Cell Growth Kinetics

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Growth Kinetics

Introduction

- <u>Autocatalytic reaction</u>: The rate of growth is directly related to cell concentration

substrates + cells → extracellular products + more cells

$$\sum S + X \rightarrow \sum P + nX$$

S: substrate concentration (g/L); X: cell mass concentration (g/L); P: product concentration (g/L); n: increased number of biomass.

<u>Net specific growth rate</u> (1/time):

$$\mu_{\text{net}} \equiv \frac{1}{X}\frac{dX}{dt}$$

t: the time

Growth Kinetics

Introduction

Net specific growth rate (1/time):

$$\mu_{\mathrm{net}} = \mu_g - k_d$$

μ_g : Gross specific growth rate (1/time)

k_d : The rate of loss of cell mass due to cell death or endogenous metabolism

Endogenous metabolism: during the stationary phase, the cell catabolizes cellular reserves for new building blocks and for **energy-producing monomers**.

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Introduction

Net specific replication rate (1/time):

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

$$\mu_R = \mu_R' - k_d$$

N : Cell number concentration (cell number /L)

μ_R' : Gross specific replication rate (1/time)

k_d : The rate of cell death (1/time)

Growth Kinetics

Introduction

- Quantifying cell concentration:

- direct: no suspended solid and interference compounds.

cell mass concentration – preferred

dry weight, optical density (turbidity) (600-700nm Wave Length)

cell number density:

Petroff-Hausser slide (hemocytometer), plate counts, etc.

Growth Kinetics

- Quantifying cell concentration:

  - indirect: direct method is inapplicable. (mold solid state fermentation)

  Cell mass can be determined by measurement of protein, DNA or ATP. e.g. 1mg ATP/g dry weight bacterial cell.

If 100 mg ATP/L is measured, then the cell mass:

100 mg (ATP/L)/1 mg ATP/g dry cells=100 (g dry weight cells/L)

Batch Growth Kinetics

Lag phase

A period of adaptation for the cells to their new environment

- New enzymes are synthesized.
- A slight increase in cell mass and volume, but no increase in cell number
- Prolonged by low inoculum volume, poor inoculum condition (high % of dead cells), age of inoculum, nutrient-poor medium

- Multiple lag phases: (diauxic growth) medium contains more than one carbon source

Batch Growth Kinetics

<u>Exponential growth phase</u>

In this phase, the cells have adjusted to their new environment and multiply rapidly (exponentially)

- <u>Balanced growth</u> –all components of a cell grow at the same rate.
- Growth rate is independent of nutrient concentration, as

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Exponential growth phase

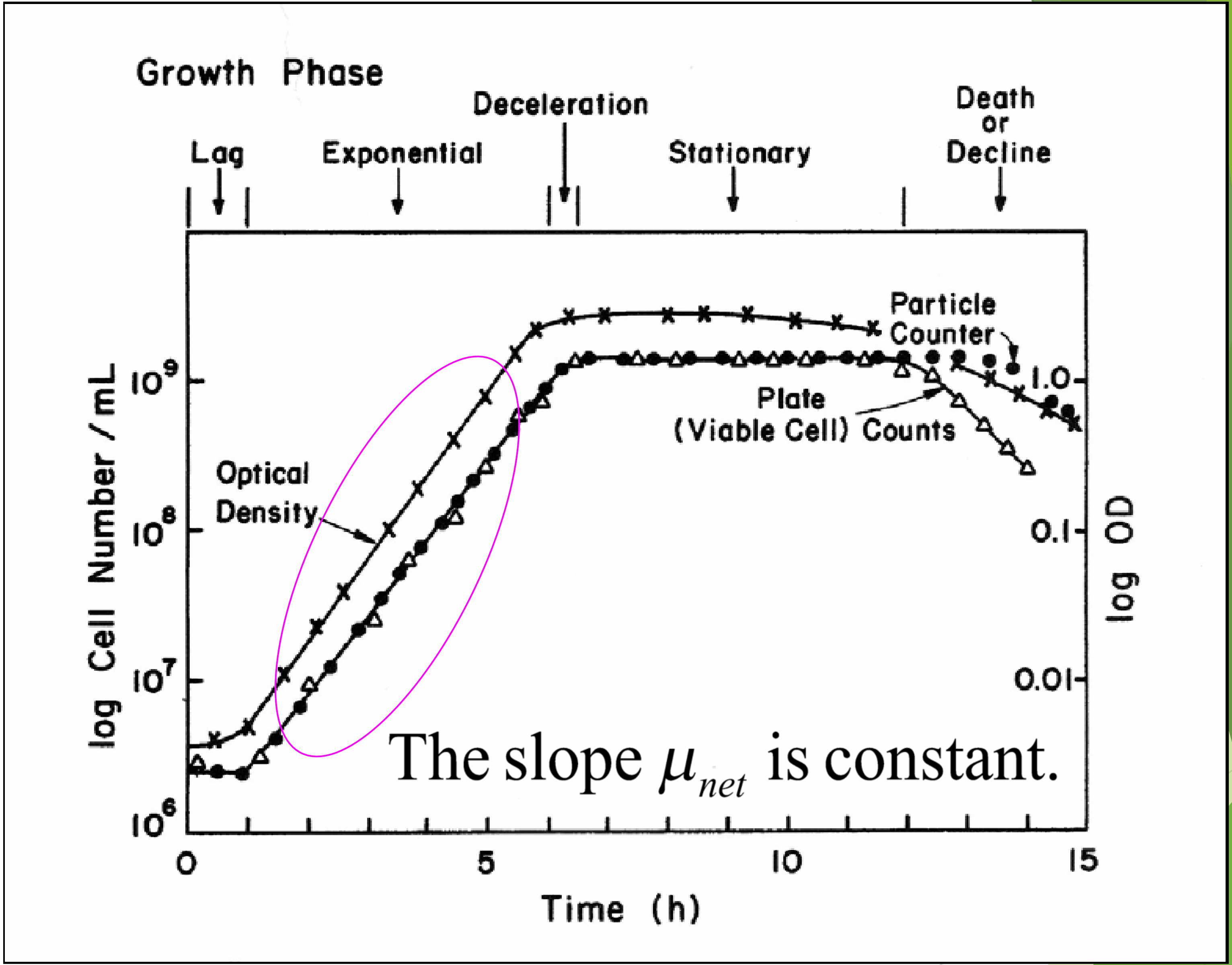
The balance of cell mass in a batch culture gives:

$$\frac{dX}{dt} = \mu_{net} X, \quad X = X_0 \;\; at \;\; t = 0$$

Integration of the above equation yields:

$$\ln \frac{X}{X_0} = \mu_{net} t \text{ , or } X = X_0 e^{\mu_{net} t}$$

X and X_0 are cell concentrations at time t and t = 0



Typical growth curve for a bacterial population

Batch Growth Kinetics

Exponential growth phase

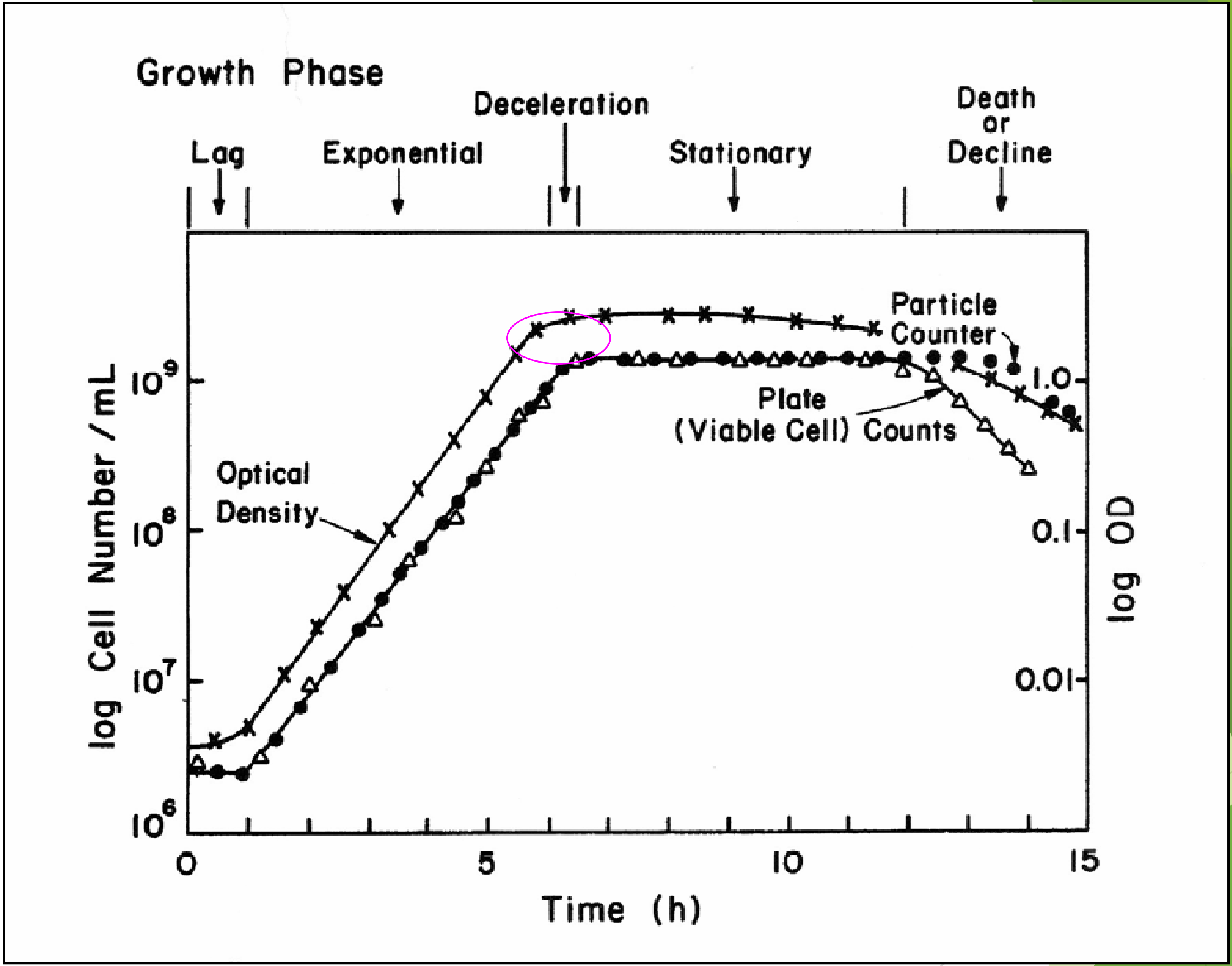
$$\mu_{net} = \mu_R = \mu_m$$

μ_m is the maximum specific growth rate (1/time)

Doubling time of cell mass: the time required to double the microbial mass:

At $t=t_d$, the time for doubling of biomass, X=2Xo

$$\tau_d = \frac{\ln X / X_0}{\mu_{net}} = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}}$$



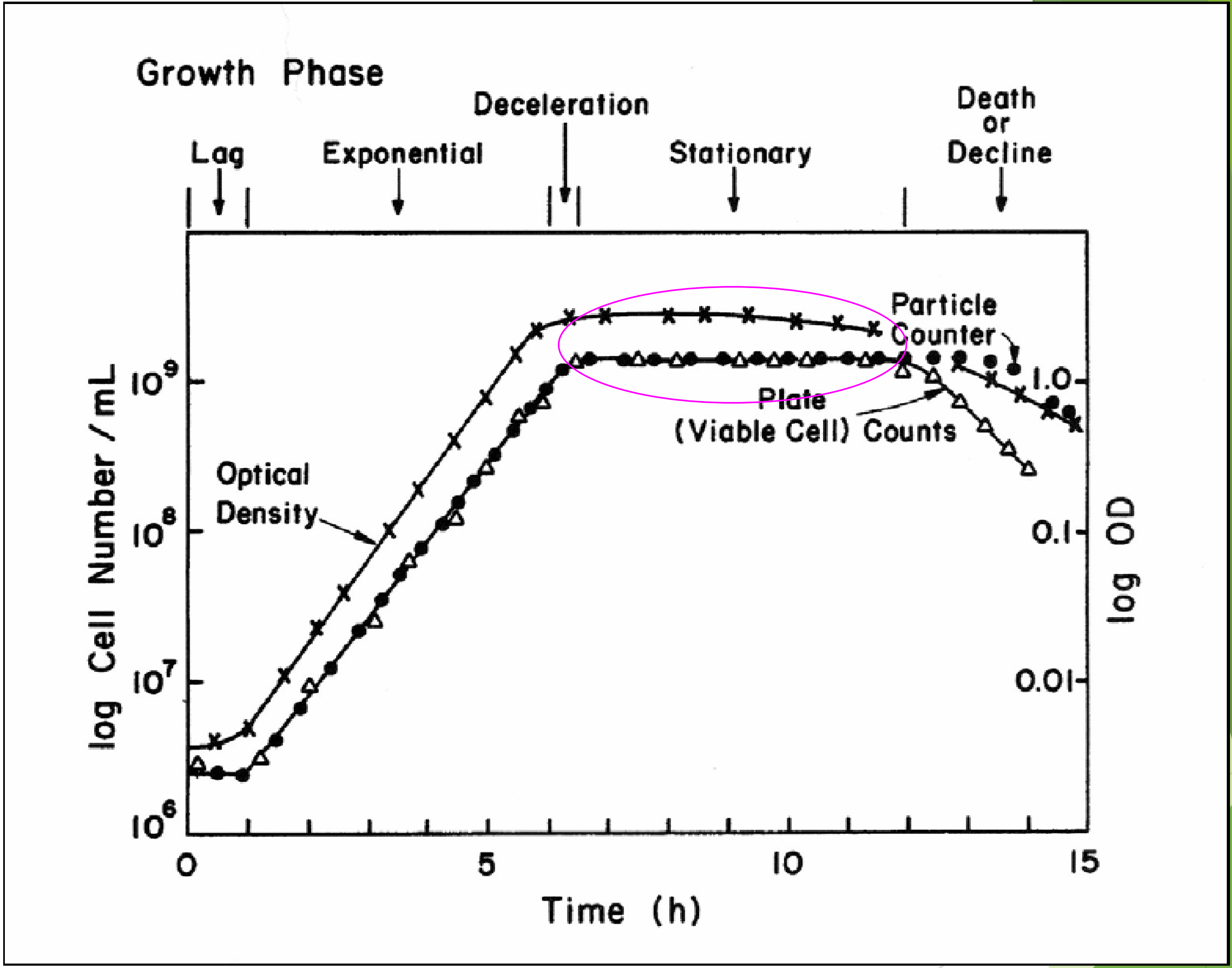
Typical growth curve for a bacterial population

Batch Growth Kinetics

<u>Deceleration growth phase</u>

Very short phase, during which growth decelerates due to either:

- Depletion of one or more essential nutrients
- The accumulation of toxic by-products of growth (e.g. Ethanol in yeast fermentations)
- Period of unbalanced growth: Cells undergo internal restructuring to increase their chances of survival



Typical growth curve for a bacterial population

Batch Growth Kinetics

<u>Stationary Phase:</u>

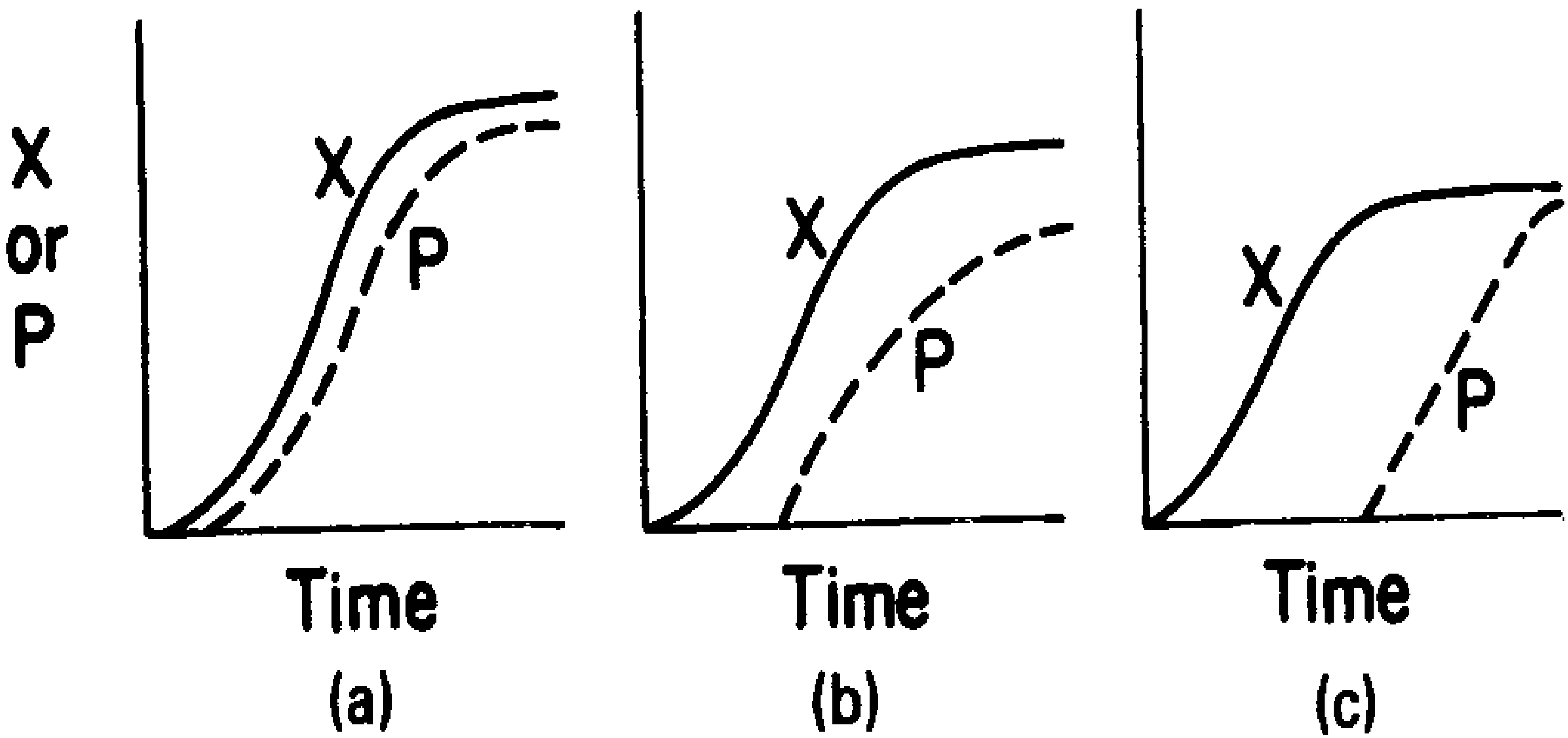
With the exhaustion of nutrients (S≈0) and build-up of waste and secondary metabolic products

- The growth rate equals the death rate.
- There is no net growth in the organism population.
- Cells may have active metabolism to produce secondary metabolites.

<u>Primary metabolites</u> are growth-related: ethanol by *S. cerevisae.*

<u>Secondary metabolites</u> are non-growth-related: antibiotics, pigments.

Kinetic Pattern of Growth



Growth-associated Mixed-growth-associated Non growth-associated

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Stationary phase

- Cell lysis(defected) may occur and viable cell mass may drop.

  A second growth phase may occur and cells may grow on lysis products of lysed cells (cryptic growth)

- Endogenous metabolism occurs by catabolizing cellular reserves for new building blocks and energy-producing monomer (maintenance energy).

  $$\frac{dX}{dt} = -k_d X$$

  k_d is the rate constant for endogenous metabolism.

  The rate describing the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis:

Stationary phase

- $dX/ dt = - K_d X$
- $dX/X= -k_d \ dt$
- Integrating and using limits as $x=X_{so}$ at =0 and X=X at t=t
- $X= X_{so} e^{- kd\, t}$
- k_d = First order rate constant
- Cell mass conc at the start of stationary phase $=X_{so}$

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Death Phase:

Death phase follows the stationary phase

There is no clear demarcation between St. phase and Death phase. Since some cells die during St. phase due to exhaustion of nutrients and accumulation of toxic metabolic by-products.

$$\frac{dN}{dt} = -k_d^{'} N$$

The living organism population decreases with time

$k_d^{'}$ is the first-order death rate constant.

The rate of death usually follows:

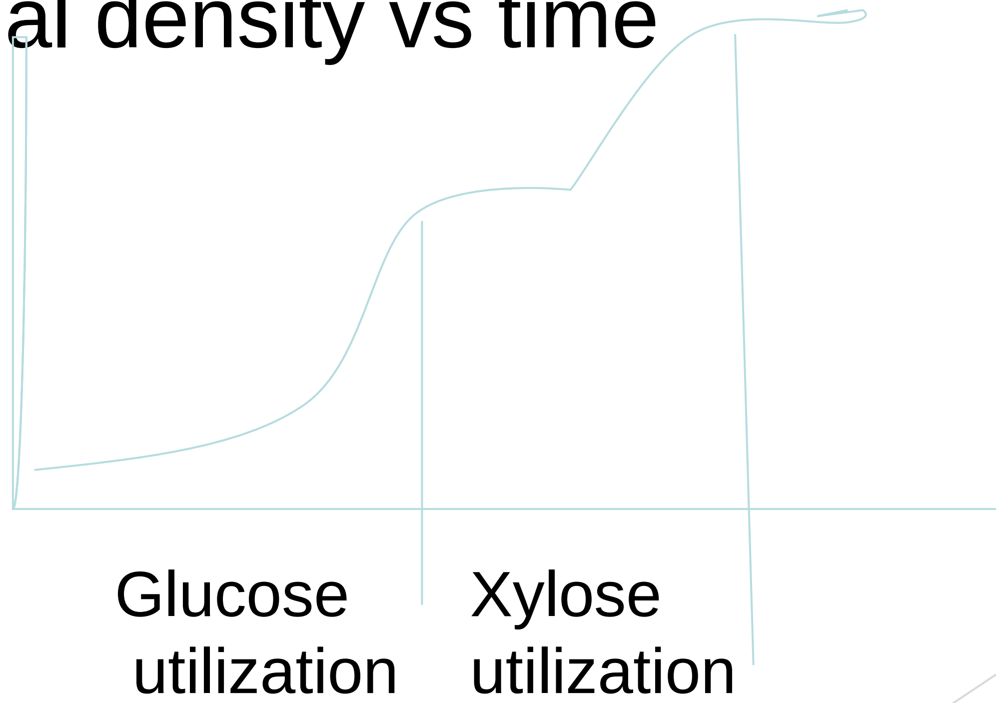
- $N = N_s e^{-k_d' t}$
- First order death rate constant= k_d'
- Multiple lag phases

due to multiple carbon sources in the form of glucose, xylose, sucrose etc.,

during cell growth being fed by one type of carbon source near its exhaustion , the cell must divert its energy from growth to retool for the new source of carbon (phenomenon is diauxic growth)

diauxic growth

- Bacterial density vs time



Precautions to avoid diauxic growth

- Inoculating Culture should be highly and grown in exponential phase
- Composition of culture medium and fermenter should be almost same
- Use of large inoculum (5-10%) of new medium volume to avoid diffusion of intermediates and

Batch Growth Kinetics

Yield coefficients: defined based on the amount of consumption of another material.

Growth yield : $$Y_{X/S} \equiv -\frac{\Delta X}{\Delta S}$$

Product yield : $$Y_{P/S} \equiv -\frac{\Delta P}{\Delta S}$$

Growth yield based on consumption of oxygen :

$$Y_{X/O_2} \equiv -\frac{\Delta X}{\Delta O_2}$$

$$\Delta S = \Delta S_{\substack{\text{assimilation}\\ \text{into biomass}}} + \Delta S_{\substack{\text{assimilation}\\ \text{into an}\\ \text{extracellular}\\ \text{product}}} + \Delta S_{\text{growth energy}} + \Delta S_{\substack{\text{maintenance}\\ \text{energy}}}$$

Batch Growth Kinetics

Yield coefficients: defined based on the amount of consumption of another material.

For most bacteria and yeast:

$$Yx/s = 0.4\text{-}0.6 \text{ g/g glucose}$$

$$Yx/_{O_2} = 0.9 – 1.4 \text{ g/g } O_2$$

At the end of the batch growth period, the measured Yields are apparent as of endogenous metabolism occurring, $K_d > 0$, which changes the metabolic pathways of the substrate. e.g.

$$Y_{X/S}^{M} > Y_{X/S}^{App}$$