

## Overview and Purpose

### General Concept / Topic To Teach:

The 'spoiling' or 'rotting' of milk occurs because of chemical and physical changes brought about by single-celled organisms called bacteria which actually live in milk, using its sugars for food.

### Standards Addressed: ( from the Archdiocese of Louisville Curriculum Framework)

"Students learn that there are certain characteristics associated with living systems including the need for food, the production of waste..."

"Students learn that their environment will be affected by chemical and physical changes."

"Students understand scientific ways of thinking and working and use those methods to come to an understanding of the world around them."

### General Goal / Specific Objectives:

- Students will learn that changes in pH are hallmarks of the 'spoiling' of milk and that these pH changes are brought about by the digestion and processing of food into energy and waste by living bacteria.
- Students will learn that decreases in the pH of milk, created by living bacteria, are associated with a cascade of events which give rise to the physical and chemical changes which occur with rotting; curds, whey, and odor.
- Students will observe that bacteria are microscopic organisms which are smaller than eukaryotic cells, lack nuclei and respond to temperature differentials and antibiotics.

### Required Materials:

- |  |   |                  |
|--|---|------------------|
| • Whole or 2% milk   | • Antibiotic tablet (cipro or any broad spectrum) | • Safety goggles |
| • Sterile 50ml centrifuge tubes (or sterile urine specimen cups) | • Packets of lemon juice                          | • Notebooks      |
| • Small balloons   | • Microscope                                      | • Graph paper    |
| • Permanent marker   | • Microscope slides                               | • Pen            |
| • pH strips  | • Immersion oil                                   |                  |
| • Incubator  | • Pasteur pipette                                 |                  |
| • Refrigerator   | • Sterile gloves                                  |                  |
|  | • Tongue depressor                                |                  |

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### Winning Lesson Plan from Louisville, Kentucky

*CSI-MILK: What made the  
milk go SO bad?*

by Fred Whittaker  
St. Francis of Assisi

Subject: Microbiology  
Grade Level: 6<sup>th</sup>& 7<sup>th</sup>  
Duration: 3-5 50-Minute  
Class Periods

## Anticipatory Set (Lead-In / Main Ideas)

Students should already have a working knowledge of the concepts of pH and physical and chemical changes. This is a culminating lab which ties in the following lecture concepts and ideas:

- Milk is a complex, colloidal substance containing, amongst other ingredients; proteins ( such as whey and casein), lipids, carbohydrates and minerals such as calcium.
- Lactose is milk sugar; specifically a disaccharide composed of glucose and galactose.
- When organisms 'eat', they are actually 'breaking open' or disrupting the bonds of atoms in molecules such as glucose to release energy. The atoms of food metabolized for energy do not just 'disappear'; they remain as new, but smaller molecules which may have different chemical properties than their precursors.
- Antibiotics are compounds which will kill or inhibit the growth and metabolism of cells.
- Temperature will affect the rate at which cells metabolize.

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## Step-By-Step Procedures

Students are told that they must determine and prove why their milk 'goes bad'. Students are divided into tetrad groupings. Milk will be divided into 5–30ml samples per group. Students should always wear goggles and sterile gloves during this lab so as to not contaminate the samples.

- 5-30ml samples of fresh whole milk are decanted from the same 1-gallon source. Lids are replaced loosely on the top of the tubes to allow venting except where indicated. Students label each sample with a permanent marker according to treatment.
- 1 sample will be stored at room temperature.
- 1 sample will be stored in the incubator set at 37' C.
- 1 sample will be stored in the refrigerator at 15' C.
- 1 sample will have its lid replaced with a balloon which fits snugly over the mouth of the tube. This sample is placed in the incubator.
- ¼ of a 250 mg Cipro (antibiotic) tablet is added to one tube and shaken. This tube is stored in the incubator.
- Students will then use pH strips to monitor pH changes in all tubes, except for the balloon tube, three times per day for 5 days. These changes are recorded in log books and are graphed.
- The balloon tube is observed for changes in the balloon. It should partially inflate.
- Students will also observe changes in the physical appearance ( separation of curds and whey) and odor of the milk in the tubes and record these in their log book.
- On day five, students will discuss observations and deduce that something alive which is affected by temperature lives in the milk. This assumption will be challenged when students are given 1–30ml sample of fresh cold milk in a 50ml tube.
- Lemon juice will be added to this tube until the milk also curdles.
- Students will be asked how definitive proof of the presence of bacteria could be ascertained, since the addition of a non-living compound, lemon juice, also produced some of the same 'evidence' for life ( curd and whey separation) observed in their samples.
- Samples of spoiled milk will be loaded onto a slide with coverslip and viewed under oil immersion. Billions of cells, absolute proof for life in milk, will be seen moving about. Everyone cheers!
- OPTIONAL: A tongue depressor can be used by the instructor to scrape samples of cheek cells from his/her mouth. These can be loaded onto a slide simultaneously with milk bacteria for a size comparison between prokaryotic and eukaryotic cells. Methylene blue stain can be used for contrast.

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1. Students will observe that pH decreases other and physical changes occur most quickly in the incubator, thus students will note that temperature affects the rate of metabolism.
2. Students will observe the precipitation of casein proteins (curds) from solution ( whey proteins remain in liquid) and understand that great changes can occur when the pH of substance drops.
3. Students will observe that the balloon fills, partially, with a bad smelling gas phase waste product.
4. Students will observe little change in the tube containing antibiotics and will thus learn that antibiotic skill or reduce the metabolism of living cells.
5. Students will observe that bacteria are small, numerous and contain no nuclei or organelles.
6. Students will learn that energy production breaks down molecules, such as lactose, into other smaller molecules such as lactic acid and carbon dioxide.
7. Students will learn that waste products contain the same atoms which were present in the parent molecules.
8. Students will learn that the 'rotting' of milk is a complex chemical process caused by the metabolism of living cells present in the milk.

**Closure (Reflect Anticipatory Set)**

Students will be asked to reflect upon one or more of the following ideas:

1. Why did the rate of spoiling depend so much upon temperature? If warmer temperatures speed spoiling up, what do you expect the milk would do if it was kept at 50°C? What about near 100 °C?
2. If lower pH means that there will be more free hydrogen released into the milk, what can we guess about the structure of casein molecules which tend 'clump' up and precipitate out of the milk when the pH is low (while whey proteins do not)?
3. Why did the milk with Cipro not spoil? Why would it be a good or bad idea to put antibiotics into all of our milk?
4. Do you think that the bacteria in the milk were there when the milk was at the store? Where do you think bacteria in milk come from?
5. If there are bacteria in our milk why do we not get sick when we drink milk? Would you be surprised to find out that there are bacteria living inside of you? Why or why not?

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Students will be assessed upon the following criteria:

- Student worked cooperatively, performing his or her assigned duty and also interacting coherently with the group sharing data and insight which illuminated the big picture.
- Student learned to measure milliliters accurately into centrifuge tubes, and to use pH strips and graph paper to precisely measure and track the chemical and physical changes in the milk.
- Student demonstrated organization and focus; replacing samples into their proper temperatures with lids replaced for 5 days.
- Students practiced safety in the lab, always wearing goggles, disposing of pH strips in proper containers and cleaning and reporting any spills to the instructor.
- Students learned lecture content related to the spoilage of milk as revealed in a written exam.
- Student learned that chemical changes will precipitate other chemical and physical events.
- Students understand that larger molecules are broken down for food; to release energy.
- Students understand the origin of the atoms in waste products ( from precursors broken open for energy)
- Students understand that bacteria are single-celled organisms with no nucleus or organelles.
- Students understand that eukaryotic cells are larger cells than bacteria and that they do contain a nucleus.

**Possible Connections to Other Subjects**

Bacteria lend themselves well as gateways to understanding other topics such as:

- Genetic engineering ( plasmids etc) /Cloning
- Antibiotic resistance
- Food production; cheese, yogurt
- Beneficial Bacteria; human coliforms, decomposers, nitrogen fixation, antibiotic production
- Cloning
- Disease and Immunology
- Environmental considerations such as bioremediation
- Bacterial Morphology and Classification
- Gene expression
- Gene transfer /conjugation