Introduction

1.1 – Batch Reactor (BSTR)

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1.1.5 – Reactor Mass Balances

$$\frac{dx}{dt} = (\mu - k_d)x \quad (13)$$

Specific cell growth rate

Specific cell death rate

For the exponential phase $\mu=\mu_{max}$

$$\frac{dx}{dt} = (\mu_{max} - k_d)x \quad (14)$$

$$x = x_0\, e^{(\mu_{max}-k_d)t} \quad (15)$$

$$t_b = \frac{1}{\mu_{max} - k_d} \ln \frac{x_t}{x_0} \quad (16)$$

t_b is the time needed to reach the maximum cell concentration ($x_t \Rightarrow x_{max}$)

Total operating time

Batch time

$$t_T = t_p + t_r + t_{lag} + t_b \quad (18)$$

preparation

emptying, cleaning

If death rate is negligible :

$$t_b = \frac{1}{\mu_{max}} \ln \frac{x_t}{x_0} \quad (17)$$

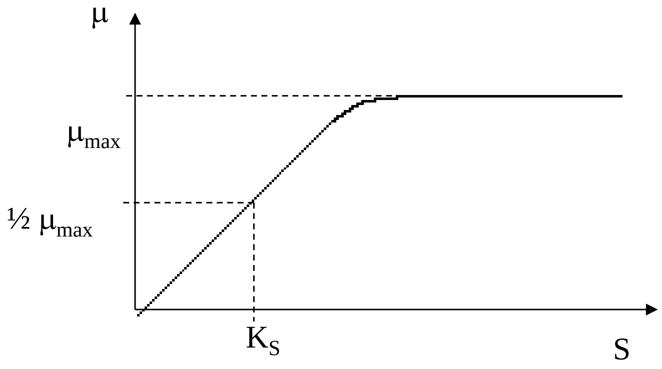
1.1.6 – Relationship between Cell Growth and Substrate Consumption

Growth **Monod Model**

$$\mu = \frac{\mu_{\max} S}{K_S + S} \quad (19)$$

$$S \gg K_s \Rightarrow \mu = \mu_{max} \quad (21)$$

$$S \ll K_s \Rightarrow \mu = \frac{\mu_{max} S}{K_s} \quad (22)$$



Substrate consumption **eq. de Michaelis-Menten**

$$-\frac{ds}{dt} = r_S = \frac{v_{\max} S}{K_m + S} \quad (23)$$

$$Y_{x/s} = \frac{x - x_0}{s_0 - s} \quad ou \quad Y_{x/s} = \frac{\Delta x}{\Delta S} \quad (24)$$

if $Y_{x/s}$ is constant:

$$r_s = \frac{1}{Y_{x/s}} \mu x \quad (25)$$

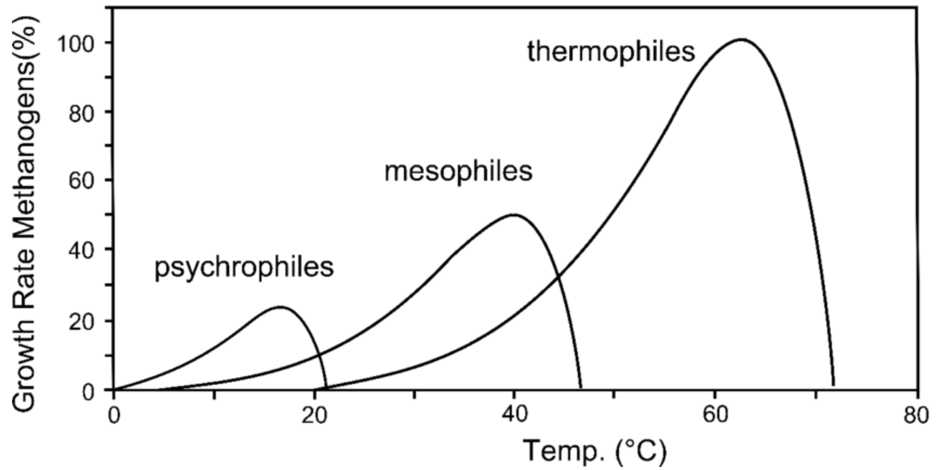
$$r_s = \frac{1}{Y_{x/s}} \frac{\mu_{\max} s}{K_s + s} x \quad (26)$$

1.1.7 – Effect of Temperature and pH on growth

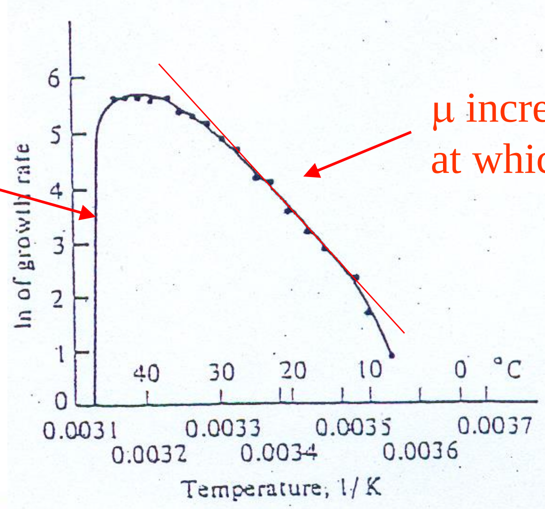
Effect of temperature

$$\mu = A\,e^{-\frac{E}{RT}}$$

$$\ln \mu = \ln A - \frac{E}{R}\,\frac{1}{T}$$



μ decreases with the temperature (enzymatic deactivation)



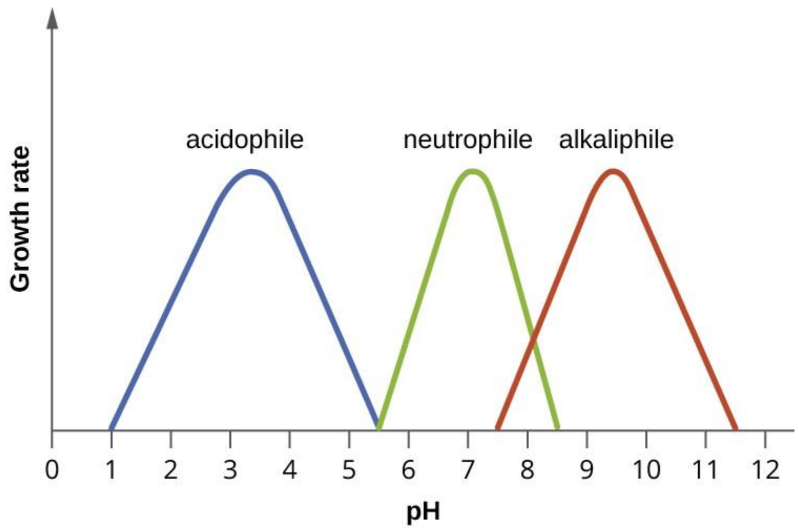
μ increases linearly with the temperature at which the Arrhenius equation is valid

1.1.7 – Effect of Temperature and pH on growth

Effect of pH

The pH influences:

- type of metabolism
- enzyme activity
- substrate or product inhibition
- biomass (cell wall) and morphological (fungi) composition



- Most bacteria grow at pH 6.5-7.5
- Yeasts grow at pH 4-5
- Algae grow at pH=10 (contain cytoplasmic membranes that are not permeable to H+ or OH)

1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

- Cell maintenance:

The energy (ATP) formed in the primary metabolism is used for:

a) cellular synthesis (substrate consumption for the production of cellular material);

b) maintain chemical gradients (intracellular pH regulation);

c) transport nutrients from the environment through the cell membrane to the cytoplasm;

d) DNA repair and replication;

e) product formation (very low value).

→ The energy spent in processes b), c) and d) is called **maintenance energy**.

1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

In this case, substrate consumption can be described by:

$$\Delta S = \Delta S_{1-assimilation/reserves} + \Delta S_{2-growth/energy} + \Delta S_{3-maintenance/energy}$$

The observed or apparent yield coefficient:

$$Y_{x/s} = \frac{\Delta x}{\Delta S_1 + \Delta S_2 + \Delta S_3}$$

$Y_{x/s}$ takes into account the amount of substrate used for all cell processes.

The true yield coefficient:

$$Y'_{x/s} = \frac{\Delta x}{\Delta s_2}$$

$Y'_{x/s}$ only takes into account the amount of substrate used for cell formation.

1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

Maintenance effect on substrate consumption rate:

$$r_s = \frac{1}{Y_{x/s}} \mu x$$

$\mu x = r_x$

$$\Leftrightarrow r_s = \frac{1}{Y_{x/s}} r_x$$

r_S – volumetric rate of substrate consumption

r_x – volumetric growth rate

1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

Maintenance effect on substrate consumption rate:

$$r_s = \frac{1}{Y_{x/s}} \mu x$$

$$\mu = \frac{\mu_{\max} S}{K_S + S}$$

$$r_s = \frac{1}{Y'_{x/s}} r_x + mx$$

m – maintenance coefficient (gS/gX h)

$Y'_{x/s}$ – true growth yield (gX/gS)

$$\boxed{r_s = \frac{1}{Y'_{x/s}} \frac{\mu_{max} S}{k_s + S} x + mx}$$

1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

The relation between$Y'_{x/s}$e $Y_{x/s}$ is given by:

$$\left.\begin{aligned} r_s &= \frac{1}{Y_{x/s}}\mu x \\ r_s &= \frac{1}{Y'_{x/s}}\mu x + mx \end{aligned}\right\}$$

$$\frac{1}{Y_{x/s}}\mu x = \frac{1}{Y'_{x/s}}\mu x + mx$$

$$\Leftrightarrow Y_{x/s} = \frac{Y'_{x/s}\,\mu}{\mu + mY'_{x/s}}$$

$Y_{x/s} << Y'_{x/s}$

The value of **m** depends on the type of substrate and environmental conditions (temperature and pH)

Note: *Many of the maintenance needs are to maintain osmotic gradients (**m** increases if the salinity or pH of the medium increases).*

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1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

- Endogenous Metabolism or Endogenous Respiration :

Reactions in cells that consume cellular substances (decrease in cell mass over time)

Cellular mass consumption rate (r_x) is given by:

$$r_x = \underbrace{\frac{\mu_{max} S}{k_s + S} x}_{\text{Cell growth}} - \underbrace{k_e x}_{\text{Endogenous respiration}}$$

K_e – endogenous specific rate (h^{-1})

Note: the term K_e can be interpreted as a death rate

In this case the relation between $Y'_{x/s}$ and $Y_{x/s}$ is given by:

$$Y_{x/s} = \frac{Y'_{x/s}\,\mu}{\mu + k_e}$$

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1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

- Calculation of m and $Y'_{x/s}$

$$V_s = \frac{r_s}{x}$$

V_S – specific substrate consumption rate (gS/gX h)

r_S – volumetric rate of substrate consumption (mgS/l.h)

$$r_s = \frac{1}{Y_{x/s}}\mu x \quad \Rightarrow \quad V_s = \frac{1}{Y_{x/s}}\mu$$

Taking into account the maintenance effects :

$$r_s = \frac{1}{Y'_{x/s}}\mu x + mx \quad \Rightarrow \quad \boxed{V_s = \frac{1}{Y'_{x/s}}\mu + m}$$

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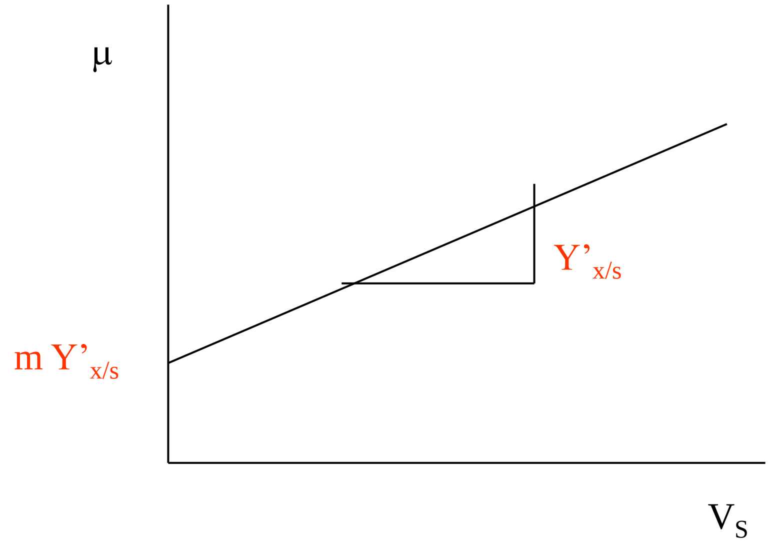
1.1.8 – Effect of Endogenous Breathing and Maintenance on Biological Reaction Kinetics

$$V_s = \frac{1}{Y'_{x/s}}\mu + m \iff \mu = \underbrace{V_s\, Y'_{x/s}}_{\text{Slope}} - \underbrace{m\, Y'_{x/s}}_{\text{Intercept}}$$



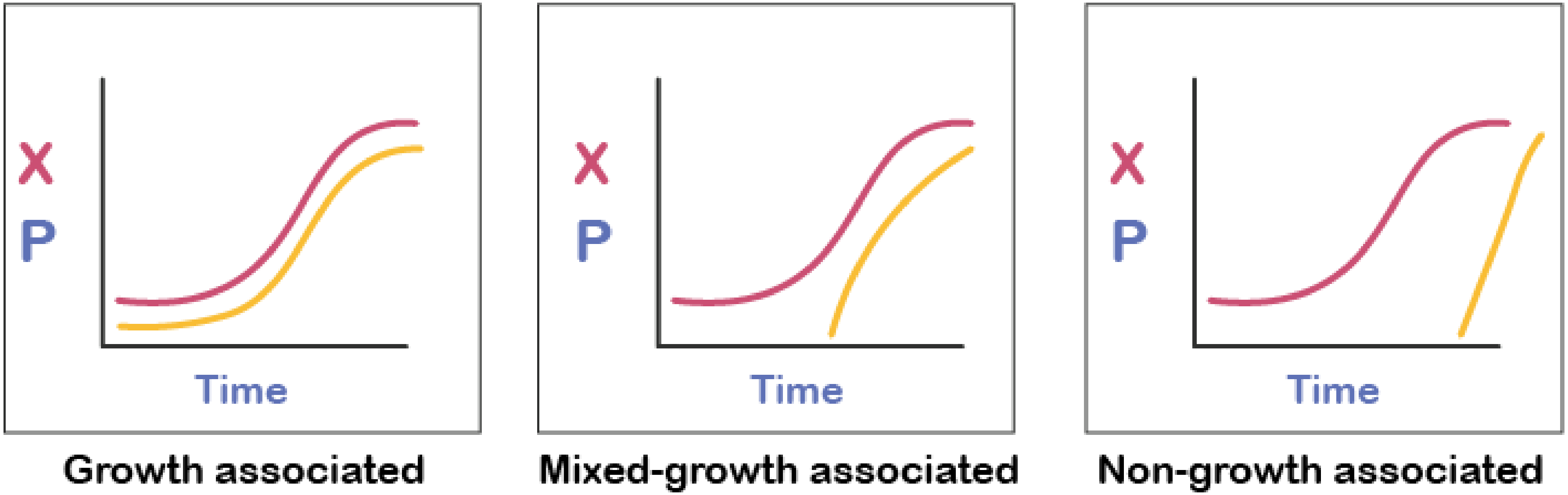
1.1.9 – Product production

- Classification of Product Types according to Gaden:

  Type I - Product associated with growth

  Type II - Product partially associated with growth

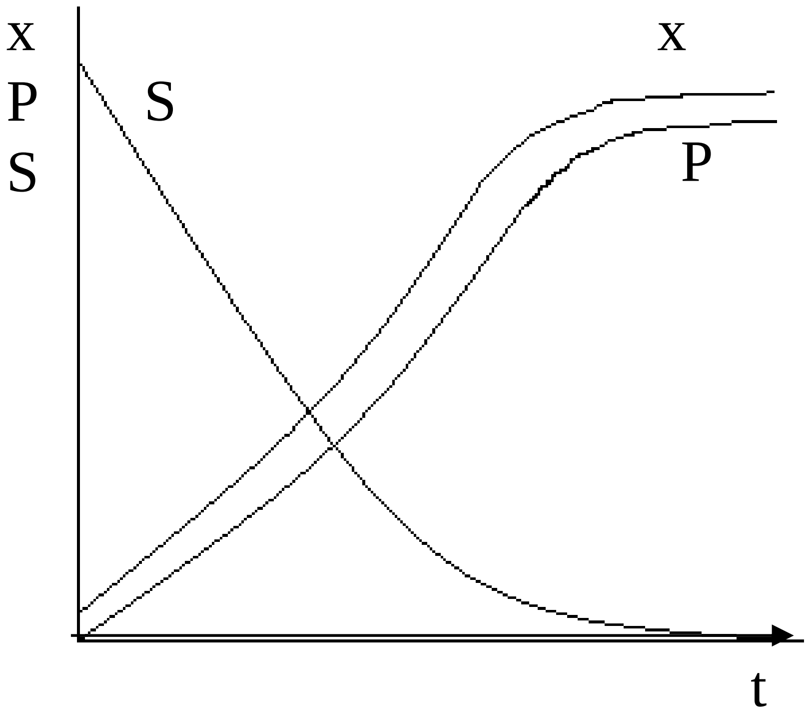
  Type III - Product not associated with growth



1.1.9 – Product production

I – <u>Product associated with growth</u>

It results directly from energy metabolism.



Examples of type I processes:
- Ethanol production
- Acetic acid production
- Production of gluconic acid

1.1.9 – Product production

I – Product associated with growth

The rate of product formation is associated with the rate of growth:

$$r_p = \frac{dP}{dt} = Y_{p/x}\, \mu\, x$$

r_p – volumetric product production rate (gP/l.h)

$Y_{p/x}$ – Yield coefficient of product formation to cells (gP/gX)

$$V_p = \frac{1}{x}\frac{dP}{dt} = Y_{p/x}\, \mu$$

V_p - specific product production rate (gP/gX.h) $\quad V_p = \frac{r_p}{x}$

Linear relationship with the substrate :

$$r_p = -Y_{p/s}\, r_s$$

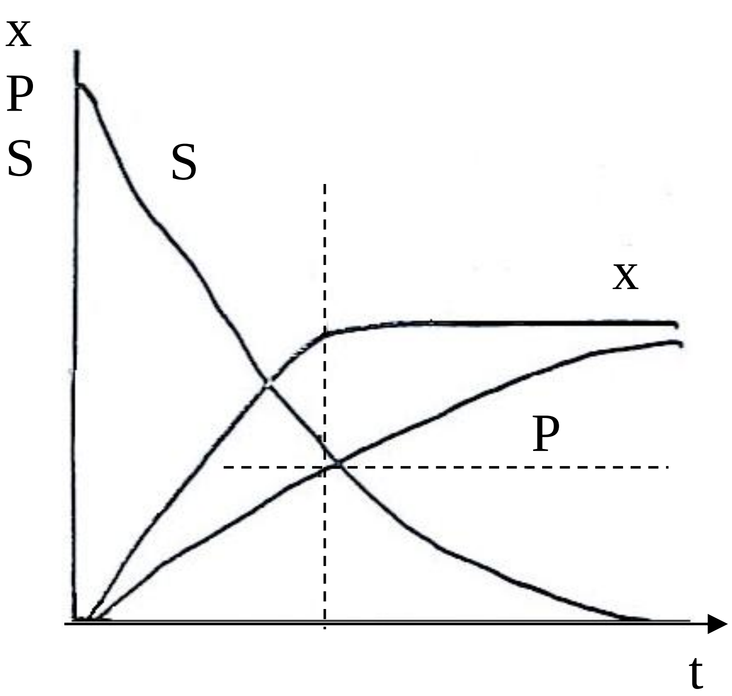
$Y_{p/s}$ – Yield coefficient of product formation to substrate (gP/gS)

where: $$-r_S = \frac{1}{Y'_{X/S}}\mu x + m x$$

1.1.9 – Product production

II – <u>Product partially associated with growth</u>

It results indirectly from energy metabolism - The rate of product formation is partially associated with growth:



Examples of type II processes:
- Production of lactic and propionic acid
- Production of amino acids
- Citric acid production
- Production of extracellular polymers (xanthan, pullulan)

1.1.9 – Product production

II – Product partially associated with growth

Model of Luedking Piret:

$$r_p = \frac{dP}{dt} = \underbrace{\alpha \, \mu \, x}_{\text{Term associated to growth}} + \underbrace{\beta \, x}_{\text{Term not associated with growth}}$$

Term associated
to growth

Term not associated
with growth

1.1.9 – Product production

II – Product partially associated with growth

Model of Luedking Piret:

$$r_p = \frac{dP}{dt} = \alpha\, \mu\, x + \beta\, x$$

$$V_p = \frac{1}{x}\frac{dP}{dt} = \alpha\, \mu + \beta$$

$$V_p = Y'_{p/x}\, \mu + m_p$$

$\alpha = Y'_{p/x}$ – true yield coefficient of product production (gP/gX)

β = specific product formation rate due to maintenance (gP/gX.h) $m_{p} = \beta$

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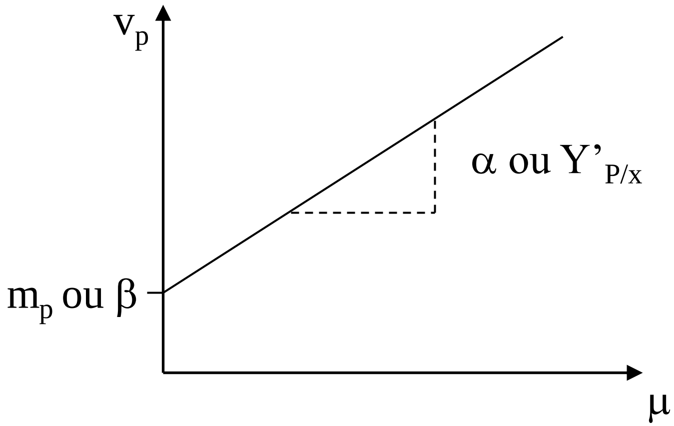
1.1.9 – Product production

II – <u>Product partially associated with growth</u>

$$V_p = Y'_{p/x}\,\mu + m_p$$

Vp = f(μ)



In Type II processes, the substrate consumption rate is a function of 3 factors:

- growth
- product formation
- substrate consumption rate for maintenance

$$r_S = \frac{1}{Y'_{x/S}}\mu x + \frac{1}{Y'_{P/S}} r_p + mx$$

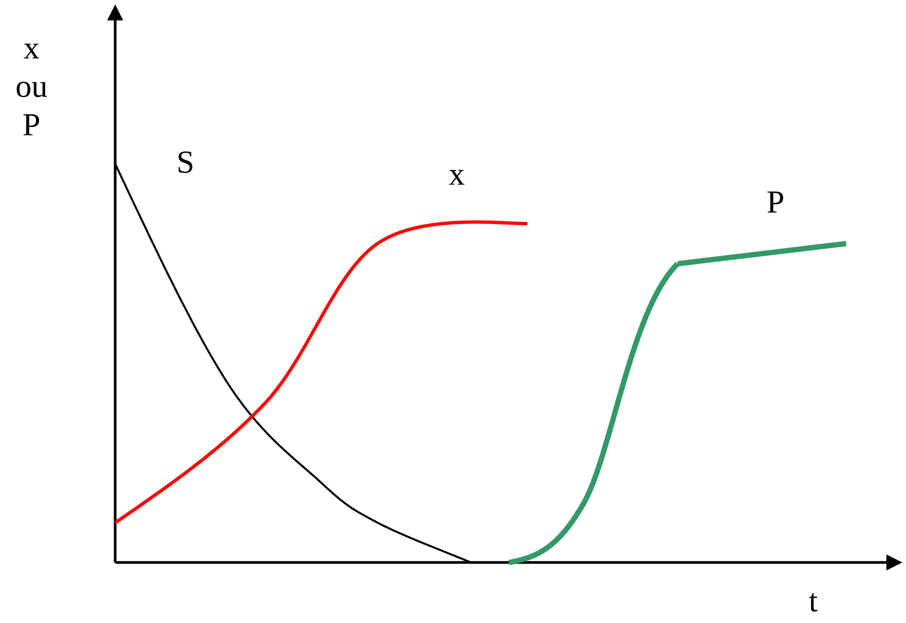
$$\boxed{Y'_{P/S} = Y'_{x/S} \times Y'_{P/x}}$$

1.1.9 – Product production

III – Non-Growth Associated Product

Product production kinetics do not depend on growth rate

Rate is proportional to cell concentration and not to growth rate.



$$r_P = Ax \quad (50)$$

Examples of type III processes:
- Production of secondary metabolites
Ex: Antibiotics: Penicillin

1.1.10 – Inhibition Models

Possible actions of an Inhibitor:

- Modifies the chemical potential of substrates, intermediates and products;
- Changes cell permeability;
- Changes the enzymatic activity;
- Dissociates enzymatic aggregates;
- Affects enzymatic synthesis;
- Influences the functional activity of cells

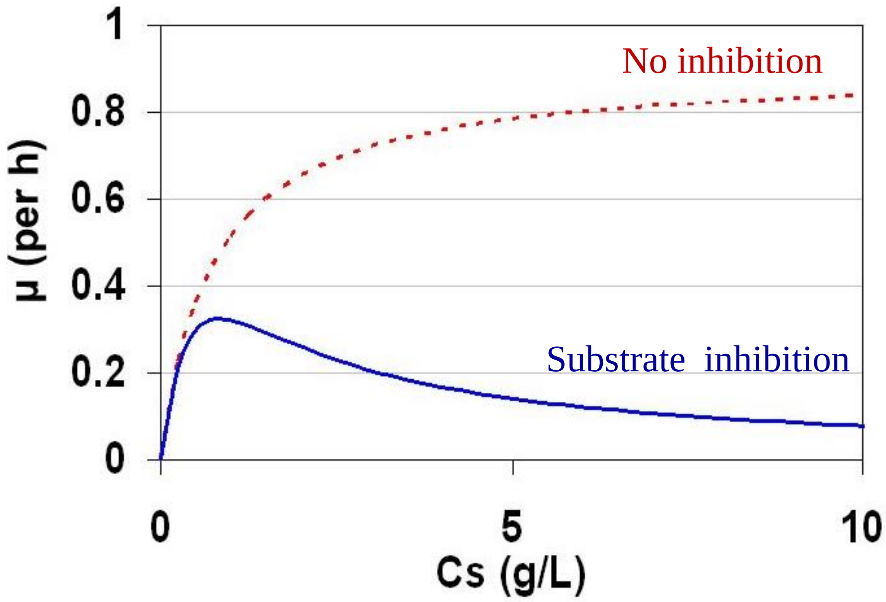
1.1.10 – Inhibition Models

- Inhibition by substrate:

(Andrews Model)

$$\frac{dx}{dt} = \mu_{max} \frac{S}{K_S + S + \frac{S^2}{K_i}} x$$

K_i – inhibition constant



Critical Substrate Concentration:

$$S_{crít} = \sqrt{K_S K_i}$$

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1.1.10 – Inhibition Models

- <u>Inhibition by substrate:</u>

  (Andrews Model)

$$\frac{dx}{dt} = \mu_{\max} \frac{S}{K_S + S + \frac{S^2}{K_i}} x$$

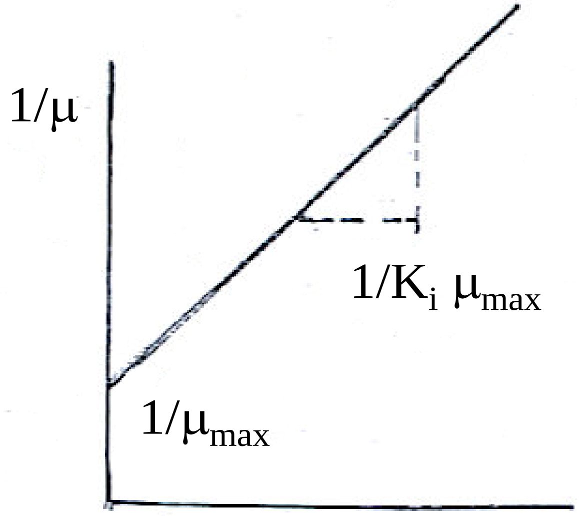
K_i – inhibition constant

$$\mu\, x = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}} x$$

$$\Leftrightarrow \mu = \mu_{max} \frac{S}{S + \frac{S^2}{K_i}}$$

for $S >> K_S$

$$\Leftrightarrow \mu = \mu_{max} \frac{1}{1 + \frac{S}{K_i}} \quad \Leftrightarrow \frac{1}{\mu} = \underbrace{\frac{1}{\mu_{max}}}_{\text{intercept}} + \underbrace{\frac{S}{K_i\, \mu_{max}}}_{\text{slope}}$$



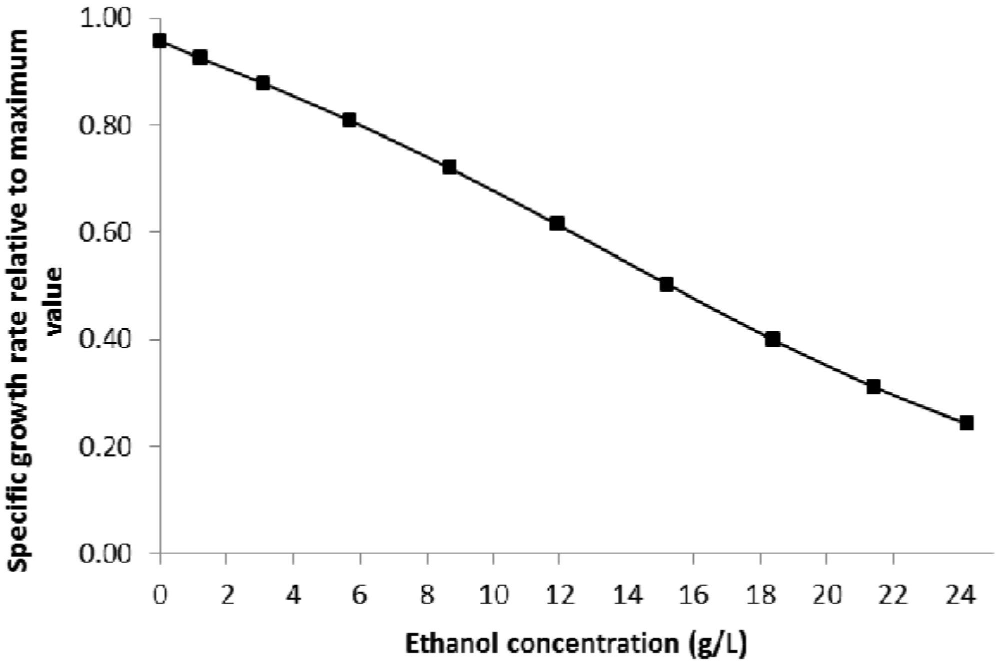
1.1.10 – Inhibition Models

- Inhibition by Product:

Aiba Model:

$$\mu = \mu_{\max} \frac{s}{K_s + s} \frac{K_P}{K_P + P}$$

K_P – Product inhibition constant



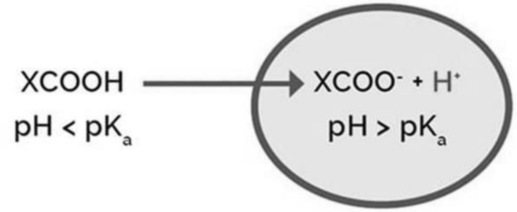
Exponential model:

$$\mu = \frac{\mu_{\max} S}{K_S + S} e^{-K_P P}$$

1.1.10 – Inhibition Models

- <u>Inhibition by undissociated acid:</u>

(Model of Han & Levenspiel)



$$\mu = \frac{\mu_{max}}{1 + \frac{\left[H^{+}\right]}{K_H} + \frac{K_{OH}}{\left[H^{+}\right]}}$$

K_H – inhibition constant