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Chapter 1

Fermentation

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. In food production, it may more broadly refer to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage. The science of fermentation is known as zymology.

In microorganisms, fermentation is the primary means of producing adenosine triphosphate (ATP) by the degradation of organic nutrients anaerobically. The word equation for fermentation is: glucose → ethanol + carbon dioxide, or $C_6H_{12}O_6$ (aq) $\rightarrow 2C_2H_5OH$ (l) + CO_2 (g). Humans have used fermentation to produce foodstuffs and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid found in such sour foods as pickled cucumbers, kombucha, kimchi, and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation also occurs within the gastrointestinal tracts of all animals, including humans.

Definitions

Below are some definitions of fermentation. They range from informal, general usages to more scientific definitions.

- Preservation methods for food via microorganisms (general use).
- Any large-scale microbial process occurring with or without air (common definition used in industry).
- Any process that produces alcoholic beverages or acidic dairy products (general use).
- Any energy-releasing metabolic process that takes place only under anaerobic conditions (somewhat scientific).
- Any metabolic process that releases energy from a sugar or other organic molecule, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (most scientific).

Biological role

Along with aerobic respiration, fermentation is a method to extract energy from molecules. This method is the only one common to all bacteria and eukaryotes. It is therefore considered the oldest metabolic pathway, suitable for primeval environments – before plant life on Earth, that is, before oxygen in the atmosphere.

Yeast, a form of fungus, occurs in almost any environment capable of supporting microbes, from the skins of fruits to the guts of insects and mammals to the deep ocean. Yeasts convert (break down) sugar-rich molecules to produce ethanol and carbon dioxide.

Basic mechanisms for fermentation remain present in all cells of higher organisms. Mammalianmuscle carries out

fermentation during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid. In invertebrates, fermentation also produces succinate and alanine.

Fermentative bacteria play an essential role in the production of methane in habitats ranging from the rumens of cattle to sewage digesters and freshwater sediments. They produce hydrogen, carbon dioxide, formate and acetate and carboxylic acids. Then consortia of microbes convert the carbon dioxide and acetate to methane. Acetogenic bacteria oxidize the acids, obtaining more acetate and either hydrogen or formate. Finally, methanogens (in the domain *Archea*) convert acetate to methane.

Biochemical overview

Fermentation reacts NADH with an endogenous, organicelectron acceptor. Usually this is pyruvate formed from sugar through glycolysis. The reaction produces NAD and an organic product, typical examples being ethanol, lactic acid, and hydrogen gas (H_2), and often alsocarbon dioxide. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Fermentation products are considered waste products, since they cannot be metabolized further without the use of oxygen.

Fermentation normally occurs in an anaerobic environment. In the presence of O_2, NADH, and pyruvate are used to generate ATP in respiration. This is calledoxidative phosphorylation. This generates much more ATP than glycolysis alone. It releases the chemical energy of O_2. For this reason,

fermentation is rarely used when oxygen is available. However, even in the presence of abundant oxygen, some strains of yeast such as *Saccharomyces cerevisiae* prefer fermentation to aerobic respiration as long as there is an adequate supply of sugars (a phenomenon known as the Crabtree effect). Some fermentation processes involve obligate anaerobes, which cannot tolerate oxygen.

Although yeast carries out the fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, this is not the only possible agent: bacteria carry out the fermentation in the production of xanthan gum.

Products of fermentation

Ethanol

In ethanol fermentation, one glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules. It is used to make bread dough rise: the carbon dioxide forms bubbles, expanding the dough into a foam. The ethanol is the intoxicating agent in alcoholic beverages such as wine, beer and liquor. Fermentation of feedstocks, including sugarcane, corn, and sugar beets, produces ethanol that is added to gasoline. In some species of fish, including goldfish and carp, it provides energy when oxygen is scarce (along with lactic acid fermentation).

The figure illustrates the process. Before fermentation, a glucose molecule breaks down into two pyruvate molecules (Glycolysis). The energy from this exothermic reactionis used to bind inorganic phosphates to ADP, which converts it to ATP,

and convert NAD to NADH. The pyruvates break down into two acetaldehyde molecules and give off two carbon dioxide molecules as waste products. The acetaldehyde is reduced into ethanol using the energy and hydrogen from NADH, and the NADH is oxidized into NAD so that the cycle may repeat. The reaction is catalyzed by the enzymes pyruvate decarboxylase and alcohol dehydrogenase.

Lactic acid

Homolactic fermentation (producing only lactic acid) is the simplest type of fermentation. Pyruvate from glycolysis undergoes a simple redox reaction, forming lactic acid. Overall, one molecule of glucose (or any six-carbon sugar) is converted to two molecules of lactic acid:

- $C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$

It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some kinds of bacteria (such as lactobacilli) and some fungi. It is the type of bacteria that convert lactose into lactic acid in yogurt, giving it its sour taste. These lactic acid bacteria can carry out either homolactic fermentation, where the end-product is mostly lactic acid, or *heterolactic fermentation*, where some lactate is further metabolized to ethanol and carbon dioxide (via the phosphoketolase pathway), acetate, or other metabolic products, e.g.:

- $C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

If lactose is fermented (as in yogurts and cheeses), it is first converted into glucose and galactose (both six-carbon sugars with the same atomic formula):

- $C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6$

Heterolactic fermentation is in a sense intermediate between lactic acid fermentation and other types, e.g. alcoholic fermentation. Reasons to go further and convert lactic acid into something else include:

The acidity of lactic acid impedes biological processes. This can be beneficial to the fermenting organism as it drives out competitors that are unadapted to the acidity. As a result, the food will have a longer shelf life (one reason foods are purposely fermented in the first place); however, beyond a certain point, the acidity starts affecting the organism that produces it.

- The high concentration of lactic acid (the final product of fermentation) drives the equilibrium backwards (Le Chatelier's principle), decreasing the rate at which fermentation can occur and slowing down growth.
- Ethanol, into which lactic acid can be easily converted, is volatile and will readily escape, allowing the reaction to proceed easily. CO_2 is also produced, but it is only weakly acidic and even more volatile than ethanol.
- Acetic acid (another conversion product) is acidic and not as volatile as ethanol; however, in the presence of limited oxygen, its creation from lactic acid releases additional energy. It is a lighter

molecule than lactic acid, forming fewer hydrogen bonds with its surroundings (due to having fewer groups that can form such bonds), thus is more volatile and will also allow the reaction to proceed more quickly.

- If propionic acid, butyric acid, and longer monocarboxylic acids are produced (see mixed acid fermentation), the amount of acidity produced per glucose consumed will decrease, as with ethanol, allowing faster growth.

Hydrogen gas

Hydrogen gas is produced in many types of fermentation as a way to regenerate NAD from NADH. Electronsare transferred to ferredoxin, which in turn is oxidized by hydrogenase, producing H_2. Hydrogen gas is a substrate for methanogens and sulfate reducers, which keep the concentration of hydrogen low and favor the production of such an energy-rich compound, but hydrogen gas at a fairly high concentration can nevertheless be formed, as in flatus.

For example, *Clostridium pasteurianum* ferments glucose to butyrate, acetate, carbon dioxide, and hydrogen gas: The reaction leading to acetate is:

- $C_6H_{12}O_6 + 4\ H_2O \rightarrow 2\ CH_3COO + 2\ HCO_3 + 4\ H + 4\ H_2$

Alternative protein

Fermentation can be applied to generate alternative protein sources. For instance, plant based protein foods such as tempeh are produced using fermentation. However,

fermentation can also be used to culture animal products made from non-living material in vitro. Eggs, honey, cheese and milk are all examples which are made of various proteins. These proteins can be produced using this particular application of fermentation. Substances that are made using fermentation and which resemble milk are called milk substitutes. Substances that resemble cheese are calledcheese analogue and substances that resemble eggs are called egg substitutes.

Some companies have started providing fermentation services to farmers (Farming as a Service).

Heme is a protein which gives meat its characteristic texture, flavour and aroma. Impossible Foods used fermentation to generate a particular strand of heme derived from soybean roots, called soy leghemoglobin, which was integrated into the Impossible Burger to mimic meat flavor and appearance.

Other

Other types of fermentation include mixed acid fermentation, butanediol fermentation, butyrate fermentation, caproate fermentation, acetone–butanol–ethanol fermentation, and glyoxylate fermentation.

Modes of operation

Most industrial fermentation uses batch or fed-batch procedures, although continuous fermentation can be more economical if various challenges, particularly the difficulty of maintaining sterility, can be met.

Batch

In a batch process, all the ingredients are combined and the reactions proceed without any further input. Batch fermentation has been used for millennia to make bread and alcoholic beverages, and it is still a common method, especially when the process is not well understood. However, it can be expensive because the fermentor must be sterilized using high pressure steam between batches. Strictly speaking, there is often addition of small quantities of chemicals to control the pH or suppress foaming.

Batch fermentation goes through a series of phases. There is a lag phase in which cells adjust to their environment; then a phase in which exponential growth occurs. Once many of the nutrients have been consumed, the growth slows and becomes non-exponential, but production of *secondary metabolites* (including commercially important antibiotics and enzymes) accelerates. This continues through a stationary phase after most of the nutrients have been consumed, and then the cells die.

Fed-batch

Fed-batch fermentation is a variation of batch fermentation where some of the ingredients are added during the fermentation. This allows greater control over the stages of the process. In particular, production of secondary metabolites can be increased by adding a limited quantity of nutrients during the non-exponential growth phase. Fed-batch operations are often sandwiched between batch operations.

Open

The high cost of sterilizing the fermentor between batches can be avoided using various open fermentation approaches that are able to resist contamination. One is to use a naturally evolved mixed culture. This is particularly favored in wastewater treatment, since mixed populations can adapt to a wide variety of wastes. Thermophilic bacteria can produce lactic acid at temperatures of around 50 °Celsius, sufficient to discourage microbial contamination; and ethanol has been produced at a temperature of 70 °C. This is just below its boiling point (78 °C), making it easy to extract. Halophilic bacteria can produce bioplastics in hypersaline conditions. Solid-state fermentation adds a small amount of water to a solid substrate; it is widely used in the food industry to produce flavors, enzymes and organic acids.

Continuous

In continuous fermentation, substrates are added and final products removed continuously. There are three varieties: chemostats, which hold nutrient levels constant; turbidostats, which keep cell mass constant; and plug flow reactors in which the culture medium flows steadily through a tube while the cells are recycled from the outlet to the inlet. If the process works well, there is a steady flow of feed and effluent and the costs of repeatedly setting up a batch are avoided. Also, it can prolong the exponential growth phase and avoid byproducts that inhibit the reactions by continuously removing them. However, it is difficult to maintain a steady state and avoid contamination, and the design tends to be complex. Typically

the fermentor must run for over 500 hours to be more economical than batch processors.

History of the use of fermentation

- The use of fermentation, particularly for beverages, has existed since the Neolithic and has been documented dating from 7000 to 6600 BCE in Jiahu, China, 5000 BCE in India, Ayurveda mentions many Medicated Wines, 6000 BCE in Georgia, 3150 BCE in ancient Egypt, 3000 BCE in Babylon, 2000 BCE in pre-Hispanic Mexico, and 1500 BC in Sudan. Fermented foods have a religious significance in Judaism and Christianity. The Baltic godRugutiswas worshiped as the agent of fermentation.

In 1837, Charles Cagniard de la Tour, Theodor Schwann and Friedrich TraugottKützing independently published papers concluding, as a result of microscopic investigations, that yeast is a living organism that reproduces by budding. Schwann boiled grape juice to kill the yeast and found that no fermentation would occur until new yeast was added. However, a lot of chemists, including Antoine Lavoisier, continued to view fermentation as a simple chemical reaction and rejected the notion that living organisms could be involved. This was seen as a reversion to vitalism and was lampooned in an anonymous publication by Justus von Liebig and Friedrich Wöhler.

The turning point came when Louis Pasteur (1822–1895), during the 1850s and 1860s, repeated Schwann's experiments and showed fermentation is initiated by living organisms in a

series of investigations. In 1857, Pasteur showed lactic acid fermentation is caused by living organisms. In 1860, he demonstrated how bacteria cause souring in milk, a process formerly thought to be merely a chemical change. His work in identifying the role of microorganisms in food spoilage led to the process of pasteurization.

In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, "*Etudes sur la Bière*", which was translated into English in 1879 as "Studies on fermentation". He defined fermentation (incorrectly) as "Life without air", yet he correctly showed how specific types of microorganisms cause specific types of fermentations and specific end-products.

Although showing fermentation resulted from the action of living microorganisms was a breakthrough, it did not explain the basic nature of fermentation; nor, prove it is caused by microorganisms which appear to be always present. Many scientists, including Pasteur, had unsuccessfully attempted to extract the fermentation enzyme from yeast.

Success came in 1897 when the German chemist Eduard Buechner ground up yeast, extracted a juice from them, then found to his amazement this "dead" liquid would ferment a sugar solution, forming carbon dioxide and alcohol much like living yeasts.

Buechner's results are considered to mark the birth of biochemistry. The "unorganized ferments" behaved just like the organized ones. From that time on, the term enzyme came to be applied to all ferments. It was then understood fermentation is

caused by enzymes produced by microorganisms. In 1907, Buechner won the Nobel Prize in chemistry for his work.

Advances in microbiology and fermentation technology have continued steadily up until the present. For example, in the 1930s, it was discovered microorganisms could be mutated with physical and chemical treatments to be higher-yielding, faster-growing, tolerant of less oxygen, and able to use a more concentrated medium. Strain selection and hybridization developed as well, affecting most modern food fermentations.

Etymology

The word "ferment" is derived from the Latin verb *fervere*, which means to boil. It is thought to have been first used in the late 14th century in alchemy, but only in a broad sense. It was not used in the modern scientific sense until around 1600.

Chapter 2

Characteristics of Fermentation

Bioconversion

Bioconversion, also known as *biotransformation*, is the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain microorganisms. One example is the industrial production of cortisone, which one step is the bioconversion of progesterone to 11-alpha-Hydroxyprogesterone by *Rhizopusnigricans*. Another example is the bioconversion of glycerol to 1,3-propanediol, which is part of scientific research for many decades.

Another example of bioconversion is the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain microorganisms, some detritivores or enzymes.

In the US, the Bioconversion Science and Technology group performs multidisciplinary R&D for the Department of Energy's (DOE) relevant applications of bioprocessing, especially with biomass. Bioprocessing combines the disciplines of chemical engineering, microbiology and biochemistry. The Group 's primary role is investigation of the use of microorganism, microbial consortia and microbial enzymes in bioenergy research. New cellulosic ethanol conversion processes have enabled the variety and volume of feedstock that can be bioconverted to expand rapidly. Feedstock now includes

materials derived from plant or animal waste such as paper, auto-fluff, tires, fabric, construction materials, municipal solid waste (MSW), sludge, sewage, etc.

Three different processes for bioconversion

1 - **Enzymatic hydrolysis** - a single source of feedstock, switchgrass for example, is mixed with strong enzymes which convert a portion of cellulosic material into sugars which can then be fermented into ethanol. Genencor and Novozymes are two companies that have received United States government Department of Energy funding for research into reducing the cost of cellulase, a key enzyme in the production cellulosic ethanol by this process.

2 - **Synthesis gas fermentation** - a blend of feedstock, not exceeding 30% water, is gasified in a closed environment into a syngas containing mostly carbon monoxide and hydrogen. The cooled syngas is then converted into usable products through exposure to bacteria or other catalysts. BRI Energy, LLC is a company whose pilot plant in Fayetteville, Arkansas is currently using synthesis gas fermentation to convert a variety of waste into ethanol. After gasification, anaerobic bacteria (*Clostridium ljungdahlii*) are used to convert the syngas (CO, CO_2, and H_2) into ethanol. The heat generated by gasification is also used to co-generate excess electricity.

3 - **C.O.R.S. and Grub Composting** are sustainable technologies that employ organisms that feed on organic matter to reduce and convert organic waste in to high quality

feedstuff and oil rich material for the biodiesel industry. Organizations pioneering this novel approach to waste management are EAWAG, ESR International, Prota Culture and BIOCONVERSION that created the *e*-CORS® system to meet large scale organic waste management needs and environmental sustainability in both urban and livestock farming reality. This type of engineered system introduces a substantial innovation represented by the automatic modulation of the treatment, able to adapt conditions of the system to the biology of the scavenger used, improving their performances and the power of this technology.

Butanediol fermentation

2,3-**Butanediol fermentation** is anaerobicfermentation of glucose with 2,3-butanediol as one of the end products. The overall stoichiometry of the reaction is

- 2 pyruvate + NADH --> $2CO_2$ + 2,3-butanediol.

Butanediol fermentation is typical for the facultative anaerobes *Klebsiella* and *Enterobacter* and is tested for using the Voges–Proskauer (VP) test. There are other alternative strains that can be used, talked about in details in the Alternative Bacteria Strains section below.

The metabolic function of 2,3-butanediol is not known, although some have speculated that it was an evolutionary advantage for these microorganisms to produce a neutral product that's less inhibitory than other partial oxidation products and doesn't reduce the pH as much as mixed acids.

There are many important industrial applications that butanediol can be used for, including antifreeze, food additives, antiseptic, and pharmaceuticals. It also is produced naturally in various places of the environment.

Comparison with mixed acid fermentation

2,3-butanediol fermentation produces smaller amounts of acid than mixed acid fermentation, and butanediol, ethanol, CO_2 and H_2 are the end products. While equal amounts of CO_2 and H_2 are created during mixed acid fermentation, butanediol fermentation produces more than twice the amount of CO_2 because the gases are not produced only by formate hydrogen lyaselike they are in the mixed acid fermentation

2,3Butanediol is produced at varying levels in aerated fermentations as long as the dissolved oxygen level is limiting (i.e., the culture is trying to consume more oxygen than is available). The degree of oxygen limitation dictates the ratios of 2,3-butanediol to by-products produced

Butanediol properties

Butanediol has various properties that help contribute to its many industrial applications and effect how it is processed during manufacturing. Butanediol is an odorless and colorless liquid. It has a high boiling point, 180-184 °C that can impact downstream processes and cause problems while recovering

the fermented slurry. It also has a low freezing point, which allows it to be used for industrial applications.

Industrial applications

2,3-butanediol has a variety of industrial applications and products it can produce. The levo isomer of butanediol has a low freezing point of -60 °C, which allows it to work as an antifreeze agent. Through catalytic dehydrogenation, butanediol can form diacetyl. Diacetyl is a food additive that can be used to add flavor. 0.1% butanediol will kill most pathogenic bacteria due to its antiseptic properties. Through esterifation, forms of precursors of polyurethane foams are produced. These can be used in various applications, including in pharmaceuticals, cosmetics, lotions, ointments, and antiperspirants. Butanediol itself even has applications in the pharmaceutical industry as a drug carrier.

Natural occurrences

2,3-butanediol can be produced naturally in various places in the environment. A few places are sweet corn, fermented soybean curds, whole and ground grains, rotten mussels, and during the fermentation of fruits and grains.

Alternative bacteria strains

Using mesophilic bacteria requires the fermentation process to occur below 40 °C, which can cause bacterial contamination due to the low temperature. On the industrial scale, this

requires sterilization steps which means a special facility must be built, more employees are needed to run this extra step, and more energy is consumed at the plant. A novel aerobic *Geobacillus* strain XT15 has been shown to produce 2,3-butanediol at a temperature between 45 and 55 °C. This higher temperature will avoid the risk of contamination because microorganisms that live in normal environments cannot reproduce above 45 °C. The *Geobacillus* strain XT15 is theromophilic, which allows it to be able to operate fermentation at this higher temperature. Sterilization would not be necessary using this alternative strain making the manufacturing process more efficient and cost-effective.

Fermentation crock

A **fermentation crock**, also known as a **gärtopf crock** or **Harsch crock**, is a crock for fermentation. It has a gutter in the rim which is then filled with water so that when the top is put on an airlock is created, which prevents the food within from spoiling due to the development of surface molds. Ceramic weights may also be used to keep the fermenting food inside submerged.

Fermentative hydrogen production

Fermentative hydrogen production is the fermentative conversion of organic substrates to H_2. Hydrogen produced in this manner is often calledbiohydrogen. The conversion is effected by bacteria and protozoa, which employ enzymes. Fermentative hydrogen production is one of several anaerobic conversions.

Dark vs photofermentation

Dark fermentation reactions do not require light energy. These are capable of constantly producing hydrogen from organic compounds throughout the day and night. Typically these reactions are coupled to the formation of carbon dioxide or formate. Important reactions that result in hydrogen production start with glucose, which is converted to acetic acid:

- $C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3CO_2H + 2\ CO_2 + 4\ H_2$

A related reaction gives formate instead of carbon dioxide:

- $C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3CO_2H + 2\ HCO_2H + 2\ H_2$

These reactions are exergonic by 216 and 209 kcal/mol, respectively.

Using synthetic biology, bacteria can be genetically altered to enhance this reaction.

Photofermentation differs from dark fermentation, because it only proceeds in the presence of light. Electrohydrogenesisis used in microbial fuel cells.

Bacteria strains

For example, photo-fermentation with *Rhodobactersphaeroides* SH2C can be employed to convert small molecular fatty acids into hydrogen.

Enterobacteraerogenes is an outstanding hydrogen producer. It is an anaerobic facultative and mesophilic bacterium that is able to consume different sugars and in contrast to cultivation of strict anaerobes, no special operation is required to remove all oxygen from the fermenter. *E. aerogenes* has a short doubling time and high hydrogen productivity and evolution rate. Furthermore, hydrogen production by this bacterium is not inhibited at high hydrogen partial pressures; however, its yield is lower compared to strict anaerobes like *Clostridia*. A theoretical maximum of 4 mol H_2/mol glucose can be produced by strict anaerobic bacteria. Facultative anaerobic bacteria such as *E. aerogenes* have a theoretical maximum yield of 2 mol H_2/mol glucose.

Liebig–Pasteur dispute

Liebig–Pasteur dispute is the dispute between Justus von Liebig and Louis Pasteur on the processes and causes of fermentation.

Dispute overview

Louis Pasteur a French chemist, supported the idea that fermentation was a biological process. Justus von Liebig, a German chemist, supported the idea that fermentation was a mechanical process. Both chemists had different methods of experimentation, and they focused on different aspects of fermentation because they had different ideas about where the fermentation began in an organism.

The Liebig–Pasteur feud started in 1857 when Pasteur said that fermentation can occur in the absence of oxygen. The two were aware of the other's works, but continued working with their own theories. The two mention each other's names, as well as other scientists' names, in articles and other publications about the processes and causes of fermentation.

Pasteur's position

Pasteur observed that fermentation does not require oxygen, but needs the yeast, which is alive. The fermentation process is a biological process, not a reduction and oxygen chemical process. He used two slender bottles. One of the bottles had a curved neck; this is called a Swan neck duct. Pasteur poured liquid broth into the two bottles, and heated in the bottom of the bottles. When the bottles of liquid boiled, he let them cool. Pasteur observed that the broth in the curved bottle stayed clear, except when the bottle was shaken.

Pasteur explained that the two bottles were filled with air, but the curved bottle could stop most of the particles in the air, and it kept its nature. However, the liquid in the other bottle degenerated. Therefore, he concluded that fermentation does not require oxygen, but needs the yeast. When yeast is allowed to grow over time, the substance will spoil or rot.

Pasteur's view of fermentation can be said to fall under the vitalism point of view. He observed that living organisms were responsible for the process of fermentation.

Liebig's position

Liebig formulated his own theory claiming that the production of alcohol was not a biological process but a chemical process, discrediting the idea that fermentation could occur due to microscopic organisms. He believed that vibrations emanating from the decomposition of organic matter would spread to the sugar resulting in the production of solely carbon dioxide and alcohol.

The change was facilitated by ferment or yeast, which has the characters of a compound of nitrogen in the state of putrefaction. Given that the ferment's susceptibility to change, it is submitted to decomposition, by the action of air (from which oxygen is provided), water (from which moisture is obtained), and a favorable temperature. Prior to contact with oxygen, the constituents are arranged together without action on each other. Through the oxygen, the state of rest (or equilibrium) of the attractions that keep the elements together has been disturbed. As a consequence of this disturbance, a separation or new arrangement of the elements has been formed. Fermentation occurs due to the transference of molecular instability from the ferment (atoms in motion) to the sugar molecules, and continues as long as the decomposition of the ferment continues.

Liebig's view of fermentation can be said to fall under a mechanism point of view. From his work, he saw that fermentation, as well as other catalysts happened by a chemical and mechanical process.

Liebig–Pasteur communications

Pasteur responded to Liebig's works, often through his own writings, and using results from his own experiments to support his theories. For example, in 1858, Pasteur wrote a paper trying to disprove Liebig's theory that fermentation cannot be caused by the growth of the yeast when it takes place when yeast is added to pure sugar-water. Pasteur thought that in pure sugar-water, yeast was both growing and disintegrating, and developed experiments to support his theories. Liebig, however, was not convinced, and claimed that Pasteur was not solving the questions he had about the decomposition in fermentation. In 1869, Liebig responded to Pasteur's challenge, which he had made public ten years before. Liebig still held this ground, and mentioned that some of Pasteur's experiments were difficult to replicate and use effectively. Pasteur was furious, and suggested the Royal Academy hire a third scientist who replicate his experiments and verify his results in order to support his theories. Liebig nor the academy responded.

Later on, Pasteur demanded a meeting with Liebig, but Liebig did not receive cordially, and refused to discuss the topic of fermentation.

Eduard Büchner

Discovery of the active agent of fermentation

The famous controversy between Pasteur and Liebig over the nature of alcoholic fermentation was uncovered by Eduard

Büchner, a German chemist and zymologist. Influenced by his brother Hans, who became the famous bacteriologist, Büchner developed an interest in the fermentation process in which yeast breaks down sugar into alcohol and carbon dioxide. He published his first paper in 1885 which revealed that fermentation could occur in the presence of oxygen, a conclusion contrary to the current prevailing view held by Louis Pasteur.

By 1893, Büchner was fully involved in seeking the active agent of fermentation. He obtained pure samples of the inner fluid of yeast cells by pulverizing yeast within a mixture of sand and diatomaceous earth, then squeezing the mixture through a canvas filter. This process avoided the destructive method of using solvents and high temperatures which had foiled previous investigations. He assumed that the collected fluid was incapable of producing fermentation because the yeast cells were dead. However, when he attempted to preserve the fluid in concentrated sugar, he was startled to observe carbon dioxide being released, a sign that fermentation was taking place. Büchner hypothesized that the fermentation was caused by an enzyme which he named zymase. His findings that fermentation was the result of chemical process both inside and outside cells, were published in 1897.

Legacies of the Liebig–Pasteur dispute

Neither Liebig nor Pasteur was completely right. However, each of their arguments led to more discoveries that created a lot of today's fields in science and medicine.

Berzelius had defined the word "ferment" as being an example of catalytic activity. Soon after, Schwann discovered pepsin was the substance responsible for albuminous digestion in the stomach. He believed this was what Berzelius defined as catalysts, or the force for chemical reactions of mineral, organic and living matter. Liebig opposed the idea by saying that the terms catalysts and pepsin are not supposed to be used as they are only representatives of an idea.

Charles Cagniard-Latour, Theodor Schwann and Friedrich TraugottKützing identified independently yeast as a living organism that nourishes itself by the sugar it ferments, a process which referred to the ethanol fermentation (alcoholic fermentation). Liebig, Berzelius, and Wohler rejected the ideas of Schwann, Latour and Kutzing. In 1839, Liebig and Wohler published a paper on the role of yeast in alcoholic fermentation. In 1858, Liebig's student Moritz Traube enunciated the theorem, which was used for alcoholic fermentation, that all fermentations produced by living organisms are based on chemical reactions rather than a vital force itself.

The dispute between Liebig and Pasteur had, in a way, slowed down the advances of science and medicine in the area of fermentation, alcohol fermentation, and the enzymes. On the other hand, the conflicting ideas sped up the research in the area of fermentation and enzymes through other scientists and chemists. Through Büchner and his experiment in fermentation, the world of science and medicine went further as to pave ways in enzyme and fermentation studies and marked one of the critical points of the history of modern chemistry.

Pasteur effect

The **Pasteur effect** is an inhibiting effect of oxygen on the fermentation process. It is a sudden change from anaerobic to aerobic process.

Discovery

The effect was discovered in 1857 by Louis Pasteur, who showed that aerating yeasted broth causes yeast cell growth to increase, while conversely, fermentation rate decreases. Louis Pasteur was selected to the Académie des Sciences in 1862. He later became the professor of geology, physics, and chemistry at the École des Beaux-Art.

Explanation

The effect can be explained; as the yeast being facultative anaerobes can produce energy using two different metabolic pathways. While the oxygen concentration is low, the product of glycolysis, pyruvate, is turned into ethanol and carbon dioxide, and the energy production efficiency is low (2moles of ATP per mole of glucose). If the oxygen concentration grows, pyruvate is converted to acetyl CoA that can be used in the citric acid cycle, which increases the efficiency to 31 or 29.5 moles of ATP per mole of glucose (it depends on which shuttle is used for reducing the reducing equivalent, NADH, that is formed in the cytosol). Therefore, about 15 times as much glucose must be consumed anaerobically as aerobically to yield the same amount of ATP.

Under anaerobic conditions, the rate of glucose metabolism is faster, but the amount of ATP produced (as already mentioned) is smaller. When exposed to aerobic conditions, the ATP and Citrate production increases and the rate of glycolysis slows, because the ATP and citrate produced act as allosteric inhibitors for phosphofructokinase 1, the third enzyme in the glycolysis pathway. The Pasteur effect will only occur if glucose concentrations are low (<2 g/L) and if other nutrients, mostly nitrogen, are limited.

From the standpoint of ATP production then, it is advantageous for yeast to utilize the citric acid cycle in the presence of oxygen, as more ATP is produced from less glucose; however, Boulton et al. (1996) have maintained that yeast will follow the anaerobic, rather than aerobic, fermentative pathway if glucose is not limited, in that respiration, although capable of producing more ATP than glycolysis per molecule of glucose, also requires more energy in terms of enzymatic and mitochondrial requirements.

Practical implications

The processes used in alcohol production are commonly maintained in a low oxygen condition, under a blanket of carbon dioxide, while breeding yeast for biomass is done in aerobic conditions, the broth being aerated.

Solid-state fermentation

Solid state fermentation (SSF) is a biomolecule manufacturing process used in the food, pharmaceutical,

cosmetic, fuel and textile industries. These biomolecules are mostly metabolites generated by microorganisms grown on a solid support selected for this purpose. This technology for the culture of microorganisms is an alternative to liquid or submerged fermentation, used predominantly for industrial purposes

Processes

This process consists of depositing a solid culture substrate, such as rice or wheat bran, on flatbeds after seeding it with microorganisms; the substrate is then left in a temperature-controlled room for several days.

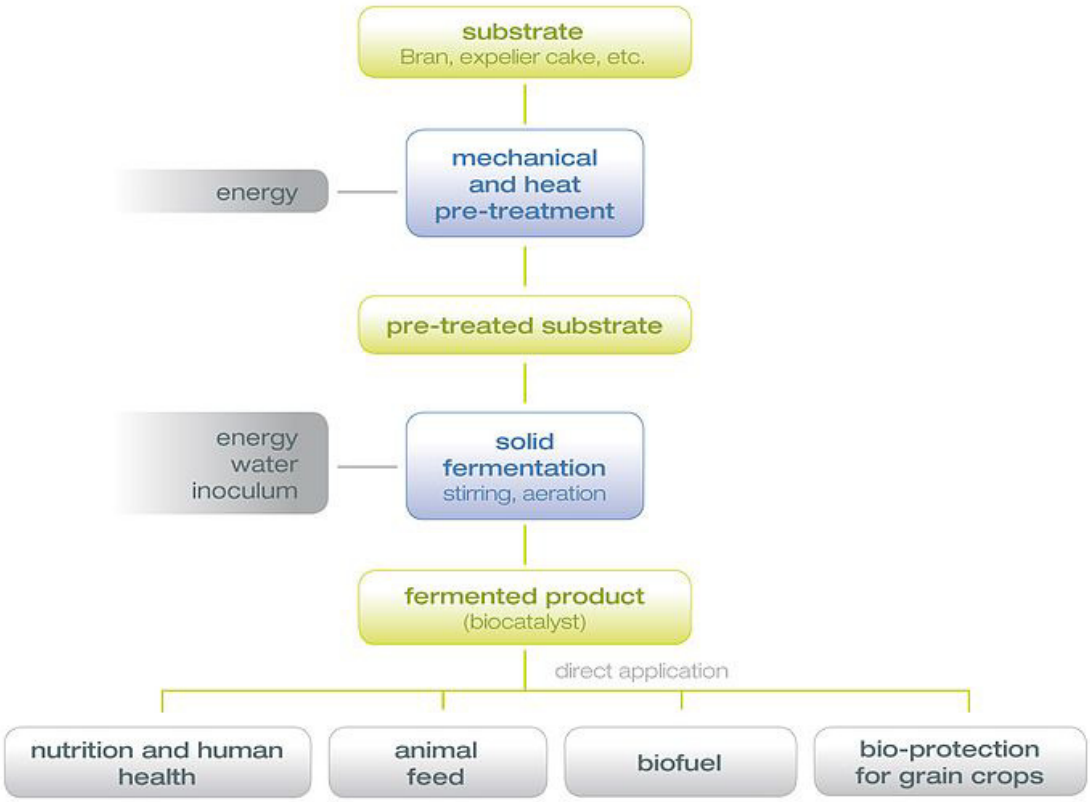
Liquid state fermentation is performed in tanks, which can reach 1,001 to 2,500 square metres (10,770 to 26,910 sq ft) at an industrial scale. Liquid culture is ideal for the growing of unicellular organisms such as bacteria or yeasts.

To achieve liquid aerobic fermentation, it is necessary to constantly supply the microorganism with oxygen, which is generally done via stirring the fermentation media. Accurately managing the synthesis of the desired metabolites requires regulating temperature, soluble oxygen, ionic strength and pH and control nutrients.

Applying this growing technique to filamentous fungi leads to difficulties. The fungus develops in its vegetative form, generating hyphae or multicellular ramous filaments, while a septum separates the cells. As this mycelium develops in a liquid environment, it generates abundant viscosity in the growing medium, reducing oxygen solubility, while stirring

disrupts the cell network increasing cell mortality. In nature, filamentous fungi grow on the ground, decomposing vegetal compounds under naturally ventilated conditions. Therefore, solid state fermentation enables the optimal development of filamentous fungi, allowing the mycelium to spread on the surface of solid compounds among which air can flow. Solid state fermentation uses culture substrates with low water levels (reduced water activity), which is particularly appropriate for mould. The methods used to grow filamentous fungi using solid state fermentation allow the best reproduction of their natural environment. The medium is saturated with water but little of it is free-flowing. The solid medium comprises both the substrate and the solid support on which the fermentation takes place. The substrate used is generally composed of vegetal byproducts such as beet pulp or wheat bran. At the beginning of the growth process, the substrates and solid culture compounds are non-soluble compounds composed of very large, biochemically complex molecules that the fungus will cut off to get essential C and N nutrients. To develop its natural substrate, the fungal organism sets forth its entire genetic potential to produce the metabolites necessary for its growth. The composition of the growth medium guides the microorganism's metabolism towards the production of enzymes that release bio-available single molecules such as sugars or amino acids by carving out macromolecules. Therefore, when selecting the components of the growth medium it is possible to guide the cells towards the production of the desired metabolite(s), mainly enzymes that transform polymers (cellulose, hemicellulose, pectins, proteins) into single moieties in a very efficient and cost-effective manner.

Compared to submerged fermentation processes, solid state fermentation is more cost-effective: smaller vessels, lower water consumption, reduced wastewater treatment costs and lower energy consumption (no need to heat up water, poor mechanical energy input due to smooth stirring). Cultivating on heterogeneous substrates requires expertise to maintain optimal growth conditions. Air flow monitoring is key because it impacts temperature, oxygen supply and moisture. In order to maintain sufficient moisture content for the growth of filamentous fungus, waterlogged air is used and may require further addition of water. In most cases, solid state fermentation does not require a completely sterile environment as the initial sterilization of the fermentation substrate associated with the rapid colonization of the substrate by the fungous microorganism limits the development of the autochthonous flora.



Uses

Traditional food production

Traditionally, SSF has been used in Asian countries to produce Koji using rice to manufacture alcoholic beverages such as Sake or Koji using soybean seeds. The latter produces sauces such as soy sauce or other foods. In Western countries, the traditional manufacturing process of many foods uses SSF. Examples include fermented bakery products such as bread or for the maturing of cheese. SSF is also widely used to prepare raw materials such as chocolate and coffee; typically cacao bean fermentation and coffee bean skin removal are SSF processes carried out under natural tropical conditions.

Enzyme production

Enzymes and enzymatic complexes able to break down difficult-to-transform macromolecules such as cellulose, hemicelluloses, pectin and proteins. Solid state fermentation is well suited for the production of various enzymatic complexes composed of multiple enzymes. Enzymatic compounds generated by SSF find outlets in all sectors where digestibility, solubility or viscosity is needed. This is why SSF enzymes are widely used in the following industries:

- fruit and vegetable transformation (pectinases)
- baking (hemicellulases)
- animal feeding (hemicellulases and cellulases)
- bio ethanol (cellulases and hemicellulases)
- brewing and distilling (hemicellulases)

Outlook

Liquid, submerged and solid state fermentation are age-old techniques used for the preservation and manufacturing of foods. During the second half of the twentieth century, liquid state fermentation developed on an industrial scale to manufacture vital metabolites such as antibiotics.

Economic changes and growing environmental awareness generate new perspectives for solid state fermentation. SSF adds value to insoluble agricultural byproducts thanks to its higher energy efficiency and reduced water consumption.

The renewal of SSF is now possible thanks to engineering firms, mainly from Asia, that have developed a new generation of equipment. Fujiwara makes vessels able to transform substrate areas up to 400 square metres (4,300 sq ft) for the production of soy sauce or sake. Other companies use solid state fermentation for enzyme complexes. In France Lyven has manufactured Pectinases and Hemicellulases on beet pulp and wheat bran since 1980. The company (now part of Soufflet Group) is now involved in a global R&D programme focusing on SSF technology.

Solventogenesis

Solventogenesis is the biochemical production of solvents (usually acetone and butanol) by *Clostridium* species. It is the second phase of ABE fermentation.

Process

Solventogenic*Clostridium* species have a biphasic metabolism composed of an acidogenic phase and a solventogenic phase. During acidogenesis, these bacteria are able to convert several carbon sources into organic acids, commonly butyrate and acetate. As acid accumulates, cells begin to assimilate the organic acids to solvents. In *Clostridium acetobutylicum*, a model solventogenic*Clostridium* species, a combination of low pH and high undisociated butyrate, referred to as the "pH-acid effect", triggers the metabolic shift from acidogenesis to solventogenesis.

Products

Acetone, butanol, and ethanol are the most common products of solventogenesis. Some species such as *Clostridium beijerinckii*, *Clostridium puniceum* and *Clostridium roseum* are able to further reduce acetone to isopropanol. Several species are able to produce additional solvents under various culture conditions. For example, glycerol fermentation results in the production of 1,3-propanediol in several species. Acetoin is produced by several species and is further reduced to 2,3-butanediol by *Clostridium beijerinckii*.

List of Solventogenic*Clostridium*

- *Clostridium acetobutylicum*
- *Clostridium aurantibutyricum*
- *Clostridium beijerinckii*

- *Clostridium butyricum*
- *Clostridium cadaveris*
- *Clostridium carboxidivorans*
- *Clostridium chauvoei*
- *Clostridium felsineum*
- *Clostridium pasteurianum*
- *Clostridium puniceum*
- *Clostridium roseum*
- *Clostridium saccharobutylicum*
- *Clostridium saccharoperbutylacetonicum*
- *Clostridium tetanomorphum*
- *Clostridium thermosaccharolyticum*
- *Clostridium tyrobutyricum*

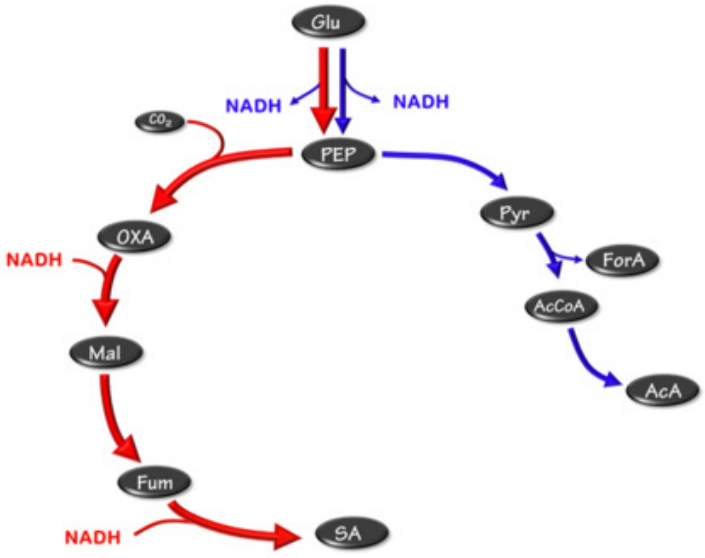
Succinic acid fermentation

Microbial production of Succinic acidcan be performed with wild bacteria like Actinobacillus succinogenes, Mannheimiasucciniciproducens and Anaerobiospirillum-succiniciproducens or genetically modified Escherichia coli, Corynebacteriumglutamicum and Saccharomyces cerevisia. Understanding of the central carbon metabolism of these organisms is crucial in determining the maximum obtainable yield of succinic acid on the carbon source employed as substrate.

Metabolic pathways

Neglecting the carbon utilised for biomass formation (known to be a small fraction of the total carbon utilised) basic biochemistry balances can be performed based on the

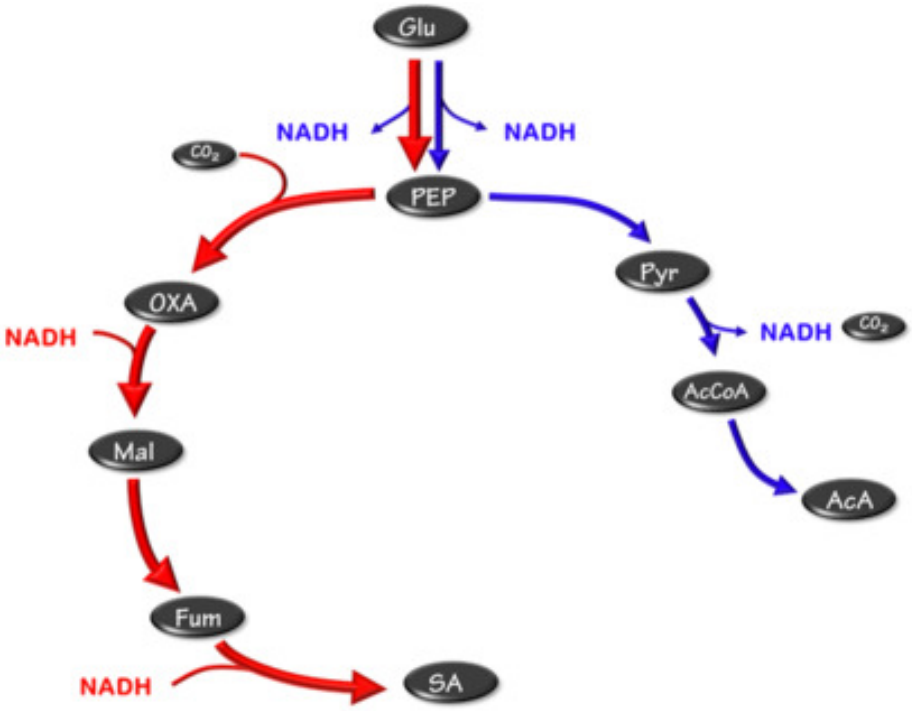
established metabolic pathways of these organisms. Using glucose as substrate the natural producing succinic acid producers are first considered. These organisms use the excretion of acetic acid (and sometimes formic acid) to balance the NADH requirement of succinic acid production. Two possible paths exist as indicated in Figure 1 and Figure 2. The difference between the two pathways lies in the pyruvate oxidation step where pyruvate formatelyaseis employed in Figure 1 and pyruvate dehydrogenase employed in Figure 2. The additional NADH generated in Figure 2 results in 66% of the molar glucose flux ending up as succinic acid compared to the 50% of Figure 1. The overall yields can be expressed on a mass basis where the pathway in Figure 1 results in a 0.66 gram succinic acid per gram of glucose consumed (g/g). The pathway in Figure 2 results in a yield of 0.87 g/g.



REDUCTIVE: $\frac{1}{2}\text{Glu} + CO_2 + \text{NADH} \rightarrow \text{SA}$

OXIDATIVE: $\frac{1}{2}\text{Glu} \rightarrow \text{NADH} + \text{AcA} + \text{ForA}$

TOTAL: $\text{Glu} + CO_2 \rightarrow \text{SA} + \text{AcA} + \text{ForA}$



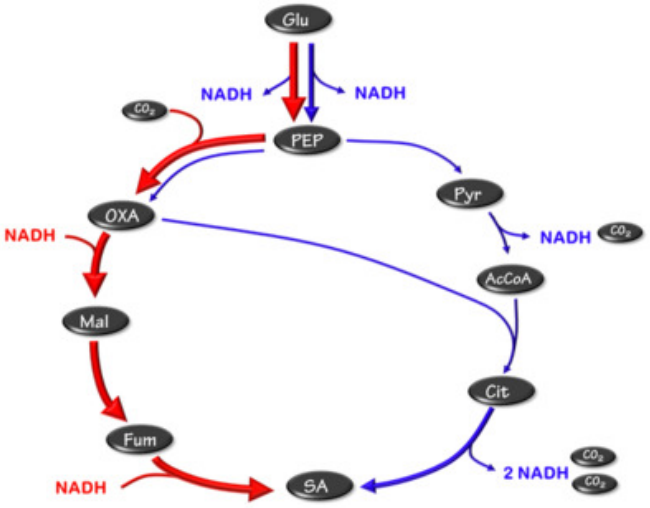
$$\textbf{REDUCTIVE:}\quad \frac{2}{3}\text{Glu} + \frac{4}{3}CO_2 + \frac{4}{3}\text{NADH} \rightarrow \frac{4}{3}\text{SA}$$

$$\textbf{OXIDATIVE:}\quad \frac{1}{3}\text{Glu} \rightarrow \frac{4}{3}\text{NADH} + \frac{2}{3}CO_2 + \frac{2}{3}\text{AcA}$$

$$\textbf{TOTAL:}\quad \text{Glu} + \frac{2}{3}CO_2 \rightarrow \frac{4}{3}\text{SA} + \frac{2}{3}\text{AcA}$$

The metabolic pathway can be genetically engineered in order to have succinic acid as the only excretion product. This can be achieved by using the oxidative section of the tricarboxylic acid cycle (TCA) under anaerobic conditions as illustrated in Figure 3.

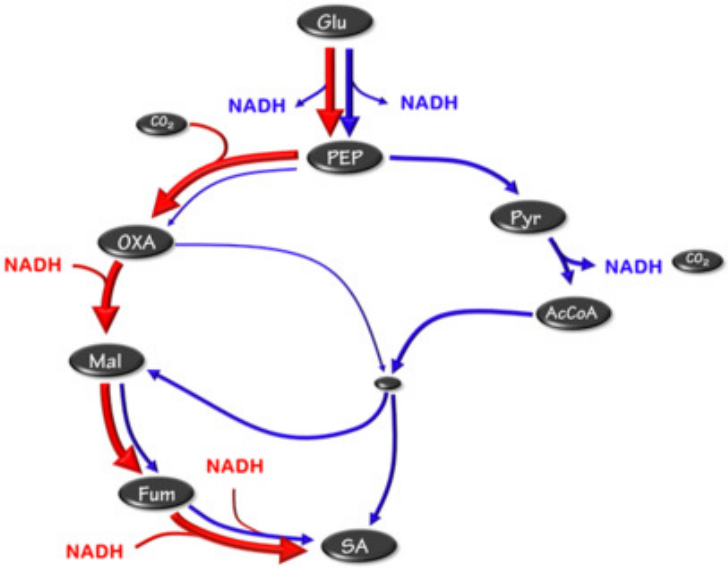
Alternatively the glyoxylate bypass can be utilised (Figure 4) to give the same result. For both these scenarios the mass based succinic acid yield is 1.12 g/g. This implies that the theoretical maximum yield is such that more succinic acid is formed than glucose consumed due to the fixation of carbon dioxide.



REDUCTIVE: $\frac{5}{7}\text{Glu} + \frac{10}{7}CO_2 + \frac{10}{7}\text{NADH} \rightarrow \frac{10}{7}\text{SA}$

OXIDATIVE: $\frac{2}{7}\text{Glu} \rightarrow \frac{10}{7}\text{NADH} + \frac{4}{7}CO_2 + \frac{2}{7}\text{SA}$

TOTAL: $\text{Glu} + \frac{6}{7}CO_2 \rightarrow \frac{12}{7}\text{SA}$



REDUCTIVE: $\frac{4}{7}\text{Glu} + \frac{8}{7}CO_2 + \frac{8}{7}\text{NADH} \rightarrow \frac{8}{7}\text{SA}$

OXIDATIVE: $\frac{3}{7}\text{Glu} \rightarrow \frac{8}{7}\text{NADH} + \frac{2}{7}CO_2 + \frac{4}{7}\text{SA}$

TOTAL: $\text{Glu} + \frac{6}{7}CO_2 \rightarrow \frac{12}{7}\text{SA}$

Symbiotic fermentation

Symbiotic fermentation is a form of fermentation in which multiple organisms (yeasts, acetic acid bacteria, lactic acid bacteria and others) interact in order to produce the desired product. For example, a yeast may produce ethanol, which is then consumed by an acetic acid bacterium. Described early on as the fermentation of sugars following saccharification in a mixed fermentation process.

History

The earliest mention of the term can be found in a lecture given by Dr. Allan Macfadyen of the Jenner Institute of Preventative Medicine in 1902. Dr. Macfadyen described symbiotic fermentation as noting "a close relationship between the organisms at work, the action of one aiding or modifying the action of the other, whilst both members are more active as a results of the partnership." Fermentative microorganisms have had a deep history as seen by Kefir and Koumiss fermentations of milk by Nomadic tribes in Russia, as well as Japanese Koji fermentation (see *Aspergillus oryzae).*

In 1927, Dr. Aldo Castellani defined symbiotic fermentation as "two microorganisms neither of which alone produces fermentation with gas in certain carbohydrates, may do so when living in symbiosis or when artificially mixed." He based this definition on the observation that ordinary bakers yeast consisted of two or more microorganisms- *Saccharomyces* and *Bacilli.* He performed experiments to show that when two different *Bacilli* species were grown in culture together with

maltose as the sugar, gas was produced as a result of symbiotic fermentation. Dr. Castellani also described symbiotic fermentation as a method to distinguish between *Bacillus dysentariae* Shiga (now *Shigelladysentariae* Shiga) and *B. dysentariae* Flexner (now *Shigellaflexneri*) by fermenting each of them with *Bacillus morgani* (now *Morganellamorganii*) in mannitol. The culture with Flexner would always produce gas and acid, while the culture with Shiga only produced acid. To summarize, one bacteria performs acid fermentation to produce acid from sugar, then the other bacteria performs gas fermentation using the acid products to produce gas. Thus creating a type of symbiotic relationship based on fermentation metabolism. More recently, symbiotic fermentation is described in a traditional sense for the fermentation of food and beverage products. Biofilm aggregates of fermentative microorganisms are commonly associated with fermentation of many products including vinegar, sake, shochu, and kefir.

In the U.S., kombucha has become a popular fermented beverage that is also a model of symbiotic fermentation. In kombucha, bacteria create the biofilm network that initiates SCOBY formation, while the yeast produce invertase that makes sugars available to the bacteria and yeast for fermentation.

Examples of Symbiotic Fermentation

- Kefir
- In Kefir, the lactose in milk is fermented by lactic acid bacteria to produce lactic acid, further

breakdown to propionic acid is done by propionibacteria. Yeast in Kefir ferment to produce ethanol, which is consumed by other bacteria to make acids and aldehydes that contribute to flavor.

- Sake
- In the making of Sake, Koji molds are used to ferment rice producing free sugars that are then fermented by lactic acid bacteria and yeast, providing ethanol and flavor active compounds.
- Lambic Beer
- Wheat is fermented by yeast and LAB.
- Shochu
- Rice, wheat, and batata are fermented by mold, yeast, and LAB.
- Vinegar
- Rice is fermented by mold, yeast, LAB, and acetic acid bacteria.
- Soy Sauce
- Soy bean and wheat are fermented by mold, yeast, and LAB.
- Whiskey
- Barely, corn, and rye are fermented by yeast and LAB.
- Wine
- Grapes are fermented by yeast and LAB.
- Kombucha
- Tea and sucrose are fermented by yeast and acetic acid bacteria from a SCOBY.

Chapter 3

Mixed Acid Fermentation

Mixed acid fermentation is the biological process by which a six-carbon sugar e.g. glucoseis converted into a complex and variable mixture of acids. It is an anaerobicfermentation reaction that is common in bacteria. It is characteristic for members of the Enterobacteriaceae, a large family of Gram-negative bacteria that includes *E. coli*.

The mixture of end products produced by mixed acid fermentation includes lactate, acetate, succinate, formate, ethanol and the gases H_2 and CO_2. The formation of these end products depends on the presence of certain key enzymes in the bacterium. The proportion in which they are formed varies between different bacterial species. The mixed acid fermentation pathway differs from other fermentation pathways, which produce fewer end products in fixed amounts. The end products of mixed acid fermentation can have many useful applications in biotechnology and industry. For instance, ethanol is widely used as a biofuel. Therefore, multiple bacterial strains have been metabolically engineered in the laboratory to increase the individual yields of certain end products. This research has been carried out primarily in *E. coli* and is ongoing.

Mixed acid fermentation in *E. coli*

E. coli use fermentation pathways as a final option for energy metabolism, as they produce very little energy in comparison to

respiration. Mixed acid fermentation in *E. coli* occurs in two stages. These stages are outlined by the biological database for *E. coli*, EcoCyc.

The first of these two stages is a glycolysis reaction. Under anaerobic conditions, a glycolysis reaction takes place where glucose is converted into pyruvate:

glucose→ 2 pyruvate

There is a net production of 2ATP and 2 NADH molecules per molecule of glucose converted. ATP is generated by substrate-level phosphorylation. NADH is formed from the reduction of NAD.

In the second stage, pyruvate produced by glycolysis is converted to one or more end products via the following reactions. In each case, both of the NADH molecules generated by glycolysis are reoxidized to NAD. Each alternative pathway requires a different key enzyme in *E. coli*.

After the variable amounts of different end products are formed by these pathways, they are secreted from the cell.

Lactate formation

Pyruvate produced by glycolysis is converted to lactate. This reaction is catalysed by the enzyme lactate dehydrogenase (LDHA).

pyruvate + NADH + H →**lactate** + NAD

Acetate formation

- Pyruvate is converted into acetyl-coenzyme A (acetyl-CoA) by the enzyme pyruvate dehydrogenase. This acetyl-CoA is then converted into acetate in *E. coli*, whilst producing ATP by substrate-level phosphorylation. Acetate formation requires two enzymes: phosphate acetyltransferase and acetate kinase.

acetyl-CoA + phosphate → acetyl-phosphate + CoA

acetyl-phosphate + ADP →**acetate** + ATP

Ethanol formation

Ethanolis formed in *E. coli* by the reduction of acetyl coenzyme A using NADH. This two-step reaction requires the enzyme alcohol dehydrogenase (ADHE).

acetyl-CoA + NADH + H → acetaldehyde + NAD + CoA

acetaldehyde + NADH + H →**ethanol** + NAD

Formate formation

Formateis produced by the cleavage of pyruvate. This reaction is catalysed by the enzyme pyruvate-formatelyase (PFL), which plays an important role in regulating anaerobic fermentation in *E. coli*.

pyruvate + CoA → acetyl-CoA + **formate**

Succinate formation

Succinateis formed in *E. coli* in several steps.

Phosphoenolpyruvate (PEP), a glycolysis pathway intermediate, is carboxylated by the enzyme PEP carboxylase to form oxaloacetate. This is followed by the conversion of oxaloacetate to malate by the enzyme malate dehydrogenase. Fumarate hydratase then catalyses the dehydration of malate to produce fumarate.

phosphoenolpyruvate + HCO_3→ oxaloacetate + phosphate

oxaloacetate + NADH + H → malate + NAD

malate→ fumarate + H_2O

The final reaction in the formation of succinate is the reduction of fumarate. It is catalysed by the enzyme fumarate reductase.

fumarate + NADH + H →**succinate** + NAD

This reduction is an anaerobic respiration reaction in *E. coli*, as it uses electrons associated with NADH dehydrogenase and the electron transport chain. ATP is generated by using an electrochemical gradient and ATP synthase. This is the only case in the mixed acid fermentation pathway where ATP is not produced via substrate-level phosphorylation.

Vitamin K_2, also known as menaquinone, is very important for electron transport to fumarate in *E. coli*.

Hydrogen and carbon dioxide formation

Formatecan be converted to hydrogen gas and carbon dioxide in *E. coli*. This reaction requires the enzyme formate-hydrogen lyase. It can be used to prevent the conditions inside the cell becoming too acidic.

formate→$\mathbf{H_2}$ and $\mathbf{CO_2}$

Methyl red test

The methyl red (MR) test can detect whether the mixed acid fermentation pathway occurs in microbes when given glucose. A pH indicatoris used that turns the test solution red if the pH drops below 4.4. If the fermentation pathway has taken place, the mixture of acids it has produced will make the solution very acidic and cause a red colour change.

The methyl red test belongs to a group known as the IMViC tests.

Metabolic engineering

Multiple bacterial strains have been metabolically engineered to increase the individual yields of end products formed by mixed acid fermentation. For instance, strains for the increased production of ethanol, lactate, succinate and acetate have been developed due to the usefulness of these products in biotechnology. The major limiting factor for this engineering is the need to maintain a redox balance in the mixture of acids produced by the fermentation pathway.

For ethanol production

Ethanol is the most commonly used biofuel and can be produced on large scale via fermentation. The maximum theoretical yield for the production of ethanol was achieved around 20 years. A plasmid that carried the pyruvate decarboxylase and alcohol dehydrogenase genes from the bacteria *Z. mobilis* was used by scientists. This was inserted into *E. coli* and resulted in an increased yield of ethanol. The genome of this *E. coli* strain, KO11, has more recently been sequenced and mapped.

For acetate production

The *E. coli* strain W3110 was genetically engineered to generate 2 moles of acetate for every 1 mole of glucose that undergoes fermentation. This is known as a homoacetate pathway.

For lactate production

Lactate can be used to produce a bioplastic called polylactic acid (PLA). The properties of PLA depend on the ratio of the two optical isomers of lactate (D-lactate and L-lactate). D-lactate is produced by mixed acid fermentation in *E. coli*. Early experiments engineered the *E. coli* strain RR1 to produce either one of the two optical isomers of lactate.

Later experiments modified the *E. coli* strain KO11, originally developed to enhance ethanol production. Scientists were able to increase the yield of D-lactate from fermentation by performing several deletions.

For succinate production

Increasing the yield of succinate from mixed acid fermentation was first done by overexpressing the enzyme PEP carboxylase. This produced a succinate yield that was approximately 3 times greater than normal. Several experiments using a similar approach have followed.

Alternative approaches have altered the redox and ATP balance to optimize the succinate yield.

Related fermentation pathways

- There are a number of other fermentation pathways that occur in microbes. All these pathways begin by converting pyruvate, but their end products and the key enzymes they require are different. These pathways include:

Propionic acid

Propionic acid (/proʊpiˈɒnɪk/, from the Greek words *protos*, meaning "first", and *pion*, meaning "fat"; also known as **propanoic acid**) is a naturally occurring carboxylic acid with chemical formula $CH_3CH_2CO_2H$. It is a liquid with a pungent and unpleasant smell somewhat resembling body odor. The anion $CH_3CH_2CO_2$ as well as the salts and esters of propionic acid are known as **propionates** or **propanoates**.

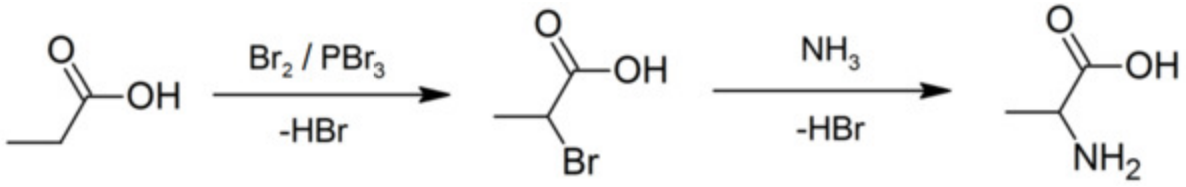
History

Propionic acid was first described in 1844 by Johann Gottlieb, who found it among the degradation products of sugar. Over the next few years, other chemists produced propionic acid by different means, none of them realizing they were producing the same substance. In 1847, French chemist Jean-Baptiste Dumas established all the acids to be the same compound, which he called propionic acid, from the Greek words πρῶτος (prōtos), meaning *first*, and πίων (piōn), meaning *fat*, because it is the smallest $H(CH_2)_nCOOH$ acid that exhibits the properties of the other fatty acids, such as producing an oily layer when salted out of water and having a soapy potassiumsalt.

Properties

Propionic acid has physical properties intermediate between those of the smaller carboxylic acids, formic and acetic acids, and the larger fatty acids. It is miscible with water, but can be removed from water by adding salt. As with acetic and formic acids, it consists of hydrogen bonded pairs of molecules in both the liquid and the vapor.

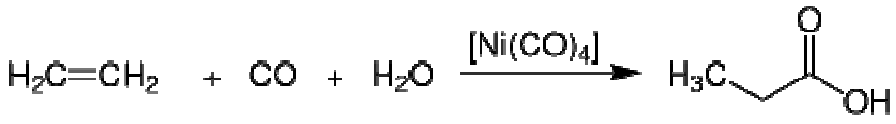
Propionic acid displays the general properties of carboxylic acids: it can form amide, ester, anhydride, and chloride derivatives. It undergoes the Hell–Volhard–Zelinsky reaction that involves α-halogenation of a carboxylic acid with bromine, catalysed by phosphorus tribromide, in this case to form 2-bromopropanoic acid, $CH_3CHBrCOOH$. This product has been used to prepare a racemic mixture of alanine by ammonolysis.



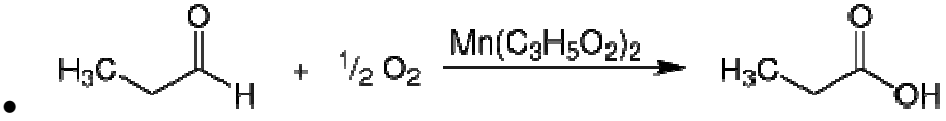
Manufacture

Chemical

In industry, propionic acid is mainly produced by the hydrocarboxylation of ethylene using nickel carbonyl as the catalyst:



It is also produced by the aerobic oxidation of propionaldehyde. In the presence of cobalt or manganese salts (manganese propionate is most commonly used), this reaction proceeds rapidly at temperatures as mild as 40–50 °C:



Large amounts of propionic acid were once produced as a byproduct of acetic acid manufacture. At the current time, the world's largest producer of propionic acid is BASF, with approximately 150 kt/a production capacity.

Biotechnological

Biotechnological production of propionic acid mainly uses *Propionibacterium* strains. However, large scale production of propionic acid by *Propionibacteria* faces challenges such as severe inhibition of end-products during cell growth and the formation of by-products (acetic acid and succinic acid). One approach to improve productivity and yield during fermentation is through the use of cell immobilization techniques, which also promotes easy recovery, reuse of the cell biomass and enhances microorganisms' stress tolerance. In 2018, 3D printing technology was used for the first time to create a matrix for cell immobilization in fermentation. Propionic acid production by *Propionibacteriumacidipropionici* immobilized on 3D-printed nylon beads was chosen as a model study. It was shown that those 3D-printed beads were able to promote high density cell attachment and propionic acid production, which could be adapted to other fermentation bioprocesses. Other cell immobilization matrices have been tested, such as recycled-glass Poraver and fibrous-bed bioreactor.

Alternative methods of production have been trialled, by genetically engineering strains of *Escherichia coli* to incorporate the necessary pathway, the Wood-Werkman cycle.

Industrial uses

Propionic acid inhibits the growth of mold and some bacteria at levels between 0.1 and 1% by weight. As a result, some propionic acid produced is consumed as a preservative for both

animal feed and food for human consumption. For animal feed, it is used either directly or as its ammonium salt. The antibiotic monensin is added to cattle feed to favor propionibacteria over acetic acid producers in the rumen; this produces less carbon dioxide and feed conversion is better. This application accounts for about half of the world production of propionic acid. Another major application is as a preservative in baked goods, which use the sodium and calcium salts. As a food additive, it is approved for use in the EU, USA, Australia and New Zealand.

Propionic acid is also useful as an intermediate in the production of other chemicals, especially polymers. Cellulose-acetate-propionate is a useful thermoplastic. Vinyl propionate is also used. In more specialized applications, it is also used to make pesticides and pharmaceuticals. The esters of propionic acid have fruit-like odors and are sometimes used as solvents or artificial flavorings.

In biogas plants, propionic acid is a common intermediate product, which is formed by fermentation with propionic acid bacteria. Its degradation in anaerobic environments (e.g. biogas plants) requires the activity of complex microbial communities.

Biology

Propionic acid is produced biologically as its coenzyme A ester, propionyl-CoA, from the metabolic breakdown of fatty acids containing odd numbers of carbon atoms, and also from the breakdown of some amino acids. Bacteria of the genus *Propionibacterium* produce propionic acid as the end-product of

their anaerobic metabolism. This class of bacteria is commonly found in the stomachs of ruminants and the sweat glands of humans, and their activity is partially responsible for the odor of Emmental cheese, American "Swiss cheese" and sweat.

The metabolism of propionic acid begins with its conversion to propionylcoenzyme A, the usual first step in the metabolism of carboxylic acids. Since propionic acid has three carbons, propionyl-CoA cannot directly enter either beta oxidation or the citric acid cycles. In most vertebrates, propionyl-CoA is carboxylated to D-methylmalonyl-CoA, which is isomerised to L-methylmalonyl-CoA. A vitamin B_{12}-dependent enzyme catalyzes rearrangement of L-methylmalonyl-CoA to succinyl-CoA, which is an intermediate of the citric acid cycle and can be readily incorporated there.

Propionic acid serves as a substrate for hepaticgluconeogenesis via conversion to succinyl-CoA. Additionally, exogenous propionic acid administration results in more endogenous glucose production than can be accounted for by gluconeogenic conversion alone. Exogenous propionic acid may upregulate endogenous glucose production via increases in norepinephrine and glucagon, suggesting that chronic ingestion of propionic acid may have adverse metabolic consequences.

In propionic acidemia, a rare inherited genetic disorder, propionate acts as a metabolic toxin in liver cells by accumulating in mitochondria as propionyl-CoA and its derivative, methylcitrate, two tricarboxylic acid cycle inhibitors. Propanoateis metabolizedoxidatively by glia, which suggests astrocytic vulnerability in propionic acidemia when intramitochondrialpropionyl-CoA may accumulate. Propionic

acidemia may alter both neuronal and glial gene expression by affecting histone acetylation. When propionic acid is infused directly into rodents' brains, it produces reversible behavior (e.g., hyperactivity, dystonia, social impairment, perseveration) and brain changes (e.g., innate neuroinflammation, glutathione depletion) that may be used as a means to model autism in rats.

Human occurrence

The human skin is host of several species of *Propionibacteria*. The most notable one is the *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*), which lives mainly in the sebaceous glands of the skin and is one of the principal causes of acne. Propionate is observed to be among the most common short-chain fatty acids produced in the large intestine of humans by gut microbiota in response to indigestible carbohydrates (dietary fiber) in the diet. The role of the gut microbiota and their metabolites, including propionate, in mediating brain function has been reviewed.

A study in mice suggests that propionate is produced by the bacteria of the genus *Bacteroides* in the gut, and that it offers some protection against *Salmonella* there. Another study finds that fatty acid propionate can calm the immune cells that drive up blood pressure, thereby protecting the body from damaging effects of high blood pressure.

Bacteriology

The Bacteria species *Coprothermobacter platensis* produces propionate when fermenting gelatin.

Propionate salts and esters

The **propionate**/ˈproʊpiəneɪt/, or **propanoate**, ion is C_2H_5COO, the conjugate base of propionic acid. It is the form found in biological systems at physiological pH. A propionic, or propanoic, compound is a carboxylate salt or ester of propionic acid. In these compounds, propionate is often written in shorthand, as $CH_3CH_2CO_2$ or simply $EtCO_2$.

Propionates should not be confused with propenoates (commonly known as acrylates), the ions/salts/esters of propenoic acid (also known as 2-propenoic acid or acrylic acid).

Examples

Salts

- Sodium propionate $NaC_2H_5CO_2$
- Potassium propionate $KC_2H_5CO_2$
- Calcium propionate $Ca(C_2H_5CO_2)_2$
- Zirconium propionate$Zr(C_2H_5CO_2)_4$

Esters

- Methyl propionate $(C_2H_5(CO)OCH_3)$
- Ethyl propionate $(C_2H_5(CO)OC_2H_5)$
- Propyl propionate $(C_2H_5(CO)OC_3H_7)$
- Pentyl propionate $(C_2H_5(CO)OC_5H_{11})$
- Fluticasone propionate $C_{25}H_{31}F_3O_5S$

Butanol

Butanol (also called **butyl alcohol**) is a four-carbon alcohol with a formula of C_4H_9OH, which occurs in five isomeric structures (four structural isomers), from a straight-chain primary alcohol to a branched-chain tertiary alcohol; all are a butyl or isobutyl group linked to a hydroxyl group (sometimes represented as **BuOH**, ***n*-BuOH**, ***i*-BuOH,** and ***t*-BuOH**). These are *n*-butanol, 2 stereoisomers of sec-butanol, isobutanol and *tert*-butanol. Butanol is primarily used as a solvent and as an intermediate in chemical synthesis, and may be used as a fuel. Biologically produced butanol is called**biobutanol**, which may be *n*-butanol or isobutanol.

Isomers

- The unmodified term *butanol* usually refers to the straight chain isomer with the alcohol functional group at the terminal carbon, which is also known as *n*-butanol or 1-butanol. The straight chain isomer with the alcohol at an internal carbon is *sec*-butanol or 2-butanol. The branched isomer with the alcohol at a terminal carbon is isobutanol or 2-methyl-1-propanol, and the branched isomer with the alcohol at the internal carbon is *tert*-butanol or 2-methyl-2-propanol.

The butanol isomers have different melting and boiling points. *n*-butanol and isobutanol have limited solubility, *sec*-butanol has substantially greater solubility, while *tert*-butanol is miscible with water. The hydroxyl group makes the molecule

polar, promoting solubility in water, while the longer hydrocarbon chain mitigates the polarity and reduces solubility.

Toxicity

Butanol exhibits a low order of toxicity in single dose experiments with laboratory animals and is considered safe enough for use in cosmetics. Brief, repeated overexposure with the skin can result in depression of the central nervous system, as with other short-chain alcohols. Exposure may also cause severe eye irritation and moderate skin irritation. The main dangers are from prolonged exposure to the alcohol's vapors. In extreme cases this includes suppression of the central nervous system and even death. Under most circumstances, butanol is quickly metabolized to carbon dioxide. It has not been shown to damage DNA or cause cancer.

Uses

Primary uses

Butanol is used as a solvent for a wide variety of chemical and textile processes, in organic synthesis, and as a chemical intermediate. It is also used as a paint thinner and a solvent in other coating applications where a relatively slow evaporating latent solvent is preferable, as with lacquers and ambient-cured enamels. It is also used as a component of hydraulic and brake fluids.

A 50% solution of butanol in water has been used since the 20th century to retard the drying of fresh plaster in fresco painting. The solution is usually sprayed on the wet plaster after the plaster has been trowelled smooth and extends the working period during which frescos can be painted up to 18 hours.

Butanol is used in the synthesis of 2-butoxyethanol. A major application for butanol is as a reactant with acrylic acid to produce butyl acrylate, a primary ingredient of water based acrylic paint.

It is also used as a base for perfumes, but on its own has a highly alcoholic aroma.

Salts of butanol are chemical intermediates; for example, alkali metal salts of *tert*-butanol are *tert*-butoxides.

Recreational Use

Butanol is a central nervous system depressant. It can have effects similar to ethanol when ingested or drunk by living beings such as humans.

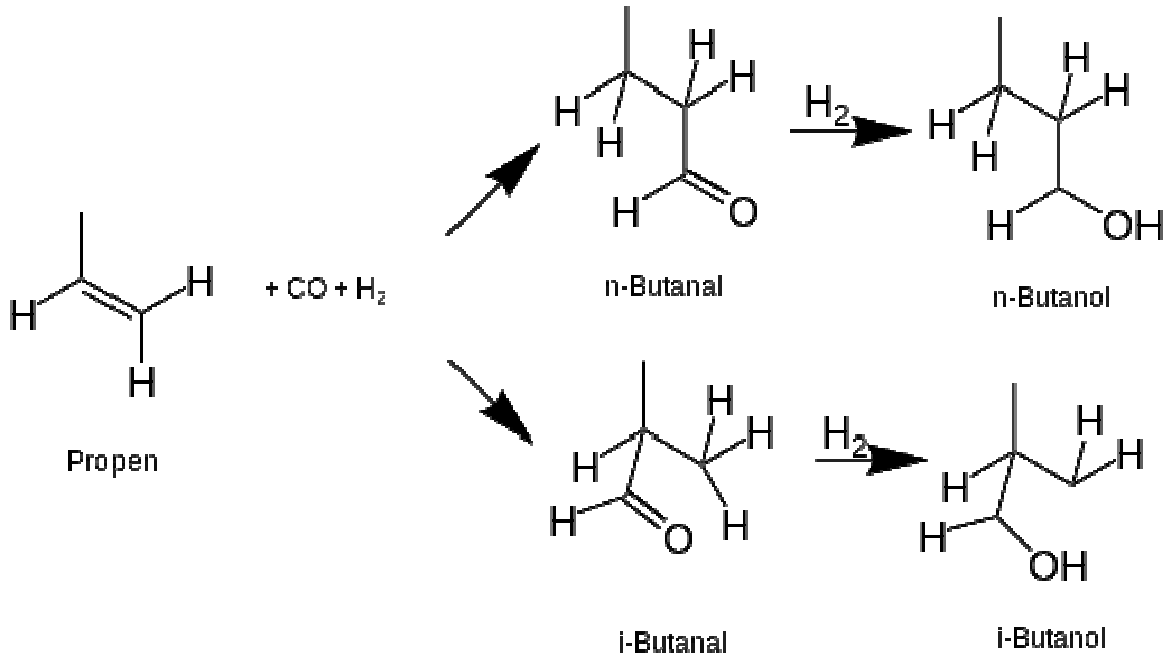
Biobutanol

Butanol (*n*-butanol or isobutanol) is a potential biofuel (butanol fuel). Butanol at 85 percent concentration can be used in cars designed for gasoline (petrol) without any change to the engine (unlike 85% ethanol), and it contains more energy for a given volume than ethanol and almost as much as

gasoline, and a vehicle using butanol would return fuel consumption more comparable to gasoline than ethanol. Butanol can also be added to diesel fuel to reduce soot emissions.

Production

Since the 1950s, most butanol in the United Statesis produced commercially from fossil fuels. The most common process starts with propene (propylene), which is put through a hydroformylation reaction to form butanal, which is then reduced with hydrogen to 1-butanol and/or 2-butanol. *tert*-butanol is derived from isobutane as a co-product of propylene oxide production.



Butanol can also be produced by fermentation of biomass by bacteria.

Prior to the 1950s, *Clostridium acetobutylicum*was used in industrial fermentation to produce *n*-butanol. Research in the past few decades showed results of other microorganisms that can produce isobutanol through fermentation.

Butanol fuel

Butanolmay be used as a fuel in an internal combustion engine. It is more similar to gasoline than it is to ethanol. A C4-hydrocarbon, butanol is a drop-in fuel and thus works in vehicles designed for use with gasoline without modification. Both *n*-butanol and isobutanolhave been studied as possible fuels.

Both can be produced from biomass (as "biobutanol") as well as from fossil fuels (as "petrobutanol"). The chemical properties.depend on the isomer (*n*-butanol or isobutanol), not on the production method.

Although intriguing in many ways, butanol fuel is rarely economically competitive.

Genetically modified bacteria

This method of production offers a way to produce liquid fuels from sustainable sources.

Fermentation however remains inefficient. Yields are low and separation is very expensive. Obtaining higher yields of butanol involves manipulation of the metabolic networks using metabolic engineering and genetic engineering.

Escherichia coli

Escherichia coli, or *E. coli*, is a Gram-negative, rod-shapedbacterium. *E. coli* is the microorganism most likely to move on to commercial production of isobutanol. In its engineered form*E. coli* produces the highest yields of isobutanol of any microorganism. Methods such as elementary mode analysishave been used to improve the metabolic efficiency of *E. coli* so that larger quantities of isobutanol may be produced. *E. coli* is an ideal isobutanol bio-synthesizer for several reasons:

- *E. coli* is an organism for which several tools of genetic manipulation exist, and it is an organism for which an extensive body of scientific literature exists. This wealth of knowledge allows *E. coli* to be easily modified by scientists.
- *E. coli* has the capacity to use lignocellulose (waste plant matter left over from agriculture) in the synthesis of isobutanol. The use of lignocellulose prevents *E. coli* from using plant matter meant for human consumption, and prevents any food-fuel price relationship which would occur from the biosynthesis of isobutanol by *E. coli*.
- Genetic modification has been used to broaden the scope of lignocellulose which can be used by *E. coli*. This has made *E. coli* a useful and diverse isobutanol bio-synthesizer.

The primary drawback of *E. coli* is that it is susceptible to bacteriophages when being grown. This susceptibility could potentially shut down entire bioreactors. Furthermore, the

native reaction pathway for isobutanol in *E. coli* functions optimally at a limited concentration of isobutanol in the cell. To minimize the sensitivity of *E. coli* in high concentrations, mutants of the enzymes involved in synthesis can be generated by random mutagenesis. By chance, some mutants may prove to be more tolerant of isobutanol which will enhance the overall yield of the synthesis.

Clostridia

n-Butanol can be produced by fermentation of biomass by the A.B.E. process using *Clostridium acetobutylicum*, *Clostridium beijerinckii*. *C. acetobutylicum*was once used for the production of acetone from starch. The butanol was a by-product of fermentation (twice as much butanol was produced). The feedstocks for biobutanol the same as those for ethanol: energy crops such as sugar beets, sugar cane, corngrain, wheat and cassava, prospective non-food energy crops such as switchgrass and even guayule in North America, as well as agricultural byproducts such as bagasse, straw and cornstalks. According to DuPont, existing bioethanol plants can cost-effectively be retrofitted to biobutanol production. Additionally, butanol production from biomass and agricultural byproducts could be more efficient (i.e. unit engine motive power delivered per unit solar energy consumed) than ethanol or methanol production.

A strain of *Clostridium* can convert nearly any form of cellulose into butanol even in the presence of oxygen.

A strain of *Clostridium cellulolyticum*, a native cellulose-degrading microbe, affords isobutanol directly from cellulose.

A combination of succinate and ethanol can be fermented to produce butyrate (a precursor to butanol fuel) by utilizing the metabolic pathways present in *Clostridium kluyveri*. Succinate is an intermediate of the TCA cycle, which metabolizes glucose. Anaerobic bacteria such as *Clostridium acetobutylicum* and *Clostridium saccharobutylicum* also contain these pathways. Succinate is first activated and then reduced by a two-step reaction to give 4-hydroxybutyrate, which is then metabolized further to crotonyl-coenzyme A (CoA) .Crotonyl-CoA is then converted to butyrate. The genes corresponding to these butanol production pathways from *Clostridium*were cloned to *E. coli*.

Cyanobacteria

Cyanobacteria are a phylum of photosynthetic bacteria. Cyanobacteria are suited for isobutanol biosynthesis when genetically engineered to produce isobutanol and its corresponding aldehydes. Isobutanol producing species of cyanobacteria offer several advantages as biofuel synthesizers:

- Cyanobacteria grow faster than plants and also absorb sunlight more efficiently than plants. This means they can be replenished at a faster rate than the plant matter used for other biofuel biosynthesizers.
- Cyanobacteria can be grown on non-arable land (land not used for farming). This prevents competition between food sources and fuel sources.
- The supplements necessary for the growth of Cyanobacteria are CO_2, H_2O, and sunlight. This presents two advantages:

- Because CO_2 is derived from the atmosphere, Cyanobacteria do not need plant matter to synthesize isobutanol (in other organisms which synthesize isobutanol, plant matter is the source of the carbon necessary to synthetically assemble isobutanol). Since plant matter is not used by this method of isobutanol production, the necessity to source plant matter from food sources and create a food-fuel price relationship is avoided.
- Because $CO_2$is absorbed from the atmosphere by Cyanobacteria, the possibility of bioremediation (in the form of Cyanobacteria removing excess CO_2 from the atmosphere) exists.

The primary drawbacks of cyanobacteria are:

- Cyanobacteria are sensitive to environmental conditions when being grown. Cyanobacteria suffer greatly from sunlight of inappropriate wavelength and intensity, CO_2 of inappropriate concentration, or H_2O of inappropriate salinity though a wealth of cyanobacteria are able to grow in brackish and marine waters. These factors are generally hard to control, and present a major obstacle in cyanbacterial production of isobutanol.
- Cyanobacteria bioreactors require high energy to operate. Cultures require constant mixing, and the harvesting of biosynthetic products is energy intensive. This reduces the efficiency of isobutanol production via Cyanobacteria.

Cyanobacteria can be re-engineered to increase their butanol production, showing the importance of ATP and cofactor driving forces as a design principle in pathway engineering. Many organisms have the capacity to produce butanol utilizing an acetyl-CoA dependent pathway. The main problem with this pathway is the first reaction involving the condensation of two acetyl-CoA molecules to acetoacetyl-CoA. This reaction is thermodynamically unfavorable due to the positive Gibbs free energy associated with it (dG = 6.8 kcal/mol).

Bacillus subtilis

Bacillus subtilis is a gram-positive rod-shaped bacteria. *Bacillus subtilis* offers many of the same advantages and disadvantages of *E. coli*, but it is less prominently used and does not produce isobutanol in quantities as large as *E. coli*. Similar to *E. coli*, *Bacillus subtilis* is capable of producing isobutanol from lignocellulose, and is easily manipulated by common genetic techniques. Elementary mode analysis has also been used to improve the isobutanol-synthesis metabolic pathway used by *Bacillus subtilis*, leading to higher yields of isobutanol being produced.

Saccharomyces cerevisiae

Saccharomyces cerevisiae, or *S. cerevisiae*, is a species of yeast. *S. cerevisiae* naturally produces isobutanol in small quantities via its valinebiosynthetic pathway. *S. cerevisiae* is an ideal candidate for isobutanol biofuel production for several reasons:

- *S. cerevisiae*can be grown at low pH levels, helping prevent contamination during growth in industrial bioreactors.
- *S. cerevisiae*cannot be affected by bacteriophages because it is a eukaryote.
- Extensive scientific knowledge about *S. cerevisiae* and its biology already exists.

Overexpression of the enzymes in the valine biosynthetic pathway of *S. cerevisiae*has been used to improve isobutanol yields. *S. cerevisiae*, however, has proved difficult to work with because of its inherent biology:

- As a eukaryote, *S. cerevisiae*is genetically more complex than *E. coli* or *B. subtilis*, and is harder to genetically manipulate as a result.
- *S. cerevisiae* has the natural ability to produce ethanol. This natural ability can "overpower" and consequently inhibit isobutanol production by *S. cerevisiae*.
- *S. cerevisiae* cannot use five carbon sugars to produce isobutanol. The inability to use five-carbon sugars restricts *S. cerevisiae* from using lignocellulose, and means *S. cerevisiae* must use plant matter intended for human consumption to produce isobutanol. This results in an unfavorable food/fuel price relationship when isobutanolis produced by *S. cerevisiae*.

Ralstoniaeutropha

Ralstoniaeutropha is a gram-negativesoil bacterium of the betaproteobacteria class. *Ralstoniaeutropha* is capable of converting electrical energy into isobutanol. This conversion is completed in several steps:

- Anodesare placed in a mixture of H_2O and CO_2.
- An electric current is run through the anodes, and through an electrochemicalprocess H_2O and CO_2 are combined to synthesize formic acid.
- A culture of *Ralstoniaeutropha* (composed of a strain tolerant to electricity) is kept within the H_2O and CO_2 mixture.
- The culture of *Ralstoniaeutropha* then converts formic acid from the mixture into isobutanol.
- The biosynthesized isobutanolis then separated from the mixture, and can be used as a biofuel.

Feedstocks

High cost of raw material is considered as one of the main obstacles to commercial production of butanols. Using inexpensive and abundant feedstocks, e.g., corn stover, can enhance the process economic viability. Metabolic engineeringcan be used to allow an organism to use a cheaper substrate such as glycerol instead of glucose. Because fermentation processes require glucose derived from foods, butanol production can negatively impact food supply (see food vs fuel debate). Glycerol is a good alternative source for butanol production. While glucose sources are valuable and

limited, glycerol is abundant and has a low market price because it is a waste product of biodiesel production. Butanol production from glycerol is economically viable using metabolic pathways that exist in *Clostridium pasteurianum* bacterium.

Improving efficiency

A process called cloud point separation could allow the recovery of butanol with high efficiency.

Producers and distribution

DuPont and BP plan to make biobutanol the first product of their joint effort to develop, produce, and market next-generation biofuels. In Europe the Swiss company Butalco is developing genetically modified yeasts for the production of biobutanol from cellulosic materials. Gourmet Butanol, a United States-based company, is developing a process that utilizes fungi to convert organic waste into biobutanol. Celtic Renewables makes biobutanol from waste that results from the production of whisky, and low-grade potatoes.

Properties of common fuels

Isobutanol

Isobutanol is a second-generation biofuel with several qualities that resolve issues presented by ethanol.

Isobutanol's properties make it an attractive biofuel:

- relatively high energy density, 98% of that of gasoline.
- does not readily absorb water from air, preventing the corrosion of engines and pipelines.
- can be mixed at any proportion with gasoline, meaning the fuel can "drop into" the existing petroleum infrastructure as a replacement fuel or major additive.
- can be produced from plant matter not connected to food supplies, preventing a fuel-price/food-price relationship.
- assuming that it is produced from residual lignocellulosicfeedstocks, blending isobutanol with gasoline may reduce GHG emissions considerably.

n-Butanol

Butanol better tolerates water contamination and is less corrosive than ethanol and more suitable for distribution through existing pipelines for gasoline. In blends with diesel or gasoline, butanol is less likely to separate from this fuel than ethanol if the fuel is contaminated with water. There is also a vapor pressure co-blend synergy with butanol and gasoline containing ethanol, which facilitates ethanol blending. This facilitates storage and distribution of blended fuels.

The octane rating of n-butanol is similar to that of gasoline but lower than that of ethanol and methanol. n-Butanol has a RON (Research Octane number) of 96 and a MON (Motor octane number) of 78 (with a resulting "(R+M)/2 pump octane number" of 87, as used in North America) while t-butanol has octane ratings of 105 RON and 89 MON. t-Butanol is used as an

additive in gasoline but cannot be used as a fuel in its pure form because its relatively high melting point of 25.5 °C (79 °F) causes it to gel and solidify near room temperature. On the other hand, isobutanol has a lower melting point than n-butanol and favorable RON of 113 and MON of 94, and is thus much better suited to high fraction gasoline blends, blends with n-butanol, or as a standalone fuel.

A fuel with a higher octane rating is less prone to knocking (extremely rapid and spontaneous combustion by compression) and the control system of any modern car engine can take advantage of this by adjusting the ignition timing. This will improve energy efficiency, leading to a better fuel economy than the comparisons of energy content different fuels indicate. By increasing the compression ratio, further gains in fuel economy, power and torque can be achieved. Conversely, a fuel with lower octane rating is more prone to knocking and will lower efficiency. Knocking can also cause engine damage. Engines designed to run on 87 octane will not have any additional power/fuel economy from being operated with higher octane fuel.

Butanol characteristics: air-fuel ratio, specific energy, viscosity, specific heat

Alcohol fuels, including butanol and ethanol, are partially oxidized and therefore need to run at richer mixtures than gasoline. Standard gasoline engines in cars can adjust the air-fuel ratio to accommodate variations in the fuel, but only within certain limits depending on model. If the limit is exceeded by running the engine on pure ethanol or a gasoline blend with a high percentage of ethanol, the engine will run

lean, something which can critically damage components. Compared to ethanol, butanol can be mixed in higher ratios with gasoline for use in existing cars without the need for retrofit as the air-fuel ratio and energy content are closer to that of gasoline.

Alcohol fuels have less energy per unit weight and unit volume than gasoline. To make it possible to compare the net energy released per cycle a measure called the fuels specific energy is sometimes used. It is defined as the energy released per air fuel ratio. The net energy released per cycle is higher for butanol than ethanol or methanol and about 10% higher than for gasoline.

The viscosity of alcohols increase with longer carbon chains. For this reason, butanol is used as an alternative to shorter alcohols when a more viscous solvent is desired. The kinematic viscosity of butanol is several times higher than that of gasoline and about as viscous as high quality diesel fuel.

The fuel in an engine has to be vaporized before it will burn. Insufficient vaporization is a known problem with alcohol fuels during cold starts in cold weather. As the heat of vaporization of butanol is less than half of that of ethanol, an engine running on butanol should be easier to start in cold weather than one running on ethanol or methanol.

Butanol fuel mixtures

Standards for the blending of ethanol and methanol in gasoline exist in many countries, including the EU, the US, and Brazil. Approximate equivalent butanol blends can be calculated from the relations between the stoichiometric fuel-air ratio of

butanol, ethanol and gasoline. Common ethanol fuel mixtures for fuel sold as gasoline currently range from 5% to 10%. It is estimated that around 9.5 gigaliter (Gl) of gasoline can be saved and about 64.6 Gl of butanol-gasoline blend 16% (Bu16) can potentially be produced from corn residues in the US, which is equivalent to 11.8% of total domestic gasoline consumption.

Consumer acceptance may be limited due to the potentially offensive banana-like smell of n-butanol. Plans are underway to market a fuel that is 85% Ethanol and 15% Butanol (E85B), so existing E85 internal combustion engines can run on a 100% renewable fuel that could be made without using any fossil fuels. Because its longer hydrocarbon chain causes it to be fairly non-polar, it is more similar to gasoline than it is to ethanol. Butanol has been demonstrated to work in vehicles designed for use with gasoline without modification.

Butanol in vehicles

Currently no production vehicle is known to be approved by the manufacturer for use with 100% butanol. As of early 2009, only a few vehicles are approved for even using E85 fuel (i.e. 85% ethanol + 15% gasoline) in the USA. However, in Brazil all vehicle manufacturers (Fiat, Ford, VW, GM, Toyota, Honda, Peugeot, Citroen and others) produce "flex-fuel" vehicles that can run on 100% Gasoline and or any mix of ethanol and gasoline up to 85% ethanol (E85). These flex fuel cars represent 90% of the sales of personal vehicles in Brazil, in 2009. BP and Dupont, engaged in a joint venture to produce and promote butanol fuel, claim that "biobutanol can be blended up to 10%v/v in European gasoline and 11.5%v/v in

US gasoline". In the 2009 Petit Le Mans race, the No. 16 Lola B09/86 - Mazda MZR-R of Dyson Racing ran on a mixture of biobutanol and ethanol developed by team technology partner BP.

Solvent

A **solvent** (from the Latin*solvō*, "loosen, untie, solve") is a substance that dissolves a solute, resulting in a solution. A solvent is usually a liquid but can also be a solid, a gas, or a supercritical fluid. Water is a solvent for polar molecules and the most common solvent used by living things; all the ions and proteins in a cell are dissolved in water within the cell.

The quantity of solute that can dissolve in a specific volume of solvent varies with temperature. Major uses of solvents are in paints, paint removers, inks, dry cleaning. Specific uses for organic solvents are in dry cleaning (e.g. tetrachloroethylene); as paint thinners (toluene, turpentine); as nail polish removers and solvents of glue (acetone, methyl acetate, ethyl acetate); in spot removers (hexane, petrol ether); in detergents (citrus terpenes); and in perfumes (ethanol). Solvents find various applications in chemical, pharmaceutical, oil, and gas industries, including in chemical syntheses and purification processes.

Solutions and solvation

When one substance is dissolved into another, a solution is formed. This is opposed to the situation when the compounds are insoluble like sand in water. In a solution, all of the

ingredients are uniformly distributed at a molecular level and no residue remains. A solvent-solute mixture consists of a single phase with all solute molecules occurring as *solvates* (solvent-solute complexes), as opposed to separate continuous phases as in suspensions, emulsions and other types of non-solution mixtures. The ability of one compound to be dissolved in another is known as solubility; if this occurs in all proportions, it is called miscible.

In addition to mixing, the substances in a solution interact with each other at the molecular level. When something is dissolved, molecules of the solvent arrange around molecules of the solute. Heat transfer is involved and entropyis increased making the solution more thermodynamically stable than the solute and solvent separately. This arrangement is mediated by the respective chemical properties of the solvent and solute, such as hydrogen bonding, dipole moment and polarizability. Solvation does not cause a chemical reaction or chemical configuration changes in the solute. However, solvation resembles a coordination complex formation reaction, often with considerable energetics (heat of solvation and entropy of solvation) and is thus far from a neutral process.

When one substance dissolves into another, a solution is formed. A solution is a homogeneous mixture consisting of a solute dissolved into a solvent. The solute is the substance that is being dissolved, while the solvent is the dissolving medium. Solutions can be formed with many different types and forms of solutes and solvents.

Solvent classifications

Solvents can be broadly classified into two categories: *polar* and *non-polar*. A special case is mercury, whose solutions are known as amalgams; also, other metal solutions exist which are liquid at room temperature.

Generally, the dielectric constant of the solvent provides a rough measure of a solvent's polarity. The strong polarity of water is indicated by its high dielectric constant of 88 (at 0 °C). Solvents with a dielectric constant of less than 15 are generally considered to be nonpolar.

The dielectric constant measures the solvent's tendency to partly cancel the field strength of the electric field of a charged particle immersed in it. This reduction is then compared to the field strength of the charged particle in a vacuum. Heuristically, the dielectric constant of a solvent can be thought of as its ability to reduce the solute's effective internal charge. Generally, the dielectric constant of a solvent is an acceptable predictor of the solvent's ability to dissolve common ionic compounds, such as salts.

Other polarity scales

Dielectric constants are not the only measure of polarity. Because solvents are used by chemists to carry out chemical reactions or observe chemical and biological phenomena, more specific measures of polarity are required. Most of these measures are sensitive to chemical structure.

The *Grunwald–Winsteinm**Y** scale* measures polarity in terms of solvent influence on buildup of positive charge of a solute during a chemical reaction.

*Kosower's**Z** scale* measures polarity in terms of the influence of the solvent on UV-absorption maxima of a salt, usually pyridiniumiodide or the pyridiniumzwitterion.

Donor number and donor acceptor scale measures polarity in terms of how a solvent interacts with specific substances, like a strong Lewis acid or a strong Lewis base.

The *Hildebrand parameter* is the square root of **cohesive energy density**. It can be used with nonpolar compounds, but cannot accommodate complex chemistry.

Reichardt's dye, a solvatochromic dye that changes color in response to polarity, gives a scale of $E_T(30)$ values. E_T is the transition energy between the ground state and the lowest excited state in kcal/mol, and (30) identifies the dye. Another, roughly correlated scale ($E_T(33)$) can be defined with Nile red.

The polarity, dipole moment, polarizability and hydrogen bonding of a solvent determines what type of compounds it is able to dissolve and with what other solvents or liquid compounds it is miscible. Generally, polar solvents dissolve polar compounds best and non-polar solvents dissolve non-polar compounds best: "like dissolves like". Strongly polar compounds like sugars (e.g. sucrose) or ionic compounds, like inorganicsalts (e.g. table salt) dissolve only in very polar solvents like water, while strongly non-polar compounds like oils or waxes dissolve only in very non-polar organic solvents like hexane. Similarly, water and hexane (or vinegar and

vegetable oil) are not miscible with each other and will quickly separate into two layers even after being shaken well.

Polarity can be separated to different contributions. For example, the **Kamlet-Taft parameters** are dipolarity/polarizability (π^*), hydrogen-bonding acidity (α) and hydrogen-bonding basicity (β). These can be calculated from the wavelength shifts of 3–6 different solvatochromic dyes in the solvent, usually including Reichardt's dye, nitroaniline and diethylnitroaniline. Another option, Hansen's parameters, separate the cohesive energy density into dispersion, polar and hydrogen bonding contributions.

Polar protic and polar aprotic

Solvents with a dielectric constant (more accurately, relative static permittivity) greater than 15 (i.e. polar or polarizable) can be further divided into protic and aprotic. Protic solvents solvate anions (negatively charged solutes) strongly via hydrogen bonding. Water is a protic solvent.

Aprotic solvents such as acetone or dichloromethane tend to have large dipole moments (separation of partial positive and partial negative charges within the same molecule) and solvate positively charged species via their negative dipole. In chemical reactions the use of polar protic solvents favors the $S_N1$reaction mechanism, while polar aprotic solvents favor the S_N2 reaction mechanism.

These polar solvents are capable of forming hydrogen bonds with water to dissolve in water whereas non-polar solvents are not capable of strong hydrogen bonds.

Multicomponent solvents

- Multicomponent solvents appeared after World War II in the USSR and continue to be used and produced in post-Soviet States. These solvents may have one or more applications, but they are not universal preparations . Also, any of these solvents is suitable as fuel for lighting firewood in bonfires , barbecues and stoves . Toluene-containing solvents are used as absolutely legal and fairly cheap hallucinogenic substances, with long inhalation of which powerful hallucinations begin .

Hansen solubility parameter values

- The Hansen solubility parameter values are based on dispersion bonds (δD), polar bonds (δP) and hydrogen bonds (δH). These contain information about the inter-molecular interactions with other solvents and also with polymers, pigments, nanoparticles, etc. This allows for rational formulations knowing, for example, that there is a good HSP match between a solvent and a polymer. Rational substitutions can also be made for "good" solvents (effective at dissolving the solute) that are "bad" (expensive or hazardous to health or the environment). The following table shows that the intuitions from "non-polar", "polar aprotic" and "polar protic" are put numerically – the "polar" molecules have higher levels of δP and the protic solvents have higher levels of δH. Because numerical values are used,

comparisons can be made rationally by comparing numbers. For example, acetonitrile is much more polar than acetone but exhibits slightly less hydrogen bonding.

If, for environmental or other reasons, a solvent or solvent blend is required to replace another of equivalent solvency, the substitution can be made on the basis of the Hansen solubility parameters of each. The values for mixtures are taken as the weighted averages of the values for the neat solvents. This can be calculated by trial-and-error, a spreadsheet of values, or HSP software. A 1:1 mixture of toluene and 1,4dioxane has δD, δP and δH values of 17.8, 1.6 and 5.5, comparable to those of chloroform at 17.8, 3.1 and 5.7 respectively. Because of the health hazards associated with toluene itself, other mixtures of solvents may be found using a full HSP dataset.

Boiling point

The boiling point is an important property because it determines the speed of evaporation. Small amounts of low-boiling-point solvents like diethyl ether, dichloromethane, or acetone will evaporate in seconds at room temperature, while high-boiling-point solvents like water or dimethyl sulfoxide need higher temperatures, an air flow, or the application of vacuum for fast evaporation.

- Low boilers: boiling point below 100 °C (boiling point of water)
- Medium boilers: between 100 °C and 150 °C
- High boilers: above 150 °C

Density

Most organic solvents have a lower density than water, which means they are lighter than and will form a layer on top of water. Important exceptions are most of the halogenated solvents like dichloromethane or chloroform will sink to the bottom of a container, leaving water as the top layer. This is crucial to remember when partitioning compounds between solvents and water in a separatory funnel during chemical syntheses.

Often, specific gravityis cited in place of density. Specific gravity is defined as the density of the solvent divided by the density of water at the same temperature. As such, specific gravity is a unitless value. It readily communicates whether a water-insoluble solvent will float (SG < 1.0) or sink (SG > 1.0) when mixed with water.

Safety

Fire

Most organic solvents are flammable or highly flammable, depending on their volatility. Exceptions are some chlorinated solvents like dichloromethane and chloroform. Mixtures of solvent vapors and air can explode. Solvent vapors are heavier than air; they will sink to the bottom and can travel large distances nearly undiluted. Solvent vapors can also be found in supposedly empty drums and cans, posing a flash fire hazard; hence empty containers of volatile solvents should be stored open and upside down.

Both diethyl ether and carbon disulfide have exceptionally low autoignitiontemperatures which increase greatly the fire risk associated with these solvents. The autoignition temperature of carbon disulfide is below 100 °C (212 °F), so objects such as steam pipes, light bulbs, hotplates, and recently extinguished bunsen burners are able to ignite its vapours.

In addition some solvents, such as methanol, can burn with a very hot flame which can be nearly invisible under some lighting conditions. This can delay or prevent the timely recognition of a dangerous fire, until flames spread to other materials.

Explosive peroxide formation

Ethers like diethyl ether and tetrahydrofuran (THF) can form highly explosive organic peroxides upon exposure to oxygen and light. THF is normally more likely to form such peroxides than diethyl ether. One of the most susceptible solvents is diisopropyl ether, but all ethers are considered to be potential peroxide sources.

The heteroatom (oxygen) stabilizes the formation of a free radical which is formed by the abstraction of a hydrogen atom by another free radical. The carbon-centred free radical thus formed is able to react with an oxygen molecule to form a peroxide compound. The process of peroxide formation is greatly accelerated by exposure to even low levels of light, but can proceed slowly even in dark conditions.

Unless a desiccant is used which can destroy the peroxides, they will concentrate during distillation, due to their higher boiling point. When sufficient peroxides have formed, they can

form a crystalline, shock-sensitive solid precipitate at the mouth of a container or bottle. Minor mechanical disturbances, such as scraping the inside of a vessel or the dislodging of a deposit, merely twisting the cap may provide sufficient energy for the peroxide to explode or detonate. Peroxide formation is not a significant problem when fresh solvents are used up quickly; they are more of a problem in laboratories which may take years to finish a single bottle. Low-volume users should acquire only small amounts of peroxide-prone solvents, and dispose of old solvents on a regular periodic schedule. To avoid explosive peroxide formation, ethers should be stored in an airtight container, away from light, because both light and air can encourage peroxide formation.

A number of tests can be used to detect the presence of a peroxide in an ether; one is to use a combination of iron(II) sulfate and potassium thiocyanate. The peroxide is able to oxidize the Fe ion to an Fe ion, which then forms a deep-red coordination complex with the thiocyanate. Peroxides may be removed by washing with acidic iron(II) sulfate, filtering through alumina, or distilling from sodium/benzophenone. Alumina degrades the peroxides but some could remain intact in it, therefore it must be disposed of properly. The advantage of using sodium/benzophenone is that moisture and oxygen are removed as well.

Health effects

General health hazards associated with solvent exposure include toxicity to the nervous system, reproductive damage, liver and kidney damage, respiratory impairment, cancer, and dermatitis.

Acute exposure

Many solvents can lead to a sudden loss of consciousness if inhaled in large amounts. Solvents like diethyl ether and chloroformhave been used in medicine as anesthetics, sedatives, and hypnotics for a long time. Ethanol (grain alcohol) is a widely used and abused psychoactive drug. Diethyl ether, chloroform, and many other solvents e.g. from gasoline or glues are abused recreationally in glue sniffing, often with harmful long term health effects like neurotoxicity or cancer. Fraudulent substitution of 1,5-pentanediol by the psychoactive 1,4-butanediol by a subcontractor caused the Bindeez product recall. If ingested, the so-called toxic alcohols (other than ethanol) such as methanol, propanol, and ethylene glycol metabolize into toxic aldehydes and acids, which cause potentially fatal metabolic acidosis. The commonly available alcohol solvent methanol can cause permanent blindness or death if ingested. The solvent 2-butoxyethanol, used in fracking fluids, can cause hypotension and metabolic acidosis.

Chronic exposure

Some solvents including chloroform and benzene a common ingredient in gasolineare known to be carcinogenic, while many others are considered by the World Health Organization to be likely carcinogens. Solvents can damage internal organs like the liver, the kidneys, the nervous system, or the brain. The cumulative effects of long-term or repeated exposure to solvents are calledchronic solvent-induced encephalopathy (CSE).

Chronic exposure to organic solvents in the work environment can produce a range of adverse neuropsychiatric effects. For example, occupational exposure to organic solvents has been associated with higher numbers of painters suffering from alcoholism. Ethanol has a synergistic effect when taken in combination with many solvents; for instance, a combination of toluene/benzene and ethanol causes greater nausea/vomiting than either substance alone.

Many solvents are known or suspected to be cataractogenic, greatly increasing the risk of developing cataracts in the lens of the eye. Solvent exposure has also been associated with neurotoxic damage causing hearing loss and color vision losses.

Environmental contamination

A major pathway to induce health effects arises from spills or leaks of solvents that reach the underlying soil. Since solvents readily migrate substantial distances, the creation of widespread soil contamination is not uncommon; this is particularly a health risk if aquifers are affected. Vapor intrusion can occur from sites with extensive subsurface solvent contamination.

Acetone–butanol–ethanol fermentation

Acetone–butanol–ethanol (ABE) fermentation is a process that uses bacterial fermentation to produce acetone, n-Butanol, and ethanol from carbohydrates such as starch and

glucose. It was developed by chemistChaim Weizmann and was the primary process used to produce acetone, which was needed to make cordite, a substance essential for the British war industry during World War I.

The process

The process may be likened to how yeast ferments sugars to produce ethanol for wine, beer, or fuel, but the organisms that carry out the ABE fermentation are strictly anaerobic (obligate anaerobes). The ABE fermentation produces solvents in a ratio of 3 parts acetone, 6 parts butanol to 1 part ethanol. It usually uses a strain of bacteria from the Class Clostridia (Family Clostridiaceae). *Clostridium acetobutylicum* is the most well-studied and widely used. Although less effective, *Clostridium beijerinckii* and *Clostridium saccharobutylicum* bacterial strains have shown good results as well.

For gas stripping, the most common gases used are the off-gases from the fermentation itself, a mixture of carbon dioxide and hydrogen gas.

History

The production of butanol by biological means was first performed by Louis Pasteur in 1861. In 1905, Austrian biochemist Franz Schardinger found that acetone could similarly be produced. In 1910AugusteFernbach (1860–1939) developed a bacterial fermentation process using potato starch as a feedstock in the production of butanol.

Industrial exploitation of ABE fermentation started in 1916, during World War I, with Chaim Weizmann's isolation of Clostridium acetobutylicum, as described in U.S. patent 1315585.

The Weizmann process was operated by Commercial Solvents Corporation from about 1920 to 1964 with plants in the US (Terre Haute, IN, and Peoria, IL), and Liverpool, England. The Peoria plant was the largest of the three. It used molasses as feedstock and had 96 fermenters with a volume of 96,000 gallons each.

After World War II, ABE fermentation became generally non-profitable, compared to the production of the same three solvents (acetone, butanol, ethanol) from petroleum. During the 1950s and 1960s, ABE fermentation was replaced by petroleum chemical plants. Due to different raw material costs, ABE fermentation was viable in South Africa until the early 1980s, with the last plant closing in 1983. The last operational plant was operated by Green Biologics Ltd. in Minnesota until it shut down in June 2019.

Improvement attempts

In order to be competitive with the petrochemical industry and to replace part of it as soon as possible, the bio-processes need to be able soon to cover a substantial part of the market demand and to be flexible with respect to the market needs and the raw material properties.

The most critical aspect in biomass fermentation processes is related to its productivity. The ABE fermentation via *Clostridium beijerinckii* or *Clostridium acetobutylicum* for

instance is characterized by product inhibition. This means that there is a product concentration threshold that cannot be overcome, resulting in a product stream highly diluted in water.

For this reason, in order to have a comparable productivity and profitability with respect to the petrochemical processes, cost and energy effective solutions for the product purification sections are required to provide a significant product recovery at the desired purity. The main solutions adopted during the last decades have been as follows:

- The employment of less expensive raw materials, and in particular lignocellulosic waste or algae;
- The microorganisms modifications or the research of new strains less sensitive to the butanol concentration poisoning to increase productivity and selectivity towards the butanol species;
- The fermentationreactor optimization aimed at increasing the productivity;
- The reduction of the energy costs of the separation and purification downstream processing and, in particular, to carry out the separation in-situ in the reactor;
- The use of side products such as hydrogen and carbon dioxide, solid wastes and discharged microorganisms and carry out less expensive process wastewater treatments.

In the second half of the 20th century, these technologies allowed an increasement in the final product concentration in the broth from 15 to 30 g/L, an increasement in the final

productivity from 0.46 to 4.6 g/(L*h) and an increasement in the yield from 15 to 42%. From a compound purification perspective, the main criticalities in the ABE/W product recovery are due to the water–alcohol mixture's non-ideal interactions leading to homogeneous and heterogeneous azeotropic species, as shown by the ternary equilibrium diagram. This causes the separation by standard distillation to be particularly impractical but, on the other hand, allows the exploitation of the liquid–liquid demixing region both for analogous and alternative separation processes.

Therefore, in order to enhance the ABE fermentation yield, mainly in situ product recovery systems have been developed. These include gas stripping, pervaporation, liquid–liquid extraction, distillation via Dividing Wall Column, membrane distillation, membrane separation, adsorption, and reverse osmosis. Green Biologics Ltd. has implemented this at an industrial scale.

Moreover, differently from crude oil feedstocks, biomasses nature fluctuates over the year's seasons and according to the geographical location. For this reasons, biorefinery operations need not only to be effective but also to be flexible and to be able to switch between two operating conditions rather quickly.[*citation needed*]

Current perspectives

ABE fermentation is attracting renewed interest with an eye on butanol as a renewable biofuel.

Sustainability is by far the topic of major concern over the last years. The energy challenge is the key point of the

environmental friendly policies adopted by all the most developed and industrialized countries worldwide. For this purpose Horizon 2020, the biggest EU Research and Innovation programme, was funded by the European Union over the 2014-2020 period.

The International Energy Agency defines renewables as the centre of the transition to a less carbon-intensive and more sustainable energy system. Biofuelsare believed to represent around 30% of energy consumption in transport by 2060. Their role is particularly important in sectors which are difficult to decarbonise, such as aviation, shipping and other long-haul transport. That is why several bioprocesses have seen a renewed interest in recent years, both from a research and an industrial perspective.

For this reason, the ABE fermentation process has been reconsidered from a different perspective. Although it was originally conceived to produce acetone, it is considered as a suitable production pathway for biobutanol that has become the product of major interest. Biogenic butanol is a possible substitute of bioethanol or even better and it is already employed both as fuel additive and as pure fuel instead of standard gasoline because, differently from ethanol, it can be directly and efficiently used in gasoline engines. Moreover, it has the advantage that it can be shipped and distributed through existing pipelines and filling stations.

Finallybiobutanol is widely used as a direct solvent for paints, coatings, varnishes, resins, dyes, camphor, vegetable oils, fats, waxes, shellac, rubbers and alkaloids due to its higher energy density, lower volatility, and lower hygroscopicity. It can be

produced from different kinds of cellulosic biomass and can be used for further processing of advanced biofuels such as butyl levulinate as well.

The application of n-butanol in the production of butyl acrylate has a wide scope for its expansion, which in turn would help in increasing the consumption of n-butanol globally. Butyl acrylate was the biggest n-butanol application in 2014 and is projected to be worth US$3.9 billion by 2020.

Chapter 4

Industrial Fermentation

Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi as well as eukaryotic cells like CHO cells and insect cells, to make products useful to humans. Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanolare made by fermentation. The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH and for aerobic fermentation oxygen. Product recovery frequently involves the concentration of the dilute solution. Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes. In some cases, production of biomass itself is the objective, like Single-cell protein and as in the case of baker's yeast and lactic acid bacteria starter cultures for cheesemaking. In general, fermentations can be divided into four types:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, algae, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved oxygen level, nutrient levels, and temperature.

General process overview

In most industrial fermentations, the organisms or eukaryotic cells are submerged in a liquid medium; in others, such as the fermentation of cocoa beans, coffee cherries, and miso, fermentation takes place on the moist surface of the medium. There are also industrial considerations related to the fermentation process. For instance, to avoid biological process contamination, the fermentation medium, air, and equipment are sterilized. Foam control can be achieved by either mechanical foam destruction or chemical anti-foaming agents. Several other factors must be measured and controlled such as pressure, temperature, agitator shaft power, and viscosity. An important element for industrial fermentations is scale up. This is the conversion of a laboratory procedure to an industrial process. It is well established in the field of industrial microbiology that what works well at the laboratory scale may work poorly or not at all when first attempted at large scale. It is generally not possible to take fermentation conditions that have worked in the laboratory and blindly apply them to industrial-scale equipment. Although many parameters have been tested for use as scale up criteria, there is no general formula because of the variation in fermentation

processes. The most important methods are the maintenance of constant power consumption per unit of broth and the maintenance of constant volumetric transfer rate.

Phases of growth

Fermentation begins once the growth medium is inoculated with the organism of interest. Growth of the inoculum does not occur immediately. This is the period of adaptation, called the lag phase. Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase.

After a phase of exponential growth, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentrations of toxic substances. This phase, where the increase of the rate of growth is checked, is the deceleration phase.

After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state. The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells (chemolysis). Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged.

If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become scenescent and begin to die off. The total amount of biomass may not decrease, but the number of viable organisms will decrease.

Fermentation medium

The microbes or eukaryotic cells used for fermentation grow in (or on) specially designed growth medium which supplies the nutrients required by the organisms or cells. A variety of media exist, but invariably contain a carbon source, a nitrogen source, water, salts, and micronutrients. In the production of wine, the medium is grape must. In the production of bio-ethanol, the medium may consist mostly of whatever inexpensive carbon source is available.

Carbon sources are typically sugars or other carbohydrates, although in the case of substrate transformations (such as the production of vinegar) the carbon source may be an alcohol or something else altogether. For large scale fermentations, such as those used for the production of ethanol, inexpensive sources of carbohydrates, such as molasses, corn steep liquor, sugar cane juice, or sugar beet juice are used to minimize costs. More sensitive fermentations may instead use purified glucose, sucrose, glycerol or other sugars, which reduces variation and helps ensure the purity of the final product. Organisms meant to produce enzymes such as beta galactosidase,invertase or other amylases may be fed starch to select for organisms that express the enzymes in large quantity.

Fixed nitrogen sources are required for most organisms to synthesize proteins, nucleic acids and other cellular components. Depending on the enzyme capabilities of the organism, nitrogen may be provided as bulk protein, such as soy meal; as pre-digested polypeptides, such as peptone or tryptone; or as ammonia or nitrate salts. Cost is also an

important factor in the choice of a nitrogen source. Phosphorus is needed for production of phospholipids in cellular membranes and for the production of nucleic acids. The amount of phosphate which must be added depends upon the composition of the broth and the needs of the organism, as well as the objective of the fermentation. For instance, some cultures will not produce secondary metabolites in the presence of phosphate.

Growth factors and trace nutrients are included in the fermentation broth for organisms incapable of producing all of the vitamins they require. Yeast extract is a common source of micronutrients and vitamins for fermentation media. Inorganic nutrients, including trace elements such as iron, zinc, copper, manganese, molybdenum and cobalt are typically present in unrefined carbon and nitrogen sources, but may have to be added when purified carbon and nitrogen sources are used. Fermentations which produce large amounts of gas (or which require the addition of gas) will tend to form a layer of foam, since fermentation broth typically contains a variety of foam-reinforcing proteins, peptides or starches. To prevent this foam from occurring or accumulating, antifoaming agentsmay be added. Mineral buffering salts, such as carbonates and phosphates, may be used to stabilize pH near optimum. When metal ions are present in high concentrations, use of a chelating agent may be necessary.

Developing an optimal medium for fermentation is a key concept to efficient optimization. One-factor-at-a-time (OFAT) is the preferential choice that researchers use for designing a medium composition. This method involves changing only one factor at a time while keeping the other concentrations

constant. This method can be separated into some sub groups. One is Removal Experiments. In this experiment all the components of the medium are removed one at a time and their effects on the medium are observed. Supplementation experiments involve evaluating the effects of nitrogen and carbon supplements on production. The final experiment is a replacement experiment. This involves replacing the nitrogen and carbon sources that show an enhancement effect on the intended production. Overall OFAT is a major advantage over other optimization methods because of its simplicity.

Production of biomass

Microbialcells or biomass is sometimes the intended product of fermentation. Examples include single cell protein, bakers yeast, lactobacillus, E. coli, and others. In the case of single-cell protein, algae is grown in large open ponds which allow photosynthesis to occur.

If the biomass is to be used for inoculation of other fermentations, care must be taken to prevent mutations from occurring.

Production of extracellular metabolites

Metabolitescan be divided into two groups: those produced during the growth phase of the organism, called **primary metabolites** and those produced during the stationary phase, called **secondary metabolites**. Some examples of primary

metabolites are ethanol, citric acid, glutamic acid, lysine, vitamins and polysaccharides. Some examples of secondary metabolites are penicillin, cyclosporin A, gibberellin, and lovastatin.

Primary metabolites

Primary metabolites are compounds made during the ordinary metabolism of the organism during the growth phase. A common example is ethanol or lactic acid, produced during glycolysis. Citric acid is produced by some strains of *Aspergillus niger* as part of the citric acid cycle to acidify their environment and prevent competitors from taking over. Glutamate is produced by some *Micrococcus*species, and some *Corynebacterium*species produce lysine, threonine, tryptophan and other amino acids. All of these compounds are produced during the normal "business" of the cell and released into the environment. There is therefore no need to rupture the cells for product recovery.

Secondary metabolites

Secondary metabolites are compounds made in the stationary phase; penicillin, for instance, prevents the growth of bacteria which could compete with *Penicillium* molds for resources. Some bacteria, such as *Lactobacillus* species, are able to produce bacteriocins which prevent the growth of bacterial competitors as well. These compounds are of obvious value to humans wishing to prevent the growth of bacteria, either as antibiotics or as antiseptics (such as gramicidin S). Fungicides, such as griseofulvinare also produced as secondary metabolites. Typically secondary metabolites are not produced

in the presence of glucose or other carbon sources which would encourage growth, and like primary metabolites are released into the surrounding medium without rupture of the cell membrane.

In the early days of the biotechnology industry, most biopharmaceutical products were made in *E. coli*; by 2004 more biopharmaceuticals were manufactured in eukaryotic cells, like CHO cells, than in microbes, but used similar bioreactor systems. Insect cell culture systems came into use in the 2000s as well.

Production of intracellular components

Of primary interest among the intracellular components are microbial enzymes: catalase, amylase, protease, pectinase, cellulase, hemicellulase, lipase, lactase, streptokinase and many others. Recombinant proteins, such as insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase and others are also made this way.

The largest difference between this process and the others is that the cells must be ruptured (lysed) at the end of fermentation, and the environment must be manipulated to maximize the amount of the product. Furthermore, the product (typically a protein) must be separated from all of the other cellular proteins in the lysate to be purified.

Transformation of substrate

Substrate transformation involves the transformation of a specific compound into another, such as in the case of phenylacetylcarbinol, and steroidbiotransformation, or the transformation of a raw material into a finished product, in the case of food fermentations and sewage treatment.

Food fermentation

Ancient fermented food processes, such as making bread, wine, cheese, curds, idli, dosa, etc., can be dated to more than seven thousand years ago. They were developed long before man had any knowledge of the existence of the microorganisms involved. Some foods such as Marmite are the byproduct of the fermentation process, in this case in the production of beer.

Ethanol fuel

Ethanol fuel is ethyl alcohol, the same type of alcohol found in alcoholic beverages, used as fuel. It is most often used as a motor fuel, mainly as a biofuel additive for gasoline. The first production car running entirely on ethanol was the Fiat 147, introduced in 1978 in Brazil by Fiat. Ethanol is commonly made from biomass such as corn or sugarcane. World ethanol production for transport fuel tripled between 2000 and 2007 from 17×10 liters (4.5×10 U.S. gal; 3.7×10 imp gal) to more than 52×10 liters (1.4×10 U.S. gal; 1.1×10 imp gal). From 2007 to 2008, the share of ethanol in global gasoline type fuel use increased from 3.7% to 5.4%. In 2011 worldwide ethanol fuel production reached 8.46×10 liters (2.23×10 U.S. gal;

1.86×10 imp gal) with the United States of America and Brazil being the top producers, accounting for 62.2% and 25% of global production, respectively. US ethanol production reached 57.54×10 liters (1.520×10 U.S. gal; 1.266×10 imp gal) in 2017–04.

Ethanol fuel has a "gasoline gallon equivalency" (GGE) value of 1.5, i.e. to replace the energy of 1 volume of gasoline, 1.5 times the volume of ethanol is needed.

Ethanol-blended fuel is widely used in Brazil, the United States, and Europe (see also Ethanol fuel by country). Most cars on the road today in the U.S. can run on blends of up to 10% ethanol, and ethanol represented 10% of the U.S. gasoline fuel supply derived from domestic sources in 2011. Some flexible-fuel vehicles are able to use up to 100% ethanol.

Since 1976 the Brazilian government has made it mandatory to blend ethanol with gasoline, and since 2007 the legal blend is around 25% ethanol and 75% gasoline (E25). By December 2011Brazil had a fleet of 14.8 million flex-fuel automobiles and light trucks and 1.5 million flex-fuel motorcycles that regularly use neat ethanol fuel (known as E100).

Bioethanol is a form of renewable energy that can be produced from agricultural feedstocks. It can be made from very common crops such as hemp, sugarcane, potato, cassava and corn. There has been considerable debate about how useful bioethanol is in replacing gasoline. Concerns about its production and use relate to increased food prices due to the large amount of arable land required for crops, as well as the energy and pollution balance of the whole cycle of ethanol production, especially from corn.

Chemistry

During ethanol fermentation, glucose and other sugars in the corn (or sugarcane or other crops) are converted into ethanol and carbon dioxide.

- $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$ + heat

Ethanol fermentation is not 100% selective with side products such as acetic acid and glycols. They are mostly removed during ethanol purification. Fermentation takes place in an aqueous solution. The resulting solution has an ethanol content of around 15%. Ethanol is subsequently isolated and purified by a combination of adsorption and distillation. During combustion, ethanol reacts with oxygen to produce carbon dioxide, water, and heat:

- $C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O$ + heat

Starch and cellulose molecules are strings of glucose molecules. It is also possible to generate ethanol out of cellulosic materials. That, however, requires a pretreatment that splits the cellulose into glucose molecules and other sugars that subsequently can be fermented. The resulting product is calledcellulosic ethanol, indicating its source. Ethanol is also produced industrially from ethylene by hydration of the double bond in the presence of a catalyst and high temperature.

- $C_2H_4 + H_2O \rightarrow C_2H_5OH$

Most ethanol is produced by fermentation.

Sources

About 5% of the ethanol produced in the world in 2003 was actually a petroleum product. It is made by the catalytic hydration of ethylene with sulfuric acid as the catalyst. It can also be obtained via ethylene or acetylene, from calcium carbide, coal, oil gas, and other sources. Two million short tons (1,786,000 long tons; 1,814,000 t) of petroleum-derived ethanol are produced annually. The principal suppliers are plants in the United States, Europe, and South Africa. Petroleum derived ethanol (synthetic ethanol) is chemically identical to bio-ethanol and can be differentiated only by radiocarbon dating.

Bio-ethanolis usually obtained from the conversion of carbon-based feedstock. Agricultural feedstocks are considered renewable because they get energy from the sun using photosynthesis, provided that all minerals required for growth (such as nitrogen and phosphorus) are returned to the land. Ethanol can be produced from a variety of feedstocks such as sugar cane, bagasse, miscanthus, sugar beet, sorghum, grain, switchgrass, barley, hemp, kenaf, potatoes, sweet potatoes, cassava, sunflower, fruit, molasses, corn, stover, grain, wheat, straw, cotton, other biomass, as well as many types of cellulose waste and harvesting, whichever has the best well-to-wheel assessment.

An alternative process to produce bio-ethanol from algae is being developed by the company Algenol. Rather than grow algae and then harvest and ferment it, the algae grow in sunlight and produce ethanol directly, which is removed without killing the algae. It is claimed the process can produce

6,000 U.S. gallons per acre (5,000 imperial gallons per acre; 56,000 liters per hectare) per year compared with 400 US gallons per acre (330 imp gal/acre; 3,700 L/ha) for corn production.

Currently, the first generation processes for the production of ethanol from corn use only a small part of the corn plant: the corn kernels are taken from the corn plant and only the starch, which represents about 50% of the dry kernel mass, is transformed into ethanol. Two types of second generation processes are under development. The first type uses enzymes and yeastfermentation to convert the plant cellulose into ethanol while the second type uses pyrolysis to convert the whole plant to either a liquid bio-oil or a syngas. Second generation processes can also be used with plants such as grasses, wood or agricultural waste material such as straw.

Production

Although there are various ways ethanol fuel can be produced, the most common way is via fermentation.

The basic steps for large-scale production of ethanol are: microbial (yeast) fermentation of sugars, distillation, dehydration (requirements vary, see Ethanol fuel mixtures, below), and denaturing (optional). Prior to fermentation, some crops require saccharification or hydrolysis of carbohydrates such as cellulose and starch into sugars. Saccharification of cellulose is calledcellulolysis (see cellulosic ethanol). Enzymes are used to convert starch into sugar.

Fermentation

Ethanol is produced by microbial fermentation of the sugar. Microbial fermentation currently only works directly with sugars. Two major components of plants, starch and cellulose, are both made of sugars—and can, in principle, be converted to sugars for fermentation. Currently, only the sugar (e.g., sugar cane) and starch (e.g., corn) portions can be economically converted.

There is interest in cellulosic ethanol obtained from breaking down plant cellulose to sugars and converting the sugars to ethanol. However, cellulosic ethanol is currently uneconomical and not practiced commercially. According to a 2006 International Energy Agency report, cellulosic ethanol could be important in the future.

Distillation

For the ethanol to be usable as a fuel, the yeast solids and the majority of the water must be removed. After fermentation, the mashis heated so that the ethanol evaporates. This process, known as distillation, separates the ethanol, but its purity is limited to 95–96% due to the formation of a low-boiling water-ethanol azeotrope with maximum (95.6% m/m (96.5% v/v) ethanol and 4.4% m/m (3.5% v/v) water). This mixture is called hydrous ethanol and can be used as a fuel alone, but unlike anhydrous ethanol, hydrous ethanol is not miscible in all ratios with gasoline, so the water fraction is typically removed in further treatment to burn in combination with gasoline in gasoline engines.

Dehydration

There are three dehydration processes to remove the water from an azeotropic ethanol/water mixture. The first process, used in many early fuel ethanol plants, is calledazeotropic distillation and consists of adding benzene or cyclohexane to the mixture. When these components are added to the mixture, it forms a heterogeneous azeotropic mixture in vapor–liquid-liquid equilibrium, which when distilled produces anhydrous ethanol in the column bottom, and a vapor mixture of water, ethanol, and cyclohexane/benzene.

When condensed, this becomes a two-phase liquid mixture. The heavier phase, poor in the entrainer (benzene or cyclohexane), is stripped of the entrainer and recycled to the feed—while the lighter phase, with condensate from the stripping, is recycled to the second column. Another early method, called extractive distillation, consists of adding a ternary component that increases ethanol's relative volatility. When the ternary mixture is distilled, it produces anhydrous ethanol on the top stream of the column.

With increasing attention being paid to saving energy, many methods have been proposed that avoid distillation altogether for dehydration. Of these methods, a third method has emerged and has been adopted by the majority of modern ethanol plants. This new process uses molecular sieves to remove water from fuel ethanol. In this process, ethanol vapor under pressure passes through a bed of molecular sieve beads. The bead's pores are sized to allow adsorption of water while excluding ethanol. After a period of time, the bed is regenerated under vacuum or in the flow of inert atmosphere

(e.g. N_2) to remove the adsorbed water. Two beds are often used so that one is available to adsorb water while the other is being regenerated. This dehydration technology can account for energy saving of 3,000 btus/gallon (840 kJ/L) compared to earlier azeotropic distillation.

Recent research has demonstrated that complete dehydration prior to blending with gasoline is not always necessary. Instead, the azeotropic mixture can be blended directly with gasoline so that liquid-liquid phase equilibrium can assist in the elimination of water. A two-stage counter-current setup of mixer-settler tanks can achieve complete recovery of ethanol into the fuel phase, with minimal energy consumption.

Post-production water issues

Ethanol is hygroscopic, meaning it absorbs water vapor directly from the atmosphere. Because absorbed water dilutes the fuel value of the ethanol and may cause phase separation of ethanol-gasoline blends (which causes engine stall), containers of ethanol fuels must be kept tightly sealed. This high miscibility with water means that ethanol cannot be efficiently shipped through modern pipelines, like liquid hydrocarbons, over long distances.

The fraction of water that an ethanol-gasoline fuel can contain without phase separation increases with the percentage of ethanol. For example, E30 can have up to about 2% water. If there is more than about 71% ethanol, the remainder can be any proportion of water or gasoline and phase separation does not occur. The fuel mileage declines with increased water content. The increased solubility of water with higher ethanol

content permits E30 and hydrated ethanol to be put in the same tank since any combination of them always results in a single phase. Somewhat less water is tolerated at lower temperatures. For E10 it is about 0.5% v/v at 21 °C and decreases to about 0.23% v/v at –34 °C .

Consumer production systems

While biodiesel production systems have been marketed to home and business users for many years, commercialized ethanol production systems designed for end-consumer use have lagged in the marketplace. In 2008, two different companies announced home-scale ethanol production systems. The AFS125 Advanced Fuel System from Allard Research and Development is capable of producing both ethanol and biodiesel in one machine, while the E-100 MicroFueler from E-Fuel Corporation is dedicated to ethanol only.

Engines

Fuel economy

Ethanol contains approx. 34% less energy per unit volume than gasoline, and therefore in theory, burning pure ethanol in a vehicle reduces range per unit measure by 34%, given the same fuel economy, compared to burning pure gasoline. However, since ethanol has a higher octane rating, the engine can be made more efficient by raising its compression ratio.

For E10 (10% ethanol and 90% gasoline), the effect is small (~3%) when compared to conventional gasoline, and even smaller (1–2%) when compared to oxygenated and reformulated

blends. For E85 (85% ethanol), the effect becomes significant. E85 produces lower mileage than gasoline, and requires more frequent refueling. Actual performance may vary depending on the vehicle. Based on EPA tests for all 2006 E85 models, the average fuel economy for E85 vehicles was 25.56% lower than unleaded gasoline. The EPA-rated mileage of current United States flex-fuel vehicles should be considered when making price comparisons, but E85 is a high performance fuel, with an octane rating of about 94–96, and should be compared to premium. Ethanol is not suitable for most aircraft, according to the RACQ, as well as some motorbikes and small engines, though the Embraer EMB 202 Ipanema is an example of an aircraft that has been specifically designed for use with ethanol fuel in some variants.

Cold start during the winter

High ethanol blends present a problem to achieve enough vapor pressure for the fuel to evaporate and spark the ignition during cold weather (since ethanol tends to increase fuel enthalpy of vaporization). When vapor pressure is below 45 kPastarting a cold engine becomes difficult. To avoid this problem at temperatures below 11 °C (52 °F), and to reduce ethanol higher emissions during cold weather, both the US and the European markets adopted E85 as the maximum blend to be used in their flexible fuel vehicles, and they are optimized to run at such a blend. At places with harsh cold weather, the ethanol blend in the US has a seasonal reduction to E70 for these very cold regions, though it is still sold as E85. At places where temperatures fall below −12 °C (10 °F) during the winter, it is recommended to install an engine heater system, both for gasoline and E85 vehicles. Sweden has a similar seasonal

reduction, but the ethanol content in the blend is reduced to E75 during the winter months.

Brazilian flex fuel vehicles can operate with ethanol mixtures up to E100, which is hydrous ethanol (with up to 4% water), which causes vapor pressure to drop faster as compared to E85 vehicles. As a result, Brazilian flex vehicles are built with a small secondary gasoline reservoir located near the engine. During a cold start pure gasoline is injected to avoid starting problems at low temperatures. This provision is particularly necessary for users of Brazil's southern and central regions, where temperatures normally drop below 15 °C (59 °F) during the winter. An improved flex engine generation was launched in 2009 that eliminates the need for the secondary gas storage tank. In March 2009 Volkswagen do Brasil launched the Polo E-Flex, the first Brazilian flex fuel model without an auxiliary tank for cold start.

Fuel mixtures

In many countries cars are mandated to run on mixtures of ethanol. All Brazilian light-duty vehicles are built to operate for an ethanol blend of up to 25% (E25), and since 1993 a federal law requires mixtures between 22% and 25% ethanol, with 25% required as of mid July 2011. In the United States all light-duty vehicles are built to operate normally with an ethanol blend of 10% (E10). At the end of 2010 over 90 percent of all gasoline sold in the U.S. was blended with ethanol. In January 2011 the U.S. Environmental Protection Agency (EPA) issued a waiver to authorize up to 15% of ethanol blended with gasoline (E15) to be sold only for cars and light pickup trucks with a model year of 2001 or newer.

Beginning with the model year 1999, an increasing number of vehicles in the world are manufactured with engines that can run on any fuel from 0% ethanol up to 100% ethanol without modification. Many cars and light trucks (a class containing minivans, SUVs and pickup trucks) are designed to be flexible-fuel vehicles using ethanol blends up to 85% (E85) in North America and Europe, and up to 100% (E100) in Brazil. In older model years, their engine systems contained alcohol sensors in the fuel and/or oxygen sensors in the exhaust that provide input to the engine control computer to adjust the fuel injection to achieve stochiometric (no residual fuel or free oxygen in the exhaust) air-to-fuel ratio for any fuel mix. In newer models, the alcohol sensors have been removed, with the computer using only oxygen and airflow sensor feedback to estimate alcohol content. The engine control computer can also adjust (advance) the ignition timing to achieve a higher output without pre-ignition when it predicts that higher alcohol percentages are present in the fuel being burned. This method is backed up by advanced knock sensors – used in most high performance gasoline engines regardless of whether they are designed to use ethanol or not – that detect pre-ignition and detonation.

In June 2021, India brought forward to 2025 its target to implement a 20% ethanol-blended auto fuel. India's ethanol blending rate in fuel (at the time of this target revision) is 8%, which is set to increase to 10% by 2022 based on the 'Roadmap for ethanol blending in India 2020-25' released on 5 June (World Environment Day) by Prime Minister Narendra Modi. The government expects oil marketing companies such as Indian Oil Corp (IOC) and Hindustan Petroleum Corp Ltd (HPCL) to provide 20% ethanol-blended fuel from April 2023

onward. States like Maharashtra and Uttar Pradesh, where ethanol is in surplus, are expected to be the first to adopt the higher ethanol fuel blending rate. India is also prioritizing roll-out of vehicles compatible with ethanol-blended fuel. From March 2021, auto manufacturers are required to indicate the ethanol compatibility of new vehicles and engines must be optimally designed to use 20% ethanol-blended fuel. The government expects automakers to begin production of ethanol-blended fuel compliant vehicles before April 2022. However, environmentalists worry that India's increased target for ethanol blending could incentivise water-intensive crops such as sugarcane and rice, and suggest that the government should focus on lower-water intensity crops such as millets since India is already facing an acute water shortage.

Other engine configurations

- ED95 engines

Since 1989 there have also been ethanol engines based on the diesel principle operating in Sweden. They are used primarily in city buses, but also in distribution trucks and waste collectors. The engines, made by Scania, have a modified compression ratio, and the fuel (known as ED95) used is a mix of 93.6% ethanol and 3.6% ignition improver, and 2.8% denaturants. The ignition improver makes it possible for the fuel to ignite in the diesel combustion cycle. It is then also possible to use the energy efficiency of the diesel principle with ethanol. These engines have been used in the United Kingdom by Reading Buses but the use of bioethanol fuel is now being phased out.

- Dual-fuel direct-injection

A 2004 MIT study and an earlier paper published by the Society of Automotive Engineers identified a method to exploit the characteristics of fuel ethanol substantially more efficiently than mixing it with gasoline. The method presents the possibility of leveraging the use of alcohol to achieve definite improvement over the cost-effectiveness of hybrid electric. The improvement consists of using dual-fuel direct-injection of pure alcohol (or the azeotrope or E85) and gasoline, in any ratio up to 100% of either, in a turbocharged, high compression-ratio, small-displacement engine having performance similar to an engine having twice the displacement. Each fuel is carried separately, with a much smaller tank for alcohol. The high-compression (for higher efficiency) engine runs on ordinary gasoline under low-power cruise conditions. Alcohol is directly injected into the cylinders (and the gasoline injection simultaneously reduced) only when necessary to suppress 'knock' such as when significantly accelerating. Direct cylinder injection raises the already high octane rating of ethanol up to an effective 130. The calculated over-all reduction of gasoline use and CO_2 emission is 30%. The consumer cost payback time shows a 4:1 improvement over turbo-diesel and a 5:1 improvement over hybrid. The problems of water absorption into pre-mixed gasoline (causing phase separation), supply issues of multiple mix ratios and cold-weather starting are also avoided.

- Increased thermal efficiency

In a 2008 study, complex engine controls and increased exhaust gas recirculation allowed a compression ratio of 19.5

with fuels ranging from neat ethanol to E50. Thermal efficiency up to approximately that for a diesel was achieved. This would result in the fuel economy of a neat ethanol vehicle to be about the same as one burning gasoline.

- Fuel cells powered by an ethanol reformer

In June 2016, Nissan announced plans to develop fuel cell vehicles powered by ethanol rather than hydrogen, the fuel of choice by the other car manufacturers that have developed and commercialized fuel cell vehicles, such as the Hyundai Tucson FCEV, Toyota Mirai, and Honda FCX Clarity. The main advantage of this technical approach is that it would be cheaper and easier to deploy the fueling infrastructure than setting up the one required to deliver hydrogen at high pressures, as each hydrogen fueling station cost US$1 million to US$2 million to build.

Nissan plans to create a technology that uses liquid ethanol fuel as a source to generate hydrogen within the vehicle itself. The technology uses heat to reform ethanol into hydrogen to feed what is known as a solid oxide fuel cell (SOFC). The fuel cell generates electricity to supply power to the electric motor driving the wheels, through a battery that handles peak power demands and stores regenerated energy. The vehicle would include a tank for a blend of water and ethanol, which is fed into an onboard reformer that splits it into pure hydrogen and carbon dioxide. According to Nissan, the liquid fuel could be an ethanol-water blend at a 55:45 ratio. Nissan expects to commercialize its technology by 2020.

Environment

Energy balance

All biomass goes through at least some of these steps: it needs to be grown, collected, dried, fermented, distilled, and burned. All of these steps require resources and an infrastructure. The total amount of energy input into the process compared to the energy released by burning the resulting ethanol fuel is known as the **energy balance** (or "energy returned on energy invested"). Figures compiled in a 2007 report by *National Geographic Magazine* point to modest results for corn ethanol produced in the US: one unit of fossil-fuel energy is required to create 1.3 energy units from the resulting ethanol. The energy balance for sugarcane ethanol produced in Brazil is more favorable, with one unit of fossil-fuel energy required to create 8 from the ethanol. Energy balance estimates are not easily produced, thus numerous such reports have been generated that are contradictory. For instance, a separate survey reports that production of ethanol from sugarcane, which requires a tropical climate to grow productively, returns from 8 to 9 units of energy for each unit expended, as compared to corn, which only returns about 1.34 units of fuel energy for each unit of energy expended. A 2006 University of California Berkeley study, after analyzing six separate studies, concluded that producing ethanol from corn uses much less petroleum than producing gasoline.

Carbon dioxide, a greenhouse gas, is emitted during fermentation and combustion. This is canceled out by the greater uptake of carbon dioxide by the plants as they grow to

produce the biomass. When produced by certain methods, ethanol releases less greenhouse gases than gasoline does.

Air pollution

Compared with conventional unleaded gasoline, ethanol is a particulate-free burning fuel source that combusts with oxygen to form carbon dioxide, carbon monoxide, water and aldehydes. The Clean Air Act requires the addition of oxygenates to reduce carbon monoxide emissions in the United States. The additive MTBE is currently being phased out due to ground water contamination, hence ethanol becomes an attractive alternative additive. Current production methods include air pollution from the manufacturer of macronutrient fertilizers such as ammonia. A study by atmospheric scientists at Stanford University found that E85 fuel would increase the risk of air pollution deaths relative to gasoline by 9% in Los Angeles, US: a very large, urban, car-based metropolis that is a worst-case scenario. Ozone levels are significantly increased, thereby increasing photochemical smog and aggravating medical problems such as asthma.

Brazil burns significant amounts of ethanol biofuel. Gas chromatograph studies were performed of ambient air in São Paulo, Brazil, and compared to Osaka, Japan, which does not burn ethanol fuel. Atmospheric Formaldehyde was 160% higher in Brazil, and Acetaldehyde was 260% higher.

Carbon dioxide

The calculation of exactly how much carbon dioxide is produced in the manufacture of bioethanol is a complex and

inexact process, and is highly dependent on the method by which the ethanol is produced and the assumptions made in the calculation. A calculation should include:

- The cost of growing the feedstock
- The cost of transporting the feedstock to the factory
- The cost of processing the feedstock into bioethanol

Such a calculation may or may not consider the following effects:

- The cost of the change in land use of the area where the fuel feedstock is grown.
- The cost of transportation of the bioethanol from the factory to its point of use
- The efficiency of the bioethanol compared with standard gasoline
- The amount of carbon dioxide produced at the tail pipe.
- The benefits due to the production of useful bi-products, such as cattle feed or electricity.

The graph on the right shows figures calculated by the UK government for the purposes of the Renewable transport fuel obligation.

The January 2006 Science article from UC Berkeley's ERG, estimated reduction from corn ethanol in GHG to be 13% after reviewing a large number of studies. In a correction to that article released shortly after publication, they reduce the estimated value to 7.4%. A National Geographic Magazine overview article (2007) puts the figures at 22% less CO_2 emissions in production and use for corn ethanol compared to

gasoline and a 56% reduction for cane ethanol. Carmaker Ford reports a 70% reduction in CO_2 emissions with bioethanol compared to petrol for one of their flexible-fuel vehicles.

An additional complication is that production requires tilling new soil which produces a one-off release of GHG that it can take decades or centuries of production reductions in GHG emissions to equalize. As an example, converting grass lands to corn production for ethanol takes about a century of annual savings to make up for the GHG released from the initial tilling.

Change in land use

Large-scale farming is necessary to produce agricultural alcohol and this requires substantial amounts of cultivated land. University of Minnesota researchers report that if all corn grown in the U.S. were used to make ethanol it would displace 12% of current U.S. gasoline consumption. There are claims that land for ethanol production is acquired through deforestation, while others have observed that areas currently supporting forests are usually not suitable for growing crops. In any case, farming may involve a decline in soil fertility due to reduction of organic matter, a decrease in water availability and quality, an increase in the use of pesticides and fertilizers, and potential dislocation of local communities. New technology enables farmers and processors to increasingly produce the same output using less inputs.

Cellulosic ethanol production is a new approach that may alleviate land use and related concerns. Cellulosic ethanol can be produced from any plant material, potentially doubling

yields, in an effort to minimize conflict between food needs vs. fuel needs. Instead of utilizing only the starch by-products from grinding wheat and other crops, cellulosic ethanol production maximizes the use of all plant materials, including gluten.

This approach would have a smaller carbon footprint because the amount of energy-intensive fertilisers and fungicides remain the same for higher output of usable material. The technology for producing cellulosic ethanol is currently in the commercialization stage.

Using biomass for electricity instead of ethanol

Converting biomass to electricity for charging electric vehicles may be a more "climate-friendly" transportation option than using biomass to produce ethanol fuel, according to an analysis published in Science in May 2009 Researchers continue to search for more cost-effective developments in both cellulosic ethanol and advanced vehicle batteries.

Health costs of ethanol emissions

For each billion ethanol-equivalent gallons of fuel produced and combusted in the US, the combined climate-change and health costs are $469 million for gasoline, $472–952 million for corn ethanol depending on biorefinery heat source (natural gas, corn stover, or coal) and technology, but only $123–208 million for cellulosic ethanol depending on feedstock (prairie biomass, Miscanthus, corn stover, or switchgrass).

Reduced petroleum imports and costs

One rationale given for extensive ethanol production in the U.S. is its benefit to energy security, by shifting the need for some foreign-produced oil to domestically produced energy sources. Production of ethanol requires significant energy, but current U.S. production derives most of that energy from coal, natural gas and other sources, rather than oil. Because 66% of oil consumed in the U.S. is imported, compared to a net surplus of coal and just 16% of natural gas (figures from 2006), the displacement of oil-based fuels to ethanol produces a net shift from foreign to domestic U.S. energy sources.

According to a 2008 analysis by Iowa State University, the growth in US ethanol production has caused retail gasoline prices to be US$0.29 to US$0.40 per gallon lower than would otherwise have been the case.

Motorsport

Leon Duray qualified third for the 1927 Indianapolis 500 auto race with an ethanol-fueled car. The IndyCar Series adopted a 10% ethanol blend for the 2006 season, and a 98% blend in 2007.

The American Le Mans Series sports car championship introduced E10 in the 2007 season to replace pure gasoline. In the 2008 season, E85 was allowed in the GT class and teams began switching to it.

In 2011, the three national NASCAR stock car series mandated a switch from gasoline to E15, a blend of Sunoco GTX unleaded racing fuel and 15% ethanol.

Australia's V8 Supercar championship uses Shell E85 for its racing fuel.

Stock Car Brasil Championship runs on neat ethanol, E100.

Ethanol fuel may also be utilized as a rocket fuel. As of 2010, small quantities of ethanol are used in lightweightrocket-racing aircraft.

Replacement cooking fuel

Project Gaia is a U.S. non-governmental, non-profit organization involved in the creation of a commercially viable household market for alcohol-based fuels in Ethiopia and other countries in the developing world. The project considers alcohol fuels to be a solution to fuel shortages, environmental damage, and public health issues caused by traditional cooking in the developing world. Targeting poor and marginalized communities that face health issues from cooking over polluting fires, Gaia currently works in Ethiopia, Nigeria, Brazil, Haiti, and Madagascar, and is in the planning stage of projects in several other countries.

Research

Ethanol research focuses on alternative sources, novel catalysts and production processes. INEOS produced ethanol

from vegetative material and wood waste. The bacteriumE.coli when genetically engineered with cow rumen genes and enzymes can produce ethanol from corn stover. Other potential feedstocks are municipal waste, recycled products, rice hulls, sugarcanebagasse, wood chips, switchgrass and carbon dioxide.

Sewage treatment

Sewage treatment (or **domestic wastewater treatment**, **municipal wastewater treatment**) is a type of wastewater treatment which aims to remove contaminants from sewage to produce an effluent that is suitable for discharge to the surrounding environment or an intended reuse application, thereby preventing water pollution from raw sewage discharges. Sewage contains wastewater from households and businesses and possibly pre-treated industrial wastewater. There is a high number of sewage treatment processes to choose from. These can range from decentralized systems (including on-site treatment systems) to large centralized systems involving a network of pipes and pump stations (called sewerage) which convey the sewage to a treatment plant. For cities that have a combined sewer, the sewers will also carry urban runoff (stormwater) to the sewage treatment plant.

A large number of sewage treatment technologies have been developed. Very broadly, they can be grouped into high tech (high cost) versus low tech (low cost) options, although some technologies might fall into either category. To decide which sewage treatment process to choose engineers and decision makers need to take into account technical and economical

criteria, as well as quantitative and qualitative aspects of each alternative. Often, the main criteria for selection are: desired effluent quality, expected construction and operating costs, availability of land, energy requirements and sustainability aspects. For example, broadly speaking, the activated sludge process achieves a high effluent quality but is relatively expensive and energy intensive compared to waste stabilization ponds which are a low cost treatment option but require a lot of land. In developing countries and in rural areas with low population densities, sewage is often treated by various on-site sanitation systems and not conveyed in sewers. These systems include septic tanks connected to drain fields, on-site sewage systems (OSS), vermifilter systems and many more. An advanced, fairly expensive, sewage treatment plant in a high-income country may include primary treatment to remove solid material, secondary treatment to digest dissolved and suspended organic material, tertiary treatment to remove the nutrients nitrogen and phosphorus, disinfection and possibly even a fourth treatment state to remove micropollutants (although this is still rare).

At the global level, an estimated 52% of sewage is treated. However, sewage treatment rates are highly unequal for different countries around the world. For example, while high-income countries treat approximately 74% of their sewage, developing countries treat an average of just 4.2%.

The treatment of sewage is part of the field of sanitation. Sanitation also includes the management of human waste and solid waste as well as stormwater (drainage) management. The term "sewage treatment plant" is often used interchangeably with the term "wastewater treatment plant".

Terminology

The term "sewage treatment plant" (or "sewage treatment works" in some countries) is nowadays often replaced with the term wastewater treatment plant or wastewater treatment station. Strictly speaking, the latter is a broader term that can also refer to industrial wastewater.

Purposes and overview

The aim of treating sewage is to produce an effluent that will do as little harm as possible when discharged to the surrounding environment, thereby preventing water pollution. Improving sewage treatment across the globe is crucial for reducing pollution to the environment and achieve water quality improvements. Sewage treatment removes the contaminants from sewage to produce liquid and solid (sludge) suitable for discharge to the environment or for reuse. It is a form of waste management. Sewage treatment results in sewage sludge which requires sewage sludge treatment before safe disposal or reuse. Under certain circumstances, the treated sewage sludge might be termed "biosolids" and can be used as a fertilizer.

With regards to biological treatment of sewage, the objectives are more specific: transform dissolved and particulate biodegradable components into acceptable end products, incorporate colloidal solids into a biological floc or biofilm, transform and remove nutrients (nitrogen and phosphorus), and in some cases remove specific trace organic constituents (micropollutants).

In most countries, sewage collection and treatment is typically subject to local and national regulations and standards.

Sewage characteristics

Sewage (or domestic sewage, domestic wastewater, municipal wastewater) is a type of wastewater that is produced by a community of people. It is typically transported through a sewer system. Sewage consists of wastewater discharged from residences and from commercial, institutional and public facilities that exist in the locality. Sub-types of sewage are greywater (from sinks, bathtubs, showers, dishwashers, and clothes washers) and blackwater (the water used to flush toilets, combined with the human waste that it flushes away). Sewage also contains soaps and detergents. Food waste may be present from dishwashing, and food quantities may be increased where garbage disposal units are used. In regions where toilet paperis used rather than bidets, that paper may be added to sewage rather than placed with municipal solid waste. Sewage may contain micro-pollutants and pollutants from industrial wastewater.

Sewage usually travels from a building's plumbing either into a sewer, which will carry it elsewhere, or into an onsite sewage facility. Collection of sewage of several households together usually takes places in either sanitary sewers or combined sewers. The former is designed to exclude stormwater flows whereas the latter is designed to also take stormwater. The production of sewage generally corresponds to the water consumption. A range of factors influence water consumption and hence the sewage flowrates per person. These include: Water availability (the opposite of water scarcity), water supply

options, climate (warmer climates may lead to greater water consumption), community size, economic level of the community, level of industrialization, metering of household consumption, water cost, water pressure and system losses in the water supply network.

- The main parameters in sewage that are measured to assess the sewage strength or quality as well as treatment options include: solids, indicators of organic matter, nitrogen, phosphorus, and indicators of fecal contamination. The following four types of pathogens from fecal matterare found in sewage: bacteria, viruses, protozoa, helminths and their eggs. In order to quantify the organic matter, indirect methods are commonly used: mainly the Biochemical Oxygen Demand (BOD) and the Chemical Oxygen Demand (COD). Typical values for physical–chemical characteristics of raw sewage in developing countries have been published as follows: 180 g/person/d for total solids (1100 mg/L concentration), 50 g/person/d for BOD (300 mg/L), 100 g/person/d for COD (600 mg/L), 8 g/person/d for total nitrogen (45 mg/L), 4.5 g/person/d for ammonia-N (25 mg/L) and 1.0 g/person/d for total phosphorus (7 mg/L).

Collection

Sewerage (or sewage system) is the infrastructure that conveys sewage or surface runoff (stormwater, meltwater, rainwater) using sewers. It encompasses components such as receiving drains, manholes, pumping stations, storm overflows, and

screening chambers of the combined sewer or sanitary sewer. Sewerage ends at the entry to a sewage treatment plant or at the point of discharge into the environment. It is the system of pipes, chambers, manholes, etc. that conveys the sewage or storm water.

In many cities, sewage (or municipal wastewater) is carried together with stormwater, in a combined sewer system, to a sewage treatment plant. In some urban areas, sewage is carried separately in sanitary sewers and runoff from streets is carried in storm drains. Access to these systems, for maintenance purposes, is typically through a manhole. During high precipitation periods a sewer system may experience a combined sewer overflow event or a sanitary sewer overflow event, which forces untreated sewage to flow directly to receiving waters. This can pose a serious threat to public health and the surrounding environment.

- The system of sewers is called*sewerage* or *sewerage system* in British English and *sewage system* in American English.

Types of treatment processes

Sewage can be treated close to where the sewage is created, which may be called a "decentralized" system or even an "on-site" system (on-site sewage facility, septic tanks, etc.). Alternatively, sewage can be collected and transported by a network of pipes and pump stations to a municipal treatment plant. This is called a "centralized" system (see also sewerage and pipes and infrastructure).

A large number of sewage treatment technologies have been developed (see List of wastewater treatment technologies). Very broadly, they can be grouped into high tech (high cost) versus low tech (low cost) options, although some technologies might fall into either category.

High tech

Examples for more high-tech, expensive sewage treatment systems (some of them are energy intensive as well); many of them provide a very high level of treatment:

- Activated sludge systems
- Aerobic granulation
- Aerobic treatment system
- Enhanced biological phosphorus removal
- Expanded granular sludge bed digestion
- Extended aeration
- Filtration
- Membrane bioreactor
- Moving bed biofilm reactor
- On-site sewage facility
- Reverse osmosis
- Rotating biological contactor
- Sequencing batch reactor
- Trickling filter
- Ultrafiltration
- Ultraviolet disinfection
- Upflow anaerobic sludge blanket digestion

Low tech

Examples for more low-tech, less expensive sewage treatment systems, often using very little energy but also often not providing a high level of treatment; some of these only treat part of the sewage, for example only the toilet wastewater, or they only provide pre-treatment, like septic tanks.

- Anaerobic digester types
- Anaerobic digestion
- Biofilters
- Composting toilet
- Constructed wetland
- Decentralized wastewater system
- Imhoff tank
- Nature-based solutions
- Oil–water separator
- Reed bed
- Retention basin
- Sand filter
- Screen filter
- Septic tank
- Waste stabilization pond
- Urine-diverting dry toilet
- Vermifilter
- Vermifilter toilet

Waste stabilization ponds are a low cost treatment option but require a lot of land. The following systems for these ponds (also called lagoons) can be distinguished:

- Facultative ponds

- Anaerobic pond – facultative ponds systems
- Facultative aerated lagoons
- Complete-mix aerated lagoon sedimentation pond systems
- High rate ponds
- Maturation ponds

Disposal or treatment options

There are other process options which are classified as disposal options, although earlier publications might have referred to some of them as treatment options. These include: Application of sludge, irrigation, soak pit, leach field, fish pond, floating plant pond, water disposal/groundwater recharge, surface disposal and storage.

Application of sewage to land can be considered as a form of final disposal or of treatment, or both. It leads to groundwater recharge and/or to evapotranspiration. Land application include slow-rate systems, rapid infiltration, subsurface infiltration, overland flow. Applying the sewage can be done by flooding, furrows, sprinkler and dripping. It is a treatment/disposal system that requires a large amount of land per person.

Design aspects

Process selection

To decide which sewage treatment process to choose engineers and decision makers need to take into account technical and economical criteria, as well as quantitative and qualitative

aspects of each alternative. A life cycle assessment (LCA) can be used, and criteria or weightings can be attributed to the various aspects. The final decision may have a degree of subjectivity. A range of publications exist to help with technology selection.

In industrialized countries, the critical items in a decreasing order of importance usually are: efficiency, reliability, sludge disposal aspects and land requirements. In developing countries, the main critical items might be different and revolve more around construction costs, sustainability, simplicity and operational costs.

Choosing the most suitable treatment process is complicated and requires expert inputs, often in the form of feasibility studies. This is because the main important factors to be considered when evaluating and selecting sewage treatment processes are numerous: process applicability, applicable flow, acceptable flow variation, influent characteristics, inhibiting or refractory compounds, climatic aspects, process kinetics and reactor hydraulics, performance, treatment residuals, sludge processing, environmental constraints, chemical product requirements, energy requirements, requirements of other resources, personnel requirements, operating and maintenance requirements, ancillary processes, reliability, complexity, compatibility, area availability.

With regards to environmental impacts the following aspects are included in the selection process: Odors, vector attraction, sludge transportation, sanitary risks, air contamination, soil and subsoil contamination, surface water pollution or

groundwater contamination, devaluation of nearby areas, inconvenience to the nearby population.

For example, broadly speaking, the conventional activated sludge process achieves a high effluent quality but is relatively expensive and energy intensive. This can be contrasted to waste stabilization ponds which have lower capital and operating costs as well as practically no energy requirements but require a lot of land.

Odor control

Odors emitted by sewage treatment are typically an indication of an anaerobic or "septic" condition. Early stages of processing will tend to produce foul-smelling gases, with hydrogen sulfide being most common in generating complaints. Large process plants in urban areas will often treat the odors with carbon reactors, a contact media with bio-slimes, small doses of chlorine, or circulating fluids to biologically capture and metabolize the noxious gases. Other methods of odor control exist, including addition of iron salts, hydrogen peroxide, calcium nitrate, etc. to manage hydrogen sulfide levels.

Energy requirements

For conventional sewage treatment plants, around 30 percent of the annual operating costs is usually required for energy. The energy requirements vary with type of treatment process as well as sewage strength. For example, constructed wetlands have a lower energy requirement than activated sludge plants, as less energy is required for the aeration step. Sewage

treatment plants that produce biogas in their sewage sludge treatment process with anaerobic digestion can produce enough energy to meet most of the energy needs of the sewage treatment plant itself.

In conventional secondary treatment processes, most of the electricity is used for aeration, pumping systems and equipment for the dewatering and drying of sewage sludge. Advanced sewage treatment plants, e.g. for nutrient removal, require more energy than plants that only achieve primary or secondary treatment.

Small rural plants using trickling filters may operate with no net energy requirements, the whole process being driven by gravitational flow, including tipping bucket flow distribution and the desludging of settlement tanks to drying beds. This is usually only practical in hilly terrain and in areas where the treatment plant is relatively remote from housing because of the difficulty in managing odors.

Co-treatment of industrial effluent

In highly regulated developed countries, industrial effluent usually receives at least pretreatment if not full treatment at the factories themselves to reduce the pollutant load, before discharge to the sewer. This process is calledindustrial wastewater treatment or pretreatment. The same does not apply to many developing countries where industrial effluent are often not treated and enter sewers, or even receiving water bodies, without (pre-)treatment.

Industrial wastewater may contain pollutants which cannot be removed by conventional sewage treatment. Also, variable flow

of industrial waste associated with production cycles may upset the population dynamics of biological treatment units, such as the activated sludge process.

Design aspects of secondary treatment processes

Non-sewered areas

Most urban residents in sub-Saharan Africa rely on on-site sanitation systems without sewers, such as septic tanks and pit latrines, and fecal sludge management in these cities is an enormous challenge.

For sewage treatment the use of septic tanks and other On-Site Sewage Facilities (OSSF) is widespread in some rural areas, for example serving up to 20 percent of the homes in the U.S.

Available process steps

The main processes involved in advanced sewage treatment are designed to remove as much of the solid material as possible, and then using biological processes to digest and remove the remaining soluble material. Often, this is done by using "activated sludge" or biofilm processes. The biological floc and remaining fine solids can then be settled as a sludge, leaving a liquid substantially free of solids, and with a greatly reduced concentration of pollutants. Advanced sewage treatment generally involves three main stages, called primary, secondary and tertiary treatment but may also include intermediate stages and final polishing processes.

Bar screens can remove large solid debris from sewage, and primary treatment can remove floating and settleable matter.

Secondary treatment can reduce biochemical oxygen demand from concentrated sewage, but is less efficient for dilute sewage, or sewage containing toxic materials. Water disinfectionmay be attempted to kill pathogens prior to disposal, and is increasingly effective after more elements of the foregoing treatment sequence have been completed.

Different types of sewage treatment may utilize some or all of the process steps listed below.

Pretreatment

Pretreatment removes all materials that can be easily collected from the raw sewage before they damage or clog the pumps and sewage lines of primary treatment clarifiers. Objects commonly removed during pretreatment include trash, tree limbs, and other large objects.

The influent in sewage water passes through a bar screen to remove all large objects like cans, rags, sticks, plastic packets, etc. carried in the sewage stream. This is most commonly done with an automated mechanically raked bar screen in modern plants serving large populations, while in smaller or less modern plants, a manually cleaned screen may be used. The raking action of a mechanical bar screen is typically paced according to the accumulation on the bar screens and/or flow rate. The solids are collected and later disposed in a landfill, or incinerated. Bar screens or mesh screens of varying sizes may be used to optimize solids removal. If gross solids are not removed, they become entrained in pipes and moving parts of the treatment plant, and can cause substantial damage and inefficiency in the process.

Grit removal

Grit consists of sand, gravel, cinders, and other heavy materials. Pretreatment may include a sand or grit channel or chamber, where the velocity of the incoming sewage is adjusted to allow the settlement of sand and grit. Grit removal is necessary to (1) reduce formation of heavy deposits in aeration tanks, aerobic digesters, pipelines, channels, and conduits; (2) reduce the frequency of digester cleaning caused by excessive accumulations of grit; and (3) protect moving mechanical equipment from abrasion and accompanying abnormal wear.

The removal of grit is essential for equipment with closely machined metal surfaces such as comminutors, fine screens, centrifuges, heat exchangers, and high pressure diaphragm pumps. Grit chambers come in 3 types: horizontal grit chambers, aerated grit chambers, and vortex grit chambers. Vortex type grit chambers include mechanically induced vortex, hydraulically induced vortex, and multi-tray vortex separators.

Given that traditionally, grit removal systems have been designed to remove clean inorganic particles that are greater than 0.210 millimetres (0.0083 in), most grit passes through the grit removal flows under normal conditions. During periods of high flow deposited grit is resuspended and the quantity of grit reaching the treatment plant increases substantially. It is, therefore important that the grit removal system not only operate efficiently during normal flow conditions but also under sustained peak flows when the greatest volume of grit reaches the plant.

Flow equalization

Clarifiers and mechanized secondary treatment are more efficient under uniform flow conditions. Equalization basinsmay be used for temporary storage of diurnal or wet-weather flow peaks. Basins provide a place to temporarily hold incoming sewage during plant maintenance and a means of diluting and distributing batch discharges of toxic or high-strength waste which might otherwise inhibit biological secondary treatment (including portable toilet waste, vehicle holding tanks, and septic tank pumpers). Flow equalization basins require variable discharge control, typically include provisions for bypass and cleaning, and may also include aerators. Cleaning may be easier if the basin is downstream of screening and grit removal.

Fat and grease removal

- In some larger plants, fat and greaseare removed by passing the sewage through a small tank where skimmers collect the fat floating on the surface. Air blowers in the base of the tank may also be used to help recover the fat as a froth. Many plants, however, use primary clarifiers with mechanical surface skimmers for fat and grease removal.

Primary treatment

Primary treatment is the "removal of a portion of the suspended solids and organic matter from the sewage". It consists of temporarily holding the sewage in a basin where heavy solids can settle to the bottom while oil, grease and

lighter solids float to the surface. The settled and floating materials are removed and the remaining liquid may be discharged or subjected to secondary treatment. Some sewage treatment plants that are connected to a combined sewer system have a bypass arrangement after the primary treatment unit. This means that during very heavy rainfall events, the secondary and tertiary treatment systems can be bypassed to protect them from hydraulic overloading, and the mixture of sewage and storm-water only receives primary treatment.

In the primary sedimentation stage, sewage flows through large tanks, commonly called "pre-settling basins", "primary sedimentation tanks" or "primary clarifiers". The tanks are used to settle sludge while grease and oils rise to the surface and are skimmed off. Primary settling tanks are usually equipped with mechanically driven scrapers that continually drive the collected sludge towards a hopper in the base of the tank where it is pumped to sludge treatment facilities.

Secondary treatment

- Secondary treatment is the removal of biodegradableorganic matter (in solution or suspension) from sewage or similar kinds of wastewater. The aim is to achieve a certain degree of effluent quality in a sewage treatment plant suitable for the intended disposal option. This is achieved with physical phase separation to remove settleable solids followed by a biological process to remove dissolved and suspended organic compounds. Secondary treatment is the portion of a sewage treatment sequence removing dissolved and colloidal

compounds measured as biochemical oxygen demand (BOD). Secondary treatment is traditionally applied to the liquid portion of sewage after primary treatment has removed settleable solids and floating material. Secondary treatment is usually performed by microorganisms in a managed aerobic habitat or less commonly by an anaerobic process. Bacteria and protozoa consume biodegradable soluble organic contaminants (e.g. sugars, fats, and organic short-chain carbon molecules from human waste, food waste, soaps and detergent) while reproducing to form cells of biological solids. Secondary treatment by biochemical oxidation of dissolved and colloidalorganic compounds is widely used in sewage treatment and is applicable to some agricultural and industrial wastewaters.

Tertiary treatment

The purpose of tertiary treatment (also called "advanced treatment") is to provide a final treatment stage to further improve the effluent quality before it is discharged to the receiving water body or reused. More than one tertiary treatment process may be used at any treatment plant. If disinfection is practiced, it is always the final process. It is also called "effluent polishing". Tertiary treatment may include biological nutrient removal (alternatively, this can be classified as secondary treatment), disinfection and removal of micropollutants, such as environmental persistent pharmaceutical pollutants.

Tertiary treatment is sometimes defined as anything more than primary and secondary treatment in order to allow discharge into a highly sensitive or fragile ecosystem such as estuaries, low-flow rivers or coral reefs. Treated water is sometimes disinfected chemically or physically (for example, by lagoons and microfiltration) prior to discharge into a stream, river, bay, lagoon or wetland, or it can be used for the irrigation of a golf course, greenway or park. If it is sufficiently clean, it can also be used for groundwater recharge or agricultural purposes.

Sand filtration removes much of the residual suspended matter. Filtration over activated carbon, also called *carbon adsorption,* removes residual toxins. Micro filtration or synthetic membranes are also used. After membrane filtration, the treated sewage is nearly indistinguishable from waters of natural origin of drinking quality (without its minerals).

Settlement and further biological improvement of treated sewage may be achieved through storage in large man-made ponds or lagoons. These lagoons are highly aerobic and colonization by native macrophytes, especially reeds, is often encouraged. Small filter-feeding invertebratessuch as *Daphnia* and species of *Rotifera* greatly assist in treatment by removing fine particulates.

Disinfection

The purpose of disinfection in the treatment of sewage is to substantially reduce the number of microorganisms in the water to be discharged back into the environment or to be reused. The effectiveness of disinfection depends on the quality of the water being treated (e.g., cloudiness, pH, etc.), the type

of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Cloudy water will be treated less successfully, since solid matter can shield organisms, especially from ultraviolet light or if contact times are low. Generally, short contact times, low doses and high flows all militate against effective disinfection. Common methods of disinfection include ozone, chlorine, ultraviolet light, or sodium hypochlorite. Monochloramine, which is used for drinking water, is not used in the treatment of sewage because of its persistence.

Chlorination remains the most common form of treated sewage disinfection in North America due to its low cost and long-term history of effectiveness. One disadvantage is that chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual chlorine or chloramines may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated, adding to the complexity and cost of treatment.

Ultraviolet (UV) light can be used instead of chlorine, iodine, or other chemicals. Because no chemicals are used, the treated water has no adverse effect on organisms that later consume it, as may be the case with other methods. UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens, making them incapable of reproduction. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement and the need for a highly treated effluent to ensure that the target microorganisms are not shielded from the UV radiation (i.e., any solids present in

the treated effluent may protect microorganisms from the UV light). In the United Kingdom, UV light is becoming the most common means of disinfection because of the concerns about the impacts of chlorine in chlorinating residual organics in the treated sewage and in chlorinating organics in the receiving water. Some sewage treatment systems in Canada and the US also use UV light for their effluent water disinfection.

Ozone (O_3) is generated by passing oxygen (O_2) through a high voltage potential resulting in a third oxygen atom becoming attached and forming O_3. Ozone is very unstable and reactive and oxidizes most organic material it comes in contact with, thereby destroying many pathogenic microorganisms. Ozone is considered to be safer than chlorine because, unlike chlorine which has to be stored on site (highly poisonous in the event of an accidental release), ozone is generated on-site as needed from the oxygen in the ambient air. Ozonation also produces fewer disinfection by-products than chlorination. A disadvantage of ozone disinfection is the high cost of the ozone generation equipment and the requirements for special operators. Ozone sewage treatment requires the use of an ozone generator, which decontaminates the water as ozone bubbles percolate through the tank.

Biological nutrient removal

Sewage may contain high levels of the nutrients nitrogen and phosphorus. Typical values for nutrient loads of raw sewage in developing countrieshave been published as follows: 8 g/person/d for total nitrogen (45 mg/L), 4.5 g/person/d for ammonia-N (25 mg/L) and 1.0 g/person/d for total phosphorus (7 mg/L). Excessive release to the environment can lead to

nutrient pollution, which can manifest itself in eutrophication. This process can lead to algal blooms, a rapid growth, and later decay, in the population of algae. In addition to causing deoxygenation, some algal species produce toxins that contaminate drinking water supplies.

Phosphorus removal is important as phosphorus is a limiting nutrient for algae growth in many fresh water systems. Therefore, an excess of phosphorus can lead to eutrophication. It is also particularly important for water reuse systems where high phosphorus concentrations may lead to fouling of downstream equipment such as reverse osmosis.

A range of treatment processes are available to remove nitrogen and phosphorus. Biological nutrient removal (BNR) is regarded by some as a type of secondary treatment process, and by others as a tertiary (or "advanced") treatment process.

Nitrogen removal

Nitrogen is removed through the biological oxidation of nitrogen from ammonia to nitrate (nitrification), followed by denitrification, the reduction of nitrate to nitrogen gas. Nitrogen gas is released to the atmosphere and thus removed from the water.

Nitrification itself is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia (NH_3) to nitrite (NO_2) is most often facilitated by *Nitrosomonas* spp. ("nitroso" referring to the formation of a nitroso functional group). Nitrite oxidation to nitrate (NO_3), though traditionally believed to be facilitated by *Nitrobacter* spp. (nitro referring the formation of a nitro functional group),

is now known to be facilitated in the environment almost exclusively by *Nitrospira* spp.

Denitrification requires anoxic conditions to encourage the appropriate biological communities to form. It is facilitated by a wide diversity of bacteria. The activated sludge process, sand filters, waste stabilization ponds, constructed wetlands and other processes can all be used to reduce nitrogen. Since denitrification is the reduction of nitrate to dinitrogen (molecular nitrogen) gas, an electron donoris needed. This can be, depending on the wastewater, organic matter (from feces), sulfide, or an added donor like methanol. The sludge in the anoxic tanks (denitrification tanks) must be mixed well (mixture of recirculated mixed liquor, return activated sludge [RAS], and raw influent) e.g. by using submersible mixers in order to achieve the desired denitrification.

Over time, different treatment configurations for activated sludge processes have evolved to achieve high levels of nitrogen removal. An initial scheme, the Ludzack–Ettinger Process, placed an anoxic treatment zone before the aeration tank and clarifier, using the return activated sludge (RAS) from the clarifier as a nitrate source.

The sewage (either raw or as effluent from primary clarification) serves as the electron source for the facultative bacteria to metabolize carbon, using the inorganic nitrate as a source of oxygen instead of dissolved molecular oxygen. This denitrification scheme was naturally limited to the amount of soluble nitrate present in the RAS. Nitrate reduction was limited because RAS rate is limited by the performance of the clarifier.

The "Modified Ludzak–Ettinger Process" (MLE) is an improvement on the original concept, for it recycles mixed liquor from the discharge end of the aeration tank to the head of the anoxic tank to provide a consistent source of soluble nitrate for the facultative bacteria. In this instance, raw sewage continues to provide the electron source, and sub-surface mixing maintains the bacteria in contact with both electron source and soluble nitrate in the absence of dissolved oxygen.

Phosphorus removal

Studies of United States sewage in the late 1960s estimated mean per capita contributions of 500 grams (18 oz) in urine and feces, 1,000 grams (35 oz) in synthetic detergents, and lesser variable amounts used as corrosion and scale control chemicals in water supplies. Source control via alternative detergent formulations has subsequently reduced the largest contribution, but the phosphorus content of urine and feces will remain unchanged.

Phosphorus can be removed biologically in a process called enhanced biological phosphorus removal. In this process, specific bacteria, called polyphosphate-accumulating organisms (PAOs), are selectively enriched and accumulate large quantities of phosphorus within their cells (up to 20 percent of their mass).

Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g. ferric chloride), aluminum (e.g. alum), or lime. This may lead to excessive sludge production as hydroxides precipitate and the added

chemicals can be expensive. Chemical phosphorus removal requires significantly smaller equipment footprint than biological removal, is easier to operate and is often more reliable than biological phosphorus removal. Another method for phosphorus removal is to use granular laterite or zeolite.

Some systems use both biological phosphorus removal and chemical phosphorus removal. The chemical phosphorus removal in those systems may be used as a backup system, for use when the biological phosphorus removal is not removing enough phosphorus, or may be used continuously. In either case, using both biological and chemical phosphorus removal has the advantage of not increasing sludge production as much as chemical phosphorus removal on its own, with the disadvantage of the increased initial cost associated with installing two different systems.

Once removed, phosphorus, in the form of a phosphate-rich sewage sludge, may be sent to landfill or used as fertilizer in admixture with other digested sewage sludges. In the latter case, the treated sewage sludge is also sometimes referred to as biosolids.

Fourth treatment stage

Micropollutants such as pharmaceuticals, ingredients of household chemicals, chemicals used in small businesses or industries, environmental persistent pharmaceutical pollutants (EPPP) or pesticides may not be eliminated in the conventional treatment process (primary, secondary and tertiary treatment) and therefore lead to water pollution. Although concentrations of those substances and their decomposition products are

quite low, there is still a chance of harming aquatic organisms. For pharmaceuticals, the following substances have been identified as "toxicologically relevant": substances with endocrine disrupting effects, genotoxic substances and substances that enhance the development of bacterial resistances. They mainly belong to the group of EPPP.

Techniques for elimination of micropollutants via a fourth treatment stage during sewage treatment are implemented in Germany, Switzerland, Sweden and the Netherlands and tests are ongoing in several other countries. Such process steps mainly consist of activated carbon filters that adsorb the micropollutants. The combination of advanced oxidation with ozone followed by granular activated carbon (GAC) has been suggested as a cost-effective treatment combination for pharmaceutical residues. For a full reduction of microplasts the combination of ultrafiltration followed by GAC has been suggested. Also the use of enzymes such as laccase secreted by fungi is under investigation. Microbial biofuel cells are investigated for their property to treat organic matter in sewage.

To reduce pharmaceuticals in water bodies, "source control" measures are also under investigation, such as innovations in drug development or more responsible handling of drugs. In the US, the National Take Back Initiative is a voluntary program with the general public, encouraging people to return excess or expired drugs, and avoid flushing them to the sewage system.

Sludge treatment and disposal

Sewage sludge treatment describes the processes used to manage and dispose of sewage sludge produced during sewage treatment. Sludge is mostly water with lesser amounts of solid material removed from liquid sewage. Primary sludge includes settleable solids removed during primary treatment in primary clarifiers. Secondary sludge separated in secondary clarifiers includes treated sewage sludge from secondary treatmentbioreactors.

- Sludge treatment is focused on reducing sludge weight and volume to reduce disposal costs, and on reducing potential health risks of disposal options. Water removal is the primary means of weight and volume reduction, while pathogen destruction is frequently accomplished through heating during thermophilic digestion, composting, or incineration. The choice of a sludge treatment method depends on the volume of sludge generated, and comparison of treatment costs required for available disposal options. Air-drying and composting may be attractive to rural communities, while limited land availability may make aerobic digestion and mechanical dewatering preferable for cities, and economies of scale may encourage energy recovery alternatives in metropolitan areas.

Disposal of treated sewage

Effects on surface waters

Sewage treatment plants can have significant effects on the biotic status of receiving waters. Nutrients concentrations are typically elevated (unless the sewage treatment included a nutrient removal step) and can cause eutrophication of receiving water bodies..

A phytoplankton study found high nutrient concentrations linked to sewage effluents. High nutrient concentration leads to high chlorophyll a concentrations, which is a proxy for primary production in marine environments. High primary production means high phytoplankton populations and most likely high zooplankton populations, because zooplankton feed on phytoplankton.

However, effluent released into marine systems also leads to greater population instability.

Scientific studies have demonstrated that very low levels of specific contaminants in treated sewage, including hormones (from animal husbandry and residue from human hormonal contraception methods) and synthetic materials such as phthalates that mimic hormones in their action, can have an unpredictable adverse impact on the natural biota and potentially on humans if the water is re-used for drinking water.

Reuse

Irrigation

Increasingly, people use treated or even untreated sewage for irrigation to produce crops. Cities provide lucrative markets for fresh produce, so are attractive to farmers. Because agriculture has to compete for increasingly scarce water resources with industry and municipal users, there is often no alternative for farmers but to use water polluted with sewage directly to water their crops. There can be significant health hazards related to using water loaded with pathogens in this way. The World Health Organization developed guidelines for safe use of wastewater in 2006. They advocate a 'multiple-barrier' approach to wastewater use, where farmers are encouraged to adopt various risk-reducing behaviors. These include ceasing irrigation a few days before harvesting to allow pathogens to die off in the sunlight, applying water carefully so it does not contaminate leaves likely to be eaten raw, cleaning vegetables with disinfectant or allowing fecal sludge used in farming to dry before being used as a human manure.

Reclaimed water

- Water reclamation (also called water reuse or water recycling) is the process of converting municipal wastewater (sewage) or industrial wastewater into water that can be reused for a variety of purposes. Types of reuse include: urban reuse, agricultural reuse (irrigation), environmental reuse, industrial reuse, planned potable reuse, de facto wastewater

reuse (unplanned potable reuse). For example, reuse may include irrigation of gardens and agricultural fields or replenishing surface water and groundwater (i.e., groundwater recharge). Reused water may also be directed toward fulfilling certain needs in residences (e.g. toilet flushing), businesses, and industry, and could even be treated to reach drinking water standards. Treated municipal wastewater reuse for irrigation is a long-established practice, especially in arid countries. Reusing wastewater as part of sustainable water management allows water to remain as an alternative water source for human activities. This can reduce scarcity and alleviate pressures on groundwater and other natural water bodies.

Global situation

Before the 20th century in Europe, sewers usually discharged into a body of water such as a river, lake, or ocean. There was no treatment, so the breakdown of the human wastewas left to the ecosystem. Today, the situation in urban areas of industrialized countries is usually that sewers route their contents to a sewage treatment plant rather than directly to a body of water. In many developing countries, the situation is still different. Few reliable figures exist on the share of the wastewater collected in sewers that is being treated in the world. A global estimate by UNDP and UN-Habitat in 2010 was that 90% of all wastewater generated is released into the environment untreated. In many developing countries the bulk of domestic and industrial wastewater is discharged to rivers

and the ocean without any treatment or after primary treatment only. Doing so can lead to serious pollution of the receiving water.

Another study in 2021 estimated that globally, about 52% of sewage is treated. However, sewage treatment rates are highly unequal for different countries around the world. For example, while high-income countries treat approximately 74% of their sewage, developing countries treat an average of just 4.2%.

In Latin America about 15 percent of collected wastewater passes through treatment plants (with varying levels of actual treatment). In Venezuela, a below average country in South America with respect to wastewater treatment, 97 percent of the country's sewage is discharged raw into the environment.

Global targets

Sustainable Development Goal 6 has a Target 6.3 which is formulated as follows: "By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally." The corresponding Indicator 6.3.1 is the "proportion of wastewater safely treated".

History

- The history of sewage treatment had the following developments: It began with land application (sewage farms) in the 1840s in England, followed by chemical

treatment and sedimentation of sewage in tanks, then biological treatment the late 19th century, which led to the development of the activated sludge process starting in 1912.

It was not until the late 19th century that it became possible to treat the sewage by biologically decomposing the organic components through the use ofmicroorganisms and removing the pollutants. Land treatment was also steadily becoming less feasible, as cities grew and the volume of sewage produced could no longer be absorbed by the farmland on the outskirts.

Edward Frankland conducted experiments at the sewage farm in Croydon, England, during the 1870s and was able to demonstrate that filtration of sewage through porous gravel produced a nitrified effluent (the ammonia was converted into nitrate) and that the filter remained unclogged over long periods of time. This established the then revolutionary possibility of biological treatment of sewage using a contact bed to oxidize the waste. This concept was taken up by the chief chemist for the London Metropolitan Board of Works, William Libdin, in 1887:

- ...in all probability the true way of purifying sewage...will be first to separate the sludge, and then turn into neutral effluent... retain it for a sufficient period, during which time it should be fully aerated, and finally discharge it into the stream in a purified condition. This is indeed what is aimed at and imperfectly accomplished on a sewage farm.
- From 1885 to 1891 filters working on this principle were constructed throughout the UK and the idea

was also taken up in the US at the Lawrence Experiment Station in Massachusetts, where Frankland's work was confirmed. In 1890 the LES developed a 'trickling filter' that gave a much more reliable performance.

Chapter 5

Fermentation in Food Processing

In food processing, **fermentation** is the conversion of carbohydrates to alcohol or organic acids using microorganisms—yeasts or bacteria—under anaerobic (oxygen-free) conditions. Fermentation usually implies that the action of microorganisms is desired. The science of fermentation is known as zymology or zymurgy.

The term, "fermentation" sometimes refers specifically to the chemical conversion of sugars into ethanol, producing alcoholic drinks such as wine, beer, and cider. However, similar processes take place in the leavening of bread (CO_2 produced by yeast activity), and in the preservation of sour foods with the production of lactic acid, such as in sauerkraut and yogurt.

Other widely consumed fermented foods include vinegar, olives, and cheese. More localised foods prepared by fermentation may also be based on beans, grain, vegetables, fruit, honey, dairy products, and fish.

History and prehistory

Natural fermentation precedes human history. Since ancient times, humans have exploited the fermentation process. The earliest archaeological evidence of fermentation is 13,000-year-old residues of a beer, with the consistency of gruel, found in a cave near Haifa in Israel. Another early alcoholic drink, made from fruit, rice, and honey, dates from 7000 to 6600 BC, in the

Neolithic Chinese village of Jiahu, and winemaking dates from ca. 6000 BC, in Georgia, in the Caucasus area. Seven-thousand-year-old jars containing the remains of wine, now on display at the University of Pennsylvania, were excavated in the Zagros Mountains in Iran. There is strong evidence that people were fermenting alcoholic drinks in Babylon ca. 3000 BC, ancient Egypt ca. 3150 BC, pre-Hispanic Mexico ca. 2000 BC, and Sudan ca. 1500 BC.

The French chemist Louis Pasteur founded zymology, when in 1856 he connected yeast to fermentation. When studying the fermentation of sugar to alcohol by yeast, Pasteur concluded that the fermentation was catalyzed by a vital force, called "ferments", within the yeast cells. The "ferments" were thought to function only within living organisms. "Alcoholic fermentation is an act correlated with the life and organization of the yeast cells, not with the death or putrefaction of the cells", he wrote.

Nevertheless, it was known that yeast extracts can ferment sugar even in the absence of living yeast cells. While studying this process in 1897, the German chemist and zymologistEduard Buchner of Humboldt University of Berlin, Germany, found that sugar was fermented even when there were no living yeast cells in the mixture, by an enzyme complex secreted by yeast that he termed *zymase*. In 1907 he received the Nobel Prize in Chemistry for his research and discovery of "cell-free fermentation".

One year earlier, in 1906, ethanol fermentation studies led to the early discovery of NAD.

Uses

Food fermentation is the conversion of sugars and other carbohydrates into alcohol or preservative organic acids and carbon dioxide. All three products have found human uses. The production of alcohol is made use of when fruit juices are converted to wine, when grains are made into beer, and when foods rich in starch, such as potatoes, are fermented and then distilled to make spirits such as gin and vodka. The production of carbon dioxideis used to leaven bread. The production of organic acids is exploited to preserve and flavor vegetables and dairy products.

Food fermentation serves five main purposes: to enrich the diet through development of a diversity of flavors, aromas, and textures in food substrates; to preserve substantial amounts of food through lactic acid, alcohol, acetic acid, and alkaline fermentations; to enrich food substrates with protein, essential amino acids, and vitamins; to eliminate antinutrients; and to reduce cooking time and the associated use of fuel.

Fermented foods by region

- **Worldwide:**alcohol (beer, wine), vinegar, olives, yogurt, bread, cheese
- **Asia**
- **East and Southeast Asia:**amazake, atchara, belacan, burongmangga, com ruou, doenjang, douchi, lambanog, kimchi, kombucha, leppet-so, narezushi, miso, nata de coco, nattō, oncom, prahok,

ruou nep, sake, soju, soy sauce, stinky tofu, tape, tempeh, zhacai

- **Central Asia:**kumis, kefir, shubat
- **South Asia:**achar, appam, dosa, dhokla, dahi (yogurt), idli, mixed pickle, ngari, sinki, tongba, paneer
- **Africa:**garri, injera, laxoox, mageu, ogi, ogiri, iru
- **Americas:**chicha, chocolate, vanilla, hot sauce, tibicos, pulque, muktuk, kiviak , parakari
- **Middle East:**torshi, boza
- **Europe:**sourdough bread, elderberry wine, kombucha, pickling, rakfisk, sauerkraut, pickled cucumber, surströmming, mead, salami, sucuk, prosciutto, cultured milk products such as quark, kefir, filmjölk, crème fraîche, smetana, skyr, rakı, tupí.
- **Oceania:**poi, kaangapirau

Fermented foods by type

Beans

Cheonggukjang, doenjang, fermented bean curd, miso, natto, soy sauce, stinky tofu, tempeh, oncom, soybean paste, Beijing mung bean milk, kinama, iru

Grain

Amazake, beer, bread, choujiu, gamju, injera, kvass, makgeolli, murri, ogi, rejuvelac, sake, sikhye, sourdough, sowans, rice

wine, malt whisky, grain whisky, idli, dosa, Bangla (drink)vodka, boza, and chicha, among others.

Vegetables

Kimchi, mixed pickle, sauerkraut, Indian pickle, gundruk, tursu

Fruit

Wine, vinegar, cider, perry, brandy, atchara, nata de coco, burongmangga, asinan, pickling, vişinată, chocolate, rakı, araghsagi, chacha (brandy)

Honey

Mead, metheglin

Dairy

Some kinds of cheese also, kefir, kumis (mare milk), shubat (camel milk), cultured milk products such as quark, filmjölk, crème fraîche, smetana, skyr, and yogurt

Fish

Bagoong, faseekh, fish sauce, Garum, Hákarl, jeotgal, rakfisk, shrimp paste, surströmming, shidal

Meat

Chorizo, salami, sucuk, pepperoni, nemchua, som moo, saucisson, fermented sausage

Tea

Pu-erh tea, Kombucha, Lahpet, Goishicha

Risks

Sterilization is an important factor to consider during the fermentation of foods. Failing to completely remove any microbes from equipment and storing vessels may result in the multiplication of harmful organisms within the ferment, potentially increasing the risks of food borne illnesses like botulism. The production of off smells and discoloration may be indications that harmful bacteria may have been introduced to the food.

Alaska has witnessed a steady increase of cases of botulism since 1985. It has more cases of botulism than any other state in the United States of America. This is caused by the traditional Eskimo practice of allowing animal products such as whole fish, fish heads, walrus, sea lion, and whale flippers, beaver tails, seal oil, and birds, to ferment for an extended period of time before being consumed. The risk is exacerbated when a plastic container is used for this purpose instead of the old-fashioned, traditional method, a grass-lined hole, as the *Clostridium botulinum* bacteria thrive in the anaerobic conditions created by the air-tight enclosure in plastic.

The World Health Organization has classified pickled foods as possibly carcinogenic, based on epidemiological studies. Other research found that fermented food contains a carcinogenic by-product, ethyl carbamate (urethane). "A 2009 review of the existing studies conducted across Asia concluded that

regularly eating pickled vegetables roughly doubles a person's risk for esophageal squamous cell carcinoma."

Baker's yeast

Baker's yeast is the common name for the strains of yeast commonly used in bakingbread and other bakery products, serving as a leavening agent which causes the bread to rise (expand and become lighter and softer) by converting the fermentablesugars present in the dough into carbon dioxide and ethanol. Baker's yeast is of the species *Saccharomyces cerevisiae*, and is the same species (but a different strain) as the kind commonly used in alcoholic fermentation, which is calledbrewer's yeast. Baker's yeast is also a single-cell microorganism found on and around the human body.

The use of steamed or boiled potatoes, water from potato boiling, or sugar in a bread dough provides food for the growth of yeasts; however, too much sugar will dehydrate them. Yeast growth is inhibited by both salt and sugar, but more so by salt than sugar. Some sources say fats, such as butter and eggs, slow down yeast growth; others say the effect of fat on dough remains unclear, presenting evidence that small amounts of fat are beneficial for baked bread volume.

Saccharomyces exiguus (also known as *S. minor*) is a wild yeast found on plants, grains, and fruits that is occasionally used for baking; however, in general, it is not used in a pure form but comes from being propagated in a sourdough starter.

History

It is not known when yeast was first used to bake bread; the earliest definite records come from Ancient Egypt. Researchers speculate that a mixture of flour meal and water was left longer than usual on a warm day and the yeasts that occur in natural contaminants of the flour caused it to ferment before baking. The resulting bread would have been lighter and tastier than the previous hard flatbreads. It is generally assumed that the earliest forms of leavening were likely very similar to modern sourdough; the leavening action of yeast would have been discovered from its action on flatbread doughs and would have been either cultivated separately or transferred from batch to batch by means of previously mixed ("old") dough. Also, the development of leavened bread seems to have developed in close proximity to the development of beer brewing, and barm from the beer fermentation process can also be used in bread making.

Without an understanding of microbiology, early bakers would have had little ability to directly control yeast cultures, but still kept locally interesting cultures by reusing doughs and starters to leaven later batches. However, it became possible to isolate and propagate favored yeast strains in the same manner as was done in the beer industry, and it eventually became practical to propagate yeast in a slurry with a composition similar to beer wort, usually including malted barley and wheat flour. Such cultures (sometimes referred to in old American cookery as "emptins", from their origins as the dregs of beer or cider fermentation) became the ancestors of modern baker's yeast, as, in general, they were carefully maintained to

avoid what was later discovered to be bacterial contamination, including using preservatives such as hops as well as boiling the growth medium.

In the 19th century, bread bakers obtained their yeast from beer brewers, and this led to sweet-fermented breads such as the Imperial "Kaiser-Semmel" roll, which in general lacked the sourness created by the acidification typical of *Lactobacillus*. However, beer brewers slowly switched from top-fermenting to bottom-fermenting yeast (Saccharomyces pastorianus) and this created a shortage of yeast for making bread, so the Vienna Process was developed in 1846. While the innovation is often popularly credited for using steam in baking ovens, leading to a different crust characteristic, it is notable for including procedures for high milling of grains (see Vienna grits), cracking them incrementally instead of mashing them with one pass; as well as better processes for growing and harvesting top-fermenting yeasts, known as press-yeast.

Refinements in microbiology following the work of Louis Pasteur led to more advanced methods of culturing pure strains. In 1879, Great Britain introduced specialized growing vats for the production of *S. cerevisiae*, and in the United States around the turn of the century centrifuges were used for concentrating the yeast, making modern commercial yeast possible, and turning yeast production into a major industrial endeavor. The slurry yeast made by small bakers and grocery shops became cream yeast, a suspension of live yeast cells in growth medium, and then compressed yeast, the fresh cake yeast that became the standard leaven for bread bakers in much of the Westernized world during the early 20th century.

During World War II, Fleischmann's developed a granulated active dry yeast for the United States armed forces, which did not require refrigeration and had a longer shelf-life and better temperature tolerance than fresh yeast; it is still the standard yeast for US military recipes. The company created yeast that would rise twice as fast, cutting down on baking time. Lesaffre later created instant yeast in 1973, which has gained considerable use and market share at the expense of both fresh and dry yeast in their various applications.

Modern baker's yeast is the species *Saccharomyces cerevisiae*. One of its properties is that it is not inhibited by propionates, which are commonly added to baked goods like bread dough to inhibit mold development and bacterial growth. Conversely, sorbates do inhibit yeast fermentation activity, so are not added directly to yeast-leavened doughed but may be sprayed onto finished products or even incorporated into packing materials.

Types of baker's yeast

Baker's yeast is available in a number of different forms, the main differences being the moisture contents. Though each version has certain advantages over the others, the choice of which form to use is largely a question of the requirements of the recipe at hand and the training of the cook preparing it. Dry yeast forms are good choices for longer-term storage, often lasting more than a year at room temperatures without significant loss of viability. In general, with occasional allowances for liquid content and temperature, the different forms of commercial yeast are considered interchangeable.

- **Cream yeast** is the closest form to the yeast slurries of the 19th century, in essence being a suspension of yeast cells in liquid, siphoned off from the growth medium. Its primary use is in industrial bakeries with special high-volume dispensing and mixing equipment, and it is not readily available to small bakeries or home cooks.
- **Compressed yeast** is, in essence, cream yeast with most of the liquid removed. It is a soft solid, beige in color, and best known in the consumer form as small, foil-wrapped cubes of **cake yeast**. It is also available in a larger-block form for bulk usage. It is highly perishable; though formerly widely available for the consumer market, it has become less common in supermarkets in some countries due to its poor keeping properties, having been superseded in some such markets by active dry and instant yeast. It is still widely available for commercial use, and is somewhat more tolerant of low temperatures than other forms of commercial yeast; however, even there, instant yeast has made significant market inroads.
- **Active dry yeast** is the form of yeast most commonly available to non-commercial bakers in the United States. It consists of coarse oblong granules of yeast, with live yeast cells encapsulated in a thick jacket of dry, dead cells with some growth medium. Under most conditions, active dry yeast must first be proofed or rehydrated. It can be stored at room temperature for a year, or frozen for more than a decade, which means that it has better keeping qualities than other forms, but it is generally

considered more sensitive than other forms to thermal shock when actually used in recipes.

- **Instant yeast** appears similar to active dry yeast, but has smaller granules with substantially higher percentages of live cells per comparable unit volumes. It is more perishable than active dry yeast but also does not require rehydration, and can usually be added directly to all but the driest doughs. In general, instant yeast has a small amount of ascorbic acid added as a preservative. Some producers provide specific variants for doughs with high sugar contents, and such yeasts are more generally known as **osmotolerant** yeasts.
- **Rapid-rise yeast** is a variety of dried yeast (usually a form of instant yeast) that is of a smaller granular size, thus it dissolves faster in dough, and it provides greater carbon dioxide output to allow faster rising. There is considerable debate as to the value of such a product; while most baking experts believe it reduces the flavor potential of the finished product, *Cook's Illustrated* magazine, among others, feels that, at least for direct-rise recipes, it makes little difference. Rapid-rise yeast is often marketed specifically for use in bread machines.
- **Deactivated yeast** is dead yeast which has no leavening value and is not interchangeable with other yeast types. Typically used for pizza and pan bread doughs, it is used at a rate of 0.1% of the flour weight, though manufacturer specifications may vary. It is a powerful reducing agent used to increase the extensibility of a dough.

Commercial brands

For most commercial uses, yeast of any form is packaged in bulk (blocks or freezer bags for fresh yeast; vacuum-packed brick bags for dry or instant); however, yeast for home use is often packaged in pre-measured doses, either small squares for compressed yeast or sealed packets for dry or instant. For active dry and instant yeast, in general a single dose (reckoned for the average bread recipe of between 500 g and 1000 g of dough) is about 2.5 tsp (~12 mL) or about 7 g (1/4 oz), though comparatively lesser amounts are used when the yeast is used in a pre-ferment. In general, a yeast flavor in the baked bread is not noticeable when the bakers' percent of added yeast is less than 2.5%.

Notable commercial brands of baker's yeast include Lesaffre's SAF red and SAF gold, Fleischmann's, and Red Star Yeast.

Use in research

Model organism

Because it is readily available and easy to culture, baker's yeast has long been used in chemical, biological, and genetic research as a model organism. Saccharomyces cerevisiae is a facultative anaerobe and undergoes aerobic fermentation in the presences of oxygen and sugars. In 1996, after 6 years of work, *S. cerevisiae* became the first eukaryote to have its entire genomesequenced. It has over 12 million base pairs and around 6000 genes. Since then, it has remained in the forefront of genetic research. For example, most of our

knowledge of the cell division cyclewas worked out from experiments with yeast.

Organic synthesis

Baker's yeast contains enzymes that can reduce a carbonyl group into a hydroxyl group in fairly high yield, thus making it useful for biotransformations in organic syntheses. It is known to reduce organometallic carbonyl compounds in very high yield.

Baker's yeast can also be used to produce ethanol via fermentation for use in chemical synthesis, although doing so in some places requires permits.

Industrial production

The baking industry relies on industrial production of its ingredients, including baking yeasts. Much effort has been put into developing and marketing yeasts that will perform reliably in mass production. Since the end of the nineteenth century, baker's yeast has been produced by companies that specialize in its production.

The main ingredients for industrial production are yeast cultures, sugar from cane and beet; but a number of minerals, nitrogen and vitamins are also needed.

Fermentation happens in several phases, which vary depending on the manufacturer:

- pure cultures in a laboratory flask for 2 to 4 days, then batch fermentations for 13 to 24 hours (anaerobic);
- intermediate and stock fermentation with gradual feeding and constant aeration;
- pitch and trade fermentation with large air supplies for up to 15 hours;
- filtration, blending, extrusion, and cutting, drying.

The yeast grows from hundreds kg in the intermediate and stock fermentor to tens of thousands kg in the trade fermentor, where most yeast is produced. The earlier stages produce more ethanol and other alcohols, while in the final stages ethanol production is suppressed up to 95% by controlling the amount of oxygen and sugar, in order to increase the yeast production instead.

The industry is highly concentrated, with 5 companies holding up to 80% of the worldwide market for dry yeast as of 2006. While dry yeast is exported over long distances and mostly sold in the developing countries, industrial customers often prefer to supply fresh yeast from local facilities, with a single wholesaler having up to 90% of the liquid yeast market in the UK in 2006. In USA companies like Lesaffre Group, AB Vista, DSM, GB Plange and AB Mauri, produced hundreds of thousands of metric tons of yeast in 2012.

BeerXML

BeerXML is a free, fully defined XML data description standard designed for the exchange of beer brewing recipes and other brewing data. Tables of recipes as well as other records such

as hop schedules and malt bills can be represented using BeerXML for use by brewing software.

BeerXML is an open standard and as a subset of **Extensible Markup Language** (**XML**). BeerXML is a markup language that defines a set of rules for encoding documents in a format that is both human-readable and machine-readable.

BeerXML is supported by a number of web sites, computerprogrammes and an increasing number of Android Windows Phone and iOS apps.

Plugins and extensions supporting BeerXMLhave been written for a variety of platforms including Ruby via RubyGems, WordPress, PHP and JavaScript

Many brewing hardware manufacturers incorporate BeerXML into their systems and third party plugins and patches are being developed for brewery control hardware and embedded systems allowing the automation and fine control and timing of processes such as mashing and potentially fermentation.

Common applications and examples of usage

BeerXMLis used in both amateur and professional brewing and facilitates the sharing of brewing data over the internet. Users of different applications such as the open-source softwareBrewtarget (with more than 52,000 downloads) can share data via XML with users of popular proprietary software such as Beersmith and ORRTIZ: BMS 4 Breweries or upload

their data to share on BeerXML compatible sharing sites and cloud platforms such as Brewtoad (over 50,000 registered users) or the Beersmith Recipe Cloud (with 43,000 registered users). A user of a recipe design and sharing and creation site such as Brewersfriend.com can import and export BeerXML to and from mobile apps or enter it into a brewing competition database such as The Brew Competition Online Entry & Management (BCOE&M) system.

The adoption of BeerXML as a standard is leading to new developments such as ingredients databases which attempt to standardise ingredients definitions and characteristics. Brewers can use platforms like Brewblogger.com to create recipes and log their brewday for publication as a blog and for export to databases and common spreadsheet applications.

JavaScript applications such as brauhaus.js (developed from the Malt.io recipe sharing site) allow users to run them on a local machine or web browser for execution through any standards compliant web browser.

Supported fields

The following fields form the core information of the BeerXML structure

- Recipes

Recipe name
Brewer
Brewing method (All grain, Partial Mash, Extract)
Recipe Type (Ale, Lager, Hybrid, etc.)
Recipe volume (Run length)
Boil volume (Wort size)
Boil time (duration)

Recipe efficiency
Estimated values
OG (Original Gravity)
FG (Final Gravity)
Color (SRM)
Bitterness (IBU)
Alcohol content (%abv)

- Hops

Name
Origin
Description
Alpha acids
Beta acids
Storageability (HSI)
Humulene
Caryophyllene
Cohumulone
Myrcene
Farsene (not explicitly included in BeerXML v1)
Total oil (not explicitly included in BeerXML v1)

Recipe Specific - When added (Boil, Mash, First Wort, Dry, etc.)

Amount
Time (duration)

- Fermentables

Name
Origin
Description
Type (Grain, Sugar, etc.)
Potential
Recommend Mash (true or false)
IBU gal/lb (for hopped extract)
Color (°Lovibond)
Moisture content
Protein content
Diastatic power (°Lintner)
Maximum used (% of grist)

Recipe Specific

Amount
Late Addition (true or false)

- Additives (Called MISC for miscellaneous in BeerXML v1)

Name
Description
Type (Fining, Spice, Herb, etc.)

Recipe Specific - When added (Boil, Primary, etc.)

Amount
Time (duration)

Yeasts

Name
Supplier
Catalog number
Description
Type (Ale, Lager, etc.)
Form (Dry, Liquid, etc.)
Best for
Temperature range
Flocculation
Attenuation
Max reuse

Recipe Specific

Amount
Added to secondary (true or false)
Time cultured

Limitations

BeerXML 1.0 supports no more than three fermentation steps. While this is not a real world limitation for many brewers, it does introduce a discrepancy where a software tool or web service that allows several or unlimited fermentation steps wishes to implement BeerXML as an import/export mechanism. For example; where a fermentation schedule instruction to pitch at 21 degrees Celsius, allow to drop to 17 over three days and then decrease by 1 degree per day until the wort reaches 10 degrees, hold for 12 days before racking for maturation. This could not be accommodated within the formal structure

requiring the use of informal/optional and non machine readable fields.

All units are converted to SI units internally. As a result, there is loss of precision when converting non SI units whether they be Imperial, US Customary or metric.

Hop oil contributions in the copper are not explicitly supported in the current definition.

Farsene levels are not explicitly supported in the current definition.

No distinction is made between weight and mass

Development

The BeerXML standard has a proposed second version which has been mooted and is under development. It has not been validated or published as its feature set is still under discussion.

XML Header

As in XML, all files begin with a header line as the first line. After the XML header a record set should start (for example`<RECIPES>…</RECIPES>` or `<HOPS> … </HOPS>`).

Required XML Header Example with Recipes tag:

```
<?xml version="1.0" encoding="UTF-8"?>
<RECIPES>
  ...
```

</RECIPES>

Tag Names

Tag names are always uppercase. For example, "HOP" is acceptable, but "hop" and Hop" are not.

Version

All records have a required <VERSION> tag that denotes the version of the XML standard. At present, all are set to the integer 1 for this version of the standard. It is intended that future versions of the standard will be backward compatible with older versions, but the VERSION tag allows newer programmes to check for a higher version of the standard or do conversions if required to be backward compatible.

Data Formats

- Record Set – A special tag that starts a particular set of data. For example, an XML table that consists of a set of hops records might start with a <HOPS> tag to denote that this is the start of hops records. After the last record, a </HOPS> tag would be used.
- Record - Denotes a tag that starts or ends a particular record—for example "HOP" might start a hops record or "FERMENTABLE" might start a fermentable record.
- Percentage - Denotes a percentage - all percentages are expressed as percent out of 100- for example 10.4% is written as "10.4" and not "0.104"

- List - The data has only a fixed number of values that are selected from the list in the description table for the tag. These items are case sensitive, and no other values are allowed.
- Text - The data is free format text. For multiline entries, line breakswill be preserved where possible and the text may be truncated on import if the text is too long for the importing program to store. Multiline entries may be split with either a newline (Unix format) or a carriage return – newline combination (DOS format). Importing programmes should accept either.
- Boolean - The Boolean data type may be either TRUE or FALSE, with TRUE and FALSE in capitals. A default value should be specified for optional fields - the default is used if the value is not present.
- Integer - An integer number with no decimal point. May include negative values - examples include ...-3, -2, -1, 0, 1, 2, 3,...
- Floating Point - A floating point number, usually expressed in its simplest form with a decimal point as in "1.2", "0.004", etc... Programmes should endeavor to store as many significant digits as possible to avoid truncating or losing small values.

Units

All units are fixed. It is the responsibility of the importing or exporting programme to convert to and from the units below if needed.

- Weight Units
- All weightsare measured in Kilograms (kg). For small values the exporting programme will make an effort to preserve as many significant digits as possible.
- Volume Units
- All volumes are measured in Litres (l). For small values the exporting programme will make an effort to preserve as many significant digits as possible.
- Temperature Units
- All temperaturesare measured in degrees Celsius.
- Time Units
- All times are given in minutes or fractions thereof – unless otherwise specified in the tag description.
- Specific Gravity Units
- Specific gravityare measured relative to the weight of the same size sample of water. For example, “1.035”, “1.060”, and so on.
- Pressure Units
- Pressures are measured in kilopascals (kPa)

Non-Standard Tags

As per the XML standard, all non-standard tags should be ignored by the importing programme. This allows programmes to store additional information if desired using their own tags. Any tags not defined as part of this standard may safely be ignored by the importing programme.

Optional tags

The optional 'Appendix A' adds tags for use in the display of brewing data using XML style sheets or XML compatible report generators. As the tags in the appendix are for display only and may include rounded values and varying units. These appendix tags are intended for display and not for data import.

Brewing

Brewing is the production of beer by steeping a starch source (commonly cereal grains, the most popular of which is barley) in water and fermenting the resulting sweet liquid with yeast. It may be done in a brewery by a commercial brewer, at home by a homebrewer, or communally. Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests that emerging civilizations, including ancient Egypt and Mesopotamia, brewed beer. Since the nineteenth century the brewing industry has been part of most western economies.

The basic ingredients of beer are water and a fermentable starch source such as malted barley. Most beer is fermented with a brewer's yeast and flavoured with hops. Less widely used starch sources include millet, sorghum and cassava. Secondary sources (adjuncts), such as maize (corn), rice, or sugar, may also be used, sometimes to reduce cost, or to add a feature, such as adding wheat to aid in retaining the foamy head of the beer. The most common starch source is ground cereal or "grist" - the proportion of the starch or cereal ingredients in a beer recipe may be called grist, grain bill, or

simply mash ingredients. Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. There are three main fermentation methods, warm, cool and spontaneous. Fermentation may take place in an open or closed fermenting vessel; a secondary fermentation may also occur in the cask or bottle. There are several additional brewing methods, such as Burtonisation, barrel-ageing, double dropping, and Yorkshire Square.

History

Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests emerging civilizations including ancient Egypt and Mesopotamia brewed beer. Descriptions of various beer recipes can be found in cuneiform (the oldest known writing) from ancient Mesopotamia. In Mesopotamia the brewer's craft was the only profession which derived social sanction and divine protection from female deities/goddesses, specifically: Ninkasi, who covered the production of beer, Siris, who was used in a metonymic way to refer to beer, and Siduri, who covered the enjoyment of beer. In pre-industrial times, and in developing countries, women are frequently the main brewers.

As almost any cereal containing certain sugars can undergo spontaneous fermentation due to wild yeasts in the air, it is possible that beer-like beverages were independently developed throughout the world soon after a tribe or culture had domesticated cereal. Chemical tests of ancient pottery jars reveal that beer was produced as far back as about 7,000 years ago in what is today Iran. This discovery reveals one of the

earliest known uses of fermentation and is the earliest evidence of brewing to date. In Mesopotamia, the oldest evidence of beer is believed to be a 6,000-year-old Sumerian tablet depicting people drinking a beverage through reed straws from a communal bowl. A 3900-year-old Sumerian poem honouringNinkasi, the patron goddess of brewing, contains the oldest surviving beer recipe, describing the production of beer from barley via bread. The invention of bread and beer has been argued to be responsible for humanity's ability to develop technology and build civilization. The earliest chemically confirmed barley beer to date was discovered at Godin Tepe in the central Zagros Mountains of Iran, where fragments of a jug, at least 5,000 years old was found to be coated with beerstone, a by-product of the brewing process. Beer may have been known in Neolithic Europe as far back as 5,000 years ago, and was mainly brewed on a domestic scale.

Ale produced before the Industrial Revolution continued to be made and sold on a domestic scale, although by the 7th century AD beer was also being produced and sold by European monasteries. During the Industrial Revolution, the production of beer moved from artisanal manufacture to industrial manufacture, and domestic manufacture ceased to be significant by the end of the 19th century. The development of hydrometers and thermometers changed brewing by allowing the brewer more control of the process, and greater knowledge of the results. Today, the brewing industry is a global business, consisting of several dominant multinational companies and many thousands of smaller producers ranging from brewpubs to regional breweries. More than 133 billion litres (35 billion gallons) are sold per year—producing total global revenues of $294.5 billion (£147.7 billion) in 2006.

Ingredients

The basic ingredients of beer are water; a starch source, such as maltedbarley, able to be fermented (converted into alcohol); a brewer's yeast to produce the fermentation; and a flavouring, such as hops, to offset the sweetness of the malt. A mixture of starch sources may be used, with a secondary saccharide, such as maize (corn), rice, or sugar, these often being termed adjuncts, especially when used as a lower-cost substitute for malted barley. Less widely used starch sources include millet, sorghum, and cassava root in Africa, potato in Brazil, and agave in Mexico, among others. The most common starch source is ground cereal or "grist" - the proportion of the starch or cereal ingredients in a beer recipe may be called grist, grain bill, or simply mash ingredients.

- Water

Beer is composed mostly of water. Regions have water with different mineral components; as a result, different regions were originally better suited to making certain types of beer, thus giving them a regional character. For example, Dublin has hard water well suited to making stout, such as Guinness; while Pilsen has soft water well suited to making pale lager, such as Pilsner Urquell. The waters of Burton in England contain gypsum, which benefits making pale ale to such a degree that brewers of pale ales will add gypsum to the local water in a process known as Burtonisation.

- Starch source

The starch source in a beer provides the fermentable material and is a key determinant of the strength and flavour of the beer. The most common starch source used in beer is malted grain. Grain is malted by soaking it in water, allowing it to begin germination, and then drying the partially germinated grain in a kiln. Malting grain produces enzymes that will allow conversion from starches in the grain into fermentable sugars during the mash process. Different roasting times and temperatures are used to produce different colours of malt from the same grain. Darker malts will produce darker beers.

Nearly all beer includes barley malt as the majority of the starch. This is because of its fibrous husk, which is important not only in the sparging stage of brewing (in which water is washed over the mashed barley grains to form the wort) but also as a rich source of amylase, a digestiveenzyme that facilitates conversion of starch into sugars. Other malted and unmalted grains (including wheat, rice, oats, and rye, and, less frequently, maize (corn) and sorghum) may be used. In recent years, a few brewers have produced gluten-free beer made with sorghum with no barley malt for people who cannot digest gluten-containing grains like wheat, barley, and rye.

- Hops

Hops are the female flower clusters or seed cones of the hop vine *Humuluslupulus*, which are used as a flavouring and preservative agent in nearly all beer made today. Hops had been used for medicinal and food flavouring purposes since Roman times; by the 7th century in Carolingian monasteries in what is now Germany, beer was being made with hops, though it isn't until the thirteenth century that widespread cultivation

of hops for use in beer is recorded. Before the thirteenth century, beer was flavoured with plants such as yarrow, wild rosemary, and bog myrtle, and other ingredients such as juniper berries, aniseed and ginger, which would be combined into a mixture known as gruit and used as hops are now used; between the thirteenth and the sixteenth century, during which hops took over as the dominant flavouring, beer flavoured with gruit was known as ale, while beer flavoured with hops was known as beer. Some beers today, such as *Fraoch* by the Scottish Heather Ales company and *Cervoise Lancelot* by the French Brasserie-Lancelot company, use plants other than hops for flavouring.

Hops contain several characteristics that brewers desire in beer: they contribute a bitterness that balances the sweetness of the malt; they provide floral, citrus, and herbal aromas and flavours; they have an antibiotic effect that favours the activity of brewer's yeast over less desirable microorganisms; and they aid in "head retention", the length of time that the foam on top of the beer (the beer head) will last. The preservative in hops comes from the lupulinglands which contain soft resins with alpha and beta acids. Though much studied, the preservative nature of the soft resins is not yet fully understood, though it has been observed that unless stored at a cool temperature, the preservative nature will decrease. Brewing is the sole major commercial use of hops.

- Yeast

Yeast is the microorganism that is responsible for fermentation in beer. Yeast metabolises the sugars extracted from grains, which produces alcohol and carbon dioxide, and thereby turns

wort into beer. In addition to fermenting the beer, yeast influences the character and flavour. The dominant types of yeast used to make beer are *Saccharomyces cerevisiae*, known as ale yeast, and *Saccharomyces pastorianus*, known as lager yeast; *Brettanomyces* ferments lambics, and *Torulasporadelbrueckii* ferments Bavarian weissbier. Before the role of yeast in fermentation was understood, fermentation involved wild or airborne yeasts, and a few styles such as lambics still use this method today. Emil Christian Hansen, a Danish biochemist employed by the Carlsberg Laboratory, developed pure yeast cultures which were introduced into the Carlsberg brewery in 1883, and pure yeast strains are now the main fermenting source used worldwide.

- Clarifying agent

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids and are found only in trace amounts in the finished product. This process makes the beer appear bright and clean, rather than the cloudy appearance of ethnic and older styles of beer such as wheat beers.

Examples of clarifying agents include isinglass, obtained from swim bladders of fish; Irish moss, a seaweed; kappa carrageenan, from the seaweed *kappaphycus*; polyclar (a commercial brand of clarifier); and gelatin. If a beer is marked "suitable for Vegans", it was generally clarified either with seaweed or with artificial agents, although the "Fast Cask" method invented by Marston's in 2009 may provide another method.

Brewing process

There are several steps in the brewing process, which may include malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. The brewing equipment needed to make beer has grown more sophisticated over time, and now covers most aspects of the brewing process.

Malting is the process where barley grain is made ready for brewing. Malting is broken down into three steps in order to help to release the starches in the barley. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hours. During germination, the grain is spread out on the floor of the germination room for around 5 days. The final part of malting is kilning when the malt goes through a very high temperature drying in a kiln; with gradual temperature increase over several hours. When kilning is complete, the grains are now termed malt, and they will be milled or crushed to break apart the kernels and expose the cotyledon, which contains the majority of the carbohydrates and sugars; this makes it easier to extract the sugars during mashing.

Mashingconverts the starches released during the malting stage into sugars that can be fermented. The milled grain is mixed with hot water in a large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. During the mash, naturally occurring enzymes present in the malt convert the starches (long chain carbohydrates) in the grain into smaller molecules or simple sugars (mono-, di-, and tri-saccharides). This "conversion" is called saccharification which occurs between the temperatures

60–70 °C (140–158 °F). The result of the mashing process is a sugar-rich liquid or "wort", which is then strained through the bottom of the mash tun in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75–78 °C (167–172 °F) (known as a mashout) to free up more starch and reduce mash viscosity. Additional water may be sprinkled on the grains to extract additional sugars (a process known as sparging).

The wort is moved into a large tank known as a "copper" or kettle where it is boiled with hops and sometimes other ingredients such as herbs or sugars. This stage is where many chemical reactions take place, and where important decisions about the flavour, colour, and aroma of the beer are made. The boiling process serves to terminate enzymatic processes, precipitate proteins, isomerize hop resins, and concentrate and sterilize the wort. Hops add flavour, aroma and bitterness to the beer. At the end of the boil, the hopped wort settles to clarify in a vessel called a "whirlpool", where the more solid particles in the wort are separated out.

After the whirlpool, the wort is drawn away from the compacted hop trub, and rapidly cooled via a heat exchanger to a temperature where yeast can be added. A variety of heat exchanger designs are used in breweries, with the most common a plate-style. Water or glycol run in channels in the opposite direction of the wort, causing a rapid drop in temperature. It is very important to quickly cool the wort to a level where yeast can be added safely as yeast is unable to grow in very high temperatures, and will start to die in temperatures above 60 °C (140 °F). After the wort goes through the heat exchanger, the cooled wort goes into a fermentation

tank. A type of yeast is selected and added, or "pitched", to the fermentation tank. When the yeast is added to the wort, the fermenting process begins, where the sugars turn into alcohol, carbon dioxide and other components. When the fermentation is complete the brewer may rack the beer into a new tank, called a conditioning tank. Conditioning of the beer is the process in which the beer ages, the flavour becomes smoother, and flavours that are unwanted dissipate. After conditioning for a week to several months, the beer may be filtered and force carbonated for bottling, or fined in the cask.

Mashing

Mashing is the process of combining a mix of milled grain (typically maltedbarley with supplementary grains such as corn, sorghum, rye or wheat), known as the "grist" or "grain bill", and water, known as "liquor", and heating this mixture in a vessel called a "mash tun". Mashing is a form of steeping, and defines the act of brewing, such as with making tea, sake, and soy sauce. Technically, wine, cider and mead are not brewed but rather vinified, as there is no steeping process involving solids. Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose to create a malty liquid called wort. There are two main methods – infusion mashing, in which the grains are heated in one vessel; and decoction mashing, in which a proportion of the grains are boiled and then returned to the mash, raising the temperature. Mashing involves pauses at certain temperatures (notably 45–62–73 °C or 113–144–163 °F), and takes place in a "mash tun" – an insulated brewing vessel with a false bottom. The end product of mashing is called a

"mash". Mashing usually takes 1 to 2 hours, and during this time the various temperature rests activate different enzymes depending upon the type of malt being used, its modification level, and the intention of the brewer. The activity of these enzymes convert the starches of the grains to dextrins and then to fermentable sugars such as maltose. A mash rest from 49–55 °C (120–131 °F) activates various proteases, which break down proteins that might otherwise cause the beer to be hazy. This rest is generally used only with undermodified (i.e. undermalted) malts which are decreasingly popular in Germany and the Czech Republic, or non-malted grains such as corn and rice, which are widely used in North American beers. A mash rest at 60 °C (140 °F) activates β-glucanase, which breaks down gummy β-glucans in the mash, making the sugars flow out more freely later in the process. In the modern mashing process, commercial fungal basedβ-glucanase may be added as a supplement. Finally, a mash rest temperature of 65–71 °C (149–160 °F) is used to convert the starches in the malt to sugar, which is then usable by the yeast later in the brewing process. Doing the latter rest at the lower end of the range favoursβ-amylase enzymes, producing more low-order sugars like maltotriose, maltose, and glucose which are more fermentable by the yeast.

This in turn creates a beer lower in body and higher in alcohol. A rest closer to the higher end of the range favoursα-amylase enzymes, creating more higher-order sugars and dextrins which are less fermentable by the yeast, so a fuller-bodied beer with less alcohol is the result. Duration and pH variances also affect the sugar composition of the resulting wort.

Lautering

Lautering is the separation of the wort (the liquid containing the sugar extracted during mashing) from the grains. This is done either in a mash tun outfitted with a false bottom, in a lautertun, or in a mash filter. Most separation processes have two stages: first wort run-off, during which the extract is separated in an undiluted state from the spent grains, and sparging, in which extract which remains with the grains is rinsed off with hot water. The lautertun is a tank with holes in the bottom small enough to hold back the large bits of grist and hulls (the ground or milled cereal). The bed of grist that settles on it is the actual filter. Some lautertuns have provision for rotating rakes or knives to cut into the bed of grist to maintain good flow. The knives can be turned so they push the grain, a feature used to drive the spent grain out of the vessel. The mash filter is a plate-and-frame filter. The empty frames contain the mash, including the spent grains, and have a capacity of around one hectoliter. The plates contain a support structure for the filter cloth. The plates, frames, and filter cloths are arranged in a carrier frame like so: frame, cloth, plate, cloth, with plates at each end of the structure. Newer mash filters have bladders that can press the liquid out of the grains between spargings. The grain does not act like a filtration medium in a mash filter.

Boiling

After mashing, the beer wort is boiled with hops (and other flavourings if used) in a large tank known as a "copper" or brew kettle – though historically the mash vessel was used and

is still in some small breweries. The boiling process is where chemical reactions take place, including sterilization of the wort to remove unwanted bacteria, releasing of hop flavours, bitterness and aroma compounds through isomerization, stopping of enzymatic processes, precipitation of proteins, and concentration of the wort. Finally, the vapours produced during the boil volatiliseoff-flavours, including dimethyl sulfide precursors. The boil is conducted so that it is even and intense – a continuous "rolling boil". The boil on average lasts between 45 and 90 minutes, depending on its intensity, the hop addition schedule, and volume of water the brewer expects to evaporate. At the end of the boil, solid particles in the hopped wortare separated out, usually in a vessel called a "whirlpool".

Brew kettle or copper

Copper is the traditional material for the boiling vessel, because copper transfers heat quickly and evenly, and because the bubbles produced during boiling, and which would act as an insulator against the heat, do not cling to the surface of copper, so the wortis heated in a consistent manner. The simplest boil kettles are direct-fired, with a burner underneath. These can produce a vigorous and favourable boil, but are also apt to scorch the wort where the flame touches the kettle, causing caramelisation and making cleanup difficult. Most breweries use a steam-fired kettle, which uses steam jackets in the kettle to boil the wort. Breweries usually have a boiling unit either inside or outside of the kettle, usually a tall, thin cylinder with vertical tubes, called a calandria, through which wort is pumped.

Whirlpool

At the end of the boil, solid particles in the hopped wortare separated out, usually in a vessel called a "whirlpool" or "settling tank". The whirlpool was devised by Henry RanulphHudston while working for the Molson Brewery in 1960 to utilise the so-called tea leaf paradox to force the denser solids known as "trub" (coagulated proteins, vegetable matter from hops) into a cone in the centre of the whirlpool tank. Whirlpool systems vary: smaller breweries tend to use the brew kettle, larger breweries use a separate tank, and design will differ, with tank floors either flat, sloped, conical or with a cup in the centre. The principle in all is that by swirling the wort the centripetal force will push the trub into a cone at the centre of the bottom of the tank, where it can be easily removed.

Hopback

A hopback is a traditional additional chamber that acts as a sieve or filter by using whole hops to clear debris (or "trub") from the unfermented (or "green") wort, as the whirlpool does, and also to increase hop aroma in the finished beer. It is a chamber between the brewing kettle and wort chiller. Hops are added to the chamber, the hot wort from the kettle is run through it, and then immediately cooled in the wort chiller before entering the fermentation chamber. Hopbacks utilizing a sealed chamber facilitate maximum retention of volatile hop aroma compounds that would normally be driven off when the hops contact the hot wort. While a hopback has a similar filtering effect as a whirlpool, it operates differently: a whirlpool uses centrifugal forces, a hopback uses a layer of

whole hops to act as a filter bed. Furthermore, while a whirlpool is useful only for the removal of pelleted hops (as flowers do not tend to separate as easily), in general hopbacksare used only for the removal of whole flower hops (as the particles left by pellets tend to make it through the hopback). The hopbackhas mainly been substituted in modern breweries by the whirlpool.

Wort cooling

After the whirlpool, the wort must be brought down to fermentation temperatures 20–26 °C (68–79 °F) before yeast is added. In modern breweries this is achieved through a plate heat exchanger. A plate heat exchanger has many ridged plates, which form two separate paths. The wortis pumped into the heat exchanger, and goes through every other gap between the plates. The cooling medium, usually water, goes through the other gaps. The ridges in the plates ensure turbulent flow. A good heat exchanger can drop 95 °C (203 °F) wort to 20 °C (68 °F) while warming the cooling medium from about 10 °C (50 °F) to 80 °C (176 °F). The last few plates often use a cooling medium which can be cooled to below the freezing point, which allows a finer control over the wort-out temperature, and also enables cooling to around 10 °C (50 °F). After cooling, oxygen is often dissolved into the wort to revitalize the yeast and aid its reproduction. Some of the craft brewery, particularly those wanting to create steam beer, utilize coolship instead.

While boiling, it is useful to recover some of the energy used to boil the wort. On its way out of the brewery, the steam created during the boil is passed over a coil through which unheated water flows. By adjusting the rate of flow, the output

temperature of the water can be controlled. This is also often done using a plate heat exchanger. The water is then stored for later use in the next mash, in equipment cleaning, or wherever necessary. Another common method of energy recovery takes place during the wort cooling. When cold water is used to cool the wort in a heat exchanger, the water is significantly warmed. In an efficient brewery, cold water is passed through the heat exchanger at a rate set to maximize the water's temperature upon exiting. This now-hot water is then stored in a hot water tank.

Fermenting

Fermentation takes place in fermentation vessels which come in various forms, from enormous cylindroconical vessels, through open stone vessels, to wooden vats. After the wortis cooled and aerated – usually with sterile air – yeast is added to it, and it begins to ferment. It is during this stage that sugars won from the maltare converted into alcohol and carbon dioxide, and the product can be called beer for the first time.

Most breweries today use cylindroconical vessels, or CCVs, which have a conical bottom and a cylindrical top. The cone's angle is typically around 60°, an angle that will allow the yeast to flow towards the cone's apex, but is not so steep as to take up too much vertical space. CCVs can handle both fermenting and conditioning in the same tank. At the end of fermentation, the yeast and other solids which have fallen to the cone's apex can be simply flushed out of a port at the apex. Open fermentation vessels are also used, often for show in brewpubs, and in Europe in wheat beer fermentation. These vessels have no tops, which makes harvesting top-fermenting

yeasts very easy. The open tops of the vessels make the risk of infection greater, but with proper cleaning procedures and careful protocol about who enters fermentation chambers, the risk can be well controlled. Fermentation tanks are typically made of stainless steel. If they are simple cylindrical tanks with beveled ends, they are arranged vertically, as opposed to conditioning tanks which are usually laid out horizontally. Only a very few breweries still use wooden vats for fermentation as wood is difficult to keep clean and infection-free and must be repitched more or less yearly.

Fermentation methods

There are three main fermentation methods, warm, cool and wild or spontaneous. Fermentation may take place in open or closed vessels. There may be a secondary fermentation which can take place in the brewery, in the cask or in the bottle.

Brewing yeasts are traditionally classed as "top-cropping" (or "top-fermenting") and "bottom-cropping" (or "bottom-fermenting"); the yeasts classed as top-fermenting are generally used in warm fermentations, where they ferment quickly, and the yeasts classed as bottom-fermenting are used in cooler fermentations where they ferment more slowly. Yeast were termed top or bottom cropping, because the yeast was collected from the top or bottom of the fermenting wort to be reused for the next brew. This terminology is somewhat inappropriate in the modern era; after the widespread application of brewing mycology it was discovered that the two separate collecting methods involved two different yeast species that favoured different temperature regimes, namely *Saccharomyces cerevisiae* in top-cropping at warmer

temperatures and *Saccharomyces pastorianus* in bottom-cropping at cooler temperatures. As brewing methods changed in the 20th century, cylindro-conical fermenting vessels became the norm and the collection of yeast for both *Saccharomyces* species is done from the bottom of the fermenter. Thus the method of collection no longer implies a species association. There are a few remaining breweries who collect yeast in the top-cropping method, such as Samuel Smiths brewery in Yorkshire, Marstons in Staffordshire and several German hefeweizen producers.

For both types, yeast is fully distributed through the beer while it is fermenting, and both equally flocculate (clump together and precipitate to the bottom of the vessel) when fermentation is finished. By no means do all top-cropping yeasts demonstrate this behaviour, but it features strongly in many English yeasts that may also exhibit chain forming (the failure of budded cells to break from the mother cell), which is in the technical sense different from true flocculation. The most common top-cropping brewer's yeast, *Saccharomyces cerevisiae*, is the same species as the common baking yeast. However, baking and brewing yeasts typically belong to different strains, cultivated to favour different characteristics: baking yeast strains are more aggressive, in order to carbonate dough in the shortest amount of time; brewing yeast strains act slower, but tend to tolerate higher alcohol concentrations (normally 12–15% abv is the maximum, though under special treatment some ethanol-tolerant strains can be coaxed up to around 20%). Modern quantitative genomics has revealed the complexity of *Saccharomyces* species to the extent that yeasts involved in beer and wine production commonly involve hybrids of so-called pure species. As such, the yeasts involved in what

has been typically called top-cropping or top-fermenting ale may be both *Saccharomyces cerevisiae* and complex hybrids of *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. Three notable ales, Chimay, Orval and Westmalle, are fermented with these hybrid strains, which are identical to wine yeasts from Switzerland.

Warm fermentation

In general, yeasts such as *Saccharomyces cerevisiae* are fermented at warm temperatures between 15 and 20 °C (59 and 68 °F), occasionally as high as 24 °C (75 °F), while the yeast used by Brasserie Dupont for saison ferments even higher at 29 to 35 °C (84 to 95 °F). They generally form a foam on the surface of the fermenting beer, which is calledbarm, as during the fermentation process its hydrophobic surface causes the flocs to adhere to CO_2 and rise; because of this, they are often referred to as "top-cropping" or "top-fermenting" – though this distinction is less clear in modern brewing with the use of cylindro-conical tanks. Generally, warm-fermented beers, which are usually termed ale, are ready to drink within three weeks after the beginning of fermentation, although some brewers will condition or mature them for several months.

Cool fermentation

- When a beer has been brewed using a cool fermentation of around 10 °C (50 °F), compared to typical warm fermentation temperatures of 18 °C (64 °F), then stored (or lagered) for typically several weeks (or months) at temperatures close to freezing point, it is termed a "lager". During the lagering or

storage phase several flavour components developed during fermentation dissipate, resulting in a "cleaner" flavour. Though it is the slow, cool fermentation and cold conditioning (or lagering) that defines the character of lager, the main technical difference is with the yeast generally used, which is *Saccharomyces pastorianus*. Technical differences include the ability of lager yeast to metabolize melibiose, and the tendency to settle at the bottom of the fermenter (though ales yeasts can also become bottom settling by selection); though these technical differences are not considered by scientists to be influential in the character or flavour of the finished beer, brewers feel otherwise - sometimes cultivating their own yeast strains which may suit their brewing equipment or for a particular purpose, such as brewing beers with a high abv.

Brewers in Bavariahad for centuries been selecting cold-fermenting yeasts by storing ("lagern") their beers in cold alpine caves. The process of natural selection meant that the wild yeasts that were most cold tolerant would be the ones that would remain actively fermenting in the beer that was stored in the caves. A sample of these Bavarian yeasts was sent from the Spaten brewery in Munich to the Carlsberg brewery in Copenhagen in 1845 who began brewing with it. In 1883 Emile Hansen completed a study on pure yeast culture isolation and the pure strain obtained from Spaten went into industrial production in 1884 as Carlsberg yeast No 1. Another specialized pure yeast production plant was installed at the Heineken Brewery in Rotterdam the following year and together they began the supply of pure cultured yeast to brewers across

Europe. This yeast strain was originally classified as *Saccharomyces carlsbergensis*, a now defunct species name which has been superseded by the currently accepted taxonomic classification *Saccharomyces pastorianus*.

Spontaneous fermentation

Lambic beers are historically brewed in Brussels and the nearby Pajottenland region of Belgium without any yeast inoculation. The wort is cooled in open vats (called "coolships"), where the yeasts and microbiota present in the brewery (such as *Brettanomyces*) are allowed to settle to create a spontaneous fermentation, and are then conditioned or matured in oak barrels for typically one to three years.