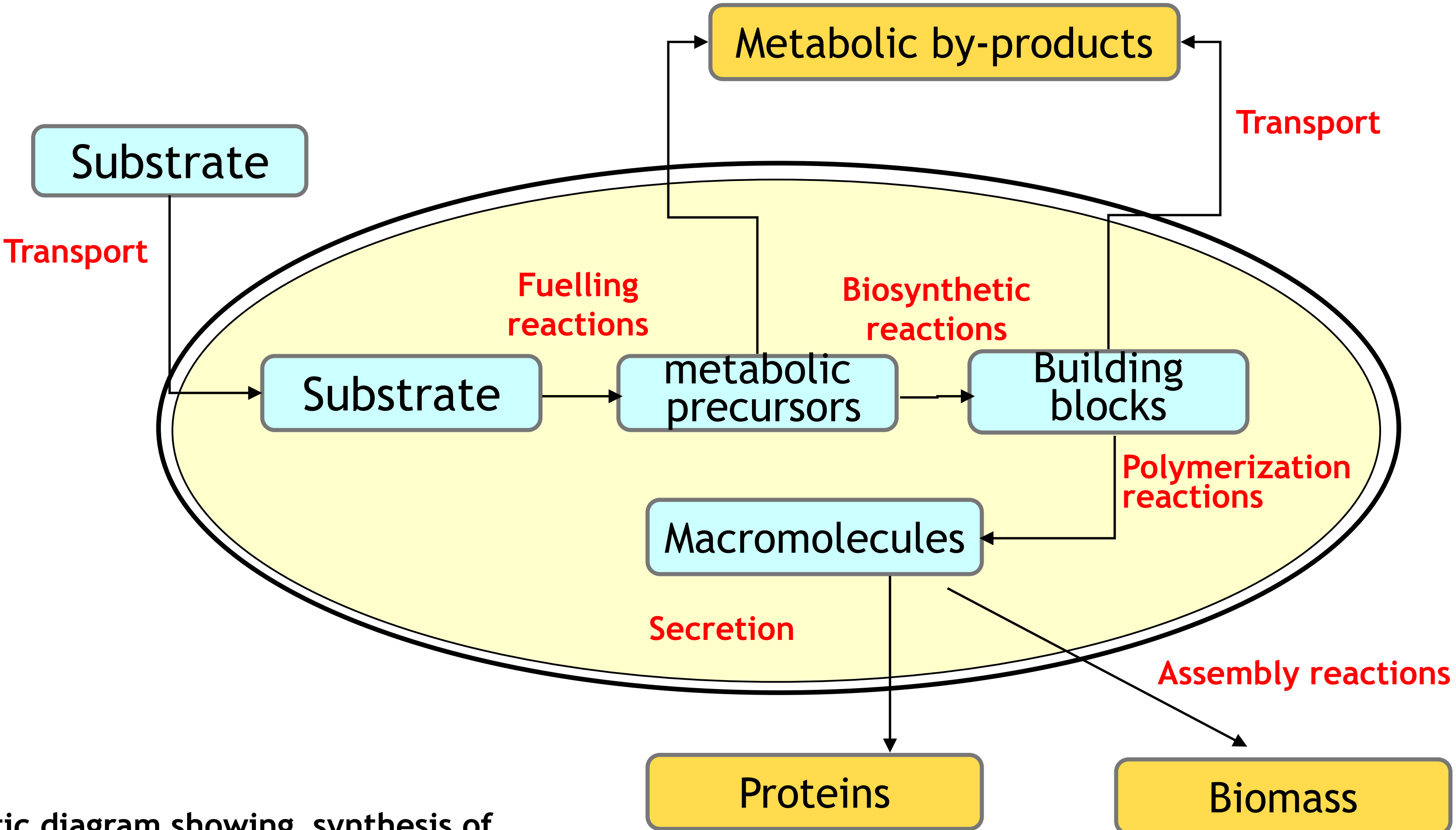
Introduction

1.1 – Batch Reactor (BSTR)

Synthesis of biological macromolecules



Schematic diagram showing synthesis of biological macromolecules from simple nutrients

Primary metabolism

Metabolism:

set of all biochemical reactions that occur in organisms

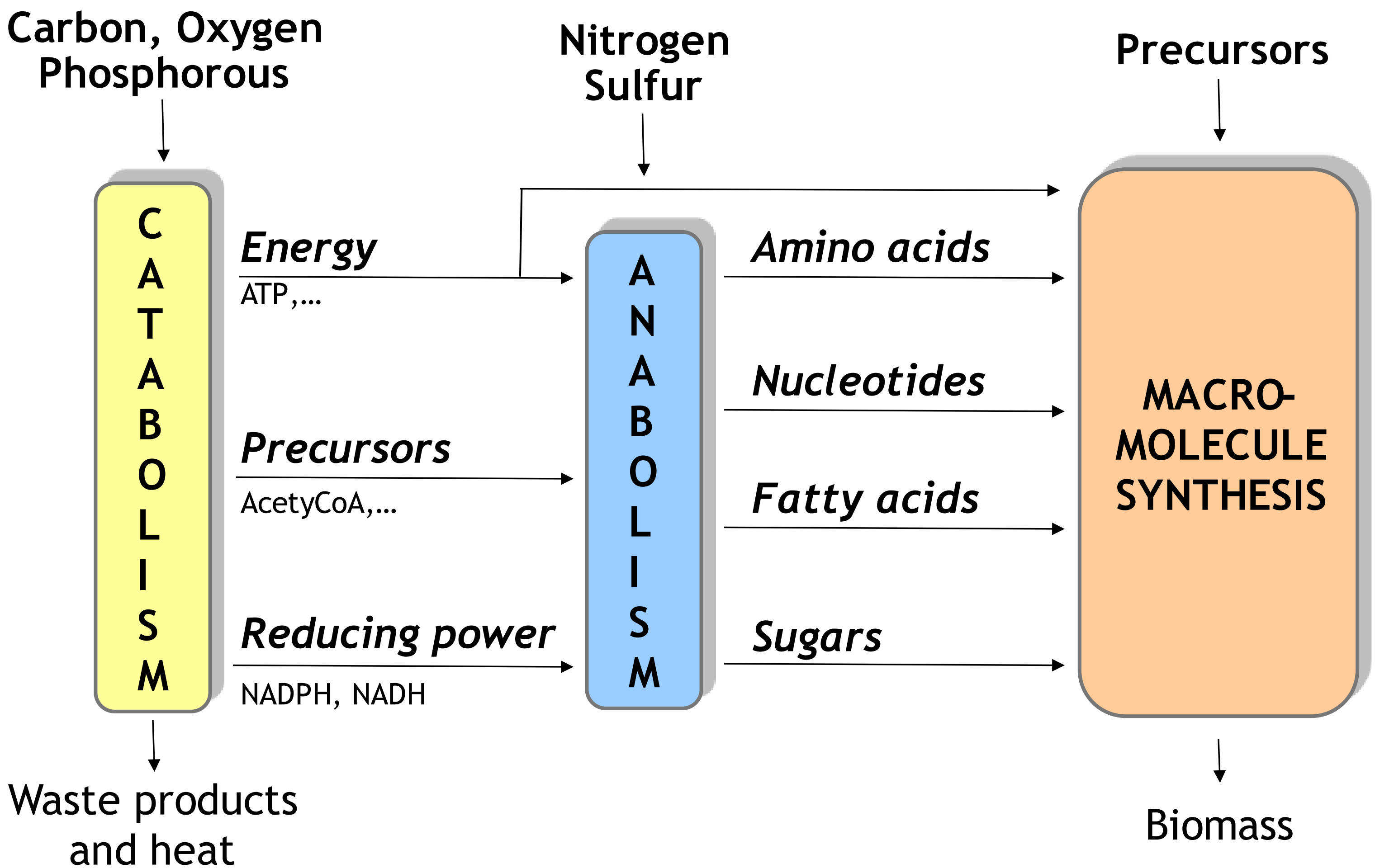
Catabolism:

reactions in which complex organic compounds are converted into simpler molecules; there is energy production

Anabolism:

reactions in which complex molecules are formed from simpler ones, with the expenditure of energy

Primary metabolism



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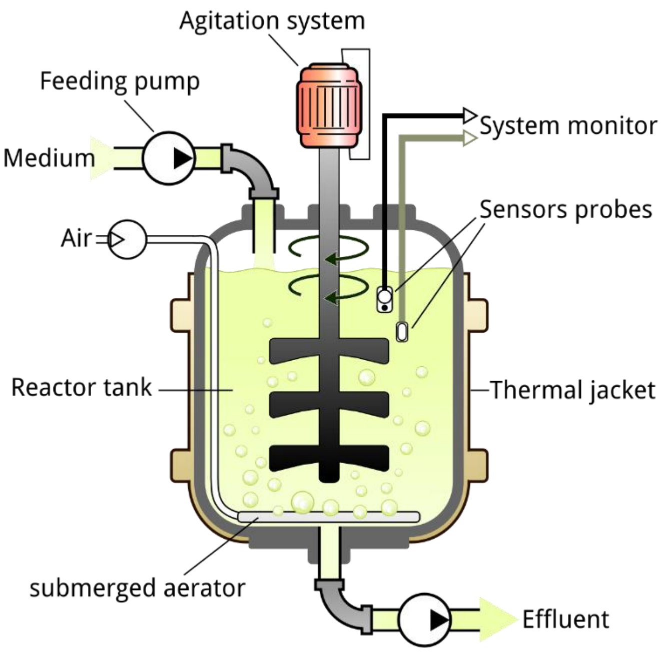
1.1.1 – Definitions

- Bioreactor

system used for the development of cultures or biological processes



1.1.1 – Definitions

- Bioreactor

system used for the development of cultures or biological processes

1.1 – Batch Reactor

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- Bioreactor

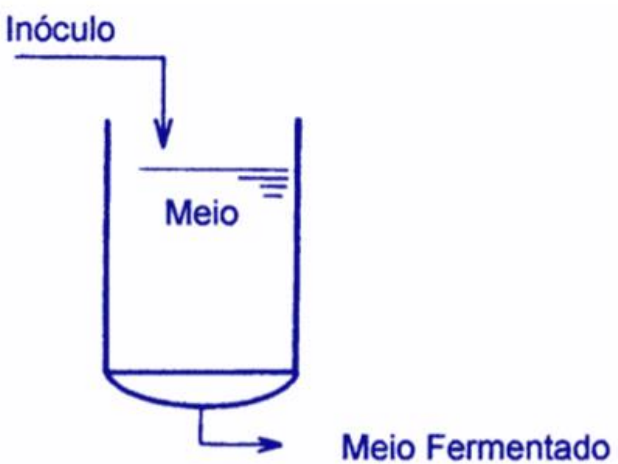
system used for the development of cultures or biological processes

- Batch Reactor

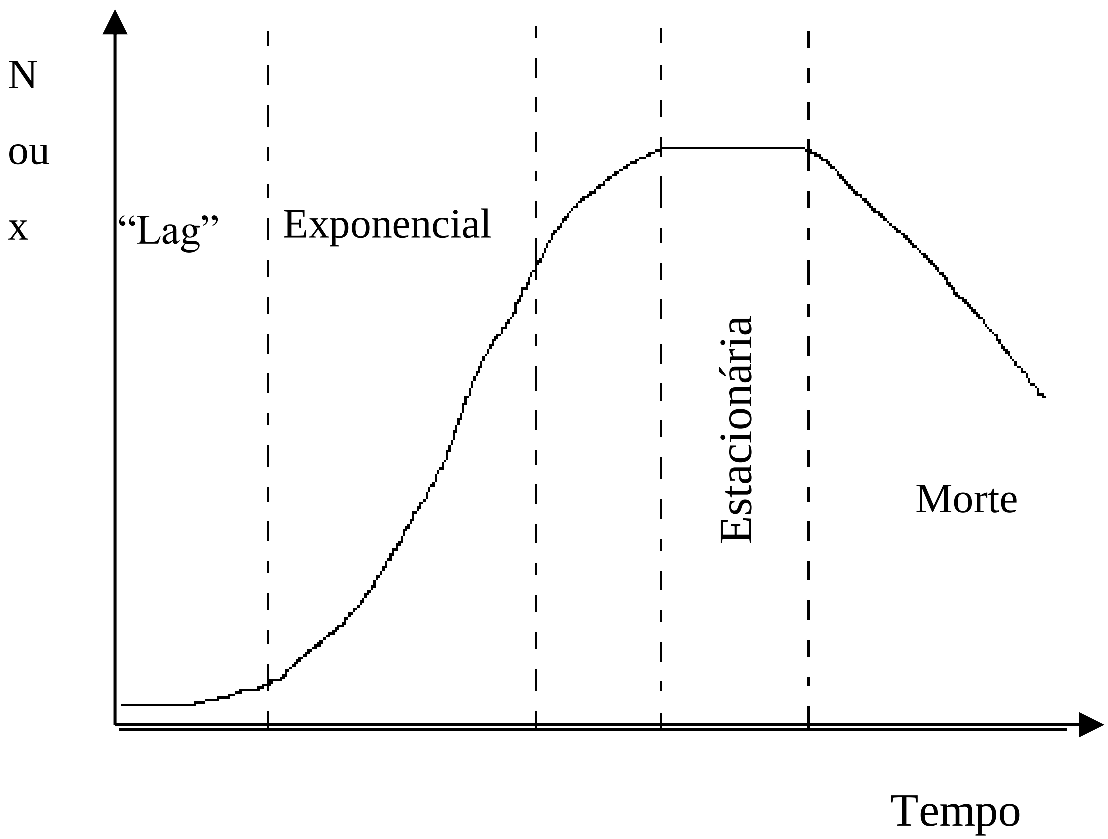
all components are inserted into the bioreactor at the beginning of the process

- Inoculum

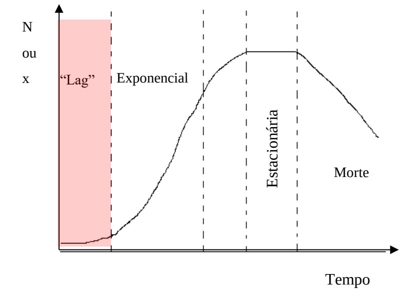
suspension of microorganisms of suitable concentration, used to start the fermentation process



1.1.2 – Cell growth phases



1.1.2 – Cell growth phases



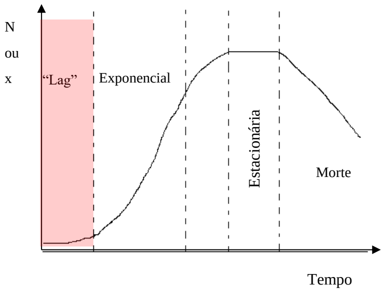
• ***lag* phase or adaptation phase** – no increase in the cell number

Factors that influence the lag phase:

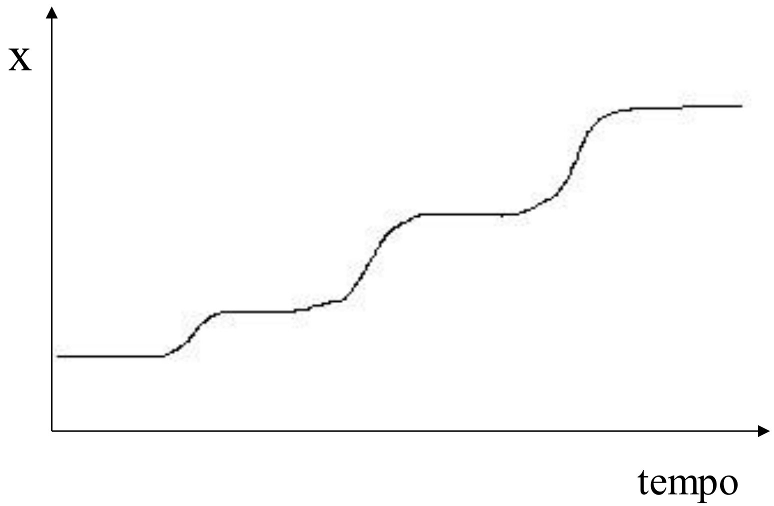
- Culture medium in the reactor should have a similar composition to the inoculum
- Quantity of inoculum– 5 - 10% of the reactor's liquid volume
- Inoculum's activity – transfer during the exponential cell growth phase

1.1.2 – Cell growth phases

- ***lag* phase or adaptation phase** – no increase in the cell number

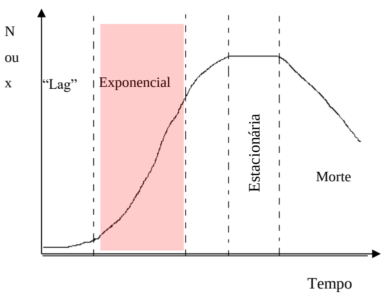


Multiple lag phases can occur: diauxic growth ➔ results from the use of various substrates



X – cell concentration

1.1.2 – Cell growth phases



• **Exponential Phase** – the cell growth rate is proportional to the number of cells (or cell concentration when x is proportional to the number of cells)

$$\frac{dx}{dt} = \mu x \quad (1) \quad ou \quad \frac{dN}{dt} = \mu N \quad (2)$$

x – cell concentration (mg/L)

N – number of cells

μ - specific cell growth rate (t^{-1})

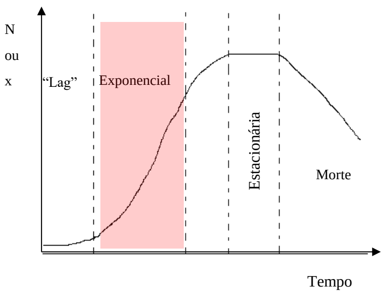
r_x – volumetric cell growth rate (mg_cel/(l.h))

"***Balanced growth***" constant cell composition

$$\frac{1}{x}\frac{dx}{dt} = \frac{1}{N}\frac{dN}{dt} \quad (3)$$

$$\frac{dx}{dt} = r_x \quad (4)$$

1.1.2 – Cell growth phases



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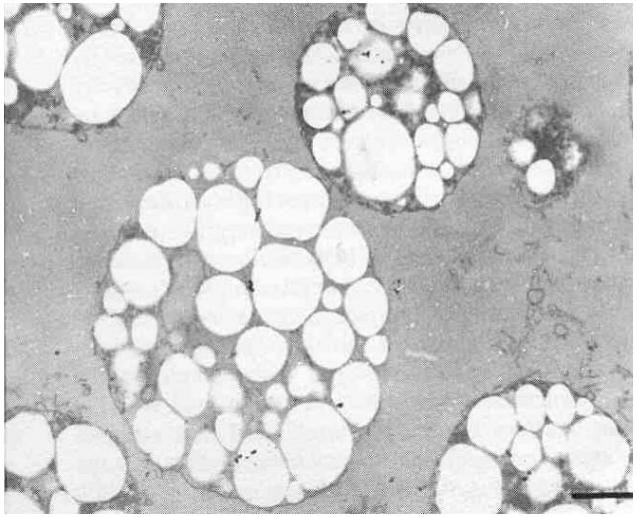
r_x – volumetric cell growth rate (mg_cel/(l.h))

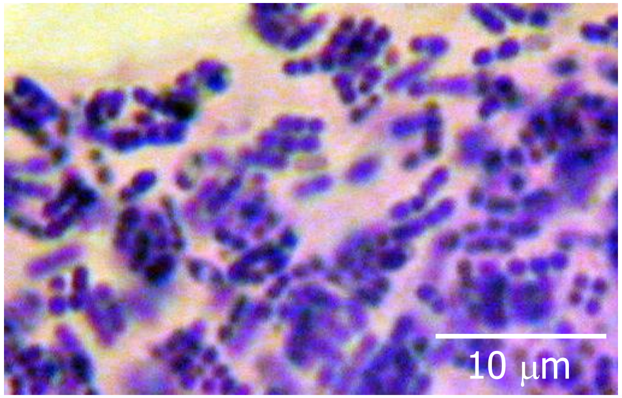
"***Unbalanced growth***" changes in cellular composition (e.g. proteins, internal reserves, etc.)

$$\frac{1}{x}\frac{dx}{dt} \neq \frac{1}{N}\frac{dN}{dt}$$

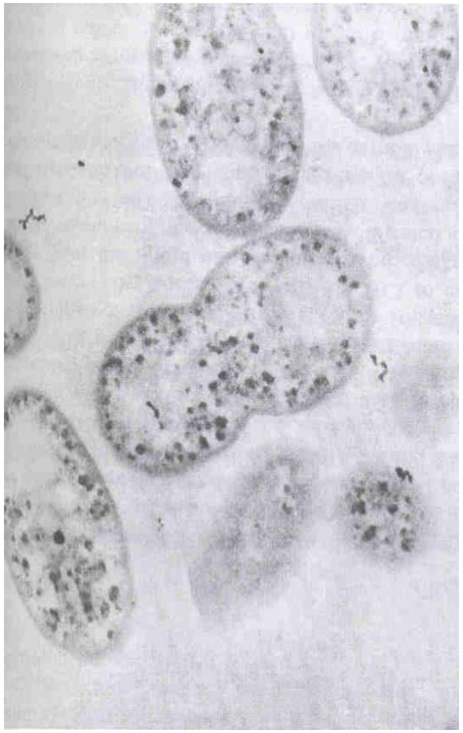
Examples of “unbalanced growth” - accumulation of internal reserves



Polyhydroxyalkanoate granules



poli-P granules

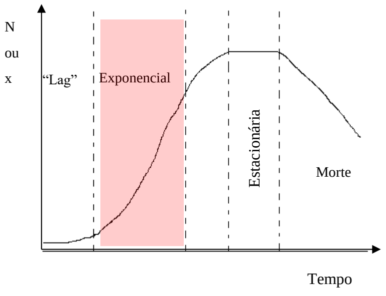


Glycogen granules

1.1.2 – Cell growth phases

- **Exponential Phase**

Integrating eq. 1 between t and t_{lag}:



$$\frac{dx}{dt} = \mu\, x \qquad (1)$$

$$\frac{dx}{x} = \mu\, dt$$

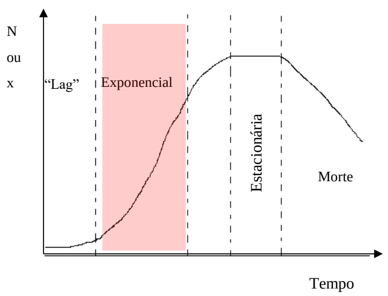
$$\int_{x_0}^{x} \frac{dx}{x} = \mu \int_{t_{lag}}^{t} dt$$

$$ln\,\frac{x}{x_0} = \mu\,(t - t_{lag})$$

$$\frac{x}{x_0} = e^{\mu\,(t - t_{lag})}$$

$$x = x_0\, e^{\mu\,(t-t_{lag})} \qquad (5)$$

1.1.2 – Cell growth phases



- **<u>Exponential Phase</u>**

$$x = x_0 e^{\mu(t - t_{lag})} \quad (5)$$

$$\ln\left(\frac{x}{x_0}\right) = \mu\,(t - t_{lag}) \quad (6)$$

$$x = x_0 \quad para \quad t = t_{lag}$$

$$se \quad t_{lag} = 0 \Rightarrow \ln x = \ln x_0 + \mu t \quad (7)$$

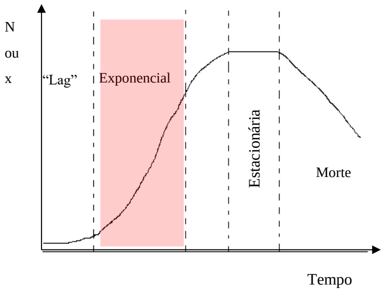
$$\Downarrow$$

$$\boxed{\mu = \mu_{\max}}$$

During the exponential growth phase

μ_{max} – maximum specific cell growth rate (h^{-1})

1.1.2 – Cell growth phases



- **Exponential Phase**

$$\ln\left(\frac{x}{x_0}\right) = \mu\,(t - t_{lag}) \quad (6)$$

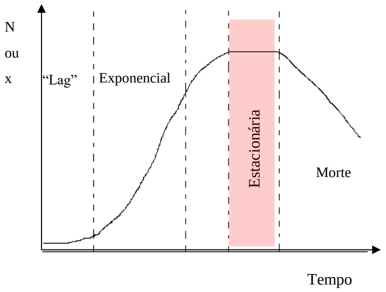
Cell duplication time (t_d):

$$t_d = \frac{\ln 2}{\mu} \quad (8)$$

$$\left(\ln \frac{2x_0}{x_0} = \mu\, t_d\right)$$

1.1.2 – Cell growth phases

- **<u>Stationary phase</u>** – constant cell concentration

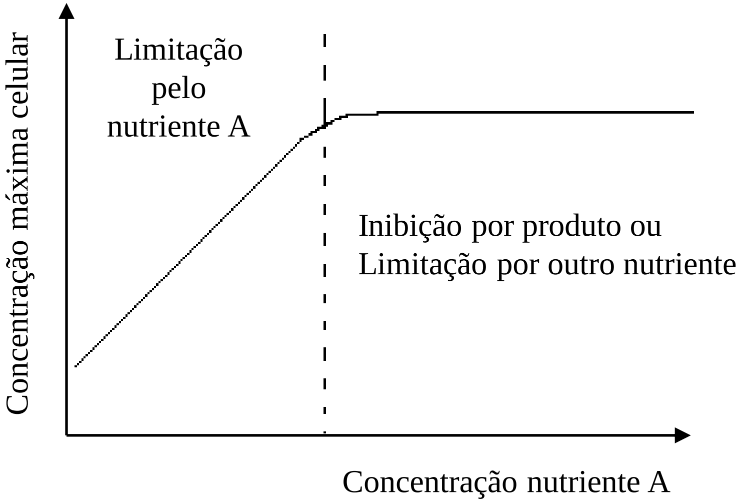


$$\boxed{\frac{dx}{dt} = 0}$$

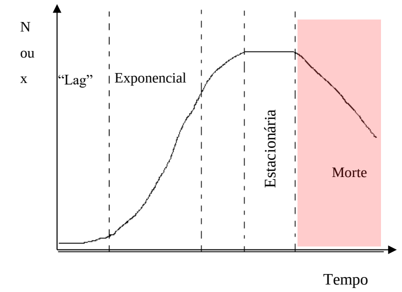
When:

- Essential substrate exhausted (O_2, carbon, nitrogen) – (Limitation)

- Acumulation of a metabolite (Inibition)



1.1.2 – Cell growth phases



- **Death phase** – the cell concentration decreases

Exponential death rate

$$\frac{dx}{dt} = -k_d\, x \qquad (9)$$

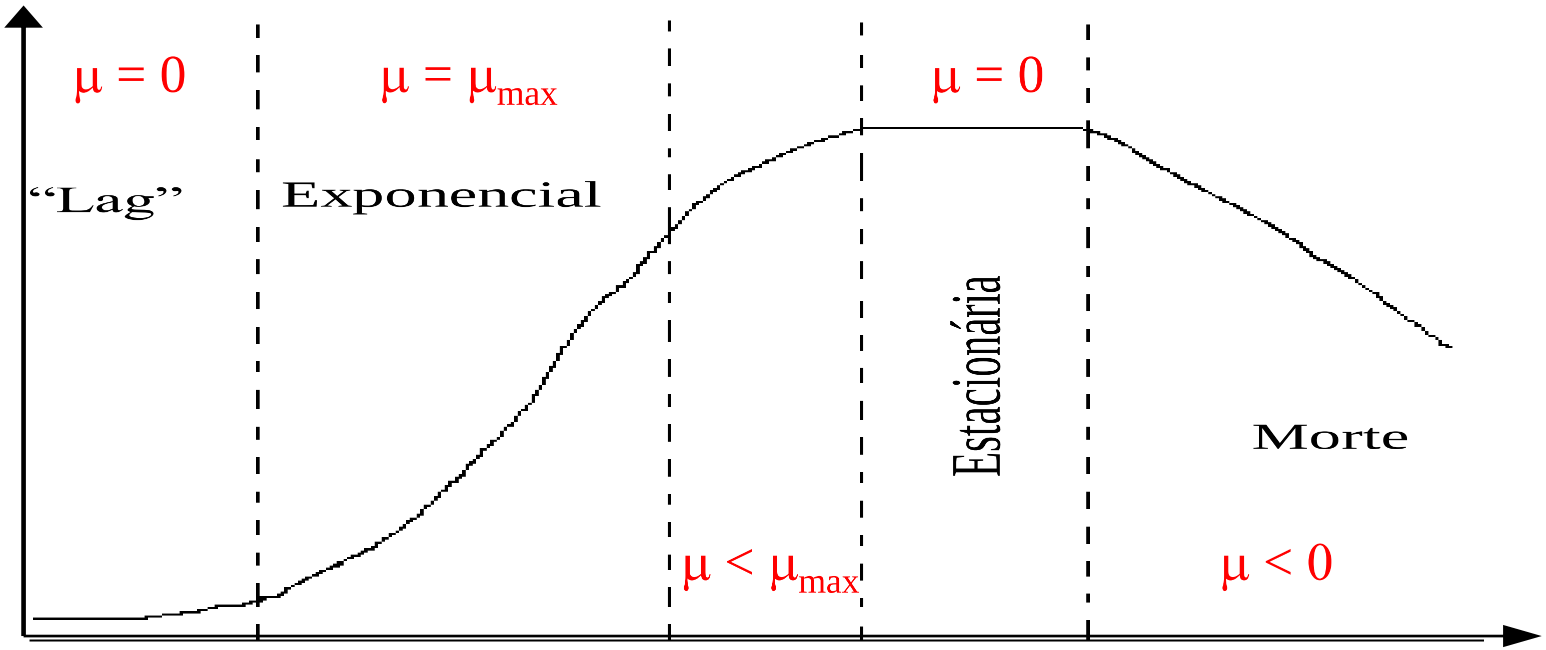
k_d – specific cell death rate (h^{-1})

t – time since onset of death phase

(Note: the number of cells that die in a given time is
a constant fraction of those that are alive)

$$x = x_s\, e^{-k_d t} \qquad (10)$$

1.1.2 – Cell growth phases



1.1.3 – Elemental composition of the biomass

- Biomass = cells (bacteria, fungi, yeasts, microalgae, etc.) → **X**

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The composition
of a bacterial cell

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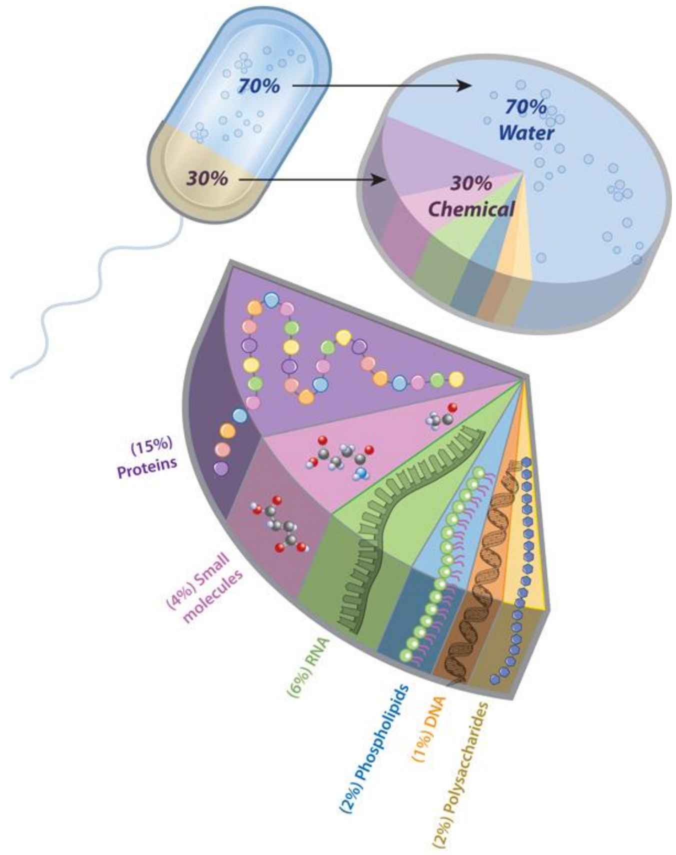
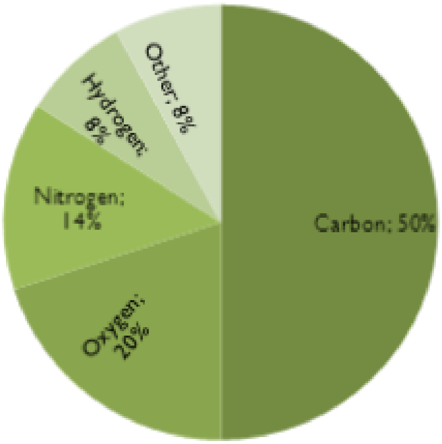


1.1.3 – Elemental composition of the biomass

- Biomass = cells (bacteria, fungi, yeasts, microalgae, etc.) → **X**

- Composition:

C **H** **O** **N** **S** **P** other elements



1.1 – Batch Reactor

1.1.3 – Elemental composition of the biomass

- Biomass = cells (bacteria, fungi, yeasts, microalgae, etc.) → **X**

- Composition:

  C **H** **O** **N** **S** **P** other elements

- Content in S, P and elements such as Ca, Mg, Na, Fe, etc. are very low comparing to the content in N

- Represented by chemical formulas:

  $\mathbf{C_i\,H_j\,O_k\,N_l}$ i, j, k, l – stoichiometric cefficients

- Dependent on the microorganism and the cultivation conditions

1.1.3 – Elemental composition of the biomass

$C_5 H_7 O_2 N$ Most commonly used formula
Simplified formula, does not represente an accurate stoichiometry

Microorganism	Elemental composition	Ash content (%)	Condition
Candida utilis	$CH_{1.83}O_{0.46}N_{0.19}$	7.0	Glucose limited, D=0.05 h^{-1}
	$CH_{1.87}O_{0.56}N_{0.20}$	7.0	Glucose limited, D=0.45 h^{-1}
	$CH_{1.83}O_{0.54}N_{0.10}$	7.0	Ammonia limited, D=0.05 h^{-1}
	$CH_{1.87}O_{0.56}N_{0.20}$	7.0	Ammonia limited, D=0.45 h^{-1}
	$CH_{1.75}O_{0.43}N_{0.22}$	3.6	Glycerol limited, D=0.10 h^{-1}

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1.1.3 – Elemental composition of the biomass

$C_5 H_7 O_2 N$ Most commonly used formula
Simplified formula, does not represente an accurate stoichiometry

Microorganism	Elemental composition	Ash content (%)	Condition
Klebsiella aerogenes	$CH_{1.73}O_{0.43}N_{0.24}$	3.6	Glycerol limited, D=0.85 h^{-1}
	$CH_{1.75}O_{0.47}N_{0.17}$	3.6	Ammonia limited, D=0.10 h^{-1}
	$CH_{1.73}O_{0.43}N_{0.24}$	3.6	Ammonia limited, D=0.85 h^{-1}
	$CH_{1.82}O_{0.58}N_{0.16}$	7.3	Glucose limited, D=0.080 h^{-1}
	$CH_{1.94}O_{0.52}N_{0.25}$	5.5	Unlimited growth

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1.1.3 – Elemental composition of the biomass

$C_5 H_7 O_2 N$ Most commonly used formula
Simplified formula, does not represente an accurate stoichiometry

Microorganism	Elemental composition	Ash content (%)	Condition
Escherichia coli	$CH_{1.77}O_{0.49}N_{0.24}$	5.5	Unlimited growth
Pseudomonas fluorescens	$CH_{1.83}O_{0.50}N_{0.22}$	5.5	
Aerobacter aerogenes	$CH_{1.96}O_{0.55}N_{0.25}$	5.5	
Penicillium chrysogenum	$CH_{1.93}O_{0.55}N_{0.25}$	5.5	
Aspergillus niger	$CH_{1.83}O_{0.55}N_{0.26}$	5.5	
	$CH_{1.64}O_{0.52}N_{0.16}$	7.9	
	$CH_{1.72}O_{0.55}N_{0.17}$	7.5	
Average	$CH_{1.81}O_{0.52}N_{0.21}$	6.0	

1.1.3 – Elemental composition of the biomass

Important for estimating the microorganims' nutrient requirements

For cell growth:

- C source (ex.: glucose, pyruvate, etc.)
- N source (ex.: NH_3)

- O_2 under aerobic conditions

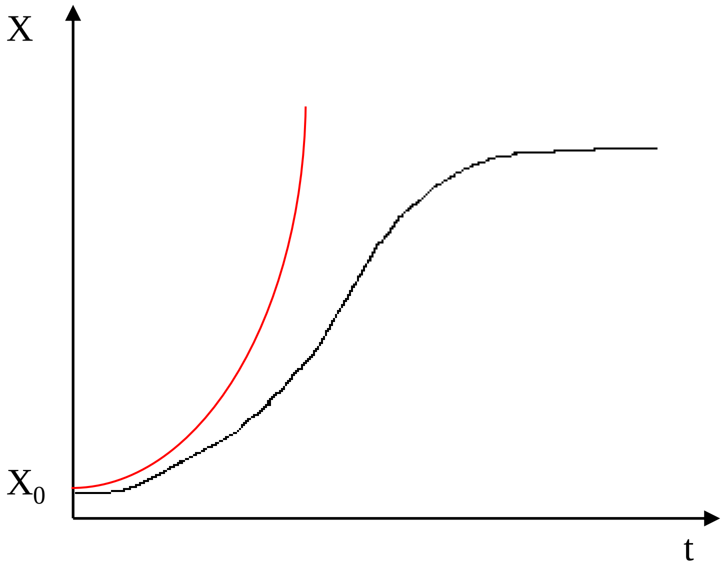
Chemical reaction for cell growth

$$\boxed{\textbf{C source} + \textbf{N source} \underbrace{(+\ O_2)}_{\text{aerobiose}} \rightarrow \textbf{Biomass} + \underbrace{CO_2 + H_2O}_{\text{respiração celular}} (+\ \textbf{Products})}$$

1.1.4 – Non strutured Models

- **Malthus Model:**

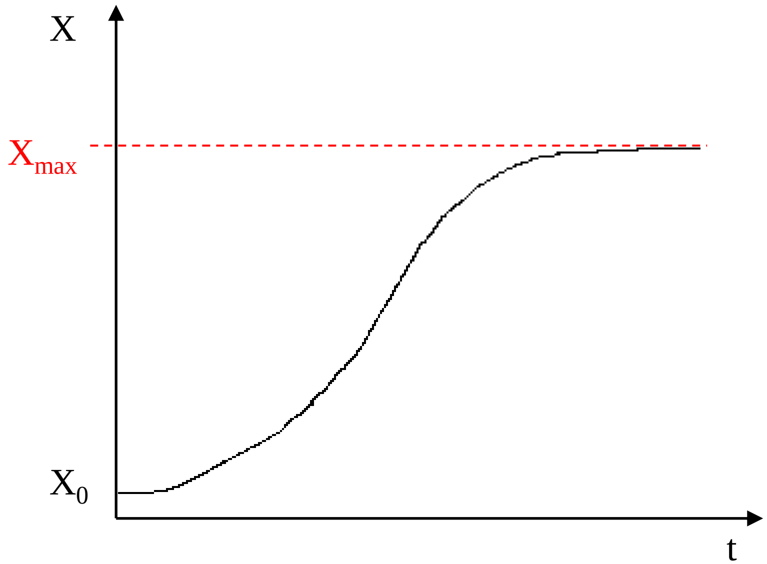
$$\frac{dx}{dt} = \mu x \quad ou \quad r_x = \mu x$$



Does not predict the appearance of the stationary phase

1.1.4 – Non strutured Models

- **Verhulst Model:** Logistic model



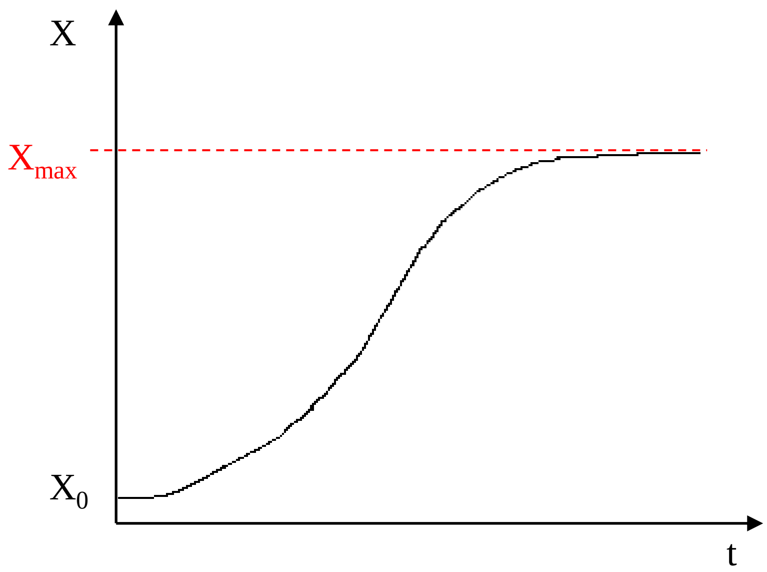
$$\frac{dX}{dt} = k\,X\,(1 - \beta\,X) \qquad (11)$$

It proposes the addition of an inhibition term that is cell concentration dependent.

Note: it does not predict the appearance of the death phase.

1.1.4 – Non strutured Models

- **Verhulst Model:** Logistic model



$$\frac{dX}{dt} = k\,X\,(1 - \beta\,X) \qquad (11)$$

Integrando, obtém-se:

$$X = \frac{X_0 e^{kt}}{1 - \beta X_0 (1 - e^{kt})} \qquad (12)$$

$$k = \mu \qquad \beta = \frac{1}{X_{max}}$$

1.1.4 – Non strutured Models

- **Verhulst Model :** Equation 12 can take a simpler form :

$$X = \frac{X_0 e^{kt}}{1 - \beta X_0 (1 - e^{kt})}$$

Multiplying by X_{max}

$$= \frac{X_{max} X_0 e^{kt}}{X_{max} - X_{max} \beta X_0 (1 - e^{kt})}$$

$X_{max}\ \beta = 1$

$$= \frac{X_{max} X_0 e^{kt}}{X_{max} - X_0 (1 - e^{kt})}$$

$k = \mu_{max}$

$$= \frac{X_{max} X_0 e^{\mu_{max} t}}{X_{max} - X_0 (1 - e^{\mu_{max} t})}$$

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1.1.4 – Non strutured Models

- **Verhulst Model :** Equation 12 can take a simpler form :

$$X = \frac{X_0 e^{kt}}{1 - \beta X_0 (1 - e^{kt})}$$

$$= \frac{X_{max} X_0 e^{kt}}{X_{max} - X_{max} \beta X_0 (1 - e^{kt})}$$

$$= \frac{X_{max} X_0 e^{kt}}{X_{max} - X_0 (1 - e^{kt})}$$

$$= \frac{X_{max} X_0 e^{\mu_{max} t}}{X_{max} - X_0 (1 - e^{\mu_{max} t})} \qquad (12a)$$

$$t = \frac{\ln\left(\frac{-(x.x_{max} - x.x_0)}{x.x_0 - x_0.x_{max}} \right)}{\mu_{max}} \qquad (12b)$$