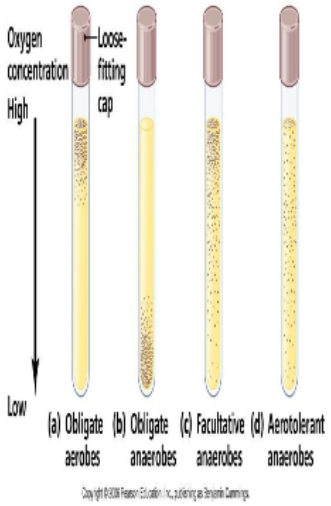
Microbiology practical – week 12

ANAEROBIC CULTIVATION

Bacteria in relation to oxygen

- Obligate aerobic -require gaseous oxygen, cannot grow without oxygen
- **Obligate anaerobic - oxygen is toxic for them. Methabolic pathway used for gaining energy is fermentation with production of foul-smelling end products.**
- Facultative anaerobic - able to adapt to aerobic or anaerobic
- Microaerophilic - require reduced oxygen tension



Anaerobic bacteria

can be divided into:

- strict anaerobes that can not grow in the presence of more than 0.5% oxygen
- moderate anaerobic bacteria that are able of growing between 2 to 8% oxygen.
- Anaerobic bacteria usually do not possess catalase
- can generate superoxide dismutase which protects them from oxygen.

- Obligate (strict) anaerobes - oxygen is toxic to these organisms, do not use oxygen as terminal electron acceptor
- e.g Clostridia, Bacteriodes etc....

Anaerobes are potentially pathogenic when displaced from normal environments (human colon, soil) and implanted in dead or dying tissue; abscesses, pneumonias, and oral and pelvic infections result.

Low or undetectable levels of superoxide dismutase and catalase allow oxygen radicals to form in anaerobic bacteria and to inactivate other bacterial enzyme systems.

Symptoms are related to the absence of oxygen from the affected area: hence, abscesses, devitalized tissue, and penetration of foreign matter lead to clinical infection.

- **Microaerophilic organisms - require low levels of oxygen for growth, but cannot tolerate the levels present in the atmosphere**
- **Aerotolerant Anaerobes: Metabolism is anaerobic but they are unaffected by the presence of oxygen.**

Environment			
Group	**Aerobic**	**Anaerobic**	**O_2 Effect**
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth	Toxic
Facultative Anaerobe (Facultative Aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

The clinically important anaerobes

- 1. Gram-negative rods (*Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*,
- 2. Gram-positive cocci (primarily *Peptostreptococcus* spp.);
- 3. Gram-positive spore-forming (*Clostridium* spp.) and nonspore-forming bacilli (*Actinomyces*, *Propionibacterium*, *Eubacterium*, *Lactobacillus* and *Bifidobacterium* spp.);
- 4. Gram-negative cocci (mainly *Veillonella*)

- Culture of anaerobes is extremely difficult due to the need to exclude oxygen, slow growth and complex growth requirements

Culture methods

- Most common adaptation of media is the addition of a reducing agent, e.g. thioglycollate, cysteine
- Acts to reduce the oxygen to water, brings down the redox potential -300mV or less.
- Can add a redox indicator such as rezazurin, pink in the presence of oyxgen - colourless in its absence

Culture media

- Blood agar
- Blood agar - enriched with growths factors and antibiotics (selective, kanamycin, neomycin, aminoglycosides - to inhibit most aerobic and facultative anaerobic bacteria)
- VL agar, VF bouillon
- Should be stored in refrigerator in plastic bag - decrease the solution of oxygen in the agar - use freshly prepared media

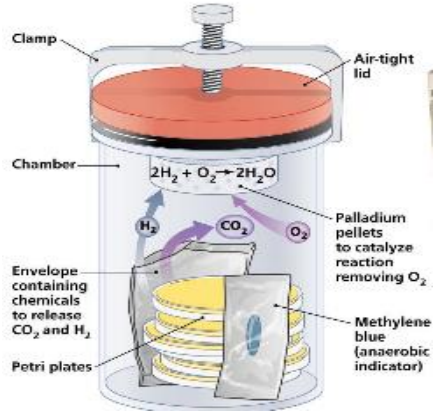
Establishing of anaerobic conditions

- Candle jar - microaerophilic, facultative anaerobic
- Anaerobic jar with atmosphere generator (foil envelopes): release hydrogen and carbon dioxide -after addition of water.
- Biological method - cultivation with *Serratia marcescens*
- Tubes of broth cultures (thioglycolate, cooked meat) - their formulations contain reducing substances - anaerobic conditions at the lower part - regeneration - 15 minutes in boiling water bath

Anaerobic jar works on the principle of evacuation and replacement, where the air inside the chamber is evacuated and replaced with mixture of gases (consisting of 5%CO2, 10%H2 and 85%N2). It is practically impossible to evacuate all the air so some amount of oxygen will still be left behind. The residual oxygen left behind is converted to water using Spongy palladium or platinum catalyst. The catalyst acts as a catalyzing agent causing slow combination of hydrogen and oxygen to form water. Reduced methylene blue is generally used as indicator (mixture of NaOH, methylene blue, and glucose). It becomes colorless anaerobically but regains blue color on exposure to oxygen.

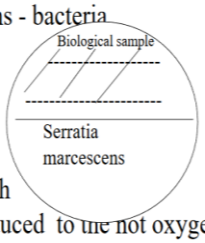
Reduced methylene blue indicator is used to check the efficacy of anaerobiasis. A tube containing reduced methylene blue solution had to kept inside the jar along with the culture plates. Methylene blue is colorless in reduced conditions and turns blue when oxidized.

ANAEROBIC JAR



Biological method

Half of medium in the Petri´s dish is innoculated with the tested sample, the other with Serratia marcescens - bacteria able to produce anaerobic environment by the consumption of oxygen. Petri dishes is sealed with the wax or parafin and introduced to the not oxygen - free incubator

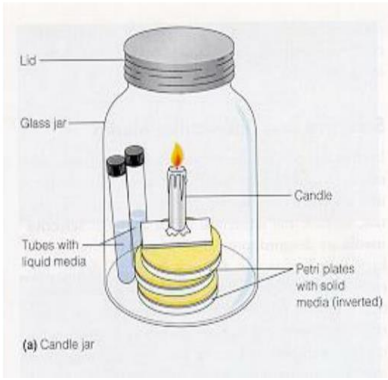


Candle jars

The candle flame will consume most of the oxygen in the jar.

Produce an elevated level of carbon dioxide.

These conditions are ideal for the growth of microaerophilic organisms.



SOURCES:

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