VIRTUAL EXPERIMENT 5A – OXYGEN RELATIONSHIPS

(REVISED FROM THE ON-LINE MANUAL)

One often sees an organism described as being a "strict aerobe," "facultative anaerobe," "strict anaerobe" or some other such designation. These terms refer to an organism's oxygen relationship – i.e., the growth relationship of an organism to the presence or absence of oxygen. Many laboratories utilize "Thioglycollate Medium" – a medium which allows for the detection of both aerobic growth and anaerobic growth in one tube, thus eliminating the necessity of incubating separate cultures of an organism under aerobic and anaerobic conditions.

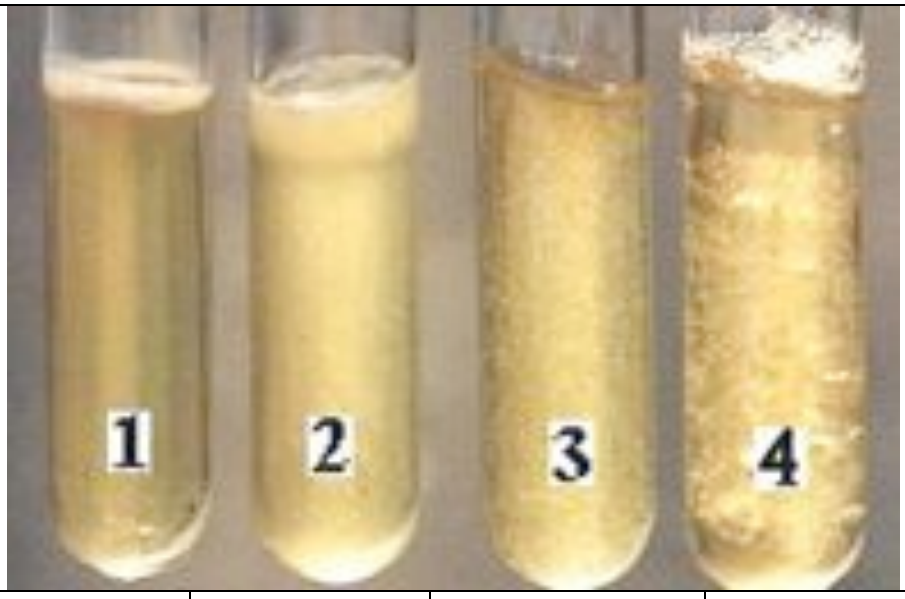
Thioglycollate Medium will support the growth of common, easily-grown chemoheterotrophic bacteria such as what are generally studied in introductory microbiology laboratory courses. These organisms include food spoilage bacteria, common contaminants and many pathogens. As seen in the illustration below, the pattern of growth of an inoculated organism will indicate whether it can respire aerobically (i.e., using oxygen) and/or ferment. The amino acids and glucose in the medium can be respired, and glucose is the only fermentable energy source in the medium (except to organisms such as certain species of *Clostridium* which can ferment amino acids).

Also, as shown in the table below, an organism's oxygen relationship designation can be determined by a combination of other methods if Thioglycollate Medium is not available: (1) testing for fermentation such as in Glucose Fermentation Broth, (2) performing the catalase test, and (3) testing if the organism can grow in the presence of oxygen. In our Microbiology 102 lab, we find these tests available in Experiments 7 and 11B, among others.

If we were to extend our study to organisms which are difficult or impossible to culture by the use of the media and incubation conditions we utilize in lab, we would find that applying an oxygen relationship designation universally would be problematic. A couple examples: (1) Quite a few organisms require special growth factors that are not present in our media. (2) Lithotrophic organisms would not be expected to grow as they would be "poisoned" by the high concentration of organic compounds in the medium.

There are other ways besides aerobic respiration and fermentation by which an organism can perform catabolic functions – namely, anaerobic respiration, anoxygenic phototrophy and oxygenic phototrophy. So, in a comprehensive classification of organisms, it may be most useful and instructive to indicate which one or more of these five catabolic methods can be accomplished by any given organism. As for anaerobic growth, fermentation is not the only way in which an organism can grow anaerobically; there are two more possibilities: anaerobic respiration (such as using nitrate in place of oxygen as an electron acceptor) and anoxygenic phototrophy (such as what we see for the purple non-sulfur photosynthetic bacteria).

The results we see in Thioglycollate Medium are shown below. The following table shows correlations of relevant reactions.

Appearance of growth in **Thioglycollate Medium**				
Oxygen relationship designation	STRICT (OBLIGATE) AEROBE	FACULTATIVE ANAEROBE	AEROTOLERANT ANAEROBE	STRICT (OBLIGATE) ANAEROBE
AEROBIC RESPIRATION	+	+	−	−
FERMENTATION	−	+	+	+
Ability to grow aerobically (oxygen tolerance)	+	+	+	−
Ability to grow anaerobically	−	+	+	+
Catalase reaction	+	+	−	−
Response to **sodium azide** in a growth medium	SENSITIVE	SENSITIVE (under aerobic conditions)	RESISTANT	RESISTANT
Reaction in **Glucose O/F Medium** (for those able to grow well in this medium)	**O or −**	**F**		

Period 1

WORK IN PAIRS

Materials

Broth cultures of *Staphylococcus epidermidis*, *Clostridium butyricum*, *Lactobacillus plantarum*, *Enterobacter aerogenes*, *Pseudomonas fluorescens* and *Alcaligenes faecalis*
6 tubes of melted Thioglycollate Medium – in 50°C water bath
4 plates of Plate Count Agar
Anaerobe jar
6 tubes of Glucose Fermentation Broth

NOTE: When labeling your plates and tubes to identify the cultures inoculated, it is best **not** to use numbers or plug/cap colors of the cultures provided. You can abbreviate the names or use initials: Se for *S. epidermidis*, Cb for *C. butyricum*, etc.

Procedure

1. Inoculate a loopful of each organism into a tube of Thioglycollate Medium. Mix without aeration as demonstrated by the instructor. (What is the reason for the pink color? Note the formula of this medium in Appendix E.) After the tubes solidify, incubate them at 30°C.

2. Divide each plate of Plate Count Agar into three sectors by the use of a wax pencil on the bottom of the plate. Label each sector such that you have two sets of duplicate plates, each plate designated for three of the organisms. Streak each organism (in a straight line) onto the approp-riate sector.

   a. Incubate one set of plates in your 30°C incubator tray.

   b. Place the other set of plates in the anaerobe jar on the stage. The atmosphere in the jar will be replaced with an oxygen-free gas mixture and then incubated at 30°C.

3. Inoculate a loopful of each organism into a tube of Glucose Fermentation Broth. Incubate at 30°C.

Period 2

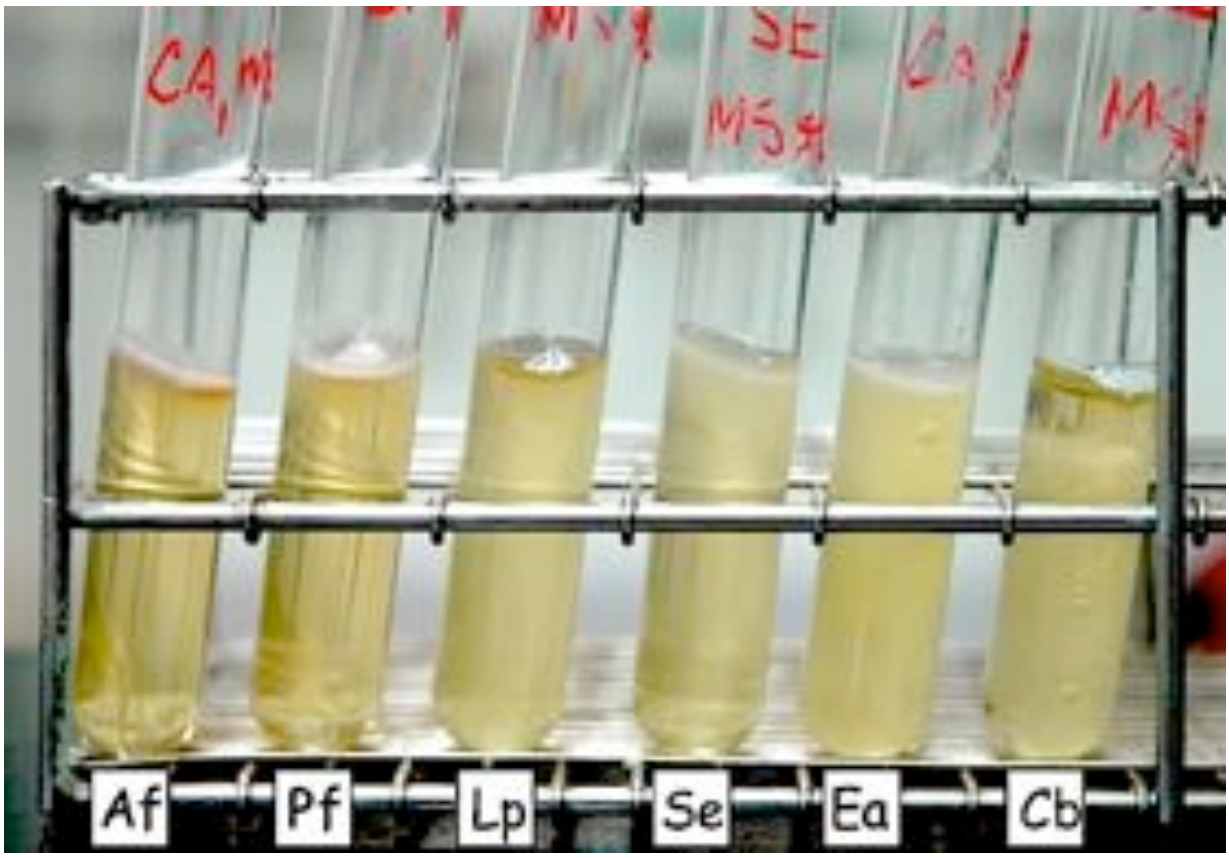
Materials

Dropper bottle of 3-5% hydrogen peroxide (H_2O_2)

Procedure

1. **Oxygen relationship.**

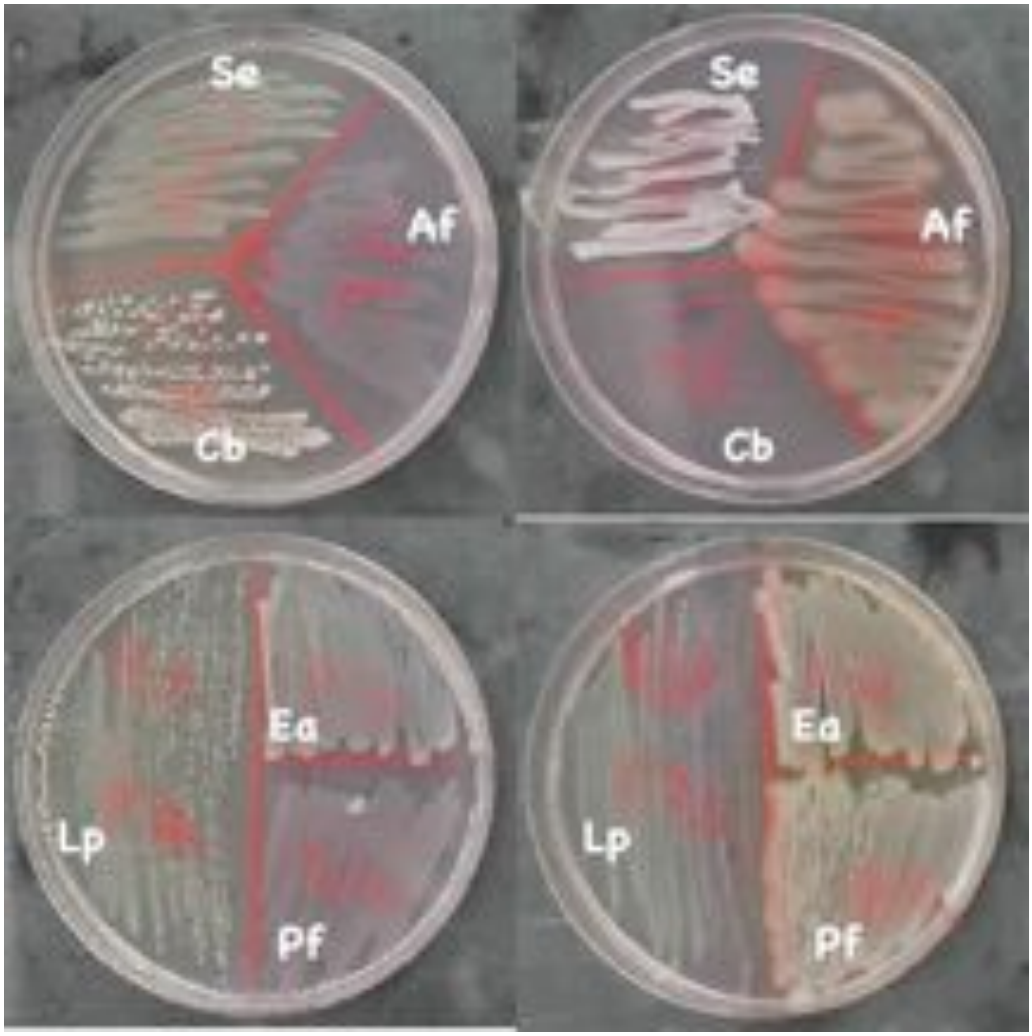
   a. Examine each **tube of Thioglycollate Medium** for the presence and relative amount of growth as illustrated in the introduction to this experiment. Growth may be detected most efficiently by holding the tubes toward the darker part of the ceiling. The dense band of growth at the top of the medium for facultative anaerobes may be very thin and somewhat difficult to see.



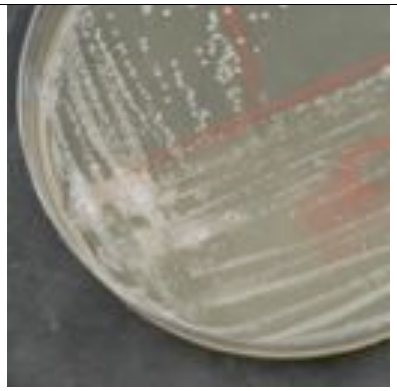
Clarification of photo: Af and Pf show only surface growth. Lp has even growth throughout the tube. Se and Ea have heavy growth toward the top and lighter growth throughout rest of tube. Cb has growth throughout the tube except in the clear area toward the top.

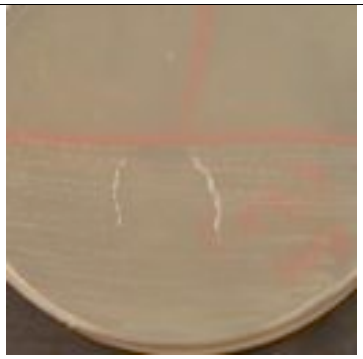
b. Examine the **plates** incubated aerobically and anaerobically for the presence and relative amount of growth. Disregard any faint appearance of growth which may appear on the plates incubated anaerobically. Sometimes a trace amount of oxygen in the jar atmosphere and/or dissolved in the medium will be enough to allow some growth of strict aerobes; this apparently happened in the plates shown below. **Hence, the Thioglycollate Medium tube tends to give a more reliable result.**

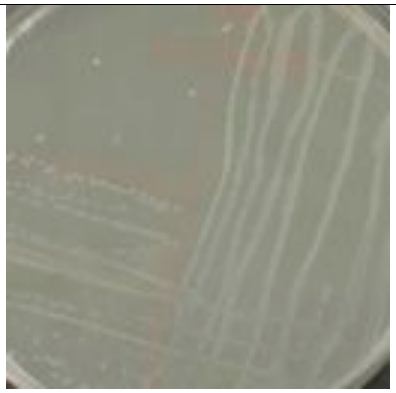
ANAEROBIC INCUBATION **AEROBIC INCUBATION**

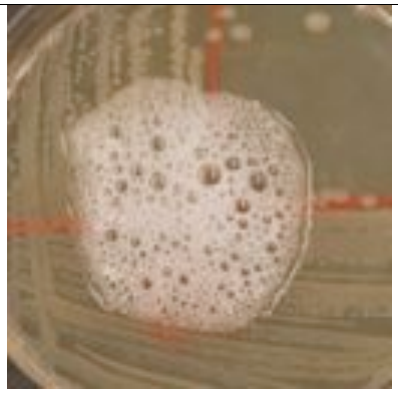


2. **Catalase test.** Add several drops of (H_2O_2) to each area of growth on the plates incubated aerobically. Observe through the top lid of the **closed** plates so you don't cause an aerosol of live cells to spread from a positive reaction! A positive reaction is indicated by the constant evolution of bubbles.

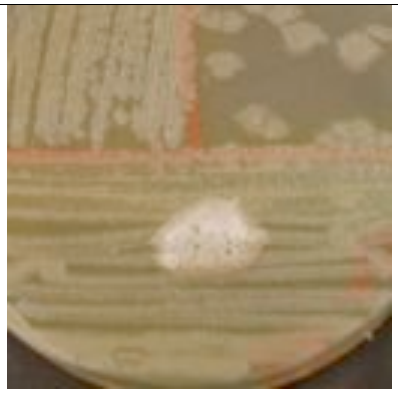

Se


Cb
(Disregard the two cracks in the medium)


Lp



Ea



Pf

Af

3. **Reactions in Glucose Fermentation Broth.** Note explanation and diagram on page 120 in the lab manual. Each tube may show some degree of growth as evidenced on the surface or by turbidity or sediment. **Any change of the pH indicator to a yellow color denotes fermentation of the glucose, and gas may also be evidenced in the Durham tube for some of the fermenters.**

Reaction seen for **Ea**

Reaction seen for **Lp** and **Se**

Reaction seen for **Af** and **Pf**

(Note: No growth seen for **Cb**. Why may this be so?)

4. Record your results in the table on page 25. **Are your results consistent with expectations**? (Refer to the table in the introduction.)