

Exercise 6: Microbial Metabolism – Fermentation of Carbohydrates

Objectives

- Describe the breakdown products of glucose, lactose, and sucrose carbohydrates.
- Interpret carbohydrate tubes and the fermentation products produced.

Reading

Metabolism can be defined as the sum total of all chemical reactions which occur inside the cell. The metabolism of carbohydrates entails the catabolism (or breakdown) of certain large molecules, such as **disaccharides** (double sugars), into smaller ones called **monosaccharides** (single sugars). For such breakdown to occur, enzymes are required. In this exercise the 2 disaccharides, sucrose and lactose, are broken down by the enzymes sucrase and lactase respectively. These reactions proceed as follows:

Lactose (disaccharide) \longrightarrow Glucose + Galactose (two monosaccharides)

Sucrose (disaccharide) \longrightarrow Glucose + Fructose (two monosaccharides)

The monosaccharides are then further degraded by a combination of glycolysis and one of two processes, fermentation or respiration. They are **fermented** by some bacteria to yield **organic acids, alcohols and/or gases**. Fermentation reactions also release a small number of energy molecules (such as ATP) which are needed to "fuel" other bacterial chemical reactions. Other organisms process monosaccharides more completely. In a process known as aerobic **respiration**, they produce abundant ATP, water, and CO₂ gas. Aerobic respiration takes place only when O₂ is available.

This exercise illustrates a simple method to detect acid and gas formation from carbohydrate breakdown. **Formation of acids** is detected by including a pH indicator in microbial growth media. In this experiment, the sugar broths contain the **pH indicator bromcresol purple (BCP)**. This pH indicator is purple at pH 6.8 (near neutral pH) and yellow at pH 5.2 (acid pH). In these broths, **gas formation** is detected by the use of a small inverted tube referred to as a Durham tube.

EX. 6: Metabolism and Fermentation –Materials per Pair

- 3 tubes of glucose-BCP broth with Durham tubes (red caps)
- 3 tubes of sucrose-BCP broth with Durham tubes (blue caps)
- 3 tubes of lactose-BCP broth with Durham tubes (green caps)

broth cultures:

- Bacillus subtilis*
- Micrococcus luteus*
- Klebsiella pneumoniae*

EX. 6: Metabolism and Fermentation - Methods

1. Inoculate a different tube of each medium with a loopful of *Bacillus subtilis*, *Micrococcus luteus*, and *Klebsiella pneumoniae*. Use aseptic technique throughout! If even a small amount of bacteria or sugar gets transferred to the next tube, it will screw up the results!

Bacillus subtilis: inoculate one tube each of glucose, sucrose and lactose.

Micrococcus luteus: inoculate one tube each of glucose, sucrose and lactose.

Klebsiella pneumoniae: inoculate one tube each of glucose, sucrose and lactose.

Note: Be sure to label each tube **on the glass** with name of bacterium, your names/seat numbers, and make certain you replace the cap on the same tube. Place tubes into rack in appropriate incubation tube. All tubes will be incubated at 37°C for 48 h.

2. Next lab period, observe the cultures for both acid and gas production.
3. Record observations in the **RESULTS** section.