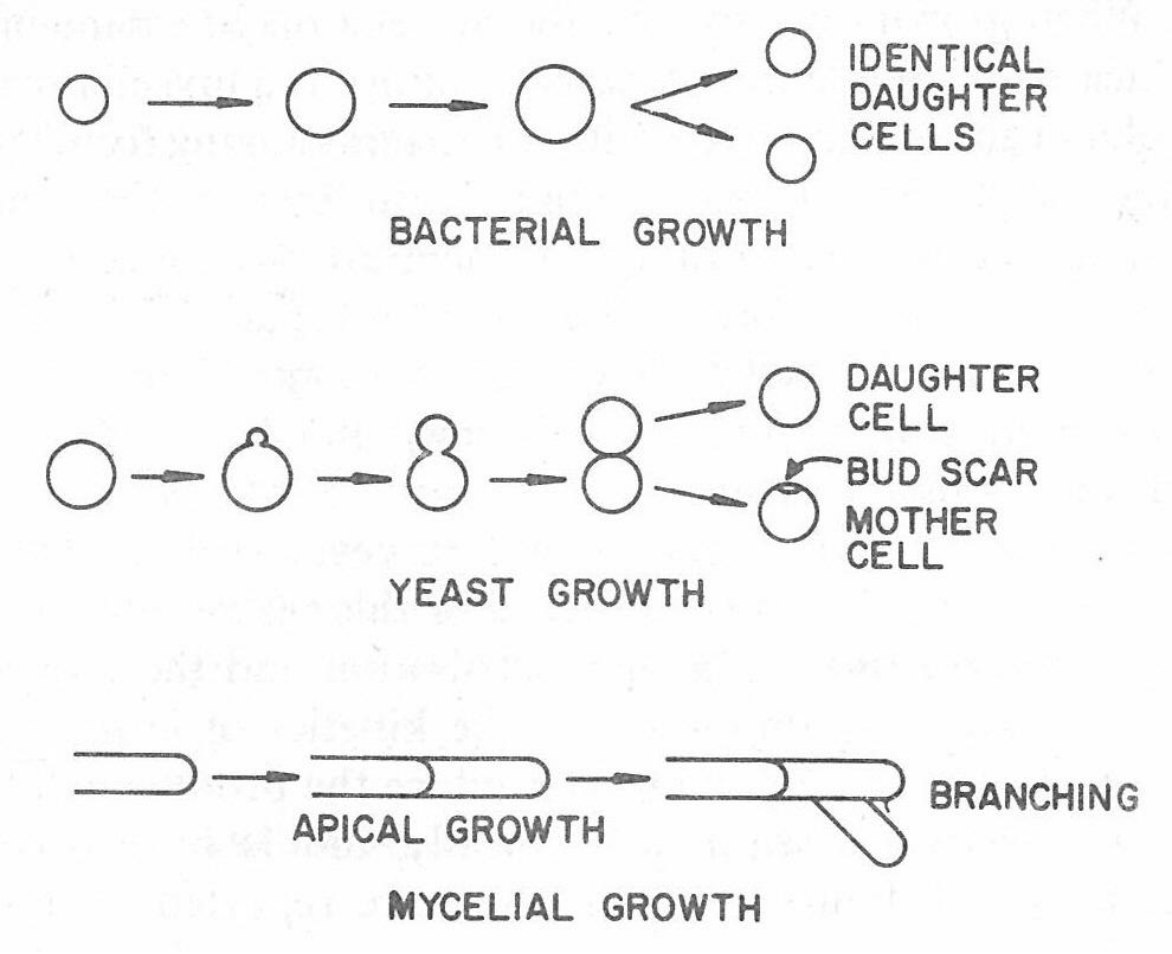
Fermentation Kinetics

Growth by bacteria, yeast and molds



Microbial Growth

$$\text{substrate} + \text{cells} \rightarrow \text{extracellular products} + \text{more cells}$$

a) In terms of biomass concentration, x

$$\frac{dX}{dt} = \mu X \qquad \mu = \frac{1}{X}\frac{dX}{dt}$$

X = cell mass concentration (g/L)

t = time (hour)

μ = specific growth rate (h^{-1})

b) In terms of cell number, N

$$\frac{dN}{dt} = \mu_n N \qquad \mu_n = \frac{1}{N}\frac{dN}{dt}$$

Measurement of cell number: Direct method

- Direct measurement of cell number
  - By using hemocytometer
  - With staining with methylene blue or trypan blue
- Viable plate count
  - Agar plate with Petri dish (1.5% - 2.5% agar)
  - 30 – 300 colony forming unit (CFU)
  - Only count the viable cells

Measurement of cell mass: Direct method

- Dry cell weight
  - Most common method for measuring total cell mass
  - After obtaining the samples of whole culture broth
    - Centrifuged
    - Washed with buffer or water
    - Dried at 80 ^{o}C for 24 hours or 110°C for 8 hours
  - Only useful for the dense cell suspension
  - Should removed the non cellular solids before using the method

Measurement of cell mass: Direct method

- Turbidity
  - The optical density or turbidity of a culture broth is proportional to the cell mass (600-700 nm)
  - A simple, fast method on a routine basis

$$-\frac{dI_t}{dL} = \varepsilon I_t C \qquad A = -\ln\frac{I_t}{I_0} = \varepsilon C L$$

I_t, I_0 : intensity of light
L : distance of light path
ε : molecular absortion coefficien t
C : concentrat ion
A : absorbance

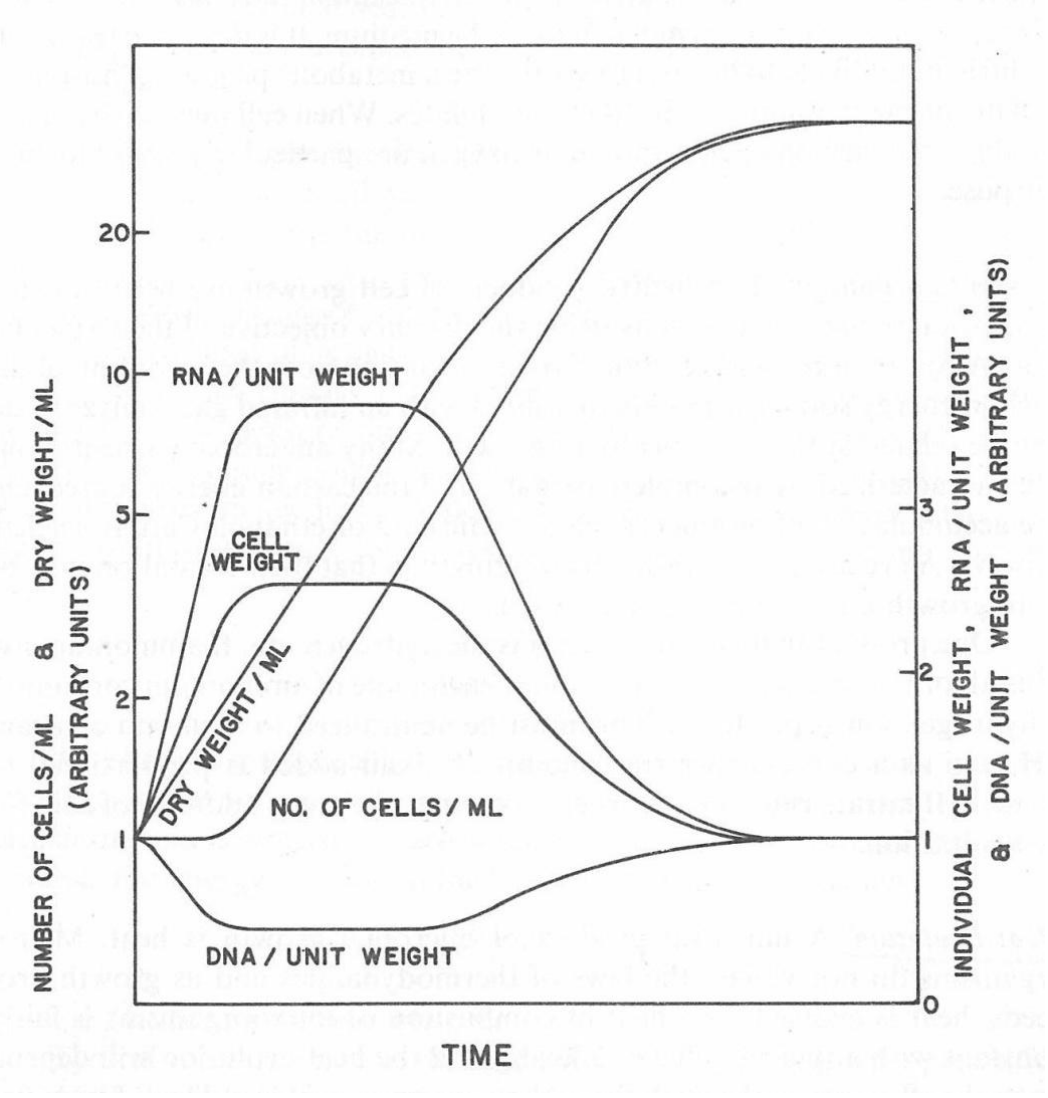
Estimation of cell mass: Indirect method

- Direct measurements can ***not be applicable to mold or mycelial cells*** with insoluble nutrients (solids or hydrocarbons) in complex media.
- Overall ***stoichiometry*** for growth and product formation

$$\text{C-source} + \text{N-source} + \text{phosphate} + O_2 \rightarrow \text{cell mass} + CO_2 + H_2O + \text{product} + \text{heat}$$

- Methods for measuring ***nutrient consumption*** and ***product formation*** related to the microbial growth

Macromolecules composition during growth



Estimation of cell mass: by measuring cell components

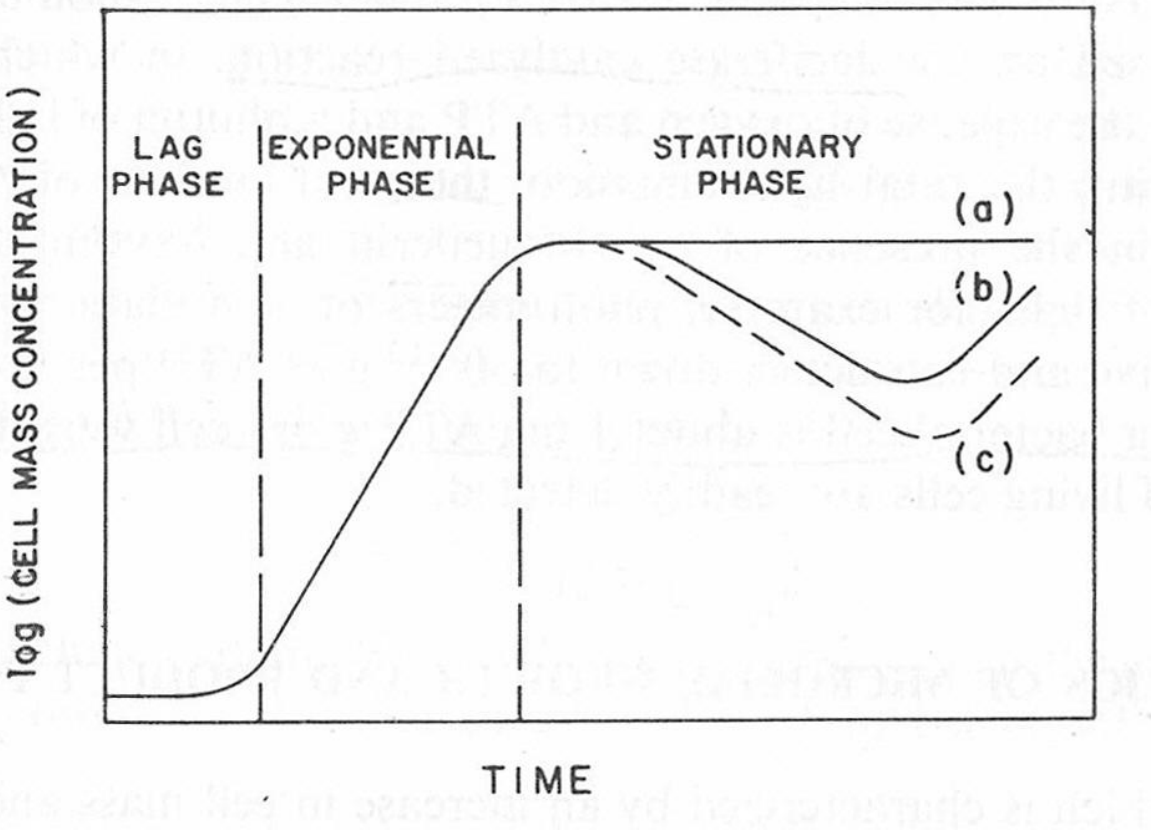
- Cell components
  - ***Proteins***
  - ***RNA*** : various with the growth phase
  - ***DNA*** : difficult to measure
- Protein measurements
  - Lowry method : peptide
  - Biuret method : peptide
  - Biorad method (Bradford) : dye complex with proteins
    - Cooassie brilliant blue G250 : shift the wave length from 465 to 595 nm when protein binding
  - Kieldahl method : total N

Estimation of cell mass: by measuring other parameters

- Nutrient uptaking
  - Carbon source
  - Nitrogen source
  - Oxygen (aerobic fermentation)
- Heat evolution
  - The universal products of microbial growth is heat
  - Heat of combustion of microorganism ~ 5 kcal/g
  - Heat balance of fermentation
- Viscosity
  - Measurement of broth viscosity
  - Mycelial growth, polysaccharide formation

Phases of microbial growth

- Lag phase : period of adaptation
  - Repressions and inductions occur
- Log phase : constant specific growth rate (μ)
- Stationary phase : stop growth, start cell lyses



Kinetics of cell growth

Growth

$$\frac{dX}{dt} = \mu X \qquad X = \text{cell mass concentration (g/L)}, \; t = \text{time (hour)}$$

$$\mu = \text{specific growth rate } (h^{-1})$$

When $t = 0, \quad X = X_0$

$$X = X_0 \exp(\mu t)$$

$$\mu = \frac{\ln 2}{t_d}, \qquad t_d = \frac{\ln 2}{\mu}$$

When there is cell death

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

$$\alpha = \text{specific cell death rate } (h^{-1})$$

Cell growth rate vs. nutrient concentration

- Monod equation, an empirical equation

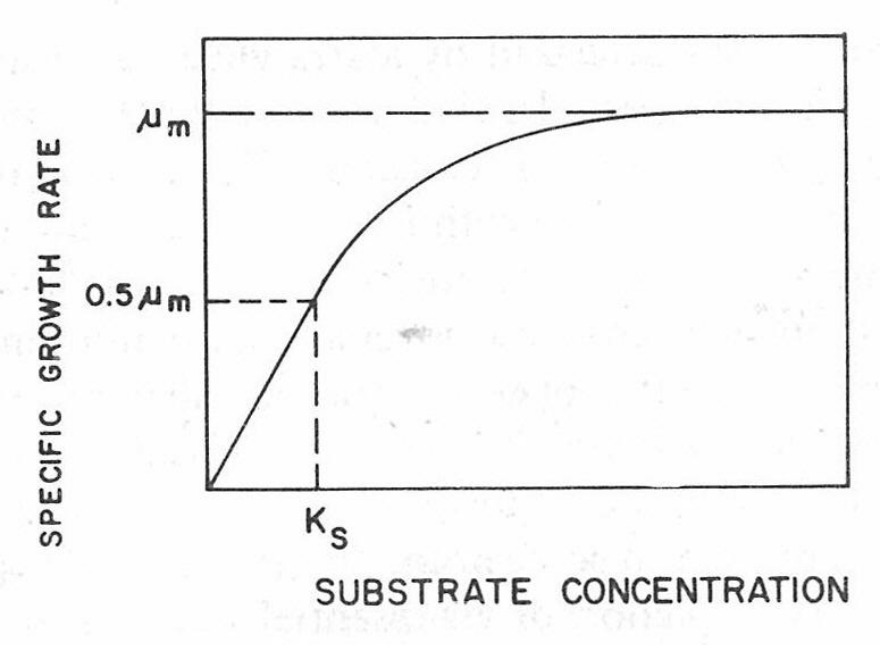
$$\mu = \mu_{\max} \frac{S}{Ks + S}$$

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

S = substrate concentration

Ks = saturation constant

when $S = Ks$, $\mu = \frac{1}{2}\mu_{\max}$



  - Typical values of Ks are quite small
    - $\mu \sim \mu_{max}$ when $S > 10Ks$

Specific rates for growth, consumption and production

$$\mu = \frac{-1}{X}\frac{dX}{dt} \quad : \text{specific growth rate}$$

$$q_s = \frac{-1}{X}\frac{dS}{dt} \quad : \text{specific substrate consumption rate}$$

$$q_s = \frac{\mu}{Y_{x/s}}$$

$$q_p = \frac{1}{X}\frac{dP}{dt} \quad : \text{specific product formation rate}$$

Kinetics of nutrient utilization and product formation

- Substrate utilization

$$\begin{matrix}\text{substrate} \\ \text{accumulation}\end{matrix} = \begin{matrix}\text{substrate} \\ \text{input}\end{matrix} - \begin{matrix}\text{substrate} \\ \text{output}\end{matrix} - \text{growth} - \begin{matrix}\text{product} \\ \text{synthesis}\end{matrix} - \text{maintenance}$$

$$\frac{dS}{dt} = \frac{F_i S_0}{V} - \frac{F_o S}{V} - \left(\frac{\mu X}{Y_{x/s}} + \frac{q_p X}{Y_{p/s}} + mX\right)$$

- Product formation

$$\begin{matrix}\text{product} \\ \text{accumulation}\end{matrix} = -\begin{matrix}\text{product} \\ \text{removal}\end{matrix} + \begin{matrix}\text{product} \\ \text{synthesis}\end{matrix} - \begin{matrix}\text{product} \\ \text{destruction}\end{matrix}$$

$$\frac{dP}{dt} = -\frac{F_o P}{V} + q_p X - KP$$

Maintenance Energy

- Observation: cells consume energy source even when not growing in number or biomass
- Hypothesis: a requirement of “maintenance energy”

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

$$\frac{1}{Y_{x/s}} = \frac{1}{Y_{\max}} + \frac{m}{\mu}$$

Description of product formation

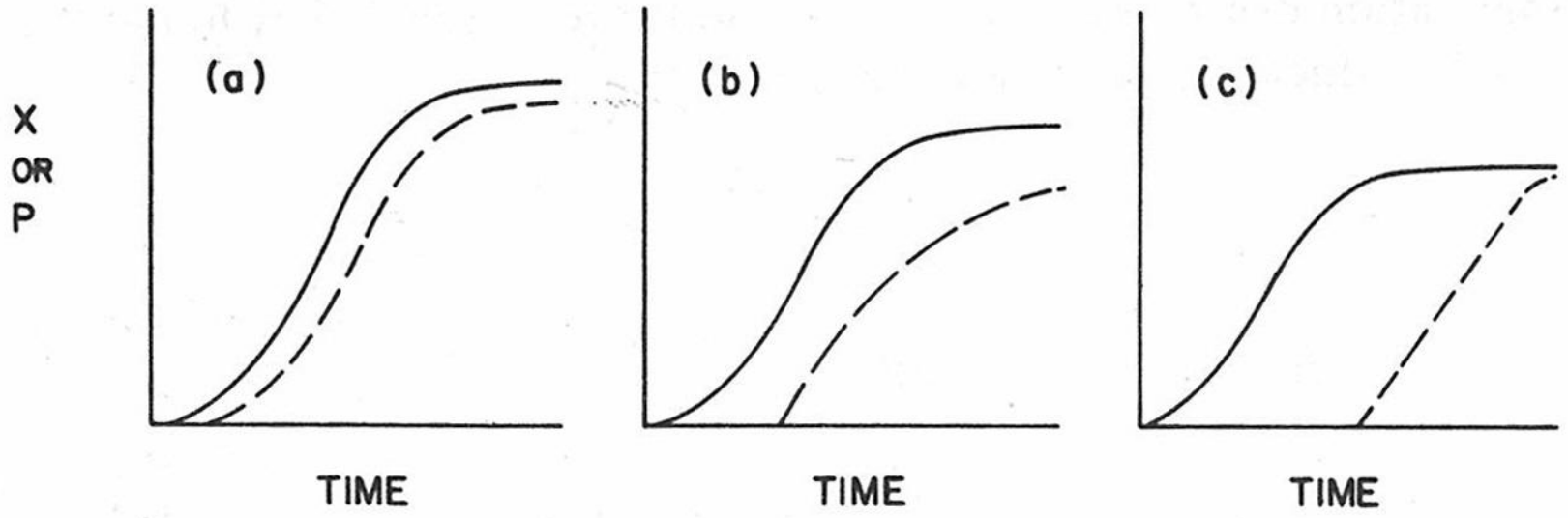
- Mixed mode of product formation
  - Ludeking –Piret model

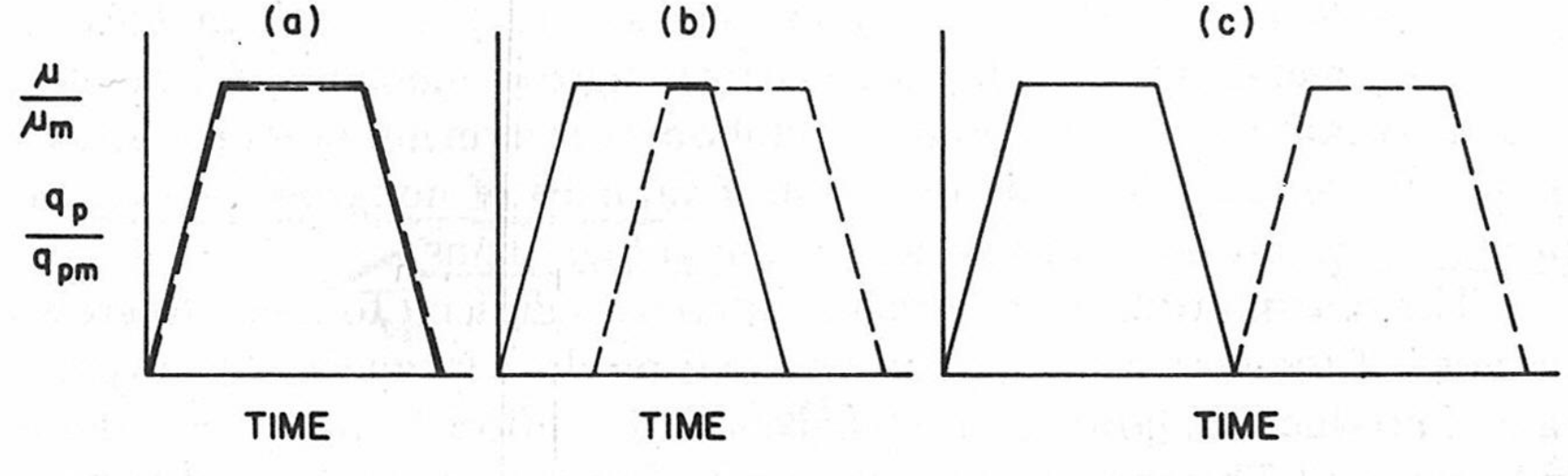
$$\frac{dP}{dt} = q_p X$$

$$q_p = \alpha\mu + \beta$$

α = growth associated specific productivity
β = non growth associated specific productivity

  - Production of growth associated: $\beta = 0$
    - Primary metabolites : amino acids, enzymes
  - Production of non-growth associated : $\alpha = 0$
    - Secondary metabolites : antibiotics, toxins


X
OR
P
(a)
(b)
(c)
TIME
TIME
TIME


μ/μm
qp/qpm
(a)
(b)
(c)
TIME
TIME
TIME

Elemental composition of animal cells and bacterium

	Mass(pg/cell)		% of Dry Mass	
	Animal cell	Bacterium	Animal cell	Bacterium
Wet weight	3500	1.5		
Dry weight	600	0.3	100	100
Protein	250	0.17	41	55
Carbohydrate	150	0.015	20	5
Lipid	120	0.015	1.5	5
DNA	10	0.015	1.5	5
RNA	25	0.075	2.5	25
Water		1.2	500	400
Volume	$4X10^{-9}cm^3$	$1.5X10^{-12}cm^3$		

Biomass Formula

- Example:

Element	C	H	N	O	ash
% of dry weight	53.0	7.3	12.0	19.0	8
Atomic number	12	1	14	16	
mole per 100gram	4.42	7.3	0.86	1.19	
Normalized to C	1	1.66	0.194	0.269	

- $CH_{1.66}N_{0.194}O_{0.269}$ constitute 0.92 of mass
- Formula weight =(12+1x1.66+14x0.194+16x0.26)/(1-ash fraction)
  =20.8

Chemical composition of cells

- Cells are consisted of proteins, carbohydrates, lipids, nucleic acids and electrolytes
- Major elemental components: C,N,H,O,P,S
- Typical cellular formula
  - Bacterium: $CH_{1.6}N_{0.20}O_{0.27}$ to $CH_2N_{0.26}O_{0.45}$
  - Yeast: $CH_{1.64}N_{0.16}O_{0.52}P_{0.01}S_{0.005}$
  - Animal Cells: $CH_{1.98}N_{0.26}O_{0.49}$

Cellular growth and product yield

$$A(C_aH_bO_c) + B(O_2) + D(NH_3) \rightarrow$$

$$M(C_\alpha H_\beta O_\gamma N_\delta) + N(C_{\alpha'} H_{\beta'} O_{\gamma'} N_{\delta'}) + P(CO_2) + Q(H_2O)$$

The cell yield on carbon source

$$Yx/s = \frac{M}{A} \frac{(12\alpha + \beta + 16\gamma + 14\delta)/(1-r)}{12a + b + 16C}$$

r is the fraction of cell mass by ash ($r \approx 0.07 - 0.10$)

The product yield on carbon source

$$Yp/s = \frac{N}{A} \frac{(12\alpha' + \beta' + 16\gamma' + 14\delta')}{(12a + b + 16C)}$$

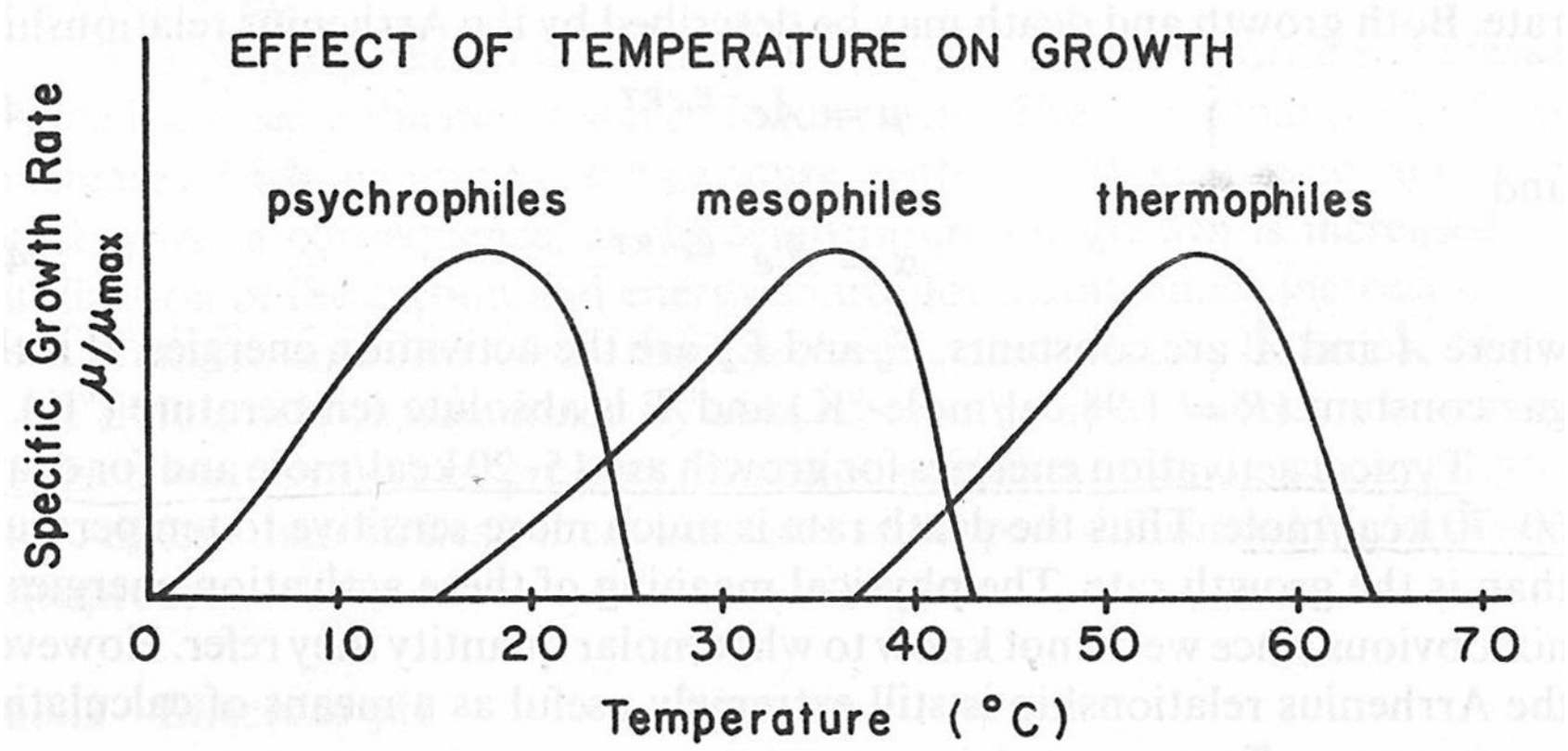
Requirements and constraints in medium design

- Nutritional and environmental requirement
  - Elemental requirements: carbon, nitrogen etc.
  - Specific nutrient requirements: vitamin, mineral, amino acids
  - Culture condition: pH, ionic strength

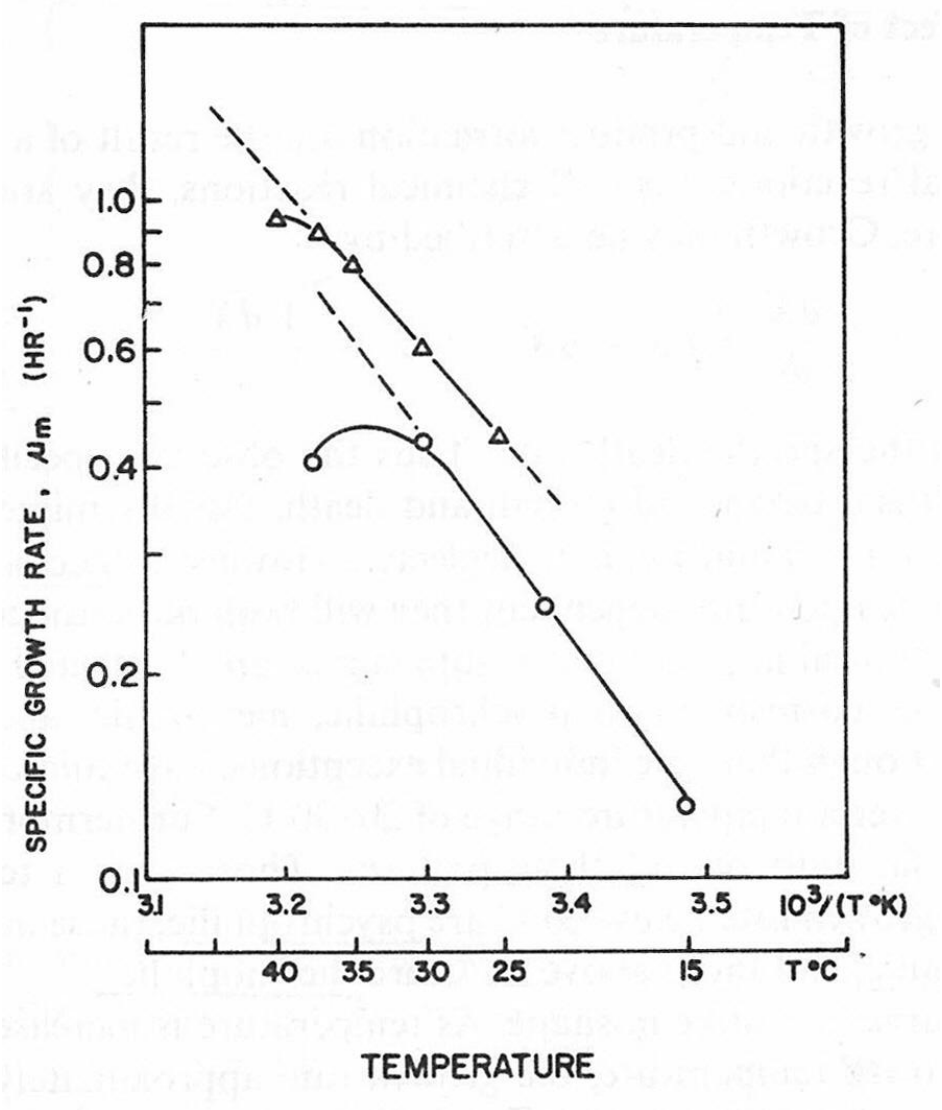
- Fermentation process objectives
  - Cell mass vs. products
  - Avoid C, N, S, PO_4 repression
  - pH or temperature

- Technoeconomical Constraints
  - Cost
  - Raw material availability
  - Product recovery
  - Pollution control

Effects of temperature on growth



Arrhenius plot of growth



Cell yield vs. temperature

