**Applications of Fermentation Technology in Biotechnology: From Food to Pharmaceuticals**

**Introduction:**

Fermentation technology, a process that utilizes microorganisms to produce desired products, has been a cornerstone of biotechnology for centuries. This chapter explores the wide-ranging applications of fermentation technology in various fields, including food and beverage production, pharmaceuticals, biofuels, and industrial biotechnology. By harnessing the metabolic capabilities of microorganisms, fermentation technology has revolutionized numerous industries, enabling sustainable and efficient production of valuable compounds. The technology of fermentation dates back as far as human civilization itself. Over the years, fermentation has evolved from its humble beginnings as a food preservation technology for households to a sophisticated one used to manufacture diverse range of products at the industrial scale [1].

Traditionally, fermentation was employed as a method of preserving and a mode of extending food shelf-life [2]. It is due to the peculiar flavor and renowned health benefits; fermented foods have become so popular. Their popularity has increased the production and consumption of fermented foods and beverages, globally in the recent years. The scientific community has paid increasing attention to the health benefits of fermented foods and beverages in the last few decades [3]. Apart from its renowned benefits on the gastrointestinal tract, fermented products have also been proven to contain anti-oxidant, anti-inflammatory, anti-diabetic and anti-fungal [4]. Probiotics can be defined live bacteria or yeast that supplements the gastrointestinal flora and improves intestinal health, enhances immune response, reduces serum cholesterol levels, lactose intolerance symptoms and prevents gut infections [5]. Fermentation involves yeasts converting sugar to alcohol and carbon dioxide in anaerobic conditions under controlled conditions, which in industrial scale produces a wide variety of substances using microorganisms or mammals. Among the many products that have been produced by fermentation are antibiotics, solvents such as ethanol, intermediary compounds such as citric acid, and probiotics such as yoghurt. In addition to drugs, fermentation is also used to produce materials for commercial use, such as diagnostic kits, monoclonal antibodies and drug delivery vehicle. As a result, the biopharmaceutical industry continues to grow rapidly thanks to fermentation technology. With the progress in novel technology, it is expected to expand even further in the days ahead [6].The popularity of industrial fermentation processes has increased in recent years. Using this technology can reduce our dependence on fossil fuels and chemicals [7]. Industrial food fermentation was possible with the discovery of microorganisms, as it became possible to understand and manage food fermentations. Although fermentation was initially used for food production, it also manufactures foods and food supplements (such as cheese, pickles), industrial chemicals (such as acetone,), pharmaceutical chemicals (such as enzymes, vaccines), alcoholic beverages (such as beer, wine), analytical products (such as citric acid), and biofuels [6]. Modern sophisticated equipment and advances in fermentation technologies have addressed many of the challenges associated with traditional fermentation methods over the years. and progressed with new approaches for fermentation of novel products [8]. As a result of the wide application associated with fermentation technology, in recent times it has witnessed a huge leap in terms of production and consumer acceptance.

**Fermentation Process Overview:**

**1.1. Microbial Fermentation:** The term fermentation originates from a Latin verb, called “fevere”, which literally means to boil. During the production of alcohol, the first truly industrial process, the gas bubbles of carbon dioxide appear at the surface of the boiling liquid, which gave the appearance of boiling liquid. Hence, it is named as fermentation. Although fermentation is defined as the anaerobic breakdown of organic substances by microorganisms such as bacteria, yeasts, and fungi. However, this definition is no longer valid since the term industrial fermentation is now used for large- scale cultivation of microorganisms, even though most of them are aerobic, for the transformation of complicated substrate into simpler products beneficial to peoplewith applications in the production of energy, pharmaceuticals, chemicals, and food industries [9], namely for the biotransformation product, metabolites, biomass, recombinant technologies, and manufacture of enzymes. During fermentation, microorganisms metabolize carbohydrates or other organic substrates, producing energy, metabolites, and desired products. In light of its lower environmental impact and lower operating costs compared to conventional chemical processes, fermentation has not only attracted attention from the food processing industry, but also the pharmaceutical and waste treatment industries. Using microorganisms, raw ingredients are transformed into foods with better storage characteristics and enhanced biochemical (nutrient) and organoleptic levels. In a fermentation process, nutrient availability, substrate concentration, pH, temperature, and aeration influence the final fermented product. [10] in order to produce primary and secondary metabolites, and perform biotransformation, oil extraction and various other fermentation process, strains of microorganisms with high yields are used to meet the market demands [11]. Fermentation technology is broadly divided into two parts; viz upstream process and downstream process. The initial process of preparing for the fermentation, including selection, screening and improvement of microbial strain, preparation and sterilization of media, inoculum preparation is called as upstream processing. The recovery of the product after the fermentation such as the filtration, extraction, product purification and packing are called as downstream processing.

**1.2. Key Factors in Fermentation:** Several factors influence fermentation processes, including microbial strain selection, nutrient availability, temperature, pH, oxygen levels, and process control parameters.

1. **Microbial strain selection**

Strain refers to the homogenous population of potent microbes. The stain should be high yielding, it should have stable biochemical characteristics, it should be easily cultivable on large scale and should not produce any undesirable substances. Desirable microbial strains can be selected through primary screening and secondary screening techniques. Further, the strain can be improved by either of the following methods: mutagenesis, genetic engineering, natural recombination, regulatory mechanisms or by protoplast fusion.

1. **Raw material:**

The type and quality of the substrate or raw material being fermented play a significant role. Fermentation can utilise a diverse range of organic products, including carbohydrates, proteins or fats. The composition, concentration and availability of nutrients in the substrate can impact fermentation efficiency and final product characteristics.

1. **Nutrient availability**

The availability of the nutrients to the microbes is dependent on the composition of the fermentation media. To achieve the technical agendas of the fermentation, the fermentation media must satisfy all the nutritional requirements of the microorganism. The formulation of nutrients should be geared towards encouraging the production of the desired end product, whether it be cellular biomass or a specific substrate. The source, type and nature of basic nutrients are the most crucial factors determining the success rate of any fermentation process. In order to reproduce, form products, and maintain cells, microorganisms require carbon sources as a source of energy. Traditional carbon sources for microbial fermentations are carbohydrates (such as malt extract, molasses, starch, whey, sulphite waste liquor, cellulose), although alcohol and alkanes can also be used. The type and amount of the product formed depends on the nature of carbon sources and rate of assimilation [12] .Similar to carbon, the source and concentration of nitrogen in the media also plays an important role. Most microorganisms can utilise both organic and inorganic source of nitrogen. In some cases, nitrogen may have retarding effects on the production of metabolite whereas in others it may have enhancer effects. Generally, the crude forms of nitrogen (such as yeast extracts, peptones, soya bean meat) supplied from the byproducts of various other industries. Phosphate is also another basic component of the fermentation media as it is required for the production of nucleic acids and for the production of phospholipids. According to the need of the organism and nature of the desired product phosphate must be added in appropriate quantity. For the production of secondary metabolites, some fermentations require specific precursors. Phenylacetic acid is used as a side chain precursor in penicillin production. It is necessary to incorporate an inducer into the culture medium during fermentation if the production of a product is dependent on the presence of the inducer. A metabolic inhibitor reduces formation of other metabolic intermediates and redirects metabolism towards a target product.Optimization techniques should be carried out not only to reduce the time for the process development but also to reduce the overall production cost [12].

1. **Temperature and pH**

Temperature and pH are the key factors that directly affect the development of microorganisms as it determines the rate of growth, multiplication, survival and death. Each microorganism has an optimum temperature for its activity. In some cases, temperature control is crucial to prevent the growth of unwanted microorganisms or to promote specific metabolic pathways. To ensure successful fermentation the pH must be kept under observation and under control at all times. as pH optimisation is important for promoting enzyme activity, microbial growth, and production of desired fermentation products.

1. **Oxygen levels**

In both the presence and lack of oxygen, fermentation can take place. The availability or absence of oxygen affects the types of microorganisms that can thrive and produce products during fermentation.

1. **Agitation**

Proper agitation enhances the distribution of nutrients, oxygen and microbial cells and helps to maintain uniform conditions throughout the fermentation vessel, improving fermentation efficiency and preventing the formation of concentration gradients.

**Food and Beverage Industry:**

**2.1. Fermented Foods:** Fermented foods are composed of the complex metabolic interaction between raw ingredients and fermenting microorganisms, resulting in a product with unique physical and biochemical characteristics. When foods are fermented, the nutritional and biochemical quality of the original raw material alter. Microorganisms play a crucial role in the fermentation process, enhancing flavor, texture, and nutritional quality of the final products. Fermenting microorganisms mainly consist of lactic acid bacteria (LAB) such as *Lactobacillus, Streptococcus,* and *Leuconostoc,* and yeasts including *Saccharomyces*, *Penicillium,* *Rhizopus*, and *Mucor* species [13], [14]*.*  Fermentation not only makes the food safer for consumption but also reduces the energy consumption when cooking [15], [16]. Several advantages can be attributed to fermented foods [4], [17]:

1. The shelf life of fermented foods is longer than that of their original counterparts
2. Enhanced of organoleptic properties. For example, cheese in comparison to its raw substrate, milk, has more enhanced organoleptic properties.
3. For obtaining raw materials that are free of harmful/unwanted ingredients. For example, Cyanide content in garri is reduced during preparation of cassava by fermentation.
4. Higher antioxidant properties in fermented foods. For example:  The proteolysis of milk proteins releases biopeptides, which give yogurt higher antioxidant properties compared to milk.
5. Cooking time period of fermented foods is greatly reduced compared to non-fermented counterparts.
6. Fermenting microorganisms enhances the nutritional properties of fermented foods. For example, Yeast increases the nutritional value of bread and garri. By lowering blood cholesterol levels, resisting infections, boosting immunity, preventing osteoporosis, combating diabetes, allergies, obesity, and atherosclerosis, in addition to soothing lactose intolerance symptoms, fermented foods have numerous positive health impacts [18]. Numerous fermented foods and beverages are produced via fermentation., including yogurt, cheese, idli, dosa, sauerkraut, kimchi, soy sauce, bread, fermented vegetables and fruits, beer, wine, vinegar, etc. Most of the fermented products are made using starter cultures of (LAB) and other microorganisms. It is the production of lactic acid that produces the preservation effect, which lowers pH and prevents harmful and spoiling organism growth [19]. Due to all these advantages of fermented foods, it has recently grown in prominence owing to consumer interest, which has significantly increased market size.

**2.1.1. Fermented milk and milk products**

Fermented milk constitutes an vital part of human nutrition due to its hypo-cholesterolemic, hypotensive, and antimicrobial effects [20]. In order to protect the nutritive value of milk and improve the shelf life most of the milk based fermented food are produced from LAB. Lactic acid fermentation enhances protein solubility and some micronutrients and amino acids' availability [21] Traditionally, fermented foods such as yogurt are prepared using microorganisms based on raw materials and local practices where, curdling of milk may be induced by adding several different curdling agents or by adding a small amount of preformed curd, with subsequent incubation at a warm temperature [22], [23]. Probiotics can be found in yogurt, a fermented dairy product made from milk that has been coagulated by lactic acid fermentation [24]. Fermented dairy products such as cheese offer high levels of fat, calcium, and vitamin B, as well as high energy values. Combined with the vitamins, minerals and bioactive peptides cheese prevents against several diseases [25]. Unpasteurized milk is fermented into koumiss, a slightly alcoholic fermented beverage [26], [27]. The distinctive sour and alcoholic flavour of kousmiss is the result of both lactic acid and alcohol fermentation [4], [28].

**2.1.2. Fermented Meat and Meat Products**

Meat fermentation is one of the earliest and most prevalent type of fermentation [29]. Several biochemical, microbiological, and chemical processes are involved in the production of fermented meat, due to which fermented meat products acquire their distinctive flavor, colour, and odour [4]. Examples of fermented meat products are Sucuk (Turkish fermented dry sausage), fermented sausage, pastırma, Nham, salami, Fermented fish products such as fish sauce, Suan yu, bagoong, paak [30], [31].

**2.1.3. Fermented Fruit and Vegetable Products**

Globally, fermented fruits and vegetables such as pickled cucumbers, and kimchi are indispensable components of human nutrition [32], [33]. The fermentation of lactic acid inhibits pathogens and spoilage microbes by converting carbohydrates to CO2, alcohol, and organic acids,and it is the primary fermentation process used for fruits and vegetables [34], [35]. Mexican pulque is made by fermenting juices from the cactus plant (Agave) and it is the earliest alcoholic beverage consumed in North America [36].

**2.1.4. Fermented Beverages**

The food fermentation sector witness rapid growth, with the beverage industry emerging as one of the swiftest expanding industry as they are regarded by today's health-conscious customers in every country as a product that is energizing, practical, and healthful as well as a source of probiotics that may enhance wellbeing and lower the chance of developing chronic and degenerative diseases [37]. Therefore, fermented beverages are gaining popularity at an escalating rate throughout the world because of their health-promoting properties. Furthermore, non-dairy probiotic fermented drinks have been created using soy milk, whey, grains in addition to conventional beverages [38]. It is Saccharomyces cerevisiae that plays a central role in producing fermented beverages and foods [39] as in the history of mankind, yeast has been the most important factor in producing alcoholic beverages and economically important of all biotechnologies [40]. There are several different types of sugar-containing materials that can be used to make fermented beverages, including vegetable juices, cereals, milk and fruits. Thus, the fermented beverages obtained from different sources includes the following: wine from grapes, beer from barley, kefir from kefir grains. Water kefir, lambic beer, kombucha, etc are the traditional Turkish fermented beverages most commonly consumed include non-alcoholic beverages in their diet [41]. Different fermented beverages have been created over time from various food matrices, giving customers a variety of ways to include bioactive ingredients in their daily diet. Science and technology have played an increasingly important role in the evolution of fermentation, from the selection and use of specific starter cultures to improved nutritional properties and characteristics [42]**.** It is anticipated that fermented beverages will gain prominence in the functional food markets, in the coming era, as a result of recent advancements [43].

**2.2. Probiotics:** Fermentation is employed to produce probiotic products containing live beneficial microorganisms. The term probiotic refers to a single strain or combination of multiple strains of living microorganisms that enhance the intestinal microbial balance of the host and provide benefits to it either directly or indirectly. Probiotics contribute to gut health, enhance digestion, and boost the immune system. In order to derive the health benefits conferred by probiotics, humans consume fermented foods, as they are the major source of probiotics [44]. Fermented dairy products are rich in probiotics. Apart from the fermented dairy products, probiotics are also available in the form of capsules, pills and tablets [45]. In addition to these, non-dairy functional foods are seen as a wise alternative for vegans and individuals with lactose intolerance [46]. Among this plethora of options rich in probiotics, the dominant source of probiotic are the dairy products [47] especially yogurts have a relatively low pH environment that is conducive to probiotic bacteria's survival. There are two genera of probiotic microorganisms that make up most of their composition: *Lactobacillus* and *Bifidobacterium.* Among which Lactic acid bacteria (LAB) are widely used as probiotics as they perform dual function; they act as both probiotic delivery vehicles as well as starter culture for fermentation [48]. In general probiotics not only serve as a starter culture but also imparts many distinctive functional and sensory characteristics to the product [49], in addition to imparting various health-promoting qualities [50]. The primary health benefits derived from probiotics include improved balance of gut microbiota that helps in alleviating resistance against gastrointestinal infections by strengthening the gut barrier function, increasing immunity, inhibiting the growth of pathogenic bacteria, preventing irritable bowel syndrome and diarrhoea, improving assimilation of serum cholesterol etc [51]. In order to derive the health benefits conferred by probiotics, strains of probiotics are incorporated in products such as yogurt, cheese, fermented milk and ice cream [52]. A probiotic fermented milk is made from a fermented milk that has been inoculated with probiotics [53]. The probiotics found in fermented fruits and vegetables containing lactic acid bacteria are effective in preventing diseases such as cirrhosis and diarrhea [54]. Therefore, probiotics have garnered considerable attention in recent decades [52]. Essentially, there are three steps involved in the manufacture of a probiotic product:

 (a) The selection of the starter culture is determined by its capacity to target a specific location within the host’s digestive system, establish a presence and deliver the probiotic benefit;

(b) The starter culture is evaluated from a technological perspective, which is based on the starter culture's ability to propagate successfully and maintain viability at industrial levels; and

(c) Incorporating probiotic cultures into products as starters or adjunct cultures. Probiotic strains tools of several strains were characterized using predictive microbiology using whole genomes to provide information related to their properties as probiotics [55], [56].

**Pharmaceuticals and Biologics:**

**3.1. Antibiotics and Pharmaceuticals**: The cultivation of specific microorganisms and optimizing fermentation conditions enable the economical manufacturing of therapeutic compounds. Antibiotics are an important group of bioactive compounds produced by different microorganisms during the process of fermentation, having the ability to selectively kill or restrict the growth of harmful pathogenic microorganisms at very low concentrations [57]. They are secondary metabolites that are produced during the idiophase. Antibiotics are widely used round the world for preventing and treating diseases [58]. With industrialization and globalisation, population is increasing at a rapid rate, leading to the increased consumption of antibiotics. Hence production of a cost-effective method for the antibiotic production is inevitable in order to meet the rising demands [59]. Many antibiotics, such as penicillin, streptomycin, and tetracycline, are produced through fermentation. Antibiotics are generally produced at the industrial scale using agro-wastes, such as sawdust; as they are rich in bioactive compounds. Different agricultural are used for the production of different antibiotics. Utilizing groundnut shells as the raw material and a strain of *Streptomyces rimosus*, *Oxytetracycline* was synthesized using SSF. [60] . This method of production of oxytetracycline was also supported by [61] and [62]. Agro-wastes are also used in the construction of antibiotic, such as neomycin [63]. Of the different agro-industrial wastes tested, coconut oil cake and ground nut shell produced the most antibiotics. In recent studies, solid state fermentation (SoSF) was used to produce oxytetracycline, neomycin, and rifamycin. Hence, antibiotics can be produced efficiently by fermenting agro-waste using appropriate fermentation techniques [57]. In addition to strain improvement, use of agricultural residues as low-cost carbon source the cost of antibiotic production can be significantly reduced. Microbial fermentation led to the production of penicillin from *Penicillium notatumoea*, the first antibiotic to be produced through this process. Mixed cultures of actinomycetes and fungi produced penicillin in Solid state fermentation (SSF). Today, SSF is more widely used than Submerged fermentation (SmF) as a result of the development of proper substrates due to the production of constant and high quantity antibiotics in SSF. Certain common antibiotics produced from SSF are Penicillin, Cephamycin C, Cyclosporin A, Cephalosporins, Iturin, and Neomycin. Through fed-batch system most antibiotics are produced, such as teicoplanin [64] daptomycin [65] tylosin and β-lactams [66]. Penicillin, cephalosporins, and monobactams are collectively referred to as -lactam antibiotics. Although antibiotics can be synthesized by synthetic processes as well, the only convenient method for creating this important medicine is still microbial fermentation. [67]. Since (a) The bacterial or fungal strain that produces the antibiotic determines the purity of the active ingredients.; (b) The raw ingredients used, especially the type of water used to cultivate the strains, can also differ.; (c) different strains may be treated under different conditions.; and (d) The selectivity of the extraction and purification procedures might be restricted [68]. Since antibiotic production has advanced dramatically, regulatory networks have evolved as well and its intracellular and environmental signals, have made it possible to discover and overproduce novel antibiotics. At present, genetic engineering is playing a vital role in strain and fermentation improvement to hasten the process of discovery and development of new antibiotics as effective drugs [69].

**3.2. Recombinant Proteins and Biologics**: In order for mankind to survive, therapeutic proteins must be produced at a large scale to treat diseases at a large scale. The production of recombinant proteins has been made possible by recent progress in recombinant DNA technologies that can be used as vaccines, diagnostic reagents, and therapeutics. Fermentation technology has an pivotal role in the production of recombinant proteins, including hormones, therapeutic enzymes, and monoclonal antibodies. Microorganisms or cell cultures are genetically engineered to express and produce these complex molecules at both the large-scale settings and at the laboratory scale. For small biologics (e.g., proteins, peptides, growth factors, cytokines, single-domain antibodies plasmid DNA, nucleic acids, and peptibodies), microbiological fermentation in bacteria, yeast, or fungi is generally preferred as the processing time required compared to cell culture, are frequently much shorter, and media expenses can be substantially lower. The use of microbial fermentations in manufacturing recombinant proteins results in accelerated progress, increased output, superior product quality, minimized batch to batch variability, enhanced scalability and reduced production expenses [70]. In the early 1980s, recombinant insulin produced from Esherichia coli became the inaugural pharmaceutical protein approved for clinical use via microbial fermentation. Since then, this field has witnessed many advances. Novel cell factories are produced through high-throughput analyses and integrative approaches, therapeutic proteins can be produced on a large scale [71]. Several studies have demonstrated that Escherichia coli has many advantages over yeast and other expression systems, it is the perfect host for the creation of non-glycosylated proteins. This is primarily because of its simple upstream process (USP) and ease of handling, which enable it to be employed in the manufacture of recombinant proteins in huge quantities at reasonable costs [72]. *Saccharomyces cerevisiae* and Pichia pastoris are the second and third most favorable microbial systems, respectively for the production of recombinant proteins after E. *coli* [73]**.** Chinese hamster ovary (CHO) cells are the fourth most common host mammalian system and account for roughly 70% of the recombinant proteins generated [74].Over the last decade the industrial scale manufacture of recombinant therapeutic proteins including monoclonal antibodies (mAbs) have undergone a sea change improvement in terms of implementation of various novel technologies [75]

**Biofuels and Renewable Energy:**

**4.1. Ethanol Production**: In recent years need for fossil fuels such as petrol and diesel has increased several folds, as a result biofuels are in high demand. This is due the fact that biofuels are an excellent substitute of fossil fuels. Various biofuels produced from biodegradable and waste materials leading to zero waste concept are biodiesels, bioethanol, butanol, biohydrogen etc [76]. Biofuel are an excellent substitute for fossil fuels their nontoxic, sulfur-free, biodegradable nature, originating from the renewable sources [77].  Furthermore, biofuels are experiencing growing utilization in the fields of transportation, heat, and power developments that require renewable energy sources [77]. The most significant benefit of biofuels is that they are a renewable source of fuel mostly derived from agriculture and essential harvesting, woods, and residue streams [78]–[80]. Biological fuels are energy sources derived from organic materials (collectively called biomass), which are renewable and can be harvested repeatedly. They are mostly derived from agricultural and essential harvesting, woods, and residue streams, which are utilised to substitute non-renewable energy fuels [78]–[80]. Biodiesel, which is derived from vegetable oils, recycled wax, or animal fats, and bioethanol, is derived from the fermentation of sugar and starch crops like maize, and biogas [77].  Utilization of agricultural leftovers such as sawdust, potato waste, rice straw, corn stalks, and sugarcane bagasse has been demonstrated in prior studies [81], [82] to produce ethanol through the action of yeast or bacteria. Fermentation is a very promising approach for the conversion of food waste into biofuels. Different biotechnologies are able to turn food waste into biofuels through , aerobic digestion, anaerobic digestion and microbial fermentation processes such as biomethane, biohydrogen, bioethanol, and biodiesel [83]. In the production of biofuel, a wide range of raw materials has been used, depending on the availability of biomass, cost-effectiveness, and their geographic location. In recent years, lignocellulosic biomass has been used as a raw material by many researchers all over the world compared to any other raw material [84]. Second-generation biofuels produced from lignocellulosic biomass were investigated in various studies [85], [86].The production of biofuels could be based on a variety of lignocellulosic residues such as straws, wood pellets, and agro-waste [87] . As a result of its low price, availability throughout the year, and wide geographical distribution, lignocellulosic biomass is regarded not only as a viable option for biofuel production, but also as a suitable replacement to fossil fuels [88]. Lignocellulosic materials have been used in many research studies to make bioethanol [89], [90]. Reference [91] discussed the use of agricultural wastes to produce second-generation bioethanol. They concentrated on the utilisation of various agro-industrial wastes' lignocellulosic content. Therefore, lignocellulosic-derived biofuels are both eco-friendly and alternative sources of energy for the production of biofuels. Due to rapid population influx and industrialization in most of the developing countries, the demand for low-priced energy source is extremely high. High demand for energy fuels can be met by using economical agricultural residues for the production of biofuels. Bioethanol was created by fermenting vegetable waste using the yeast Saccharomyces cerevisiae [92]. They made use of common vegetable scraps. Producing bioethanol might be the best alternative to eating agricultural waste. Hence, a better method to meet the need for energy while preserving limited resources is to produce valuable biofuels out of cheap, environmentally friendly agricultural waste. Hence, our reliance on woody biomass from forests is reduced by the use of agricultural residues, helping to reduce deforestation[93]. To address the industrial demand for renewable energy, metabolic engineering is pushing the boundaries of what is possible by creating microbial chassis for biofuel bio-foundries. [94]. Metabolic engineering can be used to get beyond these obstacles in the biofuel production routes, which have revolutionised the standards for producing both conventional and advanced biofuels. By changing the molecular mechanisms linked to the metabolic pathways that produce fuel, this method seeks to improve the metabolic performance of microorganisms. [94]. Hence, the environmentally-friendly and nontoxic nature of biofuels has made them a popular source of energy. Industrialization and commercialisation require accelerating laboratory-scale cycles of biofuels to enhance yields and productivities [77].

**4.2. Biogas Generation**: Anaerobic fermentation of organic waste materials, such as agricultural residues, animal manure, and food waste, produces biogas.  As a flexible energy source, biogas can be used to produce heat, power, biomaterials, and transportation fuels, as well as to ensure environmental pollution is controlled in a meaningful way [95]. Biogas, primarily composed of methane, serves as a sustainable energy source for electricity generation and heat production. The principal constituent of biogas includes CH4 (60%) and CO2 (40%) [96]. Biogas can be a useful means of achieving a number of goals relating to waste, environment, and energy management. To replace fossil fuel in an environmentally sustainable way, biogas production is the most crucial prerequisite. Biogas can be created at landfills, wastewater treatment facilities, and agricultural biogas plants under controlled conditions [97]. In spite of numerous routes of renewable energy sources available, due to the enormous supply of lignocellulosic biomass, biogas generation holds an unparalleled position. As a result, researchers from all around the world are working diligently to create low-cost, sustainable methods of producing biogas for use in power, heat, and transportation [95]. Renewable energy is expected to make up 55%-75% of total energy consumption by 2050, with an emphasis on geographic self-reliance. Thus, the expanding field of renewable energy sources should include biogas plants as facilities that transform waste into electricity. Compressed biogas can be produced from organic residues using anaerobic digestion techniques as a key renewable energy technology [98]. The efficient conversion of organic materials in biomass under the assimilation of anaerobic bacteria is known as biomass anaerobic fermentation, which finally creates economically valuable methane and some carbon dioxide that can be burned to produce electricity. Due to a number of disadvantages associated with traditional anaerobic fermentation such as long fermentation time and low gas production rate, wide application of this method for biogas production is limited. In comparison to medium temperature anaerobic fermentation, the high temperature method produces more gas and kills more pathogenic microorganisms. Hence, anaerobic fermentation technology along with high temperature is highly efficient and cost-effective [99] . It was shown that C. autoethanogenum can act as a biocatalyst to ferment carbon dioxide from synthetic biogas augmented with hydrogen to produce ethanol and acetate as biogas upgrading technologies [100]. In anaerobic fermentation to produce methane, duckweed has been proven as an excellent methane producer and can take the role of lignocellulosic plants [99]. The use of biogas as a sustainable fuel appears to be the path of rising relevance when taking into account the benefits to the environment and the economy. Utilizing waste to create biogas is undoubtedly in keeping with the circular economy movement [97].

**Industrial Biotechnology:**

**5.1. Enzyme Production**: Enzymes are efficient, sustainable and greener substitutes to the chemicals used for the industrial processes. They are the flexible biocatalysts that have the power to significantly alter the food sector and lignocellulosic biorefineries [101]. Fermentation is utilized to produce a wide range of enzymes used in various industries, including detergent, textile, paper, and biofuel production. Microorganisms are engineered to overexpress specific enzymes, resulting in high yields and cost-effective production. Enzyme-catalysed reactions offer various advantages; since these reactions are specific, produce less and low toxic by-products There are economic and environmental benefits to immobilizing and reusing enzymes, and these benefits can be achieved through enzyme inactivation [102]. There are several different applications of enzymes, which includes the food processing, technical applications, organic synthesis and biofuel production in pharmaceuticals and cosmetics [103]. Enzymes are employed in the detergent business to break down proteins, lipids, and starches in order to soften and enhance the colour of the fabric. It is used to reduce viscosity, increase softness and brightness in pulp and paper products by utilizing enzymes such as amylases, cellulases, and xylanases [104]. Enzymes utilized in the dairy sector for the manufacturing of lactose-free dairy products and cheese are lipases and lactases. Amylases are an important group of carbohydrate hydrolytic enzymes that are used for improving the stability of the dough in bakery industry and also for clarifying fruit juices in juice industry [105]. A number of enzymes are used in the production of bioethanol for the breakdown of lignocellulose, including cellulases, hemicellulases, and xylanases [106]. The collection of enzymes is made possible with both fungi and bacteria when fermentation is done on appropriate substrate. Enzymes cultivated from fungus are generally produced through solid state fermentation as they require less water potential, whereas bacterial enzyme production is best produced through submerged fermentation [107]. However, recent studies have shown that bacterial enzyme production could also be achieved by solid state fermentation. Well known enzymes produced from bacteria are amylase, cellulase, xylanase, and L-asparaginase. Several species of the fungus Aspergillus have been isolated from this procedure, which is a crucial one for the production of enzymes in industry. In the past, *Aspergillus* is used as model microorganism used to produce fungus enzymes as it is the largest fungal source of enzyme [108]. Numerous microorganisms that break down lignocellulosic material are being studied in the agroindustry as potential manufacturers of enzymes necessary for the enzymatic hydrolysis of the lignocellulosic material [109]. Worldwide, lignocellulolytic enzymes account for 20% of commercially available enzyme sales and have applications in food, textile biofuel, paper and pharmaceutical industries [110]. Engineering tools can be used to improve the strains of enzymes produced. Purity, specificity, catalytic efficiency, and expression yield of engineered enzymes are improved thanks to altered amino acid sequences and the application of potential protein engineering tools such as computational techniques, and directed evolution [111]. Thus, in the field of biotechnology, microbial enzyme production provides an invaluable resource which has a wide application.

**5.2. Specialty Chemicals and Fine Chemicals:** Fermentation enables the production of specialty chemicals and fine chemicals, including organic acids, amino acids, vitamins, and bioactive compounds. Microbial strains are optimized to generate high-value products with desired properties. Many industrial production methods rely on model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* due to their robust and desirable traits [112]. The industrial biotechnology industry has grown into a notable production method for ethanol suitable for fuel, organic acids and large quantities of amino acid, even though most items are still specialized products for food and pharmaceuticals [113].Common organic acids produced through solid state fermentation are citric acid, gallic acid, lactic acid, fumaric acid and kojic acid. Several agricultural industry wastes have been shown to be very inventive substrates for the manufacture of citric acid through solid state fermentation are wheat bran, sugarcane, coffee husk, pineapple wastes, de-oiled rice bran, grape pomace, kiwi fruit peels, and apple. For the production of citric acid from *Aspergillus*, a substrate made of pine apple waste was utilized. Secondary Metabolites are mostly produced from Fungus. Gibberellic acid is a secondary metabolite produced by a fungus using wheat bran as substrate in its stationary phase, through solid state fermentation. In SSF, you can find water-soluble vitamins like riboflavin, nicotinic acid, thiamine, vitamin B12, and vitamin B6 produced from *Rhizophus* and Klebsiella species, which produce vitamin B12 in significant amounts [113].

**Conclusion:**

Fermentation technology serves as a versatile and powerful tool in biotechnology, offering diverse applications in food production, pharmaceuticals, biofuels, and industrial biotechnology. Without a doubt, fermentation is a crucial and vital processing technique used to create new food products. It also emphasizes the significance of fermentation as a multifaceted and sustainable process for the manufacturing of a diverse array of products. This chapter underscores the crucial role of biotechnology in harnessing microbial fermentation for the benefit of society, paving the way of innovative and eco-friendly solutions to address global issues. By harnessing the metabolic capabilities of microorganisms, fermentation facilitates sustainable production processes, leading to the development of valuable compounds, renewable energy sources, and innovative solutions. Continued advancements in fermentation technology hold immense potential for addressing global challenges and shaping a more sustainable future.

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