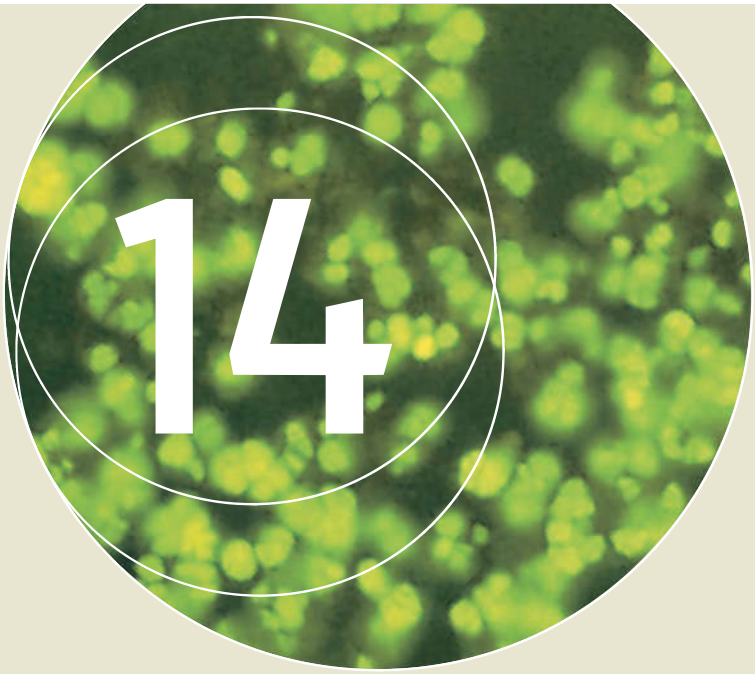

14

Catabolism of Organic Compounds

Methanogens produce natural gas (methane, CH_4) and are able to do so because they contain a series of unusual coenzymes, such as the green-fluorescing F_{420}, that participate in biochemical reactions unique to these organisms.

In Chapter 13 we considered phototrophy and chemolithotrophy, strategies for energy conservation that do not use organic compounds as electron donors. In this chapter we focus on organic compounds as electron donors and the many ways in which chemoorganotrophic microorganisms conserve energy. A major focus will be on *anaerobic* forms of metabolism, because novel strategies for anaerobic growth are a hallmark of prokaryotic diversity. We end the chapter with a consideration of the aerobic catabolism of key organic compounds, primarily monomers released from the degradation of macromolecules.

I Fermentations

Two broad metabolic processes for the catabolism of organic compounds are *fermentation* and *respiration*. These processes differ fundamentally in terms of oxidation–reduction (redox) considerations and mechanism of ATP synthesis. In respiration, whether aerobic or anaerobic, exogenous electron acceptors are required to accept electrons generated from the oxidation of electron donors. In fermentation, this is not the case. Thus in respiration but not fermentation we will see a common theme of electron transport and the generation of a proton motive force.

We begin our exploration of organic catabolism with fermentations. Compared with respirations, fermentations are typically energetically marginal. However, we will see that a little free energy can go a long way and that bacterial fermentative diversity is both extensive and innovative.

14.1 Energetic and Redox Considerations

Many microbial habitats are **anoxic** (oxygen-free). In such environments, decomposition of organic material occurs anaerobically. If adequate supplies of electron acceptors such as sulfate (SO_4^{2-}), nitrate (NO_3^-), ferric iron (Fe^{3+}), and others to be considered later are unavailable in anoxic habitats, organic compounds are catabolized by **fermentation** (Figure 14.1). In Chapter 4 we discussed some key fermentations that yield alcohol or lactic acid as products by way of the glycolytic pathway. There we emphasized how fermentations are internally balanced redox processes in which the fermentable substrate becomes both oxidized and reduced.

An organism faces two major problems when it catabolizes organic compounds for the purpose of energy conservation: (1) ATP synthesis, and (2) redox balance. In fermentations, with rare exception ATP is synthesized by *substrate-level phosphorylation*. This is the mechanism in which energy-rich phosphate bonds from phosphorylated organic compounds are transferred directly to ADP to form ATP (↻ Section 4.7). The second problem, redox balance, is solved by the production and subsequent excretion of fermentation products generated from the original substrate (Figure 14.1).

Energy-Rich Compounds and Substrate-Level Phosphorylation

Energy can be conserved by substrate-level phosphorylation from many different compounds. However, central to an understanding of substrate-level phosphorylation is the concept of

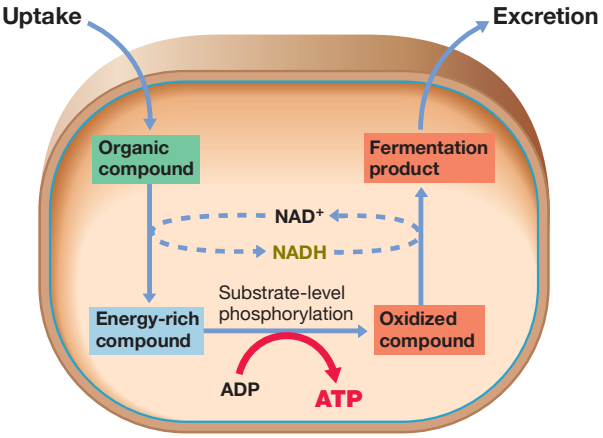

Figure 14.1 The essentials of fermentation. The fermentation product is excreted from the cell, and only a relatively small amount of the original organic compound is used for biosynthesis.

energy-rich compounds. These are organic compounds that contain an energy-rich phosphate bond or a molecule of coenzyme A; the hydrolysis of either of these is highly exergonic (↻ Figure 4.12). Table 14.1 lists some energy-rich intermediates formed during biochemical processes. The hydrolysis of most of the compounds listed can be coupled to ATP synthesis ($\Delta G^{0\prime} = -31.8$ kJ/mol). In other words, if an organism can form one of

Table 14.1 *Energy-rich compounds involved in substrate-level phosphorylation*[a]

Compound	*Free energy of hydrolysis, $\Delta G^{0\prime}$ (kJ/mol)*[b]
Acetyl-CoA	−35.7
Propionyl-CoA	−35.6
Butyryl-CoA	−35.6
Caproyl-CoA	−35.6
Succinyl-CoA	−35.1
Acetyl phosphate	−44.8
Butyryl phosphate	−44.8
1,3-Bisphosphoglycerate	−51.9
Carbamyl phosphate	−39.3
Phosphoenolpyruvate	−51.6
Adenosine phosphosulfate (APS)	−88
N^{10}-Formyltetrahydrofolate	−23.4
Energy of hydrolysis of ATP (ATP → ADP + P_i)	−31.8

[a]Data from Thauer, R. K., K. Jungermann, and K. Decker, 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev. 41:* 100–180.
[b]The $\Delta G^{0\prime}$ values shown here are for "standard conditions," which are not necessarily those of cells. Including heat loss, the energy costs of making an ATP are more like 60 kJ than 32 kJ, and the energy of hydrolysis of the energy-rich compounds shown here is thus likely higher. But for simplicity and comparative purposes, the values in this table will be taken as the actual energy released per reaction.

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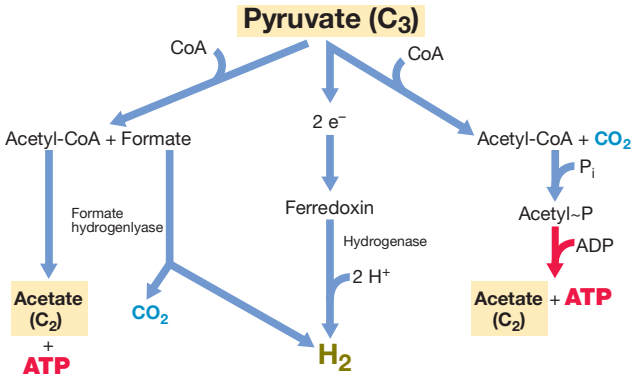


Figure 14.2 Production of H_2 and acetate from pyruvate. At least two mechanisms are known, one that produces H_2 directly and the other that makes formate as an intermediate. When acetate is produced, ATP synthesis is possible (Table 14.1).

these compounds during fermentative metabolism, it can make ATP by substrate-level phosphorylation.

Redox Balance, H_2, and Acetate Production

In any fermentation there must be atomic and redox balance; the total number of each type of atom and electrons in the products of the reaction must balance those in the substrates. This is obtained by the production and excretion from the cell of fermentation products (Figure 14.1). In several fermentations, redox balance is maintained by the production of molecular hydrogen, H_2. The production of H_2 is associated with the activity of the iron–sulfur protein ferredoxin, a very low-potential electron carrier, and is catalyzed by the enzyme **hydrogenase**, as illustrated in **Figure 14.2**. H_2 can also be produced from the C_1 fatty acid formate. Either way, the H_2 is then made available for use by other organisms.

Many anaerobic bacteria produce acetate as a major or minor fermentation product. The production of acetate and certain other fatty acids (Table 14.1) is energy conserving because it allows the organism to make ATP by substrate-level phosphorylation. The key intermediate generated in acetate production is acetyl-CoA (Table 14.1), an energy-rich compound. Acetyl-CoA can be converted to acetyl phosphate (Table 14.1) and the phosphate group of acetyl phosphate subsequently transferred to ADP, yielding ATP. One of the precursors of acetyl-CoA is pyruvate, a major product of glycolysis. The conversion of pyruvate to acetyl-CoA is a key oxidation reaction, and the electrons generated are used to form fermentation products or are released as H_2 (Figure 14.2).

MiniQuiz

- What is substrate-level phosphorylation?
- Why is acetate formation in fermentation energetically beneficial?

14.2 Lactic and Mixed-Acid Fermentations

Many fermentations are classified by either the substrate fermented or the products formed. **Table 14.2** lists some of the major fermentations classified on the basis of products formed. Note some of the broad categories, such as alcohol, lactic acid, propionic acid, mixed acid, butyric acid, and acetogenic. Some fermentations are described by the substrate fermented rather than the fermentation product. For instance, some endospore-forming anaerobic bacteria (genus *Clostridium*) ferment amino acids, whereas others ferment purines and pyrimidines. Other anaerobes ferment aromatic compounds (**Table 14.3**). Clearly, a wide variety of organic compounds can be fermented. Certain fermentations are carried out by only a very restricted group of anaerobes; in some cases this may be only a single known

Table 14.2 *Common bacterial fermentations and some of the organisms carrying them out*

Type	*Reaction*	*Organisms*
Alcoholic	Hexose → 2 ethanol + 2 CO_2	Yeast, *Zymomonas*
Homolactic	Hexose → 2 lactate$^-$ + 2 H^+	*Streptococcus*, some *Lactobacillus*
Heterolactic	Hexose → lactate$^-$ + ethanol + CO_2 + H^+	*Leuconostoc*, some *Lactobacillus*
Propionic acid	3 Lactate$^-$ → 2 propionate$^-$ + acetate$^-$ + CO_2 + H_2O	*Propionibacterium, Clostridium propionicum*
Mixed acid[a,b]	Hexose → ethanol + 2,3-butanediol + succinate^{2-} + lactate$^-$ + acetate$^-$ + formate$^-$ + H_2 + CO_2	Enteric bacteria including *Escherichia, Salmonella, Shigella, Klebsiella, Enterobacter*
Butyric acid[b]	Hexose → butyrate$^-$ + 2 H_2 + 2 CO_2 + H^+	*Clostridium butyricum*
Butanol[b]	2 Hexose → butanol + acetone + 5 CO_2 + 4 H_2	*Clostridium acetobutylicum*
Caproate/Butyrate	6 Ethanol + 3 acetate$^-$ → 3 butyrate$^-$ + caproate$^-$ + 2 H_2 + 4 H_2O + H^+	*Clostridium kluyveri*
Acetogenic	Fructose → 3 acetate$^-$ + 3 H^+	*Clostridium aceticum*

[a]Not all organisms produce all products. In particular, butanediol production is limited to only certain enteric bacteria. Reaction not balanced.
[b]Stoichiometry shows major products. Other products include some acetate and a small amount of ethanol (butanol fermention only).

Table 14.3 *Some unusual bacterial fermentations*

Type	*Reaction*	*Organisms*
Acetylene	$2\ C_2H_2 + 3\ H_2O \rightarrow$ ethanol + acetate$^-$ + H^+	*Pelobacter acetylenicus*
Glycerol	4 Glycerol + 2 $HCO_3^- \rightarrow$ 7 acetate$^-$ + 5 H^+ + 4 H_2O	*Acetobacterium* spp.
Resorcinol (aromatic)	$2\ C_6H_4(OH)_2 + 6\ H_2O \rightarrow$ 4 acetate$^-$ + butyrate$^-$ + 5 H^+	*Clostridium* spp.
Phloroglucinol (aromatic)	$C_6H_6O_3 + 3\ H_2O \rightarrow$ 3 acetate$^-$ + 3 H^+	*Pelobacter massiliensis* *Pelobacter acidigallici*
Putrescine	$10\ C_4H_{12}N_2 + 26\ H_2O \rightarrow$ 6 acetate$^-$ + 7 butyrate$^-$ + 20 NH_4^+ + 16 H_2 + 13 H^+	Unclassified gram-positive nonsporulating anaerobes
Citrate	Citrate^{3-} + 2 $H_2O \rightarrow$ formate$^-$ + 2 acetate$^-$ + HCO_3^- + H^+	*Bacteroides* spp.
Aconitate	Aconitate^{3-} + H^+ + 2 $H_2O \rightarrow$ 2 CO_2 + 2 acetate$^-$ + H_2	*Acidaminococcus fermentans*
Glyoxylate	4 Glyoxylate$^-$ + 3 H^+ + 3 $H_2O \rightarrow$ 6 CO_2 + 5 H_2 + glycolate$^-$	Unclassified gram-negative bacterium
Benzoate	2 Benzoate$^- \rightarrow$ cyclohexane carboxylate$^-$ + 3 acetate$^-$ + HCO_3^- + 3 H^+	*Syntrophus aciditrophicus*

bacterium. A few examples are listed in Table 14.3. Many of these bacteria can be considered metabolic specialists, having evolved the capacity to catabolize a substrate not catabolized by other bacteria.

We begin with two very common fermentations of sugars in which lactic acid is a major product.

Lactic Acid Fermentation

The lactic acid bacteria are gram-positive organisms that produce lactic acid as a major or sole fermentation product (↻ Section 18.1). Two fermentative patterns are observed. One, called **homofermentative**, yields a single fermentation product, lactic acid. The other, called **heterofermentative**, yields products in addition to lactate, mainly ethanol plus CO_2.

Figure 14.3 summarizes pathways for the fermentation of glucose by homofermentative and heterofermentative lactic acid bacteria. The differences observed can be traced to the presence or absence of the enzyme *aldolase*, a key enzyme of glycolysis (↻ Figure 4.14). Homofermentative lactic acid bacteria contain aldolase and produce two molecules of lactate from glucose by the glycolytic pathway (Figure 14.3*a*). Heterofermenters lack aldolase and thus cannot break down fructose bisphosphate to triose phosphate. Instead, they oxidize glucose 6-phosphate to 6-phosphogluconate and then decarboxylate this to pentose phosphate. The pentose phosphate is then converted to triose phosphate and acetyl phosphate by the key enzyme *phosphoketolase* (Figure 14.3*b*). The early steps in catabolism by heterofermentative lactic acid bacteria are those of the pentose phosphate pathway (see Figure 14.38).

In heterofermenters, triose phosphate is converted to lactic acid with the production of ATP (Figure 14.3). However, to achieve redox balance the acetyl phosphate produced is used as an electron acceptor and is reduced by NADH (generated during the production of pentose phosphate) to ethanol. This occurs without ATP synthesis because the energy-rich CoA bond is lost during ethanol formation. Because of this, heterofermenters produce only *one* ATP/glucose instead of the *two* ATP/glucose produced by homofermenters. In addition, because heterofermenters decarboxylate 6-phosphogluconate, they produce CO_2 as a fermentation product; homofermenters do not produce CO_2. Thus a simple way of differentiating a homofermenter from a heterofermenter is to observe for the production of CO_2 in laboratory cultures.

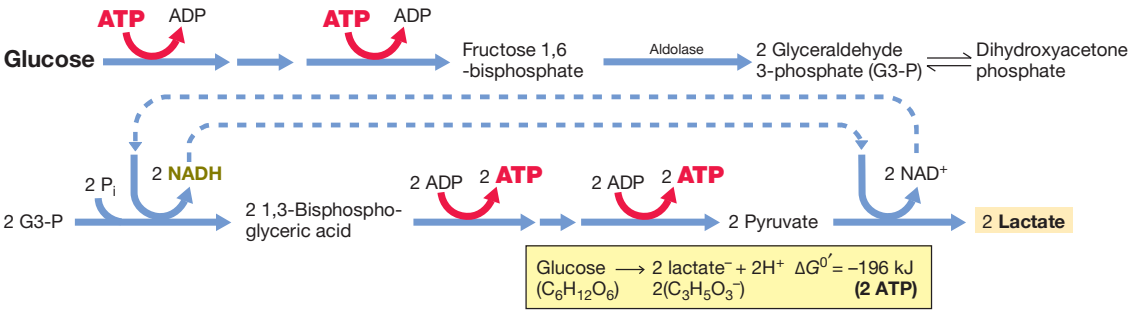
Entner–Doudoroff Pathway

A variant of the glycolytic pathway, called the *Entner–Doudoroff pathway*, is widely distributed in bacteria, especially among species of the pseudomonad group. In this pathway glucose 6-phosphate is oxidized to 6-phosphogluconic acid and NADPH; the 6-phosphogluconic acid is dehydrated and split into pyruvate and glyceraldehyde 3-phosphate (G-3-P), a key intermediate of the glycolytic pathway. G-3-P is then catabolized as in glycolysis, generating NADH and two ATP, and used as an electron acceptor to balance redox reactions (Figure 14.3*a*).

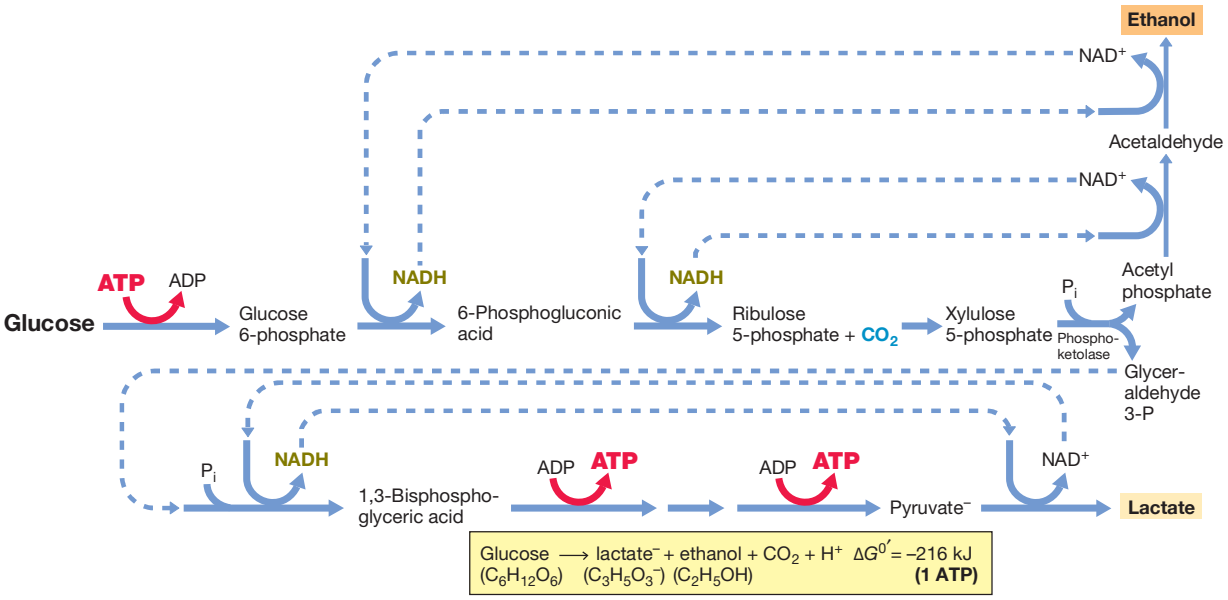
Because pyruvate is formed directly in the Entner–Doudoroff pathway and cannot yield ATP as can G-3-P (Figure 14.3), the Entner–Doudoroff pathway yields only half the ATP of the glycolytic pathway. Organisms using the Entner–Doudoroff pathway therefore share this physiological characteristic with heterofermentative lactic acid bacteria that also use a variant of the glycolytic pathway (Figure 14.3*b*). *Zymomonas*, an obligately fermentative pseudomonad, and *Pseudomonas*, a nonfermentative respiratory bacterium, are major genera that employ the Entner–Doudoroff pathway (↻ Section 17.7).

Mixed-Acid Fermentations

In mixed-acid fermentations, characteristic of enteric bacteria (↻ Section 17.11), three different acids are formed from the fermentation of glucose or other sugars—*acetic, lactic*, and *succinic*. Ethanol, CO_2, and H_2 are also formed. Glycolysis is the pathway used by mixed-acid fermenters, such as *Escherichia coli*, and we outlined the steps in that pathway in Figure 4.14.



(a) **Homofermentative**



(b) **Heterofermentative**

Figure 14.3 **The fermentation of glucose in *(a)* homofermentative and *(b)* heterofermentative lactic acid bacteria.** Note that no ATP is made in reactions leading to ethanol formation in heterofermentative organisms.

Some enteric bacteria produce acidic products in lower amounts than *E. coli* and balance redox in their fermentations by producing larger amounts of neutral products. One key neutral product is the four-carbon alcohol *butanediol*. In this variation of the mixed-acid fermentation, butanediol, ethanol, CO_2, and H_2 are the main products observed (**Figure 14.4**). In the mixed-acid fermentation of *E. coli*, equal amounts of CO_2 and H_2 are produced, whereas in a butanediol fermentation, considerably more CO_2 than H_2 is produced. This is because mixed-acid fermenters produce CO_2 only from formic acid by means of the enzyme formate hydrogenlyase (Figure 14.2):

$$HCOOH \rightarrow H_2 + CO_2$$

By contrast, butanediol producers, such as *Enterobacter aerogenes*, produce CO_2 and H_2 from formic acid but also produce two additional molecules of CO_2 during the formation of each molecule of butanediol (Figure 14.4).

Because they produce fewer acidic products, butanediol fermenters do not acidify their environment as much as mixed-acid fermenters do, and this is presumably a reflection of differences in acid tolerance in the two groups that have significance for their competitive success in nature.

MiniQuiz

- How can homo- and heterofermentative lactic acid bacteria be differentiated in pure cultures?
- Butanediol production leads to greater ethanol production than in the mixed-acid fermentation of *Escherichia coli*. Why?

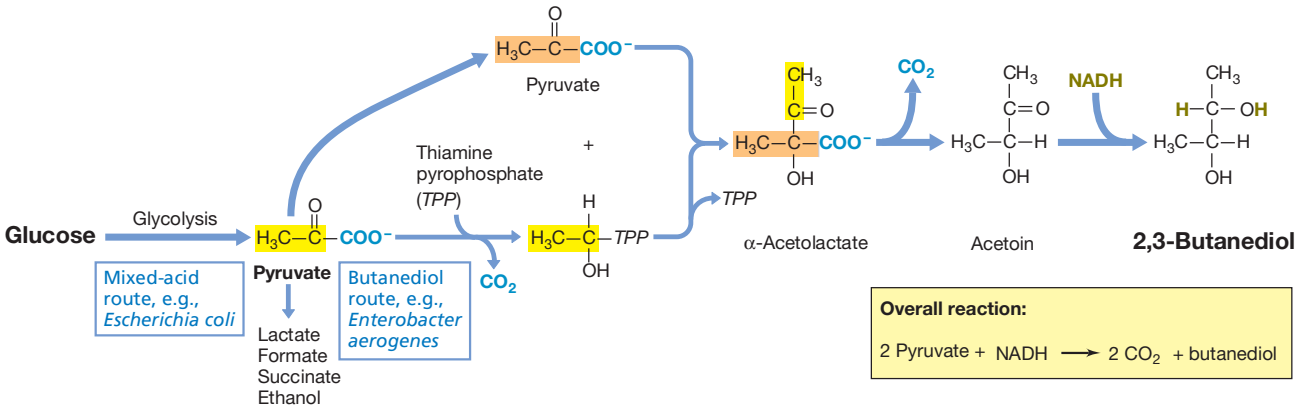


Figure 14.4 **Butanediol production and mixed-acid fermentations.** Note how only one NADH, but two molecules of pyruvate, are used to make one butanediol. This leads to redox imbalance and the production of more ethanol by butanediol producers than by mixed-acid fermenters.

14.3 Clostridial and Propionic Acid Fermentations

Species of the genus *Clostridium* are classical fermentative anaerobes (↻ Section 18.2). Different clostridia ferment sugars, amino acids, purines and pyrimidines, and a few other compounds. In all cases ATP synthesis is linked to substrate-level phosphorylations either in the glycolytic pathway or from the hydrolysis of a CoA intermediate (Table 14.1). We begin with sugar-fermenting (saccharolytic) clostridia.

Sugar Fermentation by *Clostridium* Species

A number of clostridia ferment sugars, producing *butyric acid* as a major end product. Some species also produce the neutral products acetone and butanol, and *Clostridium acetobutylicum* is a classic example of this. The biochemical steps in the formation of butyric acid and neutral products from sugars are shown in Figure 14.5.

Glucose is converted to pyruvate via the glycolytic pathway, and pyruvate is split to yield acetyl-CoA, CO_2, and H_2 (through ferredoxin) by the phosphoroclastic reaction (Figure 14.2). Some of the acetyl-CoA is then reduced to butyrate or other fermentation products using NADH derived from glycolytic reactions as electron donor. The actual products observed are influenced by the duration and the conditions of the fermentation. During the early stages of the butyric fermentation, butyrate and a small amount of acetate are produced. But as the pH of the medium drops, synthesis of acids ceases and acetone and butanol begin to accumulate. However, if the pH of the medium is kept neutral by buffering, there is very little formation of neutral products and butyric acid production continues.

The accumulation of acidic products in the *C. acetobutylicum* fermentation lowers the pH, and this triggers derepression of genes responsible for solvent production. The production of butanol is actually a consequence of the production of acetone. For each acetone that is made, two NADH produced during glycolysis are not reoxidized as they would be if butyrate were produced (Figure 14.5). Because redox balance is necessary for any

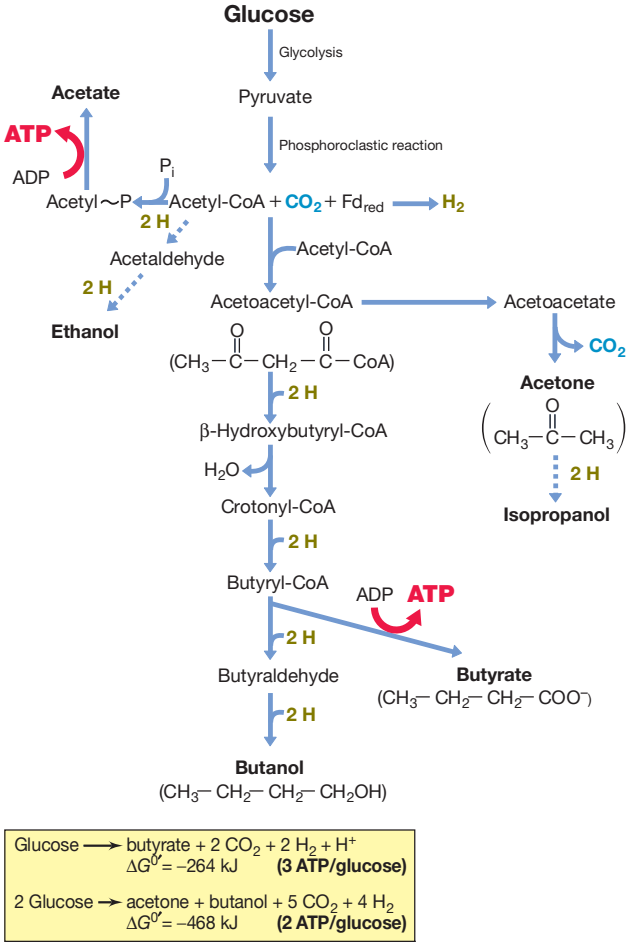


Figure 14.5 **The butyric acid and butanol/acetone fermentation.** All fermentation products from glucose are shown in bold (dashed lines indicate minor products). Note how the production of acetate and butyrate lead to additional ATP by substrate-level phosphorylation. By contrast, formation of butanol and acetone reduces the ATP yield because the butyryl-CoA step is bypassed. 2 H, NADH; Fd_{red}, reduced ferredoxin.

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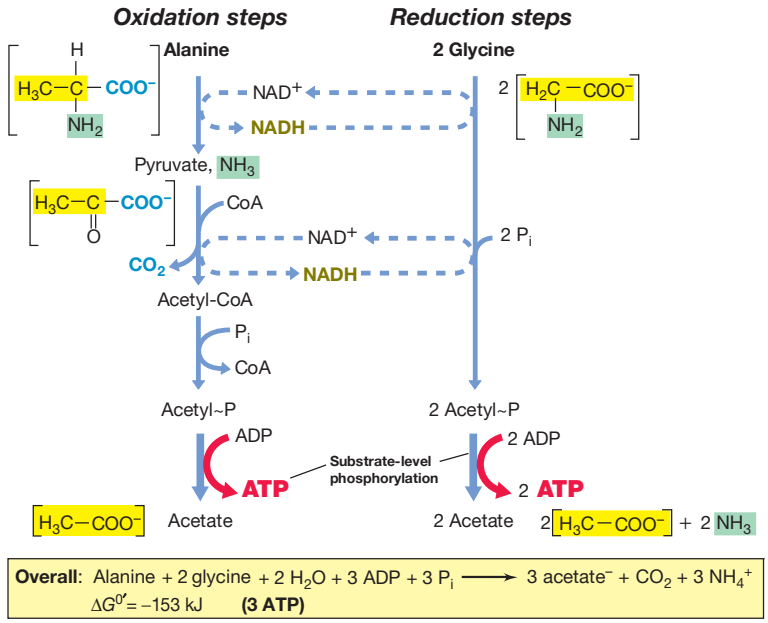

Amino acids participating in coupled fermentations (Stickland reaction)	
Amino acids oxidized:	**Amino acids reduced:**
Alanine	Glycine
Leucine	Proline
Isoleucine	Hydroxyproline
Valine	Tryptophan
Histidine	Arginine

Figure 14.6 **The Stickland reaction.** This example shows the cocatabolism of the amino acids alanine and glycine. The structures of key substrates, intermediates, and products are shown in brackets to allow the chemistry of the reaction to be followed. Note how in the reaction shown, alanine is the electron donor and glycine is the electron acceptor.

fermentation to proceed, the cell then uses butyrate as an electron acceptor. Butanol and acetone are therefore produced in equal amounts. Although neutral product formation helps the organism keep its environment from becoming too acidic, there is an energetic price to pay for this. In producing butanol, the cell loses the opportunity to convert butyryl-CoA to butyrate and thus ATP (Figure 14.5 and Table 14.1).

Amino Acid Fermentation by *Clostridium* Species and the Stickland Reaction

Some *Clostridium* species ferment amino acids. These are the "proteolytic" clostridia, organisms that degrade proteins released from dead organisms in nature. Some clostridia ferment individual amino acids, typically glutamate, glycine, alanine, cysteine, histidine, serine, or threonine. The biochemistry behind these fermentations is quite complex, but the metabolic strategy is quite simple. In virtually all cases, the amino acids are catabolized in such a way as to yield a fatty acid–CoA derivative, typically acetyl (C_2), butyryl (C_4), or caproyl (C_6). From these, ATP is produced by substrate-level phosphorylation (Table 14.1). Other products of amino acid fermentation include ammonia (NH_3) and CO_2.

Some clostridia ferment only an amino acid *pair*. In this situation one amino acid functions as the electron donor and is oxidized, whereas the other amino acid is the electron acceptor and is reduced. This coupled amino acid fermentation is known as a **Stickland reaction**. For instance, *Clostridium sporogenes* catabolizes a mixture of glycine and alanine; in this reaction alanine is the electron donor and glycine is the electron acceptor (**Figure 14.6**). Amino acids that can function as donors or acceptors in Stickland reactions are listed in Figure 14.6. The products of the Stickland reaction are NH_3, CO_2, and a carboxylic acid with one fewer carbons than the amino acid that was oxidized (Figure 14.6).

Many of the products of amino acid fermentation by clostridia are foul-smelling substances, and the odor that results from putrefaction is mainly a result of clostridial activity. In addition to fatty acids, other odoriferous compounds produced include hydrogen sulfide (H_2S), methylmercaptan (from sulfur amino acids), cadaverine (from lysine), putrescine (from ornithine), and NH_3. Purines and pyrimidines, released from the degradation of nucleic acids, lead to many of the same fermentation products and yield ATP from the hydrolysis of fatty acid–CoA derivatives (Table 14.1) produced in their respective fermentative pathway.

Clostridium kluyveri Fermentation

Another species of *Clostridium* also ferments a mixture of substrates in which one is the donor and one is the acceptor, as in the Stickland reaction. However, this organism, *C. kluyveri*, ferments not amino acids but instead ethanol plus acetate. In this fermentation, ethanol is the electron donor and acetate is the electron acceptor. The overall reaction is shown in Table 14.2.

The ATP yield in the caproate/butyrate fermentation is low, 1 ATP/6 ethanol fermented. However, *C. kluyveri* has a selective advantage over all other fermenters in its apparently unique ability to oxidize a highly reduced fermentation product (ethanol) and couple it to the reduction of another common fermentation product (acetate), reducing it to longer-chain fatty acids. The single ATP produced in this reaction comes from substrate-level phosphorylation during conversion of a fatty acid–CoA formed in the pathway to the free fatty acid. The fermentation of *C. kluyveri* is an example of a **secondary fermentation**, which is essentially a fermentation of fermentation products. We see another example of this now.

Propionic Acid Fermentation

The propionic acid bacterium *Propionibacterium* and some related bacteria produce *propionic acid* as a major fermentation product from either glucose or lactate. However, lactate, a fermentation product of the lactic acid bacteria, is probably the major substrate for propionic acid bacteria in nature, where these two groups live in close association. *Propionibacterium* is an important component in the ripening of Swiss (Emmentaler) cheese, to which the propionic and acetic acids produced give the unique bitter and nutty taste, and the CO_2 produced forms bubbles that leave the characteristic holes (eyes) in the cheese.

Figure 14.7 shows the reactions leading from lactate to propionate. When glucose is the starting substrate, it is first catabolized to pyruvate by the glycolytic pathway. Then pyruvate, produced either from glucose or from the oxidation of lactate, is carboxylated to form methylmalonyl-CoA, leading to the formation of oxalacetate and, eventually, propionyl-CoA (Figure 14.7). The latter reacts with succinate in a step catalyzed by the enzyme CoA transferase, producing succinyl-CoA and propionate. This results in a lost opportunity for ATP production from propionyl-CoA but avoids the energetic costs of having to activate succinate with ATP to form succinyl-CoA. The succinyl-CoA is then isomerized to methylmalonyl-CoA and the cycle is complete; propionate is formed and CO_2 regenerated (Figure 14.7).

NADH is oxidized in the steps between oxalacetate and succinate. Notably, the reaction in which fumarate is reduced to succinate is linked to electron transport and the formation of a proton motive force that yields ATP by oxidative phosphorylation. The propionate pathway also converts some lactate to acetate plus CO_2, which allows for additional ATP to be made (Figure 14.7). Thus, in the propionate fermentation both substrate-level *and* oxidative phosphorylation occur.

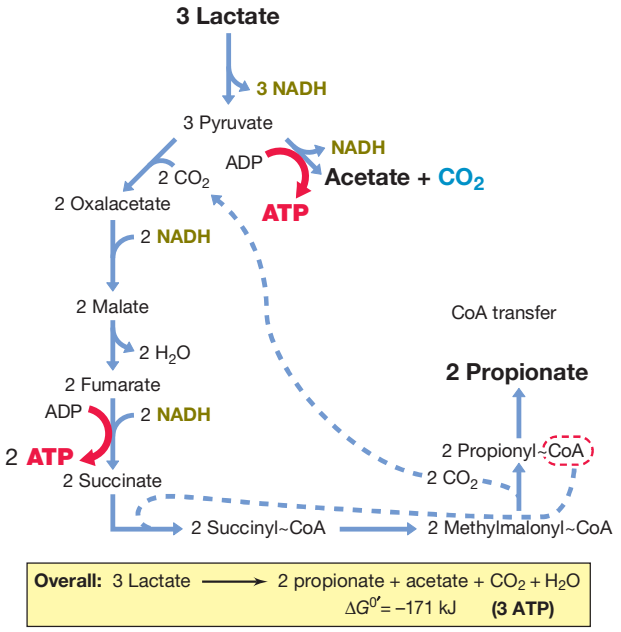

Figure 14.7 The propionic acid fermentation of *Propionibacterium*. Products are shown in bold. The four NADH made from the oxidation of three lactate are reoxidized in the reduction of oxalacetate and fumarate, and the CoA group from propionyl-CoA is exchanged with succinate during the formation of propionate.

Propionate is also formed in the fermentation of succinate by the bacterium *Propionigenium*, but by a completely different mechanism than that described here for *Propionibacterium*. *Propionigenium*, to be considered next, is phylogenetically and ecologically unrelated to *Propionibacterium*, but energetic aspects of its metabolism are of considerable interest from the standpoint of bioenergetics.

MiniQuiz

- Compare the mechanisms for energy conservation in *Clostridium acetobutylicum* and *Propionibacterium*.
- What are the substrates for the *Clostridium kluyveri* fermentation? In nature, where do these come from?

14.4 Fermentations Lacking Substrate-Level Phosphorylation

Certain fermentations yield insufficient energy to synthesize ATP by substrate-level phosphorylation (that is, less than −32 kJ, Table 14.1), yet still support growth. In these cases, catabolism of the compound is linked to ion pumps that establish a proton motive force or sodium motive force across the cytoplasmic membrane. Examples of this include fermentation of succinate by *Propionigenium modestum* and the fermentation of oxalate by *Oxalobacter formigenes*.

Propionigenium modestum

Propionigenium modestum was first isolated in anoxic enrichment cultures lacking alternative electron acceptors and fed succinate as an electron donor. *Propionigenium* inhabits marine and freshwater sediments, and can also be isolated from the human oral cavity. The organism is a gram-negative short rod and, phylogenetically, is a species of *Actinobacteria* (Section 18.4). During studies of the physiology of *P. modestum*, it was shown to require sodium chloride (NaCl) for growth and to catabolize succinate under strictly anoxic conditions:

$$\text{Succinate}^{2-} + H_2O \rightarrow \text{propionate}^{-} + HCO_3^{-}$$

$$\Delta G^{0\prime} = -20.5 \text{ kJ}$$

This decarboxylation releases insufficient free energy to support ATP synthesis by substrate-level phosphorylation (Table 14.1) but sufficient free energy to pump a sodium ion (Na^+) across the cytoplasmic membrane from the cytoplasm to the periplasm. Energy conservation in *Propionigenium* is then linked to the sodium motive force that develops from Na^+ pumping; a sodium-translocating ATPase exists that uses the sodium motive force to drive ATP synthesis (**Figure 14.8*a***).

In a related decarboxylation reaction, the bacterium *Malonomonas*, a species of *Deltaproteobacteria*, decarboxylates the C_3 dicarboxylic acid malonate, forming acetate plus CO_2. As for

UNIT 5

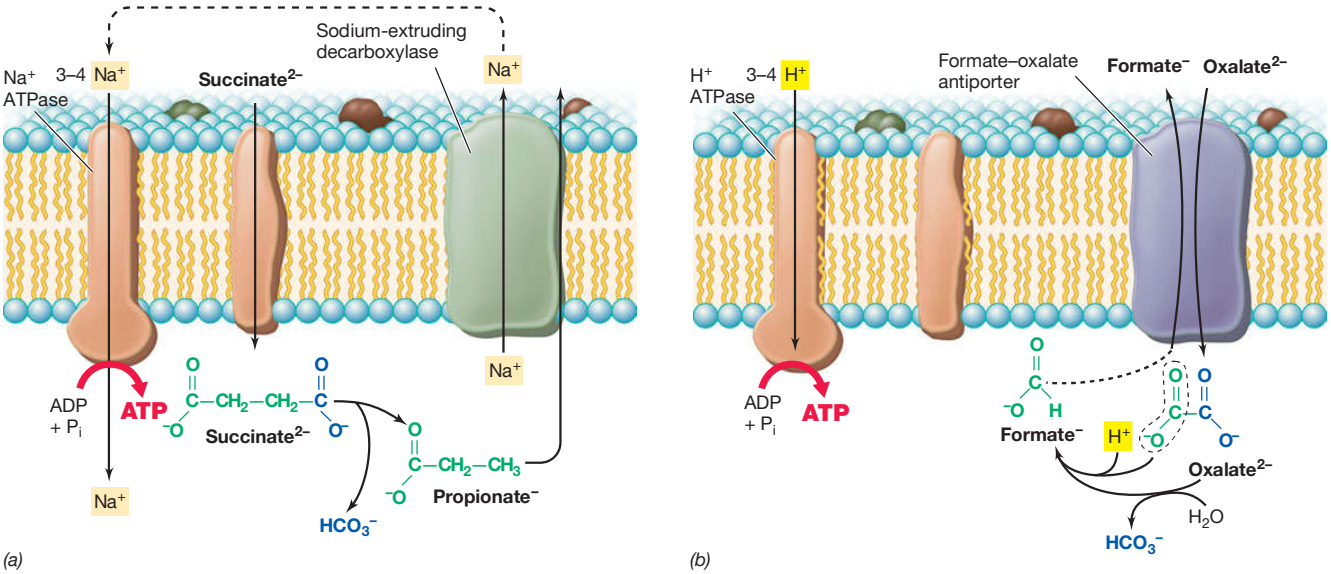

Figure 14.8 **The unique fermentations of succinate and oxalate.** *(a)* Succinate fermentation by *Propionigenium modestum*. Sodium export is linked to the energy released by succinate decarboxylation, and a sodium-translocating ATPase produces ATP. *(b)* Oxalate fermentation by *Oxalobacter formigenes*. Oxalate import and formate export by a formate–oxalate antiporter consume cytoplasmic protons. ATP synthesis is linked to a proton-driven ATPase. All substrates and products are shown in bold.

Propionigenium, energy metabolism in *Malonomonas* is linked to a sodium pump and sodium-driven ATPase. However, the mechanism of malonate decarboxylation is more complex than that of *Propionigenium* and involves many additional proteins. Interestingly, however, the energy yield of malonate fermentation by *Malonomonas* is even lower than that of *P. modestum*, −17.4 kJ. *Sporomusa*, an endospore-forming bacterium (↻ Section 18.2) and also an acetogen (Section 14.9), is also capable of fermenting malonate, as are a few other *Bacteria*.

Oxalobacter formigenes

Oxalobacter formigenes is a bacterium present in the intestinal tract of animals, including humans. It catabolizes the C_2 dicarboxylic acid oxalate, producing formate plus CO_2. Oxalate degradation by *O. formigenes* is thought to be important in humans for preventing the accumulation of oxalate in the body, a substance that can accumulate to form calcium oxalate kidney stones. *O. formigenes* is a gram-negative strict anaerobe that is a species of *Betaproteobacteria*. *O. formigenes* carries out the following reaction:

$$\text{Oxalate}^{2-} + H_2O \rightarrow \text{formate}^{-} + HCO_3^{-} \qquad \Delta G^{0\prime} = -26.7 \text{ kJ}$$

As in the catabolism of succinate by *P. modestum*, insufficient energy is available from this reaction to drive ATP synthesis by substrate-level phosphorylation (Table 14.1). However, the reaction supports growth of the organism because the decarboxylation of oxalate is exergonic and forms formate, which is excreted from the cell. The internal consumption of protons during the oxidation of oxalate and production of formate is, in effect, a proton pump. That is, a divalent molecule (oxalate) enters the cell while a univalent molecule (formate) is excreted. The continued exchange of oxalate for formate establishes a membrane potential that is coupled to ATP synthesis by the proton-translocating ATPase in the membrane (Figure 14.8*b*).

Energetics Lessons

The unique aspect of all of these decarboxylation-type fermentations is that ATP synthesis occurs *without substrate-level phosphorylation or electron transport*. Nevertheless, ATP synthesis can occur because the small amount of energy released is coupled to the pumping of an ion across the cytoplasmic membrane. Organisms such as *Propionigenium* or *Oxalobacter* thus teach us an important lesson in microbial bioenergetics: Energy conservation from reactions that yield less than −32 kJ is still possible if the reaction is coupled to an ion pump. However, a minimal requirement for an energy-conserving reaction is that it must yield sufficient free energy to pump a single ion. This is estimated to be about −12 kJ. Theoretically, reactions that release less energy than this should not be able to drive ion pumps and should therefore not be potential energy-conserving reactions. However, as we will see in the next section, there are bacteria known that push this theoretical limit even lower and whose energetics are still incompletely understood. These are the syntrophs, prokaryotes living on the energetic "edge of existence."

MiniQuiz

- Why does *Propionigenium modestum* require sodium for growth?
- Of what benefit is the organism *Oxalobacter* to human health?

14.5 Syntrophy

There are many examples in microbiology of **syntrophy**, a metabolic process in which two different organisms cooperate to degrade a substance—and conserve energy doing it—that neither can degrade alone. Most syntrophic reactions are secondary fermentations (Section 14.3) in which organisms ferment the fermentation products of other anaerobes. We will see in Section 24.2 how syntrophy is a key to the overall success of anoxic catabolism that leads to the production of methane (CH_4). Here we consider the microbiology and energetic aspects of syntrophy.

Table 14.4 lists some of the major groups of syntrophs and the compounds they degrade. Many organic compounds can be degraded syntrophically, including even aromatic and aliphatic hydrocarbons. But the major compounds of interest in freshwater syntrophic environments are fatty acids and alcohols.

Hydrogen Consumption in Syntrophic Reactions

The heart of syntrophic reactions is *interspecies H_2 transfer*, H_2 production by one partner linked to H_2 consumption by the other. The H_2 consumer can be any one of a number of physiologically distinct organisms: denitrifying bacteria, ferric iron–reducing bacteria, sulfate-reducing bacteria, acetogens, or methanogens, groups we will consider later in this chapter. Consider ethanol fermentation to acetate plus H_2 by a syntroph coupled to the production of methane (**Figure 14.9**). As can be seen, the syntroph carries out a reaction whose standard free-energy change ($\Delta G^{0\prime}$) is positive. However, the H_2 produced by the syntroph can be used as an electron donor by a methanogen in an exergonic reaction. When the two reactions are summed, the overall reaction is exergonic (Figure 14.9), and the free energy released is shared by both organisms.

Table 14.4 *Properties of major syntrophic bacteria*[a]

Genus	Number of known species	Phylogeny[b]	Substrates fermented in coculture[c]
Syntrophobacter	4	*Deltaproteobacteria*	Propionate (C_3), lactate; some alcohols
Syntrophomonas	9	*Firmicutes*	C_4–C_{18} saturated/unsaturated fatty acids; some alcohols
Pelotomaculum	2	*Firmicutes*	Propionate, lactate, several alcohols; some aromatic compounds
Syntrophus	3	*Deltaproteobacteria*	Benzoate and several related aromatic compounds; some fatty acids and alcohols

[a]All syntrophs are obligate anaerobes.
[b]See Chapters 17 and 18.
[c]Not all species can use all substrates listed.

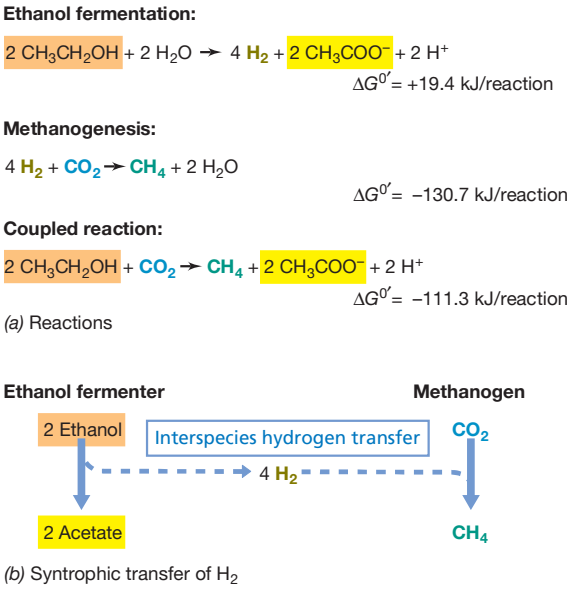

Figure 14.9 **Syntrophy: Interspecies H_2 transfer.** Shown is the fermentation of ethanol to methane and acetate by syntrophic association of an ethanol-oxidizing syntroph and a H_2-consuming partner (in this case, a methanogen). *(a)* Reactions involved. The two organisms share the energy released in the coupled reaction. *(b)* Nature of the syntrophic transfer of H_2.

Another example of syntrophy is the oxidation of a fatty acid such as butyrate to acetate plus H_2 by the fatty acid–oxidizing syntroph *Syntrophomonas* (**Figure 14.10**):

$$\text{Butyrate}^- + 2\ H_2O \rightarrow 2\ \text{acetate}^- + H^+ + 2\ H_2$$
$$\Delta G^{0\prime} = +48.2\ \text{kJ}$$

The free-energy change of this reaction is highly unfavorable, and in pure culture *Syntrophomonas* will not grow on butyrate. However, if the H_2 produced by *Syntrophomonas* is consumed by a partner organism, *Syntrophomonas* grows on butyrate in coculture with the H_2 consumer. Why is this so?

Energetics of H_2 Transfer

Because it is such a powerful electron donor for anaerobic respirations, H_2 is quickly consumed in anoxic habitats. In a syntrophic relationship, the removal of H_2 by a partner organism pulls the reaction in the direction of product formation and thereby affects the energetics of the reaction. A review of the principles of free energy given in Appendix 1 indicates that the concentration of reactants and products in a reaction can have a major effect on energetics. This is usually not an issue for most fermentation products because they are not consumed to extremely low levels. H_2, by contrast, can be consumed to nearly undetectable levels, and at these tiny concentrations, the energetics of the reactions are dramatically affected.

For convenience, the $\Delta G^{0\prime}$ of a reaction is calculated on the basis of standard conditions—one molar concentration of