F. Williams Structured Model

Williams, *J. Theoretical Biol.* 15, 190, 1967

The cell is divided into two intracellular compartments and a substrate compartment

A synthetic pool ("K-compartment")

- RNA
- Small metabolites

$$\text{total conc.} = K \Rightarrow \frac{\text{mass}}{\text{cell volume}}$$

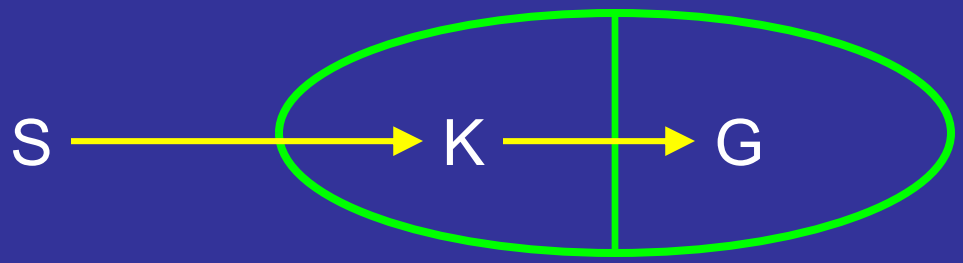
A genetic pool ("G-compartment")

- DNA
- Proteins

$$\text{total conc.} = G \Rightarrow \frac{\text{mass}}{\text{cell volume}}$$

A substrate pool ("S-compartment")

$$\text{conc.} = S \Rightarrow \frac{\text{mass}}{\text{external volume}}$$



1) Biomass (X) and Substrate (S)

Rate of generation of cell mass (X) is proportional to S and X

$$\frac{dX}{dt} = k_1 SX$$

$$\boxed{\frac{1}{X}\frac{dX}{dt} = k_1 S}$$

Rate of consumption of substrate (S) is proportional to S and X

$$\boxed{-\frac{dS}{dt} = \frac{1}{Y_{X/S}} k_1 SX}$$

2) G synthesis

G is synthesized from K at a rate proportional to the concentrations of G and K

G concentration (mass G/volume cell) = G

G concentration (mass G/mass cell) = G/ρ_{CELL}

where ρ_{CELL} = cell density (mass cell/volume cell)

Note that $G + K = \rho_{CELL}$

G concentration (mass G/reactor volume) = GX/ρ_{CELL}

Note that the term X/ρ_{CELL} converts cell conc. (mass/cell vol.) to total volume conc. (mass/reactor vol.)

Mass balance on G:

$$\frac{d(GX/\rho_{CELL})}{dt} = k_2 (GX/\rho_{CELL})K$$

$$(G)\frac{d(X/\rho_{CELL})}{dt} + (X/\rho_{CELL})\frac{dG}{dt} = k_2 (GX/\rho_{CELL})K$$

If the density of the cell is constant, then:

$$G\frac{1}{X}\frac{dX}{dt} + \frac{dG}{dt} = k_2 GK$$

Since $\frac{1}{X}\frac{dX}{dt} = k_1 S$

$$\frac{dG}{dt} = k_2 GK - k_1 SG$$

3) K synthesis

K is synthesized from S at a rate proportional to the concentration of S and to the cell density

$$\frac{d(KX/\rho_{CELL})}{dt} = \underbrace{k_1 S \rho_{CELL}(X/\rho_{CELL})}_{\text{K formed from S}} - \underbrace{k_2\, GK(X/\rho_{CELL})}_{\text{K lost to G}}$$

where ρ_{CELL} = cell density (mass cell/volume cell)

Note that $G + K = \rho_{CELL}$

If ρ_{CELL} is constant, then:

$$\frac{d(KX)}{dt} = k_1 S \rho_{CELL} X - k_2\, GKX$$

$$\frac{d(KX)}{dt} = k_1 S(G+K)X - k_2\, GKX$$

$$K\frac{dX}{dt} + X\frac{dK}{dt} = k_1 S(G+K)X - k_2\, GKX$$

$$K\underbrace{\frac{1}{X}\frac{dX}{dt}}_{k_1 S} + \frac{dK}{dt} = k_1 S(G+K) - k_2\, GK$$

$$\frac{dK}{dt} = k_1 S(G+K) - k_2\, GK - k_1 KS$$

$$\frac{dK}{dt} = k_1 S(G+K) - k_2\, GK - k_1 KS$$

$$\boxed{\frac{dK}{dt} = k_1 SG - k_2\, GK}$$

Note: $\frac{dG}{dt} = -\frac{dK}{dt}$

4) Cell Number Concentration

Cell number concentration is proportional to the mass of the genetic compartment

$N \propto$ G concentration (mass G/reactor volume)

$N \propto GX/\rho_{CELL}$

$N = k_3 GX/\rho_{CELL}$

5) Cell Volume

Cell volume is proportional to the total cell mass and inversely proportional to the genetic compartment

$$V_{CELL} \propto (K+G)/G$$

$$V_{CELL} = k_4 \rho_{CELL}/G$$

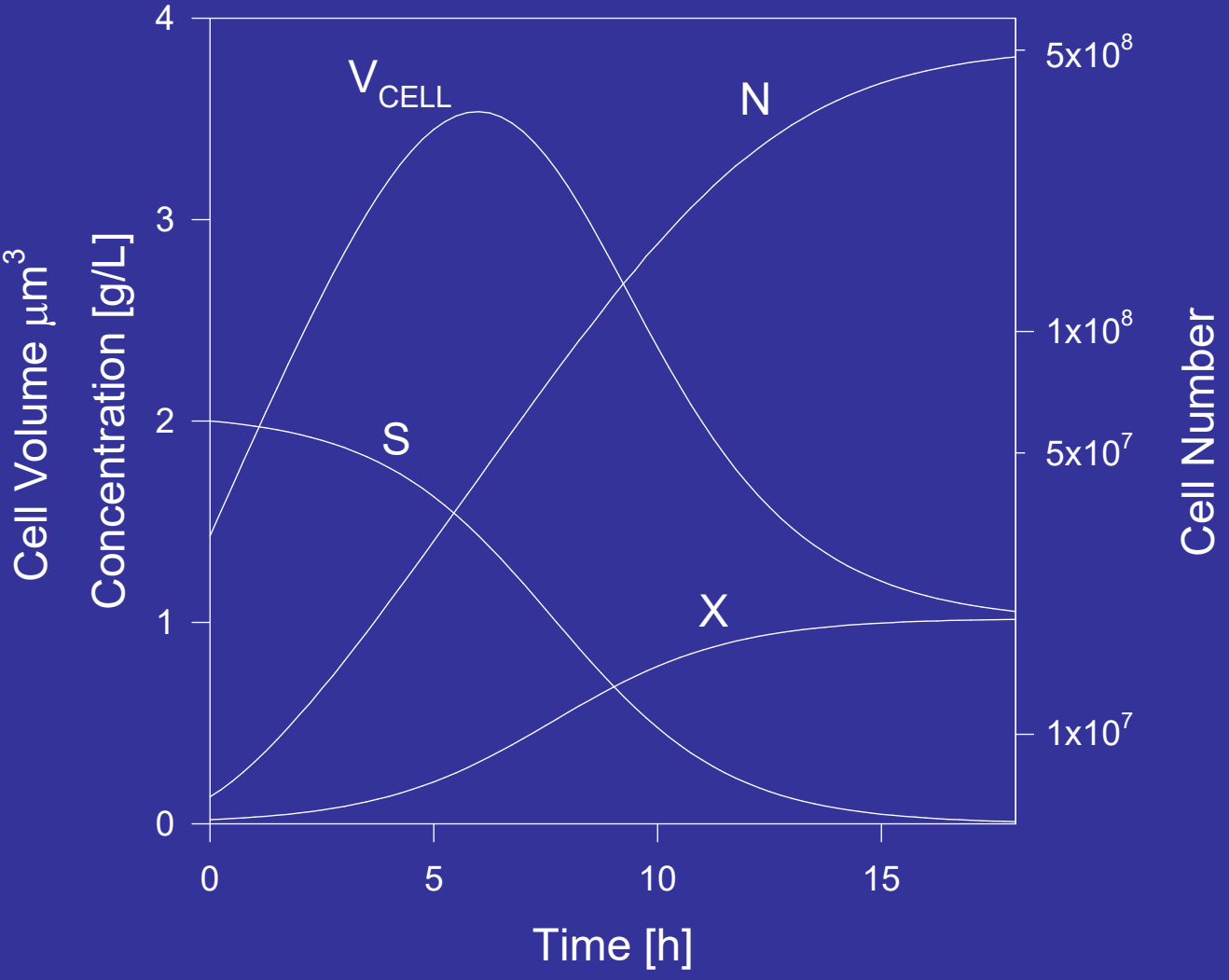
Example

$S_0 = 2$ g/L
$X_0 = 0.02$ g/L
$K_0 = 0.3$ g/L
$G_0 = 0.7$ g/L

$Y_{X/S} = 0.5$

$k_1 = 0.25$ L/gh
$k_2 = 0.50$ L/gh
$k_3 = 5 \times 10^8$ cellsL/g
$k_4 = 1.0$ μm^3

Williams Structured Model



Interesting Features of Williams Model

1) Predicts a lag phase
2) If inoculum is not fully adapted (i.e., if K_0 and G_0 deviate from values found in exponential growth phase), then cell volume will increase initially
3) Stationary phase is attained for cell mass prior to stationary phase for cell number

Potential Improvements

1) Rate of substrate uptake and growth described by Monod Equation
2) Include a maintenance term (Williams Model incorrectly predicts $K \rightarrow 0$ as $t \rightarrow \infty$)
3) Include “inhibition” that converts G & K into inactive forms

G. Heat Generation

In biological reactors, heat must be added or withdrawn to maintain the temperature at a desired value.

Usually heat is generated in a biological reactor, and therefore it must be removed. In small reactors, a jacket is commonly used to supply sufficient surface area for heat transfer. In large reactors coils are necessary.

Energy Balance:

$$Q_{MET} + Q_{AG} + Q_{GAS} = Q_{ACC} + Q_{EXCH} + Q_{EVAP} + Q_{SENS}$$

Q_{MET} = Heat generated from microbial metabolism
Q_{AG} = Heat generated from agitation
~~Q_{GAS} = Heat generated from aeration power input~~
~~Q_{ACC} = Heat accumulated by system (bioreactor)~~
Q_{EXCH} = Heat lost by exchange with surroundings
Q_{EVAP} = Heat lost by evaporation
~~Q_{SENS} = "sensible" Heat lost by flow streams (OUT-IN)~~

At steady-state

Q's have units of kcal/h (etc.),
not volumetric units (e.g., kcal/Lh)

(Typical) Design Equation:

$$Q_{MET} + Q_{AG} - Q_{EVAP} = Q_{EXCH}$$

1. Heat generated from microbial metabolism (Q_{MET})

   Find Q_{MET} either by its relationship to heats of combustion through Y_H or by its correlation with oxygen uptake.

   a) Using yield coefficient

$$Q_{MET} \propto \frac{dX}{dt} \qquad Q_{MET} \propto V$$

$$Q_{MET} = \frac{1}{Y_H} V \frac{dX}{dt}$$

$$\boxed{Q_{MET} = \frac{V}{Y_H} \mu X}$$

Where Y_H = metabolic heat yield coefficient

(g cell/kcal heat generated)

Y_H depends on substrate and the microorganism

Note units:

$$(\text{kcal/h}) = \frac{(\text{L})}{(\text{g cell/kcal heat generated})} (1/\text{h})(\text{g cells/L})$$

Find Y_H through enthalpy balance (per g cell):

Enthalpy of substrate going to cell = Enthalpy of cell + Heat evolved by cells

Enthalpy of substrate = ΔH_S (heat of combustion)

$$\text{Enthalpy of substrate going to cell} = \frac{\Delta H_S}{Y_{X/S}}$$

Enthalpy of cell = ΔH_C (heat of combustion)

$$\text{Heat evolved by cells} = \frac{1}{Y_H}$$

Result of enthalpy balance

$$\frac{\Delta H_S}{Y_{X/S}} = \Delta H_C + \frac{1}{Y_H}$$

or

$$Y_H = \frac{Y_{X/S}}{\Delta H_S - Y_{X/S}\,\Delta H_C}$$

Just use heat of combustion of cell and substrate.

The more reduced the substrate (i.e., methane vs. methanol), the more heat is generated (and the lower the value of Y_H). Using more reduced substrates generally will require more heat removal.

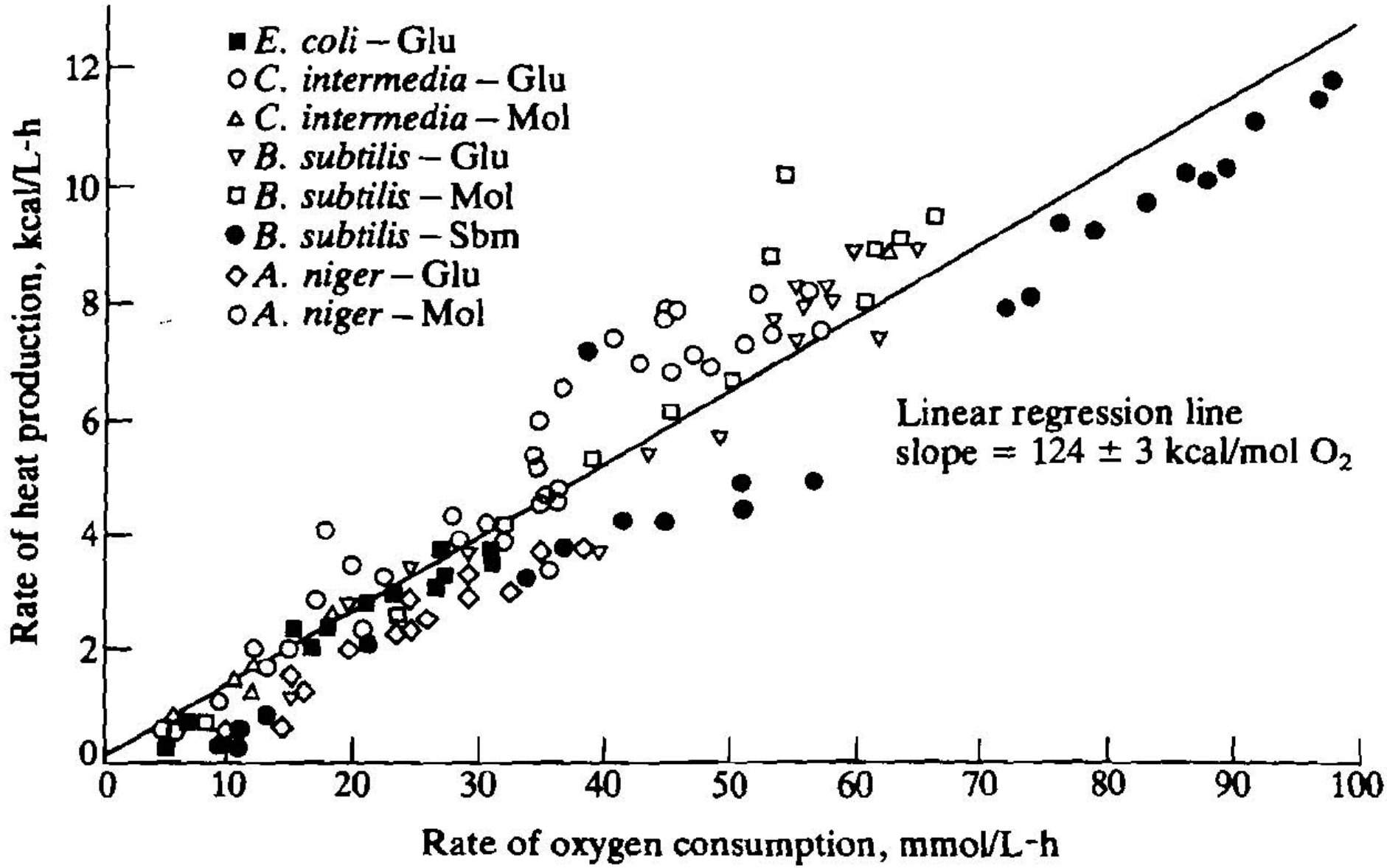
b) Correlation with Q_O

The rate of heat generated through metabolism is correlated directly with the rate of oxygen consumed (for aerobic processes). Note that more reduced substrates require more oxygen for complete oxidation.

$$Q_{MET}/V \approx 0.12Q_O$$

(kcal/h)(1/L) --- (kcal/mmol)(mmol/Lh)

Books typically don't include the volume "V" in this equation. In those cases, the units of Q_O are changed to mmol/h (I am using Q_O with more typical units of mmol/Lh). This equation can also be confusing because "Q" means heat (left side) and volumetric rate of oxygen consumption (right).



From C. L. Cooney et al., Biotechnol. Bioeng. 11:269 (1968)

Example:

E. coli has a *maximum* specific oxygen consumption rate (q_O) of about 15 mmol/gDCW h

If we grow cells to a cell density of 20 g DCW/L (OD = 50), then

$Q_O = (15 \text{ mmol/gh}) \times (20 \text{ g/L}) = 300 \text{ mmol/Lh}$

$Q_{MET}/V \approx 0.12 \times 300 =$ 36 kcal/Lh

This is VERY high! (impossibly high)

Typically a process can handle a heat generated from metabolism of 8-12 kcal/Lh

2. Heat generated from agitation (Q_{AG})

Calculate from power of motor and efficiency:

$$Q_{AG} = (\text{kW motor}) \times (860.4 \text{ kcal/kWh}) \times (\text{efficiency})$$

For example, a 15 hp motor of 92% efficiency is used to agitate a 1000 gal tank.

15 hp = 11.2 kW
1000 gal = 3785 L

$Q_{AG} = (11.2) \times (860.4) \times (0.92) =$ <u>8865 kcal/h</u>
Note: (8865 kcal/h)/3785 = 2.34 kcal/Lh

Typically, the heat generated from agitation is 2-3 kcal/Lh

3. Heat lost from evaporation (Q_{EVAP})

If gas entering bioreactor is saturated with water at the same temperature of the medium, then $Q_{EVAP} = 0$.

Typically, entering gas is compressed, cooled, condensed, then reheated. To calculate heat of vaporization, need relative humidity, and temperatures of medium and inlet air.

Easiest way to determine heat loss by evaporation is to measure mass of water evaporated (e.g., kg/h), and use steam tables at temperature of medium to measure Q. At 33°C, heat lost by evaporation is 580 kcal/kg water.

4. Heat duty of heat exchanger (Q_{EXCH})

A heat exchanger is used to remove excess heat generated in the bioreactor:

$$Q_{EXCH} = UA\Delta T$$

U = Heat transfer coefficient
A = Surface area for heat transfer
ΔT = Temperature difference
(usually log mean temperature difference is used)

As a rule of thumb, U for a clean vessel is
50-200 btu/h·ft^2°F (250-1000 kcal/h·m^2°C)
- low range applies to high viscosity (fungi)
- high range applies to low viscosity (bacteria)

Comments

- In large bioreactors, the limit of heat removal is around 15 kcal/Lh, and this requires internal coils. Internal coils can create there own problems including interferring with best mixing pattern, increased cleaning costs, leaks.

- Refrigeration tends to be too costly; freeze-ups are to be avoided.

- Impractical to use very high coolant flow rates.

- Usually impractical (cost of sterile, cleanable, non-cavitating pumping) to recirculate medium externally.

Comments

- Potential ways to reduce the heat requirement in large fermenters are:

1) slow metabolism down by operating at lower growth rate. This reduces both Q_{MET} (since $Q_{MET} \propto \mu$) and Q_{AG} because less oxygen is needed.
2) operate bioprocess at higher temperature (so ΔT can be larger)