

# OXYGEN REQUIREMENTS & CULTURING ANAEROBIC BACTERIA

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An excellent way to determine the oxygen needs of your bacterium is to grow it in different oxygen environments--- atmospheric oxygen of 22%, no oxygen at all (GasPak jar), and reduced oxygen at less than 10% (candle jar)-- and compare the quality and quantity of growth. By determining growth from the 3 environments, you can determine whether the organism is a [facultative anaerobe](#), an [anaerobe](#), or an [aerobe](#). This is also a handy way to determine if you have an aerobe which is [microaerophilic](#), since they will only grow under reduced (but present) oxygen conditions as in the candle jar.

BE SURE TO READ ABOUT **HOW** THE GASPAK JAR BECOMES ANAEROBIC, in the **Photo Atlas**.

Thioglycollate broth has a reducing agent in it---the chemical thioglycollate--- which binds any free oxygen within the medium.

You will also notice that these tubes have screw caps, allowing a tight closure, to reduce oxygen entry. However, some oxygen will be in the tube between the cap and the broth and there is no way to get rid of it. So there will be some diffusion of oxygen into the top portion of the broth, and that is where any aerobic bacteria may grow.

An indicator, [resazurin](#), in the medium will be a light pink in the area of higher oxygen. You can determine whether the bacterium is an anaerobe, facultative anaerobe, or an aerobe by checking out where the organism grows in the column of media. **DO NOT SHAKE IT! NOTE: Oxygen will permeate the broth then this medium sits around for a while. Check for the pink color: if so, boil the broth for 5 minutes (removes the oxygen).**