4 Microbial Fermentation: Basic Fundamentals and Its Dynamic Prospect in Various Industrial Applications

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Abstract

The term "fermentation" refers to the anaerobic catabolism of sugars present in the extract of fruit or malted grains by yeast to produce carbon dioxide bubbles, which give a boiling appearance to the broth. Classically, unicellular eukaryotic fungus *Saccharomyces cerevisiae*, also referred to as "brewer's yeast," has been used to produce alcohol from malt or fruit extracts on an industrial scale. Thus, alcoholic fermentation using yeast strain is an example of microbial metabolite production. Similarly, several commercially important bioproducts such as microbial enzymes, biomass, recombinant products, and other platform chemicals are produced by microbial fermentation using a wide variety of substrates. Depending on the physical state of the nutrient medium, fermentation processes are broadly divided into submerged and solid-state fermentation. A typical fermentation process has the following components: (1) formulation of nutrient medium for developing the inoculum (producer microorganism) and to be used in the production fermenter; (2) sterilization of the medium, fermenters, and accessory equipment; (3) production of metabolically active inoculum in sufficient quantity which is free from any contaminating microbes; (4) inoculation and subsequent growth of the producer microorganism in the fermenter under optimum condition; (5) product recovery and purification; and (6) safe effluent disposal. Here, in this chapter, we intend to provide a comprehensive review on

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the various fermentation processes, strategies for media formulation, and optimum growth of process microorganisms for industrial production of bioactive metabolites, recombinant proteins, and other added-value products.

Keywords

Fermentation · Aerobic · Chemostat · Lactic acid

Abbreviations

DO	Dissolved oxygen
DOE	Design of experiments
LAB	Lactic acid bacteria
OFAT	One-factor-at-a-time
PBD	Plackett-Burman design
RB	Rice bran
SmF	Submerged fermentation
SSF	Solid-state fermentation

4.1 Introduction

Fermentation is a process of generating energy where complex raw materials are converted into useful products. The definition of fermentation is different for different researchers. For some researchers, the term means any process for the production of a product by the mass culture of microorganisms, and for others, it is an energy-generating process in which organic compounds act as both electron donors and acceptors (Doelle 2014). In the fermentation process, the energy is produced by microbial enzymatic oxidation of any organic substrate such as carbohydrates, proteins, etc. The products of carbohydrate fermentation may be ethanol, lactic acid, citric acid, and butanoic acid, and fermentation like acetic acid and propanoic acid carbohydrate is not directly involved, but products of carbohydrate are used (Horton 2012). The basic raw materials used in the fermentation process act as carbon source, nitrogen source, salts, and cofactors for microorganisms. Polysaccharides are commonly used substrate for microbes used in fermentation processes, but sometimes oils and glycerol are also used as raw materials for fermentation (Glittenberg 2012). To produce maximum essential products during the fermentation process, factors such as temperature, pH, dissolved oxygen, and status of nutrients must be regulated and controlled. The most important factor is to minimize the risk of harmful contamination of microbe by carrying out under sterilized environments (Anderson 2009).

Microbial fermentation can be categorized into many criteria that may include the production of microbial biomass or microbial metabolites, enzymes, and

recombinant products (Doelle 2014). The production of different products during fermentation depends on the enzyme complex of cells and conditions of the environment of different microorganisms (Pandey 2003). But most importantly various biological functions of microbe like metabolism, reactions by enzymes, and biosynthesis result in production of fermentation products. Fermentation has diverse applications, and one of them is the improvement of organoleptic properties of food and enhancing of food safety by producing many metabolites that inhibit or kill food-borne pathogens.

4.2 Types of Fermentation Based on Substrate Used

4.2.1 Surface Fermentation

The fermentation process where the microorganisms used grow mainly on the surface of solid or liquid raw materials is known as surface fermentation. It can be easily controlled and implemented which are the main advantages of this fermentation, but its use in industries is limited because of its complexity. The aeration and agitation of media are the main basic operations of fermentation processes. The main edge of using this fermentation is needless of aeration or agitation of fermentation media. The products can be easily separated in this fermentation because microorganisms are not dispersed into the medium. Hence expenses and losses of product recovery are low. Surface fermentation has many advantages, but it also has several limitations like high investment cost on building, high personal expenses in countries with high wages, and long fermentation time (Pandey 2003).

4.2.2 Solid-State Fermentation

The fermentation technique in which microbial metabolites are produced on solid raw materials is known as solid-state fermentation (SSF). The main hallmark of solid-state fermentation is that raw materials contain enough moisture for the growth of the microorganism. The main benefits of using SSF are high product productivity, low energy consumption, low wastewater production, and relatively low moisture requirement for microbial growth and metabolism. SSF has various applications in the recently industrialized world which include bioremediation, production of microbial secondary metabolites, growth hormones for plants, organic acids, biofuel, and aromatic compounds (Pandey 1992; Agrawal and Verma 2020; Verma and Madamwar 2002).

4.2.3 Submerged Fermentation (SmF)

It is a type of bioprocess in which microbes are employed to break down the complex nutrient into value-added simple bioproducts in solution. This type of fermentation is

carried out in a closed vessel where the microbes are allowed to grow in liquid substrate like molasses, broth, etc. (Subramaniyam and Vimala 2012). In SmF, nutrients in the fermentation broth are consumed rapidly; hence it is very necessary to maintain the constant input of nutrients or replace the media with fresh media (Vidyalakshmi et al. 2009). In SmF, bacteria are the most preferred fermenting agent than any other microbes, as they grow better in high moisture conditions and exhibit high water activity (Babbar and Oberoi 2014). SmF is carried out in batch-fed fermentation and continuous fermentation. There are various factors that influence SmF such as substrate selection and fermentation agent. The selection of the right substrate is a prime matter of concern as the yield of fermentation highly relies on the nature of the substrate used. An appropriate modification of fermentation is necessary to get fruitful productivity from the particular substrate (Agrawal et al. 2019; Bhardwaj et al. 2019; Kumar et al. 2018). Substrates like soluble sugar, molasses, liquid media, sewage/waste, vegetable, and fruit juice are more frequently used (Vidyalakshmi et al. 2009). Microbes like bacterium are best suitable for this fermentation process. The SMF is mostly used for industrial purposes because of low cost, high yield, low contamination, and easy recovery of the product (Babbar and Oberoi 2014). The main limitations of SmF are high production cost, low productivity, and complexity of the medium (Fang et al. 2012). Most of the time, the submerged fermentation is used because of low expenditure, low operation time, and lesser space requirement and investments in labor, and on industrial scale sterilized conditions can be easily maintained due to simple process operations and easy to nurture property (Pandey 2003). However, SmF has various drawbacks compared to other fermentation processes which include higher funding for machinery, high consumption of electricity, high sensitivity to any irregularity in process parameters, susceptibility to microbial contaminations, etc. (Pandey 2003).

4.3 Types of Fermentation Based on the Availability of Oxygen

4.3.1 Aerobic Fermentation

Aerobic fermentation occurs at the beginning of the fermentation process and in the presence of oxygen. This is also referred to as “aerobic glycolysis” in living cells, wherein a glucose molecule is converted into two molecules of pyruvic acid through a sequential enzyme-catalyzed biochemical reaction consisting of ten steps. Following glycolysis, pyruvate is further catabolized into three molecules of carbon dioxide through the tricarboxylic acid (TCA) cycle in the cytosol and mitochondria of prokaryotes and eukaryotes, respectively. As compared to anaerobic fermentation, this process is shorter and more intense. Due to the low solubility of oxygen in water, dissolved oxygen (DO) is made available to individual microbial cells by aeration or agitation. Shake flask culture technique usually employs agitation to keep the culture at a high cell density by enhancing oxygen transfer rate in the fermentation broth to obtain higher yields of desired products (metabolites). Similarly, in bioreactors, mechanical agitation is provided by an agitator or impeller blades (such as in

continuous stirred-tank reactors) or in an airlift bioreactor, where aeration is ensured by gas purging for proper mixing of nutrients and biomass (Huang and Tang 2007).

4.3.2 Anaerobic Fermentation

Anaerobic fermentation is usually a slower process and occurs when oxygen in the fermentation broth is replaced with nitrogen, carbon dioxide, or any other by-product of the fermentation process (Huang and Tang 2007). In the absence of oxygen, pyruvic acid which is the final product of glycolysis can be directed toward two different routes depending on the type of cell. In brewer's or baker's yeast (*Saccharomyces cerevisiae*), the alcoholic fermentation pathway converts pyruvic acid into ethanol and carbon dioxide, whereas, in lactic acid bacteria (*Lactobacillus* spp.), lactate is produced by the lactic acid fermentation pathway. Certain obligate anaerobic microorganisms such as *Clostridium* spp. can produce butyric acid (short-chain fatty acid) which finds wide applications in chemical, food, and pharmaceutical industries (Xu and Jiang 2011). Several organic acids such as formate, propionate, butyrate, and acetate were produced from waste potato starch by anaerobic digestion using mixed microbial culture (Ayudthaya et al. 2018). Similarly, anaerobic fermentation has been considered as a feasible option for the generation of biogas and biomethane using microorganisms from various organic wastes including lignocellulosic biomass, animal wastes, municipal solid waste, and waste generated from food processing plants (da Silva et al. 2017; Kumar and Verma 2020a, 2021a).

Fermentation could be used in various industrial applications; some of them have been shown in Table 4.1.

4.4 Processes of Fermentation

The process of fermentation is dependent on the metabolic pathways of microorganisms involved in fermentation. Industrial fermentation is the regulated use of these metabolic pathways for the manufacture of products useful for people. Many industrially important products are manufactured using anaerobic fermentation. Some of the most important types and the kinetics involved are discussed in detail below:

4.4.1 Lactic Acid Fermentation

Lactic acid fermentation has been used for a long time for the preparation of fermented beverages and food all over the world. Many industries like dairy, cider, fermented vegetable, and meat rely on lactic acid fermentation (Taskila and Ojamo 2013). The overall relations involved in lactic acid fermentation are represented by Fig. 4.1.

Table 4.1 Microbial fermentation processes for the synthesis of various important industrial products

Substrate	Microorganisms	Type of fermentation (aerobic or anaerobic)	Product	Reference
Glucose, molasses	*Bacillus subtilis* and *bacillus licheniformis*	Aerobic fermentation	Poly-γ-glutamic acid (natural polymer)	Sirisansaneeyakul et al. 2017
Cane sugar or sugarcane molasses	*Rhodosporidium toruloides* NRRL Y-27012 and *R. Kratochvilovae* Y-43	Aerobic fermentation	Microbial lipids (single cell oil)	Boviatsi et al. 2020
Glucose	*Corynebacterium glutamicum*	Aerobic growth-arrested bioprocess	4-Hydroxybenzoic acid (aromatic compound)	Kitade et al. 2018
Waste activated sludge	Acidogenic microorganisms of the genus *Proteiniclasticum*, *Fusibacter*, *Macellibacteroides*, and *Petrimonas*	Anaerobic digestion	Volatile fatty acids	Huang et al. 2016
Glucose, Fe_3O_4 magnetic nanoparticles	*Enterobacter aerogenes* ZJU1, *Syntrophomonas*, and *Methanosarcina*	Anaerobic digestion/ dark fermentation	H_2, methane	Cheng et al. 2020
Cheese whey	*Lactobacillus acidophilus*	Anaerobic fermentation	Biogas, organic acids	Pandey et al. 2019
Cashew apple juice	*Saccharomyces cerevisiae* WUR 102 and *Hanseniaspora guilliermondii* CBS 2567	Anaerobic fermentation	Low alcoholic beverage with aroma compounds (β-phenylethanol and its acetate ester)	Gamero et al. 2019
Ovine (70%) and goat milk (30%)	Probiotic *lactobacillus paracasei* SP3 (isolated from kefir grains)	Anaerobic (fermentative lactic acid pathway)	Feta cheese	Mantzourani et al. 2018

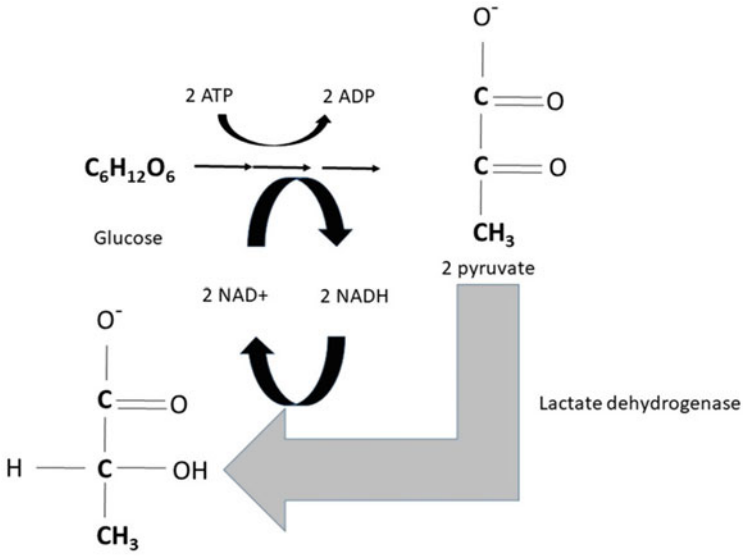

Fig. 4.1 Reactions involved in lactic acid fermentation

Lactic acid bacteria (LAB) play the most pivotal role in this type of fermentation. LAB is a group of bacteria that can utilize hexoses and produce lactic acid as the major product. There are three groups of LAB, namely, homofermentative, heterofermentative LAB, and bifidobacteria. Homofermentative LAB produces two molecules of lactic acid and ATP.

$$C_6H_{12}O_6 + 2ADP + 2Pi = 2C_3H_6O_3 + 2ATP \tag{4.1}$$

Heterofermentative LAB produces alcohol and carbon dioxide along with lactic acid.

$$C_6H_{12}O_6 + ADP + Pi = C_3H_6O_3 + C_2H_5OH + CO_2 + ATP \tag{4.2}$$

Bifidobacteria are responsible for the production of acetic acid as a by-product along with lactic acid.

$$C_6H_{12}O_6 + ADP + Pi = C_3H_6O_3 + C_2H_5OH + CO_2 + ATP \tag{4.3}$$

Since homofermentative LAB does not produce any other by-products, they are the most commonly used microorganisms for lactic acid fermentation. However, due to the ability of the heterofermentative LAB to utilize pentoses, they can be utilized for food preservation, mainly of plant origin (Zaunmüller et al. 2006). All three types of bacteria have extensive use as probiotics (Gomes and Malcata 1999).

4.4.2 Alcoholic Fermentation

Alcoholic fermentation involves the conversion of different sugars into basically ethyl alcohol and carbon dioxide by yeasts and some other organisms. The process starts with the breakdown of sugars into pyruvic acid, which is subsequently converted into acetaldehyde under anaerobic conditions. Acetaldehyde further releases two molecules of carbon dioxide and forms ethyl alcohol (Malakar et al. 2020). Figure 4.2 gives a brief idea about the reaction.

The most common ethanologenic microorganisms are yeasts which include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida* spp., *Kluyveromyces lactis*, *Pichia* spp., etc. Bacteria such as *Zymomonas mobilis*, *Clostridium sporogenes*, *Clostridium sordellii*, *Sarcina ventriculi*, and *Leuconostoc mesenteroides* are also involved in alcoholic fermentation (Binod et al. 2013; Kumar et al. 2020a, b; Kumar and Verma 2021b; Bhardwaj et al. 2020a, b).

Anaerobic Fermentation: Anaerobic fermentation occurs in certain yeast species such as *Saccharomyces*, *Schizosaccharomyces*, *Debaryomyces*, *Brettanomyces*, *Torulopsis*, Nematospora, etc. (De Deken 1966). The process of production of ethanol in presence of oxygen is known as Crabtree effect. Yeasts showing Crabtree effect can produce ethanol at high substrate concentration (Verduyn et al. 1984).

4.5 Kinetics During Fermentation

Microbial growth and product formations are interrelated events that depend upon many factors. The desired product may be some metabolic intermediates or the growing cells themselves and depending upon the nutrient requirements might differ [Clarke 2013]. Apart from nutritional requirements, other physicochemical parameters like pH, temperature, osmolarity, and oxygen availabilities also play an important role in fermentation. The discipline which deals with the optimization of

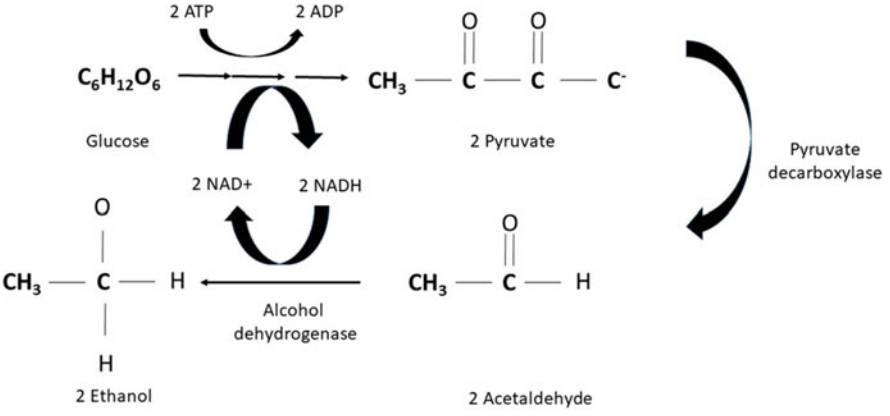

Fig. 4.2 Reactions involved in alcoholic fermentation

fermentation product yield through the utilization of different kinetic models of microbial growth is called bioprocess engineering. The product formation in a fermentation process is directly proportional to the rate of utilization of substrates. Therefore, in bioprocess engineering, different mathematical or kinetic models are developed to predict the microbial growth rate and the rate of product formation. The most important bioprocess strategies that are utilized for this purpose are batch cultivation, fed-batch cultivation, and continuous cultivation.

Batch cultivation: Batch cultures are designed for maximum product recovery during fermentation which minimizes the overall production cost [Yang et al. 2019]. It is the simplest culture technique that can be carried out on a laboratory scale under appropriate environmental conditions. The pioneering work on batch culture was done by Buchanan in 1918 and later by Monod in 1949. During batch culture, microbial growth can be classified into four phases: lag, log, stationary, and phase of decline. The lag phase defines that period of time after the time of inoculation where there is no visible sign of growth. During this phase, microbial cells adapt to the changing environment and start producing enzymes and other biomolecules required for the next phase, i.e., the log phase. The duration of the lag phase varies from organism to organism and also depends upon cultural and environmental parameters (Hill 2015). During the log phase, microbial cell division takes place and cell number increases exponentially. Exponential growth in bacteria is represented in terms of doubling time or generation time (G), in which the bacterial population doubles. Log phase cannot continue indefinitely and after a certain point of time due to the accumulation of metabolic products, cell lysis starts. When the rate of cell lysis equals the rate of cell division, the culture is termed stationary phase culture. During the stationary phase, cell culture product harvesting can be done. If the death rate exceeds the rate of cell division, then the culture is said to be in its death or decline phase.

Monod proposed a kinetic model for bacterial growth which states that bacterial growth rate is a function of limiting substrate concentration (Monod 1949).

$$\mu = \mu_{\max} \frac{[s]}{k_s + [s]} \tag{4.4}$$

where μ represents the growth rate, $\mu_{\max}$ is the specific growth rate of the microorganism, $[S]$ is the limiting substrate concentration, and k_s is the velocity constant.

The same model can also be applied for biodegradation as shown in Eq. (4.5) (Kovárová-Kovar and Egli 1998).

$$q = q_{\max} \frac{[s]}{k_s + [s]} \tag{4.5}$$

where q is the specific rate of substrate utilization (biodegradation).

Monod also coined the term yield coefficient ($Y_{x/s}$) and related to the specific rate of biomass growth (μ) and specific rate of utilization of substrate (q) by the following equation:

$$\mu = \frac{Y_{x/s}}{X} \times \frac{ds}{dt} \cong Y_{x/s} \times q \tag{4.6}$$

There are many modifications to the Monod equation, and the most common is the growth inhibition at high substrate concentration which is given by the equation below (Sokol 1986):

$$q = q_{\max} \frac{[s]}{k_s + [s] + {}^{S2}/_{K_i}} \tag{4.7}$$

where K_i is the inhibitory constant. This equation models when the substrate concentration is inhibitory for the growth of a particular microorganism.

4.6 Kinetics in Continuous Culture

A continuous culture or a chemostat is a culture that is operated with constant nutrition feed, and the parameters like the ratio between the substrate and the product and the environmental conditions are kept constant. Hence, the specific growth rate of an organism is kept constant and a steady state is maintained (Novick and Szilard 1950). In the bioreactor, at a steady state, the specific growth rate (μ) is found to be equal to the dilution rate (D). Dilution rate is the flow of the nutrient per unit time inside the bioreactor over the total volume of the bioreactor.

The mathematical model for a chemostat is represented by the formulae given below (Ziv et al. 2013):

$$\frac{dx}{dt} = \mu_{\max} \frac{S}{K_s + S} - Dx \tag{4.8}$$

$$\frac{ds}{dt} = DR - Ds - \frac{x}{y} \mu_{\max} \frac{S}{K_s + S} \tag{4.9}$$

where Eq. (4.4) denotes the change of cell density over time, assuming cell death is negligible. Eq. (4.5) describes the rate at which the limiting nutrient concentration changes with respect to the concentration of the inflowing media (R), its dilution by outflowing (s), maximum growth rate (μmax), cell density (x), and the yield constant (y).

4.6.1 Fed-Batch Culture

This type of culture can be regarded as a modification of batch culture since nutrients are supplemented after a certain period of fermentation. During batch fermentation, catabolic repression is observed due to the accumulation of products. The main aim of the fed-batch reactors is to minimize catabolic repression and enhance productivity (Özadali and Özilgen 1988). Moreover, the accumulation of biomass becomes

equal to the biomass generated and the exponential phase is maintained. For fed-batch reactors, Doran (2013) derived a formula as given below:

$$\frac{dx}{dt} = x(\mu - D) \tag{4.10}$$

where x is the biomass, μ is the specific growth rate, and D is the dilution rate.

In terms of yield, the following equation was derived:

$$\frac{ds}{dt} = D(S_i - S) - \left(\frac{\mu}{Y_{xs}} + \frac{qp}{Y_{ps}} + m_s\right)x \tag{4.11}$$

where Si is the inhibitory substrate concentration, Y_{xs} is true biomass yield, Y_{ps} is true product yield, qp is the specific rate of product formation, and m_s is the maintenance coefficient.

4.7 Different Media Formulation for Fermentation and Optimization of Media Components

As discussed earlier, the product yield in microbial fermentation depends upon many parameters, and those parameters have to be optimized for the highest yield. Some of the important parameters are discussed below.

4.7.1 Nutritional Requirements

Different media are used as growth media for microorganisms, but the basic components of most of the media include a carbon source, nitrogen source, and inorganic salts.

4.7.1.1 Carbon Source

The carbon source is the most important component of the media since different microorganisms yield energy from different sugars added to the media. The microbial biomass production and the yield of primary and secondary metabolites depend on the nature of the carbon source added (Marwick et al. 1999). Different microorganisms can utilize different sugars as carbon sources and the rate of utilization varies from microorganism to microorganism. There are also examples where a bacterium can prefer a particular carbon source over another when cultured in presence of both the sources (Monod 1949). Many carbon sources may act as interfering agents for the production of secondary metabolites such as antibiotics (Gallo and Katz 1972).

4.7.1.2 Nitrogen Source

Similar to carbon sources, the selection of nitrogen sources is also important for primary and secondary metabolite production during fermentation. There are many reports which show that use of certain amino acid repressed the production of certain metabolites (Singh et al. 2007). During wine fermentation, the production of hydrogen sulfide and ethyl carbamate was reported to be intensified by specific amino acids (Wu et al. 2018).

4.7.1.3 Inorganic Salts

Different types of inorganic salts are used in microbial culture media, and phosphate is one of the most important salts which actively participate in many vital processes of bacteria like the production of phospholipids (Singh et al. 2017). Production of many metabolites solely depends upon the concentration of phosphate ion, and above a certain concentration, the production is stopped (Sanchez and Demain 2008). Ammonium and phosphate ions were found to be promising for optimum ethanol yield, and magnesium was found to have a negative impact on alcoholic fermentation (Veljkovic et al. 1989).

4.7.2 Effect of Physical Parameters

Different physical parameters like temperature and pH play a very important role in the optimum recovery of products. Lipid production by *Fusarium solani* in batch fermentation was found to be optimum below temperature 35 °C in combination with other media components (Maia 2001). It was reported that biohydrogen production increases with temperature and decreases with pH (Xiao et al. 2013). The temperature of lipid metabolism strongly influences the odor components of wine. Fermentation of grape must be at the temperature as low as 13 °C to show better aroma components (Beltran et al. 2008).

From these reports, it is very clear that a slight change in a particular nutritional component may change the entire scenario of the fermentation process. Therefore, all the components are needed to be standardized for maximum production of a particular metabolite.

Different strategies of media optimization are utilized for the development of complete media. Optimization can be divided into two types: classical and modern (statistical) optimization (Singh et al. 2017).

Classical optimization involves a one-factor-at-a-time (OFAT) type of optimization. In OFAT optimization, at a particular time, only one factor is allowed to vary keeping other factors constant. Using OFAT, enhanced production of polysaccharides by *Stenotrophomonas daejeonensis* and *Pseudomonas geniculate* was achieved in which factors like fermentation time, carbon source, and nitrogen source were varied one at a time (Abou-Taleb and Galal 2018).

For advanced statistical methods, two main criteria are selected: finding the most significant factor and optimization of the component composition. Statistical methods or design of experiments (DOEs) suggests that changing more than one

component may be more efficient than OFAT (Fisher 1992). Moreover, DOE was found to involve less time and material to obtain similar results. Different experimental designs are available which provide a full factorial design for multiple components or factors involved in fermentation. R.L. Plackett and J.P. Burman designed a DOE known as Plackett-Burman design (PBD) (Mead et al. n.d.) which is a two-level design that is very important in differentiating the significant effects from the negligible ones. Plackett-Burman design is helpful for screening "n" number of variables using "n + 1" number of experiments (Reddy et al. 1999). Taguchi developed a method based on "orthogonal arrays" to overcome the shortcomings of PBD (Mori 2011). The Taguchi approach provides advantages such as shorter experimental time, improved quality of the product, and lesser product cost which is the primary aim for the process optimization of any fermentation method (Chanin et al. 1990).

After PBD, another model was developed by Box and Wilson (1951) which is popularly used in creating a second-order (quadratic) polynomial using the response surface methodology (RSM). An alternate to PBD is the Box-Behnken design which is independent of the quadratic model, which does not contain an embedded factorial design (Ferreira et al. 2007).

4.8 Cell Reactors Used in Fermentation

4.8.1 Immobilized Cell Reactor

The main requirement of modern bioreactor systems is biocatalyst (e.g., microorganisms, plant, or animal cells) that is immobilized into or onto a solid support matrix to prevent the cell washout, thus remarkably enhancing the concentration of biocatalyst while providing for maximum contact area with the substrate. The two most commonly used methods for cell immobilization are entrapment or encapsulation and adsorption. While the former method entraps cells into organic polymers such as polyurethane foam and absorbents like calcium alginate beads, the latter method adsorbs cells onto a solid support by physical or chemical interactions.

Immobilized cells have many different applications such as the production of pharmaceuticals and reagents, commodity chemicals, the fermentation of carbohydrates to ethanol, production of gaseous fuels such as methane and hydrogen from waste materials, production of macromolecules like enzyme, food, and beverages, and wastewater treatment (Clarke 2013).

4.8.2 Immobilized Enzyme Bioreactors

When immobilized enzymes are packed into columns and used in a flow system, they are known as immobilized enzyme reactors (IMERs). Usually, the separation of enzymes and products after reaction completion is difficult. However, the immobilized enzyme offers high catalytic efficiency, specificity, and also enhanced

tolerance to heat, pH, and organic solvents (Binod et al. 2013). Immobilized enzymes play an important role as biocatalysts in several industries like pharmaceuticals, food processing, and bioremediation purposes (Kumar and Verma 2020b; Agrawal and Verma 2020; Kumar et al. 2019). Since attachment of enzymes into or onto a support matrix (generally an inert and insoluble material) provides advantages improved stability and reusability as compared to free enzymes thereby allowing continuous use and product purification economically feasible at an industrial scale. The support matrix can be divided into two categories based on chemical compositions: inorganic and polymeric materials. The polymeric materials have sound mechanical properties and can be easily modified to fit specific requirements. Moreover, the specific activity and stability of immobilized enzymes can be improved by simply modifying the surface chemistry of the solid support. Enzyme-immobilized membrane bioreactors (EMBR) combine enzymatic catalysis with product separation. The selective membrane aims to separate the enzymes from the reaction products. The EMBR has been widely used in the chemical synthesis of antibiotics, anticancer drugs, several amino acids, vitamins, and optically pure enantiomer or even wastewater treatments and food processing (da Silva et al. 2017).

4.9 Application of Natural Substrates in Fermentation in Industrial Production of Bioactive Compounds

The metabolites comprising alkaloids, antibiotics, peptides, phenolic compounds, pigments, polysaccharides, etc. obtained from plants, algae, microorganisms, animal products, other seafood, etc., which demonstrate potential pharmacological applications, are known as bioactive compounds (Chye et al. 2018). The bioactive compounds have antimicrobial, antioxidant, antithrombotic, anti-inflammatory, and antiallergenic activities and, thus, are being used in curing chronic diseases, inhibition of carcinogenesis, anxiety, heart diseases, blood pressure, etc. to promote good health (Kris-Etherton et al. 2002; Kumar et al. 2020a, b). Due to the wide range of applications of bioactive compounds in food, and pharmaceutical industries, their extraction from natural sources has intensified using different strategies, viz., solid-liquid extraction (Tušek et al. 2016), ultrasound- and microwave-assisted extraction (Garcia-Vaquero et al. 2020), using high pressure (Alexandre et al. 2017), and supercritical fluids (Alvarez et al. 2019). Nowadays, the extraction of bioactive compounds from natural sources employing the process of fermentation is gaining attention due to its potent bioconversion property. A wide variety of natural substances have been converted to valuable products along with bioactive compounds using different processes of fermentation like solid-state fermentation and submerged fermentation (Sadh et al. 2018). Fermentation is regarded as an efficient method for the synthesis of bioactive compounds as it employs the machinery of microorganisms and microbial enzymes for the production of bioactive compounds through secondary metabolic synthetic pathways (Handa et al. 2019). Industrial production of bioactive compounds through fermentation has replaced the use of toxic solvents, hazardous chemicals, and other expensive physical methods

and, thus, serves as an eco-friendly approach to bioactive molecule synthesis (Magro et al. 2019). Agricultural residues, fermented foods, industrial effluents, vegetables, fruits, plants, cereals, and food by-products are commonly used substrates for industrial production of the bioactive compound through fermentation (Sadh et al. 2018).

Verardo et al. (2020) demonstrated that fermentation enhances the accumulation of bioactive molecules, viz., γ-aminobutyric acid (GABA), lycopene, and other phenolic compounds, and decreases the concentration of toxic substances like biogenic amines (BA). Sabater et al. (2020) described industrial production of bioactive metabolites by fermenting food wastes and by-products using lactic acid-producing bacteria, *Bacillus subtilis*, *Fusariumflocciferum*, *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., and many more. Fermentation-based succinic acid production utilizing *Saccharina latissima* as feedstock for a biorefinery approach serves as an eco-friendly alternative to petroleum-derived production of succinic acid and was demonstrated by Marinho et al. (2016). Wang et al. (2018) synthesized bioactive enzyme cellulase by submerged fungal fermentation of textile wastes using *Trichoderma reesei* ATCC 24449, an illustration of circular waste-based biorefinery. The enzyme has been used for the recovery of glucose and polyester residues from textile wastes as value-added products. Kombucha fermentation was employed for the synthesis of bioactive molecules from winery effluents using kombucha culture (*Acetobacter* sp., *Zygosaccharomyces* sp., *Saccharomyces*, *Torulopsis* sp.) (Vukmanović et al. 2020). Fermentation resulted in the increase of total phenolic content (TPC) and accumulation of other organic acids including acetic acid, oxalic acid, and a small amount of lactic acid in the beverage. The beverage showed pronounced antioxidant activity in comparison to other teas. Molinuevo-Salces et al. (2020) utilized a biorefinery approach for the production of biofuels by fermenting apple pomaces (AP) along with anaerobic co-digestion of manure.

The alcoholic fermentation study demonstrated an enhanced production of bioethanol and methane using *Kluyveromyces lactis*, *K. marxianus*, and *Lachancea thermotolerans* yeast strains and concluded that AP could be used as a potent candidate for biogas production. Olszewska-Widdrat et al. (2020) demonstrated the production of lactic acid from different renewable substrates by using *Bacillus coagulans* A534 through both batch and continuous fermentation experiments. Rice bran (RB), a by-product of rice cultivation, in general, and defatted rice bran (DRB) in particular have been used for the production of pure L-lactic acid without the addition of external supplements using *Bacillus coagulans* A107 isolate through batch fermentation (Alexandri et al. 2019). Acidogenic fermentation or anaerobic co-digestion of cheese whey and sewage sludge (used as inoculum) produced volatile fatty acids like lactic acid, acetic acid, and butyric acid (Iglesias-Iglesias et al. 2020). Agricultural wastes and by-products, forest wastes, sewage, household waste, industrial wastes, etc. could be employed for the production of various bioactive compounds beneficial for living beings. Thus, fermentation provides a cost-effective and alternative approach for the extraction of valuable bioactive molecules from natural resources which have a wide range of applications in day-

Table 4.2 Production of bioactive compounds from different biological sources through fermentation

Source/substrate	Microorganism	Bioactive compound	Fermentation type	Activity	Reference
Ecklonia cava (seaweed)	*Candida utilis*	Phlorotannin	Solid-state fermentation	Anti-inflammatory	Wijesinghe et al. 2013
Industrial wastes	*Streptomyces fradiae* NCIM 2418	Neomycin	Solid-state fermentation	Antimicrobial	Vastrad and Neelagund 2011
Black tea	Kombucha consortium	Organic acid and phenolic compounds	Solid-state fermentation	Antioxidant, anti-inflammatory, anticancer	Villarreal-Soto et al. 2019
Olive mill and winery wastes	*Aspergillus Niger* and *A. ibericus*	Xylanases, β-glucosidases, cellulases	Solid-state fermentation	Antioxidant	Filipe et al. 2020
Milk	*Lactococcus lactis*	Peptides	Solid-state fermentation	Antithrombotic and hypocholesterolemic	Rendon-Rosales et al. 2019
Opuntia ficus-indica and *Lantana camara*	*Saccharomyces cerevisiae*	Organic acid	Solid-state fermentation	Antioxidant	Tsegay 2020
Wheat straw	*Inonotus obliquus*	Phenolic compounds	Submerged fermentation	Antioxidant	Zhao et al. 2020
Celery seeds	*Bacillus subtilis*	Celery seed protein	Liquid state fermentation	Higher bile salts binding capacity	Chen et al. 2020

to-day life. Table 4.2 describes some bioactive compounds obtained from different biological sources employing potent microorganisms through different types of fermentation processes and their subsequent applications in food quality and human health.

4.10 Conclusions

In conclusion, fermentation is a process that modifies or breaks substances into simpler ones usually employing the metabolic activity of microorganisms including yeast and bacteria. Fermentation processes have been of utmost importance for human beings since time immemorial due to the wide variety of potential applications. The process of fermentation has evolved during the course of time with the development of technology and other engineering processes. Optimization of media and development of cell reactors has amplified the process of fermentation and the production of fermentation-driven products on a commercial scale. Large-scale fermentation of natural products employing microorganisms is a source of a wide variety of fermented foods and other bioactive compounds which could be used as potential functional foods and nutraceuticals. Fermentation-derived bioactive metabolites and other value-added products have been used as antimicrobial, anti-oxidant, anti-inflammatory, anticoagulant, and anticancer agents.

Declaration of Interest The authors declare that they do not have any conflict of interest.

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