

Background

There are many important factors that are essential in order for a bacterium to grow. **Oxygen** is one of those important factors. Without oxygen present, some bacteria will not grow and in the presence of oxygen, some bacteria will die.

Bacteria can be divided into several groups in accordance to use of oxygen. **Obligate aerobes** require oxygen whereas **obligate anaerobes** cannot tolerate the presence of oxygen. **Facultative anaerobes** can grow with or without oxygen. **Microaerophiles** grow at low concentrations of oxygen. **Aerotolerant** cannot use oxygen but can tolerate it well.

To test for oxygen requirements in the laboratory, we can use a semisolid media called **thioglycollate broth**. In this broth, there is a pink indicator to demonstrate that oxygen is present in the broth. When oxygen is used, it will disappear.

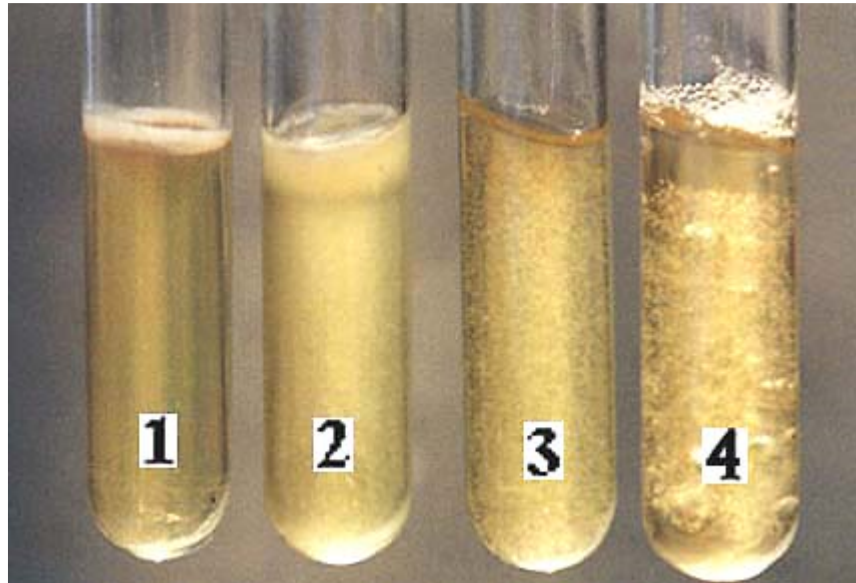
Protocol

First Lab Period

1. Break up into groups. Each group will retrieve the following:
 - ❑ 4 thioglycollate with indicator broth tubes
 - **DO NOT SHAKE THE THIOGLYCOLLATE!**
 - ❑ AF: *Alcaligenes faecalis*
 - ❑ CS: *Clostridium sporogenes*
 - ❑ EF: *Enterococcus faecalis*
 - ❑ EC: *Escherichia coli*
2. Label the four broth tubes of thioglycollate broth with group initials, culture initials, today's date, and instructor's last name.
3. Using the aseptic technique, inoculate each of the thioglycollate broths with one of the cultures listed above. (one with AF, one with CS, one with EF and one with EC)
4. Place the tubes in the incubator.

Second Lab Period

1. Retrieve thioglycollate broth tubes from the incubator.
2. Record the appearance of each broth tube in your notebook by sketching the location of the bacterial growth. Be sure to classify the tubes based on the growth of each organism. You can use the picture below to help you to classify.



<http://www.jlindquist.net/generalmicro/dfthiognf.html>

- 1 = Obligate aerobe
- 2 = Facultative anaerobe
- 3 = Aerotolerant
- 4 = Obligate anaerobe

3. Discard all materials into the biohazard.