Tema 5 – Cinética Microbiana
Microbial Kinetics

CI7115 – Biotecnología Ambiental

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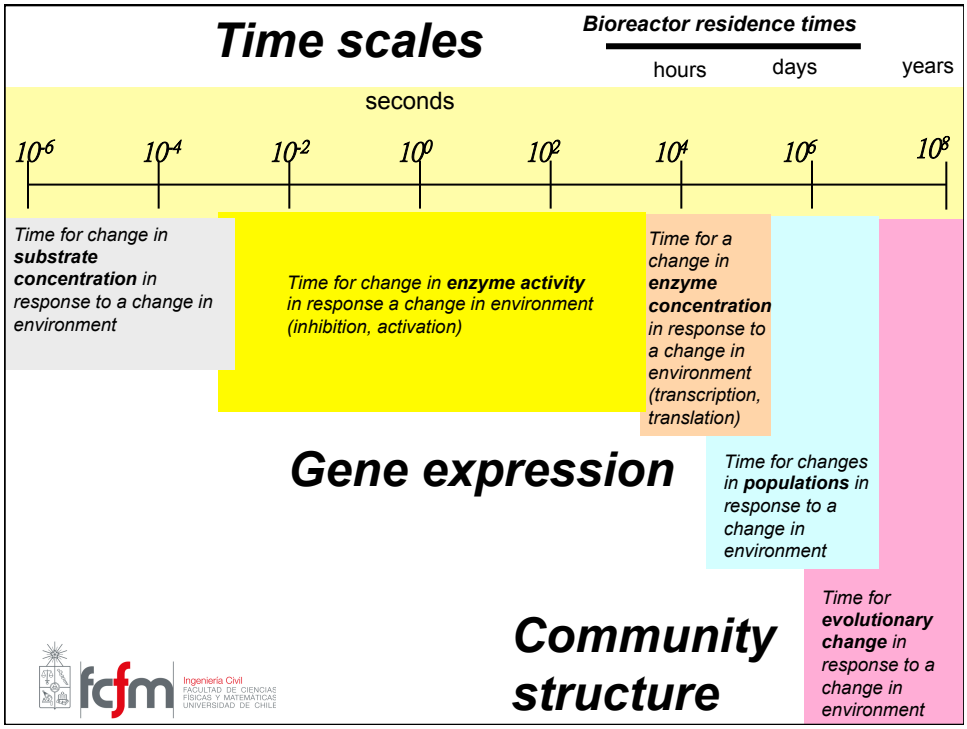

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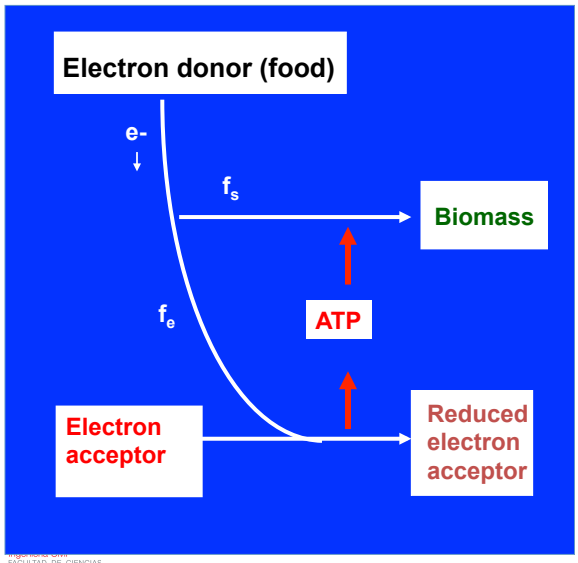
Metabolismo

What microorganisms do



Metabolismo

Includes many hazardous chemicals, such as lightly chlorinated molecules, aromatics

Electron donor (organics, H_2, NH_3)

Biomass

Electron acceptor		Reduced electron acceptor
O_2	Aerobes	H_2O
NO_3^-	Denitrifiers	N_2
SO_4^{2-}	Sulfate-reducers	H_2S
CO_2	Methanogens	CH_4
Highly chlorinated molecules	Dehalorespirers	Lightly chlorinated molecules

Reacciones cinéticas simples

Growth:

X = cells

r = dX/dt = k $X(t) = X_o + kt$ (zero order)

$r = dX/dt = kX$ $X(t) = X_o e^{kt}$ (first order)

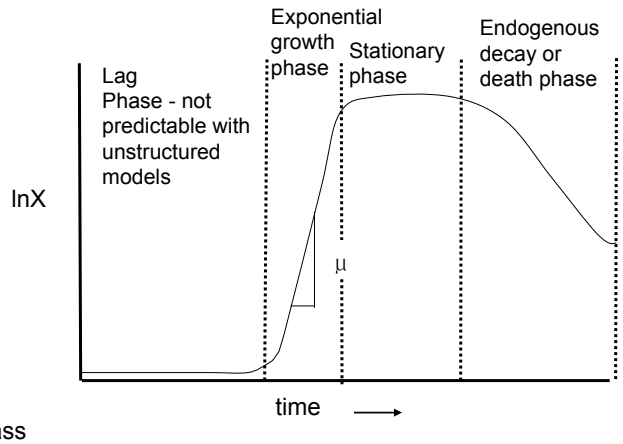
Degradation:

S = substrate

r = dS/dt = -k $S(t) = S_o - kt$ (zero order)

$r = dS/dt = -kS$ $S(t) = S_o e^{-kt}$ (first order)

Crecimiento microbiano



X = cell mass

μ = specific growth rate

How to model?

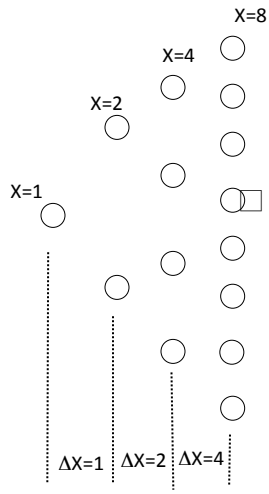
Crecimiento exponencial

$\frac{\Delta X}{\Delta t} \alpha X$ $\qquad \frac{dX}{dt} = \mu X$

$\mu = \frac{dX/dt}{X}$ Specific growth rate

If μ = constant,

X	$\Delta X/\Delta t$
1	$1/t_d$
2	$2/t_d$
4	$4/t_d$
8	$8/t_d$



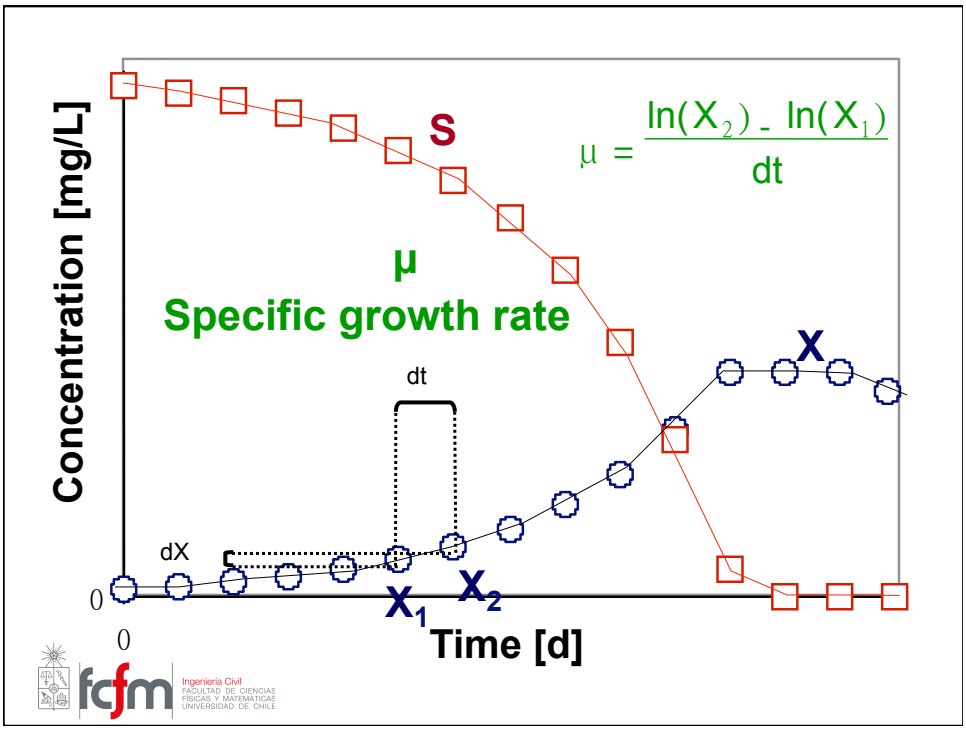
$$\int_{X_o}^{X} \frac{dX}{X} = \mu \int_{0}^{t} dt$$

$$\ln\left[\frac{X}{X_o}\right] = \mu t = \ln X - \ln X_o$$

$X = X_o e^{\mu t}$

Time to double, $t_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$

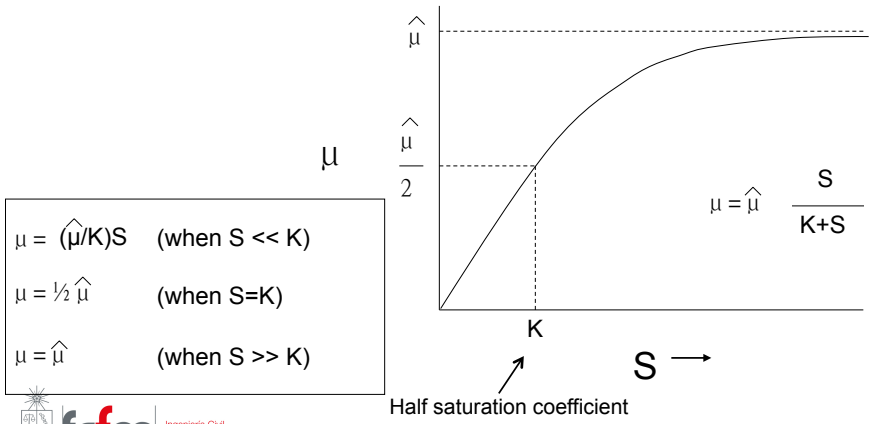
No. generations = t/t_d



Cinética del metabolismo

Monod kinetics

μ = specific growth rate [new biomass/biomass-time]



$\mu = (\hat{\mu}/K)S$ (when S << K)

$\mu = \frac{1}{2}\hat{\mu}$ (when S=K)

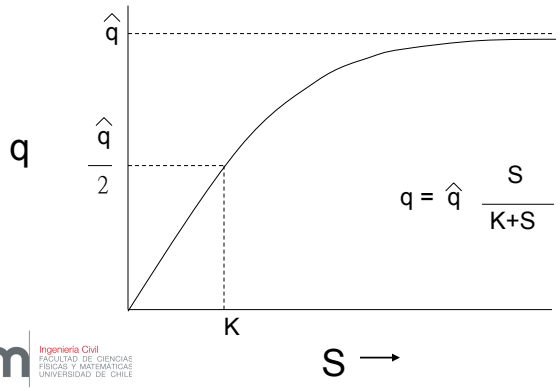
$\mu = \hat{\mu}$ (when S >> K)

Cinética del metabolismo

$\mu = Yq$

μ = specific growth rate [new biomass/biomass-time]

Y = yield = new biomass/substrate consumed
q = specific rate of substrate utilization = μ/Y
= [substrate consumed/biomass-time]



Yield (Y)

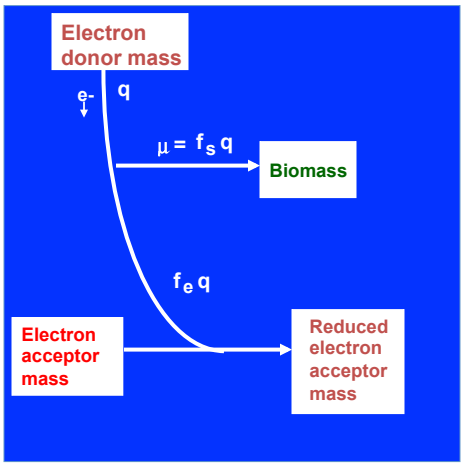
$$Y = \frac{\text{Biomass produced}}{\text{Substrate consumed}}$$

e.g., (g VSS/ g COD)

- Y can be measured and is directly related to f_s.
- Y can be changed it into f_s by converting both the numerator and the denominator into electron equivalents (or into O_2 equivalents, whichever is easier).

Y (g VSS/g COD) = f_s x (5.65 g VSS/eq)/(8 g COD/eq)
= f_s /1.42 (gCOD/gVSS)

$Y = f_s (5.65/8) = f_s /1.42$



When substrate and biomass are expressed as either electron equivalents or oxygen equivalents, **$\mu = f_s q$** and the specific rate of use of the electron acceptor is **$f_e q$**.

McCarty found that $f_e \hat{q}$ is **~1 mole e^-/g vss-d** (**8 g COD/g VSS-d**) at 25°C for a wide range of microorganisms.

McCarty found that the maximum specific rate of electron transfer <u>for energy</u> was one mole of electrons per gram VSS per day at 25°C.

<u>Given</u>: Aerobic heterotrophs have a yield of 0.42 g vss/g COD

What is $\hat{q}$, the maximum specific rate of substrate consumption ?

f_s = 0.42 g vss/g COD x 8 g COD/eq x eq/5.65 g vss = 0.60

f_e = 1- f_s = 1- 0.60 = 0.40

$f_e\hat{q}$ = 8 g COD/g VSS-d

$\hat{q} = f_e\hat{q}/f_e$ = 8/0.4 = **20 g COD/g vss-d**

0.693/8.4x24=1.98hr

Doubles approx every 2 hr

What is $\hat{\mu}$?

$\hat{\mu} = Y\hat{q}$ = 0.42 x 20 = **8.4/d**

McCarty found that the maximum specific rate of electron transfer for energy was one mole of electrons per gram VSS per day at 25°C.

<u>Given</u>: Methanogens have a maximum specific rate of substrate consumption q of 8.4 g COD/g vss-d.

What is $\hat{Y}$?

$f_e = f_e\hat{q}/\hat{q}$ = 8 COD/g vss-d ÷ <u>8.4 g COD/g vss-d</u> = 0.95

f_s = 1- f_e = 0.05 Y = 0.05 x 5.65/8 = **0.03 g vss/gCOD**

What is $\hat{\mu}$?

$\hat{\mu} = Y\hat{q}$ = (0.03)(8.4) = **0.28/d**

McCarty found that the maximum specific rate of electron transfer for energy was one mole of electrons per gram vss per day at 25°C.

<u>Given</u>: Sulfate-reducing bacteria have a maximum specific growth rate $\hat{\mu}$ of 0.5/d.

What is $\hat{q}$?

(Convert $\hat{\mu}$ from VSS to COD units)

$\hat{\mu}$ = 0.5 g vss/g vss-d x 1.42 g COD/g vss = **0.71 g COD /g vss-d**

$\hat{q}$ = 8 g COD/g vss-d (Energy) + 0.71 g COD/g vss-d (growth) = **8.71 g COD/g vss-d**

What is Y?

$f_s = \hat{\mu} / \hat{q}$ = 0.71/8.71 = 0.08

Y = 0.08 x 5.65/8 = **0.06 g vss/g COD**

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Expresiones cinéticas

$$\mu = \hat{\mu} \frac{S}{K+S}$$

$$q = \hat{q} \frac{S}{K+S}$$

How can we apply these expressions?

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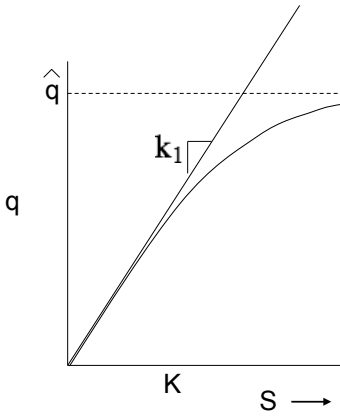
Simplificaciones

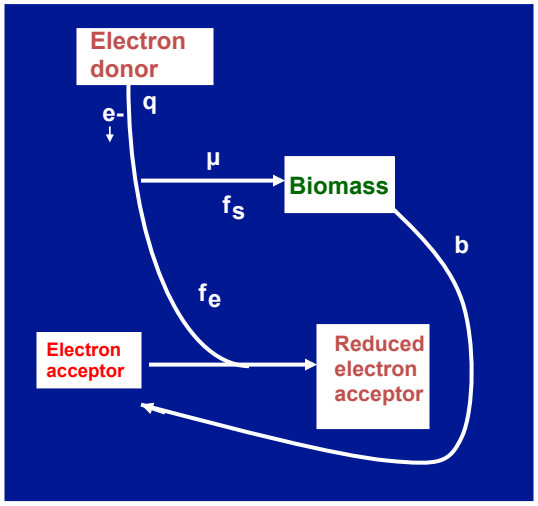


$$q = \hat{q}\frac{S}{K+S}$$

If S>>K then $q = \hat{q}$

If S<<K then $q = \frac{\hat{q}}{K}S = k_1 S$

Modelo de Herbert



Y_{eff}<<Y because of decay

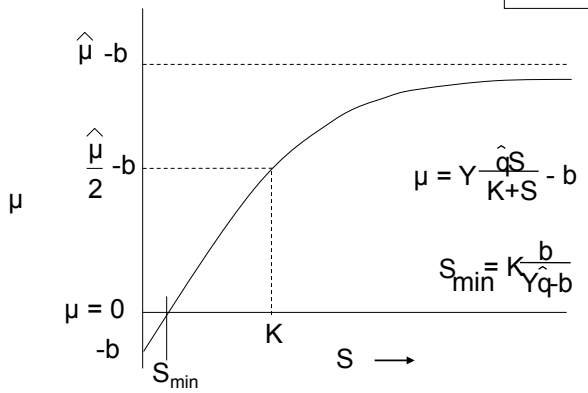
f_s<< f_s° because of decay

b = endogenous decay (T^{-1})

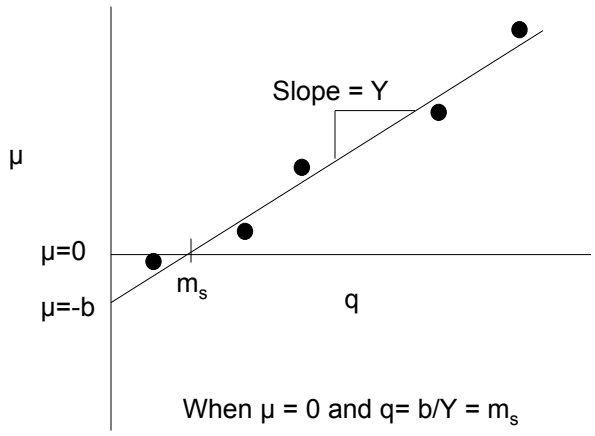
Qué pasa con el decaimiento?

Herbert: $\mu = Yq - b$

$q = \hat{q}\frac{S}{K+S}$



$\mu = Yq - b$



When $\mu = 0$ and $q = b/Y = m_s$

m_s is the maintenance coefficient [substrate mass/biomass-time]

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TYPICAL VALUES
for Y, $\hat{q}$, μ_{max}, and b

Process	Y (g cell per g of limiting substrate)	$\hat{q}$ (g substrate per g cell per day)	b (d^{-1})	μ_{max} (d^{-1})	t_d (d)
Aerobic:					
organic removal	0.45 g vss/g COD	22 g COD/g vss S-d	0.2	9.7	0.1
nitrification	0.2 g vss /g NH_3-N	2 g NH_3-N/g vss -d	0.05	0.35	2.0
S^{2-} oxidation	0.59 g vss /g S	2.5 g S/g vss -d	0.1	1.4	0.5
Fe^{2+} oxidation	0.0075 g vss /g Fe^{2+}	60 g Fe^{2+}/g vss -d	0.1	0.3	2.0
H_2 oxidation	1.38 g vss /g H_2	1.3 g H_2/g vss -d	0.1	1.7	0.4
Anaerobic:					
denitrification	0.35 g vss /g COD 0.8 g vss /g NO3-N	14 g COD/g vss -d 4 g NO3-N/g vss -d	0.1 0.1	4.8 3.1	0.14 0.22
sulfate reduction	0.1 g vss /g COD	9.3 g COD/g vss -d	0.05	0.9	0.8
methane fermentation:					
fats	0.031 g vss /g COD	8.4 g COD/g vss -d	0.02	0.2	2.9
proteins	0.081 g vss /g COD	8.4 g COD/g vss -d	0.02	0.7	1.1
carbohydrates	0.23 g vss /g COD	8.4 g COD/g vss-d	0.05	1.9	0.4
sewage sludge	0.081 g vss/gCOD	8.4 g COD/g vss -d	0.05	0.6	1.1

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Valores típicos de K

Process	K (mg substrate/L)
Aerobic:	
organic mixtures	50-150 mg COD/L
single organics	1-10 mg COD/L
nitrification	0.4 - 2 mg NH_3-N/L
Anaerobic:	
denitrification	0.06-0.20 mg NO_3-N/L
methane fermentation:	
acetate, propionate	600-900 mg COD/L
sewage sludge	2,000-3,000 mg COD/L

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Sustancias tóxicas

Naturally Occuring Biodegradable Substances
- Benzene
- Toluene
- Polycyclic aromatic hydrocarbons

Microorganisms can fortuitously transform new chemicals
- Structure is similar to enzyme's natural substrate
- Enzymes are not very specific

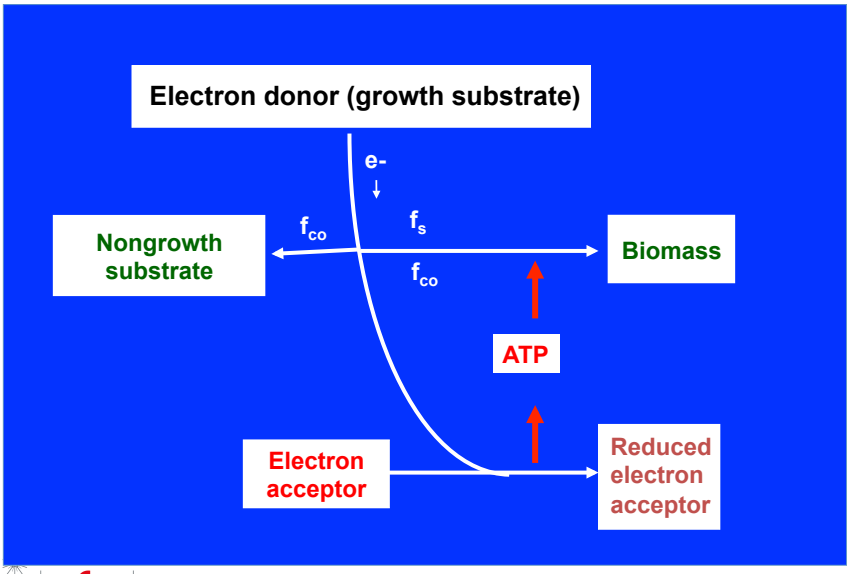
Metabolism - the organism is able to obtain energy from the reaction or make use the products of the reaction

Cometabolism - the organism cannot derive energy from the reaction or make use of the products

When a bioreactor is designed for cometabolism, growth substrate must be added

Opportunities for cometabolism because many waste sites have **mixed wastes**

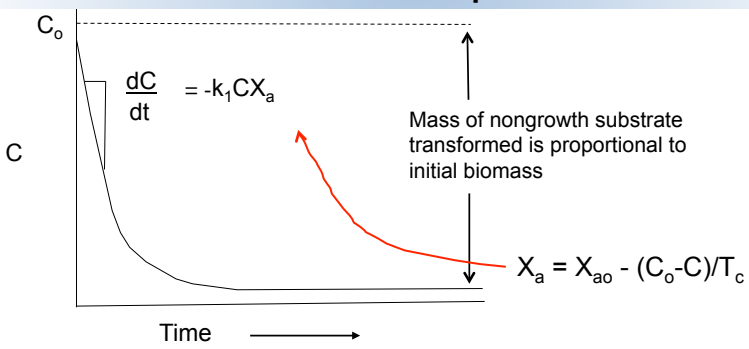
Cometabolismo



Ejemplos de cometabolismo

Growth substrates	Nongrowth substrates
Methane Toluene Phenol Propane Ammonia Cresol	Many halogenated aliphatics, alkanes, and aromatics
Napththalene	PAHs
Aliphatic hydrocarbons	Alkyl-substituted cyclic hydrocarbons
Chlorobenzoate	Monofluorobenzoates
Biphenyl, benzoate	PCBs

Cinéticas típicas de cometabolismo en ausencia de sustrato para crecimiento



T_c = transformation capacity = $\frac{dC}{dX_a}$

Integrated result:

$$C = C_o \frac{F e^{-k_1 F t}}{X_{ao} - \frac{C_o}{T_c} e^{-k_1 F t}} \quad \text{where} \quad F = X_{ao} - \frac{C_o}{T_c}$$

Elección de sustrato para crecimiento

Many growth substrates induce enzyme activity that can destroy TCE aerobically:
- ammonia (ammonia monooxygenase) ammonia oxidizers
- phenol (phenol hydroxylase)
- methane (methane monooxygenase) methanotrophs
- toluene (toluene dioxygenase)

Let's compare TCE cometabolism kinetics for the phenol degraders and the methanotrophs:

TCE kinetics for phenol-degraders:
T_c = 0.35 mg TCE/mg vs
k_1 = 0.18 L/mg vs-d

TCE kinetics for methane (methanotrophs):
T_c = 0.047 mg TCE/mg vs
k_1 = 1.4 L/mg vs-d

Which is more advantageous for sustained activity?

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Vías para biodegradación

On the web at: http://umbbd.ahc.umn.edu/

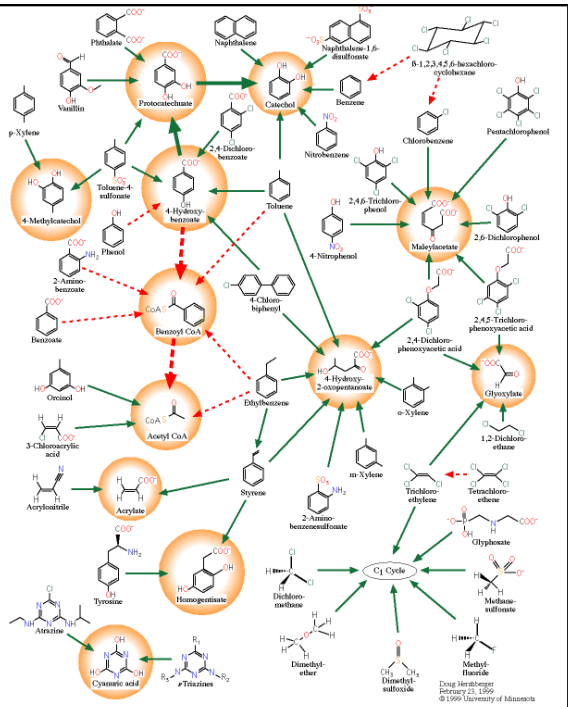
Degraded from the indicated **starting** compound to an intermediate compound, **aerobically**

Degraded from the indicated **intermediate** compound to another intermediate compound, **aerobically**

Degraded from the indicated **starting** compound to an intermediate compound, **anaerobically**

Degraded from the indicated **intermediate** compound to another intermediate compound, **anaerobically**

The circled compounds are common intermediates for trunk pathways. Trunk pathways feed into common pathways of metabolism, such as the tricarboxylic acid cycle.



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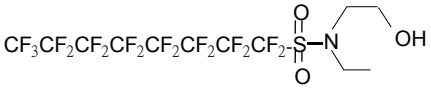
Contaminantes orgánicos persistentes

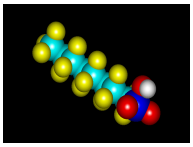
Example: the perfluorochemicals - common surfactants and coatings



Used for fabric coating

<u>Transformations</u>
atmospheric and enzymatic

Perfluorooctane sulfonate (PFOS)



No known enzyme can destroy it!

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Toxic chemicals disrupt cell physiology

This results in inhibition kinetics

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Whole cell inhibition kinetics

Competitive inhibition - apparent K increases. Observed with oxygenases used to degrade gasoline components, chlorinated solvents, ammonium.

$$q = \frac{\hat{q}\,S}{K\left(1+\frac{I}{K_i}\right)+S} \qquad \text{so that } K_{apparent} = K\left(1+\frac{I}{K_i}\right)$$

I is concentration of inhibitory compound

$K_{apparent}$ can be obtained from a Lineweaver-Burke plot or other non-linear curve fitting procedure.

Whole cell inhibition kinetics

Noncompetitive inhibition - $\hat{q}_{apparent}$ decreases. Often reported for metals.

$$q = \frac{\hat{q}\,S}{\left(1+\frac{I}{K_i}\right)(K+S)} \qquad \text{so that } \hat{q}_{apparent} = \frac{\hat{q}}{1+\frac{I}{K_i}}$$

$\hat{q}_{apparent}$ can be obtained from a Lineweaver-Burke plot or other non-linear curve fitting procedure.

Whole cell inhibition kinetics

Self inhibition (Andrews equation)

$$q = \frac{\hat{q} S}{K + S + \frac{S^2}{K_i}}$$

High levels of acetic acid show substrate inhibition in methanogenesis.

General expression for inhibition (Han and Levenspiel, 1988)

$$q = \frac{\hat{q} S}{K\left(1 - \frac{I}{I^*}\right)^m + S}\left(1 - \frac{I}{I^*}\right)^n$$

where I* is the concentration that stops growth

Tipos de inhibición

$$q = \frac{\hat{q} S}{K\left(1 - \frac{I}{I^*}\right)^m + S}\left(1 - \frac{I}{I^*}\right)^n$$

General expression of inhibition

where I* is the concentration that stops growth

Inhibition type	Effect on $\hat{q}$	Value of n	Effect on K	Value of m
competitive	none	0	increase	<0
noncompetitive	decrease	>0	none	0
uncompetitive	decrease	>0	decrease	>0
mixed	decrease	>0	increase	<0

Source: Grady et al., 2002. Biological Wastewater Treatment)

Volskay et al.(1990) found that chlorinated organic compounds were mostly mixed inhibitors of aerobic heterotrophs. The lowest value for I* was 126 mg/L for tetrachloroethylene.

For a batch system, a mass balance on substrate gives

In: 0
Out: 0
Source: 0
Sink: $qX_aV_L\Delta t$

$\Delta M = -qX_aV_L\Delta t$ (constant liquid volume)

For a nonvolatile substrate: $\Delta M/V_L = \Delta S$

$$\frac{-dS}{dt} = qX_a \qquad \frac{-dS}{dt} = \frac{\hat{q}SX_a}{K+S}$$

A mass balance on biomass gives

$$\frac{dX_a}{dt} = Y\left(\frac{-dS}{dt}\right) \qquad X_a = X_{ao} + Y(S_o - S)$$

$$t = -\frac{1}{\hat{q}}\left(\left[\frac{-K}{X_{ao}+YS_o}-\frac{1}{Y}\right]\ln\left[X_{ao}+YS_o-YS\right]+\left[\frac{K}{X_{ao}+YS_o}\right]\ln\left[\frac{SX_{ao}}{S_o}\right]+\frac{1}{Y}\left[\ln X_{ao}\right]\right)$$

- Solids retention time (SRT) = mean cell residence time (MCRT) = sludge age
- q_x = active biomass in the system/ production rate of new biomass = m^{-1}