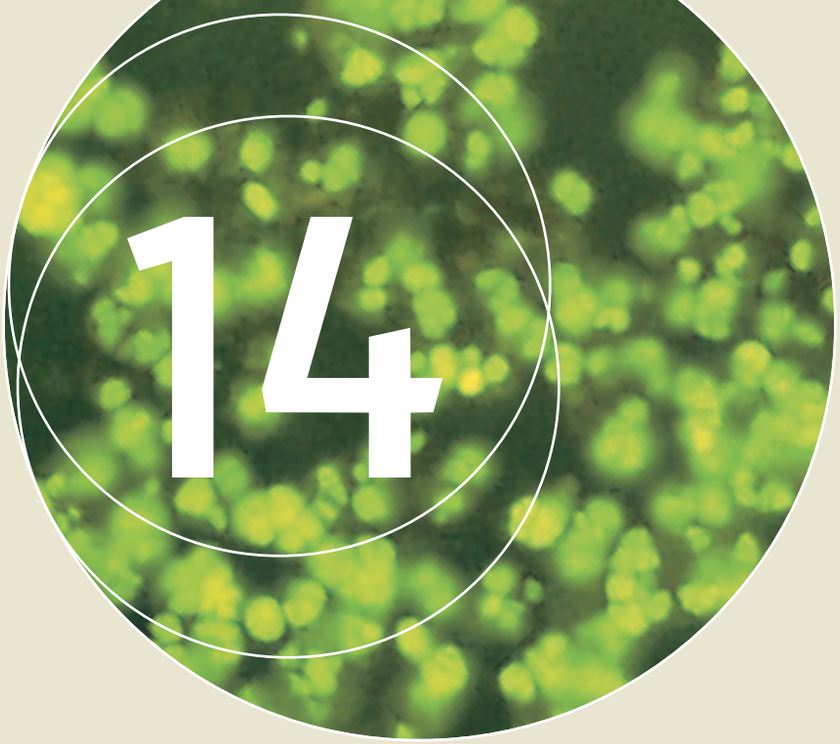

14

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.

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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.

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

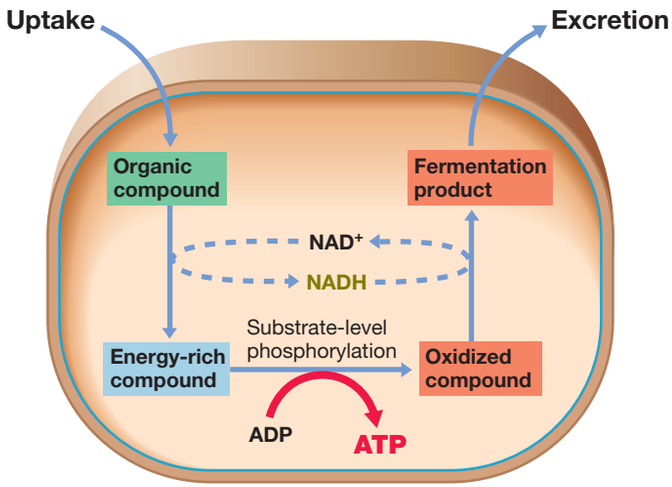


Figure 14.1 The essentials of fermentation. The fermentation product is excreted from the cell, and only a relatively small amount of the original organic compound is used for biosynthesis.

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.

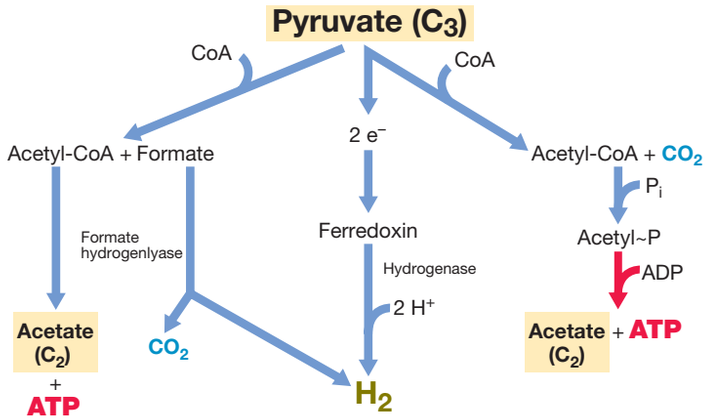


Figure 14.2 Production of H_2 and acetate from pyruvate. At least two mechanisms are known, one that produces H_2 directly and the other that makes formate as an intermediate. When acetate is produced, ATP synthesis is possible (Table 14.1).

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.

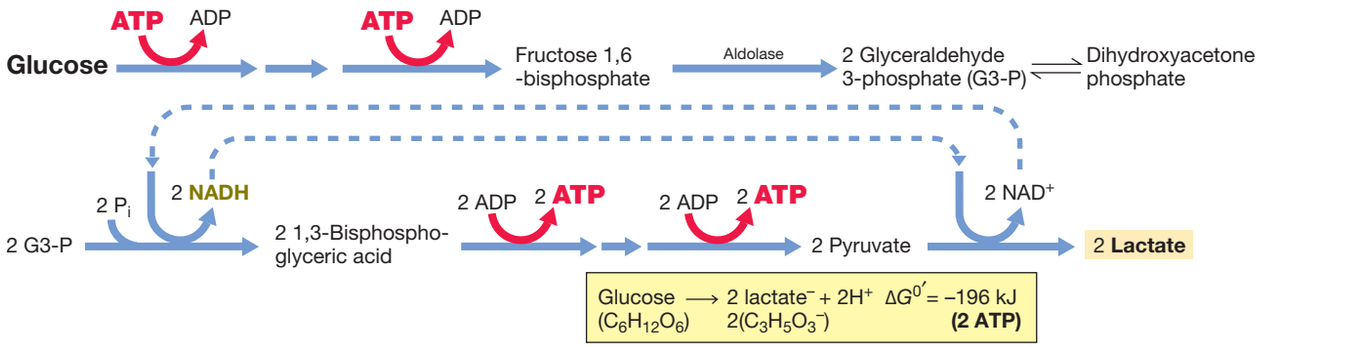
Entner–Doudoroff Pathway

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*).

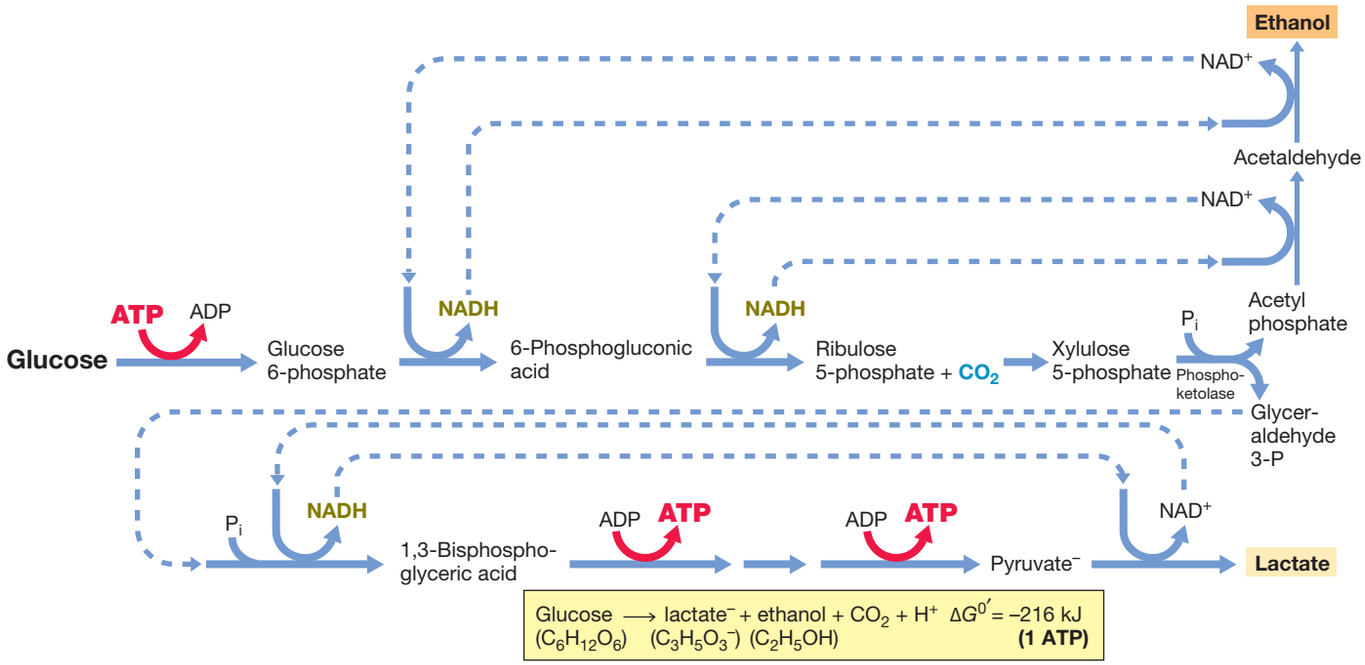
Because pyruvate is formed directly in the Entner–Doudoroff pathway and cannot yield ATP as can G-3-P (Figure 14.3), the Entner–Doudoroff pathway yields only half the ATP of the glycolytic pathway. Organisms using the Entner–Doudoroff pathway therefore share this physiological characteristic with heterofermentative 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?

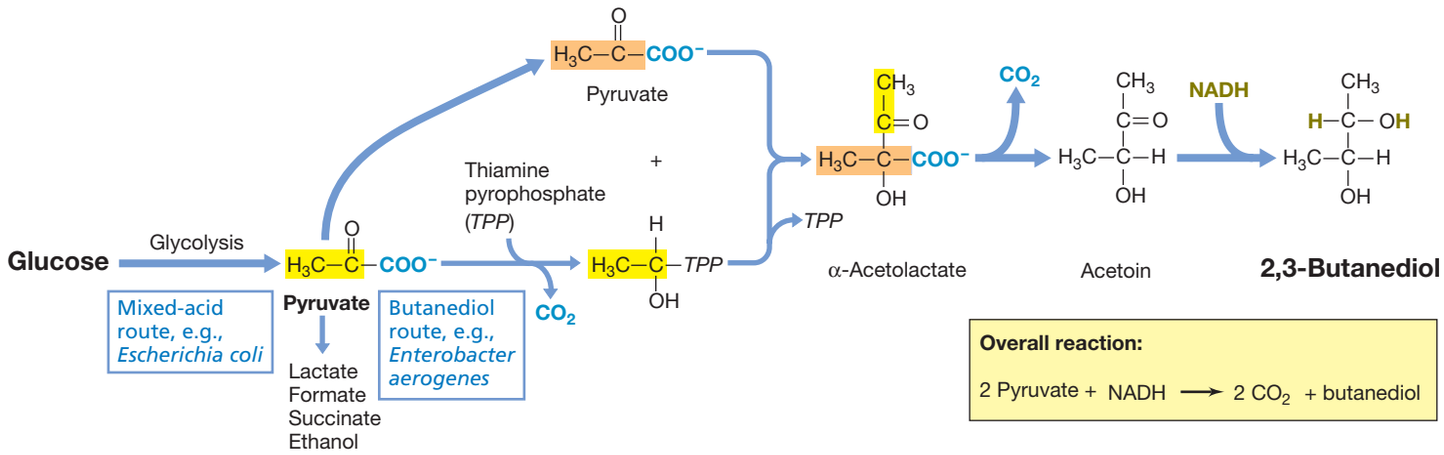


Figure 14.4 **Butanediol production and mixed-acid fermentations.** Note how only one NADH, but two molecules of pyruvate, are used to make one butanediol. This leads to redox imbalance and the production of more ethanol by butanediol producers than by mixed-acid fermenters.

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

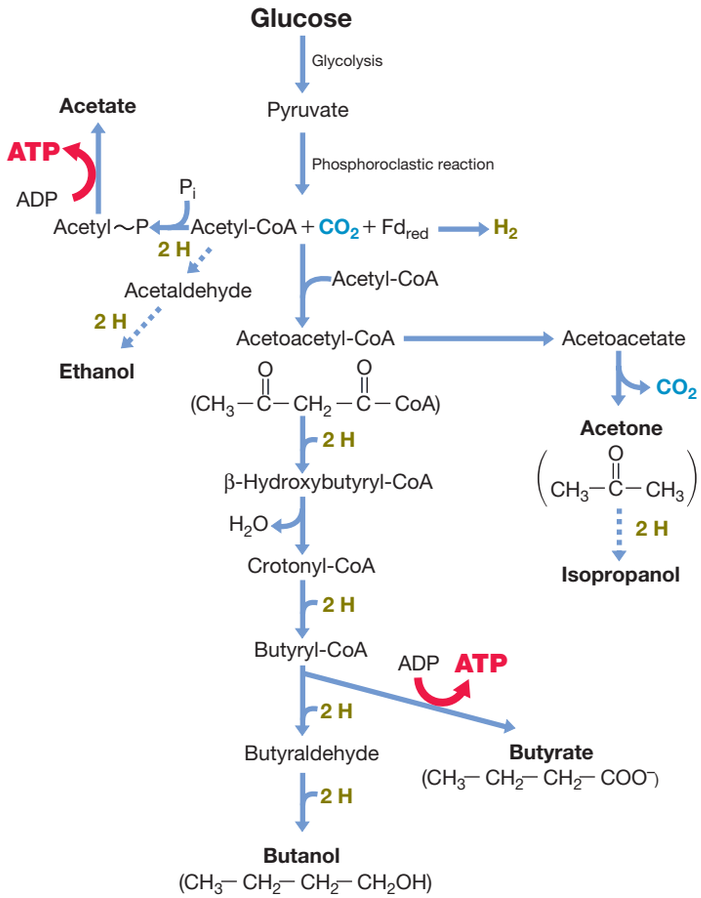
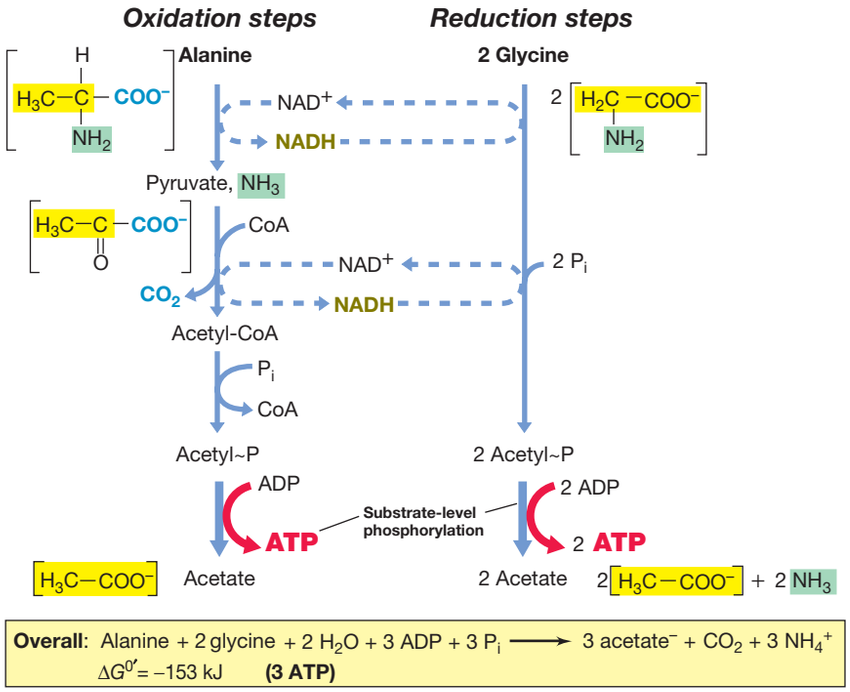


Figure 14.5 **The butyric acid and butanol/acetone fermentation.** All fermentation products from glucose are shown in bold (dashed lines indicate minor products). Note how the production of acetate and butyrate lead to additional ATP by substrate-level phosphorylation. By contrast, formation of butanol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



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.

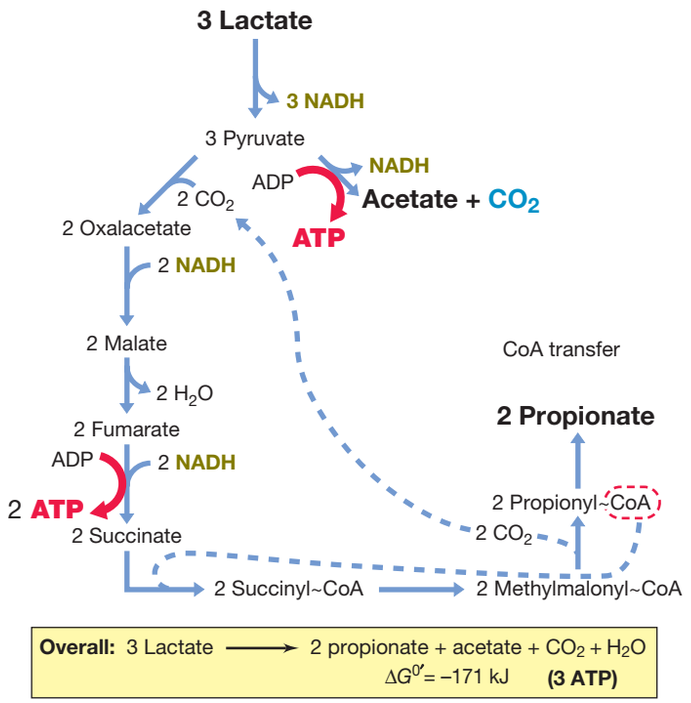


Figure 14.7 The propionic acid fermentation of *Propionibacterium*. Products are shown in bold. The four NADH made from the oxidation of three lactate are reoxidized in the reduction of oxalacetate and fumarate, and the CoA group from propionyl-CoA is exchanged with succinate during the formation of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

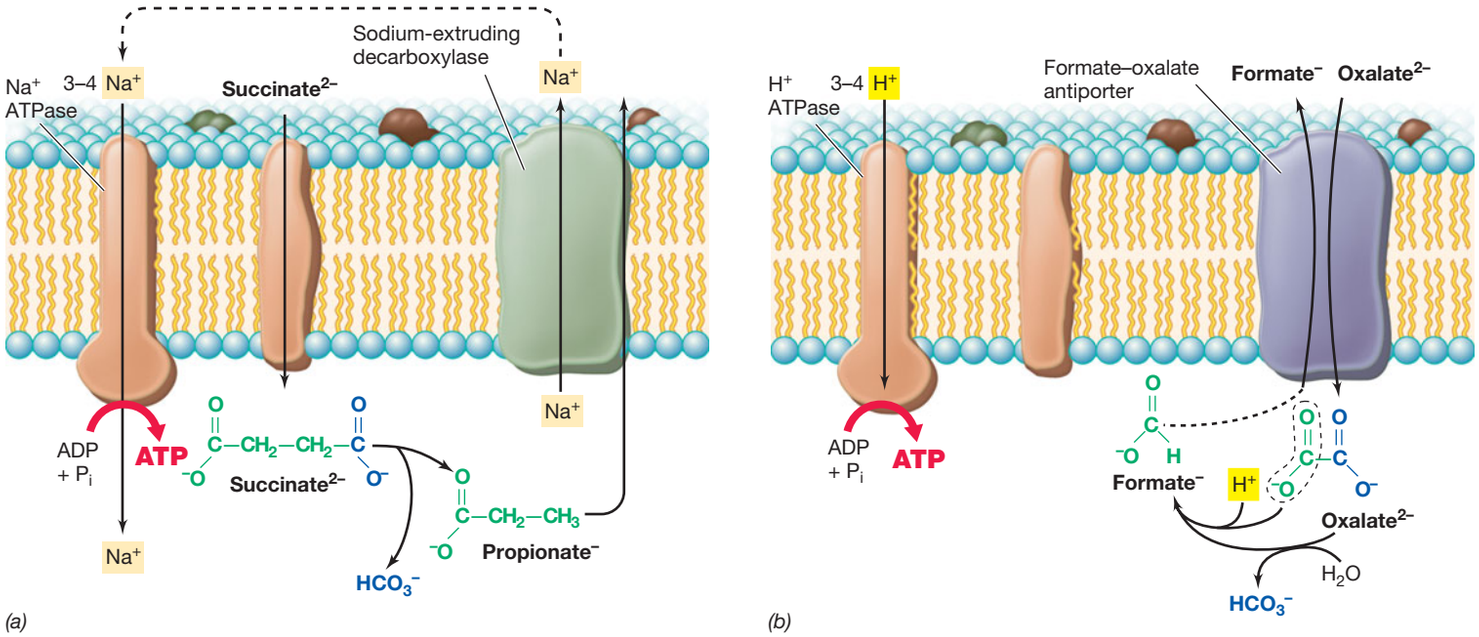


Figure 14.8 **The unique fermentations of succinate and oxalate.** *(a)* Succinate fermentation by *Propionigenium modestum*. Sodium export is linked to the energy released by succinate decarboxylation, and a sodium-translocating ATPase produces ATP. *(b)* Oxalate fermentation by *Oxalobacter formigenes*. 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.

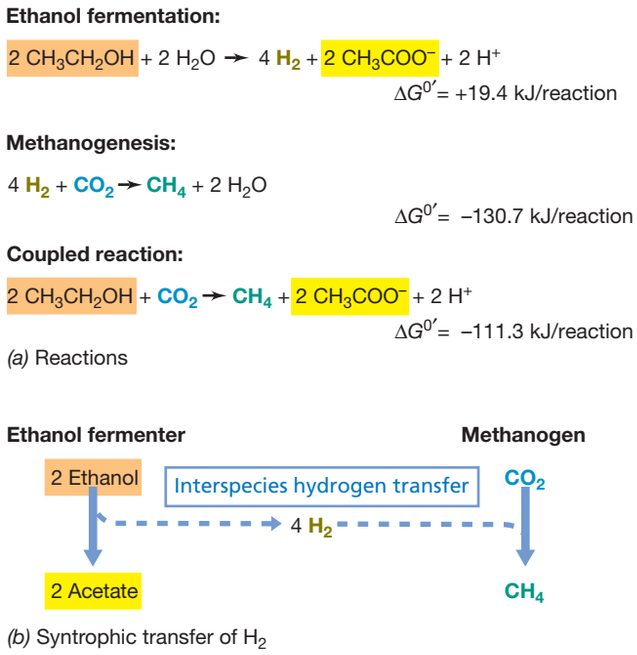


Figure 14.9 **Syntrophy: Interspecies H_2 transfer.** Shown is the fermentation of ethanol to methane and acetate by syntrophic association of an ethanol-oxidizing syntroph and a H_2-consuming partner (in this case, a methanogen). *(a)* Reactions involved. The two organisms share the energy released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

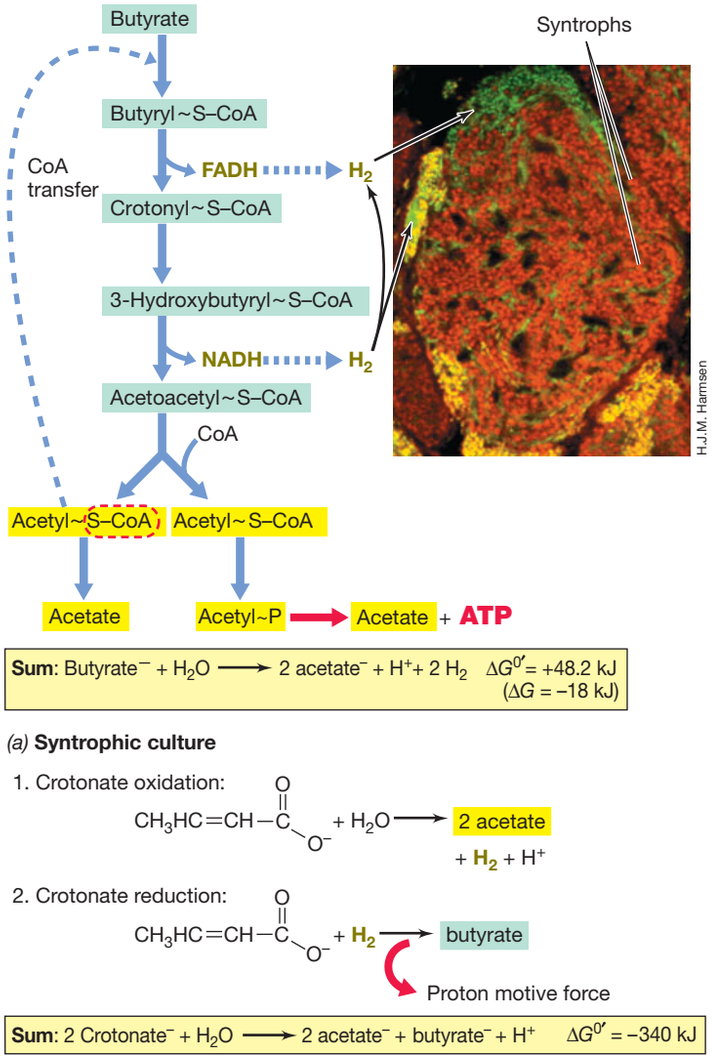


Figure 14.10 **Energetics of growth of *Syntrophomonas* in syntrophic culture and in pure culture.** *(a)* In syntrophic culture, growth requires a H_2-consuming organism, such as a methanogen. H_2 production is driven by reverse electron flow because the E_0' of the FADH and NADH couples are more electropositive than that of 2 H^+/H_2. *(b)* In pure culture, energy conservation is linked to anaerobic respiration with crotonate reduction to butyrate. Inset: photomicrograph of FISH-stained cells (↻ Section 16.9) of a fatty acid-degrading syntrophic bacterium in association with a methanogen.

products and reactants. By contrast, the related term ΔG is calculated on the basis of the actual concentrations of products and reactants present (Appendix 1 explains how to calculate ΔG). At very low levels of H_2, the energetics of the oxidation of ethanol or fatty acids to acetate plus H_2, a reaction that is endergonic under standard conditions, becomes exergonic. For example, if the concentration of H_2 is kept extremely low from consumption by the partner organism, ΔG for the oxidation of butyrate by *Syntrophomonas* yields −18 kJ (Figure 14.10*a*). As we learned in Section 14.4, this relatively low energy yield can still support growth of a bacterium.

Energetics in Syntrophs

Energy conservation in syntrophs is probably based on both substrate-level and oxidative phosphorylations. From biochemical studies of syntrophs, substrate-level phosphorylation has been shown to occur during the conversion of acetyl-CoA (generated by beta-oxidation of ethanol or the fatty acid) to acetate (Figure 14.10*a*), although the −18 kJ of energy released (ΔG) is in theory insufficient for this. However, the energy released is sufficient to produce a *fraction* of an ATP, so it is possible that two rounds of butyrate oxidation (Figure 14.10*a*) are necessary to couple to the production of one ATP by substrate-level phosphorylation.

Besides the syntrophic lifestyle, many syntrophs can carry out anaerobic respirations (Section 14.6) in pure culture by the disproportionation of unsaturated fatty acids (disproportionation is a process in which some molecules of a substrate are oxidized while some are reduced). For example, crotonate, an intermediate in syntrophic butyrate metabolism (Figure 14.10*a*), supports growth of *Syntrophomonas*. Under these conditions some of the crotonate is oxidized to acetate and some is reduced to butyrate (Figure 14.10*b*). Because crotonate reduction by *Syntrophomonas* is coupled to the formation of a proton motive force, as occurs in other anaerobic respirations that employ organic electron acceptors (such as fumarate reduction to succinate, Section 14.12), it is possible that some step or steps in syntrophic metabolism (Figure 14.10*a*) generate a proton motive force as well. Pumping protons or some other ion would almost certainly be required for benzoate- and propionate-fermenting syntrophs (Figure 14.10*a* inset) whose energy yield (ΔG) is only about −5 kJ or so.

Regardless of how ATP is made during syntrophic growth, an additional energetic burden occurs in syntrophy. During syntrophic metabolism, syntrophs produce H_2 (E_0' −0.42 V) from more electropositive electron donors such as FADH (E_0' −0.22 V) and NADH (E_0' −0.32 V), generated during fatty acid oxidation reactions (Figure 14.10*a*); this cannot occur without an energy input. Thus, some fraction of the ATP generated by *Syntrophomonas* during syntrophic growth must be consumed to drive reverse electron flow reactions (↻ Section 13.4), yielding H_2 for the H_2 consumer. When this energy drain is coupled to the inherently poor energetic yields of syntrophic reactions, it is clear that syntrophic bacteria are somehow making a living on a severely marginal energy economy. Even today syntrophs pose a significant challenge to our understanding of the minimal requirements for energy conservation in bacteria.

Ecology of Syntrophs

Ecologically, syntrophic bacteria are key links in the anoxic portions of the carbon cycle. Syntrophs consume highly reduced fermentation products and release a key product for anaerobic H_2 consumers. Without syntrophs, a bottleneck would develop in anoxic environments in which electron acceptors other than CO_2 were limiting (↻ Section 24.2). By contrast, when conditions are oxic or alternative electron acceptors are abundant, syntrophic relationships are unnecessary. For example, if O_2 or NO_3^- is available as an electron acceptor, the energetics of the fermentation of a fatty acid or an alcohol is so favorable that syntrophic relationships are unnecessary. Thus, syntrophy is charac-

teristic of anoxic catabolism in which methanogenesis or acetogenesis are the terminal processes in the microbial ecosystem. Methanogenesis is a major process in anoxic wastewater biodegradation, and microbiological studies of sludge granules that form in such systems have shown the close physical relationship that develops between H_2 producer and H_2 consumer in such habitats (Figure 14.10*a* inset).

MiniQuiz

- Give an example of interspecies H_2 transfer. Why can it be said that both organisms benefit from this process?
- Predict how ATP is made during the syntrophic degradation of ethanol shown in Figure 14.9.

II Anaerobic Respiration

In the next several sections we survey the major forms of anaerobic respiration and see the many ways by which prokaryotes can conserve energy under anoxic conditions using electron acceptors *other* than oxygen (O_2).

14.6 Anaerobic Respiration: General Principles

We examined the process of aerobic respiration in Chapter 4. As we noted there, O_2 functions as a *terminal electron acceptor*, accepting electrons that have traveled through an electron transport chain. However, we also noted that other electron acceptors could be used instead of O_2, in which case the process is called **anaerobic respiration** (↻ Section 4.12). Here we consider some of these processes.

Bacteria that carry out anaerobic respiration produce electron transport chains containing cytochromes, quinones, iron–sulfur proteins, and the other typical electron transport proteins that we have seen in aerobic respiration (↻ Section 4.9) and in photosynthesis and chemolithotrophy (Chapter 13). In some organisms, such as the denitrifying bacteria, which are for the most part facultative aerobes (↻ Section 5.17), anaerobic respiration competes with aerobic respiration. In such cases, if O_2 is present, the bacteria respire aerobically, and genes encoding proteins necessary for anaerobic respiration are repressed. However, when O_2 is depleted from the environment, the bacteria respire anaerobically, and the alternate electron acceptor is reduced. Many other organisms that carry out anaerobic respiration are obligate anaerobes and are unable to use O_2.

Alternative Electron Acceptors and the Redox Tower

The energy released from the oxidation of an electron donor using O_2 as electron acceptor is greater than if the same compound is oxidized with an alternate electron acceptor (↻ Figure 4.9). These energy differences are apparent if the reduction potentials of each acceptor are examined (**Figure 14.11**). Because the O_2/H_2O couple is most electropositive, more energy is available when O_2 is used than when another electron acceptor is used. This is why aerobic respiration is the dominant process and occurs to the exclusion of anaerobic respiration in an organism in which both processes are possible. Other electron acceptors that are near the O_2/H_2O couple are manganic ion (Mn^{4+}), ferric iron (Fe^{3+}), nitrate (NO_3^-), and nitrite (NO_2^-). Examples of more electronegative acceptors are sulfate (SO_4^{2-}), elemental sulfur (S^0), and carbon dioxide (CO_2), and organisms that use these acceptors are typically locked into an anaerobic lifestyle. A summary of the most common types of anaerobic respiration is given in Figure 14.11.

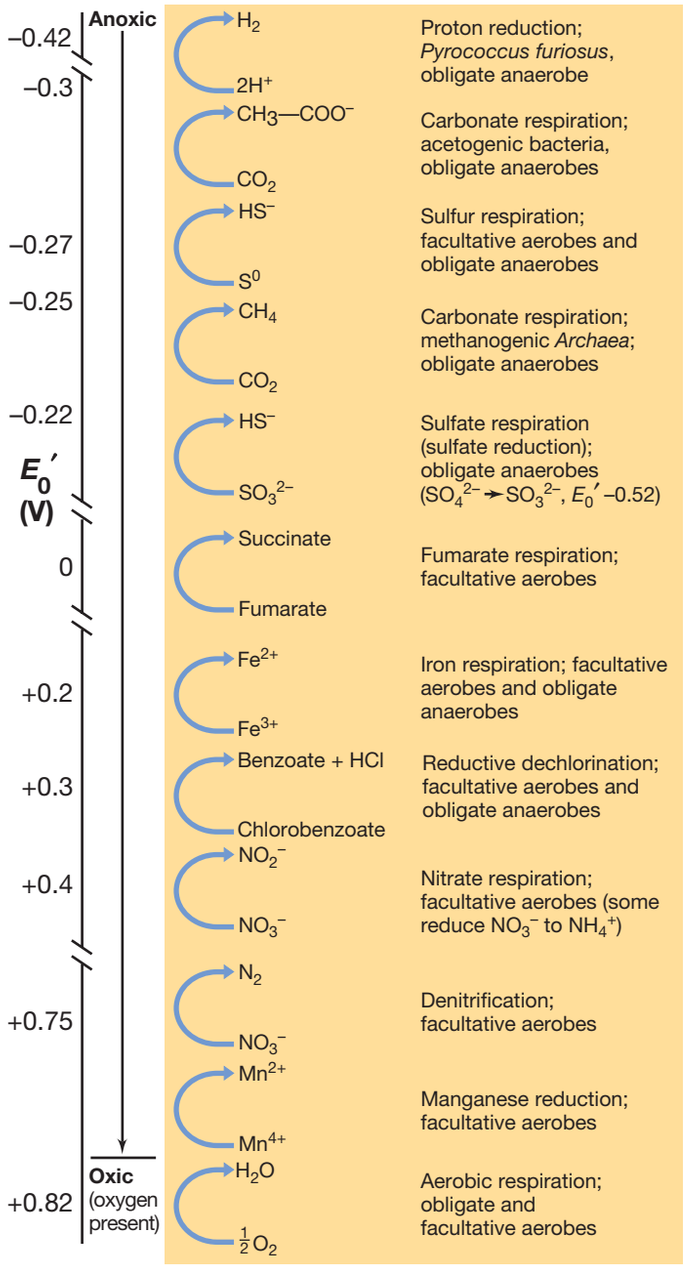


Figure 14.11 Major forms of anaerobic respiration. The redox couples are arranged in order from most electronegative E_0' (top) to most electropositive E_0' (bottom). See Figure 4.9 to compare how the energy yields of these anaerobic respirations vary.

UNIT 5

Assimilative and Dissimilative Metabolism

Inorganic compounds such as NO_3^-, SO_4^{2-}, and CO_2 are reduced by many organisms as sources of cellular nitrogen, sulfur, and carbon, respectively. The end products of such reductions are amino groups (—NH_2), sulfhydryl groups (—SH), and organic carbon compounds, respectively. When an inorganic compound such as NO_3^-, SO_4^{2-}, or CO_2 is reduced for use in biosynthesis, it is said to be *assimilated*, and the reduction process is called *assimilative* metabolism. Assimilative metabolism of NO_3^-, SO_4^{2-}, and CO_2 is conceptually and physiologically quite different from the reduction of these electron acceptors for the purposes of energy conservation in anaerobic metabolism. To distinguish these two kinds of reductive processes, the use of these compounds as electron acceptors for energy purposes is called *dissimilative* metabolism.

Assimilative and dissimilative metabolisms differ markedly. In assimilative metabolism, only enough of the compound (NO_3^-, SO_4^{2-}, or CO_2) is reduced to satisfy the needs for biosynthesis, and the products are eventually converted to cell material in the form of macromolecules. In dissimilative metabolism, a large amount of the electron acceptor is reduced, and the reduced product is excreted into the environment. Many organisms carry out assimilative metabolism of compounds such as NO_3^-, SO_4^{2-}, and CO_2, whereas a more restricted group of primarily prokaryotic organisms carry out dissimilative metabolism. As for electron donors, virtually any organic compound that can be degraded aerobically can also be degraded under anoxic conditions by one or more forms of anaerobic respiration. Moreover, several inorganic substances can also be electron donors as long as the E_0' of their redox couple is more electronegative than that of the acceptor couple in the anaerobic respiration.

MiniQuiz

- What is anaerobic respiration?
- With H_2 as an electron donor, why is the reduction of NO_3^- a more favorable reaction than the reduction of S^0?

14.7 Nitrate Reduction and Denitrification

Inorganic nitrogen compounds are some of the most common electron acceptors in anaerobic respiration. **Table 14.5** summarizes the various forms of inorganic nitrogen with their oxidation states. One of the most common alternative electron acceptors is nitrate, NO_3^-, which can be reduced to nitrous oxide (N_2O), nitric oxide (NO), and dinitrogen (N_2). Because these products of nitrate reduction are all gaseous, they can easily be lost from the environment, a process called **denitrification** (**Figure 14.12**).

Denitrification is the main means by which gaseous N_2 is formed biologically. As a source of nitrogen, N_2 is much less available to plants and microorganisms than is NO_3^-, so for agricultural purposes, at least, denitrification is a detrimental process. For sewage treatment (Section 35.2), however, denitrification is beneficial because it converts NO_3^- to N_2. This transformation decreases the load of fixed nitrogen in the sewage treatment effluent that can stimulate algal growth in receiving waters, such as rivers and streams, or lakes (Section 24.2).

Table 14.5 *Oxidation states of key nitrogen compounds*

Compound	*Oxidation state of N atom*
Organic N (—NH_2)	−3
Ammonia (NH_3)	−3
Nitrogen gas (N_2)	0
Nitrous oxide (N_2O)	+1 (average per N)
Nitric oxide (NO)	+2
Nitrite (NO_2^-)	+3
Nitrogen dioxide (NO_2)	+4
Nitrate (NO_3^-)	+5

Biochemistry of Dissimilative Nitrate Reduction

The enzyme that catalyzes the first step of dissimilative nitrate reduction is *nitrate reductase*, a molybdenum-containing membrane-integrated enzyme whose synthesis is repressed by molecular oxygen. All subsequent enzymes of the pathway (**Figure 14.13**) are coordinately regulated and thus also repressed by O_2. But, in addition to anoxic conditions, NO_3^- must also be present before these enzymes are fully expressed.

The first product of nitrate reduction is nitrite (NO_2^-), and the enzyme nitrite reductase reduces it to NO (Figure 14.13*c*). Some organisms can reduce NO_2^- to ammonia (NH_3) in a dissimilative process, but the production of gaseous products—*denitrification*—is of greatest global significance. This is because denitrification consumes a fixed form of nitrogen (NO_3^-) and produces gaseous nitrogen compounds, some of which are of environmental significance. For example, N_2O can be converted

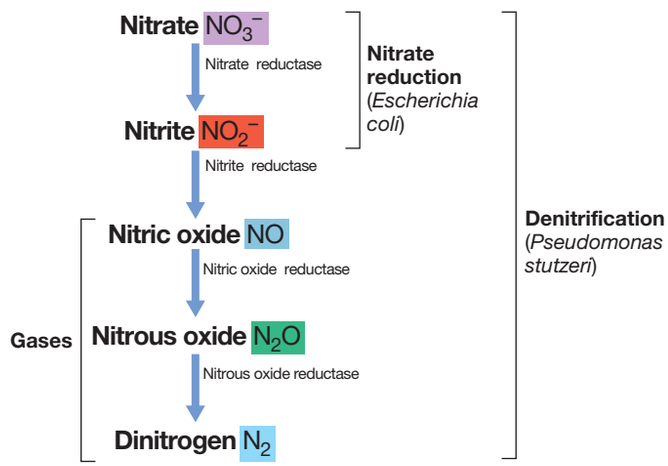


Figure 14.12 **Steps in the dissimilative reduction of nitrate.** Some organisms can carry out only the first step. All enzymes involved are derepressed by anoxic conditions. Also, some prokaryotes are known that can reduce NO_3^- to NH_4^+ in dissimilative metabolism. Note that colors used here match those used in Figure 14.13.

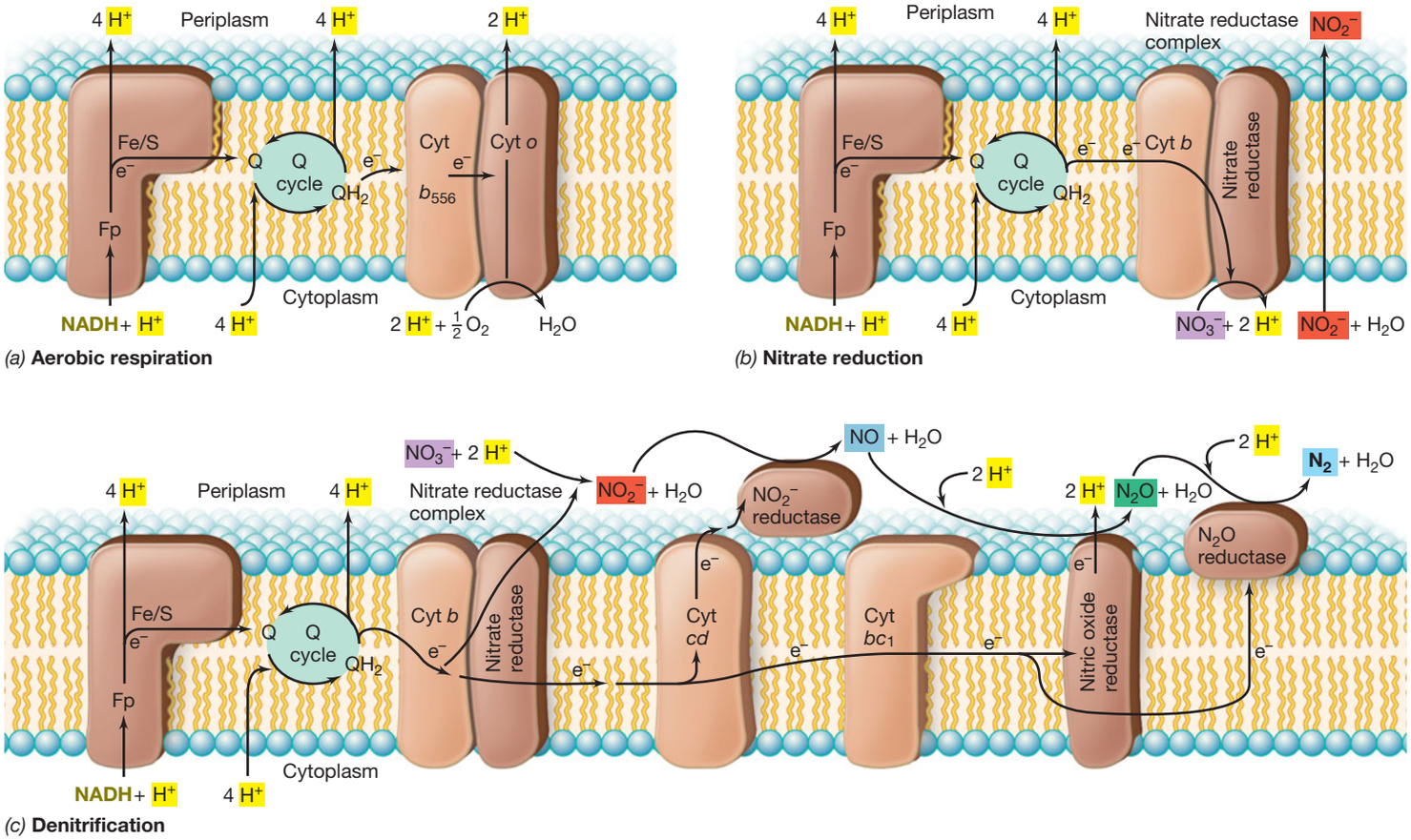


Figure 14.13 Respiration and nitrate-based anaerobic respiration. Electron transport processes in the membrane of *Escherichia coli* when *(a)* O_2 or *(b)* NO_3^- is used as an electron acceptor and NADH is the electron donor. Fp, flavoprotein; Q, ubiquinone. Under high-oxygen conditions, the sequence of carriers is cyt b_{562} → cyt *o* → O_2. However, under low-oxygen conditions (not shown), the sequence is cyt b_{568} → cyt *d* → O_2. Note how more protons are translocated per two electrons oxidized aerobically during electron transport reactions than anaerobically with NO_3^- as electron acceptor, because the aerobic terminal oxidase (cyt *o*) pumps two protons. *(c)* Scheme for electron transport in membranes of *Pseudomonas stutzeri* during denitrification. Nitrate and nitric oxide reductases are integral membrane proteins, whereas nitrite and nitrous oxide reductases are periplasmic enzymes.

to NO by sunlight, and NO reacts with ozone (O_3) in the upper atmosphere to form NO_2^-. When it rains, NO_2^- returns to Earth as nitrous acid (HNO_2) in so-called acid rain. The remaining steps in denitrification are shown in Figure 14.13*c*.

The biochemistry of dissimilative nitrate reduction has been studied in detail in several organisms, including *Escherichia coli*, in which NO_3^- is reduced only to NO_2^-, and *Paracoccus denitrificans* and *Pseudomonas stutzeri*, in which denitrification occurs. The *E. coli* nitrate reductase accepts electrons from a *b*-type cytochrome, and a comparison of the electron transport chains in aerobic versus nitrate-respiring cells of *E. coli* is shown in Figure 14.13*a*, *b*.

Because of the reduction potential of the NO_3^-/NO_2^- couple (+0.43 V), fewer protons are pumped during nitrate reduction than in aerobic respiration (O_2/H_2O, +0.82 V). In *P. denitrificans* and *P. stutzeri*, nitrogen oxides are formed from NO_2^- by the enzymes nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, as summarized in Figure 14.13*c*. During electron transport, a proton motive force is established, and ATPase functions to produce ATP in the usual fashion. Additional ATP is available when NO_3^- is reduced to N_2 because the nitric oxide reductase is linked to proton extrusion (Figure 14.13*c*).

Other Properties of Denitrifying Prokaryotes

Most denitrifying prokaryotes are phylogenetically members of the *Proteobacteria* (Chapter 17) and, physiologically, facultative aerobes. Aerobic respiration occurs when O_2 is present, even if NO_3^- is also present in the medium. Many denitrifying bacteria also reduce other electron acceptors anaerobically, such as Fe^{3+} and certain organic electron acceptors (Section 14.12). In addition, some denitrifying bacteria can grow by fermentation and some are phototrophic purple bacteria (↻ Section 13.4). Thus, denitrifying bacteria are quite metabolically diverse in alternative energy-generating mechanisms. Interestingly, at least one eukaryote has been shown to be a denitrifier. The protist *Globobulimina pseudospinescens*, a shelled amoeba (foraminifera, ↻ Section 20.11), can denitrify and likely employs this form of metabolism to survive in anoxic marine sediments where it resides.

MiniQuiz

- For *Escherichia coli*, why is more energy released in aerobic respiration than during NO_3^- reduction?
- How do the products of NO_3^- reduction differ between *E. coli* and *Pseudomonas*?
- Where is the dissimilative nitrate reductase found in the cell? What unusual metal does it contain?

14.8 Sulfate and Sulfur Reduction

Several inorganic sulfur compounds are important electron acceptors in anaerobic respiration. A summary of the oxidation states of key sulfur compounds is given in **Table 14.6**. Sulfate (SO_4^{2-}), the most oxidized form of sulfur, is a major anion in seawater and is reduced by the sulfate-reducing bacteria, a group that is widely distributed in nature. The end product of sulfate reduction is hydrogen sulfide, H_2S, an important natural product that participates in many biogeochemical processes (↻ Section 24.3). Species in the genus *Desulfovibrio* have been widely used for the study of sulfate reduction, and general properties of this and other sulfate-reducing bacteria are discussed in Section 17.18.

Assimilative and Dissimilative Sulfate Reduction

Again, as with nitrogen, it is necessary to distinguish between assimilative and dissimilative metabolism. Many organisms, including plants, algae, fungi, and most prokaryotes, use SO_4^{2-} as a source for biosynthetic sulfur needs. The ability to use SO_4^{2-} as an electron acceptor for energy-generating processes, however, involves the large-scale reduction of SO_4^{2-} and is restricted to the sulfate-reducing bacteria. In assimilative sulfate reduction, H_2S is formed on a very small scale and is assimilated into organic form in sulfur-containing amino acids and other organic sulfur compounds. By contrast, in dissimilative sulfate reduction, H_2S can be produced on a very large scale and is excreted from the cell, free to react with other organisms or with metals to form metal sulfides.

Table 14.6 *Sulfur compounds and electron donors for sulfate reduction*

Compound	*Oxidation state of S atom*
Oxidation states of key sulfur compounds	
Organic S (R—SH)	−2
Sulfide (H_2S)	−2
Elemental sulfur (S^0)	0
Thiosulfate (—S–SO_3^{2-})	−2/+6
Sulfur dioxide (SO_2)	+4
Sulfite (SO_3^{2-})	+4
Sulfate (SO_4^{2-})	+6
Some electron donors used for sulfate reduction	
H_2	Acetate
Lactate	Propionate
Pyruvate	Butyrate
Ethanol and other alcohols	Long-chain fatty acids
Fumarate	Benzoate
Malate	Indole
Choline	Various hydrocarbons

Biochemistry and Energetics of Sulfate Reduction

As the reduction potentials in Table A1.2 and Figure 14.11 show, SO_4^{2-} is a much less favorable electron acceptor than is O_2 or NO_3^-. However, sufficient free energy to make ATP is available from sulfate reduction when an electron donor that yields NADH or FADH is oxidized. Table 14.6 lists some of the electron donors used by sulfate-reducing bacteria. Hydrogen (H_2) is used by virtually all species of sulfate-reducing bacteria, whereas use of the other donors is more restricted. For example, lactate and pyruvate are widely used by species found in freshwater anoxic environments, while acetate and longer-chain fatty acids are widely used by marine sulfate-reducing bacteria. Many morphological and physiological types of sulfate reducing bacteria are known, and with the exception of *Archaeoglobus* (↻ Section 19.6), a genus of *Archaea*, all known sulfate reducers are *Bacteria* (↻ Section 17.18).

The reduction of SO_4^{2-} to H_2S requires eight electrons and proceeds through a number of intermediate stages. Sulfate is chemically quite stable and cannot be reduced without first being activated; SO_4^{2-} is activated in a reaction requiring ATP. The enzyme ATP sulfurylase catalyzes the attachment of SO_4^{2-} to a phosphate of ATP, forming *adenosine phosphosulfate* (*APS*) as shown in **Figure 14.14**. In dissimilative sulfate reduction, the SO_4^{2-} in APS is reduced directly to sulfite (SO_3^{2-}) by the enzyme APS reductase with the release of AMP. In assimilative reduction, another phosphate is added to APS to form *phosphoadenosine phosphosulfate* (*PAPS*) (Figure 14.14*a*), and only then is the SO_4^{2-} reduced. However, in both cases the product of sulfate reduction is SO_3^{2-}. Once SO_3^{2-} is formed, H_2S is generated from the activity of the enzyme sulfite reductase (Figure 14.14*b*).

During dissimilative sulfate reduction, electron transport reactions lead to a proton motive force and this drives ATP synthesis by ATPase. A major electron carrier in this process is *cytochrome* c_3, a periplasmic low-potential cytochrome (**Figure 14.15**). Cytochrome c_3 accepts electrons from a periplasmically located hydrogenase and transfers these electrons to a membrane-associated protein complex. This complex, called *Hmc*, carries the electrons across the cytoplasmic membrane and makes them available to APS reductase and sulfite reductase, cytoplasmic enzymes that generate sulfite and sulfide, respectively (Figure 14.15).

The enzyme hydrogenase plays a central role in sulfate reduction whether *Desulfovibrio* is growing on H_2, per se, or on an organic compound such as lactate. This is because lactate is converted through pyruvate to acetate (the latter is for the most part excreted because *Desulfovibrio* is a non-acetate-oxidizing sulfate reducer; ↻ Section 17.18) with the production of H_2. The H_2 produced crosses the cytoplasmic membrane and is oxidized by the periplasmic hydrogenase to electrons, which are fed back into the system, and protons, which establish the proton motive force (Figure 14.15). Growth yields of sulfate-reducing bacteria suggest

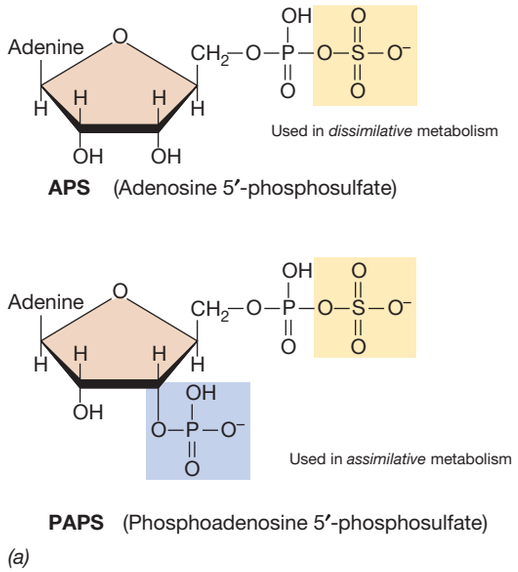


Figure 14.14 **Biochemistry of sulfate reduction: Activated sulfate.** *(a)* Two forms of active sulfate can be made, adenosine 5′-phosphosulfate (APS) and phosphoadenosine 5′-phosphosulfate (PAPS). Both are derivatives of adenosine diphosphate (ADP), with the second phosphate of ADP being replaced by SO_4^{2-}. *(b)* Schemes of assimilative and dissimilative sulfate reduction.

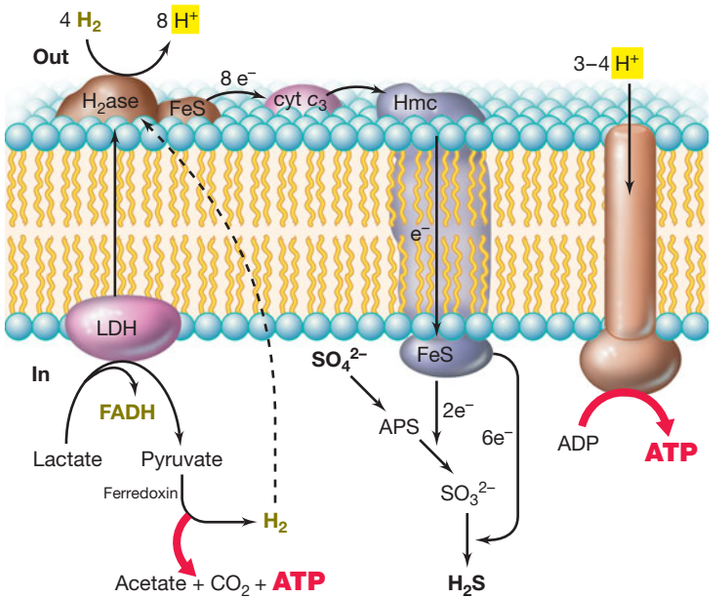


Figure 14.15 **Electron transport and energy conservation in sulfate-reducing bacteria.** In addition to external H_2, H_2 originating from the catabolism of organic compounds such as lactate and pyruvate can fuel hydrogenase. The enzymes hydrogenase (H_2ase), cytochrome (cyt) c_3, and a cytochrome complex (Hmc) are periplasmic proteins. A separate protein shuttles electrons across the cytoplasmic membrane from Hmc to a cytoplasmic iron–sulfur protein (FeS) that supplies electrons to APS reductase (forming SO_3^{2-}) and sulfite reductase (forming H_2S, Figure 14.14*b*). LDH, lactate dehydrogenase.

that a net of one ATP is produced for each SO_4^{2-} reduced to HS^-. With H_2 as electron donor, the reaction is

$$4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O \qquad \Delta G^{0\prime} = -152\ \text{kJ}$$

When lactate or pyruvate is the electron donor, not only is ATP produced from the proton motive force, but additional ATP can be produced during the oxidation of pyruvate to acetate plus CO_2 via acetyl-CoA and acetyl phosphate (Table 14.1 and Figure 14.2).

Acetate Use and Autotrophy

Many sulfate-reducing bacteria can oxidize acetate to CO_2 to obtain electrons for SO_4^{2-} reduction (↻ Section 17.18):

$$CH_3COO^- + SO_4^{2-} + 3\ H^+ \rightarrow 2\ CO_2 + H_2S + 2\ H_2O \qquad \Delta G^{0\prime} = -57.5\ \text{kJ}$$

The mechanism for acetate oxidation in most species is the *acetyl-CoA pathway*, a series of reversible reactions used by many anaerobes for acetate synthesis or acetate oxidation. This pathway employs the key enzyme *carbon monoxide dehydrogenase* (Section 14.9). A few sulfate-reducing bacteria can also grow autotrophically with H_2. When growing under these conditions, the organisms use the acetyl-CoA pathway for incorporating CO_2 into cell material. The acetate-oxidizing sulfate-reducing bacterium *Desulfobacter* lacks acetyl-CoA pathway enzymes and oxidizes acetate through the citric acid cycle (↻ Figure 4.21), but this seems to be the exception rather than the rule.

Sulfur Disproportionation

Certain sulfate-reducing bacteria can disproportionate sulfur compounds of intermediate oxidation state. Disproportionation occurs when one molecule of a substance is oxidized while a second molecule is reduced, ultimately forming two different products. For example, *Desulfovibrio sulfodismutans* can disproportionate thiosulfate ($S_2O_3^{2-}$) as follows:

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + H_2S \qquad \Delta G^{0\prime} = -21.9\ \text{kJ/reaction}$$

Note that in this reaction one sulfur atom of $S_2O_3^{2-}$ becomes more oxidized (forming SO_4^{2-}), while the other becomes more reduced (forming H_2S). The oxidation of $S_2O_3^{2-}$ by *D. sulfodismutans* is coupled to proton pumping that is used by this organism to make ATP by ATPase. Other reduced sulfur compounds such as sulfite (SO_3^{2-}) and sulfur (S^0) can also be disproportionated. These forms of sulfur metabolism allow sulfate-reducing bacteria to recover energy from sulfur intermediates produced from the oxidation of H_2S by sulfur chemolithotrophs that coexist with them in nature and also from intermediates generated in their own metabolism during SO_4^{2-} reduction.

Phosphite Oxidation

At least one sulfate-reducing bacterium can couple phosphite (HPO_3^-) oxidation to SO_4^{2-} reduction. The reaction is chemolithotrophic, and the products are phosphate and sulfide:

$$4\,HPO_3^- + SO_4^{2-} + H^+ \rightarrow 4\,HPO_4^{2-} + HS^- \qquad \Delta G^{0\prime} = -364\ kJ$$

This bacterium, *Desulfotignum phosphitoxidans*, is an autotroph and a strict anaerobe, which by necessity it must be because phosphite spontaneously oxidizes in air. The natural sources of phosphite are likely to be organic phosphorous compounds called *phosphonates*, molecules generated from the anoxic degradation of organic phosphorous compounds. Along with sulfur disproportionation (also a chemolithotrophic process) and H_2 utilization, phosphite oxidation underscores the diversity of chemolithotrophic reactions carried out by sulfate-reducing bacteria.

Sulfur Reduction

Some organisms produce H_2S in anaerobic respiration, but are unable to reduce SO_4^{2-}; these are the elemental sulfur (S^0) reducers. Sulfur-reducing bacteria carry out the reaction

$$S^0 + 2\,H \rightarrow H_2S$$

The electrons for this process can come from H_2 or from various organic compounds. The first sulfur-reducing organism to be discovered was *Desulfuromonas acetoxidans* (↻ Section 17.18). This organism oxidizes acetate, ethanol, and a few other compounds to CO_2, coupled with the reduction of S^0 to H_2S. Ferric iron (Fe^{3+}) also supports growth as an electron acceptor. The physiology of dissimilative sulfur-reducing bacteria is not as well understood as that of sulfate-reducing bacteria, but it is known that sulfur reducers lack the capacity to activate sulfate to APS (Figure 14.14), and presumably this prevents them from using SO_4^{2-} as an electron acceptor. *Desulfuromonas* contains high levels of several cytochromes, including an analog of cytochrome c_3, a key electron carrier in sulfate-reducing bacteria. Because the oxidation of acetate to CO_2 releases less energy than that needed to make an ATP by substrate-level phosphorylation, it is clear that oxidative phosphorylation plays a major role in the energetics of these organisms. A variety of other bacteria can use S^0 as an electron acceptor, including some species of the genera *Wolinella* and *Campylobacter*. In culture some sulfur reducers including *Desulfuromonas* can use Fe^{3+} as an electron acceptor, but S^0 is probably the major electron acceptor used in nature. It is the production of H_2S that connects the sulfur- and sulfate-reducing bacteria in an ecological sense.

MiniQuiz

- How is SO_4^{2-} converted to SO_3^{2-} during dissimilative sulfate reduction? Physiologically, how does *Desulfuromonas* differ from *Desulfovibrio*?
- Why is H_2 of importance to sulfate-reducing bacteria?
- Give an example of sulfur disproportionation.

14.9 Acetogenesis

Carbon dioxide, CO_2, is common in nature and typically abundant in anoxic habitats because it is a major product of the energy metabolisms of chemoorganotrophs. Two major groups of strictly anaerobic prokaryotes use CO_2 as an electron acceptor in energy metabolism. One of these groups is the *acetogens*, and we discuss them here. The other group, the *methanogens*, will be considered in the next section. H_2 is a major electron donor for both of these organisms, and an overview of their energy metabolism, **acetogenesis** and **methanogenesis**, is shown in **Figure 14.16**. Both processes are linked to ion pumps, of either protons (H^+) or sodium ions (Na^+), as the mechanism of energy conservation, and these pumps fuel ATPases in the membrane. Acetogenesis also conserves energy in a substrate-level phosphorylation reaction.

Organisms and Pathway

Acetogens carry out the reaction

$$4\,H_2 + H^+ + 2\,HCO_3^- \rightarrow CH_3COO^- + 4\,H_2O \qquad \Delta G^{0\prime} = -105\ kJ$$

In addition to H_2, electron donors for acetogenesis include C_1 compounds, sugars, organic and amino acids, alcohols, and certain nitrogen bases, depending on the organism. Many acetogens can also reduce nitrate (NO_3^-) and thiosulfate ($S_2O_3^{2-}$). However, CO_2 reduction is probably the major reaction of ecological significance.

A major unifying thread among acetogens is the pathway of CO_2 reduction. Acetogens reduce CO_2 to acetate by the **acetyl-CoA pathway**, the major pathway in obligate anaerobes for the production or oxidation of acetate. **Table 14.7** lists the major groups of organisms that produce acetate or oxidize acetate via the acetyl-CoA pathway. Acetogens such as *Acetobacterium woodii* and *Clostridium aceticum* can grow either chemoorganotrophically by fermentation of sugars (reaction 1) or

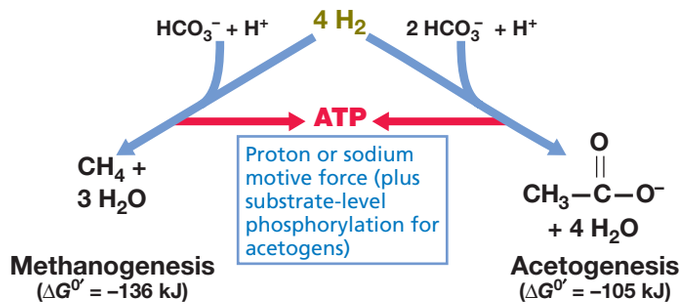


Figure 14.16 **The contrasting processes of methanogenesis and acetogenesis.** Note the difference in free energy released in the reactions.

Table 14.7 *Organisms employing the acetyl-CoA pathway*

I. Pathway drives acetate synthesis for energy purposes
Acetoanaerobium noterae
Acetobacterium woodii
Acetobacterium wieringae
Acetogenium kivui
Acetitomaculum ruminis
Clostridium aceticum
Clostridium formicaceticum
Clostridium ljungdahlii
Moorella thermoacetica
Desulfotomaculum orientis
Sporomusa paucivorans
Eubacterium limosum (also produces butyrate)
Treponema primitia (from termite hindguts)
II. Pathway drives acetate synthesis for cell biosynthesis
Acetogens
Methanogens
Sulfate-reducing bacteria
III. Pathway drives acetate oxidation for energy purposes
Reaction: Acetate + 2 H_2O → 2 CO_2 + 8 H
Group II sulfate reducers (other than *Desulfobacter*)
Reaction: Acetate → CO_2 + CH_4
Acetotrophic methanogens (*Methanosarcina, Methanosaeta*)

chemolithotrophically and autotrophically through the reduction of CO_2 to acetate with H_2 (reaction 2) as electron donor. In either case, the sole product is acetate:

$$(1)\quad C_6H_{12}O_6 \rightarrow 3\ CH_3COO^- + 3\ H^+$$

$$(2)\quad 2\ HCO_3^- + 4\ H_2 + H^+ \rightarrow CH_3COO^- + 4\ H_2O$$

Acetogens catabolize glucose by way of glycolysis, converting glucose to two molecules of pyruvate and two molecules of NADH (the equivalent of 4 H). From this point, two molecules of acetate are produced:

$$(3)\quad 2\ \text{Pyruvate}^- \rightarrow 2\ \text{acetate}^- + 2\ CO_2 + 4\ H$$

The third acetate of reaction (1) comes from reaction (2), using the two molecules of CO_2 generated in reaction (3), plus the four H generated during glycolysis and the four H generated from the oxidation of two pyruvates to two acetates [reaction (3)]. Starting from pyruvate, then, the overall production of acetate can be written as

$$2\ \text{Pyruvate}^- + 4\ H \rightarrow 3\ \text{acetate}^- + H^+$$

Most acetogenic bacteria that produce and excrete acetate in energy metabolism are gram-positive *Bacteria*, and many are species of *Clostridium* or *Acetobacterium* (Table 14.7). A few other gram-positive and many different gram-negative *Bacteria* and *Archaea* use the acetyl-CoA pathway for autotrophic purposes, reducing CO_2 to acetate as a source of cell carbon.

The acetyl-CoA pathway functions in autotrophic growth for certain sulfate-reducing bacteria and is also used by the methanogens, most of which grow autotrophically on $H_2 + CO_2$ (↻ Sections 19.3 and 14.10). By contrast, some bacteria employ the reactions of the acetyl-CoA pathway primarily in the reverse direction as a means of oxidizing acetate to CO_2. These include acetotrophic methanogens (↻ Section 19.3) and sulfate-reducing bacteria (↻ Sections 17.18 and 14.8).

Reactions of the Acetyl-CoA Pathway

Unlike other autotrophic pathways such as the Calvin cycle (↻ Section 13.12), the reverse citric acid cycle, or the hydroxypropionate cycle (↻ Section 13.13), the acetyl-CoA pathway of CO_2 fixation is not a cycle. Instead it catalyzes the reduction of CO_2 along two linear pathways; one molecule of CO_2 is reduced to the methyl group of acetate, and the other molecule of CO_2 is reduced to the carbonyl group. The two C_1 units are then combined at the end to form acetyl-CoA (**Figure 14.17**).

A key enzyme of the acetyl-CoA pathway is *carbon monoxide* (*CO*) *dehydrogenase*. CO dehydrogenase contains the metals Ni, Zn, and Fe as cofactors. CO dehydrogenase catalyzes the reaction

$$CO_2 + H_2 \rightarrow CO + H_2O$$

and the CO produced ends up as the *carbonyl* carbon of acetate (Figure 14.17). The methyl group of acetate originates from the reduction of CO_2 by a series of reactions requiring the coenzyme *tetrahydrofolate* (Figure 14.17). The methyl group is then

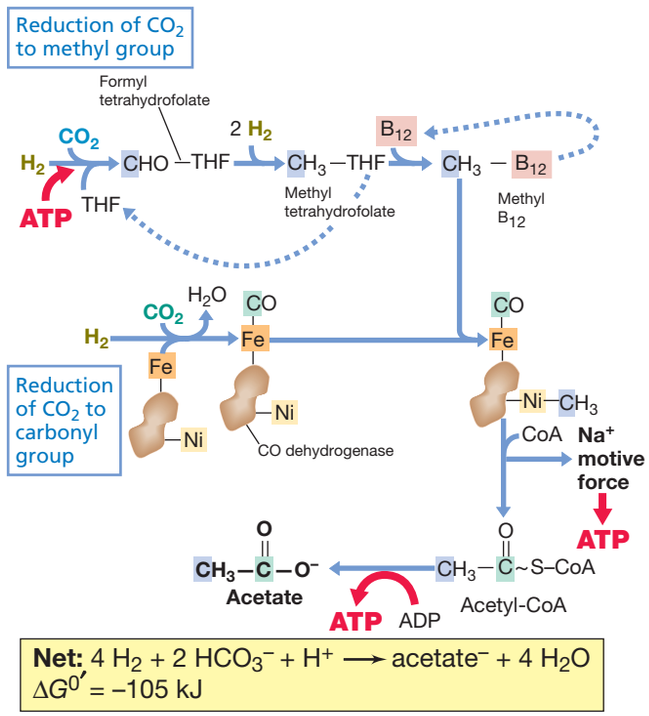


Figure 14.17 **Reactions of the acetyl-CoA pathway.** Carbon monoxide is bound to Fe and the CH_3 group to nickel in carbon monoxide dehydrogenase. Note that the formation of acetyl-CoA is coupled to the generation of a Na^+ motive force that drives ATP synthesis, and that ATP is also synthesized in the conversion of acetyl-CoA to acetate. THF, tetrahydrofolate; B_{12}, vitamin B_{12} in an enzyme-bound intermediate.

transferred from tetrahydrofolate to an enzyme that contains vitamin B_{12} as cofactor, and in the final step of the pathway, the methyl group is combined with CO by the enzyme CO dehydrogenase to form acetyl-CoA. Conversion of acetyl-CoA to acetate plus ATP completes the reaction series (Figure 14.17).

Energy Conservation in Acetogenesis

Energy conservation in acetogenesis is the result of substrate-level phosphorylation during the conversion of acetyl-CoA to acetate plus ATP (Section 14.1). There is also an energy-conserving step when a sodium motive force (analogous to a proton motive force) is established across the cytoplasmic membrane during the formation of acetyl-CoA. This energized state of the membrane allows for energy conservation from a Na^+-driven ATPase. Recall that we saw a similar situation in the succinate fermenter *Propionigenium*, where succinate decarboxylation was linked to Na^+ export and a Na^+-driven ATPase (Section 14.4). Acetogens need the ATP resulting from this reaction since the single ATP made by substrate-level phosphorylation is consumed in the first step of the acetyl-CoA pathway (Figure 14.17).

MiniQuiz

- Draw the structure of acetate and identify the carbonyl group and the methyl group. What key enzyme of the acetyl-CoA pathway produces the carbonyl group of acetate?
- How do acetogens make ATP from the synthesis of acetate?
- If fructose catabolism by glycolysis yields only two acetates, how does *Clostridium aceticum* produce three acetates from fructose?

14.10 Methanogenesis

The biological production of methane—*methanogenesis*—is carried out by a group of strictly anaerobic *Archaea* called the **methanogens**. The reduction of CO_2 by H_2 to form methane (CH_4) is a major pathway of methanogenesis and so we focus on this process and compare it with other forms of anaerobic respiration. We consider the basic properties, phylogeny, and taxonomy of the methanogens in Section 19.3; here we focus on their biochemistry and bioenergetics. Methanogenesis is a unique series of biochemical reactions that employs novel coenzymes. Because of this, we begin by considering these coenzymes and then move on to the actual pathway itself.

C_1 Carriers in Methanogenesis

Methanogenesis from CO_2 requires the input of eight electrons, and these electrons are added two at a time. This leads to intermediary oxidation states of the carbon atom from +4 (CO_2) to −4 (CH_4). The key coenzymes in methanogenesis can be divided into two classes: (1) those that carry the C_1 unit along its path of enzymatic reduction (C_1 carriers) and (2) those that donate electrons (redox coenzymes) (**Figure 14.18**, and see Figure 14.20). We consider the carriers first.

The coenzyme methanofuran is required for the first step of methanogenesis. Methanofuran contains the five-membered furan ring and an amino nitrogen atom that binds CO_2 (Figure 14.18*a*). Methanopterin (Figure 14.18*b*) is a methanogenic coenzyme that resembles the vitamin folic acid and plays a role analogous to that of tetrahydrofolate (a coenzyme that participates in C_1 transformations, see Figure 14.17) by carrying the C_1 unit in the intermediate steps of CO_2 reduction to CH_4. Coenzyme M (CoM) (Figure 14.18*c*) is a small molecule required for the terminal step of methanogenesis, the conversion of a methyl group (CH_3) to CH_4. Although not a C_1 carrier, the nickel (Ni^{2+})-containing tetrapyrrole coenzyme F_{430} (Figure 14.18*d*) is also needed for the terminal step of methanogenesis as part of the methyl reductase enzyme complex (discussed later).

Redox Coenzymes

The coenzymes F_{420} and 7-mercaptoheptanoylthreonine phosphate (also called coenzyme B, CoB), are electron donors in methanogenesis. Coenzyme F_{420} (Figure 14.18*e*) is a flavin derivative, structurally resembling the flavin coenzyme FMN (↻ Figure 4.15). F_{420} plays a role in methanogenesis as the electron donor in several steps of CO_2 reduction (see Figure 14.20). The oxidized form of F_{420} absorbs light at 420 nm and fluoresces blue-green. Such fluorescence is useful for the microscopic identification of a methanogen (**Figure 14.19**). CoB is required for the terminal step of methanogenesis catalyzed by the *methyl reductase enzyme complex*. As shown in Figure 14.18*f*, the structure of CoB resembles the vitamin pantothenic acid (which is part of acetyl-CoA) (↻ Figure 4.12).

Methanogenesis from $CO_2 + H_2$

Electrons for the reduction of CO_2 to CH_4 come primarily from H_2, but formate, carbon monoxide (CO), and even certain alcohols can also supply the electrons for CO_2 reduction in some methanogens. **Figure 14.20** shows the steps in CO_2 reduction by H_2:

1. CO_2 is activated by a methanofuran-containing enzyme and reduced to the formyl level. The immediate electron donor is the protein ferredoxin, a strong reductant with a reduction potential (E_0') near −0.4V.
2. The formyl group is transferred from methanofuran to an enzyme containing methanopterin (MP in Figure 14.20). It is subsequently dehydrated and reduced in two separate steps (total of 4 H) to the methylene and methyl levels. The immediate electron donor is reduced F_{420}.
3. The methyl group is transferred from methanopterin to an enzyme containing CoM by the enzyme methyl transferase. This reaction is highly exergonic and linked to the pumping of Na^+ across the membrane from inside to outside the cell.
4. Methyl-CoM is reduced to methane by methyl reductase; in this reaction, F_{430} and CoB are required. Coenzyme F_{430} removes the CH_3 group from CH_3–CoM, forming a Ni^{2+}–CH_3 complex. This complex is reduced by CoB, generating CH_4 and a disulfide complex of CoM and CoB (CoM-S—S-CoB).
5. Free CoM and CoB are regenerated by the reduction of CoM-S—S-CoB with H_2.

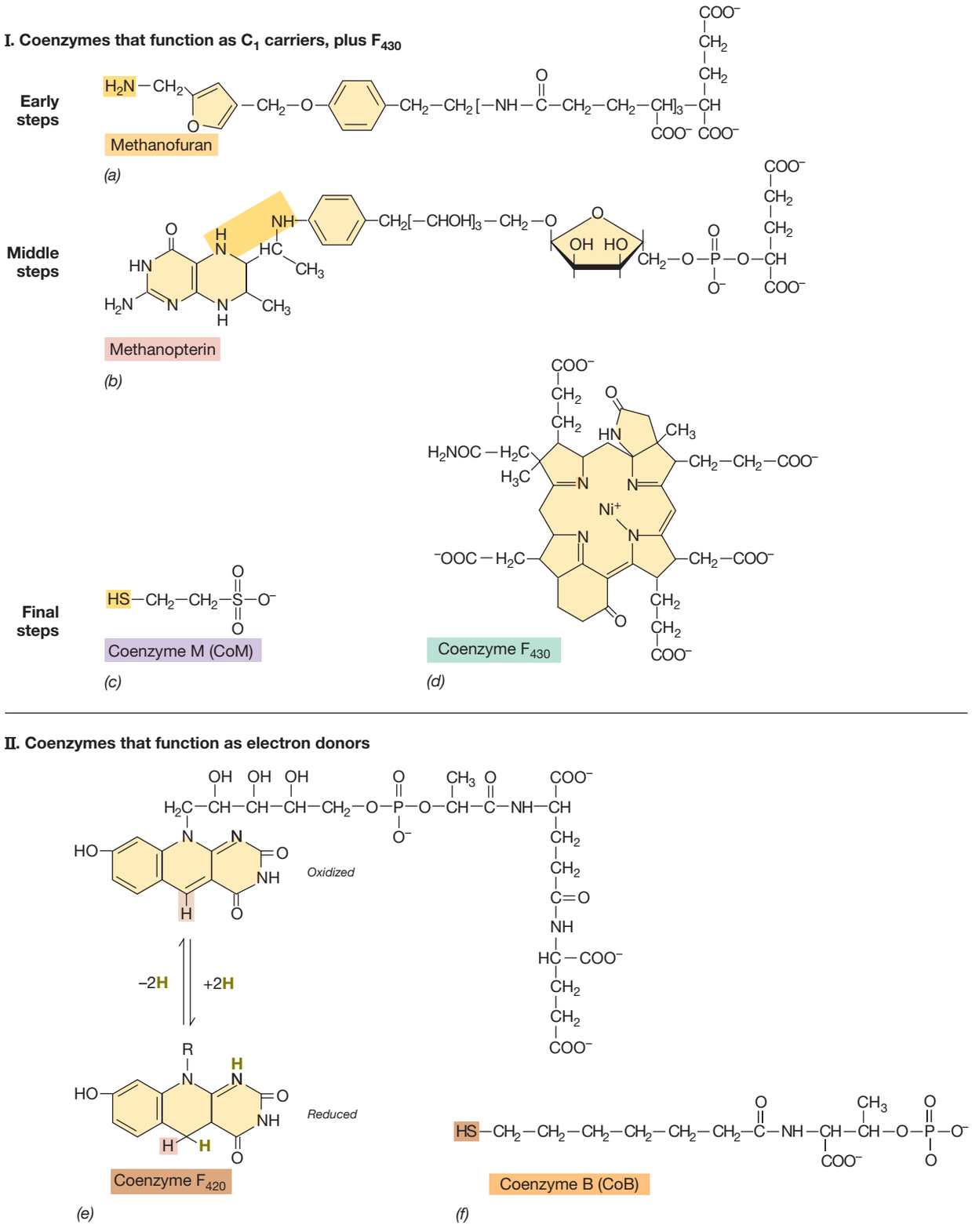


Figure 14.18 **Coenzymes of methanogenesis.** The atoms shaded in brown or yellow are the sites of oxidation–reduction reactions (brown in F_{420} and CoB) or the position to which the C_1 moiety is attached during the reduction of CO_2 to CH_4 (yellow in methanofuran, methanopterin, and coenzyme M). The colors used to highlight a particular coenzyme (CoB is orange, for example) are also in Figures 14.20–14.21 to follow the reactions in each figure.

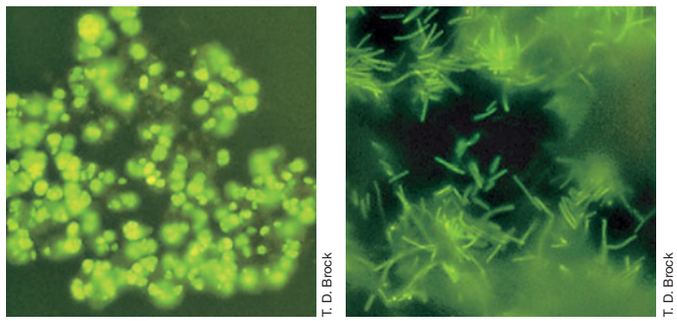


Figure 14.19 **Fluorescence due to the methanogenic coenzyme F_{420}.** *(a)* Autofluorescence in cells of the methanogen *Methanosarcina barkeri* due to the presence of the unique electron carrier F_{420}. A single cell is about 1.7 μm in diameter. The organisms were made visible by excitation with blue light in a fluorescence microscope. *(b)* F_{420} fluorescence in cells of the methanogen *Methanobacterium formicicum*. A single cell is about 0.6 μm in diameter.

Methanogenesis from Methyl Compounds and Acetate

We will learn in Section 19.3 that methanogens can form CH_4 from methylated compounds such as methanol and acetate, as well as from $H_2 + CO_2$. Methanol is catabolized by donating methyl groups to a corrinoid protein to form CH_3–corrinoid (**Figure 14.21**). Corrinoids are the parent structures of compounds such as vitamin B_{12} and contain a porphyrin-like corrin ring with a central cobalt atom (↻ Figure 15.8*a*). The CH_3–corrinoid complex then transfers the methyl group to CoM, yielding CH_3–CoM from which methane is formed in the same way as in the terminal step of CO_2 reduction (compare Figures 14.20 and 14.21*a*). If H_2 is unavailable to drive the terminal step, some of the methanol must be oxidized to CO_2 to yield electrons for this purpose. This occurs by reversal of steps in methanogenesis (Figures 14.20 and 14.21*a*).

When acetate is the substrate for methanogenesis, it is first activated to acetyl-CoA, which interacts with CO dehydrogenase from the acetyl-CoA pathway (Section 14.9). The methyl group of acetate is then transferred to the corrinoid enzyme to yield CH_3–corrinoid, and from there it goes through the CoM-mediated terminal step of methanogenesis. Simultaneously, the CO group is oxidized to yield CO_2 (Figure 14.21*b*).

Autotrophy

Autotrophy in methanogens occurs via the acetyl-CoA pathway (Section 14.9). As we have just seen, parts of this pathway are already integrated into the catabolism of methanol and acetate by methanogens (Figure 14.21). However, methanogens lack the tetrahydrofolate-driven series of reactions of the acetyl-CoA pathway that lead to the production of a methyl group (Figure 14.17). But this is not a problem because methanogens either derive methyl groups directly from their electron donors (Figure 14.21) or make methyl groups during methanogenesis from $H_2 + CO_2$ (Figure 14.20). Thus methanogens have abundant methyl groups, and the removal of some for biosynthesis is of little consequence. The carbonyl group of the acetate produced during autotrophic growth of methanogens is derived from the activity of CO dehydrogenase, and the terminal step in acetate synthesis is as described for acetogens (Section 14.9 and Figure 14.17).

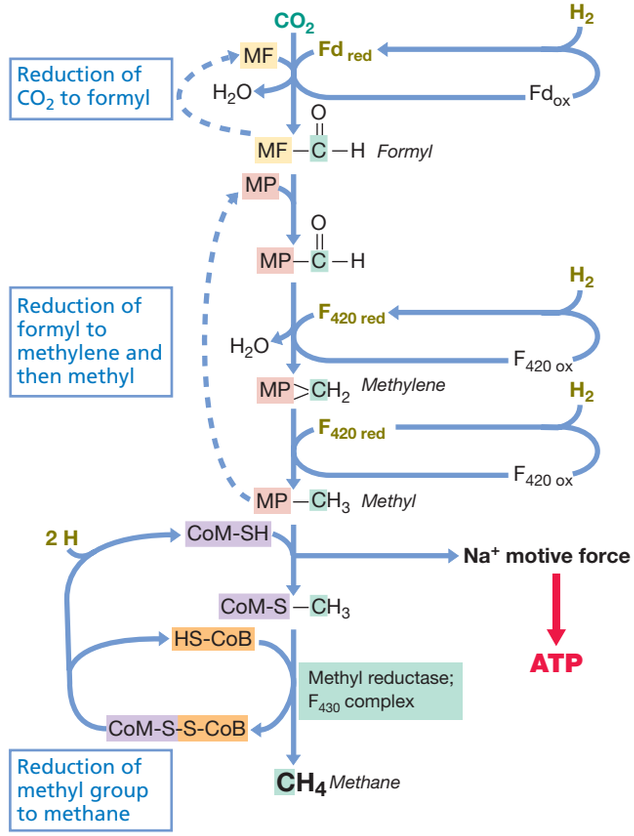


Figure 14.20 **Methanogenesis from CO_2 plus H_2.** The carbon atom reduced is shown in blue, and the source of electrons is highlighted in brown. See Figure 14.18 for the structures of the coenzymes. MF, Methanofuran; MP, methanopterin; CoM, coenzyme M; F_{420red}, reduced coenzyme F_{420}; F_{430}, coenzyme F_{430}; Fd, ferredoxin; CoB, coenzyme B.

Energy Conservation in Methanogenesis

Under standard conditions the free energy from the reduction of CO_2 to CH_4 with H_2 is −131 kJ/mol, which is sufficient for the synthesis of at least one ATP. Energy conservation in methanogenesis occurs at the expense of a proton or sodium motive force, depending on the substrate used; substrate-level phosphorylation (Section 14.1) does not occur. When methanogenesis is supported by $CO_2 + H_2$, ATP is produced from the sodium motive force generated during methyl transfer from MP to CoM by the enzyme methyl transferase (Figure 14.20). This energized state of the membrane then drives the synthesis of ATP, probably by way of an H^+-linked ATPase following conversion of the sodium motive force into a proton motive force by exchange of Na^+ for H^+ across the membrane.

In some methanogens, such as *Methanosarcina*, a nutritionally versatile organism that can make methane from acetate or methanol as well as from CO_2, a different mechanism of energy

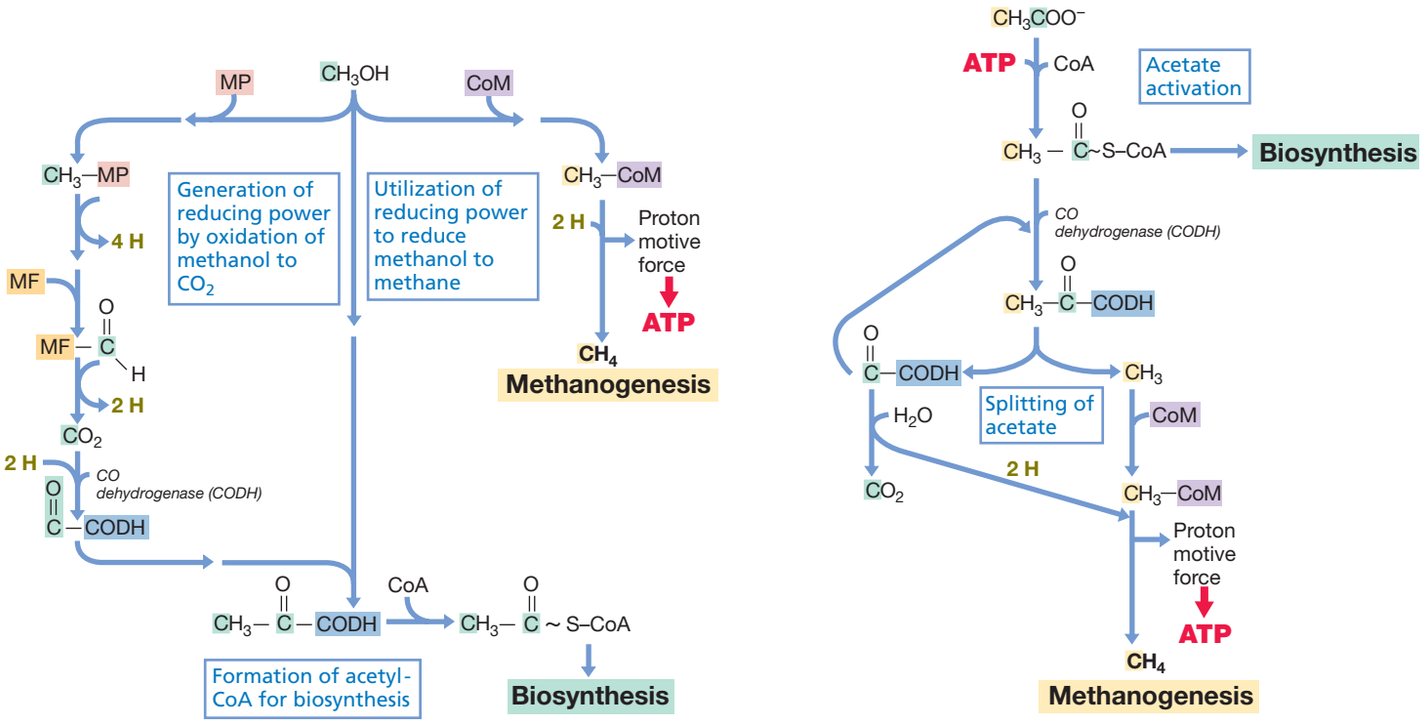


Figure 14.21 **Methanogenesis from methanol and acetate.** Both reaction series contain parts of the acetyl-CoA pathway. For growth on CH_3OH, most CH_3OH carbon is converted to CH_4, and a smaller amount is converted to either CO_2 or, via formation of acetyl-CoA, is assimilated into cell material. Abbreviations and color-coding are as in Figures 14.18 and 14.20; Corr, corrinoid-containing protein; CODH, carbon monoxide dehydrogenase.

conservation occurs in acetate- and methanol-grown cells, since the methyl transferase reaction cannot be coupled to the generation of a sodium motive force under these conditions. Instead, energy conservation in acetate- and methanol-grown cells is linked to the terminal step in methanogenesis, the methyl reductase step (Figure 14.20). In this reaction, the interaction of CoB with CH_3–CoM and methyl reductase forms CH_4 and a heterodisulfide, CoM-S—S-CoB. The latter is reduced by F_{420} to regenerate CoM-SH and CoB-SH (Figure 14.20). This reduction, carried out by the enzyme *heterodisulfide reductase*, is exergonic and is coupled to the pumping of H^+ across the membrane (**Figure 14.22**). Electrons from H_2 flow to the heterodisulfide reductase through a unique membrane-associated electron car-

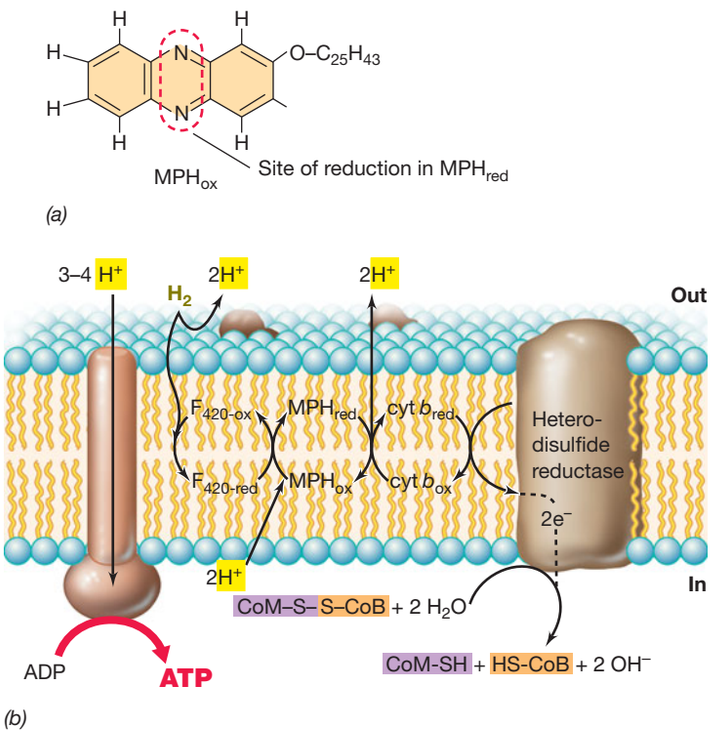


Figure 14.22 **Energy conservation in methanogenesis from methanol or acetate.** *(a)* Structure of methanophenazine (MPH in part b), an electron carrier in the electron transport chain leading to ATP synthesis; the central ring of the molecule can be alternately reduced and oxidized. *(b)* Steps in electron transport. Electrons originating from H_2 reduce F_{420} and then methanophenazine. The latter, through a cytochrome of the *b* type, reduces heterodisulfide reductase with the extrusion of H^+ to the outside of the membrane. In the final step, heterodisulfide reductase reduces CoM-S—S-CoB to HS-CoM and HS-CoB. See Figure 14.18 for the structures of CoM and CoB.

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rier called *methanophenazine*. This compound is reduced by F_{420} and then oxidized by a *b*-type cytochrome, and the latter is the electron donor to the heterodisulfide reductase (Figure 14.22). Cytochromes and methanophenazine are lacking in methanogens that use only $H_2 + CO_2$ for methanogenesis.

In methanogens we thus see at least two mechanisms for energy conservation: (1) a proton motive force linked to the methylreductase reaction and used to drive ATP synthesis in acetate- or methanol-grown cells, and (2) a sodium motive force formed during methanogenesis from $H_2 + CO_2$.

MiniQuiz

- What coenzymes function as C_1 carriers in methanogenesis? As electron donors?
- In methanogens growing on $H_2 + CO_2$, how is carbon obtained for cell biosynthesis?
- How is ATP made in methanogenesis when the substrates are $H_2 + CO_2$? Acetate?

14.11 Proton Reduction

Perhaps the simplest of all anaerobic respirations is one carried out by the hyperthermophile *Pyrococcus furiosus*. *P. furiosus* is a species of *Archaea* and grows optimally at 100°C (↻ Section 19.5) on sugars and small peptides as electron donors. *P. furiosus* was originally thought to use the glycolytic pathway because typical fermentation products such as acetate, CO_2, and H_2 were produced from glucose. However, analyses of sugar metabolism in this organism revealed an unusual and enigmatic situation.

During a key step of glycolysis, the oxidation of glyceraldehyde 3-phosphate forms 1,3-bisphosphoglyceric acid, an intermediate with two energy-rich phosphate bonds, each of which eventually yields ATP. In *P. furiosus*, this step is bypassed, yielding 3-phosphoglyceric acid directly from glyceraldehyde 3-phosphate (**Figure 14.23**). This prevents *P. furiosus* from making ATP by substrate-level phosphorylation at the 1,3-bisphosphoglyceric acid to 3-phosphoglyceric acid step, one of two sites of energy conservation in the glycolytic pathway (↻ Figure 4.14). This yields *P. furiosus* a net of zero ATP from glycolytic steps that normally yield 2 ATP in other organisms. How can *P. furiosus* ferment glucose and ignore the most important energy-yielding steps?

Protons as Electron Acceptors

The riddle of energy conservation in *P. furiosus* revolves around the oxidation of 3-phosphoglyceric acid. In glycolysis this acceptor is NAD^+, but in *P. furiosus* the protein ferredoxin is the electron acceptor (Figure 14.23). Ferredoxin has a much more negative E_0' than that of NAD^+/NADH, about the same as that of the 2 H^+/H_2 couple, –0.42 V. Ferredoxin is oxidized by transferring electrons to protons to form H_2 (Figure 14.23).

H_2 is typically produced during the oxidation of pyruvate to acetate plus CO_2 (Figure 14.2). This allows for ATP to be synthesized by substrate-level phosphorylation, and this also occurs in *P. furiosus* (Figure 14.23). But in addition, the H_2 released from ferredoxin is coupled to the pumping of protons (H^+) across the

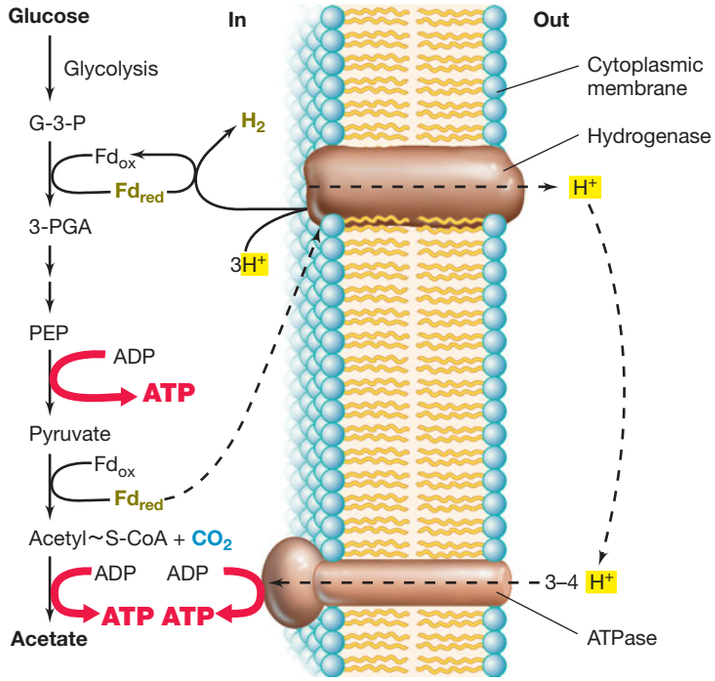


Figure 14.23 Modified glycolysis and proton reduction in anaerobic respiration in the hyperthermophile *Pyrococcus furiosus*. Hydrogen (H_2) production is linked to H^+ pumping by a hydrogenase that receives electrons from reduced ferredoxin (Fd_{red}). All intermediates from G-3-P downward in the pathway are present in two copies. Compare this figure with classical glycolysis in Figure 4.14. G-3-P, glyceraldehyde 3-phosphate; 3-PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate.

membrane by a membrane-integrated hydrogenase. This establishes a proton motive force that drives ATP synthesis by ATPase (Figure 14.23).

Although H^+ reduction by *P. furiosus* does not employ an electron transport chain per se, it can still be considered a form of anaerobic respiration because protons function as a net electron acceptor. The process differs from the proton pumping associated with decarboxylation reactions, such as those of *Oxalobacter*, where the free energy released during decarboxylation is coupled directly to H^+ translocation (Figure 14.8*b*). In *P. furiosus*, a proton is pumped during hydrogenase activity, analogous to how terminal electron carriers pump protons in aerobic or anaerobic respiratory processes (↻ Figures 4.19 and 14.13).

Growth Yields and Evolution

Measurements of growth yields of *P. furiosus* on glucose indicate that, despite being unable to conserve energy from the main reactions in glycolysis, the organism actually synthesizes more ATP from glucose than most other glucose fermenters! Two ATP are produced by substrate-level phosphorylation during the conversion of two acetyl-CoA to acetate, and about one additional ATP is produced from H_2 production by hydrogenase (Figure 14.23).

Whether H^+ reduction by prokaryotes is more widespread than that in *P. furiosus* is unknown. However, the ancient phylogeny of *Pyrococcus* (↻ Figure 19.1), coupled to its hot, anoxic habitat, similar to that of early Earth (↻ Section 16.3), suggests

that proton reduction, a bioenergetic mechanism that requires only a single membrane protein other than ATPase, might have been a very early form of anaerobic respiration, perhaps even nature's first proton pump.

MiniQuiz

- When fermenting glucose, how does *Pyrococcus furiosus* overcome the loss of most of the ATP produced by other glucose fermenters?

14.12 Other Electron Acceptors

In addition to the electron acceptors for anaerobic respiration discussed thus far, ferric iron (Fe^{3+}), manganic ion (Mn^{4+}), chlorate (ClO_3^-), perchlorate (ClO_4^-), and various organic compounds are important electron acceptors for bacteria in nature (**Figure 14.24**). Diverse bacteria are able to reduce these acceptors, especially Fe^{3+}, and many are able to reduce other acceptors, such as nitrate (NO_3^-) and elemental sulfur (S^0) (Sections 14.7 and 14.8), as well.

Ferric Iron Reduction

Ferric iron is an electron acceptor for energy metabolism in certain chemoorganotrophic and chemolithotrophic prokaryotes. Because Fe^{3+} is abundant in nature, its reduction supports a major form of anaerobic respiration. The reduction potential of the Fe^{3+}/Fe^{2+} couple is somewhat electropositive ($E_0' = +0.2$ V at pH 7), and thus, Fe^{3+} reduction can be coupled to the oxidation of several organic and inorganic electron donors. Electrons travel through an electron transport chain that generates a proton motive force and terminates in a ferric iron reductase system, reducing Fe^{3+} to ferrous iron (Fe^{2+}).

Much research on the energetics of Fe^{3+} reduction has been done with the gram-negative bacterium *Shewanella putrefaciens*, in which Fe^{3+}-dependent anaerobic growth occurs with various organic electron donors. Other important Fe^{3+} reducers include *Geobacter, Geospirillum*, and *Geovibrio*, and several hyperthermophilic *Archaea* (Chapters 17–19). *Geobacter metallireducens* has been a model for study of the physiology of Fe^{3+} reduction. *Geobacter* oxidizes acetate with Fe^{3+} as an acceptor in a highly exergonic reaction as follows:

$$Acetate^- + 8\ Fe^{3+} + 4\ H_2O \rightarrow 2\ HCO_3^- + 8\ Fe^{2+} + 9\ H^+$$
$$\Delta G^{0\prime} = -809\ kJ$$

Geobacter can also use H_2 or other organic electron donors, including the aromatic hydrocarbon toluene (see the Microbial Sidebar "Microbially Wired" in Chapter 24). This is of environmental significance because toluene from accidental spills or leakage from hydrocarbon storage tanks often contaminates iron-rich anoxic aquifers, and organisms such as *Geobacter* may be natural cleanup agents in such environments. Anoxic hydrocarbon metabolism is discussed in more detail shortly (Section 14.13).

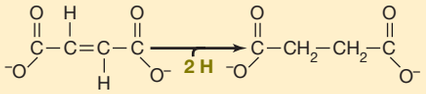
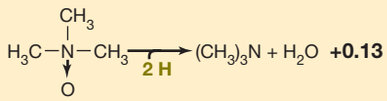
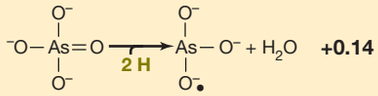
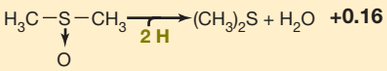
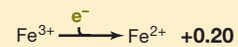
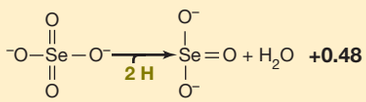
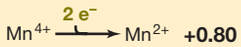
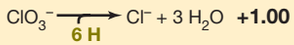
Couple	Reaction	E_0'
Fumarate/ Succinate		+0.03
Trimethylamine-*N*-oxide (TMAO)/ Trimethylamine (TMA)		+0.13
Arsenate/ Arsenite		+0.14
Dimethyl sulfoxide (DMSO)/ Dimethyl sulfide (DMS)		+0.16
Ferric ion/ Ferrous ion		+0.20
Selenate/ Selenite		+0.48
Manganic ion/ Manganous ion		+0.80
Chlorate/ Chloride		+1.00

Figure 14.24 Some alternative electron acceptors for anaerobic respirations. Note the reaction and E_0' of each redox pair.

Reduction of Manganese and Other Inorganic Substances

Manganese has several oxidation states, of which manganic (Mn^{4+}) and manganous (Mn^{2+}) are the most relevant to microbial energetics. *S. putrefaciens* and a few other bacteria grow anaerobically on acetate or several other carbon sources with Mn^{4+} as electron acceptor. The reduction potential of the Mn^{4+}/Mn^{2+} couple is extremely high (Figure 14.24); thus, several compounds can donate electrons to Mn^{4+} reduction. This is also the case for chlorate (Figure 14.24). Several chlorate and perchlorate-reducing bacteria have been isolated, and most of them are facultative aerobes and thus also capable of aerobic growth.

Other inorganic substances can function as electron acceptors for anaerobic respiration. These include selenium and arsenic compounds (Figure 14.24). Although usually not abundant in natural systems, arsenic and selenium compounds are occasional pollutants and can support anoxic growth of various bacteria. The reduction of selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) and eventually to metallic selenium (Se^0) is an important method of selenium removal from water and has been used as a means of cleaning—a process called *bioremediation* (↻ Section 24.8)—selenium-contaminated soils. By contrast, the reduction of arsenate (AsO_4^{2-}) to arsenite (AsO_3^{2-}) can actually create a toxicity problem. Some groundwaters flow through rocks containing insoluble arsenate minerals. However, if the arsenate is reduced to arsenite by bacteria, the arsenite becomes more mobile and can contaminate groundwater. This has caused a serious problem of arsenic contamination of well water in some developing countries, such as Bangladesh, in recent years.

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Figure 14.25 **Biomineralization during arsenate reduction by the sulfate-reducing bacterium *Desulfotomaculum auripigmentum*.** Left, appearance of culture bottle after inoculation. Right, following growth for two weeks and biomineralization of arsenic trisulfide, As_2S_3. Center, synthetic sample of As_2S_3.

Other forms of arsenate reduction are beneficial. For example, the sulfate-reducing bacterium *Desulfotomaculum* can reduce AsO_4^{3-} to AsO_3^{3-}, along with sulfate (SO_4^{2-}) to sulfide (HS^-). During this process a mineral containing arsenic and sulfide (As_2S_3, orpiment) precipitates spontaneously (**Figure 14.25**). The mineral is formed both intracellularly and extracellularly, and the process is an example of *biomineralization*, the formation of a mineral by bacterial activity. In this case As_2S_3 formation also functions as a means of detoxifying what would otherwise be a toxic compound (arsenic), and such microbial activities may have practical applications for the cleanup of arsenic-containing toxic wastes and groundwater.

Organic Electron Acceptors

Several organic compounds can be electron acceptors in anaerobic respirations. Of those listed in Figure 14.24, the compound that has been most extensively studied is *fumarate*, a citric acid cycle intermediate, which is reduced to succinate. The role of fumarate as an electron acceptor for anaerobic respiration derives from the fact that the fumarate–succinate couple has a reduction potential near 0 V (Figure 14.24), which allows coupling of fumarate reduction to the oxidation of NADH, FADH, or H_2. Bacteria able to use fumarate as an electron acceptor include *Wolinella succinogenes* (which can grow on H_2 as electron donor using fumarate as electron acceptor), *Desulfovibrio gigas* (a sulfate-reducing bacterium that can also grow under non-sulfate-reducing conditions), some clostridia, *Escherichia coli*, and many other bacteria.

Trimethylamine oxide (TMAO) (Figure 14.24) is an important organic electron acceptor. TMAO is a product of marine fish, where it functions as a means of excreting excess nitrogen. Various bacteria can reduce TMAO to trimethylamine (TMA), which has a strong odor and flavor (the odor of spoiled seafood is due primarily to TMA produced by bacterial action). Certain facultatively aerobic bacteria are able to use TMAO as an alternate electron acceptor. In addition, several phototrophic purple nonsulfur bacteria are able to use TMAO as an electron acceptor for anaerobic metabolism in darkness.

A compound similar to TMAO is dimethyl sulfoxide (DMSO), which is reduced by bacteria to dimethyl sulfide (DMS). DMSO is a common natural product and is found in both marine and freshwater environments. DMS has a strong, pungent odor, and bacterial reduction of DMSO to DMS is signaled by this characteristic odor. Bacteria, including *Campylobacter*, *Escherichia*, and many phototrophic purple bacteria, are able to use DMSO as an electron acceptor in energy generation.

The reduction potentials of the TMAO/TMA and DMSO/DMS couples are similar, near +0.15 V. This means that electron transport chains that terminate with the reduction of TMAO or DMSO must be rather short. As in fumarate reduction, in most instances of TMAO and DMSO reduction cytochromes of the *b* type (reduction potentials near 0 V) have been identified as terminal oxidases.

Halogenated Compounds as Electron Acceptors: Reductive Dechlorination

Several chlorinated compounds can function as electron acceptors for anaerobic respiration in the process called **reductive dechlorination** (also called *dehalorespiration*). For example, the bacterium *Desulfomonile* grows anaerobically with H_2 or organic compounds as electron donors and chlorobenzoate as an electron acceptor that is reduced to benzoate and hydrochloric acid (HCl):

$$C_7H_4O_2Cl^- + 2\ H \rightarrow C_7H_5O_2^- + HCl$$

The benzoate produced in this reaction can then be catabolized as an electron donor in energy metabolism. Besides *Desulfomonile*, which is also a sulfate-reducing bacterium (Table 14.6), several other bacteria can reductively dechlorinate, and some of these are restricted to chlorinated compounds as electron acceptors (**Table 14.8**).

Many of the chlorinated compounds used as electron acceptors are toxic to fish and other animal life; by contrast, the products of reductive dechlorination are often less toxic or even completely nontoxic. For example, the bacterium *Dehalococcoides* reduces tri- and tetrachloroethylene to the harmless gas ethene and *Dehalobacterium* converts dichloromethane (CH_2Cl_2) into acetate and formate (Table 14.8). Species of *Dehalococcoides*, which can only use chlorinated compounds as electron acceptors for anaerobic respiration, also reduce polychlorinated biphenyls (PCBs). PCBs are widespread organic pollutants that contaminate the sediments of lakes, streams, and rivers, where they accumulate in fish and other aquatic life. But removal of the chlorine groups from these molecules reduces their toxicity and makes the molecules available to further catabolism by other groups of anaerobic bacteria, such as sulfate-reducing and denitrifying bacteria. Thus, reductive dechlorination is not only a form of energy metabolism, but also an environmentally significant process of bioremediation. Many reductive dechlorinators are also capable of reducing nitrate or various reduced sulfur compounds (Table 14.8), and thus the group consists of both specialist and opportunist species.

Table 14.8 *Characteristics of some major genera of bacteria capable of reductive dechlorination*

	Genus				
Property	**Dehalobacter**	**Dehalobacterium**	**Desulfitobacterium**	**Desulfomonile**	**Dehalococcoides**
Electron donors	H_2	Dichloromethane (CH_2Cl_2) only	H_2, formate pyruvate, lactate	H_2, formate, pyruvate, lactate, benzoate	H_2, lactate
Electron acceptors	Trichloroethylene, tetrachloroethylene	Dichloromethane (CH_2Cl_2) only	Ortho-, meta-, or para-chlorophenols, NO_3^-, fumarate, SO_3^{2-}, $S_2O_3^{2-}$, S^0	Metachlorobenzoates, tetrachloroethylene, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$	Trichloroethylene, tetrachloroethylene
Product of reduction of tetrachloroethylene	Dichloroethylene	Not applicable	Trichloroethylene	Dichloroethylene	Ethene
Other properties[a]	Contains cytochrome *b*	Grows only on CH_2Cl_2 and by disproportionation as follows: $CH_2Cl_2 \rightarrow$ formate + acetate + HCl ATP is formed by substrate-level phosphorylation	Can also grow by fermentation	Contains cytochrome c_3; requires organic carbon source; can grow by fermentation of pyruvate	Lacks peptidoglycan
Phylogeny[b]	Gram-positive *Bacteria*	Gram-positive *Bacteria*	Gram-positive *Bacteria*	*Deltaproteobacteria*	Green nonsulfur *Bacteria* (*Chloroflexi*)

[a]All organisms are obligate anaerobes.
[b]See Chapters 16–18.

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MiniQuiz

- With H_2 as electron donor, why is reduction of Fe^{3+} a more favorable reaction than reduction of fumarate?
- Give an example of biomineralization.
- What is reductive dechlorination and why is it environmentally relevant?

14.13 Anoxic Hydrocarbon Oxidation Linked to Anaerobic Respiration

Hydrocarbons are organic compounds that contain only carbon and hydrogen and are highly insoluble in water. We will see later in this chapter that *aerobic* hydrocarbon oxidation is a common microbial process in nature (Section 14.14). However, both aliphatic and aromatic hydrocarbons can be oxidized to CO_2 under anoxic conditions as well. Anoxic hydrocarbon oxidation occurs by way of various anaerobic respirations but has been best studied in denitrifying and sulfate-reducing bacteria.

Aliphatic Hydrocarbons

Aliphatic hydrocarbons are straight-chain saturated or unsaturated compounds, and many are substrates for denitrifying and sulfate-reducing bacteria. Saturated aliphatic hydrocarbons as long as C_{20} have been shown to support growth, although shorter-chain hydrocarbons are more soluble and readily catabolized. The mechanism of anoxic hydrocarbon degradation has been well studied for hexane (C_6H_{14}) metabolism in denitrifying bacteria. However, the mechanism appears to be the same for anoxic catabolism of longer-chain hydrocarbons and for hydrocarbon degradation by other anaerobic bacteria.

Hexane is a saturated aliphatic hydrocarbon. In anoxic hexane metabolism by *Azoarcus*, a species of *Proteobacteria*, hexane is attacked on carbon atom 2 by an *Azoarcus* enzyme that attaches a molecule of fumarate, an intermediate of the citric acid cycle (Section 4.11), forming the intermediate *1-methylpentylsuccinate* (**Figure 14.26*a***). This compound now contains oxygen atoms and can be further catabolized anaerobically. Following the addition of coenzyme A, a series of reactions occurs that includes beta-oxidation (see Figure 14.42) and regeneration of fumarate. The electrons generated during beta-oxidation travel through an electron transport chain and generate a proton motive force. At the end of the chain, either nitrate (NO_3^-, in denitrifying bacteria) or sulfate (SO_4^{2-}, in sulfate-reducing bacteria) is reduced (Sections 14.7 and 14.8, respectively).

Aromatic Hydrocarbons

Aromatic hydrocarbons can be degraded anaerobically by some denitrifying, ferric iron-reducing, and sulfate-reducing bacteria. For anoxic catabolism of the aromatic hydrocarbon toluene, oxygen needs to be added to the compound to begin catabolism. Obviously this cannot come from O_2 if conditions are anoxic and occurs instead by the addition of fumarate, just as in aliphatic hydrocarbon catabolism (Figure 14.26). The reaction series eventually yields benzoyl-CoA, which is then further degraded by ring reduction (see Figure 14.27). Benzene (C_6H_6) can also be catabolized by nitrate-reducing bacteria, likely by a mechanism similar to that of toluene. Aromatic hydrocarbons containing multiple rings such as naphthalene ($C_{10}H_8$) can be degraded by certain sulfate-reducing and denitrifying bacteria. Growth on these substrates is very slow, and oxygenation of the hydrocarbon occurs by the addition of a molecule of CO_2 to the ring to form a carboxylic acid derivative.

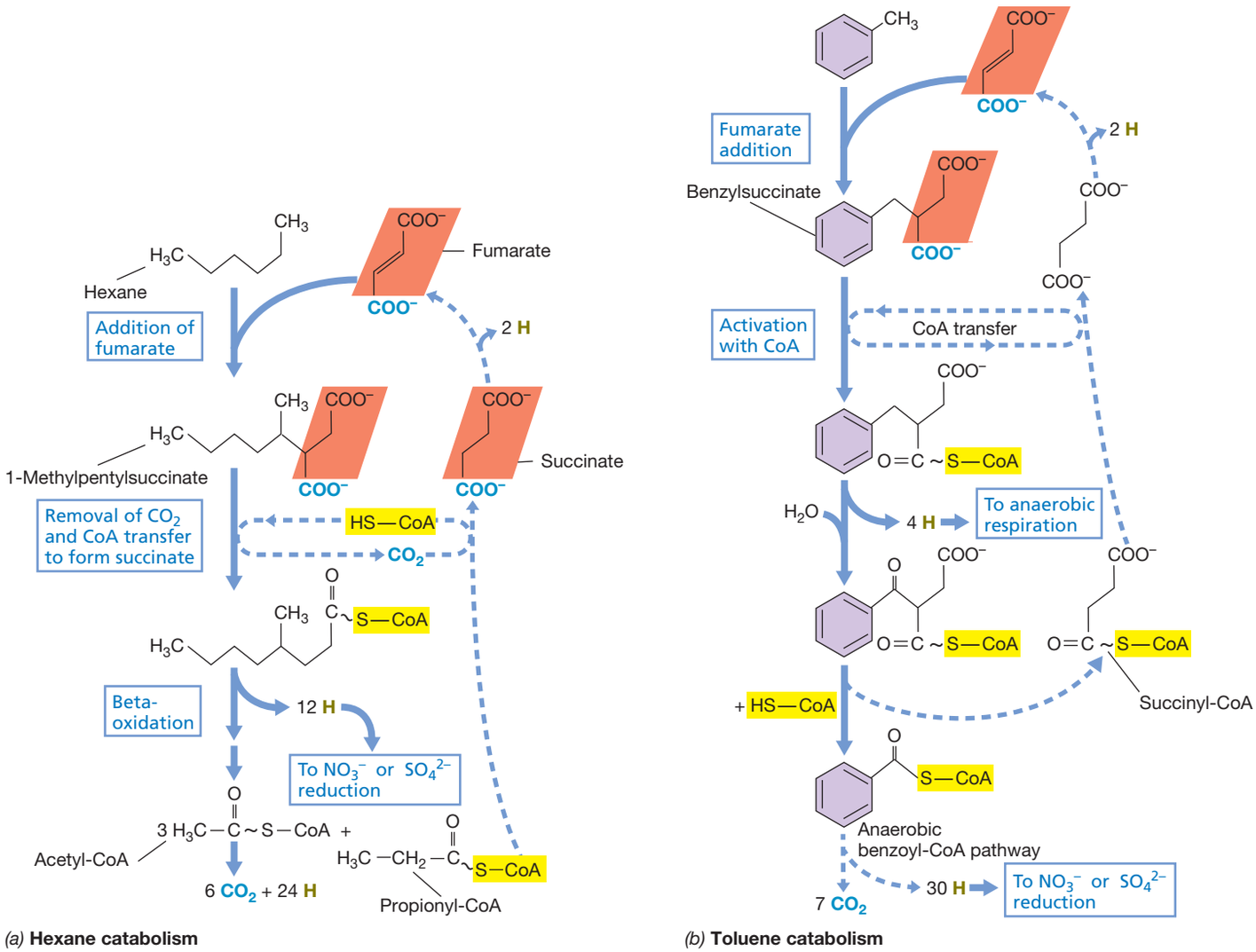

Figure 14.26 **Anoxic catabolism of two hydrocarbons.** *(a)* In anoxic catabolism of the aliphatic hydrocarbon hexane, the addition of fumarate provides the oxygen atoms necessary to form a fatty acid derivative that can be catabolized by beta-oxidation (see Figure 14.42) to yield acetyl-CoA. Electrons (H) generated from hexane catabolism are used to reduce sulfate or nitrate in anaerobic respirations. *(b)* Fumarate addition during the anoxic catabolism of the aromatic hydrocarbon toluene forms benzylsuccinate.

Besides the groups of anaerobes listed above, many other groups of bacteria can catabolize aromatic hydrocarbons anaerobically, including fermentative and phototrophic bacteria. However, except for toluene, only aromatic compounds that contain an O atom are degraded, and they are typically degraded by a common mechanism. When we examine the aerobic catabolism of aromatic compounds (Section 14.14), we will see that the biochemical mechanism occurs by way of ring *oxidation* (see Figure 14.30). By contrast, under anoxic conditions, the catabolism of aromatic compounds proceeds by ring *reduction*. Benzoate catabolism by the "benzoyl-CoA pathway" has been the focus of much of the work in this area, and the purple phototrophic bacterium *Rhodopseudomonas palustris*, an organism capable of catabolizing a wide variety of aromatic compounds, has been a model experimental organism (**Figure 14.27**). Benzoate catabolism in this pathway begins by forming the coenzyme A derivative followed by ring cleavage to yield fatty or dicarboxylic acids that can be further catabolized to intermediates of the citric acid cycle.

Anoxic Oxidation of Methane

Methane (CH_4) is the simplest hydrocarbon. In freshwater ecosystems, methane is produced in anoxic sediments by methanogens and then oxidized to CO_2 by methanotrophs when it reaches oxic zones. These methanotrophs require O_2 for the catabolism of CH_4 because the first step in CH_4 oxidation employs a monooxygenase enzyme (see Section 14.14 and Figure 14.30). However, CH_4 can also be oxidized under *anoxic* conditions in marine and freshwater sediments.

In marine sediments, the anoxic oxidation of methane (AOM) is catalyzed by cell aggregates that contain both sulfate-reducing

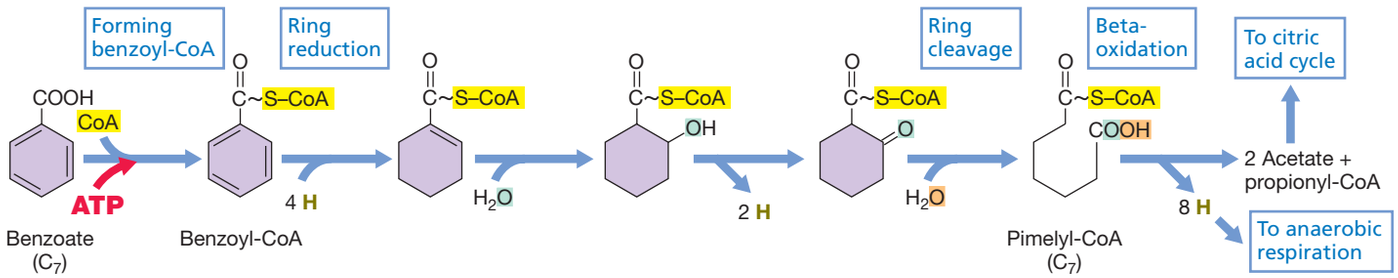

Figure 14.27 Anoxic degradation of benzoate by the benzoyl-CoA pathway. This pathway operates in the purple phototrophic bacterium *Rhodopseudomonas palustris* and many other facultative bacteria, both phototrophic and chemotrophic. Note that all intermediates of the pathway are bound to coenzyme A. The acetate produced is further catabolized in the citric acid cycle.

bacteria and *Archaea* phylogenetically related to methanogens (**Figure 14.28**). However, the archaeal component, called ANME (*an*oxic *me*thanotroph), of which there are several types, does not function in the consortium as a methano*gen*, but instead as a methano*troph*, oxidizing CH_4 as an electron donor. Electrons from methane oxidation are transferred to the sulfate reducer, which uses them to reduce SO_4^{2-} to H_2S (Figure 14.28*b*).

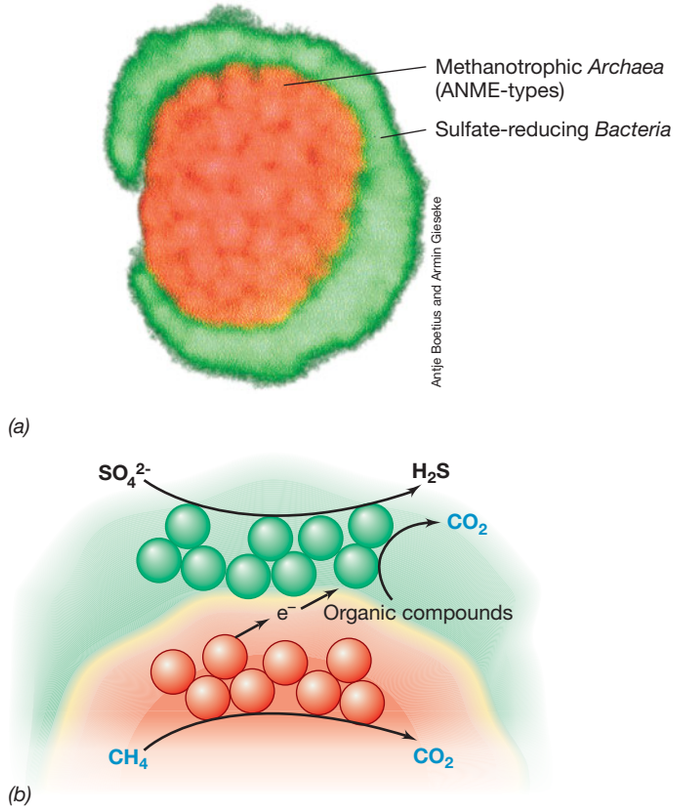

Figure 14.28 Anoxic methane oxidation. *(a)* Methane-oxidizing cell aggregates from marine sediments. The aggregates contain methanotrophic *Archaea* (red) surrounded by sulfate-reducing bacteria (green). Each cell type has been stained by a different FISH probe (↻ Section 16.9). The aggregate is about 30 μm in diameter. *(b)* Mechanism for the cooperative degradation of CH_4. An organic compound or some other carrier of reducing power transfers electrons from methanotroph to sulfate reducer.

Details of the mechanism of AOM by the two organisms in the consortia are unclear, but it is thought that the methanotroph first activates CH_4 in some way and then oxidizes it to CO_2 by reversing the steps of methanogenesis, a series of reactions that would be highly endergonic (Section 14.10). Electrons are generated during the oxidative steps, but in what form the electrons are released to the sulfate reducer is unknown. Electrons are not released as H_2. Instead, electrons from the oxidation of CH_4 are shuttled from the methanotroph to the sulfate reducer in some organic intermediate, such as acetate, formate, or possibly as an organic sulfide (Figure 14.28*b*).

Regardless of mechanism, AOM yields only a very small amount of free energy:

$$CH_4 + SO_4^{2-} + H^+ \rightarrow CO_2 + HS^- + 2\,H_2O \qquad \Delta G^{0\prime} = -18\ \text{kJ}$$

How this energy is split between the methanotroph and the sulfate reducer is unknown. Substrate-level phosphorylation is unlikely, but as we have seen several times in this chapter, ion pumps can operate at these low energy yields and probably play a role in the energetics of AOM. In addition to oxidizing CH_4, the methanotrophic component of this consortium has been shown to fix nitrogen (↻ Section 13.14), and it is possible that this fixed nitrogen supports the nitrogen needs of the entire consortium.

AOM is not limited to sulfate-reducing bacteria consortia. Methane-oxidizing denitrifying consortia are active in anoxic environments where CH_4 and NO_3^- coexist in significant amounts, such as certain freshwater sediments. In laboratory enrichments of these consortia some contain ANME-type methanotrophs while others are totally free of *Archaea*. AOM linked to ferric iron (Fe^{3+}) or manganic ion (Mn^{4+}) reduction also occurs. In both of these cases ANME-type methanotrophs have been identified, but in each system different ANME groups seem to predominate. Notably, however, the free-energy yield of AOM using Mn^{4+} or Fe^{3+} as electron acceptors is considerably more favorable than that of SO_4^{2-}, as would be expected from comparison of the reduction potentials of these different redox couples (Figure 14.11).

A newly discovered denitrifying bacterium employs a remarkable mechanism for anoxic methanotrophy not seen in any other methanotrophic system. The organism, provisionally named *Methylomirabilis oxyfera* because it is not yet in pure culture, oxidizes CH_4 with NO_3^- as an electron acceptor. During CH_4 oxidation, electrons reduce NO_3^- in steps we have previously seen in

denitrifying bacteria such as *Pseudomonas* (Section 14.7). These steps include the reduction of NO_3^- to NO_2^-, and further on to N_2 (Figure 14.13*c*). But unlike *Pseudomonas*, in *M. oxyfera* NO_2^- is reduced to N_2 by way of nitric oxide (NO) without first producing nitrous oxide (N_2O) as an intermediate. Instead, *M. oxyfera* splits NO into N_2 and O_2 ($2\ NO \rightarrow N_2 + O_2$) using an enzyme called NO dismutase and then uses the O_2 produced as an electron acceptor for CH_4 oxidation. That is, the organism *produces its own* O_2 as an oxidant for electrons generated during the oxidation of CH_4 to CO_2.

The discovery of AOM by *Methylomirabilis oxyfera* has added a new twist to an already very intriguing story. The link between AOM and sulfate reduction was the first to be discovered and would naturally predominate in marine sediments because sulfate reduction is the dominant form of anaerobic respiration that occurs there (↻ Section 24.3). But the list of alternative electron acceptors in anaerobic respiration is a very long one (Figure 14.11), and thus the discovery of AOM linked to oxidants other than SO_4^{2-}, NO_3^-, Mn^{4+}, or Fe^{3+} would not be surprising.

MiniQuiz

- Why is toluene a hydrocarbon whereas benzoate is not?
- How is hexane oxygenated during anoxic catabolism?
- What is AOM and which organisms participate in the process?

III Aerobic Chemoorganotrophic Processes

Many organic compounds are catabolized aerobically, and we survey some major aerobic processes here. We begin with a consideration of the oxygen requirements for some of these reactions.

14.14 Molecular Oxygen as a Reactant and Aerobic Hydrocarbon Oxidation

We previously discussed the role of molecular oxygen (O_2) as an *electron acceptor* in energy-generating reactions (↻ Sections 4.9 and 4.10). Although this is by far the most important role of O_2 in cellular metabolism, O_2 plays an important role as a *reactant* in certain anabolic and catabolic processes as well.

Oxygenases

Oxygenases are enzymes that catalyze the incorporation of O_2 into organic compounds. There are two classes of oxygenases: *dioxygenases*, which catalyze the incorporation of both atoms of O_2 into the molecule, and *monooxygenases*, which catalyze the incorporation of one of the two oxygen atoms of O_2 into an organic compound; the second atom of O_2 is reduced to H_2O. For most monooxygenases, the required electron donor is NADH or NADPH (**Figure 14.29**). In the example of ammonia monooxygenase discussed previously (↻ Section 13.10), the electron donor was cytochrome *c*, but this seems to be an exception.

Several types of reactions in living organisms require O_2 as a reactant. One of the best examples is O_2 in sterol biosynthesis.

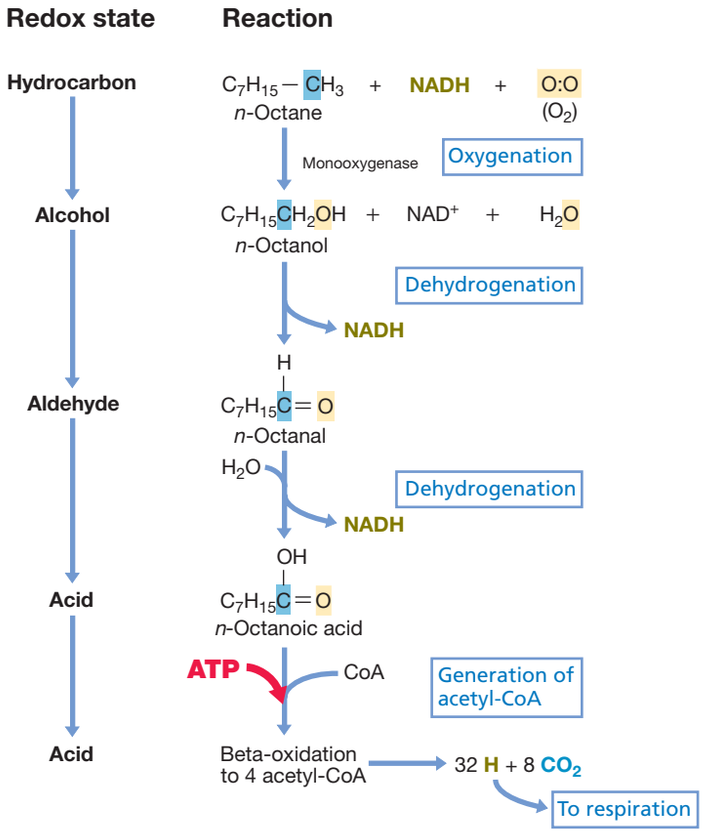

Figure 14.29 Monooxygenase activity. Steps in oxidation of an aliphatic hydrocarbon, the first of which is catalyzed by a monooxygenase. Some sulfate-reducing and denitrifying bacteria can degrade aliphatic hydrocarbons under anoxic conditions. For a description of beta-oxidation, see Figure 14.42.

Sterols are planar ring structures present in the membranes of eukaryotic cells and a few bacteria, and their biosynthesis requires O_2. Such a reaction obviously cannot take place under anoxic conditions, so organisms that grow anaerobically must either grow without sterols or obtain the needed sterols preformed from their environment. The requirement of O_2 in biosynthesis is of evolutionary significance, as O_2 was originally absent from the atmosphere of Earth when life first evolved. Oxygen became available on Earth only after the proliferation of cyanobacteria, approximately 2.7 billion years before the present (↻ Section 16.3). A second example of O_2 as a reactant in biochemical processes is with aerobic hydrocarbon oxidation, and we consider this now.

Aerobic Hydrocarbon Oxidation

We saw in Section 14.13 how hydrocarbons could be catabolized under anoxic conditions; however, the aerobic oxidation of hydrocarbons is probably a much more extensive process in nature. Low-molecular-weight hydrocarbons are gases, whereas those of higher molecular weight are liquids or solids. Hydrocarbon consumption can be a natural process or can be a directed process for cleaning up spilled hydrocarbons from human activities (bioremediation, ↻ Section 24.7). Either way, the aerobic

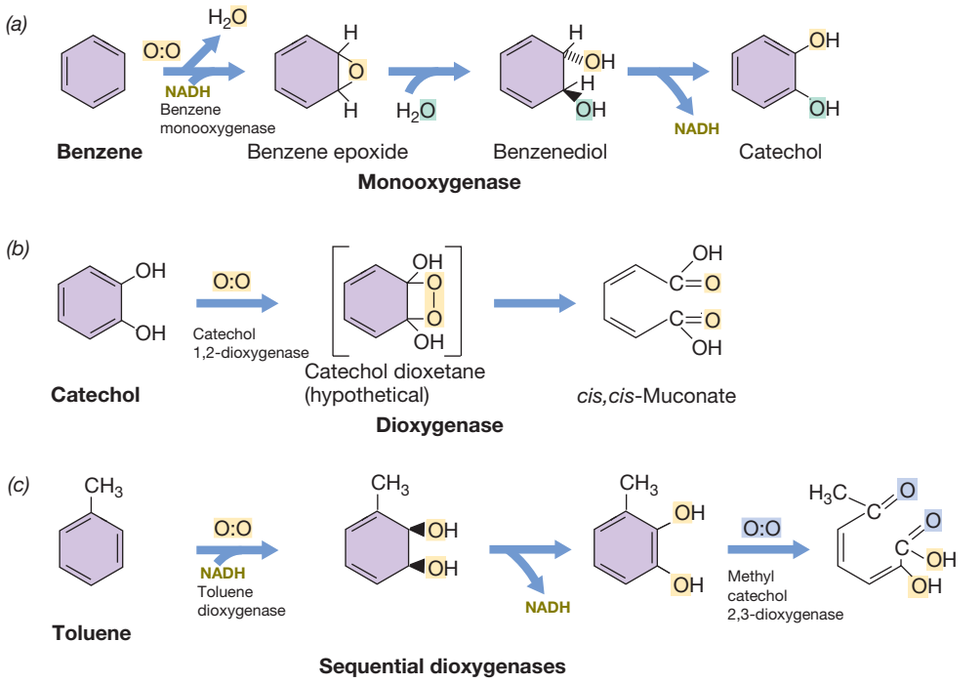

Figure 14.30 Roles of oxygenases in catabolism of aromatic compounds. Monooxygenases introduce one atom of oxygen from O_2 into a substrate, whereas dioxygenases introduce both atoms of oxygen. *(a)* Hydroxylation of benzene to catechol by a monooxygenase in which NADH is an electron donor. *(b)* Cleavage of catechol to *cis,cis*-muconate by an intradiol ring-cleavage dioxygenase. *(c)* The activities of a ring-hydroxylating dioxygenase and an extradiol ring-cleavage dioxygenase in the degradation of toluene. The oxygen atoms that each enzyme introduces are distinguished by different colors. Catechol and related compounds are common intermediates in aerobic aromatic catabolism. Compare aerobic toluene catabolism to anoxic toluene catabolism shown in Figure 14.26*b*.

catabolism of hydrocarbons can be very rapid owing to the metabolic advantage of having O_2 available as an electron acceptor compared with other acceptors of less positive reduction potential (Figure 14.11).

Several bacteria and fungi can use hydrocarbons as electron donors to support growth under aerobic conditions. The initial oxidation step of saturated aliphatic hydrocarbons by these organisms requires O_2 as a reactant, and one of the atoms of the oxygen molecule is incorporated into the oxidized hydrocarbon, typically at a terminal carbon atom. This reaction is carried out by a monooxygenase and a typical reaction sequence is shown in Figure 14.29. The end product of the reaction sequence known as beta-oxidation (see Figure 14.42) is acetyl-CoA, and this is oxidized in the citric acid cycle along with the production of electrons for the electron transport chain. The sequence is repeated to progressively degrade long hydrocarbon chains, and in most cases the hydrocarbon is oxidized completely to CO_2.

Aromatic Hydrocarbons

Many aromatic hydrocarbons can also be used as electron donors aerobically by microorganisms. The metabolism of these compounds, some of which contain several rings such as naphthalene or biphenyls, typically has as its initial stage the formation of catechol or a structurally related compound via catalysis by oxygenase enzymes, as shown in **Figure 14.30**. Once catechol is formed it can be further degraded and cleaved into compounds that can enter the citric acid cycle: succinate, acetyl-CoA, and pyruvate.

Several steps in the aerobic catabolism of aromatic hydrocarbons require oxygenases. Figure 14.30*a–c* shows four different oxygenase-catalyzed reactions, one using a monooxygenase, two using a ring-cleaving dioxygenase, and one using a ring-hydroxylating dioxygenase. As in aerobic aliphatic hydrocarbon catabolism, aromatic compounds, whether single or multiple ringed, are typically oxidized completely to CO_2 and electrons enter an electron transport chain terminating with the reduction of O_2 to H_2O.

MiniQuiz

- How do monooxygenases differ in function from dioxygenases?
- What is the final product of catabolism of a hydrocarbon?
- What fundamental difference exists in the anaerobic degradation of an aromatic compound compared with its aerobic metabolism?

14.15 Methylotrophy and Methanotrophy

Methane (CH_4) and many other C_1 compounds can be catabolized aerobically by **methylotrophs**. Methylotrophs are organisms that use organic compounds that lack C—C bonds as electron donors and carbon sources (Section 17.6). The catabolism of compounds containing only a single carbon atom, such as methane and methanol (CH_3OH), have been the best studied of these substrates. We focus here on the physiology of methylotrophy, using CH_4 as an example.

Biochemistry of Methane Oxidation

The steps in CH_4 oxidation to CO_2 can be summarized as

$$CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow HCOO^- \rightarrow CO_2$$

Methanotrophs are those methylotrophs that can use CH_4. Methanotrophs assimilate either all or one-half of their carbon (depending on the pathway used) at the oxidation state of formaldehyde (CH_2O). We will see later that this affords a major energy savings compared with the carbon assimilation of autotrophs, which also assimilate C_1 units, but exclusively from CO_2 rather than organic compounds.

Reactions and Bioenergetics of Aerobic Methanotrophy

The initial step in the aerobic oxidation of CH_4 is carried out by the enzyme *methane monooxygenase* (MMO). As we discussed in Section 14.14, monooxygenases catalyze the incorporation of oxygen atoms from O_2 into carbon compounds (and into some nitrogen compounds, ↻ Section 13.10), thereby preparing them for further degradation. Methanotrophy has been especially well studied in the bacterium *Methylococcus capsulatus*. This organism contains two MMOs, one cytoplasmic and the other membrane-integrated. The electron donor for the cytoplasmic MMO is NADH, and NADH is probably the electron donor for the membrane-integrated MMO as well (**Figure 14.31**).

In the MMO reaction, an atom of oxygen is introduced into CH_4, and CH_3OH and H_2O are the products. Reducing power for the first step comes from later oxidative steps in the pathway. CH_3OH is oxidized by a periplasmic dehydrogenase, yielding formaldehyde and NADH (Figure 14.31). Once CH_2O is formed it is oxidized to CO_2 by either of two different pathways. One pathway uses enzymes that contain the coenzyme tetrahydrofolate, a coenzyme widely involved in C_1 transformations. The second and totally independent pathway employs the coenzyme methanopterin. Recall that methanopterin is a C_1 carrier in intermediate steps of the reduction of CO_2 to CH_4 by methanogenic *Archaea* (Section 14.10 and Figure 14.20). Methanotrophs use a methanopterin-containing reaction to drive the oxidation of CH_2O to formate plus NADH; formate is then oxidized to CO_2 by the enzyme formate dehydrogenase. However, regardless of the CH_2O oxidation pathway employed, electrons from the oxidation of CH_2O enter the electron transport chain, generating a proton motive force from which ATP is synthesized (Figure 14.31).

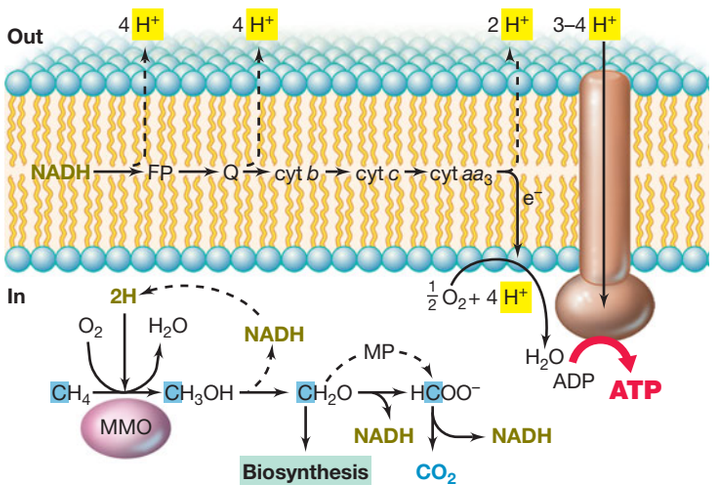

Figure 14.31 **Oxidation of methane by methanotrophic bacteria.** CH_4 is oxidized to CH_3OH by the enzyme methane monooxygenase (MMO). A proton motive force is established from electron flow in the membrane, and this fuels ATPase. Note how carbon for biosynthesis comes from CH_2O. Although not depicted as such, MMO is actually a membrane-associated enzyme and methanol dehydrogenase is periplasmic. FP, flavoprotein; cyt, cytochrome; Q, quinone; MP, methanopterin.

C_1 Assimilation into Cell Material

As will be discussed in Chapter 17, three phylogenetic groups of methanotrophs are known and at least two distinct pathways for C_1 incorporation into cell material exist. The **serine pathway**, utilized by type II methanotrophs, is outlined in **Figure 14.32**. In this pathway, a two-carbon unit, acetyl-CoA, is synthesized from one molecule of CH_2O (produced from the oxidation of CH_3OH, Figure 14.31) and one molecule of CO_2. The serine pathway requires reducing power and energy in the form of two molecules each of NADH and ATP, respectively, for each acetyl-CoA synthesized. The serine pathway employs a number of enzymes of the citric acid cycle and one enzyme, *serine transhydroxymethylase*, unique to the pathway (Figure 14.32).

The **ribulose monophosphate pathway**, used by type I methanotrophs, is outlined in **Figure 14.33**. This pathway is more efficient than the serine pathway because *all* of the carbon for cell material is derived from CH_2O. And, because CH_2O is at the same oxidation level as cell material, no reducing power is needed. The ribulose monophosphate pathway requires one molecule of ATP for each molecule of glyceraldehyde 3-phosphate (G-3-P) synthesized (Figure 14.33). Two G-3-Ps can be converted into glucose by the glycolytic pathway. Consistent with the lower

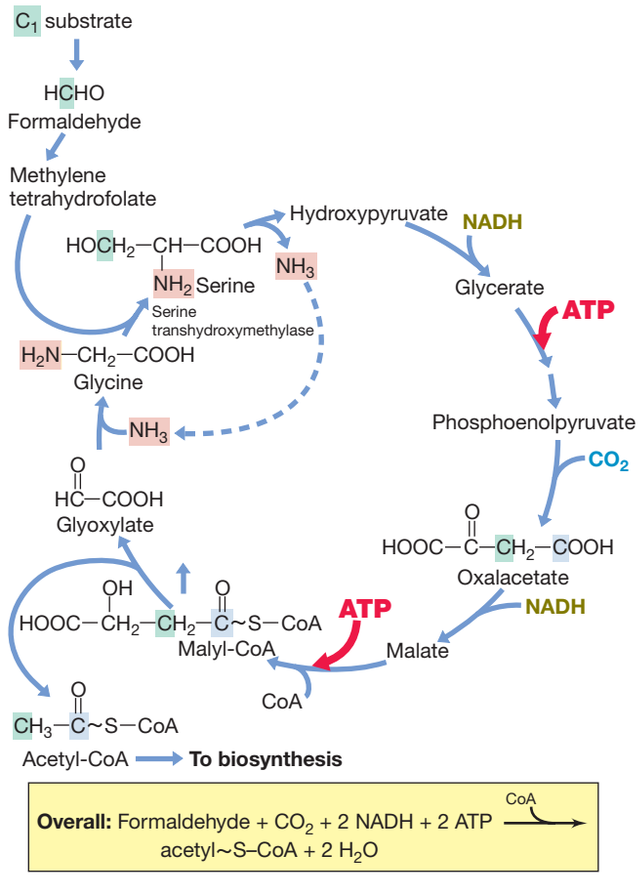

Figure 14.32 **The serine pathway for the assimilation of C_1 units into cell material by methylotrophic bacteria.** The product of the pathway, acetyl-CoA, is used as the starting point for making new cell material. The key enzyme of the pathway is serine transhydroxymethylase.

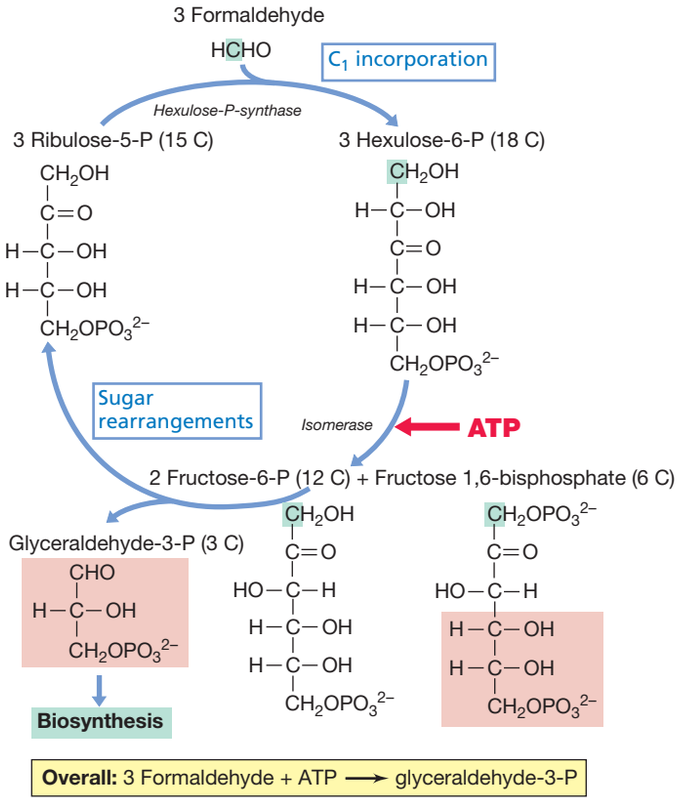

Figure 14.33 The ribulose monophosphate pathway for assimilation of C_1 units by methylotrophic bacteria. Three molecules of CH_2O are needed to complete the cycle, with the net result being one molecule of glyceraldehyde 3-phosphate. The key enzyme of this pathway is hexulose P-synthase. The sugar rearrangements require enzymes of the pentose phosphate pathway (Figure 14.38).

energy requirements of the ribulose monophosphate pathway, the cell yield (grams of cells produced per mole of CH_4 oxidized) of type I methanotrophs is higher than for type II methanotrophs.

The enzymes *hexulosephosphate synthase*, which condenses one molecule of formaldehyde with one molecule of ribulose 5-phosphate, and *hexulose 6-P isomerase* (Figure 14.33) are unique to the ribulose monophosphate pathway. The remaining enzymes of this pathway are widely distributed in bacteria. Finally, it should also be noted that the substrate for the initial reaction in this pathway, ribulose 5-phosphate, is very similar to the C_1 acceptor in the Calvin cycle, ribulose 1,5-bisphosphate (↻ Section 13.12), a signal that these two cycles likely share common evolutionary roots.

MiniQuiz

- Why are the energy and reducing power requirements for the ribulose monophosphate pathway different from those of the serine pathway?
- Why does the oxidation of CH_4 to CH_3OH require reducing power?
- Which pathway, the Calvin cycle or the ribulose monophosphate pathway, requires the greater energy input? Why?

14.16 Sugar and Polysaccharide Metabolism

Sugars and polysaccharides are common substrates for chemoorganotrophs, and we briefly consider their catabolism here.

Hexose and Polysaccharide Utilization

Sugars containing six carbon atoms, called *hexoses*, are the most important electron donors for many chemoorganotrophs and are also important structural components of microbial cell walls, capsules, slime layers, and storage products. The most common sources of hexose in nature are listed in **Table 14.9**, from which it can be seen that most are polysaccharides, although a few are disaccharides. Cellulose and starch are two of the most abundant natural polysaccharides.

Although both starch and cellulose are composed entirely of glucose, the glucose units are bonded differently (Table 14.9), and this profoundly affects their properties. Cellulose is more insoluble than starch and is usually less rapidly digested. Cellulose forms long fibrils, and organisms that digest cellulose are often found attached directly to these fibrils (**Figure 14.34**). In this way cellulase, the enzyme required to degrade cellulose, can contact its substrate and begin the digestive process. Many fungi are able to digest cellulose, and these are mainly responsible for the decomposition of plant materials on the forest floor. Among bacteria, however, cellulose digestion is restricted to relatively few groups, of which the gliding bacteria *Sporocytophaga* and *Cytophaga* (Figure 14.34 and **Figure 14.35**), clostridia, and actinomycetes are the most common.

Anoxic digestion of cellulose is carried out by a few *Clostridium* species, which are common in lake sediments, animal intestinal tracts, and systems for anoxic sewage digestion. Cellulose digestion is also a major process in the rumen of ruminant animals where *Fibrobacter* and *Ruminococcus* species actively degrade cellulose (↻ Section 25.9).

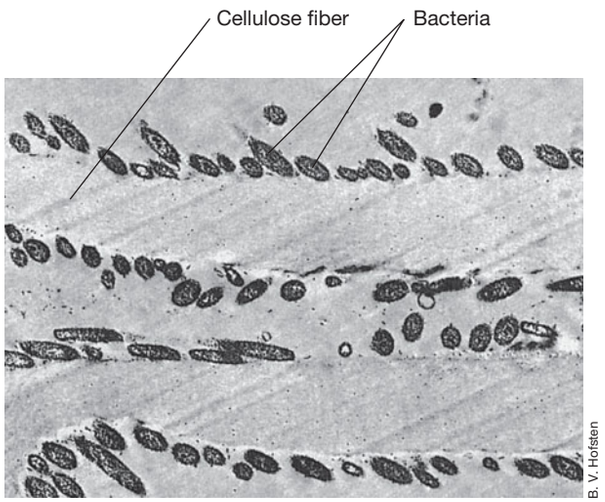

Figure 14.34 Cellulose digestion. Transmission electron micrograph showing attachment of the cellulose-digesting bacterium *Sporocytophaga myxococcoides* to cellulose fibers. Cells are about 0.5 μm in diameter.

UNIT 5

Table 14.9 *Naturally occurring polysaccharides yielding hexose and pentose sugars*[a]

Substance	*Composition*	*Sources*	*Catabolic enzymes*
Cellulose	Glucose polymer (β-1,4-)	Plants (leaves, stems)	Cellulases (β, 1-4-glucanases)
Starch	Glucose polymer (α-1,4-)	Plants (leaves, seeds)	Amylase
Glycogen	Glucose polymer (α-1,4- and α-1,6-)	Animals (muscle) and microorganisms (granules)	Amylase, phosphorylase
Laminarin	Glucose polymer (β-1,3-)	Marine algae (*Phaeophyta*)	β-1,3-Glucanase (laminarinase)
Paramylon	Glucose polymer (β-1,3-)	Algae (*Euglenophyta* and *Xanthophyta*)	β-1,3-Glucanase
Agar	Galactose and galacturonic acid polymer	Marine algae (*Rhodophyta*)	Agarase
Chitin	*N*-Acetylglucosamine polymer (β-1,4-)	Fungi (cell walls) Insects (exoskeletons)	Chitinase
Pectin	Galacturonic acid polymer (from galactose)	Plants (leaves, seeds)	Pectinase (polygalacturonase)
Dextran	Glucose polymer	Capsules or slime layers of bacteria	Dextranase
Xylan	Heteropolymer of xylose and other sugars (β-1,4- and α-1,2 or α-1,3 side groups)	Plants	Xylanases
Sucrose	Glucose–fructose disaccharide	Plants (fruits, vegetables)	Invertase
Lactose	Glucose–galactose disaccharide	Milk	β-Galactosidase

[a]Each of these is subject to degradation by microorganisms.

Starch is digestible by many fungi and bacteria; this is illustrated for a laboratory culture in **Figure 14.36**. Starch-digesting enzymes, called *amylases*, are of considerable practical utility in industrial situations where starch must be digested, such as the textile, laundry, paper, and food industries, and fungi and bacteria are the commercial sources of these enzymes (↻ Section 15.8).

All polysaccharides catabolized to support growth are first enzymatically hydrolyzed to monomeric or oligomeric units. In contrast, polysaccharides formed within cells as storage products are broken down not by *hydro*lysis, but by *phosphoro*lysis. This involves the addition of inorganic phosphate and results in the formation of hexose phosphate rather than free hexose. It may be summarized as follows for the degradation of starch, an α-1,4 polymer of glucose:

$$(C_6H_{12}O_6)_n + P_i \rightarrow (C_6H_{12}O_6)_{n-1} + \text{glucose 1-phosphate}$$

Because glucose 1-phosphate can be easily converted to glucose 6-phosphate—a key intermediate in glycolysis (↻ Section 4.8)—with no energy expenditures, phosphorolysis represents a net energy savings to the cell.

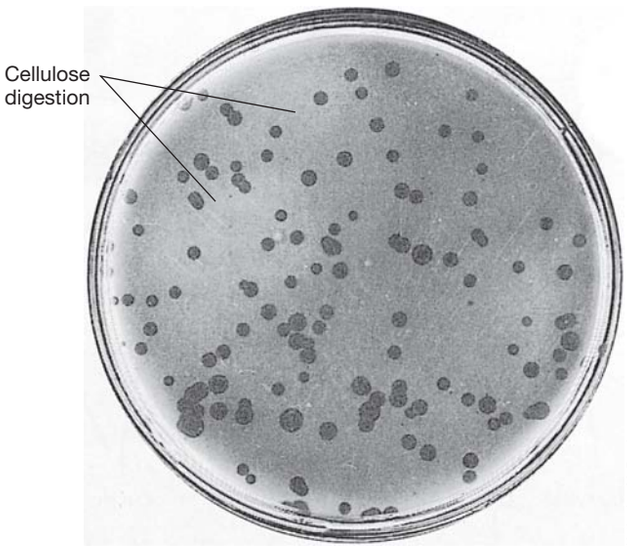

Figure 14.35 ***Cytophaga hutchinsonii*** **colonies on a cellulose–agar plate.** Areas where cellulose has been hydrolyzed are more translucent.

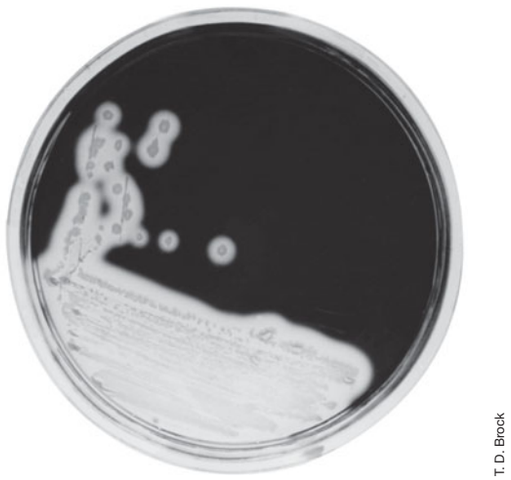

Figure 14.36 **Hydrolysis of starch by** ***Bacillus subtilis*****.** After incubation, the starch–agar plate was flooded with Lugol's iodine solution. Where starch has been hydrolyzed, the characteristic purple-black color of the starch–iodine complex is absent. Starch hydrolysis extends some distance from the colonies because cells of *B. subtilis* produce the extracellular enzyme (exoenzyme) amylase, which diffuses into the surrounding medium.

Disaccharides

Many microorganisms can use disaccharides for growth (Table 14.9). *Lactose* utilization by microorganisms is of considerable economic importance because milk-souring organisms produce lactic acid from lactose. *Sucrose*, the common disaccharide of higher plants, is usually first hydrolyzed to its component monosaccharides (glucose and fructose) by the enzyme invertase, and the monomers are then metabolized by the glycolytic pathway. *Cellobiose* (β-1,4-diglucose), a major product of cellulose digestion by cellulase, is degraded by cellulolytic bacteria but can also be degraded by many bacteria that are unable to degrade the cellulose polymer itself.

The microbial polysaccharide *dextran* is synthesized by some bacteria using the enzyme dextransucrase and sucrose as starting material:

$$n \text{ Sucrose} \rightarrow \underset{\text{dextran}}{(\text{glucose})_n} + n \text{ fructose}$$

Dextran is formed in this way by the bacterium *Leuconostoc mesenteroides* and a few others, and the polymer formed accumulates around the cells as a massive slime layer or capsule (**Figure 14.37**). Because sucrose is required for dextran formation, no dextran is formed when the bacterium is cultured on a medium containing glucose or fructose (↻ Section 27.3). In nature, when cells that contain dextran or other polysaccharide capsules die, these materials once again become available for attack by fermentative or other chemoorganotrophic microorganisms.

The Pentose Phosphate Pathway

Pentose sugars are often available in nature. But if they are not available, they must be synthesized, because they form the backbone of the nucleic acids. Pentoses are made from hexose sugars, and the major pathway for this process is the **pentose phosphate pathway**.

Figure 14.38 summarizes the pentose phosphate pathway. Several important features should be noted. First, glucose can be oxidized to a pentose by loss of one carbon atom as CO_2. This generates NADPH and the key intermediate of the pathway, *ribulose 5-phosphate* (Figure 14.38). From the latter, ribose and from it deoxyribose are formed to supply the cell with nucleic acid precursors. Pentose sugars as electron donors can also feed into the pentose phosphate pathway, typically becoming phosphorylated to form ribose phosphate or a related compound (Figure 14.38*b*) before being further catabolized.

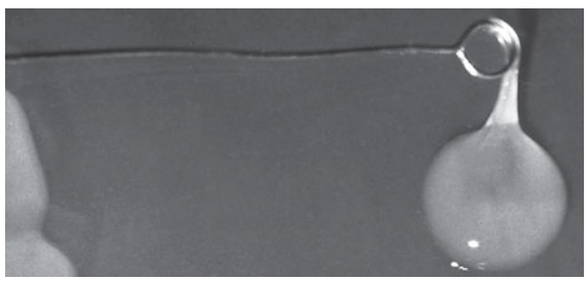

Figure 14.37 **Slime formation.** A slimy colony formed by the dextran-producing bacterium *Leuconostoc mesenteroides* growing on a sucrose-containing medium. When the same organism is grown on glucose, the colonies are small and not slimy because synthesis of dextran (a branched polysaccharide of glucose) specifically requires sucrose.

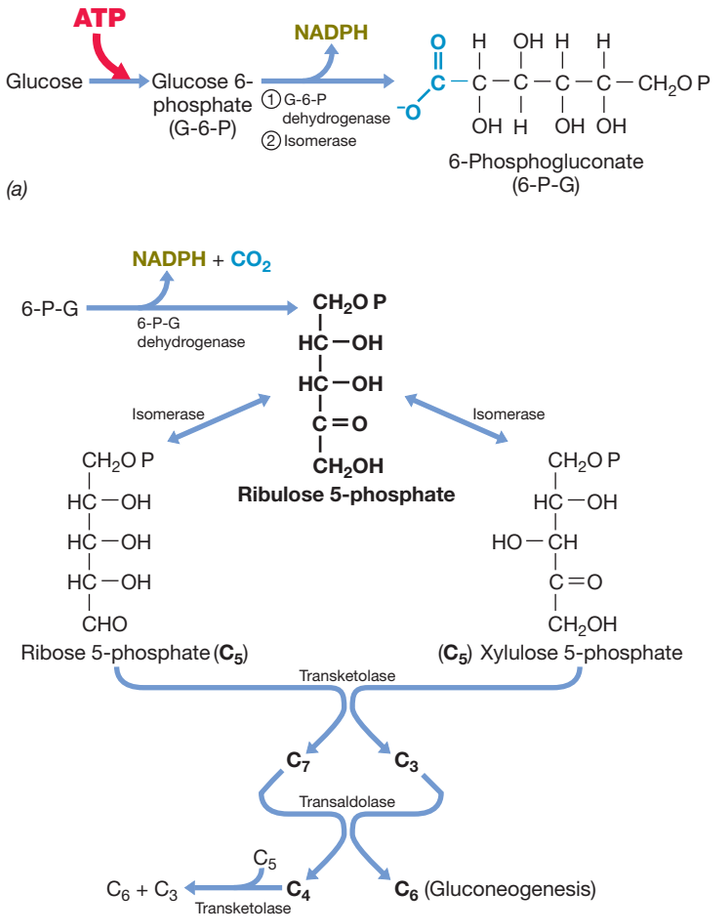

Figure 14.38 **The pentose phosphate pathway.** *(a)* The formation of 6-phosphogluconate. *(b)* The formation of pentoses from 6-phosphogluconate. The pathway is used to: (1) form pentoses from hexoses; (2) form hexoses from pentoses (gluconeogenesis); (3) catabolize pentoses as electron donors; and (4) generate NADPH. Some key enzymes of the pathway are indicated.

A second important feature of the pentose phosphate pathway is the generation of sugar diversity. A variety of sugar derivatives, including C_4, C_5, C_6, and C_7, are formed in reactions of the pathway (Figure 14.38). This allows for pentose sugars to eventually yield hexoses for either catabolic purposes or for biosynthesis (gluconeogenesis, ↻ Section 4.13).

A final important aspect of the pentose phosphate pathway is that it generates the redox coenzyme NADPH (Figure 14.38), and NADPH is used by the cell for many reductive biosyntheses; an important example would be ribonucleotide reductase, the enzyme that uses NADPH to convert ribonucleotides into deoxyribonucleotides (↻ Section 4.14). Although most cells have an exchange mechanism for converting NADH into NADPH, the pentose phosphate pathway is the major means for direct synthesis of this important coenzyme.

MiniQuiz

- What is phosphorolysis?
- What functions does the pentose phosphate pathway play in the cell?

14.17 Organic Acid Metabolism

Various organic acids can be metabolized as carbon sources and electron donors by microorganisms. The intermediates of the citric acid cycle, *citrate, malate, fumarate*, and *succinate*, are common natural products formed by plants and are also fermentation products of microorganisms. Because the citric acid cycle has major biosynthetic as well as energetic functions (↻ Section 4.11), the complete cycle or major portions of it are nearly universal in microorganisms. Thus, it is not surprising that many microorganisms are able to use citric acid cycle intermediates as electron donors and carbon sources.

Glyoxylate Cycle

Unlike the utilization of organic acids containing four to six carbons, two- or three-carbon acids cannot be used as growth substrates by the citric acid cycle alone. The same is true for substrates such as hydrocarbons and lipids, degraded via beta-oxidation to acetyl-CoA (Section 14.18). The citric acid cycle can continue to operate only if the acceptor molecule, the four-carbon acid oxalacetate, is regenerated at each turn; any removal of carbon compounds for biosynthetic purposes would prevent completion of the cycle (↻ Figure 4.21).

When acetate is used, the oxalacetate needed to continue the cycle is produced through the **glyoxylate cycle** (**Figure 14.39**), so named because the C_2 compound glyoxylate is a key intermediate. This cycle is composed of citric acid cycle reactions plus two additional enzymes: *isocitrate lyase*, which splits isocitrate into succinate and glyoxylate, and *malate synthase*, which converts glyoxylate and acetyl-CoA to malate (Figure 14.39).

Biosynthesis through the glyoxylate cycle occurs as follows. The splitting of isocitrate into succinate and glyoxylate allows the succinate molecule (or another citric acid cycle intermediate derived from it) to be removed for biosynthesis because glyoxylate (C_2) combines with acetyl-CoA (C_2) to yield malate (C_4). Malate can be converted to oxalacetate to maintain the citric acid cycle after the C_4 intermediate (succinate) has been drawn off. Succinate is used in the production of porphyrins (needed for cytochromes, chlorophyll, and other tetrapyrroles). Succinate can also be oxidized to oxalacetate as a carbon skeleton for C_4 amino acids, or it can be converted (via oxalacetate and phosphoenolpyruvate) to glucose.

Pyruvate and C_3 Utilization

Three-carbon compounds such as pyruvate or compounds that can be converted to pyruvate (for example, lactate or carbohydrates) also cannot be catabolized through the citric acid cycle alone. Because some of the citric acid cycle intermediates are used for biosynthesis, the oxalacetate needed to keep the cycle going is synthesized from pyruvate or phosphoenolpyruvate by the addition of a carbon atom from CO_2. In some organisms this step is catalyzed by the enzyme *pyruvate carboxylase*:

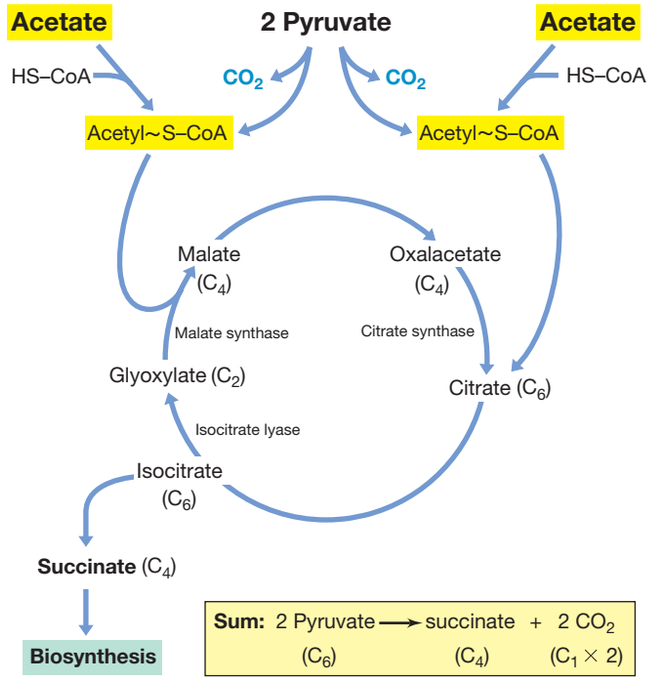

Figure 14.39 The glyoxylate cycle. Two unique enzymes, isocitrate lyase and malate synthase, operate along with most citric acid cycle enzymes. In addition to growth on pyruvate, the glyoxylate cycle also functions during growth on acetate.

$$\text{Pyruvate} + \text{ATP} + CO_2 \rightarrow \text{oxalacetate} + \text{ADP} + P_i$$

whereas in others it is catalyzed by *phosphoenolpyruvate carboxylase*:

$$\text{Phosphoenolpyruvate} + CO_2 \rightarrow \text{oxalacetate} + P_i$$

These reactions replace oxalacetate that is lost when intermediates of the citric acid cycle are removed for use in biosynthesis, and the cycle can continue to function.

MiniQuiz

- Why is the glyoxylate cycle necessary for growth on acetate but not on succinate?

14.18 Lipid Metabolism

Lipids are abundant in nature. The cytoplasmic membranes of all cells contain lipids, and many organisms produce lipid storage materials and contain lipids in their cell walls. These substances are biodegradable and are excellent substrates for microbial energy-yielding metabolism. When cells die, their lipids are thus catabolized, with CO_2 being the final product.

Fat and Phospholipid Hydrolysis

Fats are esters of glycerol and fatty acids and are readily available from the release of lipids from dead organisms. Microorganisms

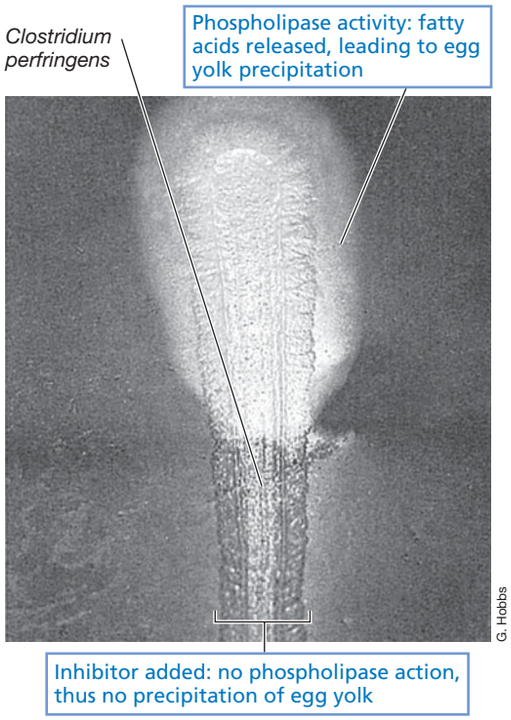

Figure 14.40 **Phospholipase activity.** Enzyme activity of phospholipase around a streak of *Clostridium perfringens* growing on an agar medium containing egg yolk. On half of the plate an inhibitor of phospholipase was added, preventing activity of the enzyme.

use fats only after hydrolysis of the ester bond, and extracellular enzymes called *lipases* are responsible for the reaction (**Figures 14.40** and **14.41**). Lipases attack fatty acids of various chain lengths. Phospholipids are hydrolyzed by enzymes called *phospholipases*, each of which is given a different letter designation depending on which ester bond it cleaves in the lipid (Figure 14.41). Phospholipases A and B cleave *fatty acid* esters, whereas phospholipases C and D cleave *phosphate* esters and hence are different classes of enzymes. The result of lipase activity is the release of free fatty acids and glycerol, and these substances can then be metabolized by chemoorganotrophic microorganisms.

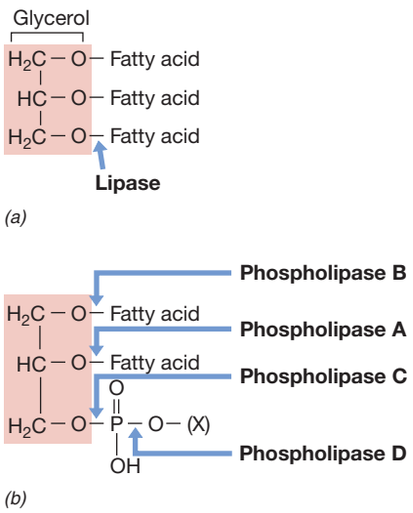

Figure 14.41 **Lipases.** *(a)* Activity of lipases on a fat. *(b)* Phospholipase activity on phospholipid. The cleavage sites of the four distinct phospholipases A, B, C, and D are shown. X refers to a number of small organic molecules that may be at this position in different phospholipids.

Fatty Acid Oxidation

Fatty acids are oxidized by *beta-oxidation*, a series of reactions in which two carbons of the fatty acid are split off at a time (**Figure 14.42**). The fatty acid is first activated with coenzyme A; oxidation results in the release of acetyl-CoA by cleavage between the α and β carbons of the original fatty acid along with the formation of a new fatty acid two carbon atoms shorter (Figure 14.42). The process of beta-oxidation is then repeated, and another acetyl-CoA molecule is released.

There are two separate dehydrogenation reactions in beta-oxidation. In the first, electrons are transferred to flavin adenine dinucleotide (FAD), forming FADH, whereas in the second they are transferred to NAD^+, forming NADH. Most

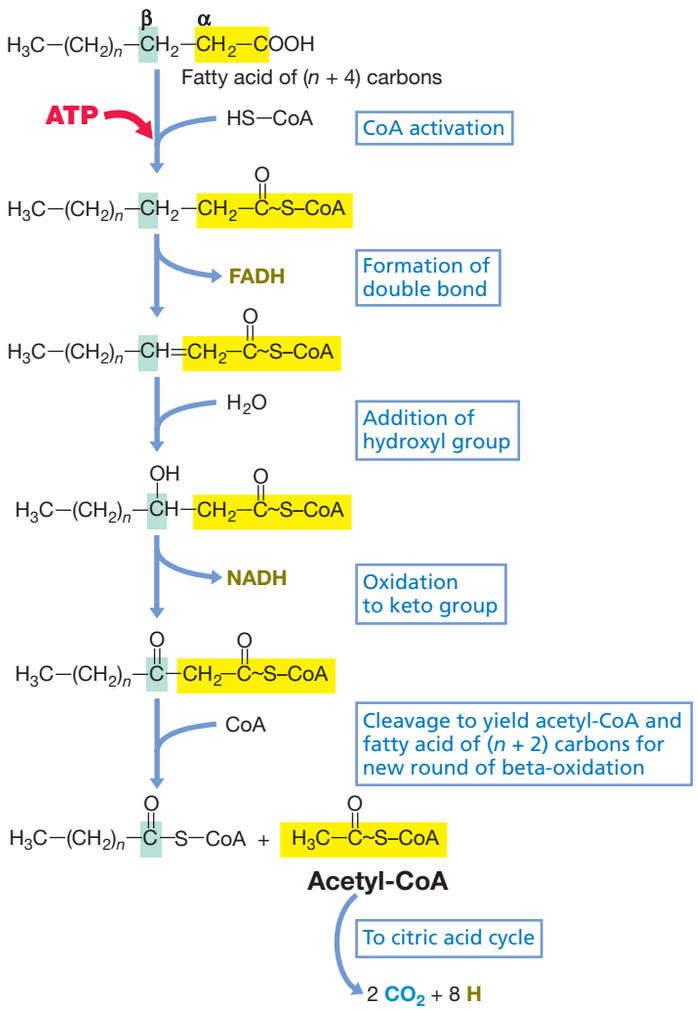

Figure 14.42 **Beta-oxidation.** Beta-oxidation of a fatty acid leading to the successive formation of acetyl-CoA.

fatty acids in a cell have an even number of carbon atoms, and complete oxidation yields acetyl-CoA. If odd-chain or branched-chain fatty acids are catabolized, propionyl-CoA or a branched-chain fatty acid–CoA remains after beta-oxidation, and these are either further metabolized to acetyl-CoA by ancillary reactions or excreted from the cell. The acetyl-CoA formed is then oxidized by the citric acid cycle or is converted to hexose and other cell constituents via the glyoxylate cycle (Figure 14.39).

Because they are highly reduced, fatty acids are excellent electron donors. For example, the oxidation of the 16-carbon saturated fatty acid palmitic acid can in theory generate 129 ATP molecules. These include oxidative phosphorylation from electrons generated during the formation of acetyl-CoA from beta-oxidations and from oxidation of the acetyl-CoA units themselves through the citric acid cycle.

MiniQuiz

- What are phospholipases and what do they do?
- How many electrons are released for every acetyl-CoA produced by beta oxidation of a fatty acid? For every acetyl-CoA oxidized to CO_2?

Big Ideas

14.1
In the absence of external electron acceptors, organic compounds can be catabolized anaerobically only by fermentation. A requirement for most fermentations is formation of an energy-rich organic compound that can yield ATP by substrate-level phosphorylation. Redox balance must also be achieved in fermentations, and H_2 production is a key means of disposing of excess electrons.

14.2
The lactic acid fermentation is carried out by homofermentative and heterofermentative species. The mixed-acid fermentation results in acids plus neutral products (ethanol, butanediol), depending on the organism.

14.3
Clostridia ferment sugars, amino acids, and other organic compounds. *Propionibacterium* produces propionate and acetate in a secondary fermentation of lactate.

14.4
The energy physiology of *Propionigenium*, *Oxalobacter*, and *Malonomonas* is linked to decarboxylation reactions that pump Na^+ or H^+ across the membrane. The reactions catalyzed by these organisms yield insufficient energy to make ATP by substrate-level phosphorylation.

14.5
In syntrophy two organisms cooperate to degrade a compound that neither can degrade alone. In this process H_2 produced by one organism is consumed by the partner. H_2 consumption affects the energetics of the reaction carried out by the H_2 producer, allowing it to make ATP where it otherwise could not.

14.6
Although O_2 is the most widely used electron acceptor in energy-yielding metabolism, certain other compounds can be used as electron acceptors. Anaerobic respiration yields less energy than aerobic respiration but can proceed in environments where O_2 is absent.

14.7
Nitrate is a common electron acceptor in anaerobic respiration. Nitrate reduction is catalyzed by the enzyme nitrate reductase, reducing NO_3^- to NO_2^-. Many bacteria that use NO_3^- in anaerobic respiration reduce it past NO_2^- to produce gaseous nitrogen compounds (denitrification).

14.8
Sulfate-reducing bacteria reduce SO_4^{2-} to H_2S. This process requires activation of SO_4^{2-} by ATP to form adenosine phosphosulfate (APS) and reduction by H_2 or organic electron donors. Disproportionation is an additional energy-yielding strategy for certain species. Some organisms, such as *Desulfuromonas*, cannot reduce SO_4^{2-} but produce H_2S from the reduction of S^0.

14.9
Acetogens are anaerobes that reduce CO_2 to acetate, usually with H_2 as electron donor. The mechanism of acetate formation is the acetyl-CoA pathway, a pathway widely distributed in obligate anaerobes for either autotrophic purposes or acetate catabolism.

14.10
Methanogenesis is the production of CH_4 from $CO_2 + H_2$ or from acetate or methanol by strictly anaerobic methanogenic *Archaea*. Several unique coenzymes are required for methanogenesis, and energy conservation is linked to either a proton or a sodium motive force.

14.11
The hyperthermophile *Pyrococcus furiosus* ferments glucose in an unusual fashion, reducing protons in an anaerobic respiration linked to ATPase activity.

14.12
Besides inorganic nitrogen and sulfur compounds and CO_2, several other substances can function as electron acceptors for anaerobic respiration. These include Fe^{3+}, Mn^{4+}, fumarate, and certain organic and chlorinated organic compounds.

14.13
Hydrocarbons can be oxidized under anoxic conditions, but oxygen must first be added to the molecule. This occurs by the addition of fumarate. Aromatic compounds are catabolized anaerobically by ring reduction and cleavage to form intermediates that can be catabolized in the citric acid cycle. Methane can be oxidized under anoxic conditions by consortia containing sulfate-reducing or denitrifying bacteria and methanotrophic *Archaea*.

14.14
In addition to its role as an electron acceptor, O_2 can also be a substrate; enzymes called oxygenases introduce atoms of oxygen from O_2 into a biochemical compound. Aerobic hydrocarbon oxidation is widespread in nature, and oxygenase enzymes are key to these catalyses. Unlike in anaerobic aromatic catabolism, the aerobic degradation of aromatic compounds proceeds by ring oxidation.

14.15
Methanotrophy is the use of CH_4 as both carbon source and electron donor, and the enzyme methane monooxygenase is a key enzyme in the catabolism of methane. In methanotrophs C_1 units are assimilated into cell material by either the ribulose monophosphate pathway or the serine pathway.

14.16
Polysaccharides are abundant in nature and can be broken down into hexose or pentose monomers and used as sources of both carbon and electrons. Starch and cellulose are common polysaccharides. The pentose phosphate pathway is the major means for generating pentose sugars for biosynthesis.

14.17
Organic acids are typically metabolized through the citric acid cycle or the glyoxylate cycle. Isocitrate lyase and malate synthase are the key enzymes of the glyoxylate cycle.

14.18
Fats are hydrolyzed by lipases or phospholipases to fatty acids plus glycerol. The fatty acids are oxidized by beta-oxidation reactions to acetyl-CoA, which is then oxidized to CO_2 by the citric acid cycle.

Review of Key Terms

Acetogenesis energy metabolism in which acetate is produced from either H_2 plus CO_2 or from organic compounds

Acetyl-CoA pathway a pathway of autotrophic CO_2 fixation and acetate oxidation widespread in obligate anaerobes including methanogens, acetogens, and sulfate-reducing bacteria

Anaerobic respiration respiration in which some substance, such as SO_4^{2-} or NO_3^-, is used as a terminal electron acceptor instead of O_2

Anoxic oxygen-free

Denitrification anaerobic respiration in which NO_3^- or NO_2^- is reduced to nitrogen gases, primarily N_2

Fermentation anaerobic catabolism of an organic compound in which the compound serves as both an electron donor and an electron acceptor and in which ATP is usually produced by substrate-level phosphorylation

Glyoxylate cycle a series of reactions including some citric acid cycle reactions that are used for aerobic growth on C_2 or C_3 organic acids

Heterofermentative producing a mixture of products, typically lactate, ethanol, and CO_2, from the fermentation of glucose

Homofermentative producing only lactic acid from the fermentation of glucose

Hydrogenase an enzyme, widely distributed in anaerobic microorganisms, capable of oxidizing or evolving H_2

Methanogen an organism that produces methane (CH_4)

Methanogenesis the biological production of CH_4

Methanotroph an organism that can oxidize CH_4

Methylotroph an organism capable of growth on compounds containing no C—C bonds; some methylotrophs are methanotrophic

Oxygenase an enzyme that catalyzes the incorporation of oxygen from O_2 into organic or inorganic compounds

Pentose phosphate pathway a major metabolic pathway for the production and catabolism of pentoses (C_5 sugars)

Reductive dechlorination (dehalorespiration) an anaerobic respiration in which a chlorinated organic compound is used as an electron acceptor, usually with the release of Cl^-

Ribulose monophosphate pathway a reaction series in certain methylotrophs in which formaldehyde is assimilated into cell material using ribulose monophosphate as the C_1 acceptor molecule

Secondary fermentation a fermentation in which the substrates are the fermentation products of other organisms

Serine pathway a reaction series in certain methylotrophs in which CH_2O plus CO_2 are assimilated into cell material by way of the amino acid serine

Stickland reaction the fermentation of an amino acid pair

Syntrophy a process whereby two or more microorganisms cooperate to degrade a substance neither can degrade alone

Review Questions

1. Define the term substrate-level phosphorylation. How does it differ from oxidative phosphorylation? Assuming an organism is facultative, what cultural conditions dictate whether the organism obtains energy from substrate-level rather than oxidative phosphorylation (Section 14.1)?
2. Although many different compounds are theoretically fermentable, in order to support a fermentative process, most organic compounds must eventually be converted to one of a relatively small group of molecules. What are these molecules and why must they be produced (Sections 14.1–14.3)?
3. Give an example of a fermentation that does not employ substrate-level phosphorylation. How is energy conserved in this fermentation (Section 14.4)?
4. Why is syntrophy also called "interspecies H_2 transfer" (Section 14.5)?
5. Why is NO_3^- a better electron acceptor for anaerobic respiration than is SO_4^{2-} (Section 14.6)?
6. In *Escherichia coli*, synthesis of the enzyme nitrate reductase is repressed by O_2. On the basis of bioenergetic arguments, why do you think this repression phenomenon might have evolved (Section 14.7)?
7. Why is hydrogenase a constitutive enzyme in *Desulfovibrio* (Section 14.8)?
8. Compare and contrast acetogens with methanogens in terms of (1) substrates and products of their energy metabolism, (2) ability to use organic compounds as electron donors in energy metabolism, and (3) phylogeny (Sections 14.9 and 14.10).
9. Why can it be said that in glycolysis in *Pyrococcus furiosus*, both fermentation and anaerobic respiration are occurring at the same time (Section 14.11)?
10. Compare and contrast ferric iron reduction with reductive dechlorination in terms of (1) product of the reduction and (2) environmental significance (Section 14.12).
11. How do denitrifying and sulfate-reducing bacteria degrade hydrocarbons without the participation of oxygenase enzymes (Sections 14.13–14.14)?
12. How do monooxygenases differ from dioxygenases in the reactions they catalyze? Why are oxygenases necessary for the aerobic catabolism of hydrocarbons (Section 14.14)?
13. How does a methano*troph* differ from a methano*gen*? How do type I and type II methanotrophs differ in their carbon assimilation patterns (Section 14.15)?
14. Compare and contrast the conversion of cellulose and intracellular starch to glucose units. What enzymes are involved and which process is the more energy efficient (Section 14.16)?
15. What is the major function of the glyoxalate cycle (Section 14.17)?
16. What is the product of the beta-oxidation of a fatty acid? How is this product oxidized to CO_2 (Section 14.18)?

Application Questions

1. When methane is made from CO_2 (plus H_2) or from methanol (in the absence of H_2), various steps in the pathway shown in Figures 14.20 and 14.21 are used. Compare and contrast methanogenesis from these two substrates and discuss why they must be metabolized in opposite directions.
2. Although dextran is a glucose polymer, glucose cannot be used to make dextran. Explain. How is dextran synthesis important in oral hygiene (↻ Section 27.3)?
3. A fatty acid such as butyrate cannot be fermented in pure culture, although its anaerobic catabolism under other conditions occurs readily. How do these conditions differ, and why does the latter allow for butyrate catabolism? How then can butyrate be fermented in mixed culture?