### Fermentation Biotechnology

A metabolic process called fermentation transforms sugar into acids, fumes, or alcohol. It happens in bacteria and yeast, as well as in muscle cells that are oxygen-starved, as in the case of lactic acid fermentation. In a broader sense, the term "fermentation" can also refer to the mass growth of microorganisms on a growth medium, frequently with the intention of creating a particular chemical product, such as an enzyme, vaccine, antibiotic, food product, or food additive. Louis Pasteur, a French microbiologist, is well known for his contributions to our understanding of fermentation and its microbiological origins. The field of zymology studies fermentation.

When the electron transport chain is inoperable due to a lack of oxygen, fermentation occurs, and it then serves as the cell's main source of ATP generation. Depending on the type of fermentation, it converts the pyruvate and NADH produced during the glycolysis process into NAD+ and a variety of other small molecules. NADH and pyruvate are used in respiration to produce ATP when O2 is present. This process, known as oxidative phosphorylation, produces a lot more ATP than just glycolysis. Because of this, cells normally benefit from avoiding fermentation when oxygen is present. Obligate anaerobes are the exception because they cannot tolerate oxygen.

Glycolysis is the initial process that all fermentation pathways share:

C6H12O6 + 2 NAD+ + 2 ADP + 2 Pi → 2 CH3COCOO− + 2 NADH + 2 ATP + 2 H2O + 2H+

[Pyruvate](https://en.wikipedia.org/wiki/Pyruvate) is CH3COCOO−. Pi is [inorganic phosphate](https://en.wikipedia.org/wiki/Inorganic_phosphate). Two [ADP](https://en.wikipedia.org/wiki/Adenosine_diphosphate) molecules and two Pi are converted to two [ATP](https://en.wikipedia.org/wiki/Adenosine_triphosphate) and two water molecules via [substrate-level phosphorylation.](https://en.wikipedia.org/wiki/Substrate-level_phosphorylation) Additionally, two NAD+ molecules are converted to NADH. Energy for ATP synthesis in oxidative phosphorylation is produced electrochemically across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) through the electron transport chain. Substrate-level phosphorylation occurs during glycolysis, where ATP is produced right away.

Since the Neolithic era, humans have employed fermentation to create food and drinks. For instance, fermentation is used to preserve food by creating lactic acid, which is present in sour foods like pickled cucumbers, kimchi, and yoghurt, as well as to make alcoholic drinks like wine and beer. Even animals like humans, who have stomachs, can experience fermentation.

### Definitions of Fermentation

### For many people, fermentation merely refers to the process through which alcoholic beverages are made from grains and fruits, such as beer and wine. A food that has soured or fermented is considered to be 'off'. These definitions of fermentation are provided. The definitions range from more informal, generic usage to more precise ones.

### 1. Generally used techniques for food preservation using microbes.

### 2. Any procedure that results in the creation of alcoholic beverages or acidic dairy products (broad application).

### 3. Any massive microbiological process that takes place in the presence or absence of air (typical term applied in industry).

### 4. Any metabolic process that releases energy and only occurs in anaerobic environments (getting more technical).

### 5. Any metabolic activity that uses an organic molecule as the final electron acceptor, releases energy from a sugar or other organic molecules, and does not require oxygen or an electron transport mechanism.

### Examples of Fermentation

### It's not necessary to carry out fermentation in an anaerobic setting. For instance, yeast cells vastly prefer fermentation to aerobic respiration even in the presence of sufficient oxygen, provided that sugars are readily available for consumption (a phenomenon known as the Crabtree effect). Hops' antibacterial properties also prevent yeast from utilising its aerobic metabolism. NADH is reacted during fermentation with an organic, endogenous electron acceptor. This is often pyruvate, which is created from the sugar during the glycolysis process. Pyruvate is transformed into a variety of chemicals during fermentation by a number of mechanisms, including:

1. Alcoholic fermentation, also known as ethanol fermentation, produces ethanol and carbon dioxide.

2. The production of lactic acid can be accomplished in two ways:

* + **Homolactic fermentation** is the production of lactic acid exclusively
  + **Heterolactic fermentation** is the production of lactic acid as well as other acids and alcohols.

The most frequent starting material for fermentation is sugar, and typical fermentation products include ethanol, lactic acid, carbon dioxide, and hydrogen gas (H2). However, fermentation can create more unusual substances like acetone and butyric acid. Beer, wine, and other alcoholic beverages that contain ethanol are fermented by yeast, which also produces a significant amount of carbon dioxide. Lactic acid is produced when fermentation takes place in mammalian muscle during periods of intensive activity when oxygen supply is reduced.

**Fermentation of Ethanol**

The alcohol fermentation of glucose, with the molecular formula C6H12O6, is depicted in the following chemical equation. Two ethanol molecules and two carbon dioxide molecules are produced from one glucose molecule:

C6H12O6 → 2 C2H5OH + 2 CO2

C2H5OH is the [chemical formula](https://en.wikipedia.org/wiki/Chemical_formula) for [ethanol](https://en.wikipedia.org/wiki/Ethanol).

One glucose molecule is split into two pyruvate molecules prior to fermentation. This process is called glycolysis.

**Fermentation of Lactic acid**

The simplest type of fermentation is homolactic fermentation, which solely yields lactic acid. A straightforward redox reaction causes the pyruvate from glycolysis to transform into lactic acid. Being one of the few respiration processes that does not result in the production of a gas as a byproduct, it is distinctive. In all, two molecules of lactic acid are produced from one molecule of glucose (or any other six-carbon sugar):

C6H12O6→2CH3CHOHCOOH

Animal muscles experience it when they require oxygen and energy more quickly than the blood can provide. Additionally, certain fungi and some types of bacteria (such Lactobacilli) exhibit it. This particular species of bacteria is responsible for the yogurt's sour taste by converting lactose into lactic acid. These lactic acid bacteria are capable of homolactic fermentation, which results in a mostly lactic acid-containing end product, or

**Heterolactic fermentation**, where some lactate is further metabolised to produce ethanol and carbon dioxide, acetate, or other metabolic products (via the phosphoketolase pathway), for example:

C6H12O6→CH3CHOHCOOH+C2H5OH+CO2

Galactose and glucose, both six-carbon sugars with the same atomic formula, are produced when lactose is fermented (as in yoghurts and cheeses): C12H22O11 + H2O → 2 C6H12O6 Heterolactic fermentation sits somewhere in between other types of fermentation, such alcoholic fermentation, and lactic acid fermentation. There are several reasons to proceed and change lactic acid into anything else, including:

• Lactic acid's acidity hinders biological processes; this can be advantageous to the fermenting organism by driving out competitors who are unaccustomed to the acidity, resulting in a longer shelf life for the food (part of the reason foods are purposefully fermented in the first place); however, beyond a certain point, the acidity starts to affect the organism that produces it.

• Le Chatelier's principle states that a high concentration of lactic acid, the result of fermentation, causes the equilibrium to shift backward, delaying growth and reducing the rate at which fermentation may take place.

• Because lactic acid is easily changed to ethanol, which is volatile and easily ejected, the reaction can proceed without difficulty. Additionally, CO2 is created, however it is considerably more volatile than ethanol and only mildly acidic.

• Although acetic acid (another conversion result) is sour and less volatile than ethanol, its synthesis from lactic acid releases a significant amount of extra energy when oxygen is scarce. It is a lighter molecule than lactic acid, more flammable, establishes fewer hydrogen bonds with its environment than lactic acid (as a result of having fewer groups that can form such connections), and will also enable the process to proceed more quickly.

• The quantity of acidity created per unit of ingested glucose will decrease, similar to ethanol, if propionic acid, butyric acid, and longer monocarboxylic acids are formed (see mixed acid fermentation), enabling faster growth.

### Aerobic respiration

The pyruvate formed during glycolysis is entirely oxidised during aerobic respiration, producing extra ATP and NADH through the citric acid cycle and oxidative phosphorylation. However, oxygen is required for this to happen. Oxygen is poisonous to facultative anaerobes but not to obligatory anaerobes, who do not need it. One of the fermentation processes, lactic acid fermentation, takes place in the absence of oxygen in order to renew NAD+.

### Hydrogen gas production in fermentation

To regenerate NAD+ from NADH, hydrogen gas is created via a variety of fermentation processes, including mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, and glyoxylate fermentation. Ferredoxin receives electrons, which hydrogenase then uses to oxidise it and produce H2. Methanogens and sulphate reducers use hydrogen gas as a substrate, which keeps hydrogen concentration low and promotes the formation of this energy-rich chemical. However, hydrogen gas can still develop at quite high concentrations, as in flatus.

Bacteria like Clostridium pasteurianum ferment glucose to produce butyrate, acetate, carbon dioxide, and hydrogen gas as an illustration of mixed acid fermentation: The process that produces acetate is:

C6H12O6 + 4 H2O → 2 CH3COO− + 2 HCO3− + 4 H+ + 4 H2

Although the global reaction only produces a small amount of energy, glucose might hypothetically be transformed into merely CO2 and H2.

### Methane gas production in fermentation

[Acetic acid](https://en.wikipedia.org/wiki/Acetic_acid) can also undergo a [dismutation](https://en.wikipedia.org/wiki/Dismutation) reaction to produce [methane](https://en.wikipedia.org/wiki/Methane) and [carbon dioxide](https://en.wikipedia.org/wiki/Carbon_dioxide): CH3COO− + H+ → CH4 + CO2 ΔG° = -36 kJ/reaction

### Methanogenic archaea, as part of their fermentative metabolism, catalyse this disproportionation reaction. From the carboxylic acid's carbonyl function (e donor), one electron is transferred. methyl group (acetic acid's e acceptor) to form CO2 and methane gas, respectively.

### History of Fermentation

### As early as 7000–6600 BCE in Jiahu, China, 6000 BCE in Georgia, 3150 BCE in ancient Egypt, 3000 BCE in Babylon, 2000 BCE in pre–Hispanic Mexico, and 1500 BC in Sudan, fermentation has been used, primarily for beverages. In Judaism and Christianity, foods that have undergone fermentation have religious significance. Rugutis, a god of the Baltics, was revered as the cause of fermentation.

### Through a series of experiments, Louis Pasteur (1822–1895) demonstrated that fermentation is started by live organisms in the 1850s and 1860s. Pasteur demonstrated that live organisms are responsible for lactic acid fermentation in 1857. His work in recognizing the function of microorganisms in food spoiling resulted in the technique of pasteurization. In 1860, he established that bacteria cause souring in milk, a process that was previously assumed to be solely a chemical change. In order to advance the French brewing sector, Pasteur wrote the renowned study on fermentation known as "Etudes sur la Bière" in 1877. This work was then translated into English as "Studies on fermentation" in 1879. He mistakenly stated that fermentation is "Life without air," but properly demonstrated that certain types of bacteria lead to certain forms of fermentation.

### Although it was a breakthrough to demonstrate that living microbes generate fermentation, it did not explain the fundamentals of the process or establish that the bacteria that seem to be present at all times are responsible for it. Pasteur was one of several scientists who tried in vain to remove the fermentation enzyme from yeast.[26] The German chemist Eduard Buechner had success in 1897 when he crushed up yeast, extracted a juice from them, and then, to his astonishment, discovered that this "dead" liquid would ferment a sugar solution, creating carbon dioxide and alcohol much like living yeasts. The discoveries of Buechner are regarded as the beginning of biochemistry. The "unorganised ferments" exhibited the same behaviours as the organised ones. From that point forward, all ferments were referred to as enzymes. It was later established that enzymes created by microbes are what drive fermentation. Buechner's work earned him the chemistry Nobel Prize in 1907.

Technology developments in fermentation and microbiology have been steady up to the present. For instance, it was shown in the late 1970s that microbes may undergo physical and chemical modifications to undergo mutations that would make them more productive, faster-growing, less oxygen-tolerant, and able to use a more concentrated media. The majority of modern food fermentations were impacted by the development of strain selection and hybridization. Companies like BioTork, a biotechnology firm that uses naturally evolving microbes to enhance fermentation processes, have taken alternative approaches to expanding the fermentation business. This method is distinct from the more well-liked genetic alteration, which is now the accepted practice in the sector.

### Industrial fermentation

Industrial fermentation is the purposeful utilisation of microbes like bacteria and fungus to ferment in order to produce goods that are beneficial to humans. Products that have undergone fermentation can be used in both food and general business. Fermentation is used to create a number of common compounds, including acetic acid, citric acid, and ethanol. The number of microorganisms, cells, cellular components, and enzymes, as well as temperature, pH, and oxygen for aerobic fermentation, all affect the rate of fermentation. Concentrating the diluted solution is typically included in the product recovery process. Almost all commercially available enzymes, including rennet, invertase, and lipase, are created by fermentation with genetically altered bacteria. In other circumstances, like the creation of baker's yeast and lactic acid bacteria starting cultures for cheese manufacture, the goal is the production of biomass itself. In general, fermentations fall into one of four categories:

• The creation of viable cellular biomass

• Creation of chemical molecules known as extracellular metabolites

• Creation of intracellular components (including proteins and enzymes)

• Substrate transformation (where the product is the transformed substrate)

These categories offer a foundation for comprehending the variations in approach, however they are not always mutually exclusive from one another. It's possible to utilise bacteria, yeast, mould, animal cells, or plant cells as the organisms. The particular organisms utilised in the fermentation require certain considerations, such as the dissolved oxygen level, nutrition levels, and temperature.

**Overview of industrial fermentation in general**

The organisms are typically immersed in a liquid medium during most industrial fermentations, however in some cases, like with the fermentation of cocoa beans, coffee cherries, and miso, the organisms are only exposed to the moist surface of the media. The fermentation process is also relevant to industrial factors. For instance, the fermentation medium, air, and equipment are sterilised to prevent biological process contamination. Chemical anti-foaming chemicals or mechanical foam destruction can both be used to control foam. Pressure, temperature, agitator shaft power, and viscosity are just a few other variables that need to be monitored and managed. Scaling up is crucial for industrial fermentations. Here, a laboratory operation is transformed into an industrial process. In the field of industrial microbiology, it is well known that what is successful in the lab may not be successful at all when initially applied at a big scale. In general, laboratory-tested fermentation conditions cannot be blindly applied to machinery designed at industrial scales. Because fermentation processes vary, even though numerous parameters have been investigated for use as scale-up criteria, there is no universal formula. The two most crucial techniques are to maintain a steady volumetric transfer rate and a constant power usage per unit of soup.

**Stages of microbiological development**

A chosen growing medium is "inoculated" with a specific organism when a specific organism is added to it. The inoculum grows gradually over time rather than right once. The lag phase, during which adaptation takes place, is at present. The organism's rate of growth increases gradually for a while after the lag phase—this time is known as the log or exponential phase. After a particular amount of time in the exponential phase, the rate of growth slows down as a result of continuously decreasing nutrient concentrations and/or continuously rising (accumulating) hazardous chemical concentrations. The deceleration phase is the one during which the rate of growth is being slowed down. The culture enters a stationary phase or steady state after the deceleration phase, where growth stops. Except when specific chemicals that have accumulated in the culture lyse the cells (chemolysis), the biomass is constant. The chemical composition of the culture does not change unless additional microbes contaminate it. The cells may turn scenescent and start to decompose if all the nutrients in the medium are used up or if the level of toxins is too high. Although the absolute amount of biomass may not change, fewer living things will exist.

**Fermenting Agent**

The bacteria that are employed in fermentation grow on (or on) a carefully formulated growth medium that provides the nutrients the organisms need to survive. There are many different types of media, but they all contain a source of carbon, a supply of nitrogen, water, salts, and micronutrients. Grape must is used as the medium in the creation of wine. Any cheap carbon source that is accessible could make up the majority of the medium used to produce bioethanol.

Typically, carbon sources are sugars or other carbohydrates, though they can also be alcohols or other substances in substrate transformations (such the creation of vinegar). To keep costs down, low-cost sources of carbohydrates like molasses, corn steep liquor, sugar cane juice, or sugar beet juice are employed in large-scale fermentations like those used to produce ethanol. Purified glucose, sucrose, glycerol, or other sugars may be used instead in more delicate fermentations to limit fluctuation and assist maintain the purity of the finished product. Starch may be fed to organisms designed to produce enzymes such as beta galactosidase, invertase, or other amylases in order to choose those that express the enzymes in a significant amount.

Most organisms need fixed nitrogen sources in order to synthesise proteins, nucleic acids, and other cellular components. Nitrogen can be given as bulk protein, like soy meal, pre-digested polypeptides, like peptone or tryptone, or as ammonia or nitrate salts, depending on the enzyme capabilities of the organism. Cost is a significant consideration when selecting a nitrogen source. Phosphorus is required for the synthesis of nucleic acids as well as the phospholipids found in cellular membranes. The quantity of phosphate that must be added is determined by the nature of the broth, the requirements of the organism, and the intended outcome of the fermentation. For instance, in the presence of phosphate, some cultures will not create secondary metabolites.

For organisms unable to synthesise all the vitamins they need, growth factors and trace elements are added to the fermentation broth. For fermentation media, yeast extract is a typical source of minerals and vitamins. While unrefined carbon and nitrogen sources generally contain inorganic nutrients, such as trace metals like iron, zinc, copper, manganese, molybdenum, and cobalt, using purified carbon and nitrogen sources may necessitate their addition. Since fermentation broth frequently contains a range of proteins, peptides, or starches that might act as foam-reinforcing agents, fermentations that produce large amounts of gas (or that require the addition of gas) will tend to form a layer of foam. Antifoaming chemicals may be applied to stop this foam from forming or building up. To keep pH close to ideal, mineral buffering salts like carbonates and phosphates can be utilised. A chelating agent may be required when metal ions are present in large quantities.

**Generating biomass**

Fermentation can occasionally result in the anticipated product of microbial cells or biomass. Examples include baker's yeast, lactobacillus, E. coli, and single cell proteins, among others. Algae are produced in sizable open ponds, which permit photosynthesis, in the case of single-cell protein. Care must be made to avoid mutations if the biomass is to be utilised as a starter culture for further fermentations.

**Extracellular metabolite production**

Secondary metabolites are those created during the stationary phase of an organism's life cycle, while primary metabolites are those produced during the organism's growth phase. Ethanol, citric acid, glutamic acid, lysine, vitamins, and polysaccharides are a few examples of primary metabolites. The drugs penicillin, cyclosporin A, gibberellin, and lovastatin are examples of secondary metabolites.

**First-order metabolites**

Primary metabolites are substances produced by an organism's normal metabolism throughout its growth period. Lactic acid and ethanol, which are both products of glycolysis, are frequent examples. Some Aspergillus niger strains create citric acid as a byproduct of the citric acid cycle in order to acidify their surroundings and keep out rivals. Some Corynebacterium species and some Micrococcus species produce glutamate, lysine, threonine, tryptophan, and other amino acids, respectively. All of these substances are created by the cell's routine "business" and discharged into the environment. Therefore, the cells do not need to be ruptured in order to recover the product.

**Subsequent Metabolites**

Compounds produced in the stationary phase are known as secondary metabolites. Penicillin, for example, inhibits the growth of bacteria that may compete with Penicillium moulds for nutrients. Some bacteria, including Lactobacillus species, have the ability to create bacteriocins, which also inhibit the growth of competing bacteria. These substances have apparent benefits for those who want to stop the spread of microorganisms, either as antibiotics or as antiseptics (such Grammaridin S). Secondary metabolites are also produced, some of which are fungicides like griseofulvin. Similar to main metabolites, secondary metabolites are often not produced in the presence of glucose or other carbon sources that would promote development. They are also discharged into the environment without damaging the cell membrane.

**Development of intracellular components**

The microbial enzymes, such as catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, and many others, are of particular importance among the intracellular components. This method is also used to produce recombinant proteins like insulin, the hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase, and others. The main distinction between this method and the others is the requirement to rupture (lyse) the cells at the conclusion of fermentation and to manage the environment to increase the amount of the product. In order for the result, which is often a protein, to be purified, it must also be isolated from all of the other cellular proteins in the lysate.

**Alteration of the substrate**

In the case of phenylacetylcarbinol and steroid biotransformation, for example, substrate transformation refers to the conversion of one compound into another. In the case of food fermentations and sewage treatment, it refers to the conversion of a raw material into a finished product.

**Fermenting food**

Ancient fermented food processes, such as making [bread](https://en.wikipedia.org/wiki/Bread), [wine,](https://en.wikipedia.org/wiki/Wine) [cheese,](https://en.wikipedia.org/wiki/Cheese) [curds](https://en.wikipedia.org/wiki/Curds), [idli](https://en.wikipedia.org/wiki/Idli), [dosa,](https://en.wikipedia.org/wiki/Dosa) etc., can be dated to more than [seven thousand years ago](https://en.wikipedia.org/wiki/5th_millennium_BC). They were developed long before man had any knowledge of the existence of the [microorganisms](https://en.wikipedia.org/wiki/Microorganism) involved. Some foods such as [Marmite](https://en.wikipedia.org/wiki/Marmite) are the byproduct of the fermentation process, in this case in the production of [beer.](https://en.wikipedia.org/wiki/Beer)

**Alcohol fuel**

The primary method of producing ethanol for ethanol fuel is fermentation. Yeast fermentation transforms common crops like corn, potato, cassava, and sugar cane into ethanol, which is then transformed into fuel.

**Sewage cleanup**

Sewage is broken down during the sewage treatment process by enzymes produced by microorganisms. Carbon dioxide and nontoxic, soluble chemicals are produced during the breakdown of solid organic materials. The resulting liquids can be utilised as liquid fertilisers or are cleaned to get rid of germs before being dumped into the sea or rivers. Sludge, also known as digested solids, is dried and used as fertiliser. Gaseous waste materials, such methane, can be converted into biogas and used to power electrical generators. Bacterial digestion has the benefit of decreasing the volume and smell of sewage, which minimises the requirement for dumping area. The primary drawback of bacterial digestion in sewage disposal is how slowly it works.

**Farm Animal Feed**

Fermented waste products from the agroindustrial sector can be used to feed animals, particularly ruminants. Cellulosic wastes have been broken down by fungi to increase protein content and boost in vitro digestibility. [Nitroglycerine](https://en.wikipedia.org/wiki/Nitroglycerin) is notorious.

**BIOASSAY**

A sort of scientific experiment is a bioassay, often known as a biological assay or assessment or biological standardisation. In order to ascertain a substance's biological activity, such as a hormone or medicine, a bioassay uses live animals or plants (in vivo) or cultured tissue or cells (in vitro). In order to create novel treatments and to monitor environmental pollutants, bioassays are often used to test how a material affects a living thing. Both methods involve examining a substance's effects on living things in order to assess its potency or nature. A bioassay can also be used to figure out how much of a mixture contains a certain constituent that could be hazardous to living things or the environment.

**Using bioassays**

By observing the impact on an organism, tissue, cell, enzyme, or receptor, bioassays can be used to ascertain the concentration, purity, or biological activity of a chemical such a vitamin, hormone, or plant growth factor. Quantitative or qualitative bioassays are both possible. When a substance's physical effects cannot be quantified, such as when seeds do not germinate or develop aberrant deformities, qualitative bioassays are used to evaluate the substance's effects. The well-known experiment with castrated hens conducted by Arnold Adolph Berthold is an illustration of a qualitative bioassay. According to this investigation, if a chicken's testicles were removed, it would not mature into a rooster because the endocrine signals required for this process were not present. Estimating the dose-response curve, or how the reaction changes with increasing exposure, is a key component of quantitative bioassays. Because of the dose-response relationship, it is possible to estimate the dose or concentration of a drug that will cause a particular biological response, such as the LC50 (concentration that will kill 50% of the organisms exposed). Biostatistical techniques are often used to analyse quantitative bioassays.

**The goal of bioassays**

1. Measuring the pharmacological action of novel or poorly understood chemicals

2. Examining the role of endogenous mediators

3. Calculating the side-effect profile, taking into account the level of drug toxicity

4. Measuring the concentration of recognised chemicals (the use of whole animals is no longer necessary due to alternatives)

5. Determining how much pollution is being emitted from a certain source, like wastewater or urban runoff.

6. Determining which substrates are particular to a given enzyme.

**Various bioassays**

There are two types of bioassays:

**Quantal:** A "all or none response" is part of a quantal assay.

**Graded:** Graded assays are based on the observation that the observed response increases proportionately as the concentration or dose is increased. The parameters used in these bioassays are determined by the type of effect the chemical is anticipated to have. For instance, the investigation of the blood pressure response to adrenaline or the contraction of smooth muscle in preparation for measuring histamine.

Any of the approaches listed below can be used to conduct a graded bioassay. The decision on the procedure is based on:

1. The needed assay precision

2. The quantity of the available sample substance

3. The accessibility of the test animals.

**Bioassay Methods**

1. Matching Bioassay, first

2. Interpolation Technique 2.

3. Using Bracketing

4. Multiple Point Bioassay (multiple of three, four, and six points)

5. Split bioassay

**Matching Bioassay:** The simplest sort of bioassay is the matching bioassay. In this kind of bioassay, the observed reaction and the response of the test chemical ingested initially are both compared to the expected response. The standard drug's responses are evaluated under various conditions until they closely resemble those of the test substance. Thus, a comparable concentration is determined. When the sample size is too small, this assay is used. The sensitivity of the preparation is not taken into account because the test does not require the recording of a concentration response curve. As a result, accuracy and dependability are poor.

**Interpolation bioassay:** In bioassays, the amount of unknown potency preparation necessary to elicit a certain impact on appropriate test animals, organs, or tissue under standard settings is calculated. A standard effect is contrasted with this one. In order to compare the amount of the test drug needed to have the same biological effect as a specific amount of a standard preparation, a simple formula is used to calculate the potency of the unknown substance as a percentage of the standard.

Both of these ultimately rely on predicating or assuming the shape of the DRC. Using this calculation frequently does not yield a trustworthy result. As a result, more accurate ways of determining potency based on observations of similar but not identical effects may be required. Statistical methods may also be used. The information (obtained from any of the employed assay procedures) that forms the basis for bioassays can be categorised as either quantal or graded response.

**Environment-based tests**

Environmental bioassays often involve a thorough toxicity survey. To identify the pertinent toxicants, a toxicity identification evaluation is carried out. Bioassays are useful for identifying the biological activity occurring within an organism, although they are frequently time-consuming and labor-intensive. Data that may not be applicable to other members of that species are a possibility due to organism-specific characteristics. For these reasons, different biological methods, such as radio-immunoassays, are frequently used.

## QUALITY CONTROL

Quality control, or QC for short, is the process by which organisations evaluate the calibre of all production-related aspects. Quality control, according to ISO 9000, is "a component of quality management focused on meeting quality requirements."

Three factors are highlighted by this strategy:

1. Components such controls, task management, clearly defined processes, performance and integrity standards, and record identification

2. Competency, including knowledge, abilities, expertise, and credentials

3. Intangible factors include people, moral character, self-assurance, organisational culture, drive, and good interpersonal interactions.

Before a product is sold into the open market, it is subjected to controls such product inspection, which involves visually inspecting each product and frequently utilising a stereo microscope for fine detail. Inspectors will be given a list of prohibited product flaws, such as cracks or surface blemishes, along with descriptions of each one. If any one of these three factors is lacking in any way, the output quality is at risk.

While quality assurance works to enhance and stabilise production (and related processes) to avoid, or at least minimise, issues that led to the defect(s), quality control emphasises testing of products to uncover defects and reporting to management who make the decision to allow or deny product release. Quality control concerns are among the main causes of not renewing a contract for contract work, especially for work given by government organisations.

**SHELVING LIFE OF GOODS**

The amount of time a product can be kept in storage before it loses its suitability for use, consumption, or sale. In other words, it might be used to describe whether a product should no longer be on a supermarket shelf (unfit for sale, but not yet unfit for use) or just no longer be on a pantry shelf (unfit for use). In addition to many other perishable things, this rule also applies to cosmetics, foods and drinks, medical equipment, medications, explosives, pharmaceutical drugs, chemicals, automobile tyres, and batteries. Packaging for packaged perishable foods may be required to have a best before, use by, or freshness date in some areas.

**Background**

The recommended maximum storage period for items or freshly harvested food during which a specific part of the products' quality remains acceptable under anticipated (or predetermined) conditions of distribution, storage, and display is known as the shelf life. The majority of expiration dates serve as suggestions based on typical and anticipated handling and temperature exposure. A food or drug's safety is not guaranteed if used before its expiration date, and a product is not always hazardous or useless after that date.

Foods in cans are safe for as long as they aren't exposed to freezing temperatures or temperatures exceeding 90 °F (32.2 °C), according to the USDA. Usefulness depends on how the cans appear. Cans with dents, rust, or swelling should be discarded. Low-acid canned goods (meats, vegetables) preserve their optimum quality for 2 to 5 years at 80 °F (27 °C), while high-acid canned foods (tomatoes, fruits) keep their greatest quality for 12 to 18 months.

An "expiration date" is sometimes referred to as a "sell by date," which is a less ambiguous term. After its expiration date, the majority of food is still edible. Even if a product has past its expiration date, quality cannot be assured. Stock rotation, which involves moving items with the earliest sell by dates from the warehouse to the sales area and then to the front of the shelf, is a common practise in grocery stores to reduce waste. Since most customers will pick these items up first, they are more likely to be sold before they reach the end of their shelf life. Consumers prefer fresher items, and some establishments may face fines for selling expired merchandise. Most, if not all, businesses would have to write such merchandise down as wasted, which would result in a loss of revenue.

The unique product's degrading mechanism determines the shelf life. Multiple factors, including exposure to light, heat, moisture, gas transport, mechanical stress, and contamination from things like microorganisms, can affect the majority. A parameter (such as a chemical compound's concentration, a microbiological index, or moisture content) is frequently used as the basis for a mathematical model of product quality.

Health concerns have a significant role in influencing the shelf life of various goods. Bacterial contamination is pervasive, and foods that are left unattended for an extended period of time are frequently contaminated with large numbers of bacterial colonies, making them unsafe to consume and causing food poisoning. However, the food's shelf life alone is not a reliable indication of how long it can be kept in a secure environment. For instance, properly stored pasteurised milk can keep its freshness for five days after its sell-by date. In contrast, the use-by dates are no longer important if the milk already contains hazardous microorganisms.

The date when the producer assures the complete potency and safety of a drug is specified by the expiration date of pharmaceuticals. After the expiration date, the majority of pharmaceuticals remain functional and secure for some time. A instance of renal tubular acidosis that was allegedly brought on by tetracycline that had expired is a rare exception. Over a hundred prescription and over-the-counter medications were covered in a study done by the U.S. Food and Drug Administration. According to the study, 90% of them were still safe and useful up to 15 years after their expiration dates. With a few notable exceptions, like nitroglycerin, insulin, and some liquid antibiotics, according to Joel Davis, a former FDA official in charge of expiration-date compliance, the majority of expired medications are likely still functional.

Drug producers have financial and liability incentives to specify shorter shelf lives so that consumers are encouraged to discard and repurchase items because shelf life is not significantly studied during drug development. One notable exception is the U.S. Department of Defense's Shelf Life Extension Programme (SLEP), which, beginning in the middle of the 1980s, asked the FDA to conduct a sizable research on pharmacological efficacy. One complaint is that, despite conducting the study, the U.S. Food and Drug Administration (FDA) refused to create regulations based on SLEP findings for routine pharmaceutical marketing. Scientific information could not be disclosed to the general public, public health departments, other governmental organisations, or pharmaceutical companies, according to a memo signed by the SLEP and the FDA. Programmes from the state and localities are not allowed to take part. Foreign governments have refused donations of due to the lack of data sharing. Prescription drugs that have expired. One exception happened in 2010 when the FDA approved Tamiflu that had gone out of date based on SLEP Data. The US military regularly uses a wide range of SLEP tested items past their declared shelf life if the drugs have been stored properly. The SLEP discovered that medications like Cipro remained effective nine years after their shelf life.

Some food and medicinal products may contain preservatives and antioxidants to increase their shelf life. To help extend the shelf life of their products where oxygen causes the loss, several businesses use induction sealing and vacuum/oxygen-barrier pouches.

**According to the DoD Shelf-Life Programme, shelf-life is:**

The total amount of time a product may be stored in the combined wholesale (including manufacturer's and retail storage systems) and retail systems, starting with the date of manufacture, date of cure (for elastomeric and rubber products only), date of assembly, or date of pack (for only subsistence), and ending with the date by which the product must be used (expiration date), or be subject to inspection, test, restoration, or disposal action. Service-life, which is not to be confused with shelf-life, is a broad phrase used to describe the typical or average life expectancy of a product or piece of equipment while in operation.

Shelf-life management ends and service life starts when a shelf-life item is unpacked and introduced to mission requirements, installed into the intended application, or simply left in storage, put in pre-expired bins, or held as bench stock.

An item's packing, distribution method, and shelf life are frequently specified together. The shelf life of an MRE field ration, for instance, is three years at 80 °F (27 °C) and six months at 100 °F (38 °C).

**Temperature regulation**

At room temperature, almost all chemical reactions are possible (although they all happen at various speeds). High temperatures, on the other hand, speed up most responses, including the deterioration of food and medicine. The conversion of numerous chemical bombs into more unstable chemicals follows the same logic.Old [explosives](https://en.wikipedia.org/wiki/Explosive_material) are thus more dangerous (i.e. liable to be triggered to explode by very small disturbances, even trivial jiggling) than more recently manufactured explosives. [Rubber](https://en.wikipedia.org/wiki/Rubber) products also degrade as [sulphur](https://en.wikipedia.org/wiki/Sulfur) [bonds](https://en.wikipedia.org/wiki/Chemical_bond) induced during [vulcanization](https://en.wikipedia.org/wiki/Vulcanization) revert; this is why old [rubber bands](https://en.wikipedia.org/wiki/Rubber_band) and other rubber products soften and get crispy, and lose their elasticity as they age.

Because activation energy barriers are more easily overcome at higher temperatures, the commonly mentioned rule of thumb states that chemical reactions double their rate for every 10 °C (18 °F) rise in temperature. There are numerous restrictions and exceptions, though, as with many generalisations. The rule is most effective for reactions with activation energies of about 50 kJ/mole, many of which are significant at the temperatures we typically experience. It is frequently and occasionally incorrectly used in shelf life estimation. Many people believe that increasing the temperature by 15 °C (27 °F) can replicate 'triple time' in practise. For example, storing a product for one month at 35 °C (95 °F) simulates three months at 20 °C (68 °F). This is inaccurate technically since the rule is simply a rough approximation and cannot always be depended upon; if it were exact, the required temperature increase would be roughly 15.8 °C (28.4 °F).

The same is true, up to a point, of the chemical reactions of living things. They are usually catalyzed by [enzymes](https://en.wikipedia.org/wiki/Enzyme) which change reaction rates, but with no variation in catalytic action, the rule of thumb is still mostly applicable. In the case of [bacteria](https://en.wikipedia.org/wiki/Bacterium) and [fungi](https://en.wikipedia.org/wiki/Fungi), the reactions needed to feed and reproduce speed up at higher temperatures, up to the point that the proteins and other compounds in their cells themselves begin to break down, or [denature,](https://en.wikipedia.org/wiki/Denaturation_%28biochemistry%29) so quickly that they cannot be replaced. This is why high temperatures kill bacteria and other micro-organisms: 'tissue' breakdown reactions reach such rates that they cannot be compensated for and the cell dies. 'Elevated' temperatures below these, on the other hand, cause greater growth and reproduction; if the organism is harmful, this growth and reproduction may reach dangerous levels.

Reactions are accelerated by temperature rises and slowed down by temperature drops. Therefore, they can be cooled to stabilise explosives for longer periods of time, maintain the springiness of rubber bands, or cause bacteria to proliferate more slowly. Since temperature control (refrigeration, insulated shipping containers, regulated cold chain, etc.) often extends shelf life, some medications and commodities require refrigeration. Due to the fact that storing such products is They are also referred to as cargo even when in special storage to emphasise the inherent time-temperature sensitivity matrix because they are temporal in nature and their shelf life depends on the temperature controlled environment.

The temperature history of a shipment can be recorded using temperature data loggers and temporal temperature indicators to assist determine how long it will stay fresh.

The USDA states that "foods kept frozen continuously are safe indefinitely."

**Merchandise Packaging**

Barrier packaging frequently aids in regulating or extending shelf life. Packaging with a low moisture vapour transmission rate and the use of desiccants help keep the moisture in the package below acceptable ranges when moisture content is a mechanism for product deterioration. Packaging with a low oxygen transmission rate and the use of oxygen absorbers can assist increase shelf life when oxidation is the main issue. Produce and other products that breathe frequently need packaging with well calibrated barrier qualities. For some products, using a modified environment in the box might increase the shelf life. There is also active packaging that has antibacterial qualities.

**Similar names**

Numerous foods, including those that are frozen, dried, tinned, and other foods, have best before or best by dates. Unlike use-by dates, which signal that a product may no longer be safe to consume beyond the designated date, these dates are merely advisory and speak to the quality of the product. Keeping food past its "best before" date won't necessarily be hazardous, but it could cause it to lose its best flavour and texture. Since salmonella is a particular example that can multiply over time, eggs should be consumed before the best before date, which in the USA is up to 45 days after the eggs are packed.

Sometimes pre-printed labels are used throughout the packing process, making it impractical to write the best before date in an obvious place. In this instance, the label may be printed with the words "best before see bottom" or "best before see lid" and the date may be marked as indicated in a different area. In general, food that has a use-by date listed on the label shouldn't be consumed after that date. This is due to the fact that such meals typically degrade rapidly and could be harmful to health. For certain meals, it's also crucial to closely adhere to the storage recommendations (for instance, if they state that the item needs to be refrigerated)

Toiletries and bathroom items typically have a use-by date expressed in months from the day the item was first opened. A visual of an open tub with the number of months written within is frequently used to represent this (e.g., "12M" implies use the product within 12 months of opening).[19] In a similar vein, several food items specify "eat within X days of opening."

**Open dating**

To establish how long to keep a food product on sale, open dating refers to the use of a date stamped on the box. By guaranteeing that the product is of the highest quality when it is sold, this benefits the consumer. If a use-by date is indicated, it should still be observed and does not overrule an open date.

#### Sell by / display until

These dates are meant to make it easier to manage the inventory in stores. Food that has reached its use by or best before date but has past its sell by or display till date will still be edible if it has been stored properly. Large retailers frequently discard such products because it facilitates stock control; also, wholesalers frequently buy back the expired goods and resell them to discount retailers at significantly reduced clearance sale prices. These procedures lessen the possibility that consumers may purchase food without checking the expiration date and then discover the following day that it cannot be used. Changing the stated date is prohibited in several nations.

To encourage customers to buy the products first and hopefully prevent them from having to be either marked down or thrown away, both of which would result in financial loss, most retailers rotate their inventory by placing the products with the earliest dates to the front of shelving units.

# Beer

#### Freshness date

In the American brewing industry, a freshness date is used to specify the date the beer was bottled or the deadline by which it should be consumed. Beer spoils quickly. Light, air, or bacterial activity can all have an impact on it. Freshness dates serve a similar purpose and are employed as a marketing strategy for beer, despite the fact that it is not legally required to have a shelf life in the United States.

#### Beginnings of freshness dating

As early as late 1935, the General Brewing Company of San Francisco began marketing their Lucky Lager Beer as "Age Dated". Each can lid had a date stamped on it to show that the beer was made before that time. This was done to make sure the beer had been aged properly, not to make sure it was "fresh." When Prohibition ended, so many breweries hurried beer to market before it was ready that consumers were wary of purchasing "green" beer. In 1985, The Boston Beer Company, the company behind Samuel Adams, was one of the first modern brewers to begin including freshness dates on their product labels. Brewers have been gradually increasing the freshness dates they apply to their beer for the past ten years.

Following the Anheuser-Busch company's aggressive marketing of "Born-On dates" beginning in 1996, the practise gained popularity very quickly. There is no consensus on what the date implies, despite the fact that many other brewers have begun to include freshness dates on their products. Some businesses may put the date the beer was bottled on the bottle or can, while others will put the date the beer should be consumed.