about 4 to 8 (Mozumder, 10), many different trials have had different conclusions, there are even trials that have concluded that lactase works best around a pH of 5 (Gray, 729), (Ho, 1). When enzymes are exposed to environments that are either more acidic or more basic than their optimum, the 3D configuration of both the enzyme (lactase in this case) and the substrate (lactose) can change, making them unrecognisable to each other and unable to bond. This prevents the enzyme from carrying out its function (Sandhyarani, Effect of). In the human body, lactase is produced around the beginning of the small intestine. The organ has varying pHs as you move through it (Collins, Anatomy, Abdomen), but lactase is found at the beginning, right as the acidic contents from the stomach are passed over. The stomach has an incredibly low pH (2-4) so while the small intestine is more basic, this region must be able to function at lower pHs (Fallingbord, Intraluminal pH). The lactase enzyme is most commonly functioning in a pH of about 5.5 to 6, so it makes sense that this should be its optimum pH.

In an experiment to test at which pH lactase works best, several pH solutions will need to be prepared. To prepare a pH solution, buffer solutions must be used. A buffer solution is a mixture of a weak acid and its conjugate base (or vice versa). It is necessary when preparing pH solutions because of its ability to maintain its current pH, even when

a strong acid or strong base is introduced to the aqueous mixture. Buffer solutions equations for the pHs of 4, 5, 6, 7, and 8 can be seen further down.

Independent Variable		
pH (4, 5, 6, 7, 8)		
Dependent Variable		
The amount of glucose in the solution (mg/dL)		
Control Variables	**How to control**	**Why to control**
Temperature (°C)	Keep the solution at room temperature (20°C). Use thermometer. (± 0.1°C)	Increase or decrease in temperature can affect the efficiency of the enzyme. Use thermometer. (± 0.1°C)
Mass of enzyme	Measure out the same number of lactase balls for each experimental group with a digital balance (± 0.01g).	If there is more enzyme but the same amount of substrate, these enzymes will be more efficient at carrying out their function.
Concentration of substrate	Measure out the same amount of lactose for each experimental group using a digital balance (± 0.01g).	More substrate will allow for a greater concentration of the product in the final solution.
Time (minutes)	For each experimental group, take data on the amount of glucose present after 24 hours using a phone timer.	Data must be taken in the same time increments to measure the rate of efficiency of the enzyme.
Same technique for making enzyme balls	To make the balls, hold your elbow to the table to keep a constant height and push on the back of the syringe so that one drop falls per second	This will make all the balls the same size, ensuring that they all carry the same amount of the enzyme and all the solutions are exposed to the same amount of enzyme

Same stirring for alginate balls	Have the magnetic stirrer stir at a constant rate for the alginate balls	This is to ensure that all the balls end up the same size, and carry the same amount of enzyme
Same $CaCl_2$ solution for balls	Measure the solution concentration you want to use and use this amount for every batch of alginate balls you make	Since CaCl2 reacts with the alginate to produce the balls, different amounts of the compound would cause different size and concentration of enzyme of the balls
Same size beakers for lactose exposure	Use the same 250 ml flasks for each solution so that the lactase is exposed to equal amounts of lactose	By having the same size beakers and the same amount of each solution, this allows for equal amounts of each reactant to be exposed to each other
Same technique for checking glucose	Use the same blood glucometer and same brand of testing strips for every reading	This allows all the data to be taken with the same machine, so that if there are systematic errors they are widespread over the entire experiment and are a constant, allowing us to still have relative readings
Contamination of solutions	Use a permanent marker to mark each flask and each beaker with an indication of the contents inside	This prevents against human error and mixing two substances that aren't supposed to be mix and forcing a redo of the experiment
Volume control of solutions	Measure out the same amount of lactose solution and pH solution using a 50 ml graduated cylinder (± 1 ml)	This allows for each substance to be exposed to the same amount of reactant so that all the solutions start out equal to give conclusive results
Evaporation of solutions	Stopper each flask when you leave it in the fume cupboard overnight	This protects the solution from evaporation which could potentially evaporate one of the reactants causing unequal numbers of reactants which could alter one of the data sets