CHAPTER III: CARBOHYDRATE CATABOLISM

2.3. Anaerobic metabolism of pyruvate

Various microorganisms, particularly strict or facultative anaerobic bacteria, are capable of metabolizing pyruvate under anaerobic conditions. This metabolism occurs through different pathways that constitute the ***fermentative metabolism***, which is species-specific and characterized by the nature of the end products of each fermentation pathway. *During fermentation, energy is produced via substrate-level phosphorylation*.

2.3.1. Alcoholic fermentation

Alcoholic fermentation is widespread in yeasts (*Saccharomyces*, *Kluyveromyces*, *Brettanomyces*, etc.). Bacteria capable of performing alcoholic fermentation are relatively few (e.g., ***Zymomonas mobilis***) (Fig. 14).

Glycolysis constitutes the first major step in yeast alcoholic fermentation. In the case of *Zymomonas mobilis,* glucose is degraded through the ***Entner–Doudoroff pathway***. Both pathways lead to pyruvate, which is then decarboxylated to acetaldehyde and CO_2. The reduction of acetaldehyde results in the formation of ethanol. Other substances may be produced in small amounts, particularly glycerol (Fig. 15) and acetic acid. The conversion of one glucose molecule to ethanol by yeasts yields 2 molecules of ATP.

$$C_6H_{12}O_6 + 2\ ADP + 2\ Pi \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP$$

Under anaerobic conditions, yeasts do not convert all glucose into ethanol and carbon dioxide; small amounts of pyruvate and $NADH_2$ are used for cell maintenance. Reoxidation of $NADH_2$ is essential for the completion of alcoholic fermentation. This occurs via the reduction of acetaldehyde, which is initially absent. In this case, dihydroxyacetone phosphate (DHAP) acts as a hydrogen acceptor, converting into L-α-glycerol-phosphate, which is subsequently transformed into glycerol (Fig. 15). These reactions constitute ***glyceropyruvic fermentation***, which always occurs at the beginning of alcoholic fermentation. This process accounts for only about 10% of the fermented sugars.

It is important to note that many sugars are fermentable into ethanol by yeast strains from breweries and distilleries (*Saccharomyces cerevisiae*, etc.):

- *S. carlsbergensis*: ferments sucrose, fructose, glucose, maltose, and maltotriose;
- *Saccharomyces diastaticus*: ferments dextrins;
- *Saccharomyces luarum*: ferments melibiose;
- Kluyveromyce:s ferments lactose and inulin;
- *Schwanniomyces*: ferments starch;
- *Candida wickerhamii* and *C. molischiana*: ferment cellobiose and cellodextrins. Additionally, certain species of *Pachysolen*, *Pichia*, and *Candida* ferment xylose.

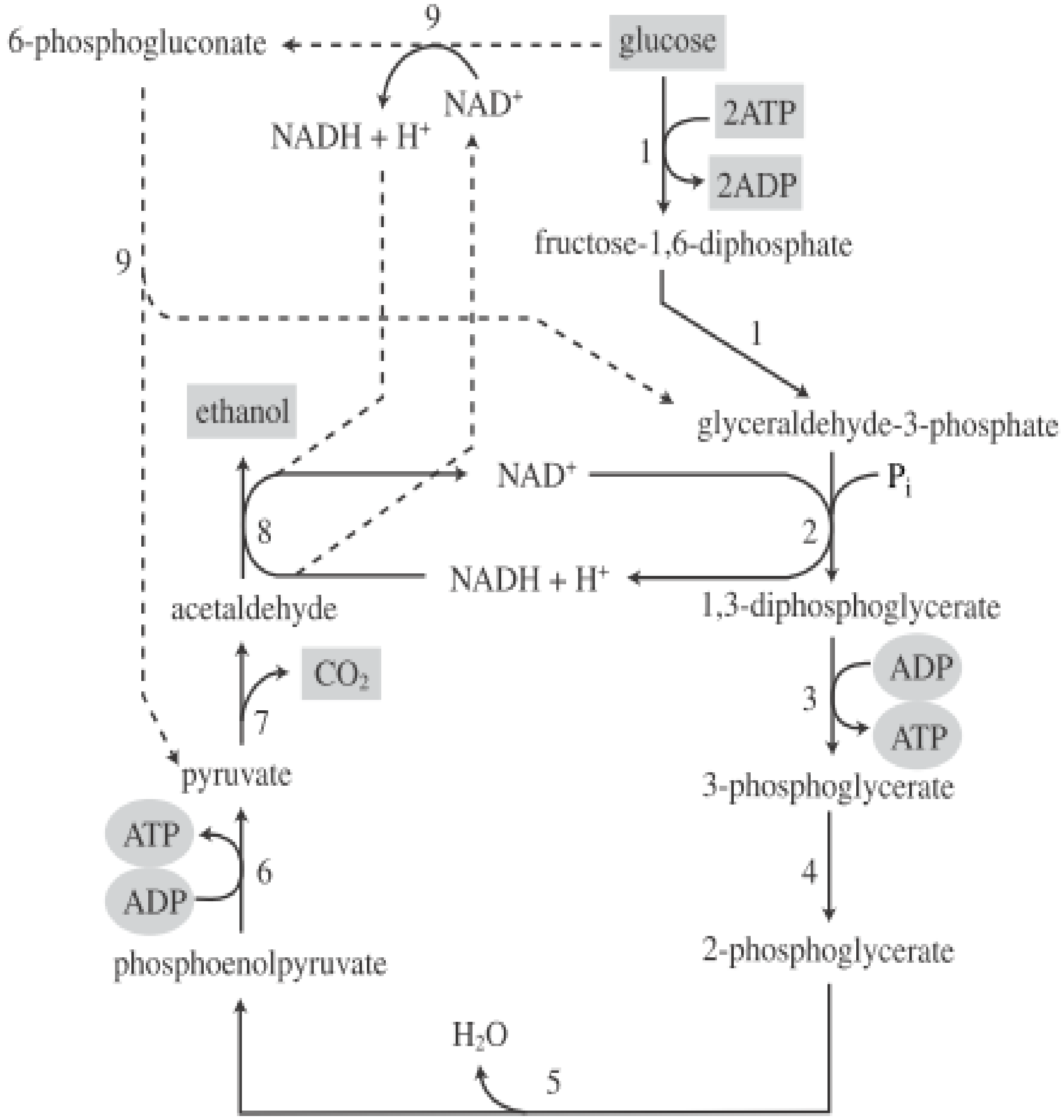

Figure 14: Alcoholic fermentation in *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

(1–6: EMP pathway, solid lines; 7: pyruvate decarboxylase; 8: alcohol dehydrogenase; 9: Entner–Doudoroff pathway, dashed lines).

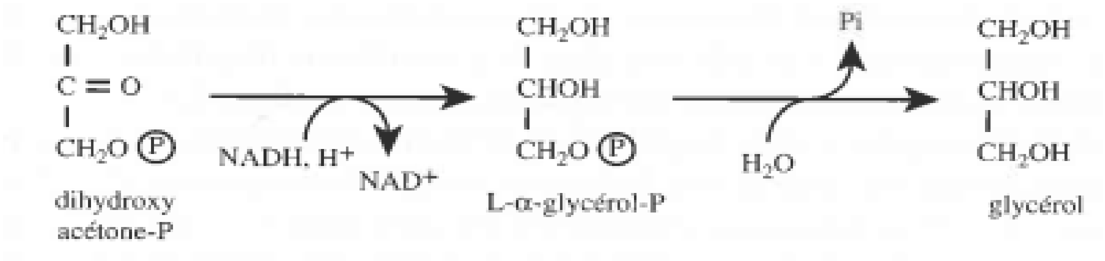

Figure 15: Formation of glycerol during alcoholic fermentation.

2.3.2. Lactic fermentation

Pyruvate from glycolysis is reduced to lactate by NAD-dependent lactate dehydrogenase. Here, the electron acceptor is pyruvate, and no CO_2 is released. Two types of lactic fermentation are distinguished (Tab. 01):

Table 01: Representative lactic acid bacteria and their fermentation modes.

Bacterial strain	**Fermentation type**	
	Homofermentation	**Heterofermentation**
Lactobacillus		
L. delbrueckii	+	-
L. lactis	+	-
L. bulgaricus	+	-
L. casei	+	-
L. curvantus	+	-
L. plantarum	+	-
L. brevis	-	+
L. fermentum	-	+
Sporolactobacillus		
S. inulinus	+	-
Enterococcus		
E. faecalis	+	-
Lactococcus		
L. cremoris	-	+
L. lactis	+	-
Leuconostoc		
L. mesenteroides	-	+
L. dextranicum	-	+
Pediococcus		
P. damnosus	+	-
Bifidobacterium	-	+
B. bifidum		

a. Homolactic fermentation

Lactic acid is the primary product of this type of fermentation (>90% of the products), unlike heterolactic fermentation (25–90% lactic acid) (Fig. 16). Small amounts of glycerol may form, but more often acetoin and diacetyl are produced (e.g., via acetolactate). Under basic pH conditions, increasing amounts of formate, acetate, and ethanol can also be produced.

Lactic acid is produced by the reduction of pyruvic acid catalyzed by lactate dehydrogenase. It may exist in the D-, L-, or DL- form, depending on the stereospecificity of the lactate dehydrogenase and the presence or absence of racemase. A microorganism may possess L-lactate dehydrogenase, D-lactate dehydrogenase, or both.

Homolactic fermentation is carried out by all members of the bacterial genera *Streptococcus*, *Pediococcus*, and *Microbacterium*, by many *Lactobacillus* species, by certain *Bacillus* species, and some molds (Phycomycetes: Oomycetes) (Tab. 01).

Chemical equation:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{Pi} \rightarrow CH_3\text{-CHOH-COOH (lactic acid)} + 2\ \text{ATP}$$

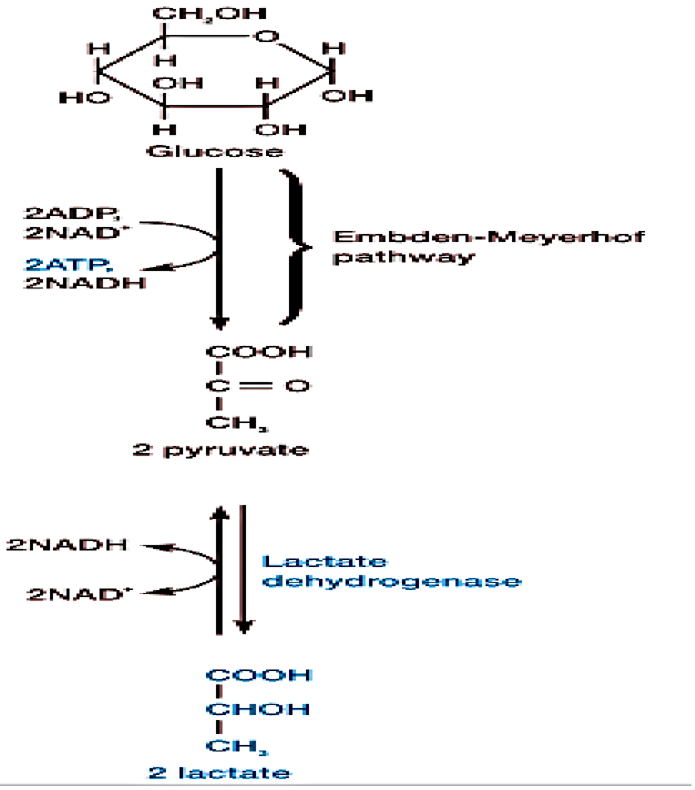

Figure 16: Homolactic fermentation.

b. Heterolactic fermentation (Phosphoketolase pathway)

This fermentation produces ethanol, acetate, and CO_2 in addition to lactate. This pathway uses the ***pentose phosphate pathway*** to generate ***xylose-5-phosphate***, which is split into **glyceraldehyde-3-phosphate (G3P) and acetyl phosphate**. G3P is converted to lactate, and acetyl phosphate is converted to acetate or ethanol. This fermentation is less efficient than homolactic fermentation, ***yielding only one ATP*** per glucose instead of 2 or 3.

Reaction equation:

$$\text{Glucose} + \text{ADP} + \text{Pi} \rightarrow \text{lactate} + \text{ethanol} + CO_2 + 1\ \text{ATP}$$

- **Fungal heterolactic fermentation**

Among molds, *Rhizopus oryzae* represents a special case (Fig. 18). When cultivated aerobically, it produces a mixture of lactic acid, acetic acid, and CO_2, whereas under anaerobic conditions, it produces a mixture of lactic acid, ethanol, and CO_2. These products are the same as those obtained in heterolactic fermentation by *Leuconostoc*, but the formation mechanism is different: glucose degradation occurs via glycolysis. In aerobic conditions, part of the pyruvate is converted into lactic acid, while the other part is oxidized.

$$\underset{\text{(Pyruvic acid}}{2\ CH_3\text{-}CO\text{-}COOH} + H_2O \rightarrow \underset{\text{Lactic acid}}{CH_3\text{-}CHOH\text{-}COOH} + CO_2 + \underset{\text{Acetic acid)}}{CH_3COOH}$$

Under anaerobic conditions, part of the pyruvate is converted into ethanol and CO_2, while the other part is converted into lactic acid. In both cases, the lactic acid produced is in the D-form.

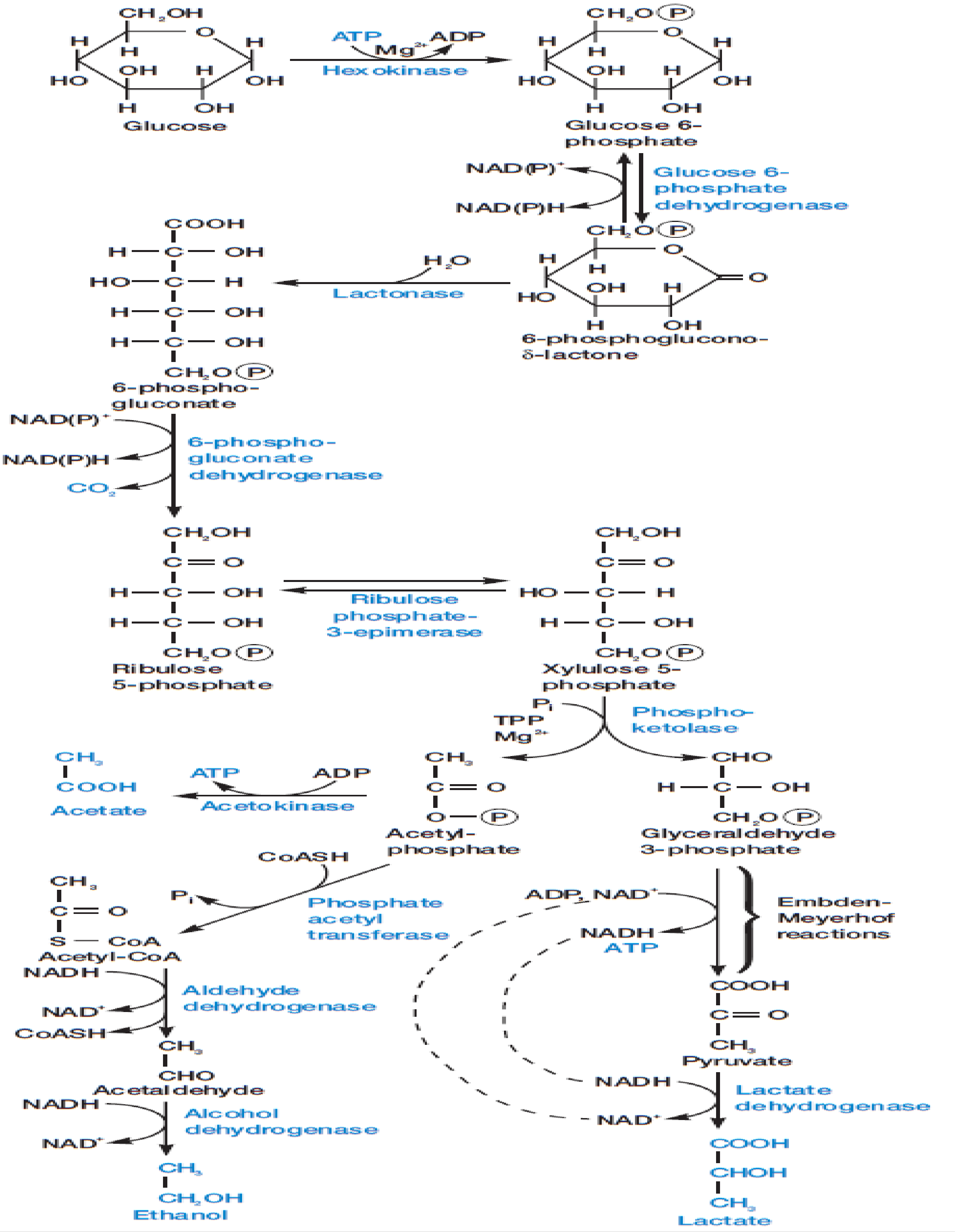

Figure 17 : Heterolactic fermentation.

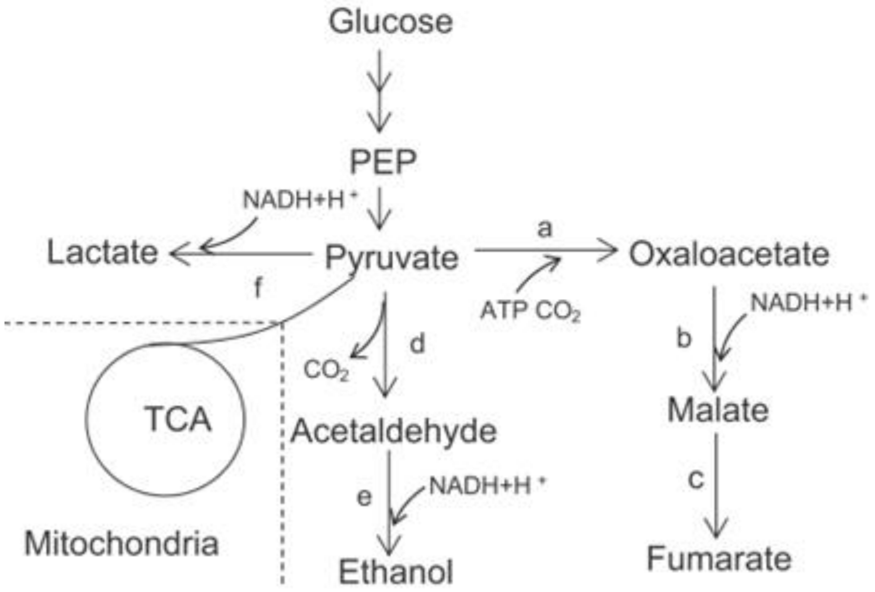

Figure 18: Metabolic pathways for formic acid, lactic acid, and ethanol biosynthesis from glucose in *R. oryzae*.

[(a) pyruvate carboxylase; (b) malate dehydrogenase; (c) fumarase; (d) pyruvate decarboxylase; (e) alcohol dehydrogenase; (f) lactate dehydrogenase and (g) phosphoenolpyruvate carboxylase].

2.3.3 Mixed acid and Butanediol fermentation

Mixed acid fermentation is performed by *Enterobacteria* belonging to the genera *Escherichia*, *Salmonella*, *Proteus*, *Shigella*, and *Yersinia*. It is also observed in *Vibrio* and some *Aeromonas* species. This type of fermentation is characterized by the production of ***ethanol*** and ***several organic acids***: lactic, acetic, succinic, and formic acids.

$$\text{Glucose} + 3\ \text{ADP} + 4\ \text{Pi} + CO_2 + 2\ H^+ \rightarrow 3\ \text{ATP} + \text{succinate} + \text{lactate} + \text{ethanol} + \text{acetate} + \text{formate}$$

Some species (*Escherichia coli*, *Proteus*, and certain *Salmonella*) possess formic hydrogen lyase and immediately decompose formic acid into H_2 and CO_2 at neutral or acidic pH:

$$HCOOH \rightarrow H_2 + CO_2$$

Butanediol fermentation is carried out by members of the genera *Enterobacter*, *Klebsiella*, *Serratia* (*Enterobacteriaceae*), as well as some *Aeromonas* and *Bacillus*. It produces the same products as mixed acid fermentation, but additionally forms ***2,3-butanediol*** (or 2,3-butylene glycol), which along with ethanol, is the most abundant product.

$$\text{Glucose} + 3\text{ADP} + 4\text{Pi} + CO_2 + 2H^+ \rightarrow 3\text{ATP} + \text{succinate} + \text{lactate} + \text{ethanol} + \text{acetate} + \text{formate} + 2,3\text{-butanediol}$$

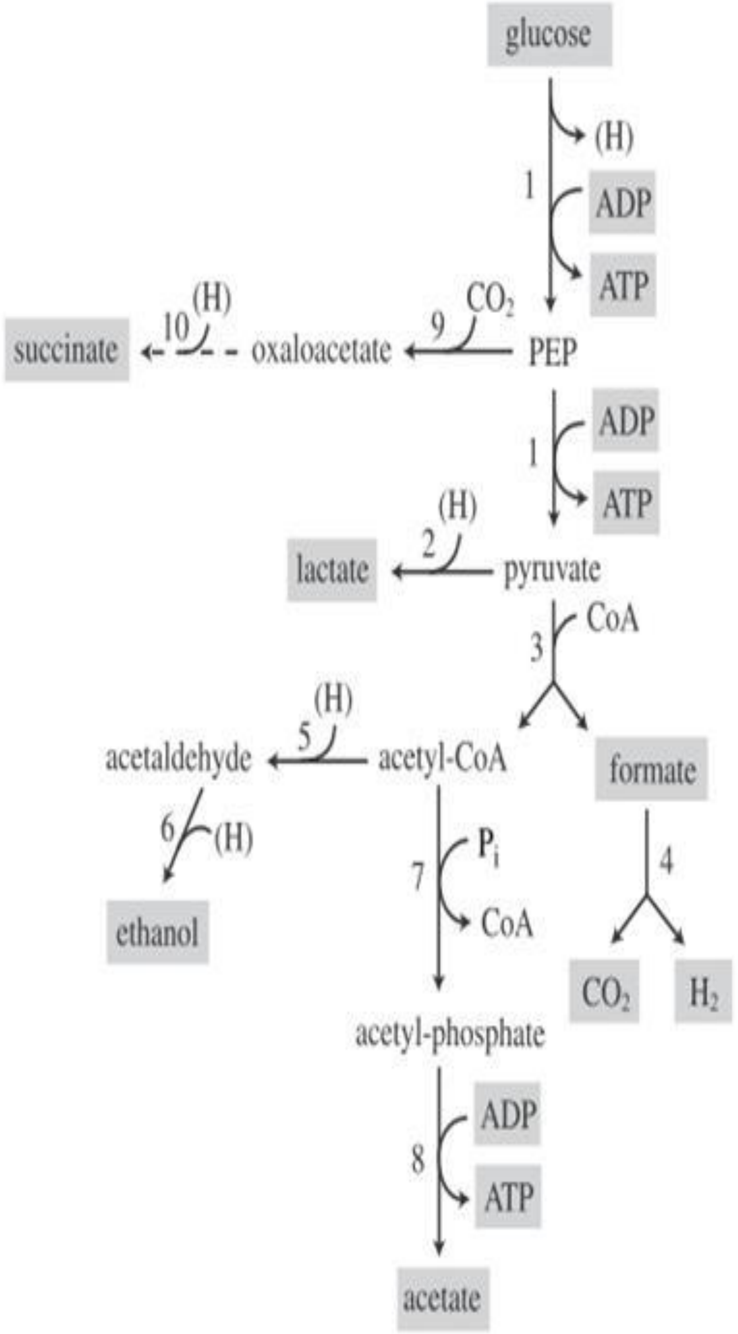

Figure 8.11 **Mixed acid fermentation by some Gram-negative facultative anaerobic bacteria.**

(Gottschalk, G. 1986, *Bacterial Metabolism*, 2nd edn., Figure 8.15. Springer, New York)

Facultative anaerobes belonging to the genera *Escherichia*, *Salmonella*, *Shigella*, *Enterobacter* and others ferment sugars to lactate, acetate, formate, succinate and ethanol in the absence of electron acceptors.

1, EMP pathway; 2, lactate dehydrogenase; 3, pyruvate:formate lyase; 4, formate:hydrogen lyase; 5, acetaldehyde dehydrogenase; 6, alcohol dehydrogenase; 7, phosphotransacetylase; 8, acetate kinase; 9, phosphoenolpyruvate (PEP) carboxylase; 10, enzymes of the TCA cycle.

Figure 19: Mixed acid fermentation by some facultatively anaerobic Gram-negative bacteria.

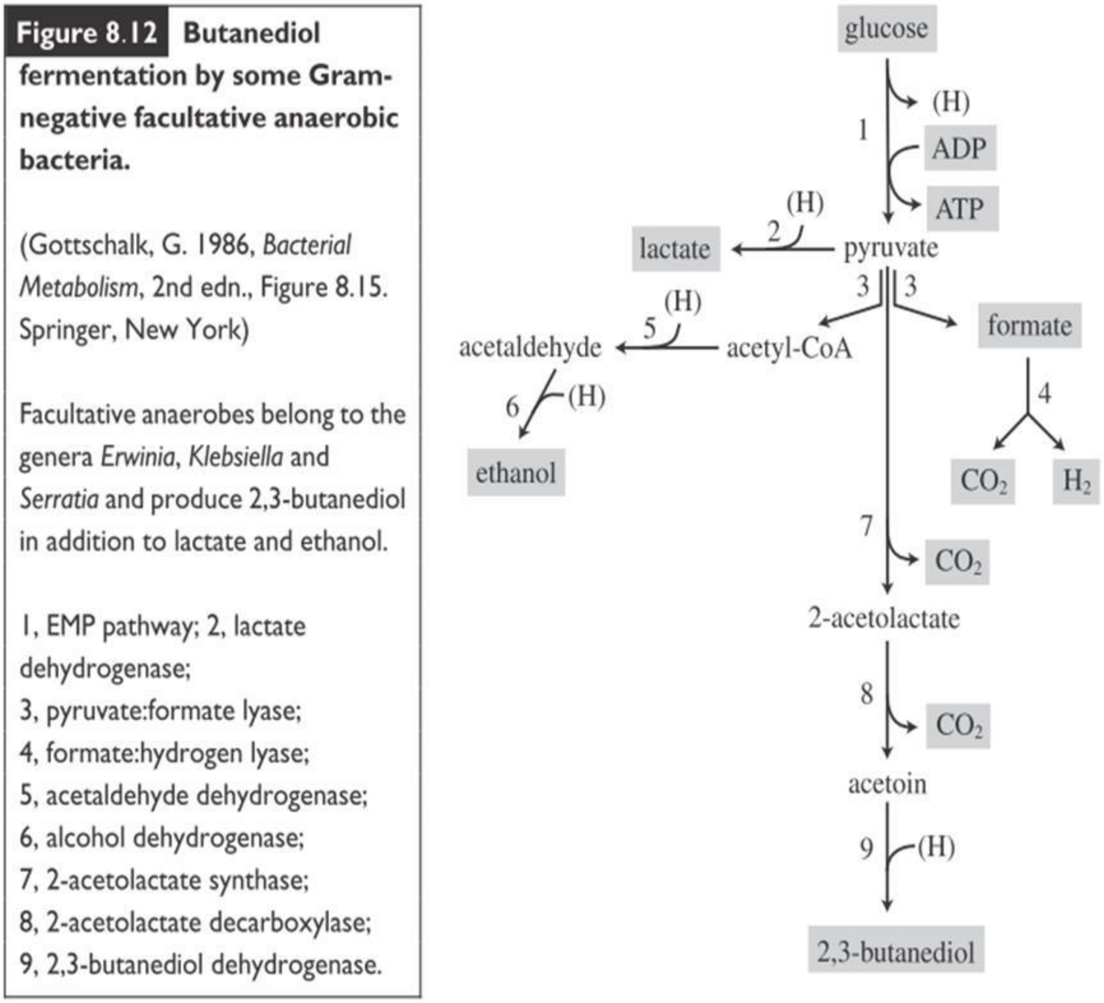

Figure 8.12 Butanediol fermentation by some Gram-negative facultative anaerobic bacteria.

(Gottschalk, G. 1986, *Bacterial Metabolism*, 2nd edn., Figure 8.15. Springer, New York)

Facultative anaerobes belong to the genera *Erwinia*, *Klebsiella* and *Serratia* and produce 2,3-butanediol in addition to lactate and ethanol.

1, EMP pathway; 2, lactate dehydrogenase;
3, pyruvate:formate lyase;
4, formate:hydrogen lyase;
5, acetaldehyde dehydrogenase;
6, alcohol dehydrogenase;
7, 2-acetolactate synthase;
8, 2-acetolactate decarboxylase;
9, 2,3-butanediol dehydrogenase.

Figure 20: Butanediol fermentation by some facultatively anaerobic Gram-negative bacteria.

2,3-Butanediol is formed by the reduction of acetylmethylcarbinol (or acetoin), a pyruvate-derived intermediate via acetolactate (Fig. 20). Acetoin and diacetyl are produced under aerobic conditions. Acids are usually produced in small amounts, though *Serratia* produces substantial formic acid. In other butylene glycol-fermenting Enterobacteria, the presence of formic hydrogen lyase leads to the formation of H_2 and CO_2, with CO_2 being more abundant as it is also formed during 2,3-butanediol synthesis. Under neutral or basic pH, the proportion of acidic products increases.

2.3.4. Butyric and acetone–butanol fermentation

a. Butyric Fermentation

Certain *Clostridium* species (*C. butyricum*, *C. perfringens*), *Butyribacterium*, some *Serratia*, and *Zymosarcina* produce butyric acid, along with acetic acid, CO_2, and hydrogen (Fig. 21). Butyric acid is formed by condensation of two acetyl-CoA molecules into acetolactate, which is then reduced to β-hydroxybutyrate and finally to butyrate. Part of the acetyl-CoA formed from pyruvate contributes to ATP and acetic acid formation.

Reaction balance:

$$\text{Glucose} + 3\ \text{ADP} + 3\ \text{Pi} \rightarrow \text{Butyrate} + CO_2 + 2\ H_2 + 3\ \text{ATP} + \text{Acetate}$$

b. Acetone–butanol fermentation

In addition to the products of butyric fermentation, some *Clostridium* species (*C. acetobutylicum*) can produce alcohols (butanol, ethanol, isopropanol) and acetone (Fig. 22).

Reaction balance:

$$2\ \text{Glucose} + 4\ \text{ADP} + 4\ \text{Pi} \rightarrow \text{Butanol} + \text{Acetone} + 4\ H_2 + 5\ CO_2 + 4\ \text{ATP}$$

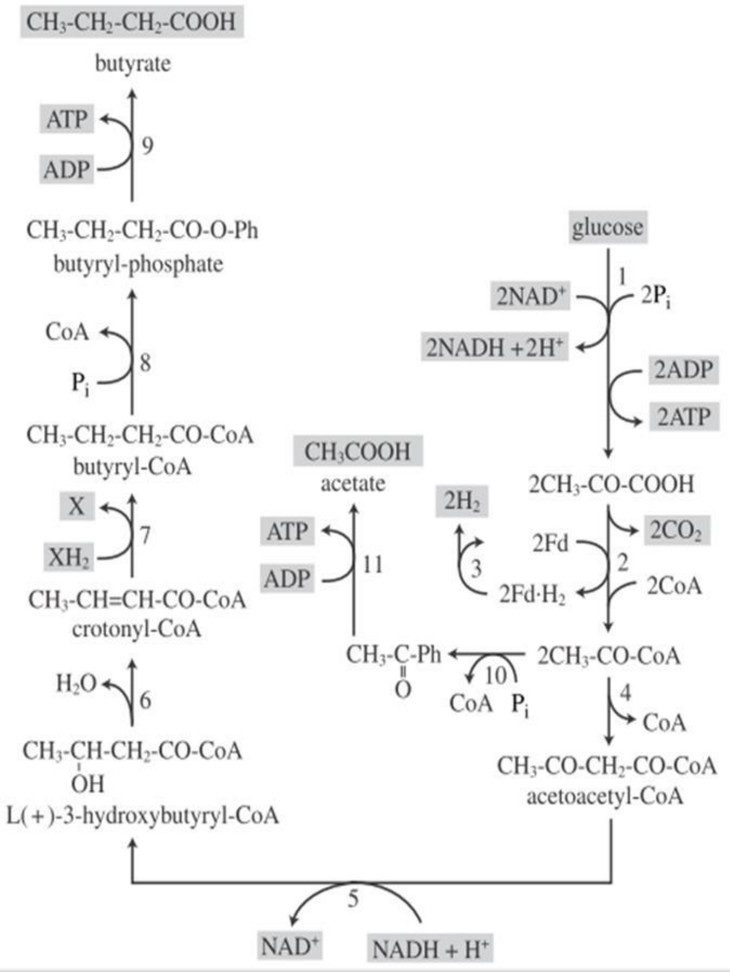

Figure 8.6 Butyrate-acetate fermentation of glucose.

(Gottschalk, G. 1986, *Bacterial Metabolism*, 2nd edn., Figure 8.9. Springer, New York)

1, EMP pathway; 2, pyruvate:ferredoxin oxidoreductase; 3, hydrogenase; 4, acetyl-CoA-acetyltransferase (thiolase); 5, L(+)-3-hydroxybutyryl-CoA dehydrogenase; 6, 3-hydroxyacyl-CoA hydrolase (crotonase); 7, butyryl-CoA dehydrogenase; 8, phosphotransbutyrylase; 9, butyrate kinase; 10, phosphotransacetylase; 11, acetate kinase.

Figure 21: Butyric fermentation.

Table 2: Sugar fermentation by selected *Clostridium* species (mmol products/100 mmol sugar consumed)

Products	***C. butyricum***	***C. perfringens***	***C. acetobutylicum***
Butyrate	76	34	4
Acetate	42	60	14
Lactate	-	33	-
CO_2	188	176	221
H_2	235	214	135
Ethanol	-	26	7
Butanol	-	-	56
Acetone	-	-	22

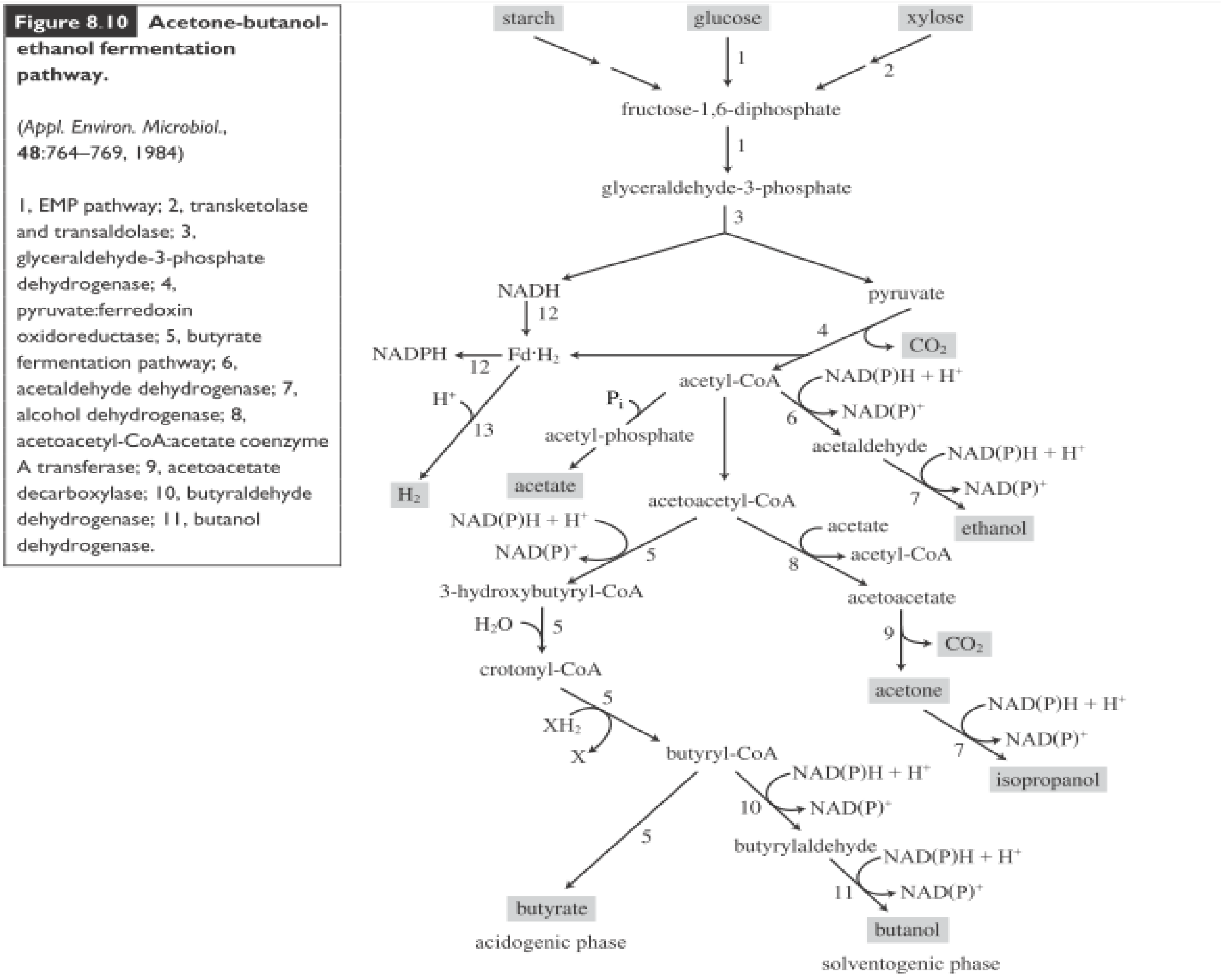

Figure 8.10 Acetone-butanol-ethanol fermentation pathway.

(*Appl. Environ. Microbiol.*, 48:764–769, 1984)

1, EMP pathway; 2, transketolase and transaldolase; 3, glyceraldehyde-3-phosphate dehydrogenase; 4, pyruvate:ferredoxin oxidoreductase; 5, butyrate fermentation pathway; 6, acetaldehyde dehydrogenase; 7, alcohol dehydrogenase; 8, acetoacetyl-CoA:acetate coenzyme A transferase; 9, acetoacetate decarboxylase; 10, butyraldehyde dehydrogenase; 11, butanol dehydrogenase.

Figure 22: Acetone–butanol fermentation (*Clostridium acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum*, *C. saccharoperbutylacetonicum*)

2.3.5. Propionic Fermentation

Various strict or facultative anaerobic bacteria (*Propionibacterium*, some *Clostridium*, *Corynebacterium*, *Neisseria*, *Veillonella*) produce propionic acid, acetic acid, CO_2, and succinic acid via fermentation (Fig. 23).

Propionic acid is formed by pyruvate reduction (with lactic acid as an intermediate) or by decarboxylation of succinic acid (*Propionibacterium pentosaceum*). Propionic fermentation can also proceed from lactate, with pyruvate as an intermediate.

Reaction balance:

$$3\ \text{Lactate} + \text{NADH,H}^+ + 3\ \text{ADP} + 3\ \text{Pi} \rightarrow 2\ \text{Propionate} + \text{NAD}^+ + 3\ \text{ATP} + \text{Acetate} + CO_2 + 2\ H_2O$$

Except in *Clostridium propionicum*, where the intermediate is acrylic acid (Fig. 24):

$$3\ \text{Lactate} + \text{ADP} + \text{Pi} \rightarrow 2\ \text{Propionate} + \text{Acetate} + CO_2 + \text{ATP} + H_2O$$

Propionibacterium plays an important role in the ruminant digestive tract and in the production of cooked-curd cheeses.

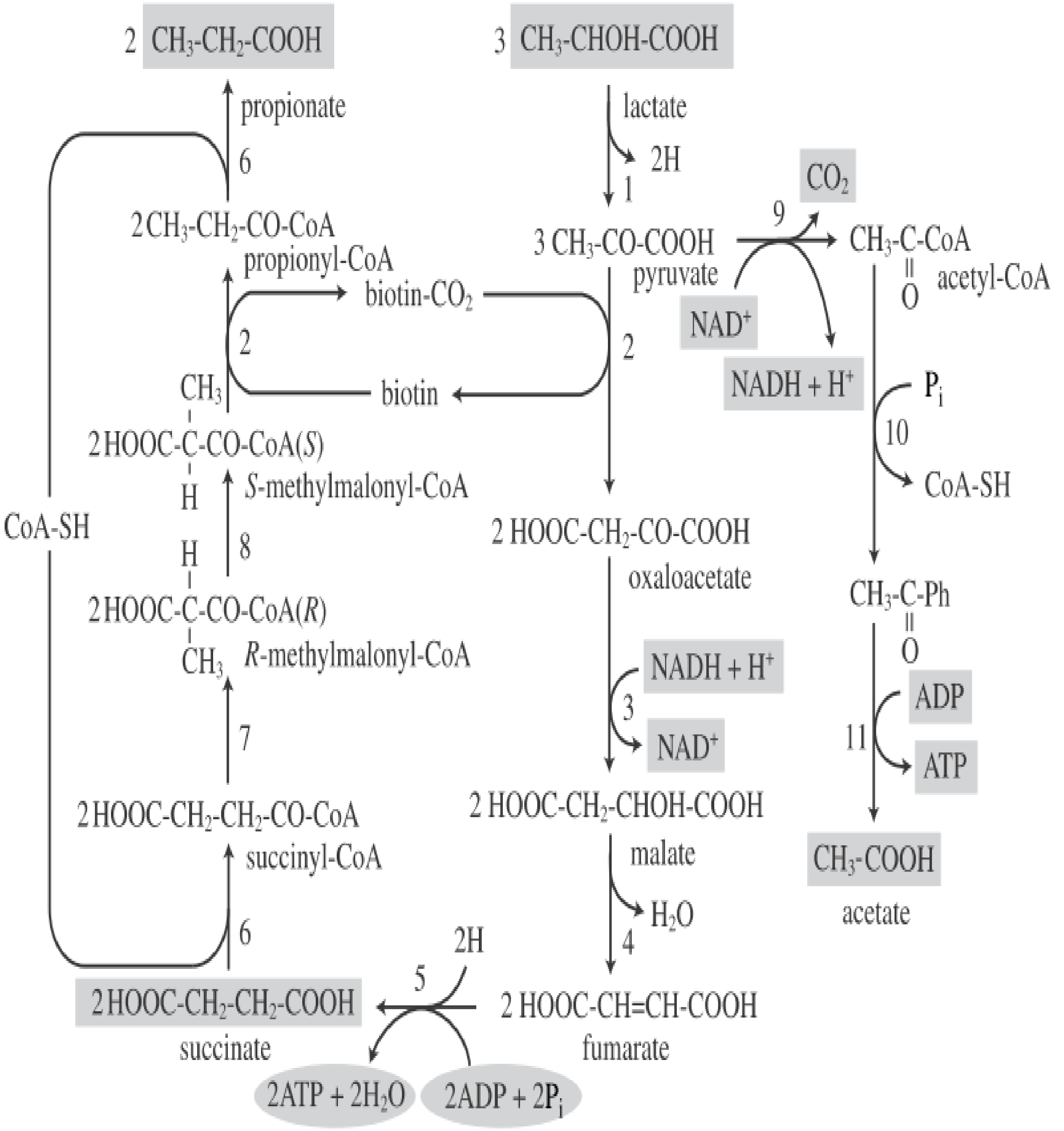

Figure 23: Propionic fermentation via the Succinate–Propionate pathway in the genus *Propionibacterium.*

[(1) Lactate dehydrogenase; (2) Methylmalonyl-CoA:pyruvate transcarboxylase; (3) Malate dehydrogenase; (4) Fumarase; (5) Fumarate reductase; (6) Coenzyme A transferase; (7) Methylmalonyl-CoA mutase; (8) Methylmalonyl-CoA racemase; (9) Pyruvate dehydrogenase; (10) Phosphotransacetylase; (11) Acetate kinase].

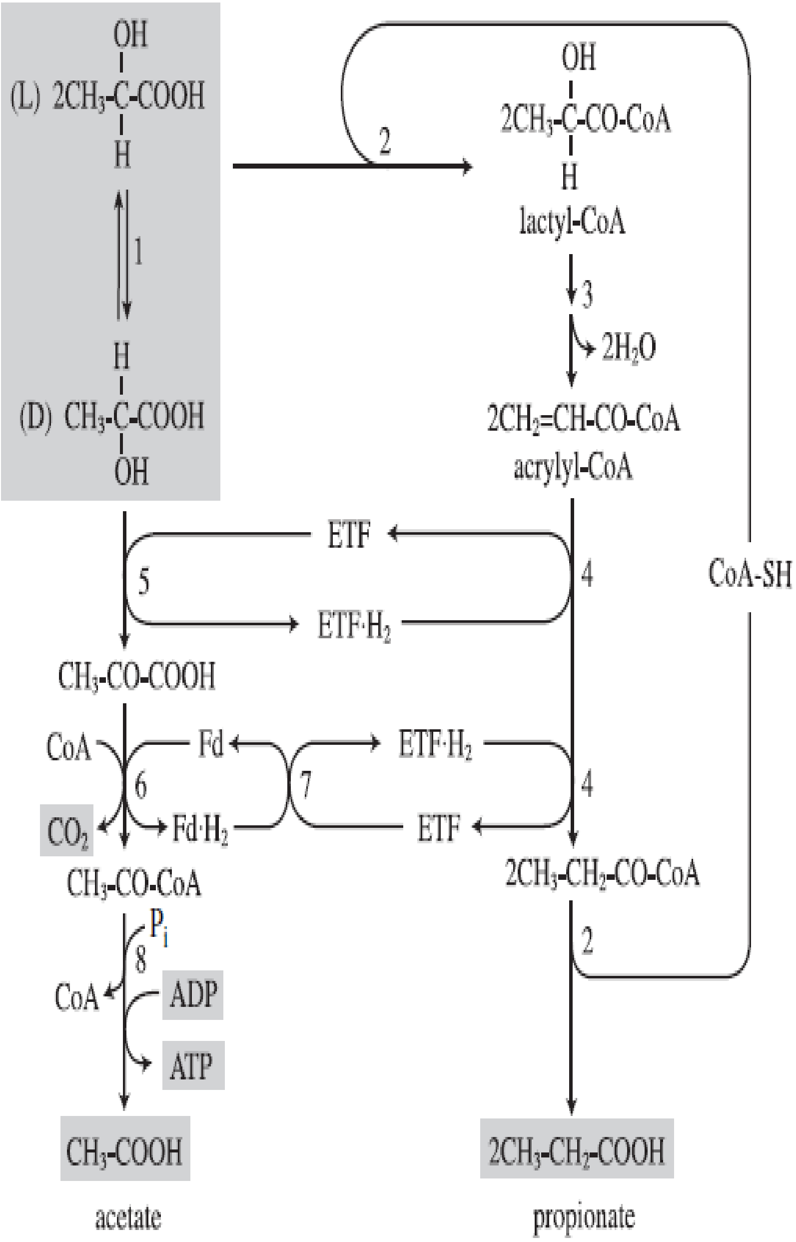

Figure 8.18 Acrylate pathway in *Megasphaera elsdenii* and *Clostridium propionicum* fermenting lactate to propionate.

(Gottschalk, G. 1986, *Bacterial Metabolism*, 2nd edn., Figure 8.19. Springer, New York)

1, lactate racemase; 2, coenzyme A transferase; 3, lactyl-CoA dehydratase; 4, acrylyl-CoA reductase; 5, lactate dehydrogenase;
6, pyruvate:ferredoxin oxidoreductase;
7, transhydrogenase;
8, phosphotransacetylase and acetate kinase.

ETF, unknown electron transfer factor, probably NAD^+.

Figure 24: Acrylate pathway in *Megasphaera elsdenii* and *Clostridium propionicum*, fermenting lactate into propionate.