Exercise 8
(Ed. Fall 2010)
Oxygen Requirements of Bacteria
Your Pet Project for the semester

INTRODUCTION:
Student Learning Objectives: After completing this exercise students will:

1. Define the terms used to describe the different categories of bacteria based on their oxygen requirements.
2. Identify four species of bacteria based on their oxygen requirements.
3. Determine the oxygen requirements of specific bacteria by observing the growth patterns in thioglycollate broth.
4. Demonstrate the use of the candle jar to grow microaerophilic organisms.
5. Demonstrate the growth of organisms in an environment of variable oxygen and nutrient availability, the Winogradsky column.

Activities for today:

- Culturing a microaerophilic bacterium, *S. pyogenes*, in a candle jar.
- Determining the oxygen requirements of *E. coli, P. aeruginosa, Clostridium sporogenes,* and *Enterococcus faecalis* by growth in thioglycollate broth.
- Assembling a Winogradsky column.

Materials
Work in groups of 4 per table. This is a group activity. Each group needs the following:

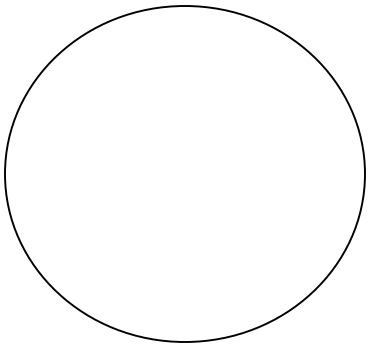
Slant cultures of:
E. coli, M. luteus, P. aeruginosa, Enterococcus faecalis and *S. pyogenes.*
Broth culture of *Clostridium sporogenes*
Candle Jar, candle, matches
Brewer Anaerobic Jar (Demonstration)
TSA plates or Blood agar plates (2 plates/table)
Thioglycollate broth tubes (3 tubes, 10 ml screwcap)
Winogradsky column components:
250 ml graduated cylinder
Calcium sulfate (Hard boiled egg yolks alternative)
Calcium carbonate (Chalk alternative)
Aluminum foil, plastic wrap, rubber band
Wooden dowels for tamping
Shredded newspaper
Mud, water from same source of mud
Light source (60 watt bulb/lamp)

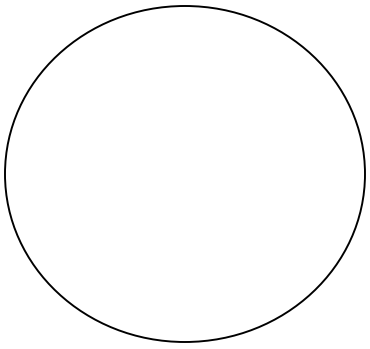
1. Growing Microaerophilic Bacteria in a Candle Jar

Microaerophilic conditions (10% Carbon Dioxide) can be created for the growth of microaerophiles by placing the culture plates inside a jar, lighting a candle inside, and sealing the lid shut. As the candle burns, most of the oxygen is used up and a 10% carbon dioxide atmosphere is generated. The jar is placed in the incubator for 24 – 48 hours to grow the organisms. Microaerophiles grow best in a reduced oxygen, increased carbon dioxide atmosphere. Follow the directions below.

Procedure:

- Streak for isolation two TSA plates (or Blood agar) with *S. pyogens* from the broth culture
- Place one plate in the candle jar
- Light the candle and close the lid shut (must be sealed shut)
- Observe the candle flame flicker and go out as the oxygen inside the jar is depleted and carbon dioxide is generated.
- Place the jar in the incubator and incubate at 37 C for 48 hours.
- Incubate the other plate on the incubator shelf at 37 C for 48 hours
- Observe for growth. Note your findings below.





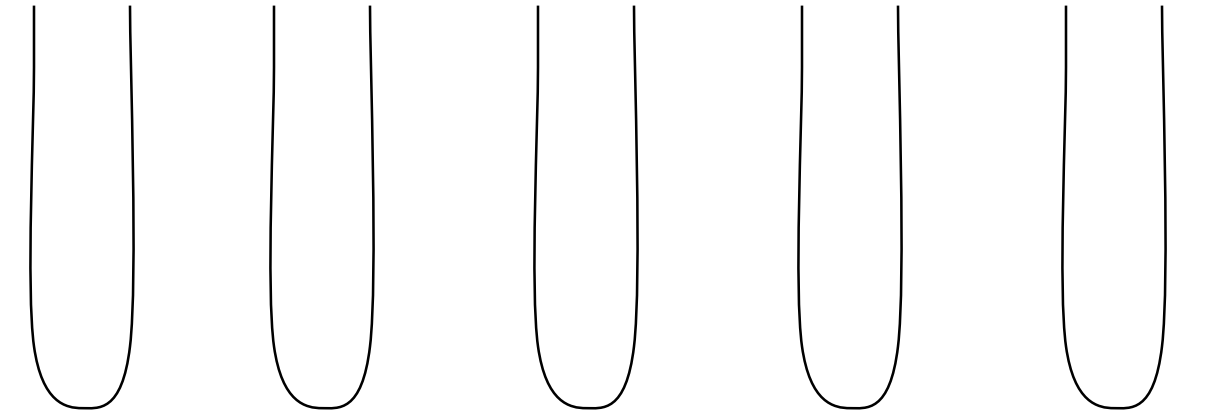
_____ _____

2. Determining the oxygen requirements of bacteria using thioglycollate broth

Procedure:

- Label and inoculate 4 thioglycollate tubes with the following organisms:
  E. coli
  P. aeruginosa
  Clostridium sporogenes
  Enterococcus faecalis
- Incubate at 37 C for 24 – 48 hours.
- Do not shake or agitate the tubes as you remove them from the incubator to your desk.
- Observe for growth in different areas of the tubes, and draw your findings below

Thioglycollate tubes



a. Which of these is a obligate aerobe? Why

_____

b. Which of these is a facultative organism? Why

_____

c. Which of these is an obligate anaerobe? Why

_____

d. Name a disease caused by an obligate anaerobe.

_____

3. The Winogradsky Column Setup: A Study in Soil Ecology

The Winogradsky column in this lab exercise is actually a vertical pond environment in which diverse microbial populations are studied. The column is named after Sergei Winogradsky, a soil microbiologist. In the column, three main attributes of microorganisms are studied; oxygen, sulfur, and photosynthetic ability. An oxygen gradient is formed by packing mud and essentail sources of carbon and sulfur in a tall container, such as a graduated cylinder, kept in the dark for some time, then allowed to remain under a constant source of light and some heat. After a long time (several weeks) the column is continuously observed for changes in color of the medium/suspension of mud, water, and essential nutrients. Both aerobes and anaerobes are observed growing in colonies which have characteristic colors, such as red, green, and purple. This exercise will help demonstrate the different oxygen needs as well as the ability of bacteria to obtain energy from different organis and inorganic

nutrients. The set up will begin today but will continuously be monitored for the remainder of the semester. Students will note their observations, and eventually their conclusions regarding the microorganisms growing in the columns.

Work to be done in groups per table

Procedure:

1. Rock-free mud is provided for you as well as water from the same pond.
2. Obtain a 250 ml graduated cylinder and add about 3 inches of shredded newspaper (or paper towels) mixed with the pond water (or distilled water).
3. Mix about 250 grams of mud with 25 grams of calcium sulfate (or mashed hard-boiled egg yolk) and 25 grams of calcium carbonate (or powdered chalk).
4. Add the above mixture to the shredded paper in the column till the cylinder is about two-thirds full.
5. Using a wooden dowel or rod, tamp or pack the surface of the mixture tightly to eliminate any air bubbles. Trapped air bubbles can alter the anaerobic environment in the cylinder.
6. Add enough pond water to fill the cylinder to about 1 inch from the top and cover with a piece of plastic wrap and a rubber band to keep it in place.
7. Wrap the cylinder entirely with aluminum foil to prevent any light from reaching the contents. This will prevent the over-growth of algae and allow other organisms to grow.
8. Store the cylinder at room temperature for about 7 –10 days.
9. Remove the aluminum foil cover from the cylinder and place near a 60 watt bulb light source for several weeks. Do not use fluorescent light sources.
10. Observe the column for growth. Different patches of color will appear. Note the color and position in the column.
11. Using a pipette, remove some of these colonies and observe them under a microscope. You may make a wet mount and Gram stain these colonies. Note your findings in the final Winogradsky Column report.