

## **Growth of Anaerobic Microorganisms**

Some organisms require oxygen for growth and cannot grow in its absence. These are called **obligate (strict) aerobes**.

Other organisms do not require oxygen for growth and cannot grow in its presence. These organisms are called **obligate (strict) anaerobes**.

Other organisms do not require oxygen for growth, but will grow better if oxygen is available. Such organisms are called **facultative anaerobes**.

Other organisms grow equally well in the presence or absence of oxygen and such organisms are called **aerotolerant anaerobes**.

**Microaerophiles** are those organisms that require low levels of oxygen, but they cannot grow at atmospheric levels of oxygen.

Many obligate anaerobes will not grow in the presence of oxygen, but they can tolerate exposure to oxygen without growth. Endospore-forming anaerobes such as members of the genus *Clostridium* cannot grow under aerobic conditions, but their spores can survive even long exposures to oxygen. Upon endospore germination under anaerobic conditions, the vegetative cells of such organisms can only grow anaerobically. Some of the organisms you will use in this exercise only grow anaerobically, under anoxic (oxygen-free) conditions, but they can survive exposure to oxygen since we can handle these organisms with a loop out in the open atmosphere and the use of an anaerobic jar (Fig. 1-35 of the Atlas, p. 11) does not achieve anoxic conditions for several hours after activating the Gas Pack. Other organisms, such as the methanogenic Archaea cannot tolerate even a brief exposure to oxygen. Such organisms must be transferred in a special chamber called an anaerobic hood under an atmosphere totally devoid of oxygen. They can be incubated in gas tight glassware or canisters that have been filled with oxygen free gas (typically a blend of hydrogen and nitrogen)

### **Refer to Pages 9-12 of the Atlas.**

Procedure:

To demonstrate the effect of different oxygen concentrations on the growth of various microorganisms we will use *Escherichia coli*, *Clostridium sporogenes*, and *Pseudomonas fluorescens* as the test organisms. Note that *Pseudomonas fluorescens* does not grow at 35 °C, but it does grow at 30 °C, so any culture plate containing *Pseudomonas fluorescens* needs to be incubated at 30 °C. *Escherichia coli* and *Clostridium sporogenes* can also grow at 30 °C although this is not their ideal growth temperature.

1. Divide two TSA plates into thirds. Inoculate each organism to be tested into a separate section of both plates. Proper labeling is important. Label one of the plates (with all three test organisms) as aerobic and the other plate as anaerobic.
2. Label one of the plates (with all three test organisms) as “aerobic” and the other plate (with all three test organisms) as “anaerobic”.

3. Place the plate labeled “anaerobic” in the anaerobic jar along with similar plates you’re your class. When all the plates have been placed in the jar, your instructor or GA will activate a GasPack to make the atmosphere inside the sealed jar anaerobic. The GasPack is activated by the addition of 10 mls of water. The GasPack system will generate both carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>) gas. In the presence of a palladium catalyst attached to the GasPack, H<sub>2</sub> will react with oxygen in the jar to form H<sub>2</sub>O (Fig. 1-36 of the Atlas, P. 11). Anaerobic conditions will be achieved in about 3 hours, as demonstrated by the conversion of a methylene blue indicator test strip from blue to white color.
4. Incubate the anaerobic jar containing the plates at 30 °C. Also incubate the plate labeled “aerobic” at 30 °C.
5. During the next laboratory period, observe the sectors of both the aerobic and anaerobically incubated plates for the extent of growth. Record your results on the next page.

You will also be using Eugon Deep or Thioglycollate semisolid tryptic soy agar tubes to grow the same organisms. Eugon or thioglycollate act as reducing agents. The tryptic soy agar components provide the necessary nutrients. When autoclaved, the oxygen in the medium is expelled, but after autoclaving, oxygen is reintroduced to the top of the tube. Eugon or thioglycollate help to maintain anaerobic conditions near the bottom of the tube. An oxygen gradient is established in the tube with the highest concentration near the top of the tube and the lowest near the bottom. The semisolid agar also helps in maintaining the oxygen gradient. (See the section on Aerotolerance starting on p. 9 of the Atlas and Fig. 1-30, 1-33 and, 1-34 of the Atlas).

1. Using an inoculating loop, inoculate each organism to be tested into separate Eugon Deep or Thioglycollate tubes. Do not shake or handle the tubes excessively. You want to keep the semisolid medium in the tubes as undisturbed as possible. Inoculate as far down into the medium as the loop will allow without touching the handle of the loop holder to the medium.
2. Incubate the tubes containing *Escherichia coli* and *Clostridium sporogenes* at 35 °C. Incubate the tube containing *Pseudomonas fluorescens* at 30 °C.
3. During the next laboratory period, observe tubes for growth and patterns of growth. Do not shake or handle the tubes excessively. You want to keep the semisolid medium in the tubes as undisturbed as possible.
4. Record your results on the next page.

