2-29-00

Theory of CC

Discuss calc. of yields and m

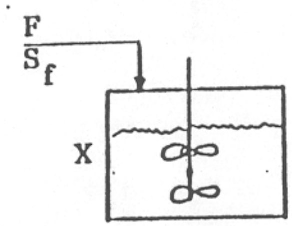
TOPIC 10:

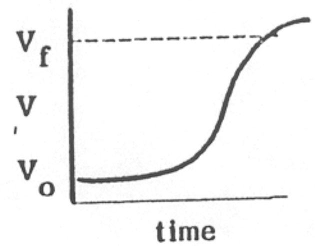
THEORY AND APPLICATIONS OF CONTINUOUS CULTURE

BY:

CHARLES L. COONEY

FED-BATCH FERMENTATION KINETICS





Growth in batch culture:

$$\frac{V d\,[X(t)]}{dt} = V\,\mu(t)\,[X(t)]$$

Fed-batch culture:

$$\frac{d\,[(X(t)\,V(t)]}{dt} = \mu(t)\,[X(t)\,V(t)]$$

$$\frac{d\,[V(t)]}{dt} = F_1(t) + \ldots + F_n(t)$$

Substrate balance:

$$\frac{d\,[S(t)\,V(t)]}{dt} = F_s(t)\,S_f - \frac{\mu(t)\,[X(t)\,V(t)]}{Y(t)}$$

Operating objective: $\frac{dS(t)}{dt} = 0$ when $S(t)$ is near 0

Therefore $\frac{S(t)\,d\,V(t) + V(t)\,dS(t)}{dt} \simeq 0$

And $F_s(t) = \frac{\mu(t)\,[X(t)\,V(t)]}{Y(t)\,S_f}$

COMPUTER CONTROL OF YEAST PRODUCTION

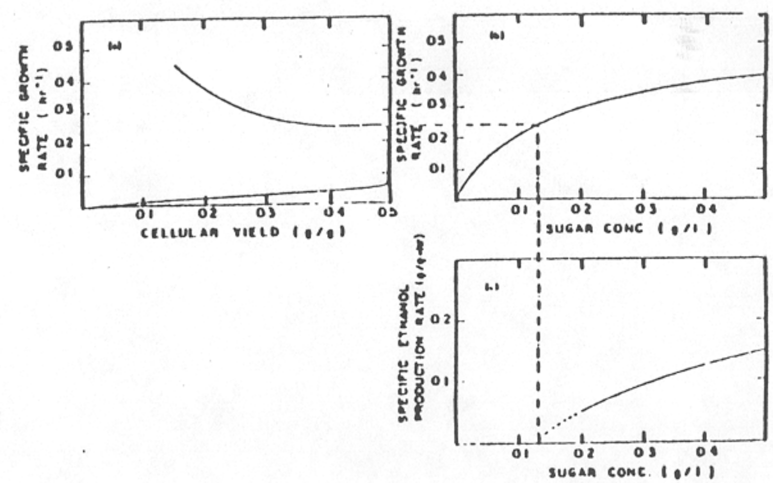
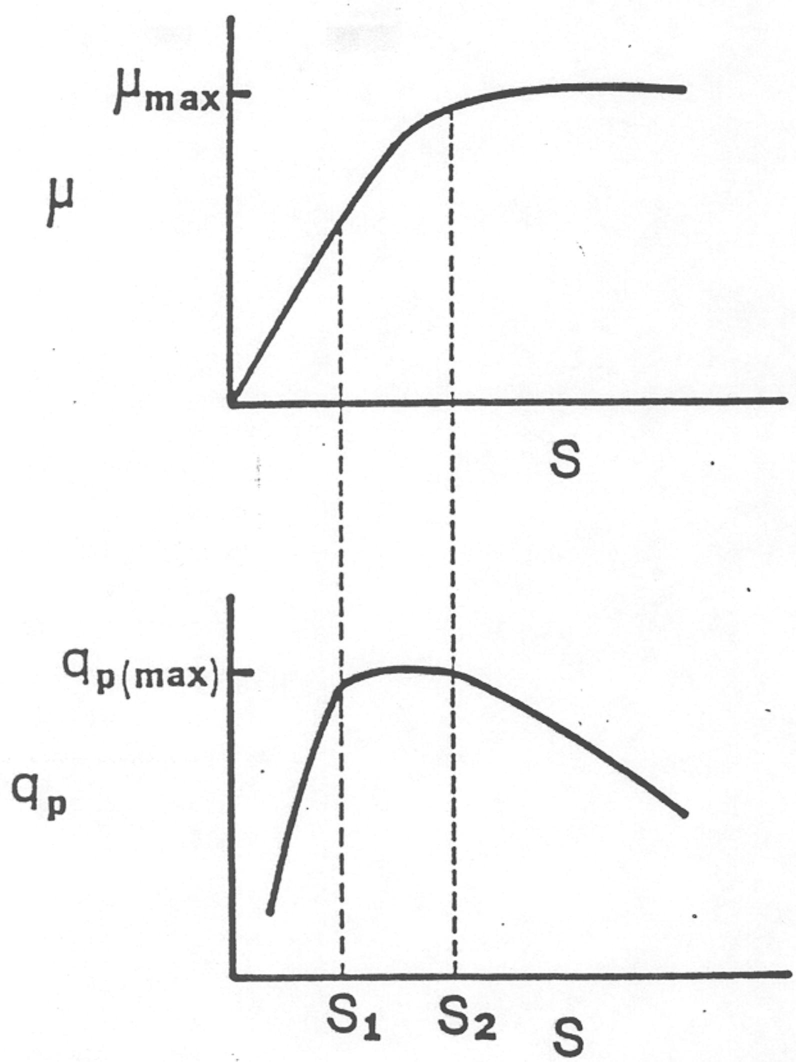


Fig. 3. Relationships between (a) specific growth rate and cell yield, (b) sugar concentration and specific growth rate, and (c) sugar concentration and ethanol production rate in a bakers' yeast fermentation.

Microbial Growth and Product Formation



μ=specific growth rate

S=substrate concentration

q_p=specific productivity

Types of Continuous Culture Systems

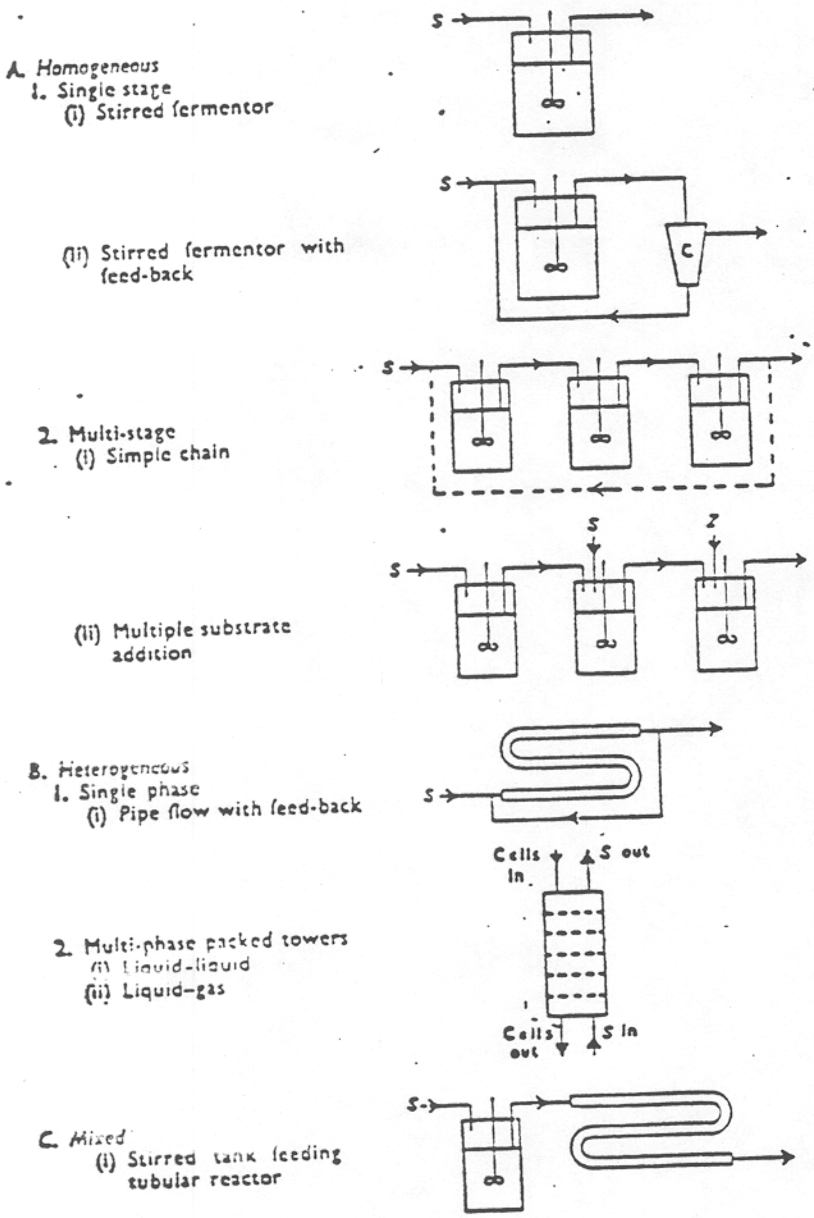


FIG. 1. *Types of 'open' continuous culture systems*
S indicates substrate addition. C indicates continuous centrifuge or settling tank

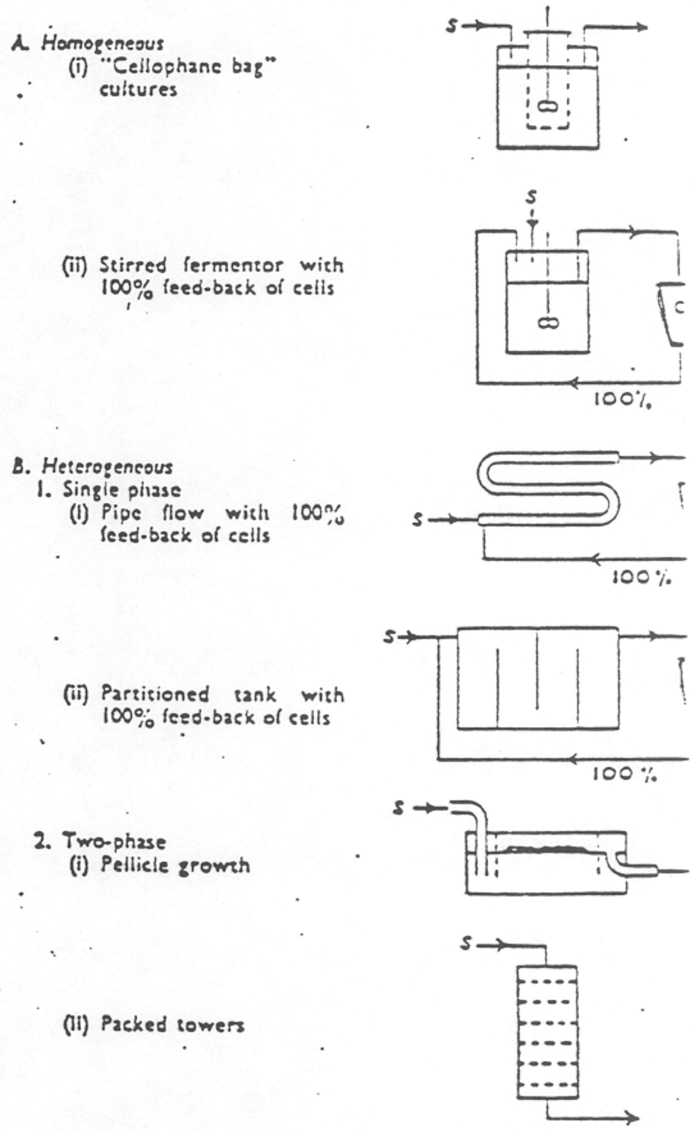
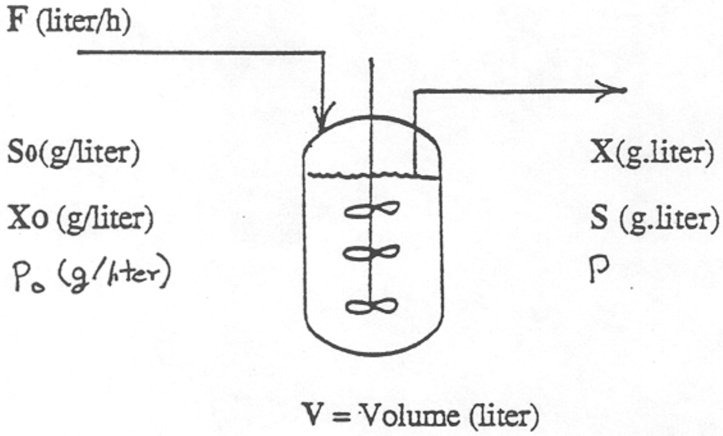


FIG. 2 *Types of 'closed' continuous culture systems*
S indicates substrate addition. C indicates continuous centrif[illegible] settling tank

Taken from Herbert (1961) Continuous Culture of Microorganisms

Society of Chemical Industries Monograph No. 12, pp. 21.

Analytical Description of a Single-Stage Chemostat



Material balance on cell mass

Accumulation = Cells In - Cells Out + Growth - Lysis

$$\frac{VdX}{dt} = F\ X_0 - F\ X + \mu XV - \alpha XV$$

At steady state (dX/dt = 0) , assume: Medium feed is sterile ($X_o = 0$) and growth is much greater than lysis ($\mu >> \alpha$)

$$\frac{VdX}{dt} = 0 = \mu XV - FX$$

$$\frac{F}{V} = D \text{ the Dilution Rate}$$

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

Specific growth rate = Dilution rate

Material balance on limiting nutrient

Accumulation = Nutrient In - Nutrient Out - (Consumption)

$$\frac{VdS}{dt} = F\ S_0 - F\ S - \frac{\mu XV}{Y_{x/s}} - mXV - \frac{q_p XV}{Y_{p/s}}$$

Substrate Allocation Model

At steady state ($dS/dt = 0$), assume that $\mu/Y \gg m$ and $q_P = 0$

$$0 = F\,S_0 - F\,S - \frac{\mu X V}{Y}$$

Substituting $F/V = D$ and $D = \mu$

$$\Rightarrow \quad X = Y\,(S_0 - S)$$

Assuming a growth model $\mu = f(S)$:

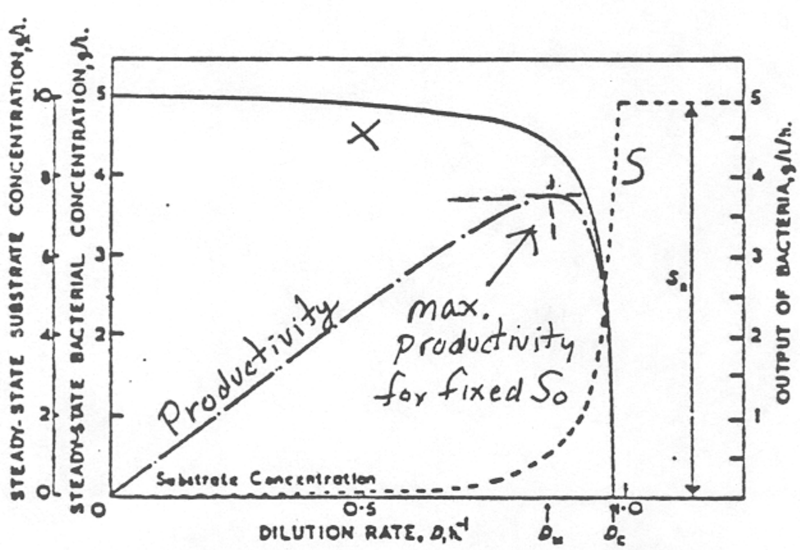
$$\mu = \mu_m \left(\frac{S}{K_s + S} \right)$$

then in continuous culture,

$$S = K_s \left(\frac{D}{D_c - D} \right)$$

and substituting

$$\Rightarrow \quad X = Y \left(S_0 - K_s \frac{D}{D_c - D} \right)$$

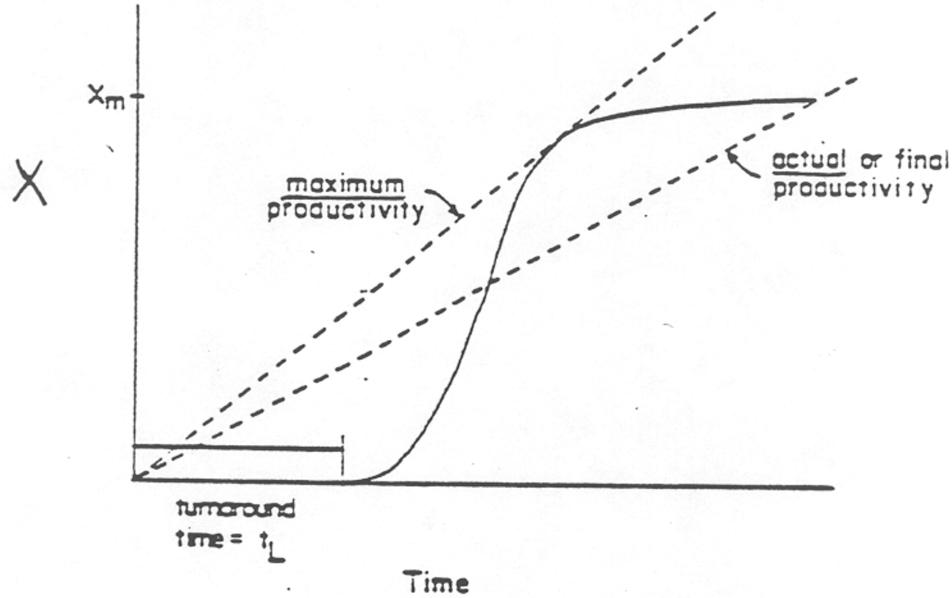


$\mu_m = 1\ h^{-1}$
$Y = 0.5\ g/g$
$K_s = 0.2\ g/L$
$S_0 = 10\ g/L$

FIG. 2 *Steady-state relationships in a single-stage continuous culture (theoretical)* The steady-state values of substrate concentration, cell concentration and output at different dilution rates are calculated from equations (14) and (15) for an organism with the following growth constants: μ_m = 1·0 h.⁻¹, Y = 0·5 and K_s = 0·2 g./l.; and a substrate concentration in the inflowing medium of s_R = 10 g./l.

——— bacterial concentration
—·—· output of bacteria
– – – substrate concentration.

5



$$\text{Productivity} = \frac{\mu m\, Y \left(\frac{x_m - x_o}{x_m}\right)}{\ln \frac{x_m}{x_o} + \mu_m t_L}$$

x_m = final cell mass

x_o = inoculum site

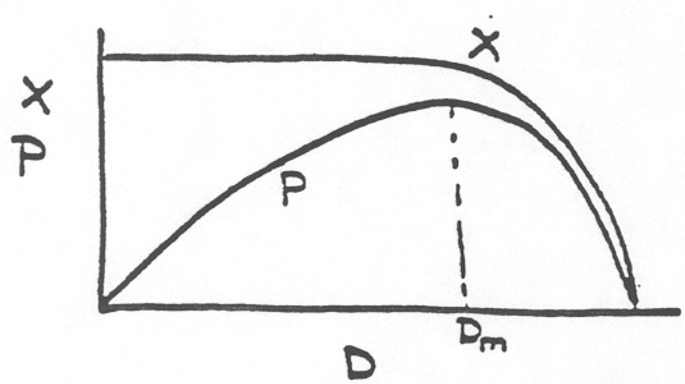
t_L = turnaround time

(Herbert et al., 1956. J. Gen. Microbiol. 5:698)

Typical Turnaround Times:

	t_2	t_{ferm}
Yeast Fermentation	4- 8	18- 24
Antibiotic Fermentation	12-18	120-240

Productivity in Continuous Culture



Productivity = P = DX (g cell/liter-h)

$$\frac{\text{Continuous Productivity}}{\text{Batch Productivity}} = \ln \frac{X_m}{X_i} + t_1 \mu_{max}$$

[x_m = max. cell density
[x_i = initial cell density
[t_1 = turnaround time
[μ_m = max. specific growth rate

ASSUMPTIONS IN THE DEVELOPMENT OF THE THEORY OF A SINGLE-STAGE CHEMOSTAT

1. Liquid volume, V, is a constant
2. Vessel is well-mixed
3. Single growth limiting nutrient, S_0
4. Steady state, $dX/dt = dS/dt = 0$
5. Medium feed is sterile, $X_0 = 0$
6. Growth is much faster than cell lysis, $\mu >> \alpha$
7. No product formation, $q_p = 0$
8. Cell maintenance is low, $\mu/Y >> m$
9. No mass or heat transfer limitations
10. Cell Yield , Y, is constant
11. Growth model, $\mu = F(s)$, e.g. the Monod Model

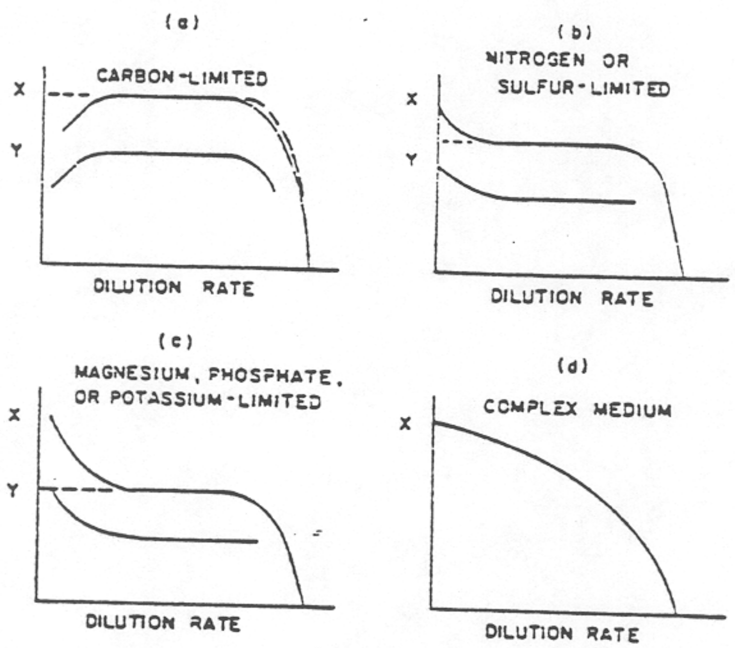
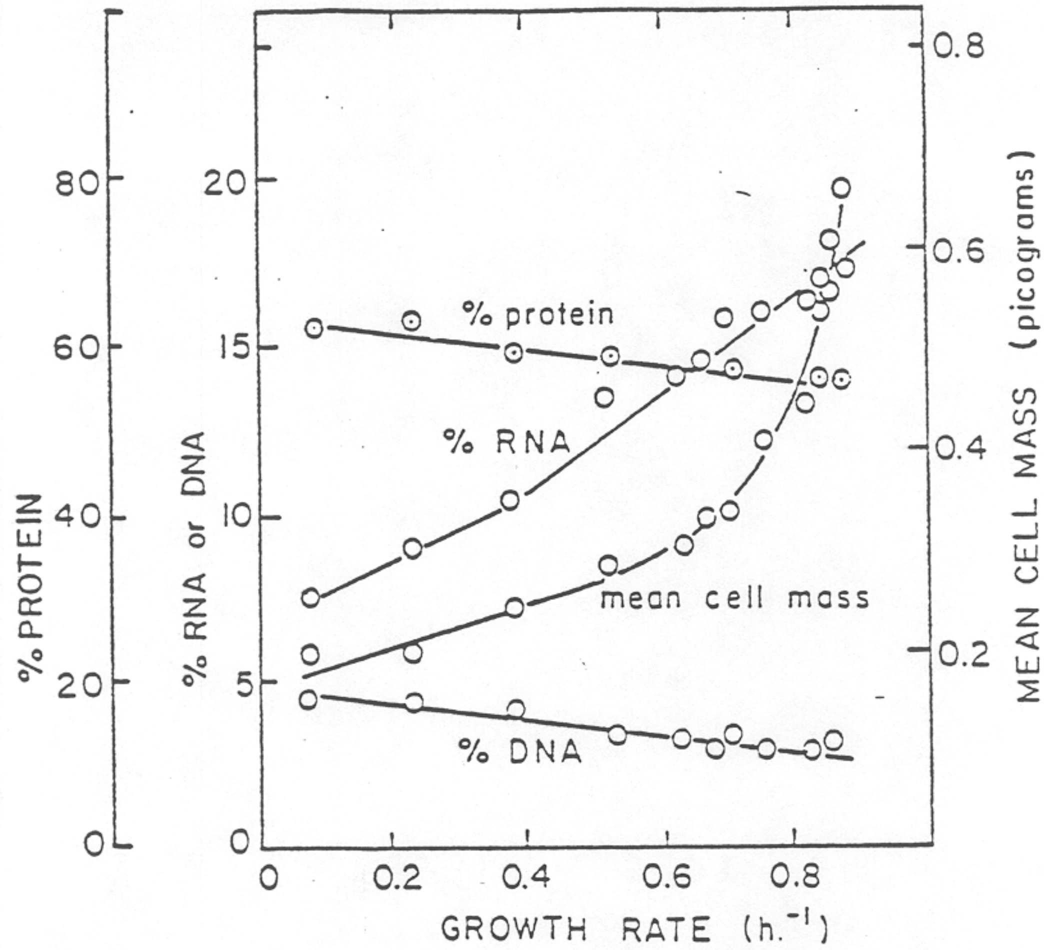
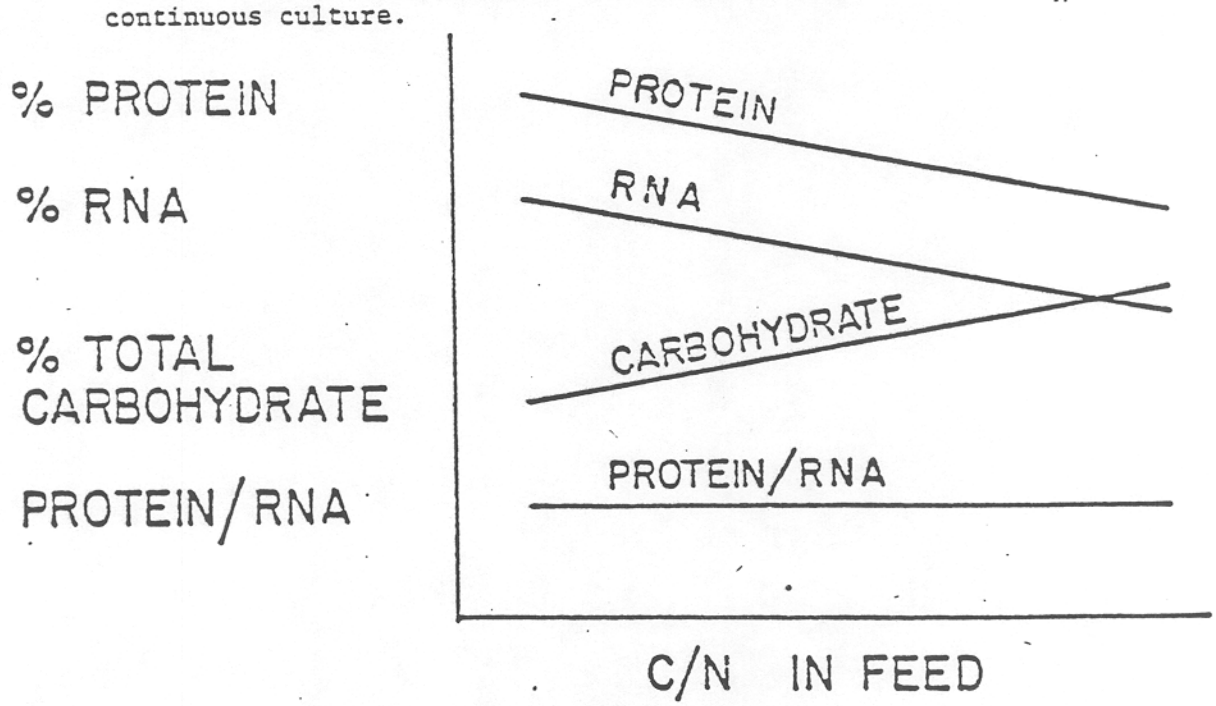


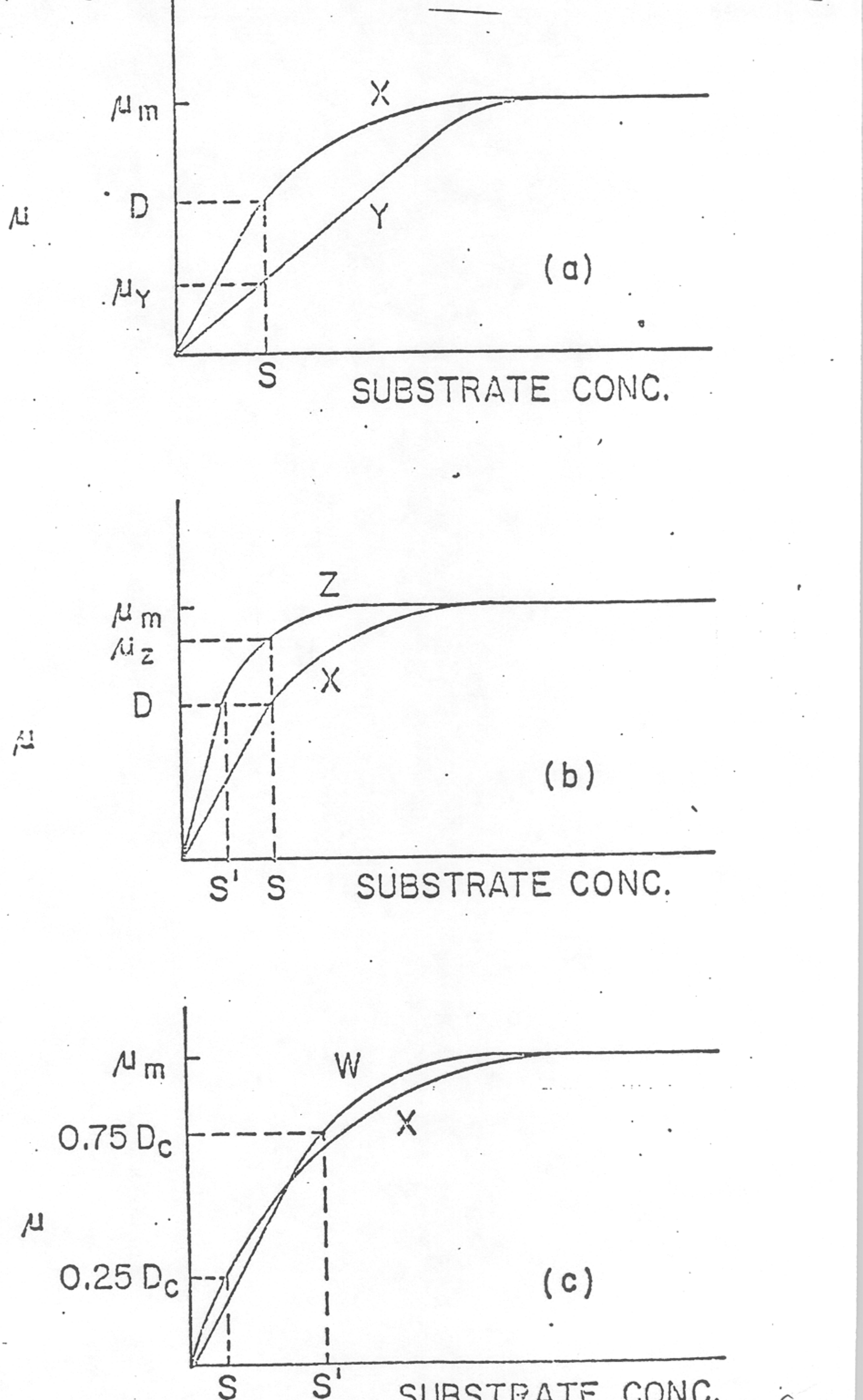
Figure 7.6 Examples of nonideal behavior of the single-stage, well-mixed chemostat; explanations are in the text. Dotted lines represent expected behavior.



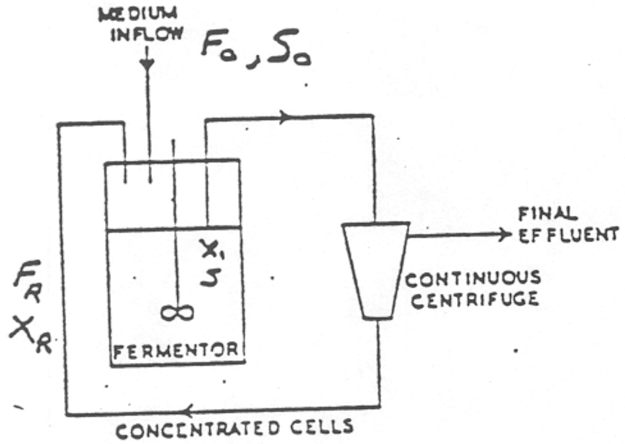
Cell composition of Aerobacter aerogenes under N-limitation at different growth (dilution) rates in continuous culture.



Variations of cell composition with dual nutrient limitation in continuous culture.


X
μ_m
D
Y
μ
μ_Y
(a)
S
SUBSTRATE CONC.
Z
μ_m
μ_z
X
D
μ
(b)
S' S
SUBSTRATE CONC.
μ_m
W
0.75 D_c
X
μ
0.25 D_c
(c)
S S'
SUBSTRATE CONC.

CELL RECYCLE IN CONTINUOUS CULTURE



Single-Stage Stirred Fermentor with

Single-Stage Stirred Fermentor with Feed-Back of Cells

$$\alpha = \frac{F_R}{F_o}$$

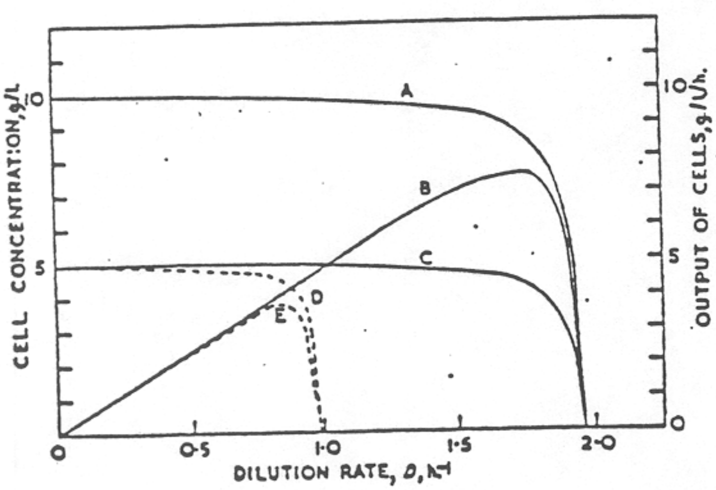
$$\beta = \frac{X_R}{X_1}$$

Steady State Equations:

$$\mu = D\,[1 - \alpha\,(\beta - 1)]$$

$$\therefore \quad \mu < D$$

$$X_1 = Y/(1 - \alpha\,(\beta-1)\,(S_o - S)$$



Steady-State Relationships in A Single-Stage Continuous Culture with Feed-Back (Theoretical)

Curves are plotted for an organism with the following growth-constants: μ_m = 1.0 h^{-1}; Y = 0.5; K_o = 0.2 g/l; and a substrate concentration in the inflowing medium of S_o = 10 g/l. Continuous curves: with feed-back (volumetric feed-back ratio α = 0.5; cell concentration factor β = 2.0). Dotted curves: without feed-back. Curve A, cell concentration in fermentor; Curve B, output of cells; Curve C, cell concentration in outflow; Curve D, cell concentration without feed-back; Curve E, output without feed-back.

CELL RECYCLE

REF: Gold, Mohagheghi, Cooney and Wang,
Biotech. Bioeng. 23:2105-2116 (1981)

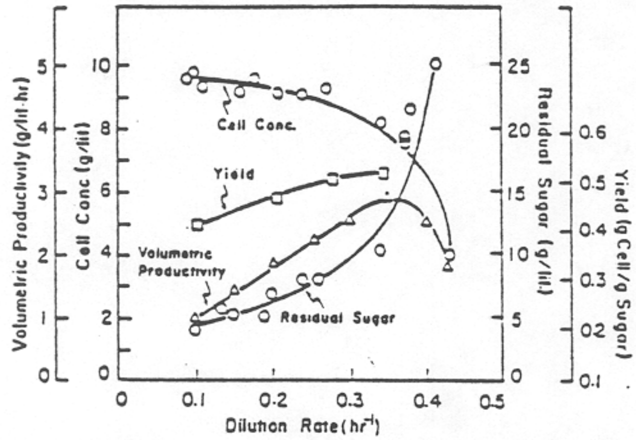


Fig. 3. Continuous culture of *C. utilis* on SSL at 30°C and pH 4.5.

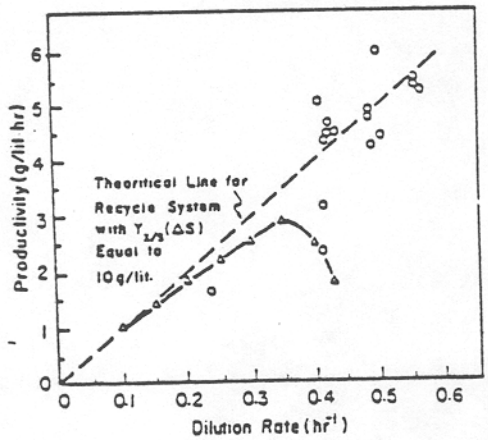


Fig. 5. Comparison of productivity for continuous culture (△) with and (○) without recycle.

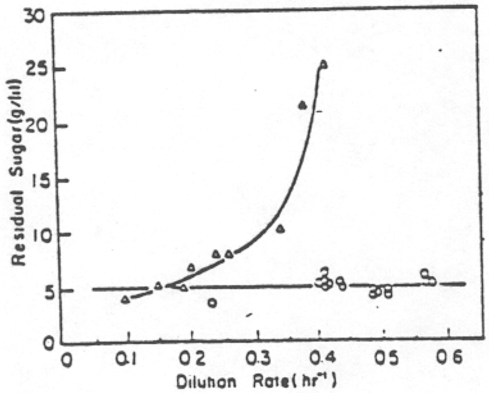
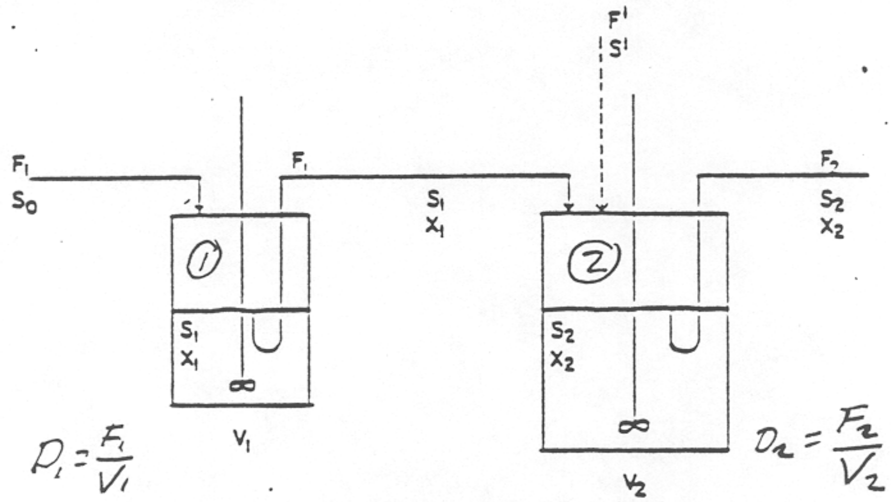


Fig. 6. Comparison of effluent sugar for continuous culture (△) with and (○) without recycle.

MULTISTAGE CONTINUOUS CULTURE



Steady State Solutions for Material Balances on
First and Second Stages

	Cell Mass Balance	Substrate Balance
First Stage	$\mu_1 = D_1$	$X_1 = Y(S_0 - S_1)$
Second Stage	$\mu_2 = D_2\left(1 - \frac{X_1}{X_2}\right)$	$X_2 = \frac{D_2}{\mu_2} Y (S_1 - S_2)$
Second Stage with additional feed system	$\mu_2 = D_2 - \frac{F_1 X_1}{V_2 X_2}$	$X_2 = \frac{Y}{\mu_2}\left(\frac{F_1 S_1}{V_2} + \frac{FS}{V_2} - D_2 S_2\right)$

PRODUCT ACCUMULATION IN CONTINUOUS CULTURE

PROBLEM:

Many investigators have proposed that penicillin be produced in continuous culture. To address this question, examine the effect of dilution rate on product concentration and yield.

DATA:

q_p	=	6 units penicillin G/mg-cell-h
X (steady state)	=	20 g/liter
M	=	0.022 g/g cell-h
$Y_{p/s}$	=	1.1 g/pen-G/g glucose
$Y_{x/s}$	=	0.5 g/g glucose

PRODUCTION OF PENICILLIN IN CONTINUOUS CULTURE

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

Substrate $$\frac{dS}{dt} = D S_0 - D S - \frac{\mu X}{Y_{x/s}} - m X - \frac{q_p X}{Y_{p/s}}$$

Product $$\frac{dP}{dt} = q_p X - D P$$

SUGAR CONSUMPTION (GM/L-HR)

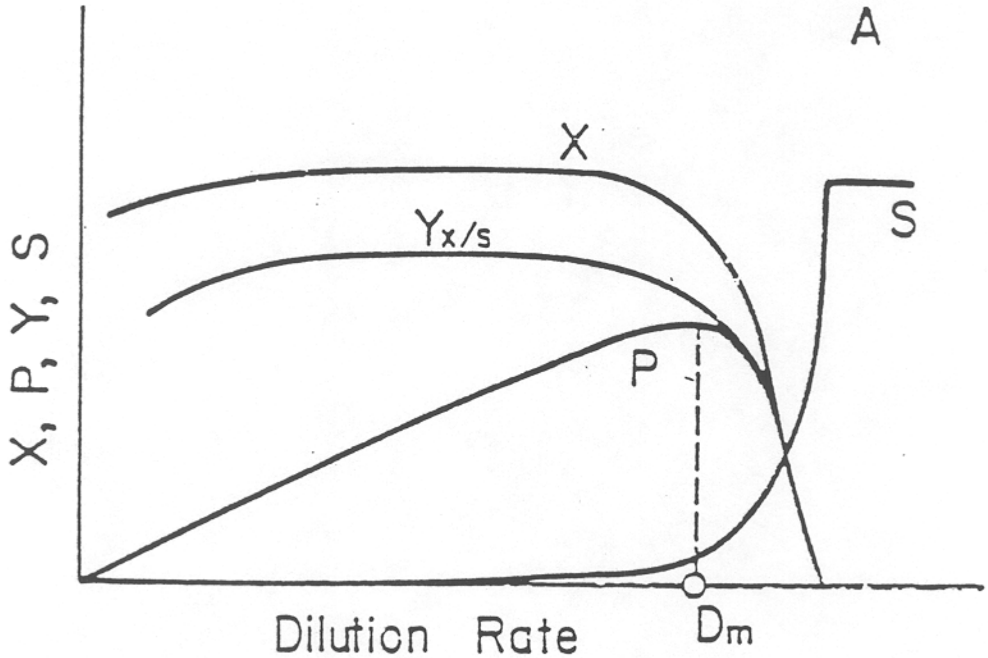
Dilution Rate (1/h)	0.05	0.1	0.15
Cell Mass	2	4	6
Maintenance	0.44	0.44	0.44
Penicillin	0.06	0.06	0.06
TOTAL	2.5	4.5	6.5

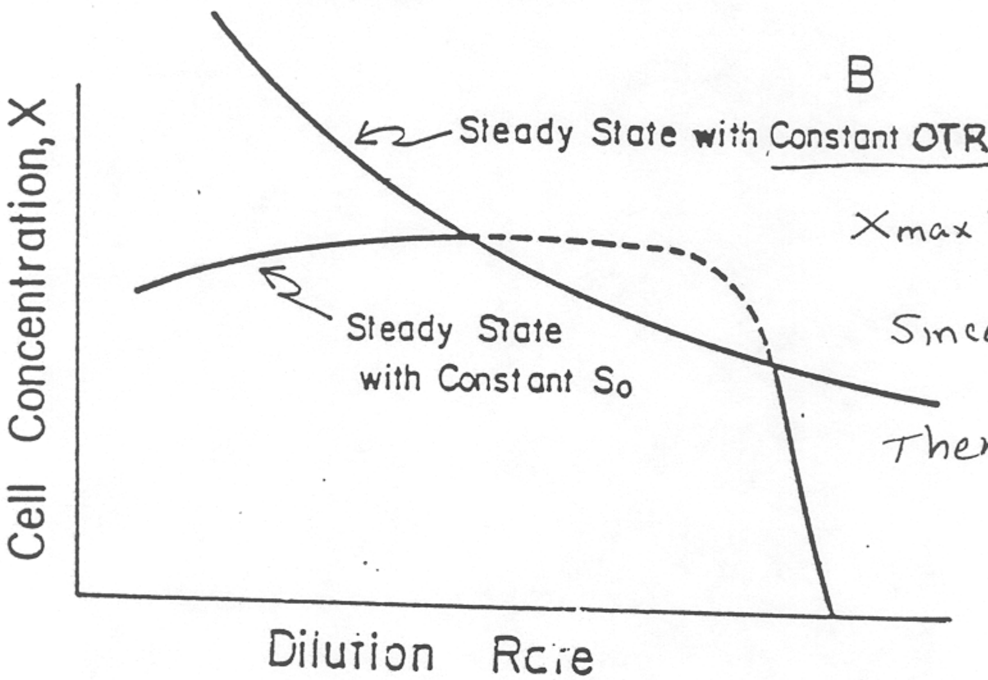
X = 20 g/liter; m = 0.022 g/g cell-h

Y = 1.1 g pen/g glucose; $Y_{x/s}$ = 0.5 g/g

q_p = 6 units/mg cell-h

Selection of Optimum Dilution Rate for Biomass Production





$X_{max} = Y_{O_2}(OTR)/D$

Since $X = Y_s(S_o - S)$

Then $S_o = \frac{(X_m + Y_s S)}{Y_c}$

COMMERCIAL APPLICATION OF CONTINUOUS CULTURE

Food and Feed Processes

- Single-Cell Protein:
  - Mycoprotein
  - Methanol
    - (ICI, *Mehtylotrophus methylophillus*)
  - Spent Sulfite Liquor
    - (*Candida utilis*)
  - Cheese whey
  - Molasses
  - n-Parafins
  - Wood hydrolyzate
- Beverage:
  - Wine
  - Beer
  - Potable alcohol
- Vinegar
- Baker's Yeast
- Yeast extract

Waste Water Treatment

- Aerobic
- Anaerobic
  - Single Stage
  - Multiple Stage - acidification/methanation

Chemicals

- Gluconic Acid
- Acetone/Butanol
- Ethanol
- Hyaluronic acid

Enzymes/Proteins

- Glucose Isomerase
- Diagnostic Enzymes
- Insulin
- Protein A

Immobilized Whole Cells

- Aspartic Acid
- Malic Acid
- 6-Aminopenicillanic Acid
- Glucose Isomerization
- Amino Acid Resolution
- Ethanol
- Waste Treatment systems

Animal Cell

- tPA (Recombinant)
- MAB (Hollow-Fiber)
- TPA/MAB (microcarrier)