Batch Bioreactor

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Introduction*

For microbes, growth is their most essential response to their physiochemical environment. Growth is a result of both replication and change in cell size. Microorganisms can grow under a variety of physical, chemical and nutritional conditions. In a suitable nutrient medium, organisms extract nutrients from the medium and convert them into biological compounds. Part of these nutrients are used for energy production and part are used for biosynthesis and product formation. As a result of nutrient utilization, microbial mass increases with time and can be described simply by

$$substrates + cells \rightarrow extracellular\ products + more\ cells$$

$$\Sigma S + X \rightarrow \Sigma \mathrm{P} + \mathrm{nX}$$

Microbial growth is a good example of an autocatalytic reaction. The rate of growth is directly related to cell concentration, and cellular reproduction is the normal outcome of this reaction.

The rate of microbial growth can be described in terms of cell number concentration, *N*:

$$\mu_R \equiv \frac{1}{N}\frac{dN}{dt}$$

Where μ_R is the net specific replication rate (h^{-1}).

Batch Growth

Batch growth refers to culturing cells in a vessel with an initial charge of medium that is not altered by further nutrient addition or removal. This form of cultivation is simple and widely used both in the laboratory and industrially.

Determining cell mass concentration

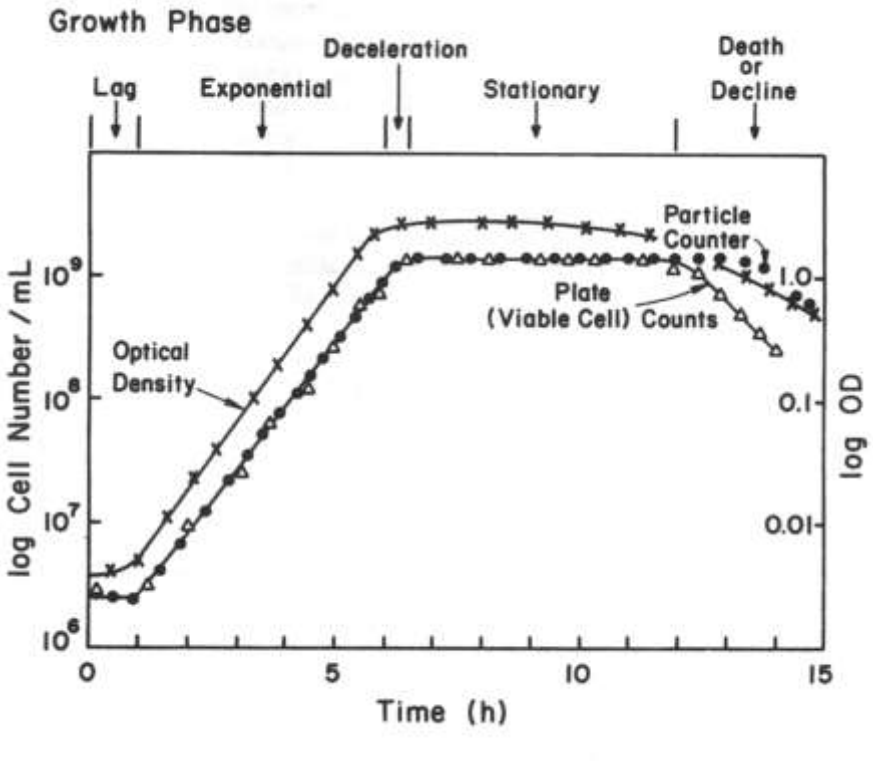
The quantification of cell concentration in a culture medium is essential for the determination of the kinetics and stoichiometry of microbial growth. Either cell number or cell mass can be quantified depending in on the type of information needed and the properties of the system. Cell mass concentration is often preferred to the measurement of cell number density when only one is measured, but the combination of the two measurements is often desirable.

One rapid method of cell number measurement is based on the absorption of light by suspended cells in sample culture media. The intensity of the transmitted light is measured using a visible light spectrometer. Turbidity or optical density (OD) 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 curves. The calibration curve relates OD to dry-weight measurement. Such calibration curves can become nonlinear at high OD values (>0.3) and depend to some extent on the physiological state of the cells.

Growth patterns and Kinetics in Batch Culture

When a liquid nutrient medium is inoculated with a seed culture, the organisms selectively take up dissolved nutrients from the medium and convert them into biomass. A typical batch growth curve includes the following phases: (1) lag phase, (2) logarithmic or exponential growth phase, (3) deceleration phase, (4) stationary phase, and (5) death phase:



The lag phase occurs immediately after inoculation and is a period of adaptation of cells to a new environment. Microorganisms reorganize their molecular constituents when they are transferred to a new medium. Depending on the composition of nutrient, new enzymes are synthesized, the synthesis of some other enzymes is repressed, and the internal machinery of cells is adapted to the new environmental conditions. These changes reflect the intracellular mechanisms for the regulation of the metabolic processes. During this phase cell mass may increase a little, without an increase in cell number density. When the inoculum is small and has a low fraction of cells that are viable, there may be a pseudolag phase, which is a result, not of adaptation, but of small inoculum size or poor condition of the inoculum.

The exponential growth phase is also known as the logarithmic growth phase. In this phase, the cells have adjusted to their new environment. After this adaptation period, cells can multiply rapidly, and cell mass number and cell number density increase exponentially with time. This is a period of balanced growth, in which all components of a cell grow at the same rate. That is the average composition of a single cell remains approximately constant during this phase of growth. During balanced growth, the net specific growth rate determined from either cell number or cell mass would be the same. Since the nutrient concentrations are large in this phase, the growth rate is independent of nutrient concentration. The exponential growth rate is first order:

$$\frac{dN}{dt} \equiv \mu_R N, \qquad N=N_0 \text{ at } t = 0$$

$$ln\frac{N}{N_0} = \mu_R t \qquad \text{or} \qquad N = N_0\, e^{\mu_R t}$$

where N and N_0 are the cell number concentrations at time t and t=0.

The time required to double the microbial cell concentration is:

$$\tau_d = \frac{ln2}{\mu_R}$$

The exponential growth is characterized by a straight line on a semilogarithm plot of ln N versus time.

The deceleration growth phase follows the exponential phase. In this phase growth decelerates due to either depletion of one or more essential nutrients or the accumulation of toxic by-products of growth. For a typical bacterial culture, these changes occur over a very short period of time. The rapidly changing environment results in unbalanced growth. During unbalanced growth, cell composition and size will change. In the exponential phase, the cellular metabolic control system is set to achieve maximum rates of reproduction. In the deceleration phase, the stresses induced by nutrient depletion or waste accumulation cause a restructuring of the cell to increase the prospects of cellular survival in a hostile environment. These observable changes are the result of the molecular mechanisms of repression and induction. Because of the rapidity of these changes, cell physiology under conditions of nutrient limitation is more easily studied in continuous culture.

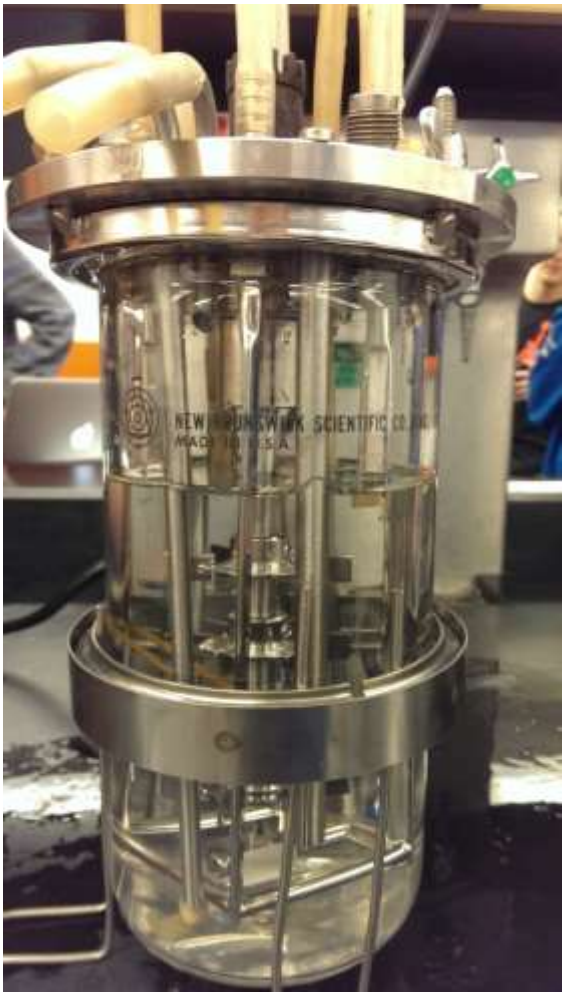
The stationary phase starts at the end of the deceleration phase, when the net growth rate is zero (no cell division) or when the growth rate is equal to the death rate. Even though the net growth rate is zero during the stationary phase, cells are still metabolically active and produce secondary metabolites. Primary metabolites are growth-related products and secondary metabolites are nongrowth-related. In fact, the production of certain metabolites is enhanced during the stationary phase (e.g. antibiotics, some hormones) due to metabolite deregulation. During the course of the stationary phase, one or more of the following phenomena may take place:

1. Total cell mass concentration may stay constant, but the number of viable cells may decrease.
2. Cell lysis may occur and viable cell mass may drop. A second growth phase may occur and cell may grow on lysis products of lysed cells (Cryptic growth).

3. Cells may not be growing but may have active metabolism to produce secondary metabolites. Cellular regulation changes when concentrations of certain metabolites (carbon, nitrogen, phosphate) are low. Secondary metabolites are produced as a result of metabolite deregulation.

*Abstracted from Ch 6, Bioprocess Engineering – Basic Concepts, 2nd Ed by Michael Shuler and Fikret Kargi

Apparatus



Vessel

Water bath incubator

Experiment A

Objective

- To observe the bacterial growth pattern under anaerobic condition in a batch bioreactor

Procedure

1. Inoculate 10% *E. Coli.* seed culture (125ml) into sterile growth medium at 37°C in the bioreactor using sterile technique. The bioreactor was filled with 1.25L of lysogeny broth (LB medium) and has been sterilized by autoclaving by the instructor.
2. Close all outlets.
3. Turn on stirrer to maximum speed.
4. Using sterile technique, obtain 20 mls of sample culture every 10 minutes until stationary growth phase is observed, or after 2 hours of incubation.
5. Blank light spectrophotometer with LB medium. The cuvette has a 1 cm pathlength and 2 ml capacity.
6. Measure OD of the sample cultures. Check if OD measurement is within linear range by standard dilutions.
7. Pre-weigh a weighing dish. Transfer 10 mls of LB medium into the weighing dish and dry at the 60°C incubator. Measure the mass of 10ml LB medium during next lab session.
8. Label and pre-weigh some weighing dishes. Transfer 10 mls of each of the sample culture into a weighing dish and dry cultures at the 60°C incubator. Measure dry cell mass and calculate cell mass density for the samples collected during next lab session.
9. At the end of experiment, autoclave bioreactor and used containers in ET 949. Spray counter top, gloves, incubator surface and other contacted area with 70% ethanol.
10. Dispose autoclaved culture, wash vessel with soap, rinse with city water and then lastly with DI water.

Experiment B

Objective

- To observe the bacterial growth pattern under aerobic condition in a batch bioreactor

Procedure

- Repeat experiment A with oxygen supply to the bioreactor through filtered air pipeline. Skip measurement of dry cell mass due to time constraint.

Data Analysis

Plot (1) OD versus time and (2) cell mass concentration versus time (for anaerobic condition only) in a semi-log plot. Identify the various growth phases and compare the growth rate between experiment A and B.