I – Bioreactor Kinetics

Introduction

1.1 – Batch Reactor (BSTR)

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- ✓ 1.1.2 – Cell growth phases
- ✓ 1.1.3- Elementary Composition of the Biomass
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INTRODUCTION

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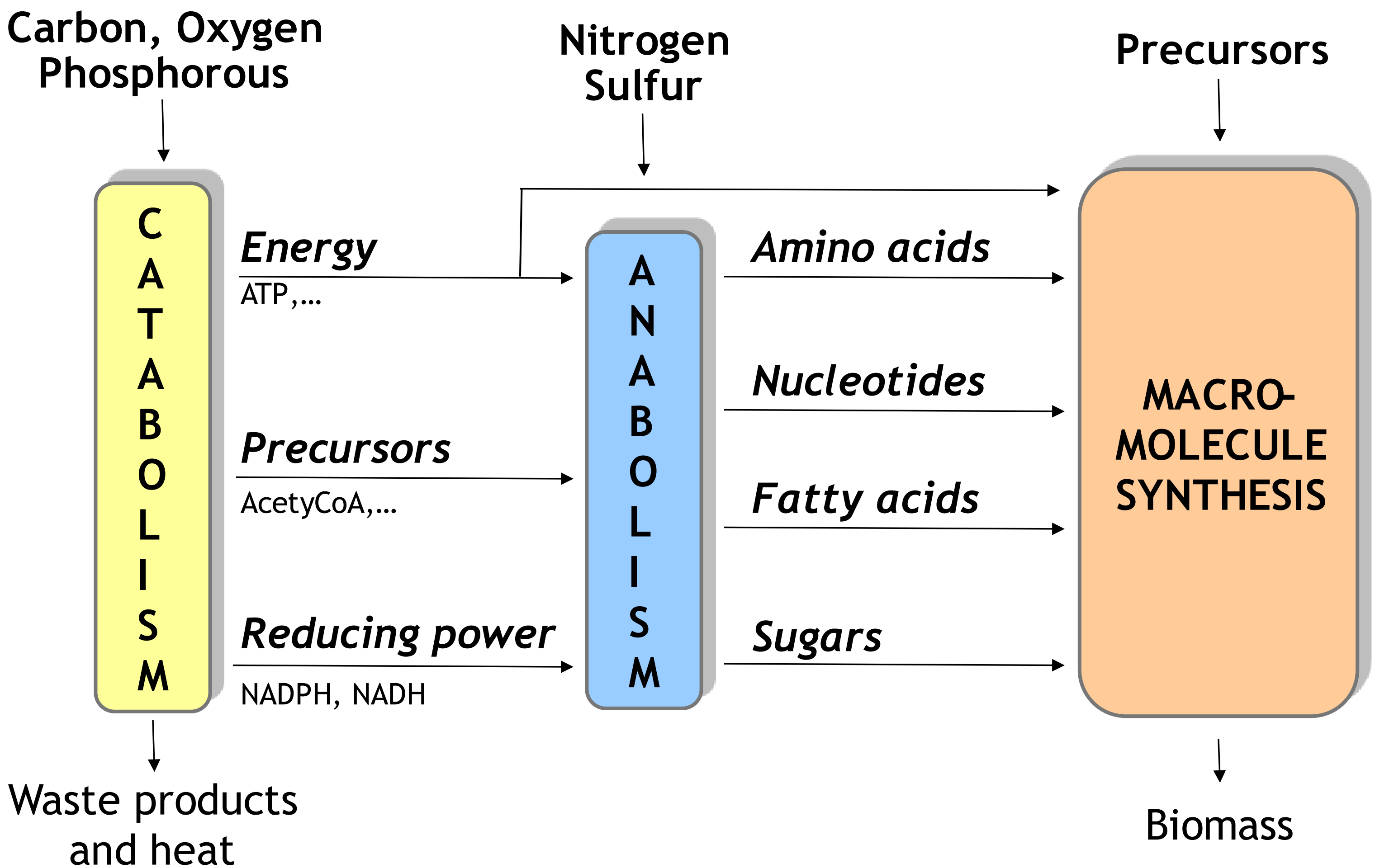
Primary metabolism



1.1 – Batch Reactor

1.1.1 – Definitions

- 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

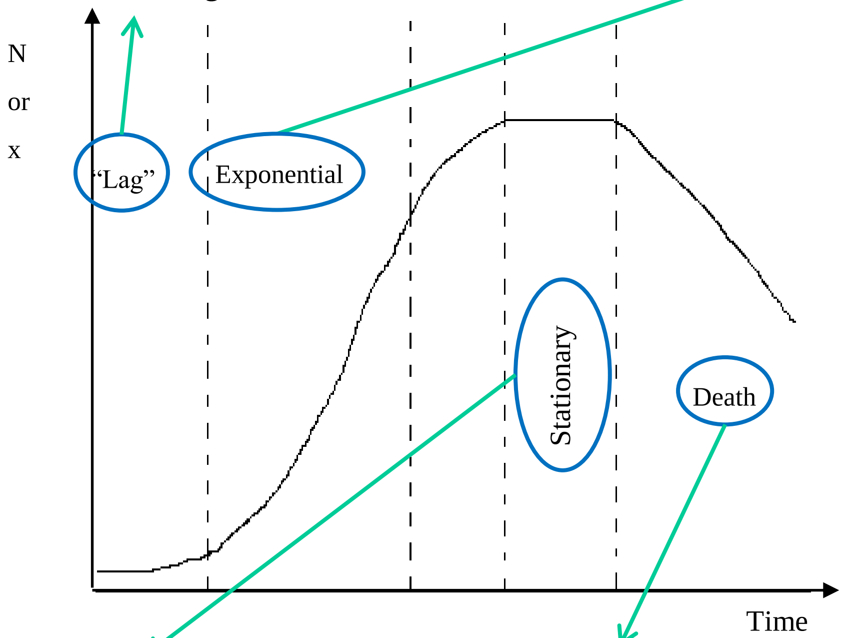
suspension of microorganisms of suitable concentration, used to start the fermentation process

1.1 – Batch Reactor

1.1.2 – Cell growth phases

"Lag" phase (adaptation)

No cell growth



stationary phase

No cell growth

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

Death phase

Decrease cell concentration

$$\boxed{\frac{dx}{dt} = -k_d\, x}$$

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

Exponential phase

The cell growth rate is proportional to the cell concentration

x –cell concentration (mg/L)

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

$\mathbf{r_x}$ – volumetric cell growth rate (mg cel/l.h)

$$\boxed{\frac{dx}{dt} = \mu x = r_x}$$

"Balanced growth"
Constant cell composition

$$\boxed{\ln x = \ln x_0 + \mu t}$$

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

$\mathbf{\mu_{max}}$ – maximum specific cell growth rate (h^{-1})

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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

- Represented by chemical formulas:

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

1.1.3 – Elemental composition of the biomass

Important for estimating the microorganims' nutrient requirements

For cell growth:

- C source
- N source

(- O_2 under aerobic conditions)

Chemical reaction for cell growth

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

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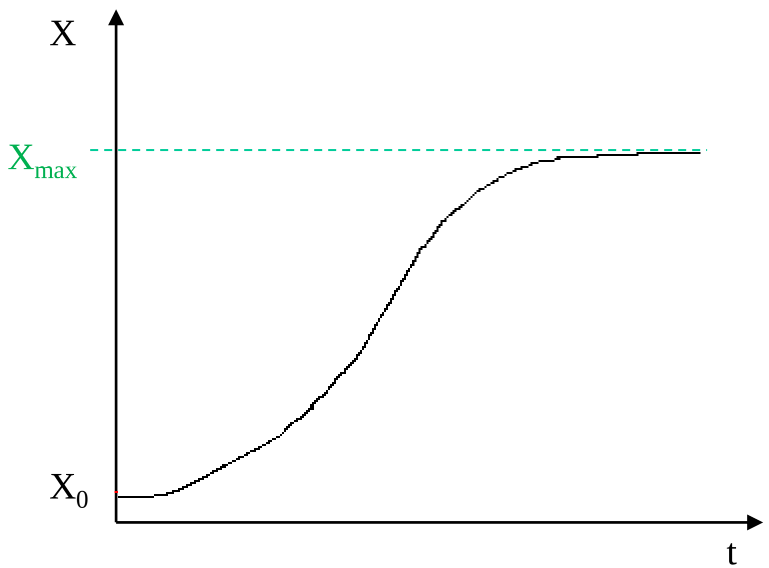
1.1.4 – Non strutured Models

- **Malthus Model:** $\frac{dx}{dt} = \mu x \quad ou \quad r_x = \mu x$ Does not predict the appearance of the stationary phase



- **Verhulst Model:** Logistic model

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

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

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

1.1 – Batch Reactor

1.1.5 – Reactor Mass Balances

Cell growth $$\frac{dx}{dt} = \mu x \quad (1)$$

Cell death $$\frac{dx}{dt} = -k_d\, x \quad (9)$$

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

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

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

$$\frac{dx}{x} = (\mu_{max} - k_d)dt \Leftrightarrow \int_{x_0}^{x} \frac{dx}{x} = \int_0^t (\mu_{max} - k_d)dt$$

$$\Leftrightarrow \ln\frac{x}{x_0} = (\mu_{max} - k_d)t \Leftrightarrow \boxed{x = x_0\, e^{(\mu_{max}-k}} \quad (15)$$

1.1.5 – Reactor Mass Balances

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

$$\Leftrightarrow \int_{x_0}^{x_t} \frac{dx}{x} = \int_0^{t_b} (\mu_{max} - k_d)dt$$

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

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

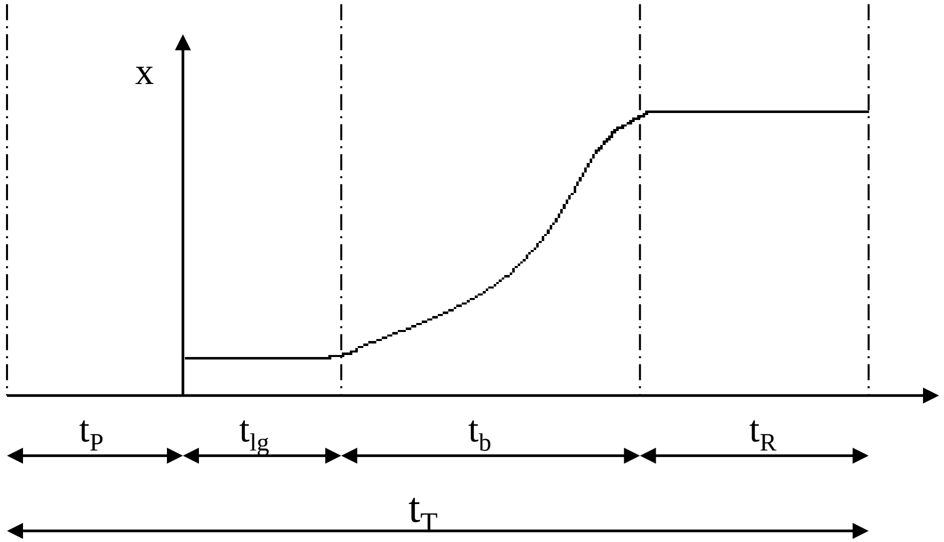
If death rate is negligible :

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

1.1.5 – Reactor Mass Balances

Total operating time (t_T) of a Batch reactor:

$$\underbrace{t_T = t_p + t_r}_{\text{preparation}} + t_{lag} + t_b \qquad (18)$$



t_p – reactor preparation time (cleaning, sterilization, addition of medium);

t_r – time to empty the reactor;

t_{lag} – "lag" phase time

1.1.6 – Relationship between Cell Growth and Substrate Consumption

<u>Monod Model</u>

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

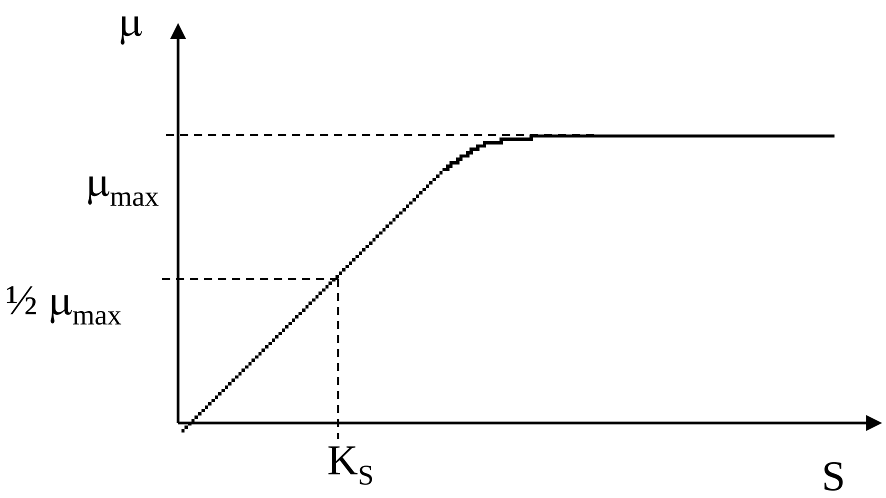
K_s - saturation constant or affinity constant (mgS/L)

S - limiting substrate concentration (mgS/L)

Assumes that only one nutrient limits growth
- limiting substrate

Graphic representation
of the Monod Model

$$\mu = f(s)$$



1.1.6 – Relationship between Cell Growth and Substrate Consumption

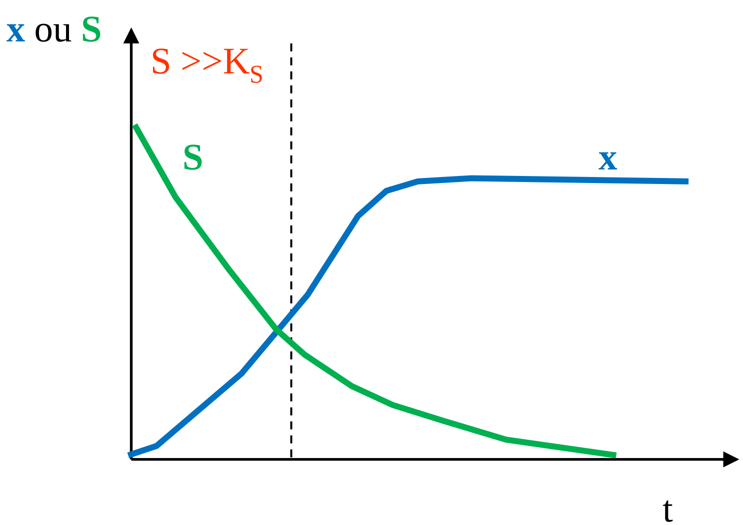
Typical values of μ_{max} and K_s for various organisms and substrates
(at the optimum growth temperatures)

Organism (temperature)	Limiting nutrient	μ_{max} (mg/L)	K_s (mg/L)
Escherichia coli (37 ºC)	Glucose Glycerol Lactose	0.8 – 1.4 0.87 0.80	2 – 4 2 20
Saccharomyces cerevisiae (30 ºC)	Glucose	0.5 – 0.6	25
Candida tropicalis (30 ºC)	Glucose	0.5	25 - 75
Candida sp.	Oxygen Hexadecane	0.5 0.5	0.045 – 0.45
Klebsiella aerogenes	Glycerol	0.85	9
Aerobacter aerogenes	Glucose	1.22	1 - 10

1.1.6 – Relationship between Cell Growth and Substrate Consumption

Representation of Model Monod X or S = f (t)

Two different zones: $S \gg K_s \Rightarrow K_s + S \approx S$



$$\mu = \frac{\mu_{max}\, S}{K_s + S} \quad \Rightarrow \mu \approx \frac{\mu_{max}\, S}{S}$$

$$\Leftrightarrow \mu \approx \mu_{max}$$

S >> $K_s \Rightarrow$ zero order rate $\Rightarrow$ μ does not depend on S

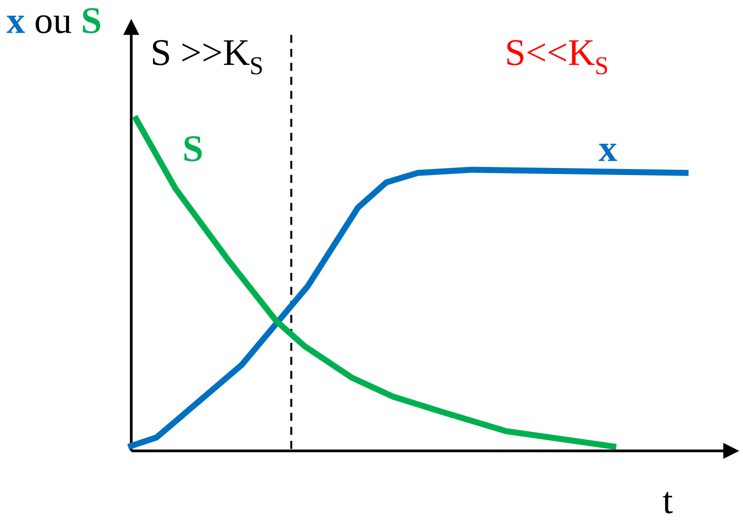
$$\boxed{\text{For } S >> K_s \quad \Rightarrow \mu = \mu_{max}} \qquad (21)$$

Note: *The K_s value depends on the type of microorganism, and for each microorganism it depends on the type of substrate and conditions operating time of the reactor.*

1.1.6 – Relationship between Cell Growth and Substrate Consumption

Representation of Model Monod X or S = f (t)

Two different zones: $S \ll K_s \Rightarrow K_s + S \approx K_s$



$$\mu = \frac{\mu_{max}\, S}{K_s + S} \quad \Rightarrow \mu \approx \frac{\mu_{max}\, S}{K_s}$$

$S \ll K_s \Rightarrow$ first order rate $\Rightarrow$ μ depends on S

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

Note: *The K_s value depends on the type of microorganism, and for each microorganism it depends on the type of substrate and conditions operating time of the reactor.*

1.1.6 – Relationship between Cell Growth and Substrate Consumption

Growth

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

(eq. de Monod)

Substrate consumption

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

(eq. de Michaelis-Menten)

v_{max} - maximum rate of substrate consumption (mg/l.h)

K_m – afinity constant (mgS/l)

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

1.1.6 – Relationship between Cell Growth and Substrate Consumption

Growth yield $Y_{x/s}$

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

$Y_{x/s}$= gX/gS

***Note**: Yield Coefficient may vary for the same medium and microorganism; may vary with μ*

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

$$r_s = \frac{1}{Y_{x/s}} \mu x \quad (25) \quad ou \quad r_s = \frac{1}{Y_{x/s}} \frac{\mu_{max}\, s}{K_s + s} x \quad (26)$$

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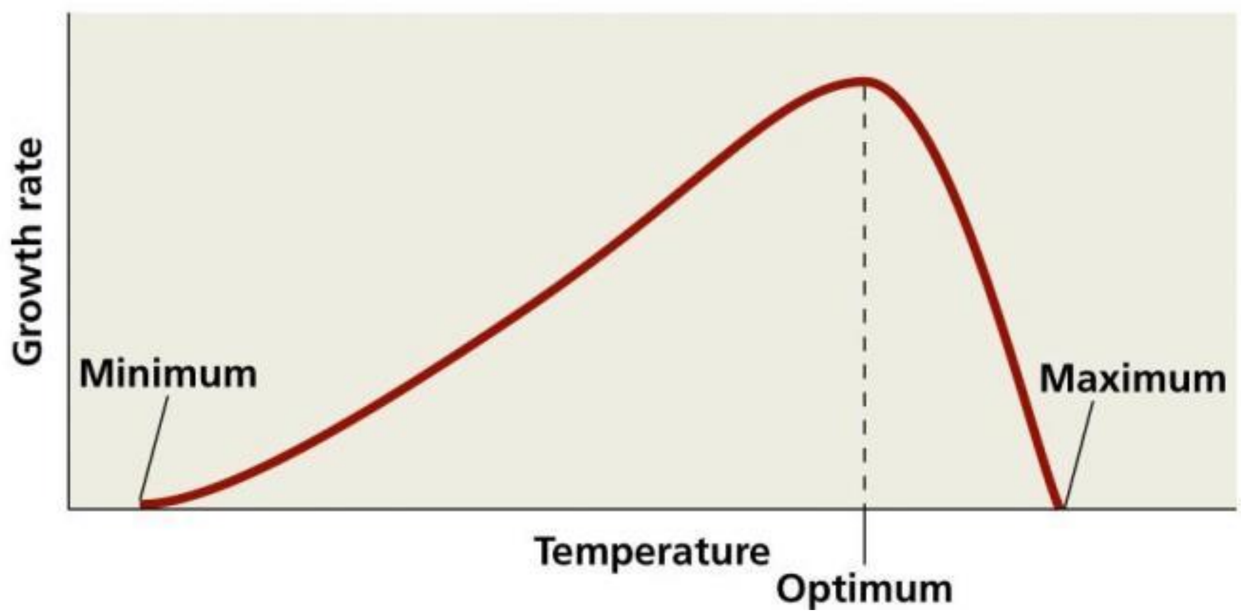
1.1.7 – Effect of Temperature and pH on growth

Effect of temperature



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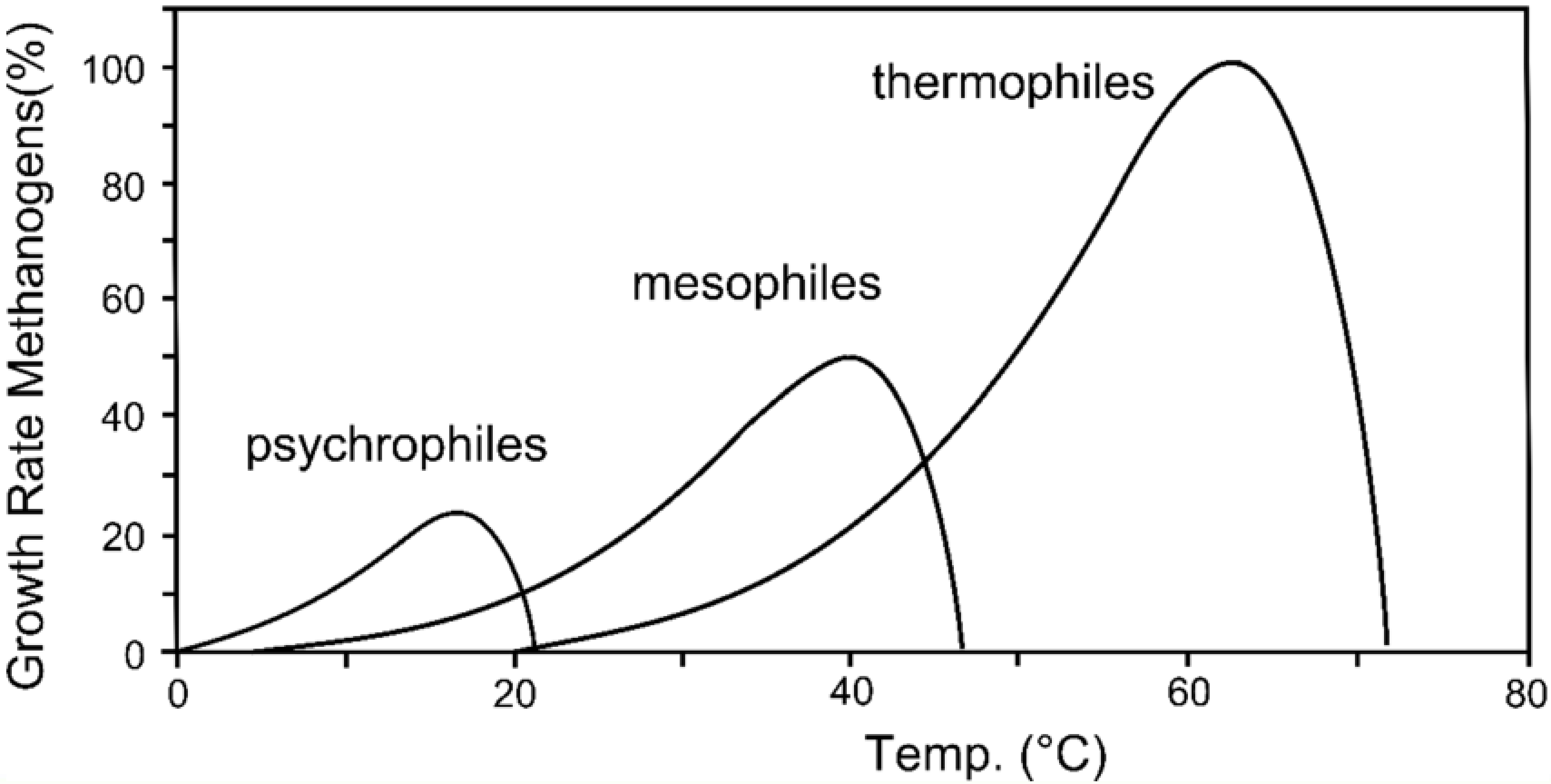


1.1.7 – Effect of Temperature and pH on growth

Effect of temperature

Classification of m.o. in function of temperature:

Group	Temperature (ºC)		
	Minimum	Optimum	Maximum
Termophiles	40-45	55-75	60-80
Mesophiles	10-15	30-45	35-47
Psycrophiles			
Obligate	-5 a 5	15-18	19-22
Facultatives	-5 a 5	25-30	30-35

1.1.7 – Effect of Temperature and pH on growth

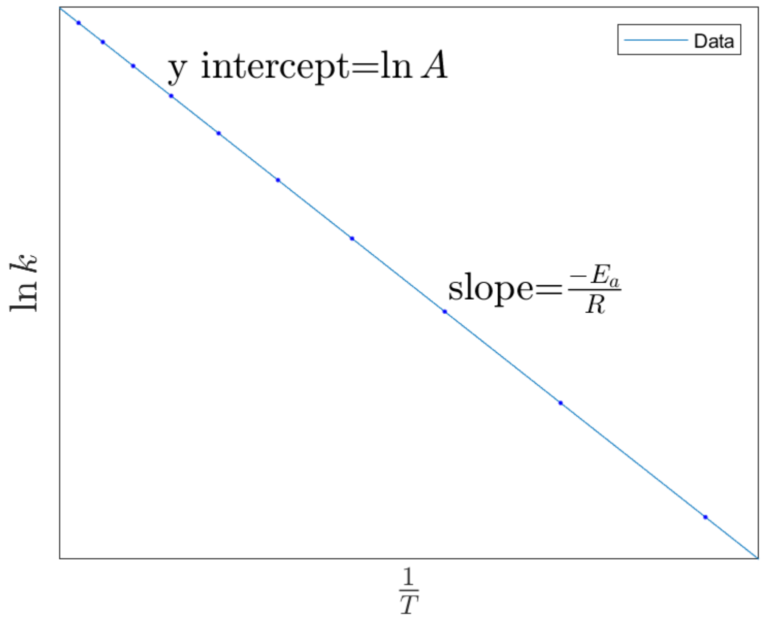
Effect of temperature

The effect of temperature on growth can be described by an equation that encompasses the Arrhenius equation and enzymatic deactivation

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

$$\ln \mu = \underbrace{\ln A}_{\text{intercept}} \underbrace{- \frac{E}{R}}_{\text{slope}} \frac{1}{T}$$

$\ln \mu$ *vs* $1/T$



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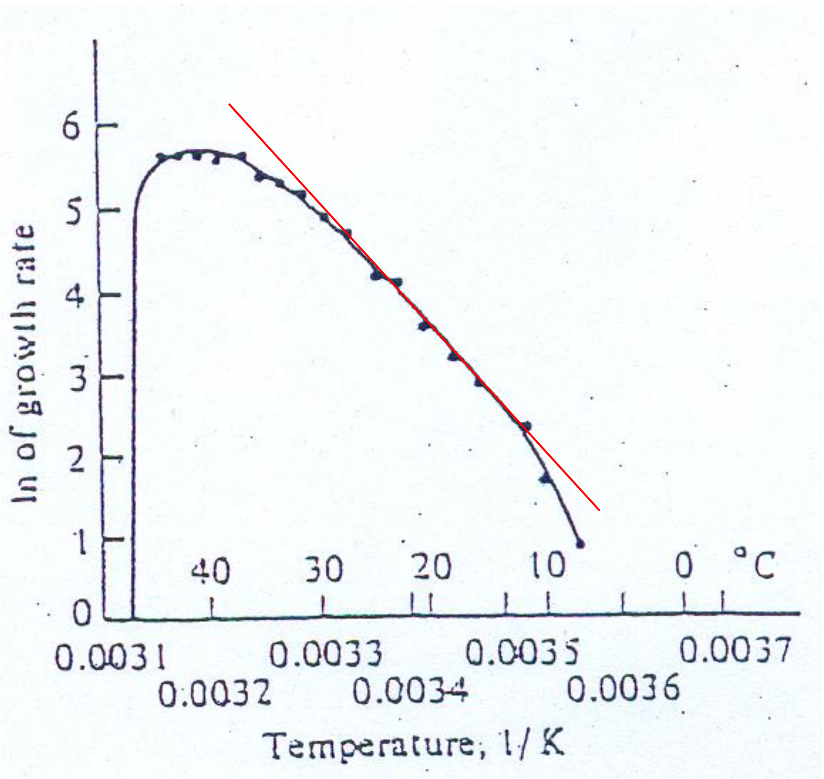


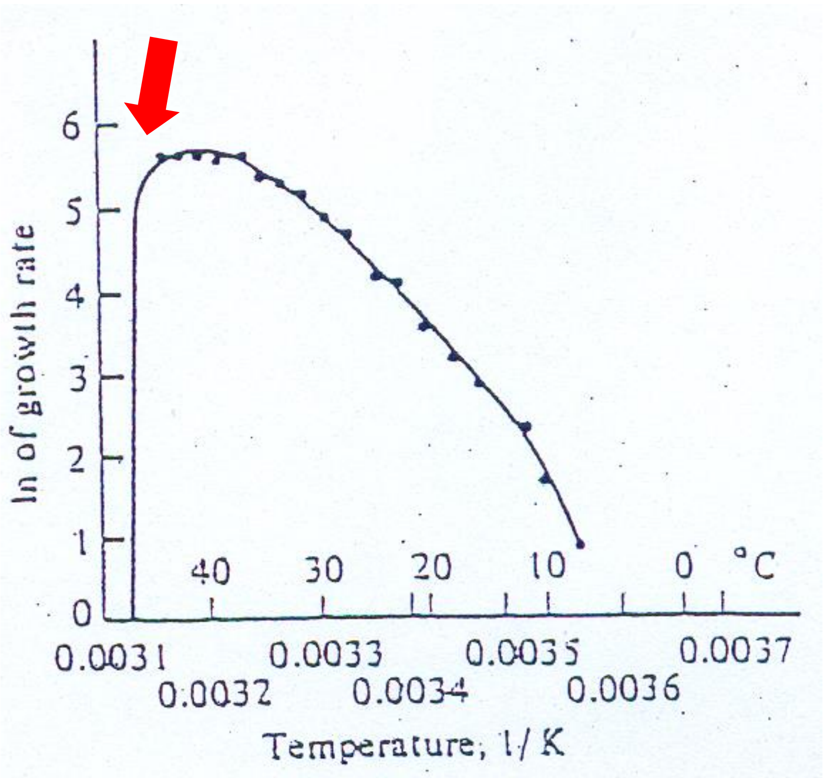
Two distinct zones:

- zone where 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 temperature

The effect of temperature on growth can be described by an equation that encompasses the Arrhenius equation and enzymatic deactivation



Two distinct zones:

- zone where μ increases linearly with the temperature at which the Arrhenius equation is valid:

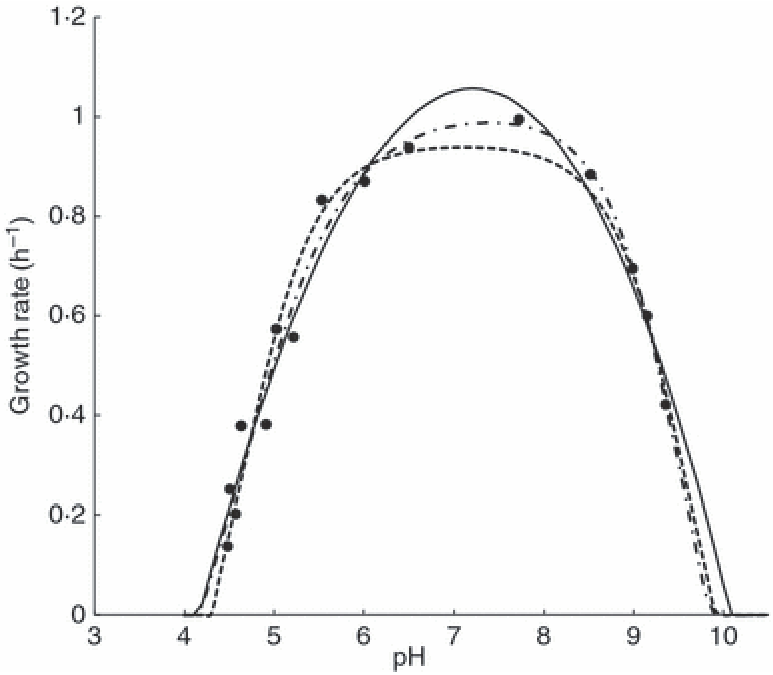
- zone in which μ decreases with the increase in temperature that corresponds to the enzymatic deactivation.

1.1.7 – Effect of Temperature and pH on growth

Effect of pH

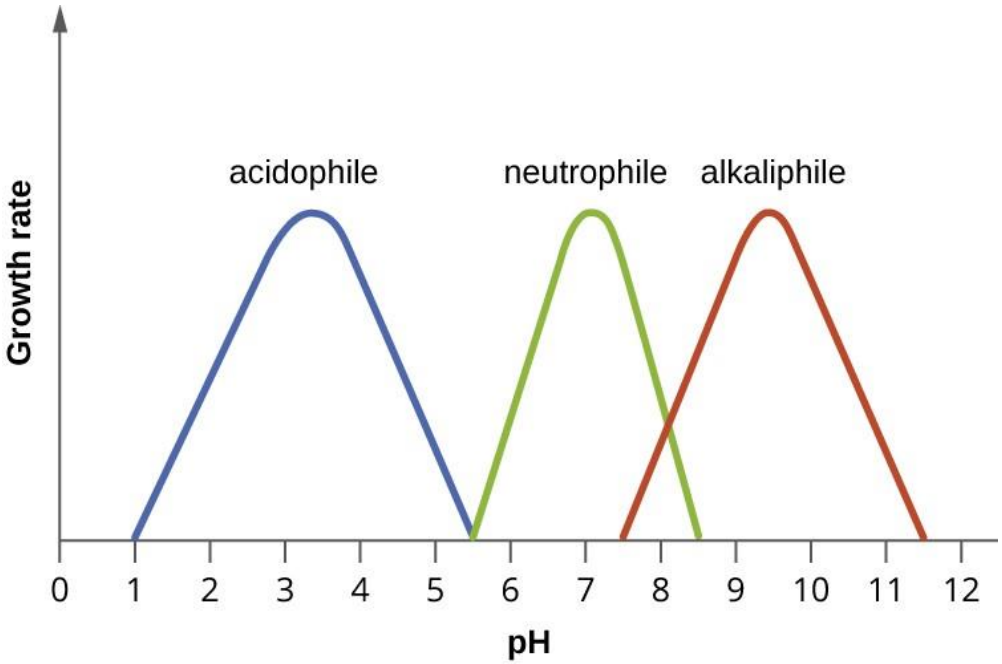
The pH influences:

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



1.1.7 – Effect of Temperature and pH on growth

Effect of pH



- 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)