Experiment No. 1

Determination of Biomass Concentration by Dry Weight Method

Objective: To determine cell biomass concentration by optical density and dry weight method

Introduction

The cell density can be quantified in two basic ways: as grams of dry or wet weight per liter of sample, or as number of viable/dead cells per ml. The cells in a sample can be separated from the broth and weighed while they are wet, or the cells may be thoroughly dried before weighing. The dry weight measurement usually gives a much more consistent result than the wet weight. Alternatively, the number of cells can be counted either by successively diluting the original sample and plating on a Petri dish, with the help of a microscope and a counting chamber, or with an automated cell counter such as a Coulter counter or a cytoflowmeter. The plating method detects only the viable cells; whereas, the automated cell counters can only detect the total number of cells.

All of the above methods either require the availability of expensive equipment or the substantial investment of time. In reality, the most often used method simply monitors the optical density of the sample. The absorbance of the sample measured in a spectrophotometer is correlated to either the dry weight or the number of cells per volume.

Biomass concentration is one of the most critically needed measurements in fermentation studies. It is also one of the most difficult and unreliable ones. For example, all the above dry/wet weight methods and all the automated counting equipment fail completely if the broth contains other insoluble particulate matter, which is often the case in a practical fermentor. Similarly, the optical density measurement only has limited usefulness if the fermentation broth is not clear. In addition, these methods cannot distinguish the viable cells from the dead ones. On the other hand, the standard plate count can detect viable cells among other particulate matters. However, the method requires elaborate preparations, and it takes 24-48 hours for the cells to be incubated and counted; the cost of Petri dishes and media can also be prohibitive. Consequently, the direct plate count is useless in feedback control of a fermentation process; it is mainly used industrially to countercheck other measurements, especially the optical density.

In this experiment, the cell density of a given sample will be measured with the dry weight and optical density methods.

List of Reagents and Instruments

A. Equipment

- Test tubes

- Eppendorfs
- Flasks
- Graduated cylinder
- Centrifuge
- Oven, 100 ºC
- Balance
- Spectrophotometer
- Aluminum Foil

B. Reagents

- Flask of culture
- Growth media
- Sterile water

C. Organism

- *Bacillus licheniformis* NRRL B-642

D. Media composition

- **For culture maintenance (Slant and/or Plate)**

  - Nutrient agar medium, 28 g/l

- **For Growth media in flask (Minimal Salt medium)**

Chemical name	Composition (g/l)
Glucose	2.0
Potassium Dihydrogen Phosphate	0.2
Di –Potassium hydrogen phosphate	0.8
Magnesium Sulphate Hepta hydrate	0.5
Ammonium Sulphate	1.0
Calcium Chloride	0.05

Procedures

1. *Dry/Wet Weight Measurement:*
    - Dry in an oven an empty weighing pan made of aluminum foil/eppendorf. Weigh them and store them in a desiccator lined with Drierite (anhydrous $CaSO_4$).
    - Stir the flask to suspend the culture evenly.
    - Separate the cells from the broth either by centrifugation at 10,000 g for 5 minutes or by filtration. In the case of centrifugation, carefully discard the clear broth and scrape the cell paste from the centrifuge tube into a weighing pan. Rinse the centrifuge tube with a few ml of water. Pour the

rinse water into the weighing pan, as well. In the case of filtration, the culture is poured into the holding reservoir fitted on the filter membrane. A vacuum is applied to pull the liquid through the membrane. Rinse the reservoir with a few ml of water and scrape any paste adhering to the glassware. The wet weight of the culture is measured immediately after all the water has been pulled through.

- Dry the cell paste in an oven set at 100ºC. The cells will be charred and the filter membrane will be burned if the temperature of the oven is set too high. Measure the weight of the pan/filter/eppendorf plus the cell paste periodically until there is no further decrease in the dry weight. It will take 6-24 hours to dry the sample completely, depending on the oven temperature and the thickness of the paste. Calculate the difference in the weight, and express the dry weight in g/l.

2. *OpticalDensity:*
   Dilute the sample to appropriate concentrations as needed, and measure the absorbance of the sample with a spectrophotometer at its λ_{max}. Generate a calibration curve to relate the absorbance with cell dry weight. The usual rules of operating a spectrophotometer apply here, as well. For example, the accuracy of the method is the highest when the absorbance is between 0.1 and 0.5. For a given culture sample, a good spectrophotometer should yield a linear relationship between the number of cells and the absorbance. However, the optical density is also a function of cell morphology such as size and shape, because the amount of transmitted or scattered light depends strongly on these factors. Consequently, an independent calibration curve is required for each condition in accurate research work, as the cell size and shape depend on the specific growth rate and the nutrient composition. As a rule of thumb, an optical density of 1 unit corresponds to approximately 1 g/l of dry cell. This is also commonly referred to as the turbidity measurement.

Task Required

1. Plot a calibration curve showing the relationship between cell biomass dry weight and optical density.
2. Report the cell density in appropriate units for the given sample.