Grade: 12 U Biology (Biotechnology) **Grade:** 11 U Biology (Evolution)	Subject: Biology	Unit: Biochemistry & Evolution

Title: Lab Investigation - Investigate factors that affect the efficient removal of lactose from milk to create lactose-free milk

Rationale

In this lesson series, students are exposed to current research done in GlycoNet laboratories that relates to therapeutic proteins and their potential impact on human health. In addition, students are asked to consider how a mutation that resulted in lactase persistence was selected for due to selective pressures (having dairy cows). The students are then given the opportunity to immobilize lactase and create a real product that is in current use today - lactose-free milk. This enables them to see biotechnology in a real life context.

Background Information

The Wakarchuk lab at Ryerson University (under the umbrella of GlycoNet) is a carbohydrate research lab that uses enzymes for therapeutic purposes. These purposes include:

- The use of enzymes to add sugars to protein drugs to make these drugs last longer (persist) in the bloodstream, thereby reducing dosage, side effects and cost
- Adding sugar molecules to cultured neuronal cell surfaces using enzymes, so that these modified neurons will find their way to where they are needed when given to Parkinson's patients
- Researching how to make these proteins using bacteria (rather than maintaining expensive mammalian cell lines)

Lactose is a disaccharide found in milk. Lactose cannot be absorbed by the digestive system; it needs to be broken down for absorption. Lactase is a naturally occurring enzyme found in the digestive system of human babies. It separates lactose (a disaccharide) into two individual monosaccharides, glucose and galactose. In the following investigation, students mix sodium alginate (found in seaweed) with a lactase solution and react it with calcium chloride to produce gel beads. When milk is poured over these beads and is allowed to react, one can test for one of the breakdown products using glucose urinalysis strips.

Time Required

Pre-lab preparation: Prepare lactase and sodium alginate solutions. Gather the other materials for the demonstration. Watch the 1 minute Adobe Spark instructional video for a general idea of how to do the lab at https://spark.adobe.com/video/vWSiVLSvwzPCm.

Period 1

- Students listen to and discuss the GlycoNet researchers and their areas of research http://glyconetlactaselab.weebly.com
- Students watch three one (1) minute videos from HHMI Biointeractive and discuss the answers together during class:
    - Lactose digestion in infants (https://www.biointeractive.org/classroom-resources/lactose-digestion-infants)
    - Natural selection for lactose tolerance (https://www.biointeractive.org/classroom-resources/natural-selection-lactose-tolerance)
    - Regulation of the Lactase Gene (https://www.biointeractive.org/classroom-resources/regulation-lactase-gene)
- The teacher demonstrates 1) how to create the lactase/sodium alginate spheres, 2) how to fill the column (syringe) with the gel spheres and 3) how to pour the milk through and test the product with the glucose strips
- The teacher will invite students to wonder/brainstorm/plan how to change the basic method to create the most efficient process of creating lactose free milk (using a modified Smarter Science framework promoted by Youth Science Canada)
- Students create a list of extra supplies needed for the inquiry day (some materials may be brought from home)
- Homework: The teacher can post an HHMI Biointeractive 15 min video to Google classroom (or other web-based platform) for them to watch for homework. Every 3-5 minutes there are comprehension questions to answer. See Student Video Worksheet. All video clips and student questions are all posted on the Weebly website.

Period 2 – Student inquiry day

- Students create lactose-free milk

Period 3 – Lab write up day

- Students who need to repeat laboratory work have an opportunity to do so
- Groups that are finished can use iPads or laptops to work on their write up
- Write up can be in a four (4) question narrative report

Curriculum Connections

Grade 11 University Biology – Evolution Unit

- C2.1 use appropriate terminology related to evolution, including, but not limited to: extinction, natural selection, phylogeny, speciation, niche, mutation, mimicry, adaptation, and survival of the fittest [C]

- C2.2 use a research process to investigate some of the key factors that affect the evolutionary process (e.g., genetic mutations, selective pressures, environmental stresses) [IP, PR]
- C3.1 explain the fundamental theory of evolution, using the evolutionary mechanism of natural selection to illustrate the process of biological change over time
- C3.2 explain the process of adaptation of individual organisms to their environment (e.g., some disease-causing bacteria in a bacterial population can survive exposure to antibiotics due to slight genetic variations from the rest of the population, which allows successful surviving bacteria to pass on antibiotic resistance to the next generation)

<u>Grade 12 University Biology – Biotechnology Unit</u>

Overall expectations:

- B1. analyse technological applications of enzymes in some industrial processes, and evaluate technological advances in the field of cellular biology;
- B2. investigate the chemical structures, functions, and chemical properties of biological molecules involved in some common cellular processes and biochemical reactions

Specific Expectations - Biotechnology

- B1.1 analyse technological applications related to enzyme activity in the food and pharmaceutical industries (e.g., the production of dairy products; breadmaking; the use of enzymes to control reaction rates in pharmaceuticals) [AI, C]
- B2.4 conduct biological tests to identify biochemical compounds found in various food samples (e.g., use Benedict's solution to test for carbohydrates in food samples) [PR, AI, C]
- B2.5 plan and conduct an investigation related to a cellular process (e.g., factors that affect enzyme activity; factors that affect transport of substances across cell membranes), using appropriate laboratory equipment and techniques, and report the results in an appropriate format [IP, PR, C]

Specific Expectations - Molecular Genetics

- D3.3 explain the steps involved in the process of protein synthesis and how genetic expression is controlled in prokaryotes and eukaryotes by regulatory proteins (e.g., the role of operons in prokaryotic cells; the mechanism of gene expression in eukaryotic cells)

<u>SIS (Scientific Investigation Skills) – both grade 11 and 12 U Biology</u>

Initiating and Planning [IP]

- A1.1 formulate relevant scientific questions about observed relationships, ideas, problems, or issues, make informed predictions, and/or formulate educated

hypotheses to focus inquiries or research

Performing and Recording [PR]

- A1.5 conduct inquiries, controlling relevant variables, adapting or extending procedures as required, and using appropriate materials and equipment safely, accurately, and effectively, to collect observations and data
- A1.6 compile accurate data from laboratory and other sources, and organize and record the data, using appropriate formats, including tables, flow charts, graphs, and/or diagrams

Analysing and Interpreting [AI]

- A1.8 synthesize, analyse, interpret, and evaluate qualitative and/or quantitative data to determine whether the evidence supports or refutes the initial prediction or hypothesis and whether it is consistent with scientific theory; identify sources of bias and/or error; and suggest improvements to the inquiry to reduce the likelihood of error
- A1.10 draw conclusions based on inquiry results and research findings, and justify their conclusions with reference to scientific knowledge

Lesson Objectives/Concepts

- Students will learn about the role of enzymes in an example of current Canadian research with GlycoNet at Ryerson University, and about future careers
- Students will develop an understanding of how lactase persistence resulted from selective pressures and evolutionary adaptation
- Students will explore the inquiry process to discover the most efficient method of making milk lactose-free using the enzyme lactase

Materials

For the teacher demonstration:

- Lactase (either purchased in the form of Lactacid from the pharmacy or lactase as a powder from a science supply company)
- Calcium chloride
- Sodium alginate (purchased from Boreal Science or another science supply store)
- Milk (could be 1%, 2%, etc.)
- Sieve/strainer
- Filter paper (for filtering the lactase solution if you use a tablet form)
- Erlenmeyer flask
- Funnel
- Electronic balance
- Graduated cylinder (1 L)

- Volumetric flask (1 L)
- Beaker (250 mL)
- Syringes (10 mL and 25 mL)
- Food colouring (optional)
- Bottle of Urinalysis glucose strips (comes in bottles of 100) – can order from science supply company – Benedict's solution could be used if the glucose strips are not available

Safety: Lactaid, milk, calcium chloride and sodium alginate are edible (these substances are used in molecular gastronomy). Calcium chloride is classified as green (general use). Safety goggles are not required, although teachers can ask students to wear them (good practice). Teachers should caution students not to eat or drink materials, regardless of their everyday use (milk).

Part B: See Student Video worksheet (clips of Canadian carbohydrate researchers and some background information about lactose, lactase and co-evolution and culture.)

Part C: Teacher Demonstration and Student Initiating and Planning (I &P)

Activities/Procedure

Lab preparation for the teacher demonstration:

- Prepare the sodium alginate solution/gel: use 0.5 tsp per 175 ml of distilled water in an Erlenmeyer flask. Swirl until the solid is dissolved. Add a couple of drops of food colouring (optional) to help visualize the gel spheres when you are making them.
- Prepare the lactase solution. If using the tablets, crush 4 tablets with the mortar and pestle and transfer to an Erlenmeyer flask. Add 60 ml of distilled water and mix. Filter the solution to remove the cellulose filler. Skip this step if using the lactase powder.
- Prepare the 2% calcium chloride solution by weighing out 20.0 g of $CaCl_2$ (with a balance) and transferring it into an Erlenmeyer flask. Use a graduated cylinder to measure out the water. Add 500 mL of water to the flask, transfer to a volumetric flask using a funnel and top up the solution to the 1000 mL/1L line. Invert to mix. This is enough calcium chloride solution for the whole activity.

Procedure for the teacher demonstration

- Show the class the sodium alginate solution, the lactase solution and the calcium chloride solution.
- Mix the sodium alginate and the lactase solution in a 3:1 ratio in a beaker.
- Draw gel mixture into a syringe.
- Place some calcium chloride solution into a beaker, 2 inches deep. Place beaker on top of a piece of white paper for better visualization. Slowly and carefully, drop the sodium alginate/lactase gel into the calcium chloride. It will make spheres. Let sit for

at least 2 minutes for the spheres to become firm.

- Rinse the spheres with distilled water, using the sieve.
- Place a small amount of cotton into the bottom of the syringe to prevent clogging. ***Important step***
- Using a spoon, place spheres into the large syringe (25 ml). Students will have to decide how much they are going to use in the planning stages. For the purposes of the demonstration, 20 mL can be used. Space needs to be left at the top to pour the milk in.
- Clamp the sphere-filled syringe onto the retort stand. Place a test tube directly underneath. One may decide to attach tubing to the end of the syringe to direct the flow of milk (optional).
- Pour the milk through the column. Once all of the milk has passed through, test it with the glucose strip, following the directions on the label. Record your observations. You may get a negative glucose reading on the first pass.
- Pour the milk through the syringe at least three times. You should be able to see the glucose strip show a positive result on the second try. It is too time consuming to demonstrate three passes through the column so just guide the students to not become discouraged when it takes more than one pass.

<u>Student Discussion</u>

Invite the students to discuss with their partners how they could change the procedure to influence the result. How could they make the process more efficient?

Some variables that they might consider:

- increasing the diameter of the syringe to increase the surface area of the beads that come into contact with the milk
- decreasing the diameter of the syringe so that the milk will travel more slowly through the column and have more contact time with the lactase
- not use the syringe at all – let the spheres sit with the milk in a stoppered test tube, inverting it at regular intervals
- having a set time of constant, gentle inversions of the stoppered test tube
- lay the stoppered test tube on its side and let the reaction happen (the spheres float so placing the test tube on its side allows for more of the milk to react with the beads)
- not using the beads at all but mixing the lactase solution with the milk directly
- changing the amount of lactase to water - preparing a different concentration of lactase
- changing the pH of one of the solutions (milk, lactase, sodium alginate solution)

Students can compare this data with data the teacher provides or, if there is time, students can do one “regular” preparation just like the teacher showed as well as one preparation that

showed the change.

Extra challenge: students can calculate the cost (and consider the time involved) of making lactose-free milk using their method. A method such as placing the beads in the milk, letting it sit for a certain period of time and then passing it through a sieve might be cost effective and not too onerous for someone who is lactose intolerant.

Notes for purchasing:
Sodium alginate, calcium chloride, lactase, urinalysis paper strip tests for glucose (packs of 100) and syringes are easily and cheaply available from the chemical supply company of your choice. Lactase can be purchased, if desired, from the pharmacy in tablet or liquid form. Urinalysis strips for testing glucose are also available from some pharmacies but may not be easy to find as diabetics are typically using electronic kits at the present time.

Assessment: Use the accompanying Lab Investigation Evaluation rubric.

Extensions/connections

This lab can be used as part of the IB Practical Scheme of Work as the rubric is very similar to the IB expectations. Also, one could search for more written material about the evolution of the lactase persistence gene for students to read about. Much is written about this subject since many people do not have this particular mutation.

Works Cited

- "Better Milk for Cats: Immobilised Lactase Used to Make Lactose-Reduced Milk." Science in School, European Journal for Science Teachers, 6 Aug. 2017, www.scienceinschool.org/2008/issue10/catmilk.
- "Milk Makes Me Sick." Exploratorium, Exploratorium, 31 July 2017, www.exploratorium.edu/snacks/milk-makes-me-sick.
- "Milk-How Sweet Is It? | HHMI's BioInteractive." HHMI BioInteractive, HHMI BioInteractive, https://www.biointeractive.org/classroom-resources/milk-how-sweet-it

Answer to Video Questions

Part B: Video Worksheet
Video clip #1: Farah Choudhary, Lab Technician (video clip, 1:22 min)

1. What is the focus of Farah's research?
   She uses bacterial enzymes to add a polymer called polysialic acid to the surface of mammalian cells. This enables cells to integrate into damaged areas.

2. What is the ultimate goal of her project?
   The idea is to replace dead or damaged cells in the nervous system with new compatible cells. This technology could allow people with diseases such as Parkinson's to recover their former abilities and lead healthier lives.

3. Why use bacterial enzymes to modify the cells? Why not mammalian cells?
   The mammalian cells are not very active and they are hard to express and purify.

Video clip #2: Dr. Lisa Willis, Post-doctoral fellow (video clip – 1:13 minutes)

1. According to Lisa, what does the attachment of polysialic acid to the cell surface allow certain cells to do?
   Polysialic acid prevents cells from sticking to their neighbours, allowing them to move more freely around the body.

2. What does polysialic acid attachment allow cancer cells to do? How does Lisa's research address the problem?
   If able to move freely, cancer cells can *metastasize*, or spread to other parts of the body and form new tumors. Lisa is working towards finding a way to prevent cancer cells from forming polysialic acid, which would prevent the cancer from spreading in the body.

Video clip #3: Alison Mark, Co-op student (video - 40 seconds)

1. What does the enzyme that Alison is working on do?
   This enzyme helps to attach multiple copies of a certain sugar onto a target protein.

2. Why is Alison trying to find a heat stable protein?
   Enzymes work faster at a higher temperature but also lose function if the temperature is too high. A heat stable protein will result in a more efficient enzyme.

Video clip #4: Ray Martinez-Rodriguez - Summer student (1:10 minutes)

1. What does the enzyme that Ray works with do?

It moves sugars from a donor molecule to an acceptor molecule.

2. What are two problems with this protein (enzyme)?
   One problem is that it is hard to purify and the other is that it is not very active.

3. Ray describes how a His tag can help fix one of the two problems mentioned above. What does it help with? Do an Internet search for His-tag (histidine tag) purification and IMAC (immobilized metal affinity column). He mentions using a nickel column. Draw a picture of an IMAC column and explain how it works.
   Scientists add histidine to proteins to help to tag them for a purification process. When trying to scale up a substance for large scale production, it is important to be able to identify and purify the final product. This helps with the purification process. See question #4 for the rest of the answer.

4. Immobilizing lactase in a calcium alginate gel and pouring milk through it to break down the lactase is a similar idea to the IMAC mentioned above. How are these two procedures similar and how are they different?

   The repeating his (histidine) tag has a certain affinity for metals such as nickel which are embedded in the column. A protein that is histidine-tagged can be poured through the Ni column and those molecules will be removed from the other substances. The his-tagged sample can be later recovered by eluting with another substance such as imidazole, which competes with histidine for the nickel. In this way, a recombinant protein can be recovered.

   This is different from using immobilized lactase since lactose passes through and the enzyme breaks it down. No eluting is necessary since the goal is to get rid of the offending lactose by breaking it down. The breakdown products are still present in the lactose free milk. The two processes are different because the goals are different. IMAC uses affinity to remove a certain molecule from other molecules in the lysate. Immobilized lactase carries out a hydrolysis reaction on one component of the mixture and then releases the products back into solution.

   The two processes are similar because in both cases, a mixture of substances is poured through the column. With IMAC, one component is removed. With immobilized lactase, lactose is removed by breaking it down into other molecules.

   Reference: “How does His-tag purification work?” http://www.bio-rad.com/featured/en/his-tag-purification.html

Homework: HHMI (Howard Hughes Medical Institute) Video Clips

Video clip: Lactose Digestion in Infants – animation (53 seconds)
https://www.biointeractive.org/classroom-resources/lactose-digestion-infants

1. Why do babies have the gene to make the lactase enzyme?
   Babies drink their mother's milk and require the enzyme to break down the milk into smaller, single sugars (glucose and galactose) which can then be absorbed in the small intestine.

Video clip: Natural Selection of Lactose Tolerance - animation (46 seconds)
https://www.biointeractive.org/classroom-resources/natural-selection-lactose-tolerance

1. Why is it biologically "expensive" for people to have this enzyme as an adult?
   In communities where dairy is not consumed, lactase in adults would have no substrate to act on. The energy that the body puts into making it could be used elsewhere.

2. How is the lactase gene selected for?
   The lactase gene is selected for in communities where they have dairy cows. The mutation itself is random but in this situation, it is a positive mutation and is therefore selected for and passed onto the next generation where it is present in a larger amount.

Regulation of the Lactase Gene – PowerPoint (includes 2 minute video by Dr. Sarah Tishkoff) https://www.biointeractive.org/classroom-resources/regulation-lactase-gene

1. What is lactase persistence?
   Lactase persistence is where the gene to make lactase is not turned off (which it should do as it is no longer needed) as a child grows up to adulthood.

2. What does lactase do?
   Lactase breaks down lactose (a disaccharide) into two monosaccharides (glucose and galactose) for proper absorption in the digestive system.

Got Lactase? The Co-evolution of Genes and Culture (15 minute video with interactive multiple choice questions)
https://www.biointeractive.org/classroom-resources/interactive-assessment-got-lactasecoevolution-genes-and-culture

1. What happens if a lactose intolerant adult drinks milk?
   The milk goes straight through to the large intestine, where bacteria break it down. This intestinal breakdown results in cramps and diarrhoea.

2. What percentage of the world's population is lactose tolerant?
   About 33%

3. In which regions of the world do people have the highest levels of lactase persistence?

Europe, some African countries

4. In which regions of the world do they have the lowest levels of this mutation?
   The mutation is present in low amounts in Asia, as well as many other countries.

5. What did researchers find out about the mutations in Europe vs. the one in Eastern African countries?
   Researchers found out that there were two independent chromosomal mutations in the non-coding regions near the lactase gene that act like a switch. Both mutations resulted in the same effect (lactase persistence).

6. How long ago did the pastoralists in Europe and Eastern Africa start using milk in their diet?
   Europe – 9000 years ago and Eastern African 5,000 years ago.

7. How does milk consumption promote an evolutionary advantage to those who are lactase persistent?
   It is very nutritious, has fewer problems with bacterial contamination (unlike water), and is another food source in times of famine.

Narrative Laboratory Report[1]

Rationale: The idea behind a narrative laboratory report is to move students beyond a question and answer style of analysis or even a formal laboratory report. They have to ask themselves the core questions: What did I look for? How did I look for it? What did I find? What did it mean?

It is hoped that by asking students to step back and take a wider view of their work, it will fit better with moving towards open inquiry where they are also wondering and generating their own questions to answer (and being comfortable with that). After the initial uncertainty about marks and stepping out of their comfortable zone, students generally like the freedom to work in this way, if the topic captivates them. It is the responsibility of the teacher to select opportunities for the students that have an interesting context or some aspect that is fascinating.

What was I looking for? (Describe your research question here along with your hypothesis) Should be 1-2 sentences, includes a testable question & hypothesis
How did I look for it? (Describe your method) Should be reproducible and step by step
What did I find? (Show any observations that you had) Should include tables (succinct & organized with headings) and graphs (label axes, include title, legend if necessary)
What does this mean? (Analysis and Conclusion) Should include analysis of scientific area and should discuss results and why they occurred Written in paragraph form

Rubric for Narrative Report

	Level 1	Level 2	Level 3	Level 4
What was I looking for?	Research question did not include a properly testable question	Research question was somewhat focused with a testable question	Research question was well focused with a testable question	Research problem/question was highly focused with a testable question
How did I look for it?	Method was incomplete	Method was mostly there	Method was written in a generally reproducible way	Method was written in a detailed and highly reproducible way
What did I find?	Observations were present but were not organized/succinct ; graphs lacking axes, labels & title	Observations were somewhat organized & succinct with table headings, good graphs	Observations were well organized & succinct with table headings, excellent graphs	Observations were highly organized & succinct with table headings, excellent graphs
What does this mean?	Analysis did not focus on the key question and did not address scientific error	Analysis somewhat captured the key questions but did not address the scientific error well	Analysis generally captured the key questions and addressed most of the scientific error	Analysis captured the key questions and worked in scientific error in a high level way

[1] Modified from Lenape District High School's Narrative Lab Report