Oxidation Fermentation(OF) test

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Introduction

The oxidative-fermentative (OF) test was developed by Hugh and Leifson in 1953. They developed OF media to differentiate between oxidative bacteria (that produces acid from carbohydrates under aerobic condition only) and fermentative bacteria (that produces acid both under aerobic and anaerobic conditions).

Saccharolytic microorganisms degrade glucose either fermentatively or oxidatively. The end products of fermentation are relatively strong mixed acids that can be detected in a conventional fermentation test medium. However, the acids formed in oxidative degradation of glucose are extremely weak and less, and the more sensitive oxidation fermentation medium of Hugh and Leifson's OF medium is required for the detection. The medium was made by increasing the amount of glucose above that found in medium used to detect fermentation and by decreasing the amount of peptone.

The OF medium of **Hugh and Leifson** differs carbohydrate fermentation media as follows:

- The concentration of agar is decreased to 2% from 3%, making it semisolid in consistency *(This assists in the determination the motility of the organism).*
- The concentration of peptone is decreased from 11% to **2%**. (decreasing the amount of alkaline product produced by the metabolism of peptone; thus reducing the neutralizing effect of these products).
- Carbohydrate concentration is increased by 0.5% to **1.0%** (The increased concentration of glucose in the medium enhances the production of these weak acids to a level that can be detected by bromthymol blue indicator.)

Principle:

The oxidative-fermentative test determines if certain gram-negative rods metabolize glucose by fermentation or aerobic respiration (oxidatively). During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids depending on the type of fermentation. The high concentration of acid produced during fermentation will **turn the bromthymol blue indicator in OF media from green to yellow** in the presence or absence of oxygen .

Certain nonfermenting gram-negative bacteria metabolize glucose using aerobic respiration and therefore only produce a small amount of weak acids during glycolysis and Krebs cycle. The decrease amount of peptone and increase amount of glucose facilitates the detection of weak acids thus
produced. Dipotassium phosphate buffer is added to further promote acid detection

:Uses

OF Test is used to determine if gram-negative bacteria metabolize carbohydrates oxidatively, by fermentation, or are nonsacchrolytic .(have no ability to use the carbohydrate in the media)

Media:

Hugh and Leifson's OF basal medium; the constituents are as follows:

- Sodium chloride : 5.0 m
- Di-potassium phosphate : 0.3 g
- Peptone : 2.0 g
- Bromthymol blue :0.03 g
- Agar :3.0 g
- Glucose : 10 g
- Water : 1000 ml
- The pH should be adjusted to 7.1 prior to autoclaving. After the medium is autoclaved at 121°C for 15 minutes, a filter sterilized solution of 10% solution of carbohydrate is aseptically added to the medium to a final concentration of **1%.**

Procedure

- Inoculate two tubes of OF test medium with the test organism using a straight wire by stabbing “half way to the bottom” of the tube.
- Cover one tube of each pair with 1 cm layer of **sterile mineral oil or liquid paraffin** (it creates anaerobic condition in the tube by preventing diffusion of oxygen), leaving the other tube open to the air.
- Incubate both tubes at 35oC for 48 hours (Slow growing bacteria may take 3 to 4 days before results can be observed)

:Following are the reaction patterns

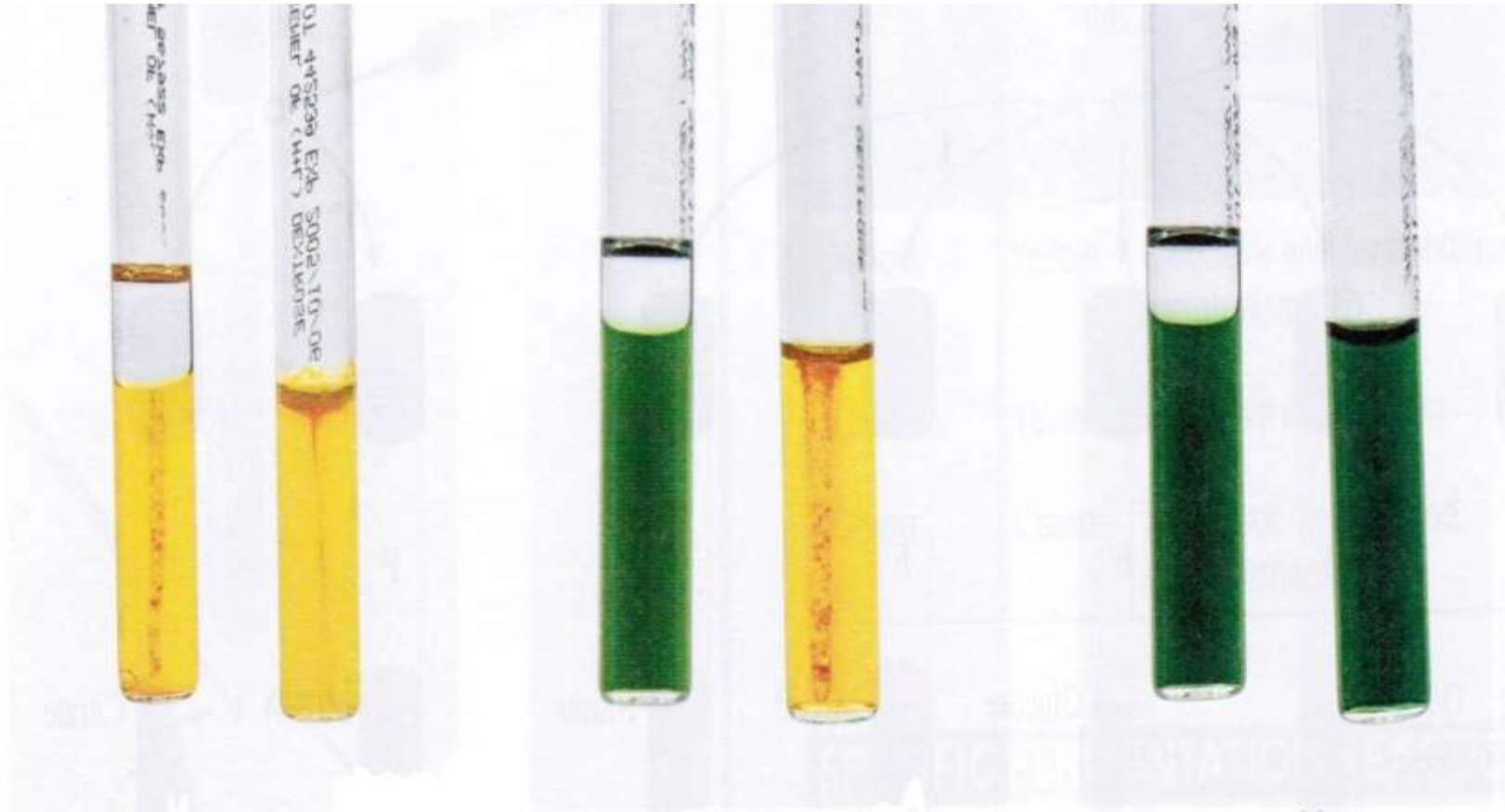
Open (Aerobic) Tube	Covered (Anaerobic) Tube	Metabolism
Acid (Yellow)	Alkaline (Green)	Oxidative
Acid (Yellow)	Acid (Yellow)	Fermentative
Alkaline (Green)	Alkaline (Green)	Non saccharolytic (glucose not metabolised)

Fermentative result: Acid production on both (open and covered) tubes. The acid produced changes the pH indicator, bromthymol blue, from green to yellow. e.g. *Escherichia coli*

Oxidative result: Acid production in the open tube (aerobic) and not the oil-covered tube (anaerobic) indicates an oxidative result. Nonfermenting bacteria that metabolize glucose via oxidative metabolism give an oxidative result. e.g. *Pseudomonas aeruginosa*

Non saccharolytic (Negative OF result): Nonsacchrolytic bacteria give a negative OF result. The negative result is indicated by no color change in the oil-covered tube and in some cases an increase in pH (pH 7.6) changing the bromthymol blue from green to blue in the top of the open tube. The increase in pH is due to amine production by bacteria that break down the peptone (protein) in the medium.

e.g. Alcaligenes faecalis.

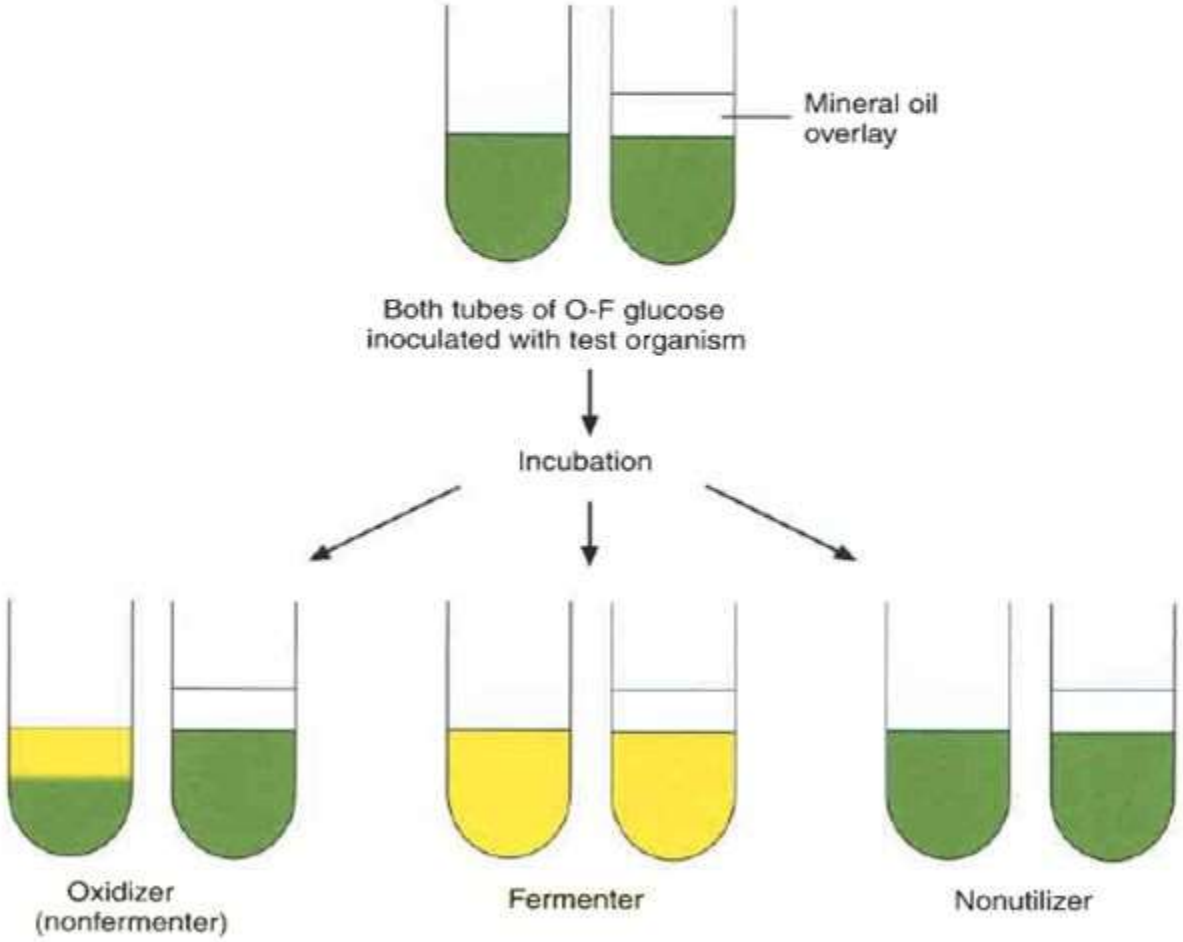


O/F glucose test. (a) Fermentative and oxidative; (b) oxidative; (c) glucose not metabolised or inert.

OF (Oxidation Fermentation) Test

Indicator: Bromothymol blue

PH: 7.1



Thank you