Depolymerase Exercise

 Many microbes are capable of breaking down polymers in their environment. Neither bacteria nor fungi are capable of ingesting food. These microbes can obtain nutrients by secreting enzymes that break polymers down into smaller molecules which can be taken up by the microbes. Since the enzymes break down polymers, they are often referred to as depolymerases. Such enzymes exist for all the types of macromolecules that are biologically produced. These include proteins, produced by all living things; plant polysaccharides such as cellulose, starch, and pectin; nucleic acids; and PHBV, a type of bacterial polyester. Cellulose is produced abundantly by plants, and much research is going into cheaper and faster ways of breaking it down for the purpose of making biofuels.

 Fungi are major sources of depolymerases. In additional to fungi, many kinds of bacteria in soil can break down polymers either aerobically or anaerobically. In this lab exercise, we will use several different types of soil as inocula to show polymer degradation. The following media will be inoculated:

Starch agar plates. After growth, flooding the plates with an iodine solution will reveal where degradation of starch took place. Pp 161-163 in your Manual.

Casease test. The major protein in milk, casein, is largely responsible for making milk appear white. In this test, a milk-containing medium will be inoculated. Activity of the enzyme casease will result in clearing around the microbial growth. Pp 169-170.

Gelatin liquefaction. When chilled, the gelatin in these tubes will gel. However, if the tubes are inoculated with gelatin-hydrolyzing microbes, the gelatin (a protein derived from collagen) will be broken into smaller molecules and will not gel when chilled. Pp 171-172.

Cellulose degradation. We will use a mineral salts medium containing both organic supplements (low concentrations of complex media) and powdered cellulose (with high surface area). In some tubes, we will add soil samples to the surface of the media for aerobic degradation. For others, we will get inocula from the anaerobic zone of our Winogradsky columns and attempt to exclude air from the culture.

PHBV degradation. Poly(3-hydroxybutyrate-co-hydroxyvalerate) is a polyester made by certain bacteria that has properties similar to the plastic polypropylene. Because it is a naturally occurring plastic, various fungi and bacteria can produce enzymes that will degrade it. PHBV powder will be suspended in the same medium used for cellulose degradation. The powder will make the medium slightly turbid. Degradation by microbes will cause the medium to clear.

Inoculated media will be incubated for 5 days, then examined for degradation.