the culture sample, and a phototube measures the light scattered by cells in the sample. The intensity of the scattered light is proportional to cell concentration. This method gives best results for dilute cell and particle suspensions.

6.2.1.2. Determining cell mass concentration. *Direct methods*. Determination of cellular *dry weight* is the most commonly used direct method for determining cell mass concentration and is applicable only for cells grown in solids-free medium. If noncellular solids, such as molasses solids, cellulose, or corn steep liquor, are present, the dry weight measurement will be inaccurate. Typically, samples of culture broth are centrifuged or filtered and washed with a buffer solution or water. The washed wet cell mass is then dried at 80°C for 24 hours; then dry cell weight is measured.

Packed cell volume is used to rapidly but roughly estimate the cell concentration in a fermentation broth (e.g., industrial antibiotic fermentations). Fermentation broth is centrifuged in a tapered graduated tube under standard conditions (rpm and time), and the volume of cells is measured.

Another rapid method is based on the absorption of light by suspended cells in sample culture media. The intensity of the transmitted light is measured using a spectrometer. Turbidity or *optical density* measurement of the culture medium provides a fast, inexpensive, and simple method of estimating cell density in the absence of other solids or light-absorbing compounds. The extent of light transmission in a sample chamber is a function of cell density and the thickness of the chamber. Light transmission is modulated by both absorption and scattering. Pigmented cells give different results than unpigmented ones. Background absorption by components in the medium must be considered, particularly if absorbing dissolved species are taken into cells. The medium should be essentially particle free. Proper procedure entails using a wavelength that minimizes absorption by medium components (600- to 700-nm wavelengths are often used), "blanking" against medium, and the use of a calibration curve. The calibration curve relates optical density (OD) to dry-weight measurements. Such calibration curves can become nonlinear at high OD values (> 0.3) and depend to some extent on the physiological state of the cells.

Indirect methods. In many fermentation processes, such as mold fermentations, direct methods cannot be used. In such cases indirect methods are used, which are based mainly on the measurement of substrate consumption and/or product formation during the course of growth.

Intracellular components of cells such as RNA, DNA, and protein can be measured as indirect measures of cell growth. During a batch growth cycle, the concentrations of these intracellular components change with time. Figure 6.2 depicts the variation of certain intracellular components with time during a batch growth cycle. Concentration of RNA (RNA/cell weight) varies significantly during a batch growth cycle; however, DNA and protein concentrations remain fairly constant. Therefore, in a complex medium, DNA concentration can be used as a measure of microbial growth. Cellular protein measurements can be achieved using different methods. Total amino acids, Biuret, Lowry (folin reagent), and Kjeldahl nitrogen measurements can be used for this purpose. Total amino acids and the Lowry method are the most reliable. Recently, protein determination kits from several vendors have been developed for simple and rapid protein measurements.