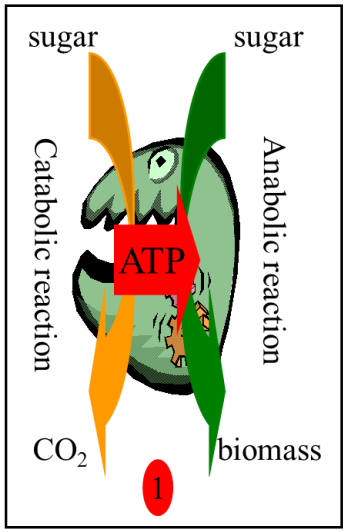
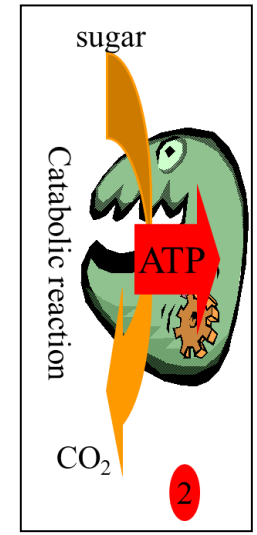
Herbert-Pirt (Extended)
Including product formation

Growth



Maintenance



Herbert-Pirt formulation
without non-catabolic product

$$q_S = \frac{1}{Y_{SX}^{\max}}\mu + m_S \qquad q_S = q_S^{\max}\frac{C_S}{K_S + C_S}$$

4 parameters: $\mathbf{Y_{SX}^{max}}$, $\mathbf{m_S}$, $\mathbf{q_S^{max}}$, $\mathbf{K_S}$

- q_P rate is a <u>linear</u> function of μ only!!!
- All q_i rates are governed by μ rate

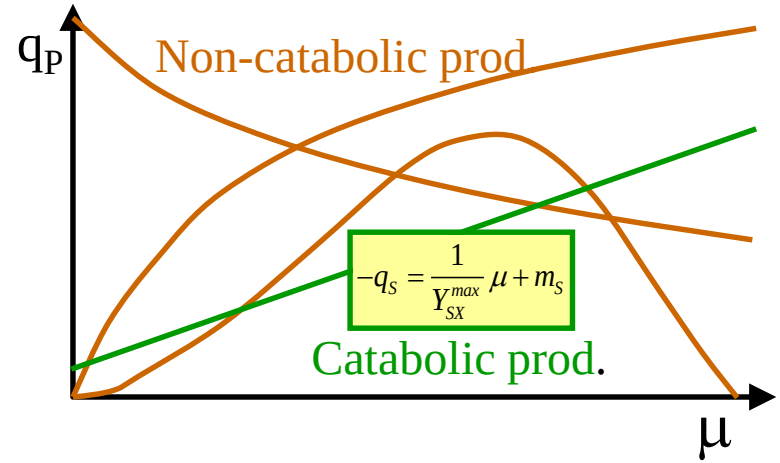
For growth **with non-catabolic product** ?

- Extension of Herbert-Pirt Eq. is needed
- An additional kinetic function for q_P is needed
- A modified $\mu = f(C_S)$ relation
- Other Herbert-Pirt relations

Growth with non-catabolic product ?

Various $q_P = f(\mu)$ functions

- Many different possibilities and case specific
- For catabolic products $q_P = f(\mu)$ is linear
- $q_P = f(\mu)$ function can easily be estimated in chemostat or fed batch, like $q_S = f(\mu)$



Kinetic functions for q_P

Assuming: single substrate limited conditions

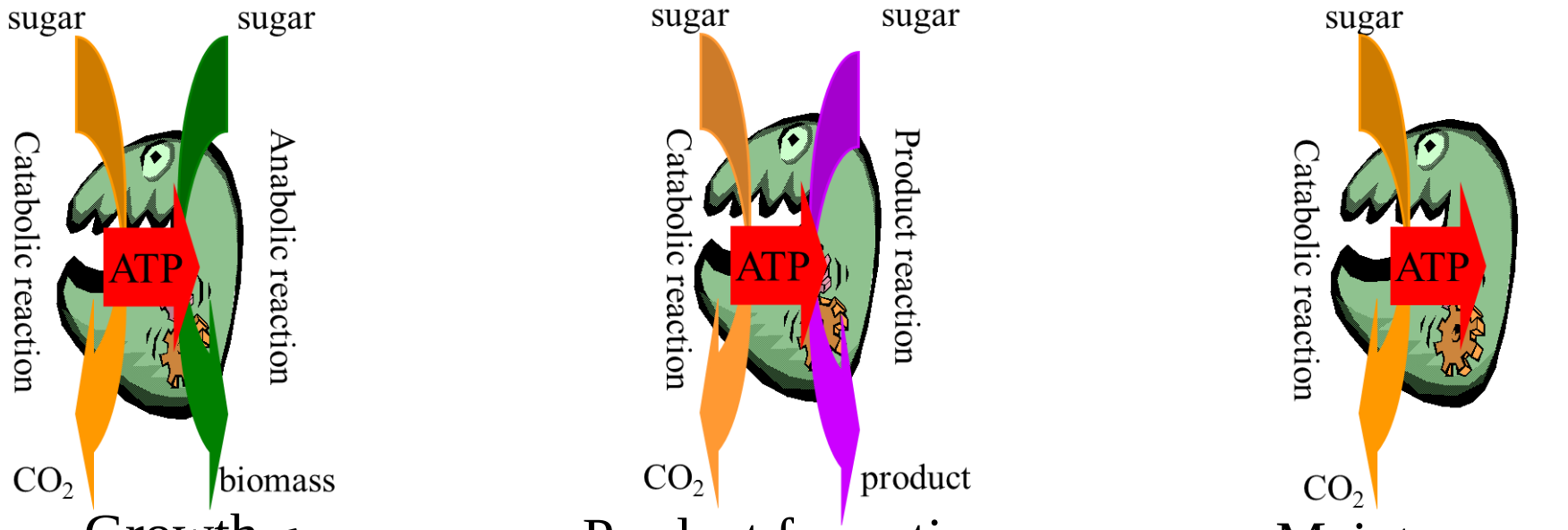
Then $q_P = f(C_S)$. But C_S is difficult to measure

However for a given, μ, C_S is also fixed as $C_S = f(\mu)$

by eliminating C_S → $q_P = f(\mu)$

This is the **$q_P = f(\mu)$** concept → Extended Herbert-Pirt Eq.

Extended Herbert-Pirt Eq.



$$-q_S = \frac{1}{Y_{SX}^{\max}}.\mu + \frac{1}{Y_{SP}^{\max}}.q_P + m_S$$

Substrate consumption:

1. Herbert-Pirt (not-extended) rate for **growth** and **catabolic** products
2. Extended Herbert-Pirt rate for **non-catabolic** products

All other q_i rates

All other rates follow from stoichiometric growth-catabolic coupling & substrate Herbert-Pirt equation. From known:

1. $q_S = f(C_S)$ hyperbolic
2. $q_P = f(\mu)$
3. Growth stoichiometry
4. Extented Herbert-Pirt Eq. for substrate

$$-q_S = \frac{1}{Y_{SX}^{max}}.\mu + \frac{1}{Y_{SP}^{max}}.q_P + m_S$$

q_{CO2} =…
q_P =…
q_{O2} =…
q_{heat} =…
q_{H2O} =…
etc…

Ex: $-q_S = 2\ \mu + 1.5\ q_P + 0.01$ [kgS.kgX^{-1}.h^{-1}]; $q_P = 0.049$ [kgP.kgX^{-1}.h^{-1}] (independent of μ)

$\mu = 0.02$ [h^{-1}]

Thus $Y_{SX}^{max} = 0.5$ [kgX.kgS^{-1}]; $Y_{SP}^{max} = 0.66$ [kgP.kgS^{-1}]; $m_S = 0.01$ [kgS.kgX^{-1}.h^{-1}] …

→ $Y_{SX} = 0.16$ [kgX.kgS^{-1}]; $Y_{SP} = 0.39$ [kgP.kgS^{-1}] (← Why & How???)

And substrate is consumed for:

- 32% → biomass production: (2*0.02) / [(2*0.02)+(1.5*0.049) + 0.01]
- 60%→ product formation: (1.5*0.049) / [0.1235]
- 8% →maintenance: 0.01 / [(2*0.02)+(1.5*0.049) + 0.01]

Extended Herbert-Pirt (non-catabolic product) q_i rates

Lysine ($C_6H_{15}N_2O_2^+$) fermentation from glucose ($C_6H_{12}O_6$) and ammonia (NH_4^+).

Available data: **$1/Y_{SX}^{max} = 0.333$** [kgC_moleX.moleS^{-1}]; **$1/Y_{SP}^{max} = 1.5$** [moleP.moleS^{-1}];

$m_S = 0.025$ [moleS.C_moleX^{-1}.h^{-1}]

Catabolic reaction: $-1\ C_6H_{12}O_6 - 6\ O_2 + 6\ CO_2 + 6\ H_2O$

Global growth reaction (which needs energy):

$-0.333\ C_6H_{12}O_6 - 0.2\ NH_4^+ - 0.95\ O_2 + 1\ C_1H_{1.8}O_{0.5}N_{0.2} + 0.2\ H^+ + 1\ CO_2 + 1.4\ H_2O$

Catabolism for growth: $0.95/6$*$(-1\ C_6H_{12}O_6 - 6\ O_2 + 6\ CO_2 + 6\ H_2O)$

→ $-0.158\ C_6H_{12}O_6 - 0.95\ O_2 + 0.95\ CO_2 + 0.95\ H_2O$ Substrate for anabolism $0.333 - 0.158 = 0.175$

Anabolism: $-0.175\ C_6H_{12}O_6 - 0.2\ NH_4^+ + 1\ C_1H_{1.8}O_{0.5}N_{0.2} + 0.2 + H^+ + 0.05\ CO_2 + 0.45\ H_2O$

Product reaction (global which needs energy):

$-1.5\ C_6H_{12}O_6 - 2\ NH_4^+ - 2\ O_2 + 1\ C_6H_{15}N_2O_2^+ + 3\ CO_2 + 2\ H^+ + 5\ H_2O$

Catabolism for product production: $2/6(-1\ C_6H_{12}O_6 - 6\ O_2 + 6\ CO_2 + 6\ H_2O)$

→ $-0.33\ C_6H_{12}O_6 - 2\ O_2 + 2\ CO_2 + 2\ H_2O$ Substrate for Product synthesis $1.5 - 0.33 = 1.17$

Product synthesis: $-1.17\ C_6H_{12}O_6 - 2\ NH_4^+ + 1\ C_6H_{15}N_2O_2^+ + 1.02 CO_2 + 1.08\ H^+ + 2.98\ H_2O$

Extended Herbert-Pirt (non-catabolic product) q_i rates

Lysine ($C_6H_{15}N_2O_2^+$) fermentation from glucose ($C_6H_{12}O_6$) and ammonia (NH_4^+).

Available data: $1/Y_{SX}^{max} = 0.333$ [moleS.kgC_moleX^{-1}]; $1/Y_{SP}^{max} = 1.5$ [moleS.moleP^{-1}];

$m_S = 0.025$ [moleS.C_moleX^{-1}.h^{-1}]

Substrate rate Herbert-Pirt (Extended) Eq.: $\mathbf{-q_S = 0.333\ \mu + 1.5\ q_P + 0.025}$

Catabolic reaction $-1\ C_6H_{12}O_6 - 6\ O_2 + 6\ CO_2 + 6\ H_2O$

Global growth reaction (which needs energy) at μ rate

$-0.333\ C_6H_{12}O_6 - 0.2\ NH_4^+ - 0.95\ O_2 + 1\ C_1H_{1.8}O_{0.5}N_{0.2} + 0.2\ H^+ + 1\ CO_2 + 1.4\ H_2O$

Product reaction (global which needs energy) at q_P rate

$-1.5\ C_6H_{12}O_6 - 2\ NH_4^+ - 2\ O_2 + 1\ C_6H_{15}N_2O_2^+ + 3\ CO_2 + 2\ H^+ + 5\ H_2O$

Thus all $\mathbf{q_i}$ rates are given by Herbert-Pirt linear expressions: $\mathbf{q_i = f(\mu, q_P)}$

$-q_S = 0.333\ \mu + 1.5\ q_P + 0.025$ with q_P = function (μ)

$-q_{O2} = 0.95\ \mu + 2\ q_P + 6\ 0.025$

$+q_{CO2} = 1\ \mu + 3\ q_P + 6\ 0.025$

$-q_N = 0.2\ \mu + 2\ q_P$

$+q_{H+} = 0.2\ \mu + 2\ q_P$

$+q_{H2O} = 1.4\ \mu + 5\ q_P + 6\ 0.025$

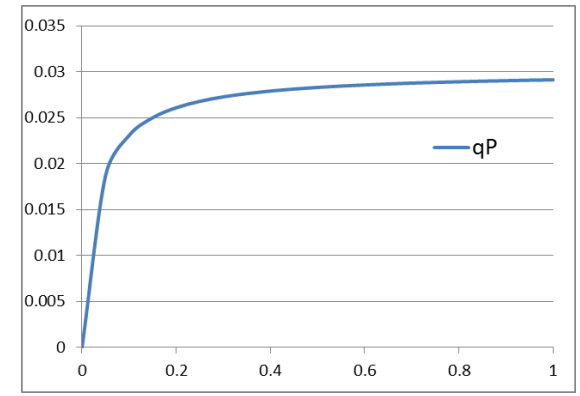
→Hence all q_i and Y_{ij} are complex functions of μ, q_P [or only μ, if ($q_P = f(\mu)$]

Extended Herbert-Pirt (non-catabolic product) Y_{ij} yields

From q_i rates are given by **Herbert-Pirt** linear expressions: $q_i = f(\mu, q_P)$

– $q_S = 0.333\ \mu + 1.5\ q_P + 0.025$

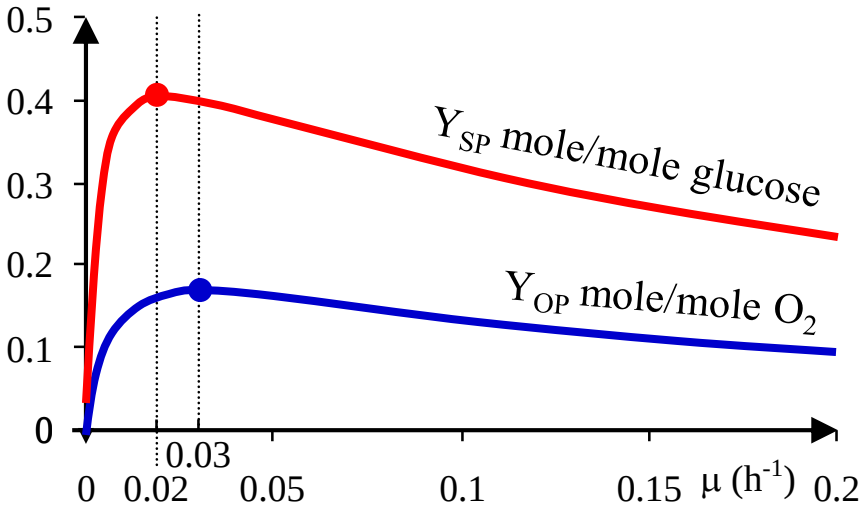
– $q_{O2} = 0.95\ \mu + 2\ q_P + 6\ 0.025$



If from measurements $q_P = f(\mu)$ is given:

$q_P = (0.03.\ \mu) / (0.03+ \mu)$ [moleP.C_moleX^{-1}.h^{-1}]

Thus: $Y_{SP} = \left|\frac{q_p}{q_s}\right| = \frac{0.03\mu/(0.03+\mu)}{0.333\mu+1.5*0.03\mu/(0.03+\mu)+0.025}$ $Y_{OP} = \left|\frac{q_P}{q_{O2}}\right| = \frac{0.03\mu/(0.03+\mu)}{0.95\mu+2*0.03\mu/(0.03+\mu)+0.15}$



Which optimal μ to choose, 0.02 or 0.03 h^{-1}?

1. For best productivity on substrate criterion, μ should be 0.02 h^{-1}
2. But often, oxygen mas transfer are limited in bioreactor, in this case, the best lysine production of Lysine per consumed O_2 should be μ = 0.03 h^{-1}

Black box kinetics
"Beyond single substrate limited growth"

Up to now, one assumption: Only one limited substrate
(all others non limiting)

What about:

- T, pH
  - What is the kinetic effect of temperature and pH?
- Multiple substrates
  - What about kinetic effects of other compounds than substrate?
- Death/inhibition/activation
  - Micro-organisms death?
  - What about the "corpses"?
- Mixed substrates
  - What if the micro-organism "sees" more than one substrate?

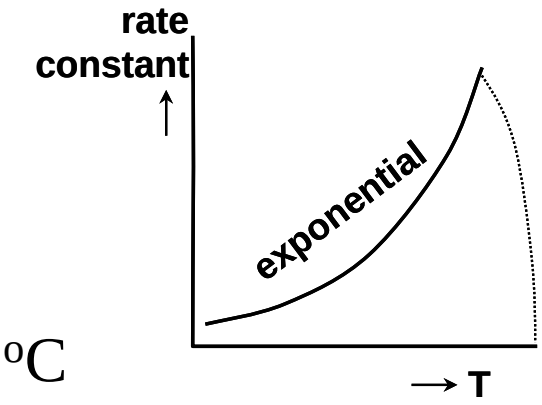
Temperature effect on kinetic parameters

- Affinity constants ($\mathbf{K_S}$, etc.) are **weakly dependent** on T
- Rate constants ($\mathbf{q_S^{max}}$, $\mathbf{m_S}$, $\mathbf{q_P^{max}}$, etc.) depend on T

→ **Arrhenius Eq.**

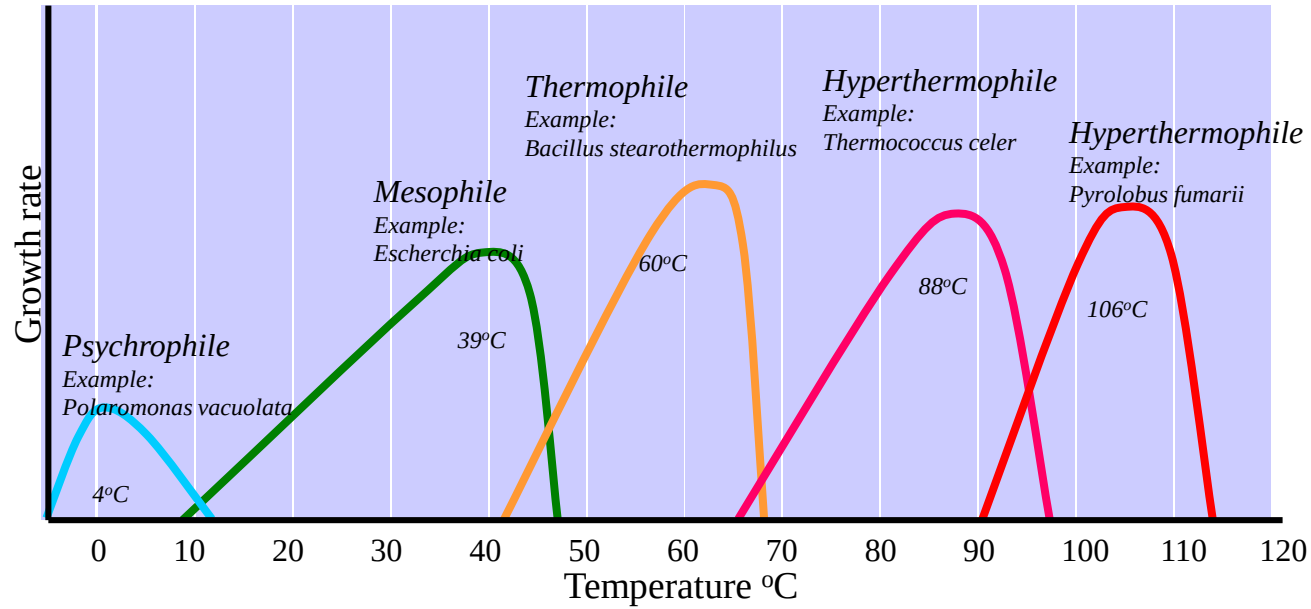
$$Rate\_Cst(T) = Rate\_Cst_{298K} * \exp\left[\frac{69000}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]$$

with $R = 8.314$ J/mol K



Increase with T: rate constants double for each $\Delta T \approx 10$ °C

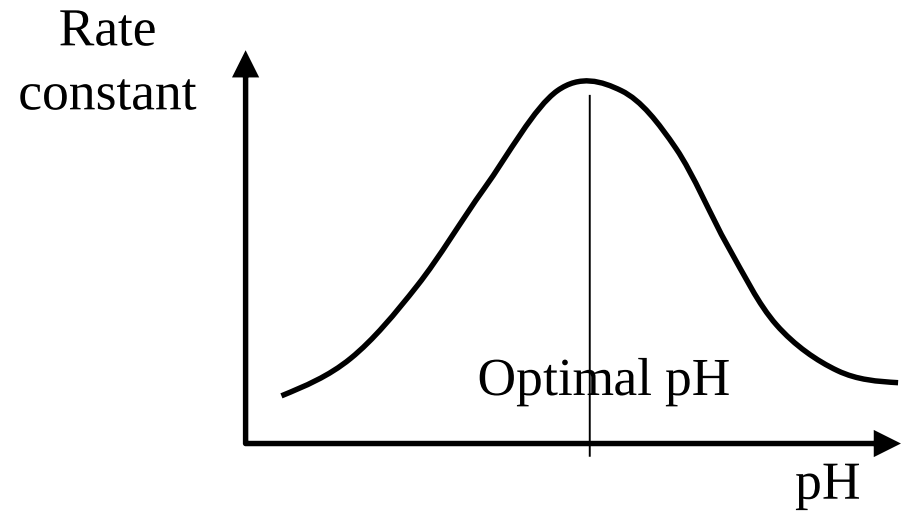
And collapse at high T



pH effect on kinetic parameters

Often empirical functions

→ see also enzymatic kinetics



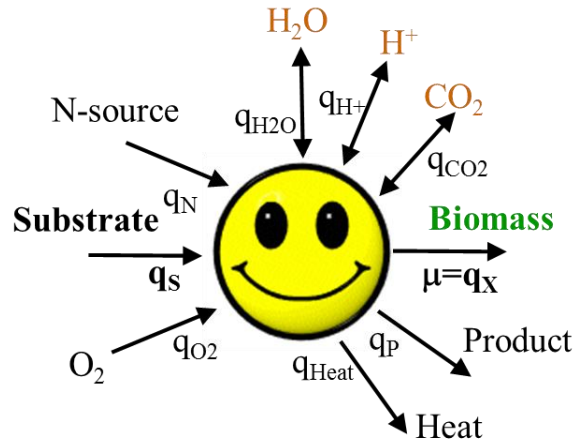
Cell Growth at pH Extremes

- Most natural environments have **pH's of 5-9**
- Most organisms have a growth **pH range of 2-3** units
- Organisms that grow at low pH are called **acidophilic**
- Organisms that grow at high pH are **alkaliphilic**
- Very few organisms grow below **pH=2** or above **pH=10**
- **Neutrophils** [pH= 7 (pH= 6-8)]

Multiple substrate limitations

What about the effect of other compound concentrations than C_S, on kinetics (C_{O2}, C_{NH4+},…)

Growth requires substrates, O_2, NH_4^+, etc…
If one is missing, $C_{O2} = 0$ or $C_{NH4+} = 0$, etc,
Rates stop! But the decrease is continuous…



The hyperbolic **Switching Function $C_i/(K_i+C_i)$** can often be used.
(Already seen in ASM modeling):
$C_i = 0$ Switching function = 0
$C_i \gg K_i$ Switching function ≈ 1

$$q_S = q_S^{max} \frac{C_S}{K_S + C_S} \frac{C_O}{K_O + C_O} \frac{C_N}{K_N + C_N}$$

Substrate term | O_2 term | Ammonia term

Multiple substrates: Inhibition

Irreversible inhibition → **Death** of biomass

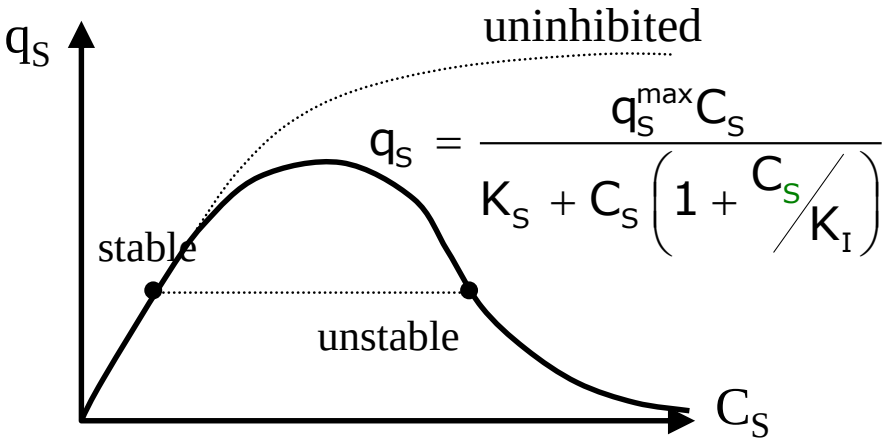
By reactive/toxic chemicals

Use of simple empirical equations!

Reversible inhibition → **Activity** of micro-organisms drops

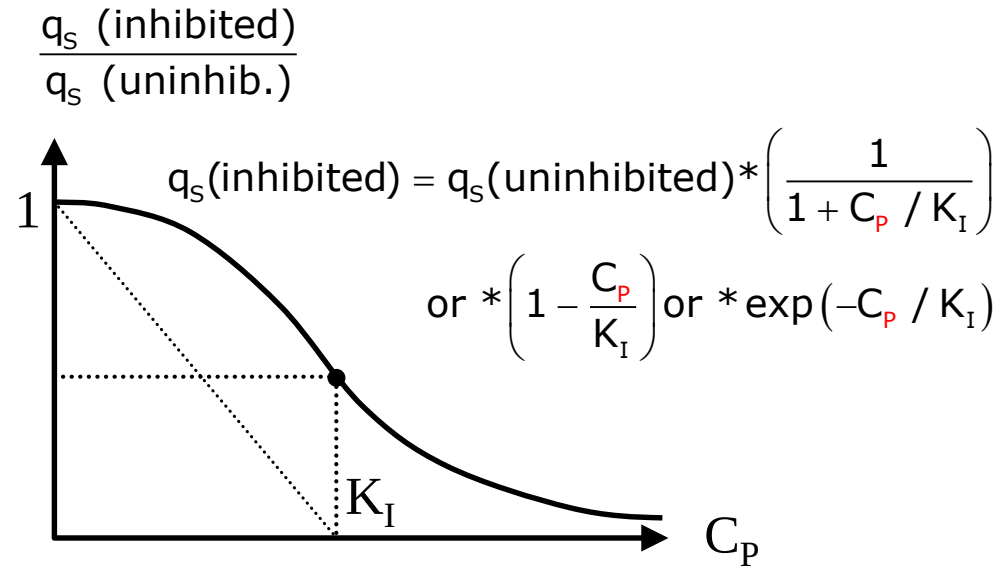
Due to high concentrations of :

- Substrates (phenol)
- Products (lactic acid, ethanol)
- Unspecific (salts, O_2 for denitrifiers)



(S)ubstrate inhibition

(Haldane kinetics)



(P)roduct inhibition

(on substrate consumption)

Inert Organic Matter production /Active Biomass (Microbial death - For more see ASM 1 Process#4 (p. 9))

Particularly relevant for : - Biological waste water treatment (high SRT)
- High cell density fermentation

Mechanism (Concerning C_X Active Biomass)

Growth: $C_S \rightarrow C_X$ (μ rate)

Death: $C_X \rightarrow C_S + C_{inert}$ (decay rate)

with $C_S = (1 - f)C_X$ Re-consumed

and $C_{inert} = f\ C_X$ Cell wall + polymeric residues

As decay rate $k_d \approx$ cst [$kgX_{(dead)}$ .kgX^{-1}.hr^{-1}]

and $f = C_{Inert}/ X_{Dead} \approx$ cst [kgC_{Inert}.$kgX_{(dead)}^{-1}$]

$1\text{-}f = C_S/X_{Dead} \approx$ cst [kgC_S.$kgX_{(dead)}^{-1}$]

Biomass lysis produces C_S and C_{inert} at cst rates:

C_S : rate $q_{S,I} = k_d.(1\text{-}f) = k_{S,I}$ [kgC_S.kgX^{-1}.hr^{-1}]

C_{Inert} : rate $q_I = k_d.f = k_I$ [kgC_{Inert}.kg X^{-1}. hr^{-1}]

Inerts from biomass lysis can be seen as a "non-catabolic" product, at rates:

q_S Ext. Herbert-Pirt kinetic

[kgC_S.kgX^{-1}.hr^{-1}]

$$-q_S = \frac{1}{Y_{SX}^{max}}\mu - k_{SI} + m_S$$

q_I ($=q_P$) kinetic (cst and independent of μ)

[kgC_{Inert}.kg X^{-1}. hr^{-1}]

$$q_I = (q_P) = k_I$$

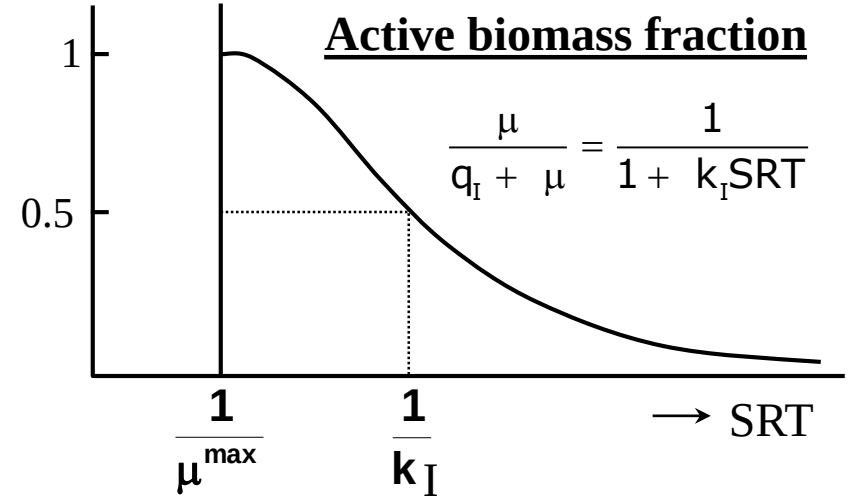
Inert Organic Matter production / Active Biomass (Microbial death - For more see ASM 1 Process#4)

$$-q_S = \frac{1}{Y_{SX}^{max}}\,\mu - k_{SI} + m_S$$

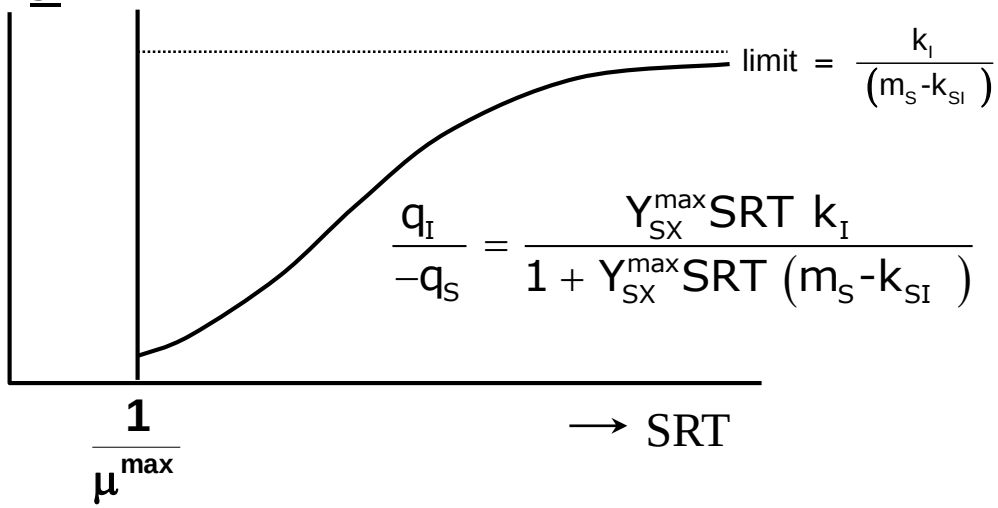
$$q_I = (q_P) = k_I$$

$$\mathbf{SRT} = \frac{\mathbf{1}}{\mu}$$

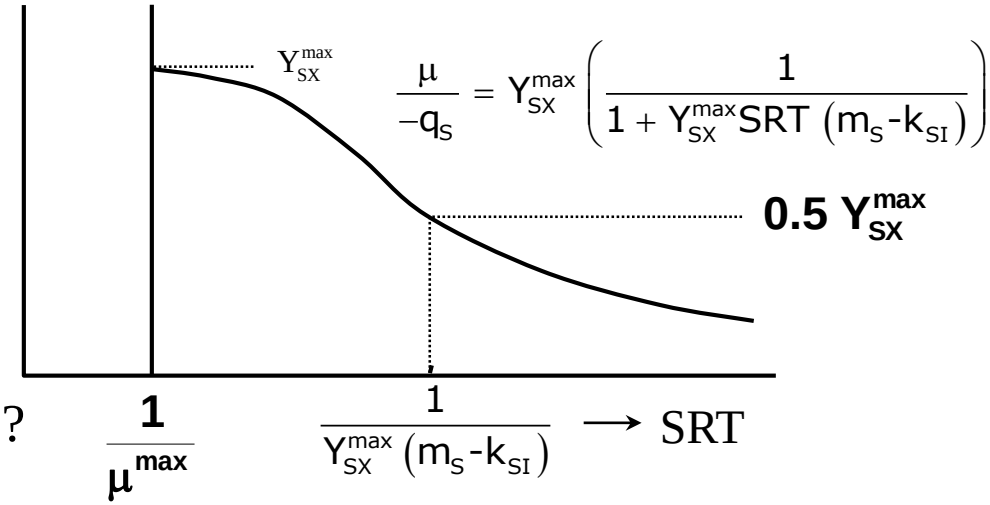
Active biomass fraction



Y_{SI} yield of Inerts I on Substrate



Yield of active biomass on substrate

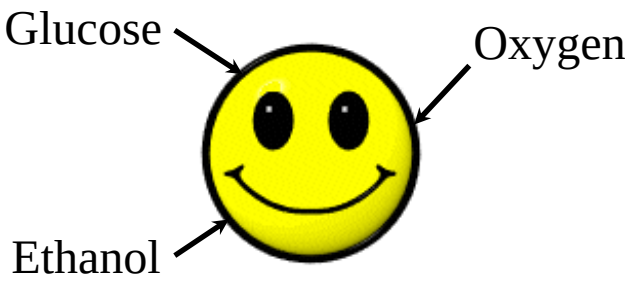


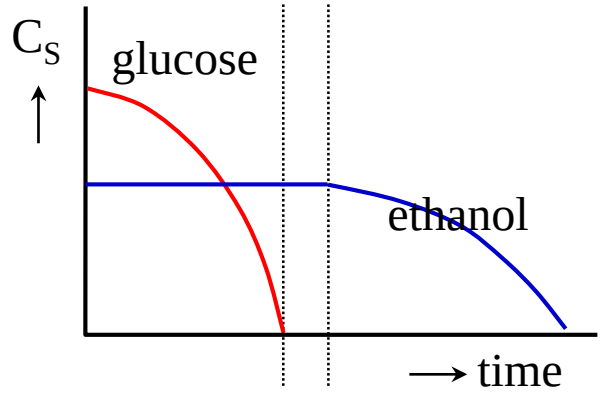
Conclusion: At high SRT

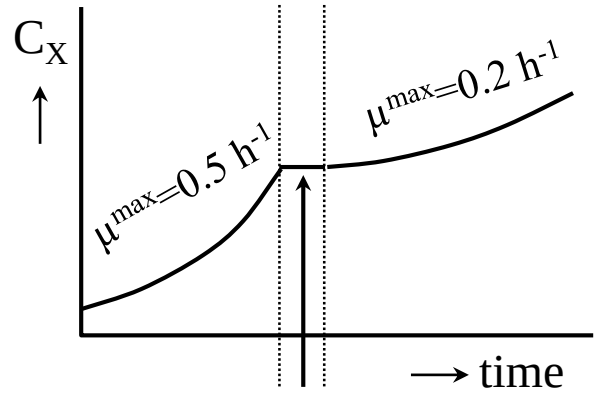
- active biomass fraction decreases strongly
- inert fraction increases strongly

? What about mineral suspended solids in WWTP ?

Mixed substrates / diauxy







time period needed to induce ethanol processing enzymes

Growth observation in batch reactor, high glucose & high ethanol:

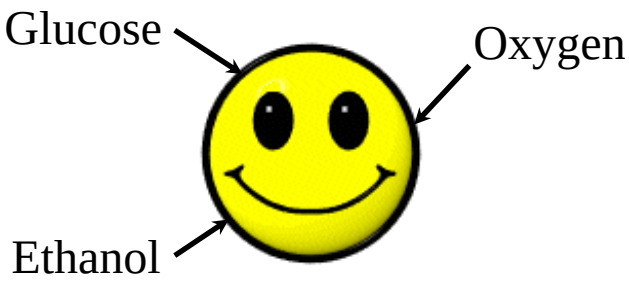
1. Substrate with highest μ^{max} is preferred, repressing the enzymes required for the 2^{cd} substrate (which are inhibited by 1st substrate).
2. At low glucose concentration ethanol consumption is initiated.

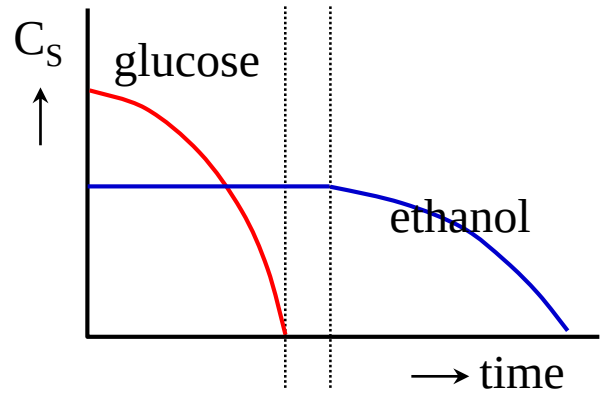
→Simple kinetic approach

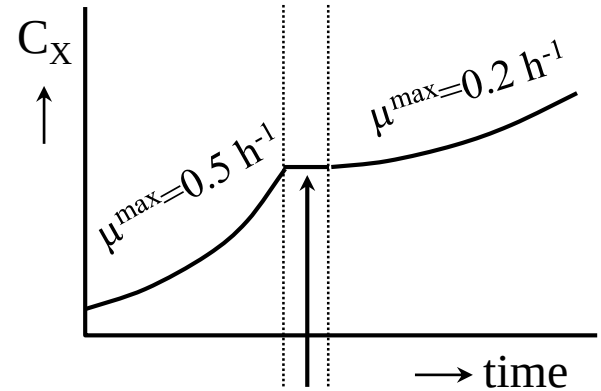
Extended Herbert-Pirt

+ Substrate inhibition

Mixed substrates / diauxy







time period needed to induce ethanol processing enzymes

Substrate-uptake rates (Substrate C_{S1} is preferred and inhibits Substrate C_{S2} consumption)

$$q_{S1} = q_{S1}^{max} \cdot \frac{C_{S1}}{K_{S1} + C_{S1}} \qquad q_{S2} = q_{S2}^{max} \cdot \frac{C_{S2}}{K_{S2} + C_{S2}} \cdot \frac{1}{1 + C_{S1}/K_I}$$

C_{S1} "Product" inhibition or Inversed Switching function

Growth rate comes from Extended Herbert-Pirt Eq:

$$\mu = Y_{S1X}^{max} . q_{S1} + Y_{S2X}^{max} . q_{S2} - K_d$$

$$-q_S = \frac{1}{Y_{SX}^{max}} \mu + m_S;\ \mu = (-q_S - m_S) Y_{SX}^{max};\ k_d = Y_{SX}^{max} m_S$$

Mixed substrates

Supposing 2 substrates C_{S1} and C_{S2} on which grows biomass

$$q_{S1} = q_{S1}^{max} \cdot \frac{C_{S1}}{K_{S1} + C_{S1}} \qquad q_{S2} = q_{S2}^{max} \cdot \frac{C_{S2}}{K_{S2} + C_{S2}}$$

Growth rate comes from Extended Herbert-Pirt Eq:

$$\mu = Y_{S1X}^{max} . q_{S1} + Y_{S2X}^{max} . q_{S2} - K_d$$

$$-q_S = \frac{1}{Y_{SX}^{max}} \mu + m_S;\ \mu = (-q_S - m_s) Y_{SX}^{max};\ k_d = Y_{SX}^{max} m_S$$

Ex: $q_{S1}{}^{max} = q_{S2}{}^{max} = 0.5$; $K_d \approx 0$; $K_{S1} = K_{S2} = 20$mg/L
Y_{S1X} = 0.5 gX/gS1; **Y_{S2X} = 0.2** gX/gS2

$\rightarrow \mu = 0.5\ q_{S1} + 0.2\ q_{S2} + 0$

C_{S1}	= 13.3 mg/l	C_{S2}	= 0 mg/l	→ μ = 0.1
q_{S1}	= 0.2	q_{S2}	= 0	
C_{S1}	= 5 mg/l	C_{S2}	= 20 mg/l	→ μ = 0.1
q_{S1}	= 0.1	q_{S2}	= 0.25	
C_{S1}	= 2.2 mg/l	C_{S2}	= 60 mg/l	→ μ = 0.1
q_{S1}	= 0.05	q_{S2}	= 0.375	

Mixed substrates / Conclusion

If:

C_{S1} = 13.3 mg/l	C_{S2} = 0 mg/l	→ μ = 0.1
q_{S1} = 0.2	q_{S2} = 0	
C_{S1} = 5 mg/l	C_{S2} = 20 mg/l	→ μ = 0.1
q_{S1} = 0.1	q_{S2} = 0.25	
C_{S1} = 2.2 mg/l	C_{S2} = 60 mg/l	→ μ = 0.1
q_{S1} = 0.05	q_{S2} = 0.375	

- The same high μ is maintained at lower C_{S1}, due to substrate C_{S2} availability
- Lower q_{S1} is compensated by q_{S2}
- Simultaneous uptake of many substrates allows high μ at very low C_{Si} individual substrate concentrations

Thus, growth under mixed substrates:

1. Gives competitive advantage because **high μ is possible even at low substrate concentrations** → ;-) ?
2. Is an **extra degree of freedom to manipulate** microbial product formation (due to changes in production of ATP, NADPH or key carbon compounds!)