BIOCHEMICAL PROCESS INDUSTRY

•MANUFACTURING OF PRODUCTS FOR MULTIPLE MARKETS

- FOOD & BEVERAGE
- HEALTH CARE
  - THERAPEUTICS
  - DIAGNOSTICS
  - DEVICE
- SPECIALTY CHEMICAL
- WASTE TREATMENT

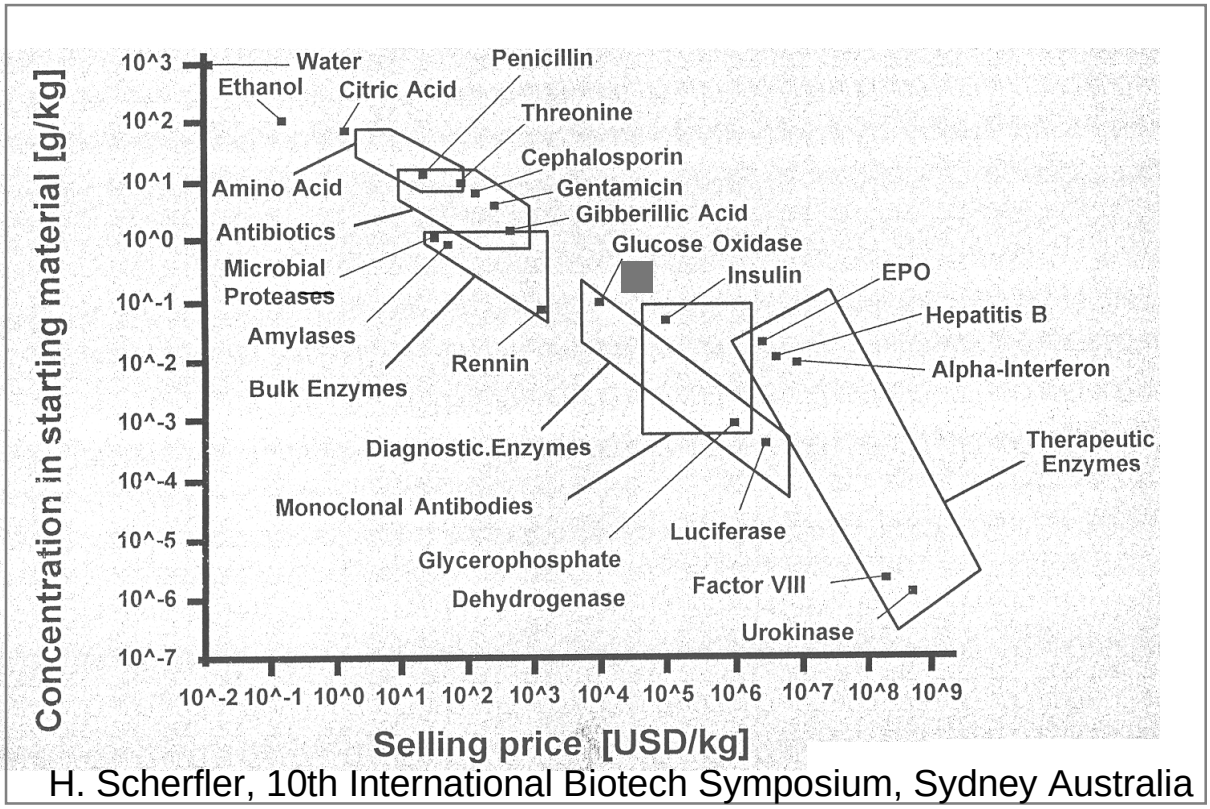
•MANUFACTURING BY MULTIPLE SYNTHETIC & EXTRACTIVE TECHNOLOGIES

- BIOSYNTHETIC - MICROBIAL, ANIMAL
- EXTRACTIVE
- CHEMICAL SYNTHESIS

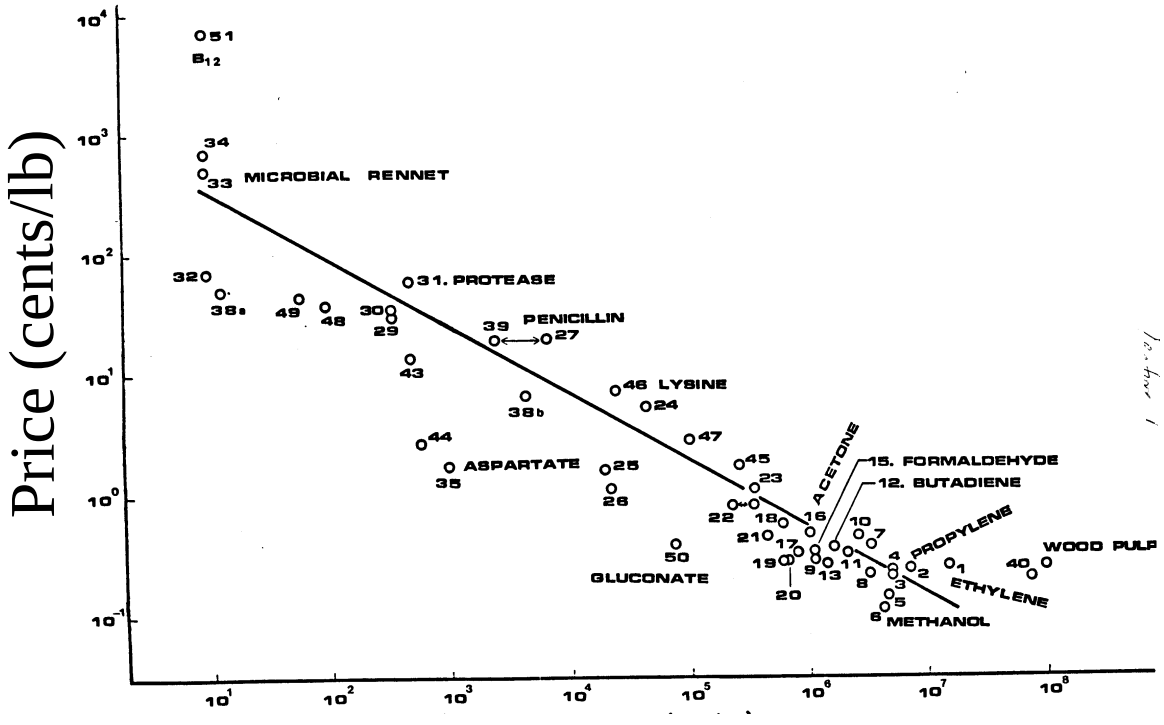
•PRODUCTS BELONG TO MULTIPLE CLASSES

- PROTEINS
- NUCLEIC ACIDS
- CARBOHYDRATES
- CATABOLITES AND ANABOLITES
- CELLS AND VIRUSES

Selling Price vs.. Volume



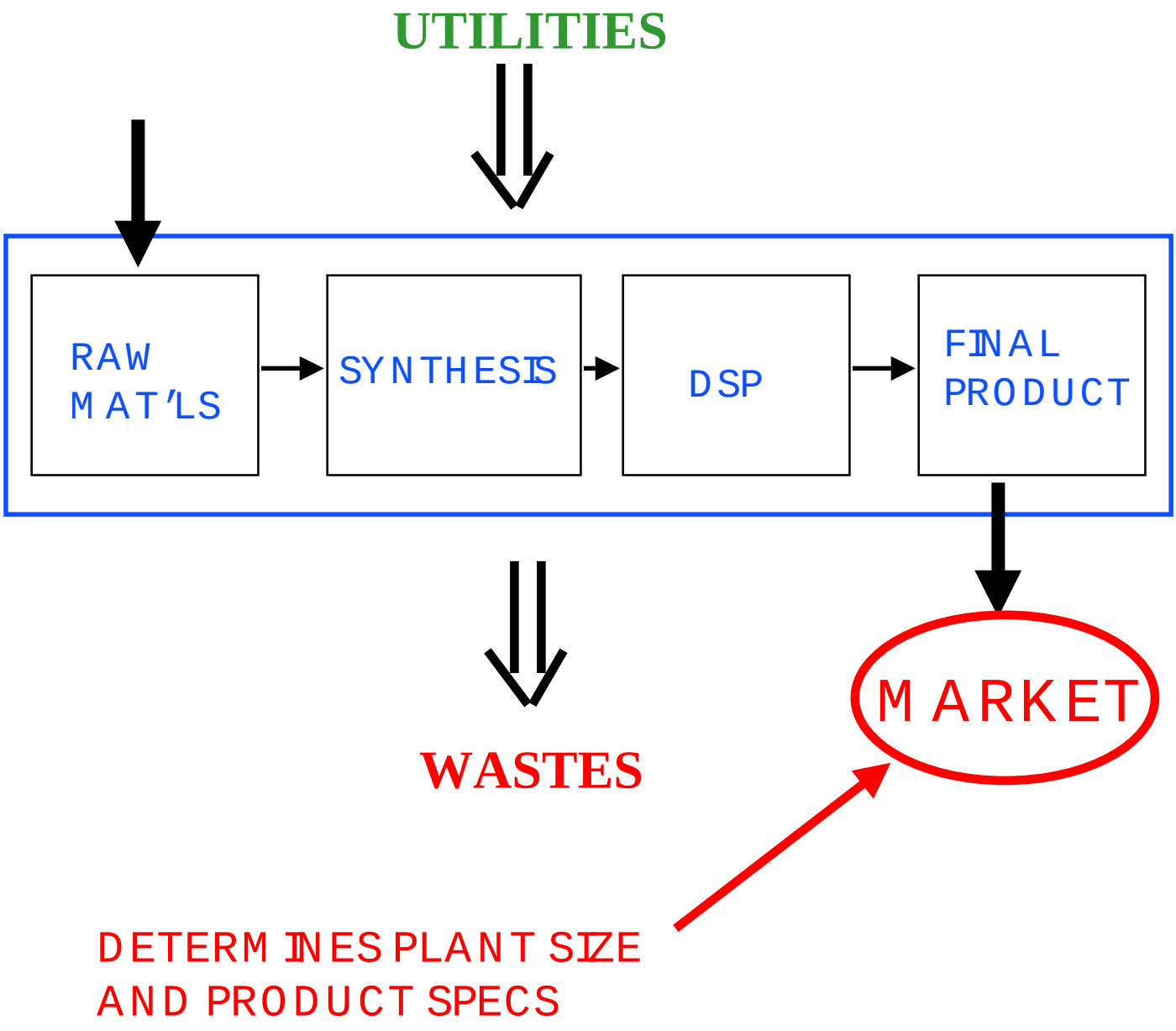
H. Scherfler, 10th International Biotech Symposium, Sydney Australia



Revenue Rate Analysis

Product	Concentration (g/liter)	Productivity (g/liter-day)	Price ($/kg)	Revenue Rate ($/liter-day)
Citric Acid	150	50	2.5	0.13
Ethanol	80	80	0.5	0.04
Glutamic Acid	120	33	5.6	0.18
Gluconic Acid	300	100	1.2	0.12
Lactic Acid	150	65	2.1	0.14
Lysine	80	33	4.8	0.16
Penicillin	60	4	18	0.07
Protease	20	7	20	0.14
Riboflavin	10	2	67	0.13
Streptomycin	30	3	150	0.45
Xanthan Gum	30	3	12.5	0.04
Vitamin B12	0.06	0.03	8750	0.26
			Average	**0.16**
			Std. Dev.	0.11

Supply Chain



Relationship of Profit to Price

Market size determined by problem solved

Selling price is Fixed by utility And competition

$$PROFIT = VF_M(S_P S_A - C_M)$$

Market share is a function of proprietary postion:

- Patents
- Marketing
- Distribution
- Innovation speed

Specific Activity

Manufacturing cost

Sensitivity of Profit to S_p, S_p & C_m

Sa (Units/lb)	Sp ($/unit)	Cm ($/lb)	Profit $ MM
200	0.45	55	210
200	0.45	35	330
200	0.35	55	90
500	0.45	55	1020
2000	0.45	55	5070

V= 30 b lb sugar & Fm = 0.2

Outline for microbial growth

GROWTH AND METABOLISM

A QUANTITATIVE OVERVIEW

CHARACTERISTICS OF CELL GROWTH

- **Bacteria, Yeast and Mycelial Forms**

MEASUREMENT OF GROWTH

- **Direct Measurement of Cell Number**
- **Direct Measurement of Cell Mass**
- **Indirect Assessment of Growth**

THE RATE LAW FOR GROWTH AND PRODUCT FORMATION

- **Exponential Growth**
- **Cell Death**
- **Cell Lysis**
- **Dependence on Cell Type**

DEPENDENCE OF GROWTH RATE ON ENVIRONMENT

- **Temperature**
- **pH**
- **Ionic Strength and Osmolality**
- **Nutrient Supply and Concentration**

REQUIREMENTS FOR GROWTH - MEDIA DESIGN

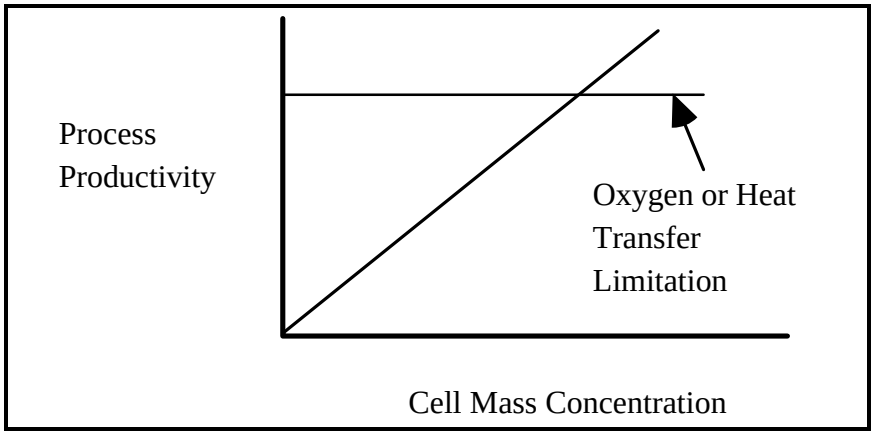
- **Process Objectives**
- **Nutritional Requirements**
- **Environmental Requirements**
- **Regulatory Constraints**
- **Techno-Economic Constraints**

THE GOAL IS TO PRODUCE AN ADEQUATE AMOUNT OF A QUALITY PRODUCT CONSISTANTLY AND AT A LOW COST

Process Constraints:

- **Product is made by_____ organism**
- **Product is intra- or extra-cellular**
- **The culture environment must meet specific conditions**
- **The equipment exists or needs to be designed**
- **FDA, EPA, OSHA constraints**
- **The cost of product in broth cannot exceed $__/kg or liter.**
- **__ kg/mo of product is needed for market**

$$P = \int Q_p \, dt = \int q_p X \, dt$$



FERMENTATION AND CELL CULTUI
PRODUCT AND PROCESS DEVELOPMENT

PROCESS DEVELOPMENT GOALS

MAXIMIZE THE AMOUNT OF "ACTIVE" CELL MA
- IN AN AFFORDABLE MANNAR

MAXIMIZE THE SPECIFIC PRODUCTIVITY, q_p, ASSOCIATED WITH THE CELL S

Genetic Control
Environmental Control

MATCH PROCESS GOALS WITH EQUIPMENT CABABILITY - OXYGEN AND HEAT TRANSFER

MINIMIZE THE PROCESS TIME

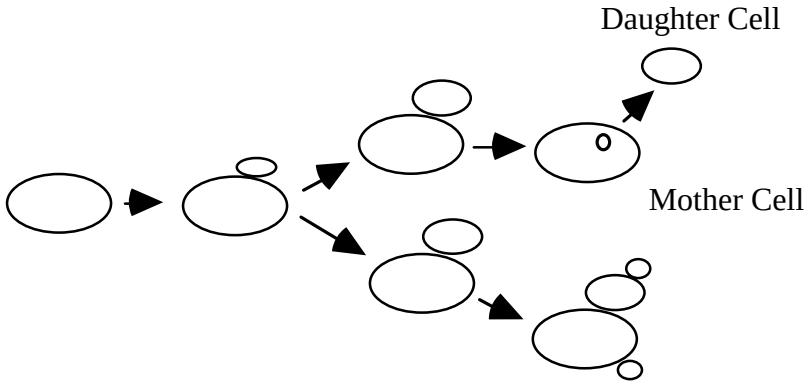
REPRODUCIBLE PERFORMANCE - ROBUSTNESS

STRATEGY:

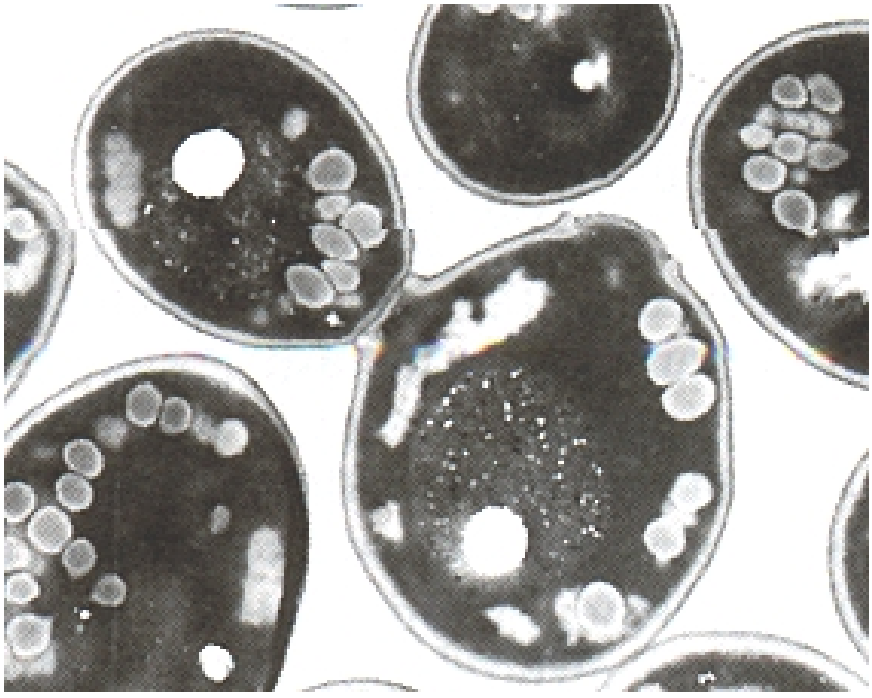
REMOVE OPERATIONAL BARRIERS
CONTROL AT CRITICAL POINTS
SYSTEMS APPRAOCH TO MEDIUM, CULTURE GROWTH, PROCESS OPERATION

CHARACTERISTICS OF CELL GROWTH

YEAST GROWTH - *CELL DIVISION BY BUDDING*



PSEUDOMYCELIA



TEM of *S. cerevisiae* grown in YEPG medium in stationary phase
(T. Srinorakutara J Ferm Bioeng 86 253 1998)

Fermentation Process Development

A +B --> C

A + C ---> D +E

Set of Enzymes and Reactions

Molecular Biology

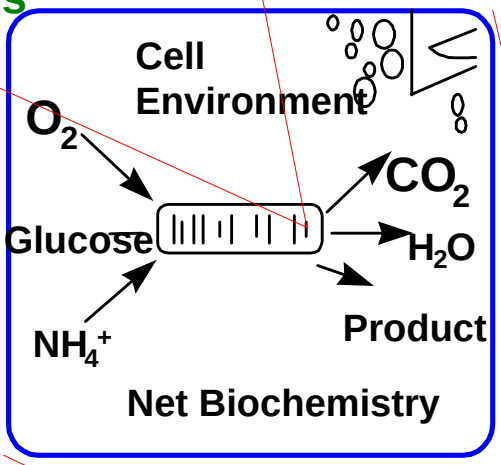
- Expression system
- Plasmid design and copy number
- Control of metabolism

Experimental Parameters

- Host cell selection
- Expression system
- Media design
- Fermentation conditions
- Aeration strategy
- Cell harvesting strategy

Metabolic pathways

Process kinetics



Elemental balances

Solubility & Equilibria

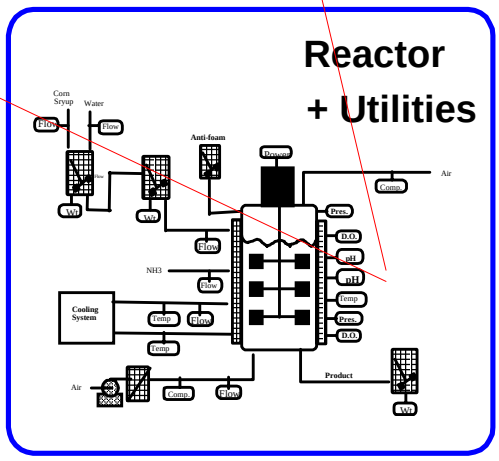
Performance Assessment

- Growth Rate
- Product Concentration
- By-Product Concentration
- Raw Materials Utilization

Mass transfer

Water balance

Equipment correlations



Cell composition

CELL PROPERTIES AND COMPOSITION*

	Bacteria	Yeast	Molds	Actino's	Animal
Size (μ)	0.5-2	2-5	5->50	2->30	5-15
Shape	rods. spheres, chains	ellipsoid, spheres, mycelia	mycelia, pellets	mycelia, pellets	variable, clumps
Volume (μ^3)	1-8	9-125			25-3000
Weight (g/cell)	10^{-12}	10^{-11}	variable	variable	$5x10^{-10}$
Density	1.05	1.05	1.05	1.05	1.05
Doubling Time (h)	0.25-2	0.75-4	3-8	2-8	15-72
Composition					
Protein	60-65	45-55	40-50	55-60	60
RNA	12-20	7-12	7-12	10-15	
DNA	2-3	1-2	1-2	2-3	
Carbohyd.	5- >30	10- >50	10- 30	5-10	
Lipid	5-10	10-	10-	5-10	
Ash	5-7	5-7	5-7	5-7	

*Composition is based on Dry Cell Weight which is approximately 20% of Wet Cell Weight.

MEASUREMENT OF CELL GROWTH

METHODS FOR MEASURING AND ESTIMATING CELL GROWTH - CRITERIA FOR TECHNIQUE SELECTION

DIRECT MEASUREMENT OF CELL NUMBER

- Applies to single cell, non-aggregating cells in suspension
- Requires separation of individual cells
- Can distinguish between viable and non-viable cells
- Opportunities for cell sorting

DIECT MASUREMENT OF CELL MASS

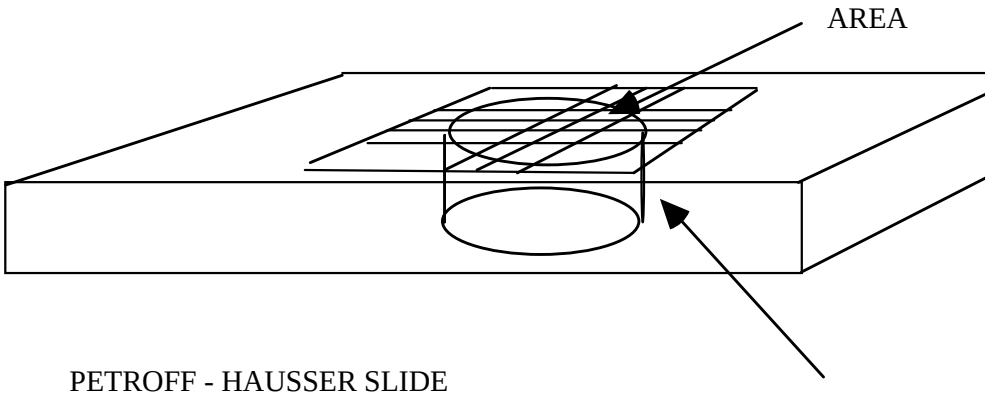
- Applicable to all cells and cell aggregates
- Requires separation of cells form non-cellular solids
- Does not distinguish between viable and non-viable cells

INDIRECT ASSESSMENT OF CELL GROWTH

- Approach is based on stoichiometry of growth and metabolism
- Accuracy depends on the technique and assumptions
- Applicable to all cell types
- Techniques can be used in systems with non-cellular solids

Direct Estimation of Cell Number

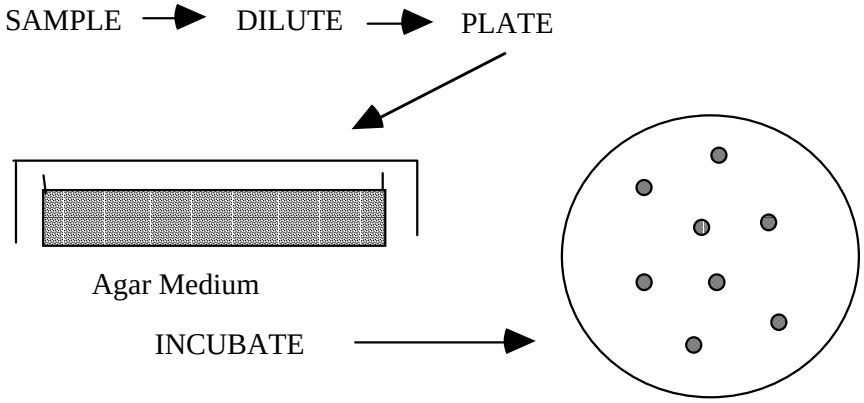
DIRECT MICROSCOPIC COUNT



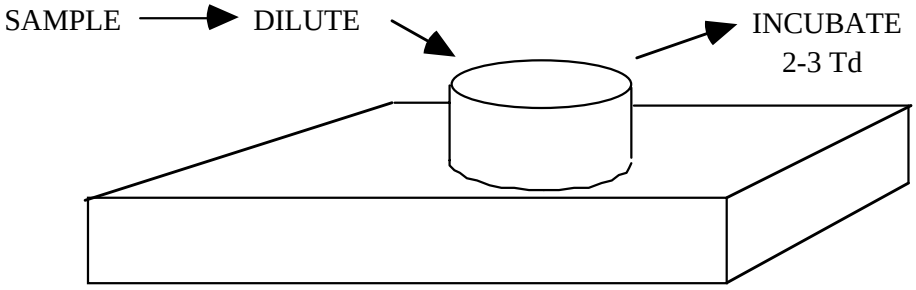
PETROFF - HAUSSER SLIDE
D = 0.02 mm

HEMOCYTOMETER SLIDE
D = 0.1 - 0.2 mm

VIABLE PLATE COUNT



SLIDE CULTURE

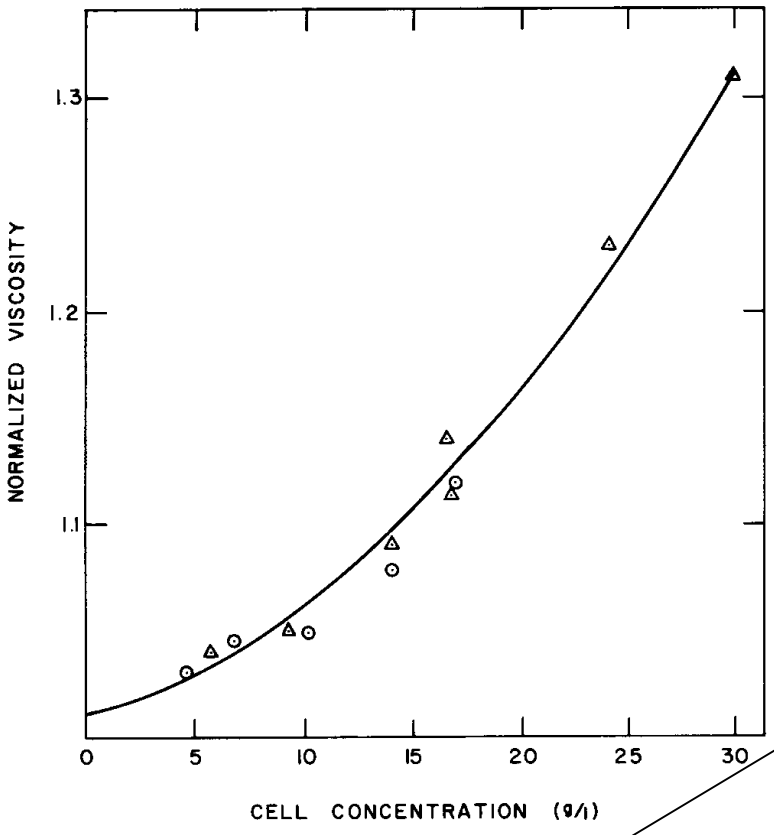


Cell Mass Estimation by Viscosity

Einstein equation for viscosity of suspensions

$$\frac{\mu_c}{\mu_r} = \frac{(1+0.5C)}{(1-C)^2}$$

μc= viscosity of cell suspension
μr = supernatant viscosity
C = volumetric particle conc.



Note: weak dependence on conc.

$$\frac{\mu_c}{\mu_r} = (2.43x10^{-4})C^2 + (2.79x10^{-3})C + 1.01$$

Perley, et. Al (Biotech. Bioeng. 21,519, 1979)

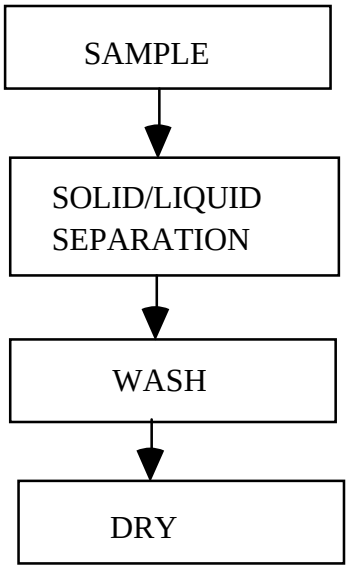
DIRECT MEASUREMENT OF CELL MASS

DRY CELL WEIGHT (DCW)

Microbial cells are typically about 20-25% solids when dried to con stai mass. Thus,

Wet Cell Weight is approximately 4.5(DCW)

PROTOCOL FOR DRY CELL WEIGHT MEASUREMENT



What is the optimal size?

Soluble and insoluble impurities

Possible leaching vs. contamination with salts

Overdrying?

CULTURE BROTH TURBIDITY

$$\frac{dI_t}{dL} = -ECI_t$$

$$\ln \frac{I_t}{I_o} = -ECL$$

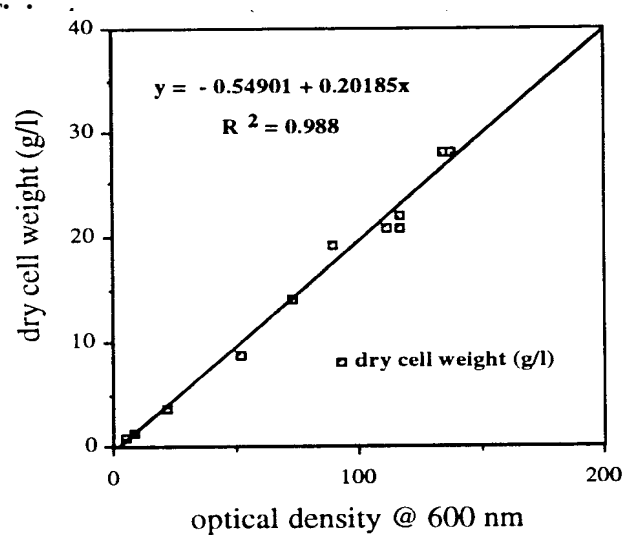
I_o = Intensity of initial light

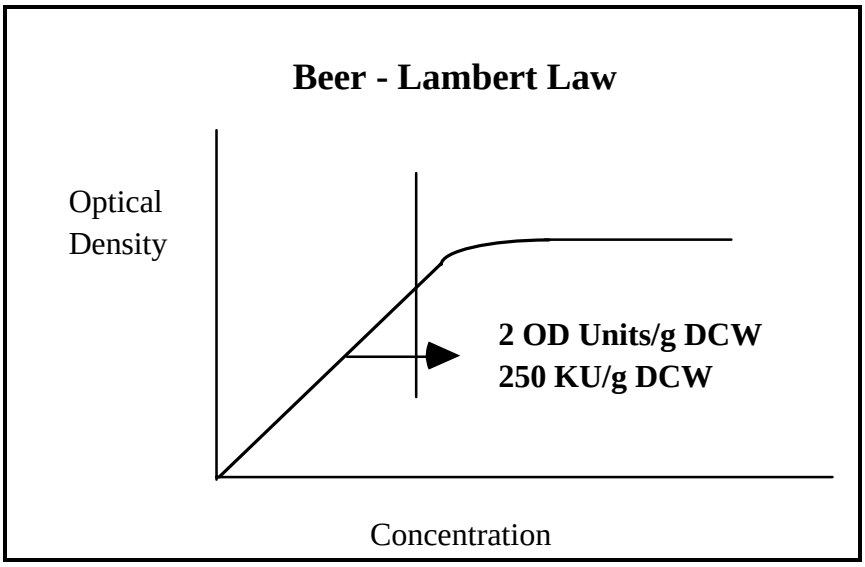
I_t = Intensity of transmited light

E = Molar extinction co

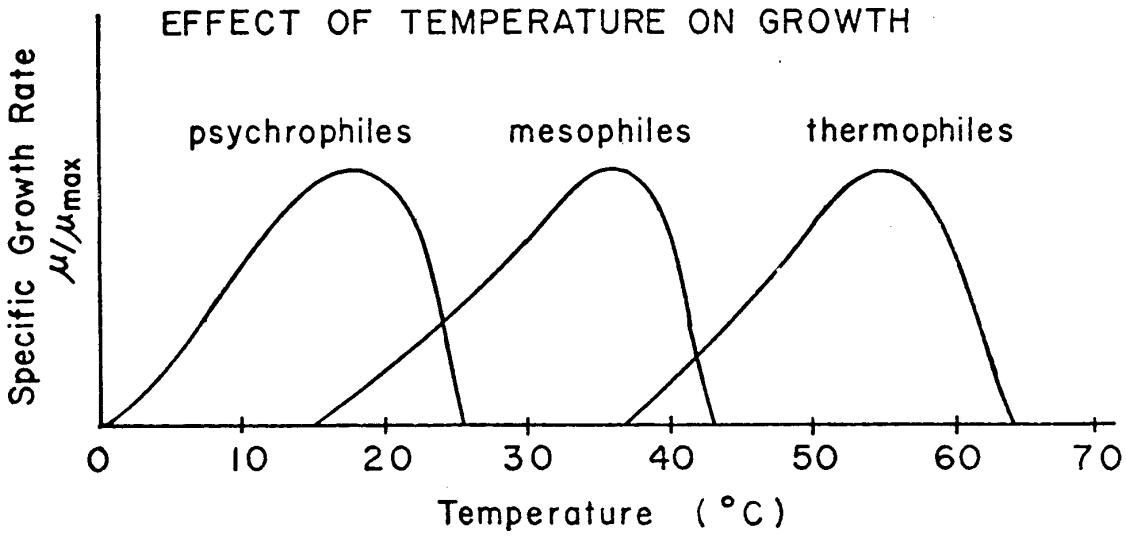
C = Concentration

L = Length of light patl





Effect of Temperature on Growth



$$\mu = Ae^{-\frac{E}{RT}}$$ E=60-70 kcal/mol

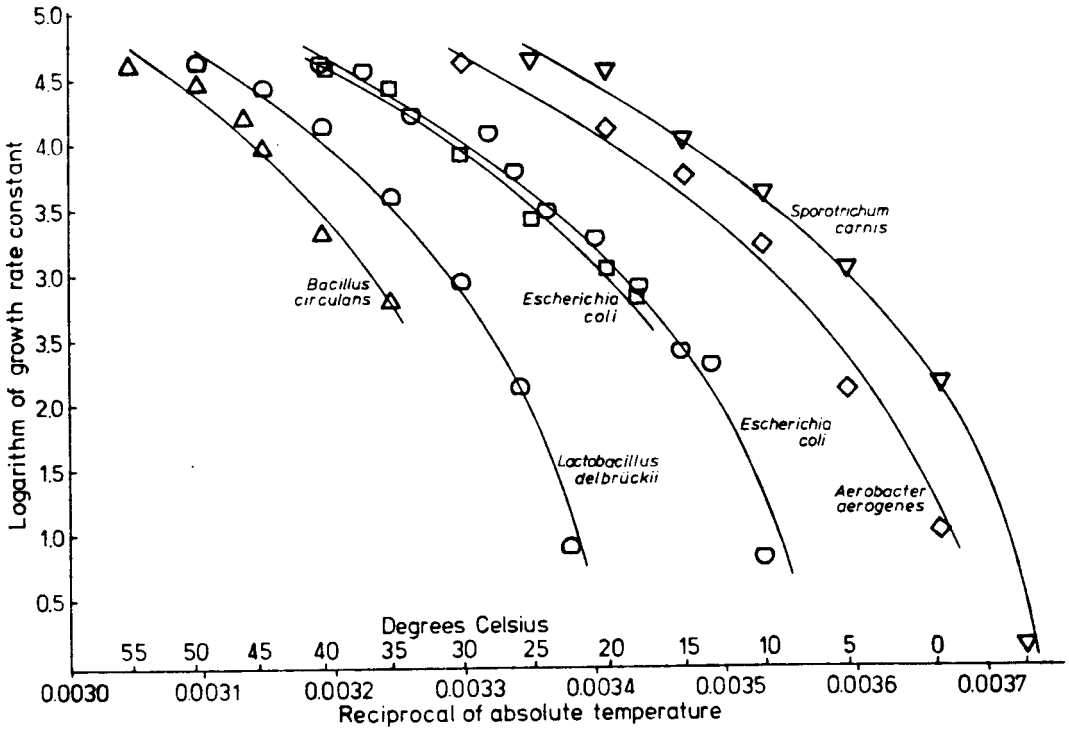


FIG. 1. Arrhenius plot of six sets of data redrawn from Johnson et al. (13). The solid curves correspond to the equation $\sqrt{r} = b\,(T - T_0)$.

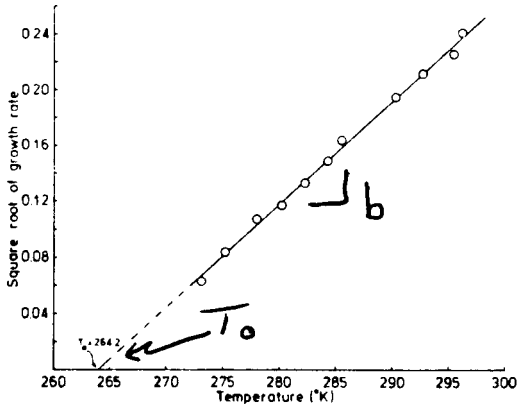
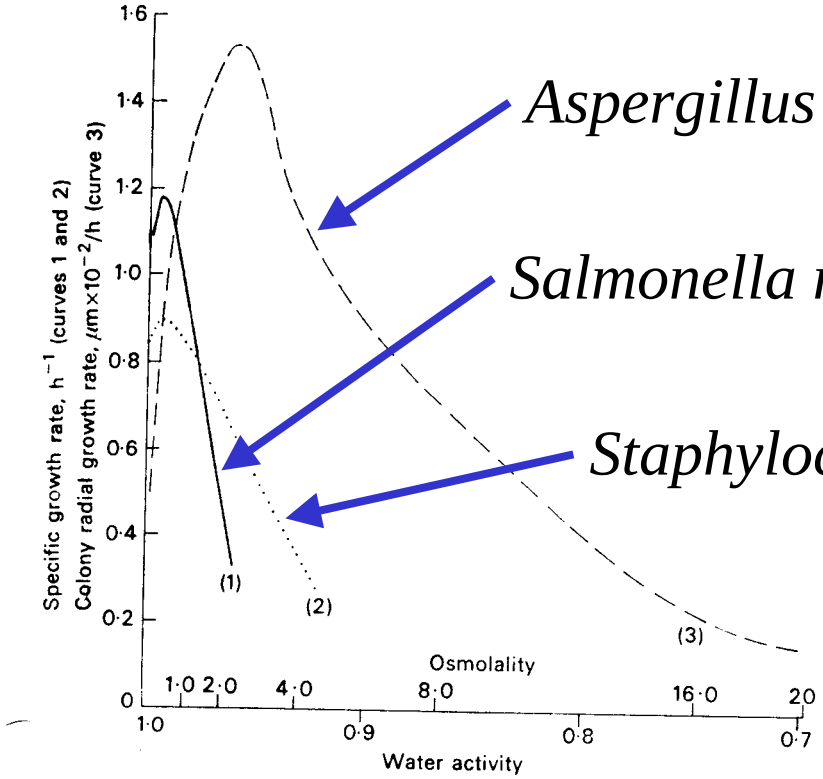


FIG. 2. Typical linear relationship for *Pseudomonas* group 1 strain 16L16 between square root of growth rate and temperature. Growth rate was measured as the reciprocal of the time to reach 25% turbidity.

$$\mu^{1/2} = bT - bT_o$$

Ratkowsky, et al., Jbacteriol <u>149</u> 1-5 (1982)



Aspergillus amstelodami

Salmonella newport

Staphyloccus aureus

$$\ln a_w = \frac{-vm\phi}{55.5}$$

a_w=water activity
v=number of solute ions
m=molar concentration
ø=molar osmotic coefficient

Allocation Model for Substrate Utilization

$$\frac{dS}{dt} = \frac{\mu X}{Y_{max}} + mX + \frac{q_p X}{Y_{p/s}}$$

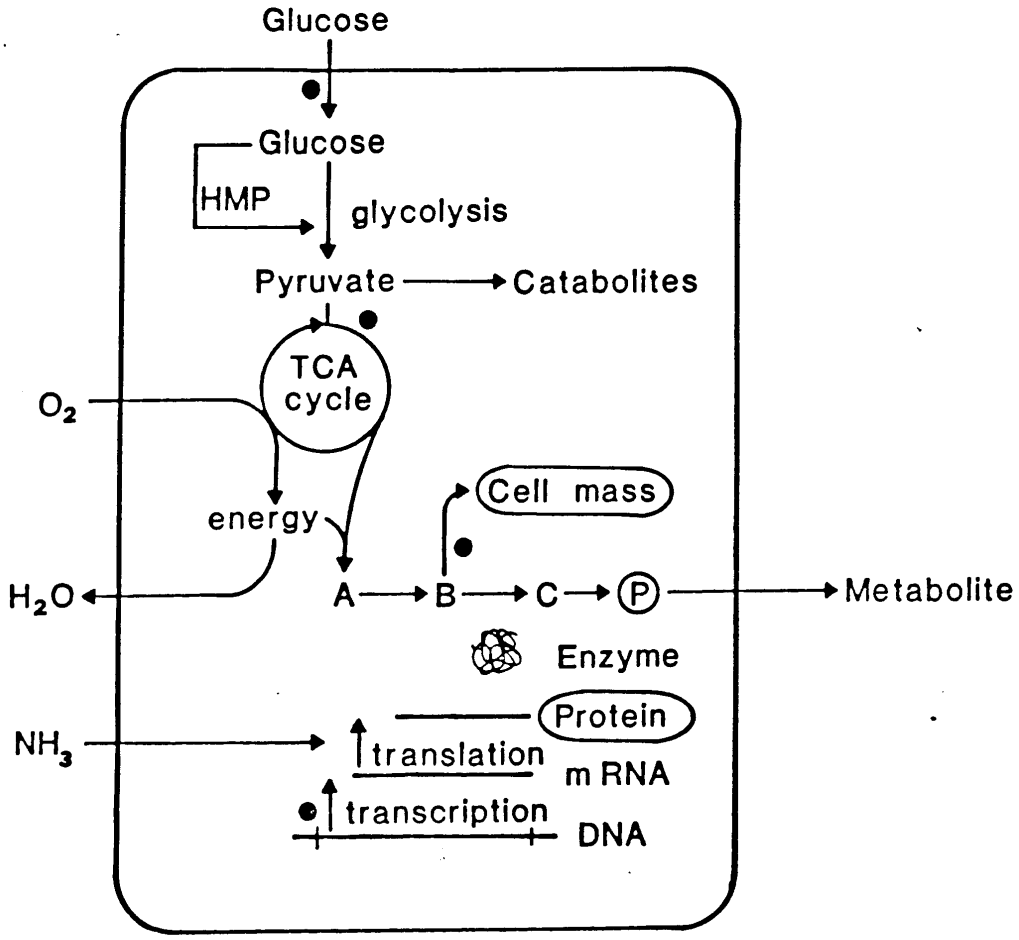
$\mu = f(cell, C_s, C_p, pH, T, a_w, t)$

Use hyperlinks to other slides

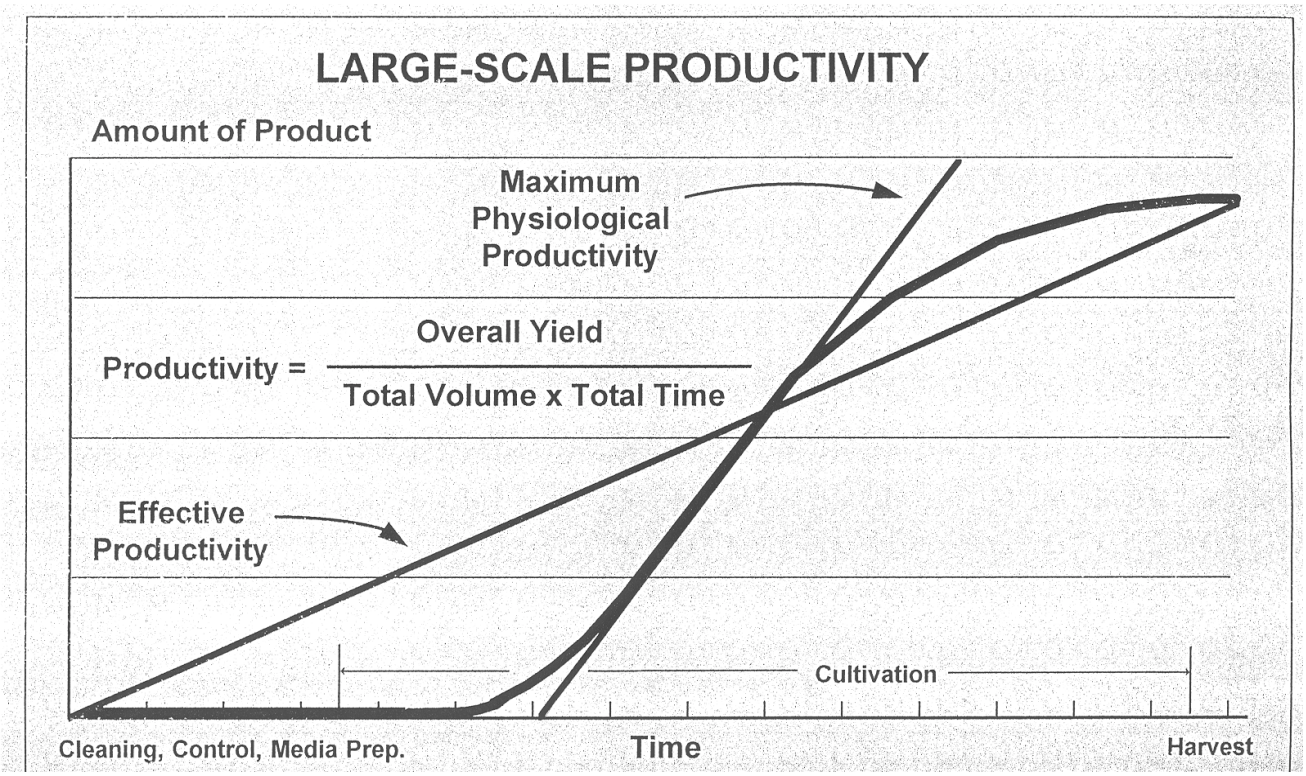
Strategies for Metabolite Overproduction

- **Random mutagenesis and selection**
  - **Example of penicillin**
- **Environmental control & optimization**
- **Metabolic engineering**
  - **Enhance or alter metabolite or electron flow**
  - **E.g. AAs, isoprenoids (kiesling), polyketide (chethan)**
- **Alteration of control**
  - **Flux control (inhibition or acceleration)**
  - **Transcriptional control**

Influence of Glucose on Fermentation



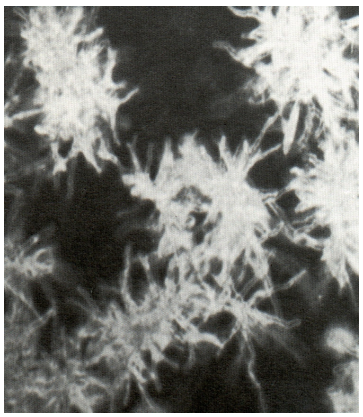
● glucose control points



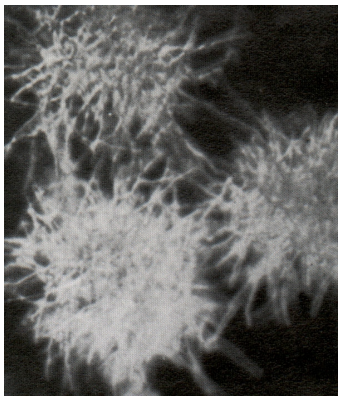
H. Scherfler, 10th International Biotech Symposium, Sydney Australia

Mycelial Pellet Growth

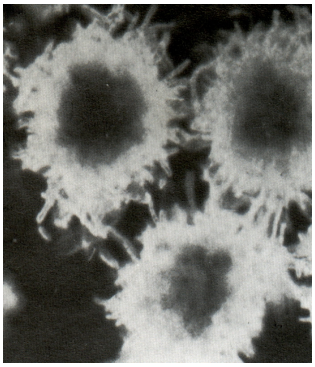
Penicillium crysogenum



t = 0 h



t = 11 h



t = 23 h

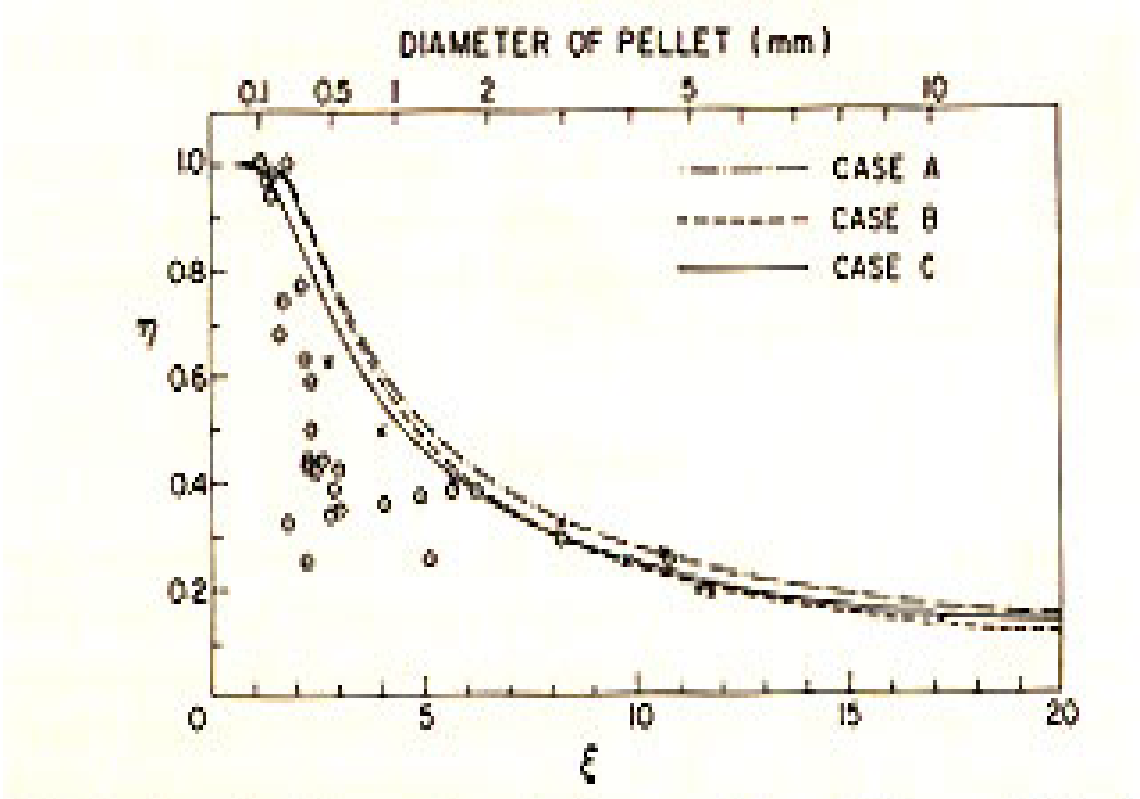
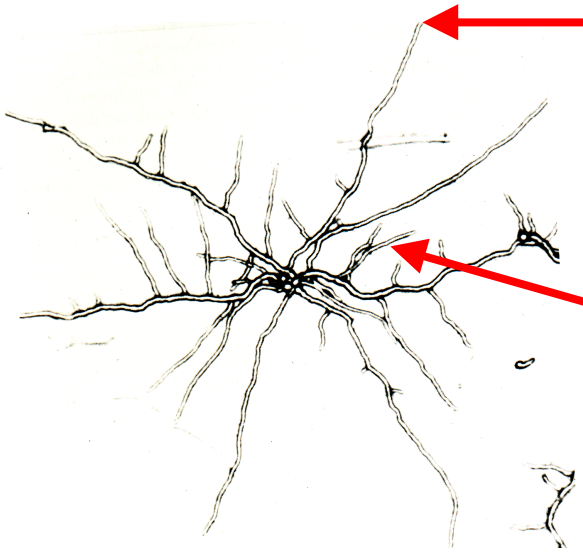


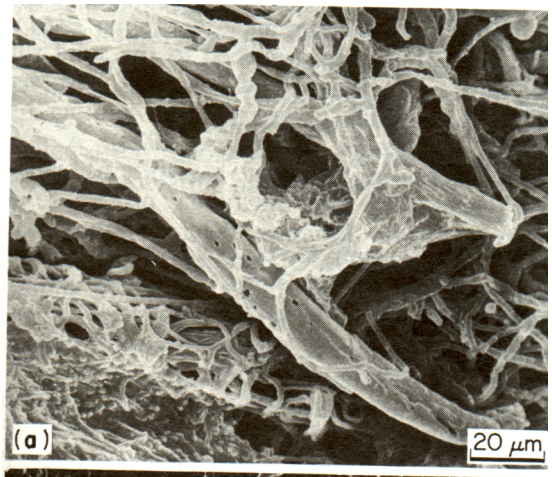
Fig. 5. Comparison between theoretical curves relating η to ξ for cases A, B, and C, and experimental data. (o) data of Yano et al.;[4] (x) this work.

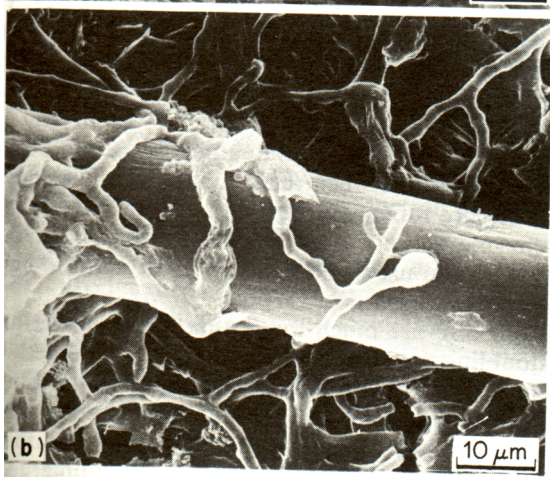
Mycelial Growth



Hyphal tip elongation

Branching

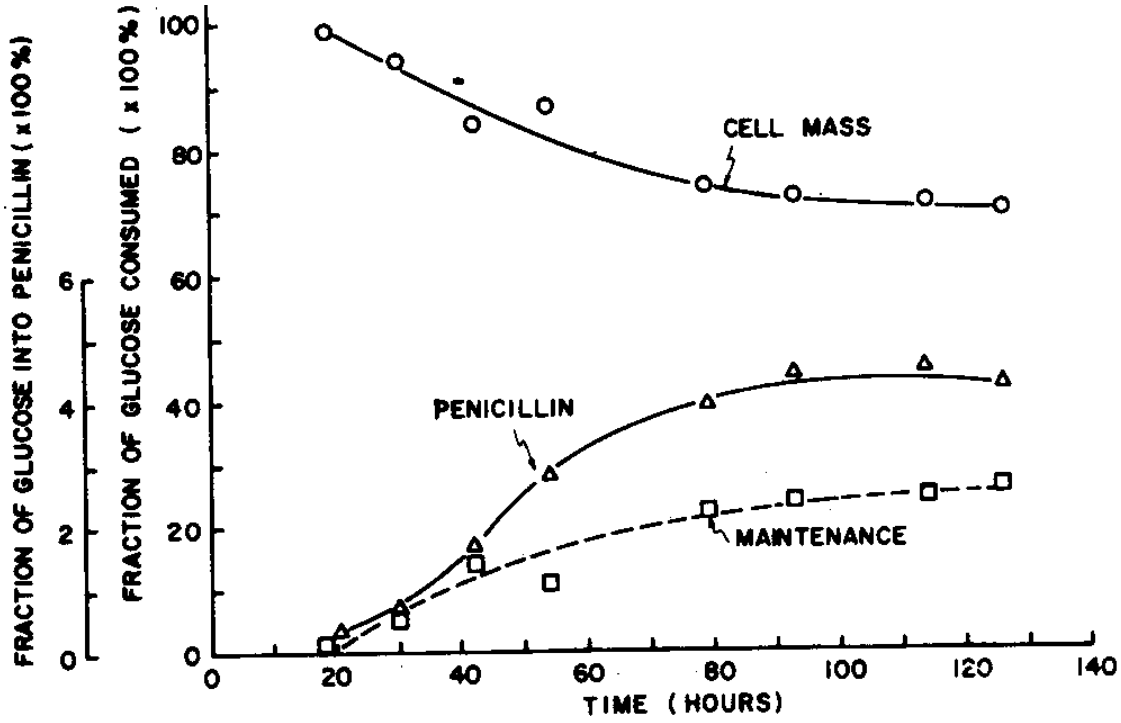


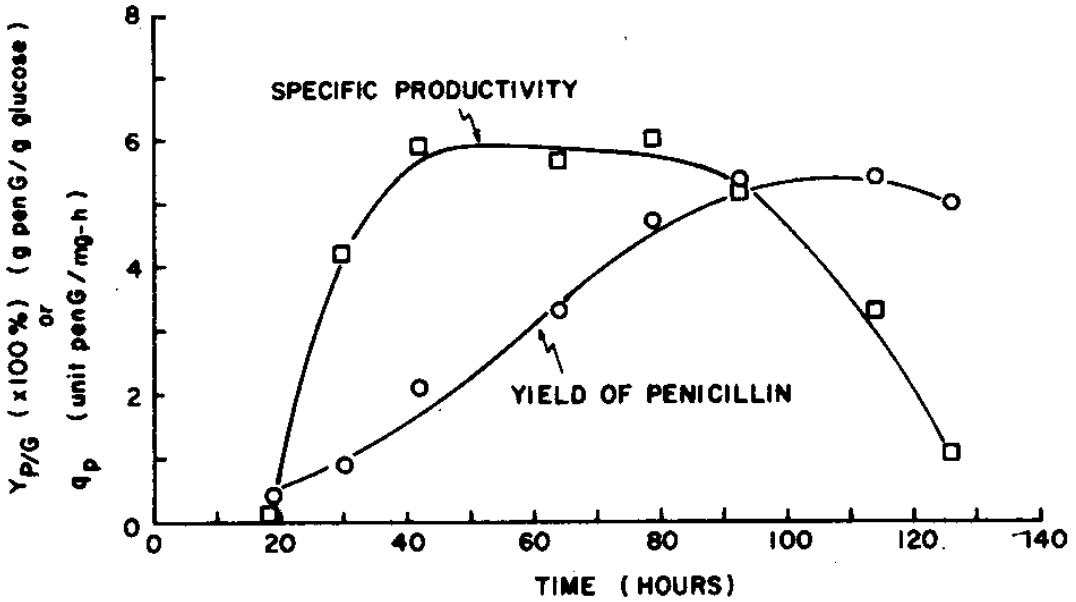


Mycelia growth on
Solid straw substrate

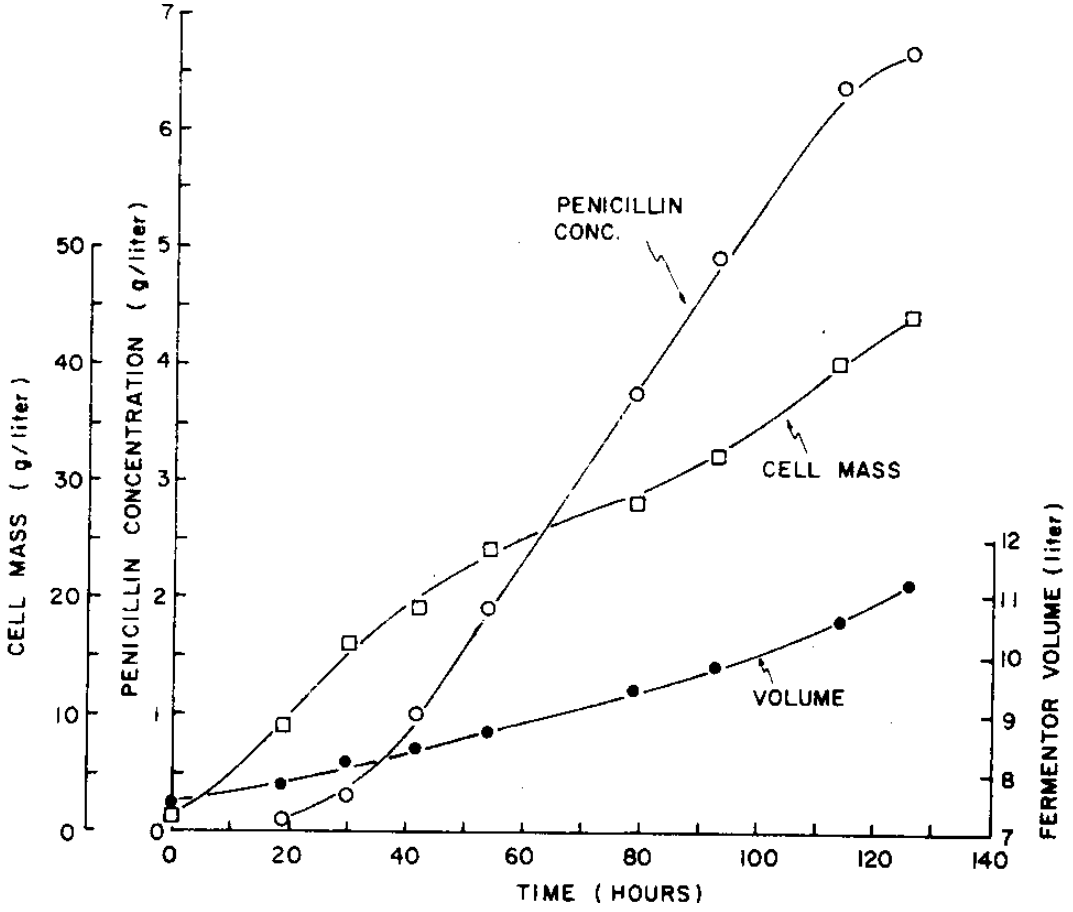
Penicillin Production

Glucose → Cell Mass 0.45 g cell/g glu
Glucose → Maintenance 0.02 g glu/g cell-h
Glucose → Penicillin 1.1 g pen/g glu

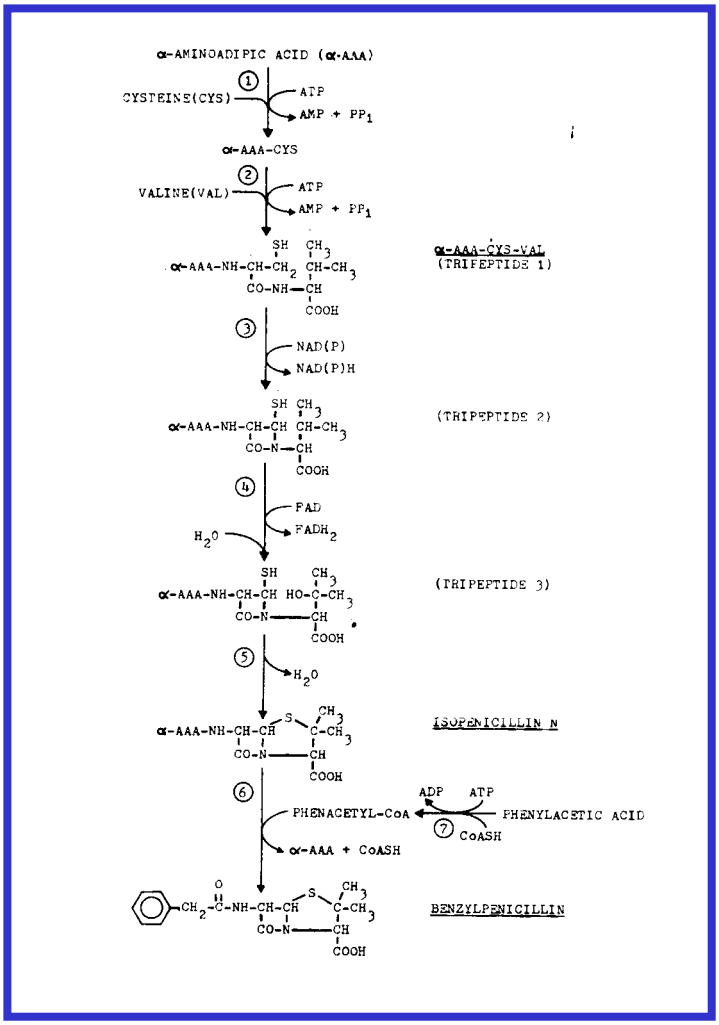




Kinetics of Penicillin Fermentation



Pathway for Penicillin Synthesis



$$Cys + Val + PAA = PenicillinG$$

$$2.5Glucose + 3NH_3 + H_2SO_4 + PAA = PenG + CO_2 + \alpha AAA$$

Component***	Weight Yield* (g Pen/g Sub.)	Molar Yield (mole Pen/mole	Unit Yield** (Bill. Units/kg S
Glucose	1.1	0.7	1.8
H2SO4	3.6	1.0	6.0
Ammonia	10.5	2.0	17.5

*MW Na-salt of benzyl penicillin is 356.4 & 6-APA is 216.3

** One international unit of penicillin is 0.6 μg sodium penicillin

*** Assuming α-AAA is recycled

MEDIA DESIGN

MEETING THE REQUIREMENTS FOR GROWTH A PRODUCT FORMATION

1. FERMENTATION PROCESS OBJECTIVES

Cell mass *vs.* Product synthesis
Substrate allocation model
Physiological Model
- **Avoid C, N, S or PO_4 catabolite repression**
- **Specific precursors, inducors, or repressors**

2. NUTRITIONAL REQUIREMENTS

Elemental requirements
Specific nutrients, e.g. vitamins. minerals, amino acids, etc.
Energy requirements - Carbon source and Oxygen
- **Growth**
- **Product Synthesis**
- **Maintenance**

3. ENVIRONMENTAL REQUIUREMENTS

pH profile
Temperature profile
Dissolved oxygen profile
Catabolite repression
Physiological constraints, e.g. ionic strength, product inhibition

MEDIA DESIGN

MEETING THE REQUIREMENTS FOR GROWTH A
PRODUCT FORMATION
(continued)

4. REGULATORY CONSTRAINTS

Qualification of vendors
Multiple sources
Traceability
Potential impurities or contaminants
Consistency

5. TECHNO-ECONOMIC CONSTRAINTS

Cost
Materials availability
Product recovery
Environmental impact

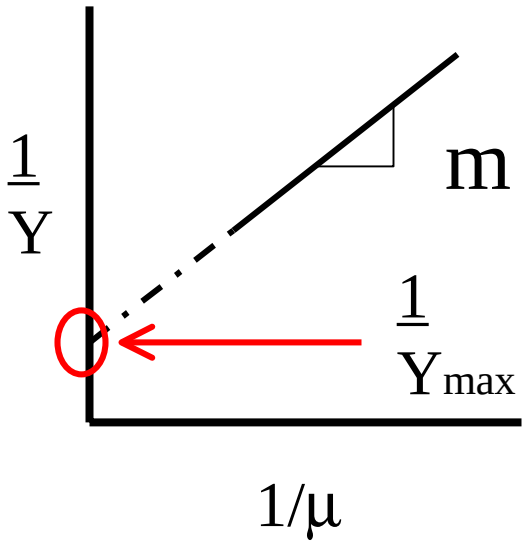
Estimation of Maintenance Energy

Substrate allocation model

$$\frac{dS}{dt} = \frac{\mu X}{Y_{\max}} + mX + \frac{q_{pX}}{Y_{p/s}}$$

Assume that q_p is small, thus

$$\frac{dS}{dt} = \frac{\mu X}{Y} = \frac{\mu X}{Y_{\max}} + mX$$



$$\frac{1}{Y} = \frac{1}{Y_{\max}} + \frac{m}{\mu}$$

Typical values of m = 0.02 to 0.1 g glucose/g cell-h

FERMENTATION MEDIUM COMPONENTS

- **PENICILLIN**
  - Molasses
  - 0.2% Soybean Oil
  - 1% Cottonseed Flour

High Impurity Content

- **STREPTOMYCIN**
  - 2.5% Cerelose
  - 4% Soybean Oil

Multiphase Components

- **LACTIC ACID BACTERIA**
  - Phosphate buffer
  - 0.5% Tryptone
  - 0.5% Yeast Extract

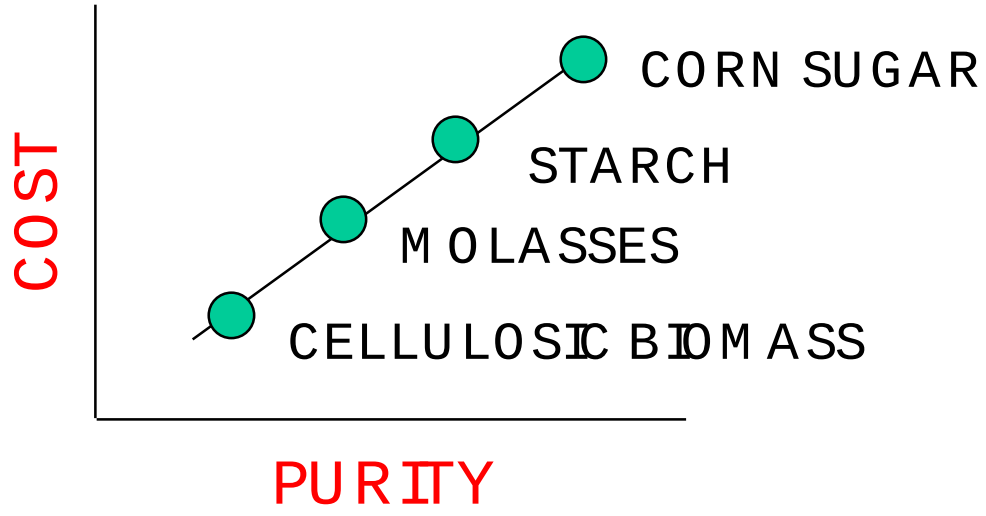
Variable Quality

- **BACITRACIN**
  - 3% Corn Steep
  - 3% Glucose

- **Baker's Yeast**
  - Molasses

FERMENTATION MEDIA

NUTRIENT	RAW MATERIAL	PRETREATMENT
CARBON SOURCE		
GLUCOSE	CERELOSE	HYDOLYZED FROM STARCH
	MOLASSES	INVERSION
		(SUCROSE TO FRUCTOSE AND GLUCOSE)
	STARCH	SOLUBILZIATION
	CELLULOSE	GRINDING AND HYDROLYSIS
FATS/OILS	SOYBEAN OIL	
	COTTONSEED OIL	
NITROGEN SOURCE		
	AMMONIA	
	PROTEIN HYDROLYSATES	ACID OR ENZYME CATALYZED
		HYDROLYSIS

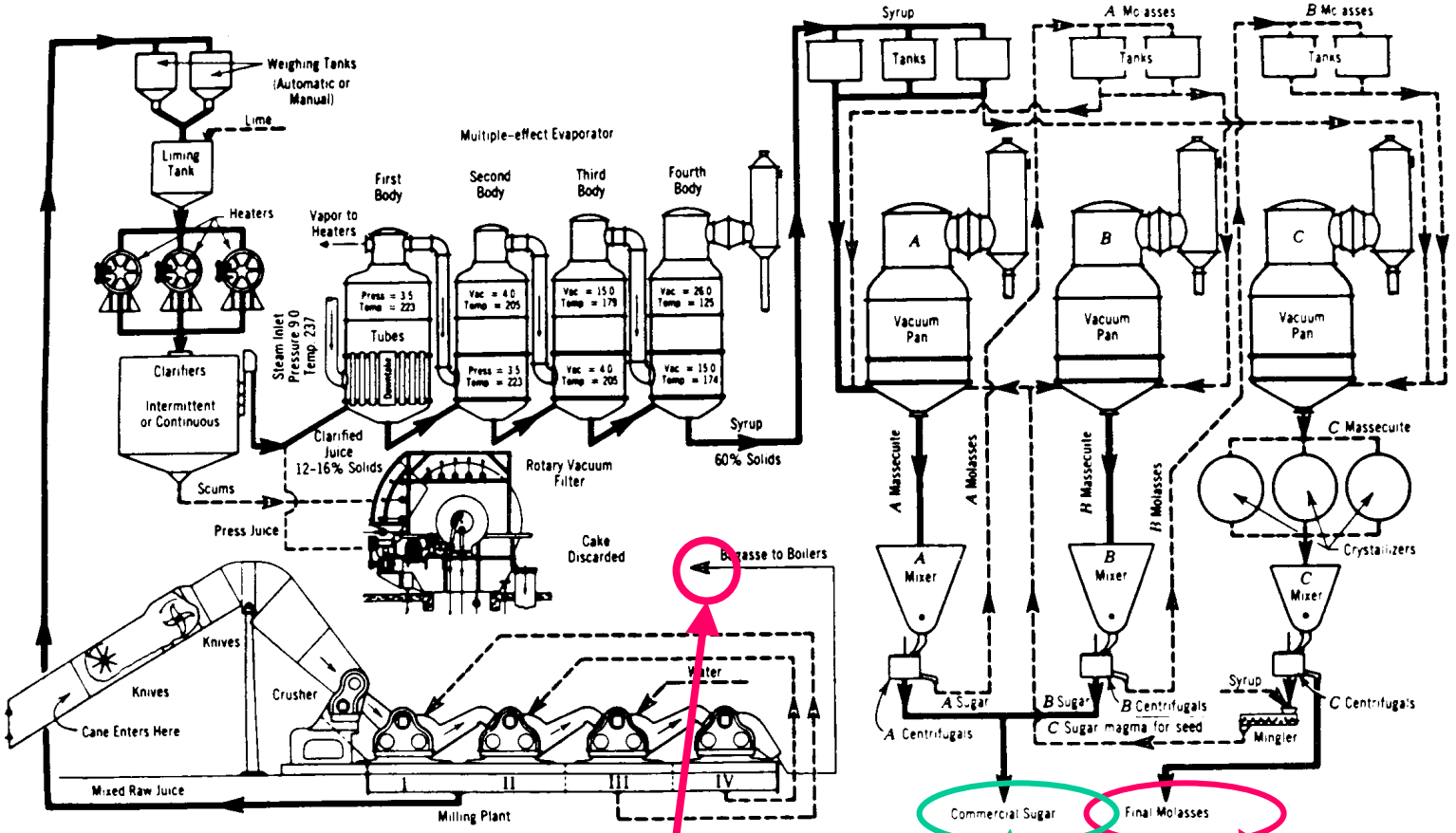


Process Water

Property	Value
pH	7.6
Temperature (°C)	21
Hardness (mg L^{-1} $CaCO_3$)	130
Total dissolved solids (mg L^{-1})	280
Turbidity (NTU)	<1
Calcium (mg L^{-1})	37
Magnesium (mg L^{-1})	4.7
Sodium (mg L^{-1})	38
Potassium (mg L^{-1})	2.0
Manganese (mg L^{-1})	<0.05
Chloride (mg L^{-1})	16
Sulfate (mg L^{-1})	48
Fluoride (mg L^{-1})	0.4
Nitrate (mg L^{-1})	2.0
Trihalomethanes (mg L^{-1})	<0.005
Conductivity (micromhos)	390
Lead (mg L^{-1})	<0.005
Iron (mg L^{-1})	<0.1
Copper (mg L^{-1})	<0.1
Zinc (mg L^{-1})	<0.03
MS-2 Bacteriophages (pfu ml^{-1})	<1
PRD-1 Bacteriophages (pfu ml^{-1})	<1

R. A. Governal & C. P. Gerba, J Ind Micro & Biotech 1999 23 166-172

Production of Molasses from Sugar Cane



From Meade and Chen (1977) by permission John Wiley & Sons, Inc.

FIG. 1.1. FLOW DIAGRAM OF RAW CANE SUGAR FACTORY

Bagasse

Sucrose

Molasses

Pancoast and Junk. Handbook of Sugars, 1980

Cane Sugar Molasses

Composition of Blackstrap Molasses (12)

% MOLASSES		
Components	Indicative average	Usual range
Water	20	17-25
Sucrose	35	30-40
Dextrose (Glucose)	7	4-9
Levulose (Fructose)	9	5-12
Other reducing substances	3	1-5
Other carbohydrates	4	2-5
Ash	12	7-15
Nitrogenous compounds	4.5	2-6
Non-nitrogenous acids	5	2-8
Wax, Sterols and Phospholipids	0.4	0.1-1
Pigments	--	--
Vitamins	--	--

OTHER CARBOHYDRATES	
	Indicated Average expressed % Molasses
Soluble Gums Xylose, Arabinose, etc.	0.72
Starch	
i-Inositol	0.20
Phytin	0.18
D-Mannitol	0.50
Uronic acid	1.80
Methoxyl	0.60

ASH (Carbonated)		
Bases	Range: % of Ash	
K_2O	30-50	4.8
CaO	7-15	1.2
MgO	2-14	0.98
Na_2O	0.3-9.0	0.10
Fe_2O_2/Al_2O_3	0.4-2.5	0.12
Acids		
SO_3	7-27	1.8
Cl	12-20	1.8
P_2O_5	1-10	0.6
SiO_2 and insolubles	1-7	0.6

NITROGENOUS COMPOUNDS		
Crude Proteins	2.5-4.5	4.0
Amino Acids	0.3-0.5	0.5
24 acids present of which:		
	Mg. per g molasses	
Alanine	0.20-0.22	
γ -Aminobutyric	0.06-0.08	
Aspartic acid	0.90-1.65	
Glutamic acid	1.02-1.04	
Glycine	0.06-0.07	
Leucine	0.03-0.05	
Lysine	0.05-0.07	
Serine	0.39-0.80	
Threonine	0.30-0.90	
Valine	0.11-0.20	
Nucleic Acid Bases		
Guanine		
Hypoxanthine		
5-Methylcytosine		
Xanthine		

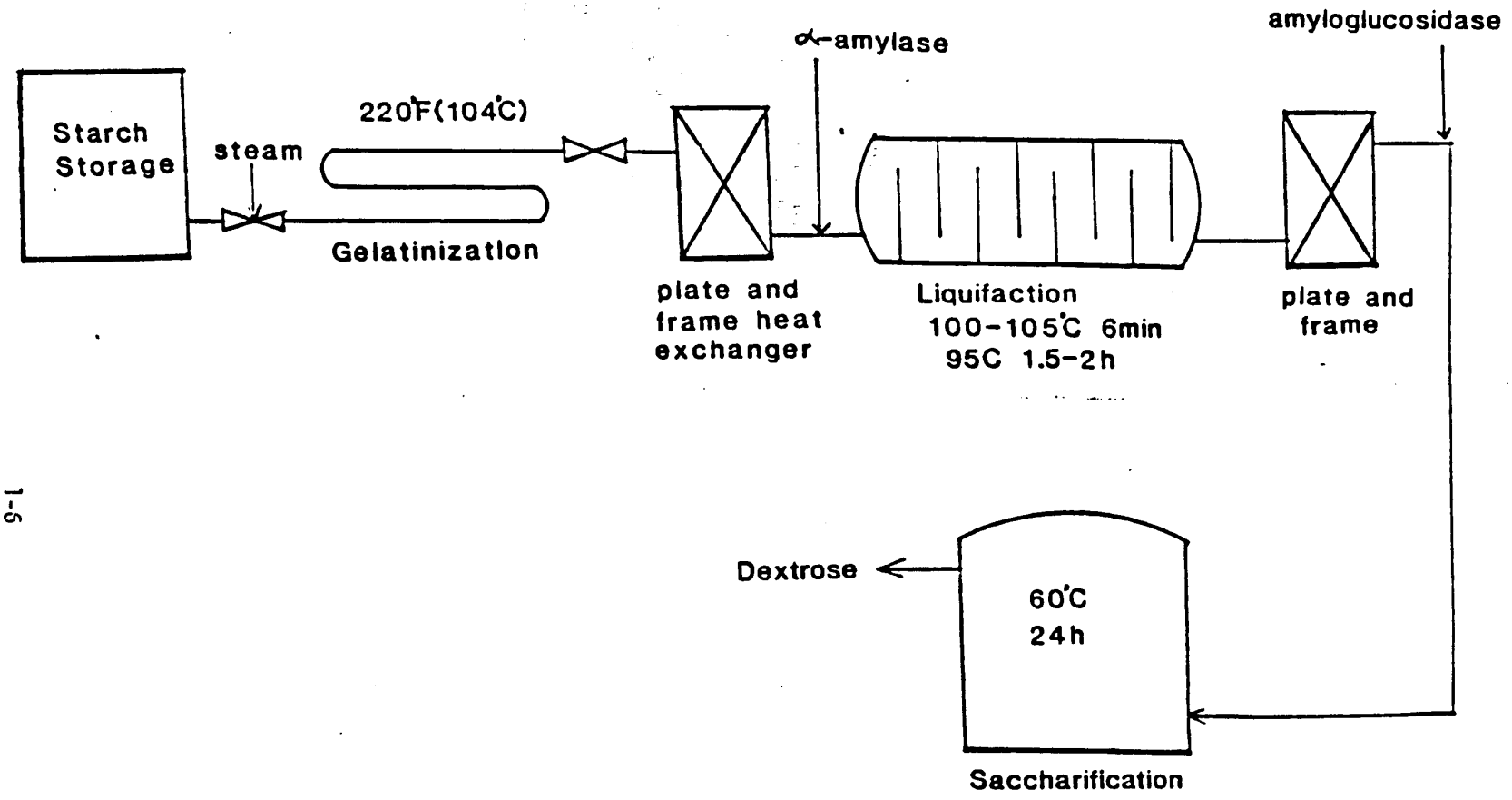
NON-NITROGENOUS ACIDS		
Aconitic	1-6	3.0
Citric, Malic, Mesaconic, Succinic, etc.	1-1-2.5	2.0

WAX, STEROLS, etc.
1-Triacontanol
Phytostérol
Stigmasterol

PIGMENTS
Chlorophyll
Tannins
Anthocyanins

VITAMINS	
	μg *per g*
Biotin (H)	1-3
Choline (B_4)	880
Folic acid (B complex)	0.3-0.4
Niacin (B complex)	17-30
Pantothenic acid (B complex)	20-60
Riboflavin (B_2)	2-3
Pyridoxine (B_4)	1-7
Thiamine (B_1)	0.6-1.0

STARCH SACCHARIFICATION



PROFLO Finished Product Standard (www.tradersprotein.com)

Net Weight as Packed:
25 Kilos ± .25 Kilos or
500 Kilos ± 5 Kilos or
1000 Kilos ± 10 Kilos

%Moisture as Packed	3.0% Maximum
% Protein as Packed (N x 6.25)	58.0% Minimum (Dry Weight Basis)
% Oil (P.E. Extract)	5.0% Maximum
Color (Minimum reflectance through a green filter on a Photovolt reflectometer)	45
Fineness (To pass No. 200 U.S. St'd Screen)	90.0%
Total Plate Count	Less than 5,000 colonies per gram

PROFLO Typical Analysis

Total	98.75%
Protein, Dry Weight Basis (N x 6.25)	58.61%
Fat	4.65%
Moisture as	1.92%
Ash	6.73%
Fiber	3.19%
Gossypol (Free)	0.037%
Gossypol (Total)	0.60%
pH (Aqueous Solution)	6.5
Total Plate Count	615 colonies per gram
Color (Reflectance)	49
Fineness (Thru 200 Mesh)	91.72%

PROFLO is a premium quality, finely ground, yellow flour made from the embryo of cottonseed. The principal component of PROFLO is nonhydrolyzed globular protein. This is a natural protein of excellent quality which comes from Traders' special oil extraction process.

PROFLO is guaranteed to contain a minimum of 58% protein on a dry weight basis and have a total plate count of less than 5,000 colonies per gram. PROFLO is made only from excellent quality, high protein cottonseed. This selectivity of raw material helps provide a greater degree of uniformity in the finished product

PROFLO OIL

PROFLO OIL is a non-edible, crude cottonseed oil. The free fatty acid content is less than 2.0% and normally runs 1.6%. PROFLO OIL contains approximately 70% unsaturated fatty acids. Its iodine value is 105. PROFLO OIL is recommended for use as a carbon source and antifoamer in production of antibiotics and steroids by submerged fermentation. PROFLO OIL is considerably more resistant to oxidation than refined oil. The remarkable stablity of PROFLO OIL is due to the presence of natural antioxidants. PROFLO OIL has been stored at room temperature for as long as seven months without appreciable deterioration or development of rancidity.

Fatty Acid	Amount
LAURIC	NONE FOUND
MYRISTIC	0.69%
PALMITIC	22.11%
STEARIC	2.43%
ARACHIDIC	NONE FOUND
MYRISTOLEIC	NONE FOUND
PALMITOLEIC	0.60%
OLEIC	17.67%
LINOLEIC	46.98%
LINOLENIC	3.15%
UNKNOWN	6.37%

PROFLO OIL is miscible at ordinary temperatures in all proportions with most organic solvents. Its solubility in water is very slight. PROFLO OIL dissolves 4-10% of its equivalent volume of air at ordinary temperatures. The solubility of most gases in PROFLO OIL increases with increasing temperatures.

Temperature (C)	Viscosity (CENTIPOISES)	Density
28	45	0.91
37	32	0.906
47	23	
67	13	0.886
94	7	
124	4	
141	3	0.838
151	3	
179	2	
203	1	0.799

The density of PROFLO OIL at 15 C. is usually between 0.918 and 0.922. The density of the oil changes about 0.000638 for each degree C. change in temperature.
The specific heat for PROFLO OIL is approximately 0.475 Cal . /G at 19.3 C. and 0.495 Cal . /G. at 50.7 C.