**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-microbial, anti-oxidant, anti-fungal, anti-diabetic and anti-inflammatory [4]. Probiotics are 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. Latest products produced through this process are anti-viral drugs, therapeutic recombinant proteins and DNA, and monoclonal antibodies. In addition to drugs, fermentation is also used to produce materials for commercial use, such as drug delivery vehicles, diagnostic kits, and medical devices. 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 (like yoghurt, cheese, pickles), agricultural products (like microbial pesticides), industrial chemicals (such as acetone, butanol), alcoholic beverages (like beer, wine), pharmaceutical chemicals (such as enzymes, vaccines), 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 word Fermentation originates from a Latin verb “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 such as yeast, bacteria, and fungi, even though most of them are aerobic, for the transformation of complicated substrate into simple compounds that are beneficial to people, such as those with applications in the production of energy, materials, 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 shelf life and protection on both a biochemical (nutrient) and organoleptic (taste, texture, odour, and appearance) level. In a fermentation process, nutrient availability, substrate concentration, pH, temperature, and aeration influence the final fermented product. [10] For the production of primary and secondary metabolites, biotransformation, oil extraction and various other fermentation process use strains of microorganisms with high yields are used in order to meet the market demands [11]. Fermentation technology is broadly divided into two parts; viz upstream processing and downstream processing. 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 wide variety of organic products such as 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 objectives of the fermentation process, the media must satisfy all nutritional requirements of the microorganism. The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific substrate. The source, type and nature of basic nutrients in the form of carbon and nitrogen source 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 inorganic and/or organic nitrogen source. In some cases, nitrogen may have inhibitory effects on the metabolite production whereas in others it may have enhancer effects. The crude forms of nitrogen supplied are usually byproducts of other industries. For example, corn steep liquor, yeast extracts, peptones, soya bean meat are examples of byproducts of 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 added 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.

In order to begin semi-pilot/pilot production plans, it is absolutely essential to optimize the fermentation media. 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 crucial for promoting microbial growth, enzyme activity and production of desired fermentation products.

1. **Oxygen levels**

In both the presence and lack of oxygen, fermentation can take place. Aerobic fermentation requires oxygen, while anaerobic fermentation do not require oxygen. 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, Enterococcus, Streptococcus, Leuconostoc, and Pediococcus* and yeasts and molds including  *Debaryomyces*, *Saccharomyces*,  *Kluyveromyces*, *Mucor*, *Rhizopus*, and *Penicillium*  species [14], [15]*.*  Fermentation not only makes the food safer for consumption but also reduces the energy consumption when cooking [16], [17]. Several advantages can be attributed to fermented foods [4], [18]:

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, and the flatulence factors in soybeans are removed by fermentation.
4. Higher antioxidant properties in fermented foods. For example:  yogurt consist of higher antioxidant properties compared to milk, as the proteolysis of milk proteins releases biopeptides such as α-casein, α-lactalbumin, and β-lactoglobulin.
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 [19]. Numerous fermented foods and beverages are produced via fermentation., including yogurt, cheese, idli, dosa, sauerkraut, kimchi, soy sauce, bread, fermented fruits, non-alcoholic beverages (boza), cereal-based fermented dishes (tarhana), fermented vegetables (mustard, pickles, and turnips), fermented fruits. beer, wine, vinegar, etc. Fermented beverages, dairy, meat, and vegetable products are all made using starter cultures of (LAB) and other microorganisms. It is the production of lactic acid and other organic acids that produces the preservation effect, which lowers pH and prevents harmful and spoiling organism growth [20]. Fermented foods are also rich in proteins. 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 important part of human nutrition due to its hypo-cholesterolemic, hypotensive, and antimicrobial effects [21]. 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 [22] 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 [23], [24]. Yogurt, a potential source of probiotics is a coagulated fermented dairy product obtained by the lactic acid fermentation of milk [25]. Fermented dairy products such as cheese offer high levels of fat, protein, calcium, and vitamin B, as well as high energy values. Combined with the vitamins, minerals and bioactive peptides cheese prevents against several diseases due to its anti-carcinogenic and anti-obesity characteristics [26]. Unpasteurized milk is fermented into koumiss, a slightly alcoholic fermented beverage [27], [28]. The distinctive sour and alcoholic flavour of kousmiss is the result of both lactic acid fermentation and alcohol fermentation [4], [29]. Kefir is a traditional fermented milk beverage with a creamy texture and sour, acidic, and faintly alcoholic flavor. to its pleasing organoleptic characteristics in addition to anti-hypertensive, anti-carcinogenic, hypocholesterolemic, anti- inflammatory, anti-mutagenic, anti-allergenic, anti-bacterial, anti-diabetic, anti-oxidant, and probiotic effects, it has become a focus of interest in recent years [30].

**2.1.2. Fermented Meat and Meat Products**

Meat fermentation is one of the earliest and most prevalent type of fermentation [31]. Several biochemical, microbiological, and chemical processes are involved in the production of fermented meat, and as a result of these changes, fermented meat products acquire their distinctive taste, colour, aroma, 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 [32], [33]

**2.1.3. Fermented Fruit and Vegetable Products**

Globally, fermented fruits and vegetables such as pickled cucumbers, sauerkraut, and kimchi are indispensable components of human nutrition [34], [35]. Lactic acid fermentation, which involves the oxidation of carbohydrates to carbon dioxide, alcohol, and organic acids that inhibit pathogen and spoilage microbes, is the primary fermentation process used for fruits and vegetables [36], [37]. Mexican pulque is made by fermenting juices from the cactus plant (Agave) and it is the earliest alcoholic beverage consumed in North America [38].

**2.1.4. Fermented Beverages**

The beverage industry is one of the fastest growing segments of the food fermentation 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 [39]. Therefore, fermented beverages are becoming increasingly popular throughout the world because of their health-promoting properties. Additionally, non-dairy probiotic fermented beverages have been developed from whey, soy milk, cereals and vegetable and fruit juices, in addition to traditional beverages [40]. In the production of fermented beverages and foods, Saccharomyces cerevisiae plays a central role [41] as in the history of mankind, yeast has been the most important factor in producing alcoholic beverages and economically important of all biotechnologies [42]. 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, cider from apples, sake from rice, mead from honey, and other fermented beverages like probiotics. Water kefir, lambic beer, kombucha, etc are the traditional Turkish fermented beverages most commonly consumed include non-alcoholic beverages in their diet [43]. Acetic acid bacteria (AAB) is commonly found in fermented beverages such as lambic beer, water kefir, kombucha, and cocoa [44]. Carrot juice supplementation fermented with Lactobacillus rhamnosus GG LGG (DFCL) may help to manage blood sugar, insulin, and antioxidant capacity [45]. 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. Throughout the evolution of fermentation, scientific and technological advances have played a crucial role, from selecting and using specific starter cultures to improving their performance through the application of novel technologies, resulting in products with improved nutritional properties and characteristics [46]**.** It is anticipated that fermented beverages will gain prominence in the functional food markets, in the coming era, as a result of recent advancements [47].

**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 are commonly classified by a widely accepted broad definition, proposed by a Joint Expert Consultation of the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) as “a live microorganism that imparts health benefits to its host when consumed in an adequate amount” [48]. 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 [49]. Fermented foods rich in probiotics are fermented dairy products like yogurt, cheese and fermented milk. Apart from the fermented dairy products, probiotics are also available in the form of capsules, pills and tablets [50]. In addition to these, non-dairy functional foods are seen as a wise alternative for vegans and individuals with lactose intolerance [51]. Among this plethora of options rich in probiotics, the dominant source of probiotic are the dairy products [52] especially yogurt as they have a relatively low pH environment for the survival of probiotic bacteria. There are two genera of probiotic microorganisms that make up most of their composition: *Lactobacillus* and *Bifidobacterium.* Among which Lactic acid bacteria (LAB) are commonly used as probiotics. LABs perform dual function; they act as both probiotic delivery vehicles as well as starter culture for fermentation [53]. In general probiotics not only serve as a starter culture but also imparts many distinctive functional and sensory characteristics to the product (for instance improved taste, aroma and textural characteristics) [54], in addition to conferring many health-promoting properties [55]. 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 [56]. 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 [57]. A probiotic fermented milk is made of fermented milk that has been inoculated with probiotics [58]. While the antioxidants in fermented fruits and vegetables can help avoid degenerative diseases brought on by damaging free radicals, the probiotics in fermented fruits and vegetables containing lactic acid bacteria can assist to prevent specific diseases like cirrhosis and diarrhoea.[59]. Hence, probiotics have gained significant prominence in the last few decades [57]. Essentially, there are three steps involved in the manufacture of a probiotic product:

(a) The starter culture is chosen based on its ability to reach a particular niche within the host's gastrointestinal tract, colonize it, and confer the probiotic action;

(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 functionality after a series of processing steps; 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 [60], [61].



**Pharmaceuticals and Biologics:**

**3.1. Antibiotics and Pharmaceuticals**: The cultivation of specific microorganisms and optimization of fermentation conditions enable the efficient production of therapeutic compounds. Antibiotics are an important group of bioactive compounds produced by different microorganisms during fermentation process, having the ability to selectively kill or inhibit the growth of harmful pathogenic microorganisms at very low concentrations [62]. They are secondary metabolites produced during the idiophase. Antibiotics are widely used round the world for the prevention and treatment of diseases [63]. 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 [64]. Many antibiotics, such as penicillin, streptomycin, and tetracycline, are produced through fermentation. Since it has been estimated that less than 1% of antimicrobial agents have any medical or commercial value despite the wide variety of antibiotics that are known, screening of useful antibiotics is an important step for antibiotic production at large scale. Antibiotics are generally produced at the industrial scale using agro-wastes, such as sawdust, corn cobs, rice hulls and groundnut shell. Agro-wastes are used as they are rich in bioactive compounds. Different agricultural are used for the production of different antibiotics. By using groundnut shell as a raw material and a strain of Streptomyces rimosus, *Oxytetracycline* was synthesized using SSF. [65] . This method of production of oxytetracycline was also supported by [66] and [67]. Agro-wastes are also used in the construction of antibiotic, such as neomycin [68]. 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. In order to reduce the operational cost and increase the yield, strain improvement is carried out for the large-scale production of antibiotics from microbial fermentations [62]. In addition to strain improvement, use of agricultural residues as low-cost carbon source the cost of antibiotic production can be significantly reduced. The first antibiotic produced through microbial fermentation was Penicillin from *Penicillium notatum,* in which wheat bran and sugarcane bagasse are used as substrate under high moisture content (70%)*.* 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 [69] daptomycin [70] tylosin and β-lactams [71]. Penicillin, cephalosporins, and monobactams are collectively referred to as -lactam antibiotics. Any research and development program aiming to manufacture fermentation products on a large scale usually ends with scaling up the fermentation process [72]. Although antibiotics can be synthesized by synthetic processes as well, the only convient method for creating this important medicine is still microbial fermentation. [73]. 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 [74]. Due to the drastic advances made in exploring the sector of antibiotic production, regulatory networks 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 [75].

**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, therapeutics, and diagnostic reagents. Fermentation technology plays a 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 faster development, higher yields, and higher quality products, reduced variation between batches, better scalability, and lower production costs. Developing bioprocessing techniques such as continuous upstream processing, continuous chromatography, integrated continuous bioprocessing, and the use of high-throughput devices for effective bioprocess optimization are crucial for the industrial production of recombinant proteins with therapeutic and preventative importance. [76]. The first recombinant pharmaceutical protein to be approved for clinical use via microbial fermentation was recombinant insulin made from Escherichia coli in the early 1980s. Since then, this field has witnessed many advances. Novel cell factories are produced for large scale production of therapeutic proteins through high throughput analysis techniques (the so-called — omics approaches) and integrative approaches (systems biology) [77]. 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 [78]. *Saccharomyces cerevisiae* and Pichia pastoris are the second and third most favorable microbial systems, respectively for the production of recombinant proteins after E. *coli* [79]**.** Given that they produce almost 70% of the recombinant proteins produced, Chinese hamster ovary (CHO) cells are the fourth most prevalent host mammalian system [80].Over the last decade the commercial production of recombinant therapeutic proteins including monoclonal antibodies (mAbs) have undergone a sea change improvement in terms of implementation of various novel technologies [81].

**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. Fermentation is used in the production of biofuels such as bioethanol, a renewable and sustainable alternative to fossil fuels. Various biofuels produced from biodegradable and waste materials leading to zero waste concept are biodiesels (produced from vegetable oils, re-used wax, or creature fats), bioethanol (alcohol produced by fermenting sugar and starch crops such as corn), butanol, biohydrogen etc [82]. Biofuel are an excellent substitute for fossil fuels their nontoxic, sulfur-free, biodegradable nature, originating from the renewable sources [83]. Moreover, they have the potential of bringing control to the ever-increasing problem of greenhouse gas emissions due to the burning of petroleum fuels. [84]. Furthermore, biofuels are increasingly being used in transportation, heat, and power developments that require renewable energy sources [83]. 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 [85]–[87]. 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 [85]–[87]. The most common types of biofuels are biodiesel, which is derived from vegetable oils, recycled wax, or animal fats, bioethanol, which is derived from the fermentation of sugar and starch crops like maize, and biogas [83].  Depending on the biomass used by the fermentation technologies four generations of biofuel production technologies have been developed. First-generation biofuels are produced from various food crops such as starch, sugars, animal fats, and vegetable oils. Second-generation biofuels are produced from the non-edible portion of crop and biological waste matter mainly lignocellulosic biomasses. Third-generation biofuels are produced from specially engineered energy crop such as algae or food waste biomass. Fourth-generation biofuel is a pretty novel idea, which aims to capture the carbon dioxide (CO2) at every stage of the biofuel production and dispose it back to earth [84],[88]. Utilization of agricultural leftovers such as sawdust, sweet potato waste, potato waste, rice straw, corn stalks, and sugarcane bagasse has been demonstrated in prior studies [89], [90] 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 [91]. 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 [92]. Second-generation biofuels produced from lignocellulosic biomass were investigated in various studies [93], [94].The production of biofuels could be based on a variety of lignocellulosic residues such as straws, crop residues, wood pellets, wood chips and agro-waste [95] . 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 alternative to fossil fuels, since it does not compete with food crops and has great potential for bioethanol production. [96]. Lignocellulosic materials have been used in many research studies to make bioethanol [97], [98]. Reference [99] 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. Using the yeast *Saccharomyces cerevisiae* bioethanol was producedfrom vegetable’s waste by fermentation [100]. They made use of common vegetable scraps including potato peel, onion peel, and carrot peel. 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[101]. 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. [102]. 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. [102]. 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 [83].

**4.2. Biogas Generation**: Anaerobic fermentation of organic waste materials, such as agricultural residues, animal manure, and food waste, produces biogas.  As a flexible source of energy, 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 [103]. Biogas, primarily composed of methane, serves as a renewable energy source for electricity generation and heat production. The principal constituent of biogas includes CH4 (60%) and CO2 (40%) [104]. 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 [105]. 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 [103]. 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 [106]. 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 [107] . 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 [108]. In anaerobic fermentation to produce methane, duckweed has been proven as an excellent methane producer and can take the role of lignocellulosic plants [107]. Another investigation into the production of biogas utilising a variety of agricultural waste products from diverse sources and two weeds, Typha angustifolia L. and Eichornia crassipes Solms, was conducted [109]. 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 [105].

**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 [110]. 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 [111]. There are several different applications of enzymes, which includes the food processing, technical applications, organic synthesis and biofuel production in pharmaceuticals and cosmetics [112]. 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 [113]. Enzymes used in the dairy industry for the manufacture of cheese and production of lactose-free dairy products 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 [114]. A number of enzymes are used in the production of bioethanol for the breakdown of lignocellulose, including cellulases, hemicellulases, and xylanases [115]. The most important technique for manufacturing enzymes is enzyme cultivation. The collection of enzymes is made possible with both fungi and bacteria when fermentation is done on appropriate substrates. Both submerged and solid-state fermentation can be used to produce enzymes. 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 [116]. However, recent studies have shown that bacterial enzyme production could also be achieved by solid state fermentation. It has been proposed that enzymes may be used for many commercial applications, including the leather industries (peptidases), degradation of plant biomass (cellulases, xylanases, esterases and ligninases), pharmaceutical industry (production of L-Asparaginase and collagenases biopharmaceuticals), and bioremediation (oxidoreductases) [117].Well known enzymes produced from bacteria are amylase, cellulase, xylanase, and L-asparaginase. Several species of fungus, Aspergillus, have been isolated from this process, which is an industrially important enzyme-producing process. In the past, *Aspergillus* is used as model microorganism used to produce fungus enzymes as it is the largest fungal source of enzyme [118]. 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 [119]. Worldwide, lignocellulolytic enzymes account for 20% of commercially available enzyme sales and have applications in food, textile biofuel, , animal feed, paper, and pharmaceutical industries [120]. 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, rational design, and directed evolution [121]. 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 [122]. The industrial biotechnology industry has grown into a significant manufacturing tool for fuel-grade ethanol, organic acids, and bulk amino acids, though most items are still specialized products for food and pharmaceuticals [123].Common organic acids produced through solid state fermentation are citric acid, gallic acid, kojic acid, fumaric acid and lactic acid. some agro-industrial wastes which are proven to be very resourceful substrates for production of citric acid in solid state fermentation are wheat bran, sugarcane, coffee husk, pineapple wastes, de-oiled rice bran, grape pomace, kiwi fruit peels, and apple. A pine apple waste substrate was used for producing citric acid from Aspergillus. 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 nicotinic acid, vitamin B12, thiamine, riboflavin, and vitamin B6 produced from Rhizophus and Klebsiella species, which produce vitamin B12 in significant amounts.

**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 production of wide range 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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