First there was the Blob, then came Godzilla. Now comes…

The Bug That Eats Toxic Waste!

(AKA: The Growing of Microbial Life in Extreme Environments)

A Integrated Teaching Module Designed for Use in
Middle School Math & Science Classrooms,
Integrating Math, Science and Chemical Engineering.

Included are the plans to build a
"Low Cost Spectrophotometer"
for use in the Middle School/High School Science Lab

Dan Dillon
Estrella Middle School
3701 N 75th Ave
Phoenix, AZ 85033

Bob Schumacher
Garrison Middle School
906 Chase
Walla Walla, WA 99362

Under the mentorship of
Dr. Brent Peyton,
Department of Chemical Engineering
Washington State University
Pullman, WA 99164-2710

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INTRODUCTION TO TEACHING MODULE

This project examines the growth and survival of bacteria from environments that are "extreme" when compared to what is usually considered typical. Students are invited to learn how to sample natural environments and grow these organisms for lab research or class demonstrations. This "hands on" project provides a unique perspective about the diversity of life on earth and develops insights regarding the search for life on other planets.

This teaching module is being presented as an "interdisciplinary unit," which integrates math and science and requires the involvement of both teachers. Part of the unit would be conducted in the science lab under the supervision of the science teacher. The other part could be conducted in the math classroom with the math teacher. A middle school teaching team consisting of the English, Social Studies, Math, and Science teachers could all work together to develop a complete integrated unit if desired. This module will only address what will take place in the science and math classrooms.

Problem Statement

Unique environmental niches harbor a wide diversity of microscopic life that is largely undiscovered. In the classroom, study of these bacteria can provide students with insight into the factors that control populations of living organisms. For example, common table salt is toxic at high concentrations to many bacteria; however, a culture taken from a high salt environment requires salt concentrations that are high enough to kill other bacteria. This example shows that populations adapt to their environments and that even substances we consider safe can be toxic to populations from different ecosystems.

Chemical Engineering Application

A chemical engineer now working at Washington State University was asked to help isolate and grow bacteria that would thrive in and destroy the toxic waste held by a large corporation. The waste solution was found to have a salt content of approximately 1.5 M or (8.8%). A media was then mixed that replicated the chemical composition of the toxic waste at the corporation. A search then began for bacteria that would grow in a solution with the high salt concentration. Engineers searched for bacteria among the salt flats at Soap Lake in Washington State. Experiments will then be conducted to determine the growth rate of the bacteria growing in the prepared media.

Experiment To Be Conducted

By using bacteria taken from a natural high salt, high pH environment, students will develop and test various methods for growing these bacteria. Growth media for these bacteria will be made from inexpensive materials and substances commonly available to middle school students. Guidance will be given in designing tests that compare the effects of changes in pH and salt concentrations on the growth of bacteria from these environments. Students will use a spectrophotometer that has been constructed prior to beginning the experiments.

BACKGROUND INFORMATION

Prior to using this teaching module in the classroom there is some information with which the teacher should be familiar. There are also some pre-requisites that the student should understand prior to beginning this lesson. The writers would first like to share some basic information with the teacher. Following this information will be a list of the skills that the students should be comfortable with prior to beginning this lesson.

NCTM Standards

When the National Council for teachers of mathematics released their standards in 1989 they advocated the implementation of 13 curriculum standards for grades 5-8. This lesson was designed to integrate several of these standards. The standards addressed in this lesson are:

Standard 1: Mathematics as Problem Solving - "Problem solving is the process by which students experience the power and usefulness of mathematics in the world around them." **http://www.enc.org/reform/journals/ENC280/nf_28075s1.htm**

Standard 4: Mathematical Connections – "For many students, mathematics in the middle grades has far too often simply repeated or extended much of the computational work covered in the earlier grades. The intent of this standard is to help students broaden their perspective, to view mathematics as an integrated whole rather than as an isolated set of topics, and to acknowledge its relevance and use both in and out of school. Students should have opportunities to observe the interaction of mathematics with other school subjects and with everyday society." **http://www.enc.org/reform/journals/ENC2280/nf_28084ss4.htm**

Standard 10: Statistics–"In this age of information and technology, a need exists to understand how information is processed and translated into usable knowledge. Because of society's expanding use of data for prediction and decision making, it is important that students develop an understanding of the processes used in analyzing data." **http://www.enc.org/reform/journals/ENC2280/nf_28010510.htm**

Many of the other standards are also incorporated into this teaching module. They include, but are not limited to, the following: "Mathematics as Communication", "Mathematics as Reasoning", "Computation and Estimation", "Patterns and Functions", and "Measurement. With regard to this module the writers have identified the above three as the dominant standards, which have been incorporated. For a complete listing of the standards visit the NCTM web page at: **http://www.nctm.org**

Science Information

Math teachers come into the classroom with varied understanding of Chemistry and other science disciplines. The following is given as an introduction to the information presented in this

teaching module. Some Internet links are provided as sources for additional information to teachers that desire to learn more prior to implementing this teaching module.

Bacteria

What are bacteria? Bacteria (often referred to as "bugs") are single-celled microorganisms that lack a nuclear membrane, are metabolically active and divide by binary fission. Medically they are a major cause of disease. Superficially, bacteria appear to be relatively simple forms of life; in fact, they are sophisticated and highly adaptable. Many bacteria multiply at rapid rates, and different species can utilize an enormous variety of hydrocarbon substrates, including phenol, rubber, and petroleum. These organisms exist widely in both parasitic and free-living forms. Because they are ubiquitous and have a remarkable capacity to adapt to changing environments by selection of spontaneous mutants, the importance of bacteria in every field of medicine cannot be overstated. **http://gsbs.utmb.edu/microbook/intobact.htm**

How do bacteria multiply? Each single celled bacterium grows until there is enough material to form two separate bacteria. The parent bacterium then splits into two progeny bacteria. This process is known as binary fission. The time that it takes one bacterium to accumulate enough material to split is known as the generation time. Generation time varies greatly among different species of bacteria, from as short as twenty minutes for E. coli to as long as twenty-four hours for M. tuberculosis. The population growth trend for bacteria is an exponential curve. Each generation doubles in number. Begin with 1 then you have 2, then 4, then 8 and so on To view a sample 6th grade problem showing exponential growth visit "The Math Forum" at: **http://forum.swarthmore.edu/dr.math/problems/keiper9.17.97.html**

How do bacteria evolve? Bacteria, like all organisms, have a set of genes that demonstrate the physical, chemical and biological characteristics of each bacterium. Mutations may

occur when there is an error in the copying of genes from parent to progeny bacterium. Statistically, random mutations may happen as often as one in every million multiplications or as seldom as one in every billion multiplications.

Viruses can attack bacteria and humans alike. Bacterial viruses are known as bacteriophages. Bacteriophages invade bacteria, and can change the DNA of host bacteria. These actions alter the genotype of the bacterium. This process is known as *Transduction*. Sometimes, through *Conjugation,* bacteria may join together and exchange DNA. This also changes the genotype of the bacteria.

The evolution of bacteria is important because it allows the bacteria to adapt to their environment. The bacteria originally used in this teaching module are bacteria from an extreme environment. The pH of the solution in which this bacteria thrives is μ 9.0. The high salt content in the environment is about 12% or 125 grams per 1000 ml of liquid. The bacteria were discovered in the area around Soap Lake in South Eastern Washington State. This extreme environment is similar to the toxic waste solution at the site of the company that contacted the Chemical Engineering Department at Washington State University. The current WSU research deals with growing bacteria that would thrive in this toxic solution and rid the solution of the elements that would be harmful to the soil and water. To learn more about bacteria and how they grow visit: **http://ireland.iol.ie/~alank/CROHNS/PRIMER/bacteria.htm**

Chemical Engineering

The term “chemical engineer” is not intended to describe the type of work a chemical engineer performs. Instead it is meant to reveal what makes the field different from the other branches of engineering.

All engineers employ mathematics, physics, and the engineering art to overcome technical problems in a safe and economical fashion. Yet, it is the chemical engineer that draws upon the vast and powerful science of chemistry to solve a wide range of problems. The strong technical and social ties that bind chemistry and chemical engineering are unique in the fields of science and technology. This marriage between chemists and chemical engineers has been beneficial to both sides and has rightfully brought the envy of the other engineering fields.

The breadth of scientific and technical knowledge inherent in the profession has caused some to describe the chemical engineer as the "universal engineer." Despite a title that suggests a profession composed of narrow specialists, chemical engineers are extremely versatile and able to address a wide range of technical problems.

You may ask, "What have chemical engineers done for you?" The American Institute of Chemical Engineers (AIChE) has compiled a list of the "10 Greatest Achievements of Chemical Engineering". These are found below:

1) The Atom – help in splitting the atom and in isolating isotopes.
2) The Plastic Age – helping to make polymers a viable economic reality
3) The Human Reactor – Helping to improve clinical care leading to mechanical wonders such as artificial organs.
4) Wonder Drugs for the Masses – Today's low price and high volumes of antibiotics owe their existence to the work of chemical engineers.
5) Synthetic Fibers – Help reduce the strain on natural sources of cotton and wool.
6) Liquefied Air – Chemical engineers can separate air cooled to below 320^{o} F below zero into different components.
7) The Environment – Chemical engineers provide economical answers to clean up yesterday's waste and prevent tomorrow's pollution.
8) Food – Chemical engineers are at the forefront of food processing where they help create better tasting and more nutritious foods.
9) Petrochemicals – Chemical engineers have helped develop processes like catalytic cracking to break down the complex organic molecules found in crude oil into much simpler species.
10) Running on Synthetic Rubber – Chemical engineers played a prominent role in developing today's synthetic rubber industry.

To see a summary of each of these and to learn more about the field of Chemical Engineering visit: **http://www.cems.umn.edu/~aiche_ug/history/h_whatis.html**

The "Big Four" engineering fields consist of civil, mechanical, electrical, and chemical engineers. Of these, chemical engineers are numerically the smallest group. However, this relatively small group holds a prominent position in many industries, and chemical engineers are, on average, the highest paid of the "Big Four". For more information on the salary of engineers see: **http://www.cems.umn.edu/~aiche_ug/history/h_wage.html)** Additionally, many chemical engineers have found their way into upper management. A chemical engineer is either currently, or has previously occupied the CEO position for: 3M, Du Pont, Union Carbide, Dow Chemical, Exxon, BASF, Gulf Oil, Texaco, and B.F. Goodrich. Even a former director of the CIA, John M. Deutch, was a chemical engineer by training.

Today, there are approximately 70,000 practicing chemical engineers in the United States (57,000 of these are AIChE members). During the history of the profession there have only been about 135,000 American chemical engineers. This means that more than half of all the chemical engineers that have ever existed are contributing to society right now!

Due to the growing need for chemical engineers in the work force, many universities offer incentives for those majoring in the field. For example, Washington State University will fund the education of the graduate students that are accepted in their program.

TEACHING MODULE

Objectives

- The student will be able to explain how bacteria grow exponentially.
- The student will construct a spectrophotometer. This apparatus will be used to test the amount of light that is able to pass through the media that is placed within it.
- The student will be able to explain how the spectrophotometer constructed in class can be used to help measure the growth of bacteria.
- The student will be able to graph the results of the data gathered on the growth of the bacteria growing in their media.
- The student will be able to determine the growth rate of given bacteria growing in various media.
- The students will be able to adjust the readings from the low cost spectrophotometer so they can see the exponential growth of the bacteria in their media.
- The student will compare the growth rate of given bacteria in various media that have been mixed.
- The student will be able to interpret the results of the information gathered from the graphs and draw conclusions as to the best media for bacteria growth.

Flow Chart

The flow chart that follows in figure 1 displays the needs, skills and processes all students should possess **before** they can successfully engage in this module.

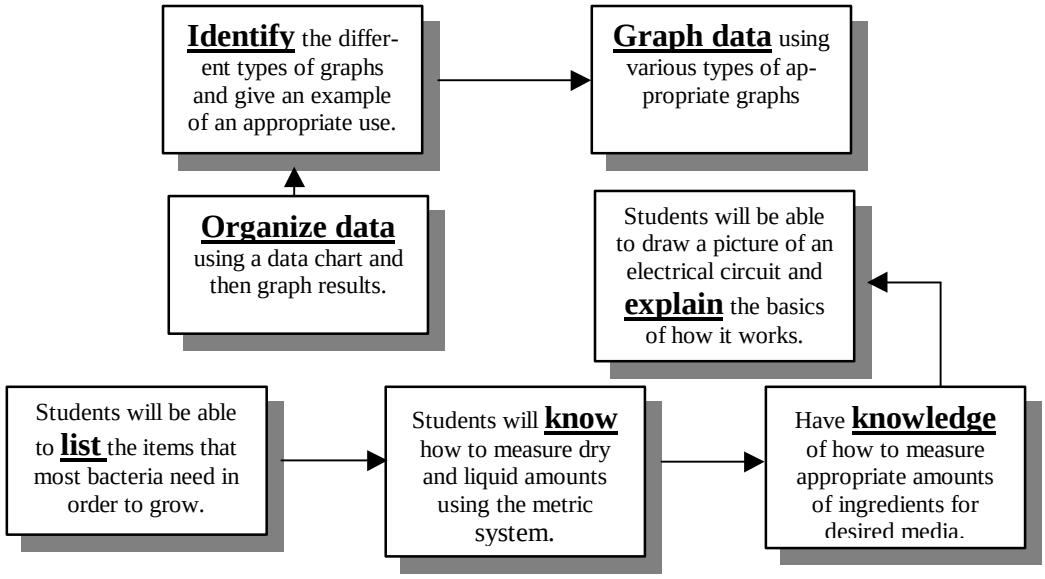


Figure 1

Prerequisite Skills

Math Skills Needed

1. Metric measurement
2. Basic operations involving decimals
3. Averaging three or more numbers
4. Organizing data in tables and charts
5. Graphing of data using scatterplot, bar and or line graphs

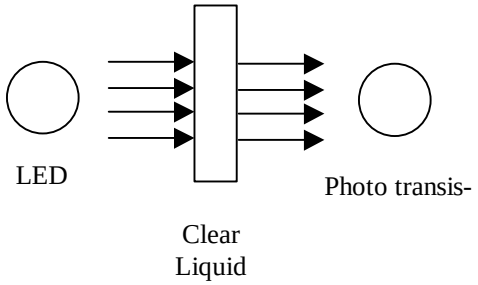
Science Skills Needed

1. Basic understanding of Bacteria and how they grow
2. Able to work with metric mass and volume measurement
3. Basic understanding of the electrical circuit
4. Safety precautions in the science laboratory

Plans for Low Cost Spectrophotometer

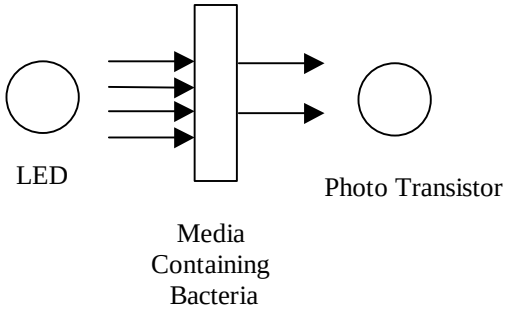
Background Information

A spectrophotometer is a device that measures the amount of light that passes through media. The spectrophotometer is extremely useful in collecting data on the growth of microorganisms that can not be seen with the naked eye. The light is emitted from an infrared LED (Light Emitting Diode) on one side of the liquid sample and is then received by a phototransistor on the other side of the liquid sample. The phototransistor gives a numerical reading in milliamps when read on a multimeter.



Negligible Light Loss

In clear liquids, little light is absorbed giving high transmission readings. Light is absorbed in media growing bacteria compared to negligible absorption when using distilled water. Since less light passes through the prepared media than clear liquids, like distilled water, the milliamp reading on the multimeter is slightly lower. As the bacteria grow, more and more light is absorbed. Correspondingly, less and less light is transmitted through the media. The spectrophotometer readings are therefore lower.



Light Loss Due To Absorption

Materials Needed for Spectrophotometer

Glue, Glue gun or epoxy
¼ inch plywood or shoe box
2"x 2" lumber under 6 inches long
D cell Battery
D cell battery holder (Radio Shack #270-403 $.99)
6 volt lantern battery ($4-$8) or power supply
4 feet #18 gauge wire
6 alligator clips (Radio Shack #270-380a $2.39)
1 high-output infrared LED (Radio Shack #276-143A $1.49)
1 infrared photo transistor (Radio Shack #276-145 $.99)
Small test tube or vial – 47-mm tall 12-mm diameter
Multimeter that reads 20-200 milliamps

(Prices may vary)

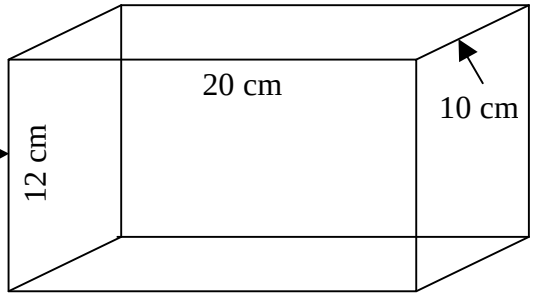
Construction of Spectrophotometer

The Case and Lid (See diagrams 1 & 2)

1. The case should be a least 12-cm tall, 10-cm deep and 20 cm wide. The case that was built is about 12 cm tall, 20 cm wide and 12 cm deep. A shoebox could be used instead, but this would not be very durable.

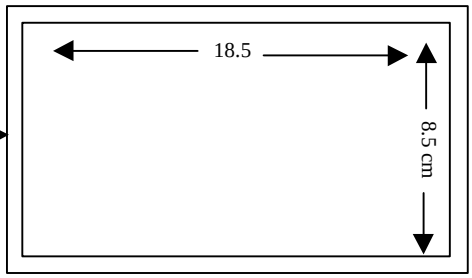
2. Cut the sides out of ¼ inch plywood and glue to form a box.

Diagram 1
Skeleton view of spectrophotometer with dimensions given.



3. Two pieces of ¼ plywood are needed for the top, one piece the same dimensions as the bottom of the box and the second piece having a length and width 1.5-cm smaller. Glue the smaller piece of wood centered on the larger piece. This will make a good, nearly light-tight seal with the sides of the case.

Diagram 2
Lid for case



The Sensor Block (See diagram 3)

1. The block is first drilled from the top, in the center, 3.7 cm deep. The hole should be 1.6 cm in diameter (5/8 inch). This size may vary if a different size vial or test tube is used. The hole should be a close fit but allowing for easy removal of the vial. The hole should be deep enough for the vial to stick up 1 cm above the wood block so it can be grasped with the fingers for easy removal.
2. The block is next drilled horizontally from the side. The hole should be the diameter of the LED (The LED and transistor are the same diameter). Drill completely through the block. It is important to also go through the center of the hole drilled for the vial.
3. Slide the LED in the horizontal hole on the left side. The LED goes as close to the vial hole as possible without hitting the vial.
4. Repeat the same process for the transistor but on the right side of the horizontal hole.
5. To prevent the two leads on each side from shorting out, push a tissue plug into each side of the horizontal hole between the separated leads or separate the leads and fill with silicon or epoxy. At least 1 cm of each lead should be exposed to solder a length of wire.
6. With the sensor completed, glue the sensor block in the center of the back wall with the leads exposed to the sides. The LED should be on the left and the transistor on the right.

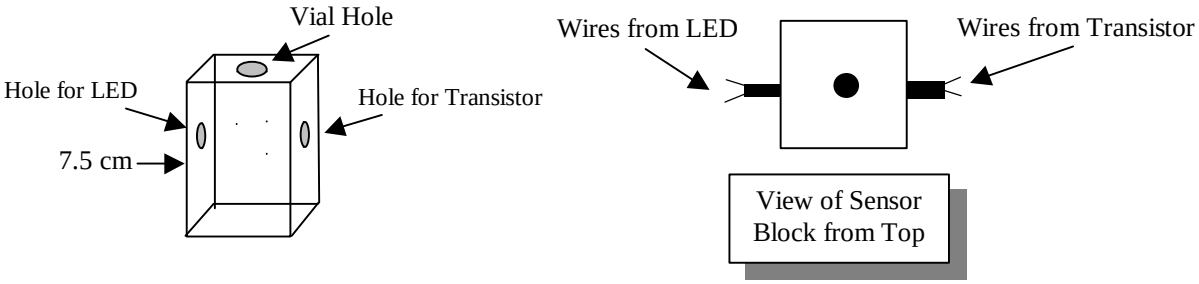
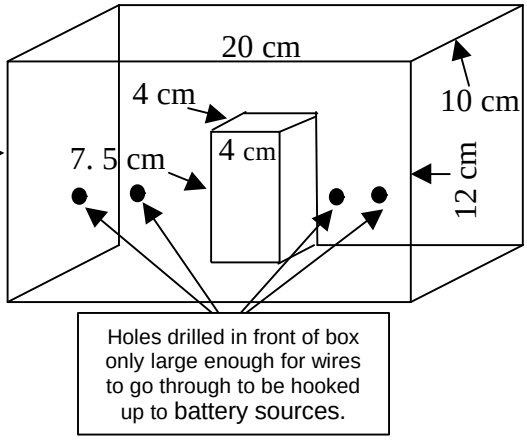


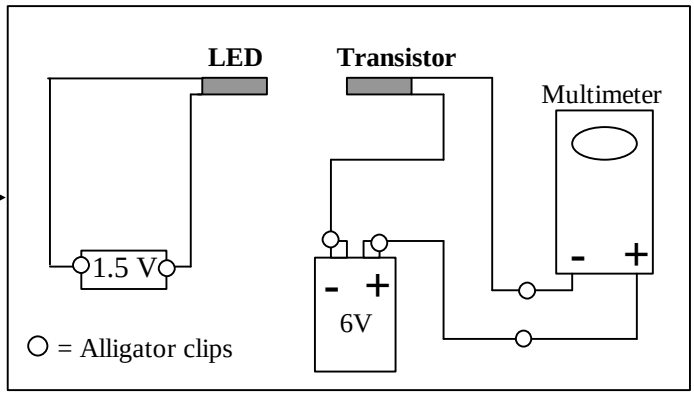
Diagram 3
Sensor Block

Diagram 4
Skeleton view spectrophotometer with sensor box in place and holes for wires to LED and transistor.



Instructions to Operate the Spectrophotometer (See Diagram 5)

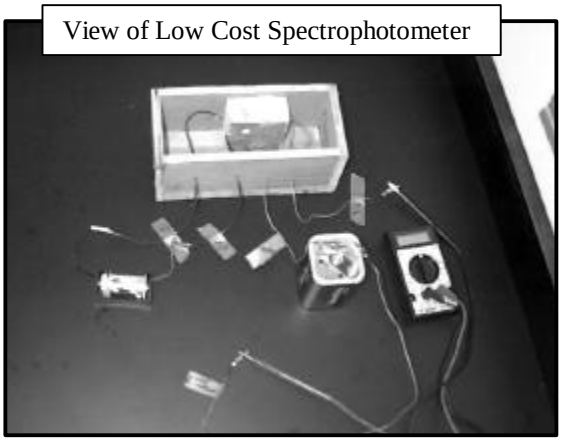
Diagram 5
Schematic is drawing of circuit connections to provide power to LED, transistor and multimeter.



1. Connect the wires on the right (LED side) to the battery holder for the 1.5-volt. If the multimeter shows no reading or negative reading, the connections to the 1.5-volt battery holder may need to be reversed.
2. On the left side, connect one transistor wire to the 6-volt battery or power source.
3. Connect the other left side wire (transistor) to the one-multimeter probe.

4. Connect the wire with the alligator clips from the battery to the other multimeter probe.
5. Turn the multimeter to the milliamp settings. The writers used a 200-milliamp setting. You need to check that you are getting a positive reading. If the multimeter reading is negative reverse the alligator clips connected to the 6-volt source.
6. If more than one low cost spectrophotometer is built, care must be taken to install the LED and transistor within the hole at the same angle (Perpendicular to the vertical hole). Different angles will result in each spectrophotometer giving different milliamp readings. One way to adjust the additional spectrophotometer units is to connect the circuit and glue the LED and transistor when adjusted to yield the same readings as the first spectrophotometer completed.

View of Low Cost Spectrophotometer



The spectrophotometer is now ready to use. Be sure to place the top on the case when getting readings. Don't rush when obtaining readings. The reading will oscillate when first media is first placed in sensor box. Wait about 30 seconds for the oscillation to stop. To prevent unnecessary battery loss disconnect a wire from each battery and turn off the multimeter when finished. Check battery strength often with a battery tester to insure consistent spectrophotometer function.

Media Formulas

Background Information

The bacteria with which the writers are working in this teaching module live in rather extreme environmental conditions. These halophytic bacteria are a diverse group of prokaryotes that require at least 1.5 M (8.8%) NaCl (salt) for growth, most require a 17-23% salt concentration for optimal growth. Their saline environment is common in hot, dry areas of the world, and such climatic conditions encourage evaporation and further concentrations of the salts.[1] The

[1] Brock, Thomas D. and Madigan, Michael T., Biology of Microorganisms, Prentice Hall Publications, Englewood Cliffs, New Jersey, 07632, 1998, p.767-771.

diagram below shows the writers collecting soil samples from salt flats around the Soap Lake area north of Moses Lake, Washington.

Bacteria from these extreme environments have not been found to be harmful to humans. This makes them relatively safe organisms to use in the classroom. However, care should be taken to wash hands after working with any microorganisms. The writers worked with the bacteria "*Halomonas campusalis*" which was found as a result of a search for a high salt, high pH bacterium that could degrade nitrate.[2] The bacteria were isolated from Soap Lake, Washington as a new unidentified species by Melanie R. Mormile, Margaret F. Romine, Thomas J. Bailey, and Brent M. Peyton. This bacterium is of special interest for two of reasons. First, these bacteria could prove commercially beneficial as they degrade nitrates, a common water pollutant. Second, organisms from extreme environments on the earth may help us to understand organisms that may exist in some of the extreme environments of other planets or their moons.

Bacteria, like all organisms, need something to metabolize in order to survive and grow. Below, you will find the media used by university researchers to grow these high salt, high pH environment bacteria. The writers have adapted this media recipe to allow common ingredients to be used. Experimentation with growing these bacteria in the "store" media has had positive results. (See Appendixes A & B)

[2] Mormile, Melanie; Romine, Margaret F.; Bailey, Thomas J.; Peyton, Brent M.; "Halomonas campusalis", International Journal of Systematic Bacteriology, in preparation for 1998 presentation.

Introductory Notes on Media

1. Molasses was tried as a source of sugar but, the media was too dark to give good results on the spectrophotometer.
2. Glucose tablets for diabetics could not be used for a sugar source because the inert material in the tablets caused an undesirable precipitate.
3. The brewers yeast will produce some precipitate. Pour off the top part of the media. Do not use the precipitate portion.
4. Pour about 100 ml media into 250 milliliter flasks and stopper each flask with a cotton ball or a piece of foam rubber. The stopper should allow air to enter in order for the bacteria to breath but should not allow air born particles to enter that could allow unwanted bacteria or fungi.

Research Media

1000 ml DI (De-ionized) water
125 g. NaCl
.5 g. KH_2PO_4
1.0 g. NH_4Cl
1.0 g. Yeast Extract
4.0 g. $Na_2B_4O_7$ x 10 H_2O
10.0 g. Glucose or 10 ml Lactic Acid Syrup
Adjust pH to 9 with 5N NaOH

Store Media #1 (See Appendix A for Data and Graph Using Media #1)

1000 ml Distilled Water
1.5 g. Miracle Grow® (Replacing the KH_2PO_4 and NH_4Cl)
1 g. Yeast Extract (Science vendor 100 g/$29.00)
4 g. Borax® (Replacing the $Na_2B_4O_7$ x 10 H_2O)
10 g. Table Sugar (Replacing the Glucose)
125 g. salt (Replacing the NaCl)
Adjust pH to 9 with NaOH

Store Media #2 (See Appendix B for Data and Graph Using Media #2)

To 1000 ml Distilled Water add 1 g. brewers yeast & boil for 15 minutes – Cool to room temperature (Use only the top portion of the mixture)
1.5 g. Miracle Grow®
4 g. Borax®
10 ml Corn Syrup
125 g. Salt
Adjust pH to 9 with NaOH

Store Media # 3 & #4

Prepare the media according to directions for Media 1 or Media 2 but use 10 grams of table sugar with the brewer's yeast for one Media 3. Media 4 could be made using yeast extract with the corn syrup.

Bacteria Preparation for Classroom Experiments

1. In order to locate extreme environment bacteria, collect soil samples from areas of known high salt, high pH or high evaporation (dry deposits may be visible on soil surface.
2. From an area that appears to have salt deposits from high evaporation collect soil about 1-2 inches below the surface.
3. Place 10 grams of dirt in 100 ml of media.
4. Allow this media & soil to sit about 5 days. Swirl the media once a day.
5. On the 5th day prior to swirling take about 10 ml of the supernatant of the media and place in a flask of fresh media. If the media becomes cloudy, you likely have some extreme environment bacteria growing.
6. The media determines the bacteria that develop. Most types of bacteria are unable to live in this high salt, high pH media.
7. To learn more about isolating and growing bacteria visit:
   http://ems.cea.wsu.edu/che/94modules/goldberg/bacteria.html

Lesson Plans

Time Required: A minimum of six, 45 minute class periods will be needed. Extended time will be needed for the actual running of the tests. At the conclusion of the testing another day will be needed for the presentations from each group of students.

Materials Required for Spectrophotometer (See section on Spectrophotometer)

Materials Required for Growth Media: (Enough for all classes)

Table Sugar – 1 LB bag
Table Salt (non iodized) – 3 containers
Miracle Grow® – Granulated, small box
Borax® Soap – Regular sized box
Distilled water – 2 Gallons per class
Yeast Extract – 100 grams ($30/100 grams – Science Vendor)

Day 1 - MATH CLASS

Students will have a chance to work with the concept of exponential growth today. Students would be given Handout #1 (D) and asked to work with a group to provide a solution. They will be asked to share with the class their answer and explain why they think their solution is reasonable. For the answers refer to Appendix F.

Suggestions for the teacher:

- Give each group a large piece of butcher paper along with crayons and/or markers and tell them to use this when they make their presentation to the class.
- You may mention to the students that they can draw pictures, create graphs, or make tables for their presentation to the class.
- Remind the students that the question is: "Which method would you choose and why? There is no right and wrong answer. After all have completed their presentations then you may ask: "If the question was, "Which method would give you the most money?" Now would there be a correct answer?

Day 1 – SCIENCE CLASS

Students should review metric mass and volume prior to preparation of the bacterial media recipes. Collect 10-20 small objects such as staples, paper clips, marbles, ½ oz. Lead weights, 5 cm wood dowels, etc. Using 10 ml to 100 ml graduated cylinders, begin with a known volume of water (about ½ full). For smaller objects, use a small graduated cylinder. One object is placed in the cylinder at a time. Record the increase in volume as the volume of the object. For a sample recording chart see Appendix J.

To review metric mass, use metric balance scales. Be sure to cover the procedure for checking the calibration of the balance scale. Balance scales can vary in mass reading from one day to the next. The same objects may be used as in the volume lab. It is important to use exact measurement. Do not round the answers. For a sample recording chart see Appendix K.

Day 2 – MATH CLASS

Continuing the study of exponential growth, the teacher needs to obtain an appropriate video showing how bacteria grow exponentially (There are many good videos available for teachers. First check with your district office media center. If one can not be found there then you may want to check with the media center at the State Department of Education.). The teacher will share with the students that the fastest growing bacteria known is the E coli bacteria. It doubles in population every 20 minutes. Beginning with one bacterium students will be given Handout #2 (Appendix F) and asked to graph the increased population of the bacteria for 9 hours. The teacher will then facilitate a discussion based on the students graphs and the following two questions: "What do you think the graph would look like after 1 day? What do you think it would look like after 2 days?" The teacher will then share with the students that in 48 hours there would be enough E. coli to occupy the entire volume of the earth (1.63×10^{24} cubic meters). (See Appendix G for math calculations) In only about two additional hours, these bacteria would weigh as much as the earth (6.6×10^{21} tons)!

The teacher would then facilitate a discussion as to "Why hasn't the E. coli bacteria taken over the world"? The point should eventually be made that the bacteria run out of things to eat, or eventually die due to their own toxic waste (lucky for us!).

Day 2 & 3 – SCIENCE CLASS

INTRODUCTION: Review the previous lesson on metric volume and mass. Some points to consider are:

- How do you find the volume of objects?
- What is the correct method of reading a volume using a graduated cylinder?
- How are volume and mass numbers labeled?
- How do you take the volume of floating objects such as a wood dowel?

- How are measurements of mass recorded in as much as most measurements will not be whole numbers?
- How do you find the mass of substances such as salt? (Substances should not be poured directly on the balance scale. Use a piece of paper and subtract it's mass.)

SPECTROPHOTOMETER: Introduce the spectrophotometer that will be used in the next class to simulate the exponential growth of bacteria. Make a transparency (Appendix L) in order to make the use of the spectrophotometer clear. Main points of discussion:

- The LED is connected to the 1.5 volt D-Cell
- The LED will not function unless connected correctly. If here is no milliamp reading, reverse the connections to the D-Cell.
- The vial or test tube needs to be marked with a marker so that the same side always faces forward. We have found that the reading will be higher or lower unless the vial always has the same orientation.
- The vial must always be wiped off before being placed in the hole in the sensor. Incorrect reading will result if the vial is wet. Even fingerprints can cause incorrect data numbers.
- The phototransistor is connected to the 6 volt with the multimeter connected in line.
- The phototransistor must be connected so as to give a positive reading. If no reading or a negative reading is observed, reverse the 6-volt connections.
- The multimeter must be on a 20 to 200 milliamp setting. Check again for a positive reading on the scale. Reverse the multimeter and/or 6 volt connections to get positive readings. Even an empty test tube will give a reading in the air of 17 to 30 milliamps. **NOTE:** It is possible to ruin a multimeter if it is connected incorrectly.
- Clean the vial with distilled water after anything is placed in the vial. Dry the vial after the last distilled water use. This will prevent water spots that will result in poor data numbers.
- The LED phototransistor will take 30 seconds or longer to get a stable reading., If the numbers are slowly increasing, wait until the highest number is reached. It is possible that after the highest number is reached, the numbers may go down several numbers and then climb again. This could result in the need to average the numbers on the multimeter.
- Suggestion - Teachers may wish to print the above discussion points and have them available for each lab group for the next science class.

VIDEO: A video on bacteria would give students background on the organisms they will soon be using in a lab.

Day 3 & 4 – MATH CLASS

Students will continue to talk about the mathematics involved in finding the volume and mass of the E Coli after 48 and 50 hours. They will also graph the data they are obtaining in the science class with the Carrot Juice experiments. Butcher paper again will be given to each group and they will be asked to present their data to the class. They will be asked to explain why they think they obtained the results that did.

Day 4 & 5 – SCIENCE CLASS

For the next two days students will run dilution labs using the "Budget Spectrophotometer". Students will be given Handouts 5 & 6 (Appendix L & M). Handout 5 is a table that can be used to help gather and organize data. Handout 6 will provide a grid to use to graph their results. Time should be spent reviewing the need for accuracy in gathering data and in graphing.

Students will start with a 10 ml of tap water. A reading will be taken using some of this 10 ml solution. Add 2 ml of carrot juice to this solution, swirling the beaker to mix solution. Another reading will then be taken. This will continue until a total of 10 ml of the carrot juice has been added. The teacher may want to also try this same procedure with tomato juice to see if the same results are obtained. Students will record the data on data sheets.

Day 5 & 6 - MATH CLASS

The math teacher can continue to work with the class on gathering and graphing data (There is an assumption that the science teacher will already have bacteria available for student use in their experiments. Directions are given in the media section for growing bacteria. The students could also mix the media if so desired). See Appendix C for a sample of data taken with the low cost spectrophotometer and adjusting the mathematical data. Refer to Appendix G for

the rational as to why the data must be "adjusted" and how it is adjusted when the data is taken using the low cost spectrophotometer.

Theoretical data of the population growth of the world could be examined and graphed. **http://www.census.gov/ipc/prod/wp96/wp96005.pdf** The teacher could then facilitate a discussion relating the population growth with the growth of the E Coli bacteria. Are there any similarities? Are there any differences?

Day 5 & 6 – SCIENCE CLASS

During the following two days the students will prepare the "media" that they will use to grow their bacteria. One of the media will be the STANDARD media given in this teaching module. The second media could contain varying amounts of sugar but the same amount of yeast, borax, miracle grow, salt and distilled water. Would the bacteria grow twice as fast with twice as much sugar? Will it grow without sugar?

Day 7 or until the conclusion – SCIENCE CLASS

Each group will be asked to stir their media at least two times each day. Students will decide in their group who has this responsibility. Students are to test their 2 growth mediums daily using the spectrophotometer. Time will be spent during the first day reviewing proper techniques for taking samples and recording data. A minimum amount of class time will be used until both the science teacher and math teacher has prearranged for the testing of media to cease.

Second to last day – MATH CLASS

Students will be asked to evaluate the data gathered in their science class with their group members. They will make several inferences based on their gathered data:

- Which of their two media provided the best growing environment for their bacteria?
- What conclusions can they draw from the results of their experiment?
- Did the bacteria growth reach a plateau during the testing period? (After testing some Mediums for up to three weeks the writers never saw the growth reach a plateau).
- If so, what happened to the data on the following days?
- What conclusions can be drawn from these observations?

Each group of four students again will be given butcher paper and markers. They will be told that they will present their data to all students the following day.

Final Day – MATH CLASS & SCIENCE CLASS

On the final day the two classes will meet together each period. During this time each group will make a 5-minute presentation to the entire group. They will explain their procedures, describe the second media they mixed, and present the results of their experiment to the group.

Possible Extensions

An obvious extension would be to allow students to gather samples of soil around your school and see if they can grow any bacteria in their media. They would need to follow the directions in the media section for obtaining soil samples and growing bacteria. They can also visit the web site given in the media section for more information on growing bacteria.

A teacher may also want to have the students work on building an even better spectrophotometer. If CBL's (Calculator Based Laboratories) are available it may be possible to incorporate a data probe into the construction of the Spectrophotometer. This would allow data to be gathered automatically and then graphed on the calculator or imported into a spreadsheet program on the computer.

Assessment of Student's Research Project

During the final presentation both the science and math teacher will assess the work that the students have done. The understanding gained through this investigation can not easily be assessed through a traditional testing method. Students should have a chance to "reflect" on their experience. Provide the students with some prompts and have them respond to the prompts. The responses can be written on paper or done orally between the teacher and student. Remember, you are not assessing the student on their writing or speaking skills. You want to find out how much the student has learned through this integrated teaching unit. You want to try to avoid questions that solicit yes or no answers.

POSSIBLE PROMPTS

- You will need to ask the student to explain what was the purpose of this project.
- Ask the student to explain why their group selected a certain media as the best growth media.
- Ask the student to try and tell you what they would change if they were to mix up a third media. Ask them how they think this would change the final results.
- Ask the student to tell you about some of the math skills they used during the project and some of the science skills they had to use.
- You may want to ask the student how Chemical Engineers could use research like they just conducted to make life better for them and their future children

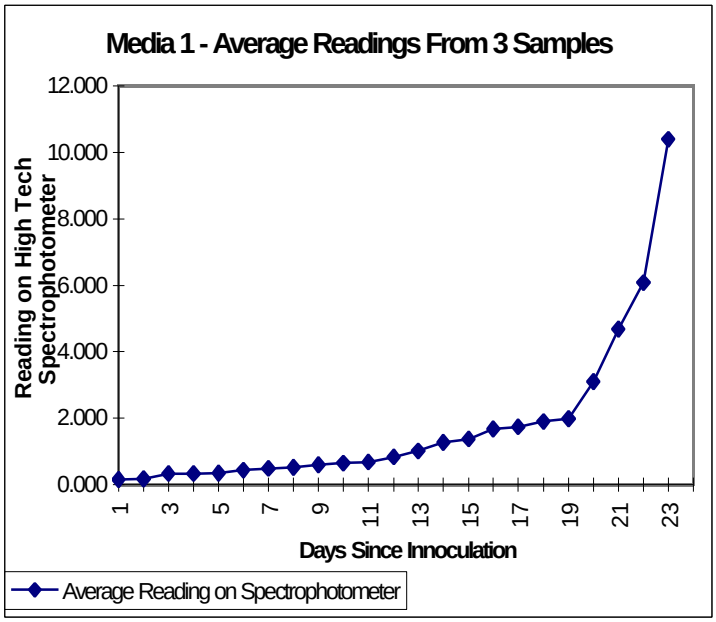
In closing Bob Schumacher and Dan Dillon would like to thank Dr. Brent Peyton for his guidance and mentorship in the writing of this teaching module.

Appendix A

Data and Graph of Bacteria Growth Using Media 1

Began on 6/30/98
Researchers: Bob Schumacher and Dan Dillon

Days	Average Reading on Spectrophotometer
1	0.160
2	0.166
3	0.334
4	0.338
5	0.351
6	0.434
7	0.482
8	0.518
9	0.591
10	0.643
11	0.682
12	0.838
13	1.010
14	1.270
15	1.378
17	1.677
18	1.743
19	1.892
20	1.990
21	3.102
22	4.692
23	6.080
24	10.400
25	



Media 1: To 500 ml DI add:

62.5g	Table Salt
.75 g	Miracle Grow
.5 g	Yeast Extract
2.0 g	Borax Soap
5 g	Table Sugar

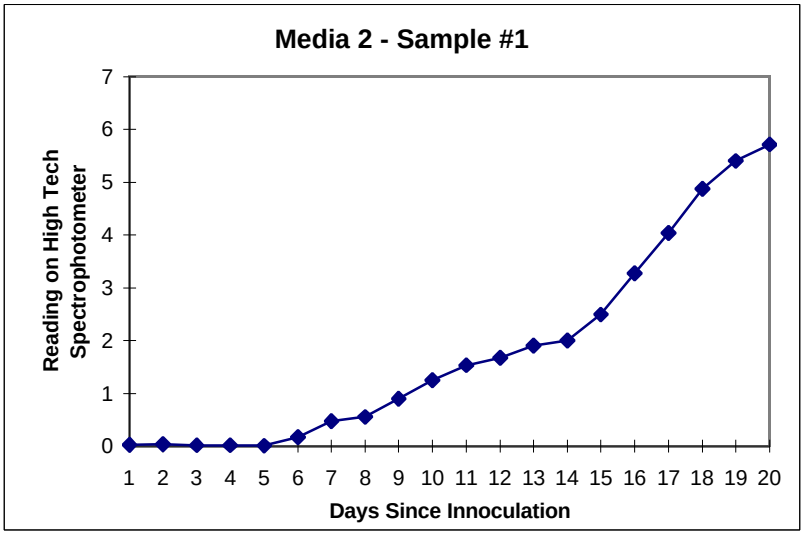
Adjust pH to 9 with NaOH

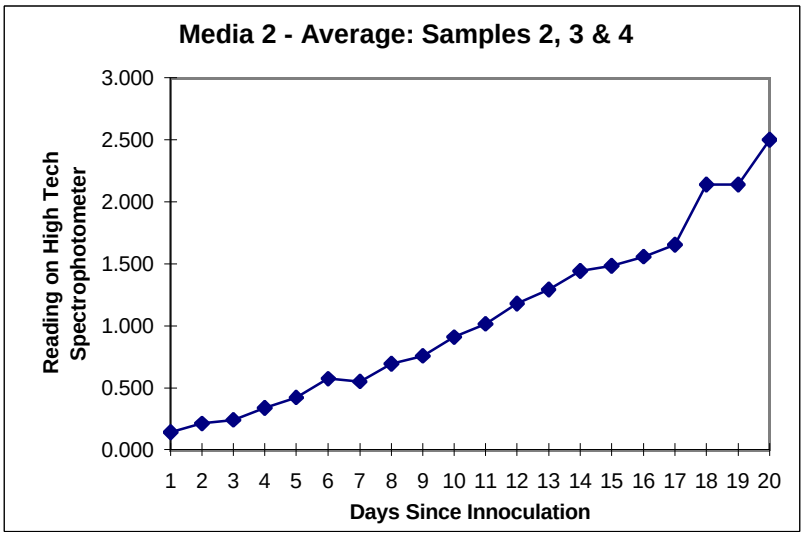
Appendix B

Data a graph of Bacteria Growth Using Media 2

Growth Media 2 Using Karo Syrup and Yeast Extract
Began on 7/1/98
Researchers: Bob Schumacher and Dan Dillon

Days	Sample #1	Average Spectrophotometer Readings
1	0.03	0.144
2	0.043	0.209
3	0.024	0.243
4	0.024	0.336
5	0.012	0.422
6	0.174	0.572
7	0.47	0.551
8	0.551	0.691
9	0.898	0.758
10	1.254	0.908
11	1.537	1.017
12	1.68	1.179
13	1.909	1.290
14	1.995	1.440
15	2.5	1.485
16	3.276	1.560
17	4.04	1.654
18	4.88	2.140
19	5.4	2.140
20	5.72	2.500
21	6.28	2.980
22		





Media 2: To 500 ml DI add:	
62.5g	Table Salt
.75 g	Miracle Grow
.5 g	Yeast Extract
2.0 g	Borax Soap
5 ml	Clear Karo Syrup

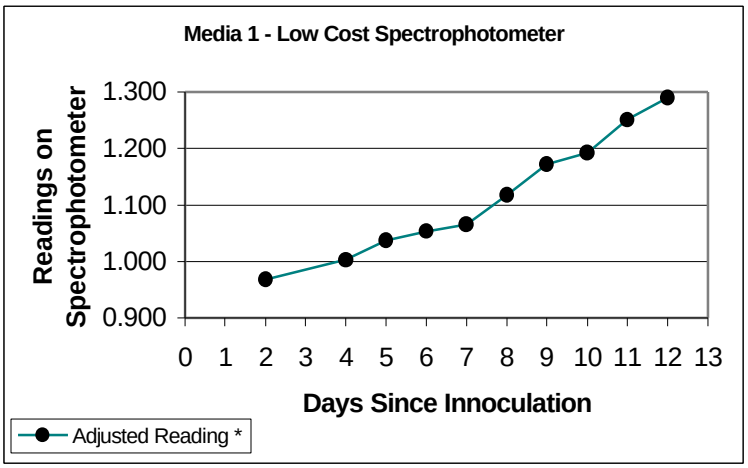
Appendix C

Data and Graph of Bacteria Growth Using Media 1

USING THE LOW COST SPECTROPHOTOMETER

Growth Media 1 - Using Table Sugar and Yeast Extract
Began on 7/6/98
Researchers: Bob Schumacher and Dan Dillon

Days	DI	Standard	Sample 1	Sample 2	Sample 3	Average	Adjusted Reading *
2	44.7	47.3	46	46.4	46.2	46.200	0.968
4	46	46.9	45.6	45.9	46.1	45.867	1.003
5	45.8	46.5	44.4	43.8	44.3	44.167	1.037
6	46	47.9	43.4	43.8	43.8	43.667	1.053
7	44.5	46.9	40.9	42.2	42.2	41.767	1.065
8	43.9	45.7	38.5	39	40.4	39.300	1.117
9	43.6	44.8	36	37.5	38.1	37.200	1.172
10	43.7	45.8	35	36.4	38.5	36.633	1.193
11	44.5	46.9	34.1	35.8	36.8	35.567	1.251
12	41.3	43.5	31.1	31.3	33.7	32.033	1.289



Media 1: to 500 ml DI add:

62.5 g	Table Salt
.75g	Miracle Grow
.5 g	Yeast Extract
2.0 g	Borax Soap
5 g	Table Sugar

Adjust pH to 9.0 with nNaOH

* Refer to Appendix G to see how to adjust the low cost spectrophotometer readings.

Appendix D

Handout 1

Rich Aunt Lucy

The Problem: You received a letter from your rich Aunt Lucy. In the letter she said she wants to give you some money. She gives you three choices as to how to receive your money. The choices are:

Choice One: She will give you 10 dollars at the end of the first week. At the end of the second week she will give you 1 ½ times the amount that she has already given you. At the end of the third week she will give you 1 ½ times the total amount that she has given you. At the end of the fourth week she will give you 1 ½ times the total amount that she gave you the previous three weeks.

Choice Two: She will give you 1 penny on day one. She will double that amount to 2 pennies on day two. On day three she will double that amount and give you 4 pennies. This will continue until the end of the 28^{th} day.

Choice Three: She will give you $100 in cash on day one.

Which of the three choices would you choose and why?

Appendix E

Answer to Rich Aunt Lucy Problem

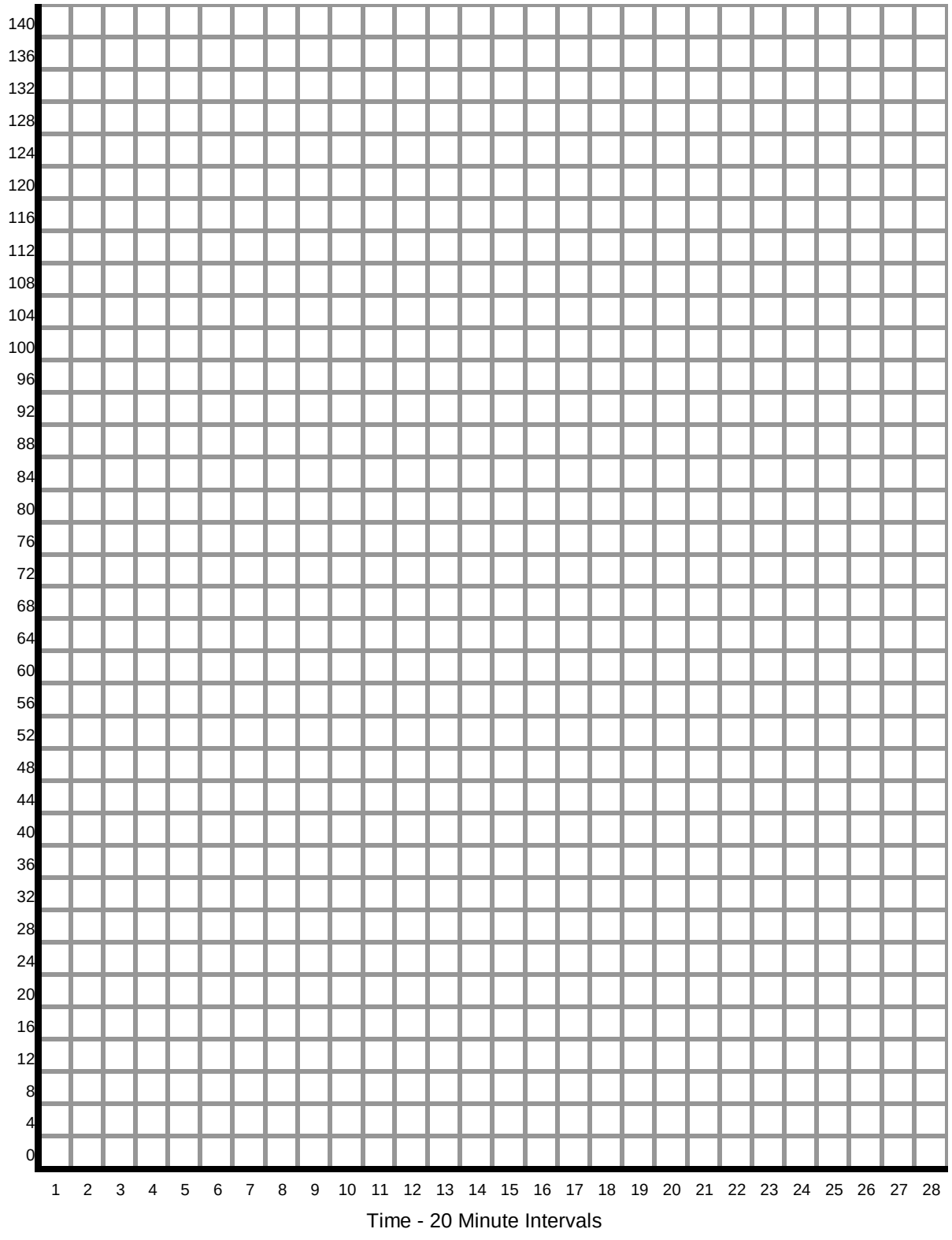
Day	Choice #1	Choice #2	Choice #3
1	$0	$0.01	$100
2	$0	$0.03	$100
3	$0	$0.07	$100
4	$0	$0.15	$100
5	$0	$0.31	$100
6	$0	$0.63	$100
7	$10	$1.27	$100
8	$10	$2.55	$100
9	$10	$5.11	$100
10	$10	$10.23	$100
11	$10	$20.47	$100
12	$10	$40.95	$100
13	$10	$81.91	$100
14	$25	$163.83	$100
15	$25	$327.67	$100
16	$25	$655.35	$100
17	$25	$1,310.71	$100
18	$25	$2,621.43	$100
19	$25	$5,242.87	$100
20	$25	$10,485.75	$100
21	$63	$20,971.51	$100
22	$63	$41,943.03	$100
23	$63	$83,886.07	$100
24	$63	$167,772.15	$100
25	$63	$335,544.31	$100
26	$63	$671,088.63	$100
27	$63	$1,342,177.27	$100
28	**$156**	**$2,684,354.55**	**$100**

Some students will come up with Choice #2 because of prior experience in dealing with exponential growth. Some students will work the problem through only the 7^{th} or 8^{th} day and then will guess and possibly select a different choice. This problem is a good example of how the spreadsheet can be used in the math classroom in helping to solve problems. Unless they have a scientific calculator they would not be able to use their calculator to reach their final answer for Choice #2. Depending on the calculator used some students would not be able to use their calculators past day 19. Some can go through day 26.

Appendix F

Handout 2

Population Growth of E. coli Bacteria



Appendix G

Math Calculations

Volume of E Coli Bacteria

You would end up with about 1.114×10^{43} bacteria after 48 hours. The volume of the bacteria is $\frac{1}{2} \circ r^2 L$ where $r \approx .25 \ell m$ and $L = 1 \ell m$

$\frac{1}{2} (3.14)(.25^2)(1) \ \ell m^3 = .098125 \ \ell m^3$

$V_{bac} = .098125 \ \ell m^3 \ (m/10^6 \ \ell m)^3 = 9.8125 \times 10^{-21} \ m^3$

Total Volume = 1.114×10^{43} bacteria x $9.8125 \times 10^{-21} \ m^3$

Approximately 1.09×10^{21} cubic meters

(ℓm = 1/1000th of a millimeter)

Adjustment of Readings on Low Cost Spectrophotometer

The low cost spectrophotometer readings are in miliamps of current that are being transmitted through the media. As the bacteria grows the amount of light being transmitted is decreased. We would like to see an exponential growth of the bacteria. To achieve our desired type of graph were required to calculate 1/n, where "n" is the reading taken from the low cost spectrophotometer. There is also some concern that as the batteries lose power it could affect the readings. To do this we decided to multiply the inverse of our readings by the value of the DI when it was tested during the readings. The actual value we are therefor looking for would be DI/Reading. An example would be:

On Monday your DI reading was 43.2 milliamps and your media reading was 36.3 milliamps. The number you would graph would be: 43.2/36.3 = 1.19. On Tuesday your DI was 42.6 and your media reading was 33.2. The number you would graph would be: 42.6/33.2 = 1.28. Even though the actual media reading was lower the second day the amount you graph shows an increase – indicating bacteria growth.

Appendix H

Handout 3

Data Gathering Chart

Media #:
Date of Innoculation:
Researcher:

Date/Time	DI	Standard	S 1	S. 2	S.3

Notes on Media:

Media #:
Date of Innoculation:
Researcher:

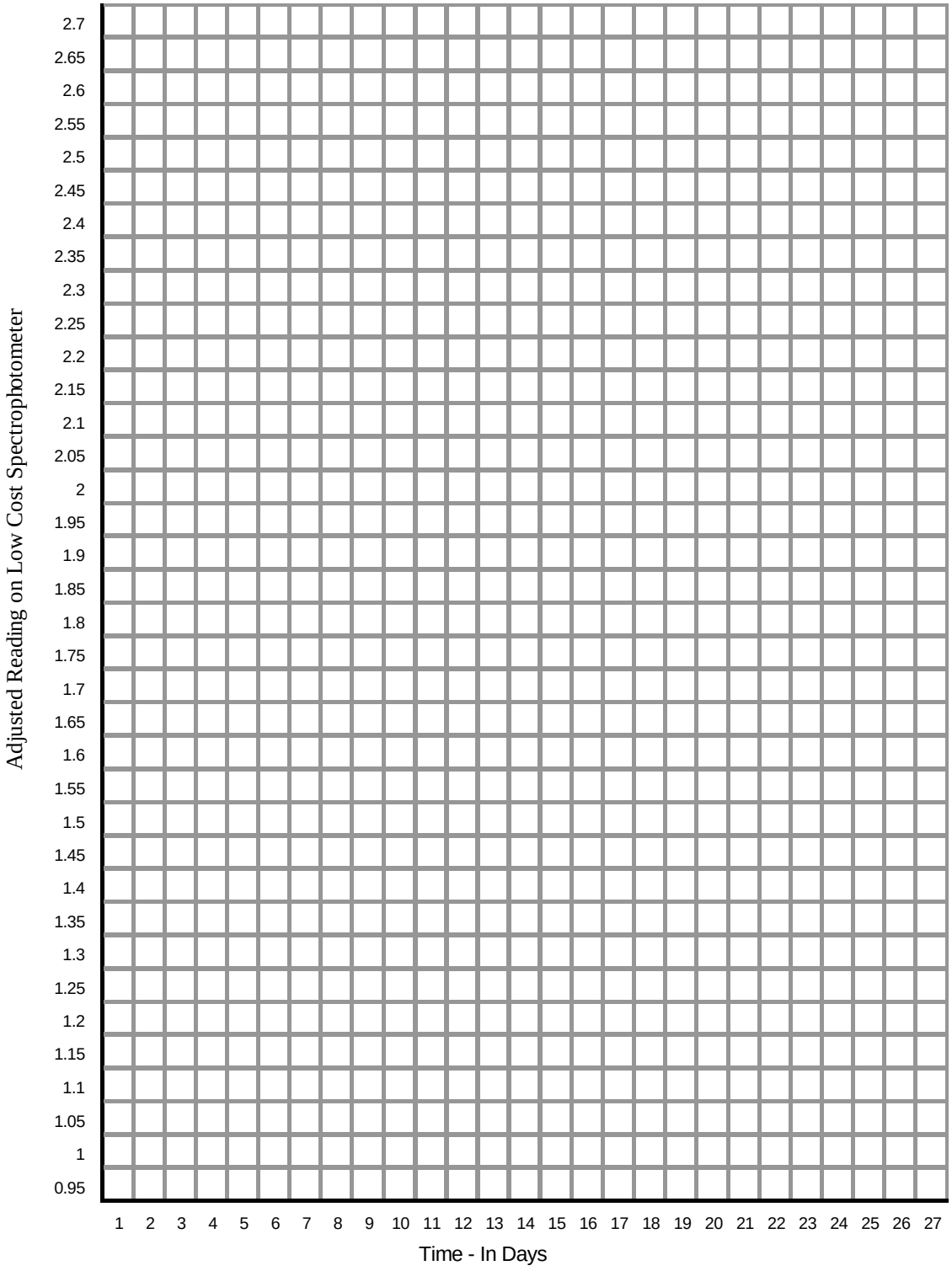
Date/Time	DI	Standard	S 1	S. 2	S. 3

Notes on Media:

Appendix I

Handout 4

Population Growth of Bacteria



Appendix J

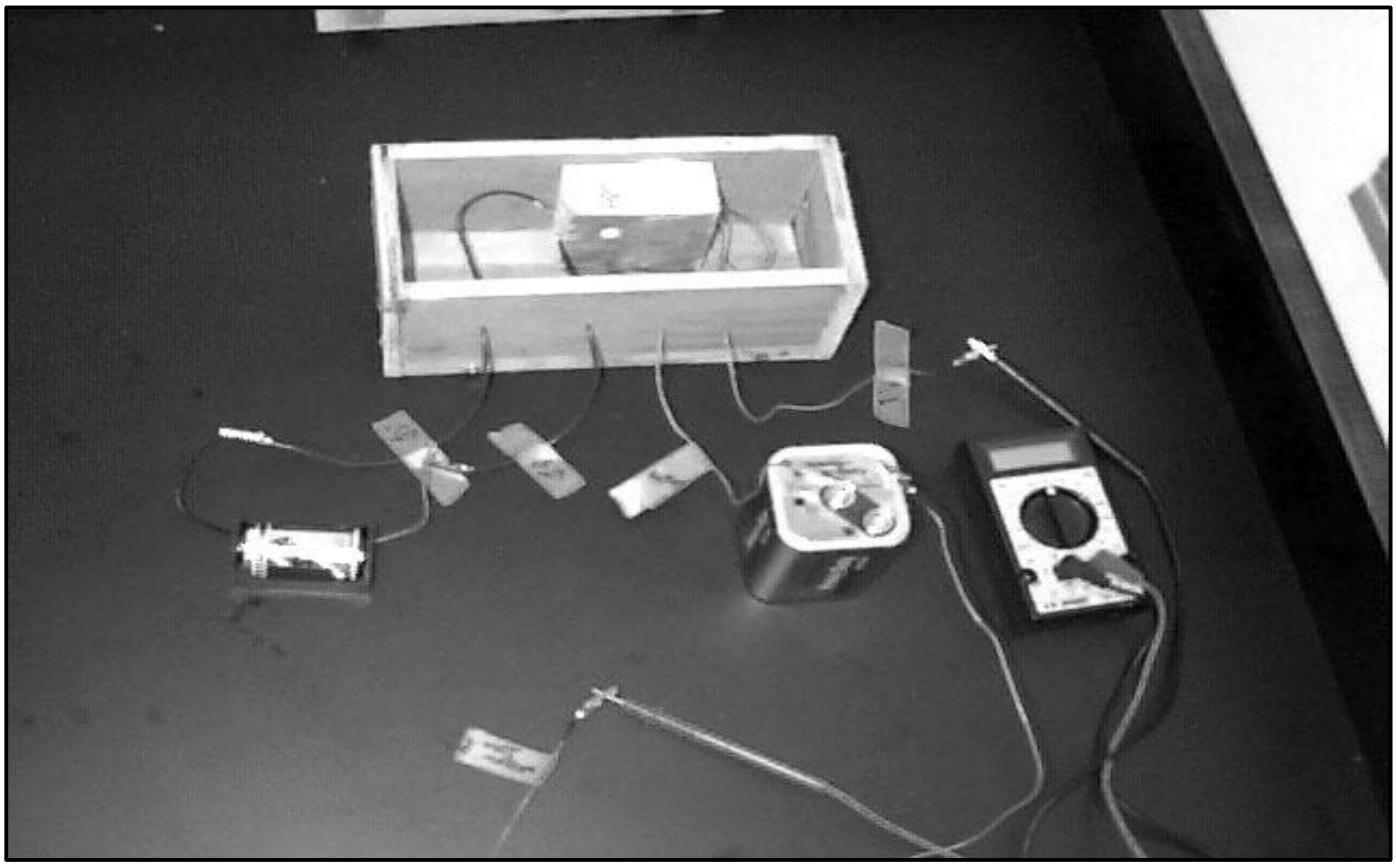
Student Sheet for Volume Data

Object	Beginning Volume	Volume of Water With Object	Volume of Object
Paper Clip			
Marble			

Appendix K

Object	Mass
Paper Clip	1.7 grams

Appendix L



Appendix M

Practice Taking Readings on the Low Cost Spectrophotometer

Experimental Liquid Used: C_____

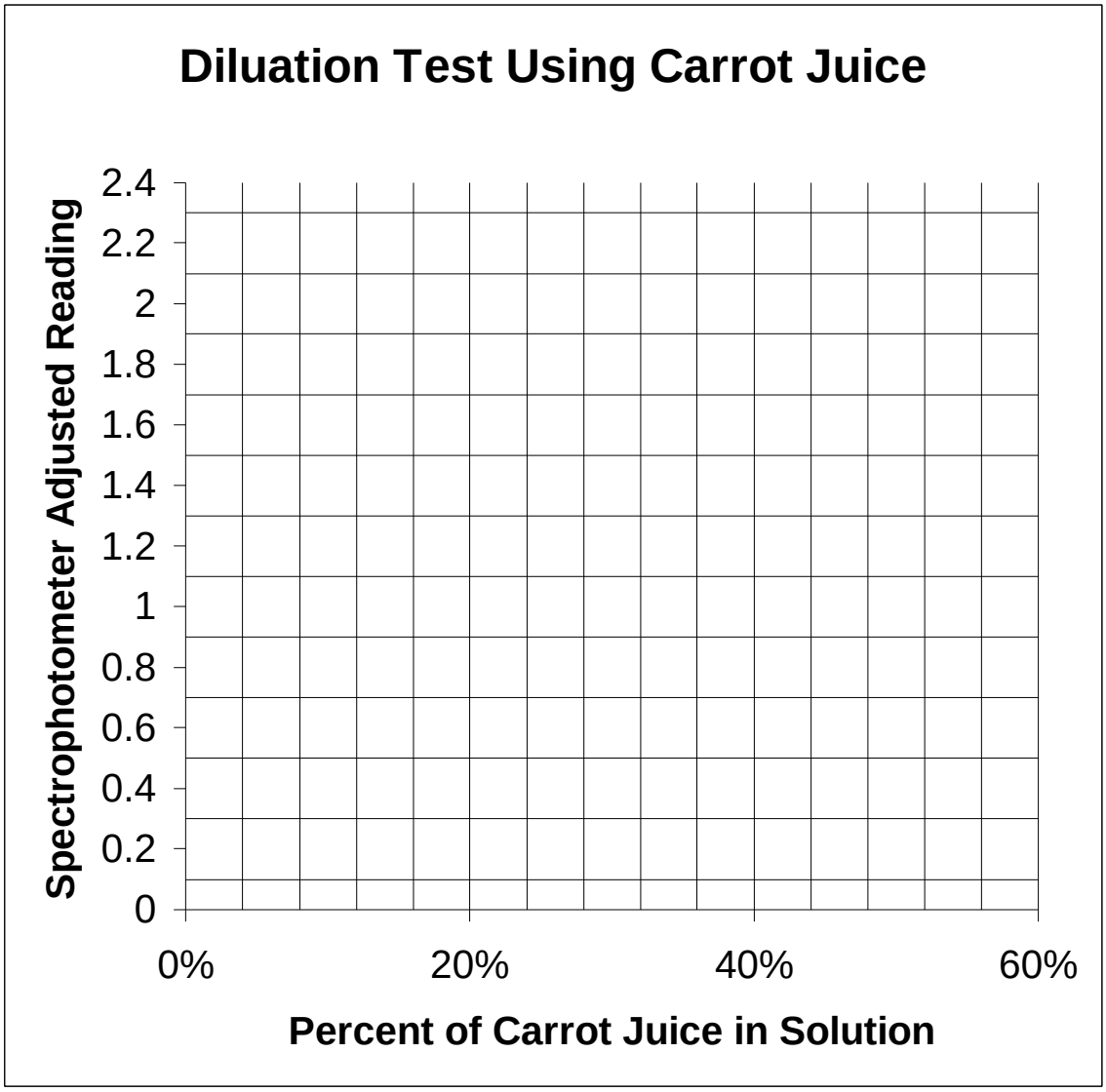
Name: _____

Date: _____

Start with 10 ml of distilled water then add 2 ml of Carrot Juice prior to each reading.

Reading #	Carrot Juice	Total Solution	Percent	Spectrophotometer Reading	Adjusted Reading
1	0	10ml			
2	2ml	12ml			
3	4ml	14ml			
4	6ml	16ml			
5	8ml	18ml			
6	10ml	20ml			

Appendix N


Diluation Test Using Carrot Juice
Spectrophotometer Adjusted Reading
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0%
20%
40%
60%
Percent of Carrot Juice in Solution

References

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Mormile, Melanie; Romine, Margaret F.; Bailey, Thomas J.; Peyton, Brent M. (1998). "Halomonas campusalis" *International Journal of Systematic Bacteriology*. In preparation for presentation.