ABE 580

Process Engineering of Renewable Resources

Chapter 4

Microbial Fermentation

Fermentation

- Microbial processes that produce various products via biochemical action
- Foods, pharmaceuticals, small molecules, proteins, “biologics”
- vs Cell Culture (mammalian or plant)
- Control
  - Temperature
  - Nutrients
  - pH
  - O_2

Industrially Important Microorgansims
Table 4.2

Name	Organism Type	Product
Industrial Chemicals		
Saccharomyces cerevisiae	Yeast	Ethanol (from Glucose)
Clostridium acetobutylicum	Bacterium	Acetone and Butanol
Aspergillus niger	Mold	Citric Acid
Amino Acids and Flavor-Enhancing Nucleotides and Acids		
Corynebacterium glutamicum	Bacterium	L-Lysine
Corynebacterium glutamicum	Bacterium	5′-Inosinic Acid and 5′-Guanylic Acid; Glutamate
Vitamins		
Eremothecium ashbyi	Yeast	Riboflavin
Pseudomonas denitrificans	Bacterium	Vitamin B_{12}
Propionibacterium	Bacterium	Vitamin B_{12}

Foods and Beverages		
Saccharomyces cerevisiae	Yeast	Baker's Yeast, Wine, Ale, Sake
Saccharomyces carlsbergensis	Yeast	Lager Beer
Candida milleri	Yeast	Sour French Bread
Lactobacillus sanfrancisco	Bacterium	Sour Bread
Streptococcus thermophilus	Bacterium	Yogurt
Lactobacillus sp.	Bacterium	Yogurt
Propionibacterium shermanii	Bacterium	Swiss Cheese
Gluconobacter suboxidans	Bacterium	Vinegar

Polysaccharides		
Leuconostoc mesenteroides	Bacterium	Dextran
Xanthomonas campestris	Bacterium	Xanthan Gum

Pharmaceuticals		
Penicillum chrysogenum	Mold	Penicillins
Cephalosporium acremonium	Mold	Cephalosporins
Streptomyces	Bacterium	Amphotericin *B*, Kanamycins, Neomycins, Streptomycin, Tetracyclines and Others
Bacillus brevis	Bacterium	Gramicidin *S*

Fermentation Processes Must Provide Nutrients (Table 4.1)

Monomers From	Total*	C	H	O	N	P	S
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
Hydrolysis	-10.97		-1.23	-9.74			
Total	94.5	46.93	6.29	23.41	14.39	2.74	0.75

Fermentation Media

- Defined media

- Rich media

Defined (Minimal) Medium (Table 4.6)

Component	Amount (g/L)
Glucose	100.0
Ammonium sulphate	5.19
Potassium dihydrogen phosphate	1.53
Magnesium sulphate 7_2HO	0.55
Calcium chloride $2H_2O$	0.13
Boric acid	0.01
Cobalt sulphate $7H_2O$	0.001
Copper sulphate $5H_2O$	0.004
Zinc sulphate $7H_2O$	0.010
Manganous suphate $7H_2O$	0.003
Aluminum sulphate	0.001

Defined Medium (additional precursors)

Component	Amount (g/L)
Biotin	0.002
Pantothenate (B5)	0.003
Inositol	0.000125
Thiamin	0.00625
Pyridoxine (B6)	0.125
p-Aminobenzoic acid	0.005
Nicotinic acid	0.00625

Rich Media

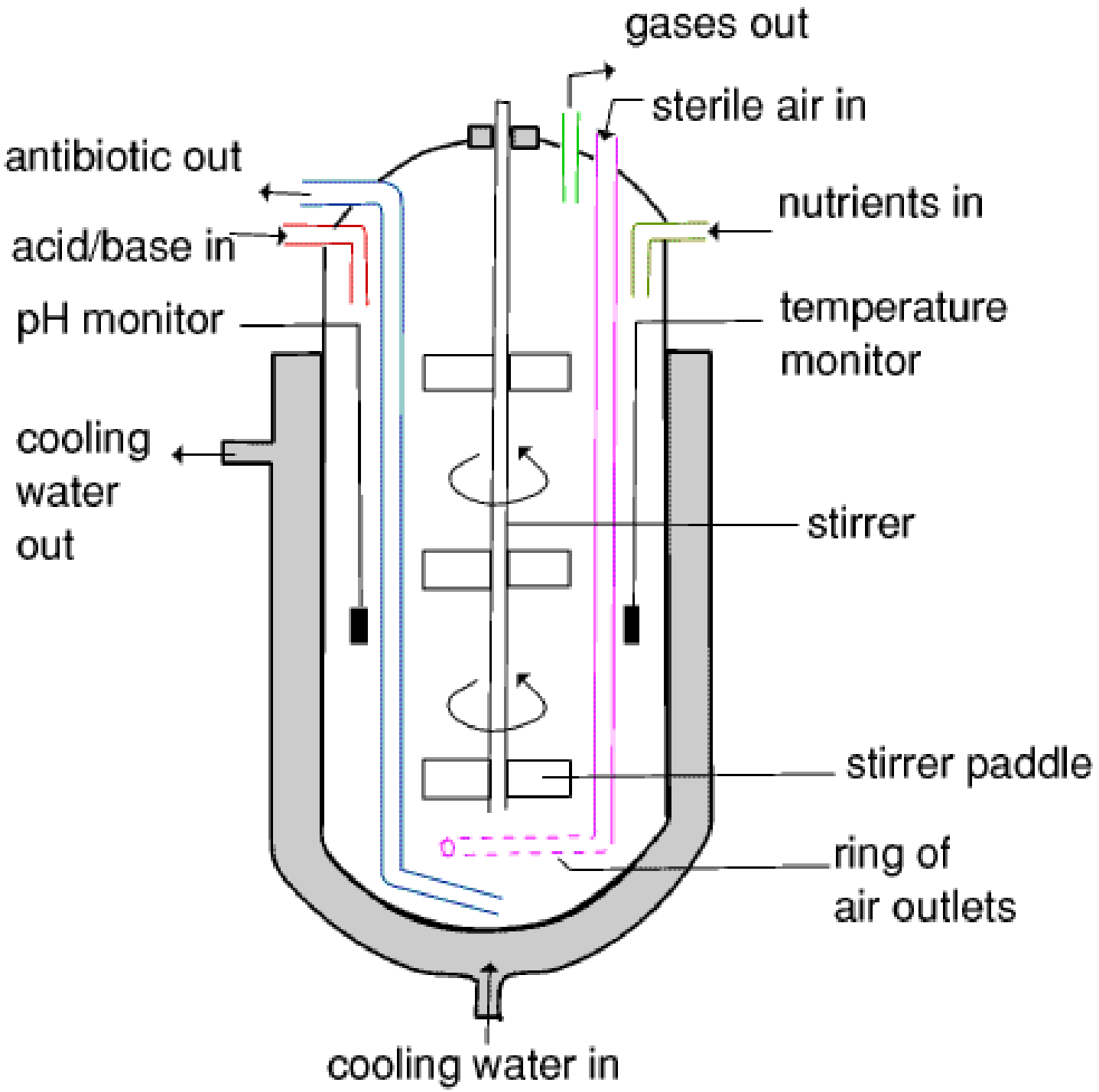
- AKA Complex media or undefined media
- Partially processed plant, animal, or microbially derived material
- Complex mixture of carbohydrates, minerals, vitamins, and biomolecules
- Laboratory media: Luria broth, trypticase soy agar, yeast extract with peptone, etc.

Industrial undefined media

- Carbon source
  - Molasses
  - Malt extract
  - Whey
  - Dextrose
  - Starch
- Lipids (rarely used)
  - Soy oil
  - Fish oil
- Nitrogen source
  - Urea
  - Diammonium Phosphate (DAP)
  - Corn steep liquor
  - Yeast extract
  - Soy meal
- Vitamins and minerals
  - Corn steep liquor
  - Yeast extract
  - Specialty products (too numerous to list

Rich Media Components

Components	Yeast Extract	Blackstrap Molasses	Corn Steep Liquor
Overall (%)			
Carbohydrate	39.5	54.0	5.8
Lipid	1.5	0.4	1.0
Ash	7.0	9.0	8.8
Vitamins (mg/kg)			
Biotin	----	----	0.9
Choline	4,840	660	----
Niacin	498	47	----
Pantothenic Acid	121	43	----
Pyridoxine	50	44.	19.4
Riboflavin	35	4.4	----
Thiamine	75	0.9	0.88
Amino Acids (%)			
Arg	2.2	----	0.4
Cys	0.6	----	0.5
Gly	3.4	0.10	1.1
His	1.3	----	0.3
Ile	2.7	----	0.9
Leu	3.3	0.01	0.1
Lys	3.4	0.01	0.2
Met	1.0	----	0.5
Phe	1.8	----	0.3


gases out
sterile air in
antibiotic out
nutrients in
acid/base in
pH monitor
temperature monitor
cooling water out
stirrer
stirrer paddle
ring of air outlets
cooling water in

1 L Fermenter

40 L
Laboratory
Fermenter

300 L Fermenter

20,000 L Fermenter


pyrex

Modeling

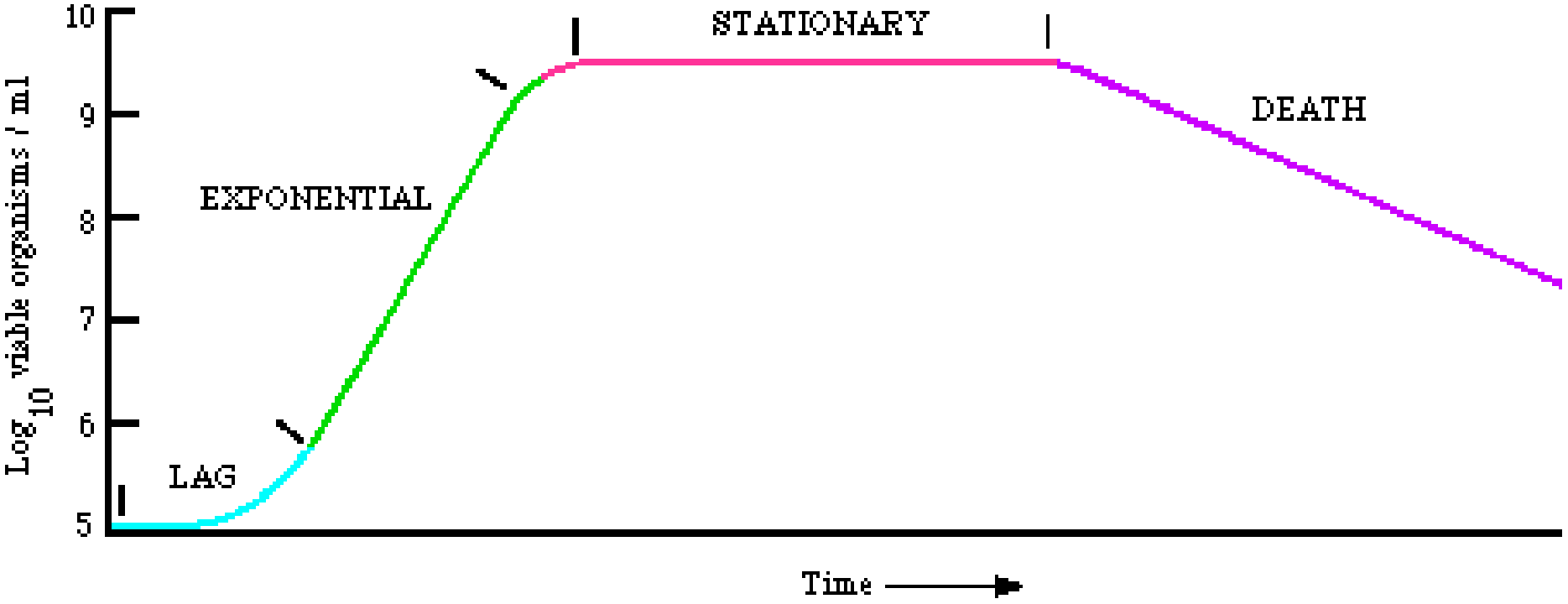
- How is the model to be used?
- What level of detail is required?
- How accurate must your model be (error)?
- Does you model need to be predictive or just descriptive?
- How robust does your model need to be?

Modeling Fermentation

- Growth of cells
  - unstructured, unsegregated model

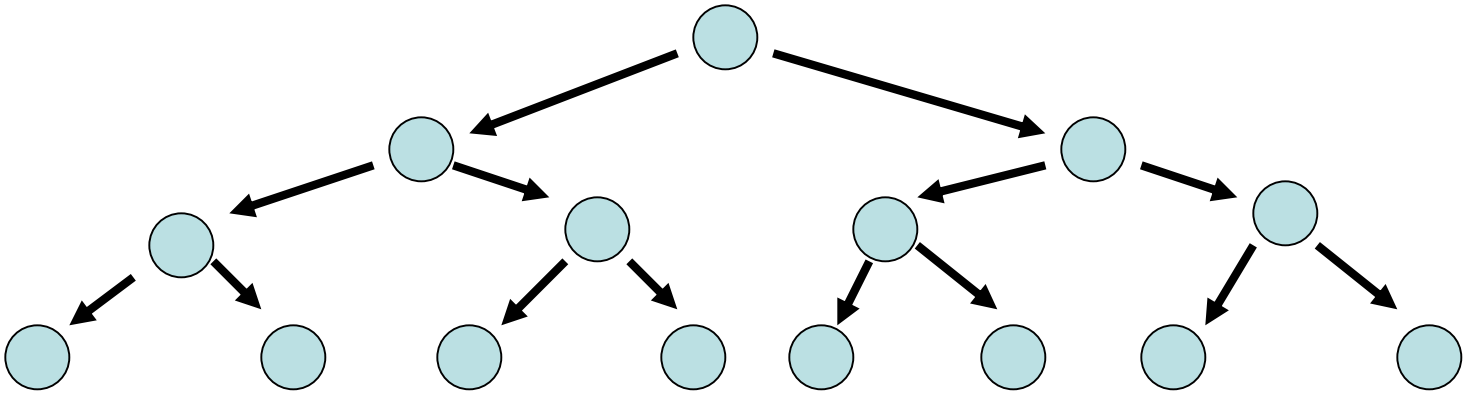
- Unstructured
  - Mechanism (biochemical reactions) not specified
- Unsegregated
  - Parameters are lumped

Phases of Fermentation



Cell Growth

- Cells reproduce through binary division



Modeling Cell Division

- How much time passes between cellular divisions?
- Doubling time, t_d

$$G = 2^{t/t_d}$$

G = number of generations

t = time that has passed

t_d = doubling time

Modeling Binary Division

$$X = X_o 2^{t/t_d}$$

$$\mu_d = 1/t_d$$

$$X = X_o 2^{\mu_d t}$$

$$\mu = \ln 2 \cdot \mu_d = \frac{\ln 2}{t_d}$$

$$X = X_o e^{\mu t}$$

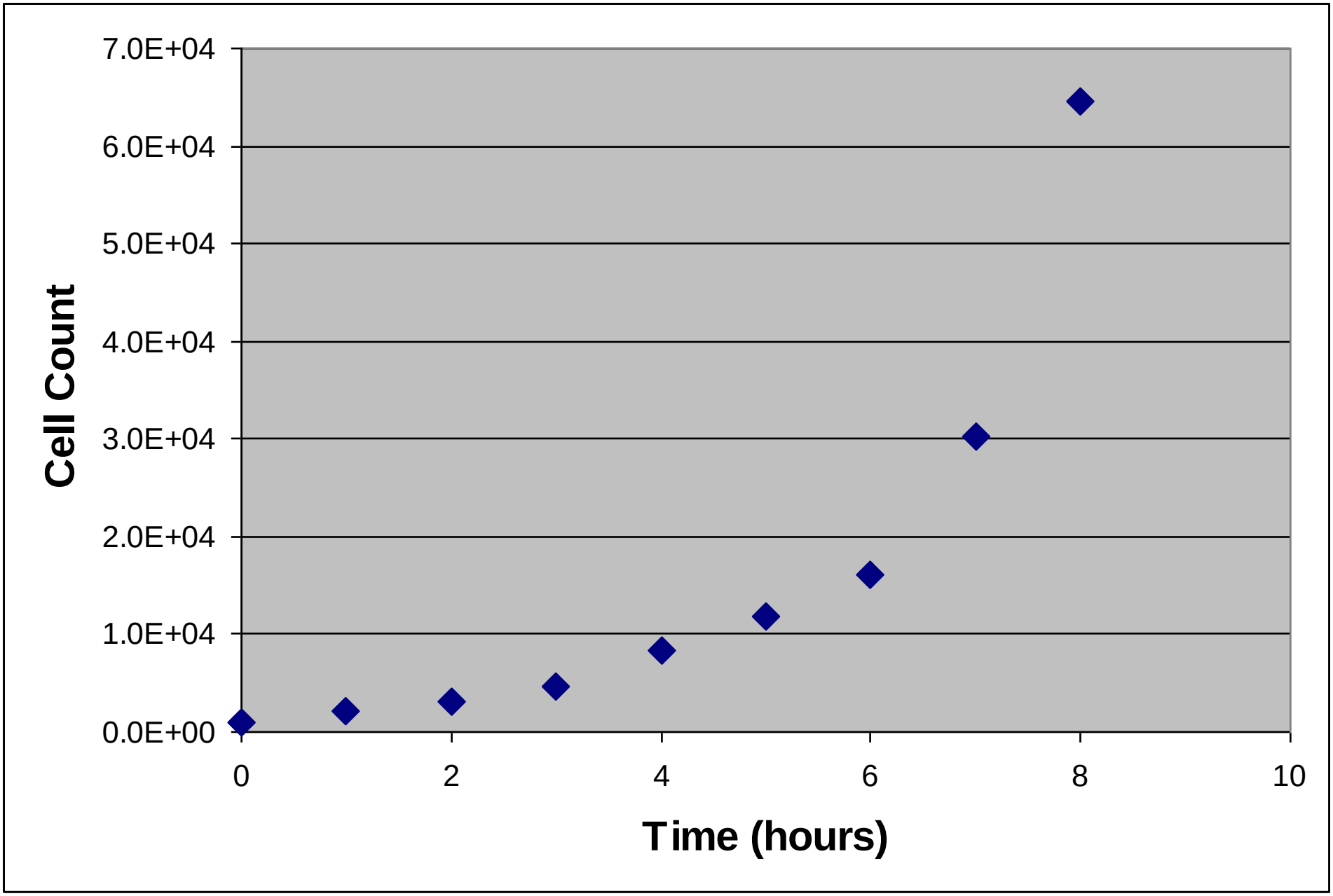
$$\ln(X) = \ln\left(X_o e^{\mu t}\right)$$

$$\ln X = \mu t + \ln(X_o)$$

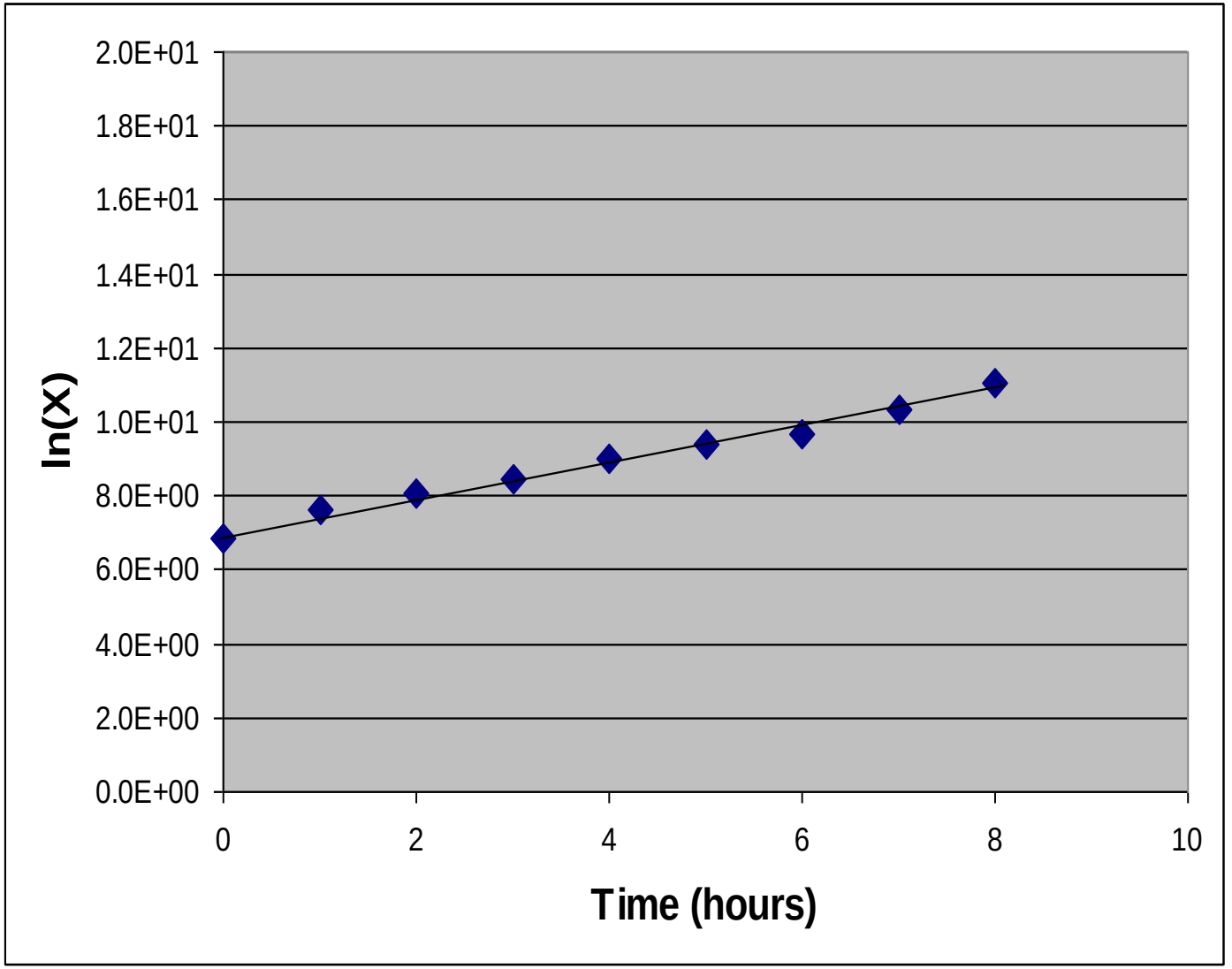
$$\frac{1}{x} dX = \mu dt + 0$$

$$\frac{1}{x}\frac{dX}{dt} = \mu$$

μ is Specific Growth Rate


7.0E+04
6.0E+04
5.0E+04
4.0E+04
3.0E+04
2.0E+04
1.0E+04
0.0E+00
Cell Count
0
2
4
6
8
10
Time (hours)

Plot ln(X/Xo) vs t



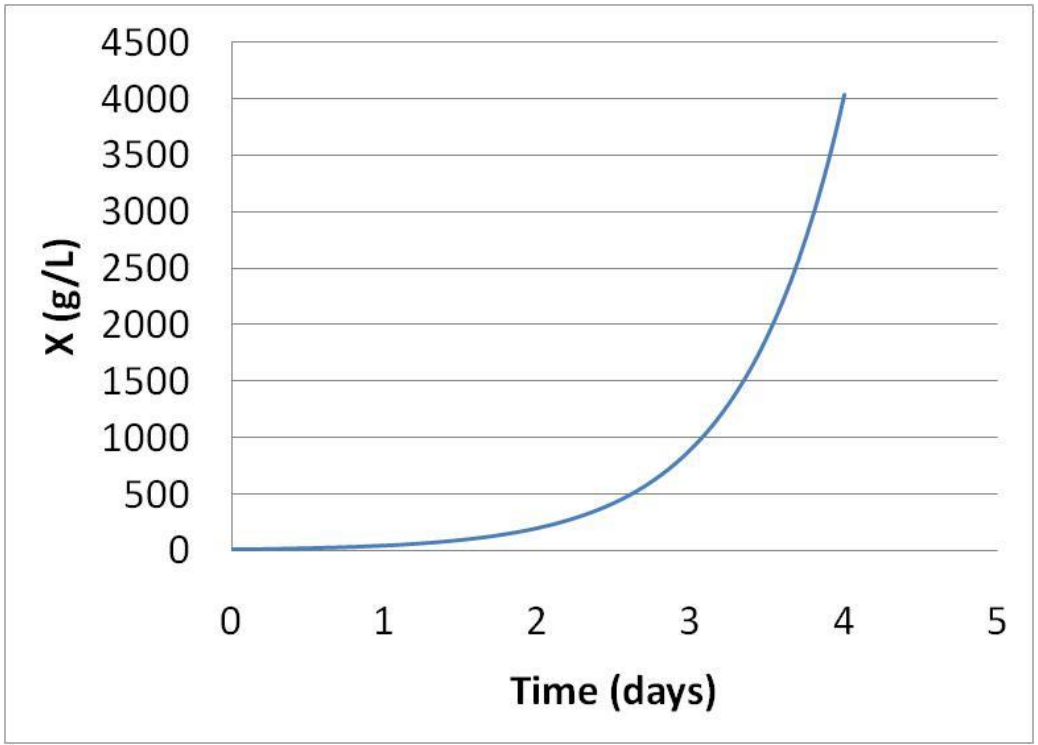
Monod Equation

$$r_X = \frac{dX}{dt} = \mu \cdot X$$

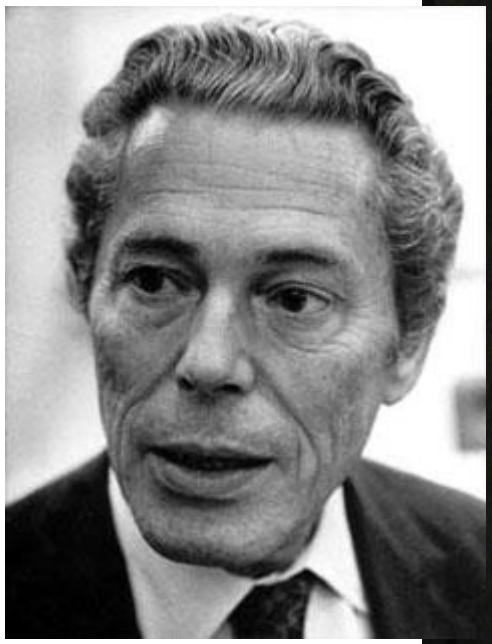
Where X = cell concentration (g/L)

μ = specific growth rate

$$X_t = X_0 \cdot e^{\mu \cdot t}$$



Research On the Growth of Bacterial Cultures



Série A, N° 1960
N° d'ordre : 2847

THÈSES 1941 (65)

PRÉSENTÉES

A LA FACULTÉ DES SCIENCES
DE L'UNIVERSITÉ DE PARIS

POUR OBTENIR

LE GRADE DE DOCTEUR ÈS SCIENCES NATURELLES

PAR

Jacques MONOD
Licencié ès Sciences
Assistant à la Faculté des Sciences
de l'Université de Paris

1re THÈSE. — Recherches sur la Croissance des Cultures bactériennes.

2e THÈSE. — Propositions données par la Faculté.

Soutenues le 17 décembre 1941, devant la Commission d'examen.

MM. PÉREZ, *Président*
PLANTEFOL
GRASSÉ } *Examinateurs.*

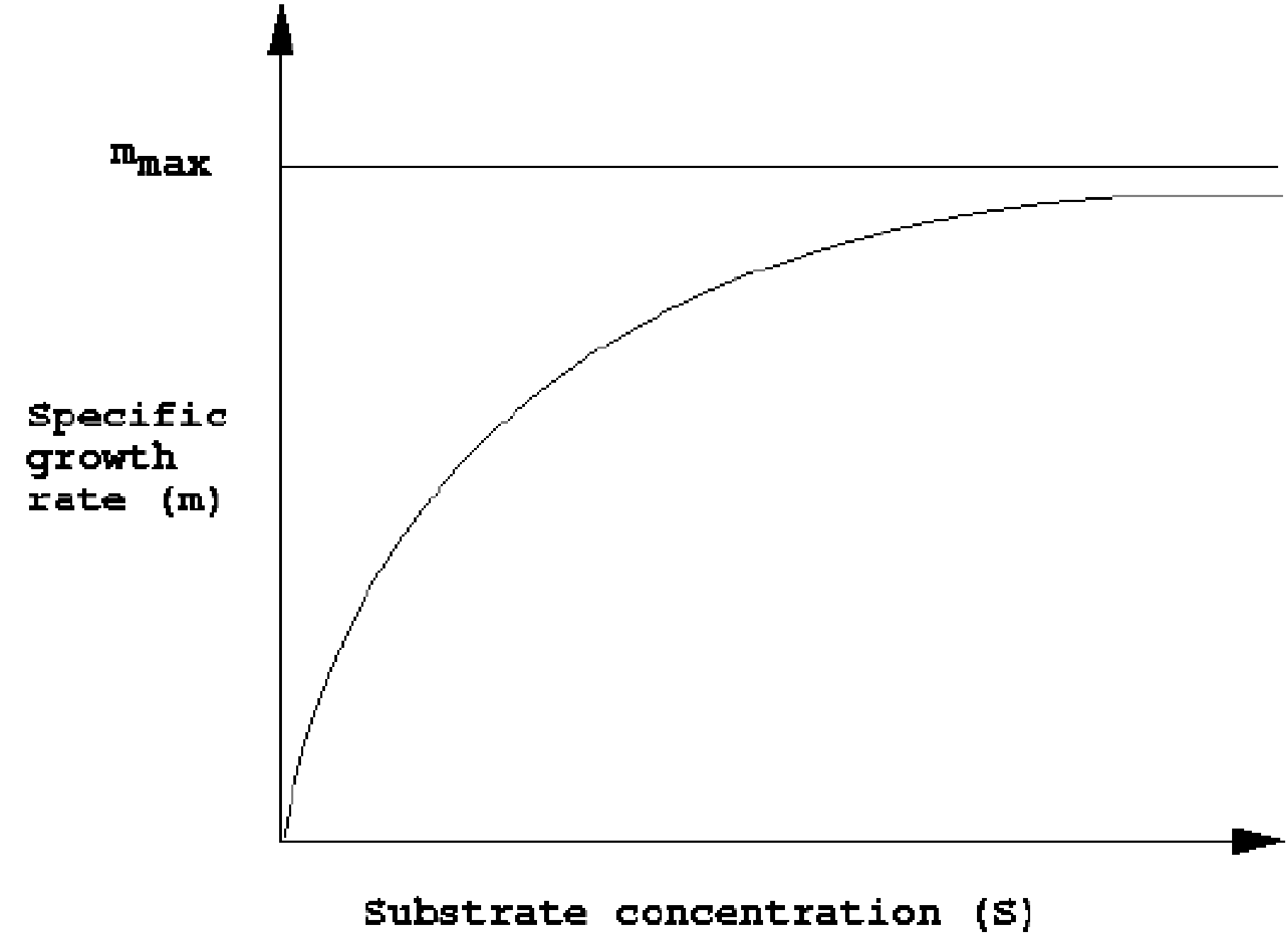
HERMANN & Cie, ÉDITEURS
6, rue de la Sorbonne
—
1941

Assumptions

- Homogeneous
- Exponential growth
  - Cells grow as quickly as possible
- Balanced growth
  - All cells are equal metabolically/physiologically
  - All cells divide at same rate: “synchronization of cell division” – Campbell 1957
  - Because all cells are the same, we can assume that the mass of each cell is the same
  - X = # cells/L becomes X = g cells/L

Assumptions

- Substrate controlled
- Saturated vs limited growth


μ_{max}
Specific growth rate (μ)
Substrate concentration (S)

Monod Equation

$$r_X = \frac{dX}{dt} = \mu \cdot X$$

Growth rate not constant

Subject to constraints
Substrate limited

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

S = substrate (g/L)
μ_{max} = maximum growth rate
K_s = Monod constant
(S where $\mu = \frac{1}{2} \mu_{max}$)

Coupling Cell Growth to Substrate Use

- Yield coefficient

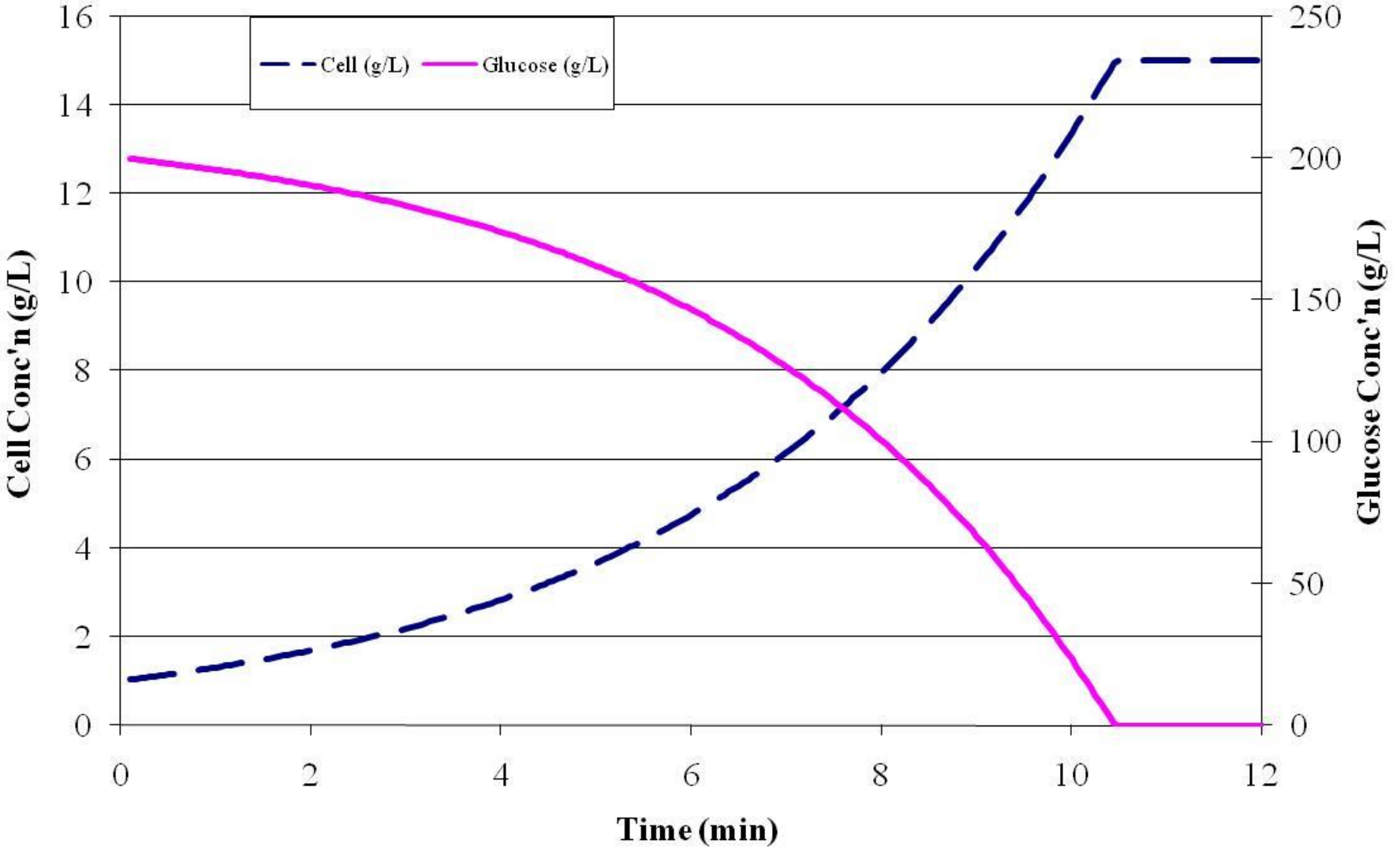
$$Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{dX}{dS}$$

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

Units = g (cells) / g (substrate)

$$Y_{X/S}\frac{dS}{dt} = \frac{dX}{\cancel{dS}}\frac{\cancel{dS}}{dt} = \frac{dX}{dt}$$

$$\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} = Y_{S/X}\frac{dX}{dt}$$

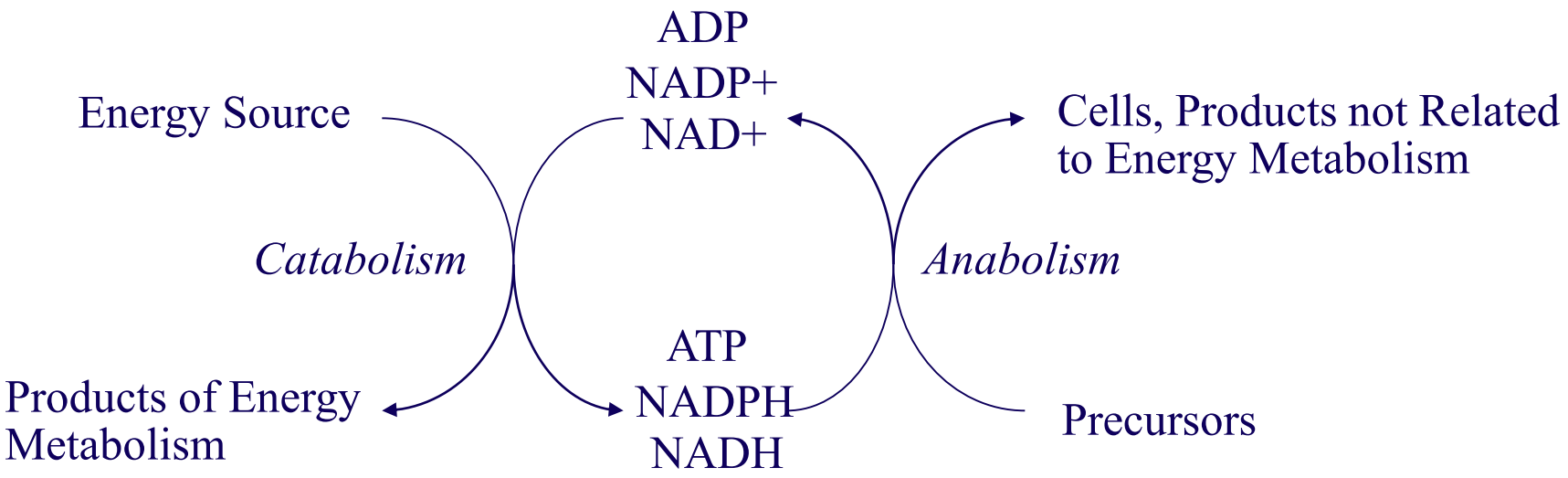

Cell (g/L)
Glucose (g/L)
Cell Conc'n (g/L)
Glucose Conc'n (g/L)
Time (min)
0
2
4
6
8
10
12
14
16
50
100
150
200
250

Modeling Product Formation

- How is product formed (biochemistry)?
- How is product formation related to cell growth?
- How is product formation related to substrate consumption?
- Are there any other factors that affect product formation?

Metabolic Context

Metabolism: The sum total of cellular processes resulting in cell maintenance & growth.



Fermentation Classification

- Aerobic vs Anaerobic vs Microaerobic
  - Is oxygen required?
  - Is oxygen necessary
- Fermentation Type
  - Relationship between cell growth, substrate utiliztation, and product formation
  - Related to metabolic pathway responsible for product

Oxidative metabolism

$$C_{C_s}H_{H_s}O_{O_s} + \mathbf{o}O_2 \rightarrow$$

$$Y_{X/S}C_{C_x}H_{H_x}O_{O_x}$$

$$\mathbf{w}H_2O + \mathbf{c}CO_2$$

Non-oxidative metabolism

$$C_{C_s}H_{H_s}O_{O_s} \rightarrow$$

$$Y_{X/S}C_{C_x}H_{H_x}O_{O_x} + Y_{P/S}C_{C_p}H_{H_p}O_{O_p}$$

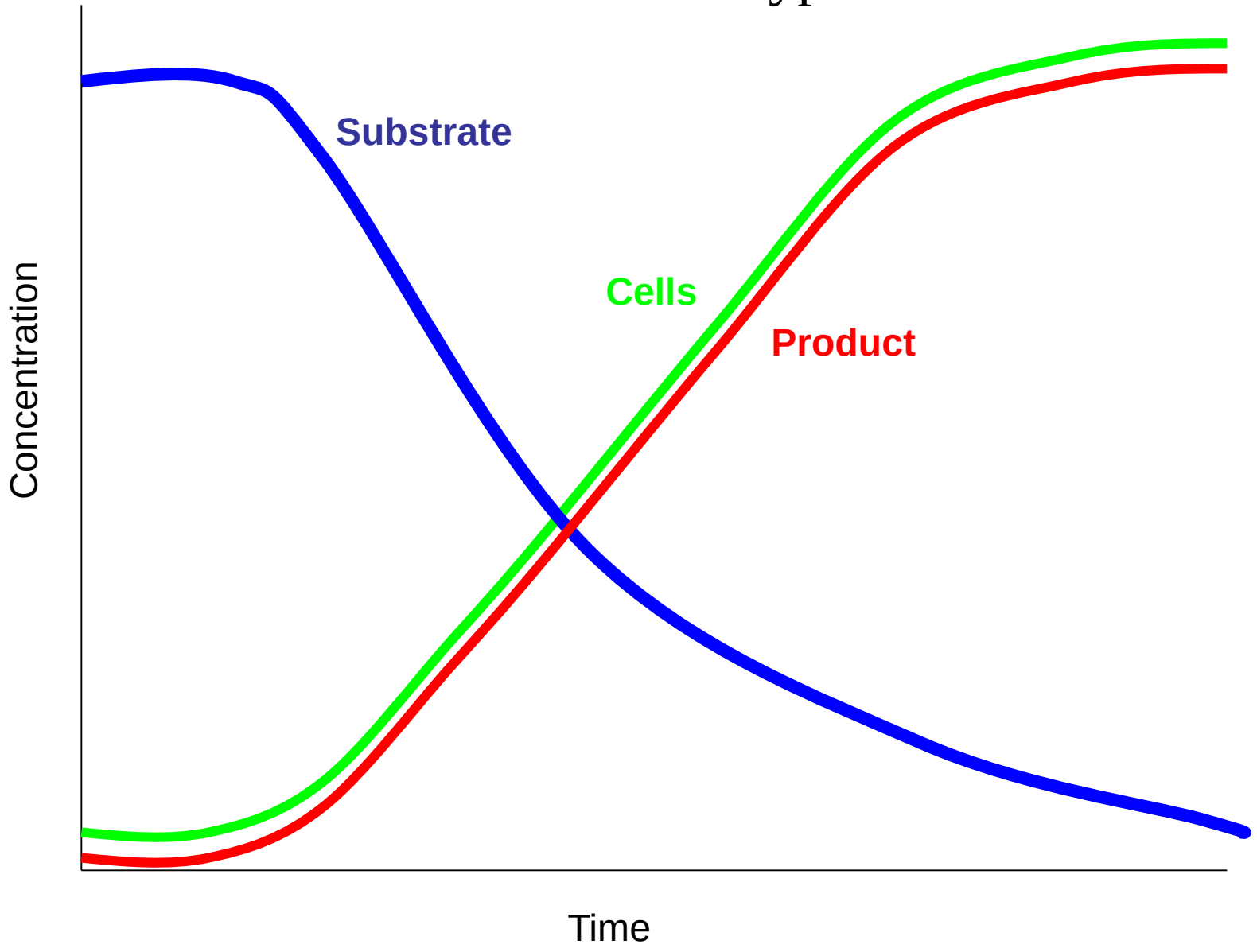
$$\mathbf{w}H_2O + \mathbf{c}CO_2$$

ATP synthesis:	Respiration, 36 ATP/glucose	Substrate-level phosphorylation, 1 to 4 ATP/glucose
Cell yield, $Y_{X/S}$:	~ 0.5	~ 0.1
Product yield, $Y_{P/S}$:	~ 0	0.5 to 0.9 common
O_2 transport:	Major design, scale-up issue	Not needed, easily prevented
Fate of feedstock energy/reducing power:	Heat, cells	> 90% typically in organic products
Heat production:	~0.5 x Feedstock heating value	~ 10% feedstock heating value

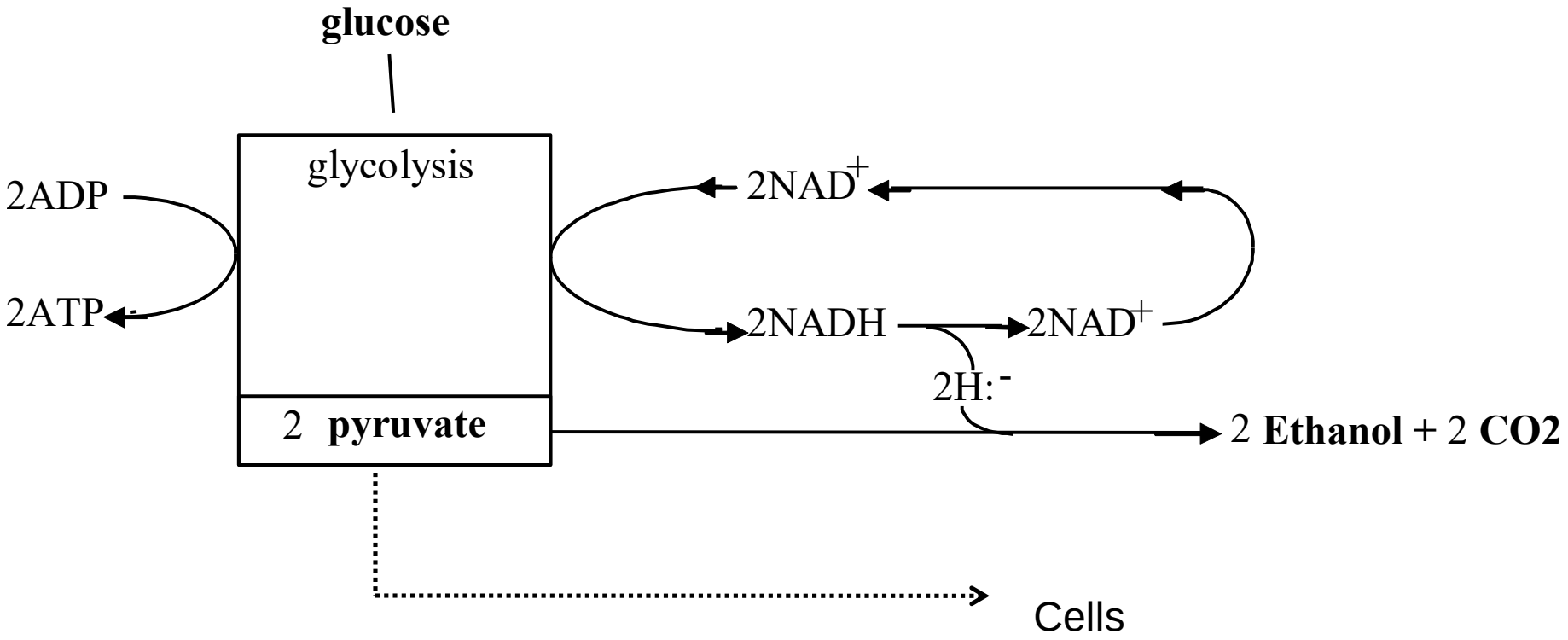
Oxidative and non-oxidative metabolism can be combined, but not without decreasing product yield.

Converting a large fraction of the feedstock mass & energy to organic products requires that most or all metabolism proceed non-oxidatively.

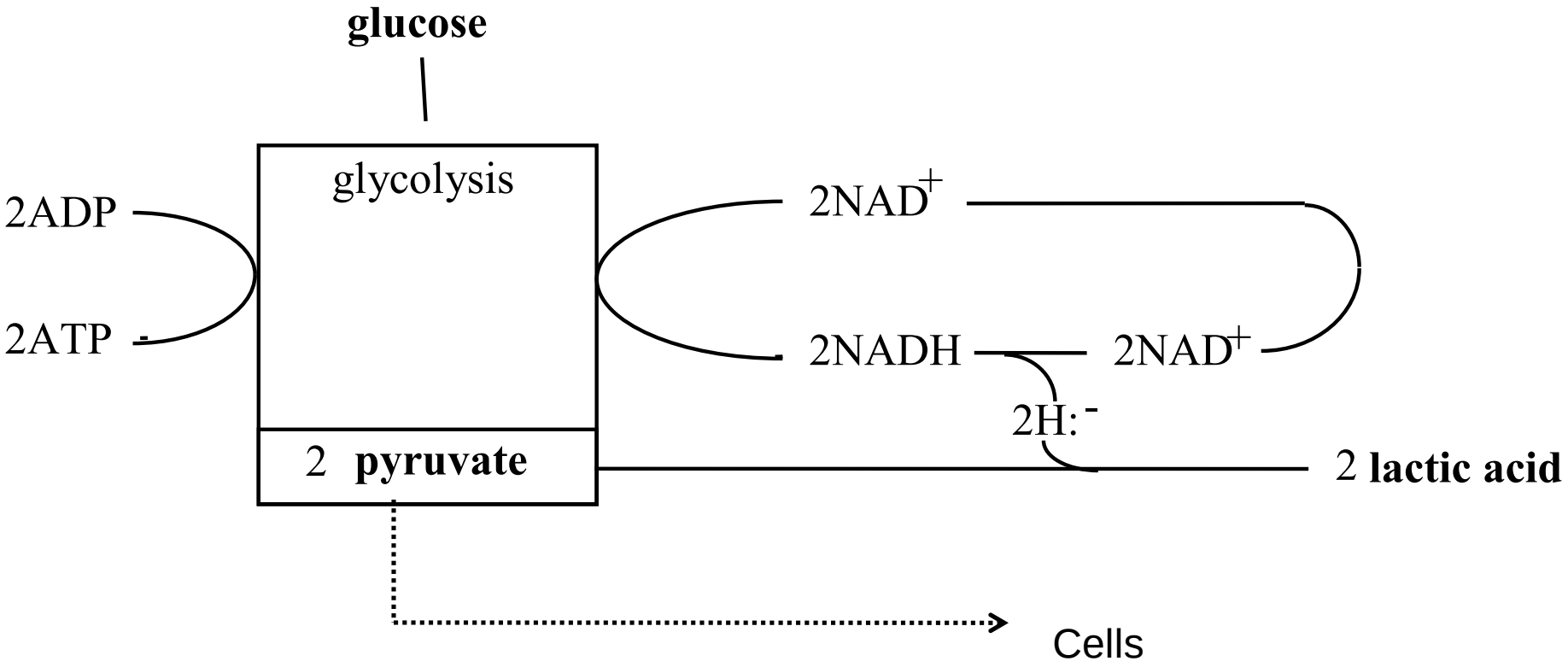
Fermentation: Type I



Ethanol Fermentation Schematic



Lactic Acid Fermentation Schematic



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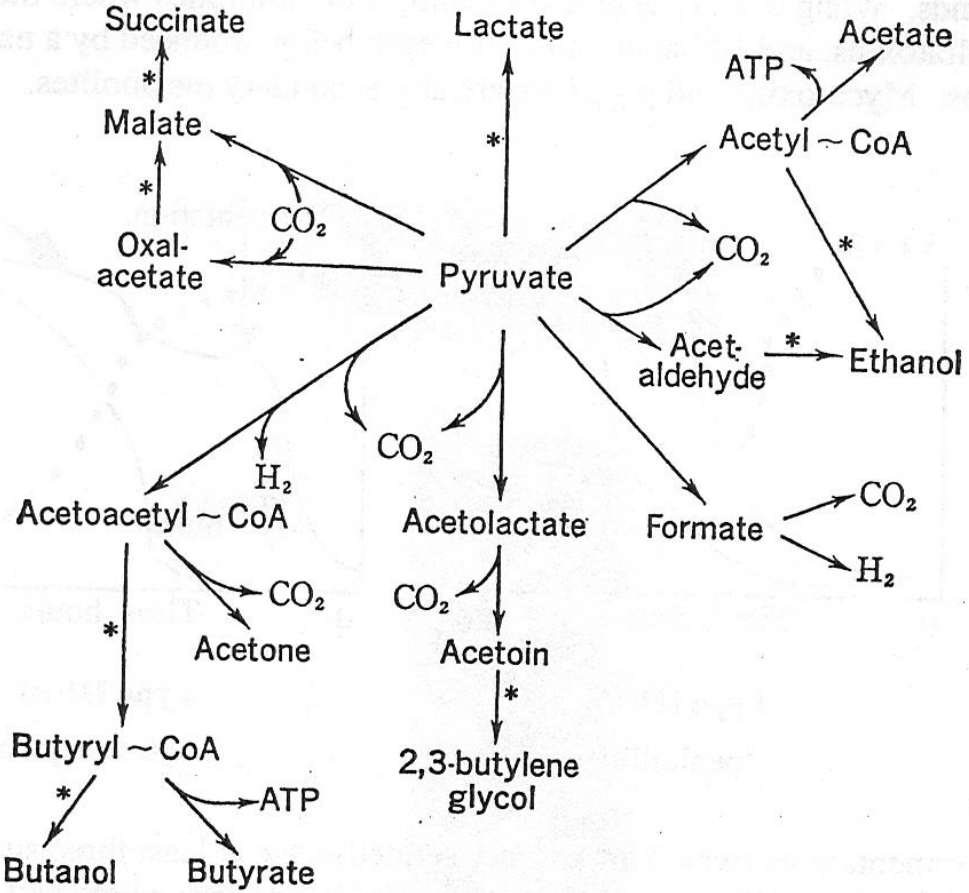
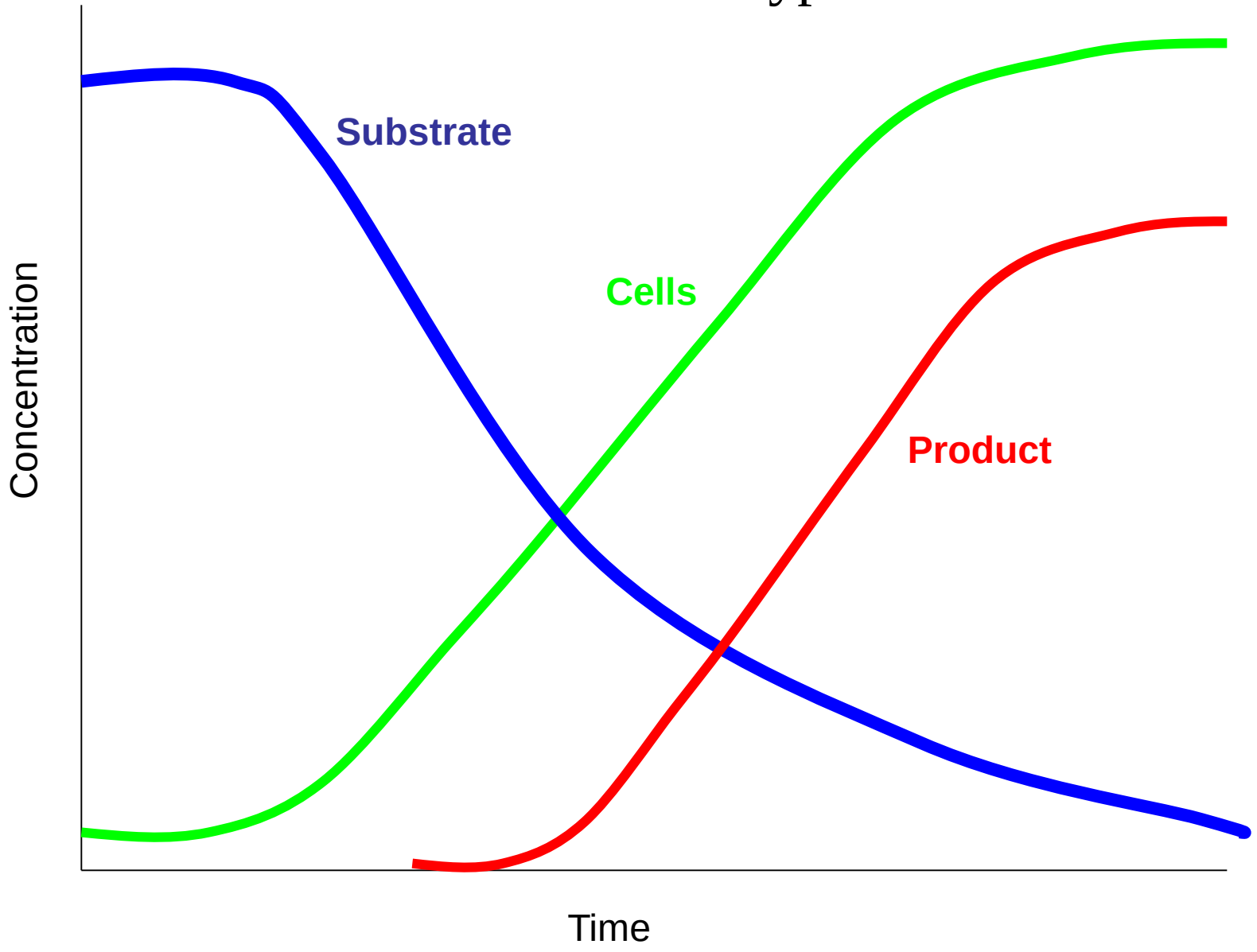


Fig. 3.7. Anaerobic metabolism of pyruvate by different organisms.

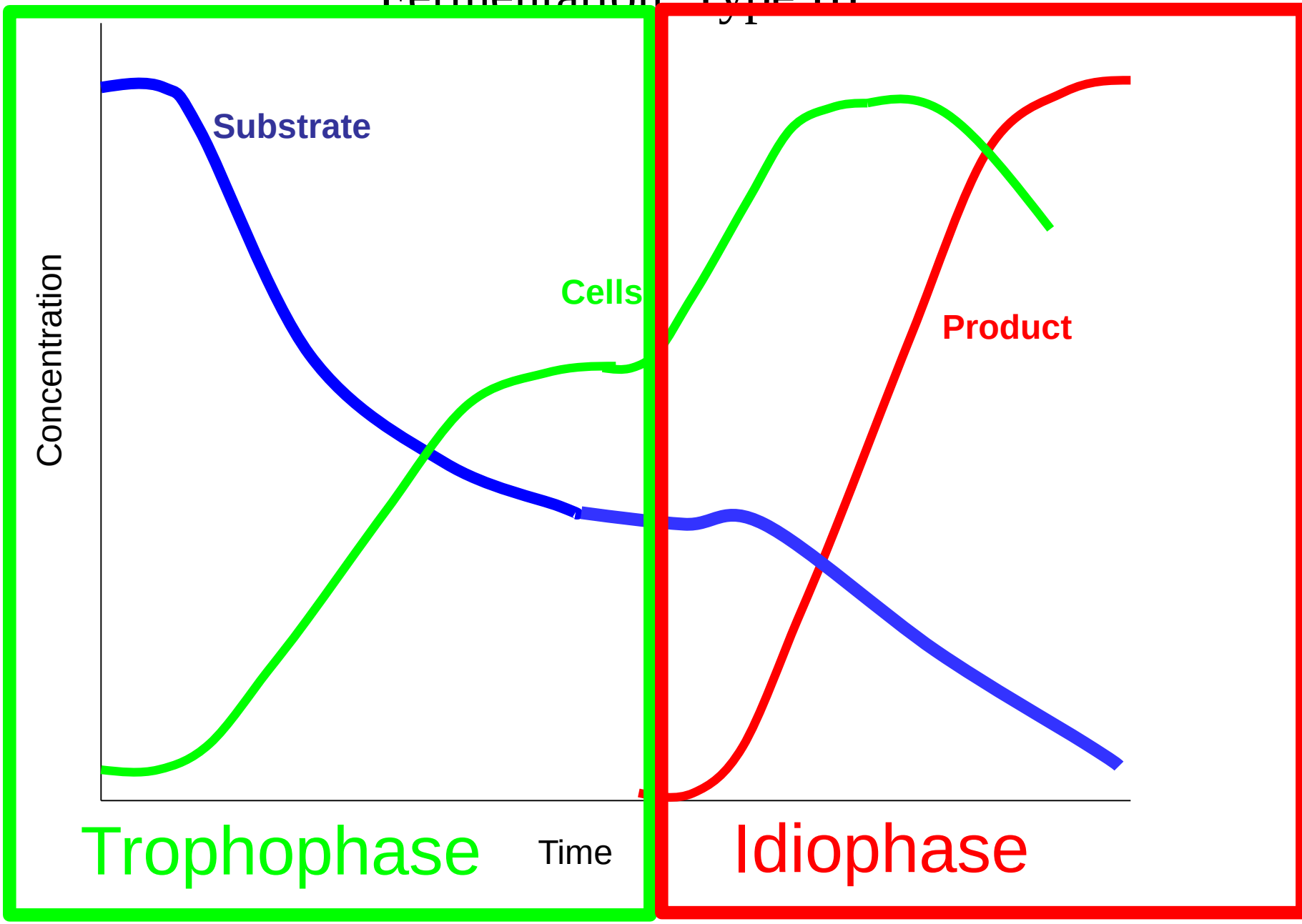
*=Reactions that oxidize $NADH_2 \longrightarrow NAD^+$

Organism	*Major Products*
Clostridia	Butyric, acetic acids, butanol, ethanol, acetone, CO_2, H_2.
Enteric bacteria	Acetic acid, ethanol, CO_2, H_2 (or formic acid), lactic acid, succinic acid, 2,3-butylene glycol.
Yeast	Ethanol, CO_2.
Homofermentative lactobacilli	Lactic acid.

Fermentation: Type II



Fermentation: Type III



Fermentation Types

- Type I
  - Product directly from central metabolism
  - Cell growth coupled to product formation
  - Ethanol, lactic acid, acetone
- Type II
  - Product not directly from central metabolism
  - Secondary or intermediate
  - Citric acid, glutamate, lysine
- Type III
  - Product not byproduct or intermediate of central metabolism
  - Product formation not related to cell growth
  - Antibiotics, antibodies, recombinant proteins