5. Cell Growth

Read: Chapter 6
pp. 155 - 200

Types of models to describe cell growth, substrate utilization and product formation:

Segregated - Models which consider differences among cells in a population and categorize different cells into individual “compartments”. Models rely on the ability to distinguish one or more characteristics which make cells in a population differ.

Non-segregated - Models which consider the entire population of cells to be identical. The system may be described by one cell concentration. These models are mathematically simple.

Structured - Models which categorize individual reactions or groups of reactions in a cell.

Unstructured - Models which view a cell as a whole entity which interacts with its environment.

Stochastic - Models which consider distributions of characteristics and the probabilities associated with each level of a characteristic. These models are statistical in nature because of their consideration of natural randomness.

Deterministic - Models which have outputs that are completely determined by the model inputs, without consideration of random variations.

Two types of “rates” are used in describing cell functions:

Volumetric Rate - A rate per volume of solution. The units of a volumetric rate are: mass/(volume•time)

Specific Rate - A rate per mass of cells. The units of a specific rate are: mass/(mass cells•time)

A. Specific Growth Rate

Define:

$X \equiv$ mass concentration of cells (g/L)

$N \equiv$ number concentration of cells (number/L)

Note: $X = f(N)$

As cell number concentration increases usually the cell mass concentration increase. This functionality may be complex, however, because cells change size and shape during growth.

A Principle of Populations Dynamics:

The growth rate of a population of living organisms is proportional to the number of organisms

$$\frac{dN}{dt} \propto N$$

For example, *in a medium with a limitless supply of nutrients*, if 4 organisms reproduce to become 8 in certain time (t_D), then those 8 will reproduce to become 16 in the same time.

Simplification: $X \propto N$

(All cells have the same mass)

Then: $$\frac{dX}{dt} \propto X$$

$$\frac{dX}{dt} = \mu X$$

$$\boxed{\frac{1}{X}\frac{dX}{dt} = \mu}$$

The proportionality constant μ is called the specific growth rate. (units are time^{-1})

Notes:

- μ can be positive (growth) or negative (death). Usually μ is used in the context of cell growth.
- μ is a function of nutrient supply and therefore can be a function of time (i.e., if nutrient supply is changing with time).

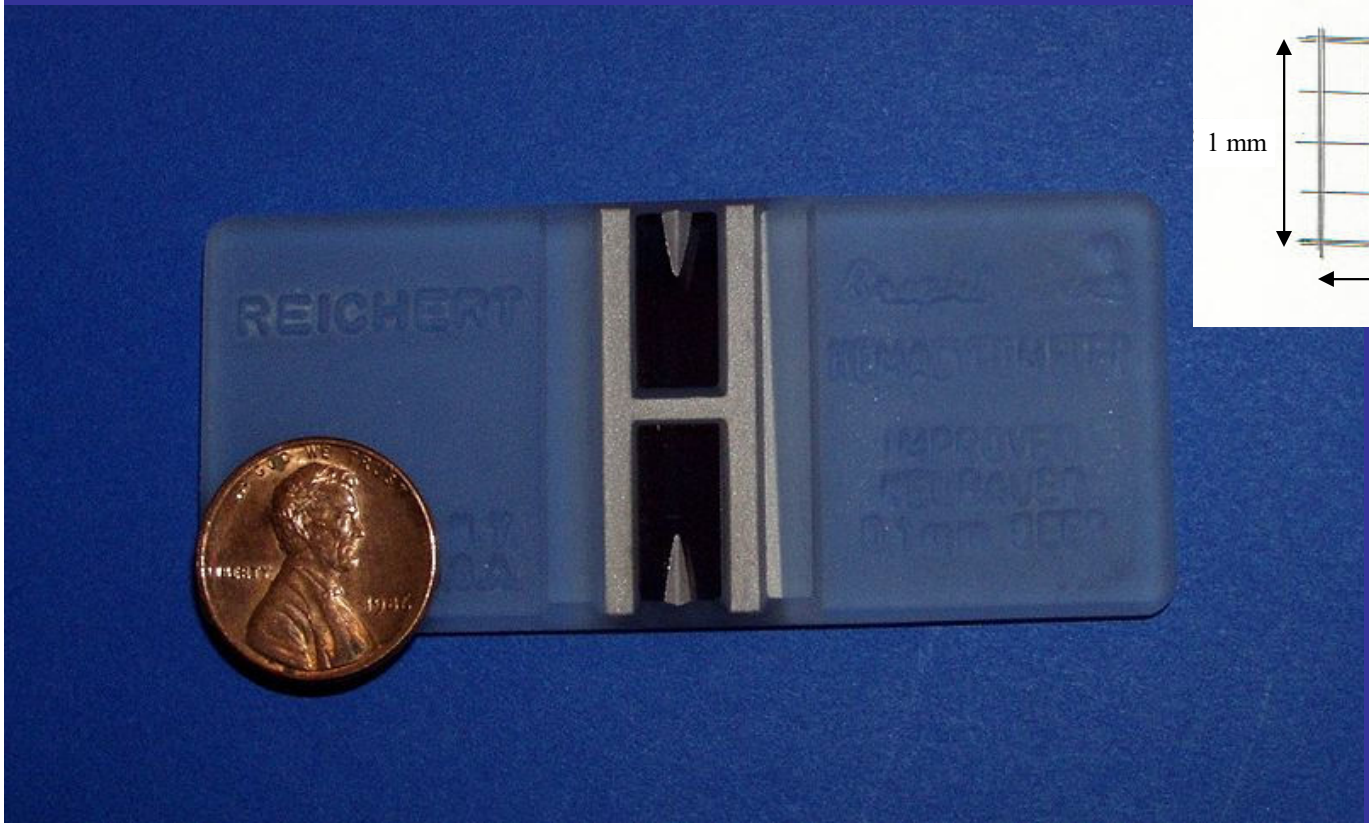
B. Measurement of Cell Concentration ("Cell Density")

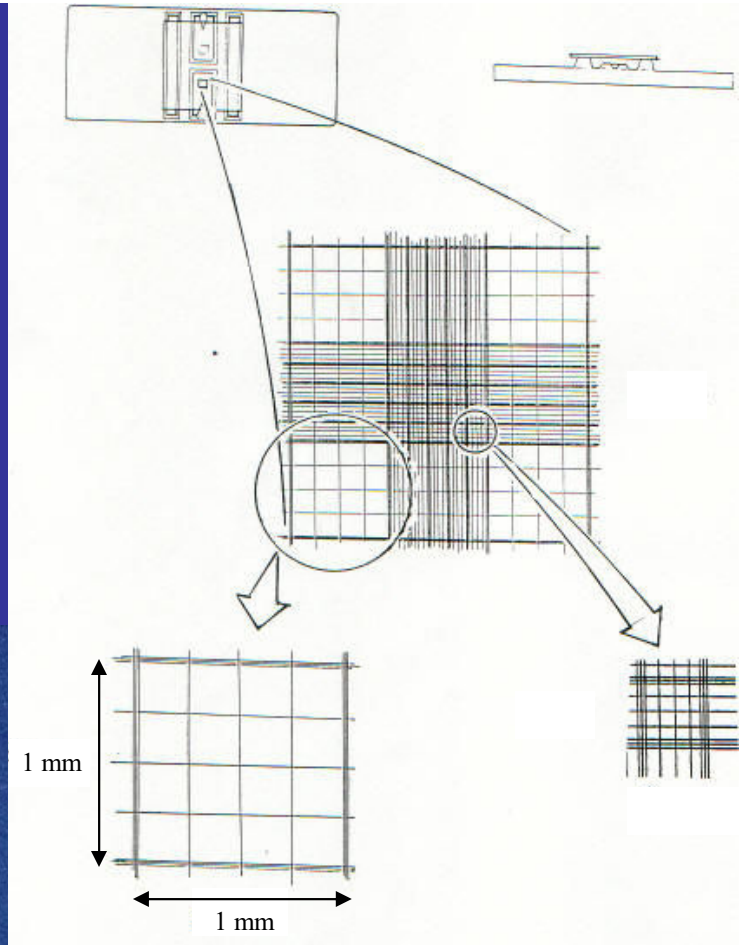
1. Counting

A) Hemocytometer, Petroff-Hauser Slide

Microbial slides with special chambers, each having a known volume. One actually counts the number of cells through the microscope, thereby determining number per volume.

- Cannot distinguish living from dead organisms unless stains are used.
- Method difficult for irregularly shaped organisms or organisms which clump.





$1\ \text{mm} \times 1\ \text{mm} \times 0.1\ \text{mm} = 10^{-4}\ \text{cm}^3$

B) Plate Counts

Spread a known volume of medium on a solid growth surface (agar). Living cells will replicate forming colonies visible to the eye. "Viable Cell Count" or "Colony Forming Units"

- Problematic for cells which clump and for highly motile organisms.
- Only measures living organisms.
- Volume of medium containing cells must have a certain range of cells to yield a reasonable number to count on the surface: 30 – 300

C) Particle Counters

Flow cell-containing liquid between two electrodes. A particle will cause a change in resistance of the fluid. Electronics used to count pulses of resistance change.

- Problematic for cells which clump.
- Cannot distinguish living from dead organisms.

2. Mass Measurements

A) Dry Cell Weight

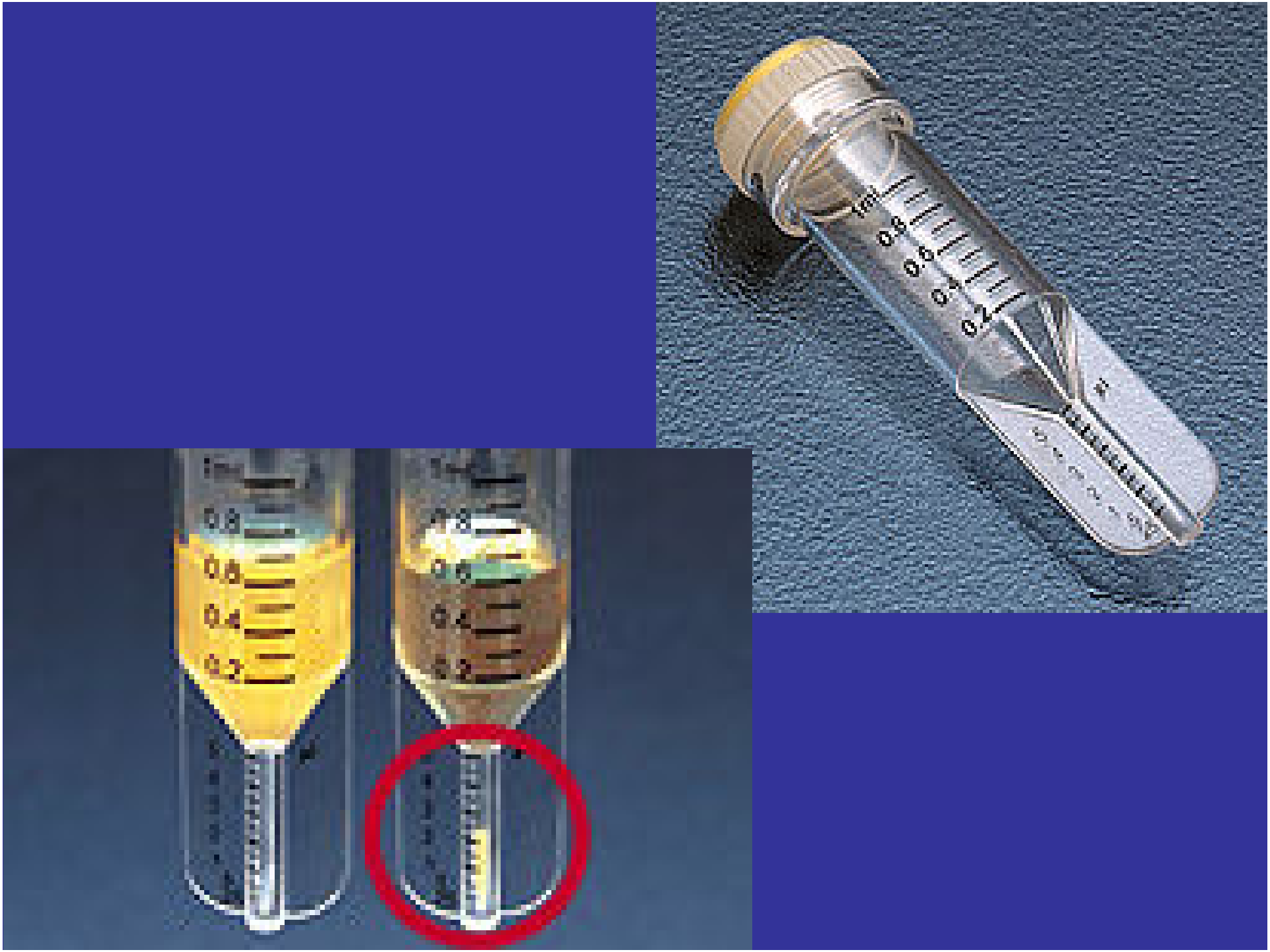
Centrifuge medium that contains cells, wash cells, recentrifuge and dry in oven at specified conditions of temperature and time.

- Since cells are >80% water, method of drying significantly affects measurement.
- Since cells are composed of readily degradable compounds, method of drying significantly affects measurement.
- Cannot distinguish living from dead organisms.

B) Packed Cell Volume

Centrifuge medium at a specified rate and duration in a graduated tube. The volume of the packed cells can be correlated with some other measurement.

- Problematic for cells which clump and for highly motile organisms.
- Cannot distinguish living from dead organisms.


0.8
0.6
0.4
0.2

C) Optical Density

A beam of light is passed through a sample containing cells. Particles in medium absorb light according to number and size of particles in medium.

- Problematic if medium (or substrate, products) absorb light.
- Cannot distinguish living from dead organisms.
- Absorption becomes markedly nonlinear at a high cell concentration.
- Problematic if morphology of cells changes.

D) DNA Measurement

DNA, RNA or total protein concentration is directly related to cell concentration. The chemical of interest is extracted from medium and analyzed with a chemical assay.

- Distinguish living from dead organisms (?).

3. Cell Activity Measurements

A) ATP Measurement

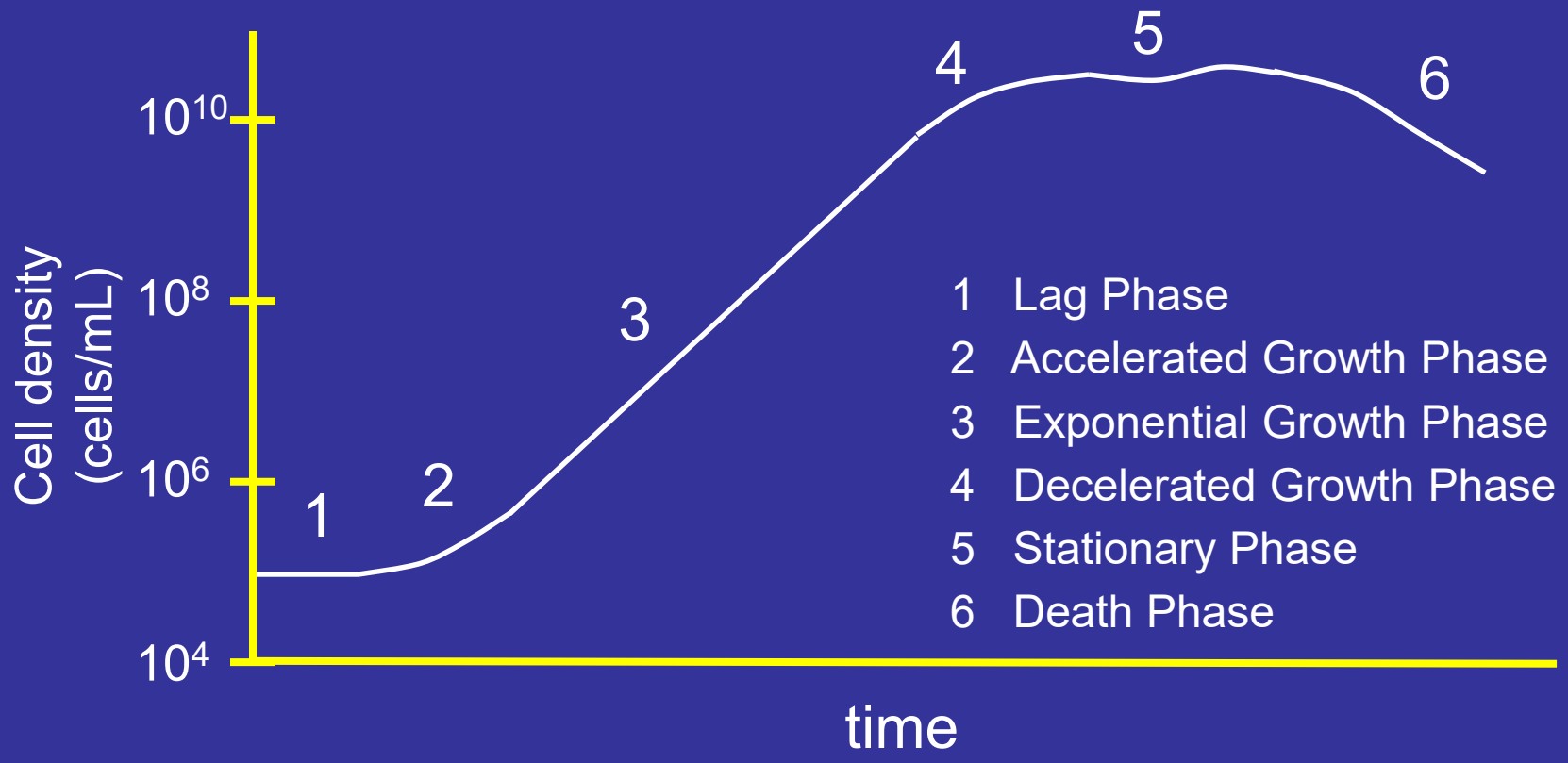
The cellular activity of a cell population can be determined by an assay with luciferase.

B) NADH Measurement

The cellular activity of a cell population can be determined by a fluorescence assay.

C. Batch Growth Phases

When a population of organisms is “grown” in a *batch* of nutrient-rich medium, the cell density will typically change with time:



Strictly speaking

$$\frac{dX}{dt} = \mu X - \alpha X$$

μ = specific growth rate

α = specific death rate

Important Phases:

1. Lag phase (1)

$\mu = 0$ $\alpha = 0$

A period of adaptation for cells, and it is a function of:

- Availability of nutrients
- Similarity of nutrients to those in inoculum
- Health of inoculum

Important Phases:

2. Exponential growth phase (3)

$\alpha \approx 0$

$$\frac{dX}{dt} = \mu X$$

a) defined media

μ is a constant that depends on the energy source (usually carbon source)

Result:

$$X = X_0 e^{\mu t}$$

where X_0 is the initial cell density

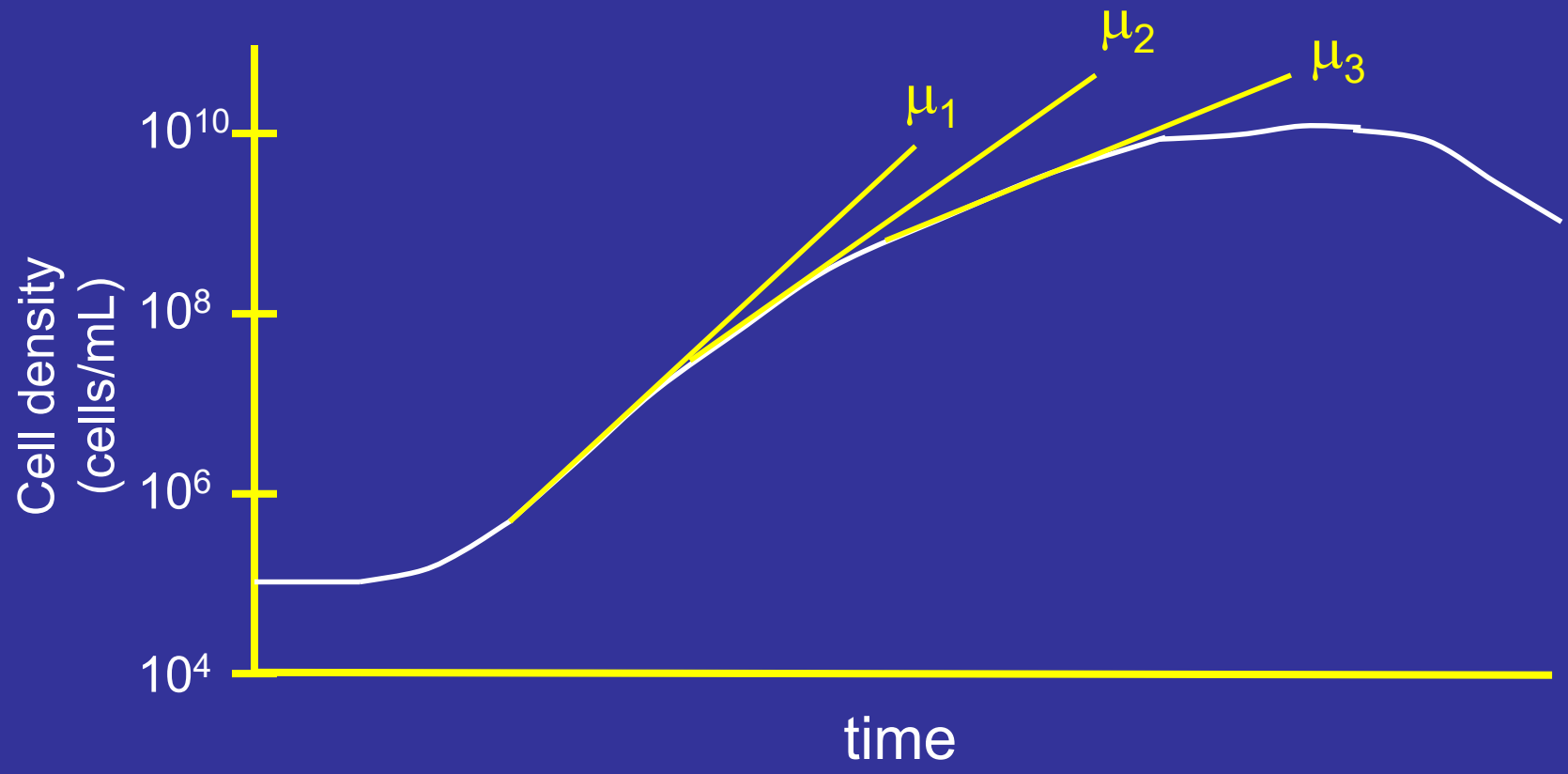
t_D = doubling time
(time at which $X = 2X_0$)

$$2 = e^{\mu t_D} \qquad \ln 2 = \mu t_D$$

$$t_D = \frac{\ln 2}{\mu}$$

b) undefined media

μ shifts from high to low values as progressively more difficult substrates are consumed



3. Stationary phase

$$\mu \approx \alpha$$

$$\frac{dX}{dt} = 0$$

Growth rate becomes zero because of exhaustion of nutrients or accumulation of toxic products.

Many organisms produce the product of interest only during the stationary phase.

D. Parameters used to describe substrate utilization

A substrate is consumed by an organism. The mass of the substrate goes towards three uses:

1. Maintenance

The substrate may be converted by the organism into energy for it to maintain its standard of living. This concept is called maintenance requirement. The majority of a cell's maintenance energy goes towards maintaining osmotic and ionic gradients across the cell membrane.

The rate of substrate consumed in order to maintain the cells is proportional to the number of cells or the mass density:

$$-\left.\frac{dS}{dt}\right|_{\text{Maintenance}} \propto X$$

or

$$-\left.\frac{dS}{dt}\right|_{\text{Maintenance}} = mX$$

Where m is the maintenance coefficient. The units of m are (for example) g substrate/g cell•h.

The higher the value of the maintenance coefficient, the more substrate goes towards maintaining the culture without contributing to cell mass or product formation. Maintenance is the "overhead" costs of the organism for doing business.

Notice that maintenance is defined in terms of any single substrate. Usually maintenance coefficients are considered for the two limiting substrates – carbon (m_S) and oxygen (m_O).

Organism	substrate	m_S	m_O
Aerobacter aerogenes	glucose	0.054 g/gh	0.054 g/gh
A. aerogenes	glycerol	0.096	0.109
A. aerogenes	citrate	0.058	0.048
Penicillium chrysogenum	glucose	0.022	0.024

2. Cell Mass

The substrate may be used for the production of new cellular components which ultimately become new cells. The rate of substrate consumed to produce more cells is proportional to the rate of new cells produced:

$$-\left.\frac{dS}{dt}\right|_{Cells} \propto \frac{dX}{dt}$$

By convention, the proportionality is written in front of the substrate consumption rate term:

$$-Y_{X/S}\left.\frac{dS}{dt}\right|_{Cells} = \frac{dX}{dt}$$

or

$$-\left.\frac{dS}{dt}\right|_{Cells} = \frac{1}{Y_{X/S}}\frac{dX}{dt}$$

where $Y_{X/S}$ is the cell yield coefficient. Cell yield coefficients may be written for any substrate:

$$Y_{X/S} = \frac{\text{g cell formed}}{\text{g substrate consumed}}$$

$$Y_{X/O} = \frac{\text{g cell formed}}{\text{g oxygen consumed}}$$

Organism	substrate	$Y_{X/S}$	$Y_{X/O}$
Candida utilis	glucose	0.51 g/g	1.30 g/g
Candida utilis	acetic acid	0.36	0.62
Candida utilis	ethanol	0.68	0.58

3. Products

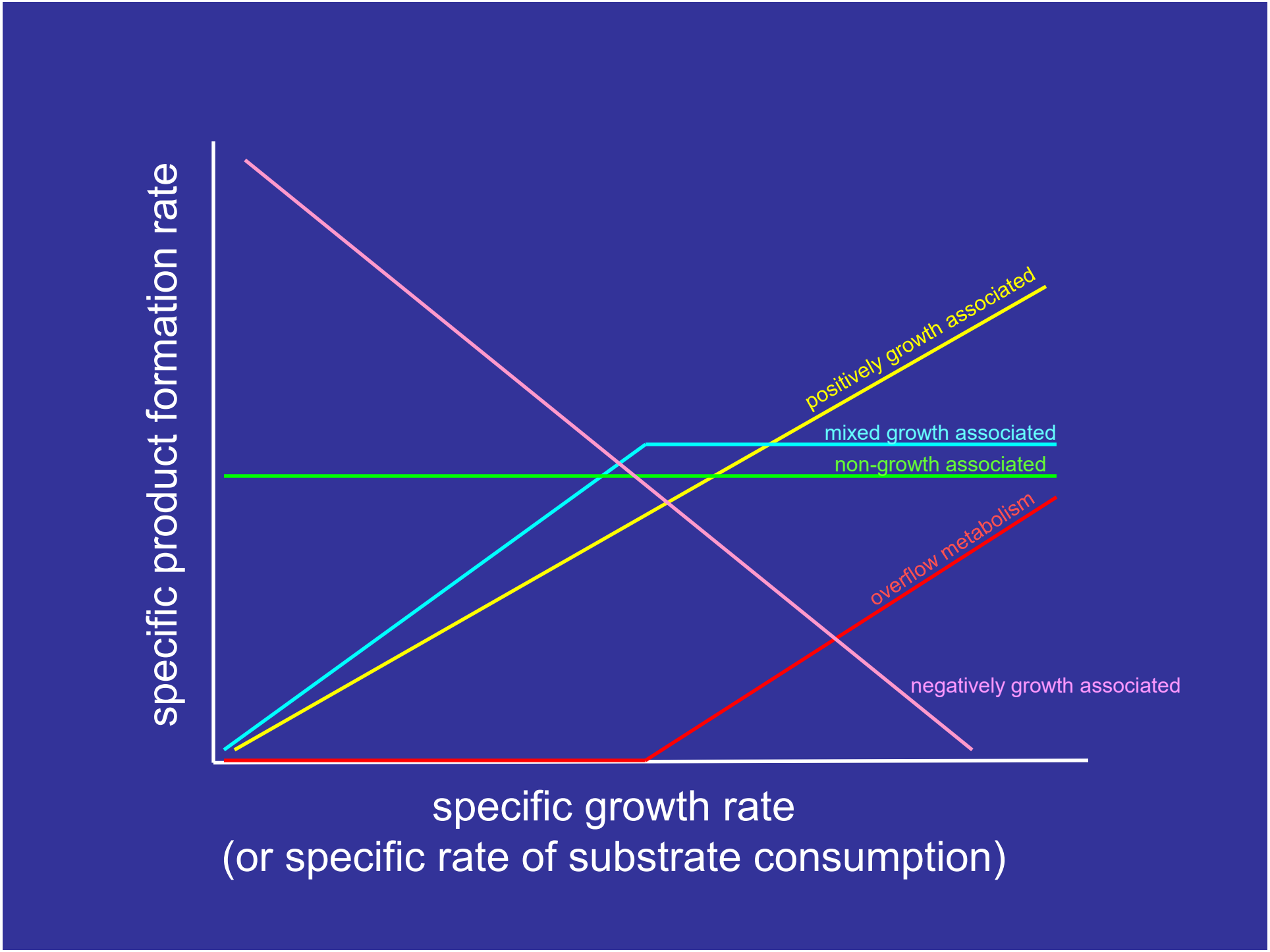
The substrate may be used for the synthesis of chemical products. These are usually at the termination of biochemical pathways.

Some useful terms:

Volumetric rate of product formation = $Q_P = \frac{dP}{dt}$

Specific rate of product formation = $q_P = \frac{1}{X}\frac{dP}{dt}$

There are several relationships which can exist between the rate or product formation (q_P) and the specific growth rate (μ).


specific product formation rate
positively growth associated
mixed growth associated
non-growth associated
overflow metabolism
negatively growth associated
specific growth rate
(or specific rate of substrate consumption)

a. Growth Associated

In this case a simple stoichiometric relationship exists between the product formation rate and substrate utilization rates.

$$-\left.\frac{dS}{dt}\right|_{Products} \propto \frac{dP}{dt}$$

$$-Y_{P/S}\left.\frac{dS}{dt}\right|_{Products} = \frac{dP}{dt}$$

$$-\left.\frac{dS}{dt}\right|_{Products} = \frac{1}{Y_{P/S}}\frac{dP}{dt}$$

where $Y_{P/S}$ is a product yield coefficient based on substrate. Such a yield coefficient can be written for each substrate (and for each product):

$$Y_{P/S} = \frac{\text{g product formed}}{\text{g substrate consumed}}$$

$$Y_{P/O} = \frac{\text{g product formed}}{\text{g oxygen consumed}}$$

Growth associated products form simultaneously with and as a natural consequence of cell growth. Thus, the rate of product formation is also proportional to growth rate:

$$\frac{dP}{dt} \propto \frac{dX}{dt}$$

$$\frac{dP}{dt} = Y_{P/X} \frac{dX}{dt}$$

where $Y_{P/X}$ is the specific product yield coefficient.

Note:

$$\frac{dP}{dt} = Y_{P/X} \, X \, \frac{1}{X} \, \frac{dX}{dt}$$

$$Q_P = Y_{P/X} \, \mu \, X$$

$$q_P = Y_{P/X} \, \mu$$

growth associated products

b. Non-Growth Associated

The specific rate of product formation is a constant.

$$q_P = \beta$$

$$Q_P = \beta X$$

c. Mixed-Growth Associated

$$q_P = \alpha\mu + \beta$$ Luedeking-Piret Equation

$$Q_P = \alpha\mu X + \beta X$$

4. Summary

Thus, the total substrate utilization may be written:

$$-\left.\frac{dS}{dt}\right|_{Total} = -\left.\frac{dS}{dt}\right|_{Maint} - \left.\frac{dS}{dt}\right|_{Cells} - \left.\frac{dS}{dt}\right|_{Products}$$

$$-\left.\frac{dS}{dt}\right|_{Total} = mX + \frac{\mu X}{Y_{X/S}} + \frac{Q_P}{Y_{P/S}} = Q_S$$

$$q_S = \frac{Q_S}{X}$$