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Fatty Acids *see* **Fermentation (Industrial)**: Production of Oils and Fatty Acids.

FERMENTATION (INDUSTRIAL)

Contents

Basic Considerations
Media for Industrial Fermentations
Control of Fermentation Conditions
Recovery of Metabolites
Production of Xanthan Gum
Production of Organic Acids
Production of Oils and Fatty Acids
Colours/Flavours Derived by Fermentation

Basic Considerations

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Introduction

Fermentation processes utilize microorganisms to convert solid or liquid substrates into various products. The substrates used vary widely, any material that supports microbial growth being a potential substrate. Similarly, fermentation-derived products show tremendous variety. Commonly consumed fermented products include bread, cheese, sausage, pickled vegetables, cocoa, beer, wine, citric acid, glutamic acid and soy sauce.

Types of Fermentation

Most commercially useful fermentations may be classified as either solid-state or submerged cultures. In solid-state fermentations, the microorganisms grow on a moist solid with little or no 'free' water, although capillary water may be present. Examples of this type of fermentation are seen in mushroom cultivation, bread-making and the processing of cocoa, and in the manufacture of some traditional foods, e.g. miso (soy paste), saké, soy sauce, tempeh (soybean cake) and gari (cassava), which are now produced in large industrial operations. Submerged fermentations may use a dissolved substrate, e.g. sugar solution, or a solid substrate, suspended in a large amount of water to form a slurry. Submerged fermentations are used for pickling vegetables, producing yoghurt, brewing beer and producing wine and soy sauce.

Solid-state and submerged fermentations may each be subdivided – into oxygen-requiring aerobic processes, and anaerobic processes that must be conducted in the absence of oxygen. Examples of aerobic fermentations include submerged-culture citric acid production by *Aspergillus niger* and solid-state koji fermentations (used in the production of soy sauce). Fermented meat products such as bologna sausage (polony), dry sausage, pepperoni and salami are produced by solid-state anaerobic fermentations utilizing acid-forming bacteria, particularly *Lactobacillus*, *Pediococcus* and *Micrococcus* species. A submerged-culture anaerobic fermentation occurs in yoghurt-making.

Fermentations may require only a single species of microorganism to effect the desired chemical change. In this case the substrate may be sterilized, to kill unwanted species prior to inoculation with the desired microorganism. However, most food fermentations are non-sterile. Typically fermentations used in food processing require the participation of several microbial species, acting simultaneously and/or sequentially, to give a product with the desired properties, including appearance, aroma, texture and taste. In non-sterile fermentations, the culture environment

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may be tailored specifically to favour the desired microorganisms. For example, the salt content may be high, the pH may be low, or the water activity may be reduced by additives such as salt or sugar.

Factors Influencing Fermentations

A fermentation is influenced by numerous factors, including temperature, pH, nature and composition of the medium, dissolved O_2, dissolved CO_2, operational system (e.g. batch, fed-batch, continuous), feeding with precursors, mixing (cycling through varying environments), and shear rates in the fermenter. Variations in these factors may affect: the rate of fermentation; the product spectrum and yield; the organoleptic properties of the product (appearance, taste, smell and texture); the generation of toxins; nutritional quality; and other physico-chemical properties.

The formulation of the fermentation medium affects the yield, rate and product profile. The medium must provide the necessary amounts of carbon, nitrogen, trace elements and micronutrients (e.g. vitamins). Specific types of carbon and nitrogen sources may be required, and the carbon : nitrogen ratio may have to be controlled. An understanding of fermentation biochemistry is essential for developing a medium with an appropriate formulation. Concentrations of certain nutrients may have to be varied in a specific way during a fermentation to achieve the desired result. Some trace elements may have to be avoided – for example, minute amounts of iron reduce yields in citric acid production by *Aspergillus niger*. Additional factors, such as cost, availability, and batch-to-batch variability also affect the choice of medium.

Submerged Fermentations

Fermentation Systems

Industrial fermentations may be carried out either batchwise, as fed-batch operations, or as continuous cultures (**Fig. 1**). Batch and fed-batch operations are quite common, continuous fermentations being relatively rare. For example, continuous brewing is used commercially, but most beer breweries use batch processes.

In batch processing, a batch of culture medium in a fermenter is inoculated with a microorganism (the 'starter culture'). The fermentation proceeds for a certain duration (the 'fermentation time' or 'batch time'), and the product is harvested. Batch fermentations typically extend over 4–5 days, but some traditional food fermentations may last months. In fed-batch fermentations, sterile culture medium is added either continuously or periodically to the inoculated fermentation batch. The volume of the fermenting broth increases with each addition of the medium, and the fermenter is harvested after the batch time.

In continuous fermentations, sterile medium is fed continuously into a fermenter and the fermented product is continuously withdrawn, so the fermentation volume remains unchanged. Typically, continuous fermentations are started as batch cultures and feeding begins after the microbial population has reached a certain concentration. In some continuous fermentations, a small part of the harvested culture may be recycled, to continuously inoculate the sterile feed medium entering the fermenter (Fig. 1(D)). Whether continuous inoculation is necessary depends on the type of mixing in the fermenter. 'Plug flow' fermentation devices (Fig. 1(D)), such as long tubes that do not allow back mixing, must be inoculated continuously. Elements of fluid moving along in a plug flow device behave like tiny batch fermenters. Hence, true batch fermentation processes are relatively easily transformed into continuous operations in plug flow fermenters, especially if pH control and aeration are not required. Continuous cultures are particularly susceptible to microbial contamination, but in some cases the fermentation conditions may be selected (e.g. low pH, high alcohol or salt content) to favour the desired microorganisms compared to potential contaminants.

In a 'well-mixed' continuous fermenter (Fig. 1(C)), the feed rate of the medium should be such that the dilution rate, i.e. the ratio of the volumetric feed rate to the constant culture volume, remains less than the maximum specific growth rate of the microorganism in the particular medium and at the particular fermentation conditions. If the dilution rate exceeds the maximum specific growth rate, the microorganism will be washed out of the fermenter.

Industrial fermentations are mostly batch operations. Typically, a pure starter culture (or seed), maintained under carefully controlled conditions, is used to inoculate sterile Petri dishes or liquid medium in the shake flasks. After sufficient growth, the pre-culture is used to inoculate the 'seed' fermenter. Because industrial fermentations tend to be large (typically 150–250 m^3), the inoculum is built up through several successively larger stages, to 5–10% of the working volume of the production fermenter. A culture in rapid exponential growth is normally used for inoculation. Slower-growing microorganisms require larger inocula, to reduce the total duration of the fermentation. An excessively long fermentation time (or batch time) reduces productivity (amount of product produced per unit time per unit volume of fermenter), and increases costs. Sometimes inoculation spores,

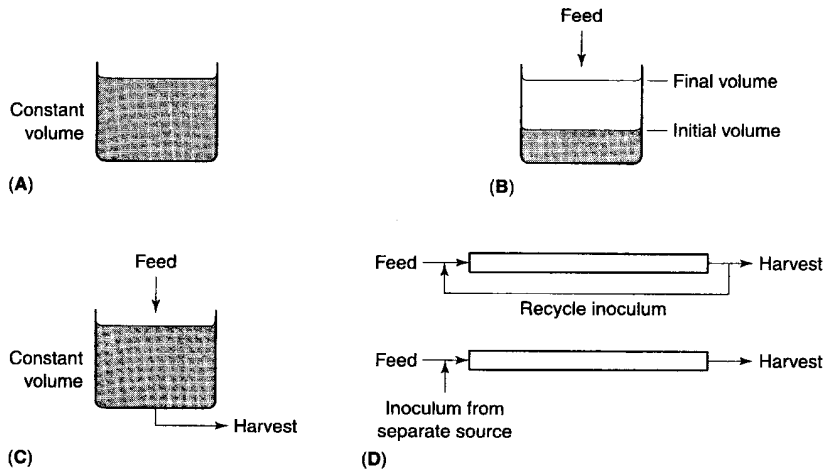

Figure 1 Fermentation methodologies. (**A**) Batch fermentation. (**B**) Fed-batch culture. (**C**) Continuous-flow well-mixed fermentation. (**D**) Continuous plug flow fermentation, with and without recycling of inoculum.

produced as seeds, are blown directly into large fermentation vessels with the ingoing air.

Microbial Growth

Microbial growth in a newly inoculated batch fermenter typically follows the pattern shown in **Figure 2**. Initially, in the lag phase, the cell concentration does not increase very much. The length of the lag phase depends on the growth history of the inoculum, the composition of the medium, and the amount of culture used for inoculation. An excessively long lag phase ties up the fermenter unproductively – hence the duration of the lag phase should be minimized. Short lag phases occur when: the composition of the medium and the environmental conditions in the seed culture and the production vessel are identical (hence less time is needed for adaptation); the dilution shock is small (i.e. a large amount of inoculum is used); and the cells in the inoculum are in the late exponential phase of growth. The lag phase is essentially an adaptation period in a new environment. The lag phase is followed by exponential growth, during which the cell mass increases exponentially. Eventually, as the nutrients are exhausted and inhibitory products of metabolism build up, the culture enters a stationary phase. Ultimately, starvation causes cell death and lysis, and hence the biomass concentration declines.

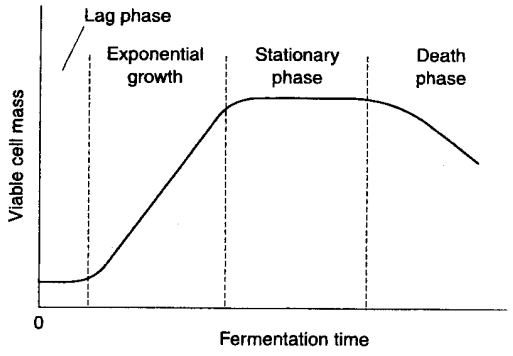

Figure 2 Typical growth profile of microorganisms in a submerged culture.

Exponential growth can be described by **Equation 1**:

$$\frac{dX}{dt} = \mu X - k_d X \qquad \text{(Equation 1)}$$

where: X is the biomass concentration at time t; μ is the specific growth rate (i.e. growth rate per unit cell mass); and k_d is the specific death rate. During exponential growth, the specific death rate is negligible and Equation 1 reduces to **Equation 2**:

$$\frac{dX}{dt} = \mu X \qquad \text{(Equation 2)}$$

For a cell mass concentration X_0 at the beginning of the exponential growth (X_0 usually equalling the concentration of inoculum in the fermenter), and taking the time at which exponential growth commences as zero, Equation 2 can be integrated to produce **Equation 3**:

$$\ln\frac{X}{X_0} = \mu t \qquad \text{(Equation 3)}$$

Using Equation 3, the biomass doubling time, t_d, can be derived (**Equation 4**):

$$t_d = \frac{\ln 2}{\mu} \qquad \text{(Equation 4)}$$

Doubling times typically range over 45–160 min. Bacteria generally grow faster than yeasts, and yeasts multiply faster than moulds. The maximum biomass concentration in submerged microbial fermentations is typically 40–50 kg m^{-3}.

The specific growth rate μ depends on the concentration S of the growth-limiting substrate, until

the concentration is increased to a non-limiting level and μ attains its maximum value μ_{max}. The dependence of the growth rate on substrate concentration typically follows Monod kinetics. Thus the specific growth rate is given as **Equation 5**:

$$\mu = \mu_{max} \frac{S}{k_s + S} \qquad \text{(Equation 5)}$$

where k_s is the saturation constant. Numerically, k_s is the concentration of the growth-limiting substrate when the specific growth rate is half its maximum value.

An excessively high substrate concentration may also limit growth, for instance by lowering water activity. Moreover, certain substrates inhibit product formation, and in yet other cases, a fermentation product may inhibit biomass growth. For example, ethanol produced in the fermentation of sugar by yeast can be inhibitory to cells. Multiple lag phases (or diauxic growth) are sometimes seen when two or more growth-supporting substrates are available. As the preferentially-utilized substrate is exhausted, the cells enter a lag phase while the biochemical machinery needed for metabolizing the second substrate is developed. Growth then resumes. Details of the kinetics of continuous culture, fed-batch fermentation, product formation and more complex phenomena, such as the inhibition of growth by substrates and products, are given in the references listed under Further Reading.

Aeration and Oxygen Demand

Submerged cultures are most commonly aerated by bubbling with sterile air. Typically, in small fermenters, the maximum aeration rate does not exceed 1 volume of air per unit volume of culture broth. In large bubble columns and stirred vessels, the maximum superficial aeration velocity tends to be $< 0.1\ m\ s^{-1}$. Superficial aeration velocity is the volume flow rate of air divided by the cross-sectional area of fermenter. Significantly higher aeration rates are achievable in airlift fermenters. In these, aeration gas is forced through perforated plates, perforated pipes or single-hole spargers located near the bottom of the fermenter. Because O_2 is only slightly soluble in aqueous culture broths, even a short interruption of aeration results in the available O_2 becoming quickly exhausted, causing irreversible damage to the culture. Thus uninterrupted aeration is necessary. Prior to use for aeration, any suspended particles, microorganisms and spores in the gas are removed by filtering through microporous membrane filters.

The O_2 requirements of a fermentation depend on the microbial species, the concentration of cells, and the type of substrate. O_2 supply must at least equal O_2 demand, or the fermentation will be O_2-limited. O_2 demand is especially difficult to meet in viscous fermentation broths and in broths containing a large concentration of O_2-consuming cells. As a general guide, the capability of a fermenter in terms of O_2 supply depends on the aeration rate, the agitation intensity and the properties of the culture broth. In large fermenters, supplying O_2 becomes difficult when demand exceeds $4–5\ kg\ m^{-3}\ h^{-1}$.

At concentrations of dissolved O_2 below a critical level, the amount of O_2 limits microbial growth. The critical dissolved O_2 level depends on the microorganism, the culture temperature and the substrate being oxidized. The higher the critical dissolved O_2 value, the greater the likelihood that O_2 transfer will become limiting. Under typical culture conditions, fungi such as *Penicillium chrysogenum* and *Aspergillus oryzae* have a critical dissolved O_2 value of about $3.2 \times 10^{-4}\ kg\ m^{-3}$. For bakers' yeast and *Escherichia coli*, the critical dissolved O_2 values are $6.4 \times 10^{-5}\ kg\ m^{-3}$ and $12.8 \times 10^{-5}\ kg\ m^{-3}$ respectively.

The aeration of fermentation broths generates foam. Typically, 20–30% of the fermenter volume must be left empty to accommodate the foam and allow for gas disengagement. In addition, mechanical 'foam breakers' and chemical antifoaming agents are commonly used. Typical antifoams are silicone oils, vegetable oils and substances based on low-molecular-weight polypropylene glycol or polyethylene glycol. Emulsified antifoams are more effective, because they disperse better in the fermenter. Antifoams are added in response to signals from a foam sensor. The excessive use of antifoams may interfere with some downstream separations, such as membrane filtrations – hydrophobic silicone antifoams are particularly troublesome.

Heat Generation and Removal

All fermentations generate heat. In submerged cultures, typically $3–15\ kW\ m^{-3}$ comes from microbial activity. In addition, mechanical agitation of the broth produces up to $15\ kW\ m^{-3}$. Consequently, a fermenter must be cooled to prevent a rise in temperature and damage to the culture. Heat removal tends to be difficult, because typically the temperature of the cooling water is only a few degrees lower than that of the fermentation broth. Therefore industrial fermentations are commonly limited by their heat-transfer capability. The ability to remove heat depends on: the surface area available for heat exchange; the temperature difference between the broth and the cooling water; the properties of the broth and the coolant; and the turbulence in these fluids. The geometry of the fermenter determines the surface area that can be provided for heat exchange. Heat generation

due to metabolism depends on the rate of O_2 consumption, and heat removal in large vessels becomes difficult as the rate of O_2 consumption approaches $5\,kg\,m^{-3}\,h^{-1}$.

A fermenter must provide for heat transfer during sterilization and subsequent cooling, as well as removing metabolic heat. Liquid medium, or a slurry, for a batch fermentation may be sterilized using batch or continuous processes. In batch processes, the medium or some of its components and the fermenter itself are commonly sterilized together in a single step, by heating the medium inside the fermenter. Steam may be injected directly into the medium, or heating may take place through the fermenter wall.

Heating to high temperatures (typically 121°C) during sterilization often leads to undesirable reactions between components of the medium. Such reactions reduce the yield, by destroying nutrients or by generating compounds which inhibit growth. This thermal damage can be prevented or reduced by sterilizing only certain components of the medium in the fermenter and adding other, separately-sterilized components, later. Sugars and nitrogen-containing components are often sterilized separately. Dissolved nutrients that are especially susceptible to thermal degradation may be sterilized by passage through hydrophilic polymer filters, which retain particles of 0.45 μm or more. Even finer filters (e.g. retaining particles of 0.2 μm) are also available.

The heating and cooling of a large fermentation batch takes time, and ties up a fermenter unproductively. In addition, the longer a medium remains at a high temperature, the greater the thermal degradation or loss of nutrients. Therefore, continuous sterilization of the culture medium en route to a presterilized fermenter is preferable, even for batch fermentations. Continuous sterilization is rapid and it limits nutrient loss – however, the initial capital expense is greater, because a separate sterilizer is necessary.

Photosynthetic Microorganisms

Photosynthetic cultures of microalgae and cyanobacteria require light and CO_2 as nutrients. Microalgae such as *Chlorella* and the cyanobacterium *Spirulina* are produced commercially as health foods in Asia. Algae are also cultivated as aquaculture feeds for shellfish.

Typically, open ponds or shallow channels are used for the outdoor photosynthetic culture of microalgae. Culture may be limited by the availability of light, but under intense sunlight, photoinhibition limits productivity. Temperature variations also affect performance.

More controlled production is achieved in outdoor tubular photobioreactors, bubble columns and airlift systems. Tubular bioreactors use a 'solar receiver', consisting of either a continuous tube looped into several U-shapes to fit a compact area, or several parallel tubes connected to common headers at either end. The continuous looped-tube arrangement is less adaptable, because the length of the tube cannot exceed a certain value: photosynthetically-produced O_2 builds up along the tube, and high levels of dissolved O_2 inhibit photosynthesis. The parallel-tube arrangement can be readily scaled up by increasing the number of tubes. Typically, the tubes are 0.05–0.08 m in diameter and the continuous-run length of any tube does not exceed 50 m. However, greater lengths may be feasible, depending on the flow velocity in the tube. The tubular solar receivers may be mounted horizontally, or horizontal tubes may be stacked in a ladder configuration, forming the rungs of the ladder. The latter arrangement reduces the area of land required.

The culture is circulated through the tubes by an airlift pump or other suitable low-shear mechanism. The maximum flow rate is limited by the tolerance of the algae to hydrodynamic stress. The flow velocity is usually $0.3–0.5\,m\,s^{-1}$. The tube diameter is limited by the need to achieve adequate penetration of light. This declines as the cell concentration increases, due to self-shading. Closed, temperature-controlled outdoor tubular systems attain significantly higher productivity than open channels. The protein content of the algal biomass, and the adequacy of the development of colour (chlorophyll) affect the acceptability of the product.

Among other types of culture system, airlift devices tend to perform better than bubble columns because only part of the airlift system is aerated and hence the penetration of light is less affected by air bubbles. Conventional external-loop airlift devices may not be suitable because of the relatively high hydrodynamic shear rates they generate. However, concentric-tube airlift devices, with gas forced into the draft tube (zone of poor light penetration), are likely to perform well. Also, split-cylinder types of airlift system may be suitable. However, the volume of the aerated zone in any airlift device for microalgal culture should not exceed approximately 40% of the total volume of the circulating zones. This way the light blocking effect of bubbles remains confined to a small zone.

Submerged-culture Fermenters

Types The major types of submerged-culture bioreactor are:

- stirred-tank fermenter
- bubble column
- airlift fermenter

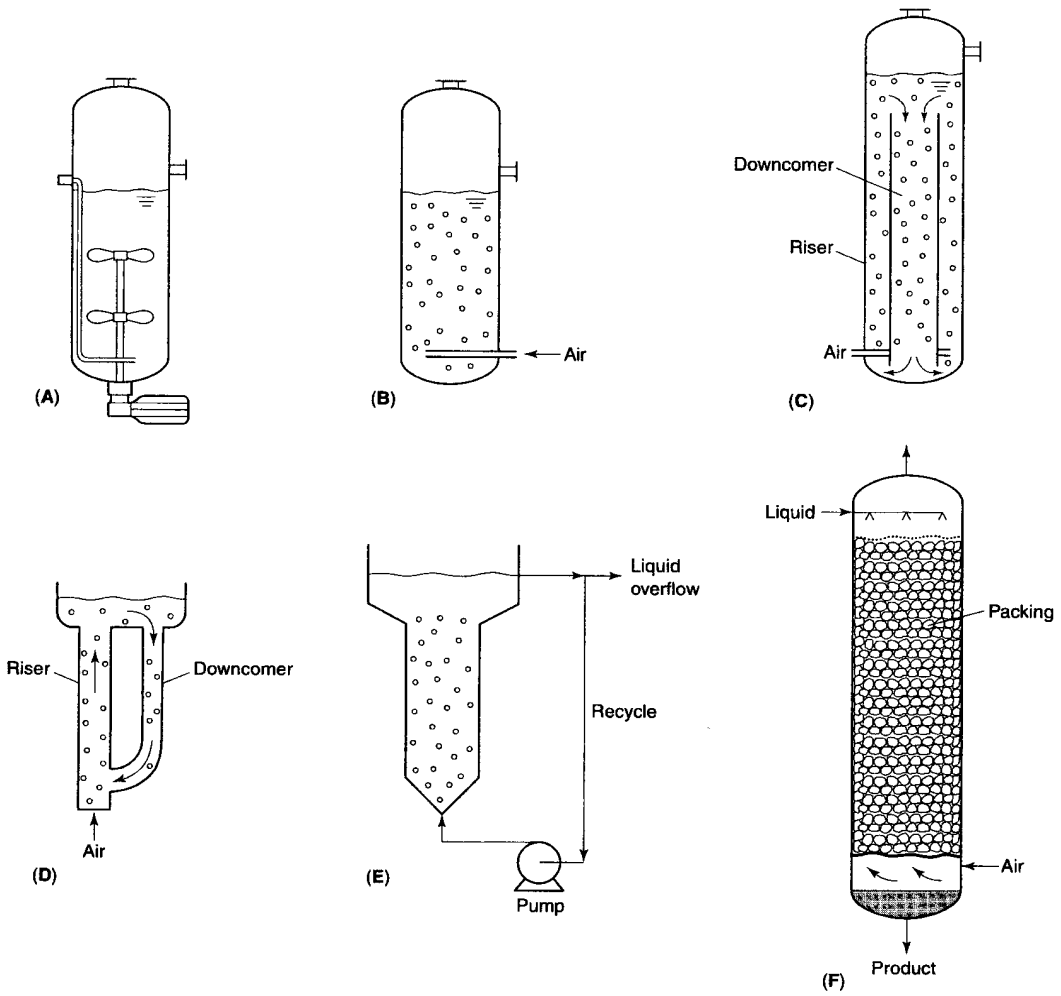

Figure 3 Types of submerged-culture fermenter. **(A)** Stirred-tank fermenter. **(B)** Bubble column. **(C)** Internal-loop airlift fermenter. **(D)** External-loop airlift fermenter. **(E)** Fluidized-bed fermenter. **(F)** Trickle-bed fermenter.

- fluidized-bed fermenter
- trickle-bed fermenter.

These are shown in **Figure 3**.

Stirred-tank Fermenter (See Fig. 3(A).) This is a cylindrical vessel with a working height-to-diameter ratio (aspect ratio) of 3–4. A central shaft supports three to four impellers, placed about 1 impeller-diameter apart. Various types of impeller, that direct the flow axially (parallel to the shaft) or radially (outwards from the shaft) may be used (**Fig. 4**). Sometimes axial- and radial-flow impellers are used on the same shaft. The vessel is provided with four equally spaced vertical baffles, that extend from near the walls into the vessel. Typically, the baffle width is 8–10% of the vessel diameter.

Bubble Column (See Fig. 3(B).) This is a cylindrical vessel with a working aspect ratio of 4–6. It is sparged at the bottom, and the compressed gas provides agitation. Although simple, it is not widely used because of its poor performance relative to other systems. It is not suitable for very viscous broths or those containing large amounts of solids.

Airlift Fermenters (See Figs. 3(C) and 3(D).) These come in internal-loop and external-loop designs. In the internal-loop design, the aerated riser and the unaerated downcomer are contained in the same shell. In the external-loop configuration, the riser and the downcomer are separate tubes that are linked near the top and the bottom. Liquid circulates between the riser (upward flow) and the downcomer (downward flow). The working aspect ratio of airlift fermenters is 6 or greater. Generally, these are very capable fermenters, except for handling the most viscous broths. Their ability to suspend solids and transfer O_2 and heat is good. The hydrodynamic shear is low. The external-loop design is relatively little-used in industry.

Fluidized-bed Fermenter (See Fig. 3(E).) These are

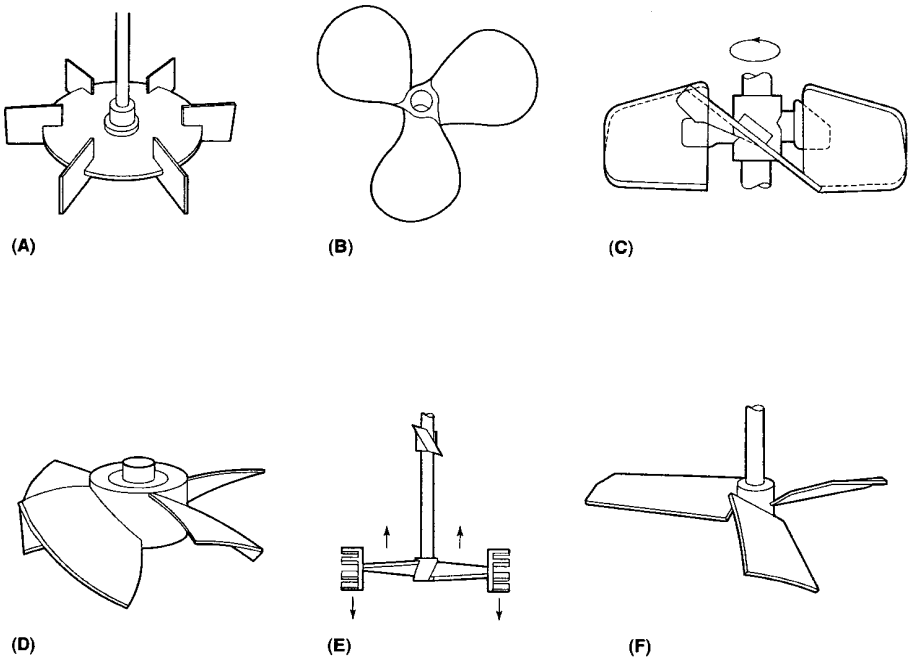

Figure 4 Impellers for stirred-tank fermenters. (**A**) Rushton disc turbine (radial flow). (**B**) Marine propeller (axial flow). (**C**) Lightnin' hydrofoil (axial flow). (**D**) Prochem hydrofoil (axial flow). (**E**) Intermig (axial flow). (**F**) Chemineer hydrofoil (axial flow).

similar to bubble columns with an expanded cross section near the top. Fresh or recirculated liquid is continuously pumped into the bottom of the vessel, at a velocity that is sufficient to fluidize the solids or maintain them in suspension. These fermenters need an external pump. The expanded top section slows the local velocity of the upward flow, such that the solids are not washed out of the bioreactor.

Trickle-bed Fermenter (See Fig. 3(F).) These consist of a cylindrical vessel packed with support material (e.g. woodchips, rocks, plastic structures). The support has large open spaces, for the flow of liquid and gas and the growth of microorganisms on the solid support. A liquid nutrient broth is sprayed onto the top of the support material, and trickles down the bed. Air may flow up the bed, countercurrent to the liquid flow. These fermenters are used in vinegar production, as well as in other processes. They are suitable for liquids with low viscosity and few suspended solids.

Design Irrespective of their configuration, industrial bioreactors for sterile operations are designed as pressure vessels, capable of being sterilized *in situ* with saturated steam at a minimum guage pressure of 0.11 MPa. Typically, the bioreactor is designed for a maximum allowable working pressure of 0.28–0.31 MPa (guage) and a temperature of 150–180°C. The vessels are designed to withstand a full vacuum. Modern commercial fermenters are predominantly made of stainless steel. Type 316L stainless steel is preferred, but the less expensive Type 304L (or 304) may be used in less corrosive situations. Fermenters are typically designed with clean-in-place capability.

A typical submerged-culture vessel has the features shown in **Figure 5**. Sight glasses in the side and top of the vessel allow for easy viewing. The top sight glass can be cleaned during fermentation, using a short-duration spray of sterile water derived from condensed steam. An external lamp is provided, to light the vessel through the sight glass or a separate window. The vessel has ports for sensors of pH, temperature and dissolved O_2. A steam-sterilizable sampling valve is provided. Connections for the introduction of acid and alkali (for pH control), antifoam agents, substrate and inoculum are located above the liquid level in the bioreactor vessel. Additional ports on the top support a foam-sensing electrode, a pressure sensor and sometimes other instruments. Filter-sterilized gas for aeration is supplied through a submerged sparger. Sometimes CO_2 or ammonia may be added to the aeration gas, for pH control.

A harvest valve is located at the lowest point on the fermenter. A mechanical agitator, entering from either the top or the bottom, may be used. The agitator shaft supports one or more impellers, of various designs (Fig. 4). A high-speed mechanical foam breaker may be provided at the top of the vessel, and waste gas may exit through the foam breaker. Commonly, the exhaust gas line also has a heat exchanger, to condense and return water in the gas to the fermenter. The top

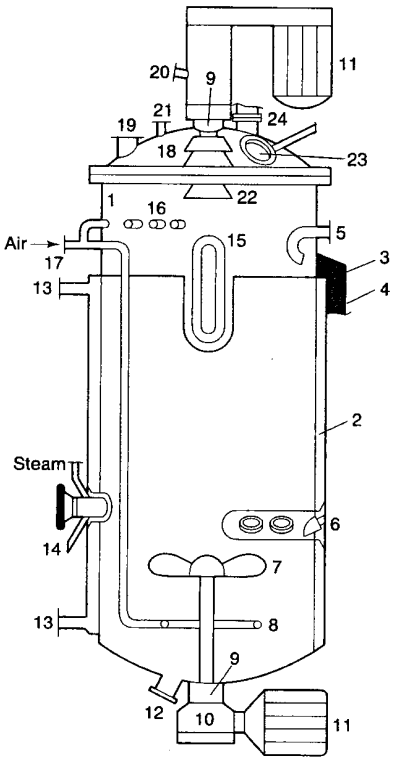

Figure 4 A typical submerged-culture fermenter. (**1**) Reactor vessel. (**2**) Jacket. (**3**) Insulation. (**4**) Protective shroud. (**5**) Inoculum connection. (**6**) Ports for sensors of pH, temperature and dissolved O_2. (**7**) Agitator. (**8**) Gas sparger. (**9**) Mechanical seal. (**10**) Reducing gearbox. (**11**) Motor. (**12**) Harvest nozzle. (**13**) Jacket connections. (**14**) Sample valve with steam connection. (**15**) Sight glass. (**16**) Connections for acids, alkalis and antifoam agents. (**17**) Air inlet. (**18**) Removable top. (**19**) Medium feed nozzle. (**20**) Air exhaust nozzle (connects to condenser, not shown). (**21**) Instrumentation ports for foam sensor, pressure gauge and other devices. (**22**) Centrifugal foam breaker. (**23**) Sight glass with light (not shown) and steam connection. (**24**) Rupture disc nozzle. Vertical baffles are not shown. Baffles are mounted on brackets attached to the wall. A small clearance remains between the wall and the closest vertical edge of the baffle.

of the fermenter is either removable or provided with a manhole. A port on the top supports a 'rupture disc' that is piped to a drain. The disc is intended to protect the vessel in the event of a pressure build-up. The fermentation vessel is jacketed for heat exchange, and the jacket may be covered with fibreglass insulation and a protective metal shroud. Additional surfaces for heat exchange, typically coils, may be located inside the vessel.

The equipment for fermenting slurries containing undissolved solid substrates is identical to that used in submerged-culture processes. Commonly-used slurry fermenters include stirred tanks, bubble columns, and airlift vessels.

Selection Considerations in selecting industrial fermenters are:

1. Nature of substrate solid, liquid, suspended slurry, water-immiscible oils).
2. Flow behaviour (rheology), broth viscosity and type of fluid (e.g. Newtonian, viscoelastic, pseudoplastic, Bingham plastic).
3. Nature and amount of suspended solids in broth.
4. Whether fermentation is aerobic or anaerobic, and O_2 demand.
5. Mixing requirements.
6. Heat-transfer needs.
7. Shear tolerance of microorganism, substrate and product.
8. Sterility requirements.
9. Process kinetics, batch or continuous operation, single-stage or multistage fermentation.
10. Desired process flexibility.
11. Capital and operational costs.
12. Local technological capability and potential for technology transfer.

Solid-state Fermentations

Substrate Characteristics

Water Activity Typically, solid-state fermentations are carried out with little or no free water. Excessive moisture tends to aggregate the substrate particles, and hence aeration is made difficult. For example steamed rice, a common substrate, becomes sticky when the moisture level exceeds 30–35% w/w. Percentage moisture by itself is unreliable for predicting growth: for a given microorganism growing on different substrates, the optimum moisture level may differ widely. This water activity correlates with microbial growth. The water activity of the substrate is the ratio of the vapour pressure of water in the substrate to the saturated vapour pressure of pure water at the temperature of the substrate. Water activity equals 1/100th of the relative humidity (RH%) of the air in equilibrium with the substrate. Typically, water activities of < 0.9 do not support bacterial growth, but yeasts and fungi can grow at water activities as low as 0.7. Thus the low-moisture environment of many solid-state fermentations favours yeasts and fungi.

The water activity depends on the concentrations of dissolved solutes, and so sometimes salts, sugars or other solutes are added to alter the water activity. Different additives may influence the fermentation differently, even though the change in water activity produced may be the same. Furthermore, the

fermentation process itself leads to changes in the water activity, as products are formed and the substrate is hydrolysed, e.g. the oxidation of carbohydrates produces water. During fermentation, the water activity is controlled by aeration with humidified air and, sometimes, by intermittent spraying with water.

Particle Size The size of substrate particles affects the extent and the rate of microbial colonization, air penetration and CO_2 removal, as well as the downstream extraction and handling characteristics. Small particles, with high surface-to-volume ratios, are preferred because they present a relatively large surface for microbial action. However, particles that are too small and shapes that pack together tightly (e.g. flat flakes, cubes) are undesirable because close packing reduces the interparticle voids that are essential for aeration. Similarly, too many fine particles in a batch of larger particles will fill up the voids.

Substrate pH The pH is not normally controlled in solid-state fermentations, but initial adjustments may be made during the preparation of the substrate. The buffering capacity of many substrates effectively checks large changes in pH during fermentation. This is particularly true of protein-rich substrates, especially if deamination of the protein is minimal. Some pH stability can be obtained by using a combination of urea and ammonium sulphate as the nitrogen source in the substrate. In the absence of other contributing nitrogen sources, an equimolar combination of ammonium sulphate and urea is expected to yield the greatest pH stability.

Aeration and Agitation

Aeration plays an important role in removing CO_2 and controlling temperature and moisture. In some cases, an increased concentration of CO_2 may be severely inhibitory, while an increase in the partial pressure of O_2 may improve productivity. Deep layers and heaps of substrate may require forced aeration and agitation. Forced aeration rates vary widely, a typical range being $(0.05–0.2) \times 10^{-3}\,m^3\,kg^{-1}\,min^{-1}$. Occasional turning and mixing improve O_2 transfer and reduce compaction and mycelial binding of the substrate particles. However, excessive agitation is undesirable because continuous agitation damages the surface hyphae – although mixing suppresses sporulation, which is often unwanted. The frequency of agitation may be purely experience-based, as in the occasional turning of a fermenting heap of cocoa beans, or it may be adjusted in response to a temperature controller.

Heat Transfer

The biomass levels in solid-state fermentations, at 10–30 $kg\,m^{-3}$, are lower than those in submerged cultures. However, because there is little water, the heat generated per unit of fermenting mass tends to be much greater in solid-state fermentations than in submerged cultures, and again because there is little water to absorb the heat, the temperature can rise rapidly. The cumulative metabolic heat generation in fermentations producing koji, for the manufacture of a variety of products, has been noted at 419–2387 kJ per kilogram solids. Higher values, up to 13 398 $kJ\,kg^{-1}$, have been observed during composting. Peak heat generation rates in koji processes lie in the range 71–159 $kJ\,kg^{-1}h^{-1}$ but average rates are more moderate, at 25–67 $kJ\,kg^{-1}\,h^{-1}$. The peak rate of production of metabolic heat during the fermentation of readily oxidized substrates, such as starch, can be much greater than that associated with typical koji processes.

The substrate temperature is controlled mostly through evaporative cooling – hence drier air provides a better cooling effect. The intermittent spraying of cool water is sometimes necessary to prevent dehydration of the substrate. The air temperature and humidity are also controlled. Occasionally, the substrate-containing metal trays may also be cooled (by circulating a coolant), even though most substrates are relatively dry and porous, and hence are poor conductors. The intermittent agitation of substrate heaps further aids heat removal. However, despite much effort, temperature gradients in the substrate do occur, particularly during peak microbial growth.

Koji Fermentations

Koji fermentations are widely practised, typical examples of solid-state fermentations. Koji comprises soybeans or grain on which mould is growing, and has been used in oriental food preparation for thousands of years. Koji is a source of fungal enzymes, which digest proteins, carbohydrates and lipids into nutrients which are used by other microorganisms in subsequent fermentations. Koji is available in many varieties, which differ in terms of the mould, the substrate, the method of preparation and the stage of harvest. The production of soy sauce, miso and saké involves koji fermentation. Koji technology is also employed in the production of citric acid in Japan. The production of soy sauce (shoyu in Japanese) koji is detailed below, as an example of a typical industrial solid-substrate fermentation.

The koji for soy sauce is made from soybeans and wheat. Soybeans, or defatted soybean flakes or grits are moistened and cooked (e.g. for 0.25 min or less,

at about 170°C) in continuous pressure cookers. The cooked beans are mixed with roasted, cracked wheat, the ratio of wheat to beans varying with the variety of shoyu. The mixed substrate is inoculated with a pure culture of *Aspergillus oryzae* (or *A. sojae*), the fungal spore density at inoculation being about 2.5×10^8 spores per kilogram of wet solids. After a 3-day fermentation, the substrate mass becomes green-yellow because of sporulation. The koji is then harvested, for use in a second submerged fermentation step. Koji production is highly automated and continuous – processes producing up to $4150\,kg\,h^{-1}$ of koji have been described. Similar large-scale operations are used to produce koji for miso and saké in Japan.

Solid-state Fermenters

Solid-state fermentation devices vary in technical sophistication, from very primitive banana-leaf wrappings, bamboo baskets and substrate heaps to the highly automated machines used mainly in Japan. Some 'less sophisticated' fermentation systems, e.g. the fermentation of cocoa beans in heaps, are quite effective at large-scale processing. Also, some of the continuous, highly mechanized processes for the fermentation of soy sauce, that are successful in Japan, are not suitable for less highly developed locations in Asia. Thus, fermentation practice must be tailored to local conditions.

The use of pressure vessels is not the norm for solid-state fermentation. The commonly used devices are:

- tray fermenter
- static-bed fermenter
- tunnel fermenter
- rotary disc fermenter
- rotary drum fermenter
- agitated-tank fermenter
- continuous screw fermenter.

These are described below. Large concrete or brick fermentation chambers, or koji rooms, may be lined with steel, typically Type 304 stainless steel. For more corrosion-resistant construction, Type 304L and 316L stainless steels are used.

Tray Fermenter This is a simple type of fermenter, widely used in small- and medium-scale koji operations in Asia (see **Fig. 6**). The trays are made of wood, metal or plastic, and often have a perforated or wire-mesh base to achieve improved aeration. The substrate is fermented in shallow (≤ 0.15 m deep) layers. The trays may be covered with cheesecloth to reduce contamination, but processing is non-sterile. Single or stacked trays may be located in chambers in which the temperature and humidity are controlled, or simply in ventilated areas. Inoculation and occasional mixing are done manually, although the handling, filling, emptying and washing of trays may be automated. Despite some automation, tray fermenters are labour-intensive, and require a large production area. Hence the potential for scaling up production is limited.

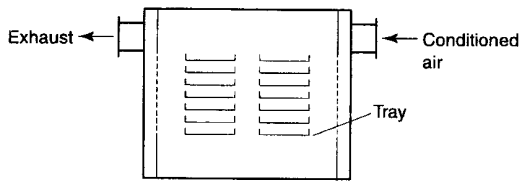

Figure 6 Tray fermenter.

Static-bed Fermenter This is an adaptation of the tray fermenter (**Fig. 7**). It employs a single, larger and deeper, static bed of substrate located in an insulated chamber. O_2 is supplied by forced aeration through the bed of substrate.

Tunnel Fermenter This is an adaptation of the static-bed device (**Fig. 8**). Typically, the bed of solids is quite long but no deeper than 0.5 m. Fermentation using this equipment may be highly automated, by way of mechanisms for mixing, inoculation, continuous feeding and harvest of the substrate.

Rotary Disc Fermenter The rotary disc fermenter consists of upper and lower chambers, each with a circular perforated disc to support the bed of substrate

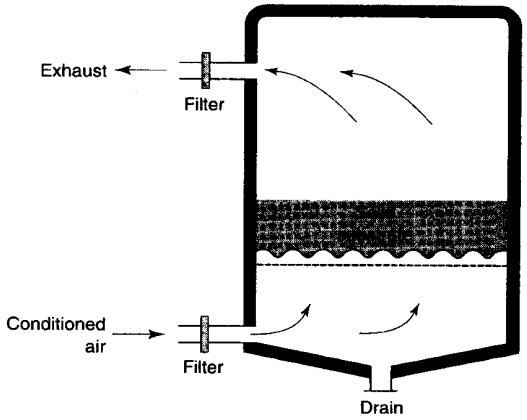

Figure 7 Static-bed fermenter.

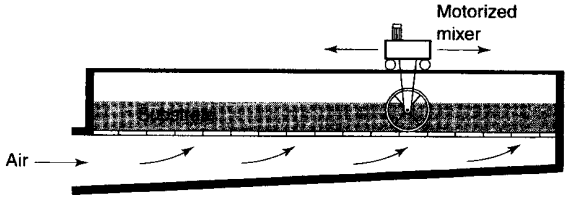

Figure 8 Tunnel fermenter.

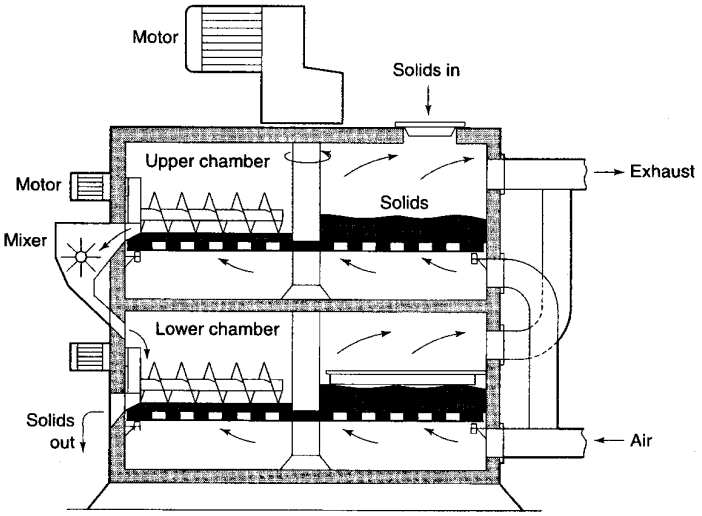

Figure 9 Rotary disc fermenter.

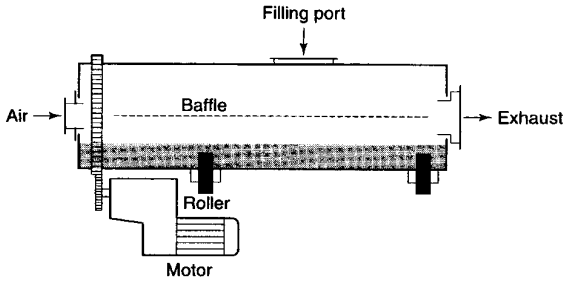

Figure 10 Rotary drum fermenter.

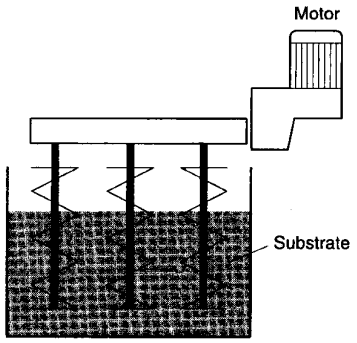

Figure 11 Agitated-tank fermenter.

(**Fig. 9**). A common central shaft rotates the discs. Inoculated substrate is introduced into the upper chamber, and slowly moved to the transfer screw. The upper screw transfers the partly fermented solids through a mixer to the lower chamber, where further fermentation occurs. The fermented substrate is harvested using the lower transfer screw. Both chambers are aerated with humidified, temperature-controlled air. Rotary disc fermenters are used in large-scale koji production in Japan.

Rotary Drum Fermenter The cylindrical drum of the rotary drum fermenter is supported on rollers, and rotated at 1–5 r.p.m. around the long axis (**Fig. 10**). Rotation may be intermittent, and the speed may vary with the fermentation stage. Straight or curved baffles inside the drum aid in the tumbling of the substrate, hence improving aeration and temperature control. Sometimes the drum can be inclined, causing the substrate to move from the higher inlet end to the lower outlet during rotation. Aeration occurs through coaxial inlet and exhaust nozzles.

Agitated-tank Fermenter In this type of fermenter, either one or more helical-screw agitators are mounted in cylindrical or rectangular tanks, to agitate the fermented substrate (**Fig. 11**). Sometimes, the screws extend into the tank from mobile trolleys, that ride on horizontal rails located above the tank. Another stirred-tank configuration is the paddle fermenter. This is similar to the rotary drum device, but the drum is stationary and periodic mixing is achieved by motor-driven paddles supported on a concentric shaft.

Continuous Screw Fermenter In this type of fermenter, sterilized, cooled and inoculated substrate is fed in through the inlet of the non-aerated chamber (**Fig. 12**). The solids are moved towards the harvest port by the screw, and the speed of rotation and the length of the screw control the fermentation time. This type of fermenter is suitable for continuous anaerobic or microaerophilic fermentations.

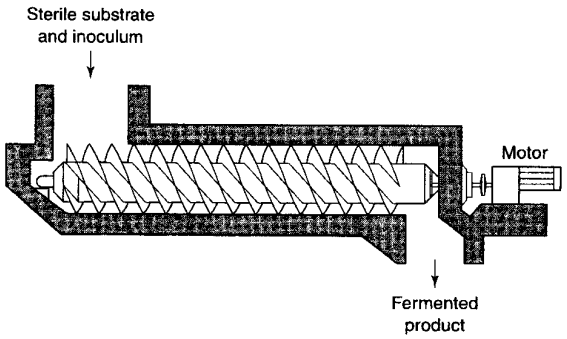

Figure 12 Continuous screw fermenter.

Safe Fermentation Practice

The microorganisms used in certain industrial fermentations are potentially harmful. Certain strains have caused fatal infections in immunocompromised individuals, and rare cases of fatal disease in previously healthy adults have also been reported. Microbial spores and fermentation products, as well as microbes, have been implicated in occupational diseases. Most physiologically active fermentation products are potentially disruptive to health, and certain products are highly toxic. The product spectrum of a given microorganism often depends on the fermentation conditions. Under certain environmental conditions, some organisms, e.g. *Aspergillus flavus* and *A. oryzae*, are known to produce lethal toxins, and specific strains of the blue-veined cheese mould *Penicillium roqueforti* also produce mycotoxins under narrowly defined environmental conditions. Poor operational practice and failings in process and plant design can increase the risks. The safety aspects of industrial fermentations are considered in some of the literature cited under Further Reading. Consumer safety, product quality and the cleanliness of a fermentation product should be ensured by compliance with Good Manufacturing Practices (GMP).

See also: **Fermentation (Industrial)**: Basic Considerations; Control of Fermentation Conditions; Recovery of Metabolites; Production of Xanthan Gum; Production of Organic Acids; Production of Oils and Fatty Acids; Colours/Flavours Derived by Fermentation. **Fermented Foods**: Origins and Applications; Fermented Vegetable Products; Fermented Meat Products; Fermentations of the Far East; Beverages from Sorghum and Millet; Fermented Fish Products. **Fermented Milks**: Range of Products; Yoghurt; Products from Northern Europe; Products of Eastern Europe and Asia.

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Media for Industrial Fermentations

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Introduction

The production of foods and beverages from fermentable carbon sources by microorganisms represents the oldest and most economically significant of all biotechnologies. A wide array of plant- and animal-based complex media for the industrial cultivation of bacteria, fungi and yeasts are employed in the food industry (**Table 1**).

The composition of a fermentation medium in terms of nutrients, their bioavailability, and the