Bacterial Fermentation

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Under anaerobic conditions, in the dark and in the absence of electron acceptors, organic compounds are catabolized by strictly anaerobic or facultatively anaerobic bacteria by internally balanced oxidation–reduction reactions, a process called fermentation. In fermentation, the organic compound serves as both electron donor and acceptor, and adenosine triphosphate is synthesized by substrate-level phosphorylation.

Advanced article

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Introduction

The first to describe anaerobic fermentative metabolism was Louis Pasteur, who described fermentation as 'la vie sans l'air'. Today, three physiological groups of anaerobic microorganisms can be distinguished: (i) the anaerobic phototrophs, (ii) the anaerobic respirers such as denitrifiers, sulfate reducers, methanogens and acetogens, which use nitrate, sulfate and carbon dioxide, respectively, as electron acceptors in the absence of oxygen and (iii) the fermentative organisms. **See also**: Pasteur, Louis

The Nature of Fermentation

Most energy-conserving reactions in living organisms are redox reactions. One substrate is oxidized with the concomitant reduction of another substrate. In chemoorganotrophic aerobes, the substrate reduced is usually oxygen. In respiring anaerobes, the electron acceptor can be either organic or inorganic. Typical examples are the sulfate-reducing or methanogenic organisms (carbon dioxide). In respiring organisms, both aerobic and anaerobic, most of the energy is produced by electron transport phosphorylation. This is in contrast to fermentations, in which most of the adenosine triphosphate (ATP) is synthesized by substrate-level phosphorylation. Fermentation is an anaerobic redox process, in which the oxidation of the substrate is coupled to the reduction of another substrate or an intermediate derived from the oxidation, with the difference in redox potential of the substrate and the end product providing energy for ATP synthesis (**Figure 1**). In most fermentations, the same substrate is used as both reductant and oxidant, whereas in some amino acid fermenting organisms, one amino acid is oxidized and another is reduced (Stickland reaction). The oxidation reaction is coupled to substrate-level phosphorylation, whereas the reduction reaction is usually not. The fermentation end products are excreted. The nature of these products is different in various species, and the various fermentation pathways are named after their main products (Schmitz *et al*., 2001; **Figure 2**). **See also**: Anaerobic Respiration; Electron Carriers: Proteins and Cofactors in Oxidative Phosphorylation; Methanogenesis Biochemistry; Oxidation–Reduction Reactions

In fermentation, the substrate is only partly oxidized, and, therefore, only a small amount of the energy stored in the substrate is conserved. In most fermentative organisms, ATP is produced only by substrate-level phosphorylation, but there are also a few examples of an additional ion-gradient-driven phosphorylation; the ion gradient is either a proton or a sodium ion gradient and is generated by electron transport (e.g. during fumarate reduction or ferredoxin-dependent nicotinamide–adenine dinucleotide $(NAD)^+$ reduction), decarboxylation (e.g. during citrate fermentation) (Dimroth, 1997), ion-coupled end product efflux (e.g. during lactate production) and substrate–product antiport (e.g. in lactic acid bacteria; Konings *et al*., 1997). **See also**: Adenosine Triphosphate

Types of Substrate Used

Fermentative organisms are nutritionally very versatile and they are the first limb of the anaerobic food chain. Polymers such as polysaccharides, proteins, deoxyribonucleic acid (DNA) and lipids are attacked by extracellular enzymes and broken into smaller units which are taken up by the initial degrader or other fermenters. Fermentable monomers include sugars (hexoses, pentoses, tetroses), polyols, organic acids, amino acids and purines and pyrimidines. Apart from these classical substrates, others such as acetylene, citrate, glyoxylate, succinate, oxalate and malonate are also fermented. Even the aromatic compounds resorcinol and phloroglucinol are fermented by pure cultures. Fermentation of aromatic hydrocarbons by pure cultures has not been reported although oxidation by mixed cultures does occur (Heider and Fuchs, 1997).

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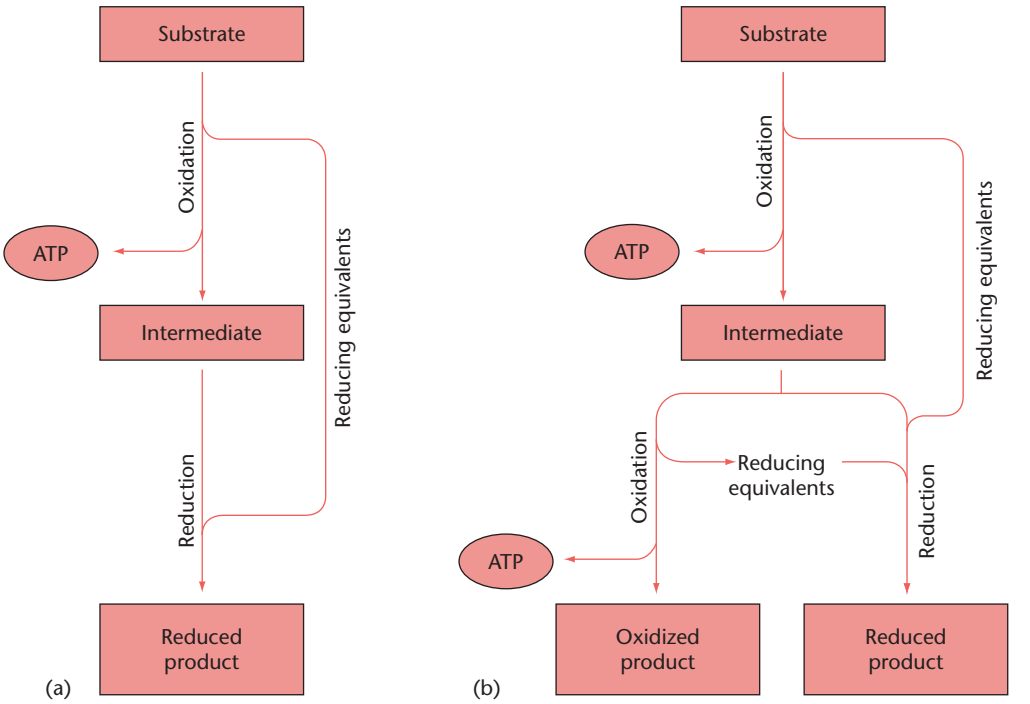

Figure 1 Generalized schemes for fermentation pathways. (a) A substrate is oxidized and the intermediate generated is reduced and excreted; an example is homolactic acid fermentation. (b) The oxidized intermediate (e.g. pyruvate) is disproportionated leading to a more complex product pattern, as observed in a variety of fermentations.

ATP Yield

Despite the large number of fermentation pathways, only a few reactions within such pathways are exergonic enough to conserve energy either by substrate-level phosphorylation or ion-gradient-driven phosphorylation. The latter contributes only a small fraction to the ATP yield of a given fermentation and, therefore, the ATP yield of a fermentation usually reflects the amount of ATP synthesized by substrate-level phosphorylation. Enzymes that are coupled to substrate-level phosphorylation are:

Phosphoglycerokinase:

$$\text{Bisphosphoglycerate} + \text{ADP} \rightarrow 3\text{-Phosphoglycerate} + \text{ATP} \quad [\text{I}]$$

Pyruvate kinase:

$$\text{Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP} \quad [\text{II}]$$

Acetate kinase:

$$\text{Acetylphosphate} + \text{ADP} \rightarrow \text{Acetate} + \text{ATP} \quad [\text{III}]$$

Butyrate kinase:

$$\text{Butyryl phosphate} + \text{ADP} \rightarrow \text{Butyrate} + \text{ATP} \quad [\text{IV}]$$

Carbamylphosphate kinase:

$$\text{Carbamylphosphate} + \text{ADP} \rightarrow \text{Carbamate} + \text{ATP} \quad [\text{V}]$$

Formyltetrahydrofolate synthetase:

$$\text{Formyltetrahydrofolate} + \text{ADP} + \text{P}_\text{i} \rightarrow \text{Formate} + \text{Tetrahydrofolate} + \text{ATP} \quad [\text{VI}]$$

Glycine reductase:

$$\text{Glycine} + 2\,[\text{H}] + \text{ADP} + \text{P}_\text{i} \rightarrow \text{Acetate} + \text{NH}_3 + \text{ATP} \quad [\text{VII}]$$

See also: Thermodynamics in Biochemistry

Reactions [I] and [II] are inherent to glycolysis and, therefore, part of most fermentation pathways. Reaction [III] is part of most fermentation pathways that are by way of glycolysis, and this reaction is the only way to generate additional ATP apart from the glycolytic ATP (reactions [I] and [II]). Reaction [IV] is found during the path of butyrate fermentation. Reaction [V] occurs during the degradation of arginine to ornithine, which is widespread in bacteria and archaea. Reaction [VI] is restricted to a limited number of bacteria growing on purines and other *N*-containing substrates as well as during methyl group oxidation in the acetyl-CoA pathway as carried out by, for example, acetogens and

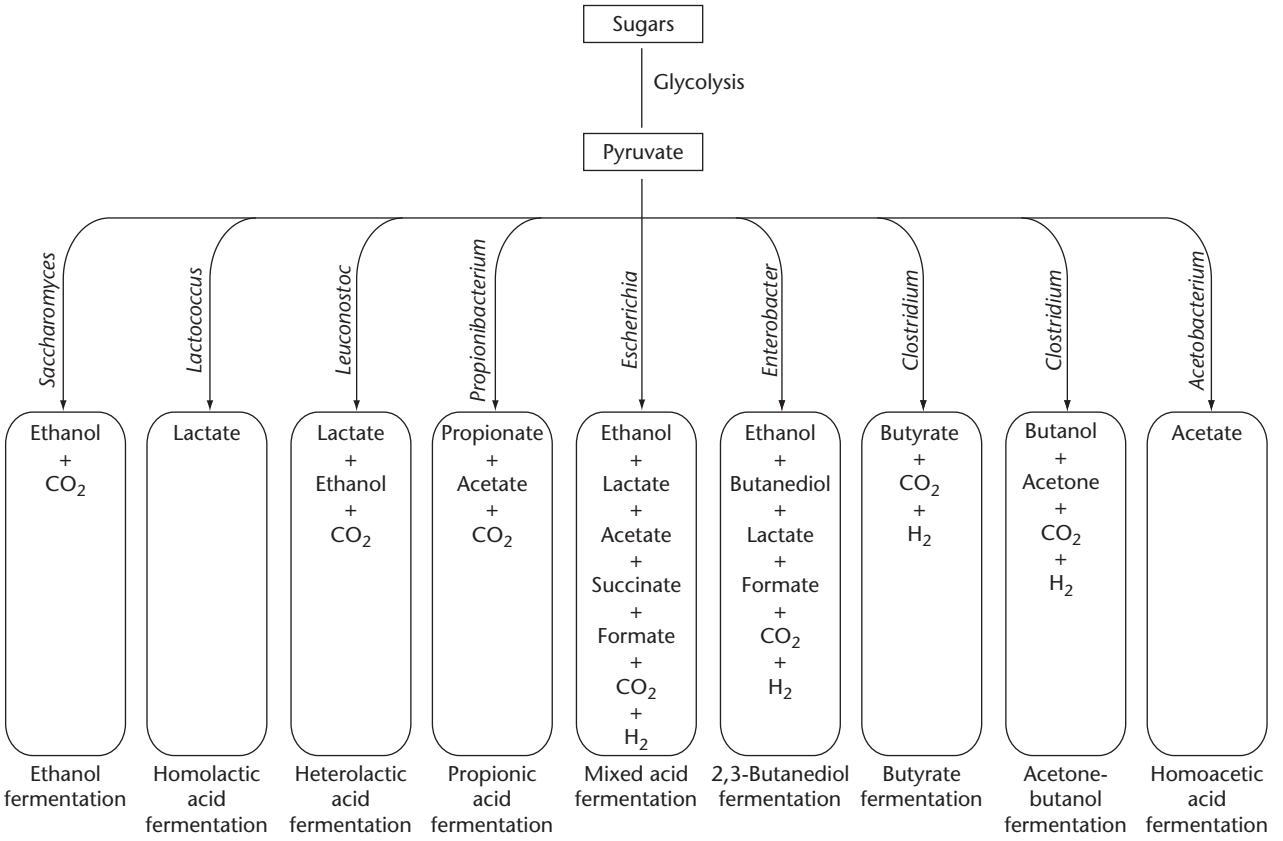

Figure 2 Major pathways for fermentation of sugars including organisms involved and end products formed.

sulfate reducers. Reaction [VII] is restricted to organisms such as *Eubacterium acidaminophilum*. The glycine reductase is the prime example of a substrate-level phosphorylation coupled to a reductive branch of a fermentation pathway. **See also**: Glycolytic Pathway

The ATP yield of a fermentation is dependent on the pathway used and can range from 0.3 to 4 mol ATP per mol substrate. This yield is considerably smaller than the one obtained during aerobic catabolism; hence the fermenters usually convert more substrate per biomass unit than aerobes. The maximum ATP yield is obtained when glucose is converted via glycolysis to pyruvate and if the organism can make use of the acetate kinase reaction in addition. However, the conversion of pyruvate to acetyl-coenzyme A (CoA) is an oxidation reaction. Therefore, the complete oxidation of glucose to acetate and carbon dioxide according to

$$1\ \text{Glucose} + 4\,\text{ADP} + 4\,P_i \rightarrow 2\ \text{Acetate} + 2\,CO_2 + 4\,H_2 + 4\,\text{ATP} \quad \text{[VIII]}$$

is only possible if the electrons generated during glycolysis and pyruvate cleavage are quantitatively released as hydrogen, or if they are used to reduce $2CO_2$ to acetate. The latter reaction is catalysed by acetogenic bacteria, which therefore convert 1 mol of glucose to 3 mol of acetate, thereby gaining 4 mol of ATP by substrate level phosphorylation.

Anaerobic bacteria have different ways to evolve hydrogen, two of them are directly coupled to pyruvate oxidation. First, the pyruvate:formate lyase system (present for example in enterobacteria) oxidizes pyruvate to acetyl-CoA and formate and the latter is then split by a hydrogen lyase into hydrogen and carbon dioxide. Second, the pyruvate:ferredoxin oxidoreductase (present for example in clostridia) oxidizes pyruvate to acetyl-CoA, carbon dioxide and reduced ferredoxin. Both systems have in common that the precursors have redox potentials low enough to allow electron transfer to protons with production of hydrogen (E_0' CO_2/HCOOH $= -432$ mV; E_0' ferredoxin ox/red $= -398$ mV; E_0' $H^+/H_2 = -414$ mV). Therefore, reducing equivalents produced during pyruvate:formate lyase and pyruvate:ferredoxin oxidoreductase reactions are easily released as hydrogen.

Recent genomic as well as biochemical analyses revealed that some anaerobes such as clostridia or acetogens have a membrane-bound electron transport chain that couples electron flow from reduced ferredoxin to NAD^+. The enzymes involved are similar to Rnf-type NADH (reduced form of nicotinamide–adenine dinucleotide) dehydrogenases that are assumed to catalyse electron transport-driven ion export from the cytoplasm. Thus, additional energy could be conserved by ferredoxin-dependent NAD^+ reduction as catalysed by Rnf-type

ferredoxin:NAD^+ oxidoreductase (FNOR) in anaerobes (Boiangiu *et al*., 2005; Müller *et al*., 2008).

During glycolysis $NADH_2$ is formed. However, the redox potential of the $NAD/NADH_2$ couple ($E_0' = -320$ mV) is too electropositive to allow reduction of protons (E_0' $H^+/H_2 = -414$ mV) but this reaction becomes thermodynamically feasible if the hydrogen formed is taken out of the equilibrium. In nature, this is achieved by interspecies hydrogen transfer in microbial consortia in which hydrogen-consuming bacteria oxidize the hydrogen generated by the fermenting organism (Thauer *et al*., 1977). Under these conditions glucose fermentation according to reaction [VIII] becomes feasible. FNOR might be involved in this reaction. **See also**: Bacterial Ecology

There are some fermentations which, in addition to substrate-level phosphorylation, gain some energy by ion-gradient-driven phosphorylation. The reactions leading to the generation of an ion gradient and an energized membrane are either decarboxylation reactions (such as oxaloacetate decarboxylase during citrate fermentation in *Klebsiella*), electron transport (i.e. FNOR fumarate reduction), and an electrogenic product/proton symport (as in lactic acid bacteria). **See also**: Ion Transport Across Nonexcitable Membranes; Membrane Potential

Types of Fermentations

Ethanol fermentation

Ethanol is the major end product of the anaerobic metabolism of yeast but also of *Zymomonas* species. In both, ethanol is fermented according to

$$\text{Glucose} \rightarrow 2\,CO_2 + 2\,\text{Ethanol} \qquad [IX]$$

Yeasts ferment glucose by way of glycolysis to pyruvate, which is decarboxylated to acetaldehyde and carbon dioxide. This reaction is catalysed by pyruvate decarboxylase, the key enzyme of alcohol fermentation by yeast. Acetaldehyde is then reduced to ethanol with $NADH_2$, generated in the course of the glyceraldehyde 3-phosphate dehydrogenase reaction, as reductant. The ATP yield is 2 mol per mol substrate, compared to 38 mol ATP per mol glucose under aerobic conditions. Therefore, it is clear that the preferred mode of life for yeast is the aerobic one, and the direction of electron and carbon flow is regulated by the energy charge of the cells via covalent modification of key enzymes (Pasteur effect). **See also**: Yeasts

Zymomonas mobilis was isolated from Mexican pulque. Alcohol fermentation by *Zymomonas* species is not via glycolysis but the Entner–Doudoroff pathway, which leads to 2 mol pyruvate per mol glucose. Pyruvate is then decarboxylated by pyruvate decarboxylase to acetaldehyde. The $NAD(P)H_2$ generated by the glucose 6-phosphate dehydrogenase and the glyceraldehyde 3-phosphate dehydrogenase is reoxidized by reduction of the 2 mol acetaldehyde to ethanol. Considering the energy balance this fermentation is particularly interesting, since the entire pathway yields only 1 mol ATP per mol glucose. Taking into account that glucose is actively transported in most organisms, only a fraction of an ATP would be available. This problem is solved by *Zymomonas*, which lives in environments with high sugar concentrations, by employing a glucose transporter that catalyses facilitated diffusion instead of active, energy-consuming transport.

In addition to being the major end product, ethanol is the byproduct of many fermentations. Many lactic acid bacteria, enterobacteria and clostridia form considerable amounts of ethanol as a reduced end product to maintain their redox balance. In these cases, acetyl-CoA is reduced via acetaldehyde to ethanol by acetaldehyde dehydrogenase and ethanol dehydrogenase, respectively.

Ethanol fermentation by yeasts is an ancient process used by humans to produce alcoholic beverages (**Table 1**). Most fruit juices undergo a spontaneous fermentation caused by wild yeasts that are present on the fruits. The most important alcoholic beverages are beer (produced from malted grains) and wine (produced from fruits). After concentration of the alcohol by distillation, various spirits are produced. For example, distillation of malt brews yields whisky and distillation of fermented grain or potato yields vodka. **See also**: Fungal Fermentation: Industrial

Ethanol is also used as a raw material in the chemical industry for various purposes and as an additive to fuel (**Table 1**). However, yeasts are very susceptible to ethanol inhibition. Concentrations of 1–2% (w/v) are sufficient to retard growth, and at 10% growth is inhibited. Therefore, for production of ethanol on an industrial-scale yeast strains have been selected for features such as high ethanol yield and glucose. Industrial strains produce 50–120 g of ethanol per litre, with high selectivity from raw materials such as sugar crops, industrial and food processing wastes such as whey and sulfite liquors, lignocellulose and starches.

Fermentation of lignocellulosic biomass is an attractive alternative for the economic production of ethanol, but *Sa. cerevisiae* is not able to ferment pentoses, the main constituents of lignocellulose. However, strains that ferments arabinose or xylose to ethanol have been generated, a promising way for biotechnological production of ethanol (van Maris *et al*., 2006).

Lactic acid fermentation

Lactate is a common end product of fermentations. Some organisms, collectively called the lactic acid bacteria, form large amounts of lactate. Lactic acid bacteria are subdivided according to their fermentation products. The homofermentative species produce a single end product, lactic acid, whereas the heterofermentative species produce other compounds, mostly ethanol and carbon dioxide, along with lactate. These differences are due to the employment of different pathways for glucose oxidation: in homofermentative organisms glucose breakdown is via glycolysis according to

$$\text{Glucose} \rightarrow 2\,\text{Lactate} \qquad [X]$$

Table 1 Industrial products from fermentations

Products	Starting material	Microorganisms involved
Foods		
Beer	Grains	*Saccharomyces* spp.
Wine	Fruits, grapes	*Saccharomyces* spp.
Bread		
Sourdough	Wheat flour	*Lactobacillus* spp.
White	Wheat flour	*Saccharomyces cerevisiae*
Sausage	Pork and beef	*Pediococcus cerevisiae*
Pickles	Cucumber	*Lactobacillus* spp.
Yoghurt	Milk	*Lactobacillus delbrueckii* ssp. *bulgaricus*
Brie	Milk	*Brevibacterium linens*, *Penicillium cambertii*, *Lactobacillus casei*, *Streptococcus cremoris*
Cheddar	Milk	*L. casei*, *St. cremoris*
Chemicals		
Ethanol	Sugar crops, whey, sulfite liquors, starches	*Saccharomyces* spp.
Lactate	Glucose, maltose, sucrose, whey	*Lactobacillus delbrueckii* ssp. *delbrueckii*, *Lactobacillus delbrueckii* ssp. *lactis*, *L. delbrueckii* ssp. *bulgaricus*
Acetone-butanol	Starches, molasses	*Clostridium* spp.

The pyruvate formed is reduced to lactate by the action of lactate dehydrogenase, which catalyses a stereospecific reduction to either L- or D-lactate. The ATP yield is 2 mol per mol glucose. Heterofermentative bacteria are devoid of aldolase but contain phosphoketolase instead. Glucose 6-phosphate is oxidized to 6-phosphogluconate and then decarboxylated to ribulose 5-phosphate. After epimerization the xylulose 5-phosphate is split by phosphoketolase to acetyl-phosphate and glyceraldehyde 3-phosphate. To maintain a proper redox balance acetyl-CoA derived from acetyl phosphate is reduced to ethanol, and the glyceraldehyde 3-phosphate is converted to lactate. The overall reaction

$$\text{Glucose} \rightarrow \text{CO}_2 + \text{Lactate} + \text{Ethanol} \qquad [\text{XI}]$$

is coupled to the net synthesis of only 1 mol ATP per mol glucose. Lactic acid bacteria are nutritionally very versatile and grow not only on glucose but also on other substrates such as fructose, galactose, mannose, saccharose and pentoses. With these substrates, certain variations of the fermentation pathways occur. For example, pentoses are fermented by facultative homofermentative organisms via the phosphoketolase pathway. Fructose can be used as carbon source but also as an electron acceptor (thus generating mannitol), thereby allowing acetate production. Citrate, an ingredient of milk, is converted to diacetyl, the typical flavour of butter (Kandler, 1983). **See also**: Glycolytic Enzymes; Gram-type Positive Bacteria

Lactic acid bacteria, which are found in dairy farms, on plants, in the intestine and various mucosal surfaces of animals and humans, are widely used in the food industry. Owing to the production of acid, the pH of their environment is lowered, which inhibits growth of other organisms. Therefore, lactic acid fermentation is an ancient way to preserve food (**Table 1**). Milk was among the first agricultural products available, and its high nutritional value makes it a good growth substrate for lactic acid bacteria. Typical products produced from milk are yoghurt (*Lactobacillus*), sour cream (*Streptococcus cremoris*), butter (*Streptococcus diacetilactis*) and cheese. Typically, starter cultures are used for the fermentations, and different cultures and also different production processes yield the various types of cheese. Lactic acid bacteria are also employed in the production of, for instance, pickles, sour dough, sauerkraut and some types of sausages.

Lactic acid is also used as a bulk chemical, produced by lactic acid bacteria (**Table 1**), but the biological production has always been in competition with chemical synthesis. Lactic acid, which is mostly used in food and pharmaceutical processes, is produced by homofermentative lactic acid bacteria such as *Lactobacillus delbrueckii* strains with a yield up to 90 g lactic acid per 100 g glucose.

Propionate fermentation

Propionate is a major end product of various fermentations, and many bacteria convert glucose to a mixture of propionate, acetate and carbon dioxide. However, most propionic acid bacteria are also able to ferment the end product of lactic acid fermentation, lactate, to propionate. There are two pathways for propionate formation from lactate, both of which have the same fermentation equation:

$$3\ \text{Lactate} \rightarrow 2\ \text{Propionate} + \text{Acetate} + \text{CO}_2 \qquad [\text{XII}]$$

The acrylate pathway as carried out by *Clostridium propionicum* consists of an oxidative and a reductive branch. In the oxidative branch 1 mol lactate is oxidized to acetate, thus giving rise to CO_2, 1 mol ATP and four reducing equivalents. The electrons are fed into the reductive branch

in which lactate is activated by a CoA transferase, the lactyl-CoA formed is dehydrated to acryloyl-CoA and then reduced to a propionyl-CoA. Per mol acetate formed 2 mol lactate have to be reduced to propionate to maintain the redox balance, and hence, the ATP yield is only 0.3 mol ATP per mol lactate consumed!

The methylmalonyl-CoA pathway, as carried out by the propionic acid bacteria, is energetically more efficient. Again, 1 mol lactate is oxidized to acetate giving rise to ATP but also to reducing equivalents. The electrons are fed into the reductive branch, which is very interesting from a biochemical point of view since it contains a number of unusual enzymes such as CoA transferases, a transcarboxylase and a B_{12}-containing enzyme. In this pathway, lactate is oxidized to pyruvate, pyruvate is carboxylated to oxaloacetate, and the latter is then reduced via the intermediates of the Krebs cycle to succinyl-CoA. Succinyl-CoA undergoes a rearrangement to methylmalonyl-CoA, which is subsequently decarboxylated to propionyl-CoA, the ultimate precursor of propionate. There is no ATP synthesis by substrate-level phosphorylation in the reductive branch, but an ion gradient is produced. Depending on the species, the ion gradient is generated during either fumarate reduction or methylmalonyl-CoA decarboxylation; the ion gradient in turn is used to drive ATP synthesis via a membrane-bound ATP synthase.

Propionate fermentation is used, for example, during the production of Swiss cheese. The holes in Swiss cheese are formed from carbon dioxide, generated by *Propionibacterium* from lactate.

Mixed acid and butanediol fermentation

Mixed acid and butanediol fermentation is carried out by the facultative anaerobic enterobacteria (Böck and Sawers, 1996). Members of the genera *Salmonella*, *Escherichia*, *Citrobacter*, *Shigella* and *Proteus* ferment glucose to a mixture of acids (acetic, lactic and formic acid), carbon dioxide and some ethanol, but not butanediol. As is evident from their names, the butanediol fermenters such as *Klebsiella*, *Enterobacter*, *Serratia*, *Erwinia* and *Hafnia* produce fewer acids but considerable amounts of butanediol, and also carbon dioxide. This difference is the basis for the diagnostic key used to differentiate *Escherichia coli* and *Enterobacter aerogenes*.

In the mixed acid fermentation glucose is converted by way of glycolysis. The fate of pyruvate is a reduction to lactate by the action of lactate dehydrogenase, a reduction to succinate after carboxylation to oxaloacetate, and a cleavage to acetyl-CoA and formate by pyruvate:formate lyase, a key enzyme of mixed acid fermentation. Pyruvate:formate lyase is a radical enzyme and subject to regulation by activation and deactivation. Interestingly, deactivation is catalysed by the *adhE* gene product, the alcohol dehydrogenase. The alcohol dehydrogenase is a polymer of a single 96-kDa subunit with a helical assemblage into rods 60–200 nm long. Formate is cleaved to hydrogen and carbon dioxide by the formate:hydrogen lyase complex, which actually consists of a molybdenum- and selenium-containing formate dehydrogenase (FdHH) and a nickel-iron hydrogenase (Hyd3), multimeric membrane-associated enzyme complexes. Typically, in mixed acid fermentations the ratio of acids to neutral products is 4:1, and hydrogen and carbon dioxide are produced in a 1:1 ratio.

During butanediol fermentation fewer acids are formed from pyruvate. Instead, two molecules of pyruvate are condensed under decarboxylation to α-acetolactate; this reaction is catalysed by α-acetolactate synthase. α-Acetolactate is then decarboxylated to acetoin, which is subsequently reduced to 2,3-butanediol. Diacetyl is a spontaneous autooxidation product of acetoin, and therefore the pathways for diacetyl formation in lactic acid bacteria and enterobacteria are different. Since butanediol formation is coupled to two decarboxylation reactions, butanediol fermenters produce much more gas than do mixed acid fermenters (note the name: *Enterobacter aerogenes*!). The ratio of carbon dioxide to hydrogen is 5:1, and the ratio of acidic to neutral products is 1:6. At present, there is no commercial use for the products of the mixed acid and butanediol fermentations. However, 2,3-butanediol is a potential fuel additive and has potential value as a chemical feedstock.

Butyrate and acetone-butanol fermentation

Butyrate and butanol are typical fermentation end products of a number of clostridial species (Bahl and Dürre, 1993). Hexoses are oxidized by way of glycolysis to pyruvate, which is oxidized by pyruvate:ferredoxin oxidoreductase to acetyl-CoA, carbon dioxide and reduced ferredoxin. Owing to its low redox potential, reduced ferredoxin can reduce protons to hydrogen or be used to reduce NAD^+ by the membrane-bound energy-conserving FNOR (see earlier). In butyrate fermenters such as *Clostridium butyricum*, acetyl-CoA is condensed in a reaction catalysed by thiolase to acetoacetyl-CoA, which is subsequently reduced to butyryl-CoA (with NADH as reductant, analogous to β-oxidation of fatty acids). The CoA of butyryl-CoA is transferred via a CoA transferase to acetate, giving rise to acetyl-CoA, which is then fed into the acetate kinase reaction to regenerate acetate, but most importantly, ATP. The overall reaction

$$\text{Glucose} \rightarrow \text{Butyrate} + 2\,CO_2 + 2\,H_2 \qquad [XIII]$$

is accompanied by the synthesis of 3 mol ATP.

During butanol fermentation the glycolytic reducing equivalents are reoxidized by reduction of butyryl-CoA to butanol via butyraldehyde. Therefore, 2 mol hexose have to be oxidized to gain the electrons required. The spare acetoacetyl-CoA is converted to acetoacetate and the CoA is transferred to acetate, giving rise to acetyl-CoA and opening the opportunity for additional ATP synthesis in the acetate kinase reaction. Acetoacetate is decarboxylated to acetone, the second product of this fermentation. The overall reaction is

$$2\ \text{Glucose} \rightarrow \text{Butanol} + \text{Acetone} + 4\,H_2 + 5\,CO_2 \quad [\text{XIV}]$$

If one takes into account the additional acetyl-CoA generated by the acetoacetyl-CoA:acetate CoA transferase then 2.5 mol ATP per mol of hexose are formed.

Species such as *Clostridium acetobutylicum* perform a so-called shift of their fermentation pathways. At first butyrate is produced, but with increasing acidification of the medium the acetoacetate decarboxylase is activated, leading to the formation of acetone. Decarboxylation of acetoacetate has the physiological consequence that less acetoacetyl-CoA can be reduced to butyryl-CoA, and, therefore, there is a shortage in electron acceptor. This problem is circumvented by taking up the butyrate again. Butyrate is activated by CoA transfer and subsequently reduced to butanol via butyraldehyde, thus maintaining the redox balance.

Acetone, butanol and 2-propanol are important solvents used as bulk chemicals in various industrial processes. During the first decade of the twentieth century, acetone-butanol fermentation became in volume the second largest fermentation process in the world, exceeded only by ethanol fermentation (**Table 1**). With the rise of the petrochemical industry in the 1950s, the biological production of acetone-butanol declined, and today there is no plant left that produces acetone-butanol on an industrial scale.

Homoacetate fermentation

Acetate is an end product of many fermentations but only a few microorganisms such as *Moorella thermoacetica* (formerly *Clostridium thermoaceticum*) and *Acetobacterium woodii* ferment organic compounds exclusively to acetate according to

$$\text{Glucose} \rightarrow 3\ \text{Acetate} \quad [\text{XV}]$$

Hexose conversion is by way of glycolysis to pyruvate, which is then converted to acetyl-CoA, carbon dioxide and reduced ferredoxin by pyruvate:ferredoxin oxidoreductase. The carbon dioxide formed is then reduced via the acetyl-CoA or Wood–Ljungdahl pathway. First, carbon dioxide is reduced to formate which is then bound under ATP hydrolysis to tetrahydrofolate (THF); the formyl-THF is subsequently reduced to methyltetrahydrofolate via methenyl- and methylene-THF. Methyl-THF condenses on the enzyme acetyl-CoA synthase with carbon monoxide to acetyl-CoA. The carbon monoxide is derived from the reduction of the second mole of carbon dioxide, catalysed by the carbon monoxide dehydrogenase activity of the acetyl-CoA synthase. Acetyl-CoA is converted via acetyl-phosphate to acetate, and 1 mol ATP is conserved. The net production of ATP by substrate-level phosphorylation is 4. In addition to substrate-level phosphorylation the acetyl-CoA pathway is coupled to ion-gradient-driven phosphorylation, and with respect to their energy metabolism homoacetogens can be divided into two groups, the proton and the sodium ion organisms. In *M. thermoacetica* a proton motive force is established, most probably by electron transport to methylene-THF. In *A. woodii* a primary sodium ion potential is generated during operation of the acetyl-CoA pathway, which in turn is used for ATP synthesis by a Na^+-translocating F_1F_0 ATP synthase (Müller, 2003). **See also**: Folates

Distribution of Fermentation among Organisms

During heavy exercise, muscle cells of higher eukaryotes encounter oxygen depletion and reduce pyruvate to lactate instead of oxidizing it, which can be a painful experience. The ability to ferment is also found in certain protozoa, fungi and worms (*Ascaris lumbricoides*). Fermentation is a very old and rather primitive metabolic route, allowing life in the absence of oxygen. Only with the evolution of oxygen by phototrophs were the energetically more favourable mechanisms of aerobic respiration invented, but the huge number of anaerobic environments demanded that the ability to ferment was kept during evolution. Among bacteria, fermentation is found in a number of organisms belonging to very different phylogenetic tribes, and the various tribes may contain aerobes as well as anaerobes. Fermentation is found in Gram-negative and Gram-positive organisms, in spore formers as well as in nonspore formers, in mesophiles as well as thermophiles, and it is not restricted to a certain morphological group, a pH range or salt concentration. For examples of organisms see the discussion of the fermentation pathways earlier. **See also**: Comparative Vertebrate Muscle Physiology; Large Fermenters

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Further Reading

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