SECTION II: KINETICS AND BIOREACTOR DESIGN:

LESSON 9.2. - Enzymatic kinetics, microbial kinetics and metabolic stoichiometry – Alive cells in bioprocesses

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AIMS FOR TODAY'S LESSON

1.- ABOUT GROWTH

How to express (microbial) growth.

2.- ABOUT MICROORGANISMS in BATCH PROCESSES:

Steps along a batch growth - Balanced growth?

Yields - Kinds of products - Oxygen necessities

3.- ABOUT MICROORGANISMS in CONTINUOUS PROCESSES:

Perfect mixing .

Chemostat / Turbidostat.

1. PROCESSES via CELLS

ALIVE CELLS IN BIOPROCESSES

STOICHIOMETRY

Many enzymatic reactions: Metabolism
Complex scheme of reactions: need simplification
ANALYSIS: stoichiometric study

$$\text{Substrates} \xrightarrow{\text{Cells}} \text{CELLS}$$

$$\text{Substrates} \xrightarrow{\text{Cells}} \text{Products}$$

$$\text{Substrates} \xrightarrow{\text{Cells}} \text{Energy}$$

KINETIC MODELS

Each **KEY COMPUND** for each reaction
Autocatalytic reactions
Slow process ➔ higher reactor volume or reaction time
Depending on cell type: chemo-, photo-, heterotroph, autotroph
O_2 (aerobic, anaerobic), T, pH
cell state: phase growth, viability, stability (GMO)
Empirical equations ➔ Problems in Scaling up
NEED OF SIMPLIFCATION: Structure, segregation

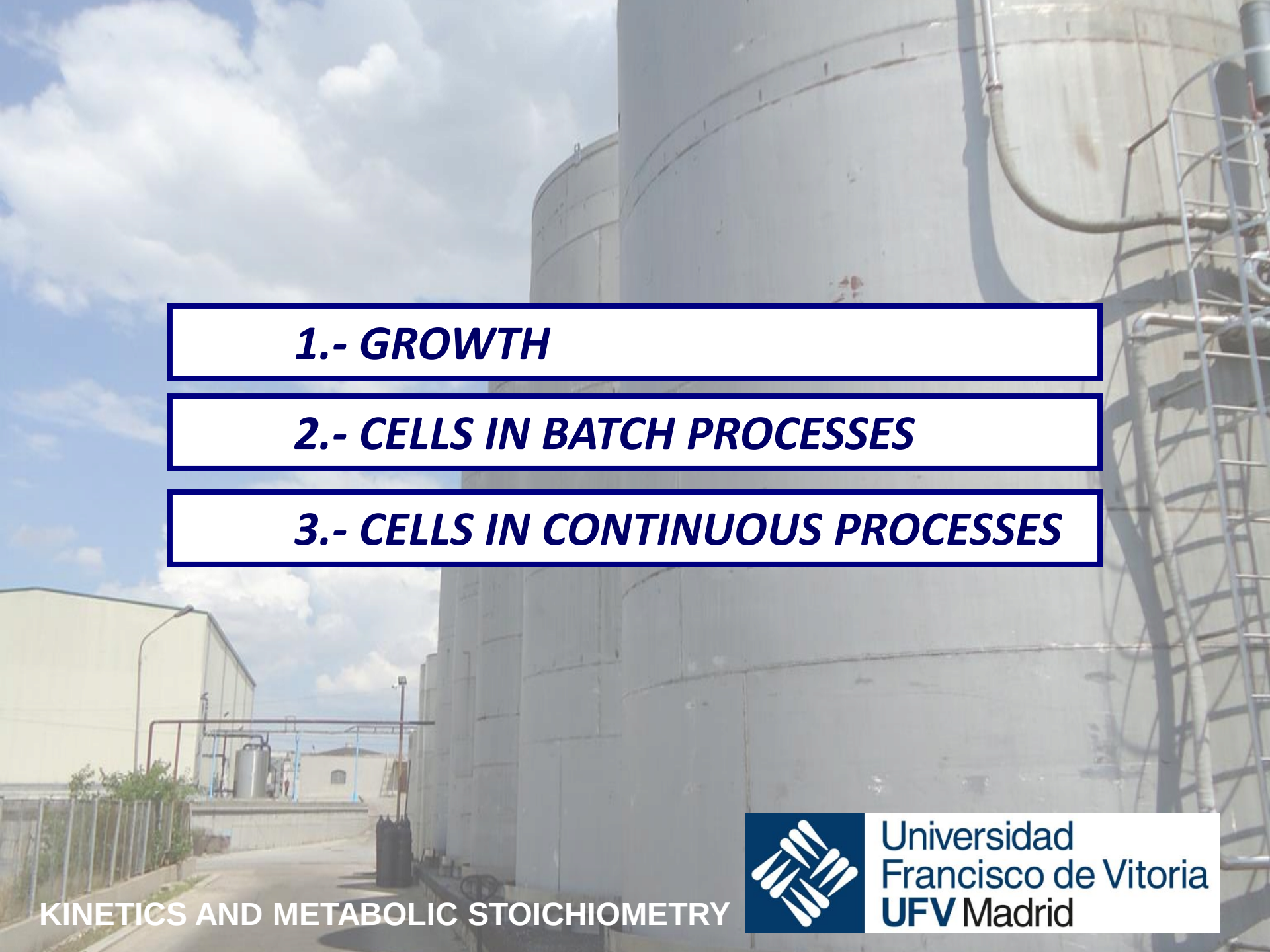
Simplified reaction scheme
Many reaction rates, kinetic parameters (macroscopic)
Empirical kinetic model: key components

1. PROCESSES via MICROORGANISMS

Changes in physico-chemical environment result in **different responses in microorganism growth**.

The propper medium allows organisms to extract necessary nutrients in order to cover different metabolic necessities:

- **Energy requirements**
- **Biosynthesis**
  - Product generation.
  - Biomass rise.



1.- GROWTH

2.- CELLS IN BATCH PROCESSES

3.- CELLS IN CONTINUOUS PROCESSES

1.- GROWTH

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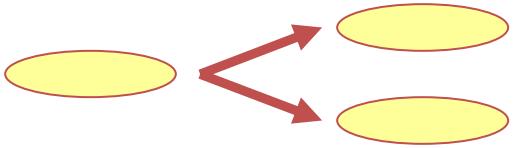
2. MICROBIAL GROWTH

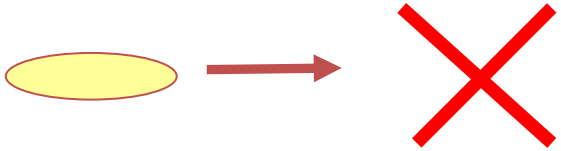
GROWTH HYPOTHESIS?:

- Each cell leads to two cells.



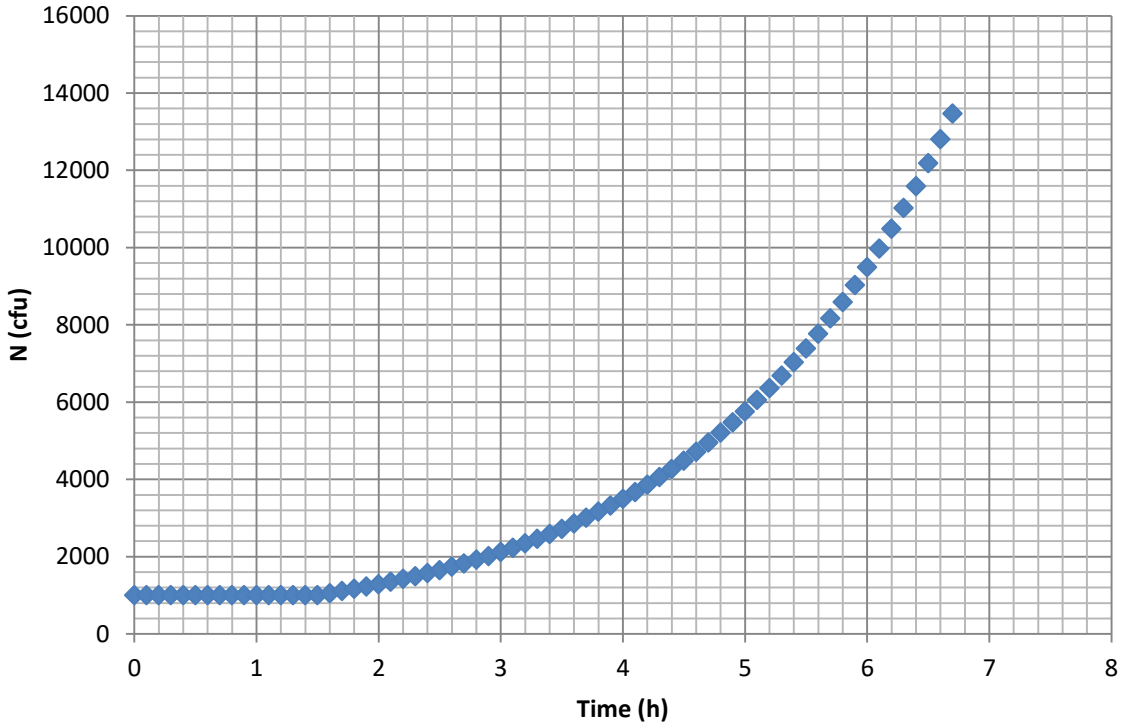
- Cells unable to reproduce are not taken into account.

- Cells dying are not taken into account



2. MICROBIAL GROWTH

GROWTH HYPOTHESIS?:



2. MICROBIAL GROWTH

$$Substrates + Cells \rightarrow Extracellular\ products + New\ Cells$$

$$\sum S \quad + \quad X \quad \rightarrow \quad \sum P \quad + \quad n \cdot X$$

It is an autocatalytic process

Rate of growth is directly proportional to cell concentration; in other words:

$$r = \frac{dX}{dt} = k \cdot X$$

2. MICROBIAL GROWTH

$$r = \frac{dX}{dt} = k \cdot X$$

First – order kinetics

Constant of proportionality **specific growth rate**:

$$r = \frac{dX}{dt} = \mu_{net} \cdot X; \quad \mu_{net} = \frac{1}{X} \cdot \frac{dX}{dt}$$

$$\mu_{net} = \mu_g - \mu_d$$

2. MICROBIAL GROWTH

$$r = \frac{dX}{dt} = \mu_{net} \cdot X; \quad \mu_{net} = \mu_g - \mu_d$$

- **X:** biomass concentration (g/L).
- **t:** time (h).
- **μ_{net}:** specific net rate (h^{-1}).

  Difference between:

  μ_g: specific growth rate (h^{-1}).

  μ_d: specific cell death rate (or endogenous metabolism) (h^{-1}).

2. MICROBIAL GROWTH

In a different way:

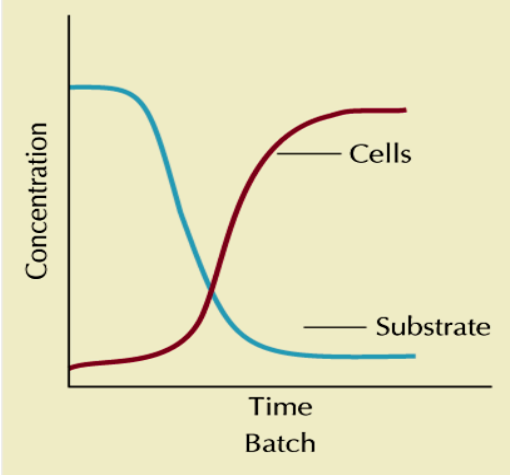
$$\mu_R = \frac{1}{N} \cdot \frac{dN}{dt}; \quad \mu_R = \mu_g - \mu_d$$

- **N:** cell concentration (cfu/L; spores/L).
- **t:** time (h).
- μ_R**:** specific net replication (or duplication) rate(h^{-1}).

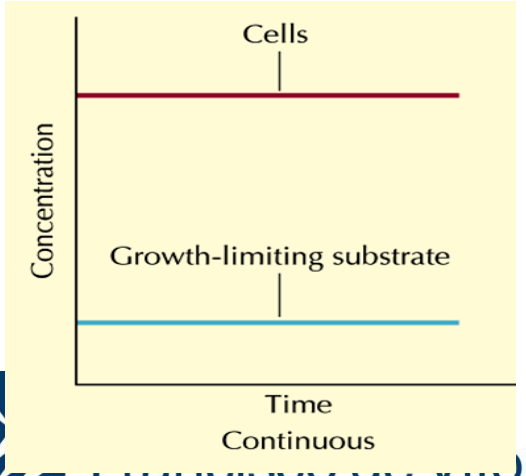
2. MICROBIAL GROWTH – Two possibilities

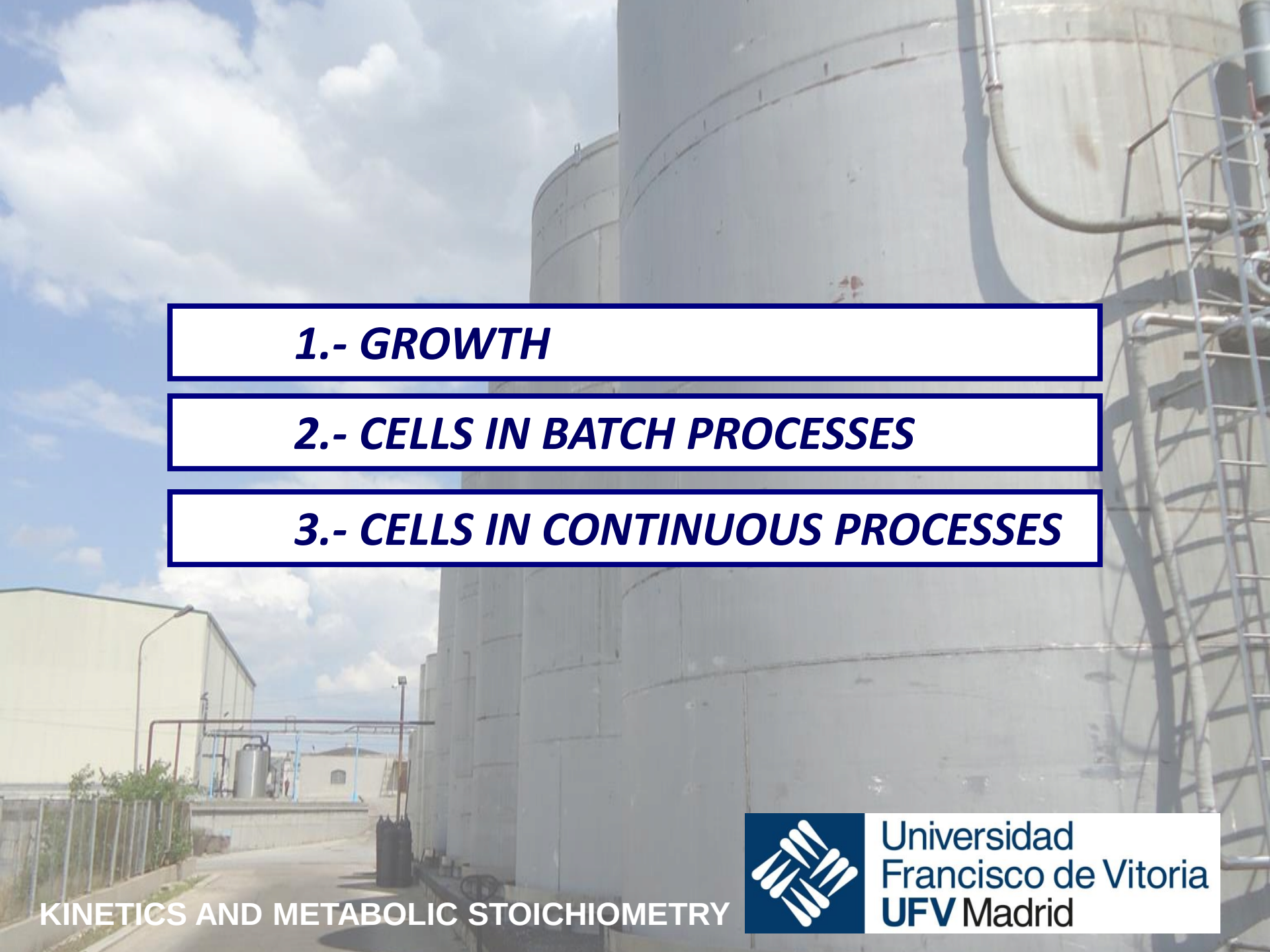
Equations describing growth kinetics change depending on:

- we are using a batch process.



- we are using a continuous process.





1.- GROWTH

2.- CELLS IN BATCH PROCESSES

3.- CELLS IN CONTINUOUS PROCESSES

2.- CELLS IN BATCH PROCESSES

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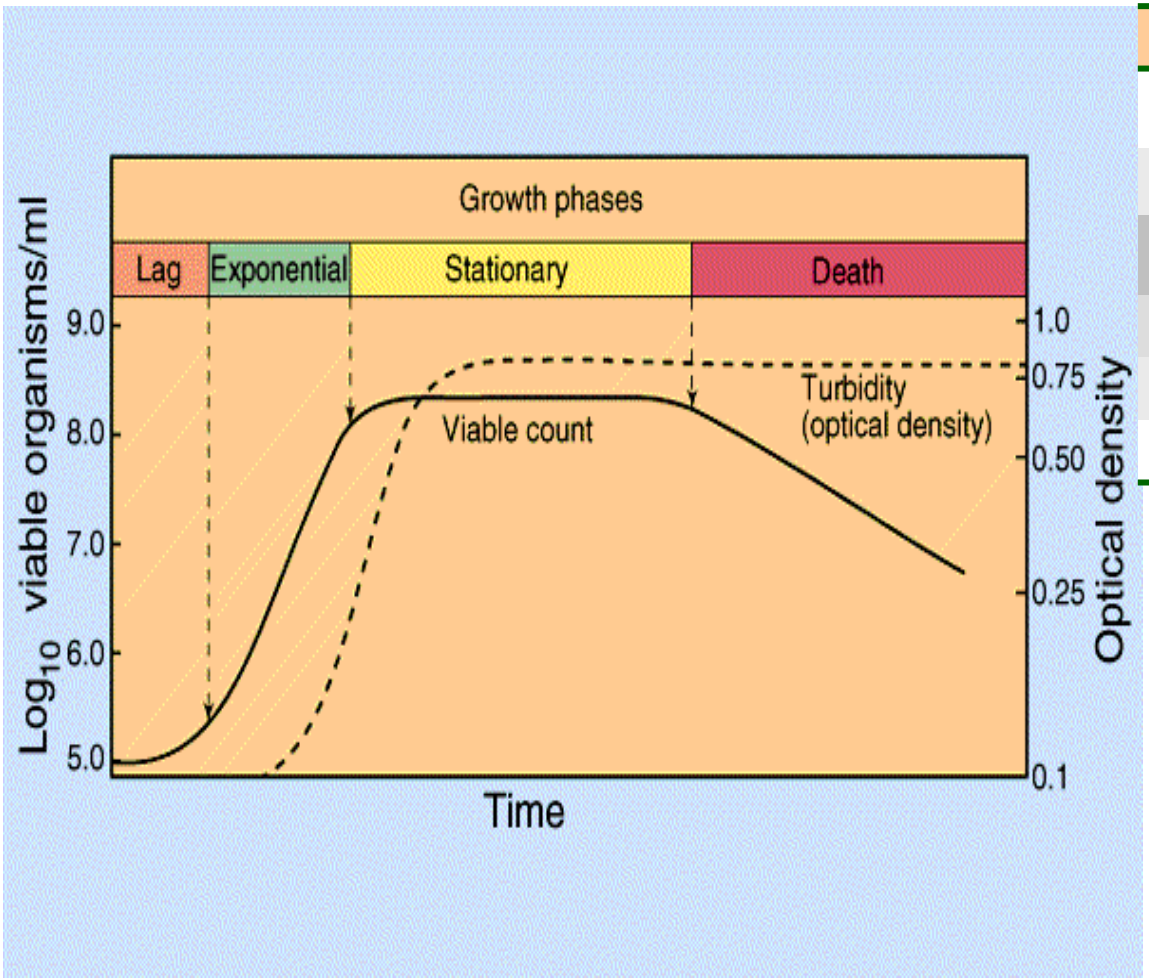
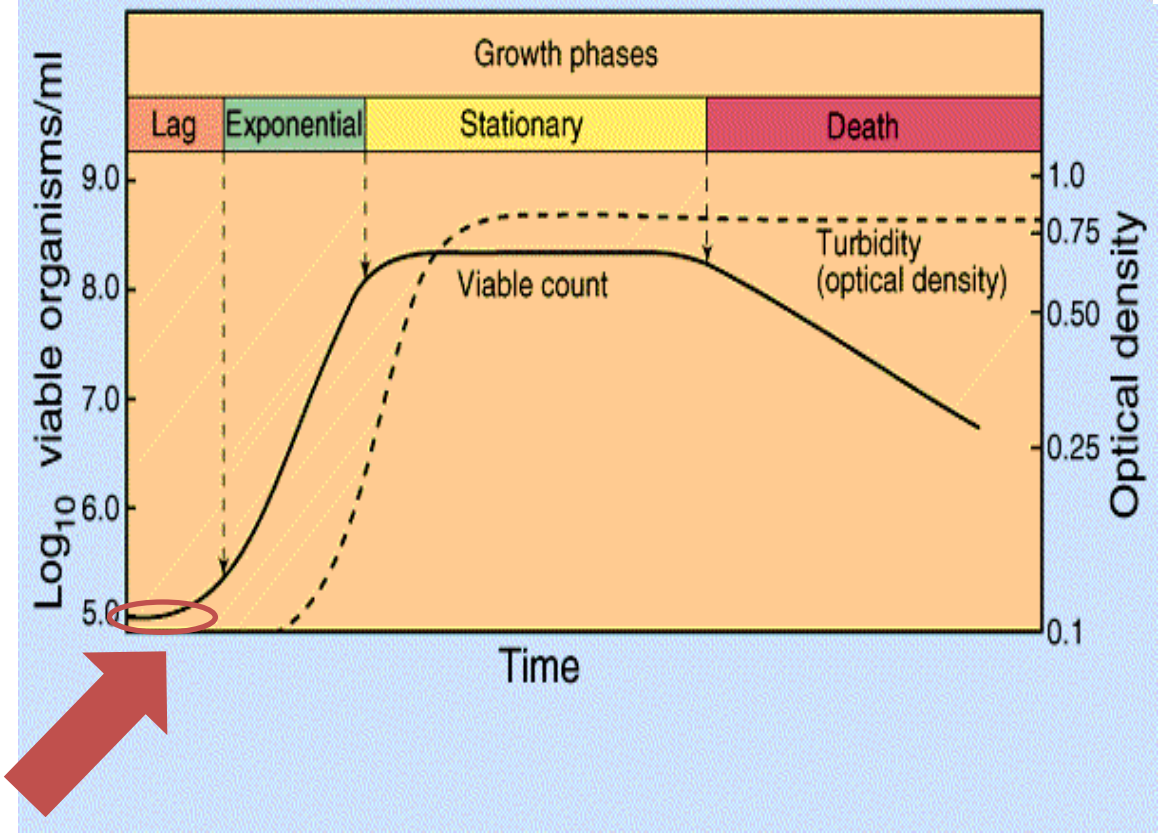
3. MICROBIAL BATCH PROCESS



	Phase
I	Lag (delay or latency)
II	Acceleratión
III	Exponential (logaríthmic)
IV	Deceleration
V	Stationary
VI	Decay or death

3. MICROBIAL BATCH PROCESS



Phase	
I	Lag (delay or latency)

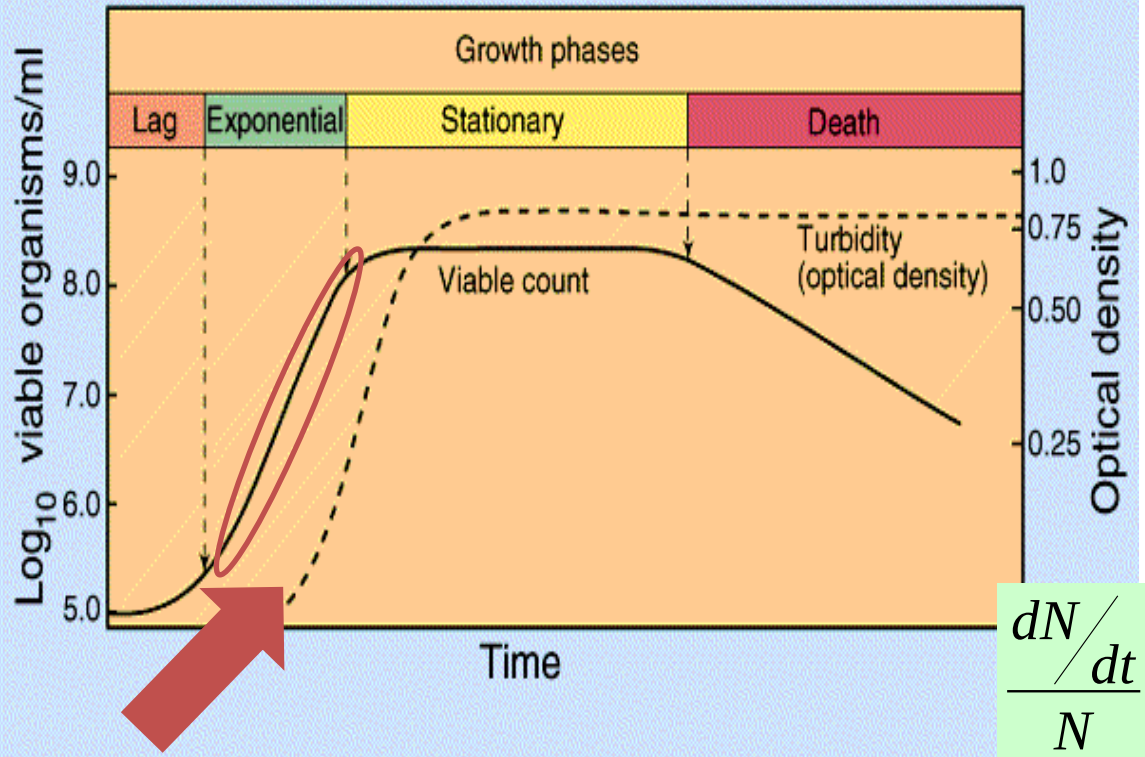
DURATION:

- Similarity between previous and current medium.
- Type of microorganism.
- This phase always exists.

DIAUXIC GROWTH:

- More than one source of C.
- Metabolic adaptations
- "Multiple phases of latency"

3. MICROBIAL BATCH PROCESS



Phase	
III	Exponential (logaríthmic)

Balanced growth

- ONLY WITHIN THIS PHASE

$$\frac{dN/dt}{N} = \frac{dDNA/dt}{DNA} = \frac{dPROT/dt}{PROT} = \mu$$

3. MICROBIAL BATCH PROCESS

Phase	
III	Exponential (logaríthmic)

Balanced growth

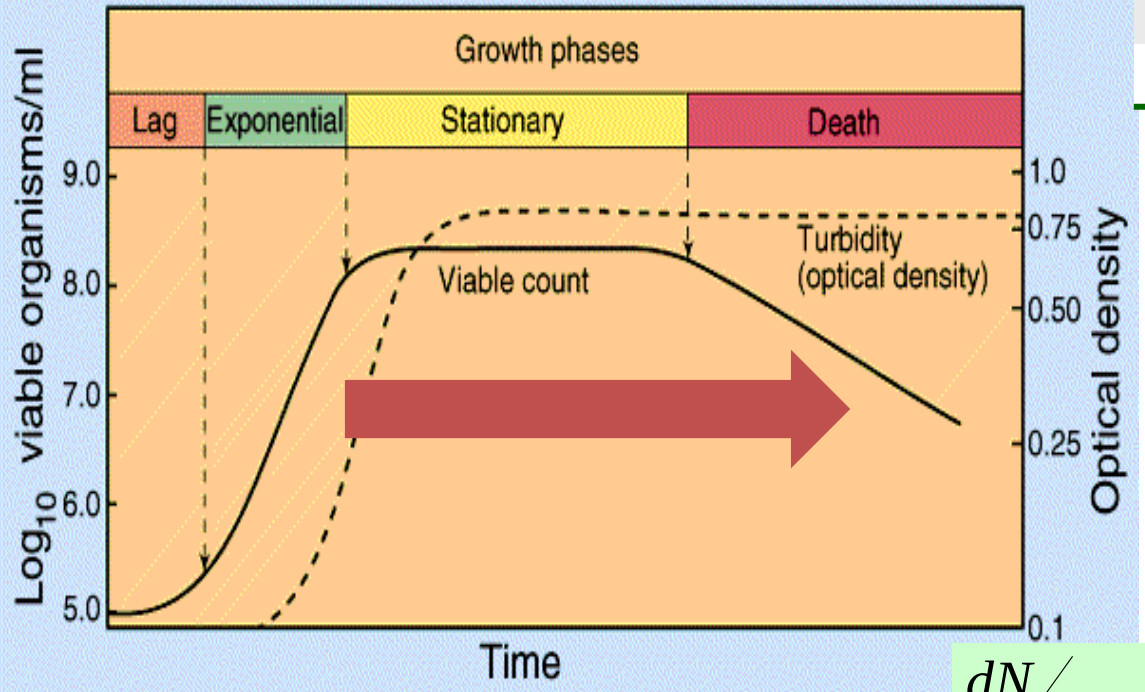
$$r = \frac{dX}{dt} = \mu_{net} \cdot X; \quad \mu_{net} = \frac{1}{X} \cdot \frac{dX}{dt} \Rightarrow X = X_0 \cdot e^{\mu_{net} \cdot t}$$

$$\mu_{net} = \mu_g - \mu_d$$

$$t_g = \frac{\ln(2)}{\mu_{net}} \; or \; t'_g = \frac{\ln(2)}{\mu_R}$$

$$\mu_R = \frac{1}{N} \cdot \frac{dN}{dt}$$

3. MICROBIAL BATCH PROCESS



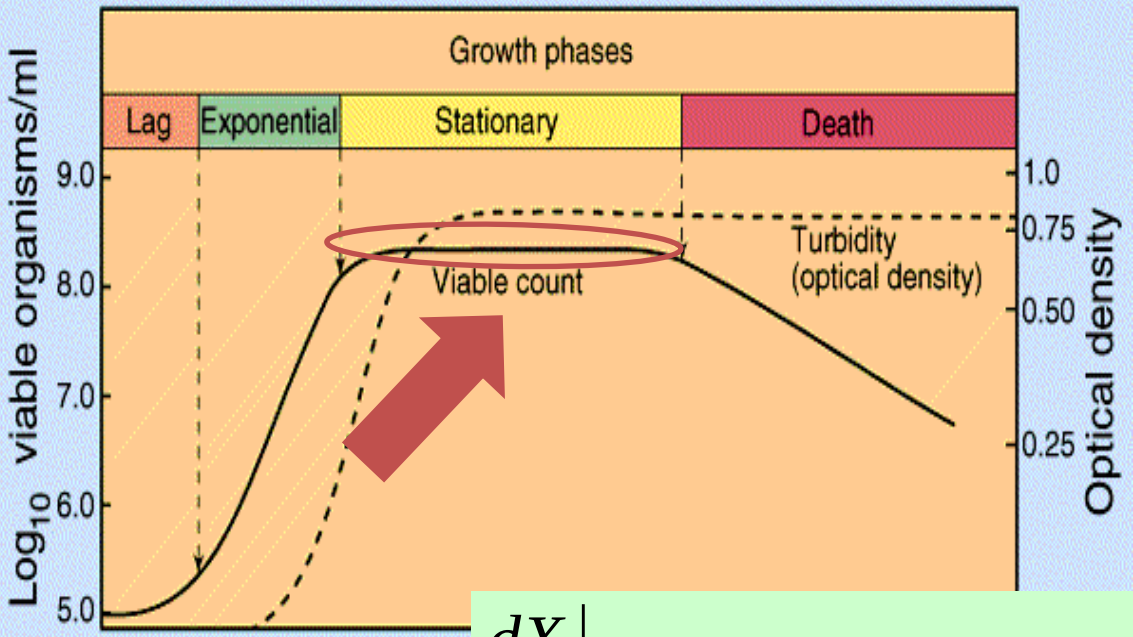
	Phase
IV	Deceleration
V	Stationary
VI	Decay or death

STRESS

$$t_g = \frac{\ln(2)}{\mu_{net}} \neq t'_g = \frac{\ln(2)}{\mu_R}$$

$$\frac{dN/dt}{N} \neq \frac{dDNA/dt}{DNA} \neq \frac{dPROT/dt}{PROT} \neq \mu$$

3. MICROBIAL BATCH PROCESS



Phase	
V	Stationary

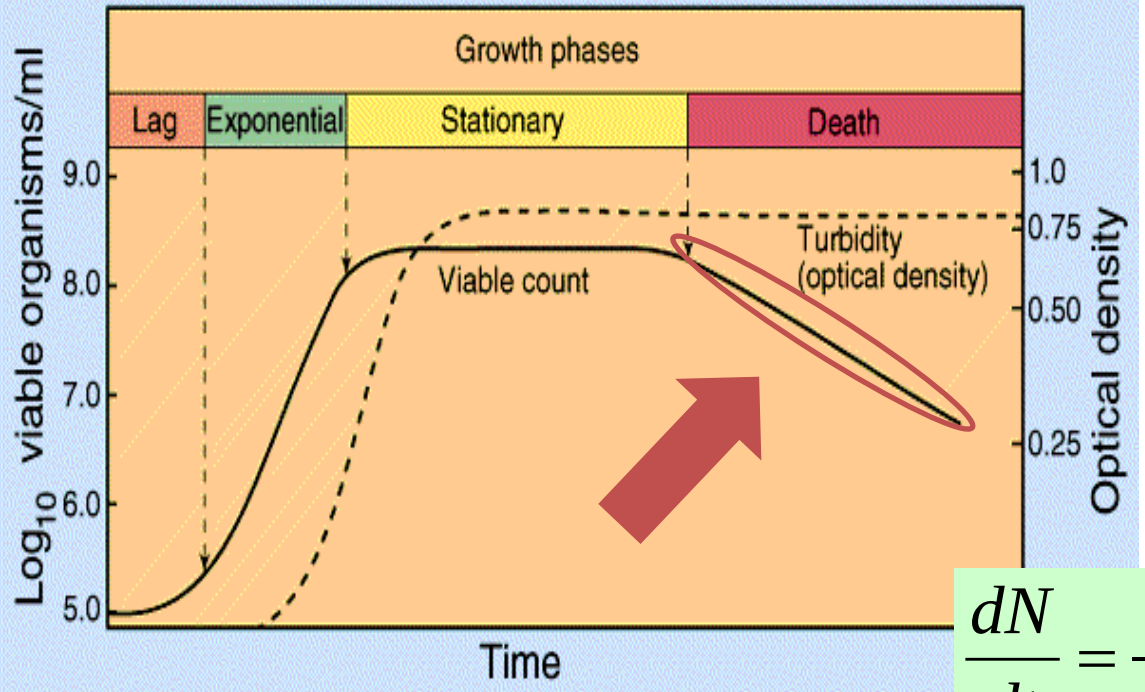
ENDOGENOUS METABOLISM

Biomass conversion into energy:

- X_{S0}: initial biomass concentration at the beginning of stationary phase (g/L).
- **t:** time (h).
- μ_d: specific rate for endogenous metabolism (h^{-1}).

$$\left.\frac{dX}{dt}\right|_{endogenous\ metabolism} = -k_d \cdot X \Leftrightarrow X = X_{S0} \cdot e^{-\mu_d \cdot t}$$

3. MICROBIAL BATCH PROCESS



Phase	
VI	Decay or death

- Absolut dependence of endogenous metabolism.
- Cell death and lysis ➔ number of viable cells falls exponentially.

$$\frac{dN}{dt} = -\mu_d \cdot N \Leftrightarrow N = N_S \cdot e^{-\mu_d \cdot t}$$

4. YIELDS

$Y_{X/S}$ Substrate to biomass yield (g cells / g substrate)

←Connects the amount of biomass produced and the amount of substrate consumed.

$$Y_{X/S} = -\frac{dX}{dS} \approx -\frac{\Delta X}{\Delta S}$$

$Y_{P/S}$ Substrate to product yield (g product / g substrate)

←Connects the amount of product generated and the amount of substrate consumed.

.

$$Y_{P/S} = -\frac{dP}{dS} \approx -\frac{\Delta P}{\Delta S}$$

4. YIELDS

$Y_{X/O2}$ Oxygen to biomass yield (g cells / g oxygen)

←Connects the amount of biomass produced and the amount of oxygen consumed.

$$Y_{X/O_2} = -\frac{dX}{dO_2} \approx -\frac{\Delta X}{\Delta O_2}$$

$Y_{P/X}$ Biomass to product yield (g product / g cells)

←Connects the amount of product generated and the amount of biomass produced.

.

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

5. MAINTENANCE COEFFICIENT

DEFINITION:

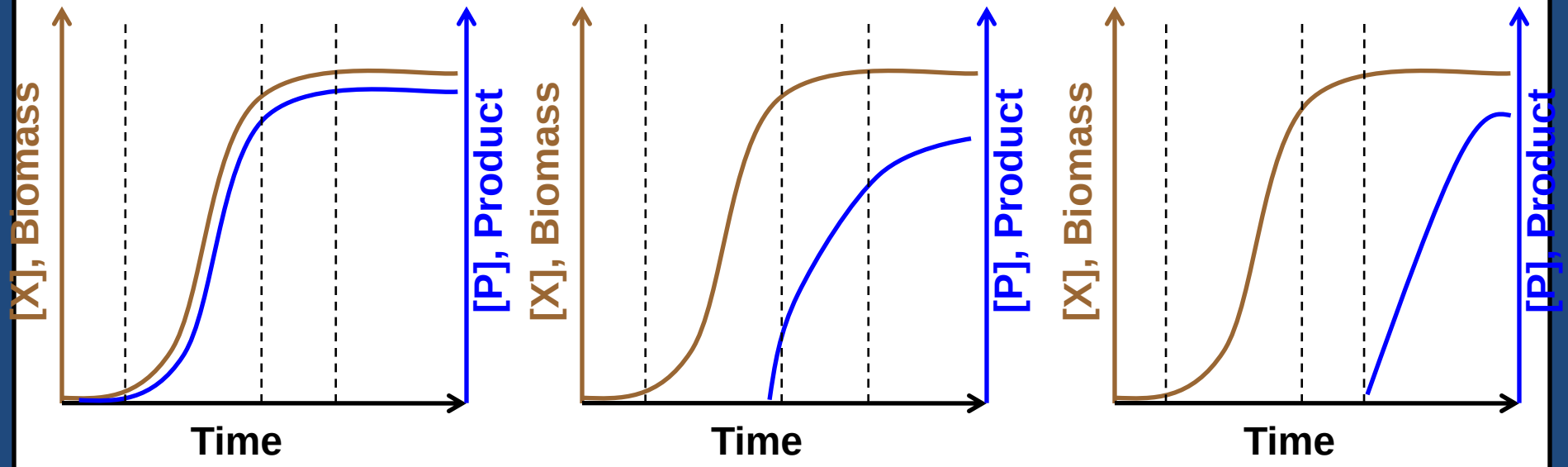
specific rate of substrate consumption for cell maintenance.

$$m = -\frac{\left[dS/dt\right]_{average}}{X}$$

It represents the energy expenditure necessary to:

- Repair cell damage
- Transport of nutrients and products through
- Motility
- Osmolarity control

6. KINDS OF PRODUCTS



ASSOCIATED WITH GROWTH

PRIMARY METABOLITE

PARTIALLY ASSOCIATED WITH GROWTH

NON ASSOCIATED WITH GROWTH

SECONDARY METABOLITE

6. KINDS OF PRODUCTS

$$q_P = \frac{1}{X}\cdot\frac{dP}{dt}$$
$$\Rightarrow q_P = Y_{P/X}\cdot\mu$$

$$q_P = \alpha\cdot\mu + \beta$$

$$q_P = \beta = cte$$

ASSOCIATED WITH GROWTH

PRIMARY METABOLITE

PARTIALLY ASSOCIATED WITH GROWTH

SECONDARY METABOLITE

NON ASSOCIATED WITH GROWTH

7. SOLVED OXYGEN

- **No oxygen limitation ➔**

Growth rate **doesn't depend** on oxygen concentration

Growth: first-order kinetics

- Oxygen limitations ➔

Growth **depends on** oxygen concentration

Saturating kinetics

7. SOLVED OXYGEN

- **OXYGEN TRANSPORT RATE (OTR)**

From gas to the liquid phase:

$$N_{O_2} = k_L a \cdot \left(C^* - C_L\right) = OTR$$

- **k_L:** coefficient of oxygen transfer (cm/h)
- **a:** interfacial surface between gas and liquid (cm^2/cm^3)
- **$k_L a$:** volumetric oefficient of oxygen transfer **(h^{-1})**
- **C*:** saturation concentration of oxygen (mg/L).
- **C_L:** concentration of oxygen within the liquid (mg/L).
- N_{O2}: OTR (mg O_2/(L·h))

7. SOLVED OXYGEN

- **OXYGEN UPTAKE RATE(OUR)**

By microorganism:

$$OUR = q_{O_2} \cdot X = \frac{\mu_g \cdot X}{Y_{X/O_2}}$$

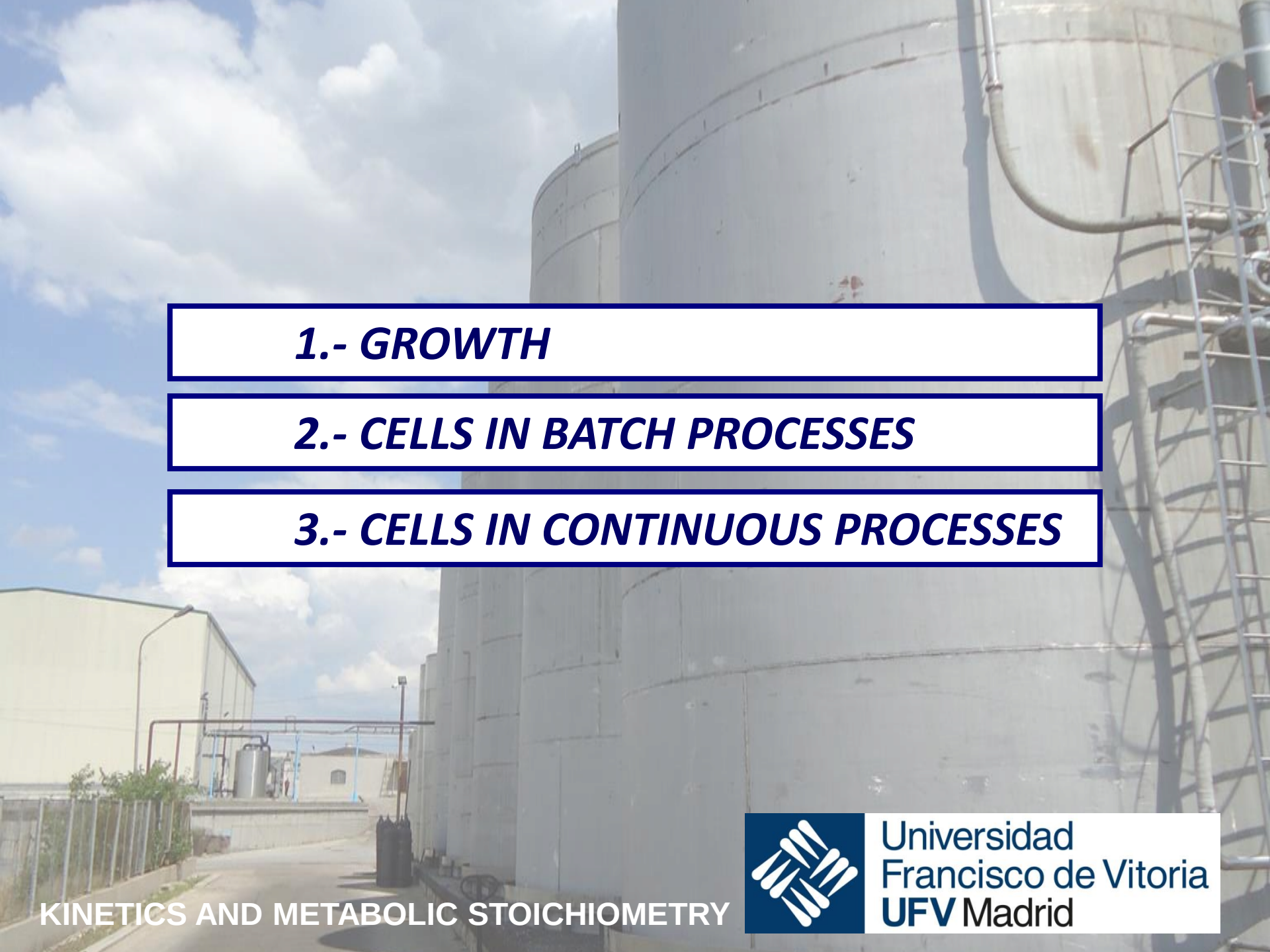
- **q_{O2}:** specific oxygen uptake rate (mg O_2/(g·h))
- **$Y_{X/O2}$:** oxygen to biomass yield (g/g)
- **X:** biomass concentration (g/L)

7. SOLVED OXYGEN

-**MASS BALANCE FOR OXYGEN**

Accumulation = Transport – Uptake

$$\frac{dO_2}{dt} = k_L a \cdot \left(C^* - C_L\right) - q_{O_2} \cdot X = OTR - OUR$$



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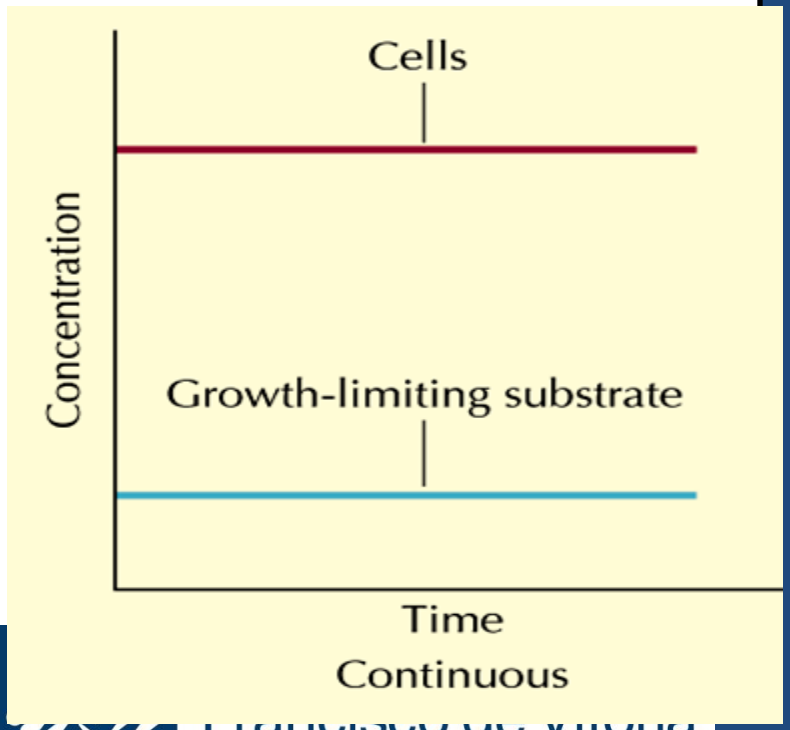
8. MICROBIAL CONTINUOUS REACTORS

- Fresh medium need to be constantly supplied ➔ nutrients
- Biomass and product constantly extracted➔ avoid inhibition
- Maintaining conditions for long periods

STEADY STATE is reached

[S]=[P]= constant

HOMOGENEITY IN PRODUCT QUALITY



8. MICROBIAL CONTINUOUS REACTORS

➔ **CULTURE extended along time**

Perfect mixing hypothesis

- **Chemostat**
- **Turbidostat**

Plug flow hypothesis

- **Tubular (PLUG FLOW) reactor**

8. MICROBIAL CONTINUOUS REACTORS

PERFECT MIX HYPOTHESIS:

Easiest approach for Tank Reactor behaviour.

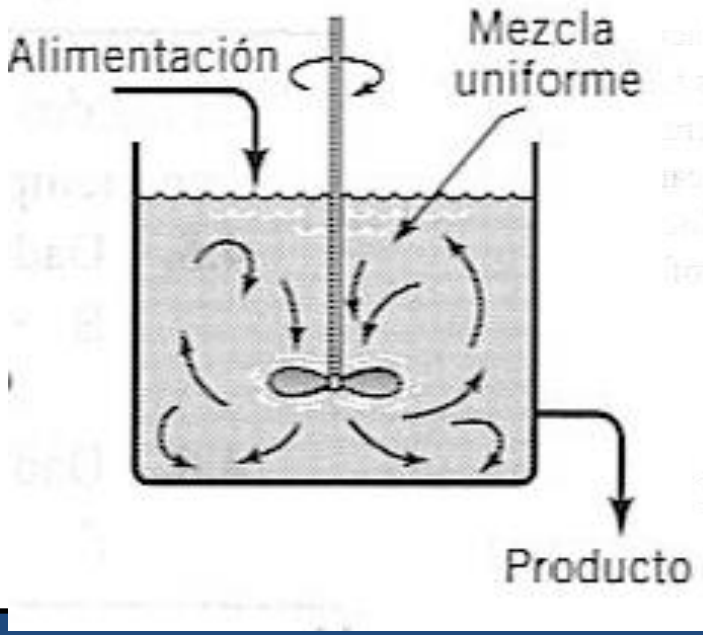
Matter entering the reactor is **instantaneously and homogeneously mixed** so that at each moment the concentration inside the vessel is exactly the same in the outlet current.

No short-circuit, nor dead zones.

8. MICROBIAL CONTINUOUS REACTORS

PERFECT MIX HYPOTHESIS :

Matter entering the reactor is **instantaneously and homogeneously mixed** so that at each moment the concentration inside the vessel is exactly the same in the outlet current.



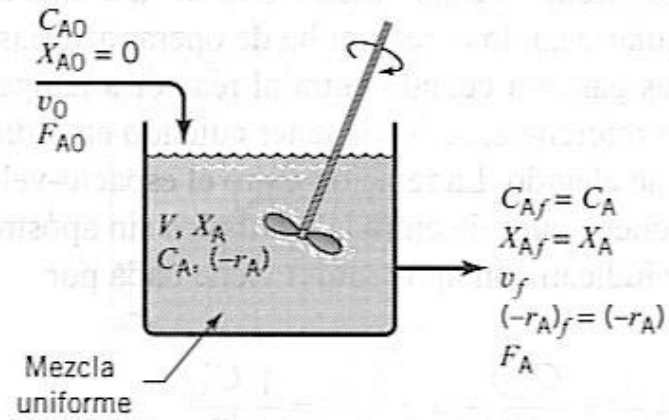


Figura 5.3. Nomenclatura utilizada en un reactor de tanque agitado

8.1 CHEMOSTAT

One limiting nutrient determines growth rate and cell density.

Growth is kept constant by supplying fresh medium with a nutrient at a fixed concentration while extracting culture containing microorganisms with the same rate.

CHEMOSTAT <> CHEMICALLY CONSTANT ENVIRONMENT

8.1 CHEMOSTAT

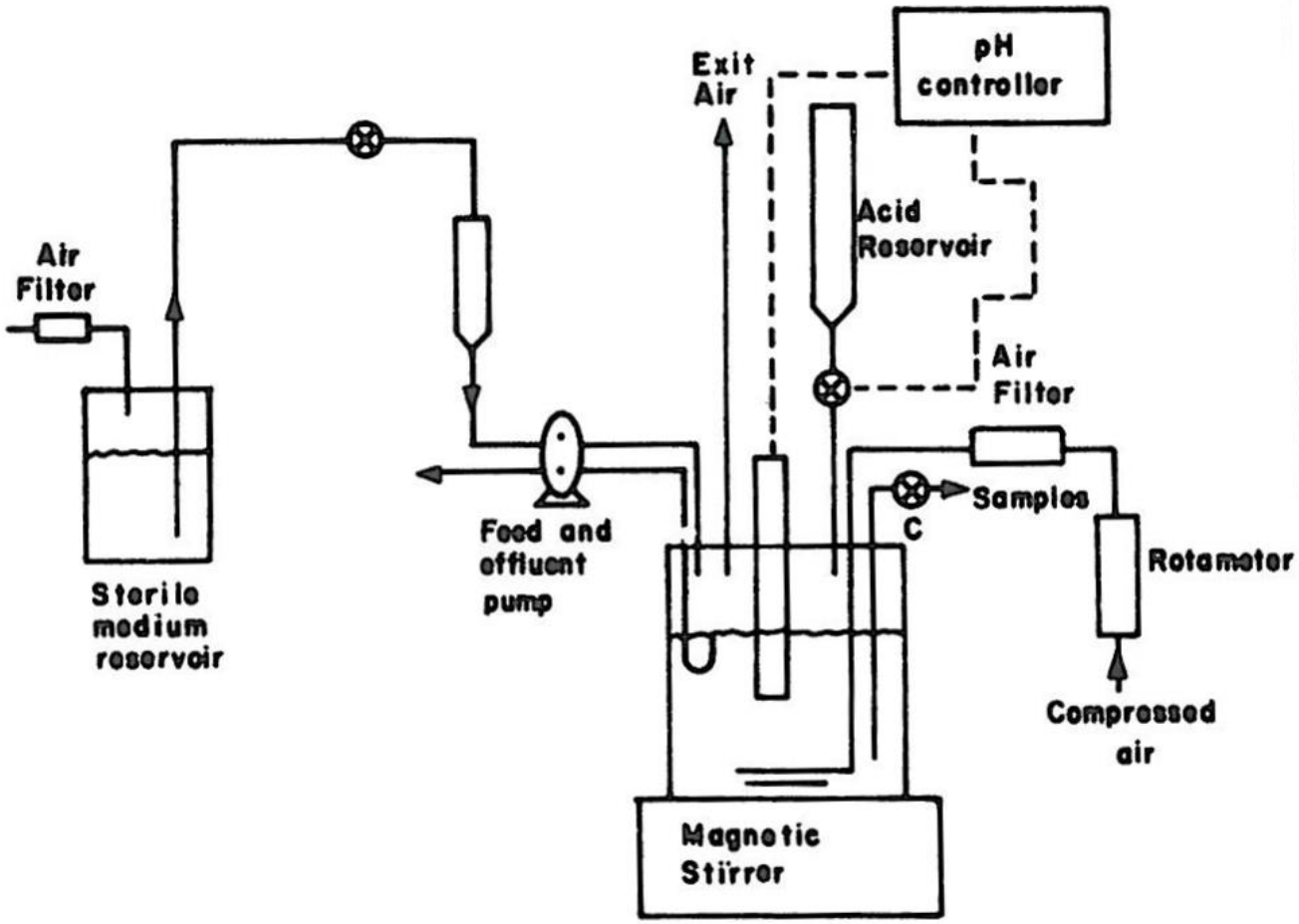


Figure 6.16. A continuous-culture laboratory setup (chemostat). (With permission, from D. I. C. Wang and others, *Fermentation and Enzyme Technology*, John Wiley & Sons, Inc., New York, 1979, p. 99.)

8.2. TURBIDOSTAT

Biomass concentration is maintained constant by measuring the optical density and controlling the inlet current.

In order to avoid changes in reactor volume, the same amounts of culture being removed and medium being added are needed.

TURBIDOSTAT <> DYNAMIC ENVIRONMENT

More difficult control than in chemostat case.

8.2. TURBIDOSTAT

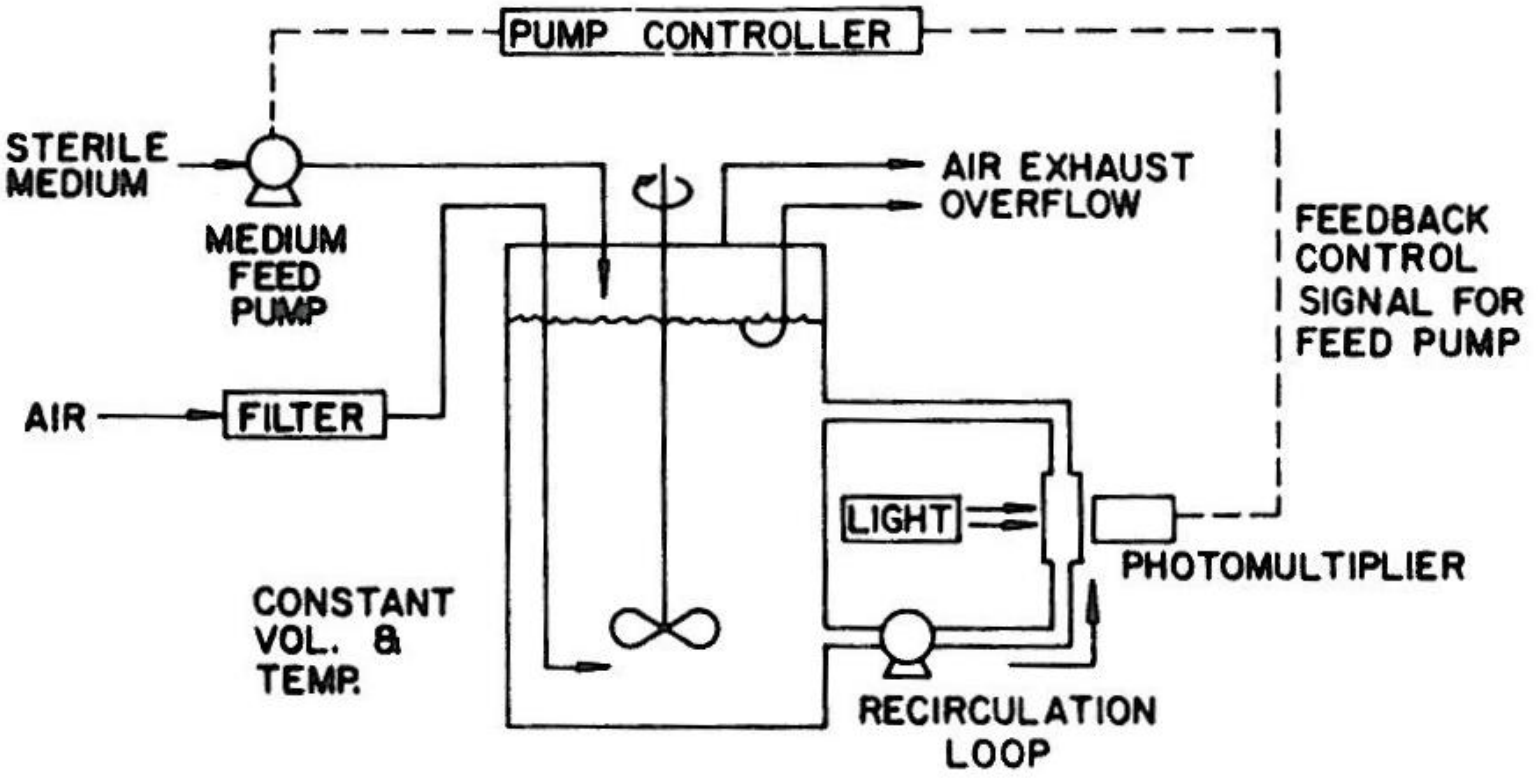


Figure 6.17. Typical laboratory setup for a turbidostat. (With permission, from D. I. C. Wang and others, *Fermentation and Enzyme Technology*, John Wiley & Sons, Inc., New York, 1979, p. 100.)

8.3. PLUG FLOW

PLUG FLOW HYPOTHESIS :

Easiest approach for Tubular Reactor behaviour.

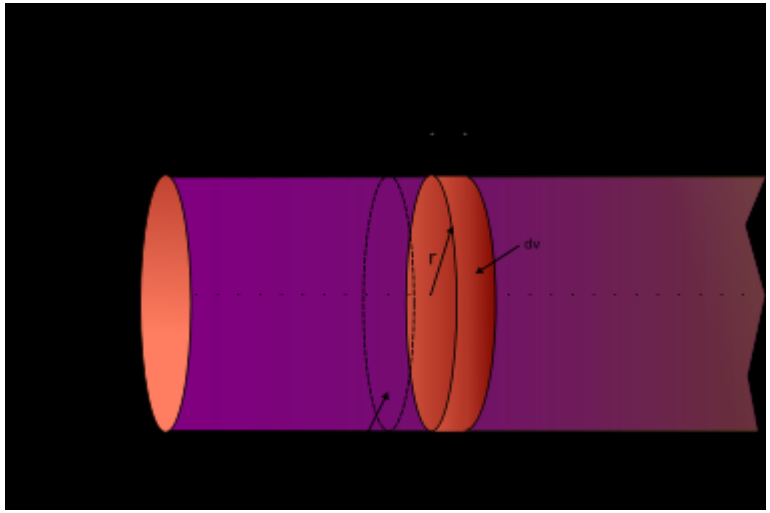
Uniformity alnog any cross-section in the reactor

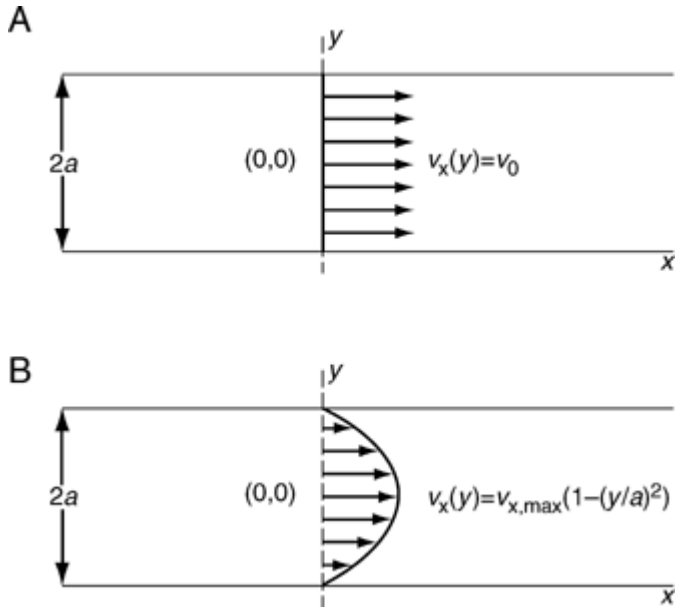
➔same speed and fluid properties

(temperature, pressure and composition) .

No axial flow.

8.3 PLUG FLOW





No mixing along this axis between cells inoculated at different times.

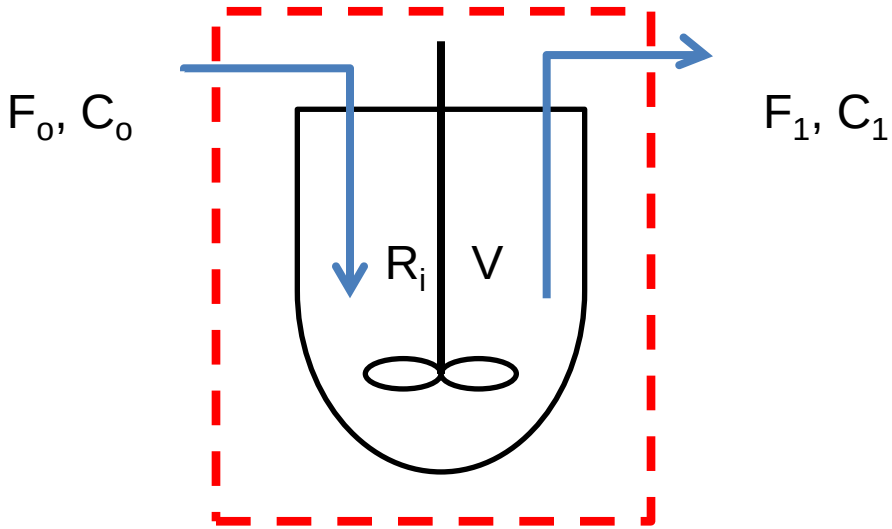
➔ Often used in waste treatment.

8.4 IDEAL CHEMOSTAT

CONDITIONS:

- Continuous Flow
- Complete mix
- Stirred tank reactor
- Control of pH, T, ...
- Feeding of a **sterile medium**, without biomass.
- Constant reaction volume

8.4 IDEAL CHEMOSTAT



$$\frac{V \cdot dC_i}{dt} = F_o \cdot C_{i,0} - F_1 \cdot C_{i,1} + V \cdot R_i$$

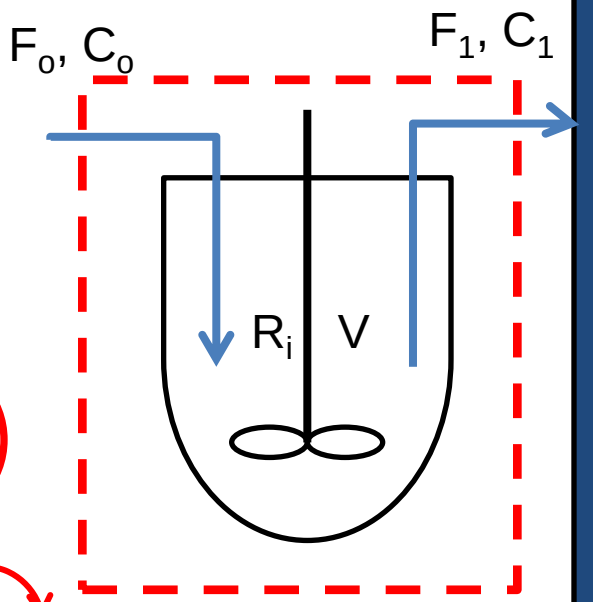
Accumulation | Inputs | Outputs | Generation

(i) MASS BALANCE

WITHIN BIOREACTOR for

***i* component**

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR para biomasa

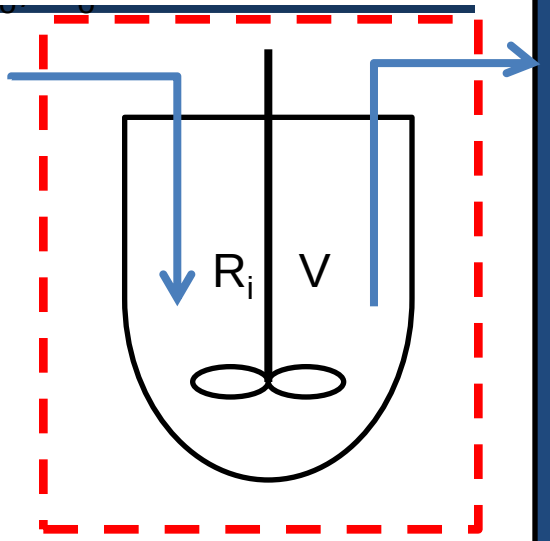
$$\frac{V \cdot dC_i}{dt} = F_o \cdot C_{i,0} - F_1 \cdot C_{i,1} + V \cdot R_i$$

$$\frac{V \cdot d[X]}{dt} = F_o \cdot [X]_0 - F_1 \cdot [X] + V \cdot R_X$$

In order to calculate R_X, which are reactions where biomass is involved?

$$Substrate \xrightarrow{Cells} Cells, \quad r_X \Rightarrow R_x = r_X$$

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

$$\frac{V \cdot dC_i}{dt} = F_o \cdot C_{i,0} - F_1 \cdot C_{i,1} + V \cdot R_i$$

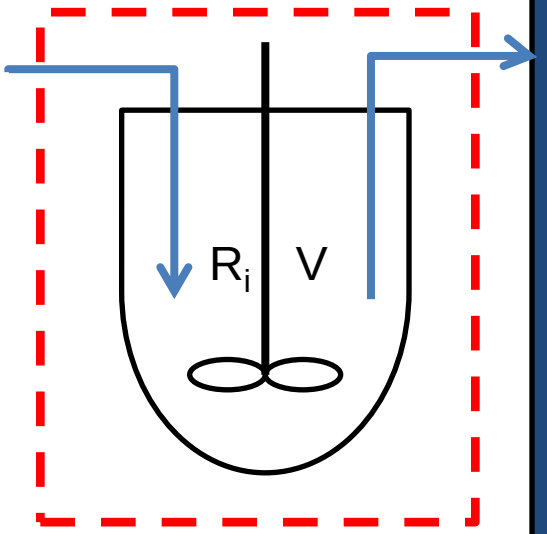
$$\frac{V \cdot d[X]}{dt} = F_o \cdot [X]_0 - F_1 \cdot [X] + V \cdot R_X$$

$$\frac{V \cdot d[X]}{dt} = F_o \cdot [X]_0 - F_1 \cdot [X] + V \cdot \mu_{net} \cdot X$$

8.4 IDEAL CHEMOSTAT

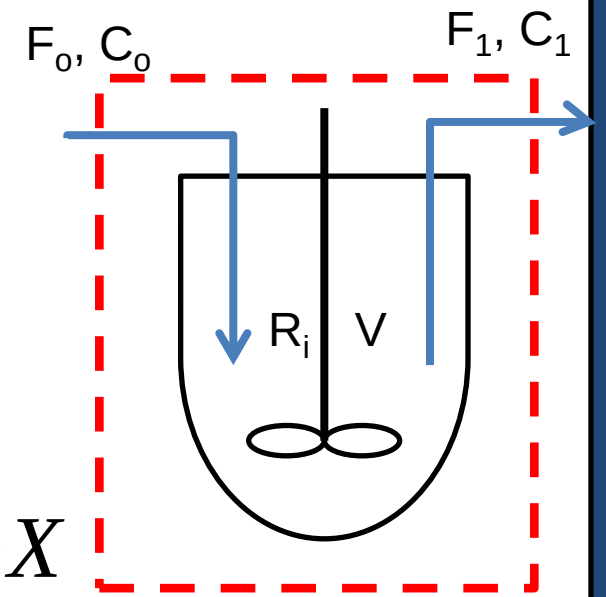
(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

$$\frac{V \cdot d[X]}{dt} = F_o \cdot [X]_0 - F_1 \cdot [X] + V \cdot \mu_{net} \cdot X$$



- **$[X]_0$:** biomass concentration within Input currents (DCW g/L)
- **F_0:** Input flow (L/h)
- **[X]:** biomass concentration within output currents (DCW g/L)
- **F:** Output flow (L/h)

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

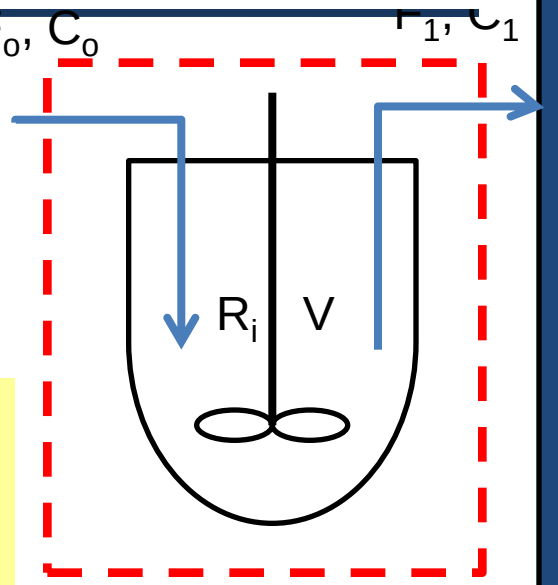
- **$F_0 = F_1 = F$** ➔ V = constant

$$\frac{V \cdot d[X]}{dt} = F_o \cdot [X]_0 - F_1 \cdot [X] + V \cdot \mu_{net} \cdot X$$

$$\Downarrow$$

$$\frac{V \cdot d[X]}{dt} = F \cdot [X]_0 - F \cdot [X] + V \cdot \mu_{net} \cdot X$$

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

DILUTION RATE:

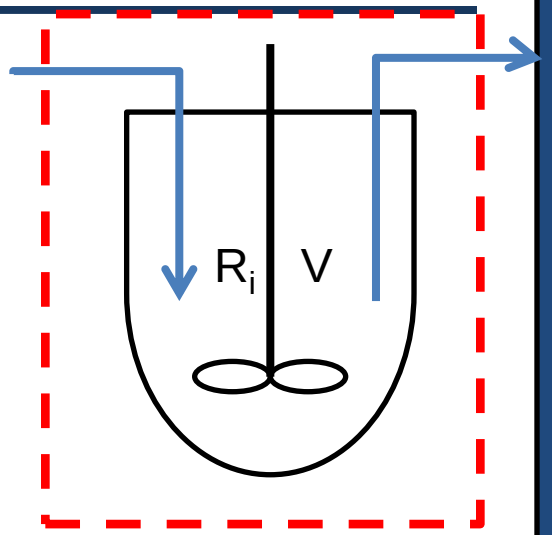
Quotien between Fed flow and volumen of reactor:

D = F/V

$$\frac{d[X]}{dt} = D\cdot[X]_0 - D\cdot[X] + \mu_{net}\cdot X$$

$$\frac{d[X]}{dt} = D\cdot[X]_0 + (\mu_{net} - D)\cdot X$$

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

HYPOTHESIS:

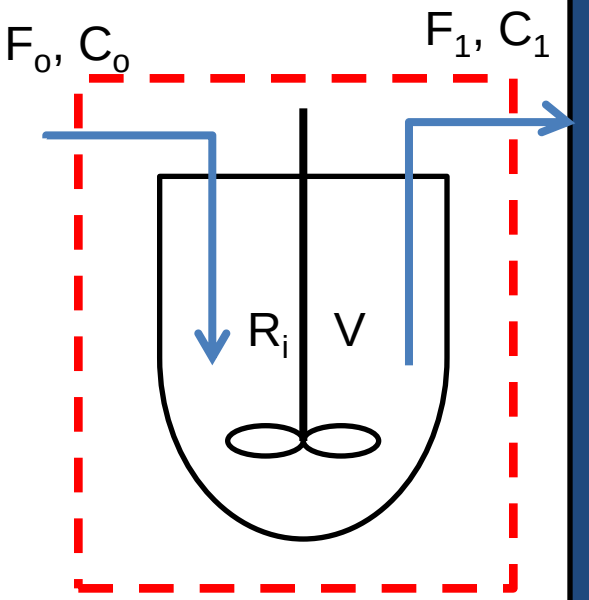
- **Sterile feeding ➔ $[X]_0 = 0$** (dw g/L)

$$\frac{d[X]}{dt} = D\cdot[X]_0 + (\mu_{net} - D)\cdot X$$

- **Insignificant endogenous metabolism ➔ $\mu_d = 0$**

$$\frac{d[X]}{dt} = (\mu_g - D)\cdot X$$

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

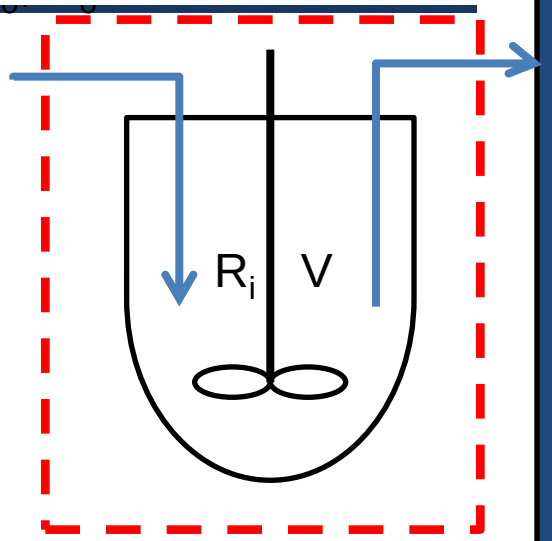
HYPOTHESIS:

- **Steady State ➔ d[X]/dt = 0**

$$\frac{d[X]}{dt} = (\mu_g - D)\cdot X$$

$$0 = (\mu_g - D)\cdot X \Rightarrow D = \mu_g$$

8.4 IDEAL CHEMOSTAT



(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

HYPOTHESIS:

- **Sterile feeding ➔ $[X]_0 = 0$** (dw g/L)
- **Insignificant endogenous metabolism ➔ $\mu_d = 0$**
- **Steady State ➔ d[X]/dt = 0**

$$D = \mu_g = \frac{\mu_m \cdot [S]}{K_S + [S]}$$

Monod equation:

Describes growth kinetics when there is a

limiting nutrient, ***S.***

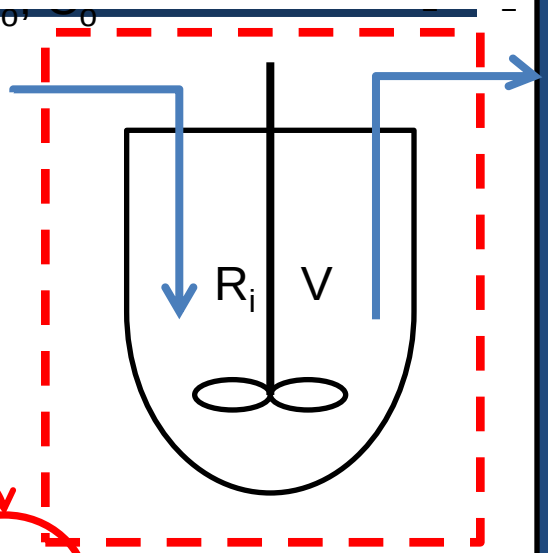
8.4 IDEAL CHEMOSTAT

(i) MASS BALANCE WITHIN BIOREACTOR for biomass:

$$D = \frac{\mu_m \cdot [S]}{K_S + [S]} \Rightarrow [S] = \frac{K_S \cdot D}{\mu_m - D}$$

$D < \mu_m$

8.4 IDEAL CHEMOSTAT



(ii) MASS BALANCE WITHIN BIOREACTOR for substrate

$$\frac{V \cdot dC_i}{dt} = F \cdot C_i - F \cdot C_i + V \cdot R_i$$

$$\frac{V \cdot d[S]}{dt} = F \cdot [S]_0 - F \cdot [S] + V \cdot R_S$$

In order to calculate R_S, ¿wich reaction need to be considered?

$$Substrate \xrightarrow{Cells} Cells, \quad r_X ; Y_{X/S} ; \mu_{net}$$

$$Substrate \xrightarrow{Cells} Products, \quad r_p ; Y_{P/S} ; q_P$$

8.4 IDEAL CHEMOSTAT

(ii) MASS BALANCE WITHIN BIOREACTOR for substrate:

$$\frac{V \cdot d[S]}{dt} = F \cdot [S]_0 - F \cdot [S] + V \cdot R_S$$

$$Substrate \xrightarrow{Cells} Cells, \quad r_X ; Y_{X/S}^{\max} ; \mu_{net}$$

$$Substrate \xrightarrow{Cells} \Pr oducts, \quad r_p ; Y_{P/S} ; q_P$$

$$R_S = -\mu_g \cdot X \cdot \frac{1}{Y_{X/S}^{\max}} - q_P \cdot X \cdot \frac{1}{Y_{P/S}}$$

(ii) MASS BALANCE WITHIN BIOREACTOR for substrate:

$$\frac{V \cdot d[S]}{dt} = F \cdot [S]_0 - F \cdot [S] - V \cdot \mu_g \cdot X \cdot \frac{1}{Y_{X/S}^{max}} - V \cdot q_P \cdot X \cdot \frac{1}{Y_{P/S}}$$

- **If extracellular generation of products is negligible ➔** $q_P = 0$.
- **Steady state ➔ d[S]/dt = 0**

$$0 = F \cdot [S]_0 - F \cdot [S] + V \cdot \mu_g \cdot X \cdot \frac{1}{Y_{X/S}^{max}}$$

$$0 = D \cdot [S]_0 - D \cdot [S] - \cdot \mu_g \cdot X \cdot \frac{1}{Y_{X/S}^{max}} \Rightarrow D \cdot ([S]_0 - [S]) = \frac{\mu_g \cdot X}{Y_{X/S}^{max}}$$

8.4 IDEAL CHEMOSTAT

(ii) MASS BALANCE WITHIN BIOREACTOR for substrate:

$$D\cdot\left([S]_0-[S]\right)=\frac{\mu_g\cdot X}{Y_{X/S}^{\max}}$$

- **No Endogenous metbolism and steady state ➔ D = μ_g**

$$X=Y_{X/S}^{\max}\cdot\left([S]_0-[S]\right)$$

$$X=Y_{X/S}^{\max}\cdot\left([S]_0-\frac{K_S\cdot D}{\mu_m-D}\right)$$

Para D < μ_m

SECTION II: KINETICS AND BIOREACTOR DESIGN:

LESSON 9.2. - Enzymatic kinetics, microbial kinetics and metabolic stoichiometry – Alive cells in bioprocesses

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