Lab #2
Bioreactors and Fermentation
Outline

• Goals of Lab
• Yeast
• Fermentation
• Bioreactor
• Analysis equipment
  – Hemacytometer, cellometer, spectrophotometer, HPLC system
Goals of Lab

• Familiarization with a bioreactor used for growing yeast under aerobic and anaerobic (fermentation) conditions and the factors that affect growth rate (biomass production) and ethanol production

• Familiarization with dilution calculations

• Familiarization with determination of absorbance (optical density) using a spectrophotometer

• Familiarization with determination of total cell count using a hemacytometer

• Familiarization with the principle of operation and capabilities of an HPLC system
Yeast

• Unicellular fungus (more than 1000 species identified)
  – Commonly used to leaven bread and ferment alcoholic beverages
  – Most yeasts belong to the division Ascomycota
  – A few yeasts (e.g., Candida albicans) can cause infection in humans
  – Saccharomyces cerevisiae (most commonly used yeast), was domesticated for wine, bread, and beer production 2000+ yrs ago

• Yeast physiology can be either obligately aerobic or facultatively anaerobic (fermentative)
  – There is no known obligately anaerobic yeast

• In absence of $O_2$, fermentative yeasts produce energy by converting sugars into carbon dioxide and ethanol (alcohol)
• In brewing, ethanol is the desired product, while in baking, carbon dioxide raises the bread and the ethanol evaporates
Yeast (contd.)

• Many yeasts can be isolated from sugar-rich environmental samples such as fruits and berries (grapes, apples, peaches etc.) and exudates from plants (such as plant saps or cacti).

• The most common mode of vegetative growth in yeast is the asexual reproduction by budding or fission:
  – A small bud (daughter cell), is formed on the parent cell
  – The nucleus of the parent cell splits into a daughter nucleus and migrates into the daughter cell
  – The bud continues to grow until it separates from the parent cell, forming a new cell
Yeast (contd.)

- In brewing beer, top-fermenting yeasts (float to the top of the beer) produce higher alcohol concentrations and prefer higher temperatures (15-25 °C)
  - Eg., Saccharomyces cerevisiae (known to brewers as ale yeast)
    - They produce fruitier, sweeter type ale beers

- Bottom-fermenting yeasts ferment more sugars, leaving a crisper taste and work well at low temperatures (5-10 °C)
  - Eg., Saccharomyces uvarum (formerly known as Saccharomyces carlsbergensis)
    - They are used in producing lager-type beers

- Brewers of wheat beers often use varieties of Torulaspora delbrueckii
Fermentation

• Conversion of carbohydrate (eg. sugar) into acid or alcohol by yeast or bacteria

• It is used in brewing and wine making for the conversion of sugars to alcohol (ethanol – CH₃CH₂OH)
  – This process, followed by distillation, can be used to obtain pure ethanol (bioethanol) for use as a transport biofuel

• It can also be viewed as the energy-yielding anaerobic metabolic breakdown (respiration) of a nutrient molecule such as glucose, without net oxidation (eg., in muscle cells)

• Fermentation typically refers to the fermentation of sugar to alcohol using yeast, but other fermentation processes include making of yogurt, souring of milk, rising of dough
Bioreactor

- An apparatus (usually jacketed cylindrical SS vessel) for growing organisms such as bacteria, viruses, or yeast that are used in the production of pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic wastes.
- Under optimum conditions of gas (air, oxygen, nitrogen, and carbon dioxide) flow rates, temperature, pH, dissolved oxygen level, and agitation speed, the microorganisms or cells will reproduce at a rapid rate.
Bioreactor

Controls
- Temperature
- Pressure
- pH
- Agitation speed
- Air flow rate
**Hemacytometer**

A device used to count cells

A thick glass microscope slide with a rectangular indentation that creates a chamber that is engraved with a laser-etched grid of perpendicular lines. The area bounded by the lines and depth of chamber are known. Thus, by counting the number of cells in that volume of fluid, we can calculate the concentration of cells in the fluid.
Cellometer

Automated cell counting – no need for hemacytometer.

Disposable Counting Chambers consist of two enclosed chambers with a precisely controlled height. 20 μl is loaded into the chamber and inserted into the Cellometer. It utilizes bright field imaging and pattern-recognition software to identify and count individual live & dead cells stained with Trypan Blue.

Green: Live cells
Red: Dead cells
Spectrophotometer

Measures amount of light reflected from an object or amount of light absorbed by an object

Biomate 3
Split beam
quartz coated
Xenon lamp
(190-1100 nm)
High Performance Liquid Chromatography (HPLC) System

HPLC: Mass transfer involving adsorption

Separate, identify, quantify components in a mixture. Pump a solvent with the sample through a solid column of adsorbent material. Each component diffuses at different rates, thereby separating. The detector identifies and quantifies each component.

Granular mtl. (silica, polymers; 2-50 μm)

UV absorbance
Fluorescence
Refractive index
Evaporative light scattering

Injected Sample Band (Appears “Black”) (Blue, Red, Yellow)

Time Zero Mobile Phase

Time +10 Minutes Mobile Phase

Analyte Bands

HPLC Column

Detector Flow Cell

Injection Start
Base-line

Computer Data Station