Chapter 5: Major Metabolic Pathways

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Presentation Outline:

- Introduction to Metabolism
- Glucose Metabolism
  - Glycolysis, Kreb's Cycle, Respiration
- Biosysthesis
- Fermentation

Introduction

Metabolism is the collection of enzyme-catalyzed reactions that convert substrates that are external to the cell into various internal products.

Introduction:
Metabolism, Genetic Engineering and Bioprocessing

Genetic Engineering allows for the alteration of metabolism by insertion or deletion of selected genes in a predetermined manner (Metabolic Engineering).

An understanding of metabolic pathways in the organism of interest is of primary importance in bioprocess development.

Characteristics of Metabolism

1. Varies from organisms to organism
2. Many common characteristics
3. Affected by environmental conditions
   - » a) O_2 availability: *Saccharomyces cerevisiae*

     Aerobic growth on glucose → more yeast cells

     Anaerobic growth on glucose → ethanol
   - » b) Control of metabolism is important in bioprocesses

Types of Metabolism

Catabolism

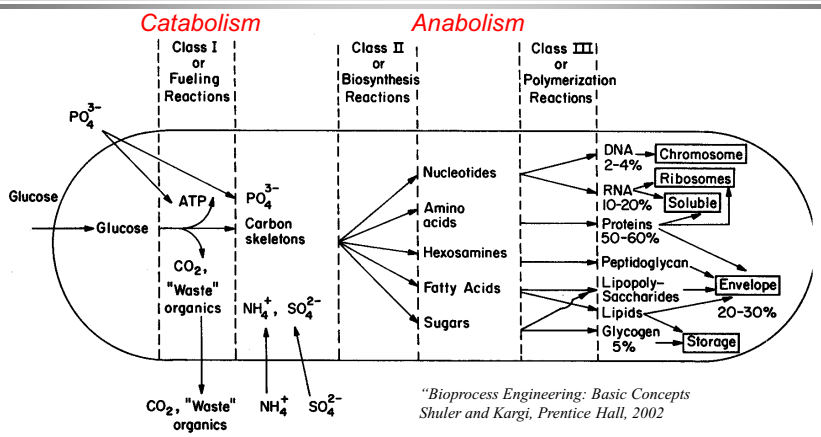
Metabolic reactions in the cell that degrade a substrate into smaller / simpler products.

Glucose → CO_2

Anabolism

Metabolic reactions that result in the synthesis of larger / more complex molecules.

Figure 5.1: Classes of Reactions (Fig. 5.1)



"Bioprocess Engineering: Basic Concepts Shuler and Kargi, Prentice Hall, 2002

Figure 5.1. Schematic diagram of reactions in a bacterial cell.

Bioenergetics

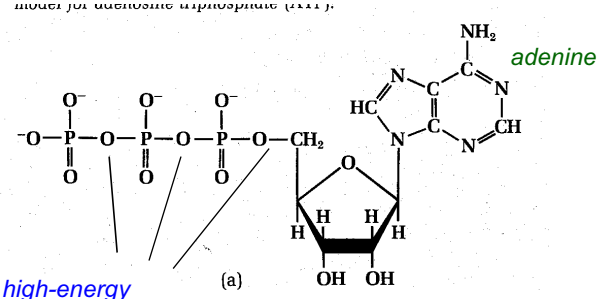
The source of energy to fuel cellular metabolsim is "reduced" forms of carbon (sugars, hydrocarbons, etc.)

The Sun is the ultimate source via the process of Photosynthesis in plants

$$CO_2 + H_2O + hv \rightarrow CH_2O + O_2$$

ATP - Adenosine Triphosphate

Catabolism of carbon-containing substrates generates high energy biomolecules



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ATP - Reactions

Release of energy

$ATP + H_2O \rightleftharpoons ADP + Pi; \Delta G^o = -7.3$ kcal/mole

Storage of energy

$ADP + H_2O \rightleftharpoons AMP + Pi; \Delta G^o = -7.3$ kcal/mole

Analogs of ATP

GTP = guanosine triphosphate

UTP = uridine triphosphate

CTP = cytidine triphosphate

ATP: Energy Currency of the Cell (Fig. 5.2)

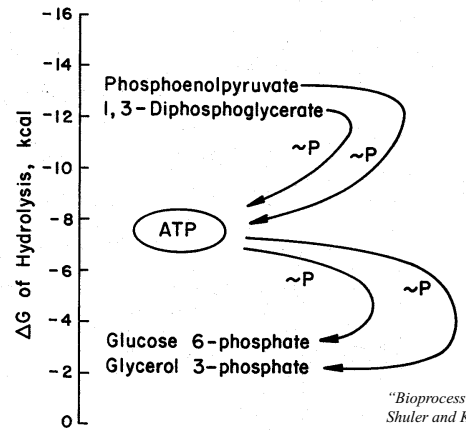

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Figure 5.2. Transfer of biological energy from high-energy to low-energy co

NAD+ and NADP + (Fig. 5.3)

- *Nucleotide derivatives that accept H+ and e- during oxidation / reduction reactions*
- *Transfer e- to O_2 during respiration*

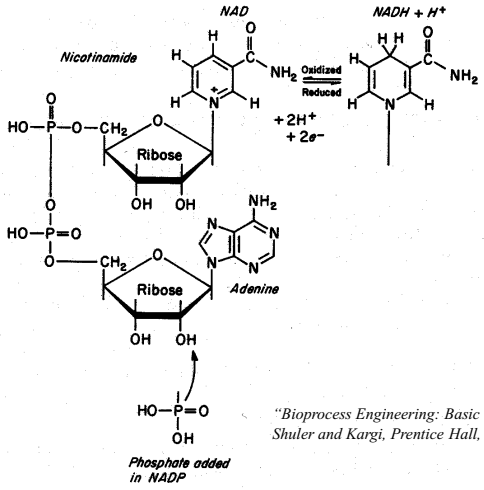

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Figure 5.3. Structure of the oxidation–reduction coenzyme nicotinamide ade

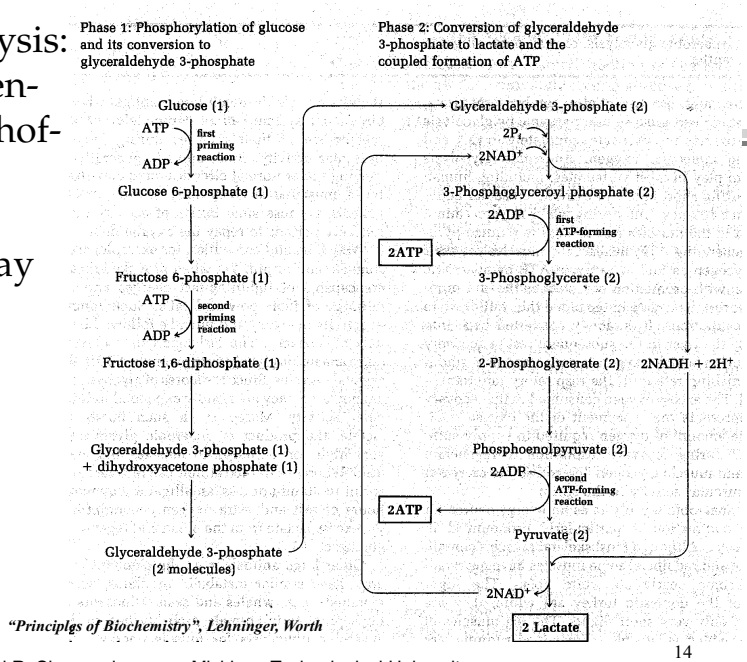
Glucose Metabolism:
Catabolic Pathways of Primary Importance

1. Glycolysis: from glucose to pyruvate.

2. Krebs or tricarboxylic acid (TCA) cycle for conversion of pyruvate to CO_2.

3. Respiration or *electron transport chain* for formation of ATP by transferring electrons from NADH to an electron acceptor (O_2 under aerobic conditions).

Glycolysis: Embden-Meyerhof-Parnas (EMP) Pathway



"Principlgs of Biochemistry", Lehninger, Worth

Glycolysis: in Eucaryotes

- *Fermentation of Glucose → Pyruvate*
- *no O_2 required*
- *Occurs in the Cytoplasm*

Glucose + 2 ADP + 2 NAD^+ + 2 P_i →
2 Pyruvate + 2 ATP + 2 (NADH + H^+)

In Eucaryotes, Cytoplasm to Mitochondria

2 (FADH + H^+)

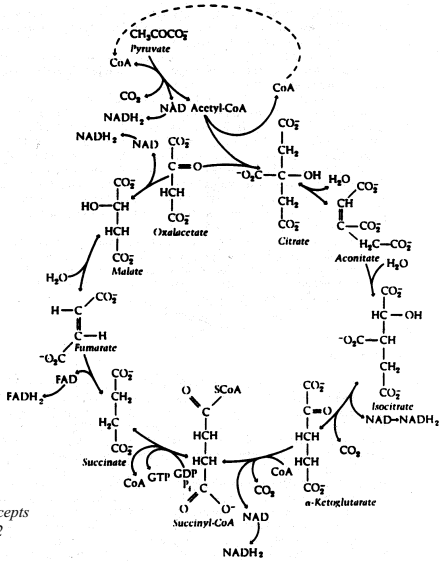
4 ATP = 6 ATP

Krebs or TCA Cycle

- In Mitochondria of eucaryotes
- provides e^- (NADH) and ultimately energy (ATP) for biosynthesis
- provides intermediates for amino acid synthesis
- generates energy (GTP)

Krebs or TCA Cycle

(Fig. 5.5)



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Overall reaction: $Pyruvate + 4NAD + FAD \rightarrow 3CO_2 + 4NADH_2 + FADH_2$

$GDP + phosphate \rightarrow GTP$

$GTP + ADP \rightarrow GDP + ATP$

Oxidative phosphorylation: $4NADH_2 \equiv 12ATP$, $FADH_2 \equiv 2ATP$ | 15ATP

Krebs or TCA Cycle

$$Pyruvate + 4\,NAD^+ + FAD \rightarrow 3\,CO_2 + 4NADH_2 + FADH_2$$

$$GDP + P_i \rightarrow GTP$$

$$GTP + ADP \rightarrow GDP + ATP$$

Yield of ATP $1 + 4(3) + 2 = 15\ ATP$

Complete Oxidation of Glucose

Glucose + 36 P_i + 36 ADP + 6 O_2 →
6 CO_2 + 6 H_2O + 36 ATP

ΔG^o = (36)(7.3 kcal/mole) = 263 kcal/mole glucose

Energetics of Glucose Oxidation

Direct Oxidation of Glucose
Glucose + 6 O_2 → 6 CO_2 + 6 H_2O

ΔG^o = 686 kcal/mole glucose

Energy Efficiency of Glycolysis/TCA Cycle
263/686(100) = 38% (standard conditions)
≈ 60% (nonstandard conditions)

ATP Yields

Eucaryotes

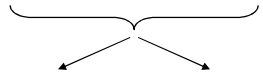
3 ATP, NADH 2 ATP FADH → 36 ATP Glucose

Procaryotes

2 ATP, NADH 1 ATP FADH → 24 ATP Glucose

Respiration

- In Mitochondria of eucaryotes
- in membrane-bound proteins in procaryotes
- e^- transport from NADH or FADH to an electron acceptor



Aerobic
O_2

Anaerobic
NO_3^-, SO_4^{2-}, Fe^{3+}, Cu^{2+}, S^o,

Respiration

(Fig. 5.6)

Goals of Respiration

1. **Regenerate NAD+**
2. **Generate ATP**

Oxidative Phosphorylation

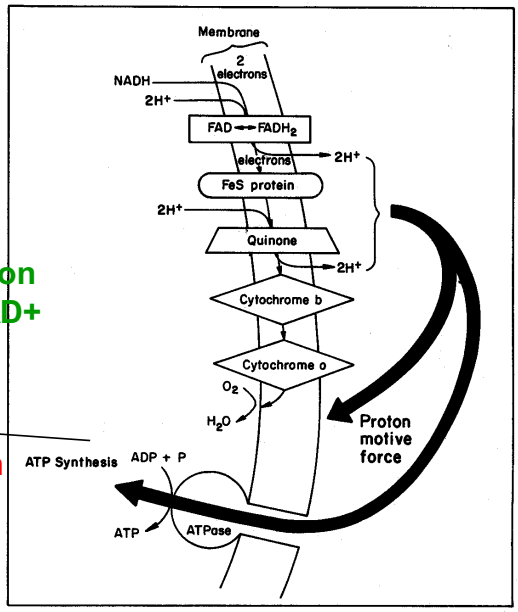


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Figure 5.6. Electron transport and electron transport phosphorylation. Top: Ox

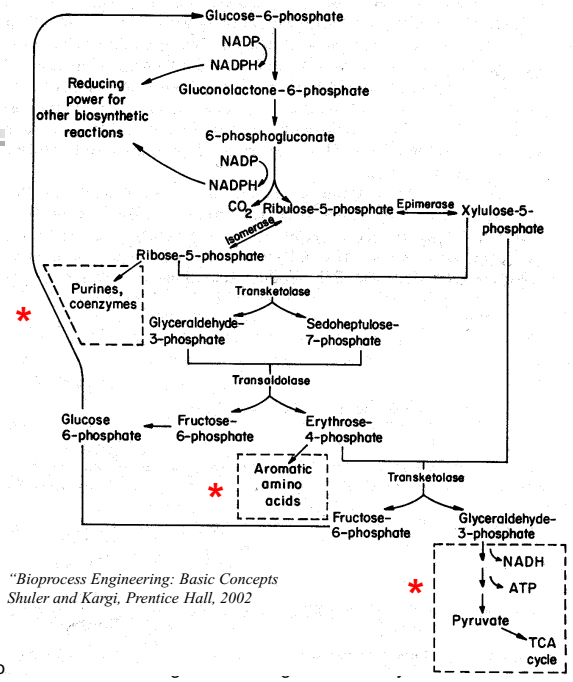
Biosynthesis

The EMP pathway and TCA cycle are used for catabolism (Glucose → CO_2 + NADH + ATP) primarily. → energy production.

• The Hexose - Monophosphate pathway (HMP) is used for biosynthesis

HMP Pathway

(Fig. 5.7)



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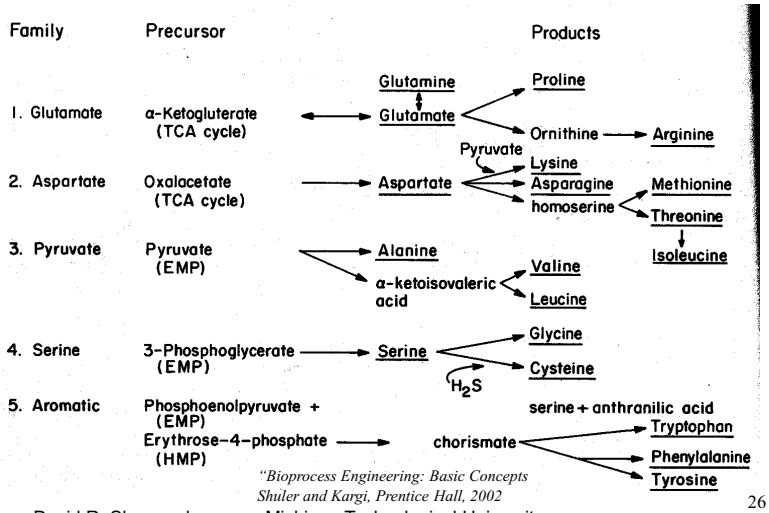
Amino Acids by Various Pathways (Fig. 5.8)



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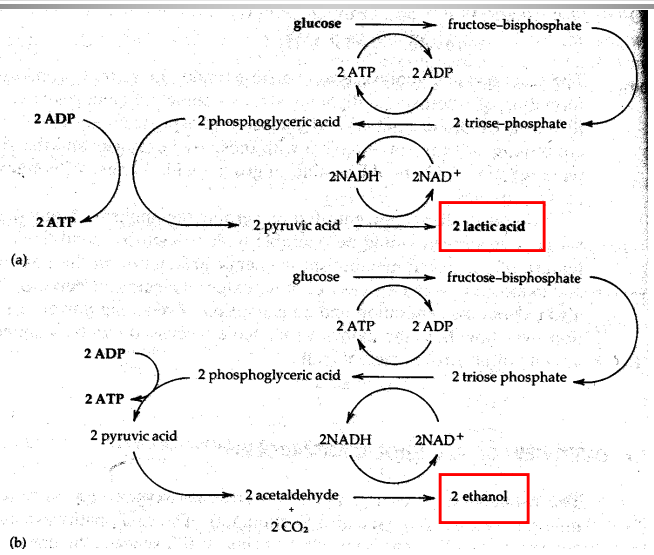
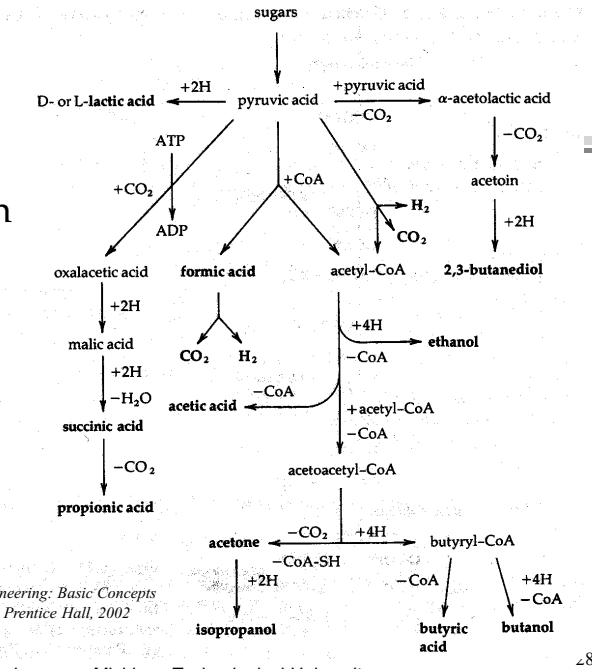
Fermentation:
No TCA Cycle or Respiration



Products from Fermentation



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Metabolic Engineering (ME)

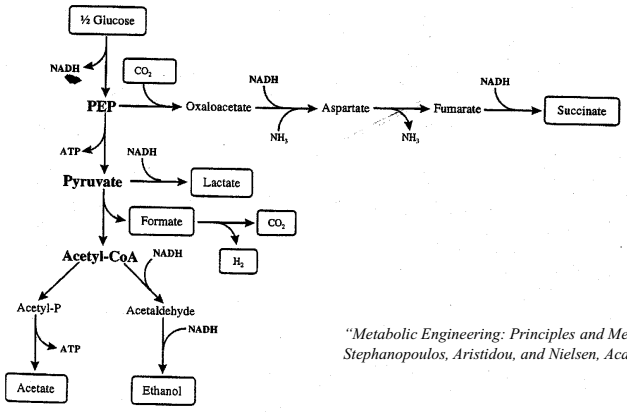
"*the directed improvement of product formation or cellular properties through the modification of specific biochemical reactions(s) or the introduction of new one(s) with the use of recombinant DNA technology*".

It is a field that employs the following skills

+ Applied molecular biology
+ Reaction Engineering
+ Systems analysis

"Metabolic Engineering: Principles and Methodologies"
Stephanopoulos, Aristidou, and Nielsen, Academic Press, 1998

Metabolic Pathway Analysis



"Metabolic Engineering: Principles and Methodologies"
Stephanopoulos, Aristidou, and Nielsen, Academic Press, 1998

FIGURE 3.1 Mixed acid fermentation by *E. coli*. The substrate (glucose) and the seven metabolic products are circled. Intracellular metabolites and cofactors included in the metabolic model are marked in boldface type. The transamination reaction where aspartate is formed from oxaloacetate is shown as a direct amination, i.e., glutamate and 2-ketoglutarate are not shown in order to reduce the complexity.

Principles of ME and Mixed Acid Fermentation

1. Rates of intra-cellular reactions can be measured by extra-cellular product accumulation. (ATP)
2. The redox balance (balance on NADH consumption and generation) must balance.

TABLE 3.1 Typical Yields of the Mixed Acid *E. coli* Fermentation[a]

Metabolic product	Moles formed per 100 mol of glucose fermented
Formate	2.4
Acetate	36.5
Lactate	79.5
Succinate	10.7
Ethanol	49.8
CO_2	88.0
H_2	75.0

[a] The data are taken from Ingraham *et al.* (1983).

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Stephanopoulos, Aristidou, and Nielsen, Academic Press, 1998

Analysis of E. Coli Mixed Acid Fermentation

1. Using a basis of 100 moles of Glucose, how many moles of NADH are generated?

2. Using the data in Table 3.1, how many moles of NADH are consumed?

3. Is a redox balance achieved during this fermentation?

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Analysis of E. Coli Mixed Acid Fermentation

1. Using a basis of 100 moles of Glucose, how many moles of NADH are generated?

2 (10.7) + 79.5 + 2 (49.8) = 200.5 moles NADH consumed

2. Using the data in Table 3.1, how many moles of NADH are consumed?

1 (2 x 100) = 200 moles NADH generated

3. Is a redox balance achieved during this fermentation?

Yes

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