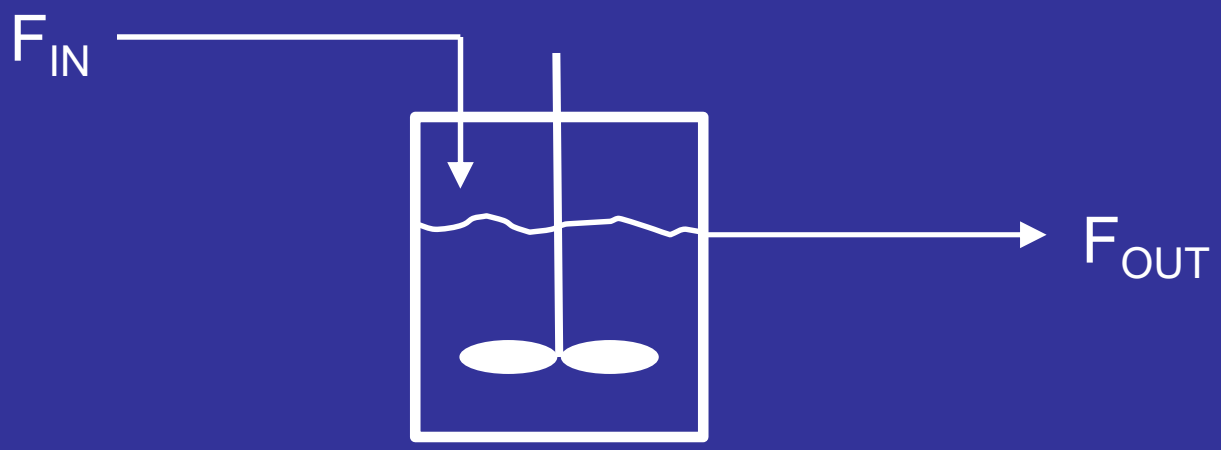
K. Continuous Culture & Fed-Batch Culture

1. General Material Balances

Consider the following well-mixed system:



F_{IN} = flowrate of fluid into system (vol/time)

F_{OUT} = flowrate of fluid out of system (vol/time)

V = volume of fluid in system

Other key variables (a reminder):

X = concentration of cells (mass/vol)

S = concentration of substrate (mass/vol)

P = concentration of product (mass/vol)

W = concentration of water (mass/vol)

r_i = mass rate of *i* generation (mass/vol·time)

Four (or more) material balances can be written:

Accumulation = In – Out + Gen

a) Cells:

$$\frac{d(VX)}{dt} = F_{IN}X_{IN} - F_{OUT}X_{OUT} + r_X V$$

b) Limiting substrate:

$$\frac{d(VS)}{dt} = F_{IN}S_{IN} - F_{OUT}S_{OUT} + r_S V$$

Note: we expect r_S to be negative

c) Product:

$$\frac{d(VP)}{dt} = F_{IN}P_{IN} - F_{OUT}P_{OUT} + r_P V$$

d) Water:

$$\frac{d(VW)}{dt} = F_{IN}W_{IN} - F_{OUT}W_{OUT} + r_W V$$

2. Common Simplifications

a) Concentration of water remains unchanged

$$W_{IN} = W_{OUT} = W$$

and insignificant water is generated

$$r_W = 0$$

b) Reactor is well-mixed

$$P_{OUT} = P$$

$$S_{OUT} = S$$

$$X_{OUT} = X$$

c) No product and no cells in feed

$P_{IN} = 0;\ X_{IN} = 0$

d) Cell growth rate is greater than cell death rate and can be expressed as:

$r_X = \mu X$

Results of these simplications:

a) Cells: $\frac{d(VX)}{dt} = -F_{OUT}X + \mu XV$

b) Substrate: $\frac{d(VS)}{dt} = F_{IN}S_{IN} - F_{OUT}S + r_S V$

c) Product: $\frac{d(VP)}{dt} = -F_{OUT}P + r_P V$

d) Water: $\frac{dV}{dt} = F_{IN} - F_{OUT}$

3. Modes of Operation - Chemostat

A chemostat occurs when $F_{OUT} = F_{IN} = F$

The volume is therefore constant and the concentrations will reach a steady-state.

Define: Dilution rate = $D = \frac{F}{V}$ $[time^{-1}]$

The material balances become….

a) Cells: $\frac{dX}{dt} = -DX + \mu X$

b) Substrate: $\frac{dS}{dt} = D(S_{IN} - S) + r_S$

c) Product: $\frac{dP}{dt} = -DP + r_P$

But, at steady-state, there will be no change in concentrations…

The material balances become….

a) Cells: $DX = \mu X$

b) Substrate: $D(S_{IN} - S) = -r_S$

c) Product: $DP = r_P$

Let us now look at each one of these results….

a. Cells $DX = \mu X$

or $\boxed{D = \mu}$!

This equation states that the cells' specific growth rate is determined by the dilution rate.

We have already defined a *maximum specific growth rate* as μ_{MAX}. If $D > \mu_{MAX}$, then the cells will not be able to grow fast enough for the flowing fluid. This condition is called wash-out. Note that:

$$\frac{dX}{dt} = -DX + \mu X$$

b. Substrate $D(S_{IN} - S) = -r_S$

Before this equation is used, we must have a model for the value of r_S. We previously have shown:

$$-r_S = m_S X + \frac{\mu X}{Y_{X/S}} + \frac{q_P X}{Y_{P/S}}$$

Commonly the term $\frac{q_P X}{Y_{P/S}}$ is taken to be small:

$$-r_S = m_S X + \frac{\mu X}{Y_{X/S}}$$

The material balance becomes:

$$D(S_{IN} - S) = m_S X + \frac{DX}{Y_{X/S}}$$

or

$$\frac{D(S_{IN} - S)}{X} = m_S + \frac{D}{Y_{X/S}}$$

We will now rename $Y_{X/S}$ as the true biomass yield or the maximum biomass yield. We do this to distinguish it from the observed biomass yield…

Define: $$\frac{X}{(S_{IN} - S)} = Y_{X/S}^{OBS}$$

$Y_{X/S}^{OBS}$ = Observed Biomass Yield or Apparent Biomass Yield

So: $$\boxed{\frac{D}{Y_{X/S}^{OBS}} = m_S + \frac{D}{Y_{X/S}}}$$

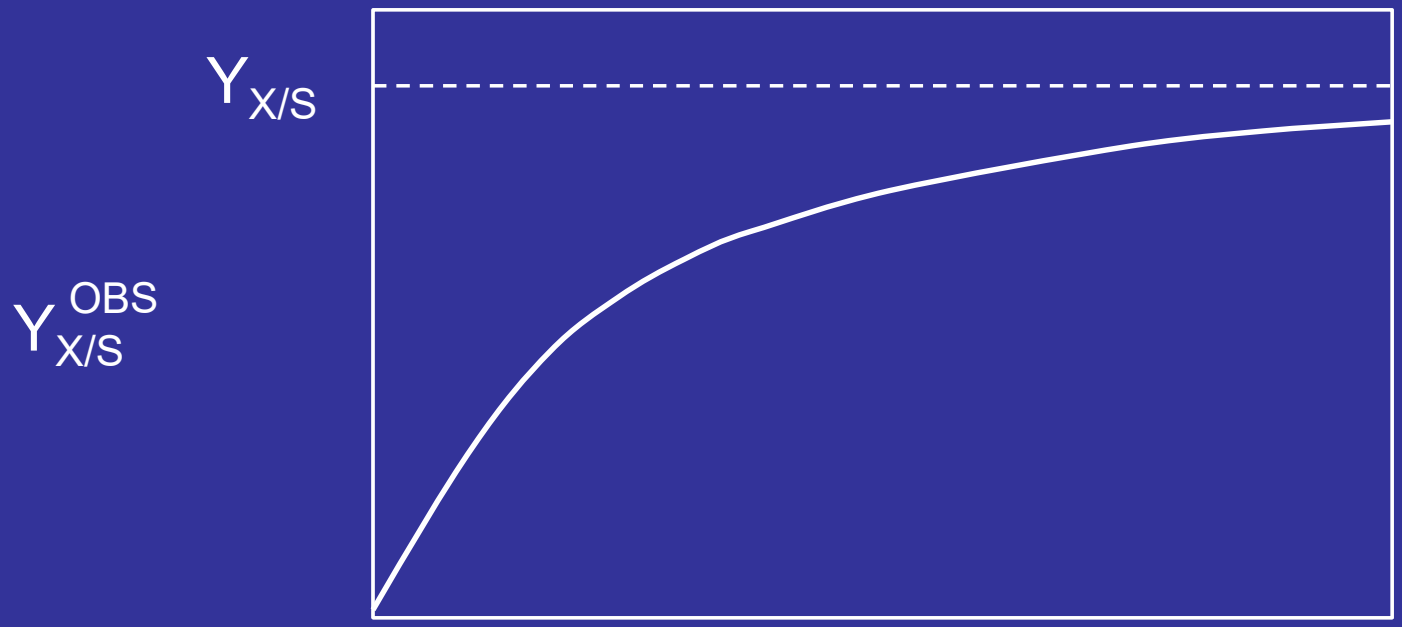
c. Biomass Yield

$$\frac{D}{Y_{X/S}^{OBS}} = m_S + \frac{D}{Y_{X/S}}$$

This equation really describes $Y_{X/S}^{OBS} = f(D)$. m_S and $Y_{X/S}$ are parameters. Note that the observed biomass yield is a function of dilution rate, but the true biomass yield is not. The equation may be written:

$$Y_{X/S}^{OBS} = \frac{DY_{X/S}}{D + m_S Y_{X/S}}$$

This equation expresses a saturation model (i.e., like Michaelis-Menten kinetics)



At high dilution rate $Y_{X/S}^{OBS} \rightarrow Y_{X/S}$

d. Calculation of Maintenance Coefficient and True Biomass Yield

The easiest way to determine the value of the maintenance coefficient and the true biomass yield is to conduct several chemostats at different dilution rates (D), and calculate the observed biomass yield. Then make one of three possible plots of data.

1) Pirt Plot (Pirt, 1965)

Rewrite equation as

$$\frac{1}{Y_{X/S}^{OBS}} = \frac{m_S}{D} + \frac{1}{Y_{X/S}}$$

$$y = mx + b$$

Plot $\frac{1}{D}$ (“x”) versus $\frac{1}{Y_{X/S}^{OBS}}$ (“y”)

$$\text{Intercept} = \frac{1}{Y_{X/S}}$$

$$\text{Slope} = m_S$$

2) Hofstee Plot

Rewrite equation as

$$Y_{X/S}^{OBS} = \frac{-m_S Y_{X/S} Y_{X/S}^{OBS}}{D} + Y_{X/S}$$

$$y = mx + b$$

Plot $\frac{Y_{X/S}^{OBS}}{D}$ ("x") versus $Y_{X/S}^{OBS}$ ("y")

Intercept = $Y_{X/S}$

Slope = $-m_S Y_{X/S}$

This plot is almost never used

3) Tempest Plot (Tempest & Neijssel, 1985)

Leave equation as

$$\frac{D}{Y_{X/S}^{OBS}} = \frac{D}{Y_{X/S}} + m_S$$

$$y = mx + b$$

Plot D ("x") versus $\frac{D}{Y_{X/S}^{OBS}}$ ("y")

Intercept = m_S

Slope = $\frac{1}{Y_{X/S}}$

Comments:

$Y_{X/S}$ is sometimes referred as "*maximum yield coefficient*" or "*true yield coefficient*"

$Y_{X/S}$ usually refers to carbon/energy source, and is sometimes given a more specific name "*biomass yield coefficient on glucose*" or "*biomass yield from glucose*", etc.

Understand the difference between $Y_{X/S}$ and $Y_{X/S}^{OBS}$

Most people calculate biomass yield from batch data. Strictly speaking, this is the observed yield, $Y_{X/S}^{OBS}$. However, at high growth rates $Y_{X/S}^{OBS} \rightarrow Y_{X/S}$

Pirt plot seems to be preferred linearization.

e. More on Maintenance

For *E. coli* on glucose:

m_S = 0.047 – 0.067 g gluc/gh ≈ 0.05 g gluc/gh

m_O = 0.014 g O_2/gh

$Y_{X/S}$ = 0.422 – 0.455 g/g gluc ≈ 0.44 g/g gluc

$Y_{X/O}$ = 1.87 g/g O_2

<u>references:</u>

Farmer and Jones, 1976
Neijssel et al., 1996
Nanchen et al., 2006

Then $$\frac{D}{Y_{X/S}^{OBS}} = m_S + \frac{D}{Y_{X/S}}$$

Becomes: $$\frac{D}{Y_{X/S}^{OBS}} = 0.05 + \frac{D}{0.44}$$

D	$Y_{X/S}$
0.05	0.306
0.10	0.361
0.20	0.396
0.40	0.417
0.80	0.428

Assuming maintenance is constant…

What is maintenance?

Protein turnover in *E.* coli during stationary phase is 5% per hour (Mandelstam & McQuillen, 1968). On the basis of a cell composition of 50% protein, 4 ATP needed per amino acid residue, an average molecular mass of an amino acid of 100, and 24 ATP generated per mole glucose, the glucose consumption rate necessary to sustain protein turnover is:

$$\frac{0.05}{h}\ \frac{0.5\ g\ protein}{g\ cells}\ \frac{4\ mol\ ATP}{mol\ Amino\ Acid}\ \frac{mol\ AA}{100\ g\ AA}$$

$$\frac{mol\ glucose}{24\ mol\ ATP}\ \frac{180\ g\ glucose}{mol\ glucose} = 0.0076\ g\ glucose/gh$$

What is maintenance?

0.0076 g glucose/gh is 15% of 0.05 g glucose/gh. One could conclude that 15% of cell maintenance is due to protein turnover.

More than 50% of maintenance is proposed to be used to sustain a membrane potential (Stouthamer & Bettenhausen, 1977)

f. Product $DP = r_P$

We'll just write $r_p = q_pX$

So, $DP = q_PX$

g. Calculating Substrate Concentration in Chemostat

We have previously shown that $D = \mu$ at steady-state.

But, several models exist for the relationship between specific growth rate μ and substrate concentration. For example, Monod model:

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

Thus, at steady-state ("SS") we would expect:

$$D = \frac{\mu_{MAX} S_{SS}}{K_S + S_{SS}}$$

Or:

$$S_{SS} = \frac{DK_S}{\mu_{MAX} - D}$$

This equation allows us to calculate the steady-state concentration of the substrate S for any dilution rate. Surprisingly, this equation states that the outlet substrate concentration is independent of the inlet substrate concentration! (as long as $S_{IN} > S_{SS}$).

Note that (unreasonably) $S_{SS} \rightarrow \infty$ as $D \rightarrow \mu_{MAX}$

What is steady-state substrate concentration?

For *E. coli* on glucose:

K_S = 9.2 mg/L – 14.3 mg/L ≈10 mg/L
(Reiling et al. 1985)

μ_{MAX} = 0.80 h^{-1}

At a dilution rate of 0.40 h^{-1}:

$$S_{SS} = \frac{DK_S}{\mu_{MAX} - D} = \frac{(0.40\ h^{-1})\ (10\ mg/L)}{(0.80\ h^{-1} - 0.40\ h^{-1})} = \underline{\underline{10\ mg/L}}$$

This value is very low. Thus, during a chemostat operation, there is essentially no limiting substrate remaining in culture and in outlet. (i.e., growth rate is limited by availability of substrate.)

h. Calculating Monod Constant, K_S

$$S_{SS} = \frac{DK_S}{\mu_{MAX} - D}$$

This equation can also be used to calculate the parameters K_S and μ_{MAX}. These parameters are calculated by running several chemostats at different dilution rates and measuring the substrate concentration in the effluent. For example, rewrite this equation as…

$$\frac{1}{D} = \frac{K_S}{\mu_{MAX}} \frac{1}{S_{SS}} + \frac{1}{\mu_{MAX}}$$

$$y = mx + b$$

Plot $\frac{1}{S_{SS}}$ (“x”) versus $\frac{1}{D}$ (“y”)

$$\text{Intercept} = \frac{1}{\mu_{MAX}}$$

$$\text{Slope} = \frac{K_S}{\mu_{MAX}}$$

The slope is actually close to zero because K_S is so small.

i. Industrial Use of a Chemostat

Disadvantages:

- Product is diluted by all that feed; product never gets a chance to accumulate.
- Contamination is fatal. Because system is open and longer-lasting, it is also more subject to contamination.
- Evolution. The desired characteristics of a microbe can be lost if they don't confer an evolutionary advantage.

So, when would a chemostat be used?

- When product concentration is not relevant, or diluted product is desirable.
- When contamination is desirable.
- When evolution/selection of characteristics is desirable.

<u>Specifically:</u>

1. Wastewater Treatment
2. Biomining

j. Why conduct a laboratory chemostat experiment?

- To vary growth rate with no other change in the environment.
- To fix growth rate while changing the environment.
- To maintain substrate-limited growth with a constant growth rate.
- To determine the best growth rate to operate a fed-batch process.

j. Why conduct a laboratory chemostat experiment? (cont'd)

- To determine how product is formed relative to substrate consumption. (e.g., is product positively growth associated, negatively growth associated, etc.)
- To study evolution at a reasonable time-scale. Or, to intentionally evolve a strain for desired characteristics.
- To measure physiological fluxes.
- To affect metabolism in cases where a particular substrate limitation is advantageous.

k. Example calculations

Data Collected from Acetate-Limited Chemostat
(Strain requires both glucose and acetate for growth)

Feed Flowrate	0.155 L/h
Volume	1.00 L
Gas Flowrate (STP)	1.00 L/min
Dry Cell Weight Concentration	2.45 g/L
Feed Concentrations:	
Glucose	35.494 g/L
Acetate	1.023 g/L
Pyruvate	0.000 g/L
O_2 (Dry Basis)	20.92 %
CO_2 (Dry Basis)	0.00 %
Effluent Concentrations	
Glucose	10.330 g/L
Acetate	0.000 g/L
Pyruvate	17.892 g/L
O_2 (Dry Basis)	20.13 %
CO_2 (Dry Basis)	0.67 %

a) Dilution Rate

$$D = \frac{F}{V}$$

$$D = \frac{0.155\ L/h}{1.00\ L} = \underline{0.155\ h^{-1}}$$

b) Residence Time

$$\theta = \frac{1}{D} = \frac{1}{0.155\ h^{-1}} = \underline{6.45\ h}$$

One should allow 4-5 residence times to pass before the system is “at” steady-state.

c) Observed Biomass Yield on Glucose

$$Y_{X/G}^{OBS} = \frac{X}{(G_{IN} - G)} = \frac{2.45\ g/L}{(35.494 - 10.330)\ g/L}$$

$$= \underline{0.097\ g\ cells/\ g\ glucose}$$

Probably makes more sense to calculate biomass yield on acetate, since it is **the** limiting substrate.

d) Observed Biomass Yield on Acetate

$$Y_{X/A}^{OBS} = \frac{X}{(A_{IN} - A)} = \frac{2.45\ g/L}{(1.023 - 0.0)\ g/L}$$

$$= \underline{2.392\ g\ cells/\ g\ acetate}$$

e) Volumetric Rate of Glucose Consumption

$$Q_G = (G_{IN} - G) \times D = (35.494 - 10.330)\ g/L \times 0.155\ h^{-1}$$

$$= \underline{3.90\ g\ glucose/Lh}$$

f) Specific Rate of Glucose Consumption

$$q_G = \frac{Q_G}{X} = \frac{3.90\ g/Lh}{2.45\ g/L}$$

$$= \underline{1.59\ g\ glucose/g\ cells\ h}$$

Note: An interesting calculation can be made from the value of q_G:

$$\frac{1.59\ \text{g gluc}}{\text{g cells h}} \times \frac{\text{mol gluc}}{180\ \text{g gluc}} \times \frac{6.02 \times 10^{23}\ \text{mlcs}}{\text{mol}}$$

$$\times \frac{2 \times 10^{-13}\ \text{g cells}}{\text{cell}} \times \frac{\text{h}}{3600\ \text{s}}$$

= 296,000 mlcs glucose/second (per cell)

g) Volumetric Rate of Pyruvate Production

$$Q_P = (P - P_{IN}) \times D = (17.892 - 0.0)\ g/L \times 0.155\ h^{-1}$$

$$= \underline{2.77\ g\ pyruvate/Lh}$$

h) Specific Rate of Pyruvate Production

$$q_P = \frac{Q_P}{X} = \frac{2.77\ g/Lh}{2.45\ g/L}$$

$$= \underline{1.13\ g\ pyruvate/g\ cells\ h}$$

i) Oxygen

The gas composition is at STP and is dry basis. Thus, the only components of the gas are N_2, O_2, & CO_2.

Inlet:

$$x_N + x_O + x_{CO2} = 1$$

$$x_N + 0.2092 + 0 = 1$$

$$x_N = 0.7908$$

$$n^{IN}_{TOTAL} = \frac{PQ_{GAS}}{RT} = \frac{(1000)(1\ atm)(1.00\ L/min)}{(0.08206\ L\ atm/molK)(273.15K)}$$

i) Oxygen (cont'd)

$n_{TOTAL}^{IN} = 44.61$ mmol/min

$n_N^{IN} = (0.7908)(44.61) = 35.28$ mmol/min

$n_O^{IN} = (0.2092)(44.61) = 9.33$ mmol/min

Outlet:

$$x_N + x_O + x_{CO2} = 1$$

$$x_N + 0.2013 + 0.0067 = 1$$

$x_N = 0.7920$ $\qquad n_N^{IN} = n_N^{OUT} = 35.28$ mmol/min

i) Oxygen (cont'd)

$n_{TOTAL}^{OUT} = n_N^{OUT} / x_N = 35.28/0.7920 = 44.55$ mmol/min

$n_O^{OUT} = (0.2013)(44.55) = 8.97$ mmol/min

$n_{CO2}^{OUT} = (0.0067)(44.55) = 0.30$ mmol/min

$OUR = (n_O^{IN} - n_O^{OUT})/V = [(9.33 – 8.97)$ mmol/min$]/1.00$ L

$= 0.36$ mmol/Lmin = <u>21.96 mmol/Lh</u>

$q_O = OUR/X = (21.96$ mmol/Lh$)/(2.45$ g/L$) =$ <u>8.96 mmol/gh</u>

j) Carbon Dioxide (CO_2 Evolution Rate, CER)

$$CER = (n_{CO2}^{OUT} - n_{CO2}^{IN})/V = [(0.30 – 0.00)\ mmol/min]/1.00\ L$$

$$= 0.30\ mmol/Lmin = \underline{17.91\ mmol/Lh}$$

$$q_{CO2} = CER/X = (17.91\ mmol/Lh)/(2.45\ g/L) = \underline{7.31\ mmol/gh}$$

k) RQ

The Respiratory Quotient (RQ) or Respiratory Coefficient is merely the molar ratio of CO_2 generated to O_2 consumed. It can also be calculated from the ratio of rates:

$$RQ = \frac{CER}{OUR}$$

$$RQ = \frac{17.91 \text{ mmol/Lh}}{21.96 \text{ mmol/Lh}} = 0.816 \text{ mol/mol}$$

Note that complete ‘combustion’ of glucose would result in an RQ of 1.00.

I) Carbon Balance

Carbon Generated

CO_2: (17.91 mmol CO_2/Lh)(1.00 L)(1 mol C/mol CO_2)

= 17.91 mmol C/h

Pyruvate: (2.77 g pyru/Lh)(mol pyru/87.06 g pyru)
(1.00 L)(1000 mmol/mol)(3 mol C/mol pyru)

= 95.45 mmol C/h

Biomass: (2.45 g DCW/L)(0.155 h^{-1})(1.00 L)
(mol DCW/24.70 g DCW†)(1000 mmol/mol)
(1 mol C/mol DCW)

= 15.37 mmol C/h

I) Carbon Balance (cont'd)

†Battley (1991, 2003) found apparent "unit carbon" molecular formula of *E. coli* to be:

$CH_{1.595}N_{0.263}O_{0.374}P_{0.023}K_{0.013}S_{0.006}$

FW = 24.70 g DCW/mol

Notes:

A "unit carbon" formula has subscript of 1.00 for C.
% C by mass is 48.6%
% N by mass is 14.9%
% P by mass is 2.9%

I) Carbon Balance (cont'd)

Carbon Consumed

Glucose: (3.90 g gluc/Lh)(mol gluc/180.16 g gluc)
(1.00 L)(1000 mmol/mol)(6 mol C/mol gluc)

= 129.88 mmol C/h

Acetate: (1.023 -0.000 g/L)(0.155 h^{-1})(1.00 L)
(mol acet/59.05 g acet)(1000 mmol/mol)
(2 mol C/mol acet)

= 5.37 mmol C/h

I) Carbon Balance (cont'd)

Carbon Balance

Total C Generated = 17.19 + 95.45 + 15.37
= 128.62 mmol C/h

Total C Consumed = 129.88 + 5.37
= 135.3 mmol C/h

$$\text{Carbon Recovery} = \frac{\text{Carbon Generated}}{\text{Carbon Consumed}} = \frac{128.62}{135.3}$$

$$= \underline{95.1\%}$$

4. Modes of Operation – Exponential Fed-Batch

a. Motivations

1) Oxygen Transfer

$$OUR = OTR$$

$$m_O + \frac{\mu X}{Y_{X/O}} = k_L a(c^*_{O2} - c^l_{O2})$$

$$0.014 + \frac{\mu X}{(1.87)} = 180(8.0 - 0.0)(0.001)$$

We examined our lab's 2.0 L fermenters, and the $k_L a$ obtained is about 180 h^{-1}

$$\mu X = 2.7 \text{ g/Lh}$$

$\underline{\mu X = \quad 2.7 \text{ g/Lh}}$

$\mu_{MAX} = \quad 0.80 \text{ h}^{-1}$

Which means that, based on using air for O_2 transfer, the maximum cell concentration that can be reached by cells growing at μ_{MAX} is 3.3 g/L (OD ≈ 9.5)

However, if we reduce the growth rate of cells to $\mu = 0.1 \text{ h}^{-1}$, we can reach a cell concentration of 26.7 g/L (OD ≈ 76)

In other words, because of oxygen transfer, the lower the growth rate allowed, the lower the oxygen transfer rate demanded.

2) Heat Transfer

$$Q_{MET}/V = 0.12Q_O = 0.12\ OUR = 0.12\left(m_O + \frac{\mu X}{Y_{X/O}} \right)$$

The maximum Q_{MET}/V which can be withdrawn in a larger fermenter is about 12 kcal/Lh, meaning that the maximum OUR is about 100 mmol/Lh = 3.2 g/Lh.

$$0.014 + \frac{\mu X}{(1.87)} = 3.2$$

$$\mu X = 6.0\ g/Lh$$

$\mu X = 6.0$ g/Lh

$\mu_{MAX} = 0.80\ h^{-1}$

Which means that, based on heat transfer constraints, the maximum cell concentration that can be reached in a larger fermenter by cells growing at μ_{MAX} is 7.5 g/L (OD ≈ 21).

However, if we reduce the growth rate of cells to $\mu = 0.1\ h^{-1}$, we can reach a cell concentration of 60 g/L.

Note that oxygen transfer would seem more stringent than heat transfer when air is used. However, when pure oxygen is used, heat transfer becomes more stringent.

3) Products

A chemostat does not allow the accumulation of a product, while a batch process does not allow a process to be run where because growth is limited, a carbon source that is in excess can be partly diverted to a desired product.

A *Nutrient-Limited* Fed-Batch operation allows the best of both worlds…products accumulate but cells' metabolism will often maximally generate a product.

b. Derivation

Recall the results of our material balance:

$$\frac{d(VX)}{dt} = -F_{OUT}X + \mu XV$$

$$\frac{d(VS)}{dt} = F_{IN}S_{IN} - F_{OUT}S + r_S V$$

$$\frac{d(VP)}{dt} = -F_{OUT}P + r_P V$$

$$\frac{dV}{dt} = F_{IN} - F_{OUT}$$

We will consider a process with $F_{OUT} = 0$ and $F_{IN} = F(t)$

$$\frac{d(VX)}{dt} = \mu XV$$

$$\frac{d(VS)}{dt} = F(t)\,S_{IN} + r_S V$$

$$\frac{d(VP)}{dt} = r_P V$$

$$\frac{dV}{dt} = F(t)$$

Obviously, volume is not constant

How should process be operated to achieve a constant $\mu = \mu_C$?

1) Find F(t)

$$\frac{d(VX)}{dt} = \mu_C XV$$

From cell balance

$$\int_{X_0V_0}^{XV} \frac{d(VX)}{(VX)} = \mu_C \int_0^t dt$$

$$XV = X_0V_0\exp(\mu_C t)$$

EQN. A

Note that $X = f_1(t)$ and $V = f_2(t)$

$$\frac{d(VS)}{dt} = F(t)\,S_{IN} + r_S V$$ From substrate balance

or $$-r_S V = F(t)\,S_{IN} - \frac{VdS}{dt} - \frac{SdV}{dt}$$

If S is limiting substrate, then it is fed slower than organism can consume it. Thus,

$$S \approx 0 \quad \text{and} \quad \frac{dS}{dt} \approx 0$$

$$-r_S V = F(t)\,S_{IN}$$ EQN. B

Recall $$-r_S = m_S X + \frac{\mu X}{Y_{X/S}}$$

or for $\mu = \mu_C$ $$-r_S = X\left(m_S + \frac{\mu_C}{Y_{X/S}}\right)$$

Inserting this equation into EQN B leads to

$$F(t)\, S_{IN} = XV\left(m_S + \frac{\mu_C}{Y_{X/S}}\right)$$

Inserting EQN A into this equation leads to

$$F(t)\, S_{IN} = X_0 V_0 \exp(\mu_C t)\left(m_S + \frac{\mu_C}{Y_{X/S}}\right)$$

$$F(t) = \frac{X_0 V_0}{S_{IN}}\left(m_S + \frac{\mu_C}{Y_{X/S}}\right)\exp(\mu_C t)$$

This equation represents the feed rate F(t) needed to maintain cell growth at a constant specific growth rate of $\mu_C < \mu_{MAX}$. Note that the equation has the form:

$$F(t) = \alpha \exp(\mu_C t)$$

Exponential Feed!

2) Find V(t)

$$\frac{dV}{dt} = F(t)$$ From 'water' balance

or $$\frac{dV}{dt} = \alpha \exp(\mu_C t)$$

Integrating from V_0 (at $t = t_0$) to V at t

$$V - V_0 = \frac{1}{\mu_C} [\, \alpha \exp(\mu_C t) - \alpha \underbrace{\exp(\mu_C t_0)}_{1} \,]$$

$$V - V_0 = \frac{\alpha}{\mu_C} [\exp(\mu_C t) - 1]$$

$$V = V_0 + \frac{\frac{X_0 V_0}{S_{IN}} \left(m_S + \frac{\mu_C}{Y_{X/S}} \right)}{\mu_C} [\exp(\mu_C t) - 1]$$

$$\boxed{V(t) = V_0 (1 + \beta[\exp(\mu_C t) - 1])}$$

where $\beta = \frac{X_0}{S_{IN}} \left(\frac{m_S}{\mu_C} + \frac{1}{Y_{X/S}} \right)$ Dimensionless

3) Find X(t)

Recall $XV = X_0V_0\exp(\mu_C t)$

$$X(t) = \frac{X_0V_0\exp(\mu_C t)}{V(t)}$$

$$\boxed{X(t) = \frac{X_0\exp(\mu_C t)}{1 + \beta[\exp(\mu_C t) - 1]}}$$

where $\beta = \frac{X_0}{S_{IN}}\left(\frac{m_S}{\mu_C} + \frac{1}{Y_{X/S}}\right)$

c. Example Calculations

What feed is necessary to grow cells at $\mu_C = 0.15\ h^{-1}$?

Data:

$V_0 = 1.5\ L$
$X_0 = 2.0\ g/L$
$S_{IN} = 500\ g/L$
$m_S = 0.05\ g/gh$
$Y_{X/S} = 0.44\ g/g$

$\beta = 0.0104$

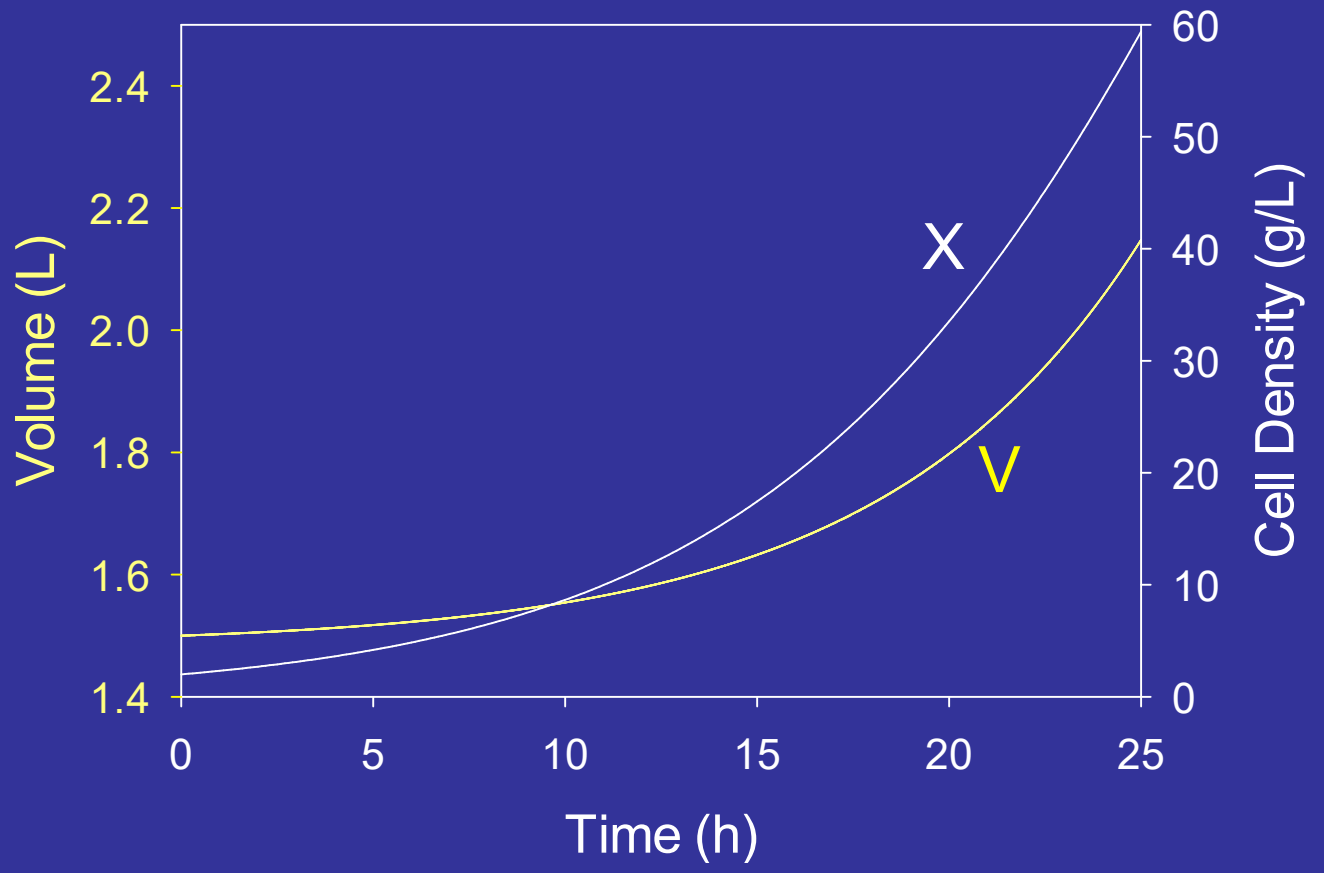
c. Example Calculations (cont'd)

$$F(t) = 2.35 \exp(0.15t) \quad [mL/h]$$

$$V(t) = 1.4844 + 0.0156 \exp(0.15t) \quad [L]$$

$$X(t) = \frac{2 \exp(0.15t)}{0.9896 + 0.0104 \exp(0.15t)} \quad [g/L]$$

Note that cell density increases less than exponentially (because the volume is increasing and diluting the exponential growth).



Note that these calculations presume that all other nutrients are present in excess.

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