## Temperature and Oxygen Requirements

### Objective

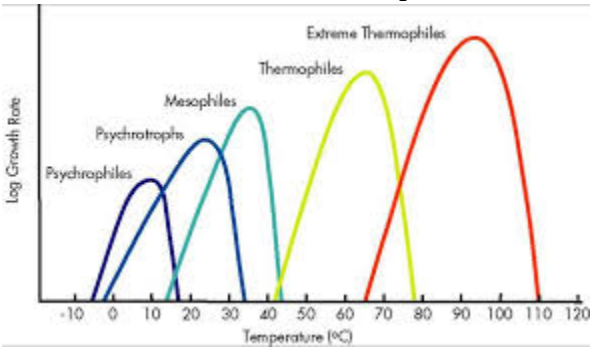
Most microorganisms can survive within a range of environmental conditions but tend to grow most optimally where conditions are most favorable. Two important resources influencing microbial growth are oxygen and temperature.

### Key Terms

**Aerotolerance**: The ability or inability to live in the presence of oxygen.
**Anaerobic**: The absence of oxygen.
**Aerobic**: The presence of oxygen.
**Obligate (strict) aerobes**: Organisms that require oxygen for aerobic respiration.
**Obligate (strict) anaerobes**: Organisms for which even small amounts of oxygen are lethal.
**Facultative anaerobes**: organisms that can grow in the presence or absence of oxygen. When oxygen is available, they respire aerobically. When oxygen is not available, they respire anaerobically or ferment an available substrate.
**Aerotolerant anaerobes**: Organisms that do not require oxygen and are not adversely affected by it.
**Micoaerophiles**: Survive in environments only containing lower than atmospheric levels of oxygen.
**Capnophiles**: Survive only if carbon dioxide levels are elevated.
**Psychrophiles**: Only grow below 20ºC. Optimal growth temperature is 15 ºC.
**Psychotrophs**: organisms adapted to cold habitats from 0ºC to above 30ºC. Optimal growth at room temperature.
**Mesophiles**: Bacteria adapted to temperatures between 15ºC and 45ºC. Optimal growth at 37 ºC.
**Thermophiles**: Are adapted to temperatures above 40ºC. Will not grow below 40ºC.
**Exteme thermophiles**: Can survive temperatures in the 65ºC to 110ºC range.

### Introduction

Bacteria have a limited control over their internal environments and are completely dependent on external factors to provide conditions suitable for their existence. One way to observe microbial responses to environmental changes is to artificially manipulate an external factor and measure its effect on growth rate after a given incubation time. In this series of laboratory exercises you will examine the effects of temperature and oxygen gas on growth rate.



**Materials**

Anaerobic Jar and Aerotolerance species: *B. cereus, E. coli, M. luteus, Clostridium sporogenes*
Temperature growth species: *Bacillus stearothermophilus, Pseudomonas fluorescens, E. coli*
Lab apron
Chemical eye protection
4 Agar deep tubes
2 TSA plates
Anaerobic (Brewer's) Jar with anaerobic gas generator packet


**Procedure**

**Anaerobic jar**

Work in your group of three. Each group does all 4 bacteria.

(1) Each group obtains 2 TSA plates

(2) Divide each plate into 4 quadrants and label each quadrant with the names of the different species. Label one plate "Aerobic" and the other "Anaerobic".

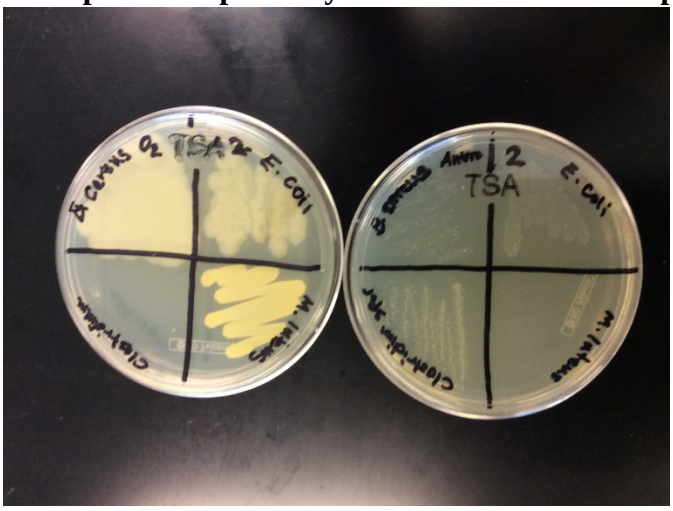
(3) Inoculate each quadrant with a different organism using a sterile loop. Inoculate with single fishtail streaks. Be careful to avoid inoculating over the quadrant line streaking into the quadrant of the next species. (NOTE: when inoculating *B. cereus* leave sufficient space from the dividing line as this species will spread significantly when grown.)

(4) The Instructor will show you how to incubate the anaerobic plates in the GasPak anaerobic (Brewer's) jar. The aerobic plates go into the 37°C incubator.

(5) Next period record the presence or absence of growth on the plate

Take Home Lesson: Describe how a GasPak anaerobic system generates an anaerobic environment. How can you tell whether an anaerobic environment has been achieved in the jar? Given a set of aerobic / anaerobic plates, determine which organisms are: strict aerobes, strict (obligate) anaerobes, and facultative anaerobes.
An indicator strip is used with the anaerobic systems to ensure that anaerobic conditions have been created. The indicator strip is soaked in methylene blue dye that is blue when oxidized and colorless when reduced.

**Examples of respectively aerobic and anaerobic plate microbes**




**Aerotolerance – Agar deep method**

We are going to perform an alternate procedure using melted sterile Brain Heart Infusion (BHI) agar deeps.

(Work in same groups; each group does all 4 bacteria).

1. You will need 4 BHI deeps. The BHI deeps have been melted for you. They are cooling in a 45 - 50°C water bath. Do not get them until you are ready to inoculate! The molten agar will solidify as it cools.

2. Label and inoculate each tube with a different organism.

3. Add two drops (equivalent to 0.2mL) of each culture to a different tube using a sterile 1mL pipette.

4. Rotate molten inoculated deep between your hands to mix.

5. Immediately pop bubbles on the surface with a HOT loop.

6. Place in rack for incubation.

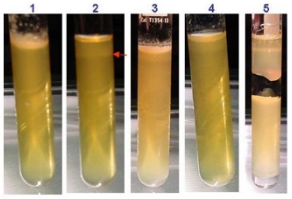
7. Next period record the distribution of growth in each tube.

Take Home Lesson: Define aerobe, strict aerobe, microaerophile, obligate-, aerotolerant- and facultative anaerobe. Know how the enzymes superoxide dismutase, peroxidase and catalase function to protect bacteria. Why are these enzymes necessary and for which groups of organisms are they necessary? Given a set of inoculated and incubated agar deeps, determine the organism's oxygen requirement.

Obligate anaerobes: Grow only at the lower regions of the medium depending upon how far into the medium oxygen has diffused.
Obligate aerobes: Grow only at the top where oxygen is most plentiful.
Facultative anaerobes: Grow throughout the medium concentrating at the top.

BHI Agar

1. Obligate aerobe
2. Microaerophile
3. Facultative anaerobe
4. Aerotolerant anaerobe
5. Obligate anaerobe




http://ws2.collin.edu/dcain/CCCCD%20Micro/bhitubes.htm

**<u>Effect of Temperature on Microbial Growth</u>**
(Work in groups of three)

1. Obtain 12 TSB tubes.

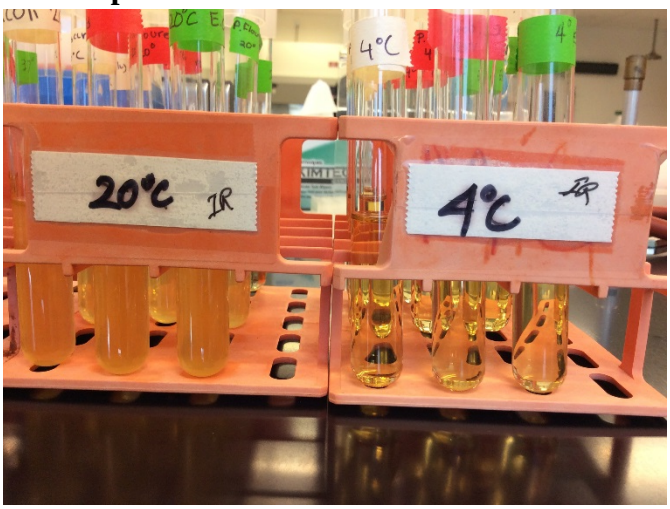
2. Label sets of 4 TSB tubes with name of each organism. (4 - E. coli, 4 - Bacillus, 4 - Pseudomonas)

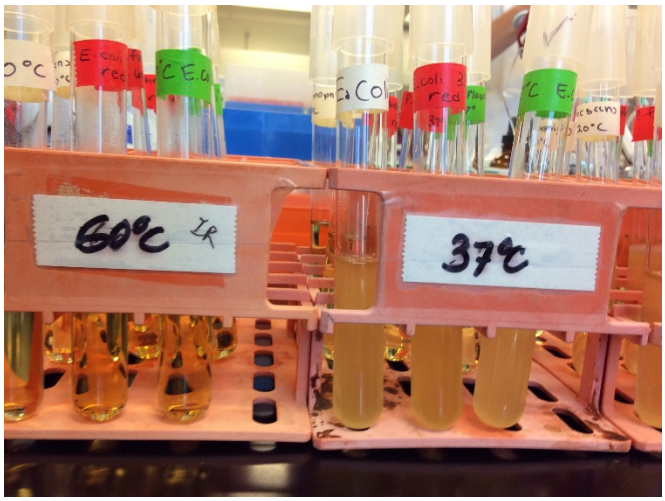
3. Label each set of tubes with each temperature: 10°C, 20°C, 37°C, 60°C.
4. Inoculate each set of tubes with a different organism using sterile swabs.
5. Put tubes into the appropriately labeled test tube rack. They will be incubated at each of the 4 temperatures.

6. Next period record the presence of growth. If growth is observed at more than one temperature for the same organism, record the relative amount of growth in each tube.

Take Home Lesson: You should be able to define psychrophile, psychrotroph, mesophile and thermophile. Given a set of inoculated and incubated broth tubes, you should be able to recognize a psycrophile, mesophile, and thermophile based on its ability to grow at the indicated temperatures.

**Examples of Bacterial Growth at Different Temperatures**



**Safety and disposal**

All used broth and agar tubes should be placed in the metal containers (baskets) on the cart at the front of the lab. Please ensure that you have removed any adhesive tape you have on your tubes before placing them on the cart.

**Observations**

This is a qualitative procedure designed for observing the effect of temperature on microbial growth. It allows an estimation of cardinal temperatures for individual species. Remember to carefully look at the bottom of the tubes for sediment and turbidity. Some non-motile species may only grow at the bottom of the tube.

**Anaerobic Jar**

| Organism | Growth on Aerobic Plate | Growth on Anaerobic Plate | Classification |
|---|---|---|---|
| *E. coli* | | | |
| *B. cereus* | | | |
| *M. luteus* | | | |
| *C. sporogenes* | | | |

**Aerotolerance Deeps**

| Organism | Location of Growth in Deep | Classification |
|---|---|---|
| *E. coli* | | |
| *B. cereus* | | |
| *M. luteus* | | |
| *C. sporogenes* | | |

**Temperature**

Indicate amount of growth: (-) = no growth, +, ++, +++ as amount of growth increases

| Organism | 10ºC | 20 ºC | 37 ºC | 60 ºC | Classification |
|---|---|---|---|---|---|
| *E. coli* | | | | | |
| *Pseudomonas fluorescens* | | | | | |
| *Bacillus stearothermophilus* | | | | | |