

SCIENCE IN THE REAL WORLD

Microbes in Action



Coming Clean With Enzymes

A Microbial Enzyme Laboratory

Science in the Real World

Microbes In Action

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At A Glance

Description

These labs show the ability of bacteria to produce extracellular enzymes. They also demonstrate the ability of these enzymes, when produced and collected through biotechnological techniques, to function as additives to household detergents and cleaners.

Time Requirements

Four days are suggested. This unit has separate activities that flow sequentially from one to another.

Curriculum Placement

This unit can be used as a part of a microbiology unit, an enzyme or biochemistry unit, an introduction to scientific method, or as an introduction to biotechnology.

Equipment

Autoclave or sterilizer, Erlenmeyer flasks, culture tubes with caps, incubator, Bunsen burner, inoculating loops

Materials

Cultures of:

Escherichia coli

and 2 from the following list (See page 23 for help in deciding which cultures to order):

Pseudomonas fluorescens

Bacillus subtilis

Bacillus cereus

Serratia liquifaciens

Media

petri plates

nutrient agar

JELL-O™ or generic gelatin

sodium carbonate (Na_2CO_3)

nonfat dry milk

egg

alcohol

Supplies

sterile transfer pipettes

plastic soda straws

Lugol's iodine stain (50% concentration)

aluminum foil

marking pens

an assortment of detergents

corn starch or soluble starch

Mello JELL-O™

Protein Digestion by Enzymes

Introduction

Foods are not only important nutrients for cells, they are also the cause of some stains in clothes. Since some of the large complex molecules in food do not dissolve well in water, they are often left in clothes after washing. Enzymes are proteins that break down complex molecules in food to produce smaller molecules that are more soluble in water. For example, enzymes can break down the protein gelatin, a major part of JELL-O™. Manufacturers take advantage of the ability of enzymes to break down food by adding them to detergents to enhance stain removal.

Purpose

You will observe the effect of enzymes in detergents and cleaning solutions on JELL-O™.

Materials

Per Student or Team of Students

1 plate containing JELL-O™

Caution: This JELL-O™ contains sodium carbonate and should NOT be eaten.

1 plastic straw section

1 toothpick

1 marking pen

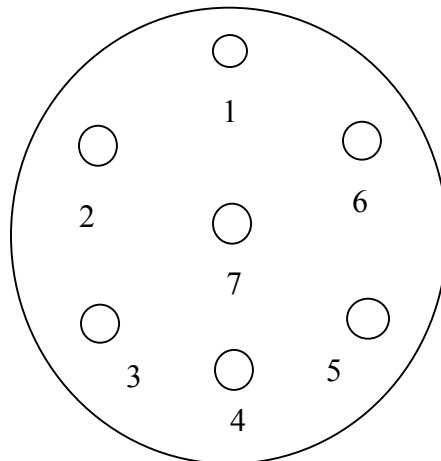
1 metric ruler

detergents and cleaning solutions each with their own pipette

distilled water with a pipette

Procedure

1. Obtain a JELL-O™ filled plate. Label the plate on the bottom by writing (near the edge and in small letters) your name, class period, and today's date.
2. Using a piece of plastic soda straw, cut wells in the JELL-O™. See the template for a pattern.



3. Remove the plugs of JELL-O™ with a toothpick. Take care not to tear the layer of JELL-O™. Number the wells on the bottom of your plate, so that when face up, the numbers are as shown on the template. (This means you write the numbers backward and counter-clockwise on the bottom of your plate.)
4. Measure the diameter of the wells (in millimeters). Record these numbers in Table 1 as “initial diameter.”
5. Decide within your group which detergents you would like to test. Record the detergent number or letter in Table 1 next to the appropriate well number.
6. Use only the dropper that is in each solution. Do not exchange droppers between tubes!
7. Load distilled water into well #7. This will be your negative control. To load the wells, place the pipette into the well and gently dispense just enough liquid to fill the well. Try not to drop any liquid onto the surface of the JELL-O™. If you do, note the location of the drop by drawing a picture in your notebook.
8. Carefully load each of the wells with one of the detergent solutions.
9. Replace the lid on the plate. Do not turn the plate upside down. You will spill the solutions that you just loaded into the wells.
10. Let the plate sit undisturbed for several hours or overnight at room temperature.

Name _____

Date _____

Observations

1. Using a pipette, remove the liquid from the wells and discard. Observe the wells in the plate. Record any physical change in the JELL-O™ that you see around any of the wells.

2. Was there any change in the JELL-O™ around well #7? Explain.

3. Measure the largest diameter of each well in millimeters. The diameter of a well is the distance from solid JELL-O™ on one side to the solid JELL-O™ on the other side. Record this number in Table 1 as the “final diameter.” Calculate the change in diameter for each detergent.

Well #	Detergent Name and Number or Letter	Initial Diameter	Final Diameter	Change in Diameter
1		mm	mm	mm
2		mm	mm	mm
3		mm	mm	mm
4		mm	mm	mm
5		mm	mm	mm
6		mm	mm	mm
7	distilled water	mm	mm	mm

4. a) Which products increased the diameter of the well? _____

b) The chemicals responsible for changing the JELL-O™ are called enzymes. Refer to your textbook and write a definition for enzyme.

5. Based on your observation and the information supplied in this lab, what ingredient in JELL-O™ do you think was changed?

6. What ingredient in the detergent is probably responsible for the breakdown of the protein, gelatin?

7. Enzyme names often end in “ase.” For example, lactase is the enzyme that break down the milk sugar, lactose. Suggest a name for the enzyme that breaks down the protein, gelatin.

In the Clear

Introduction

Most foods contain a variety of nutrients, but foods usually have large amounts of protein, carbohydrates and fat. Different foods have more or less of these three nutrients. Enzymes are special chemicals that can recognize a specific type of molecule and can break the chemical bond in that molecule. In the stomach and intestines a variety of enzymes act together to break down the complex molecules in food. The simple molecules produced by the enzyme action can pass through the intestines to the blood where they serve as food for the cells in the body.

Just as there are different classes of nutrients, there are different classes of enzymes. In general, an enzyme recognizes the particular type of molecule that it will break down, but does not recognize other molecules. For example, an enzyme that breaks down protein is called a protease. It will break down most proteins, but will not break down fats or carbohydrates. Enzymes that break down fats (lipids) are called lipases. The ability of an enzyme to act on only one class of molecules means that it is specific for that type of molecule; therefore, we say that enzymes show “specificity” for those particular molecules.

In this activity you will observe the action of enzymes on all three types of nutrients. Milk agar plates contain large amounts of the protein, casein. Egg yolk agar plates are high in lipids, while the starch plates contain the carbohydrate molecule, starch. These nutrients are all large molecules that typically do not dissolve well in water. Therefore the plates appear cloudy. Enzymes break down the large complex molecules to smaller molecules that are soluble. The result is that the cloudiness of the plates disappears wherever an enzyme has broken down the large molecule.

Purpose

This lab demonstrates that the enzymes in detergents degrade biochemical compounds such as the lipids, proteins and starches found in food. You will determine the types of enzymes that are present in various detergents and compare these products in terms of enzyme activity.

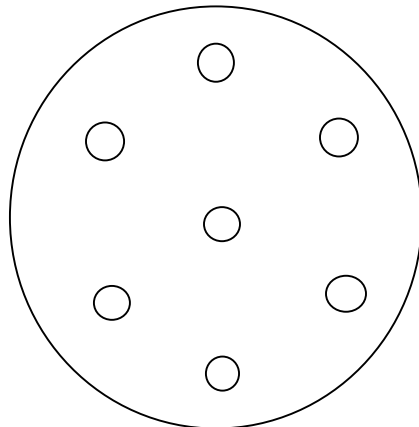
Materials

Per student or team of students:

- 1 egg yolk agar plate
- 1 milk agar plate
- 1 starch agar plate
- 1 plastic straw section
- 1 small container of alcohol
- 1 toothpick
- 1 metric ruler
- Lugol's iodine stain
- 1 marking pen
- 1 piece of aluminum foil
- detergents and cleaning solutions each with their own pipette
- distilled water with a pipette

Procedure

1. Label each of the three agar plates on the bottom by writing (near the edge and in small letters) your names, your class period and today's date.
2. Using the template for a pattern cut wells in each of the agar plates with the plastic soda straw. In this lab, we are using nutrient agar plates so it is important to sterilize in alcohol in order to minimize the risk of transferring bacteria onto the plate. To do this, dip the straw in the beaker of alcohol. Let the alcohol drain out of the straw back into the beaker. When most of the alcohol has evaporated use the straw to cut wells in the agar. Remove the agar plugs with a toothpick.



3. For each of the three plates, number the wells. Remember to write on the bottom of the plate. (Write the numbers backwards and counterclockwise on the bottom of your plate.)
4. Measure the diameter of the well (in millimeters). Record this value in the Data Table as the initial diameter.

5. Decide within your group which of the available detergents you would like to test. Choose six and record the names in your data table next to the appropriate well number. Use only the dropper that is in each solution. Do not exchange droppers between tubes.
6. Fill well #7 with distilled water. This will be your control. To fill the wells, place the pipette into the wells and gently dispense just enough liquid to fill the well. Try not to drop any liquid onto the surface of the agar. If you do, note the location of the drop by drawing a picture in your notebook.
7. Carefully load each of the wells 1-6 with one of the detergents.
8. Cover your plates and carefully place the three agar plates in a stack in the area designated by your teacher. Do not turn the plates upside-down.
9. You will make observations of the plates tomorrow.

Observations

- The yolk in egg yolk agar contains lipids and provides the yellow color of the agar. The breakdown of these lipids by enzymes called lipases leaves a clear ring around the wells of the solutions that contain these lipid digesting enzymes.
 - Similarly, milk agar contains milk proteins (casein). The white color provided by casein disappears in the presence of proteases.
 - Starch agar contains starch, a long chain carbohydrate, which can be digested by enzymes called amylases. This agar is transparent and must be stained with Lugol's iodine to show where the starch is present (the purple stained area) and where it has been digested (clear).
10. In order to observe the digestion of starch in the agar by the detergent solutions, the starch plate must be stained with the starch indicator, Lugol's iodine. Before staining, remove the liquid in each well using a dropping pipette. To stain, slowly flood the surface of the starch agar with iodine, replace the lid, cover the plate with aluminum foil, and let it sit while you observe the milk and egg yolk agar plates.
 11. Look for zones of clearing around the wells in the egg yolk, milk and starch agar plates. Clearings are areas where the agar is still present and intact but it looks much clearer or transparent than the surrounding agar. Record in your Data Table the presence and the diameter of this clearing.
 12. Compare data with your classmates.

Name _____
Date _____

Data Table

Well #	Detergent Solution	Egg Yolk Agar Initial/Final Diameter (mm)	Change in Diameter	Milk Agar Initial/Final Diameter(mm)	Change in Diameter	Starch Agar Initial/Final Diameter(mm)	Change in Diameter
1		/		/		/	
2		/		/		/	
3		/		/		/	
4		/		/		/	
5		/		/		/	
6		/		/		/	
7		/		/		/	

Analysis

- Using the data above, name the 3 detergents with the most enzyme activity among the six that you chose.

- Which, if any, of the detergents digested the following nutrients?

Protein (milk agar) _____

Lipids (egg yolk agar) _____

Carbohydrates (starch agar) _____

- Find the containers for these detergents and read the labels (always a good practice for the informed consumer!). Do the labels help you determine what might account for the differences observed among the different detergents? Explain your answer.

4. Think about food stains. Why would you want a detergent to be able to digest starches, proteins and lipids?

5. Using the class data, list below the most active detergents for each category.

Starch Digestion	Protein Digestion	Lipid Digestion

6. Look at the class data and determine any trends that may be apparent regarding the types of detergents, the names of the detergents, the ingredients listed on the labels, and name brands versus generic brands. Discuss your conclusions as a class. Summarize that discussion here.

Go to the Source

Introduction

Enzymes are biological catalysts and are produced by all living organisms. Most enzymes increase the rate of a reaction by at least a million fold. Another way to say this is that if the reaction would take one minute with an enzyme, it would require two years without an enzyme! Enzymes are also specific. Each enzyme recognizes a specific type of chemical by its shape and structure. It helps that particular chemical to react.

Enzymes are proteins that have a particular function in living cells. They break down complex molecules in food to produce smaller molecules that can be taken up by cells. All cells are surrounded by a membrane, called the cytoplasmic membrane, which does not allow large molecules to pass into the cell, but does allow small molecules to enter.

Bacteria are similar to human cells in some ways. For example, bacteria cannot use complex food sources in their environment because these complex molecules cannot pass through the bacterial cytoplasmic membrane into the cell. Some bacteria have solved this problem by making enzymes that can break down complex food molecules and then secreting those enzymes out into the environment. When these enzymes leave the bacterial cell they move into the environment around that cell and break down complex foods to simple molecules. These simple molecules then enter the bacterial cell providing it with the nutrients it needs to grow. In order to take advantage of all the complex food molecules in the environment, the bacterial cell would have to produce enzymes for all three major nutrients: proteins, carbohydrates and lipids. Because enzymes act specifically on only one class of molecules, different enzymes are required for different food molecules.

Purpose

In today's activity you will determine whether different strains of bacteria can secrete enzymes into the environment. You will see whether they produce enzymes that can break down the protein in milk agar, the lipid in egg yolk agar, or the carbohydrate in starch agar.

Materials:

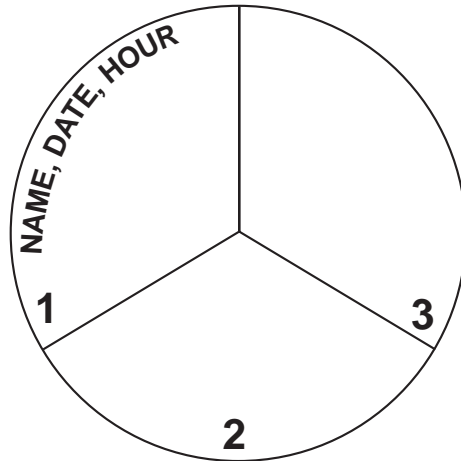
 Per student or team of students:

1 Bunsen burner	Lugol's iodine (50% concentration)
1 inoculating loop	1 metric ruler
10% bleach solution (disinfectant)	1 milk agar plate
plate cultures of 3 different bacteria	1 egg yolk agar plate
aluminum foil	1 starch agar plate

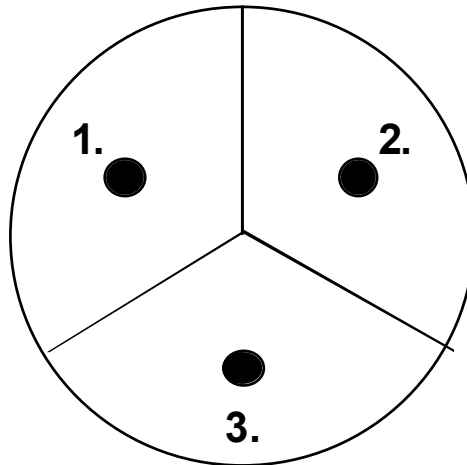
Procedure:

Although we are using species of bacteria that are harmless to humans, all bacteria should be handled with care. Wash your hands and disinfect your countertop before you begin and when you finish the procedure!

1. Label each of the three plates by writing (near the edge and in small letters) your name, class period, and today's date on the bottom. Using the marker, divide the bottom of the plate into three sections numbered 1, 2, 3 as shown below.



2. Place the plates right-side up on the counter. Using an inoculating loop, transfer a very small amount of bacteria from the plate provided to the center of the appropriate section of the plate. Keep the spot small. List the strains used in the table to the right.



4. Turn the plates upside down and place in the incubator (25°C) for 24 hours or at room temperature for 48 hours.

Name _____

Date _____

Observations

1. In order to observe the digestion of starch in the agar by bacteria, the plates must be stained with the starch indicator, Lugol's iodine. To stain, slowly flood the surface of the starch agar with iodine, replace the lid, cover the plate with aluminum foil, and let it sit while you observe the milk and egg yolk agar plates.
2. Observe the milk and egg yolk agar plates. Digestion of nutrients in the agar can be observed as a clear area around the growth of bacteria. Record your assessment of the digestive abilities of each species of bacteria on each type of nutrient agar plate. For positive results, use (+), (++) , or (+++) to indicate the degree of digestion. For negative results, use (-) to indicate no digestion of the nutrient.

Bacteria	Egg Yolk Agar	Milk Agar	Starch Agar
1.			
2.			
3.			

3. Observe the starch plate. The iodine will stain the starch purple. There will be clearing in the area where the starch was digested. Use the directions in step 3 to record starch digestion.

Questions

1. What type of chemical compound, secreted by the bacteria, causes the digestion of nutrients?

2. Do some bacteria secrete digestive enzymes outside the cell? How do you know this?

3. Milk has a high concentration of a particular protein. Which kind(s) of bacteria digested milk protein? How do you know?

4. Egg yolk has a high concentration of lipids. Which kind(s) of bacteria digested the lipid found in egg yolk? How do you know?

5. Which kind(s) of bacteria digested starch? How do you know?

6. Did any of the bacteria digest all three nutrients? If so, which?

7. You are the head chemist at a detergent company. You are to select one bacterial strain to use as the organism to produce secreted enzymes to use as an additive for a new detergent. Which of the bacterial strains that you or your classmates tested would you choose? Why?

Spot Be Gone!

Until now, all of these activities have been laboratory based. How does all of this relate to the real world and the practical application of laundry detergent? Design a real world application using this information that would test the effectiveness of detergents on food stains. Follow the steps below to begin your laboratory development.

1. Pose a question that you can answer by performing an experiment.
2. Develop a hypothesis using the if, then format.
3. Identify the variables. What is the independent variable? What is the dependent variable?
4. What would be an appropriate control?
5. Design your experiment. Be creative!

Spot Be Gone - Detailed Protocol

Introduction

When people buy laundry detergents they hope to get the best product for their dollar. Are all detergents basically the same? Do additives really help make your clothes cleaner? Do the detergents marked “Ultra” and “Super” do a better job than their “non-super” competition? Are generic brands equal to the brands that they mimic? Does using more detergent make a difference? If not, why do companies recommend using more for a pre-soak? Are they simply encouraging you to buy more?

Purpose

The purpose of this lab is to test the cleaning power of various detergents on stains of different origins.

Materials

- white 100% cotton fabric
- assorted foods to stain the cloth
- 1 quart jars or plastic containers with lids to hold the detergent solutions
- detergent solutions

Procedure

1. Cut cloth into squares of the same size (approximately 6” X 6”).
2. Stain each square with several of the foods available. Be sure to keep each stain discrete and separate from the others. Number each cloth in the upper right hand corner with ink and create a key, which records the exact location and color of the stains. Let dry overnight.
3. Place the stained cloth in a beaker or jar. Add detergent solution to cover the cloth. For one stained cloth, use plain water as a control. Record on the container which solution you chose and its concentration. Let these sit overnight.
4. Remove the cloth from the container and rinse with water. Allow to dry.

To evaluate each set of stains, create a grid with the detergent names listed vertically on the left side and the types of stains created across the top. Use a scale of +, ++, +++ or - to indicate the extent to which the stain was removed. Use the cloth soaked in water as the basis for comparison.

Name _____

Date _____

Observations

1. Which detergents removed all of the stains?

2. Which of the stains did most of the detergents remove?

3. Pool class data. Using this information, write a brief statement about the effectiveness of the detergents tested.

4. Check the labels of the most effective detergents and compare them to the others. Is there any ingredient found in the better products that is missing in the others?

Conclusion

Summarize below the results of this experiment. Now take this summary home and share your results with the person in charge of laundry.

Teacher Guide

Instructional Objectives

At the end of this unit of activities the student should be able to:

1. demonstrate the methods of scientific inquiry by
 - a. stating a problem
 - b. writing a hypothesis
 - c. performing an experiment according to given directions
 - d. gathering data
 - e. analyzing data
 - f. developing further investigations
2. demonstrate the following laboratory skills:
 - a. use a transfer pipet
 - b. use a metric ruler to measure diameters
 - c. observe zones of digestion (clearing)
 - d. sterile technique
 - e. use an inoculating loop to transfer bacteria
 - f. identify an appropriate control
 - g. construct a data table
3. demonstrate an understanding of the following scientific concepts:
 - a. enzymes:
 - I. their source
 - II. their activity
 - III. their specificity
 - IV. their use as additives to products
 - b. microbes
 - I. grow on various nutrients
 - II. may produce extracellular enzymes

Background

Through this series of lab activities, the students will investigate enzymes, microbes and biotechnology. Beginning with a simple digestion, the students will see that many detergents contain an active ingredient that breaks down JELL-O™. This ingredient is an enzyme that recognizes and breaks down proteins, a group of biological organic molecules of which gelatin is an example.

The next experiment investigates the role of enzymes in the degradation of other foods. Egg yolk, milk, and starch represent the lipids, proteins, and carbohydrates, respectively. Different enzyme additives in detergents are responsible for removing stains such as blood, coffee, grass and oils by acting on these organic molecules. Proteases digest protein, lipases digest lipid, and amylases digest starch.

Once the students recognize the variability among detergents and their enzyme additives, we go to the source of these enzyme additives, the microbes! The students inoculate bacteria on agar plates containing egg yolk, milk or starch to observe the activity of some bacterial enzymes as they digest nutrients found in the agar. The result is a clearing of the agar where the enzymes are present. Now the students can determine the industrial source of enzyme additives.

Finally, the students can apply what they have learned to determine whether detergents with enzymes remove food stains better than detergents without enzymes.

Sources of Supplies

Carolina Biological Supply
 2700 York Road
 Burlington, NC 27215
 (800) 334-5551

Description	Stock Number	Quantity
Cultures of:		
<i>Bacillus subtilis</i>	F6-15-4921	1 tube
<i>Escherichia coli</i>	F6-15-5065	1 tube
<i>Pseudomonas fluorescens</i>	F6-15-5255	1 tube
<i>Bacillus cereus</i>	F6-15-4870	1 tube
<i>Serratia liquifaciens</i>	F6-15-5448	1 tube
nichrome wire (or disposable plastic) inoculating loop	F6-70-3060	12
petri dishes (sterile, polystyrene, disposable)	F6-74-1350	500
sodium carbonate (Na ₂ CO ₃) - crystalline form		

Sigma Chemical
 PO Box 14508
 St. Louis, Missouri 63178
 (800) 325-3010

Description	Stock Number	Quantity
Lugol's iodine stock solution	L 6146	100 ml
polyethylene transfer pipettes (dropping pipettes)	213,500-3	500

Materials and Equipment Chart

Amount	Materials	Lab 1	Lab 2	Lab 3	Lab 4
15	JELL-O™ Plates	x			
15	Egg Yolk Agar Plates		x	x	
15	Milk Agar Plates		x	x	
15	Starch Agar Plates		x	x	
8	Lugol's iodine solution in dropper bottles		x	x	
*	detergent/cleaner solutions	x	x		x
**	Bacterial plate culture			x	
15	marking pens	x	x	x	x
15	metric rulers, flat	x	x		x
8	Bunsen burner			x	
15	inoculating loops			x	
15	3 cm plastic straw sections (can reuse)	x	x		
15	alcohol in beaker		x		
1 roll	aluminum foil		x	x	
1 box	toothpicks	x	x		
	incubator (if available)			x	
	refrigerator (if available)	x	x	x	
15	20cm x 20 cm squares of white cloth				x

*Make three tubes of at least 10 different detergents. Concentrations of 50% will work well. Detergent solutions should be made up fresh each day.

** Prepare bacterial plate cultures 24-48 hours before class. You will need 1 plate of each species for each group, or you can have groups share plates if you watch out for contamination.

Lab 1 “Mello JELL-O™”

This introductory activity can be set up in one class period and completed in the next class period. It is intended to be used as an exploratory activity with little introduction other than brief demonstrations of the laboratory techniques.

Background

Gelatin is a protein that is the gelling component of JELL-O™. Many detergents and cleaners have added enzymes that break down or digest a variety of organic molecules, one of which is gelatin. Enzymes called proteases digest (break bonds in) the protein chain so that the broken protein chains can no longer gel; therefore, JELL-O™ liquefies when exposed to these enzymes.

Preparations

1. JELL-O™ Plates: (for lab 1)

Mix JELL-O™ in the following proportions:

36 grams of regular JELL-O™ or generic gelatin dissolved in 100 ml boiling water.

We have had success using lime green JELL-O™, but have found that black cherry doesn't work as well.

Check pH of JELL-O™ with pH paper - Use crystalline** sodium carbonate (Na₂CO₃) to raise the pH to 8 which is the optimum for enzyme activity. * It will require a few grams of sodium carbonate, but the amount depends on the particular brand/ flavor of gelatin. As the pH becomes neutral, add sodium carbonate slowly because the pH will change quickly.

Pour 1 plate for each student or a team of 2 students. Use approximately 25 ml of JELL-O™ per plate. One 3 ounce box makes approximately 10 plates. Refrigerate. Wells are easier to cut when the JELL-O™ is firm.

2. Detergent and Cleaner Solutions: (for labs 1 and 2)

These solutions can be made once and used for both labs if used on the same day. Make certain that detergent solutions are made up fresh each day. The enzymes break down in solution.

Mix 50% solutions with tap water or distilled water. Put solutions in test tubes with a dropping pipette in each tube. Label the pipette with the name of the detergent.

*(Armour, S. and Fall, R. 1992, BioTechniques: Enzymes Made Easy. “The Science Teacher” vol. 59, #8, pps. 46-49).

**Powdered sodium carbonate is NOT recommended because it causes excessive foaming.

Detergents that have been tested include:

Dreft

Ingredients: Cleaning agents (anionic and nonionic surfactants, enzymes), water softeners, stabilizers (propylene glycol), soil suspending agent, color protection agent, dye transfer inhibition agent, suds suppressor, and perfume.

Tide

Ingredients: Cleaning agents (anionic and nonionic surfactants, enzymes), water softeners, stabilizers (propylene glycol), water, soil suspending agents, color protection agent, suds suppressor, and fabric brightener.

Cheer

Ingredients: Cleaning agents (anionic and nonionic surfactants, enzymes), water softeners, stabilizers (propylene glycol), water, soil suspending agent, color protection agent, dye transfer inhibition agent, suds suppressor, colorant and perfume.

ERA

Ingredients: Cleaning agents (anionic and nonionic surfactants, enzymes), water softeners, stabilizers, soil suspending agent, suds suppressor, fabric brightener, colorant and perfume.

Wisk

Ingredients: Cleaning agents (anionic and nonionic surfactants, enzymes), water softener (sodium citrate), stabilizer, buffering agent, and brightening agent.

All

Ingredients: Cleaning agents (anionic and nonionic surfactants), buffering agent, stabilizer and brightening agent.

Woolite

Ingredients: Not listed, but it does NOT have enzymes or bleach or phosphates or dyes. Cleaning agents are biodegradable.

Arm & Hammer

Ingredients: Cleaning agents (anionic and nonionic surfactants), buffering agents, soil suspending & stabilizing agents, fabric whitener, water, perfume and colorant.

Sample Data for Detergent Enzyme Activity

Initial Well Diameter was 6 mm. Measurements given are final cleared diameters in mm.

<u>DETERGENTS</u>	<u>JELL-O Well Diameter</u>
WOOLITE	8 mm
ALL	8 mm
TIDE LIQUID	16 mm
DREFT	7 mm

Answer Key: Mello JELL-O™ (Lab 1)

Hypothesis: A good hypothesis will identify variables in an "if, then" format. An example would be: "If I put Detergent A into a well, the jello will liquefy (get mushy)."

1. Observe the wells in the plate. Record any physical change in the JELL-O™ that you see around any of the wells.

Possible answers include: the JELL-O™ was digested, degraded, broken down (not melted).

2. Was there any change in the JELL-O™ around well #7? Explain.

No, the distilled water in well #7 should not react with the JELL-O™. This is the negative control.

3. Complete chart.

4. a) Which products increased the diameter of the well?

Answers will vary.

b) The chemicals responsible for changing the JELL-O™ are called enzymes. Refer to your textbook and write a definition for enzyme.

Answers will vary, but should indicate that enzymes are biological catalysts that bind substrates to increase the rate of a reaction.

5. Based on your observations, what ingredient in JELL-O™ do you think was changed?

gelatin

6. What ingredient in the detergent is probably responsible for the breakdown of the protein, gelatin?

Enzymes are responsible for the breakdown of the protein gelatin.

7. Suggest a name for the enzyme that breaks down the protein, gelatin.

Answers could include gelatinase or any reasonable alternative (e.g., jelloase)

Lab 2 "In The Clear"

This lab is an extension of lab 1. It demonstrates that enzymes degrade biochemical compounds such as lipids, proteins and starches found in food.

Background

The yolk in egg yolk agar contains lipids and provides the yellow color of the agar. The breakdown of these lipids by enzymes called lipases leaves a clear ring around the wells of the solutions that contain these lipid-digesting enzymes.

Similarly, milk agar contains milk proteins (casein). The white color provided by casein disappears in the presence of proteases.

Starch agar contains starch, a long chain carbohydrate, that can be digested by enzymes called amylases. This agar is transparent and must be stained with Lugol's iodine to show where the starch is present (the purple stained area) and where it has been digested (clear).

Preparations

1. Detergent and Cleaner Solutions: These solutions should be made fresh once a day.

Mix 50% solutions with tap water or distilled water. Put solutions in test tubes with a dropping pipette in each tube. Label the pipettes with the name of the detergent.

2. Lugol's iodine stock solution. Dilute one part stock solution (as purchased) with one part water. This is the Lugol's iodine students will use.

3. Preparing Agar Plates:

Use containers that are at least twice the volume of agar solution you are preparing. The agar may boil over during autoclaving if the container isn't large enough. (400 ml in a 1 liter flask is good.)

• Nutrient Agar: You may need to prepare a few nutrient agar plates to maintain bacterial cultures. Prepare nutrient agar plates as directed on the package.

Recipes below are for about 15-20 plates:

- Milk Agar: Measure 9.2 g nutrient agar powder and mix with 320 ml distilled water in a 1 liter flask. Measure 8 g nonfat dry milk powder and mix with 80 ml distilled water in a 250 ml flask. Close the opening of both flasks with aluminum foil or cotton plugs and autoclave both for 20 minutes at 15 p.s.i. Place the flask containing the agar in a water bath set at 50°C until it cools. If no water bath is available, agar is cool enough when you are able to hold the container with no discomfort. It will still feel hot to the touch. Add the milk solution to the cooled nutrient agar using sterile technique. Swirl until well mixed, but avoid bubble formation. Immediately pour 20-25 ml into each empty sterile plate. Store as directed above.

- Starch Agar: Prior to autoclaving, add 4.0 g of soluble starch powder to 400 ml nutrient agar (400mL distilled water and 8.75g nutrient agar). Autoclave 20 minutes at 15 p.s.i. Pour into plates when agar has cooled to 50°C and store as directed above.

- Egg Yolk Agar: Autoclave 400 ml nutrient agar for 20 minutes at 15 p.s.i. Allow agar to cool to 50°C before adding egg yolk so that it doesn't cook. Before adding egg yolk, surface sterilize a large egg by placing it in a beaker of alcohol for five minutes. Remove the egg and pour out the alcohol. Crack the egg on the side of the beaker from which you poured the alcohol. Carefully separate the yolk from the white in the usual way. Drop the egg yolk into the sterile 50°C nutrient agar. Swirl the broken yolk into the agar. Stir until yolk is evenly dispersed throughout the agar, but avoid bubble formation. Pour and store plates as directed above.

Allow poured plates to solidify and sit on the counter for several hours to minimize condensation on the lids of the plates. Plates should be stored upside-down in the refrigerator.

Answer Key: In The Clear (Lab 2)

1. Using the above data, name the detergents that showed the most enzyme activity among the six that you chose.
Answers will vary.
2. Which, if any, of the detergents digested the following nutrients?
Protein (milk agar)
Lipids (egg yolk agar)
Carbohydrates (starch agar)
Refer to data sheet.
3. Find the containers for these detergents and read the labels (always a good practice for the informed consumer). Do the labels help you determine what might account for the differences observed between the different detergents? Explain your answer.
Look for the label with the word "enzymes".
4. Why would you want a detergent to be able to digest starches, proteins and lipids?
Because most stains on clothes are due to foods and/or products of living things or organisms (i.e. grass stains).
5. Using the class data, list below the most active detergents for each category. (See chart on page 16.)
Answers will vary.
6. Look at the class data and determine any trends that may be apparent regarding the types of detergents, the names of the detergents, the ingredients listed on the labels, and name brands versus generic brands. Discuss your conclusions as a class. Summarize that discussion here.
Answers will vary.

Lab 3 "Go To the Source"

This lab investigation can be set up in one class period and completed in the next class period. It uses bacterial cultures to show students the source of some industrial enzymes - microbes! It also lets the students make a qualitative comparison of the enzymes produced from each species.

Background

Industry is active in the development of new and improved enzymes for use in their products. Enzymes have many industrial uses including brewing, food and drug production, paper making and textile manufacturing. They are used as additives in detergents and as cleaners for their stain removal abilities as well as the removal of fabric pills, those unsightly fabric balls that plague collars of shirts.

Enzyme manufacturers use recombinant DNA technology as well as traditional methods to develop new and improved enzymes. Traditionally, microorganisms that already make the desired enzyme are found. Through genetic engineering, the gene responsible for the production of this enzyme is modified to make even more of this product. Growth conditions for the microbe are optimized. The desired enzyme is then separated and purified for use in industry.

Preparations

1. Bacterial Cultures

Order bacterial cultures 1 to 2 weeks prior to the lab. Obtain one negative (-) species and 2 positive (+) species from the following list.

The cultures arrive on agar slants or plates. They can be stored in the refrigerator for a few weeks. Prepare sterile nutrient agar plates to subculture the bacteria for classes.

Bacterial Species	Microbial Enzyme Activity		
	Egg yolk (lipid)	Milk (protein)	Starch
<i>Escherichia coli</i>	—	—	—
<i>Bacillus cereus</i>	—	+	+
<i>Bacillus subtilis</i>	+	+	+
<i>Pseudomonas fluorescens</i>	+	+	+
<i>Serratia liquifaciens</i>	—	+	+

To subculture: use sterile technique and streak from the agar slant to the nutrient agar plates. Incubate only until plates show good growth and then refrigerate the plates.

Answer Key: Go To the Source (Lab 3)

1. What type of chemical compound, secreted by the bacteria, causes the digestion of nutrients?

Enzymes cause the digestion of nutrients.

2. Do some bacteria secrete digestive enzymes outside the cell? How do you know this?

Yes. Those bacteria that caused clearing of the medium secreted enzymes.

3. Milk has a high concentration of a particular protein. Which kind(s) of bacteria digested milk protein? How do you know?

See Microbial Enzyme chart on previous page.

4. Egg yolk has a high concentration of lipids. Which kind(s) of bacteria digested the lipid found in egg yolk? How do you know?

See Microbial Enzyme chart on previous page.

5. Which kind(s) of bacteria digested starch? How do you know?

See Microbial Enzyme chart on previous page.

6. Did any species of bacteria digest all three nutrients? If so which?

*Yes. *B. subtilis*, *P. fluorescens**

7. You are the head chemist at a detergent company. You are to select one bacterial strain to use as the organism to produce secreted enzymes to use as an additive for new detergent. Which of the 3 bacterial strains that you or your classmates tested would you choose? Why?

Answers may vary but should be one that digested all three types of nutrients.

Lab 4 "Spot Be Gone!"

Students can apply the knowledge they have gained through the three previous laboratory activities to design their own experiment to test the effectiveness of laundry detergents. However, a detailed protocol is provided on the following pages. You may wish to simply guide the students using this protocol, or you may provide the entire protocol to the students if you think they need more direction.

Background

Some manufacturers add enzymes and bleaching agents to their product in order to increase the effectiveness of the detergents. These enzyme additives are often harvested from bacteria (see background information for the lab "Go to the Source"). If these additives are present, they are simply listed on the label as "enzymes" and not specifically listed by type. Therefore, each detergent that has "enzyme" listed as an ingredient may actually include different types of enzymes and give different results in this lab.

Materials

1. White cotton (100%) squares of fabric, about 6 inches square
These can be cut from old sheets or T-shirts. While the actual type or source of cloth doesn't greatly matter, the material should be machine washable and the same material should be used with each detergent for comparisons to be valid.
2. Laundry detergents
To simulate actual laundry conditions a concentration of about 0.3% is appropriate. This is made by adding 3 ml of liquid detergent to 1 liter of water or 3 g of powder detergent to 1 liter of water (this is 0.3%).
To simulate a presoak concentration, simply increase the amount of detergent to 1.5%.
3. Food for stains. We tried the following and saw variation in stain removal among brands.

beef blood (from packaged steak or roast)	coffee
catsup	gravy
chocolate syrup	motor oil
chocolate milk	raspberry juice

Teacher Hints & Troubleshooting

A. Safety

1. Caution students that the JELL-O™ contains sodium carbonate and should not be eaten.
2. Remind students to wash their hands before and after all bacterial labs!
3. Have spray bottles of disinfectant around the room for disinfecting countertops before and after experiments.

B. Preparation

1. Not all flavors of JELL-O work well. Use lime or green. Do not use black cherry or grape.
2. If incubators are not available, bacterial cultures will grow at room temperature in 48 hours.
3. Powdered sodium carbonate can be used, but it will cause excessive foaming. If you use powdered sodium carbonate prepare the JELL-O in a very large container.
4. Allow the agar to cool to 50°C before pouring plates to reduce condensation on the lids.
5. Fresh bacterial plates work best. Make them two days before the lab.
6. To reduce your cost for the detergent labs, ask students to bring in samples from home: Be sure to have them include the name and the ingredient lists.

C. Lab Notes

1. Sterile wooden toothpicks or plastic inoculating loops may be used to streak bacteria if burners and wire loops are not available. Caution students not to dig into the agar - just lightly touch the surface of the agar.
2. Remind students to keep agar plates covered except when inoculating them.
3. Demonstrate sterile technique for transferring bacteria. If students have never handled bacteria, you may want them to practice with non-sterile plates.
4. Sometimes cloudy areas appear around the bacterial growth and wells. Do not measure these. Digestion of the nutrients will appear as a clear area.
5. If students overfill the well in the plate, the circles will not be uniform in shape, but the plates are still useable.
6. Use letter codes for the detergents. Identify the detergent when students are making observations for Lab 1 (JELL-O™ Lab). They can then write the name of the product in their data table.

During the observation of Lab 1 and Lab 2, have the detergent and cleaners available in the room so students can check ingredients.

Dear Parents:

As always, the biology classes are up to something!!

The project that your child is beginning this week includes a series of labs that test the effectiveness of laundry detergents. We will be looking at chemical reactions of detergents as they act on food stains like the ones that pop up in your laundry with unfortunate regularity. Food stains are produced by combinations of proteins, lipids and carbohydrates found in foods. What great information for them to bring home to you to make your housekeeping routines more efficient! At the completion of this project your children should be able to offer advice to you like the advice offered by consumer groups regarding detergents with various additives. Check in with them to see what they have learned.

If you are interested in having your own personal choice of detergent tested for efficiency, please feel free to send in about one scoop of powder or 1/2 cup of liquid in a zip lock bag, and we will be happy to include it in our sample. Be sure to include the name of the detergent and all ingredients listed on the container.

If you have any questions or comments feel free to contact me at school.