ABE 580

Process Engineering of Renewable Resources

Chapter 6

Aerobic Fermentations

Aerobic Fermentations

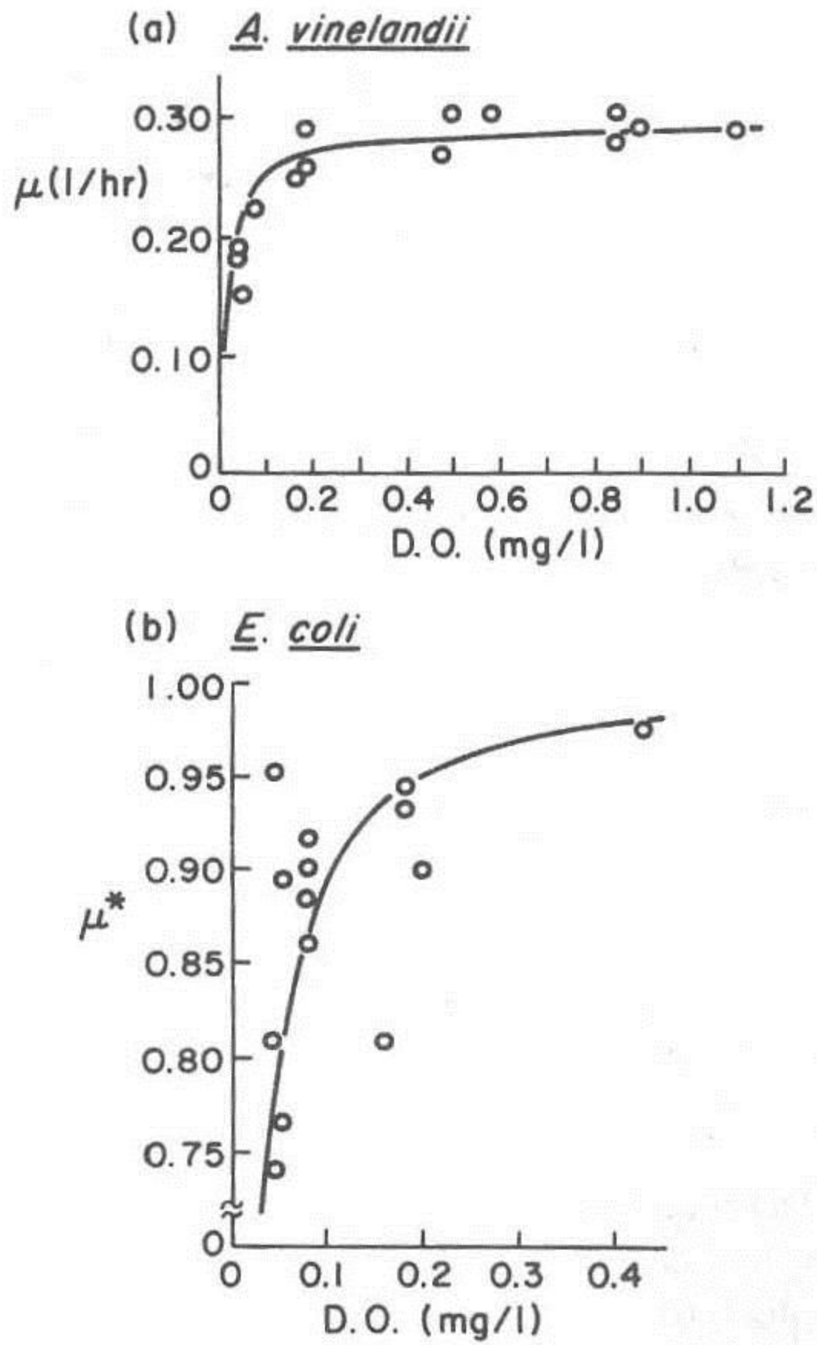
- Amino acids
- Antibiotics
- Recombinant proteins (pharmaceuticals)

Effect of DO o Cell Growth

$$\mu = \frac{\mu_{max} DO}{K_S^{DO} + DO}$$

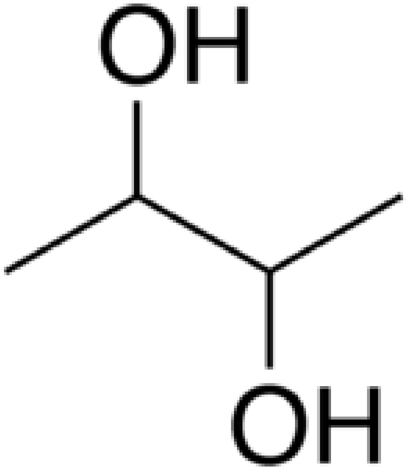
$$K_S^{DO} << DO$$

$$\mu \approx \mu_{max}$$



2,3 Butanediol

- Manufacture of
  butadiene rubber, plastics
  (ABS – Lego bricks)



Klebsiella oxytoca ATCC 8724

- Related *Klebsiella pneumoniae*
- Hans Christian Gram invented “Gram stain” to differentiate between *Klebsiella* and *Streptococcus pneumoniae*

- Of industrial interest because it readily ferments xylose and glycerol

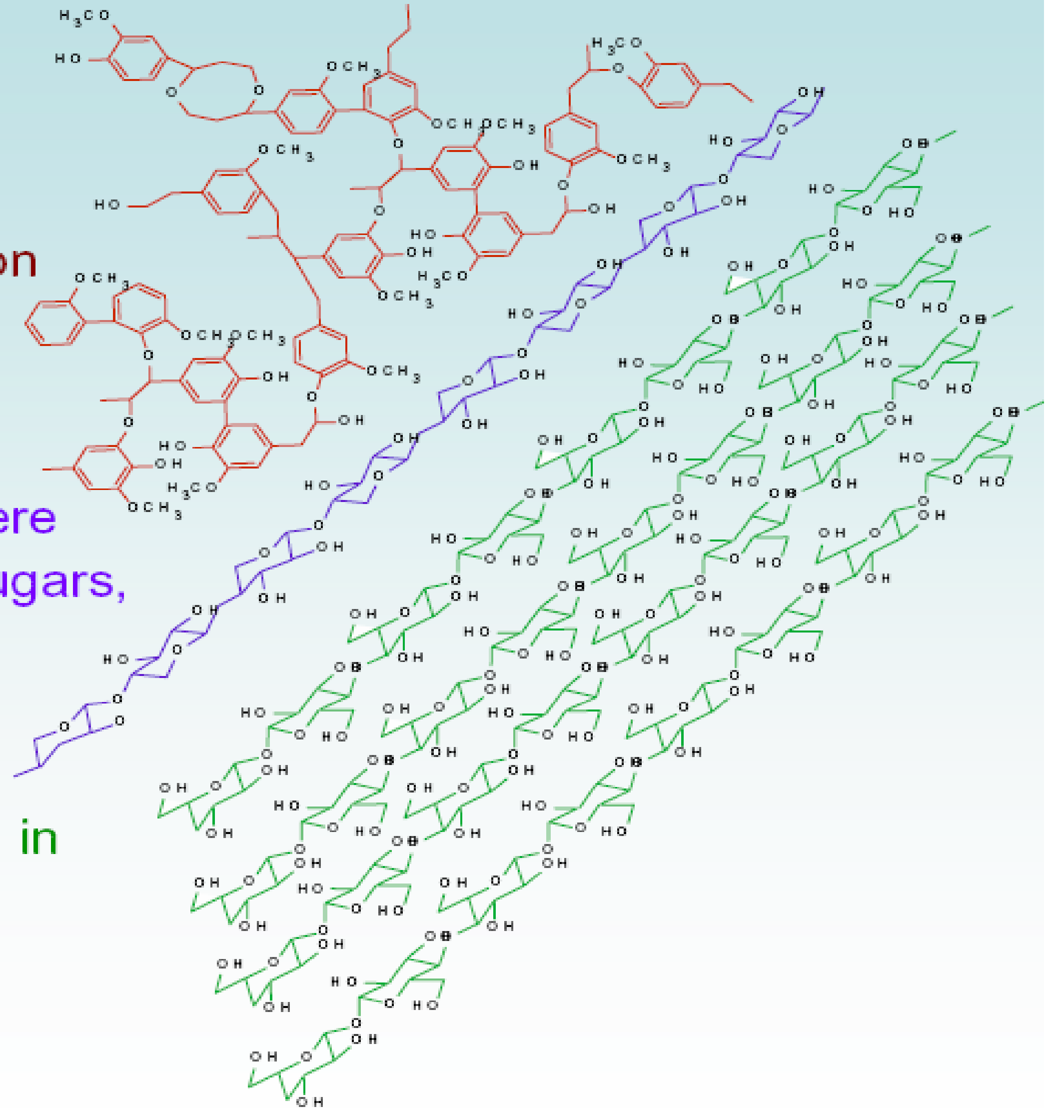
- Facultative Anaerobe

Cellulosic Biomass: Major Constituents

Lignin: 15%–25%
- Complex aromatic structure
- High energy content
- Resists biochemical conversion

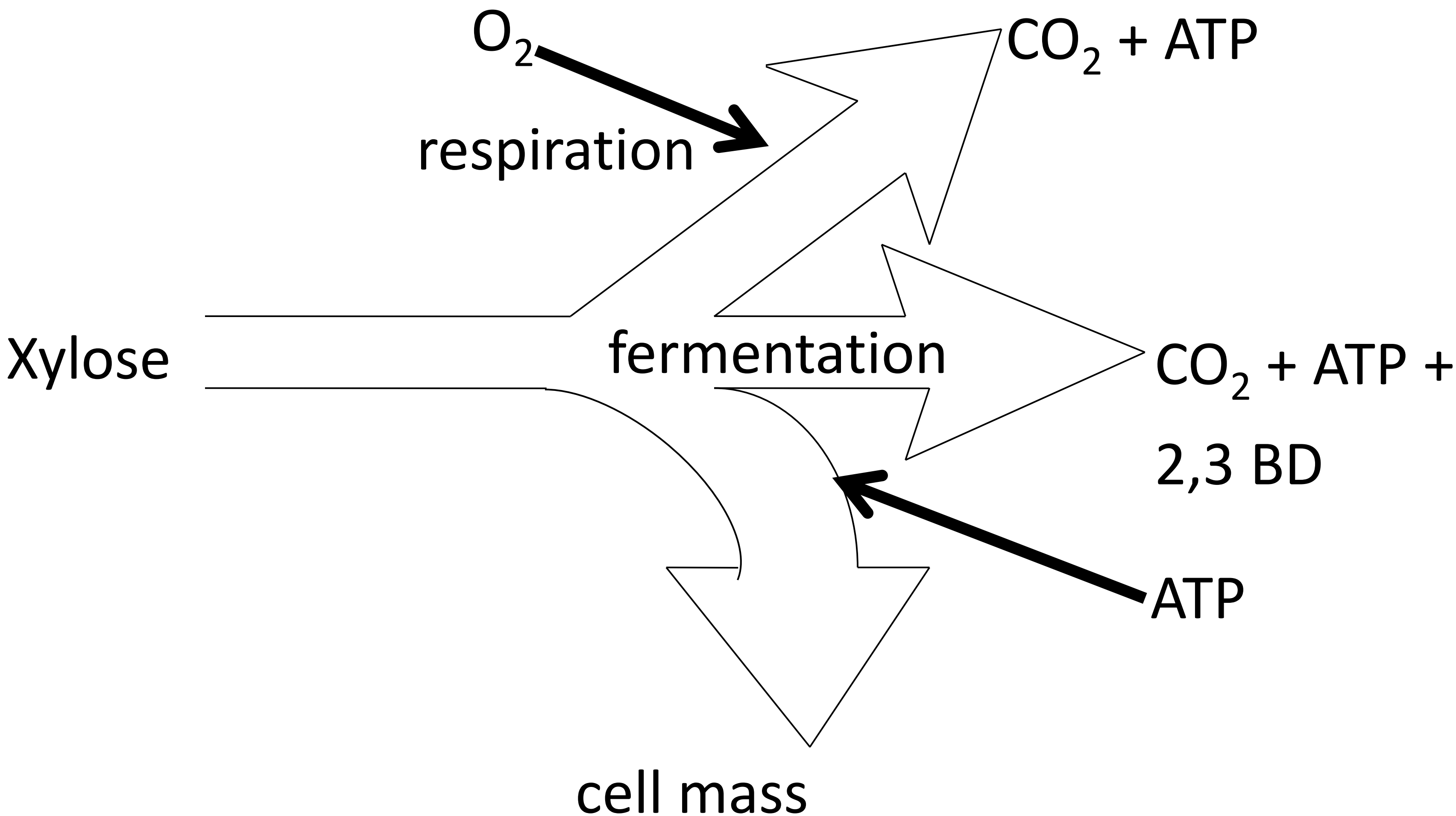
Hemicellulose: 23%–32%
- Xylose is the second most abundant sugar in the biosphere
- Polymer of 5- and 6-carbon sugars, marginal biochemical feed

Cellulose: 38%–50%
- Most abundant form of carbon in biosphere
- Polymer of glucose, good biochemical feedstock



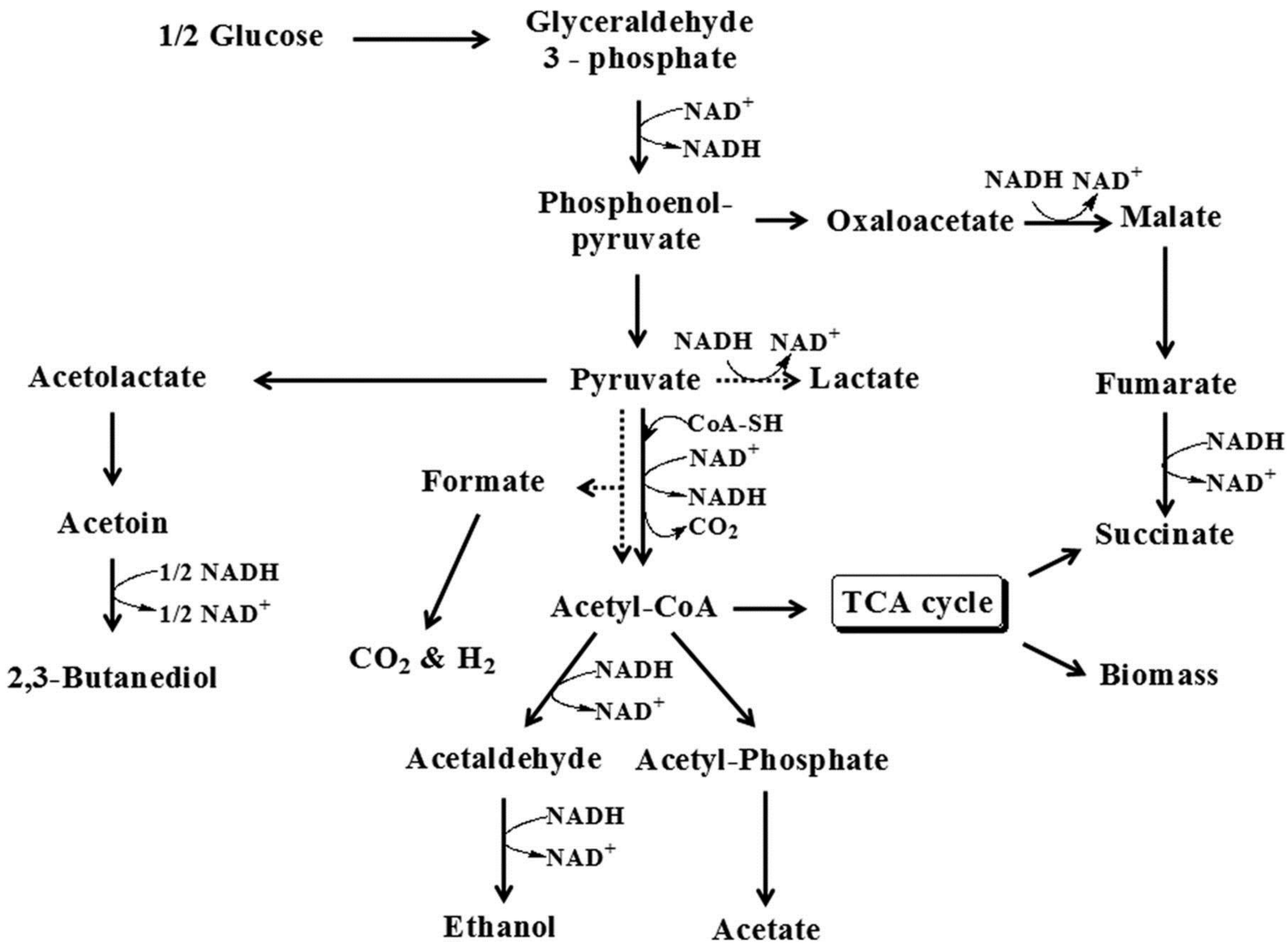
Xylose (Wood Sugar)

- $C_5H_{10}O_5$
- Pentose (5-sugar)
- In grasses (corn, wheat, rice, etc.), 40% of carbohydrate of inedible plant material



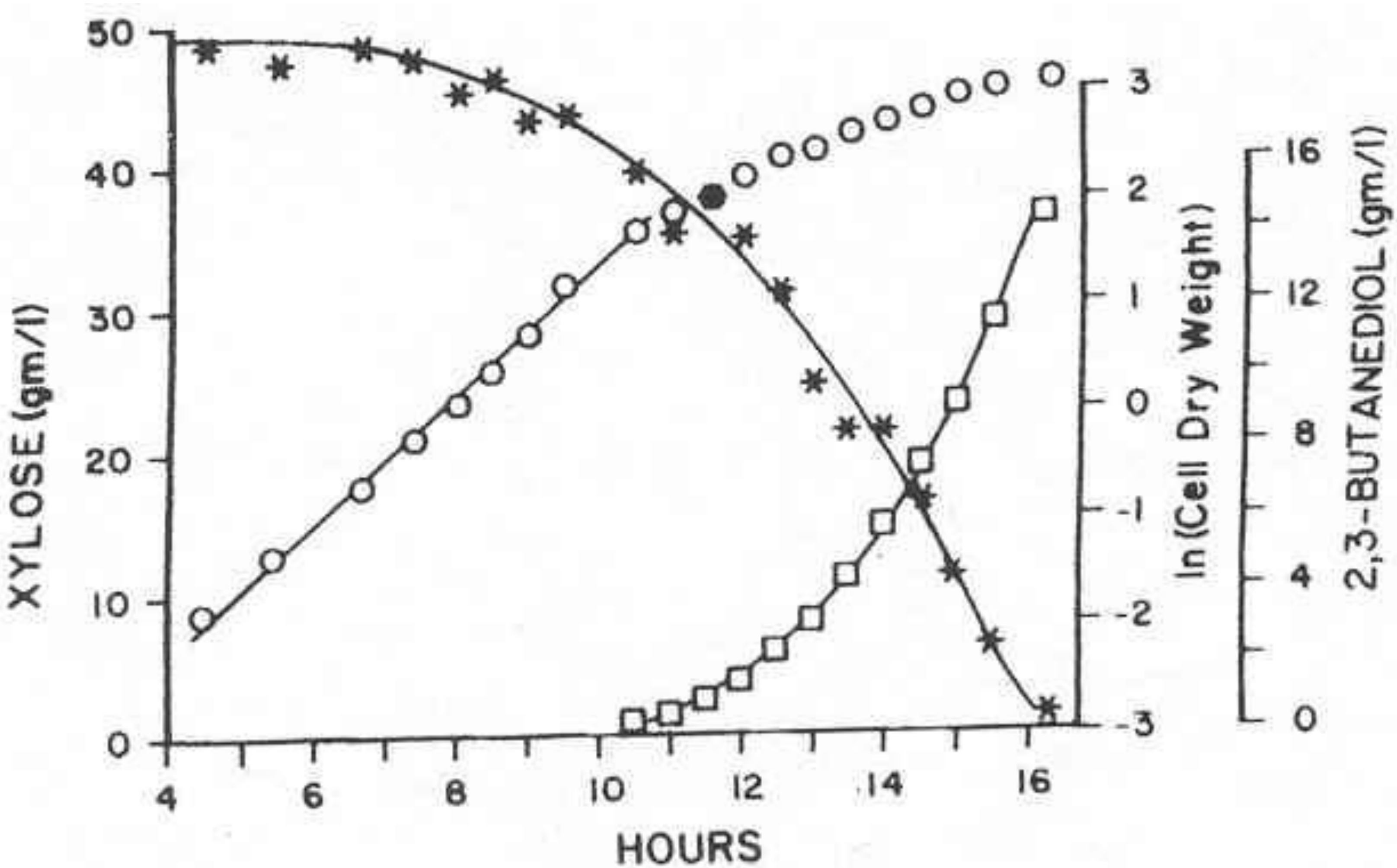
O2
CO2 + ATP
respiration
Xylose
fermentation
CO2 + ATP +
2,3 BD
ATP
cell mass

Fermentation pathways in Klebsiella pneumoniae and strategies for constructing the 2,3-butanediol-producing base strain.



Moo-Young Jung et al. Appl. Environ. Microbiol. 2014;80:6195-6203

2,3-Butanediol



Jansen, N. B. et al. Biotechnology and Bioengineering. 1984;26:362-369

Modeling Approach

- Cellular function is ATP constrained
- ATP use is prioritized: cell maintenance then cell growth
- Metabolism is regulated to maximize ATP production
- O_2 is required for maximum ATP generation
- When O_2 is limiting, anaerobic metabolism is induced to make up the ATP deficiency

2,3 Butanediol
Phases of fermentation

1. Aerobic fermentation
2. Transition from aerobic to oxygen limiting conditions
3. Oxygen limiting conditions (microaerobic or anoxic fermentation)

Terminology

$$Q_S$$

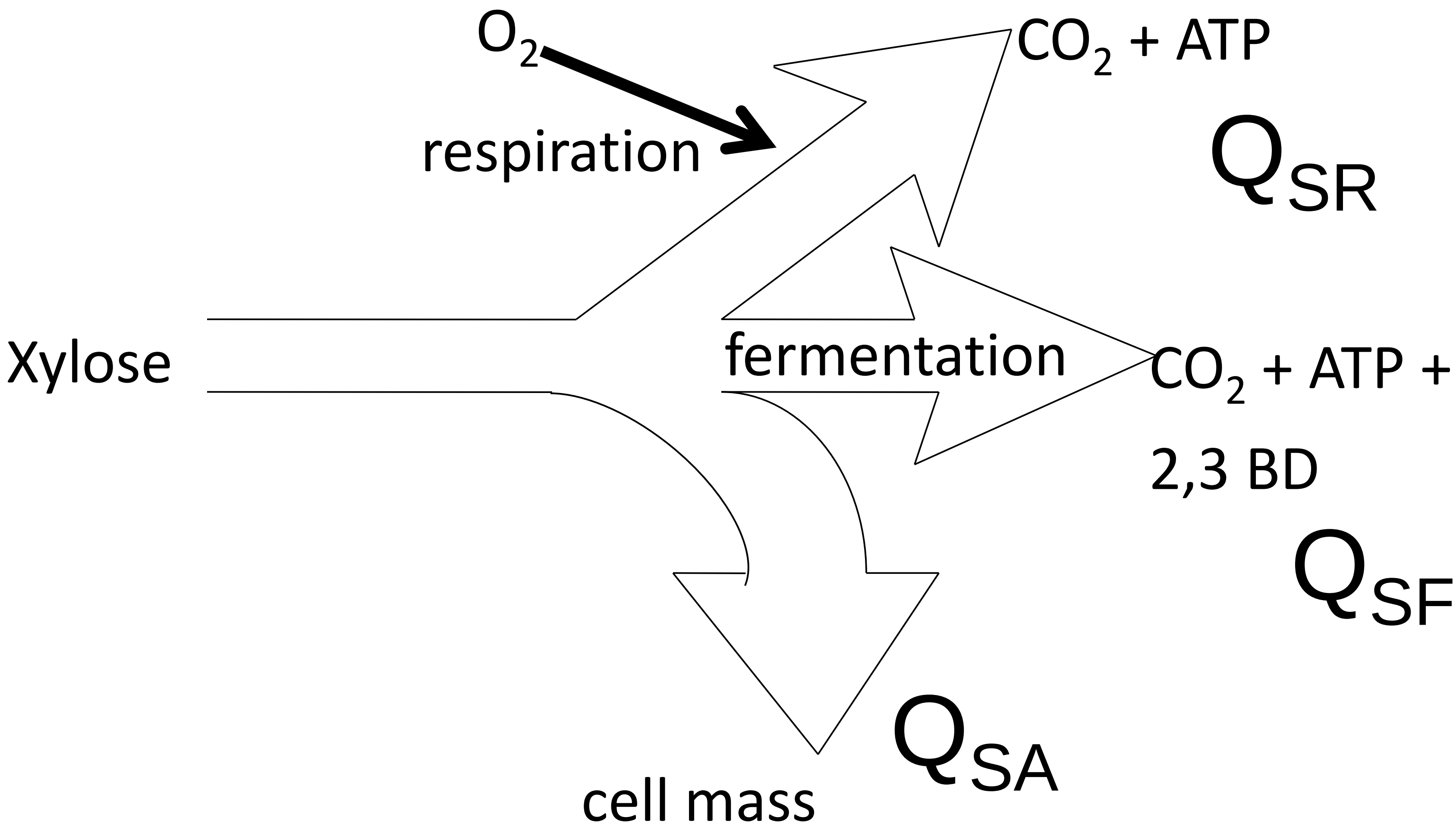
Rate of substrate utilization

(consumption) via specific metabolism

$$dS/dt = \Sigma\,(Q_{si})*\text{mol wt.}$$

(in batch fermentation)

$$Q_{STot} = Q_{SA} + Q_{SR} + Q_{SF}$$



Stoichiometry of Respiration

$$\text{xylose} \longrightarrow 5\ CO_2 + 10\ NADH + \frac{10}{3} ATP$$

$$\frac{1}{2} O_2 + NADH + H^+ \longrightarrow 2\ ATP + H_2O$$

$$\text{xylose} + 5\ O_2 \longrightarrow 5\ CO_2 + \frac{70}{3} ATP$$

$$Q_{SR} = \frac{3}{70}\left(\frac{dx}{dt}\ \frac{1}{Y_{ATP,1}} + m_{el}\ X \right)$$

Stoichiometry of Fermentation

$$\text{xylose} \longrightarrow \frac{5}{3}\ \text{CO}_2 + \frac{5}{6}\ \text{NADH} + \ \frac{5}{3}\ \text{ATP} + \ \frac{5}{6}\text{butanediol}$$

Phase 1 – Aerobic Growth

$$\frac{dx}{dt} = \mu_{max} x$$

$$\frac{dS}{dt} = -\left(Q_{SA} + Q_{SR} + Q_{SF}\right) \bullet mw\left(xylose\right)$$

$$Q_{Si} = \text{mol L}^{-1}\ \text{hr}^{-1}$$

$$Q_{Sa} = \frac{1 \text{ mol Xylose}}{120 \text{ g Cells}} \frac{dx}{dt} = \frac{1}{120} \frac{dx}{dt}$$

$$Q_{Sr} = \frac{3}{70}\left(\frac{dx}{dt} \ \frac{1}{Y_{ATP,1}} + \ m_{e,1} \ X \right)$$

$$Q_{Sa} = \frac{1}{120}\frac{dx}{dt}$$

$$Q_{SA} = \frac{mol}{L\ hr}$$

$$MW_{xylose} = 150\ {}^{g}\!/_{mol}$$

$$\frac{dX}{dt} = \frac{g}{L\ hr}$$

$$Q_{SA}\frac{150\ g}{mol} = Y_{S/X}\frac{dX}{dt} = \frac{1}{120}\frac{dX}{dt}$$

$$Y_{S/X} = \frac{150}{120} = 1.25$$

Phase 1 - Continued

$$Q_{SR} = \frac{3}{70}\left(\frac{dx}{dt}\ \frac{1}{Y_{ATP,1}} + \ m_{el}\ X\right)$$

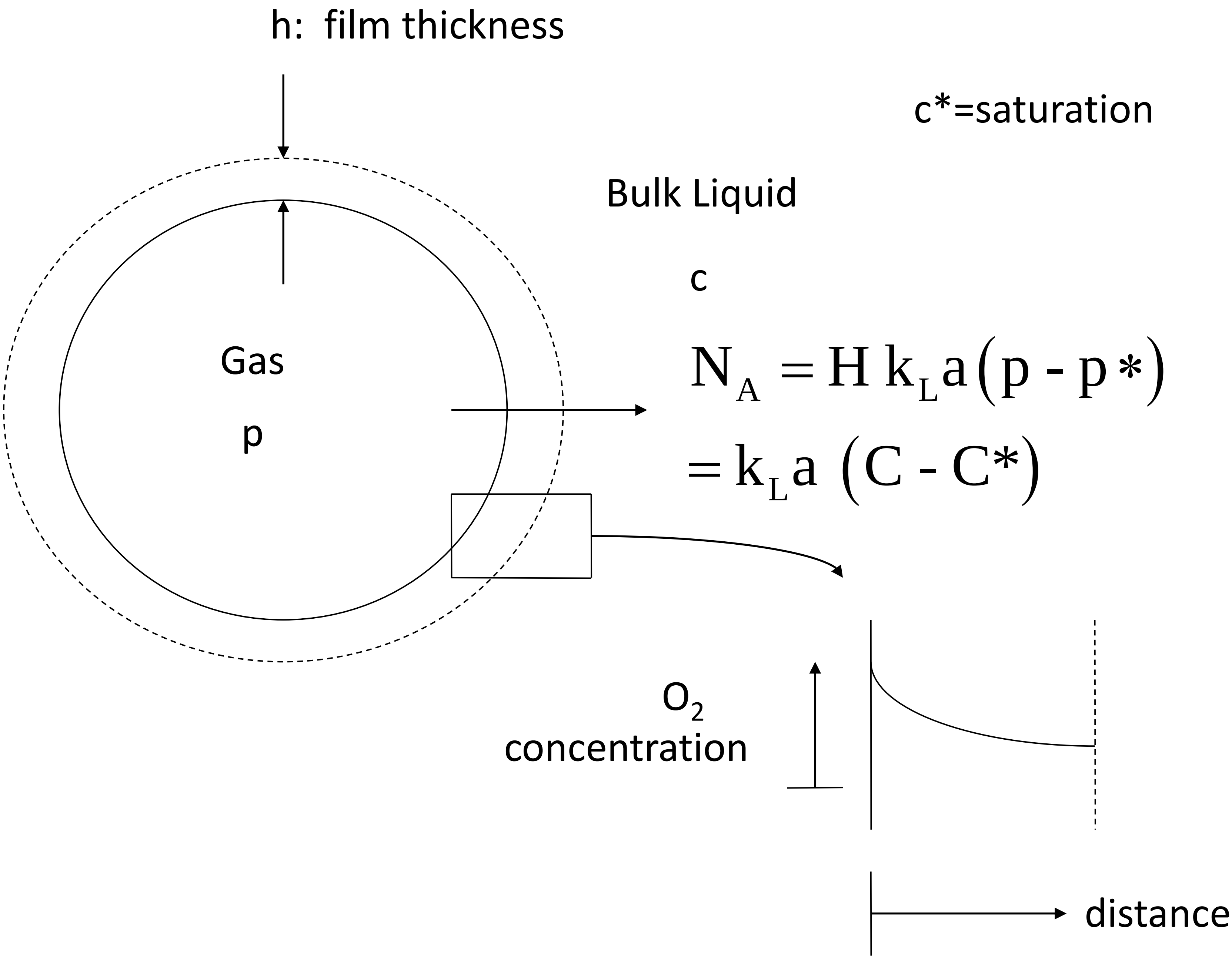
$$Q_{SF} = 0$$

$$\frac{dP}{dt} = 0$$

Phase 2 – When is O_2 Limiting?

$$\text{xylose} + 5\,O_2 \longrightarrow 5\,CO_2 + \frac{70}{3}\,\text{ATP}$$

$$5Q_{SR} > k_L a C^*$$



$$N_A = H\,k_L a\left(p - p*\right)$$

$$= k_L a\left(C - C*\right)$$

When O2 Limiting

- Metabolism is balanced so that oxidative phosphorylation (electron transport system, ETS) is saturated

$$\frac{1}{2}\mathrm{O}_2 + \mathrm{NADH} + \mathrm{H}^+ \longrightarrow 2\ \mathrm{ATP} + \mathrm{H}_2\mathrm{O}$$

$$\mathrm{Q}_{\mathrm{ETS}} = 2\mathrm{K}_L a C^*$$

$$\mathrm{Q}_{\mathrm{ETS}} = 10\mathrm{Q}_{SR} + \frac{5}{6}\mathrm{Q}_{SF}$$

ATP Balance

$$\mathrm{Q_{SR}} = \frac{1}{10}\left(Q_{ETS} - \frac{5}{6} Q_{SF} \right)$$

$$\mu_{\mathrm{max}} \mathrm{X} \ \frac{1}{\mathrm{Y}_{ATP}} + \ \mathrm{m_{e,1}} \ \mathrm{X} = \frac{10}{3} Q_{SR} + \frac{5}{3} Q_{SF} + 2 Q_{ETS}$$

$$\mathrm{Q_{Sf}} = \frac{18}{25}\left(\mu_{\mathrm{max}} \mathrm{X} \ \frac{1}{\mathrm{Y_{ATP,2}}} + \mathrm{m_{e2}} \ \mathrm{X} - \ \frac{7}{3} \ \mathrm{Q}_{ETS} \right)$$

Phase 3 - Fermentation

$$Q_{ETS} = 2K_L aC^*$$

$$Q_{Sf} = \frac{18}{25}\left(\mu_{max} X \; \frac{1}{Y_{ATP,2}} + m_{e2} \; X - \frac{14}{3} \; k_L aC^* \right)$$

$$Q_{SR} = \frac{1}{10}\left(2K_L aC^* - \frac{5}{6} Q_{SF} \right)$$

$$Q_{Sa} = \frac{1}{120} \mu_{max} X$$

$$\frac{dx}{dt} = \left(\mu_{max} - k_d \; P \right) \; X$$

$$\frac{dP}{dt} = \frac{5}{6}\ Q_{sf}\ \ MW_{2,3BD}$$

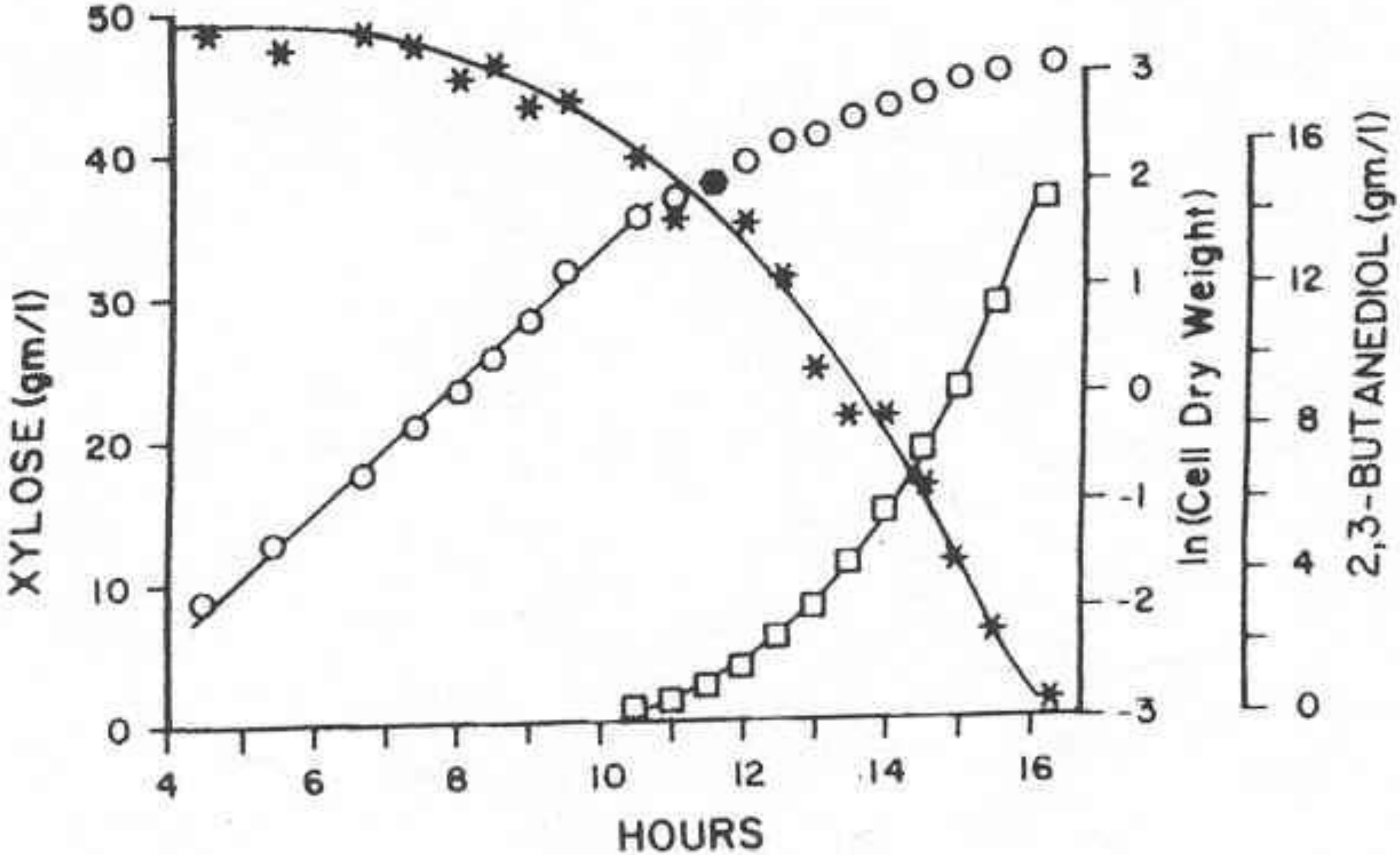
$$MW_{2,3BD} = 90\,\frac{g}{mol}$$

Table 6.2

	Oxygen Sufficient (i = l)	Oxygen Limiting (i = 2)
$Y_{ATP,i}$ (g cells /mol ATP)	11.5	10.4
μ_{max} (h^{-1})	0.6	0.6
m_{ei} (mol ATP/g cell - hr)	0.047	0.017
$K_L aC^*$ (mol O2 / L-hr)	-------	0.027
K_d	-------	0.0077

$$x_L\ (gL^{-1}) = \frac{14}{3}\ k_L ac^* / \left(m_{e1} + \mu_{max} / Y_{ATP}^{max}\right) = 1.27$$

2,3-Butanediol



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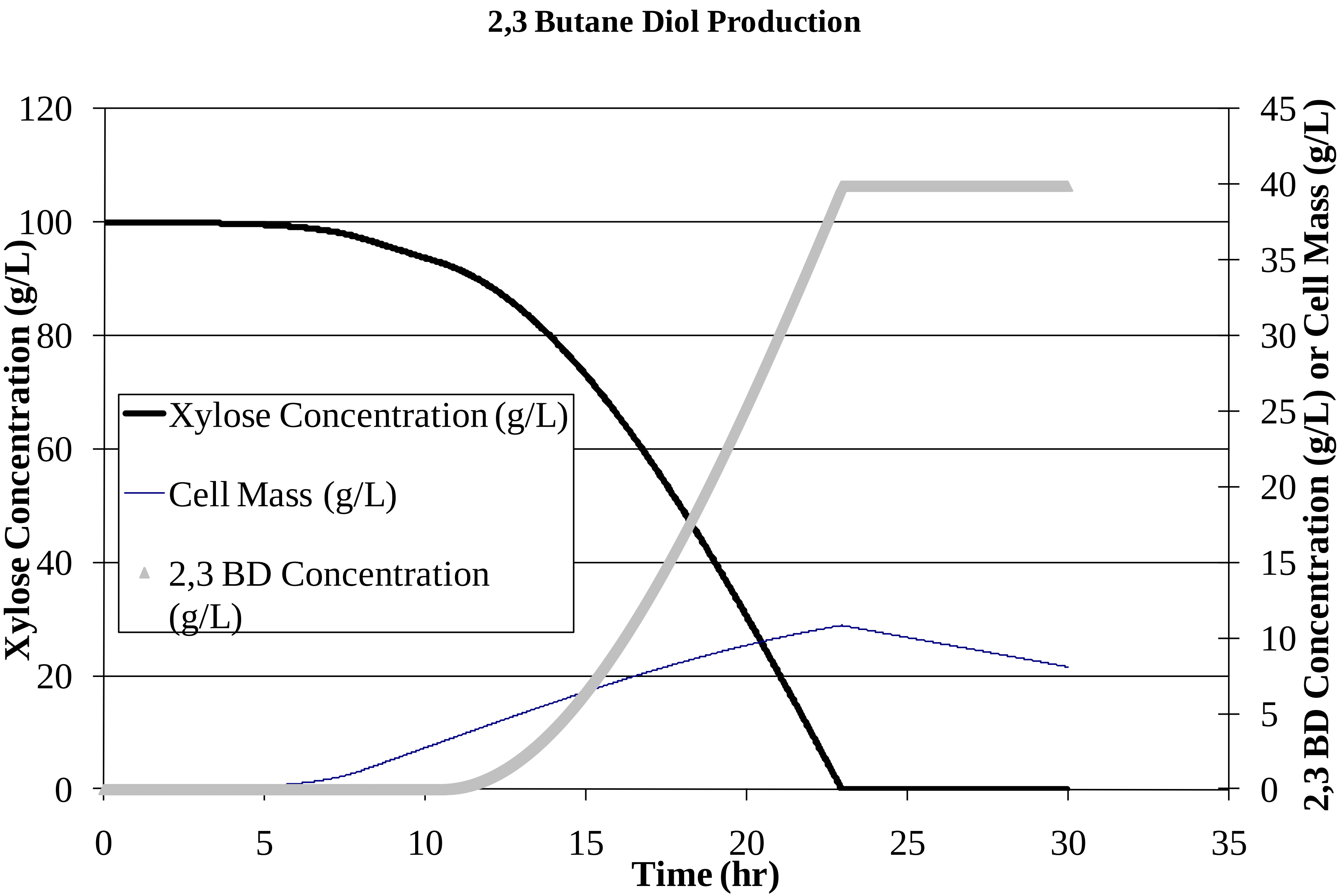
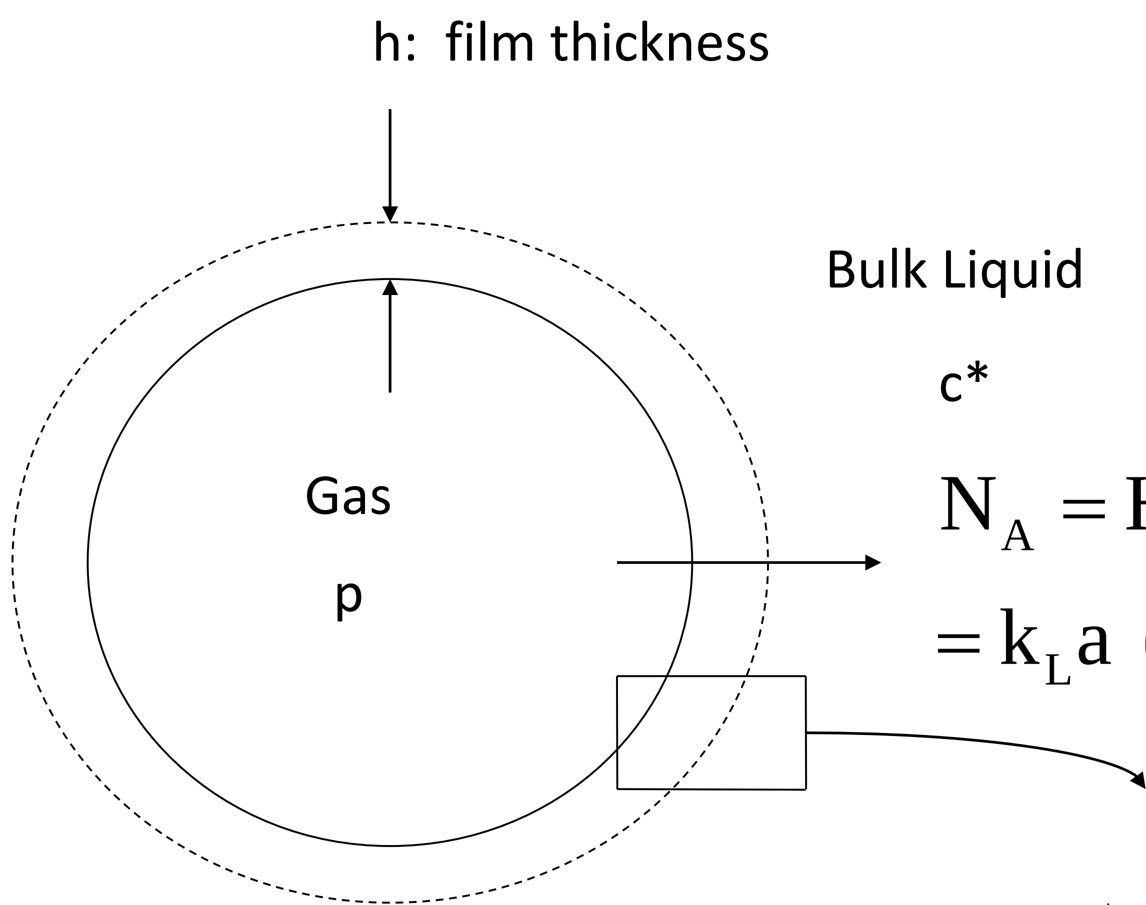
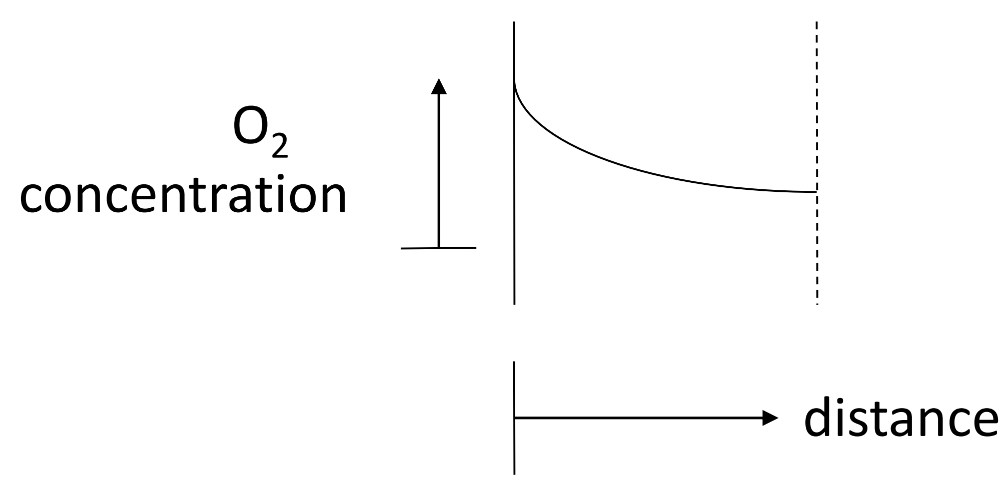

2,3 Butane Diol Production
Xylose Concentration (g/L)
2,3 BD Concentration (g/L) or Cell Mass (g/L)
Time (hr)
Xylose Concentration (g/L)
Cell Mass (g/L)
2,3 BD Concentration (g/L)
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
40
45
0
5
10
15
20
25
30
35

Aeration

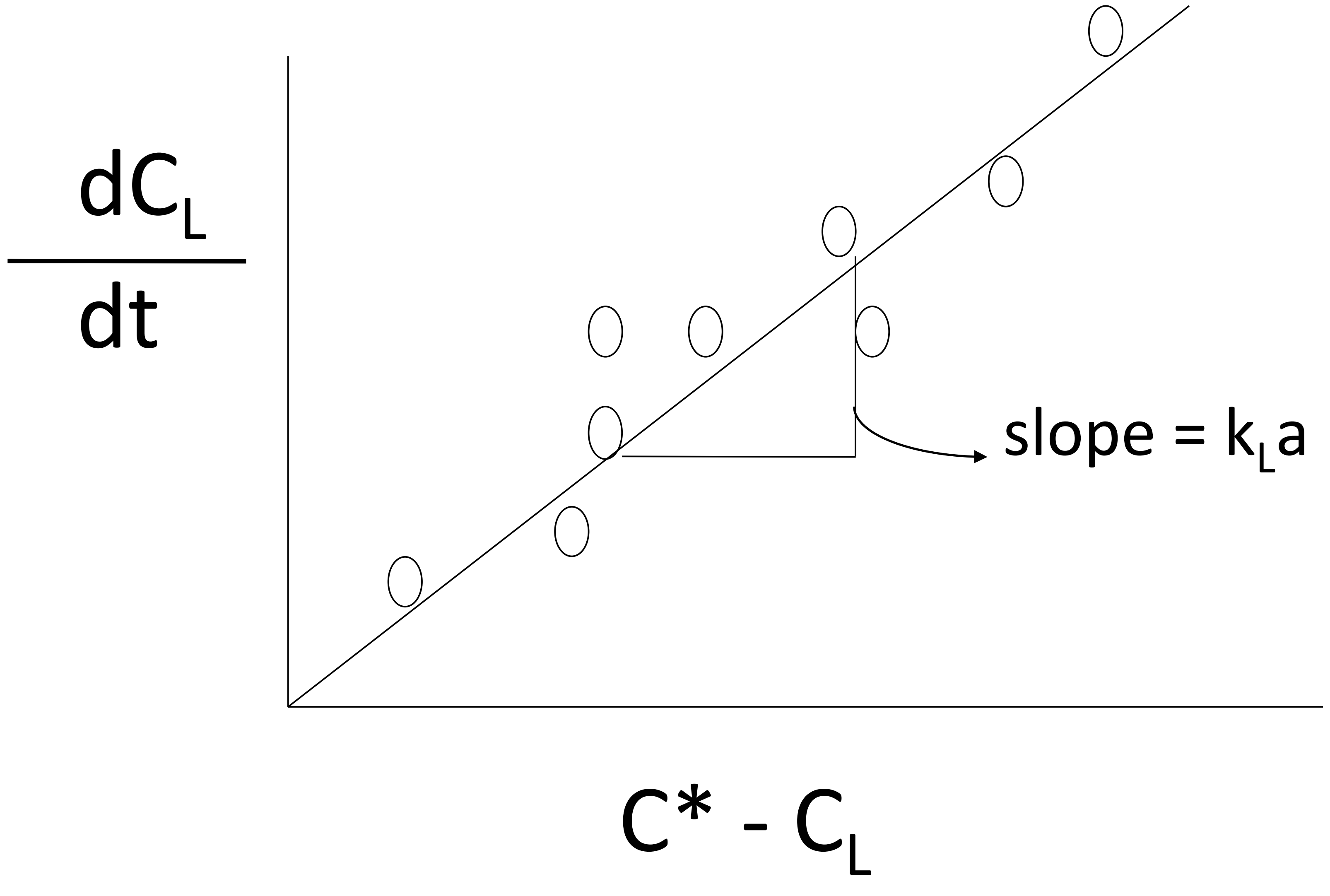
- Required for cellular reactions using respiration
- O2 transfer limited by
  - Diffusivity of O2
  - Transfer area (surface area of bubbles)
  - Concentration gradient
- Power requirements for aeration are huge!



$$N_A = H\,k_L a\left(p - p*\right)$$
$$= k_L a\,\left(C - C*\right)$$



$$-N_{O2} = \frac{dc_L}{dt} = k_L a\left(C^* - C_L\right)$$



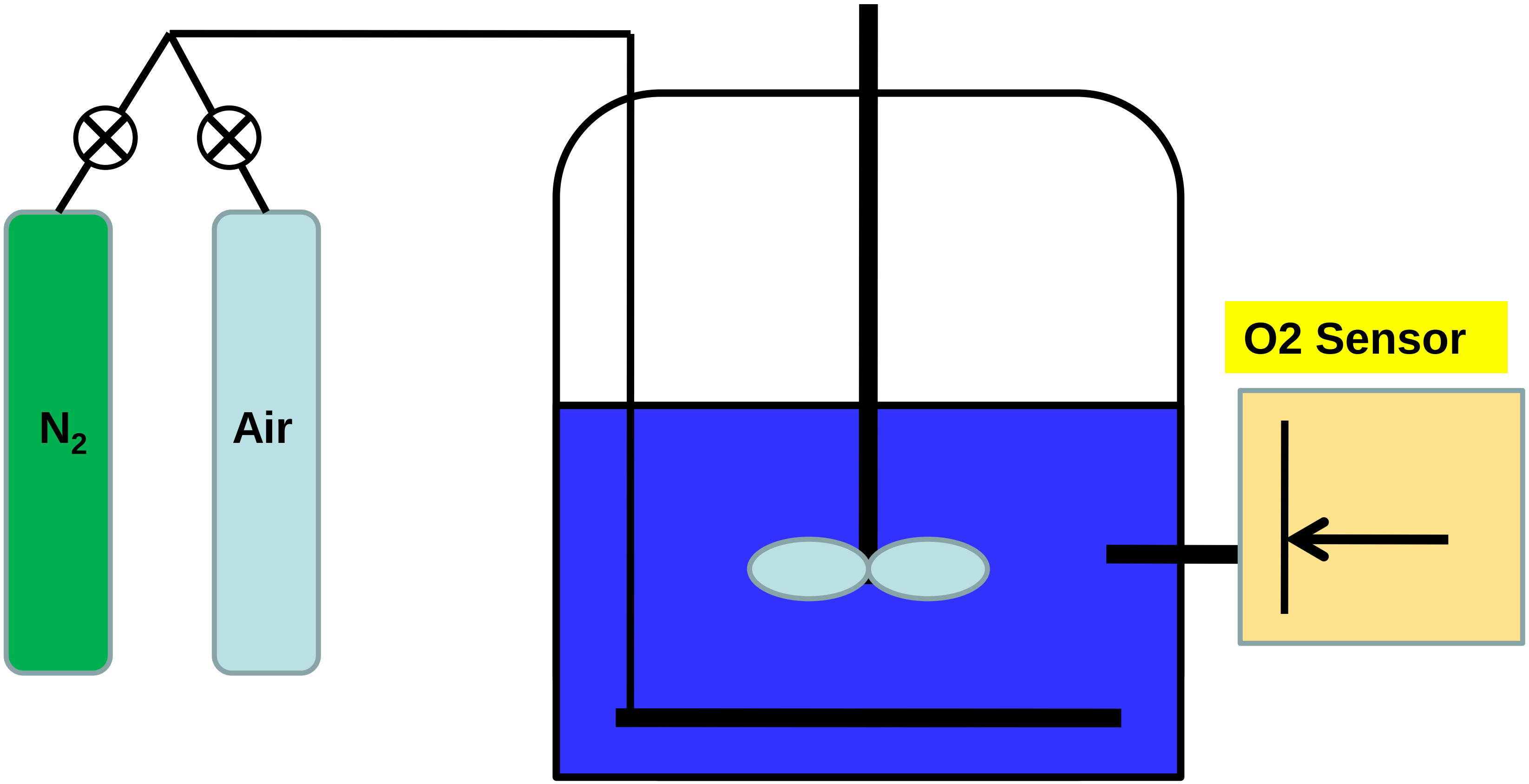
Steady State

$$k_L a\left(C^* - C_L\right) = OUR$$

OUR = oxygen uptake rate

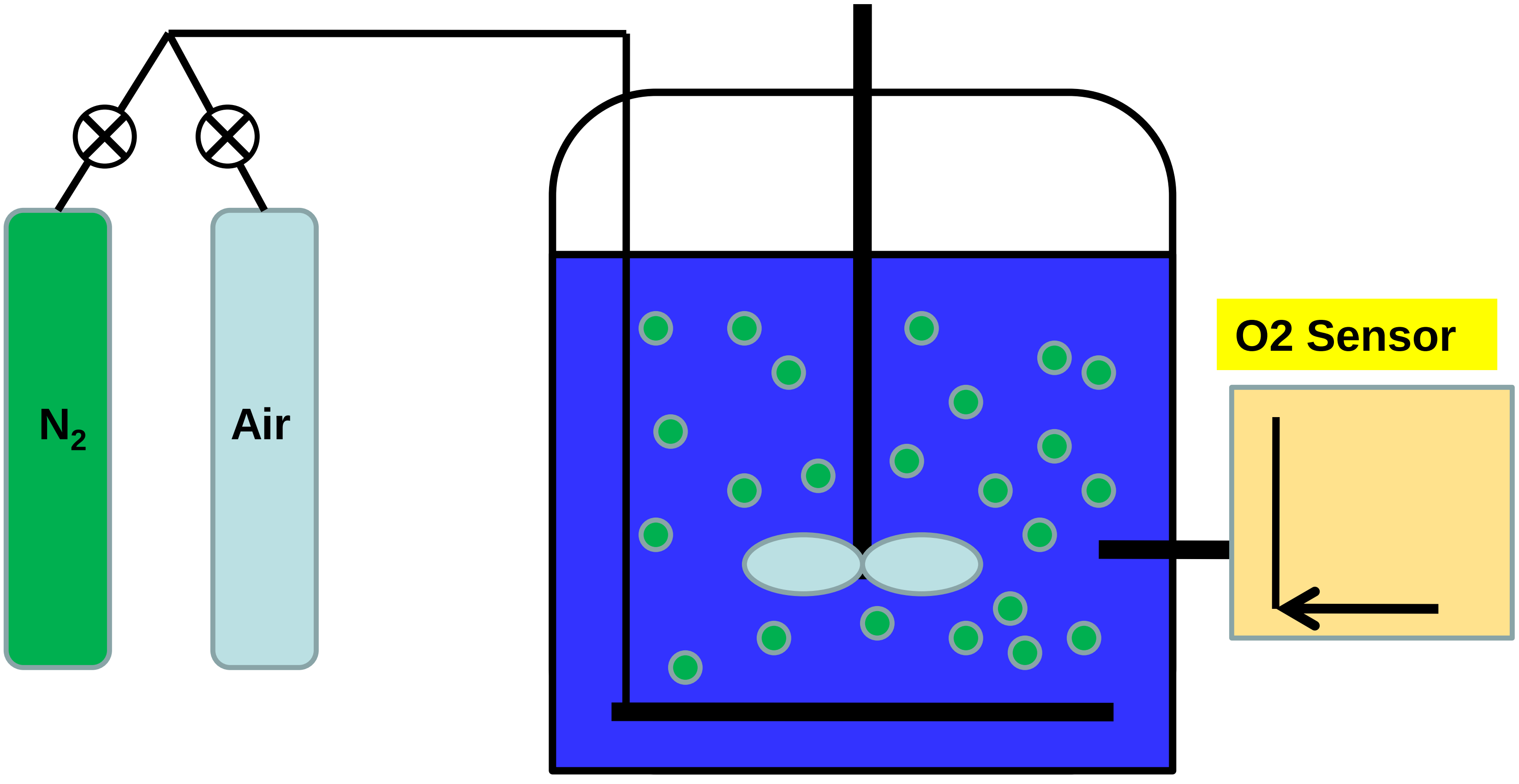
$= 5Q_{SR}$

Practical $K_L a$ Measurement



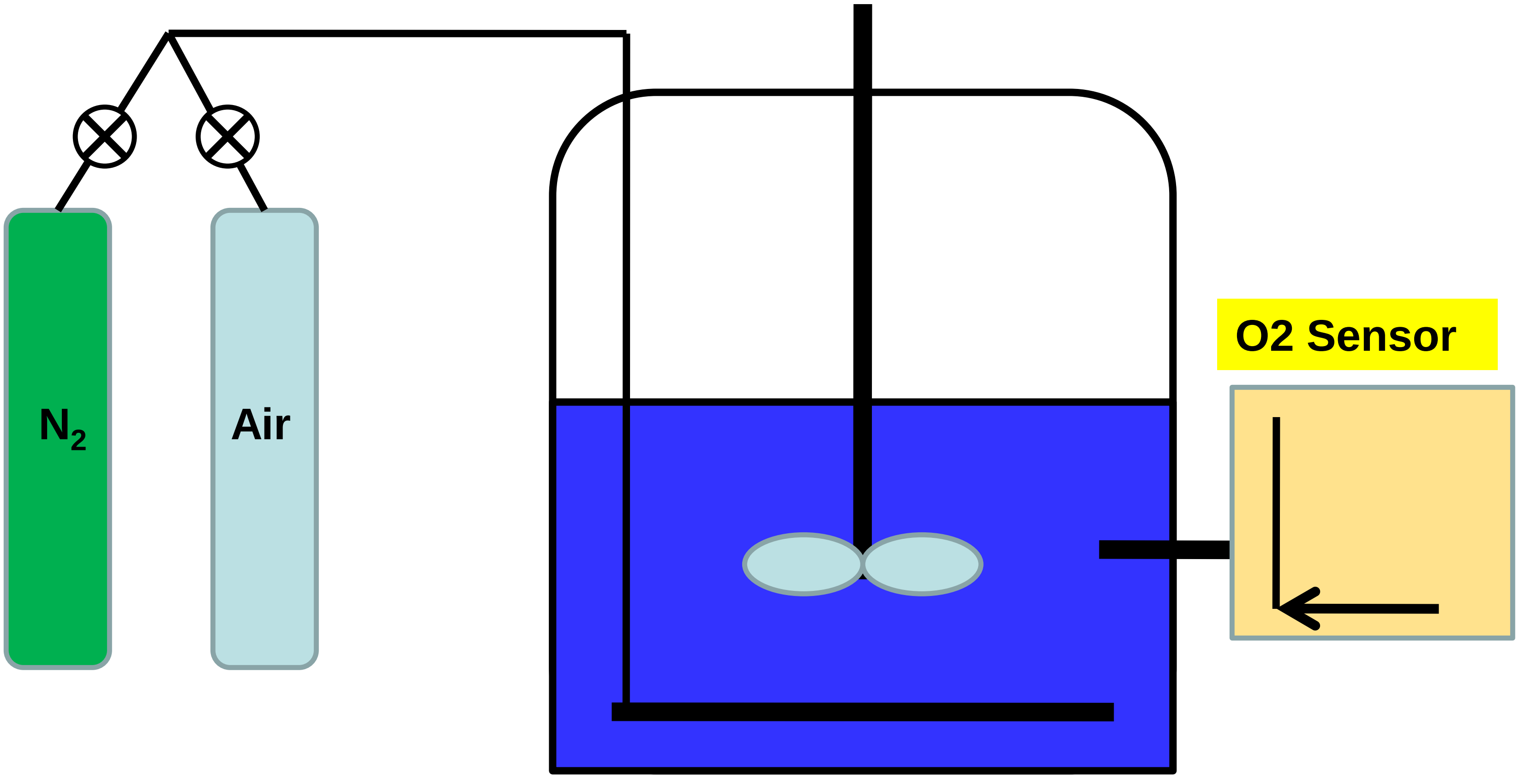
Practical $K_L a$ Measurement

1. Sparge fermenter with N_2



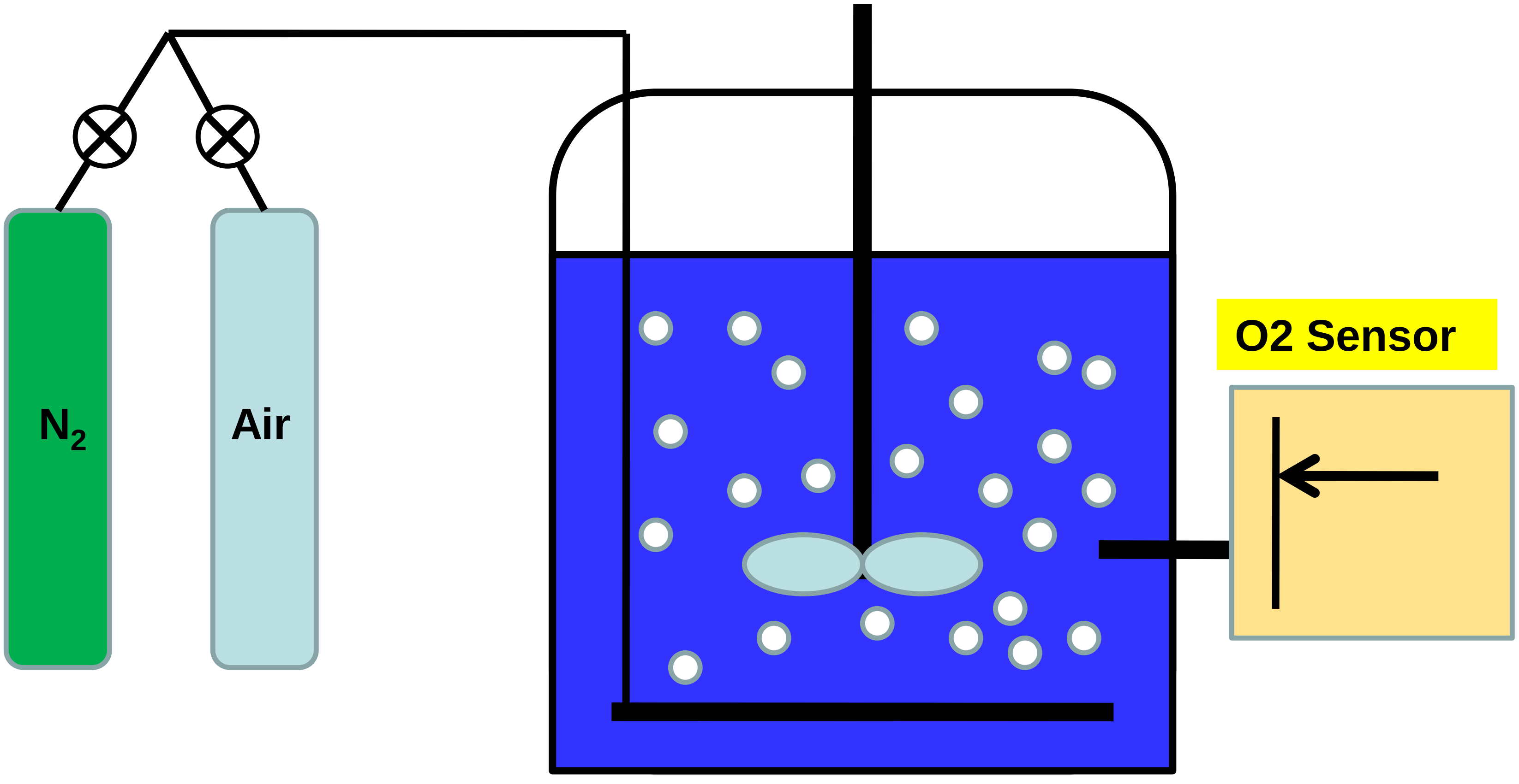
Practical K_La Measurement

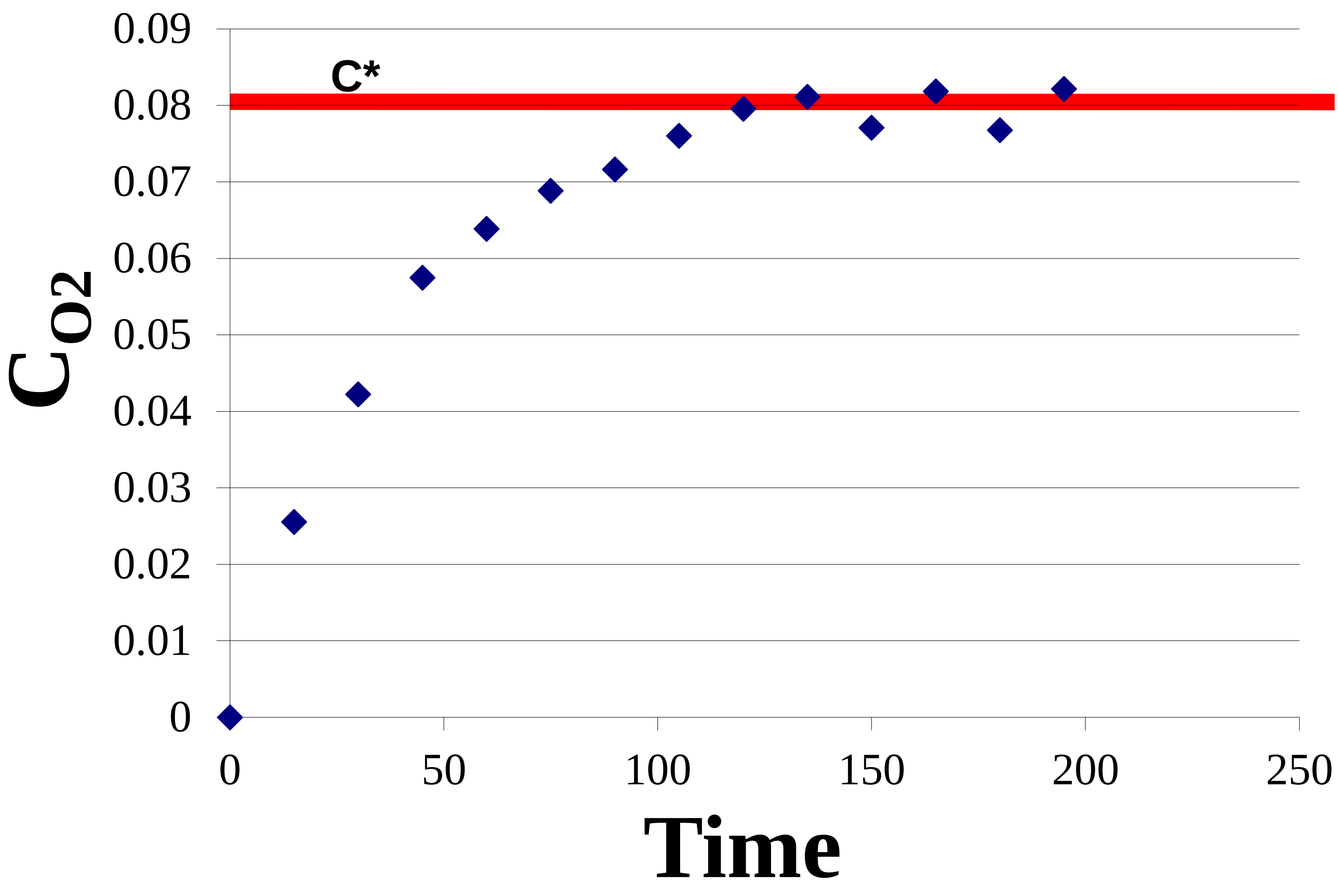
1. Stop N_2



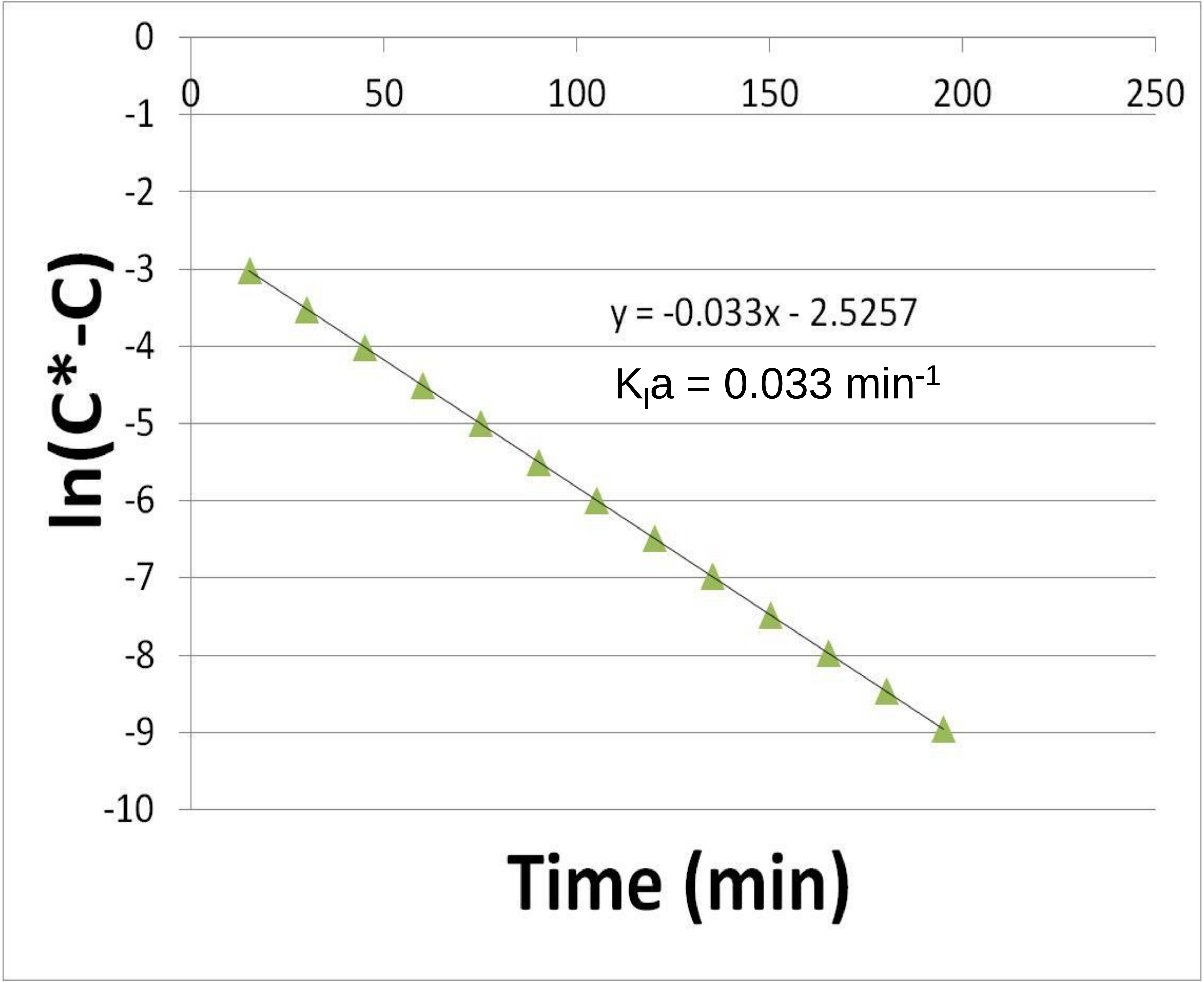
Practical K_La Measurement

1. Sparge fermenter with air – measure increase in O2





C*
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
C_{O2}
0
50
100
150
200
250
Time



ln(C*-C)
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
0
50
100
150
200
250
y = -0.033x - 2.5257
K_la = 0.033 min^{-1}
Time (min)

Dynamic Method

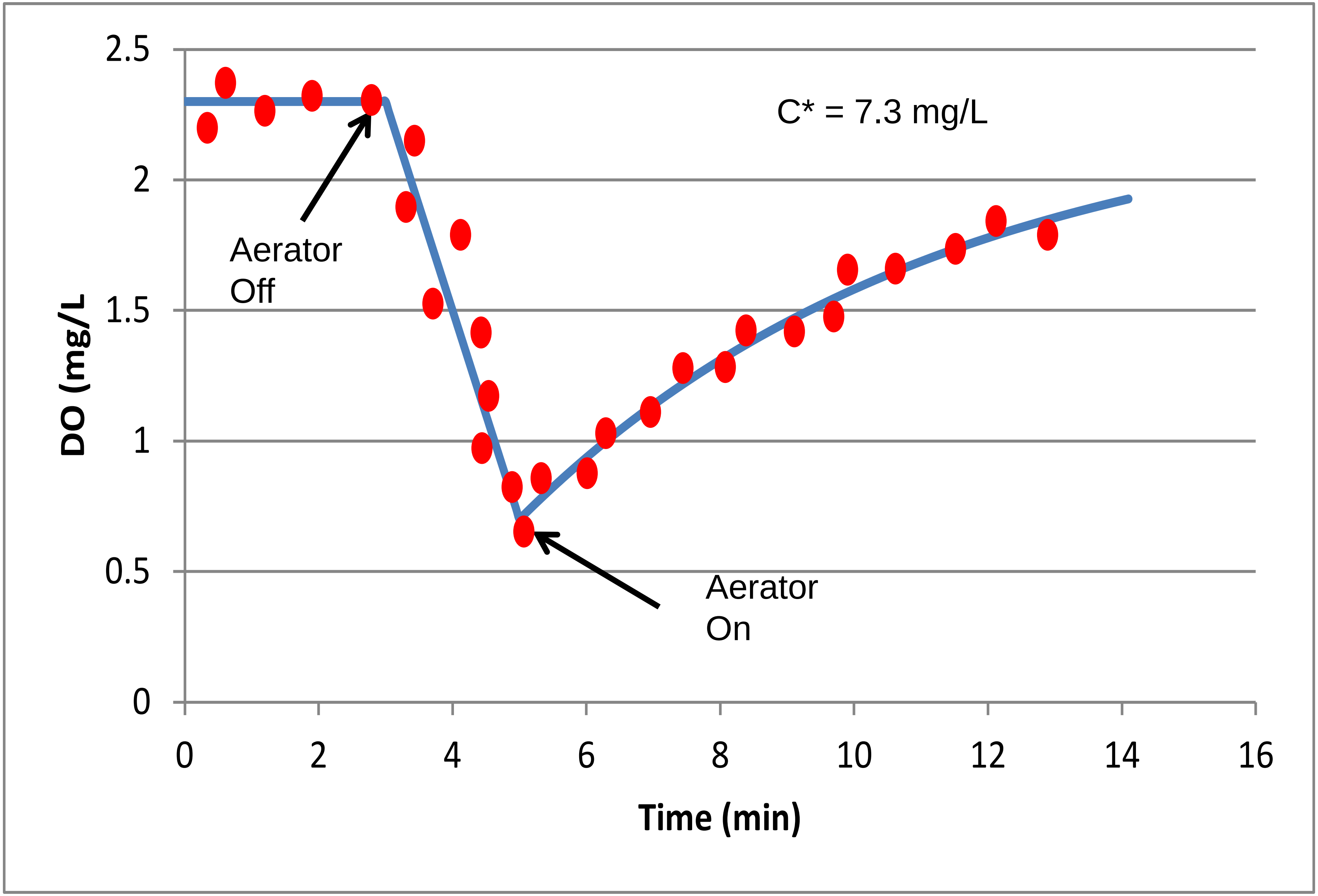
- Similar to unsteady-state method described above.

- Uses live cells in the reactor
- Procedure
  - Operate reactor aerobically
  - Turn off aeration for short period of time (2-5 minutes)
  - Turn aeration back on

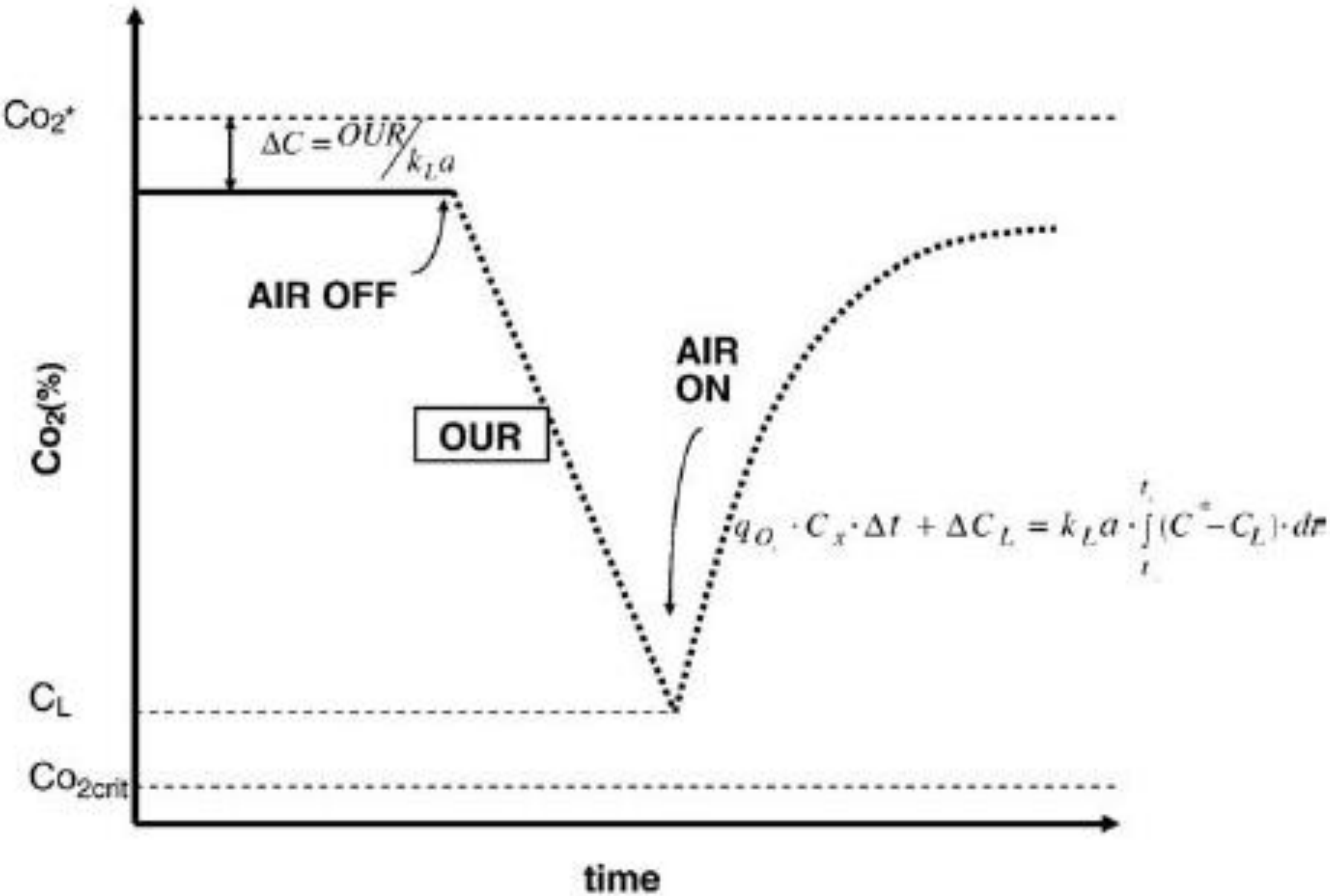
Dynamic Method

$$\frac{dC_{O2}}{dt} = OTR - OUR = k_L a\left(C_{O2}^{*} - C_{O2}\right) - q_{O2}X$$

Assume X = constant over test (<30 minutes total)



C* = 7.3 mg/L
Aerator
Off
Aerator
On
DO (mg/L)
Time (min)
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
16



Co2*
$\Delta C = OUR / k_L a$
AIR OFF
Co2(%)
OUR
AIR ON
$q_{O_2} \cdot C_x \cdot \Delta t + \Delta C_L = k_L a \cdot \int_{t}^{t_1} (C^* - C_L) \cdot dt$
CL
Co2crit
time

Surface Area of Bubbles

$$a = \frac{\text{total volume of bubbles}}{\text{total volume of broth}} \quad \frac{\text{area of bubble}}{\text{volume of bubble}}$$

$$= \frac{nF_o t_b}{V} \quad \frac{\pi D^2}{\left(\frac{\pi D^3}{6}\right)}$$

n = number of orifices in a sparging tube

F_o = volumetric air flow rate per orifice

t_b = residence time of bubble in liquid

D = average diameter of air bubble

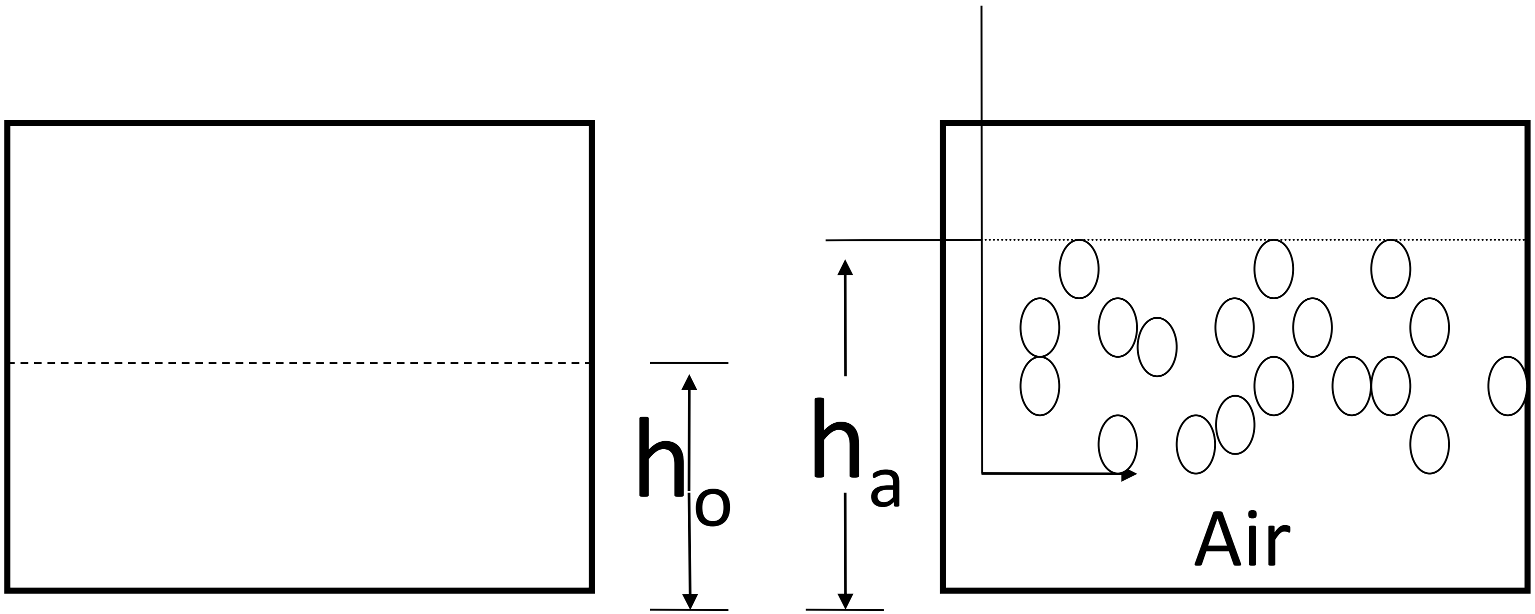
Surface Area of Bubbles

$$a = \frac{\text{total volume of bubbles}}{\text{total volume of broth}} \quad \frac{\text{area of bubble}}{\text{volume of bubble}}$$

$$= \frac{nF_o t_b}{V} \quad \frac{\pi D^2}{\left(\frac{\pi D^3}{6}\right)} = \frac{V_b}{V_l} \quad \frac{\pi D^2}{\left(\frac{\pi D^3}{6}\right)}$$

$$a = H\left(\frac{6}{D}\right)$$

$$V = \pi r^2 height$$



$$H = \frac{h_a - h_o}{h_o}$$

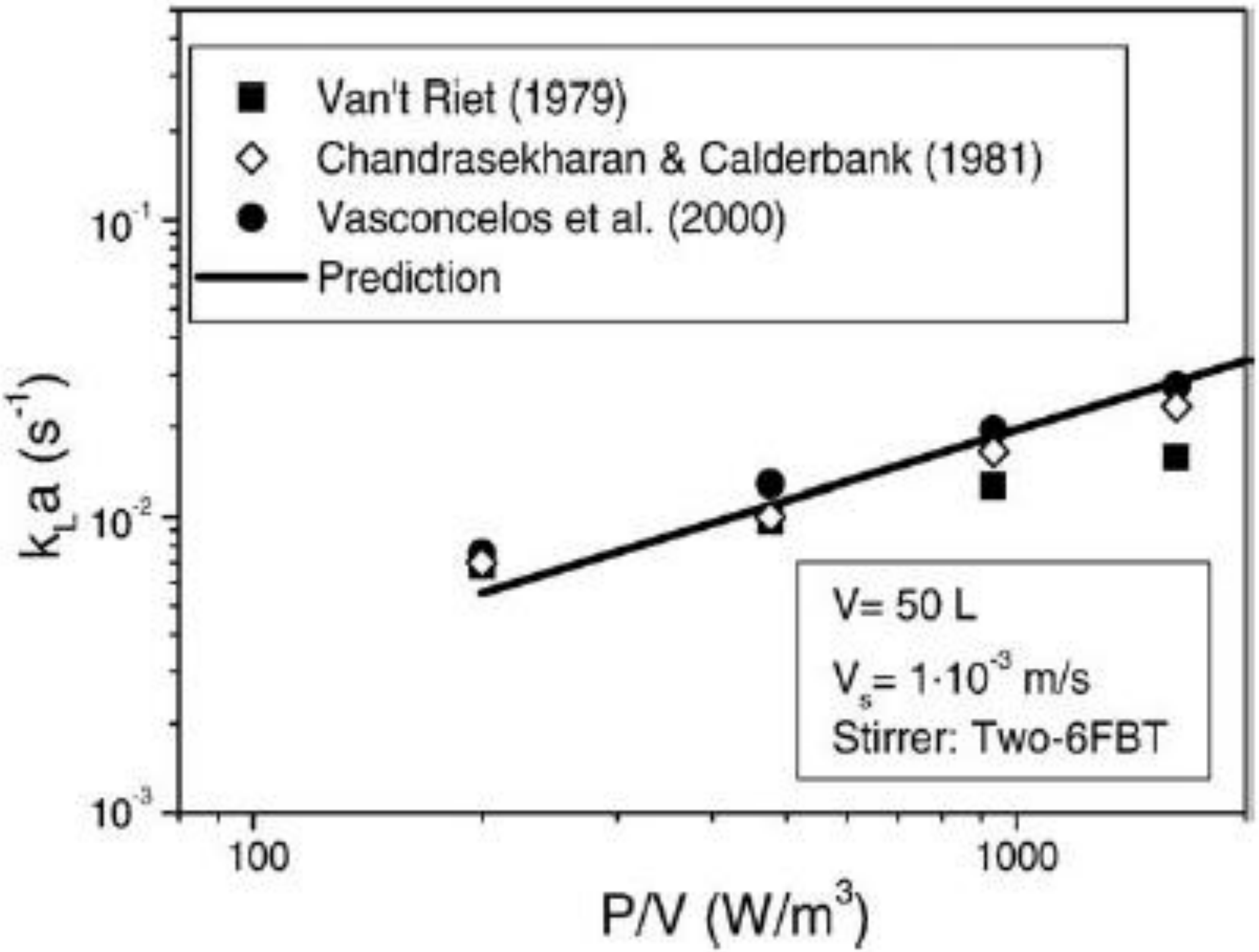
Bioreactors/Fermenters

- Mechanical Agitation
- Bubble columns
- Loop Reactors

Power for Agitation

$$k_L a \;\; \alpha \;\; \left(\frac{P_g}{V}\right)^m (v_s)^n$$

- P_g = gassed power, horsepower
- V = volume of gas-liquid dispersion (aerated solution), L
- ν_s = superficial gas velocity, cm/sec



Van't Riet (1979)
Chandrasekharan & Calderbank (1981)
Vasconcelos et al. (2000)
Prediction
V= 50 L
V_s= 1·10^{-3} m/s
Stirrer: Two-6FBT
k_La (s^{-1})
10^{-1}
10^{-2}
10^{-3}
100
1000
P/V (W/m^3)

$$P_g = 0.08\left[\frac{P_o^2 N D^3}{Q^{0.56}}\right]^{0.45}$$

- P_g = gassed power, HP
- P_o = ungassed power, HP
- N = rpm of impeller (min^{-1})
- D = impeller diameter in feet
- Q = gas flow rate in ft^3/min

Michel and Miller

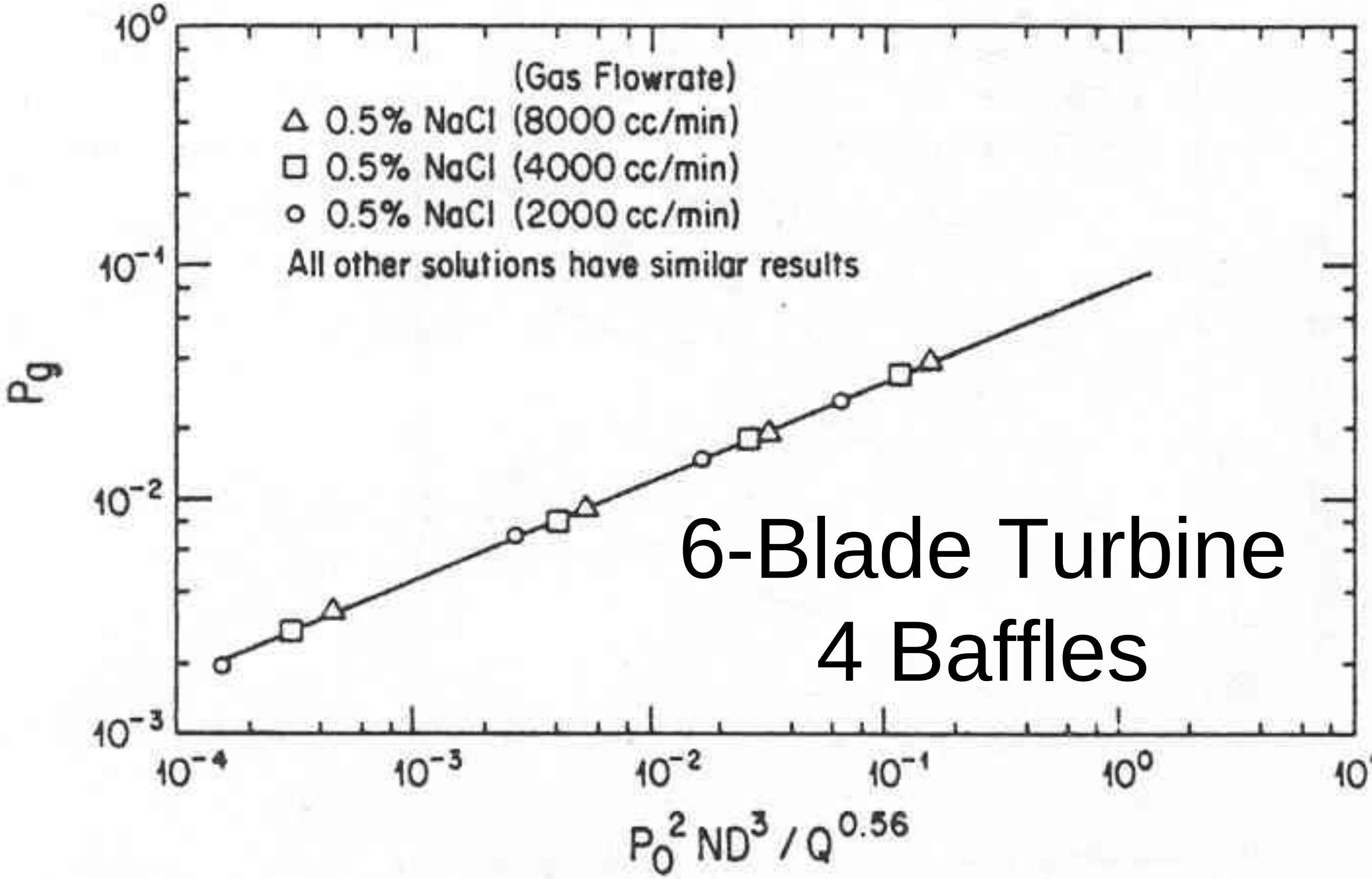
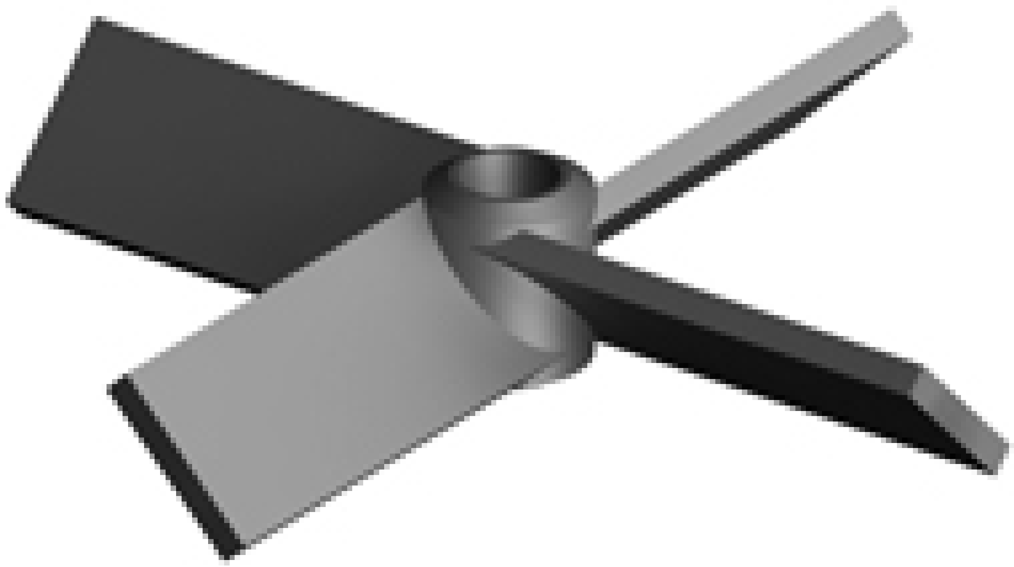
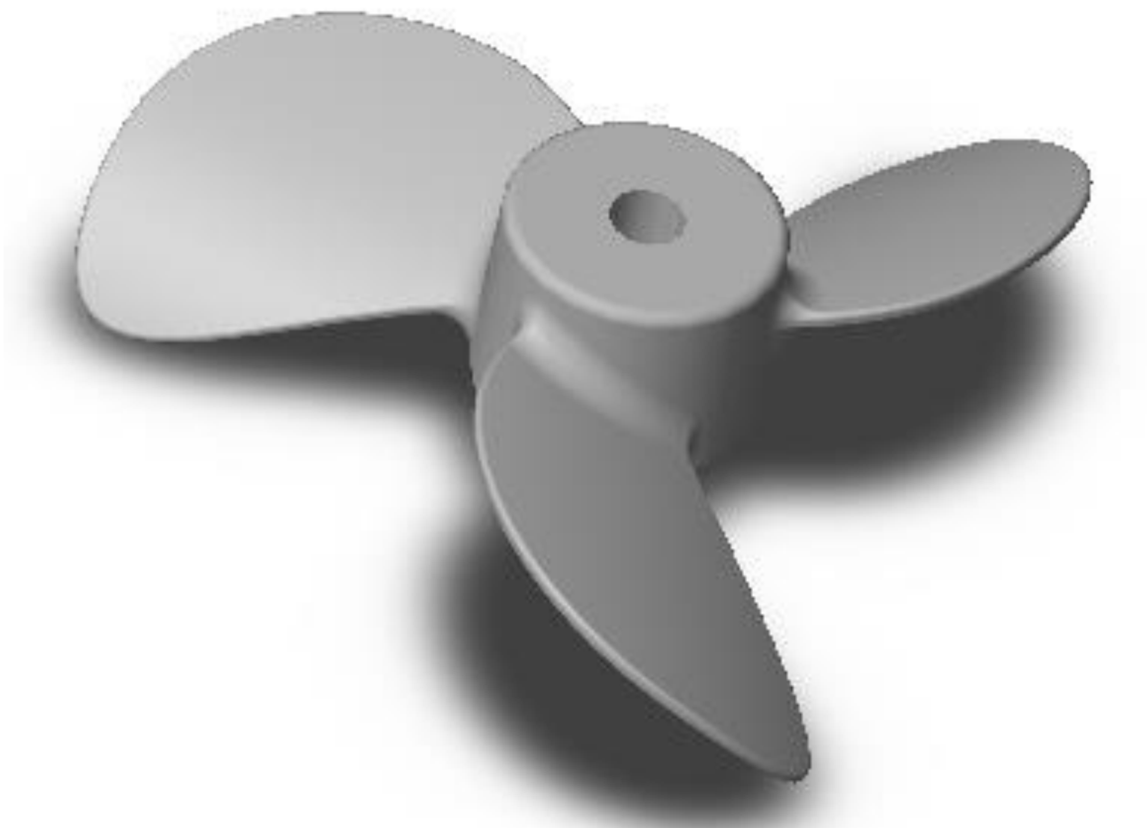


Fig. 6-11

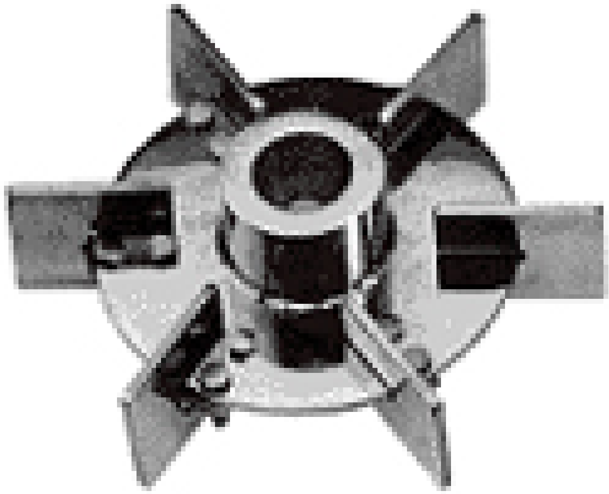
Turbine Impeller

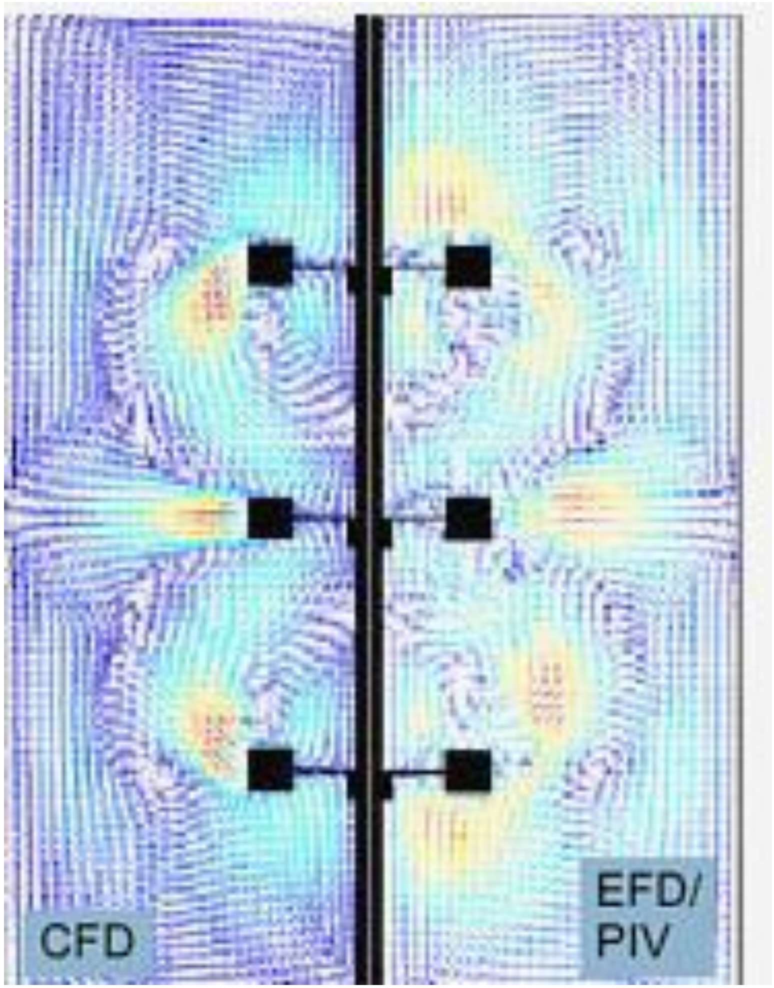


Marine Impeller



Rushton Impeller



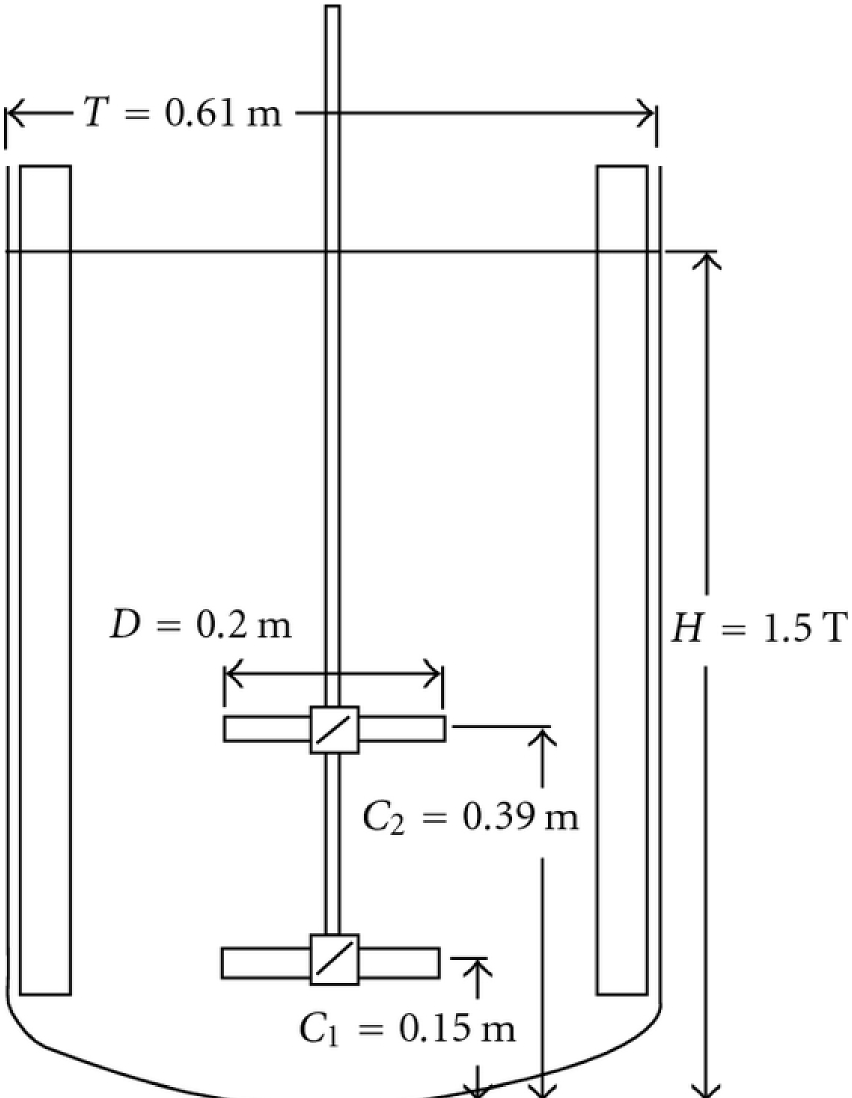


Fermenters

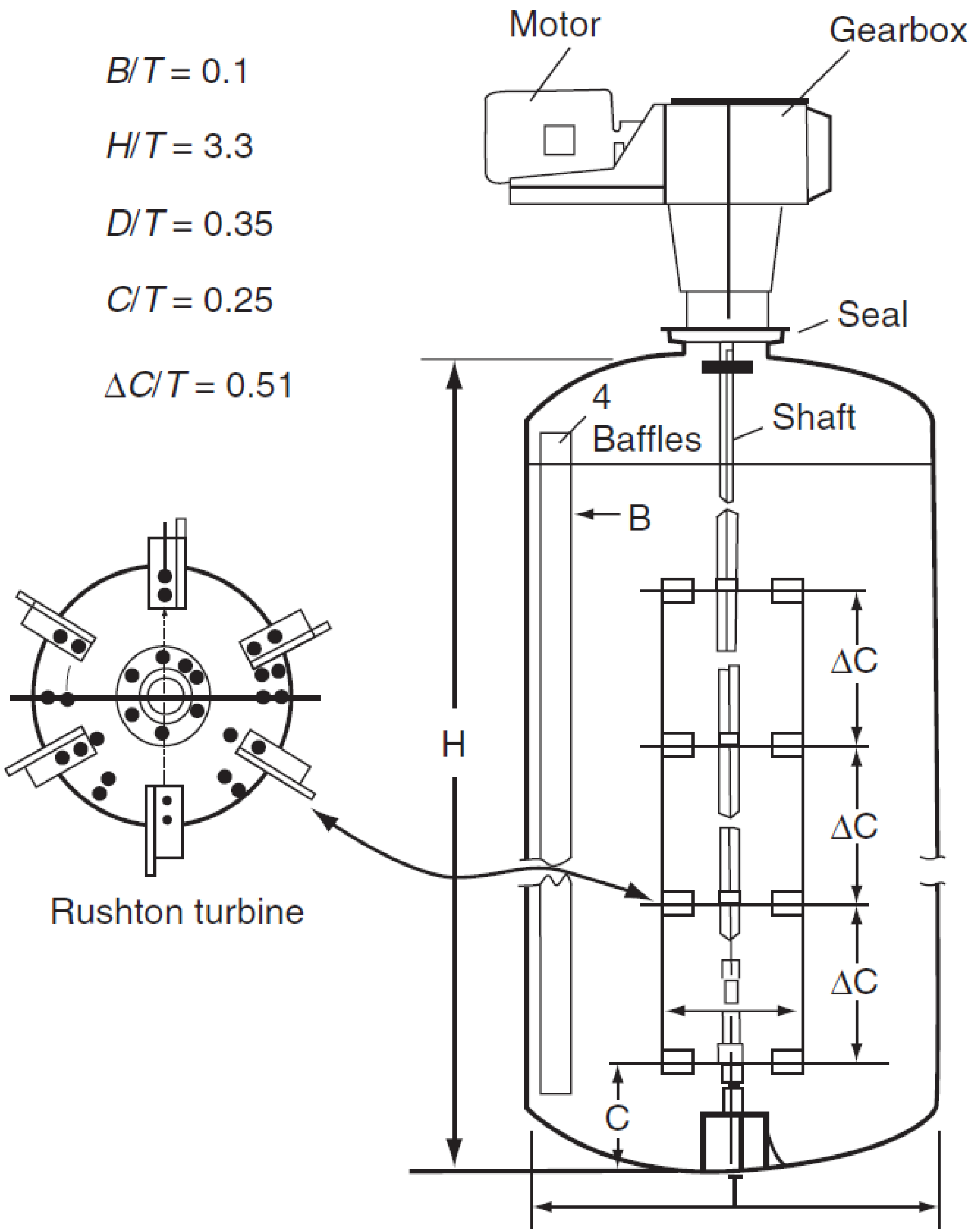
- Height : Diameter = 2:1 or 3:1
  - Animal cell reactors often 1:1
- Constructed from stainless steel to prevent corrosion
  - Plant and animal cell – 316L (low carbon)
- Foaming is an issue in aerated reactors
  - Working volume usually 60-75% of total volume

Stirred Tank Bioreactor

Stirred Tank Bioreactor



- Flexible operation
- High k_La
- High Power
  - Up to 5 kW/m^3
- 400 m^3 (400,000 L) max



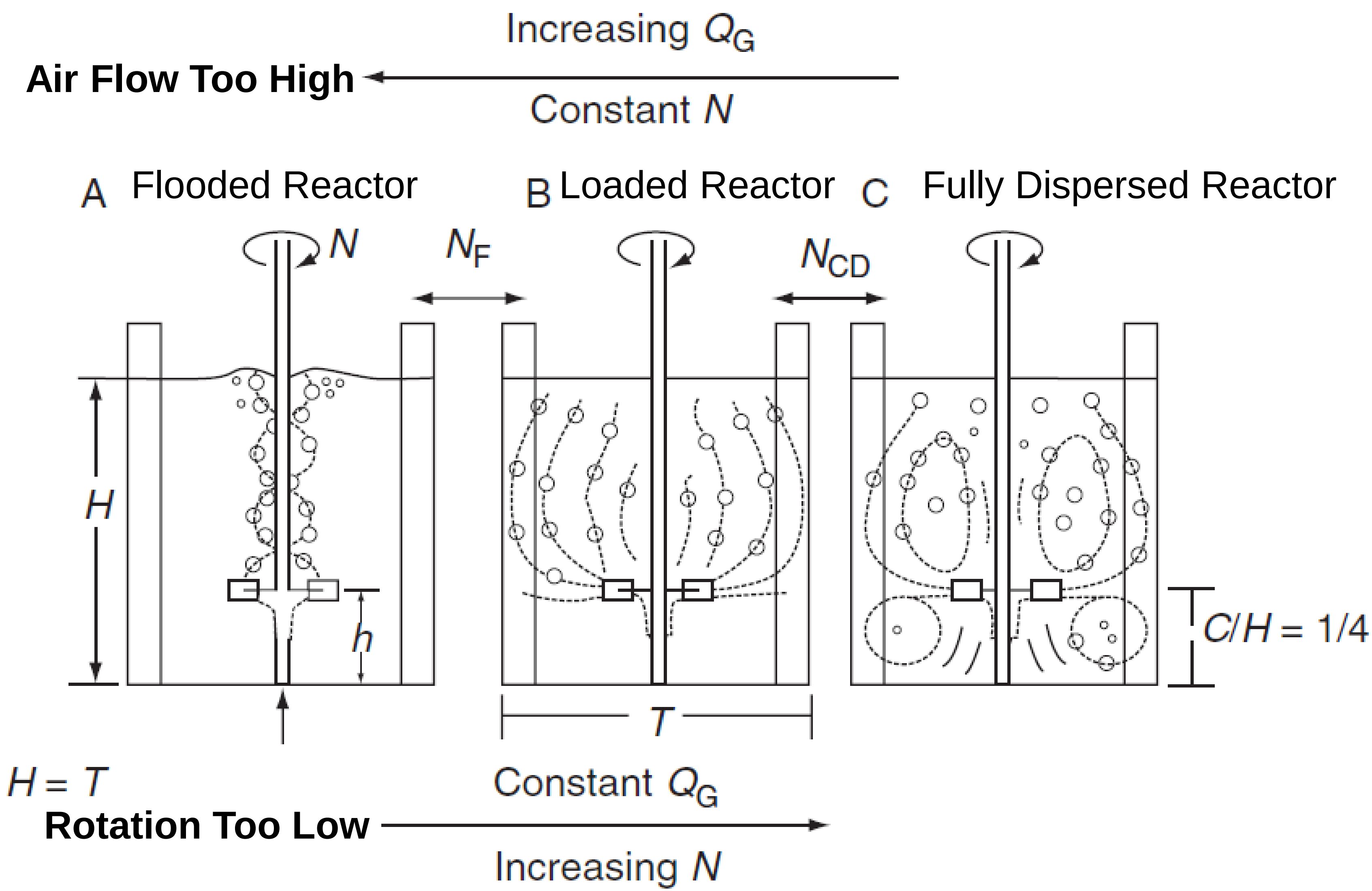
Mixing equipment CO.—1981

H = Tank Height
T = Tank Diameter
D = Impeller Diameter
C = Spacing of Impeller
B = Baffle thickness

Hewitt and Nienow, Batch and Fed-Batch Fermentation Processes, Advances in Applied Microbiology, 62, 105- 135 (2007).

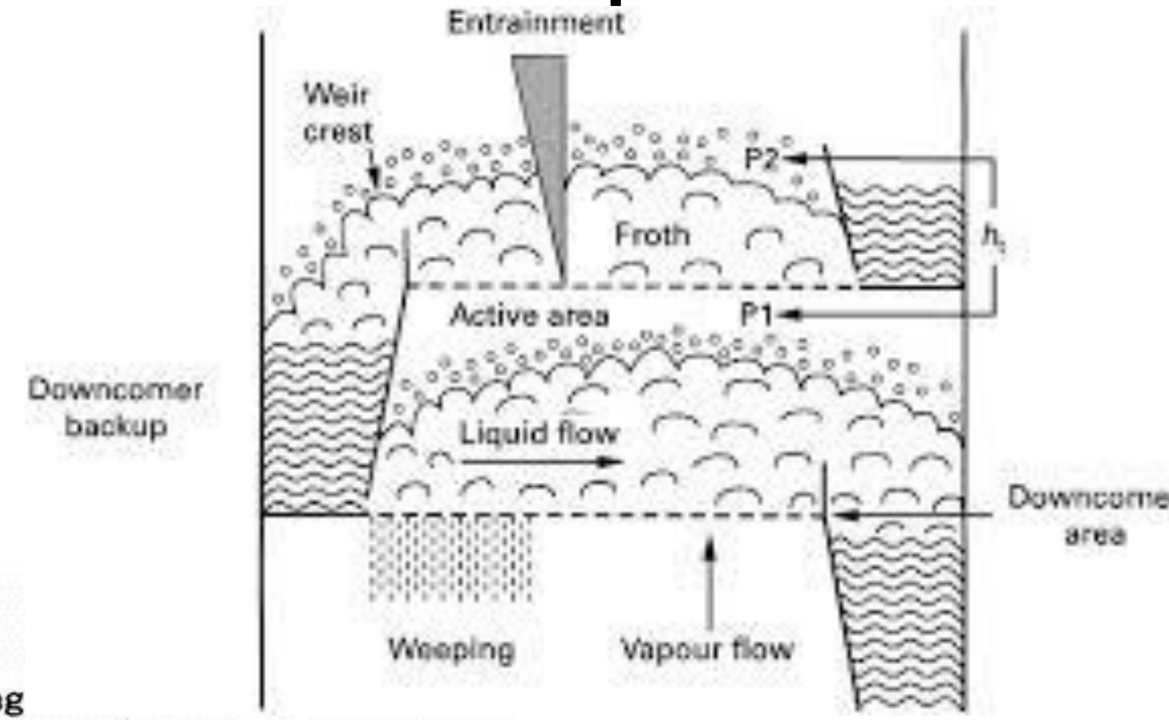
Design Parameters

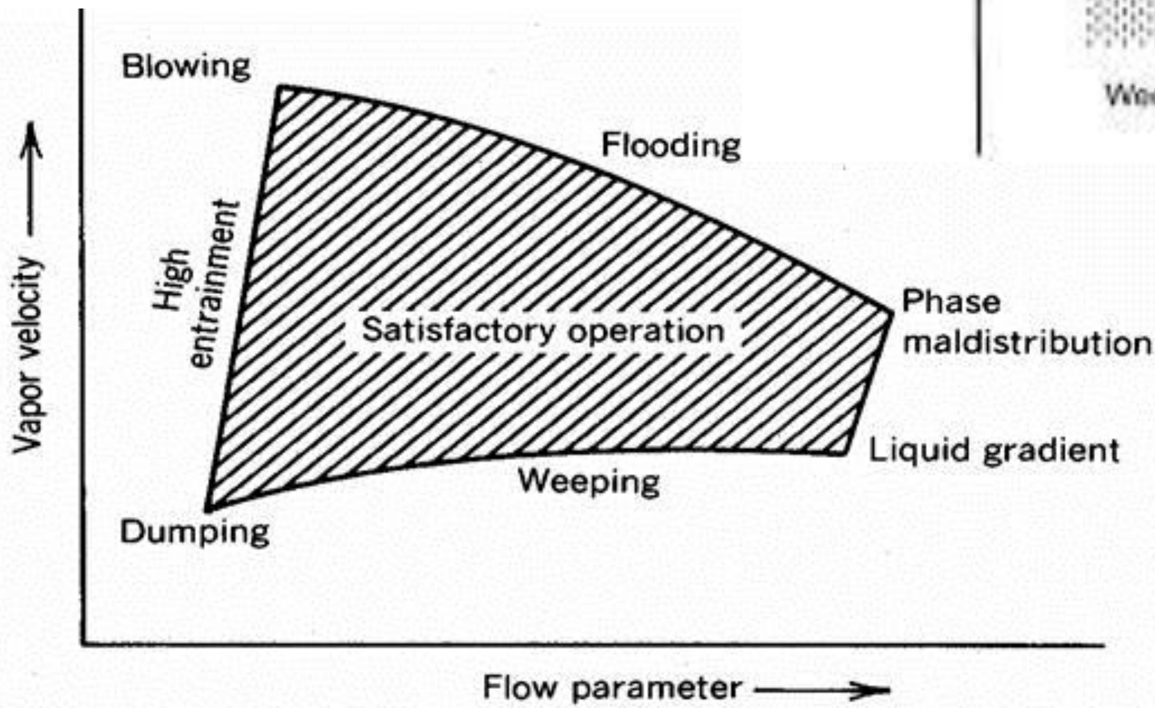
Parameter	Symbol
Power Input	P
Volume	V
Impeller Rotation	N
Impeller Diameter	D
Density of fluid	ρ
Viscosity of fluid	μ
Gas flow rate	Q_G



Increasing Q_G
Air Flow Too High
Constant N
A Flooded Reactor
B Loaded Reactor
C Fully Dispersed Reactor
N
N_F
N_{CD}
H
h
T
$C/H = 1/4$
$H = T$
Constant Q_G
Rotation Too Low
Increasing N

Similar to Distillation Operation





Rushton Impeller Flooding Limit

$Q_{Gas}/(ND^3) = 30(D/H)^{3.5}(Fr)$

Fr =Froude number = N^2D/g = ratio of the inertial to buoyancy forces

ND^3 = pumping rate of impellers = volume of liquid pushed by impellers = Q

Scale-Up Rules of Thumb

- Constant P/V = Constant $k_L a$
- Constant N*D = Constant Shear Rate
- Constant N = Constant Mixing Times

$$P/V \propto N^3 D^2$$

$$Q \propto ND^3$$

$$P \propto N^3 D^5$$

Calculating effect of scale up difficult!
A 10x increase in volume is common for empirical testing
(e.g. 1 L to 10 L)

Table 9
Different criteria for bioreactor scale-up (adapted from Oldshue, 1966)

Variable	Value of volume at model system (2 L)	Value of volume at pilot scale (20 L)			
		Scale-up criteria			
		$P/V=C$	$\pi NT=C$	$Re=C$	$k_La=C$
T	1.0	2.14	2.14	2.14	2.14
P	1.0	10.0	4.80	0.50	13.8
P/V	1.0	1.0	0.48	0.05	1.38
N	1.0	0.60	0.47	0.22	0.67
$N \cdot T$	1.0	1.28	1.0	0.47	1.43
Re	1.0	2.75	2.15	1.0	3.07
k_La	1.0	0.77	0.55	0.19	1.0

Mixing in Large Scale Bioreactors

- Mixing – for O_2 transfer <u>and</u> nutrient and pH dispersion – is the critical issue with scale up
- Mixing rate (time) is proportional to mixing speed (N)
- Power requirement quickly outpaces ability to mix reactor

Estimating Mixing Time

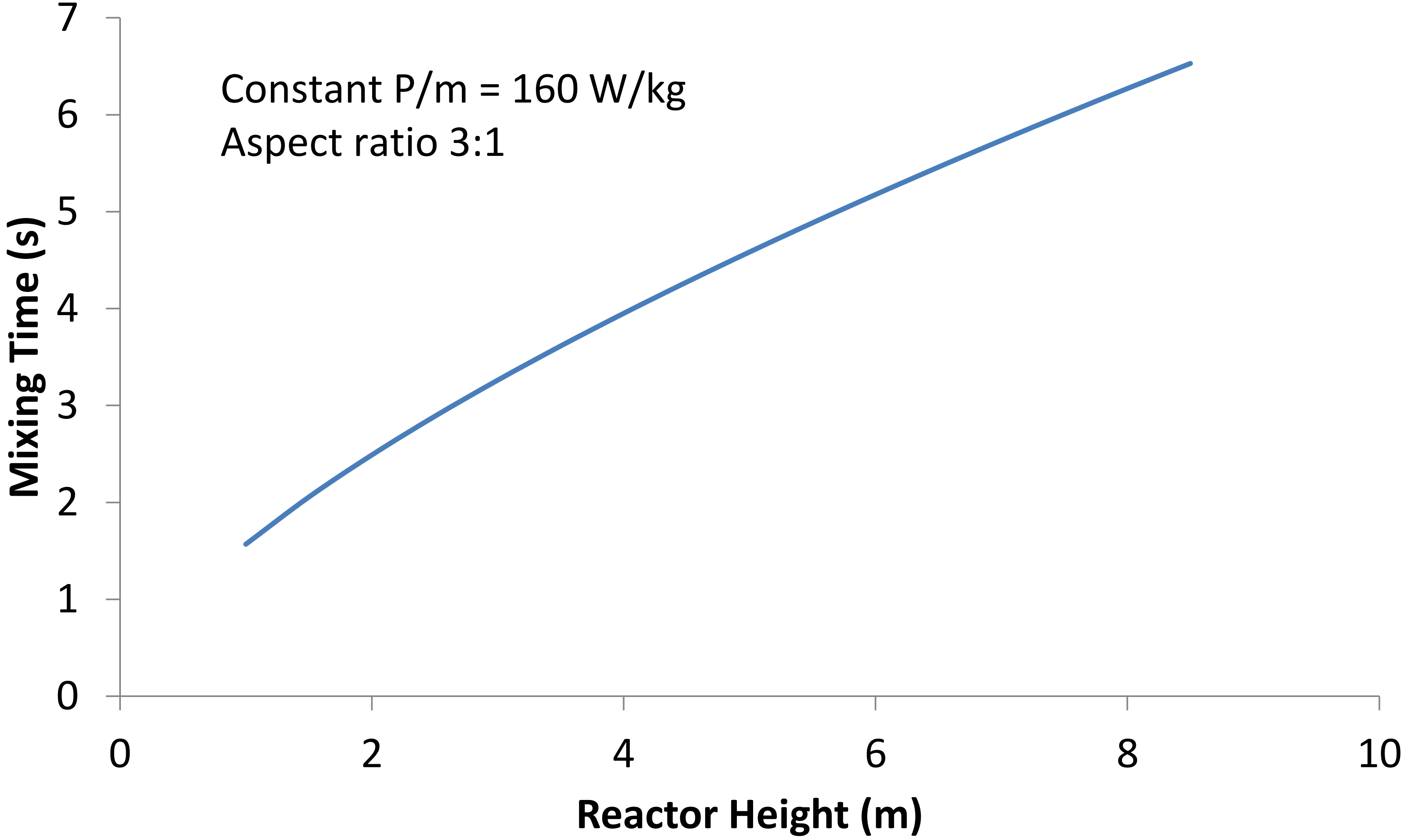
$$\theta_m(\mathrm{s}) = 5.9\mathrm{H}^{2/3}\left(\varepsilon_T\right)^{-1/3}\left(\frac{D}{H}\right)^{-1/3}$$

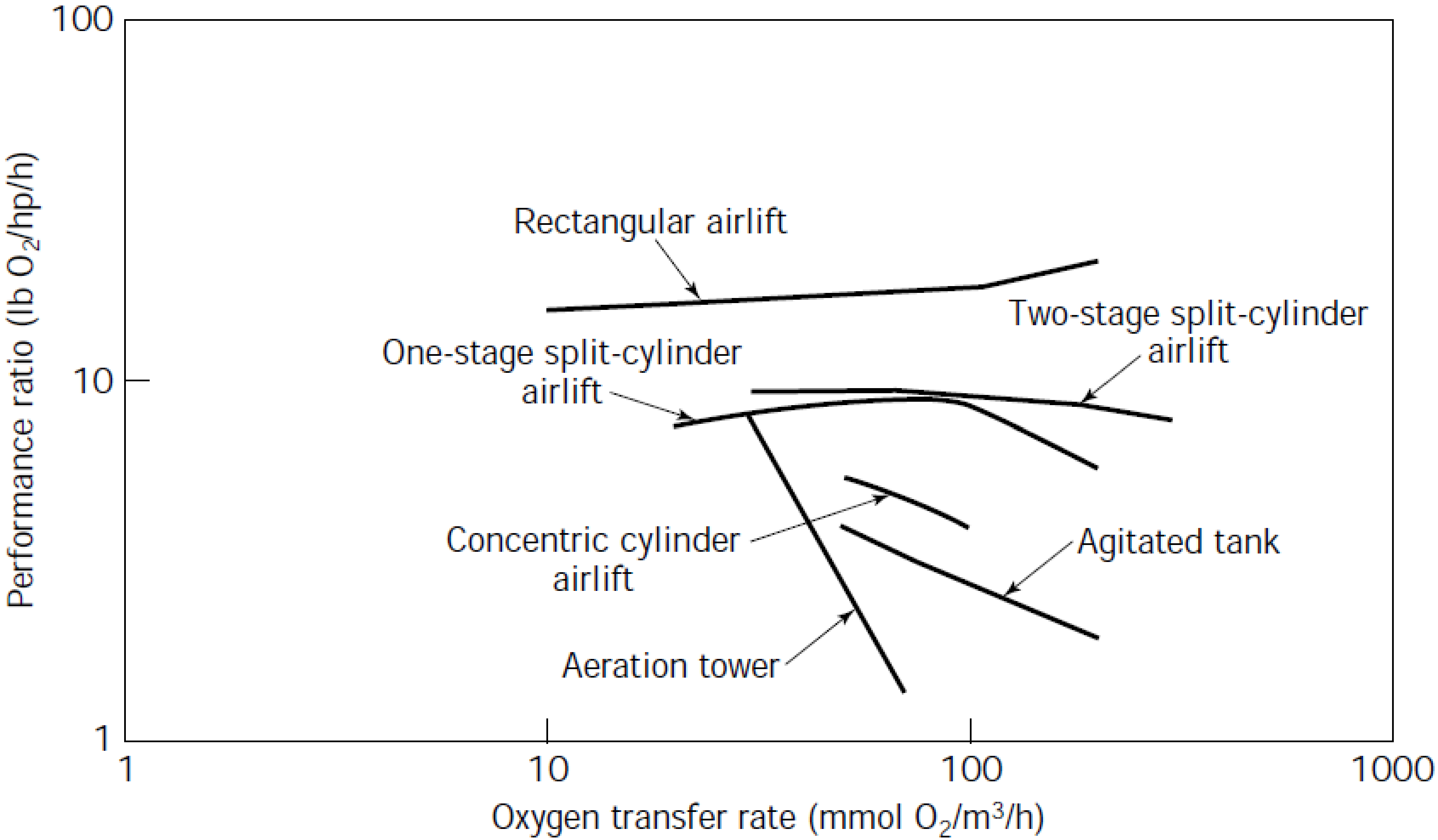
$$where$$

$$\varepsilon_T = \frac{P}{\rho \mathrm{V}}$$

ε_t has units of W/Kg

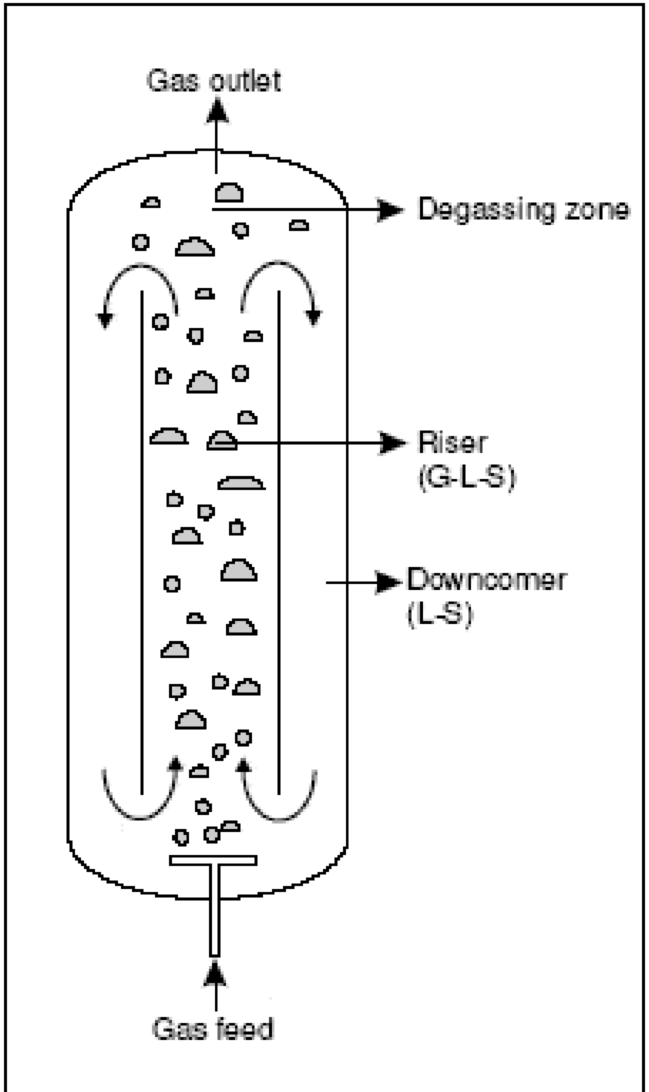
Bioreactors Mixing Time





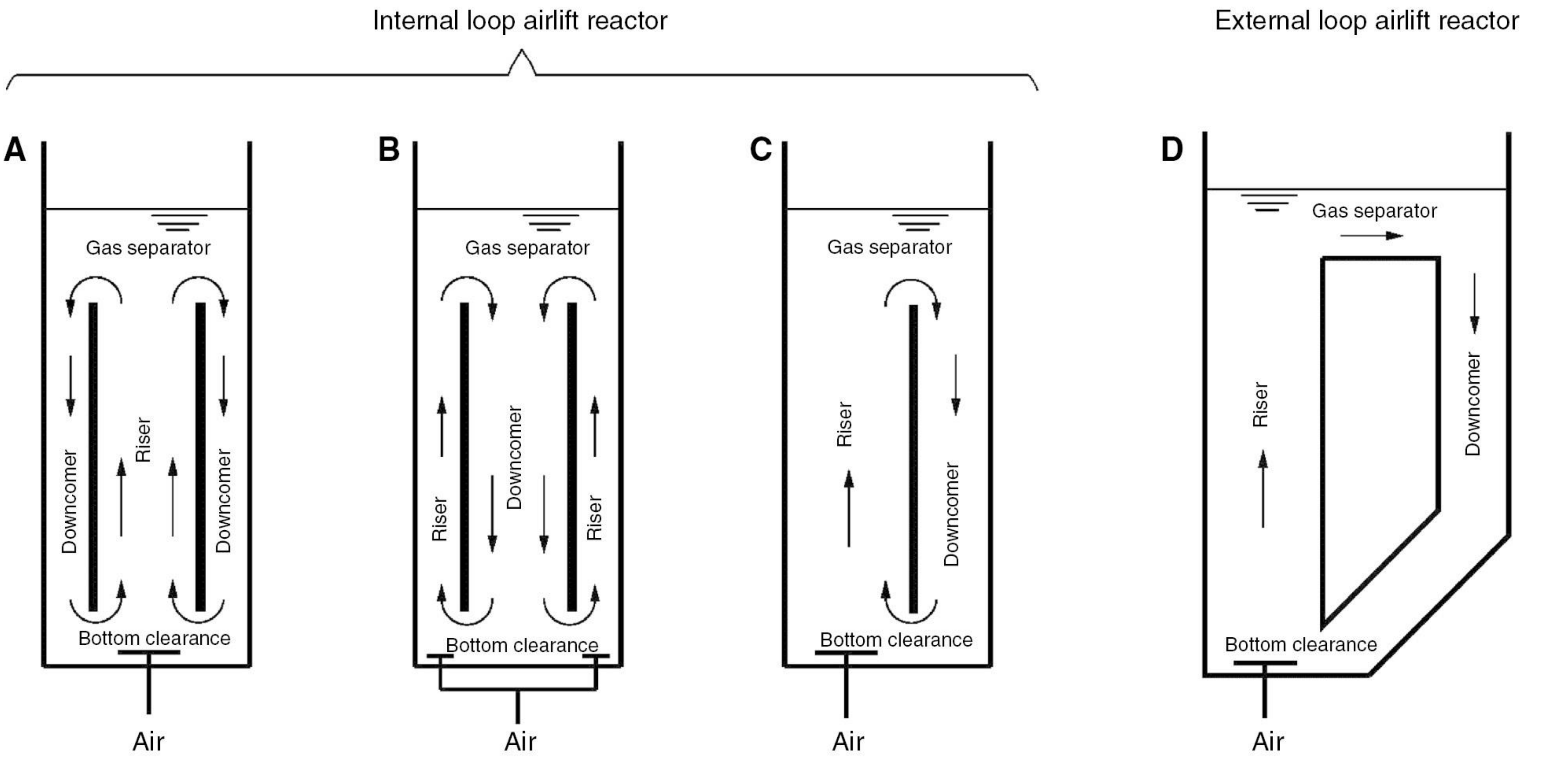
M.E. Orazem and L.E. Erickson, *Biotechnol. Bioeng.* **21**, 69–88 (1979).

Airlift Loop Reactors

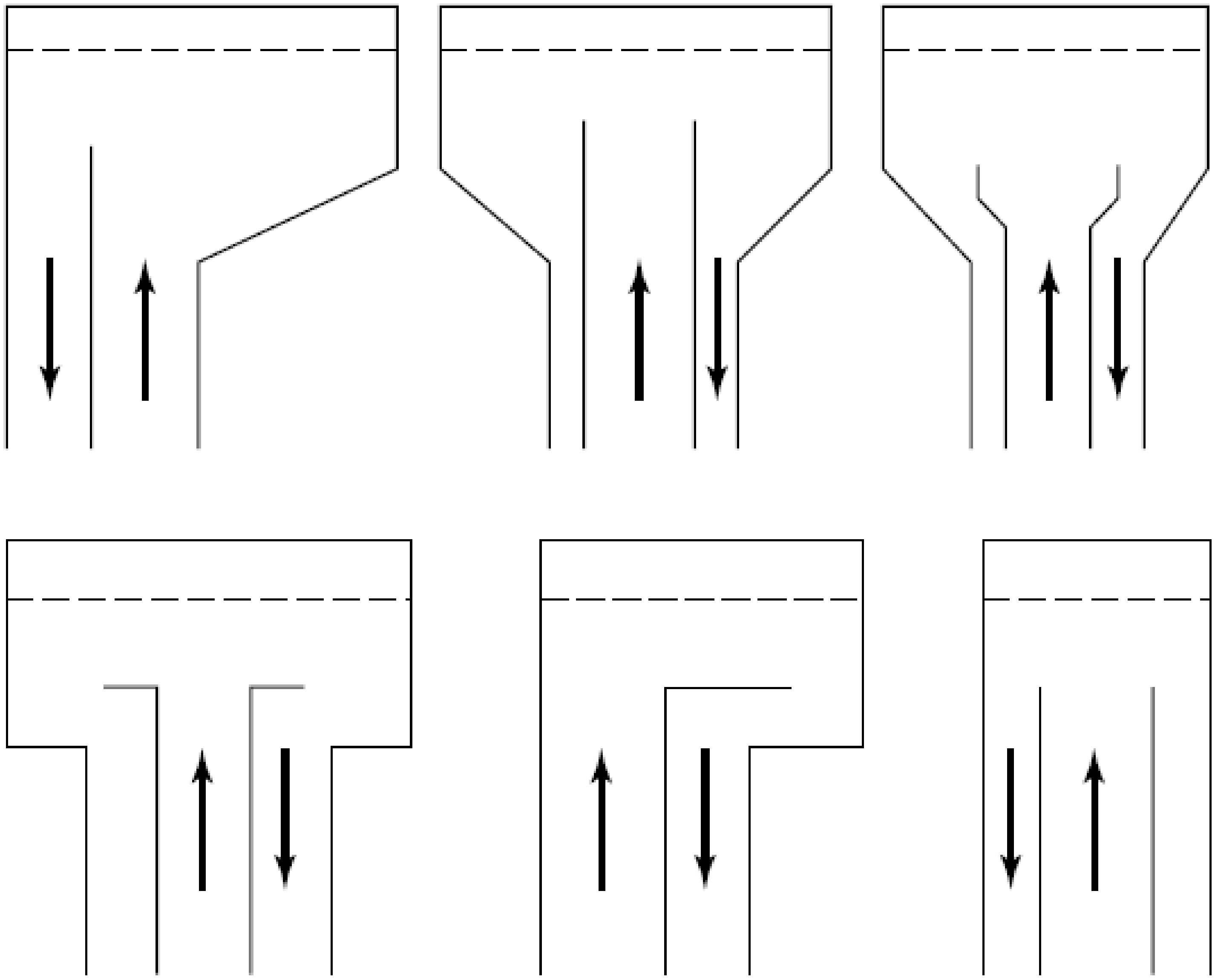


- Low shear
- Lower $k_L a$ than mechanically stirred rxtr

Design Variations



Gas separator configurations of internal-loop ALRs



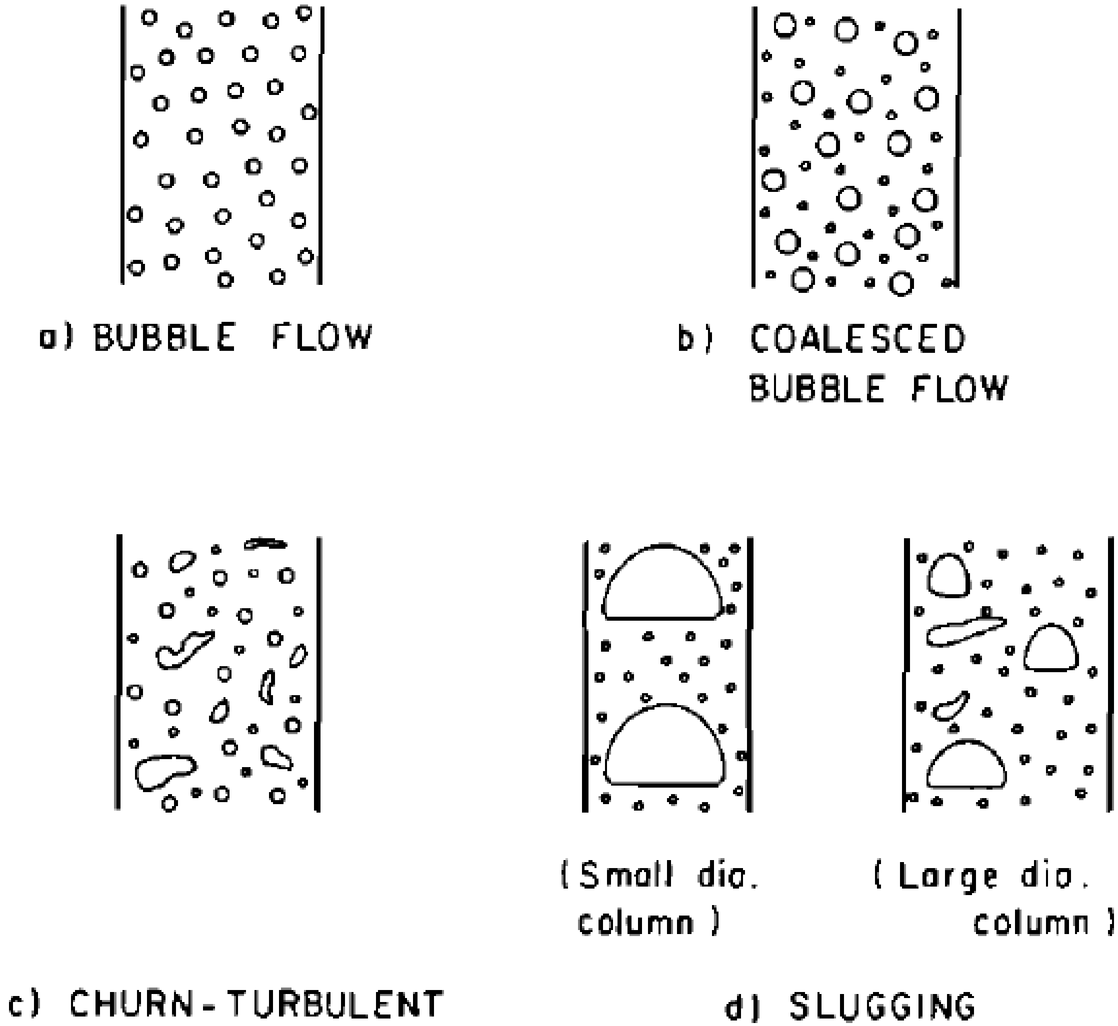


FIGURE 3 Reactor flow regimes.

M.Y. CHISTI & M. MOO-YOUNG (1987) AIRLIFT REACTORS: CHARACTERISTICS, APPLICATIONS AND DESIGN CONSIDERATIONS, CHEMICAL ENGINEERING COMMUNICATIONS, 60:1-6, 195-242, DOI: 10.1080/009864487

U_{sg} = superficial gas velocity

U_{Lr} = superficial liquid velocity

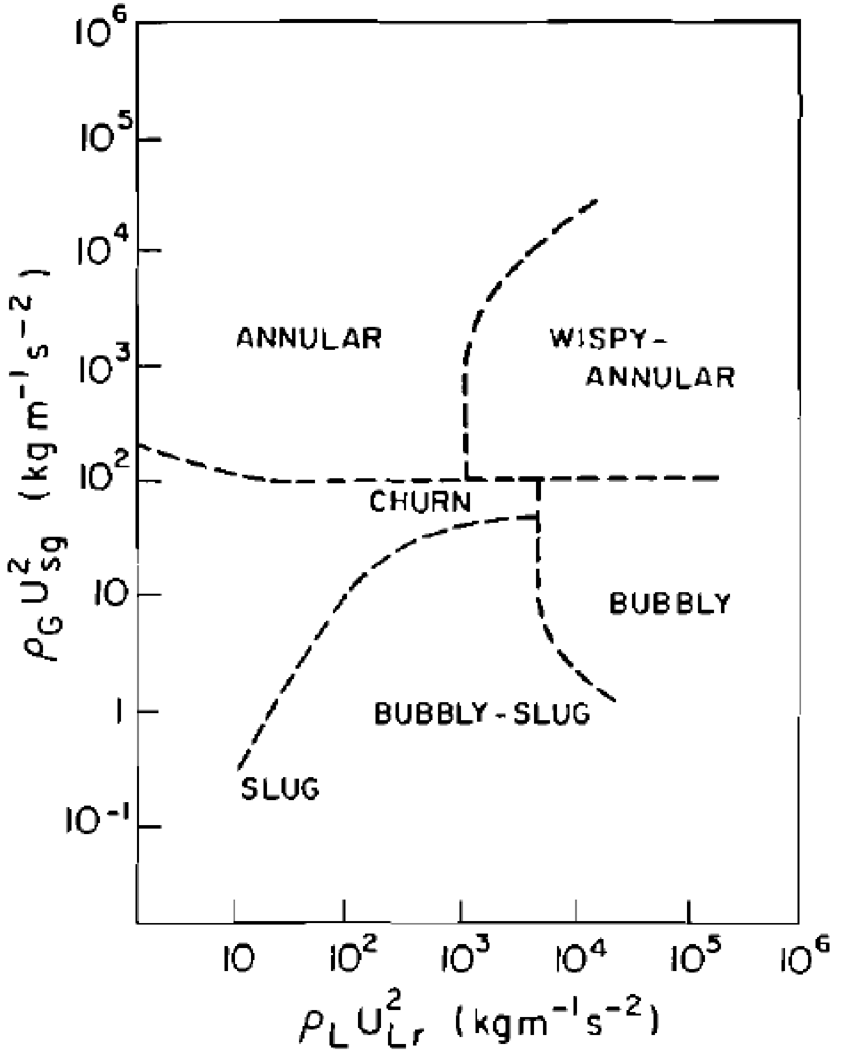
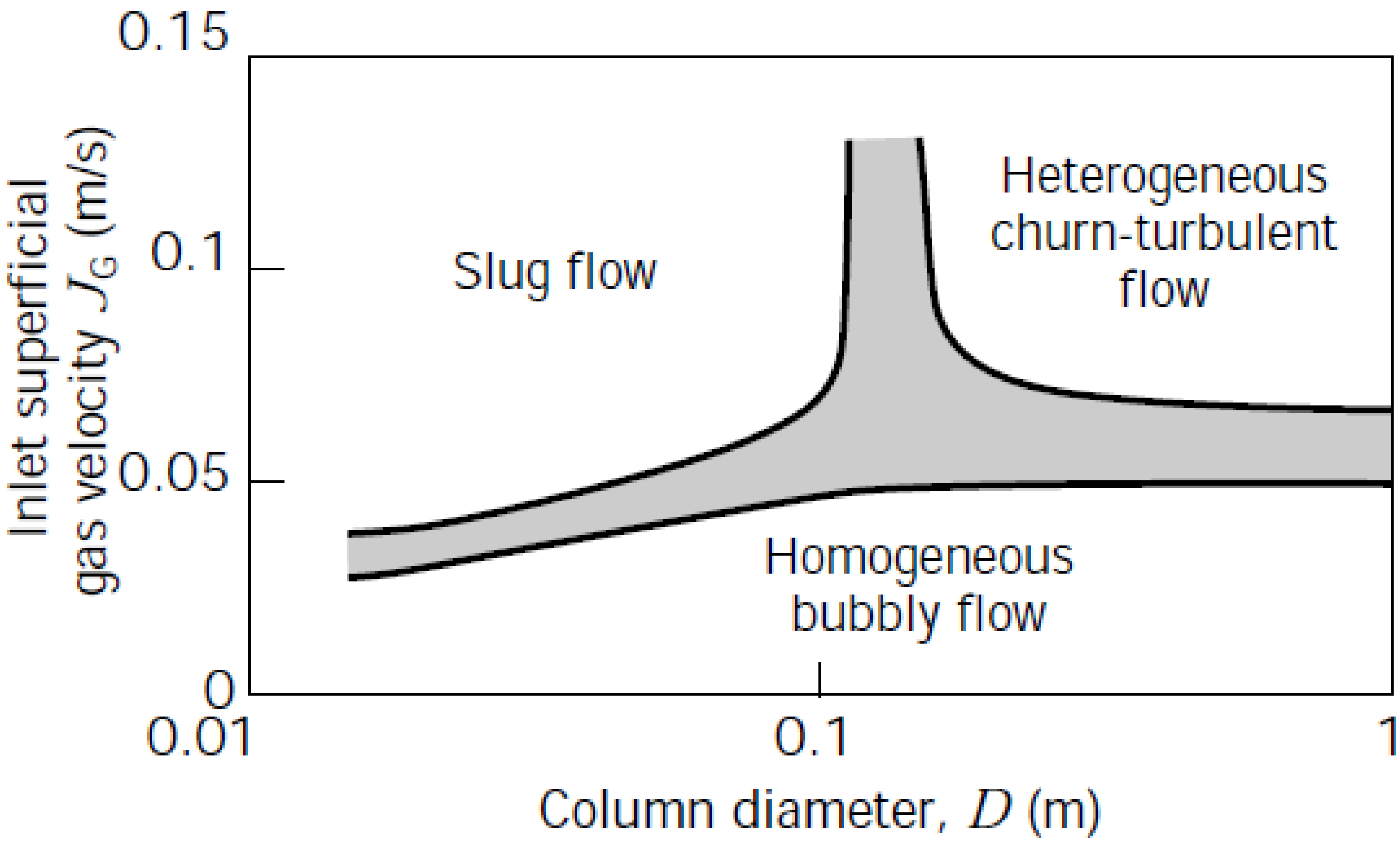


FIGURE 4b Flow pattern map for vertical gas–liquid flow for low viscosity Newtonian liquids. Adapted from Collier.[37]

M.Y. CHISTI & M. MOO-YOUNG (1987) AIRLIFT REACTORS: CHARACTERISTICS, APPLICATIONS AND DESIGN CONSIDERATIONS, CHEMICAL ENGINEERING COMMUNICATIONS, 60:1-6, 195-242, DOI: 10.1080/009864487



K. Wiswanathan, *Flow Patterns in Bubble Columns*, Gulf, Houston, TX., 1986, pp. 291–308.

Antibiotics

- Specific chemical substances derived from or produced by living organisms that are capable of inhibiting the life processes of other organisms

- Various mechanisms are known
  - Interfere with protein synthesis
  - Interfere with key enzymes needed for synthesizing cell wall/membrane

Bacteria – Gram Stain

- Method for identifying bacteria by differential staining
- Gram + bacteria hold stain when washed with solvent (purple/blue)
- Gram – bacteria do not hold stain (counter stain, pink/red)

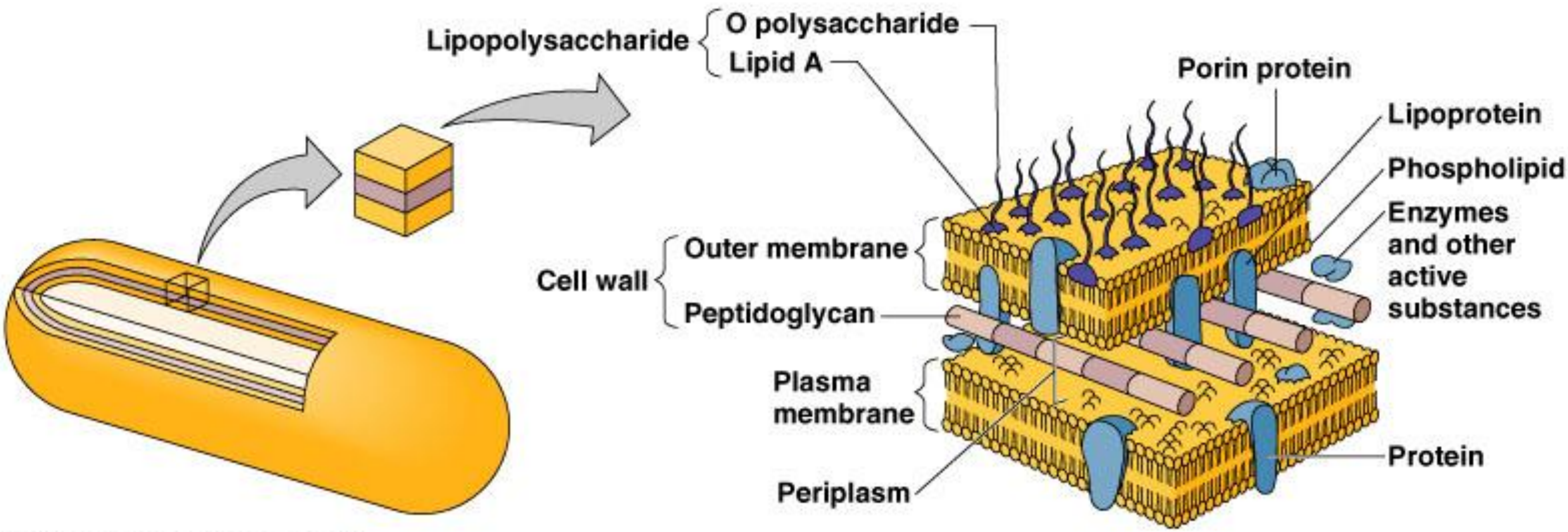
Gram (+) vs Gram (-) Bacteria

- Gram (+) Bacteria: Thick cell walls of amino acid cross-linked polysaccharides

- Gram (-) Bacteria: Thin polysaccharide cell wall coated with a lipid layer (lipopolysaccharides, LPS)

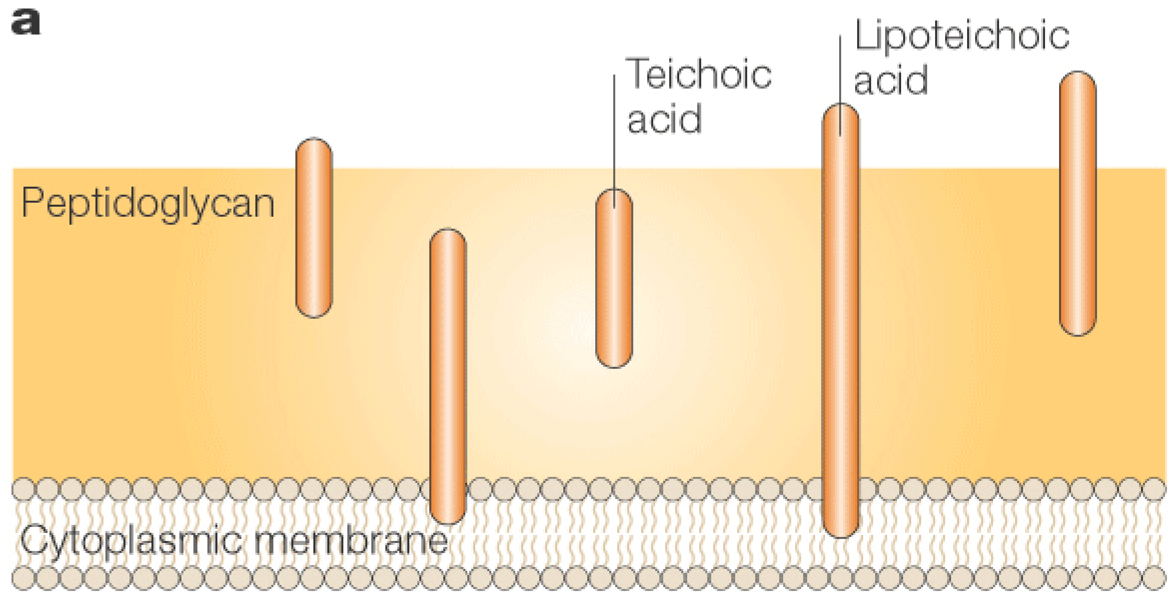
- Pathogenic forms of both are known

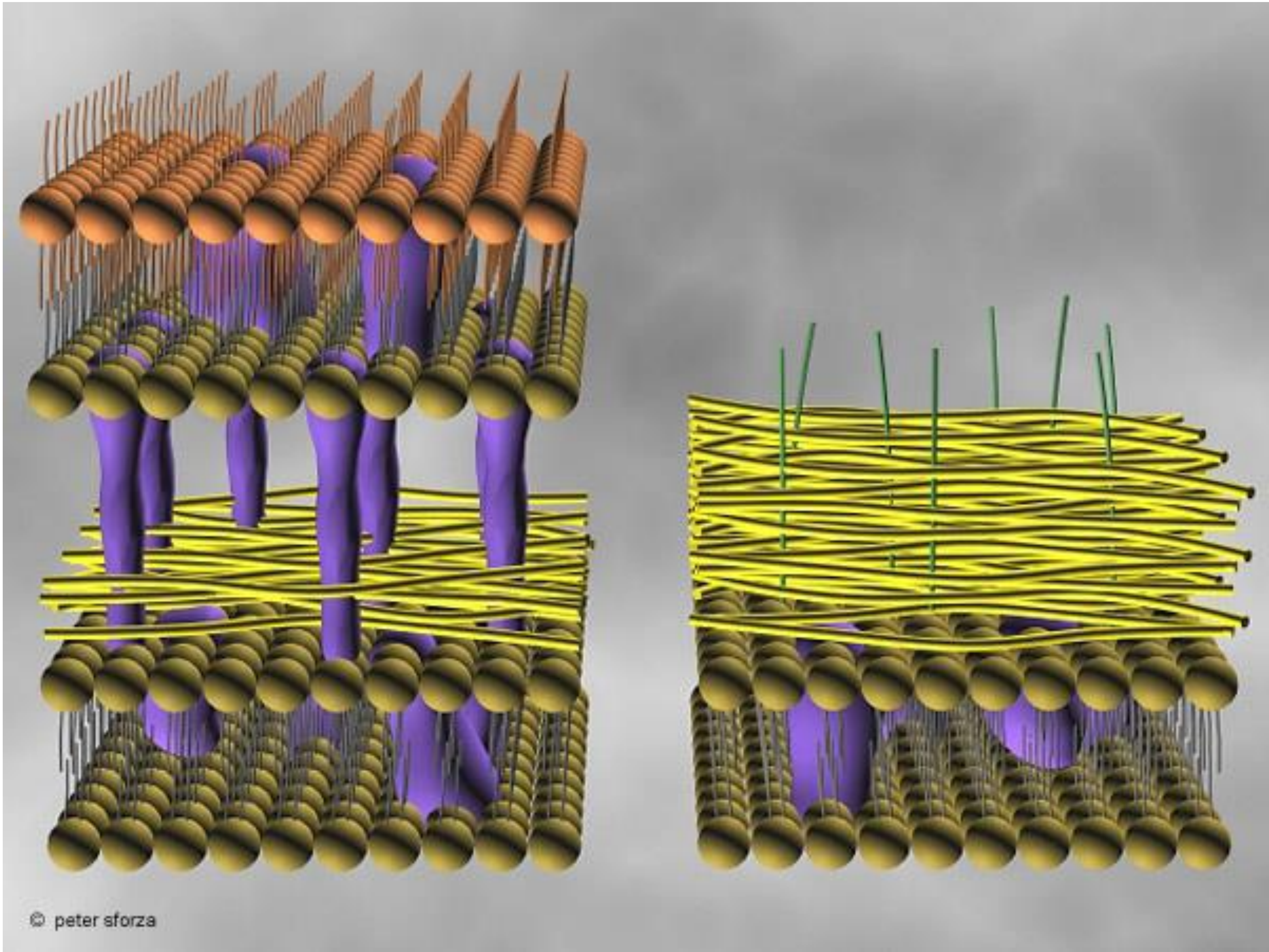
Bacterial Cell Wall

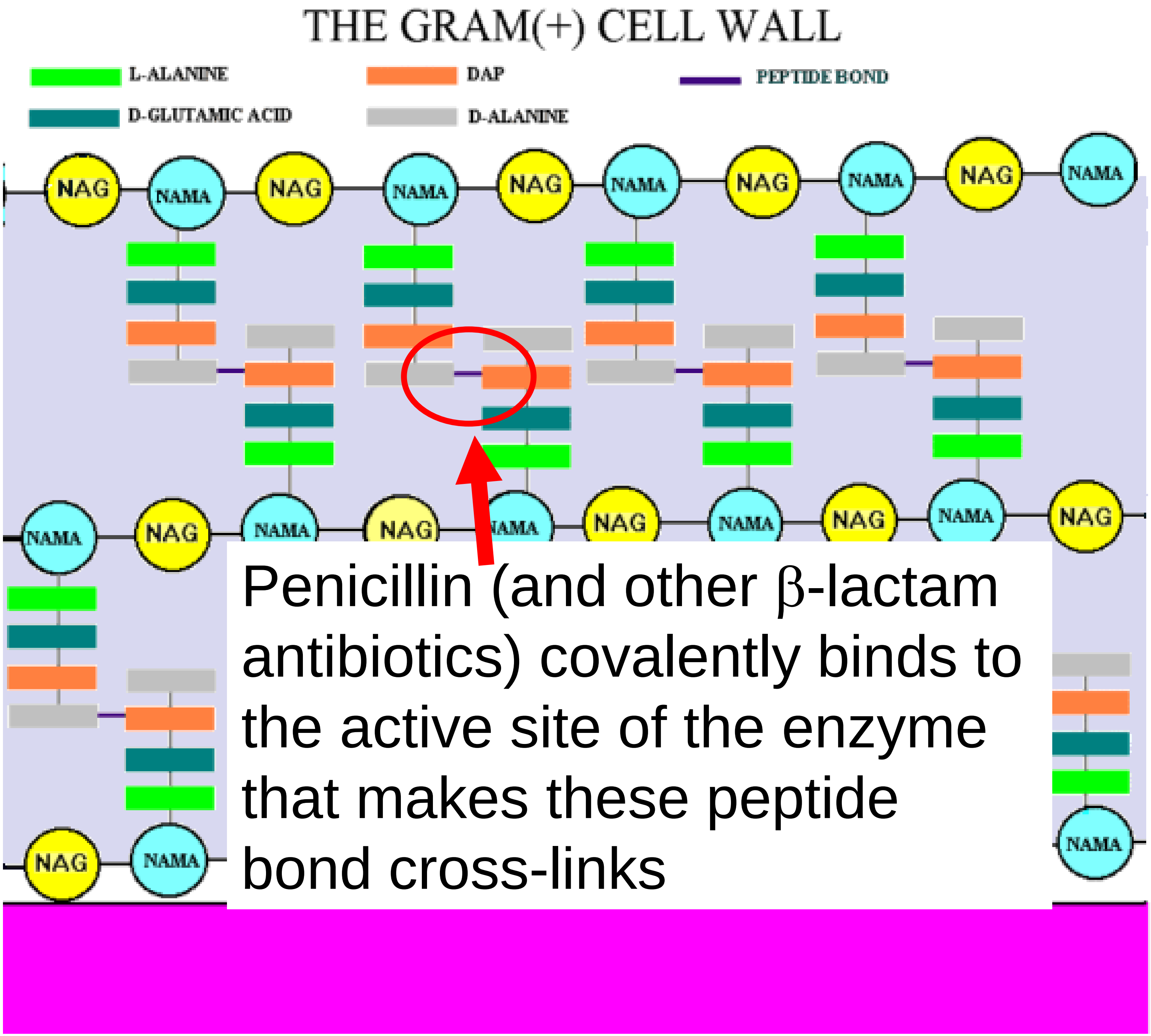


(c) Gram-negative cell wall

Gram +

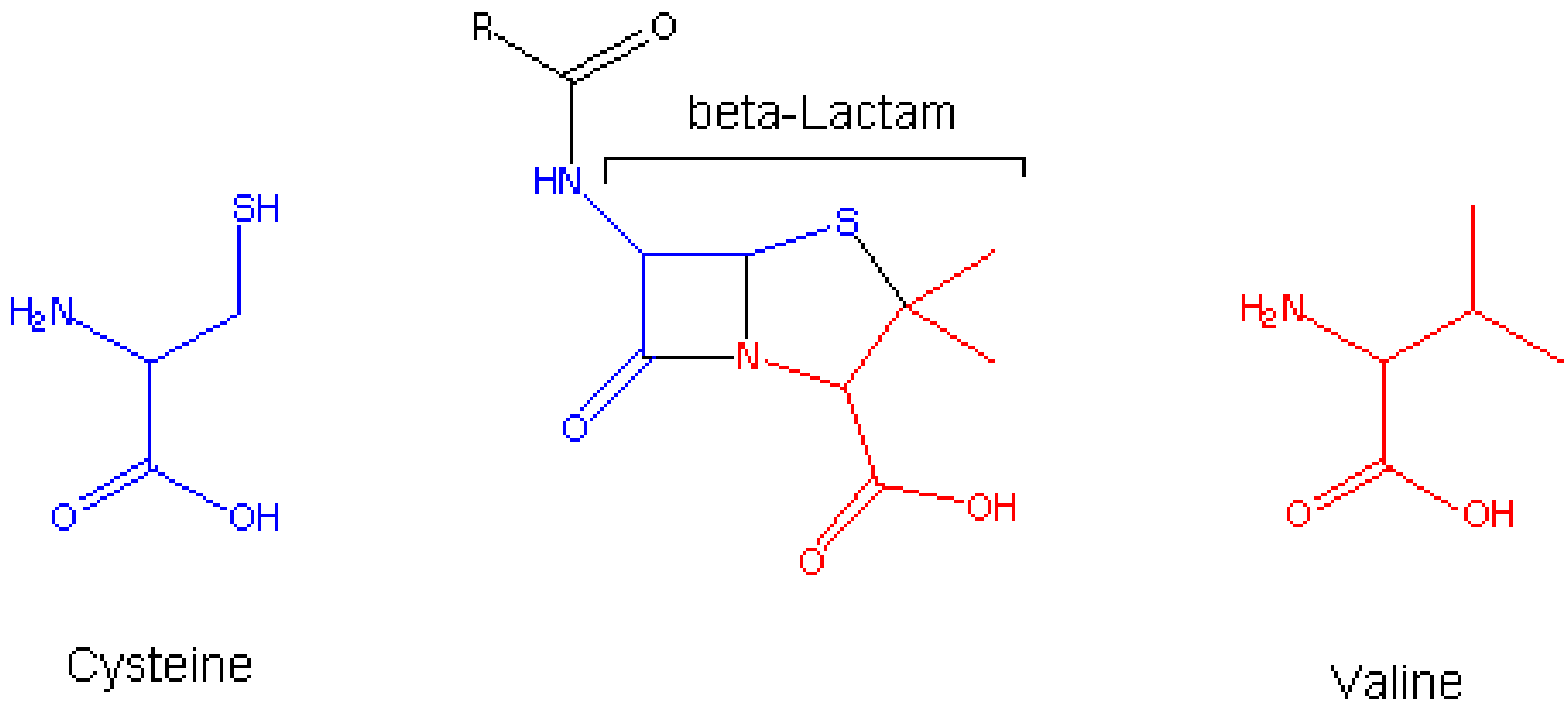

a
Teichoic acid
Lipoteichoic acid
Peptidoglycan
Cytoplasmic membrane


© peter sforza

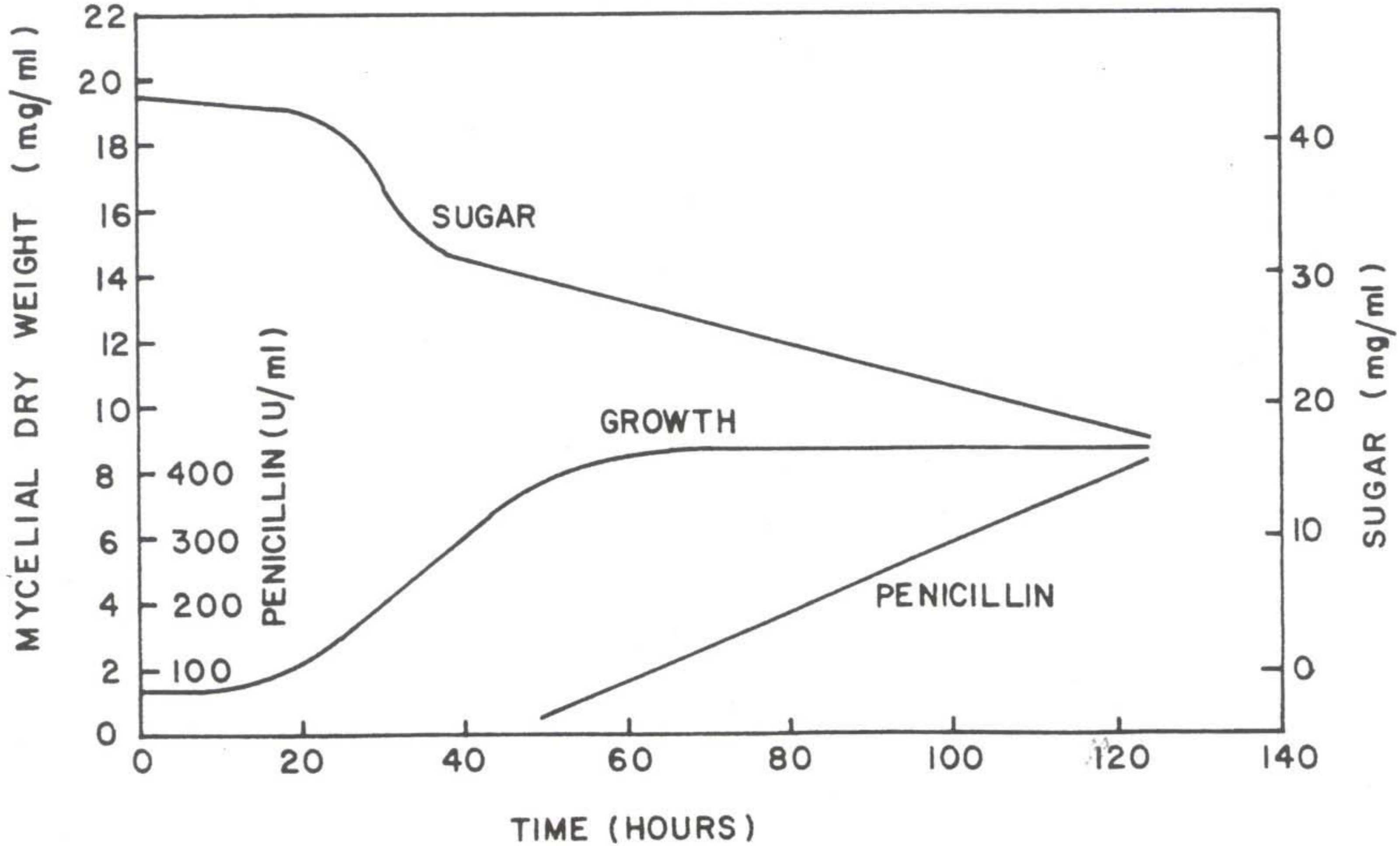


Penicillin (and other β-lactam antibiotics) covalently binds to the active site of the enzyme that makes these peptide bond cross-links

Penicillin - Beta Lactam Structure



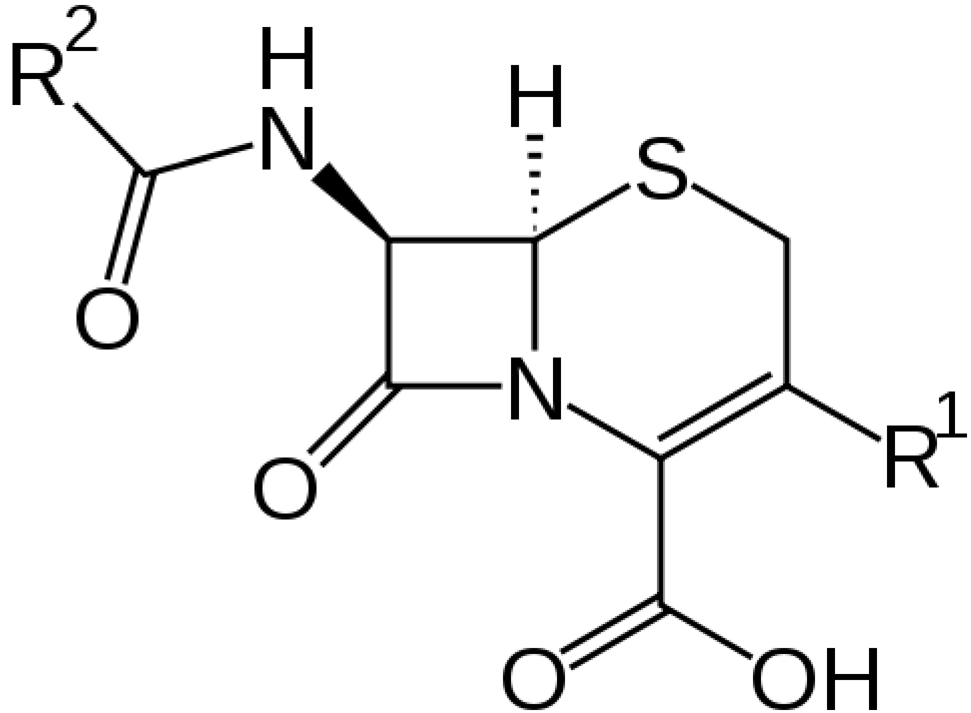
C. Ophardt, c. 2003



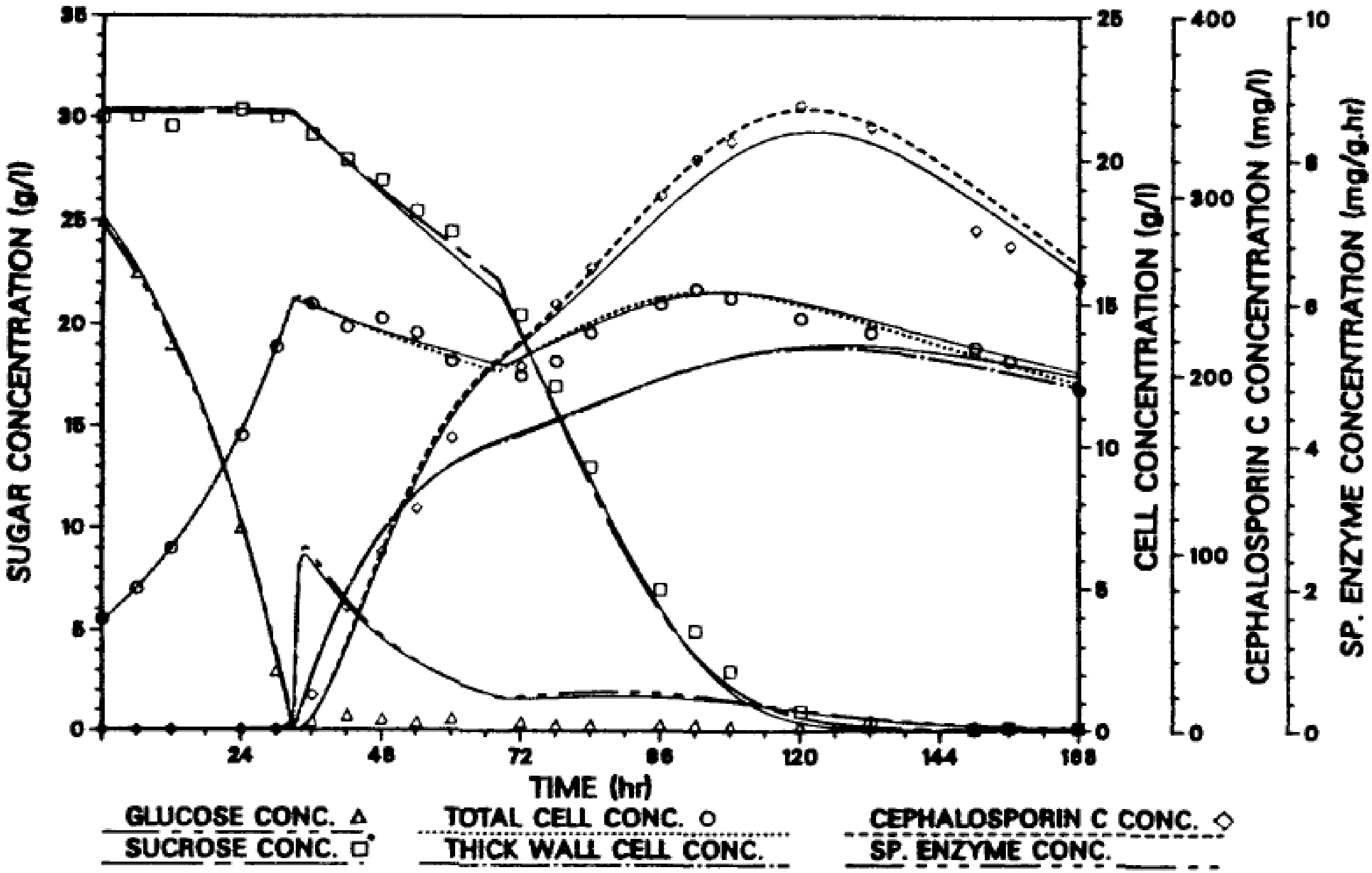
MYCELIAL DRY WEIGHT (mg/ml)
PENICILLIN (U/ml)
SUGAR (mg/ml)
TIME (HOURS)
SUGAR
GROWTH
PENICILLIN
0
2
4
6
8
10
12
14
16
18
20
22
100
200
300
400
0
10
20
30
40
0
20
40
60
80
100
120
140

Cephalosporin C

- Beta lactam antibiotic
- *Cephalosporium acremonium*
- Discovered in a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu
- First commercial product released by Eli Lilly in 1964


R2
H
N
H
S
O
N
R1
O
O
OH

CEPHALOSPORIN C FERMENTATION AT PH 6.2 AND 32°C



Vancomycin

- Gram (+)
- Binds with the substrate, not the enzyme (contrast with penicillin)
- Binds the D-alanyl-D-alanine terminal dipeptide of peptidoglycan precursors
- Prevents the reaction used to link peptidoglycan precursors together from taking place

Streptomycin

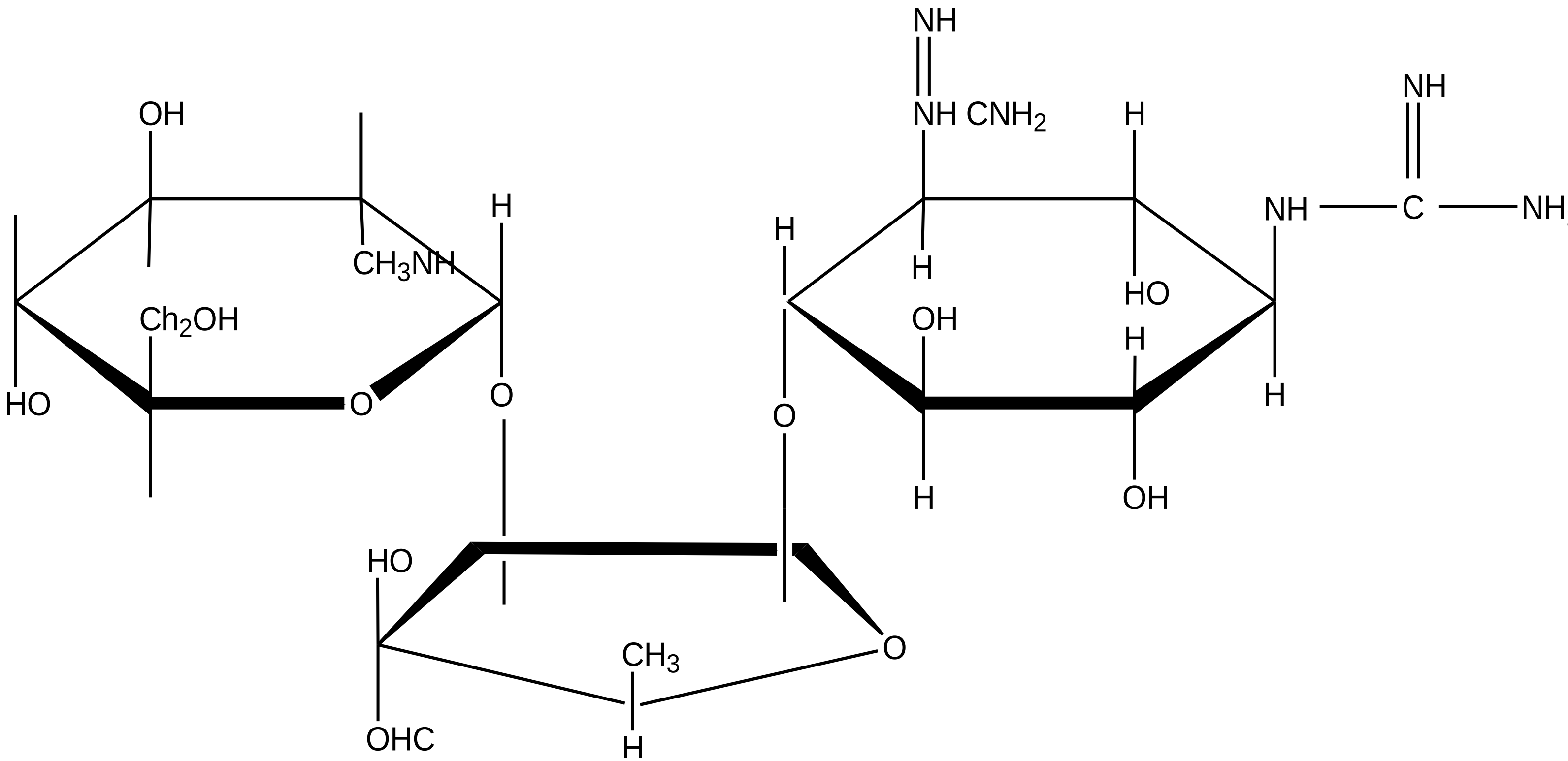


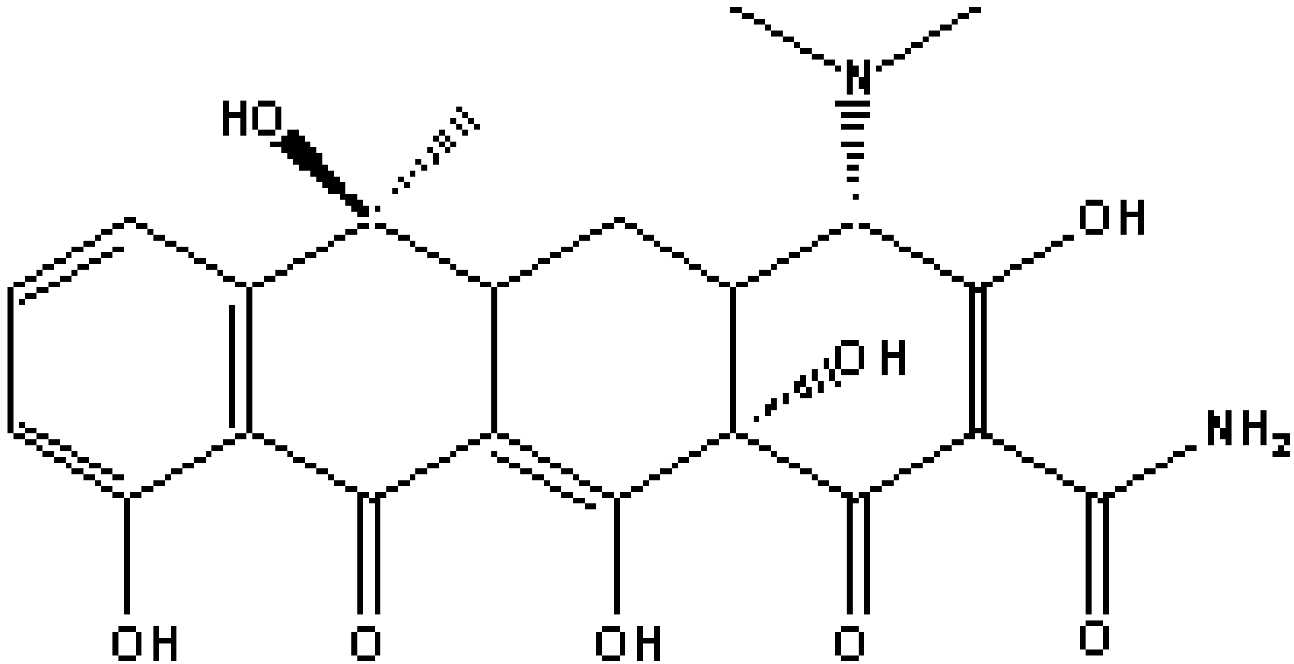
Figure 7-16

Streptomycin

- Effective against gram-negative bacteria

- Binds to the 30S ribosome - changes its shape so that it inhibits protein synthesis by causing a misreading of messenger RNA information.

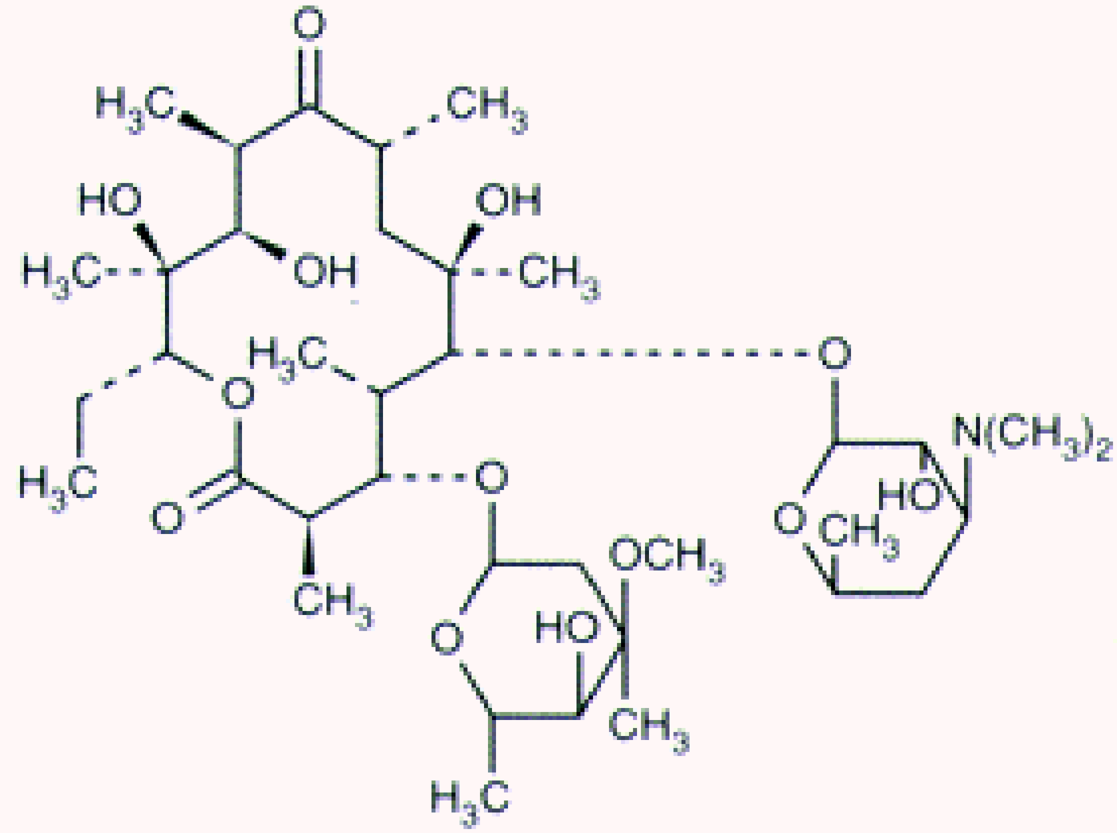
Tetracycline



Tetracycline

- Very broad spectrum - both Gram (+) and Gram (-)
- Inhibit bacterial protein synthesis by blocking the attachment of the transfer RNA-amino acid to the ribosome. More precisely they are inhibitors of the codon-anticodon interaction.

Erythromycin



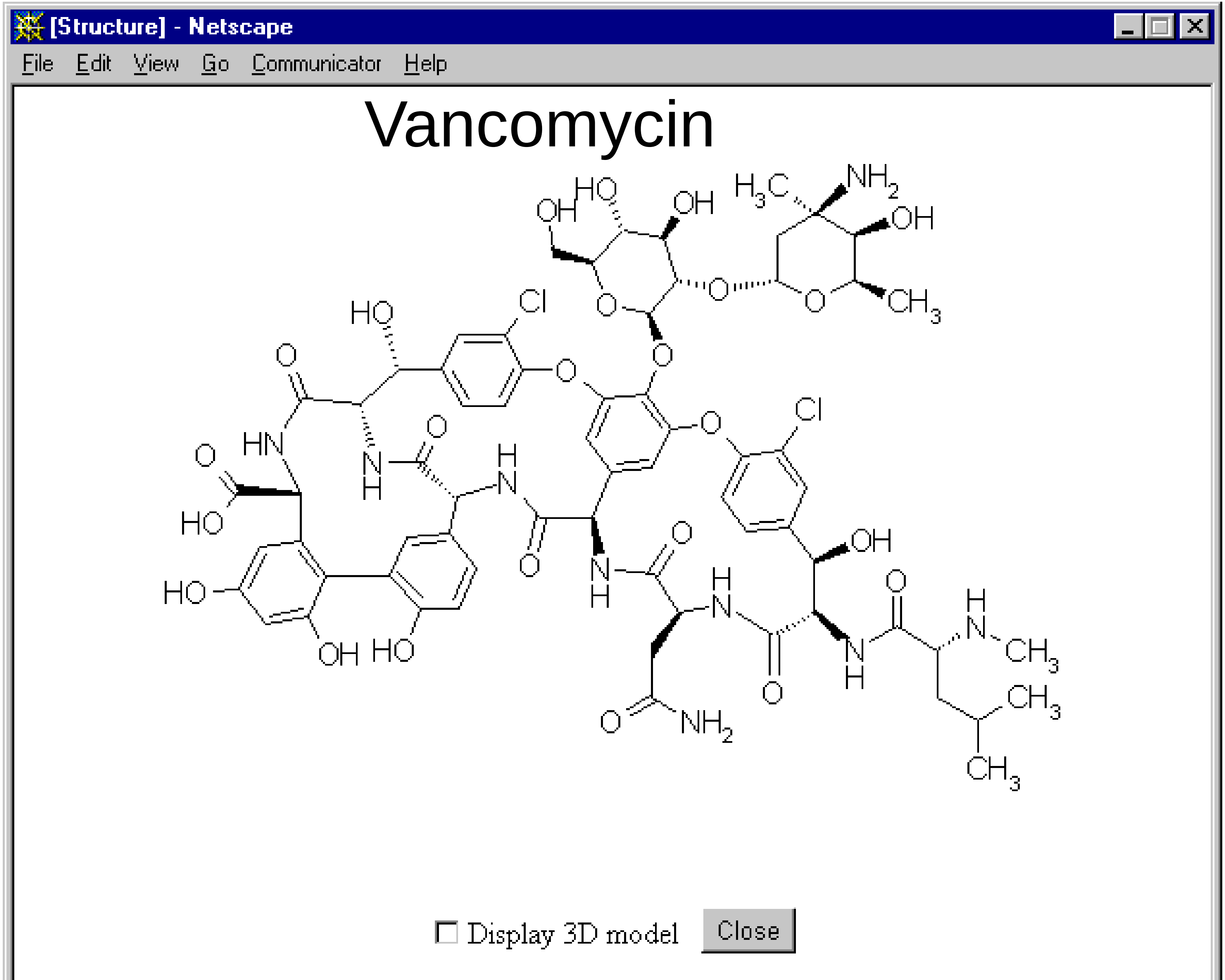
G.L. Coppoc, DVM, PhD, Purdue University

Erythromycin

- Macrolide (a product of actinomycetes - soil bacteria) or semi-synthetic derivatives of them.

- Erythromycin was discovered in 1952 in the metabolic products of a strain of *Streptocyces erythreus*, originally obtained from a soil sample

- Inhibit protein synthesis by binding to the 23S rRNA molecule (in the 50S subunit) of the bacterial ribosome blocking the exit of the growing peptide chain (Humans do not have 50 S ribosomal subunits, but have ribosomes composed of 40 S and 60 S subunits)


[Structure] - Netscape
File Edit View Go Communicator Help
Vancomycin
Display 3D model
Close