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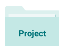

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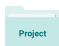

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4 Advanced Fermentation Processes

Leona Paulová, Petra Patáková, and Tomáš Brányik

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4.1 INTRODUCTION

The most common meaning of fermentation is the conversion of a sugar into an organic acid or an alcohol. Fermentation occurs naturally in many foods and humans have intentionally used it since ancient times to improve both the preservation and organoleptic properties of food. However, the term "fermentation" is also used in a broader sense for the intentional use of microorganisms such as bacteria, yeast, and fungi to make products useful to humans (biomass, enzymes, primary and secondary metabolites, recombinant products, and products of biotransformation) on an industrial scale.

Modern industrial fermentation processes used in the food and beverage industry can be described according to different perspectives. In the center of these processes are usually bioreactors, which can be classified with respect to the feeding of the bioreactor (batch, fed-batch, and continuous mode of operation), immobilization of the biocatalyst (free or immobilized cells/enzymes), the characteristic state of matter in the system (submerged or solid substrate fermentations), single strain/mixed culture processes, mixing of the bioreactor (mechanical, pneumatic, and hydraulic agitation), or the availability of oxygen (aerobic, microaerobic, and anaerobic processes). The decision as to which bioreactor or fermentation process should be implemented in any particular application involves considering the advantages and disadvantages of each setup. This includes examining the properties and availability of the primary raw materials, any necessary investment and operating costs, sustainability, availability of a competent workforce, as well as the desired productivity and return on investment (Inui et al., 2010). Since in large-scale applications, each fermentation system needs to operate efficiently and reliably, the major criterion for the selection of a bioreactor/fermentation process remains the minimum for capital costs per unit of product recovered. Simultaneously, with efficient design and operation, in large-scale processes, the issues concerning by-product and wastewater management are inevitable.

4.2 TYPES OF FERMENTATION PROCESSES

4.2.1 Submerged Cultivation

Submerged cultivation of microbial cells in bioreactors guarantees a controlled environment for the efficient production of high-quality end products and to achieve optimum productivity and yield. Industrial bioreactors operated in batch, fed-batch, or continuous mode are utilized to culture different types of microorganisms producing a wide range of products. In the following sections, different approaches to submerged cultivation of microorganisms in bioreactors are discussed briefly and the typical features, benefits, and drawbacks of each cultivation mode are highlighted. Finally, the relevant applications for batch, fed-batch, and continuous cultivation of microorganisms in liquid media used in the production of different types of food industry products are demonstrated.

4.2.1.1 Batch Cultivation

Batch culture represents a closed system in which the medium, nutrients, and inoculum are added to the bioreactor, mostly under aseptic conditions, at the beginning of cultivation (Figure 4.1a), that is, the volume of the culture broth in the bioreactor is theoretically constant during cultivation (practically, small deviations in culture volume are caused by a low feed rate of acid/base solutions to keep the pH at a desired level and by sampling or introducing air/gas into the culture; on balance, such changes are usually ignored due to their small value relative to the total working volume of the bioreactor).

Typically, at the beginning of batch cultivation, a known number of viable cells are inoculated into the bioreactor that is already filled with sterilized medium

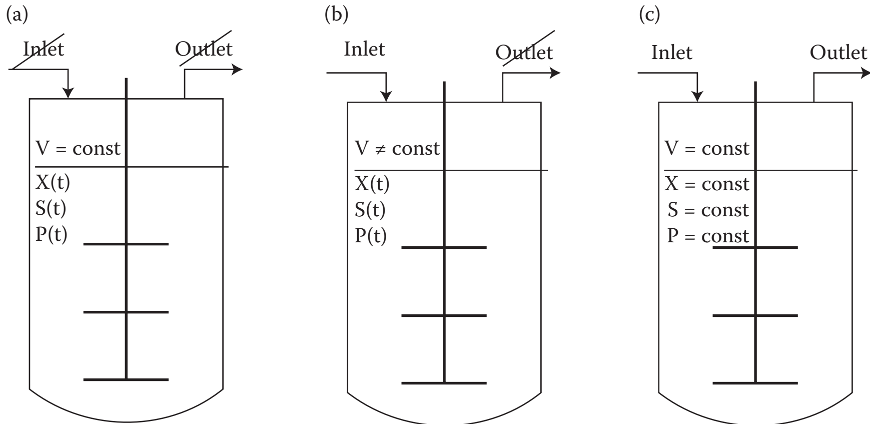

FIGURE 4.1 Simplified scheme of (a) batch, (b) fed-batch, and (c) continuous cultivation.

containing all nutrients. After inoculation, the cell culture follows the classical growth curve described by Monod (1949), which is divided into four main phases. As the lag phase is an "inefficient" stage of culture (even though the cells are metabolically active—they are adapting their enzymatic apparatus to a new environment; no significant increases in biomass concentration, substrate consumption, or product synthesis are observed), it is desirable to shorten it as much as possible. The length of the lag phase is influenced mainly by the concentration of cells in the inoculum and their physiological state, the composition of the inoculation and cultivation medium (mainly the source of carbon and energy, pH, and temperature), and the size of the inoculum. The exponential (logarithmic growth) phase is characterized by rapid cell proliferation (biomass concentration is an exponential function of time), constant specific growth rate, which is equal to the maximum specific growth rate of the culture under conditions of absence of growth limitation (growth rate is not limited because all nutrients are present in excess, while also not attaining growth-inhibiting concentrations), fast consumption of the source of carbon and energy, and a high rate of primary metabolite production. The depletion of nutrients by the end of the exponential phase (in the case of aerobically grown cultures, these are signaled by a rapid increase in dissolved oxygen concentration) causes a progressive reduction in the specific growth rate and a transition to the stationary phase, characterized by the stagnation of growth and utilization of endogenous reserves of carbon and energy; this phase is important for the synthesis of secondary metabolites. Most industrial bioreactors are operated in batch mode due to the relative simplicity of this process. The whole batch operation consists of several steps, including medium formulation, filling the bioreactor, sterilization in place (SIP systems), inoculation, cultivation, product harvesting, and bioreactor cleaning in place (CIP systems). For efficient performance of batch operation, it is important to minimize all nonproductive steps (all steps listed above except cultivation), achieve a high rate of product synthesis, optimize productivity, and maximize the yield of the end product. The performance of any particular batch operation is thus influenced by the type of end product—an

extension of exponential growth is advantageous for the efficient production of biomass (baker's yeasts, feed biomass) or primary metabolites (ethanol, acetic, citric, or lactic acids), whereas in the case of secondary metabolite production, the exponential phase is shortened (by the limitation of one nutrient, usually the source of nitrogen) and the stationary phase is prolonged to achieve the maximum yield of the product.

Submerged batch cultivation can be used for the production of alcoholic beverages (beer, wine, and distilled spirits such as whisky, brandy, rum, and others), organic acids used in the food industry either as acidifiers or as preservatives (citric, acetic (vinegar), and lactic acids), and amino acids used as flavor enhancers (e.g., monosodium glutamate) or sweeteners (e.g., aspartate).

For distilled spirits, the fermentation of wort during Scotch whisky production is taken as an example. Washbacks, simple cylindrical fermentation vessels (volume 250–500 m^3) for the production of distilled spirits are made either from wood or from stainless steel. Although wood washbacks are difficult to clean and sanitize, they are still used, especially in malt whisky distilleries. Wort to be fermented is pumped to the washback, cooled to 20°C, and inoculated with either fresh or dried yeast cells (Campbell, 2003).

The global production of citric acid reached 1.8×10^6 tonnes in 2010 (F.O. Licht data); 90% of this was produced by microbial (*Aspergillus niger*) synthesis from sugar- or starch-containing materials (sugar beet, sugarcane molasses, and corn) and about 60% of this amount was consumed in the food industry. Although citric acid can be produced at an industrial scale using surface liquid cultivation, solid-state cultivation, or submerged liquid cultivation, nowadays, the latter predominates. Submerged cultivation is carried out in stirred bioreactors (capacity 150–200 m^3) or bubble columns (capacity up to 1000 m^3), usually operating aerobically for 4–10 days until the citric acid concentration reaches 10–15% w/v (Moresi and Parente, 2000; Soccol et al., 2006).

4.2.1.2 Fed-Batch Cultivation

Fed-batch culture represents a semi-open system in which one or more nutrients are aseptically and gradually added to the bioreactor while the product is retained inside (Figure 4.1b); that is, the volume of the culture broth in the bioreactor increases within this time. The main advantages of fed-batch over batch cultures are: (a) the possibility to prolong product synthesis, (b) the ability to achieve higher cell densities and thus increase the amount of the product, which is usually proportional to the concentration of the biomass, (c) the capacity to enhance yield or productivity by controlled sequential addition of nutrients, and (d) the feature of prolonged productive cultivation over the "unprofitable periods" when the bioreactor would normally be prepared for a new batch.

Fed-batch is advantageously used in processes (a) where substrate inhibition or catabolic repression is expected; this problem can be overcome by using a "safe" concentration of the substrate in batch mode followed by feeding the remaining substrate within fed-batch operation, (b) where a Crabtree effect (repression of yeast respiratory enzymes by high concentrations of glucose) is expected (de Deken, 1966); by gradual feeding of the substrate, the production of ethanol by yeasts can be eliminated under aerobic conditions, (c) where a high cell density is required; a high

and constant specific growth rate can be maintained by exponential feeding of the substrate, (d) where a high production rate should be achieved; cell metabolism can be regulated by precise sequential feeding of nutrients, and (e) where a high viscosity of culture broth is expected (e.g., production of dextran or xanthan); a gradual dilution of the medium can overcome the problems of mixing and oxygen transfer.

There are many methods of adding a substrate to the bioreactor (either as a concentrated solution of a sole carbon and energy source or as a medium containing carbon plus other nutrients); the proper choice of the nutrient feeding rate can enhance the culture performance considerably since it influences cellular growth rate, cell physiology, and the rate of product formation. The common feeding strategies are: (a) discontinuous feeding, achieved by regular or irregular pulses of substrates and (b) regular continuous feeding of nutrients designed according to a precalculated profile (Figure 4.2) or based on the feedback control of online measured variables associated with cell growth and metabolism, for example, dissolved oxygen concentration, pH, CO_2, evolution rate, and biomass concentration.

The typical food fed-batch fermentations are large-scale production of baker's yeast, pure ethanol, which is further utilized for alcoholic beverages produced by mixing ingredients such as liquors or cordials, and submerged acetification for vinegar production.

Baker's yeast (*Saccharomyces cerevisiae)*, which is distributed as compressed, dried, or instant biomass, is valued for its dough-leavening ability.

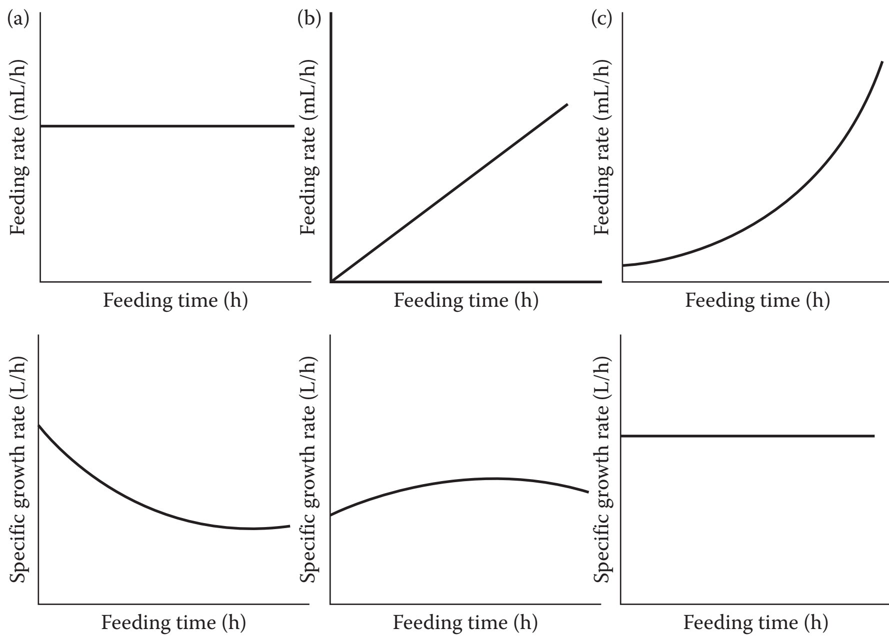

FIGURE 4.2 Relationship between precalculated profiles of substrate feeding and specific growth rates of cell culture. (a) Constant feeding rate, (b) linearly increasing feeding rate, and (c) exponential feeding rate.

Yeast production is the only technology in which the respiratory metabolism of *S. cerevisiae,* leading to high biomass yield, is stressed. Nevertheless, due to the Crabtree effect, that is, the formation of ethanol under aerobic conditions in the presence of excess substrate, the only alternative for producing baker's yeast is fed-batch cultivation. The main bottleneck of large-scale baker's yeast production is the control of nutrient medium inflow, which was traditionally based on empirical data. Currently, many feeding methods utilizing different approaches have been developed, for example, a logistic feeding profile (Borowiak et al., 2012), evolutionary optimization of genetic feeding algorithms (Yüzgeç et al., 2009), fuzzy control (Karakuzu et al., 2006), pulsed feeding optimization (Kasperski and Miśkiewitz, 2008), and others.

In the European Union (EU), the annual production of vinegar, comprising 10% (w/v) acetic acid, is estimated to be about 5×10^6 hL (García-García et al., 2009). Human knowledge of vinegar production dates back to ancient history and several distinct production methods have been used, for example, surface oxidation (Orleans process, slow vinegar production), the quick vinegar process using a trickle-bed bioreactor, or submerged acetification. The most modern process is submerged acetification, in which acetic acid (vinegar) is produced by *Acetobacter*-mediated oxidation of ethanol, and takes place in special "acetator" bioreactors. The most common acetator, a Frings Acetator®, differs from the usual bioreactors by using a special rotor/stator turbine aerator. The aerator with a double function (aeration and mixing), consists of a rotor placed under the bioreactor and connected to an air-suction pipe surrounded by a stator. The aerator is self respiring, that is, during rotation (speed about 1500 rpm), it sucks air and pumps liquid, which causes the formation of an air–liquid mixture that is radially injected into the culture medium. The foam is broken by a mechanical defoamer and because oxidation is an exothermal process, cooling is necessary. The acetators are operated in repeat fed-batch mode and one cultivation cycle in a single acetator can produce vinegar containing 15% (w/v) of acetic acid. In addition, a dual-stage high-strength fermentation process, which allows the culture to generate up to 20.5% (w/v) acetic acid, has been developed (Ebner et al., 1996; García-García et al., 2009).

4.2.1.3 Continuous Cultivation

Continuous culture represents an open system in which nutrients are aseptically and continuously added to the bioreactor, and the culture broth (containing cells and metabolites) is removed at the same time (Figure 4.1c), that is, the volume of the culture broth is constant due to a constant feed-in and feed-out rate.

Frequently, continuous culture is used as a synonym for a chemostat, represented by a constant specific growth rate of cells, which is equal to the dilution rate and is controlled by the availability of the limiting nutrient, although other types of continuous operation such as turbidistat (a constant concentration of biomass controlled by the dilution rate) or nutristat (a constant parameter related to cell growth controlled by the dilution rate) can be employed. The main advantages of continuous culture (chemostat) over the batch mode are (a) the possibility to set up optimum conditions for maximum and long-term product synthesis, (b) the ability to achieve stable product quality (the steady state is characterized by a homogeneous cell culture

represented by a constant concentration of biomass and metabolites), and (c) a distinct reduction in "unprofitable" periods of the bioreactor operation.

In spite of these advantages, there are also several problems that hamper the extensive utilization of continuous operation on a large scale. These include (a) increased risk of contamination due to the pumping of the medium in and out of the bioreactor, (b) the danger of genetic mutations in the production strain in a long-term operation, and (c) additional investments may be required for technical facilities.

Despite the producer's proclamations, only a few food or feed production systems employing microorganisms are operated in a genuine continuous mode, where continuous fermentation is defined as a process running in one or more bioreactors at a stable dilution rate. One of the rare examples of this is fodder yeast (*Kluyveromyces fragilis*) production using spent sulfite liquor as the substrate, which is operated in the Czech Republic (http://www.biocel.cz/e_html/index.htm). The main reason why this process can be performed continuously is the low sensitivity of the production medium to possible contamination. At the same time, this process can be taken as a rare example of industrial-scale production using a highly valued substrate, lignocellulosic hydrolysate, which is obtained as a waste product from pulp production.

In other food applications such as in modern distilleries, a semicontinuous fermentation operated in a series of fermentors is usually used instead of genuine continuous fermentation. The current estimates are that only about 16% of ethanol in North America is produced in a continuous mode due to problems with contamination (Ingledew and Lin, 2011). Nevertheless, both batch and semicontinuous mode fermentations permit continuous distillation, which is the reason why the whole distillery production is often considered as continuous.

Continuous fermentation systems based on immobilized cell technology have also been studied in beer production. Although continuous beer fermentation has been tested as a promising technology for several decades, the number of industrial applications is still limited. The reasons include engineering problems (excess biomass and problems with CO_2 removal, optimization of operating conditions, clogging, and channeling of the reactor) and unrealized cost benefits (carrier price, complex, and unstable operation). The major obstacle hindering the extensive industrial exploitation of this technology is the difficulty in achieving the correct balance of sensory compounds in a short time typical for continuous systems. However, recent developments in reactor design and in our understanding of immobilized cell physiology, together with applications of novel carrier materials, could provide a new stimulus to research and potential applications of this promising technology (Brányik et al., 2008).

4.2.2 Solid Substrate Fermentation

The term "solid substrate fermentation" (SSF) or "solid substrate cultivation" (SSC) is used for systems where microorganisms are cultured on the surface of a concentrated water-insoluble substrate (usually containing polysaccharides as a carbon and energy source) with a low level of free water. This technique was developed in the Eastern countries, where it has been used for centuries for the production of traditional foods such as soy sauce, koji, miso, or sake, using different substrates and

microorganisms. In the Western countries, it has not been widely exploited and its application is limited mainly to the production of industrial enzymes, certain food products, or feed supplements. In the following section, typical features, advantages, and problems of SSF together with relevant applications in both the food and feed industries are discussed.

4.2.2.1 Typical Features of Solid Substrate Fermentation

Solid substrate fermentation is characterized by very low water activity (the relative humidity of the gaseous phase in equilibrium with the moist solid is significantly below 1) (Hölker and Lenz, 2005); thus, the main features of this system are substantially different compared with classical submerged cultivation as shown in Table 4.1.

There are several advantages (Ali et al., 2011; Barrios-Gonzáles, 2012) of SSF over the conventional submerged technology such as (a) the use of a concentrated medium, resulting in a smaller reactor volume and lower capital investment costs, (b) the lower risk of contamination with yeasts and bacteria due to low moisture levels and substrate complexity, (c) the simplicity of the technology and low production of effluent water from the process, (d) the higher product yield and easier product recovery, and (e) the use of agricultural wastes as substrates for certain applications (e.g., feed supplements and cellulolytic enzymes).

4.2.2.2 Microorganisms and Substrates Used in SSF Processes

Filamentous fungi are preferable for SSF processes, mainly due to their abilities to (a) grow on substrates with reduced water activity, (b) penetrate their hyphae into

TABLE 4.1
Main Differences between Solid Substrate Fermentation and Submerged Cultivation

Solid Substrate Fermentation	Submerged Cultivation
Low water content of the cultivation medium (40–80%)	Liquid cultivation medium (~95% water content)
Three-phase system: gas–liquid–solid	Two-phase system: gas–liquid
Complex substrate insoluble in water, high local concentration of nutrients	Nutrients are dissolved in water, concentration of nutrients is lower
Nonhomogeneous system, gradient of nutrients	Homogeneous system
Microorganisms are grown on the surface of the solid substrate	Microorganisms are grown in the liquid medium
Gas–liquid and liquid–solid oxygen transfer	Gas–liquid oxygen transfer
Limitations in heat, oxygen, and nutrient transfer	Transport processes are usually not limited (exception can be oxygen transfer)
Heat is removed by using a stream of air or by placing the bioreactor into the temperature-controlled chamber	Cooling is achieved by bioreactor jacket cooling system
Process monitoring and control are difficult	Online monitoring and control of the process are common
High concentration of the product	Product is dissolved in the liquid phase

the solid substrate, and (c) produce exoenzymes (e.g., amylolytic and cellulolytic enzymes), which decompose the polysaccharides (the main carbon source often present in solid substrates). When using yeast, it is necessary to integrate a material pretreatment step (such as steam explosion, acid or alkali treatment, followed by enzymatic digestion or a combination of these) into the process (Jacob, 1991) or to use a mixed culture, wherein the complex cellulosic or starchy material is first degraded by other organisms (usually molds) that are able to produce extracellular enzymes and the released glucose is then consumed by yeasts (or less frequently by bacteria) yielding the desired product.

The efficiency of SSF is highly influenced by the selection of the solid substrate. The substrates suitable for SSF should ideally meet the following requirements: (a) have a porous solid matrix with a large surface area per unit volume (10^3–10^6 m^2/cm^3), (b) should sustain gentle compression and mixing, (c) should contain biodegradable carbohydrates, (d) its matrix should absorb water in the proportion of 1 to several times its dry weight, (e) should have relatively high water activity on the solid/gas interface to support microbial growth, and (f) should absorb the additionally added nutrients such as nitrogen sources (ammonia, urea, and peptides) and mineral salts (Orzua et al., 2009; Raimbault, 1998; Singhania et al., 2009). On the basis of these characteristics, the ideal material consists of small granular or fibrous particles that do not break or stick together. The commonly used solid substrates include wheat, wheat bran, soybean, rice barley, oats, and other cereals (Chisti, 1999).

4.2.2.3 Limitations and Challenges of Solid Substrate Fermentation

Although SSF has many advantages over liquid cultivation, the main challenges are poor heat and mass transfers within the substrate, and limited potential to monitor, online, key cultivation parameters (temperature, pH, dissolved oxygen, nutrient concentrations, or water content), and thus an inability to precisely control the microbial environment (Mitchel et al., 1999). The heat generated by microbial metabolism is a major bottleneck for process scale-up; the released heat can reach up to 3000 kcal from 1 kg of assimilated substrate, causing a radial gradient of 5°C/cm at the center of the reactor (Bellon-Maurel et al., 2003). Low water content in the system and poor heat conductivity of SSF substrates promote the heat gradient, which is accentuated by limited agitation (the molds are very sensitive to shear stress) and is reduced mostly by evaporative cooling, which can conversely exacerbate further water loss. In addition, the pH gradient caused mainly by the production of organic acids and the utilization of proteins poses another challenge; pH monitoring and control is difficult because no existing pH electrodes can operate in the absence of free water (Bellon-Maurel et al., 2003). Therefore, the variations in pH should be prevented by increasing substrate buffering capacity (e.g., by the addition of $CaCO_3$) or by the addition of urea, which can counteract acidification. Oxygen gradients can be reduced by aeration with moist air, which also plays a role in the desorption of carbon dioxide and the regulation of temperature and moisture levels.

4.2.2.4 Industrial Applications of SSF

In Europe, SSF in the food and feed contexts is usually used for the production of mold and ripening cheeses, production of fermented vegetables (e.g., sauerkraut or

pickled cucumbers) or silage (preserved cattle feed), and in Asia for the production of fermented products from soya, rice, or corn.

An example of Oriental SSF is red yeast rice (red rice, ang-kak, anka, ankak, beni-koji, and other names), which is a product obtained after SSF of rice with different *Monascus* species, in the most usual case with *Monascus purpureus*. This product has been known in various Asian countries (China, Japan, Thailand, Indonesia, Taiwan, and Philippines) for centuries and can be used for food coloring, such as red koji (for the production of Kaoliang liqueur) or as a food supplement with an anticholesterolemic effect. The cultivation conditions of *Monascus* SSC of rice differ depending on the intended use. For food coloring, high pigment content (especially oligoketide, red-colored compounds, monascorubramine and rubropunctamine, and their complexes with the amino group-containing compounds) is required; for koji production, the creation of hydrolytic enzymes is most important and in red yeast rice food supplement, a high concentration of monacolin K (lovastatin) is a necessary prerequisite (Martinkova and Patakova, 2000; Patakova, 2005). The main drawback of red yeast rice is its possible contamination with citrinin, which can be overcome by the selection of an appropriate producer strain or by its modification (Jia et al., 2010).

Traditionally, red yeast rice used for coloring of fish meals, soya products, vinegar, Beijing duck, and more recently, also frankfurters or sausages, is produced by successive washing, soaking, draining, and steaming of nonglutinous rice, which is then followed by inoculation with *Monascus*, 7-days incubation at 30°C, and product drying at 45°C or 60°C. Originally, the production took place in wobbled bamboo trays covered with banana leaves in which rice kernels were manually mixed or moistened if necessary (Lin et al., 2008). Nowadays, different types of trays, roller drums, or fluid-bed bioreactors are being used (Chiu et al., 2006).

Mushroom cultivation, which is well accepted by the public, can be considered as a product of a special single-cell protein (SCP) system, which utilizes various types of agricultural and forestry wastes and is relatively simple to perform. The special types of SSF are used for the production of edible mushrooms of *Agaricus, Pleurotus, Lentinula, Flammulina*, and other genera. These comprise the following steps: maintenance of mushroom culture, seed or spawn production, substrate pretreatment, growing, and cropping. The primary rot fungi such as oyster mushroom (*Pleurotus* spp.), shiitake (*Lentinula edodes*), winter mushroom (*Flammulina velutipes*), or paddy straw mushroom (*Volvariella* spp.) degrade the moistened lignocellulosic material such as straw, corn stover, saw dust, wood logs, or stumps as substrates (Rai, 2003). The button mushroom (*Agaricus bisporus*), unlike other species, is a secondary rot fungus that requires compost preparation prior to culture (Rai, 2003). To get high mushroom yields, and because many mushroom species need changes in the environmental conditions to form fruiting bodies, the original methods of outdoor or extensive cultivation have been replaced by intensive mushroom farming that requires the construction of specialized facilities that allow the precise control of environmental factors (temperature, humidity, light, and atmospheric gases). After thermal pretreatment, the substrate is placed into different immobile (shelves) or mobile (plastic bags or containers) systems. Substrate handling, spawn spreading (inoculation), substrate mixing, and moistening, along with cropping, can

be mechanized, for example, special combined harvesters are used for button mushrooms (Chakravarty, 2011; Sánchez, 2004).

Among a wide range of SSF applications, the processes yielding protein-enriched agro-industrial materials that can be used as animal feed play an important role (Ugwuanyi et al., 2009). In the literature, there are reports on the production of protein-enriched animal feeds by SSF using starchy materials as substrates. Besides their characteristics described above, all organisms intended for animal consumption must comply with certain nutritional requirements such as amino acid composition and digestibility, and the absence of toxins, antibiotics, and mainly mycotoxins (Ghorai et al., 2009).

SSF has also been described as a protein enhancement factor for cereal grain and potato residues (Gélinas and Barrete, 2007). The protein content of wheat bran was increased fourfold by SSF of *Aspergillus terreus* (Sabry, 1993).

4.3 INTENSIFICATION OF FERMENTATION PROCESSES

There are several strategies available for the intensification of bioprocesses. Some of them focus on engineering aspects, whereas others exploit the tools of physiological modulation (selection or adaptation of microorganisms), mutagenesis, or genetic manipulation to improve the production strains. Examples of areas where a significant improvement of the fermentation processes can be achieved by engineering approaches are improved mass and heat transfer, reduction of power consumption, high-density cell cultures, and low-shear mixing (Chisti and Moo-Young, 1996). The performance of the bioprocess is both individually and synergistically influenced by all components of the production unit and related know-how (strain, bioreactor, media composition, feeding strategy, etc.). In addition, the biological elements of the process (microorganisms, animal and plant cells, and enzymes) are subject to many processing constraints (fragility, temperature, pH range, and hygienic design of the equipment). These facts place important practical limitations on the bioreactor and bioreaction engineering.

In the last few decades, there has been a significant progress in the area of process control and instrumentation for bioreactors. This has an economic importance because the optimum operation of a fermentation process is associated with improved productivity (high product concentration, high production rate) and savings in product separation. The ability to operate a process at high productivity requires a sound understanding of the biological requirements, process kinetics (limitation, inhibition), and transport phenomena (Erickson, 2011). The following sections provide the principles and examples of some bioprocess intensification methods.

4.3.1 Immobilized Cell Technology

The increased productivity of bioprocesses can be achieved through controlled contact of substrates with a high concentration of the active biocatalyst, enzyme, or microbial cells. These high-cell-density cultures can be created by feeding strategies, cell retention/recycle, or cell immobilization (Bumbak et al., 2011; Schiraldi et al., 2003; Verbelen et al., 2006). Among the strategies to create high cell-density cultures, cell immobilization is the most widely studied and applied in the food and

TABLE 4.2
Examples of Immobilized Cell Applications in Food and Beverage Production

Application	References
Wine	Yokotsuka et al. (1997); Divies and Cachon (2005)
Vinegar	de Ory et al. (2004)
Malolactic fermentation	Kosseva and Kennedy (2004)
Cider	Nedovic et al. (2000)
Beer	Willaert and Nedovic (2006); Brányik et al. (2012)
Meat processing	McLoughlin and Champagne (1994)
Aroma compounds	Rozenbaum et al. (2006)
Pigments	Fenice et al. (2000)
Sweetener	Kawaguti and Sato (2007); Krastanov et al. (2007)
Dairy products	Dimitrellou et al. (2009)
Baking	Plessas et al. (2007)
Nutraceuticals	Tsen et al. (2004)

beverage industries (Karel et al., 1985; Kosseva, 2011). Table 4.2 lists some references of research papers on food applications of immobilized cells.

The maximum immobilized biomass concentration achieved in continuous beer fermentation was up to 10 times greater than the free cell concentration at the end of the conventional batch fermentation (Nakanishi et al., 1993). However, the immobilization of microorganisms provokes different physiological responses when compared to low-cell-density cultures of free cells (Junter et al., 2002) and therefore, their application has to be carefully considered in terms of product quality. For example, the application of immobilized microorganisms in fermentation processes induces modifications in cell physiology due to mass transfer limitations, concentration gradients created by an immobilization matrix, and by aging of the immobilized biomass.

4.3.1.1 Immobilized Cell Physiology

An important factor influencing the growth and metabolic activity of immobilized cells is the microenvironment of the solid immobilization matrix, represented by parameters such as water activity, pH, oxygen, substrate and product concentration gradients, and mechanical stress. The interplay between the appropriate production strains and immobilization methods is very important in immobilized cell reactors and their suitable combination can improve both system performance and product quality. The importance of careful matching of the chosen yeast strain with the immobilization method and the suitable reactor arrangement was demonstrated in beer production (Mota et al., 2011). Although there are a variety of methods for investigating the metabolic state of immobilized cells (monitoring of cellular activity, microscopic, noninvasive, and destructive methods), acquiring the reliable data is still the limiting factor for process optimization (Kosseva, 2011; Pilkington et al., 1998). In addition, the data concerning the physiological conditions of immobilized

microbial cells are rather complex, due to different matrices and variable system configurations, and therefore their interpretation is difficult (Junter and Jouenne, 2004).

Immobilization has been reported to activate some metabolic functions (substrate uptake, product formation, enzyme expression, and activity) of microbial cells (Lohmeier-Vogel et al., 1996; Norton and D'Amore, 1994 ; Van Iersel et al., 2000). According to some authors, the enhanced metabolic activity can also be attributed to surface-sensing responses in immobilized microbial cells (Prakasham et al., 1999) but the reasons are still a matter of controversy. Overall, conclusions should be very carefully drawn from the results, since sampling and sample treatment may also influence the measurements of immobilized cell physiology.

It has been shown that immobilized cells exhibit increased levels of deoxyribonucleic acid (DNA), structural carbohydrates (Doran and Bailey, 1986), glycogen (Galazzo and Bailey, 1990), and fatty acids (Hilge-Rotmann and Rehm, 1991), as well as modifications of cell proteome, cell wall, and cell membrane composition (Jirků, 1995; Parascandola et al., 1997). Not surprisingly, alterations in plasma membrane composition have a profound impact on several enzymes, sensor proteins, transporters, and membrane fluidity. Many reports also underline increased stress resistance of immobilized cells (Junter and Jouenne, 2004; Reimann et al., 2011). The increased resistance to inhibit substances (ethanol, pollutants, antimicrobial agents, etc.) can be ascribed to changes in the composition and organization at the level of the cell wall and plasma membrane (Jirků, 1999) and/or to the protective effect of the immobilization support (Norton et al., 1995).

4.3.1.2 Mass Transfer in Immobilized Cell Systems

The diffusional resistance to substrate transport from the bulk solution to the biocatalyst and the hindered diffusion of products in the opposite direction may represent the most significant mass transfer limitations arising from the use of immobilized cell technology. These mass transfer limitations constitute the most evident hypothesis to explain the often-observed decrease in immobilized cell growth rate and specific productivities as compared to free cell cultures (Abdel-Naby et al., 2000; Taipa et al., 1993). The typical immobilization materials exhibiting internal mass transfer limitations are polymeric matrices (Willaert and Baron, 1996). In these materials used for cell entrapment, the internal mass transfer limitations of cells by nutrients can be further influenced by the position of the cells, bead size, and structure of the polymer. Mass transfer limitations are crucial in immobilized cell systems when oxygen supply to cells and the removal of carbon dioxide are required. Oxygen transfer from the gas phase to the immobilized biocatalyst has long been recognized as the major rate-limiting step in aerobic immobilized cell processes. The most common option to improve mass transfer in these systems is to reduce the bead diameter (Groboillot et al., 1994).

Unlike polymeric matrices, the preformed porous (sintered glass) and nonporous (DEAE-cellulose, wood chips, and spent grains) carriers do not have the additional gel-diffusion barrier. However, depending on the porosity of the carrier and on the amount of biomass adsorbed in the pores, internal mass transfer limitations may also occur (Norton and D'Amore, 1994). In the case of nonporous carriers, internal

mass transfer problems vary with the thickness of the cell layer (biofilm). The yeast adhered in a single layer of DEAE-cellulose showed similar metabolic activities (Šmogrovičová and Dömény, 1999) whereas multilayers of yeast attached to spent grains had a significantly lower specific sugar consumption rate as compared to free cells (Brányik et al., 2004).

4.3.2 Engineering Aspects of Process Intensification

Fermentation processes can be divided into two main categories based on the characteristic state of matter of the medium: solid substrate fermentation (Section 4.2.2) and submerged fermentation. Among the latter, the most common bioreactor configurations used in food and beverage applications are stationary particle bioreactors, such as packed-bed/fibrous-bed, trickle-bed reactors, and mixed (particle) bioreactors, such as fluidized bed, gas lift, bubble column, and stirred tank (Kosseva, 2011; Raspor and Goranovic, 2008; Willaert and Nedovic, 2006). The stationary particle bioreactors are either operated with immobilized cells (enzymes) or a mixture of free and immobilized cells (enzymes), and their typical internal mass transfer issues are discussed in Section 4.3.1.2. Mixed bioreactors may contain solely free or immobilized cells as well as their mixture. There are also bioreactor configurations that do not fit into the two previous categories. For example, rotating biological contactors (RBCs), also classified among moving surface reactors, where a biofilm grows on rotating disks partially or completely immersed in a liquid medium. The use of RBCs in food applications is rare, and is limited to the production of citric acid (Wang, 2000).

The selection of a suitable reactor design from numerous available types and configurations (Zhong, 2011) is a complex task and depends on various factors (Table 4.3). The importance of individual factors may change depending on the process requirements and the product characteristics. However, there is a need to have a fundamental understanding of the kinetics and transport limitations when a bioreactor is selected or when a new bioreactor is designed and constructed. Two-phase bioreactors are generally limited to anaerobic processes or to processes where

TABLE 4.3
Factors Influencing the Selection of Bioreactor Type

Free Cell Bioreactors	
Nature of substrate and product	Biological requirements
Kinetics of product formation	Process conditions (T, pH, and Δp)
Mass transfer considerations	Hygienic considerations
Heat transfer considerations	Scale-up considerations
Hydrodynamic considerations	Ease of fabrication and reactor costs
Process control	Running costs
Immobilized Cell Bioreactors (Additional Factors)	
Method of immobilization	Internal mass transfer in the biocatalyst
Carrier costs	Biocatalyst replacement/regeneration

gas–liquid mass transfer plays a marginal role. Conversely, in three-phase bioreactors, efficient mass transfer usually requires an intimate mixing of all three phases. Three-phase bioreactor design is an area in which significant process intensification can be achieved through the enhancement of gas–liquid mass transfer (Chisti and Moo-Young, 1996; Suresh et al., 2009).

4.3.2.1 Gas–Liquid Mass Transfer Considerations

In aerobic bioprocesses, oxygen is the key substrate due to its low solubility in aqueous media. Consequently, a continuous supply of oxygen into aerobic bioreactors is often needed. Therefore, the oxygen transfer rate (OTR) should be predicted prior to the choice/design and scale-up of a bioreactor. Many studies have been conducted to estimate the efficiency of oxygen transfer in different bioreactors and these have been reviewed in various works (Clarke and Correia, 2008; Garcia-Ochoa and Gomez, 2009; Kantarci et al., 2005; Suresh et al., 2009). Another area where gas–liquid mass transfer rate is crucially important (CO_2 supply and O_2 removal) is the construction and operation of photobioreactors used for the cultivation of photoautotrophic microorganisms (microalgae) with nutritional potential (Carvalho et al., 2006).

The dissolved oxygen concentration in aerobic cultures depends on the rate of oxygen transfer from the gas phase (usually air bubbles) to the liquid, on the rate at which oxygen is transported into the cells, and on rate of oxygen uptake by the microorganism. The transport of oxygen from air bubbles to the site of oxygen consumption can be described in a number of steps, among which oxygen diffusion through the liquid film surrounding the bubble shows the greatest resistance. The gas–liquid mass transfer rate is usually modeled according to the two-film theory and is characterized by the volumetric (gas–liquid) mass (oxygen) transfer coefficient (k_La), while the driving force of the process is the difference between the concentration of oxygen at the interface (C^*) and that in the bulk liquid (C_L). In the case of large microbial pellets, immobilized cells, or fungal hyphae, the resistance in the liquid film surrounding the solid can also be significant (Blanch and Clark, 1997).

Oxygen transfer in aerobic bioprocesses is strongly influenced by the hydrodynamic conditions in the bioreactors. These conditions are known to be affected by the operational conditions (stirrer speed, superficial gas velocity, liquid circulation velocity, etc.), physicochemical properties of the culture (viscosity, density, and surface tension), bioreactor geometry, and also by the presence of oxygen-consuming cells (Garcia-Ochoa and Gomez, 2009).

Stirred tank bioreactors (STBRs) are widely used in a large variety of bioprocesses taking advantage of free cell (enzyme) suspensions. An industrial-scale STBR usually consists of a stainless-steel vessel, motor-driven impeller, and gas sparger positioned below the impeller. Aerated STBRs generally have high mass and heat transfer coefficients, good homogenization, and the capability of handling a wide range of superficial gas velocities. Mass transfer and mixing in STBRs are most significantly affected by stirrer speed, type and number of stirrers, and the gas flow rate (Garcia-Ochoa et al., 2011).

In bioreactors with a height-to-diameter ratio (H/T) above two, standard single-impeller systems were often found to have unsuitable operating parameters. Oxygen transfer in these geometries can be improved by using multiple-impeller

configurations (approximately one impeller per each H/T = 1) that exhibit efficient gas distribution, increased gas holdup, superior liquid-flow characteristics, and lower power consumption per impeller as compared to single-impeller systems (Gogate et al., 2000).

Pneumatic bioreactors consist of a cylindrical vessel, into the bottom (usually) of which air (gas) is introduced to ensure aeration, mixing, and liquid circulation, without any moving mechanical parts. In pneumatically agitated reactors such as bubble columns (random liquid circulation) and airlift reactors (streamlined liquid circulation), the homogeneous shear environment compared to the local shear extremes in STBRs has enabled the successful cultivation of shear-sensitive cells such as mammalian and plant cells or mycelial fungi (Guieysse et al., 2011). In contrast, the lack of mechanical agitation can cause poor mixing in a highly viscous medium and serious foaming under high aeration. Airlift and stirred tank reactors exhibit comparable mass transfer capacities; however, airlift reactors can be superior to STBRs in terms of operating costs because of lower power consumption (Chisti, 1998). A further increase in the overall volumetric gas–liquid oxygen transfer coefficient (k_La) in bubble column and airlift reactors was achieved by the installation of static mixers (Thakur et al., 2003) into the draft tubes and riser sections, respectively (Chisti et al., 1990; Goto and Gaspillo, 1992). The improvement of OTR achieved by static mixers is a result of air bubble breakup increasing the specific gas–liquid interfacial area (a). Industrial applications include the cultivation of a filamentous mold (Gavrilescu and Roman, 1995) and ethanol production in an airlift reactor (Vicente et al., 1999). However, in the second example, increased ethanol productivity was also achieved as a consequence of size reduction of yeast flocs, and thus improved liquid–solid mass transfer, provoked by the new riser design.

The predictions of OTR determined by a dynamic method in sterile culture medium in the absence of biomass, often underestimates the k_La value for the real bioprocess (Djelal et al., 2006). In fermentation processes, an enhancement of OTR was found to be due to oxygen consumed by the microorganisms, leading to a lower dissolved oxygen concentration in the bulk liquid (C_L). Simultaneously, mass transfer enhancement was also attributed to the presence of a dispersed phase (microorganisms) adsorbed onto the gas–liquid interface, influencing the oxygen adsorption rate and gas–liquid interfacial area. The extent of this enhancement was expressed as the biological enhancement factor (E). According to some studies, the E value of k_La can be up to 1.3 times that of the mass transfer coefficient determined for the system without microbial cells (Garcia-Ochoa and Gomez, 2005).

4.4 FUTURE PERSPECTIVES

The development of fermentation processes for the food and beverage industries aims at improving the productivity and product quality by means of process design, strain selection/construction, and process monitoring. In all these areas, there have emerged some very innovative ideas that could lead to economically attractive solutions.

With regard to (online) process monitoring, significant progress is required, particularly in the area of advanced instrumentation and sensor development, for solid substrate fermentations. The innovative techniques described so far include different

sensor technologies, respirometry, x-rays, image analysis, infrared spectrometry, magnetic resonance imaging, and so on. However, for some of them, the main drawback is high cost, which makes these techniques unsuitable for large-scale applications (Bellon-Maurel et al., 2003).

One of the significant challenges in the bioreactor design is the improvement of large-scale photobioreactors and phycocultures (seaweed farms) for the production of micro- and macroalgae and algae-derived food products (Carvalho et al., 2006; Luening and Pang, 2003). Another prospective strategy to increase the metabolic productivity in bioprocesses is the use of suitably controlled ultrasonication. The beneficial effects of ultrasound can be exploited at the level of biocatalysts (cells and enzymes) and their function (e.g., cross-membrane ion fluxes, stimulated sterol synthesis, altered cell morphology, and increased enzyme activity) and sonobioreactor performance (mass transfer enhancement) (Chisti, 2003; Kwiatkowska et al., 2011).

The potential for genetic engineering in the field of food fermentation is indisputable and has been reviewed (Leisegang et al., 2006). However, the nutritional status of fermented foods can also be improved by the rational choice of food-fermenting microbes based on the understanding of their interaction with diet and human gastrointestinal microbiota. In this respect, fermented foods can be regarded as an extension of the food digestion and fermentation processes and can be steered toward beneficial health attributes (Vlieg et al., 2011).

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