ABE 580

Process Engineering of Renewable Resources

Chapter 5

Modeling Fermentation

Derivation of Monod Equation

- Empirical Model
  - Useful curve for fitting measured (empirical) data
- Theoretical Approaches
  - Cellular Energy Balance (Heijnen and Remein)
  - Cellular Redox Balance (Jin and Bethke)
  - Coupled transport/reaction (Merchuk and Asenjo)

Monod Equation

- Empirically derived from curve fitting of microbial growth data
- Constants have direct relationship to physical/chemical phenomena in fermentation

$$\frac{dX}{dt} = \mu \bullet X = \frac{\mu_{\max} \bullet S}{K_S + S} \bullet X$$

X = cell concentration (g/L)

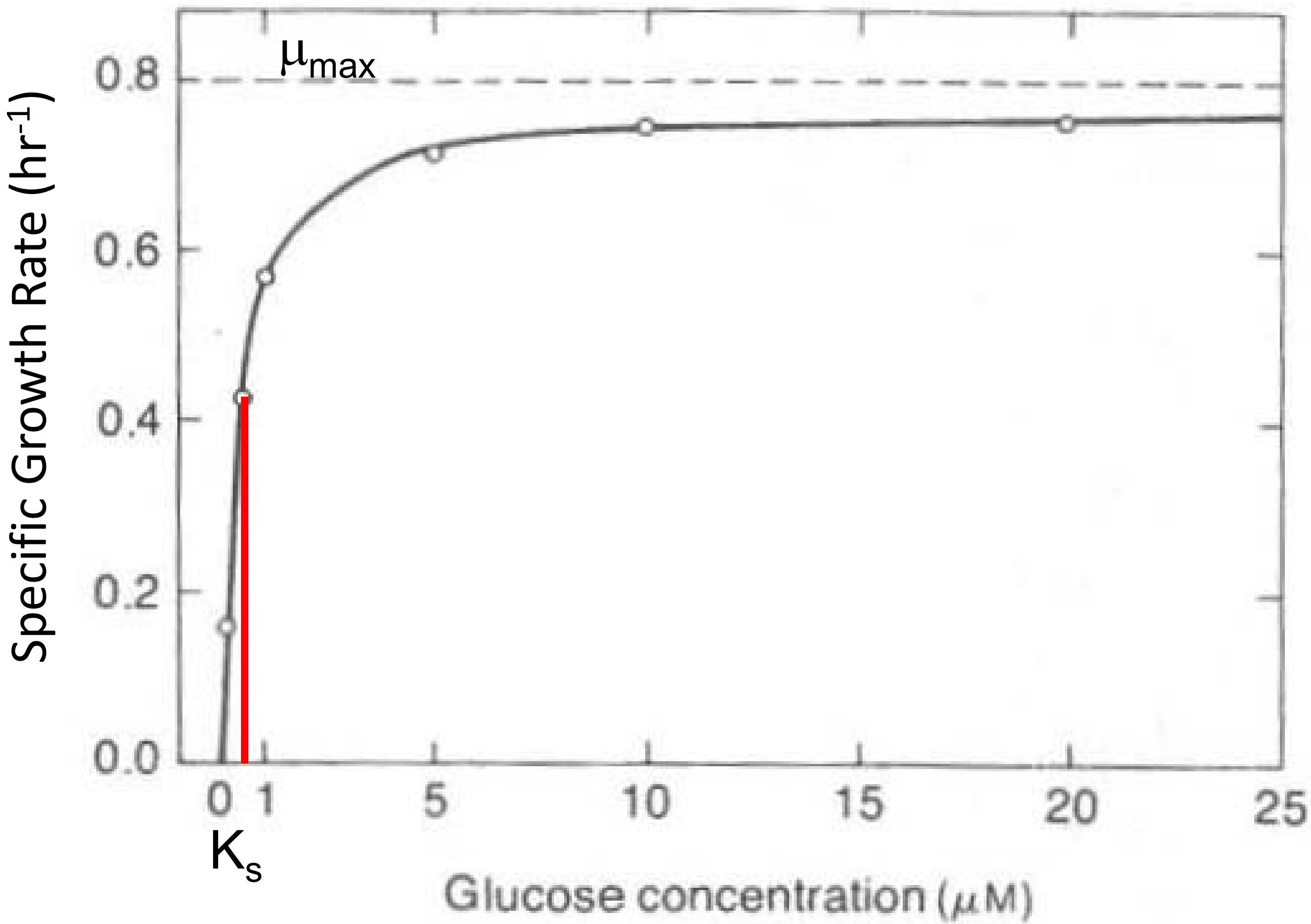
$\mu_{\max}$ = maximum specific growth rate

S = concentration of limiting nutrient

K_S = Monod coefficient

μ

$$\frac{1}{X}\frac{\Delta X}{\Delta t}$$



Linearizing the Monod Equation

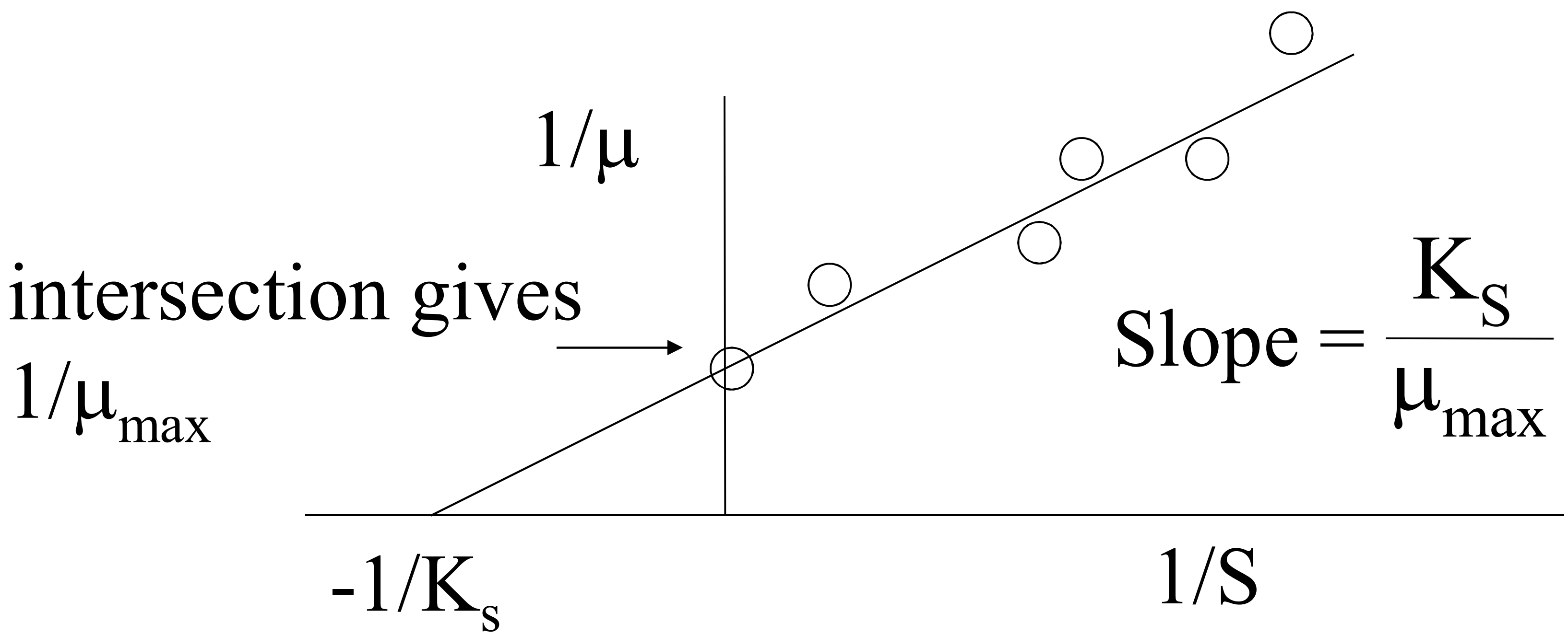
$$\mu = \frac{\mu_{\max} \bullet S}{K_S + S}$$

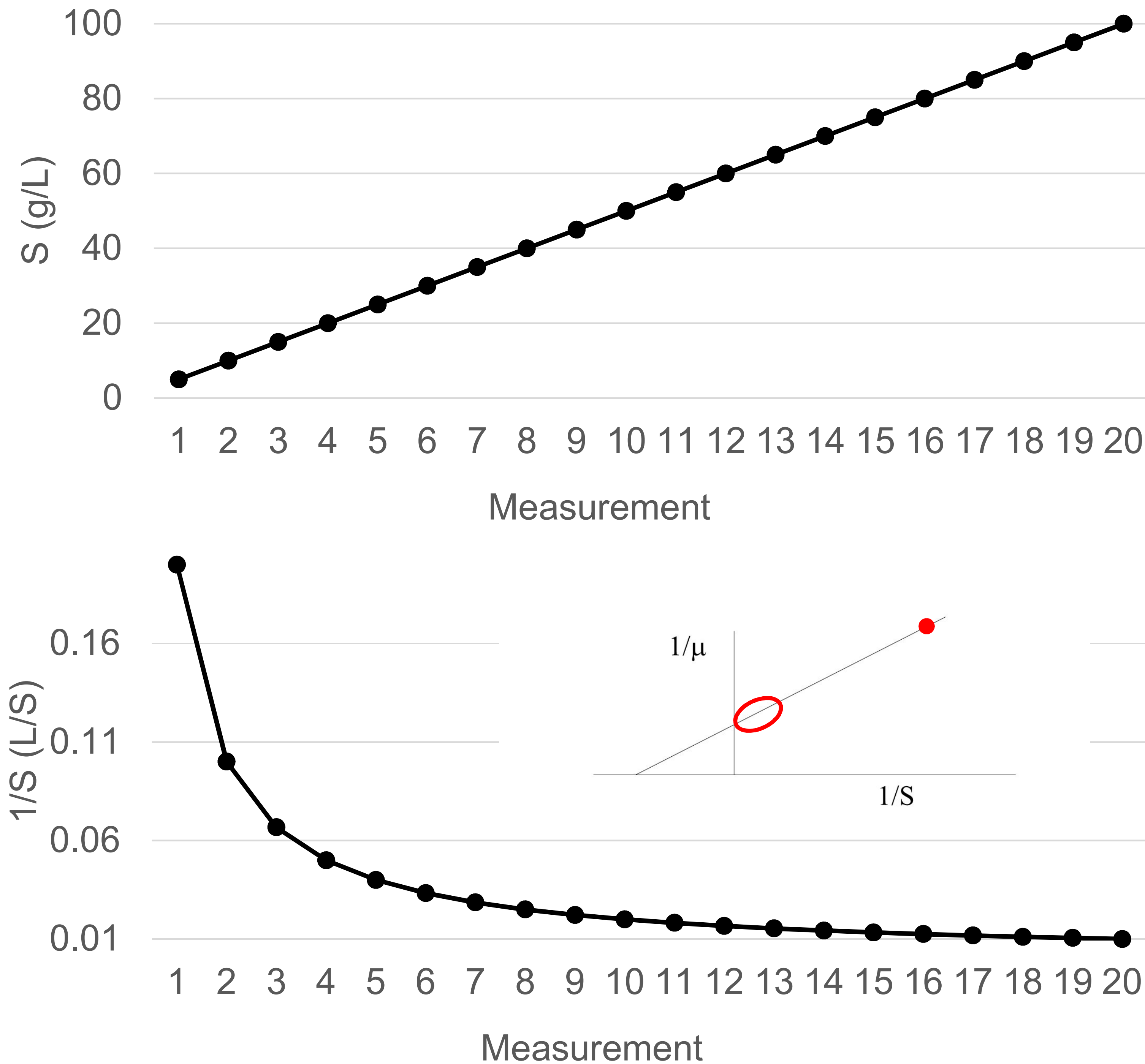
$$\frac{1}{\mu} = \frac{K_S + S}{\mu_{\max} \bullet S} = \frac{K_S}{\mu_{\max} \bullet S} + \cancel{\frac{S}{\mu_{\max} \bullet S}}$$

$$\frac{1}{\mu} = \frac{K_S}{\mu_{\max}}\left(\frac{1}{S}\right) + \frac{1}{\mu_{\max}}$$

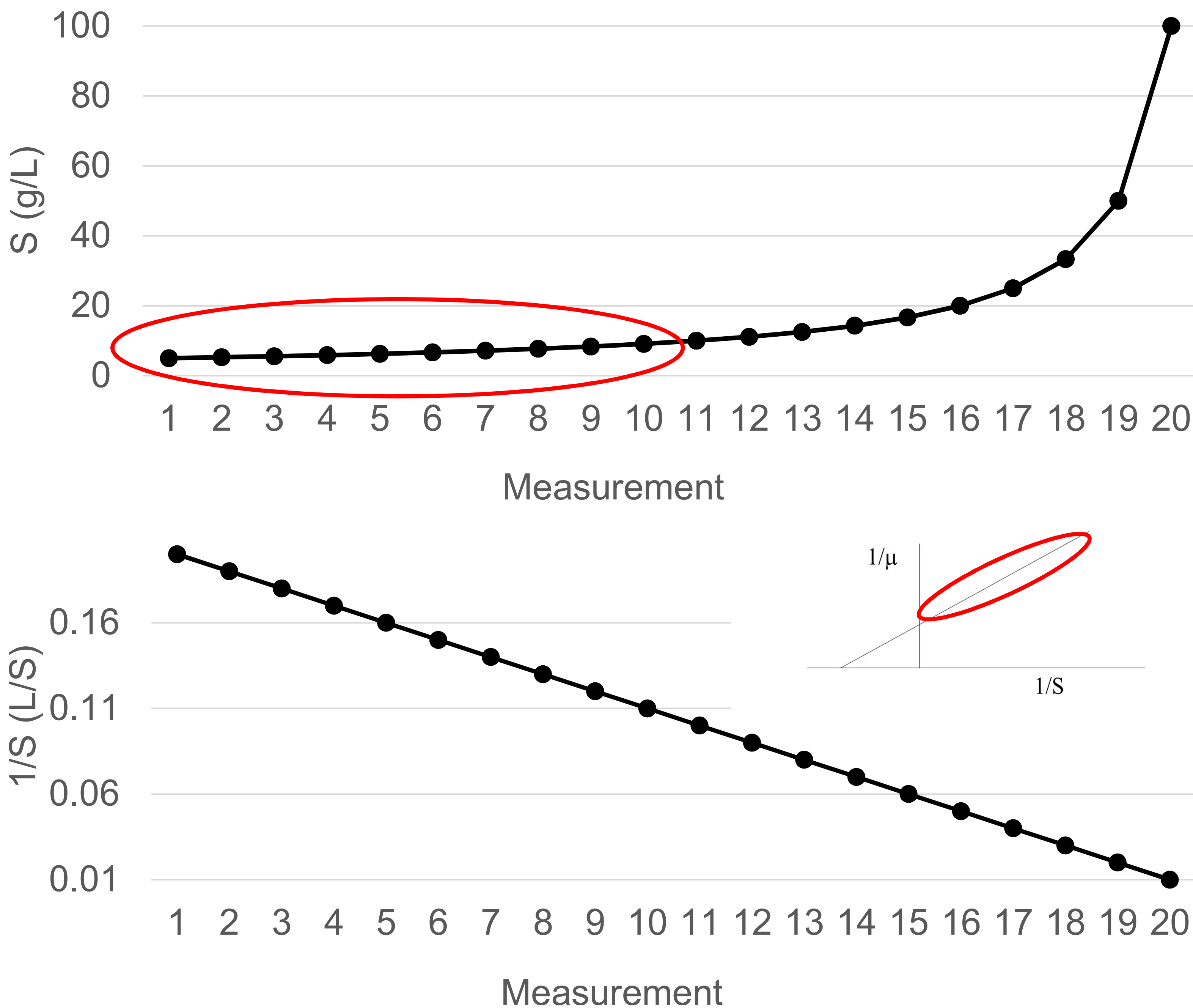
$$y = m \ (x) + b$$

Inverse Plot
(Lineweaver-Burk)





100
80
60
40
20
0
S (g/L)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Measurement
0.16
0.11
0.06
0.01
1/S (L/S)
1/μ
1/S
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Measurement



100
80
60
40
20
0
S (g/L)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Measurement
0.16
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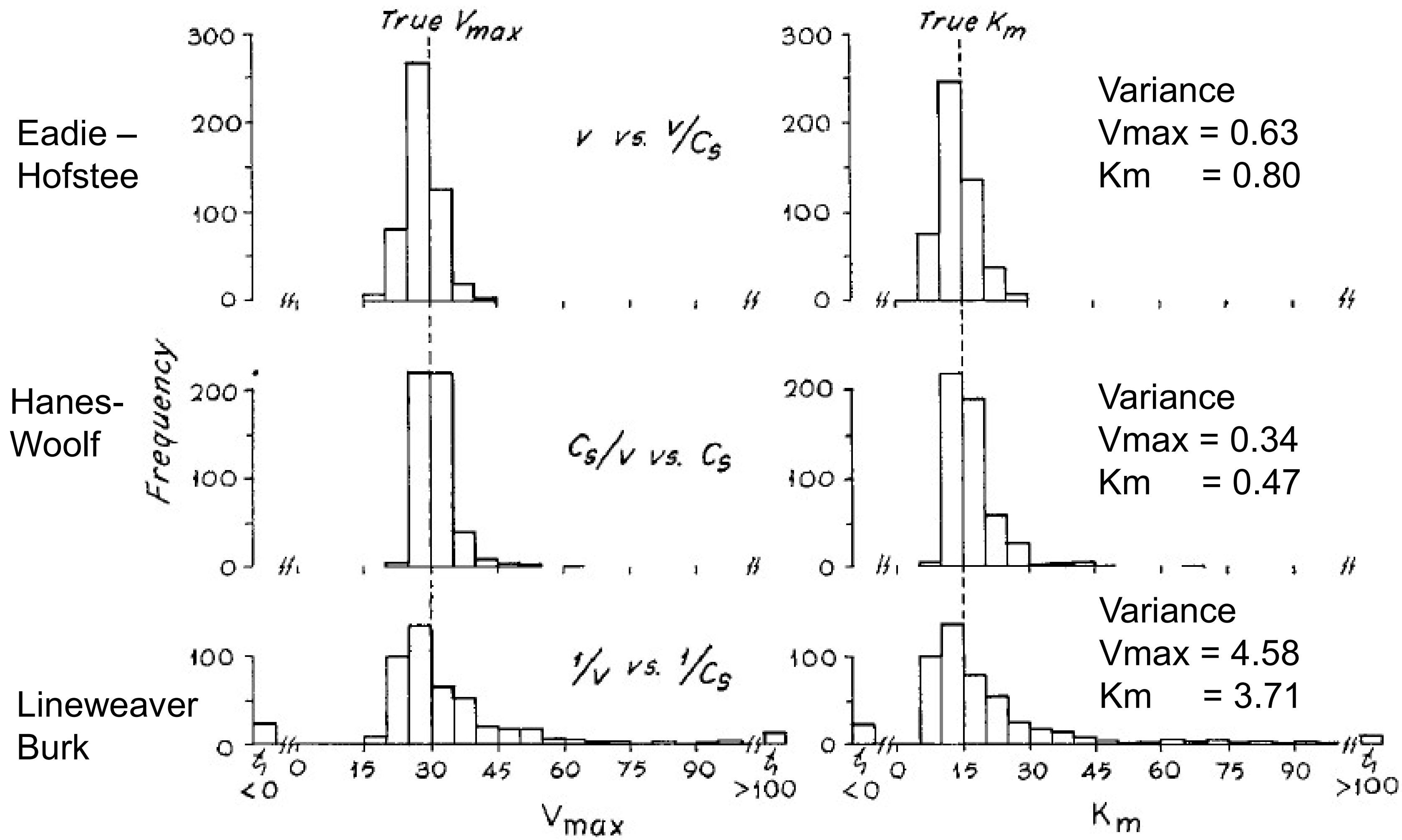
0.01
1/S (L/S)
1/μ
1/S
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Measurement



Frequency distribution for estimates of Vmax and Km for 500 "experiments" with small (4%) random, constant measurement errors

Dowd and Riggs, J. Bio. Chem., 240(2), 1965.

Coupling Substrate Use to Products (including cells)

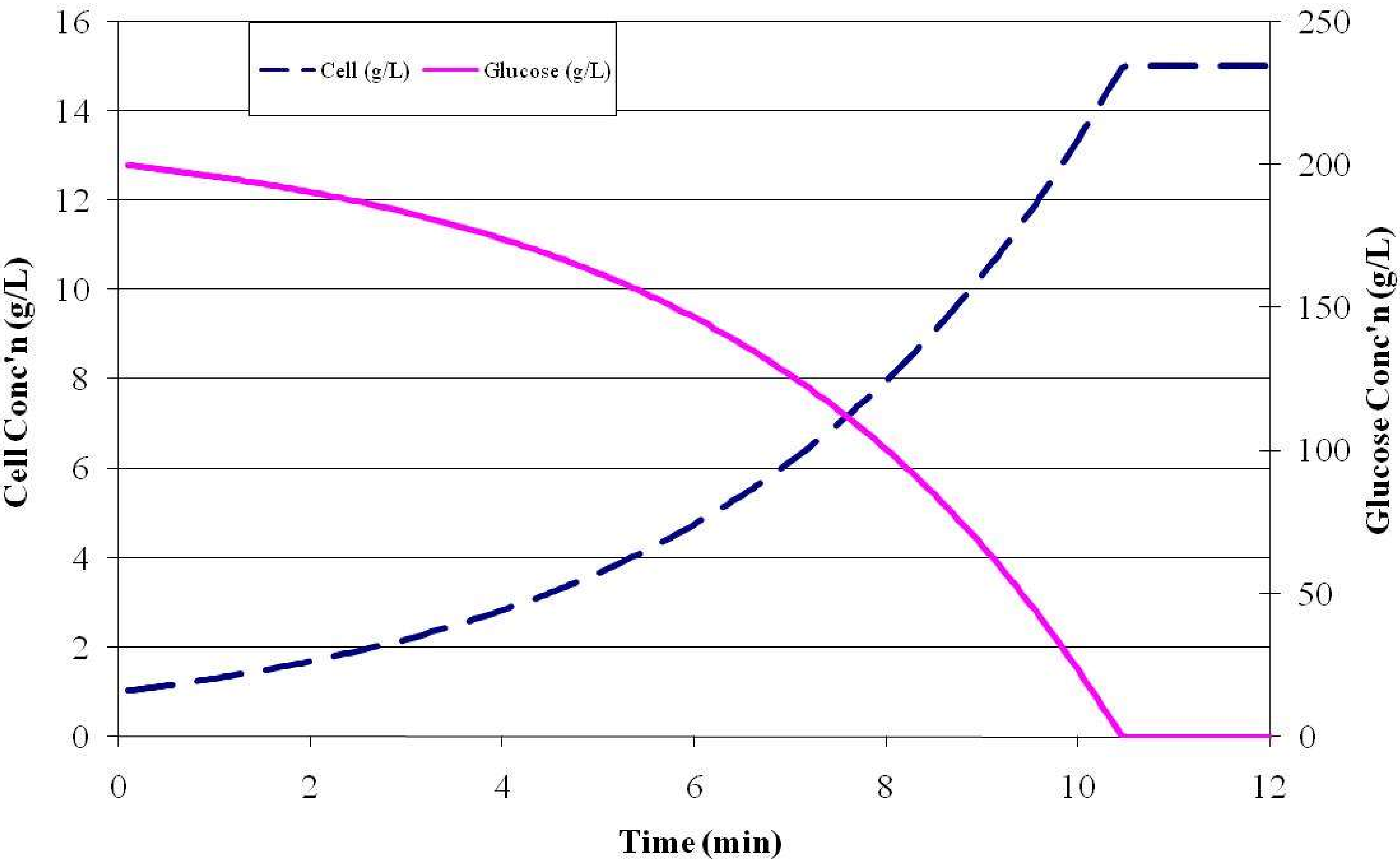
- Yield coefficient

$$Y_{X/S} = \frac{\Delta X}{\Delta S}$$

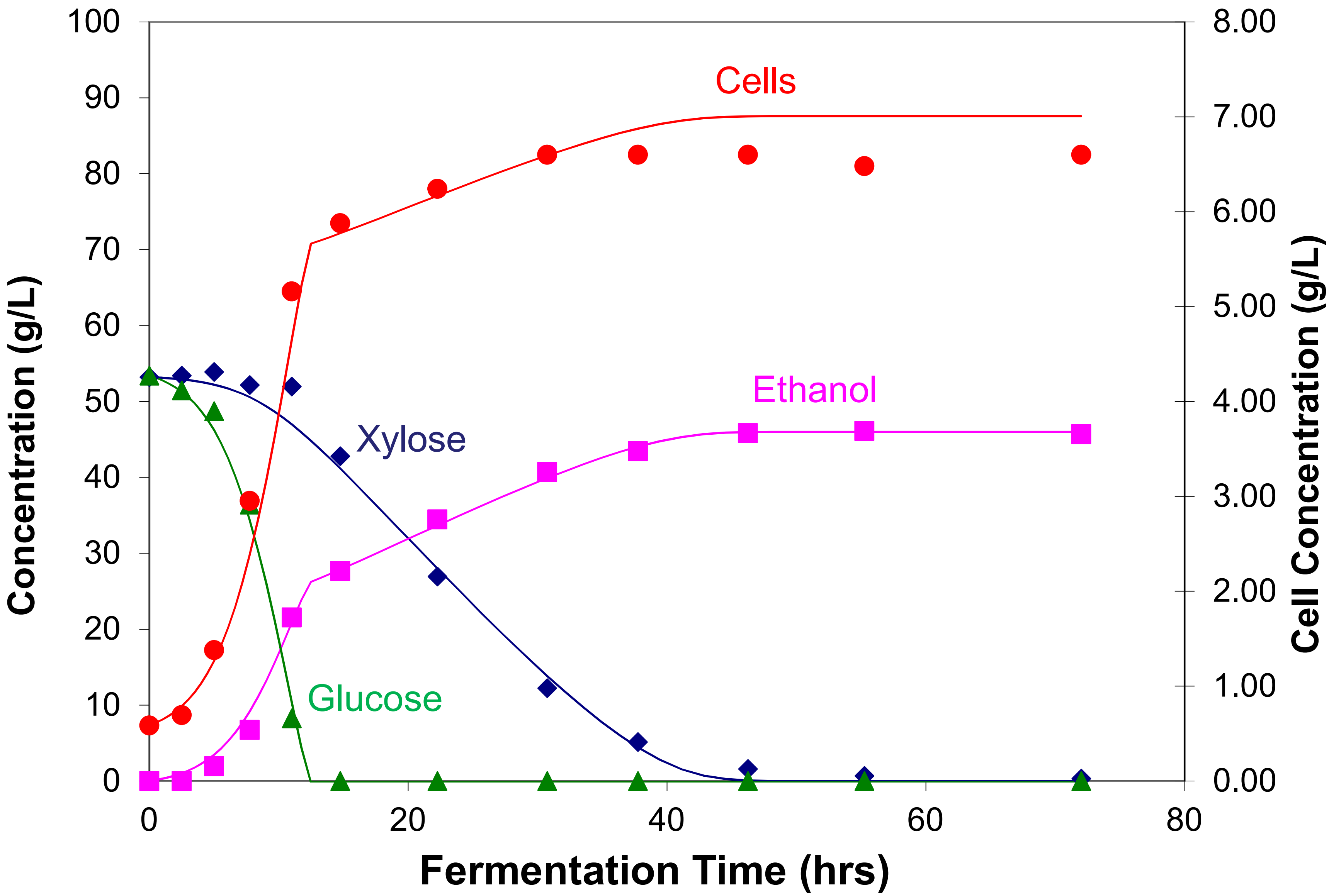
"yield of cells (X) per utilized substrate (S)"

Units = g (cells) / g (substrate)

$$\sum Y_{i/S} = \frac{\Delta X}{\Delta S} + \frac{\Delta P_1}{\Delta S} + \frac{\Delta P_2}{\Delta S} + \ldots = 1$$



Cell (g/L)
Glucose (g/L)
Cell Conc'n (g/L)
Glucose Conc'n (g/L)
Time (min)
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250



100
90
80
70
60
50
40
30
20
10
0
Concentration (g/L)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Cell Concentration (g/L)
0
20
40
60
80
Fermentation Time (hrs)
Cells
Ethanol
Xylose
Glucose

Model:
Material Balance on Cell

- Assume sugar is limiting nutrient
- Treat cell as “black box”
- True mass balance?
  - Type 1 Fermentations most amenable to mass balance approach
  - Type 3 Fermentations least amenable

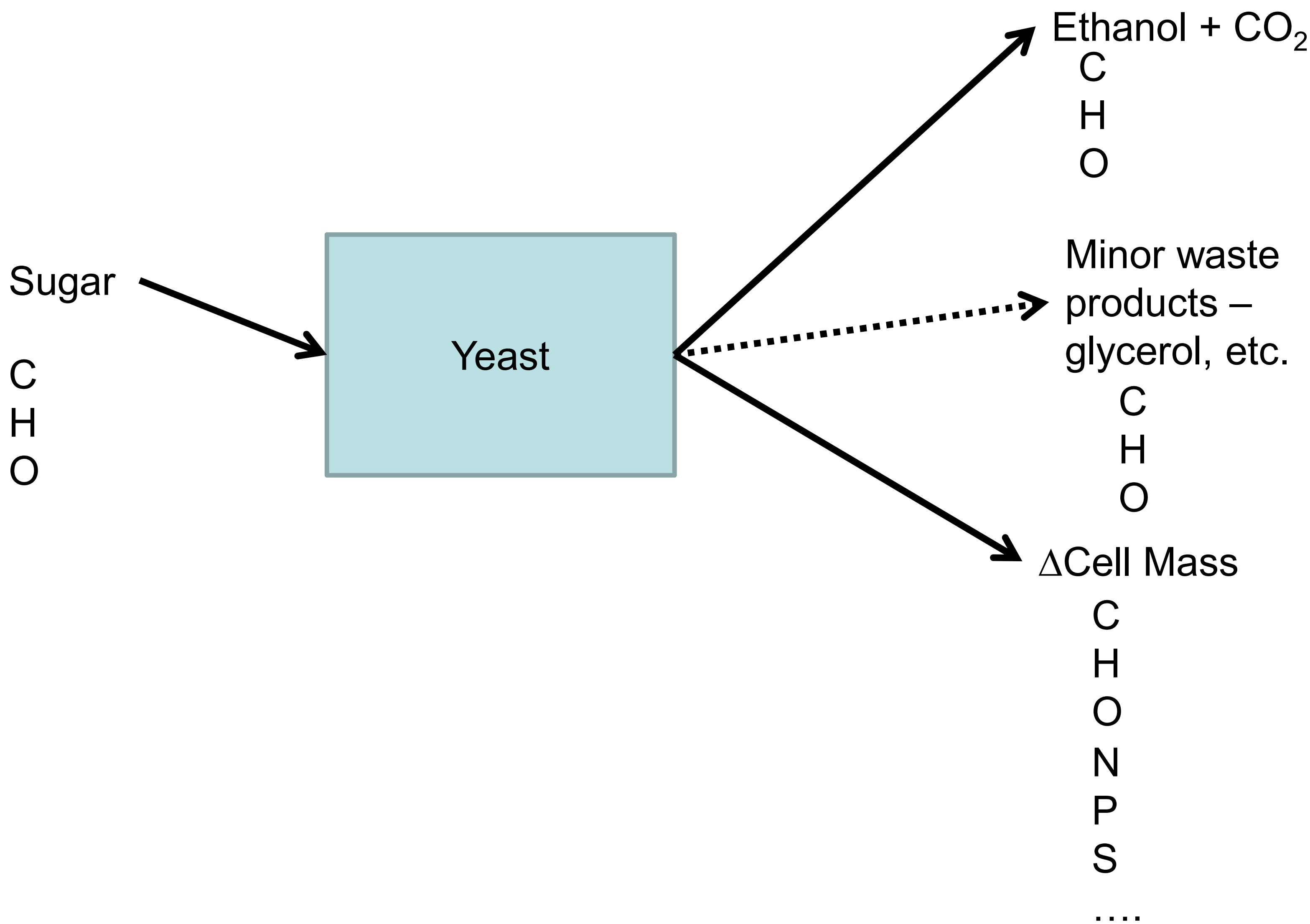

Sugar
C
H
O
Yeast
Ethanol + CO_2
C
H
O
Minor waste products – glycerol, etc.
C
H
O
ΔCell Mass
C
H
O
N
P
S
….

Table 4.1 Total Weights of Monomer Constituents which Make up Macromolecular Components in 100 g Dry Weight of *E. coli* K-12 cells (adapted from Battley, 1991)

Monomers From	Total*	C	H	O	N	P	S
		weight g/100 g cells					
Proteins	64.18	29.25	4.95	19.48	9.75	1.95	0.75
RNA	21.58	7.25	0.88	8.02	3.49	0.31	
DNA	3.27	1.17	0.14	1.12	0.53	0.40	
Lipids	9.17	5.92	0.96	1.75	0.14	0.08	
LPS	4.03	1.89	0.33	1.63	0.08		
Peptidoglycans	2.84	1.23	0.20	1.15	0.27		
Polyamines	0.40	0.22	0.05		0.13		
	105.47	46.93	7.51	33.15	14.39	2.74	0.75
Water	-10.97		-1.23	-9.74			
	94.5	46.93	6.29	23.41	14.39	2.74	0.75

76.63% **91.02%**

Generalized Mass Balance

$$\underset{\text{carbon source}}{A\ (C_aH_bO_c)} + \underset{\substack{\text{oxygen}\\\text{(optional)}}}{B\ (O_2)} + \underset{\text{nitrogen source}}{D\ (NH_3)}$$

$$\downarrow$$

$$\underset{\text{Cell Mass}}{M\ (C_\alpha H_\beta O_\gamma N_\delta)} + \underset{\text{Product}}{N\ (C_\alpha{}' H_\beta{}' O_\gamma{}' N_\delta{}')} + \underset{\text{Waste}}{P\ (CO_2)} + Q\ (H_2O) + \text{Heat}$$

Note: this is on a **molar** basis (need to convert mass of cells to number of cells to moles of cells using Avogadro's number, 6.022×10^{23})

Cell Yield

$$Y_{X/S} = \frac{\text{mass of cells produced}}{\text{mass of substrate consumed}} = \frac{X - X_o}{S_o - S} =$$

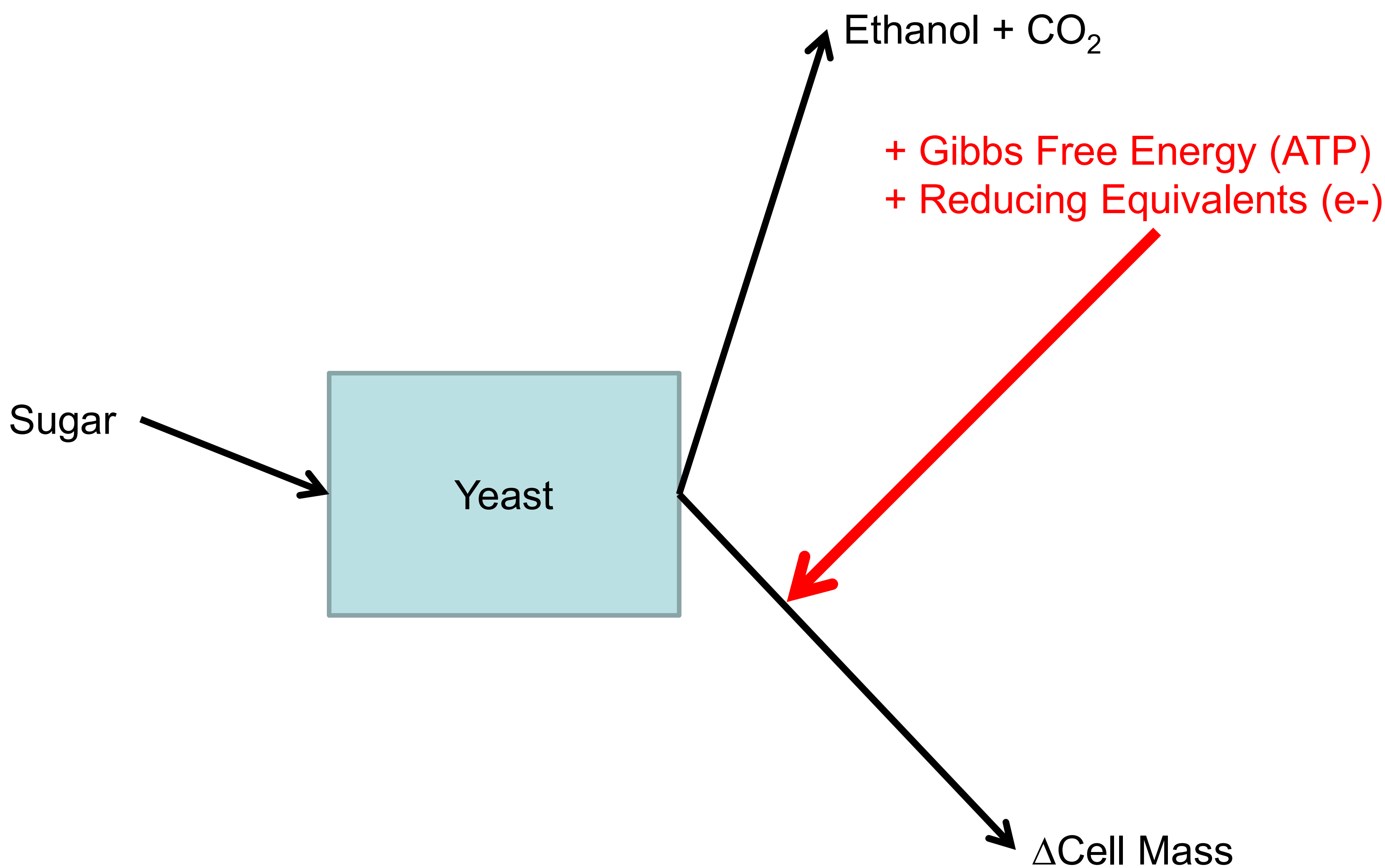
$$\frac{\Delta X}{\Delta S} \stackrel{?}{=} \frac{\left[M\left(12\alpha + 1\beta + 16\gamma + 14\delta\right)r\right]}{\mathrm{A}\left(12\mathrm{a}+1\mathrm{b}+16\mathrm{c}\right)}$$

r = CHON composition of cells (approximately 0.91)
or CHO composition of cells (approximately 0.766)

Product Yield

$$Y_{P/S} = \frac{(\Delta P)}{\Delta S} = \frac{P - P_o}{S_o - S} \stackrel{?}{=}$$

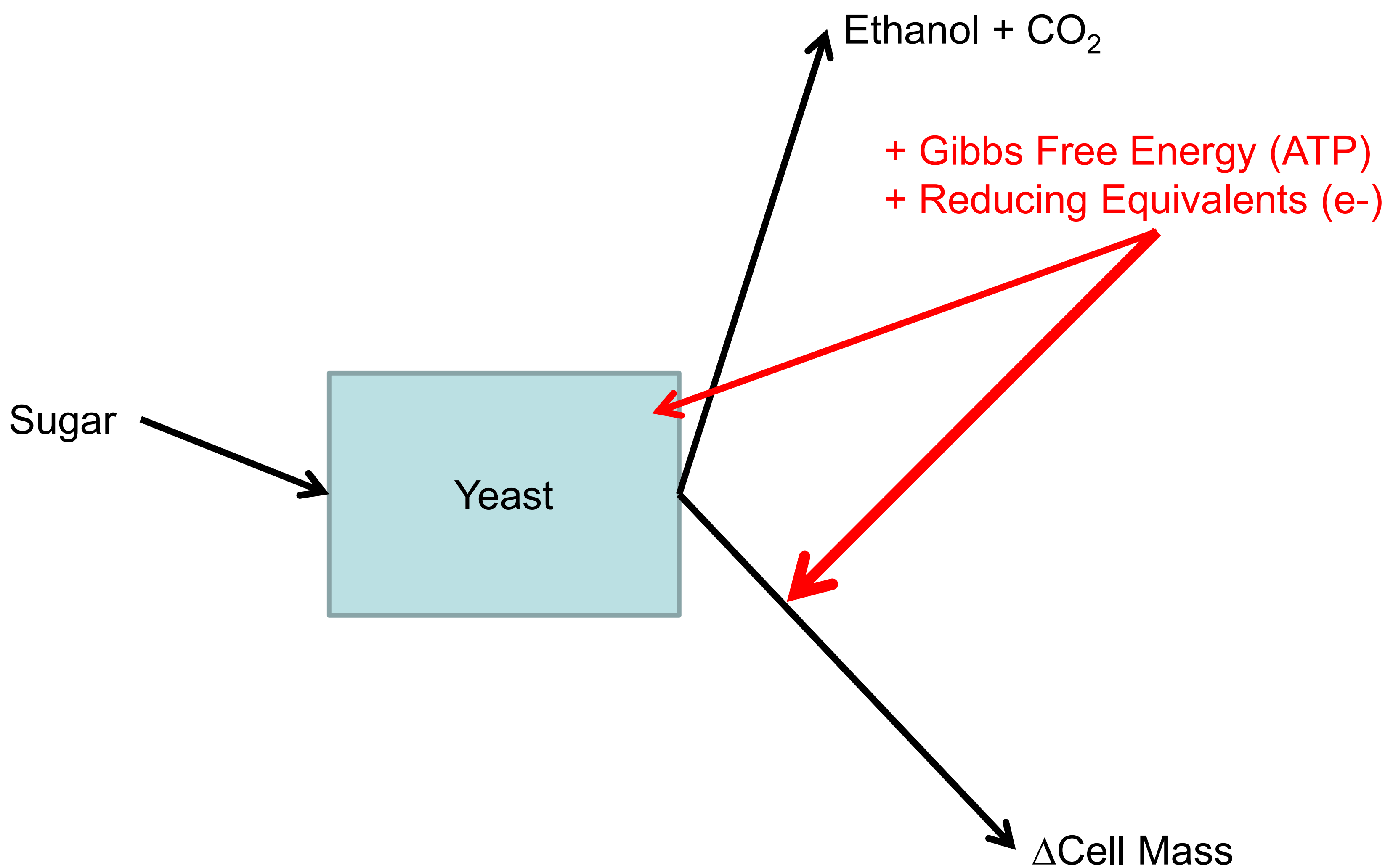
$$\frac{N}{A}\frac{(12\alpha' + 1\beta + 16\gamma' + 14\delta')}{(12a + 1b + 14c)}\left(\frac{\text{g product}}{\text{g substrate}}\right)$$


Ethanol + CO_2
+ Gibbs Free Energy (ATP)
+ Reducing Equivalents (e-)
Sugar
Yeast
ΔCell Mass

Energy

- ATP = carrier of Gibbs Free Energy
- Energy <u>also</u> needed for cell growth

$$Y_{X/ATP} = \frac{\Delta X}{ATP} = \frac{\left(\dfrac{\Delta X}{\Delta S}\right)}{\left(\dfrac{\Delta ATP}{\Delta S}\right)} = \frac{Y_{X/S}}{Y_{ATP/S}}$$


Ethanol + CO_2
+ Gibbs Free Energy (ATP)
+ Reducing Equivalents (e-)
Sugar
Yeast
ΔCell Mass

Luedeking-Piret Model

$$\frac{dS}{dt} = \alpha \frac{dx}{dt} + \beta x$$

Substrate Consumption | Growth Associated | Non-growth Associated

$$\frac{dX}{dt} = \mu X = \frac{\mu_{max}[S]}{K_S + [S]} \mathrm{X}, \text{ or other function!}$$

$$\alpha = \mathrm{Y}_{S/X} = \text{Yield coefficient}$$

$$\beta = \mathrm{m}_e = \text{ maintenance coefficient}$$

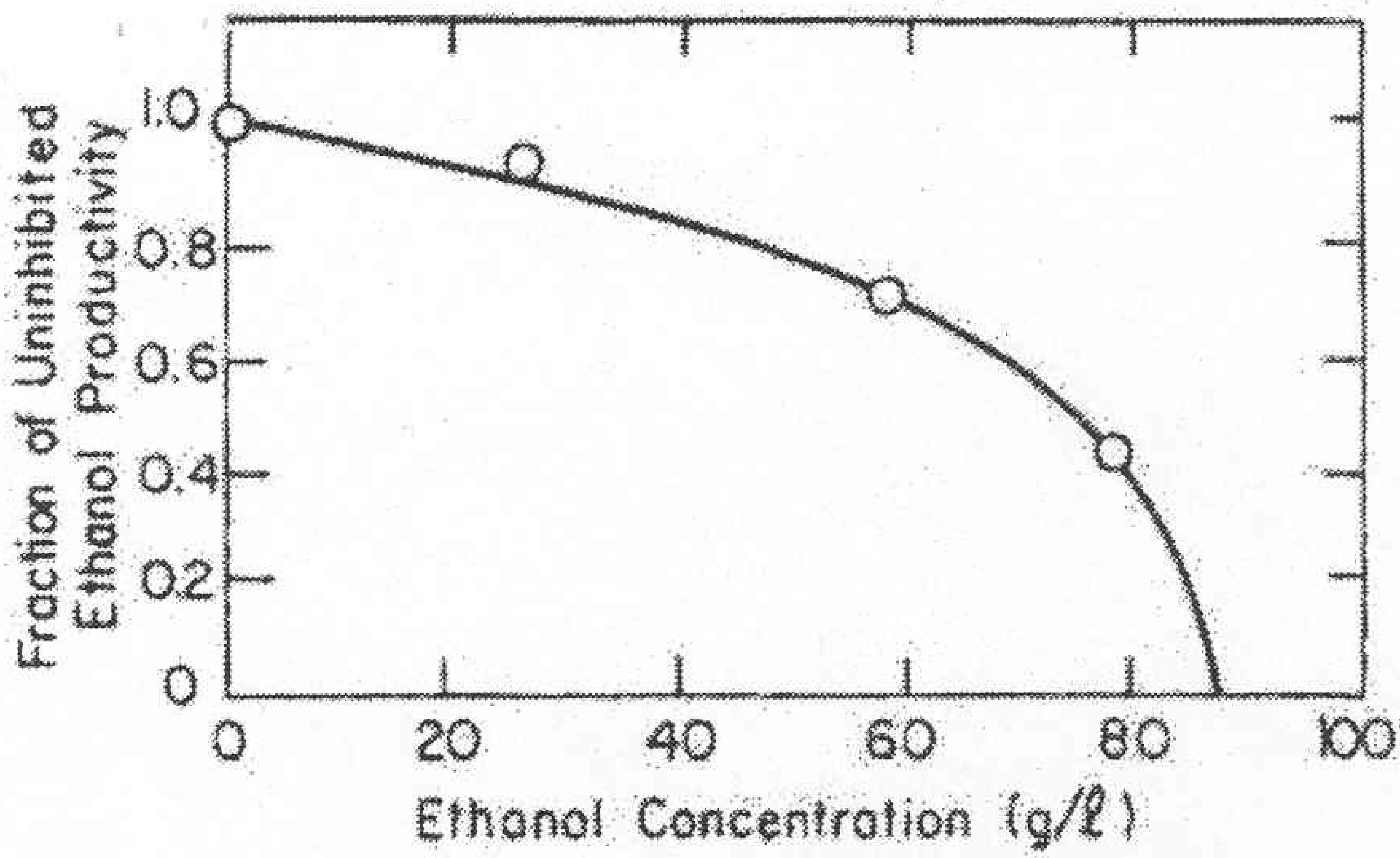
Modeling Constants

Microorganism	Substrate Concen g/L	K_s mg/L	μ_{max} 1/hr	$Y_{P/S}$ g/g	$Y_{X/S}$ g/g
S. cerevisiae	100 glucose	----	0.27	0.46	0.055
Industrial[1]	20 glucose	----	0.29	----	----
424A (LNH-ST)[2]	20 xylose	----	0.21	----	----
Unidentified[3]		250	----	----	----
ATCC 4226[4]		315	----	----	----
Bacteria					
Z. mobilis		----	0.37	0.49	0.028
E. coli[3]		2 to 4	----	----	----

Inhibition

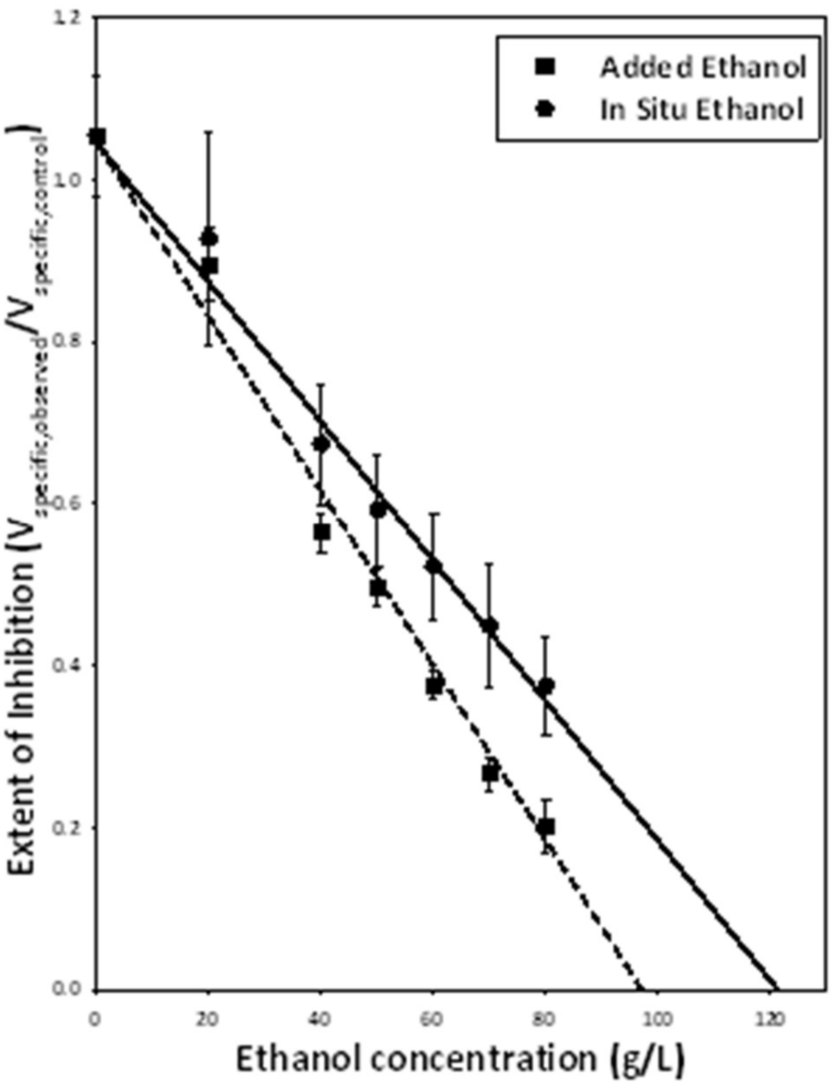
- Waste products often inhibitory to growth and metabolic function
- Disrupt cell membrane
- Inhibit cellular functions
- Inhibit enzymes

Inhibition



Fraction of Uninhibited Ethanol Productivity
1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
Ethanol Concentration (g/ℓ)

Ethanol Inhibition of Xylose Fermentation

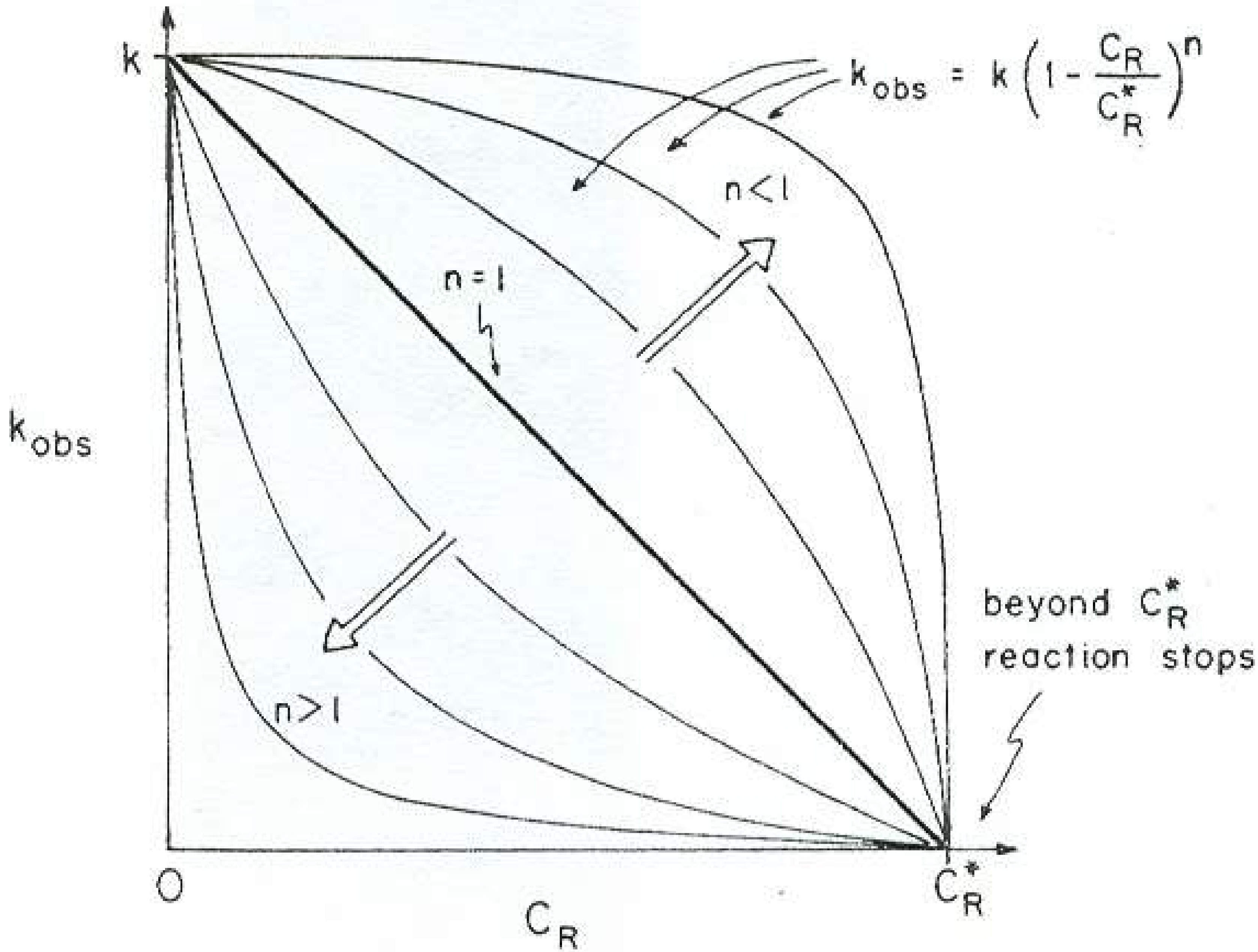


Athmanathan, A.; Sedlak, M.; Ho, N.W.Y.; Mosier, N.S. "Effect of Product Inhibition on Xylose Fermentation to Ethanol by *Saccharomyces cerevisae* 424A (LNH-ST)," Biological Engineering 3(2): 111-124 (2011).

By-Product Inhibition Summary

Table 5-2

Product	Concentration at high inhibition[a] (g/L)	Inhibition mechanism
Ethanol	70	Membrane stability/porosity
Furmic acid	2.7	Chemical interference with cell maintenance functions
Acetic acid	7.5	Chemical interference/pH
Lactic acid	38	Chemical interference
Propanol	12	Chemical interference
Methyl-1-butanol	3.5	Chemical interference
3-Butanediol	90	Chemical interference
Acetaldehyde	5.0	Chemical interference


k
$k_{obs} = k\left(1 - \frac{C_R}{C_R^*}\right)^n$
n<1
n=1
k_{obs}
beyond C_R^*
reaction stops
n>1
O
C_R
C_R^*

Extended Monod Kinetics for Substrate, Product, and Cell Inhibition

Keehyun Han* **and Octave Levenspiel**
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Accepted for publication August 6, 1987

A generalized form of Monod kinetics is proposed to account for all kinds of product, cell, and substrate inhibition. This model assumes that there exists a critical inhibitor concentration above which cells cannot grow, and that the constants of the Monod equation are functions of this limiting inhibitor concentration. Methods for evaluating the constants of this rate form are presented. Finally the proposed kinetic form is compared with the available data in the literature, which unfortunately is very sparse. In all cases, this equation form fitted the data very well.

INTRODUCTION

Although microbial growth is a very complex phenomenon, the overall growth can often be regarded as a single chemical reaction with a simple rate expression. Many equations are used for this purpose. Among these, the simplest and most popular is the one proposed by Monod who assumed that a single essential substrate is the growth limiting factor.[1] Monod kinetics can be expressed as

$$\text{Substrate } (A) \xrightarrow{\text{Cells } (C)} \text{more Cells } (C) + \text{Product } (R)$$

$$r_C = k\frac{C_A C_C}{C_A + C_M}, \qquad \left(\frac{\text{cell mass produced}}{\text{unit volume} \times \text{time}}\right) \tag{1}$$

For substrate inhibition:

$$r_C = k\left(1 - \frac{C_A}{C_A^*}\right)^n \frac{C_A C_C}{C_A + C_M(1 - C_A/C_A^*)^m} \tag{3}$$

for product inhibition:

$$r_C = k\left(1 - \frac{C_R}{C_R^*}\right)^n \frac{C_A C_C}{C_A + C_M(1 - C_R/C_R^*)^m} \tag{4}$$

for cell inhibition:

$$r_C = k\left(1 - \frac{C_C}{C_C^*}\right)^n \frac{C_A C_C}{C_A + C_M(1 - C_C/C_C^*)^m} \tag{5}$$

In the extreme where $C_I \ll C_I^*$, the above eqs. (2)–(5) all reduce to the simple Monod expression of eq. (1).

HOW TO EVALUATE THE CONSTANTS OF THE GENERALIZED MONOD EQUATION FOR PRODUCT OR CELL INHIBITION

As with Monod kinetics, the constants in eq. (2) with $C_I = C_R$ or $C_I = C_C$ can be evaluated from a Lineweaver–Burk plot of C_C/r_C vs $1/C_A$. Thus inverting eq. (2) gives

Using Levenspiel Equation

$$\frac{dX}{dt} = \mu_{max}\left[\frac{S}{S+K_m}\right]\left[1-\frac{P}{P_{max}}\right]^n X$$

Maiorella Ethanol Model

$$\mu = Ev$$

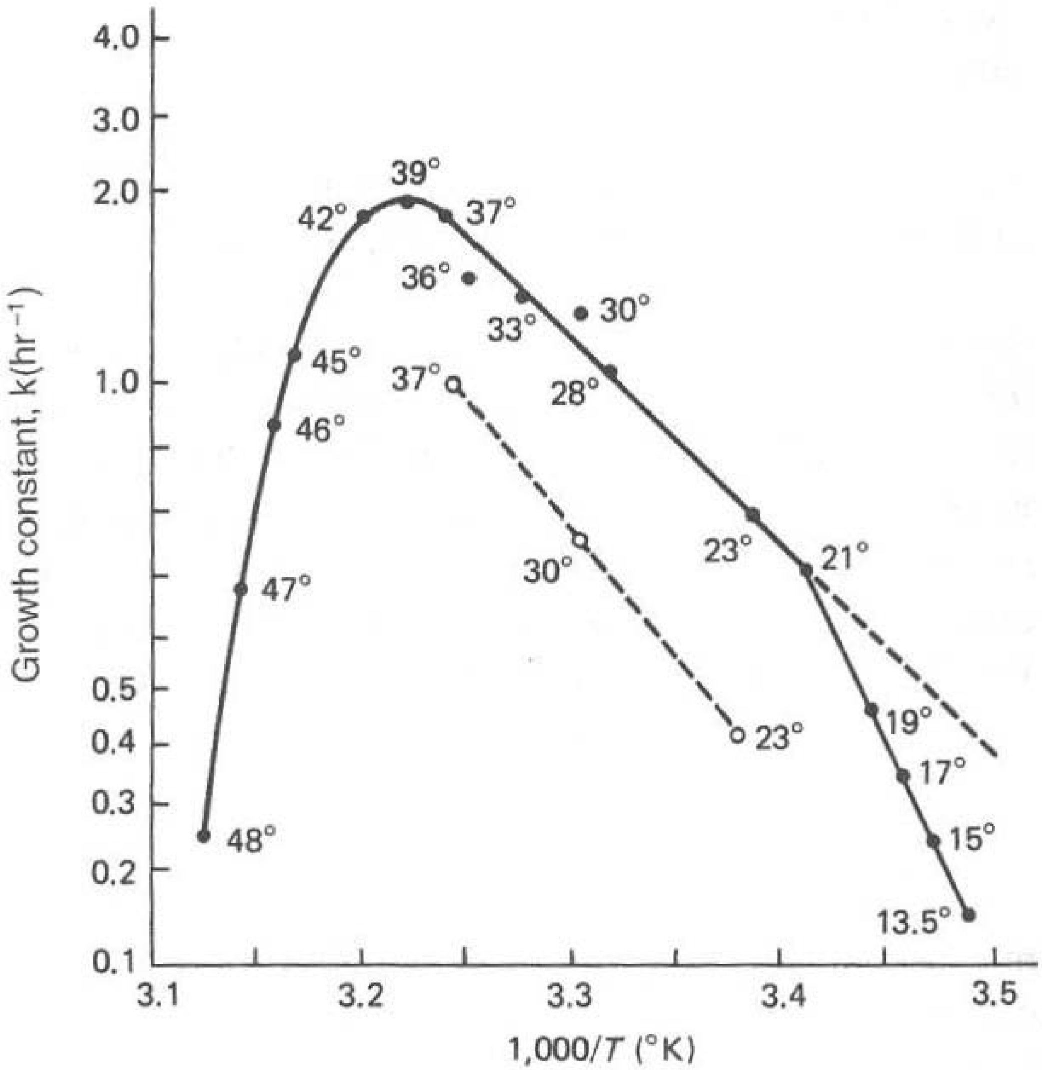
$$\nu = \nu_{max}\left[\frac{S}{S+K_m}\right]\left[1-\frac{P}{P_{max}}\right]^n$$

E	=	efficiency of cell mass production, (0.249)
v	=	specific ethanol production rate (g ethanol produced/g cells. hr)
S	=	substrate (glucose) concentration (g/L)
vmax	=	maximum specific production rate (1.15 g ethanol/g cells. hr)
Km	=	Monod constant (0.315 g/L)
n	=	toxic power constant (0.36)
Pmax	=	maximum product concentration (87.5 g/L)

Other Factors that Influence Growth

- Temperature
- pH

Influence of Temperature



$$acc = \cancel{in} - \cancel{out} + gen - con$$

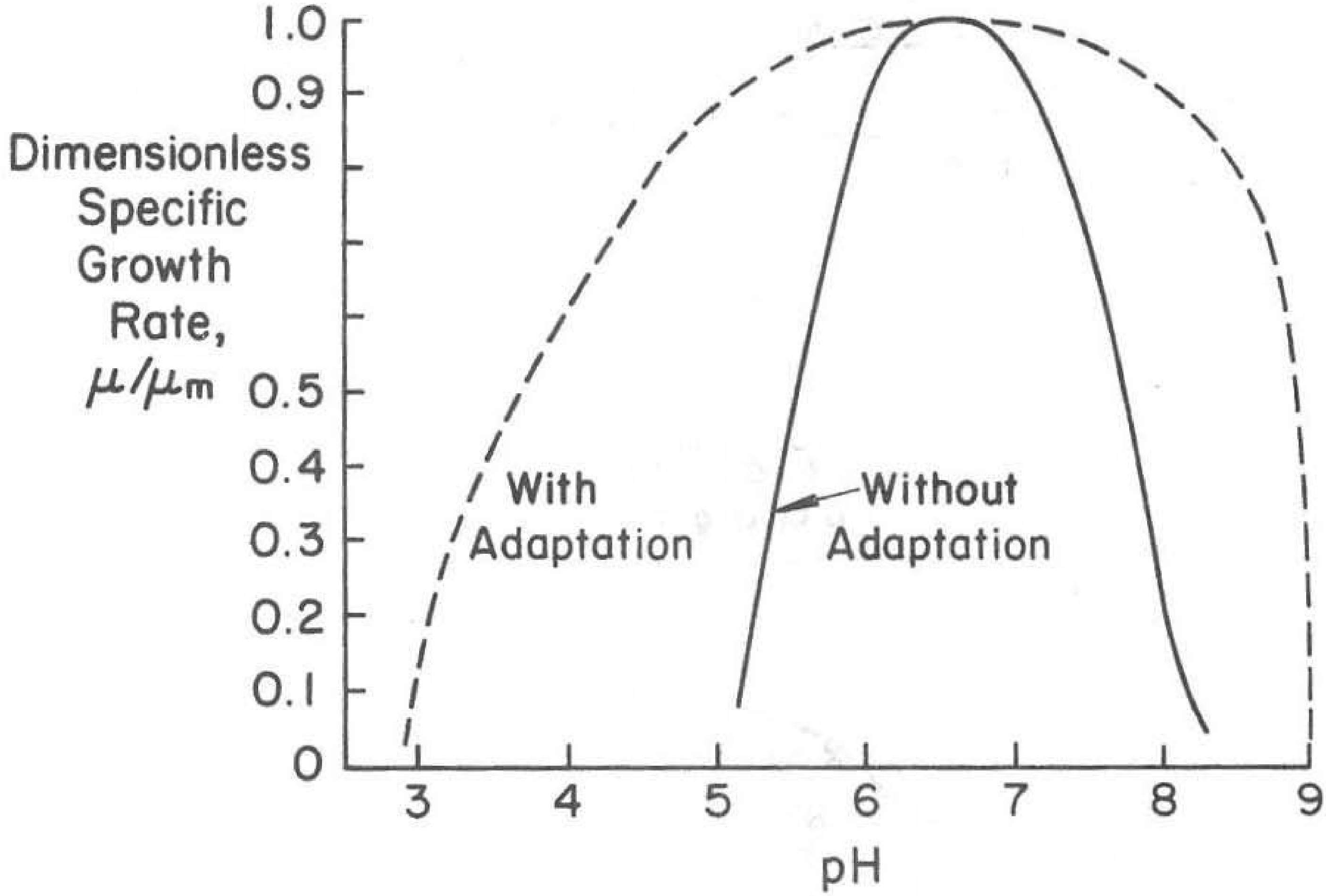
$$\frac{dX}{dt} = \mu X - k_d X$$

$$\frac{dX}{dt} = \left(\mu - k_d\right) X$$

$$\mu_{max} = \mu_0 e^{-E_{a,g}/RT}$$

$$k_d = k_0 e^{-E_{a,d}/RT}$$

Influence of pH



Continuous Stirred Tank Bioreactor

- Chemostat, turbidostat
- Tank content is homogeneous in
  - Temperature
  - Composition (concentrations)
  - pH
  - Etc.
- Inlet material instantaneously mixed into tank contents

CSTR vs CSTBR

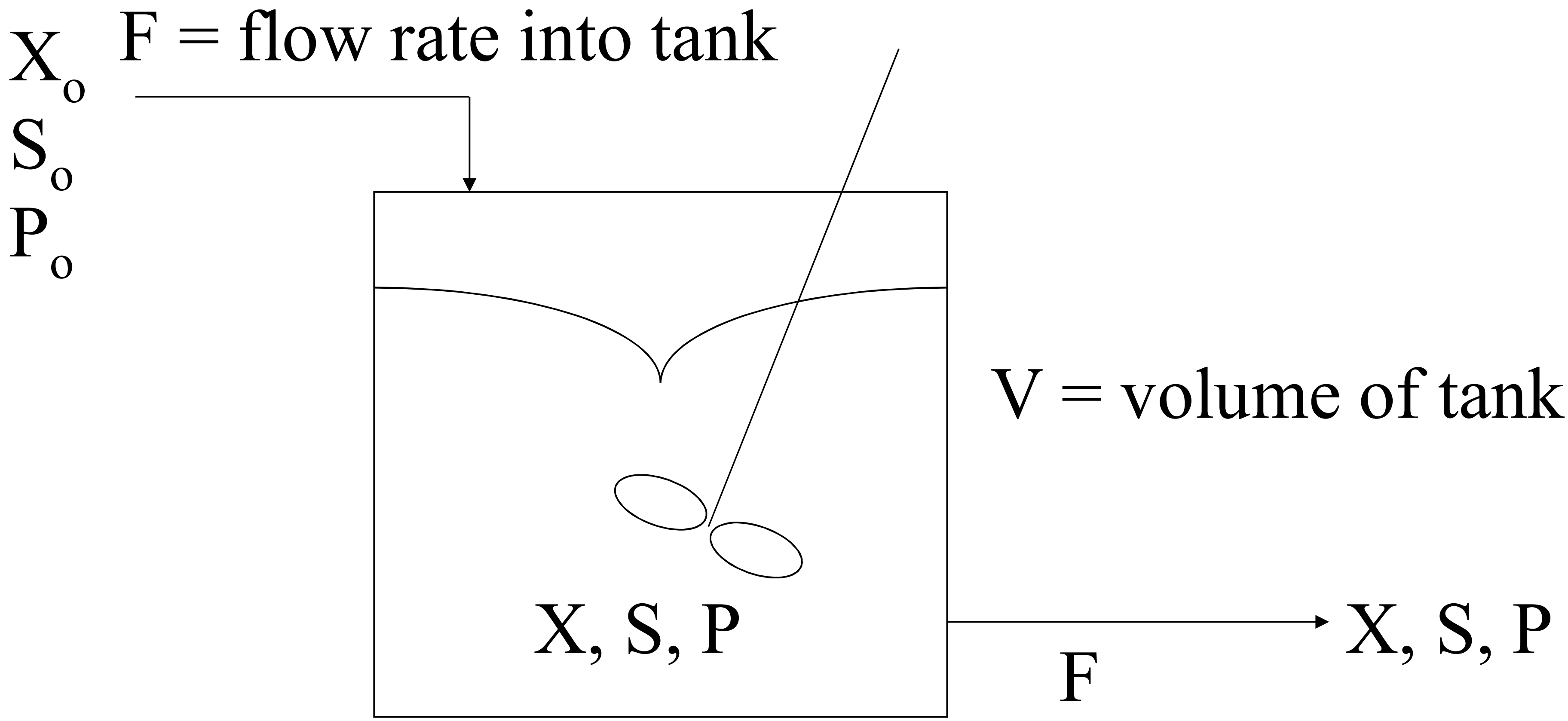
- Growth of cells (biocatalysts) in CSTBR

- Difficulty in strict stoichiometry in biochemical reactions

- Otherwise, basic equations very similar

Uses of CSTBRs

- Rare for industrial applications – why?
- Research and development applications:
  - Study effect of changes in substrate concentration on cell growth/product formation
  - Study effect of environmental parameters such as pH and temperature
  - Selective culturing method for strain development
  - Sample for metabolic flux analysis of cellular metabolism (fill in the "black box")


X_o
S_o
P_o
F = flow rate into tank
V = volume of tank
X, S, P
F
X, S, P

Derivation of Growth Expression

Accumulation = In – Out – Generation - Consumption

$$V\,dX = 0 - FX \bullet dt + V\mu X\,dt - 0$$

$$V\,dX = V\mu X\,dt - FX \bullet dt$$

Divide by V•dt

$$\frac{dX}{dt} = (\mu - D)X \quad D = \frac{F}{V} \quad D = \frac{1}{t_{residence}}$$

Coupling Substrate to Growth

Accumulation = In – Out + Generation – Consumption

$$V\,dS = FS_0 \bullet dt - FS \bullet dt - V\,\mu\,X\,Y_{sx}\,dt$$

Divide by V•dt

$$\frac{dS}{dt} = D\left(S_0 - S\right) - \mu X Y_{s/x}$$

Steady-State CSTBR

$$\frac{dX}{dt} = \frac{dS}{dt} = 0$$

$$\frac{dX}{dt} = (\mu - D)X = 0$$

$$\frac{dS}{dt} = D(S_0 - S) - \mu X Y_{s/x} = 0$$

CSTBR to Find Monod Constants

$$\frac{dX}{dt} = (\mu - D)X = 0$$

$$\mu = D$$

$$\frac{\mu_{max} S}{S + K_s} = D$$

$$S = \frac{K_s D}{\mu_{max} - D}$$

CSTBR to Find Monod Constants

$$\mu = D$$

$$\frac{dS}{dt} = D\left(S_0 - S\right) - \mu X Y_{s/x} = 0$$

$$\mu\left(S_0 - S\right) = \mu X Y_{s/x}$$

$$X = \frac{\left(S_0 - S\right)}{Y_{s/x}} = Y_{x/s}\left(S_0 - S\right)$$

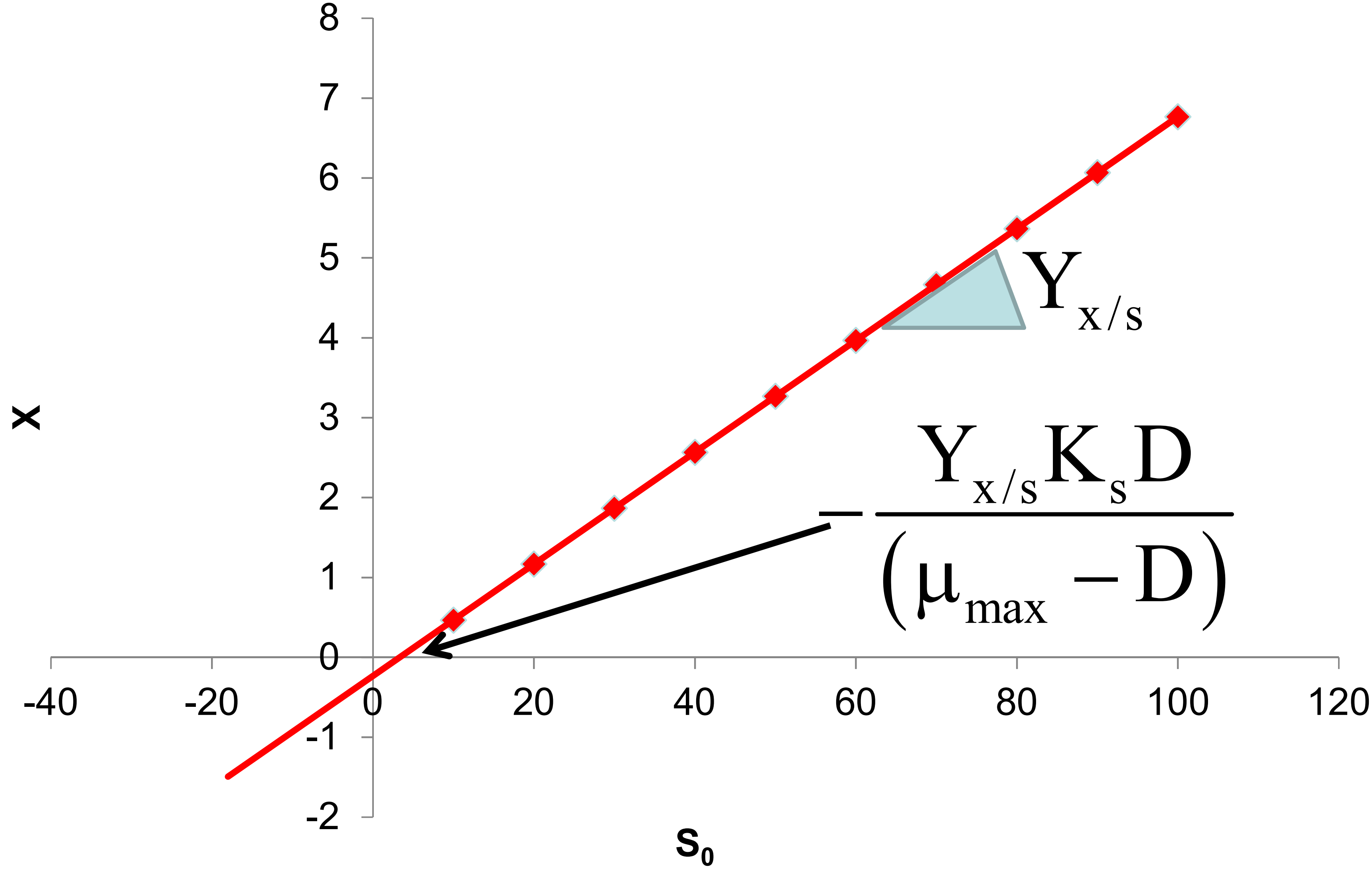
CSTBR to Find Monod Constants

$$X = \frac{(S_0 - S)}{Y_{s/x}} = Y_{x/s}(S_0 - S)$$

$$S = \frac{K_s D}{\mu_{max} - D}$$

$$X = Y_{x/s}\left(S_o - \frac{K_s D}{(\mu_{max} - D)}\right)$$

Constant Dilution Rate



Using CSTBR to find m_e

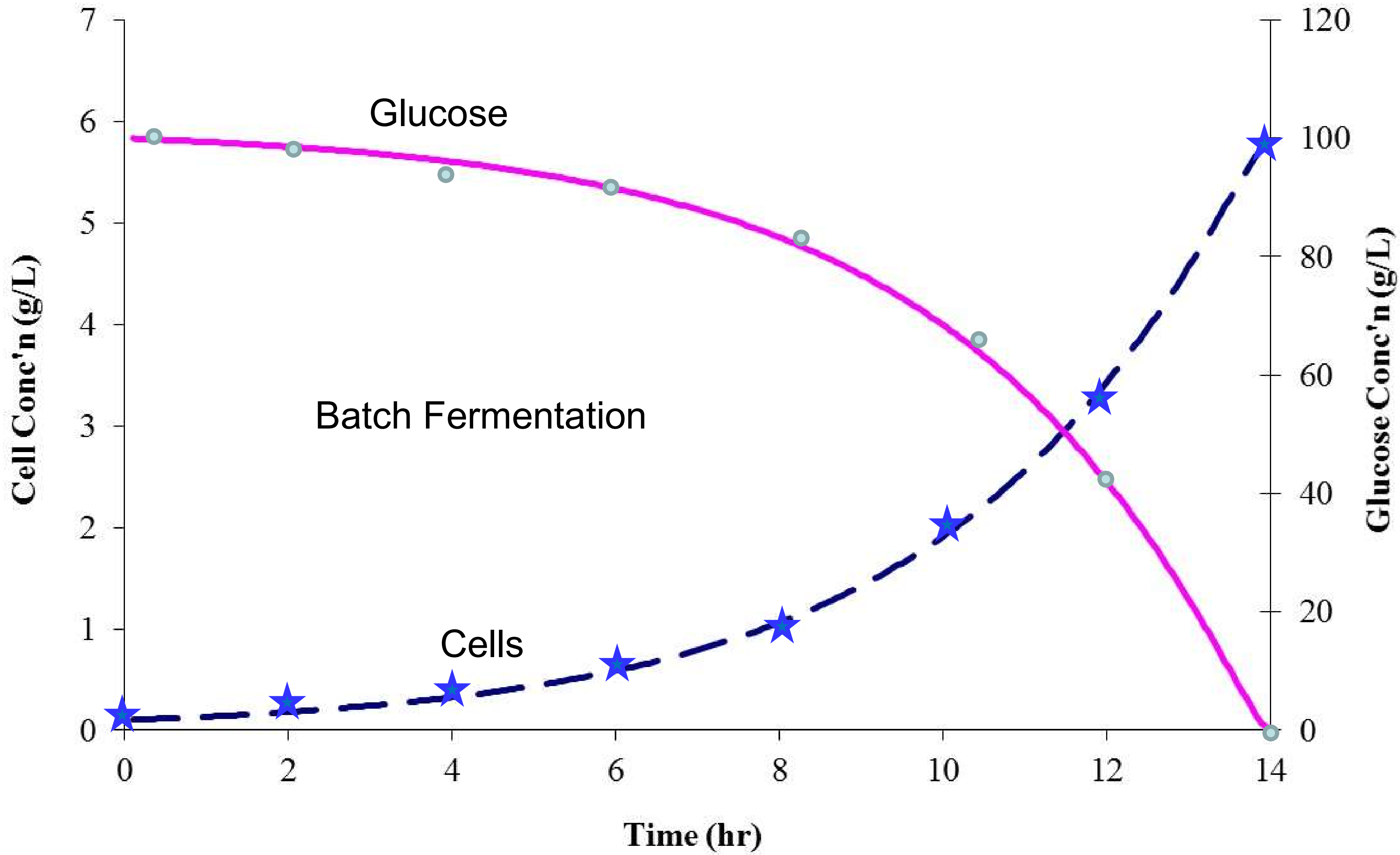
Batch $$\frac{dS}{dt} = -\left(m_e + Y_{s/x}\mu\right)X$$

Batch $$\frac{dS}{dt} = -Y_{s/x,app}\frac{dX}{dt}$$

Where Ysx,app = observed disappearance of S per appearance of X

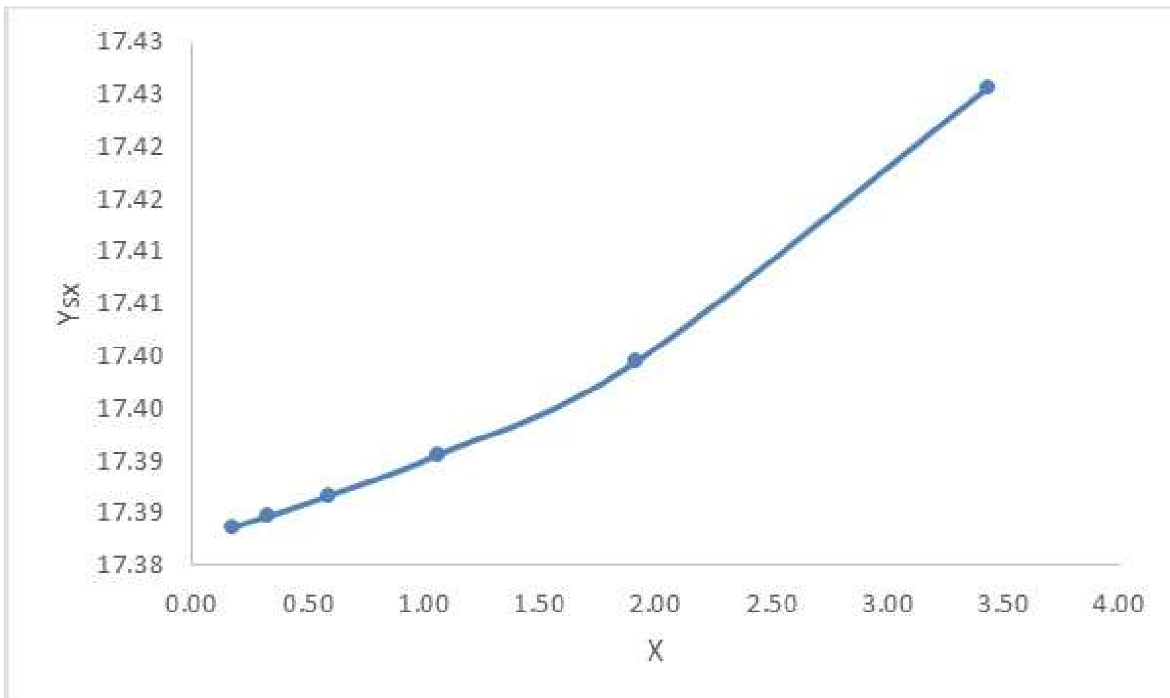
$$Y_{s/x,app} = Y_{s/x} + \frac{m_e}{\mu} = Y_{s/x} + \frac{m_e}{D} = m_e \cdot \frac{1}{D} + Y_{s/x}$$

Practical Measurement of Yield Coefficients and me



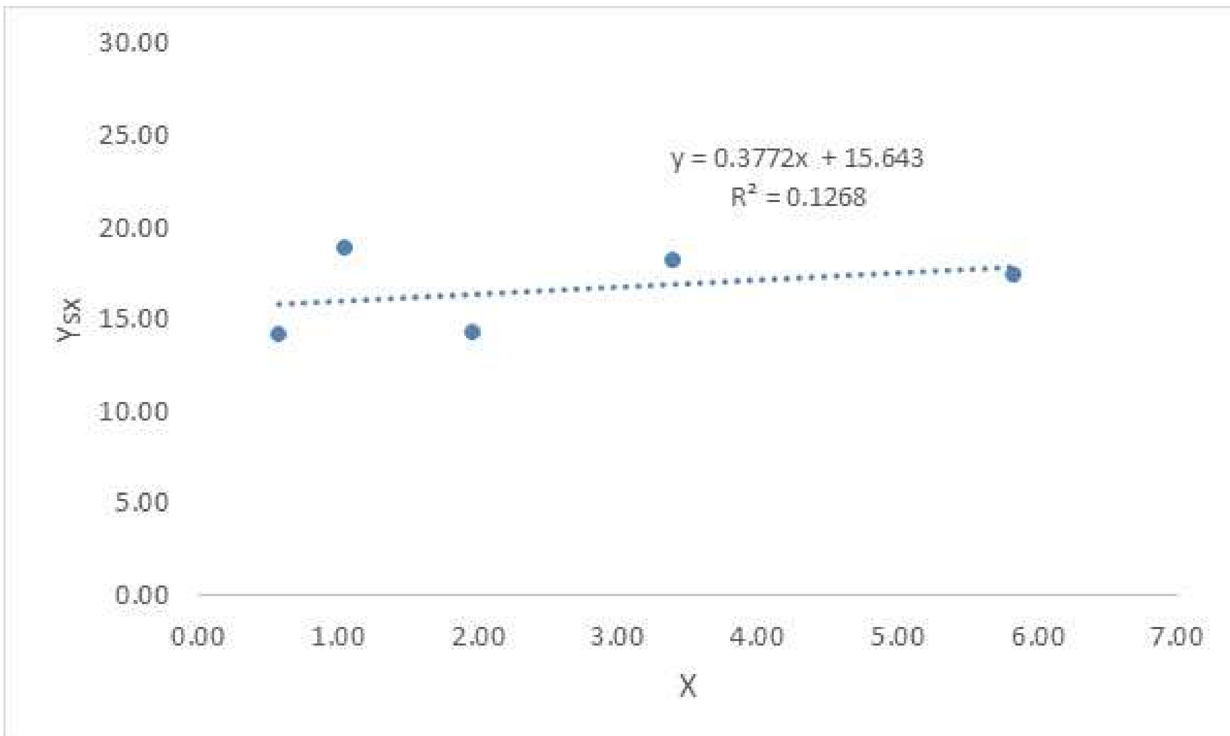
Time	X	S	ΔS/ΔX
0	0.10	100.00	n/a
2	0.18	98.60	17.38
4	0.33	96.07	17.38
6	0.59	91.51	17.39
8	1.06	83.28	17.39
10	1.91	68.48	17.40
12	3.43	42.03	17.43
14	5.77	0.05	17.93

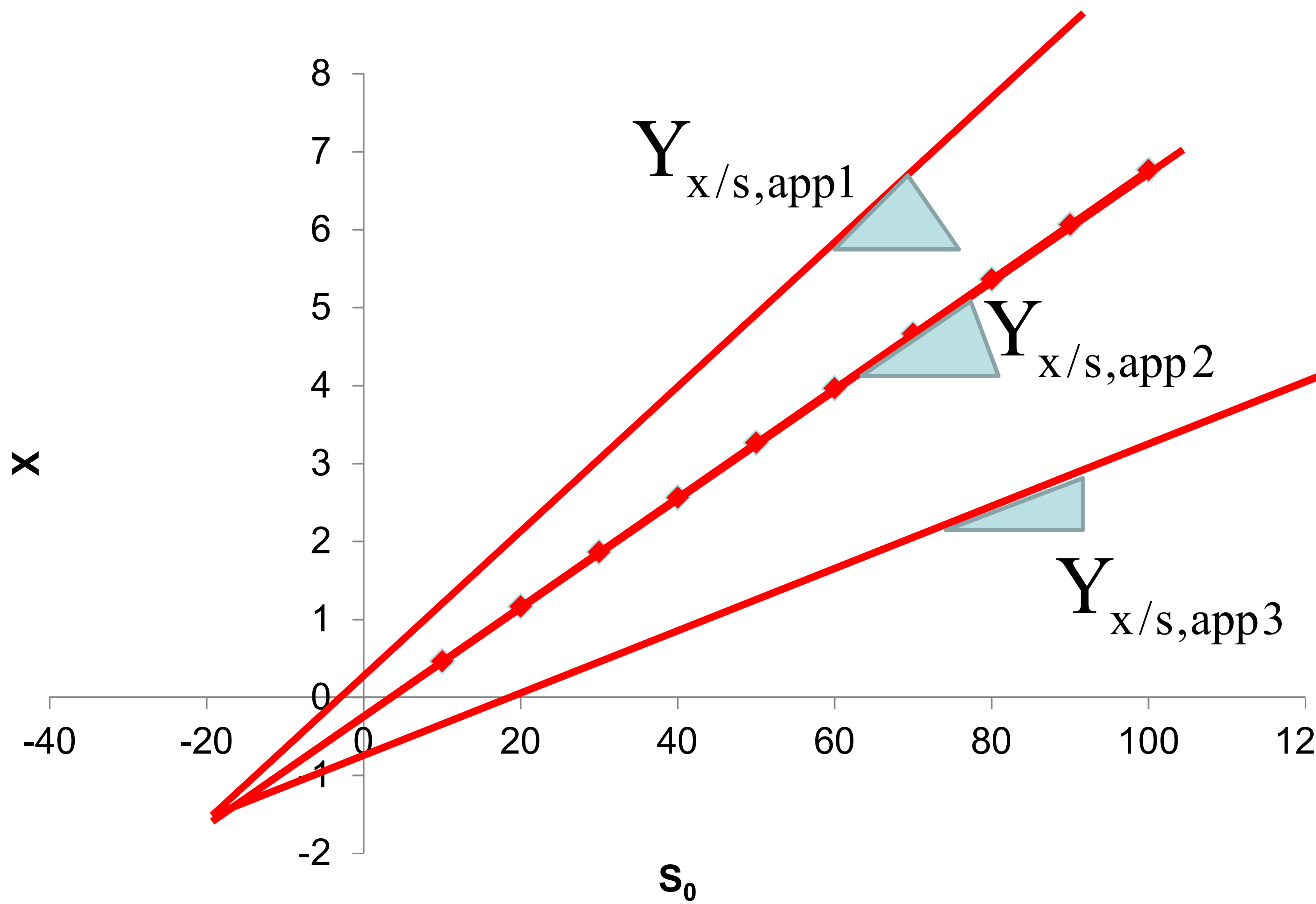
Ysx = 17.62



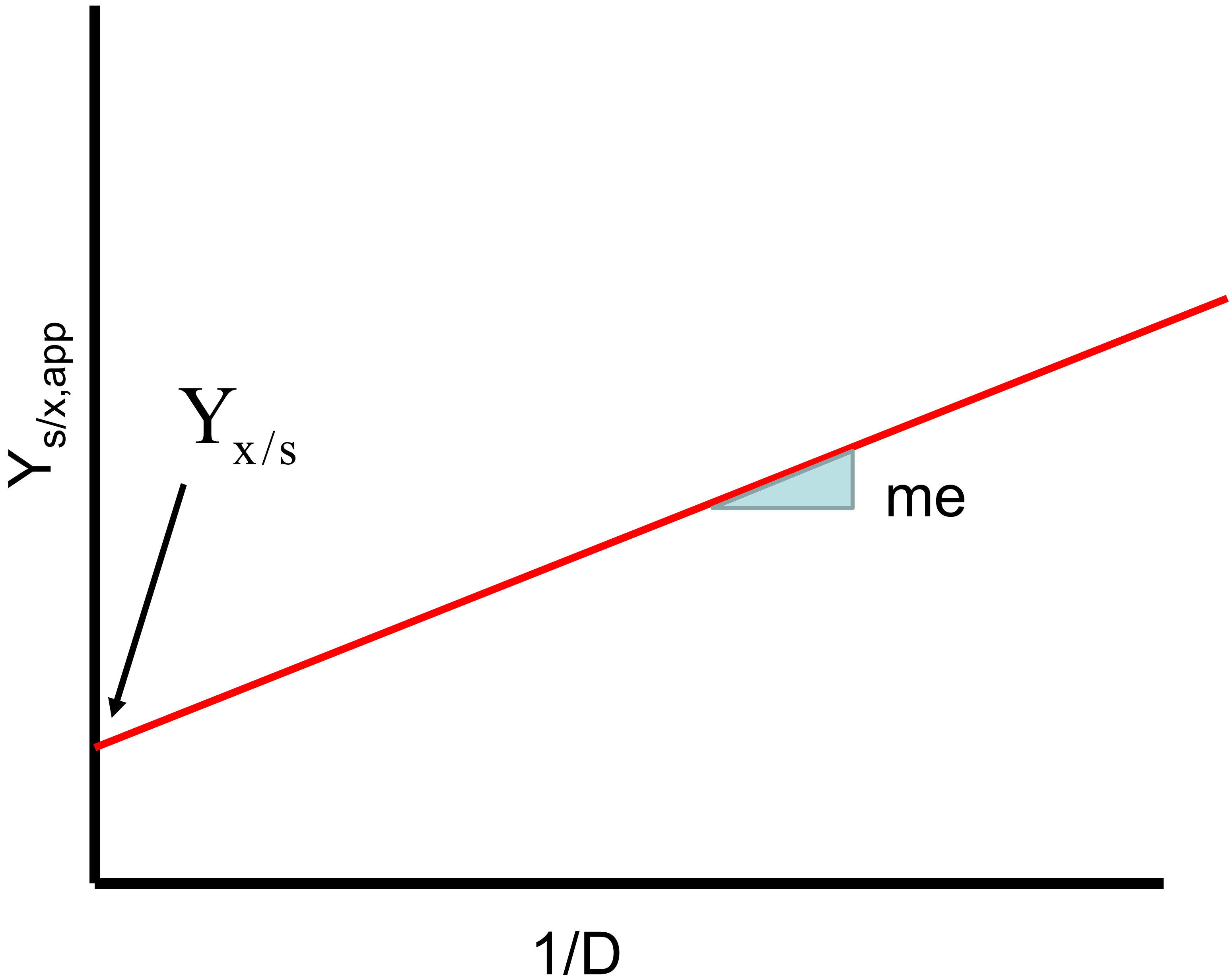
Time	X	S	ΔS/ΔX
0	0.10	98.00	n/a
2	0.19	97.61	4.09
4	0.34	94.00	24.71
6	0.58	90.59	14.15
8	1.05	81.61	18.93
10	1.97	68.48	14.30
12	3.40	42.45	18.25
14	5.83	0.05	17.42

$Y_{sx} = 17.08$

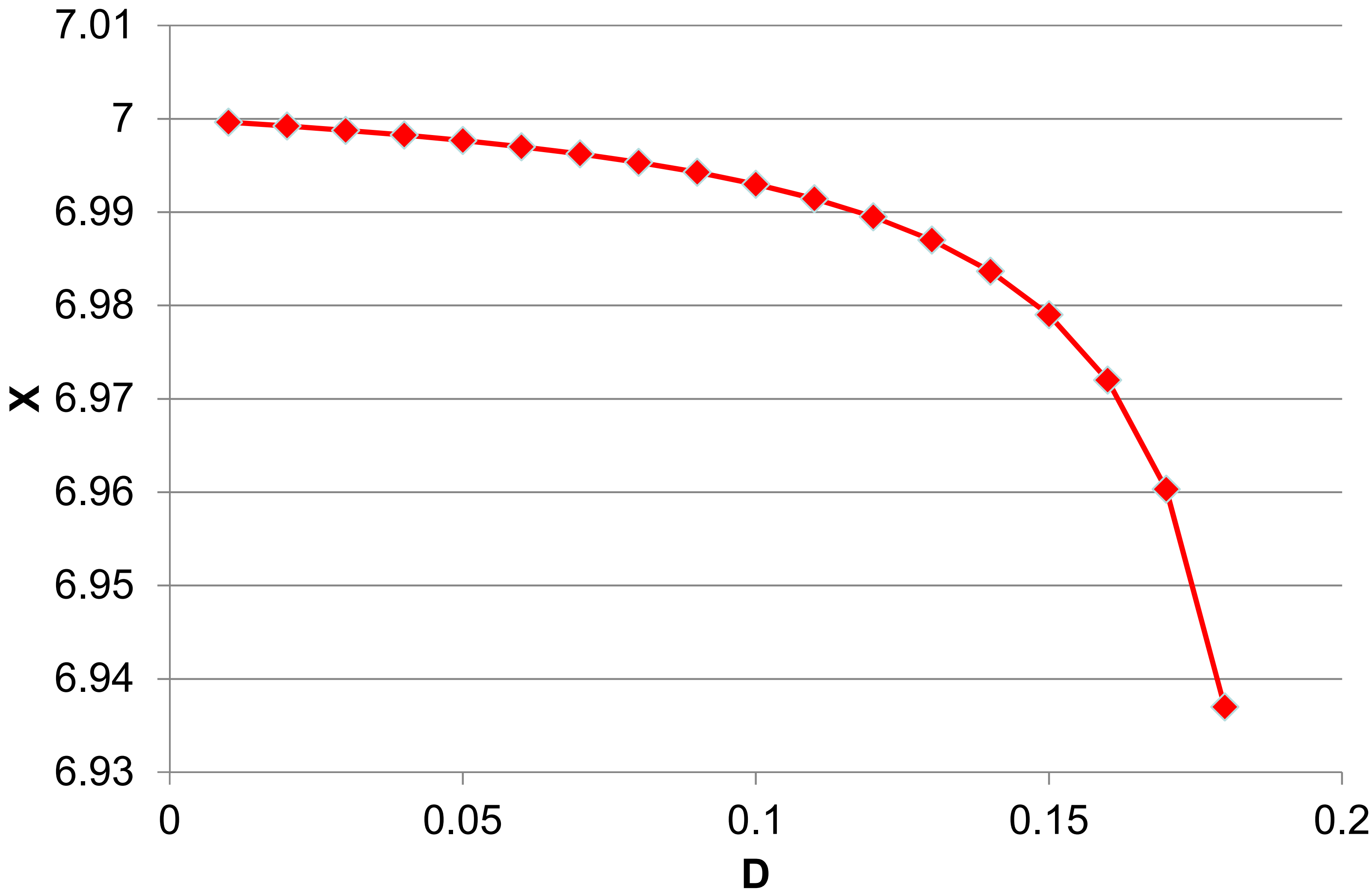


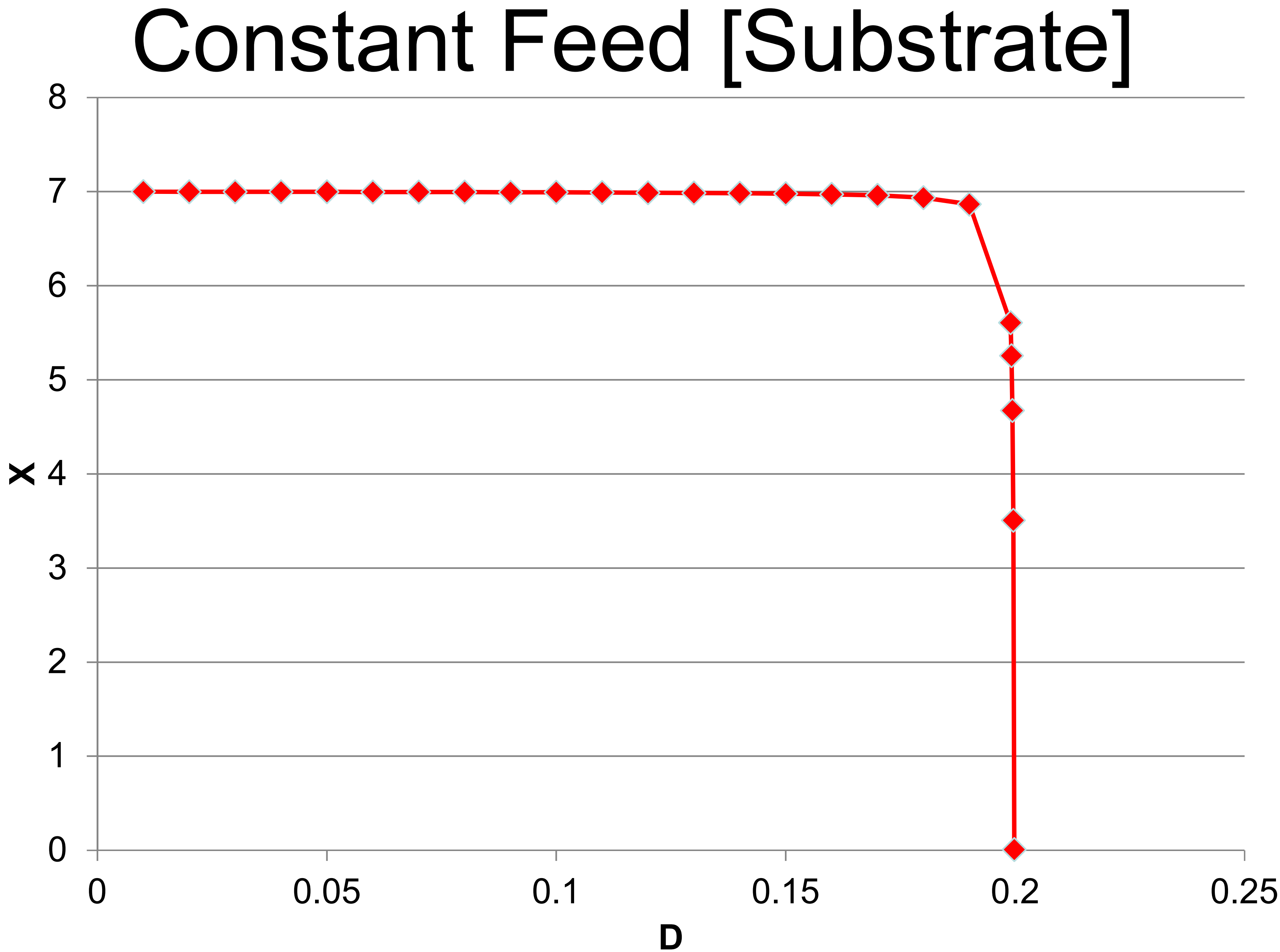


8
7
6
5
4
3
2
1
0
-1
-2
-40
-20
0
20
40
60
80
100
120
x
s_0
$Y_{x/s,app1}$
$Y_{x/s,app2}$
$Y_{x/s,app3}$


$Y_{s/x,app}$
$Y_{x/s}$
me
1/D

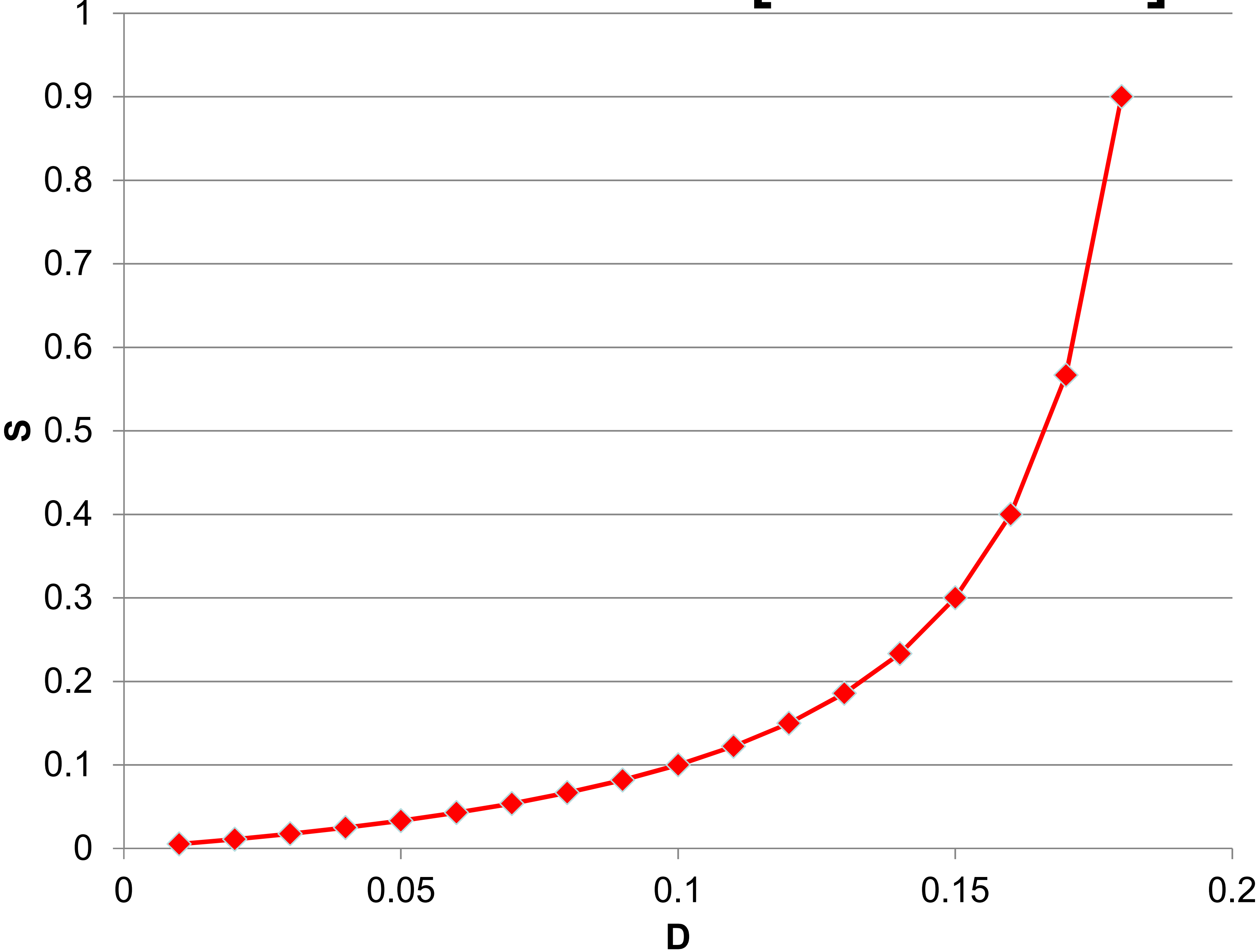
Constant Feed [Substrate]

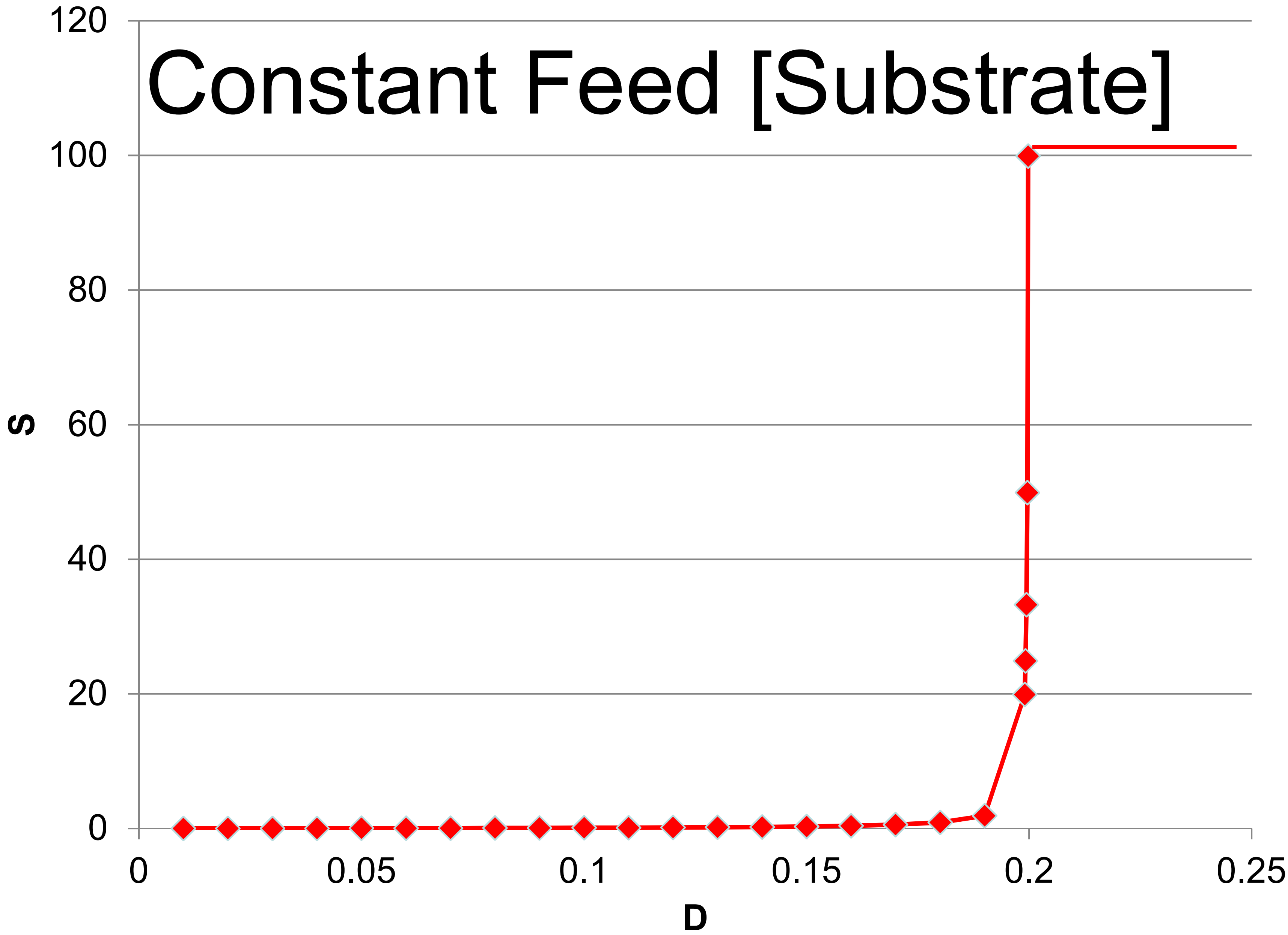




Constant Feed [Substrate]
8
7
6
5
4
3
2
1
0
X
0
0.05
0.1
0.15
0.2
0.25
D

Constant Feed [Substrate]

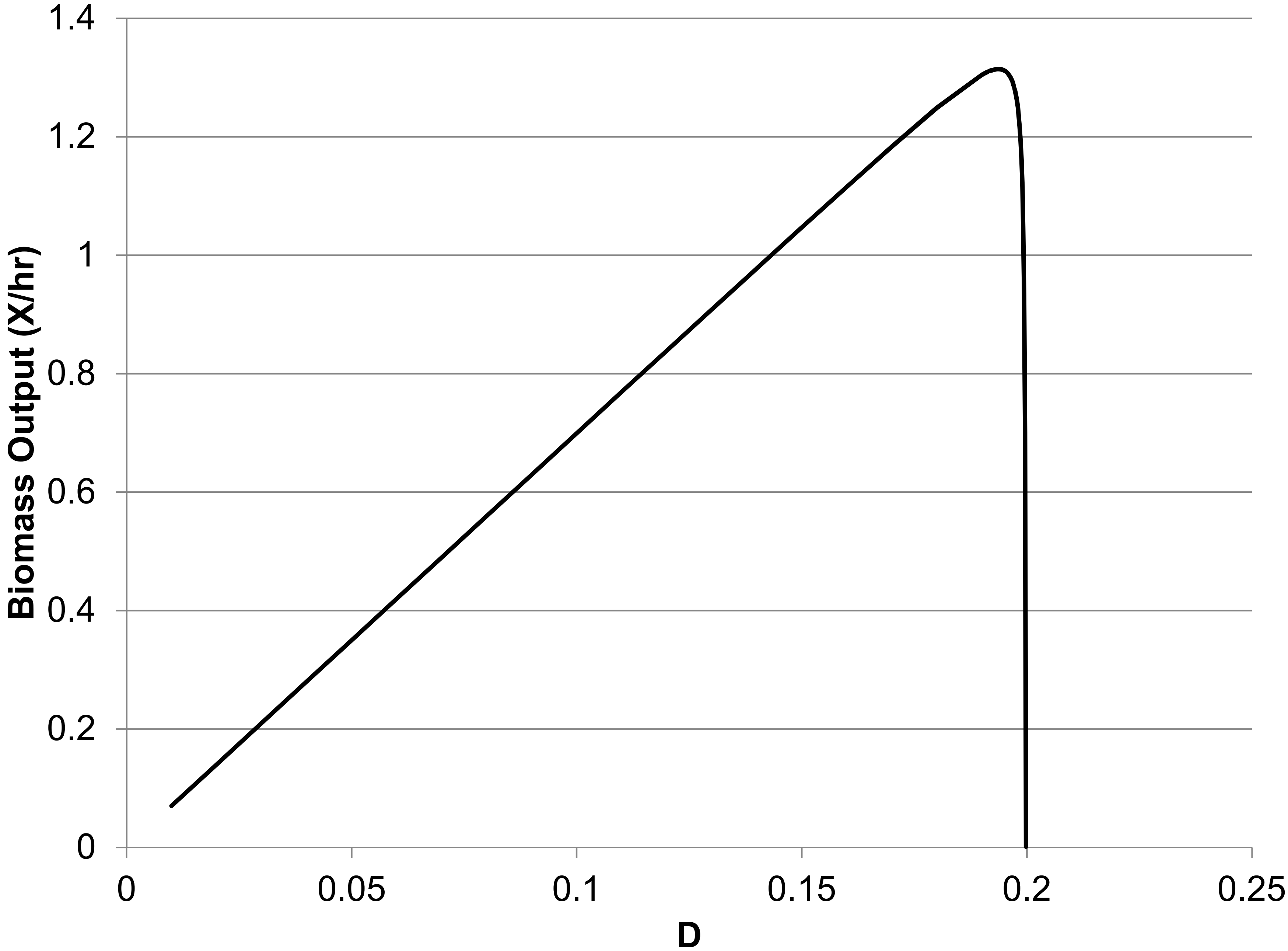



Constant Feed [Substrate]
S
D
120
100
80
60
40
20
0
0
0.05
0.1
0.15
0.2
0.25

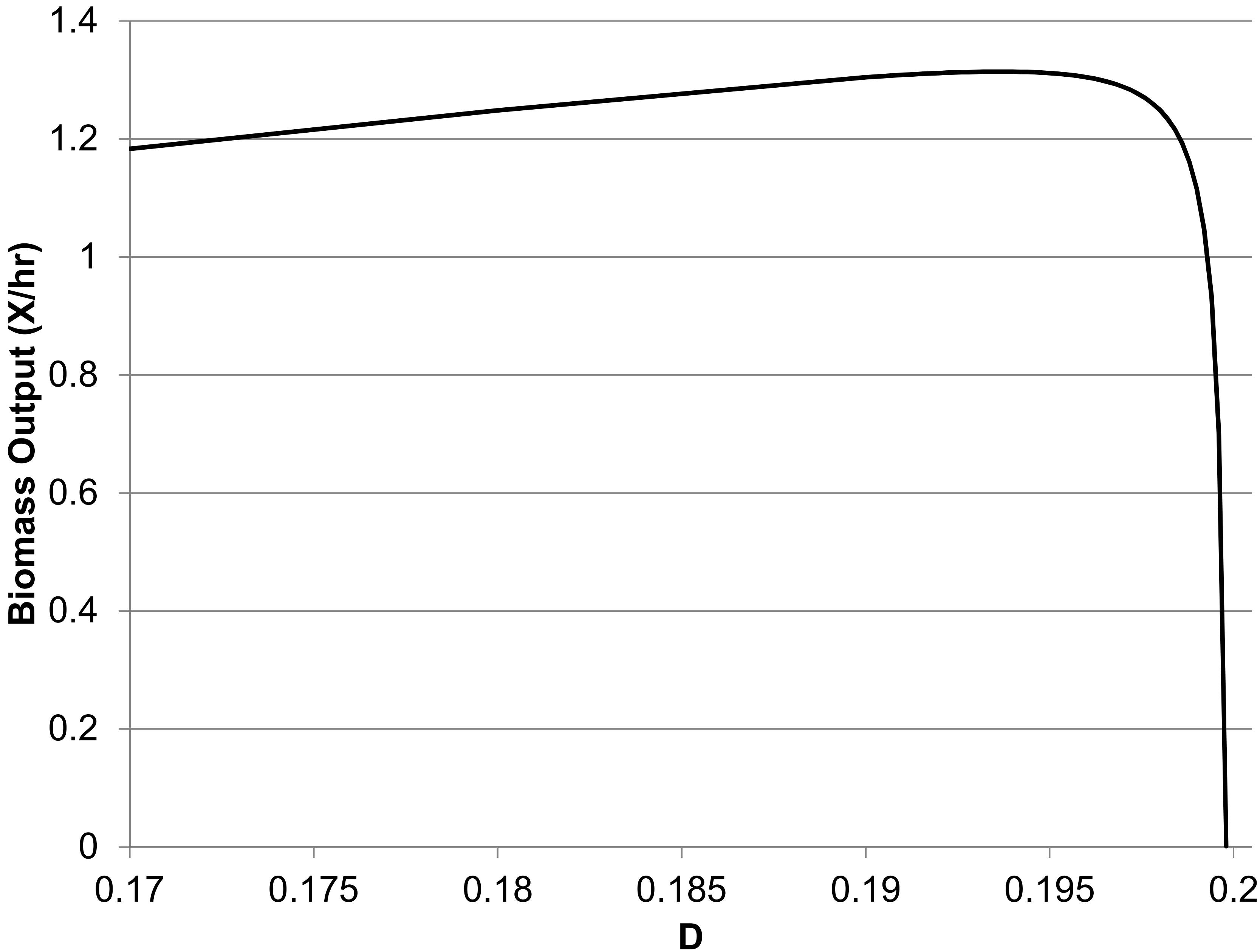
$$X = 0 = Y_{x/s}\left(S_o - \frac{K_s D}{(\mu_{max} - D)} \right)$$

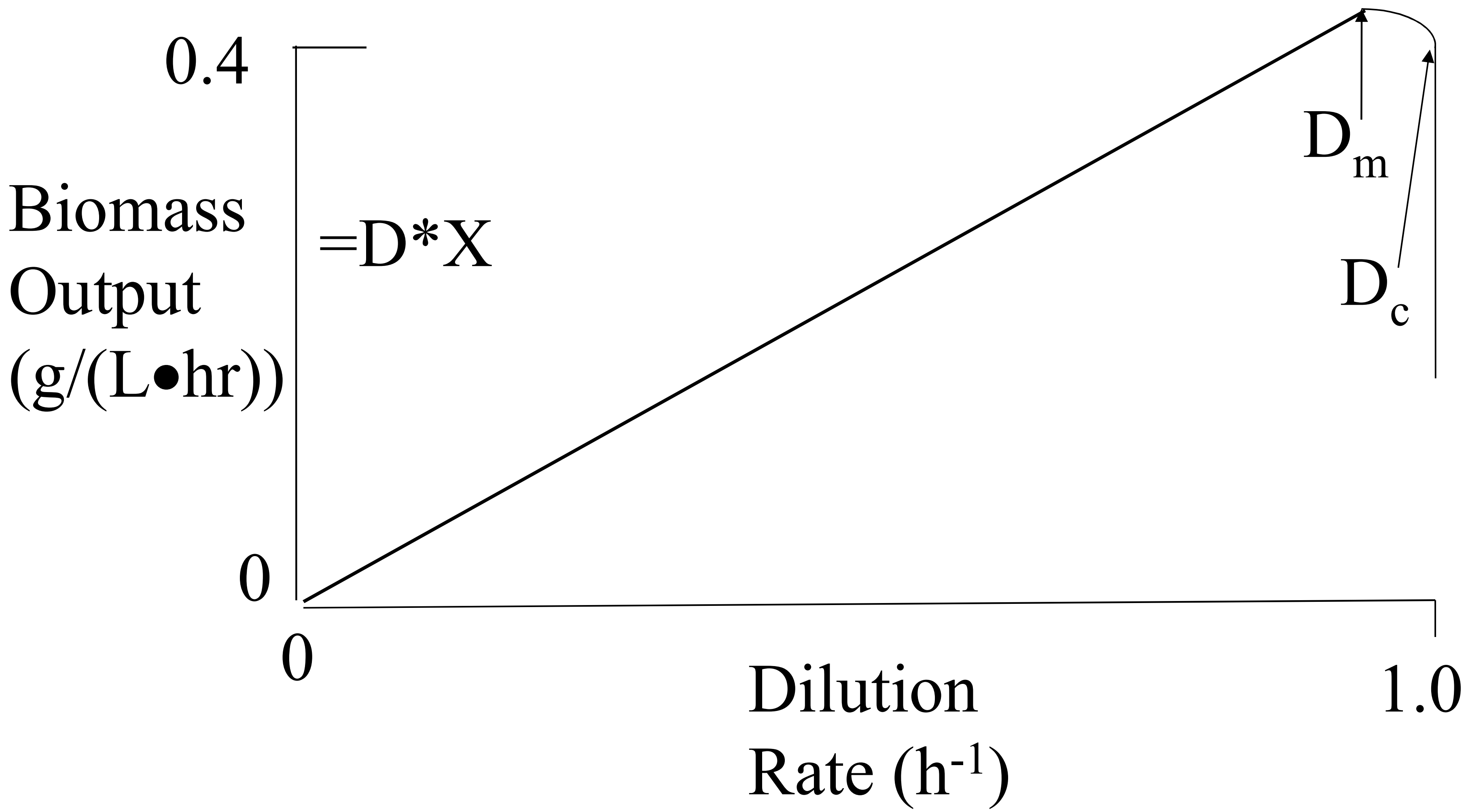
$$S_o = \frac{K_s D_C}{(\mu_{max} - D_C)} \qquad D_C = \frac{\mu_{max} S_0}{K_s + S_0}$$

$$\text{When } S_0 >> K_S, \quad \mu = D_C = \mu_{max}$$



Biomass Output (X/hr)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.05
0.1
0.15
0.2
0.25
D


Biomass Output (X/hr)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.17
0.175
0.18
0.185
0.19
0.195
0.2
D


0.4
Biomass
Output
(g/(L•hr))
=D*X
D_m
D_c
0
0
Dilution
Rate (h^-1)
1.0

Maximum Productivity

Rate of Cell Output = R = DX

$$R = DY_{x/s}\left[S_o - \frac{K_s D}{\mu_{max} - D}\right]$$

$$\frac{dR}{dD} = 0 = \frac{d}{dD}\left[DY_{x/s}\left(S_r - \frac{K_s D}{\mu_{max} - D}\right)\right]$$

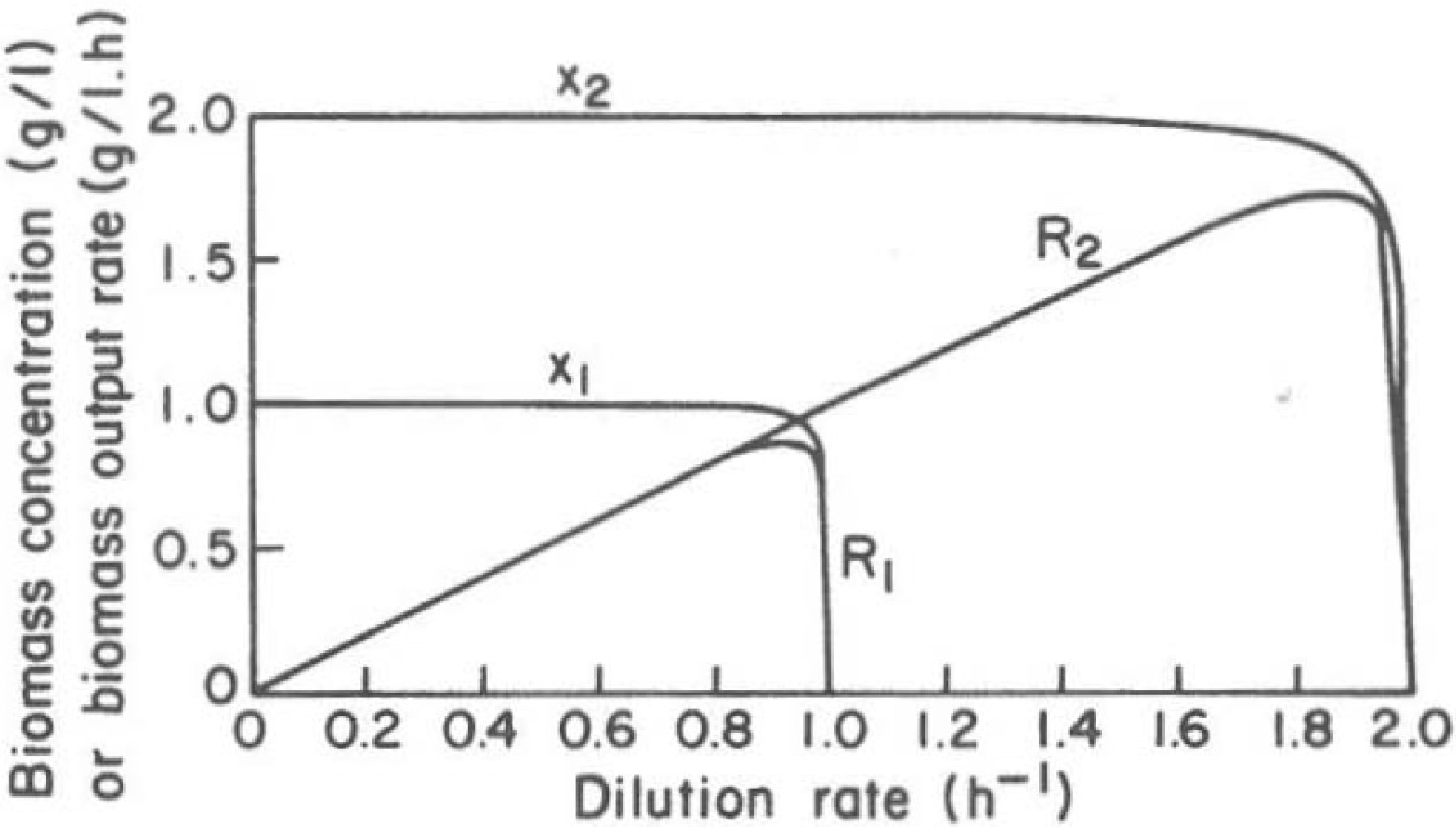
Dilution Rate at Maximum Productivity

$$D_m = \mu_{max}\left[1 - \left(\frac{K_s}{S_o + K_s}\right)^{1/2}\right]$$

Maximum Cell Concentration

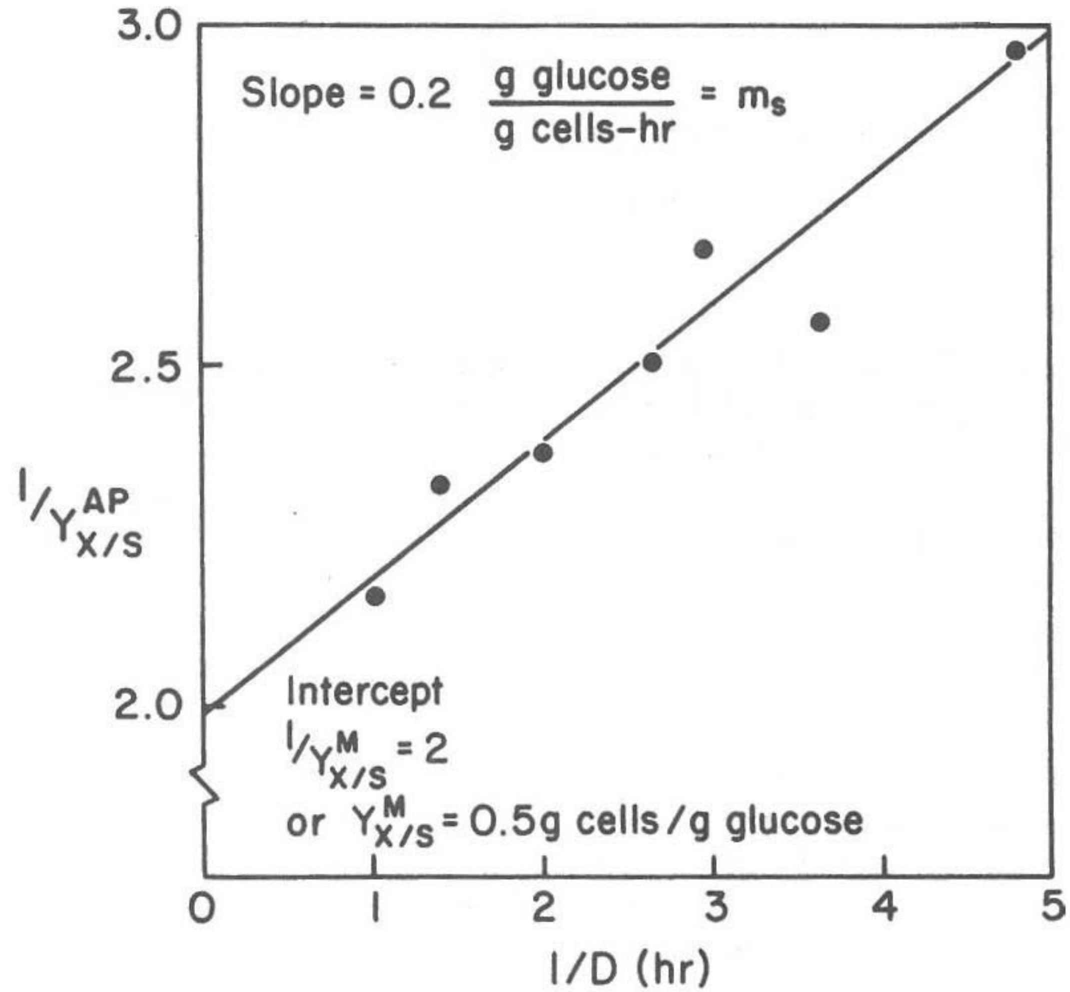
$$X_m = Y_{x/s}\left[S_o + K_s - \left\{K_s\left(S_o + K_s\right)\right\}^{1/2}\right]$$

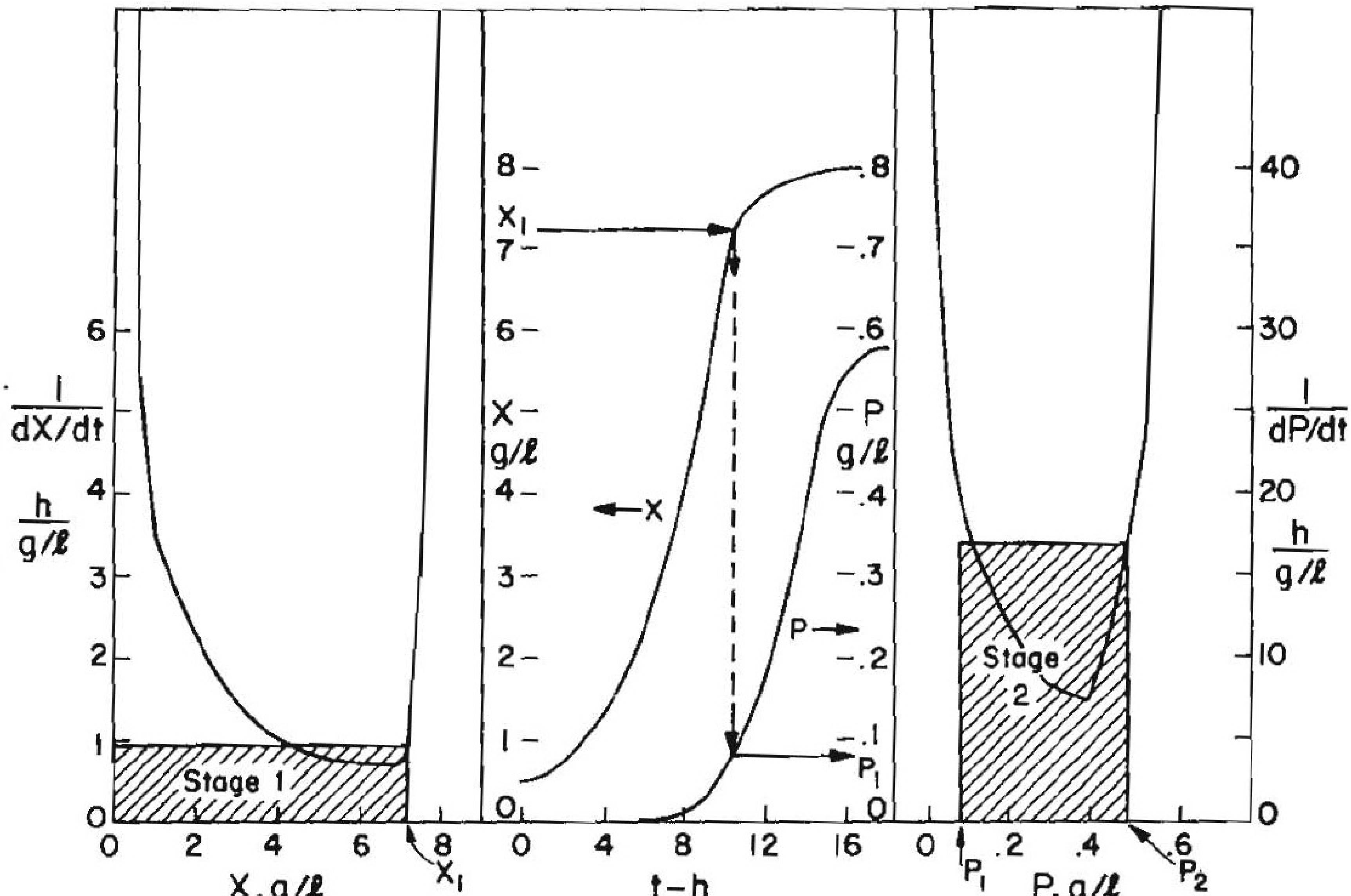
$$\overline{x}_m \cong Y_{x/s}S_o \quad \text{if } S_0 >> K_S$$



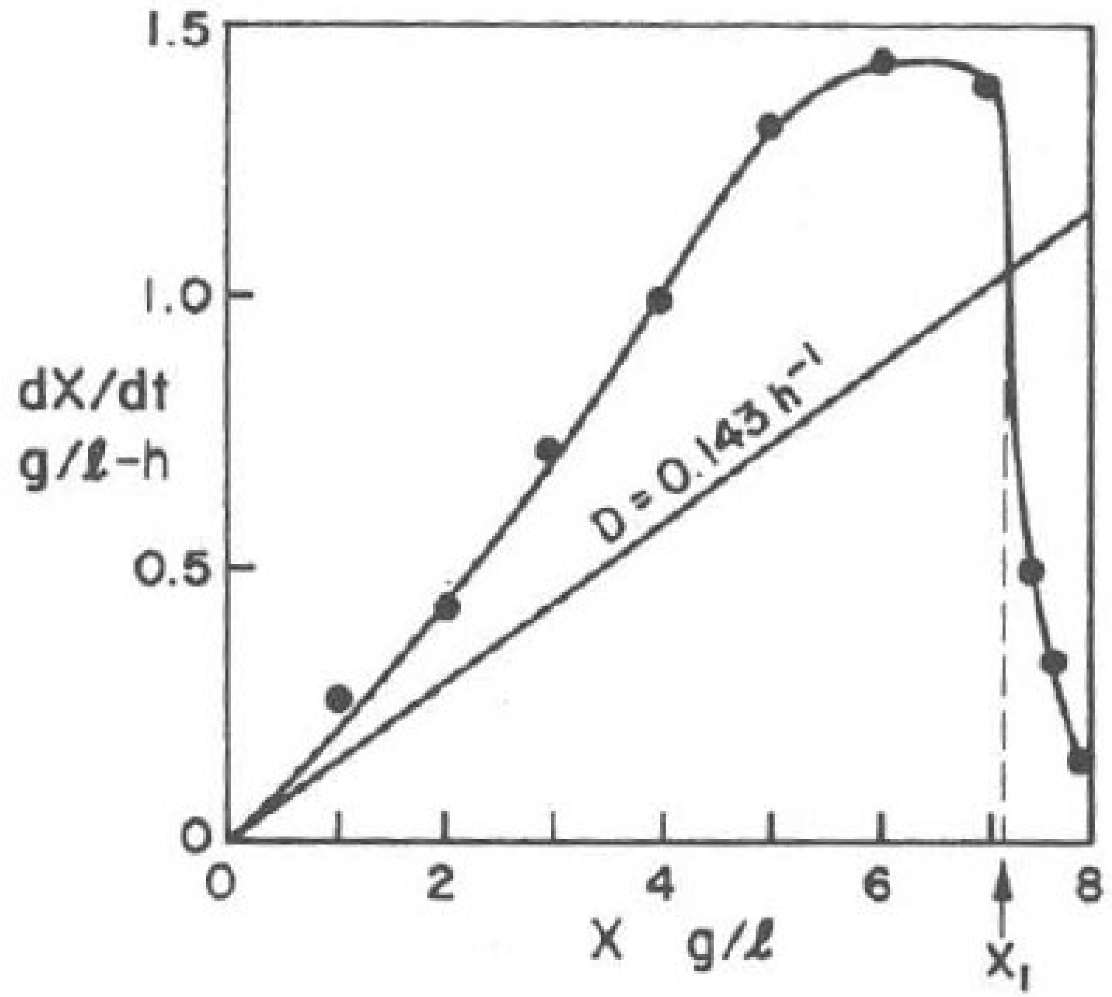
Biomass concentration (g/l)
or biomass output rate (g/l.h)
2.0
1.5
1.0
0.5
0
x_2
R_2
x_1
R_1
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Dilution rate (h^{-1})

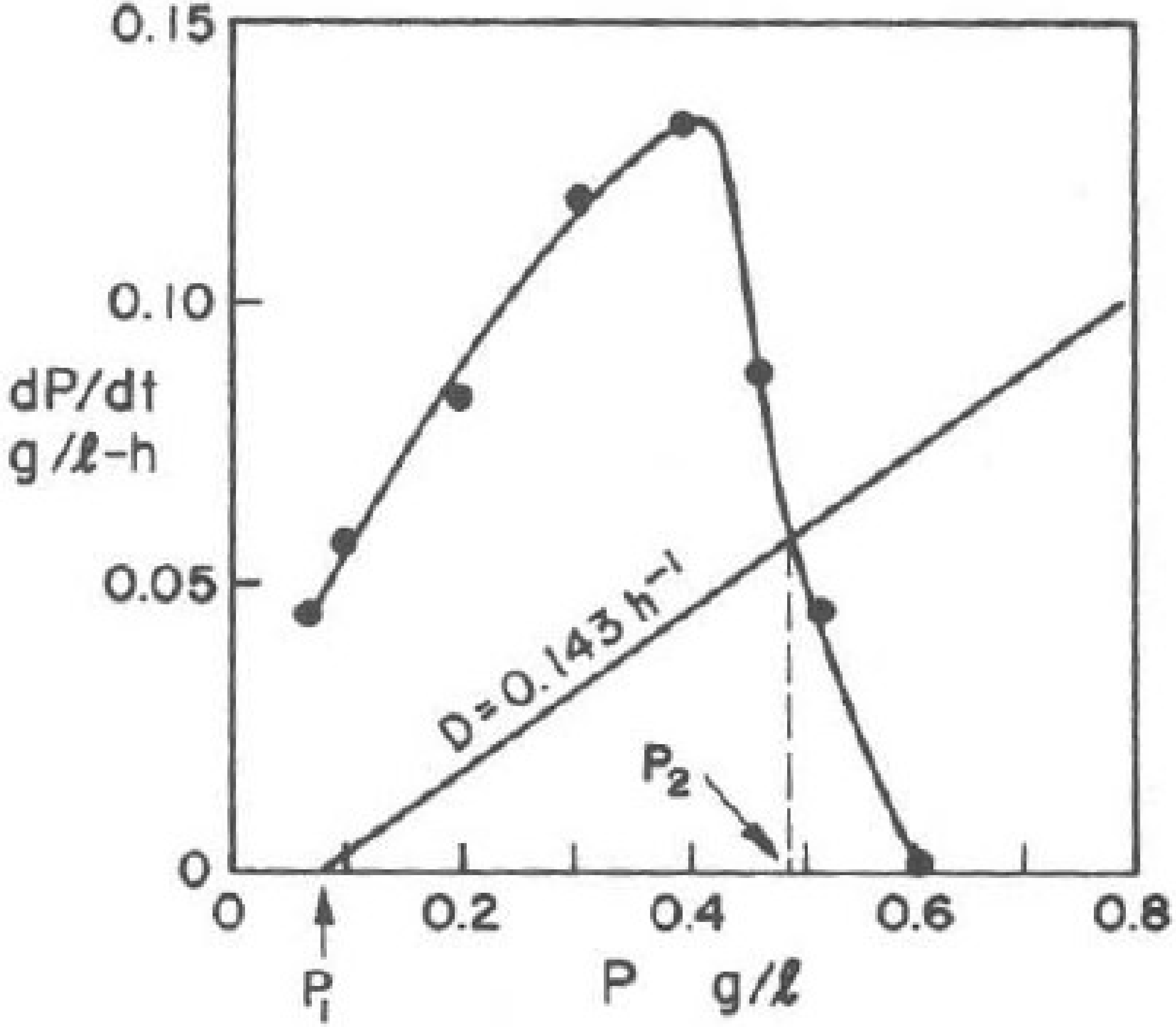
Determining Yield Constants M_e





1/(dX/dt)
h/(g/ℓ)
Stage 1
X, g/ℓ
X1
X g/ℓ
P g/ℓ
X1
← X
P →
P1
t − h
Stage 2
P1
P, g/ℓ
P2
1/(dP/dt)
h/(g/ℓ)


1.5
1.0
0.5
0
dX/dt
g/ℓ-h
D = 0.143 h⁻¹
0
2
4
6
8
X g/ℓ
X₁



0.15
0.10
0.05
0
dP/dt
g/ℓ-h
D=0.143 h⁻¹
P2
0
0.2
0.4
0.6
0.8
P1
P g/ℓ