Application of Microbes in Biotechnology, Industry, and Medical Field

4

Moises Bustamante-Torres, David Romero-Fierro, Jocelyne Estrella-Nuñez, Evelin Cuadros-Buenaventura, and Emilio Bucio

Abstract

Microbes encompass a wide range of a group of bacteria, archaea, protists, fungi, and viruses. The term microbes is commonly related to side effects. However, the advances in the biology branch have promoted the application of microbes in almost unlimited fields. Microorganisms can be classified into prokaryotes (bacteria and archaea) and eukaryotes (Protist and Fungi). Prokaryotes can survive in extreme conditions. They are employed as biofactories. However, eukaryotes have been used in the agroindustry and for some medical purposes. Besides, viruses are a type of microbes that are commonly applied in the medical industry. This chapter describes the application of microbes in several fields with great importance. Besides, new techniques with better sensibility and reduced costs are

M. Bustamante-Torres (✉)
Biomedical Engineering Department, School of Biological and Engineering, Yachay Tech University, Urcuqui, Ecuador

Department of Radiation Chemistry and Radiochemistry, Institute of Nuclear Sciences, National Autonomous University of Mexico, Mexico, Mexico
e-mail: moises.bustamante@yachaytech.edu.ec

D. Romero-Fierro · J. Estrella-Nuñez
Chemistry Department, School of Chemical and Engineering, Yachay Tech University, Urcuqui, Ecuador

E. Cuadros-Buenaventura
Biomedical Engineering Department, School of Biological and Engineering, Yachay Tech University, Urcuqui, Ecuador

E. Bucio (✉)
Department of Radiation Chemistry and Radiochemistry, Institute of Nuclear Sciences, National Autonomous University of Mexico, Mexico, Mexico
e-mail: ebucio@nucleares.unam.mx

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required to study and the understanding of the microbes. The versatility of these microbes has enhanced the study and application of them in biotechnology, industry, and medical field.

Keywords

Microbes · Application · New techniques · Biotechnology · Industry

4.1 Overview of Microorganisms

Microorganisms constitute most of the earth's biodiversity and are an integral part of the biosphere process (Amsellem et al. 2017). At first glance, the use of microorganisms is considered a wrong idea. The idea of microorganisms is related to diseases or producers of bad consequences. Throughout history, microorganisms have played fundamental roles in the evolution and the constant change of the world. Today, their applications are almost limitless, which makes them essential for the development of a huge range of industries and even the environment.

The emergence of eukaryotes in a world dominated by prokaryotes is one of the defining moments of modern microbial evolution (Bendich and Drlica 2000). Microbes are divided into two categories: prokaryotes, whose DNA interacts closely with the cytoplasm; eukaryotes, whose DNA is separated from the cytoplasm by a nuclear membrane (Murat et al. 2010). Eukaryotic chromosomes have characteristics that usually lack these characteristics in prokaryotic chromosomes, such as the presence of nuclear membrane and cytoplasmic space.

Microbes are tiny living things that despite their ubiquity usually cannot be seen with the unaided eye. This biodiverse group of organisms embraces bacteria, archaea, protist, fungi, and virus. Although microbes have commonly a negative connotation due to its infective and pathogenic nature, they also constitute an important component in the equilibrium of life in the entire environment (Tortora et al. 2019). In addition, their distinctive characteristics, which include high reproductive rate and biosynthesis capacity, make them attractive organisms for biotechnology application (Demain 2000a; Kouzuma and Watanabe 2014).

As evidence suggests, the application of microbes in biotechnology is recorded for the first time in 5000 BC, during the beginnings of large-scale winemaking activity (Borneman et al. 2013). However, microbial biotechnology began to be formally considered in the 1980s, when the first patent was granted for a genetically modified *Pseudomonas putida*. This engineered bacteria was intended for the organic digestion of compounds present in oil spills (Vitorino and Bessa 2017). This fact, together with the rapid advancement of various areas of science such as microbiology, molecular and synthetic biology, has greatly promoted the use of microbes in the different subtypes of biotechnology such as medical, agricultural, industrial, marine, food, and environmental (De Lorenzo et al. 2018; Gupta et al. 2016).

4.1.1 Prokaryotic Microorganisms

Prokaryotic microorganisms are divided into two domains: Bacteria and Archaea. These types of organisms are the smallest and simplest form of life (Tortora et al. 2019). Consequently, in comparison with eukaryotic microorganisms, prokaryotes have a short cell cycle (Harvey et al. 2000). Despite this, they constitute a large portion of the genetic diversity of life and possess an important metabolic diversity and in some cases exclusive to prokaryotes (i.e., routes in addition to those present in eukaryotes for CO_2 assimilation, anaerobic photosynthesis, fixation of $N_{2,}$ and adaptation to extreme environmental conditions) (Ward 2002; Amann and Rossello 2001; Grogan 1990). These characteristics make prokaryotic organisms a very attractive target for biotechnological manipulation. This section will describe the principal characteristics that make these type of microbes relevant in the area of biotechnology.

Bacteria

Bacteria are single-celled organisms and can be found almost anywhere on earth. They range in size from 0.2 to 2μm in diameter and 2 to 8μm in length. Morphologically, it is characterized by the lack of defined nuclear and membrane-bound organelles (Tortora et al. 2019; Rogers 2011). Furthermore, its genetic material consists of a circular chromosome made up of double-stranded DNA (free of histones) located in the nucleoid of the cell, and plasmids (extrachromosomal circular double-stranded DNA). According to their shape, bacteria can be classified as coccus (spherical shape), bacillus (rod-shaped), and spiral (Tortora et al. 2019).

However, the main criteria for classifying bacteria are based on the biochemical composition and structure of their cell walls. This classification divides the bacterial domain into Gram-positive and Gram-negative (Beveridge 2001). Gram-positive bacteria are characterized by a single cell plasma membrane and a thick cell wall composed of peptidoglycan. In contrast, gram-negative bacteria have thinner cell walls than gram-positive bacteria. In addition to the cytoplasmic membrane, the outer membrane of Gram-negative bacteria also contains carbohydrate and protein receptor sites, allowing phage to attach (Moat et al. 2002; Snyder et al. 2013). For the manipulation of microorganisms, it is important to identify and classify them properly, since several processes and characteristics, such as cell division, transformation, resistance to antibiotics and adaptability, can vary between Gram-positive and Gram-negative bacteria (Moat et al. 2002).

Other characteristics relevant to the biotechnology of bacteria include that they are haploid organisms. In other words, bacteria only have one allele of each gene. Therefore, genetic manipulation and mutation identification require simple processes. In addition, bacteria are microorganisms that reproduce asexually by binary fission and have a very short generation time. Consequently, it is possible to obtain large quantities of identical organisms in relatively short periods of time (Snyder et al. 2013).

In biotechnology, bacteria are frequently used. In the food industry they are required as metabolic agents in the production of fermented foods (Behera et al.

2019). They are also used as biofactories for nucleic acids, enzymes, and other proteins, important elements in the food and pharmaceutical industries (Nigam 2013; Ferrer-miralles and Villaverde 2013). Bacteria have relatively simple genetic characteristics; therefore, they can be genetically manipulated for different purposes, such as the improvement or introduction of metabolic processes (Singh et al. 2011).

Archaea

Archaea are widely distributed in different types of habitats. However, a large part of these microorganisms are considered extremophiles since they inhabit environments with extreme temperature, pH, and/or salinity (Snyder et al. 2013). The diameter of archaea ranges from 0.1 to 15μm, and the length does not exceed 200μm (Alquéres et al. 2007). They are divided mainly into two phyla: Euryarchaeota, which comprises the methanogens, halophiles, and hyperthermophiles, and Crenarchaeota containing only sulfur-dependent thermophile (Snyder et al. 2013).

Being a prokaryotic organism, Archaea share common characteristics with Bacteria: they are unicellular organisms, do not have a nucleus defined by a membrane, lack organelles such as mitochondria, chloroplast, Golgi apparatus, and endoplasmic reticulum, and their genetic material consists of a single circular chromosome and plasmids (Snyder et al. 2013). However, these two types of microorganisms also have important characteristic points of divergence from each other. For example, the lipids of the Archaea membrane are composed of isoprenoid chains instead of fatty acids as is the case of Bacteria. Furthermore, the most common peptidoglycan in the cell wall of Bacteria: murein, is not present in Archaea, instead S-layer protein or pseudo-murein can be found (Bräsen et al. 2014). Also, although the metabolic genes of Bacteria and Archaea have evolutionary aspects in common, the transcriptional and translational machinery of Archaea more closely resembles that of Eukarya (Alquéres et al. 2007; Barry and Bell 2006).

Archaean microorganisms occupy an important place in the biotechnology industry, because due to their extremophilic nature, archaeans are known to produce enzymes and metabolites with high biotechnological potential (Straub et al. 2018). Furthermore, the unique metabolic characteristics of Archaea, such as methane production and other unusual pathways involved in carbohydrate metabolism, present novel resources (e.g., enzymes, metabolic pathways) for their biotechnological application (Bräsen et al. 2014).

4.1.2 Eukaryotic Microorganisms

Eukaryotic microbes are mainly divided into two groups: Protist and Fungi. As is characteristic of all eukaryotic cells, eukaryotic microbes have a nucleus surrounded by a nuclear membrane, in which chromosomes are found. In addition, they have organelles such as mitochondria or chloroplast, Golgi apparatus, and endoplasmic reticulum (Tortora et al. 2019; Gross et al. 1995). The cell division of these organisms usually occurs by mitosis, so the two resulting cells are equal to each other (Tortora et al. 2019). Unlike the more highly evolved eukaryotic cells, eukaryotic microorganisms are cells of smaller size and less complexity that cannot form real tissues (Gross et al. 1995). However, these types of microbes have characteristics that may be of great interest in microbial and environmental biotechnology.

Protist

Protists are a complex group of organisms, mostly unicellular, that inhabit most terrestrial, marine and aquatic ecosystems. In addition, they can live as parasites of other Protists, Fungi, plants, and animals. In the terrestrial environment, protists are the main predators of bacteria and fungi and are an important indicator of soil condition. Morphologically, they are very diverse, but metabolically, Protists are less diverse than Bacteria. According to their ways of nutrition, they can be divided into osmo-heterotrophs, phago-heterotrophs, and phototrophs, which have the ability to fix carbon. Protists include organisms such as slime molds, protozoa, and algae (Dunlap 2001; Sergio et al. 2018).

The functional diversity of Protists in the soil microbiome makes this group of eukaryotes a rich source of tools that can be used in agricultural biotechnology. For example, they can be introduced into the plant microbiome as pathogen control agents as well as a nutrient provider and growth stimulant. In addition, they can be used to improve the fertility and productivity of crops (Jousset 2017). Also, some algae, such as dinoflagellates, constitute the most important source of natural products, which may be of great interest in medical biotechnology (Piel 2010).

Fungi

Phylogenetically, Fungi are divided into four groups: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (Dunlap 2001). Yeasts, which belong to the Ascomycota group, are unicellular organisms. However, most Fungi tend to be multicellular and filamentous. The individual filaments formed by the fungi are called hyphae, and the network formed by these are called mycelium. In some organisms, the mycelium can be divided by cell walls known as septum (Gross et al. 1995).

Fungi are heterotrophic organisms that can be found in different types of habitats. They can be saprophytes, that is, they inhabit in decomposing organic matter; plant and animal parasites; and symbiotic organisms such as mycorrhizae (Gross et al. 1995). Its reproduction is asexual (except in the Basidiomycota phylum) and occurs through the formation of spores in the hyphae. The organisms obtained from reproduction are genetically identical to their parents, and the germination of the spore is necessary for the formation of the organism (Tortora et al. 2019).

Fungi are highly used in the field of biotechnology. Organisms such as *S. cerevisiae*, *P. pastoris*, and *H. polymorpha* are highly used as a host for the production of recombinant proteins, many of which are used for pharmaceutical purposes. Furthermore, several of its secondary metabolites are of great industrial interest. For example, penicillin (antibiotic), cyclosporin A (immunosuppressive agent), lovastatin and pravastatin (hypocholesterolemic agent), carotenoid astaxanthin and b-carotene (pigment), and ergot alkaloids (mycotoxin) (Adrio and Demain 2003).

Virus

The main characteristic of viruses within the world of microbes is that they are not cellular entities. Therefore, although it remains in dispute, viruses are not considered living organisms (Herrero-Uribe 2011). Compared with other microorganisms, viruses are much smaller, ranging from 20 to 1000 nm. According to virus morphology, they can be divided into helical (rod-shaped), polyhedral (icosahedron-shaped), enveloped viruses (i.e., helical or polyhedral covered by envelope), and complex viruses (e.g., bacteriophages) (Tortora et al. 2019).

Structurally, viruses consist of nucleic acids and a capsid whose protein composition depends on the genetic information contained in the virus. In some cases, the capsid may be covered by an envelope composed of lipids, proteins, and carbohydrates. The genetic material of a virus can only exist in one form. These can be single-stranded DNA, double-stranded DNA, single-stranded RNA, or double-stranded RNA. Depending on the microorganisms, these can be linear or circular. For their replication, viruses need to host a living cell since they do not have the machinery for their replication on their own. This process is generally done in five steps: attachment, penetration, biosynthesis, maturation, and release (Tortora et al. 2019).

Viruses are easy to manipulate and in the medical industry, they have been widely used as an alternative to antibiotics for resistant bacteria, as vaccine vectors, as a delivery vehicle for gene therapy, and for the screening of libraries of antibodies and other proteins (phage display) (Haq et al. 2012). In addition, in agriculture, viruses have been used as biocontrol agents and through their genetic manipulation, the generation and growth time of seedlings has been accelerated (Maeda et al. 2020).

4.2 Principles

Microorganisms are important natural resources for the development of microbial biotechnology. The sequencing of microbial genomes, the identification of established functions, and the study of microbial metabolism are indispensable foundations for the establishment of genome sequence databases (Glazer and Nikaido 2007). The analysis and manipulation of the sequenced genomes have given rise to multiple identifications, production, optimization, and application processes.

4.2.1 Screening for Microbial Products

A screening method involves the extraction, isolation, and identification of a compound or components in a sample studied (Biniarz et al. 2017). The main goal of any microbial screening technology for biologically active compounds is to discover a new chemical entity or molecule with unreported biological activity (Monciardini et al. 2014). The discovery of these new biologically active compounds is biased toward the isolation and selection of the most common and easy-to-cultivable strains (Monciardini et al. 2014). Practically, screeninig can be considered as a isolation process or isolation of microorganisms. In the colony, inspections are conducted once for specific properties. The screening steps are critical and need to be carefully selected, because each step has its advantages and disadvantages and can give qualitative and quantitative results (Biniarz et al. 2017).

Important points in the detection of microorganisms and their respective metabolites are listed below.

– Develop and verify tests and evaluate their repeatability separately. At this point, preliminary screening can estimate the hit rate and quickly screen a large number of samples to obtain data for complete screening.
– High-performance screening, processing a large number of samples in a more simplified process by using an automated system.
– Development of a secondary screening to eliminate false positives.

Screening Methods

Appropriate isolation procedures play a vital role in preliminary screens to identify secondary metabolites (Yuan-Kun 2013). Traditional techniques sometimes lead to less than optimal results or present certain limitations. Novel screening methods with high sensitivity are needed to discover new enzymes for their application with diverse purposes. The screening methods need to be low-cost and high-throughput to be considered efficient.

Various techniques for microbial screening have been reported in different studies. Denaturing gradient gel electrophoresis (DGGE) is used as a rapid screening method for the production of biological hydrogen (Kumar et al. 2018), fluorescence technology high-throughput screening (HTS) is used for kinase screening (Morgan et al. 2004), and surface plasmon resonance (SPR biosensor is used for primary and secondary screening of acetylcholine) binding protein ligands (Retra et al. 2010), Nouws antibiotic test (NAT) screening in slaughtered animals (Pikkemaat et al. 2008), Limulus Amebocyte Lysate (LAL) screening in gram-negative bacteria (Seiter and Jay 1980), Microbial Luminescence System (MLS) test (Bottari et al. 2015) are used to assess biological contamination.

Taking into account the progress of synthetic biology, miniaturization, and automation technology, high-performance screening technology has been developed through HTS. HTS technique presents remarkable properties such as screening a significant amount of isolates, a high sensitivity, and determining the minimum inhibitory concentration of the active fraction against a series of indicator microorganisms (Walter et al. 2010; Leavell et al. 2020). Through this technique, the manual intervention is reduced, and at the same time the error rate. For this reason, the HTS technique has emerged as useful technology adopted by biotechnological fields (Sarnaik et al. 2020). There have been some reports of changes in the past, including high-throughput screening systems based on phenolic reaction transcription (Jeong et al. 2012), flow cytometry and light-sensitive sequencing (Sciarria et al. 2019), and extracellular electron transfer (ETT) (Tahernia et al. 2020).

4.2.2 Microbial Bioprocess

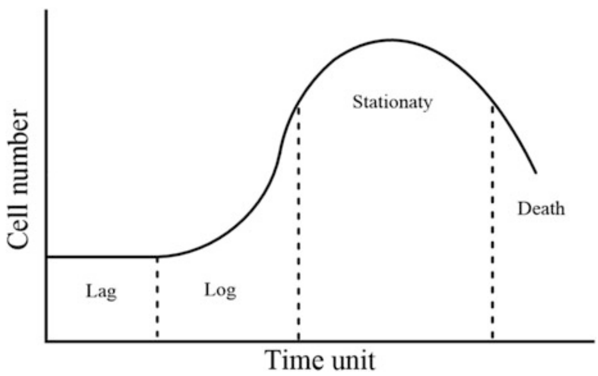
Microbial process has been useful in industrial applications through the bioprocess technology. The production strains and the culture conditions are key points in the bioprocesses of microbial agents. In microbial cultures, the mechanisms of the growth process are influenced by the activity of subcellular elements and complexes of enzymes (Panikov 1995). The main factors that influence production are the host of expression, temperature changes, acidity, the composition and tonicity of the media, among others, which must be carefully chosen for better adaptation and efficiency (Rohe et al. 2012). There are different reagents in the culture medium, which can act as restrictive precursors or promoters for the product. Precursors can lead the fermentation process to the formation of specific products (directed biosynthesis) without having to change the rate (Demain 2000b). The use of biological processes involves gentle reactions, which are more specific, more efficient, and produce biomass (renewable products).

The growth of microbial cultures is directly related to growth kinetics, which are affected by chemical and physical parameters. The growth of microorganisms is not linear. As shown in Fig. 4.1, there are different growth stages, which are represented by atypical growth curves.

Growth curves describe the density of cell populations in the media over time, which includes distinct phases, which are described below (Yuan-Kun 2013; Ram et al. 2019).

(a) *Lag phase:* During the cell adaptation phase, the cell size and weight increase.
(b) *Exponential phase:* During this period, the cells begin to grow and multiply, and the cells grow and divide rapidly at an approximately constant rate. After exponential growth, there is a decelerating growth phase.

Fig. 4.1 Microbial growth curve



(c) *Stationary phase:* The growth rate is equal to death rate. It could happen when a nutrient is exhausted, or by presence of inhibitors or changes in the medium conditions.
(d) *Death phase:* The conditions of the medium become less favorable and the number of cells begins to decline.

Optimization
Synthetic biological technology has been raised great progress through the modification of factors and processes that are involved in the biotechnological industry. Metabolic engineering and genome editing have been used to modify genomic strains and optimize the structure of producers (Wen et al. 2019). Other promising strategies are based on heterologous expression, coculture systems, improvement of culture conditions, and the designing of bioreactor (Ray and Behera 2017). The strains improvement could reduce the cost of the processes, increase productivity, and obtain specialized characteristics and conditions.

Sustainable Technologies
Microbial technology is considered as a promising element because it can contribute substantively to environmental goals. They exhibit a wide spectrum of evolutionary, functional, and metabolic diversity (Timmis et al. 2017). There are important interactions in nature, such as the interaction between microorganisms and minerals. These interactions contribute to the development of sustainable microbial coal biotechnology, acting as a warehouse for a variety of new biomolecules and acid mine drainage (Mishra et al. 2015). One of the most promising areas of interest is related to bioelectricity generation which brings economic and environmental benefits (Mateo et al. 2014; Xiaobo 2020).

4.2.3 Enzymology

Enzymes are known as a potential biocatalyst, which is demanded by industrial applications for its beneficial properties like high specificity, fast action, low toxicity, product purity, and biodegradability. However, most of them need to be redesigned to improve their catalytic performance (Brahmachari 2016). The use of enzymes has been allowed to work at high temperatures, extreme pH, with organic solvents. Enzymes interfere in most biological processes and therefore in their industrial applications, mainly in fermentation processes. For this reason, microbial biotechnology has had an exponential growth with the study and manufacture of enzymes of various types such as lipases, carbohydrases, proteases, recombinant chymosin, among others (Demain 2000b). The discovery of new microbial enzymes with new characteristics and functions imply possibilities for new applications, mainly in organic synthesis, clinical analysis, pharmaceutical products, and fermentation processes, in addition to having a feasible use in environmental remediation processes (Ogawa 1999; Bhatt 2019) and recombinant DNA technology (Eun 1996).

4.2.4 Gene Manipulation

Enzymes obtained from natural sources are generally not suitable for industrial use. They are usually genetically modified to improve their high yield characteristics. In biotechnology, when the culture medium is manipulated, it involves the testing of hundreds of additives, which are considered to be the limiting precursors of the final product (Demain 2000b).

Through DNA sequences, phylogenetic relationships are obtained between new organisms. This method has a great limitation related to the great diversity of existing species and is only used for nearby species. Based on this precedent, the use of rRNA revolutionized the techniques of recognition and identification of new species. The use of rRNA is characterized by performing an identical function in each organism and by the slight evolutionary change in its sequence, making it an ideal referential marker (Glazer and Nikaido 2007). The exploration of the complete genome sequence from a free-living organism is carried out through the whole-genome shotgun sequencing method, which is shown in Fig. 4.2. This method is traditionally used to identify genome sequences of a given organism, or it is also used to capture a representative sequence of various organisms in a simultaneous way (Venter et al. 2004).

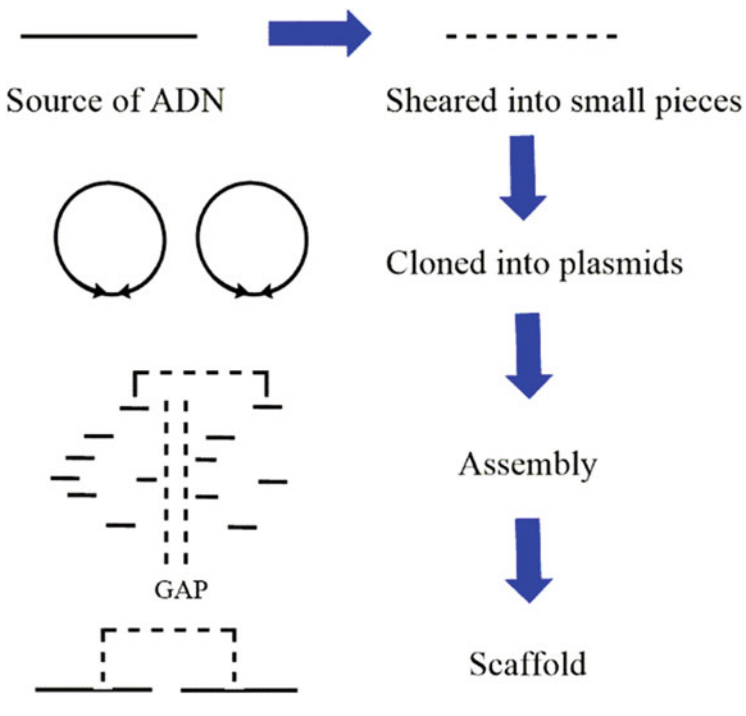


Fig. 4.2 Shotgun sequencing method

Hundreds of sequenced genomes have been collected, thanks to computer systems that have allowed better analysis of biological data.

There are certain bioinformatic parameters, which are important around the sequencing of genomes from various natural sources.

There are specific bioinformatic parameters, which are essential around the sequencing of genomes from various natural sources. Besides, there is a comparative analysis for gene identification and specific function based on genomics. Another relevant point is the visualization and cell simulation to analyze and model the organisms studied and their behaviors. Finally, the application in different areas (Bansal 2005).

Recombinant DNA Technology

In the biotechnology industry, the continuous progress of recombinant DNA technology is noteworthy. This relatively novel technology involves the production of essential and non-essential molecules. This technique is used to discover new secondary products through the introduction of genes (Demain 2000b; Murooka 1993). Recombinant DNA technology involves the modification of genetic material to obtain improved characteristics of a certain organism or its derivatives. This methodology involves inserting DNA fragments of the desired gene sequence or by blocking the expression of endogenous genes (Khan et al. 2016; Lodish et al. 2000).

4.3 Applications

Microbes have been wrongly known just because of their dangerous properties. Microbes describe a wide range of active applications. Unlike natural microorganisms, synthetic microorganisms have been genetically manipulated to perform specific activities and enhance their properties, which is beneficial due to low cost, safety, and wide range (Singh et al. 2014).

4.3.1 Industry

Food-Fermented Foods

In the food industry, microbial biotechnology is focused on improving the safety, quality, and consistency of bioprocessed products, as well as the yield, efficiency, and control of the processes adopted in this industry. Nowadays, biotechnology takes advantage of the biodiversity of described microorganisms, not only to use in important processes such as food fermentation, but also to obtain metabolites and enzymes that are required in the food industry as food ingredients, additives, and aids (Bhowmik and Patil 2018). This section describes how biotechnology, through the use of different types of microbes, has influenced the food industry in three different aspects: improvement of food quality, improvement of the efficiency and productivity of process, and the production of food additives.

Improvement of Food Quality

Fermentation is an anaerobic process which consists of a series of chemical reactions induced by different types of microorganisms or enzymes, which aims to convert sugars into alcohols or organic acids (Balaman 2019). Initially, this technique was used exclusively for the preservation of fruits and vegetables. However, nowadays fermentation is an important technique in the production of food products. Fermented products are part of the daily diet of human beings. Furthermore, the fermentation process is also used to increase the nutritional values of foods, enriching the substrates of proteins, minerals, essential amino acids, and fatty acids. It also contributes to the reduction of bacterial contamination and to eliminate anti-nutritive factors (Tamang et al. 2016; Petrova and Petrova 2020).

The improvement of the nutritional quality of foods through the fermentation process is evident in the production of yogurt. The beneficial compounds in yogurt, which include bioactive peptides, exopolysaccharides, and CLA, are obtained during the fermentation process, in which lactic acid bacteria are involved (Fernandez et al. 2017). These kind of bacteria produce an acidic environment during the fermentation of yogurt. This characteristic favors the bioavailability of the minerals present in yogurt, which are 50% more concentrated than in the raw material (milk) (El-abbadi et al. 2014).

The elimination of anti-nutritional factors from food is another way to improve the nutritional quality of the products. Oligosaccharides that cannot be metabolized by humans (e.g., raffinose, stachyose and verbascose), and whose α-D-Galactosidic bonds are not broken through the cooking process, constitute a very common anti-nutrient in foods such as soybean. Fermentation of soybeans with *Rhizopus oligosporus* molds, which are a great source of α-Galactosidases, allows the degradation of these oligosaccharides. The product obtained from this process is known as Tempeh (Bhowmik and Patil 2018). Other products, nutritionally favored in the fermentation process are described in Table 4.1.

Improvement Efficiency and Productivity of Process

The enzymatic variety that can be obtained from microbes is one of the most valuable resources that microbial biotechnology possesses. Since several enzymes, whose origin is mainly from bacteria and yeasts, are highly used to improve the efficiency and productivity of a number of processes carried out in the food industry.

A very common example is the use of xylanase and cellulase to selectively polish rice. This enzymatic treatment reduces the percentage of rice breakdown, improves nutrient retention, and increases the uptake rate of water, reducing cooking time (Das et al. 2008; Arora et al. 2007). The cellulase enzymes are mainly obtained from *Aspergillus* and *Trichoderma* (fungi) and *Bacillus* and *Paenibacillus* (bacteria). While Xylanase can be obtained from bacteria such as *Bacillus* sp. and *Pseudomonas* sp., as well as fungi such as *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. (Raveendran et al. 2018). From *Aspergillus fumigatus*, in particular, other nonlipolytic enzymes such as hemicellulase, chitanase, pectinase, and protease can also be obtained. These enzymes are used as a pre-treatment for oilseeds since they can degrade their cell wall, facilitating and increasing the performance of the oil

Table 4.1 Microorganisms involved in the improvement of nutritional and functional quality of fermented products

Fermented product	Raw product	Involved microorganism	Advantages	Source
Kimchi	Chinese cabbage 74–90%, radish 2.8–13.5%, garlic 1.4–2.0%, ginger 0.5–1.0%, onion 1.5–2.0%, green onion 1.0–3.5%, red pepper 1.8–3.0%	Starter culture: *Ln. mesenteroides*, *Ln. citreum*, and *Lb. plantarum*	Preservation of vegetables and improvement of nutritional quality	Patra et al. (2016)
Doenjang	Doenjang-meju and brine	*Bacillus, Enterococcus, Lactobacillus, Clostridium, Staphylococcus, Corynebacterium, and Oceanobacillus*	Improvement of nutritional quality	Kwak et al. (2012), Jung et al. (2016)
Kombucha	Tea	Bacteria phylum: *Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Deinococcus-Thermus, Firmicutes, Proteobacteria, and Verrucomicrobia*	Improvement of nutritional quality	Mitchell and Finn (2020)
		Yeast phylum: *Ascomycota*		
Sausages	Pork meat	Starter culture: *Lactobacillus plantarum and Pediococcus damnosus*	Improvement of nutritional and functional quality	Kim et al. (2014)
Pasta	Wheat semolina	*Lactobacillus plantarum*	Improvement of nutritional quality	Capozzi et al. (2011)
Pasta	Durum semolina	*Lactobacillus alimentarius, Lactobacillus brevis, Lactobacillus sanfranciscensis, and Lactobacillus hilgardii*	Reduction of gluten content in the pasta	Di Cagno et al. (2005)
Gari	Cassava tubers	*Leuconostoc, Lactobacillus,* and *Streptococcus*	Elimination of anti-nutritional factors	Omolara (2014)

extraction process (Sarkar et al. 2004). Consequently, a reduction in costs and pollutants is achieved.

Moreover, enzymes of microbial origin are also an essential part in the elaboration of various products. The α-amylase produced by *Bacillus amyloliquefaciens*, *Bacillus stearothermophilus,* or *Bacillus licheniformis* and the glucoamylase produced by *Aspergillus niger* and *Aspergillus awamori*, both enzymes are used in the production of glucose and fructose syrups from starch (Raveendran et al. 2018).

Another way to optimize and improve processes in the food industry, based on microorganisms is fermentation. The fermentation of cocoa and coffee is not only carried out to enhance the flavor of their products, but also because it is an important process for the separation of tissues. The microorganisms related to this fermentation process are mainly yeasts such as *Kloeckera apiculata*, *Hanseniaspora uvarum*, *Pichia kluyveri,* and *Kluyveromyces marxianus*; and LAB, Enterobacteriaceae, and *Bacillus* (Bhowmik and Patil 2018*;* Schwan and Wheals 2010).

Food Additives

In the food industry, one of the greatest challenges historically has been the preservation of food, which so far has been solved through the use of additives. Food additives are also used to improve the taste, color, texture, and aroma of foods. In addition, they are used as antioxidants, emulsifiers, and thickeners (Bhowmik and Patil 2018).

Due to growing evidence of the adverse effects of chemical additives in foods, the popularity of additives of microbial origin has been increasing. Some of these additives, such as bioflavors, can biotechnologically be obtained de novo from the metabolic capacity of microbes, since many of the bioflavors are secondary metabolites or enzymes that are produced naturally by microorganisms. However, the bioflavors used in the food industry can also be obtained by technological biotransformation through fermentation by microorganisms (Schrader 2005). In addition, recombinant technology allows the design of competent microorganisms in the synthesis of different types of additives. In Table 4.2 some types of additives generated from different types of microorganisms used in the food industry are presented.

Agroindustry

The main objective of agricultural biotechnology is to provide the necessary tools to improve the yield of different agronomic practices, while reduce the negative environmental impact that the agroindustry actually produces (Gupta et al. 2016). Based on this objective, two approaches are identified in which biotechnology is currently working and microbial diversity plays an important role. These are: combat pests in crops, increase crop yields and quality of agricultural products.

Pest in Crops

Insects, weeds, and pathogenic microorganisms are the main cause of the loss of crops worldwide (Manosathiyadevan et al. 2017). Chemical pesticides are usually used to avoid pests that tend to attack crops. However, in addition to increasing the

Table 4.2 Different types of additives produced by microbes used in food industries

Additive type	Function	Compound	Features	Microorganism	Source
Bacteriocins	Preserve foods by inhibition or kill undesired microorganisms	Nisin	Inhibit: Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus*)	*Lactococcus lactis*	Bhowmik and Patil (2018), Silva et al. (2018)
		Pediocin	Inhibit: LAB, clostridia, *Listeria*, *Staphylococci*	*Pediococcus pentosaceus*	
		Lacticin	Inhibit: LAB, clostridia, *Listeria monocytogenes*	*Lactococcus lactis*	
		Subtilin	Inhibit: Gram-positive bacteria	*Bacillus subtilis*	
		Microgard	Inhibit: Gram-negative bacteria, some yeast and mold	*Propionibacterium shermanii*	
		Sakacin	Inhibit: Gram-negative bacteria, some yeast and mold	*Lactobacillus bake*	
		Enterocin	Inhibit: *Listeria monocytogenes and Staphylococcus aureus*	*Enterococcus faecalis*	
		Aureocin	Inhibit: *Listeria monocytogenes*	*Staphylococcus aureus*	
Bioflavors	Diverse group of molecules with distinctive structure and functional groups that contribute to flavor and aroma of food	Pyrazines	Nutty and roasted flavor	Fungi: *Aspergillus* sp. *and Kluyveromyces lactis*	Bhowmik and Patil (2018), Bhari and Singh (2019)
				Bacteria: *Bacillus* sp., *Penicillium* sp., *Pseudomonas* sp., *Streptomyces* sp., *Streptococcus* and *Corynebacterium glutamicum*	
		Vanillin	Vanilla flavor	Fungi: *Phanerochaete chrysosporium, Pycnoporus cinnabarinus*	
		Methyl anthranilate	Fruity type flavor	Fungi: *Pycnoporus cinnabarinus and Trametes* sp.	

(continued)

Table 4.2 (continued)

Additive type	Function	Compound	Features	Microorganism	Source
		Limonin	Citric flavor	Bacteria: *Arthrobacter globiformis*	
		Lactone	Fruity type flavor	Fungi: *Cladosporium* sp.	
				Ceratocystis sp., *Saccharomyces* sp. and *Candida* sp.	
				Bacteria: *Sarcina* sp.	
		Terpene	Fruity type flavor	Fungi: *Ceratocystis* sp., *Kluyveromyces* sp., *Phellinus* sp. and *Lentinus* sp.	
		Diacetyl	Butter-like flavor	Bacteria: *Leuconostoc* sp., *Streptococcus* sp. and *Lactobacillus lactis*	
		Citronellol	Floral flavor	Fungi: *Ceratocystis variospora, Ceratocystis moniliformis* and *Trametes odorata*	
		Methyl ketones	Butter-like flavor	Bacteria: *Pseudomonas oleovorans*	
		Benzaldehyde	Fruity type flavor	Fungi: *Ischnoderma benzoinum*	
				Bacteria: *Pseudomonas putida*	
Pigments	Compounds that can serve as food colorants	Lutein	Yellow color	Protist: *Chlorella* and others *Microalgae*	Sen et al. (2019)
		Zeaxanthin	Yellow color	Bacteria: *Staphylococcus aureus, Flavobacterium* spp., *Paracoccus zeaxanthinifaciens,* and *Sphingobacterium multivorum*	

		Ankaflavin	Yellow color	Fungi: *Monascus* sp.	
		β-Carotene	Yellow-orange color	Protist: *Dunaliella salina*	
				Fungi: *Blakeslea trispora, Fusarium sporotrichioides, Mucor, circinelloides, Neurospora crassa, Phycomyces* and *Blakesleeanus*	
		Phycocyanin	Blue and green color	Protist: *Arthrospira* sp. and *Cyanobacteria*	
				Bacteria: *Pseudomonas* spp.	
		Phycoerythrin	Red color	Protist: *Porphyridium cruentum* and *Cyanobacteria*	
		Heptyl prodigiosin	Red color	Bacteria: *α-Proteobacteria*	
		Prodigiosin	Red color	Bacteria: *Serratia marcescens* and *Pseudoalteromonas rubra*	
		Lycopene	Red color	Fungi: *Fusarium sporotrichioides,* and *Blakeslea trispora*	
		Melanin	Black color	Fungi: *Saccharomyces* and *Neoformans*	
		Canthaxanthin	Orange and pink color	Fungi: *Monascus* spp.	
		Violacein	Purple color	Bacteria: *Janthinobacterium lividum, Pseudoalteromonas tunicata,* and *Chromobacterium violaceum*	
Enzymes		Proteases	Are essential in the production of dough and soy sauce and help in the degradation of plant proteins	Bacteria: *Bacillus* spp.	Raveendran et al. (2018)
				Fungi: *Aspergillus* spp. and *Saccharomyces* spp.	

(continued)

Table 4.2 (continued)

Additive type	Function	Compound	Features	Microorganism	Source
		Pectinases	Participate in: Clarification of fruit juices and wine	Fungi: *Aspergillus* spp.	
		Amylases	Participate in: Saccharification of starch for alcohol production and starch hydrolysis into dextrin, maltose, and glucose	Bacteria: *Bacillus* sp.	
				Fungi: *Aspergillus* spp.	
		ß-Glucanase	Participate in: Reduction of viscosity for mash filtration in brewing	Fungi: *Trichoderma*	
		Lactase	Participate in: Lactose intolerance reduction in people	Fungi: *Kluyveromyces lactis* and *Kluyveromyces fragilis*	
		Peroxidase	Participate in: Development of flavor, color, and nutritional quality of food	Fungi: *Phanerochaete chrysosporium*	
		Cellulase	Participate in: Cellulose degradation and ethanol production	Fungi: *Aspergillus* and *Trichoderma*	
				Bacteria: *Bacillus* and *Paenibacillus*	

price of agricultural production, it has been exposed that pesticides can have negative effects on human and environmental health (Dahab et al. 2017). Therefore, several alternatives have been proposed through microbial biotechnology.

In the case of the control of microorganisms, one of the alternatives consists in the use of microbial biological control agents (MBCA). MBCA consists of a group of microorganisms selected specifically for against pathogens, but also contain antimicrobial metabolites produced by these selected microbes. MBCAs protect cultures from pathogens in four ways. The first is that MBCAs release molecules that stimulate the immune system of the plant (MAMP), inducing resistance (priming). Microorganism-associated molecular patterns (MAMPs) can vary a lot, but some receptors for glucan, chitin, and xylan produced by Phytophthora megacephalus and Trichoderma have been identified. The second form of action of MBCAs is to create an environment of competition for resources with pathogens. For this mechanism to be efficient, MBCAs must contain highly competitive microbes under environmental conditions. The third and fourth mechanisms of action are direct interactions between MBCAs and pathogens, i.e. hyperparasitism and antibiosis through antimicrobial metabolites (Köhl et al. 2019).

For the control of insects, the spores of the Fungi microorganisms as bio-insecticides are a very promising option, since they are responsible for causing diseases to more than 200 different insects (Mostafiz 2012). For its action as an insecticide, the spores of selected Fungi are applied in the fields affected by insects. The spores adhere to the pathogen's cuticle, germinate, and penetrate the insect and invade the interior of the insect spreading its hyphae, which causes mechanical failure and the insect dies (Altinok et al. 2019). The use of the spore combination of *Beauveria bassiana* and *Trichoderma lignorum* as a bioinsecticide for the control of *Atta cephalotes* was recently reported (Felipe et al. 2019).

Nowadays, recombinant technology has allowed the development of new techniques for pest control. One of the most representative examples are Bt cultures. They are named in this way because they are genetically modified cultures that contain one or more genes that encode for one or more *Bacillus thuringiensis* (Bt) proteins. *Bacillus thuringiensis* is one of the most effective microorganisms as pesticide, since its spores contain crystal proteins (Cry and Cyt), which are highly insecticidal. These proteins are those produced by Bt cultures, therefore they are not usually invaded by pathogens (Koch et al. 2015).

Crop Yield and Product Quality

Fertilizers are a mixture of substances assimilable by plants that is widely used in the agricultural industry in order to increase soil nutrients and thus obtain crops with better performance and quality of their products. However, the excessive use of these can bring negative consequences to the environment such as air and water pollution, increased greenhouse gas emissions, and degraded soils (Chandini et al. 2019). Therefore, more environmentally friendly biotechnological alternatives such as bio-fertilizers have been proposed.

The metabolic capacity of some microorganisms has positioned them as powerful fertilizing agents. For example, microorganisms such as *Penicillium bilaii*,

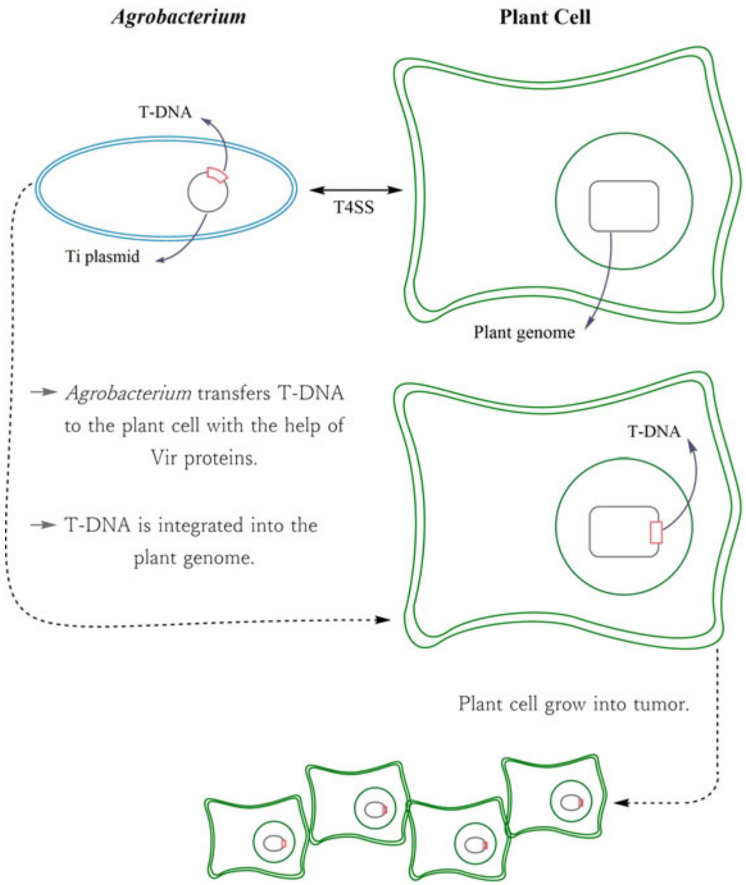


Fig. 4.3 Mechanism of Agrobacterium for gene transfer to plants

Arthrobacter chlorophenolicus, *Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus* are considered as fertilizing agents because they have the ability to solubilize the phosphate fixed in the soil so that it can be assimilated by plants. Other microorganisms of interest are N fixers such as *Rhizobium leguminosarum*, *Staphylococcus* sp., *Bacillus subtilis*, and *Gluconacetobacter diazotrophicus* used as inoculums (Schütz et al. 2018). In addition, some microorganisms (*Actinobacteria*) solubilize phosphate, fix nitrogen, and produce plant growth hormone, which stimulates the spread of roots horizontally, allowing the passage of more nutrients (Anilkumar et al. 2017).

On the other hand, as in the first approach, modern biotechnology makes genetic recombination available, which allows the introduction of genes of interest into the plant. For this, the pathogenicity mechanism of *Agrobacterium* as shown in Fig. 4.3 has been quite useful in the transformation process. They contain T-plasmid as an infectious agent that is used as the vector for exogenous genes (Krebs et al. 2014).

Today, this genus of bacteria, especially *Agrobacterium tumefaciens*, is the basis of one of the most used techniques in the transformation of plants (Baloglu et al. 2018). Some Agrobacterium-based transformations report an increase in the yield of blueberry crops (Song and Gao 2017), and early kiwifruit in vitro flowering (Moss et al. 2018). Although to a lesser extent, viruses have also been used in the transformation of plants. The use of Apple latent spherical vector virus was recently reported to accelerate breeding of Grapevine, reducing its generation time (Maeda et al. 2020).

Construction

In the construction area, microorganisms have been associated with the deterioration of the different materials used in this industry. However, microbial biotechnology has changed this connotation about microorganisms, through taking advantage of their innate metabolic characteristics in the development of construction processes and construction materials elaboration (Dapurkar and Telang 2017).

One of the earliest indications of construction biotechnology was the biotechnological production from microorganisms of polysaccharides and other metabolic products. In this context, microbial polysaccharides are used as admixtures for concrete, dry-mix mortars, injection grouts, and wall plasters (Stabnikov et al. 2015). The resulted characteristics of the admixtures vary depending on the polymer and the material in which it is added. For example, high molecular weight additives such as dextran (produced by *Leuconostoc mesenteroides* or *Streptococcus mutans*) and welan gum (produced by *bacteria Alcaligenes* sp.) when added to concrete reduce the porosity of concrete and can increase its viscosity and water retention capacity. This results in the improvement of the mechanical properties of the concrete, specifically the strength (Stabnikov et al. 2015; Ivanov 2017). In addition to modifying the viscosity and water retention, some admixtures such as Diutan gum (produced by *Sphingomonas* sp.) can generate pseudoplasticity to the material. Other metabolic products derived from microorganisms such as protein hydrolysates, lipids, sophorolipid, lignosulfate, and antioxidants are also used as additives in several of the materials already mentioned. They can have an effect on the shear resistance, mechanical properties, water retention, and fluidity of the material. In addition, these admixtures are used as detoxifiers, anti-corrosive agents, and deodorants (Dapurkar and Telang 2017).

Bio-cementation or also known as microbially induced carbonate precipitation (MICP) is another of the applications of microbial biotechnology in the construction industry. Bio-cementation is a process that occurs due to the precipitation of carbonate produced by the alkalinization of the medium caused by the metabolic activity of the microorganisms involved (Dapurkar and Telang 2017). Several microorganisms such as *Shewanella* (Ghosh et al. 2005), *Sporosarcina pasteurii* (Achal et al. 2011a), and *Bacillus* (Achal et al. 2011b) sp. have been used for bio-cementation processes to improve the strength and permeability of concrete and cement mortar that positively affect the durability of the material. In addition, this process allows the repair of cracks in concrete, either manually (Bang et al. 2001) or even by self-healing (Jonkers et al. 2010).

Microbes in the construction industry are also used to seek for more ecofriendly production alternatives, since concrete production is one of the largest contributors to greenhouse gas emissions. A recent study proposes a new method of bio-cementation using the metabolism of bacteria, which reduces the emission of CO_2 (Myhr et al. 2019).

Chemical Industry

Microbial biotechnology offers to be a good ally of the chemical industry, since the processes used in the production of industrial chemicals are linked to several disadvantages. These include: high production costs, use of non-renewable resources as raw material, high risk level, and poor waste management. Microbial biotechnology, for its part, offers highly competent alternatives for the production of chemicals, which have shown to reduce or eliminate most of these disadvantages. The potential of microbial biotechnology is based on four points. The first is the feedstock flexibility of microorganisms, since they can assimilate and process a wide range of materials. The second is based on the metabolic diversity of microorganisms. This allows all the processes necessary for the elaboration of a chemical product to be carried out within a single cell. Third, microorganisms are simple for their genetic manipulation, which allows any bioprocess to be efficiently designed. Fourth, the culture conditions of the microorganisms are moderate and do not require the use of toxic or flammable products (Burk and Van Dien 2015).

1,3-Propanediol was the first commercially produced compound by a genetically modified microorganism (Barton et al. 2015). Naturally some microorganisms of species such as *Citrobacter*, *Clostridium*, *Enterobacter*, *Klebsiella,* and *Lactobacillus* produce 1,3-propanediol from glycerol. Several genes of these and other species that produce 1,3-propanediol from glycerol were collected and analyzed. Then, through metabolic engineering, a recombinant strain that produces glycerol from D-glucose, the cheapest feedstock, was obtained (Nakamura and Whited 2003). In the chemical industry, other compounds such as 1,4-butanediol (Barton et al. 2015), succinic acid (Zeikus 1999), cis, cis-muconic acid (Yoshikawa et al. 1990), aromatic alcohols (Ghosh et al. 2008), and fine chemicals (Hara 2014) (physiologically active) have been produced from biotechnological techniques. In Fig. 4.4, differences between conventional production and bio-production of a chemical such as 1,4-butanediol are shown.

Cleaning

Bioremediation

Bioremediation is the process in which microorganisms are used for pollutant removal, is a highly promising method, and is cost-effective and efficient technology (Kumar et al. 2011; Azubuike et al. 2016).

Bacteria, microbes, archaea, and fungi are those microorganisms used for bioremediation, in this way they are called bioremediators. The design, control, and optimization of the bioremediation process is a complex system composed of multiple internal and external factors (Zouboulis and Moussas 2011).

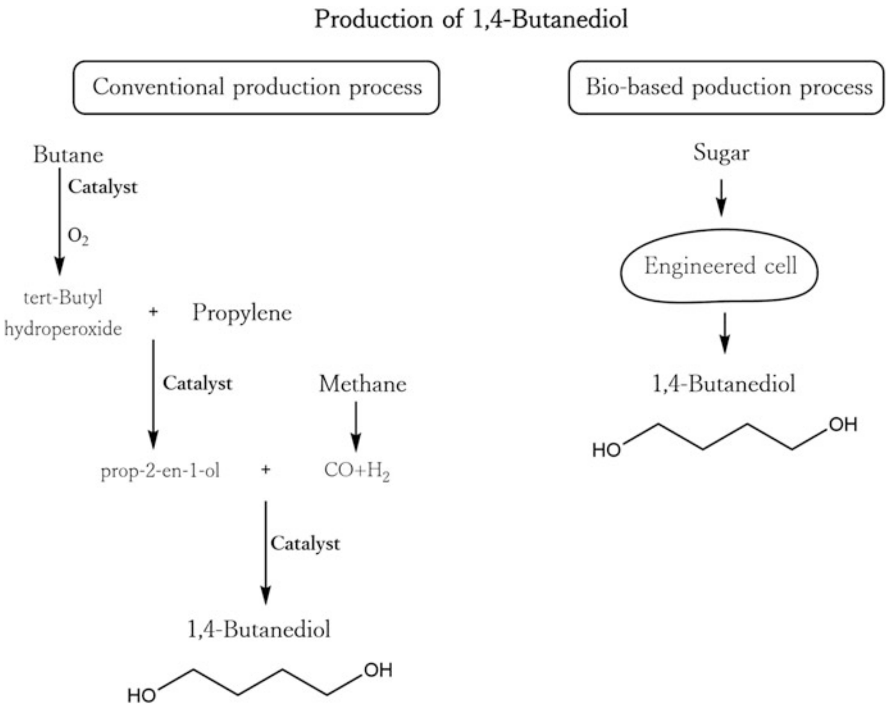


Fig. 4.4 Comparison between the conventional and bio-based production method of 1,4-butanediol

Some microorganisms are used to produce extracellular enzymes due to their properties, which can reduce the amount of pollutants in the environment. These microorganisms are called "microbial cleaners." Microbial cleaners are specially designed from several microorganisms for bioremediation (Spök 2009). Some microbes used for bioremediation including *Bacillus, Mycobacterium, Penicillium, Pseudomonas, Rhizoctomia,* among others (Gupta et al. 2017). However, they work synergistically to enhance their properties of biodegradation.

Chemical-Based Cleaning Products

The employing of chemical-based cleaning products is common in the whole world. The cleaning is a habit and lifestyle adopted for the population throughout the years. However, certain compounds used as cleaners can be harmful. EPA categorizes many of these chemicals as "volatile organic compounds" which can be harmful in different ways. These chemicals include phosphorus, nitrogen, and ammonia (Alton 2020).

The principal cleaners or disinfectants used are based on chlorine (Friedman et al. 2013), formaldehyde, phenolics (Bruins and Dyer 1995), hydrogen peroxide (Akuji and Chambers 2017), peracetic acid (Walters et al. 2019). However, they produce side effects to the day-to-day living, health, and the environment. Therefore, the use of new ways for cleaning arises in order to reduce the negative consequences by the normal cleaners.

As mentioned above, enzymes produced by microorganisms are potentially biodegradable and can be used in detergent products. Bacillus subtilis strains have been engineered to express modified genes and recombinant lipases (Hasan et al. 2010). Saccharomyces cerevisiae and Candida are fungal species used as cleaners (Gupta et al. 2017). Achromobacter, Actinobacillus, Alcaligenes, Arthrobacter, Rhodopseudomonas, Rhodobacter, and Lactobacillus are also used as detergents (Wassenaar 2008).

4.3.2 Environment

Microorganisms play an important role in their environment and contribute to the metabolism of all kinds of compounds, with important consequences for the functioning and maintenance of ecosystems. Thus, microorganisms can adapt to environments contaminated by toxic agents and transform them into harmless agents using the energy used in the process for their benefit (Kumar and Pal 2017). The main advantage of bioremediation mechanisms is that they can be applied in different settings, restoring the environment and preventing potential future contamination (Abatenh et al. 2017). These scenarios include wastewater treatment, solid treatment, metal recovery, and even the production of environmentally friendly fuels.

Wastewater Treatment

Humans have developed treatment systems that use microorganisms found in nature to neutralize household and industrial waste. The microorganisms in the wastewater treatment system remove the organic matter (dissolved in the form of particles), thereby converting it into new cell growth and by-products, that is, they are the main decomposition products (Adebayo and Obiekezie 2018). The microorganisms selected for wastewater treatment must not only use the capacity of organic matter, but also settle after the degradation process is completed, because bacteria and certain protozoa can aggregate to form flocs. It is easy to settle and a clear supernatant is obtained. The weight of filamentous bacteria and fungi is very small, and their surface area is large, so the sedimentation is poor, causing the problem of bulking (foam) (Wagner et al. 2002).

In a wastewater treatment plant, three stages of biodegradation take place during the degradation process:

- **Transfer:** Process by which organic matter comes into contact with microorganisms. It can be by absorption, the dissolved organic matter is transported inside the cell to be used as a source of nutrients, or by adsorption: the microbes adsorbed to the colloidal particles secrete enzymes that break them into particles that can be transported inside the cells (Rani et al. 2019).
- **Conversion:** The microbes are metabolizing their nutrients.

- **Stabilization:** When the microbes complete their capacity, their activity decreases and they sediment or flocculate easily (Rani et al. 2019).

Microbes in a biological waste treatment system are sensitive to many parameters. A very high organic load means that the microorganisms in the system are not enough to consume all the existing nutrients, or extreme temperatures can slow down the metabolism of bacteria in such a way that the decrease in organic matter does not meet the discharge requirements of the effluent (Coelho and Rezende 2015).

Therefore, for an effective treatment the addition of microorganisms is necessary to increase or restore the degradation process in biological treatments. The use of biotechnological products will not only increase the microbial population but will also allow the use of microorganisms that are better adapted to varying conditions of temperature, pH, salinity, etc. (Daims et al. 2006)

Solid Hazardous Treatment

Organic substances belonging to household or commercial solid waste can be biodegraded under controlled conditions until they reach a sufficiently stable state that they can be stored and used without adverse side effects (Shalaby 2011). Controlled conditions give the process a higher speed, reduce its uncertainty, and obtain a uniform final product (Mondal et al. 2019). The solid waste decomposition process can basically be carried out in two ways:

- Under aerobic conditions (in the presence of oxygen), organic matter is directly degraded into carbon dioxide, the most difficult to degrade organic matter is stabilized, and organic fertilizer products (compost) with stable quality are obtained.
- Under anaerobic conditions (in the absence of O_2), organic matter is partially degraded into CH_4 and CO_2 (biogas), and partially stabilized organic matter (Kobayashi and Rittmann 1982).

The two technologies can be implemented independently or in combination. There are experiences in which, in a first stage, anaerobic digestion is applied to obtain biogas and a composting process (maturation) is followed to completely stabilize the organic matter and obtain a high-quality compost (Rastogi et al. 2020).

Regardless of whether the process is aerobic or anaerobic, a biological treatment system consists of the following stages:

- **Pre-treatment:** Operations prior to the biological process, to adapt the waste and allow an adequate development of the process. Depending on the type of waste and the technology applied, the pre-treatment can be more or less intense. The pre-treatment normally includes the removal of unsuitable, crushing, mixing with additives (structuring material, co-substrates, etc.), homogenization, humidity adjustment, etc.
- **Biological treatment/s.**

- **Post-treatment:** Its objective is to refine the characteristics of the product obtained. Some of the possible operations are the classification according to size, the elimination of impurities, the humidity adjustment, the mixtures with inorganic fertilizers, etc.

Composting

Composting is the aerobic biological decomposition under controlled conditions to obtain a product with a high quality and sufficiently stable for storage and use without secondary effects (compost).

As the definition indicates, it is an aerobic biological process. For this reason, it is necessary to maintain optimal conditions so that the microorganisms responsible for the decomposition process can develop (Rastogi et al. 2020). The presence of oxygen is, in this case, the essential condition for the process to take place. Another important point to highlight from the definition is that the objective of the process is to obtain a stable quality product, compost. They indicated that all efforts have to focus on obtaining a quality compost, which can be useful in agriculture as a soil amendment and source of nutrients (Pan et al. 2012; Partanen et al. 2010).

The composting process can be used for a variety of wastes: biosolids (sludge) from sewage and industrial purifiers, livestock waste, plant re-waste from parks and gardens, waste from agri-food industries, and organic fraction of solid waste. As can be seen, composting can be applied to waste with very diverse characteristics (different C/N ratios, moisture content, nutrients, etc.). In many cases, however, it is necessary to mix residues with complementary properties for the process to develop properly (Pan et al. 2012).

For a compost to be considered of quality, it must have the following characteristics: (1) acceptable appearance and color, (2) correct sanitation, (3) low level of impurities and contaminants, (4) high level of agronomically useful components (N, P, K, etc.), and (5) constant composition (Rastogi et al. 2020).

Anaerobic Digestion

Anaerobic digestion is defined as anaerobic microbiological process (total oxygen absence) where organic matter is progressively degraded, by a heterogeneous bacterial population, to methane and carbon dioxide (Wang et al. 2018).

This type of decomposition is nothing more than a fermentation catalyzed by specific bacteria, which occurs sporadically in nature. It is the source of gas from swamps, natural gas from underground deposits, and even gas produced in the stomachs of ruminants.

In general, anaerobic digestion can be applied to any waste. However, the higher its organic matter content, the greater the biogas production and the more appropriate this treatment will be (Kumar and Sharma 2019).

The main advantages of anaerobic digestion include: (1) partially stabilizes and mineralizes organic matter, (2) has a positive energy balance, (3) homogenizes the composition of the waste. Likewise, it should be noted that this process is more sensitive than composting, so it is necessary to better understand the process and control more parameters, and it has a higher cost of implementation (Reineke 2005).

Metal Recovery

Industrial activities generate large-scale contamination with heavy metals (Cu, Zn, Pb, Cd, Cr, Ni, Hg, Co, Ag, Au) and radionuclides (U, Th) in the environment (Krebs et al. 1997). In the particular case of soils, they tend to affect fertility and/or their subsequent use, while in the case of aquifers and surface waters, they can seriously compromise the use of this resource as a source of water for human consumption (Krebs et al. 1997). The remediation of these contaminated environments through the use of chemical methods involves excessively high cost processes due to the specificity required. In addition, this type of solution is not suitable for in-situ repair processes because some metals cannot be processed due to competition from other metals (Ojuederie and Babalola 2017).

The application of effective remedial methods depends on the understanding of site hydrological and geological factors, the solubility and form of heavy metals, the attenuation and fixation process, and the degree to which metals can be dispersed horizontally and vertically when they migrate in the horizontal direction along the ground (Bal et al. 2019). On the other hand, the use of biological methods to repair the contaminated environment has high operational flexibility in both in-situ and ex-situ systems and can easily remove target metals.

The toxicity of heavy metals is very significant. The effects occur directly on organisms by preventing biological activity; that is to say, the inactivation of enzymes is caused by forming the bond between the metal and the -SH (sulfhydryl) group of the protein, thereby causing inactivation in different organisms. Reversal of destruction. In order for heavy metals to be toxic to organisms, they must be able to be captured, that is, the metals must be bioavailable (Ojuederie and Babalola 2017).

All interactions between microorganisms and metals or other elements (such as carbon, nitrogen, sulfur, and phosphorus) are fundamental components of the biogeochemical cycle (Rawlings 2002). Metal–microbiota interactions are studied in depth in the context of environmental biotechnology, in order to implement removal, recovery or detoxification methods for heavy metals and radionuclides (Gadd and Metals 2010).

Depending on the oxidation state of the metal and the species it forms, microorganisms can perform two possible transformations. The transformation will correspond to the mobilization of metals, that is, from the initial insoluble state corresponding to the solid phase (for example, metals related to soil, sulfide, or metal oxide) to the final soluble state (Gadd and Metals 2010). This process is called microbial leaching. The other corresponds to the immobilization of metals, that is, the transition from the initial soluble state in the aqueous phase to the final insoluble state in the solid phase. Conversely, there are multiple mechanisms in nature through which metal fixation can occur (Krebs et al. 1997; Rawlings 2002).

Microbial Biofuels

Microorganisms convert biomass into chemical compounds that can be used in the production of biofuels. This activity has been exploited for many years in the production of methanol, ethanol, and butanol, and more recently interest in the production of hydrogen, biodiesel, among other alternatives, has increased (Kumar and Kumar 2017). The main cost in the production of biofuels, in economic and environmental terms, is the raw material (biomass). The selection of the raw material is fundamental for the conversion to biofuel; the hydrolysis of biomass is required to produce a fermentable substrate. This step can involve physical, chemical, and enzymatic treatments (Sindhu et al. 2019; Bokinsky and Groff 2013).

In a natural way, a large number of metabolic processes occur in microorganisms that generate different compounds, both gaseous and liquid, where energy is stored that can be used as fuel (Speight 2011).

Biomethanol

Methanol has been obtained as an intermediate for degradation of methanotrophic bacteria, which use methane as an energy source to produce carbon dioxide. Methane is a type of biogas, which is produced by the action of microbes called methanogens. It is a very large and diverse group with three basic characteristics: (1) They form a large amount of methane as the main product of energy metabolism; (2) They are strictly anaerobic bacteria, (3) They belong to the field of archaea (Demirbaş 2008).

Methanogenic bacteria gain energy for growth by converting a limited amount of substrate into methane gas (Nakagawa et al. 2011). The synthesis of methane is the main energy source for the growth of methanogens. For this reason, methane production can be regarded as a form of anaerobic respiration, in which the methyl CO_2 from the carbon atom compound or the methyl carbon from the acetate is the electron acceptor. It should be mentioned that methanogens are very sensitive to oxygen (Kumar and Kumar 2017).

The large-scale methanol production process by microbial cells has several technical limitations. This is mainly because the metabolic processes of microorganisms usually produce a variety of products, by-products, and intermediates, which hinder the control and regulation of the global process that generates specific end products. This can be controlled by the use of specific enzymes that direct the reaction to the path required to obtain the desired product (Demirbaş 2008).

Bioethanol

Ethyl alcohol is a chemical product obtained from the fermentation of sugars found in plant products such as: cereals, beets, sugarcane, sorghum, or biomass. These sugars are combined in the form of sucrose, starch, hemicellulose, and cellulose (Ingale et al. 2014).

In the fermentation process of the sugar contained in the organic matter of plants, a hydrated alcohol is obtained. The water content is about 5%. After dehydration, it can be used as fuel and is called bioethanol. It is mixed with gasoline to produce a high-energy biofuel whose characteristics are very similar to gasoline, but significantly reduce the pollution emissions of traditional internal combustion engines (Prasad et al. 2019).

Ethanolic fermentation is by far the most exploited microbial process, and although there are several possible microorganisms responsible, it is undoubtedly the yeast Saccharomyces cerevisiae that is of the greatest industrial importance. However, it has been seen that the bacterium *Zymomonas mobilis* is the other microorganism that produces ethanol through homoethanolic fermentation. Among the by-products obtained from fermentation are: CO_2, low concentrations of methanol, glycerol, and water (Prasad et al. 2019).

Butanol

Butyl alcohol is one of the four-carbon primary alcohols having the molecular formula C_4H_9OH. It is a colorless liquid that produces irritating vapors that have an effect on the mucous membranes and at high concentrations it produces a narcotic effect (Singh and Nigam 2014).

Due to its properties it can directly replace gasoline, or it can serve as a fuel additive. Industrial production is based on a fermentation process carried out by *Clostridium acetobutylicum*, a microorganism that ferments carbohydrates and produces mainly butanol and acetone. However, different clostridia are capable of producing butanol, acetone, and isopropanol (Bokinsky and Groff 2013).

A characteristic of the process is that it is a biphasic fermentation. The first phase is acidogenic and it is an exponential phase, where acetate, butyrate, hydrogen, and CO_2 are formed as main products. The second phase is solvent-borne, the acids are re-assimilated and used in the production of acetone, butanol, and ethanol (Berezina et al. 2012).

Biodiesel

The oil used to make biodiesel is composed of triglycerides, and three fatty acids are esterified with one molecule of glycerol. Then, triglycerides and methanol react in a reaction called transesterification or alcoholysis. Transesterification reaction produces fatty acid methyl esters, called diesel and glycerol (Singh and Nigam 2014).

Unlike the production of biodiesel based on corn, soybean, or palm oil, obtaining with microorganisms has some advantages: (1) the production of oil per area is much higher than 4000 gallons/per year while that of plants is 50–60 gallons/acre per year, (2) microorganisms require much less water than terrestrial plants, (3) can be grown without using topsoil and do not compete for resources from conventional agriculture, (4) biomass production microalgae can be combined with waste CO_2 biofixation (1 kg of dry biomass requires about 1.8 kg of CO_2), (5) fertilizers, mainly nitrogen and phosphorus, can be supplemented by wastewater, (6) the cultivation of microorganisms does not need pesticides, (7) the residual biomass after extraction of the oils can be used as food, fertilizer, as a source for alcoholic fermentation or for methane production, and (8) the composition of the biomass. It can be modulated by varying the growing conditions (Sindhu et al. 2019). However, there are some limitations that must be considered: (1) selecting microalgae or microorganisms with high biomass production and high lipid production, (2) keeping the algae in laboratory conditions or in production systems, (3) carrying out large-scale production of the microorganism, and (4) the energy required to pump water, transfer the CO_2, mix the culture suspension, harvest and drain the biomass of the microorganisms (Speight 2011).

Some microalgae and other microorganisms can generate oils or biodiesel in a renewable way, they can be derived from fatty acids, diacyl or triacylglycerides. Some microalgae like *Botryococcus* can naturally accumulate long-chain terpenoids that can also be used in the production of biodiesel (Singh and Nigam 2014).

Medical Biotechnology

Microbiology plays a significant role in general medical devices, pharmacology and medicine. The main purpose during the use of microbes is to minimize risks. For long years, microbes have been studied as causes of disease. The lack of appropriate analytical methods showed a late comprehension about the importance of them.

Many microorganisms, such as viruses, have been studied through vaccines as antibiotics for drug-resistant bacteria. In the USA, methicillin-resistant Staphylococcus aureus (MRSA) and Clostridium difficile are the most common drug-resistant strains that cause disease. Many gram-negative bacilli with multiple drug resistance also fit the description of super bacteria (antibiotic-resistant microorganisms), and they have become the main last line of defense against these gram-negative super bacteria.

Microbiota is defined as the number of microorganisms into the human body and other multicellular organisms. The microbiota is associated with important functional roles, such as vitamin synthesis, digestion, and colonization resistance to intestinal pathogens (Autenrieth 2017).

The participation of microorganisms in the production of medical products or services involves the biological control of diseases and vaccine production (Vitorino and Bessa 2017). Vaccines are classified as attenuated, inactivated, DNA, or recombinant vaccines as shown in Table 4.3. In an attenuated vaccine, the pathogen (virus or bacteria) is alive and can induce an immune response similar to a real infection

Table 4.3 Types of vaccines

Vaccine	Properties	Application
Attenuated vaccine (A.V.)	High immunogenicity	Influenza, polio, rubella, tuberculosis, dengue, yellow fever
	Humoral immunity	
	One dose	
Inactivated vaccine	Safer than A.V.	Influenza, hepatitis A, cholera, pertussis
	Humoral immunity	
	Multiple doses	
DNA vaccines	Immunogenicity	Influenza, hepatitis B, HIV, HPV
	Use bacteria as carries of DNA plasmid	
Recombinant vaccines	Antibody production	Not yet available clinically
	Immunization	
	Intrinsically safe	

(Plotkin et al. 2008). In contrast, inactivated vaccines consist of the entire or fractioned part of a pathogen completely inactivated. Inactivated vaccine is safer than attenuated because the pathogen is dead. The DNA vaccine then consists of an expression plasmid that contains genes encoding one or more immunogenic antigens of interest (Robinson 1997). Finally, recombinant (gene) vaccines are prepared from viruses engineered with genes encoding antigens, inducing antibody production and immunity (Vitorino and Bessa 2017).

In addition, microorganisms have become an important factor in disease diagnosis. Microbiological assays describe controlled conditions for the growth of microorganisms with an appropriate antibiotic sensitivity pattern, which will work at a specific antibiotic concentration. Escherichia coli, Lactobacillus casei, and other microorganisms have been used to chemically detect the anti-tumor activity of microorganisms, because compounds with anti-tumor activity also often inhibit the growth of test microorganisms (Thayer et al. 1971; Abbott 1976). Although microbiological assays are cheaper, they are less sensitive.

Bacteriocins are a heterogeneous group of biologically active bacterial peptides or proteins synthesized by ribosomes, which show antibacterial activity against other bacteria (Karpiński and Szkaradkiewicz 2016). Bacteriocins are antibiotics produced by certain strains of microorganisms that are active against other strains of the same or related species (Gundogan 2014).

4.4 Conclusions

Advances in technology have promoted the development of new technologies related to microbial research. News and convenient methods with high sensitivity, such as DGGE, NAT, LALA MLS, SPR, and HTS, tend to study the importance of microorganisms. However, HTS (high-throughput screening) describes an effective technique that not only can isolate a large number of strains, but also has high sensitivity, limited manual interaction, and reduced error rates. It is important to consider that microbial production and research depend on growth conditions (hysteresis, exponential, static and death stages).

Microorganisms are essential for maintaining ecosystems. For a long time, microorganisms produced through biotechnology have been an important part of the world's largest industries, such as food, agriculture, construction, and chemical industries. The versatility of microorganisms allows it to be used in simple processes (such as food fermentation), but also in more complex processes (such as the production of recombinant proteins for therapeutic purposes). In addition, the application of genetically modified enzymes produced through microbial technology will work together to improve its performance. Microbial biotechnology has affected the reduction of production costs, the optimization of processes, and the improvement of product quality. At the same time, it has created a potential alternative method for reducing pollutant emissions in the industry. Environmental bioremediation is an area where microorganisms can also be applied. Because of all the advantages associated with these fields, microbes and environmental biotechnology have aroused great interest among researchers. However, there are still many options and areas to explore.

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