

# Chapter 1

## Efficient Utilization and Bioprocessing of Agro-Industrial Waste



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**Abstract** The rising population and industrialisation produce a huge amount of waste, in particular from agro-industries, thus inducing environmental pollution. Therefore, waste should be better recycled into materials and energy, e.g. by incineration, gasification, and pyrolysis. Valuable by-products include enzymes, pigments, biofertilizers and edibles. Here we review the conversion of agro-waste, with focus on fermentation, composting, anaerobic digestion, enzymes, bioethanol, biofertilizers, microbial treatment and greenhouse gases.

**Keywords** Agro-industrial waste · Bioenergy · Biofertilizer · Sustainable · Bioconversion · Renewable

### 1.1 Introduction

The rapid growth of the population generated significant investment in the food and agricultural industries. Every year, approximately 1.3 billion tonnes of agro-industrial food waste is produced worldwide. Fruits, vegetables, roots, and tubers account for the bulk of this (40–50%), making for 520–650 million tonnes (Ravindran et al. 2018), and destined for landfills or uncontrolled disposal, resulting in damage to the environment and financial loss. Therefore, sustainable

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management of them must be established. Population rise also calls for increased global energy demand, currently supported by fossil-fuels, which prevents long-term sustainability. The biggest and most often used source of renewable energy globally is biomass, which is increasingly used. The world biomass production is expected to 1500 Exajoules in 2050 and can be used sustainably from 200 to 500 Exajoules, accounting for 40–50% of the estimated primary energy demand (Popp et al. 2014). The energy demand is expected to rise by about 48 percent between 2012 and 2040 (Avcioğlu et al. 2019). Government assistance is a pillar in accessing funding and technology and reducing the cost of producing green energy to promote the application of biomass-based energy technologies (Gutiérrez et al. 2020).

High-strength residues like livestock slurry, manure, crop remains, by-products of the agri-food such as coffee dregs, bagasse, degummed fruit, legumes, milk serum, sludge from wool, cellulose generated from agricultural and industrial sectors may be used to produce electricity, composting, biogas, biofuels and many other by-products by the action of microbes. (Yusuf 2017). For this purpose, advanced biorefinery becomes a promising option with several outputs (biofuels, biomaterials). The circular economy is based on a biorefining concept and approach to reducing, reusing, and recycling waste to recover waste products considered renewable resources (Peng and Pivato 2017).

This chapter discussed various kinds of waste generated from the agro-industrial sectors, such as agricultural residues, industrial residues, and different forms of wastewaters generated from agro-food industries. Well-established methods such as solid-state fermentation, Anaerobic digestion, and the composting process can be used to treat these various organic wastes. The action of different microbial communities can result in the production of various value-added products such as enzymes (alpha and beta-amylase,  $\beta$ -glucanase, cellulase, hemicellulase, xylanase, mannase, pectinase, inulinase, laccase, phytase, invertase, protease, pullulanase, and lipase), bioethanol, biodiesel, biogas and microbial biofertilizers with their potential applications in industrial or agricultural sectors. Increased industrialization and improper agro-waste management lead to greenhouse gas generation at a high rate, which has a toxic effect on the atmosphere and the human race. So, proper management practices can be helpful in the reduction of greenhouse gases and also dependency on fossil fuels.

## 1.2 Generation and Utilization of Agro-Industrial Biomass

A vast amount of waste is generated from agriculture-based industries in the form of agriculture and industrial residues, and wastewaters. Poor and unorganized disposal of these wastes adversely affects the environment and human health. These agro-based wastes have great potential to generate various kinds of value-added products such as biofuels, enzymes, and biofertilizers. Different type of biomass wastes generated from these industries are discussed below.

### ***1.2.1 Agricultural Residues***

Agriculture residues can be classified into field residues and process residues. Field residues are residues present in the field during the crop harvesting process, such as seed pods, stems, leaves, and stalks. Process residues are present till the crop has been turned into a profitable replacement commodity. These residues consist of husks, nuts, straw, leaves, shells, peel, pulp, roots, molasses, bagasse, and other waste biomass. They are used for animal feed, soil enhancement, fertilizers production, and various other uses (Sadh et al. 2018). Five million metric tons of agricultural biomass are generated annually from agro-based industries (Bharathiraja et al. 2017), which is underutilized, and their controlled use will improve irrigation abilities and erosion management. Barley and wheat are the main crops in the Middle East region. Moreover, many other crops are also produced worldwide, such as Fruits, vegetables, rice, corn, lentils, and chickpeas. The availability and characteristics of agricultural residues distinguished them from other solid fuels such as charcoal, char briquette, and wood.

### ***1.2.2 Industrial Wastes***

An immense quantity of organic waste and associated effluents are produced annually by food processing industries such as fruits, chips, garments, juices, and meat. These organic residues may be used as multiple sources of energy. The continually increasing population has raised the demand for food and its uses. Thus, in most of the nations, numerous food and beverage industries in this region have greatly expanded to satisfy food needs. These food and fruit industries lost around 20–40% of fruit and vegetable production annually (Dora et al. 2020). These agro-food-based waste materials show different compositions of lignin, ash, moisture, carbon, nitrogen, cellulose, and hemicellulose, which are useful for biogas production, bio-fuels, and some other valuable products by biochemical conversion. With increasing production by food industries, the percentage of waste generated also increased with a high value of chemical oxygen demand (COD), biological oxygen demand (BOD), and other suspended solids in it. Many of these wastes are discarded or untreated, resulting in harmful natural, human, and animal health effects, but their composition involves many organic compounds providing a range of value-added goods and reducing the manufacturing costs (Madureira et al. 2020).

After extracting oil from the seeds, enormous quantities of refined residues are made in oil industries called oil-cakes. Different varieties of oil cakes are generated after processing, such as coconut oil cake, mustard oil cake, soybean cake, cottonseed cake, rapeseed cake, palm kernel cake, olive oil cake, groundnut oil cake, sunflower oil cake, sesame oil cake, and canola oil cake. (Ramachandran et al. 2007). These industries pollute water, air, and other solid waste because these residues have vast amounts of grease, oil, suspended solids, fat, and dissolved solids. These

agro-industrial residues are comparatively inexpensive, containing vast quantities of components, and can be used as substitute substrates for fermentation in an infinite prospect.

### ***1.2.3 Drainage from Agro-Food Industries***

In India, roughly 65–70% of organic contaminants are released into water bodies by food and agricultural sectors, such as distilleries, sugar mills, milk, fruit canning, and pulp and paper milling industries. The production of wine has also reached prominence in other parts of the world and is one of the leading food-processing industries in Mediterranean countries such as Australia, South Africa, Chile, China, and The US with its rising economic impact (Ganesh et al. 2010). According to the international organization of vine and wine (OIV), in 2020, global wine production was estimated at approximately 258 million hectoliters. Wine produces large amounts of wastewaters primarily from various washing procedures, e.g., when the grapes were crushed and squeezed, fermentation tanks, barrels, other machinery, and the surfaces were washed (Badshah et al. 2012). Moreover, for many Mediterranean countries, the olive oil industry has become of fundamental economic value. Malaysia currently represents 28% of the world's palm oil production and 33% of world exports (MPOC 2020). Because of its surplus production, a large amount of contaminated wastewater is generated, commonly referred to as palm oil mill effluent (POME). An estimated 1750 million metric tons of olive oil are produced worldwide annually, with Portugal, Greece, Italy, Tunisia, and Spain are the leading producers. About 30 million cubic meters of waste oils are produced annually in the Mediterranean region in seasonal olive oil extraction (Meksi et al. 2012). The final effluent produced after the processing of coffee industries in the regions such as Colombia, Brazil, and Vietnam has created a huge environmental impact, which requires low-cost and efficient techniques for treating wastewater generated (Fia et al. 2012). Also, some agro-food wastes such as yogurt waste, milled apple waste, beverages waste, fat and oil from dairy wastewater treatment, and cattle manure have great potential for the production of methane and other biofuels.

## **1.3 Different Methods Used for Bioconversion of Agro-Industrial Waste**

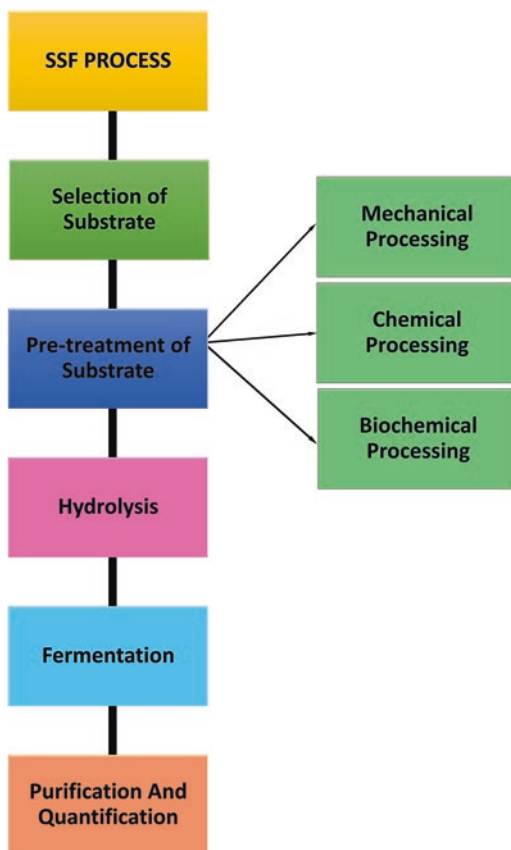
Various microbial treatments such as solid-state fermentation, composting, anaerobic digestion may employ to manage waste generated from the agro-industries. These treatment technologies are eco-friendly, cost-effective, and produce many value-added products to overcome future demand. Some of the treatment methods are discussed below:

### 1.3.1 Solid-State Fermentation

When non-soluble or solid organic wastes are processed in biotechnological ways in the absence or lack of water by microorganisms, it is called solid-state fermentation (Soccol et al. 2017). Residues of rice bran, rice straw, wheat bran, wheat straw, barley straw, corn, legume seeds, sawdust, wood shavings, fruits and vegetable peels, and other plant and animal products constitute the substrate for the solid-state fermentation (Singhania et al. 2009). Though these substrates are polymeric and barely soluble in water or barely soluble, they are available easily and cheaply. The process of solid-state fermentation has been given in Fig. 1.1.

Both at the lab and pilot scale, the solid-state fermentation process can occur, which results in bioconversion of organic agro-industrial waste leading to the production of active biological metabolites. The bio-products produced by the solid-state fermentation method are organic acids, aromatic compounds, enzymes, biosurfactants, bio-pesticides, bio-fertilizers, feed for animals, vitamins, antibiotics, and pigments (Thomas et al. 2013). Different industries like agriculture, paper, beer

**Fig. 1.1** Solid-state fermentation (SSF). The first step in solid-state fermentation is the selection of substrate and its pretreatment, which can be done through mechanical, chemical, and biochemical processes. The substrate undergoes hydrolysis followed by fermentation by the action of the microorganism. The fermented by-product is purified and quantified for further usage. (Modified from Sadh et al. 2018)



and wine, detergent, textile, food, animal feed generate large substrates for solid-state fermentation. Though the substrates used in solid-state fermentation are solids, the moisture level is low. The low-level moisture is sufficient for the fermentation process. Because of their high-water absorbing capacity, many agro-industrial wastes are being used as carriers in solid-state fermentation that help in the immobilization of fungus (Orzua et al. 2009). This helps in the efficacious growth of required microorganisms.

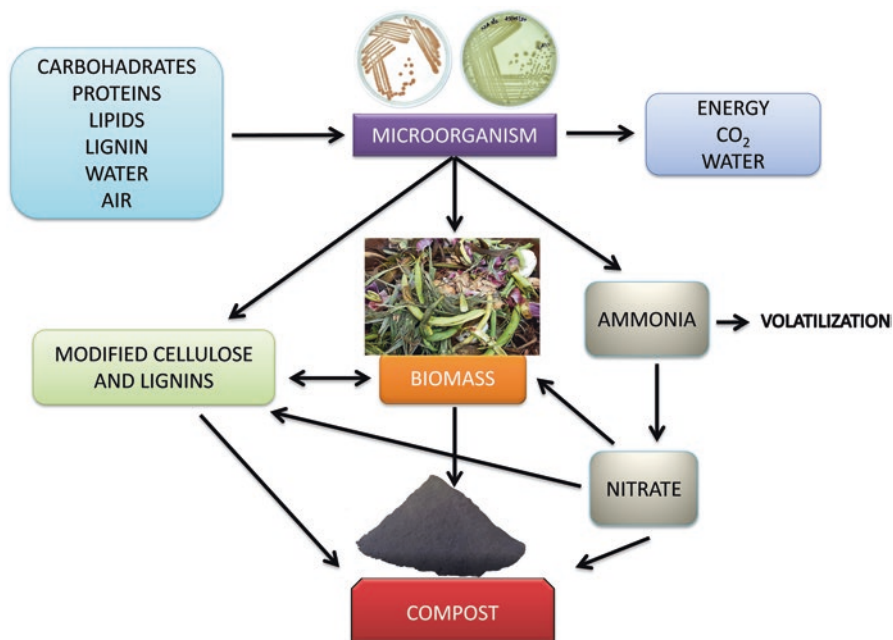
Through the process of solid-state fermentation, from the cheap substrates of agro-industrial solid wastes, many varieties of value-added products like tempeh, oncom, animal feed, biofuels, biogas are being prepared and sold for economic benefits (Fig. 1.2).

### 1.3.2 Composting

It is a managed process of converting organic matter into nutrient-rich and pathogen-free end product, i.e., compost (Fig. 1.3) (Onwosi et al. 2017). The end product is dark brown with a humus-like substance that may be stored and used as a beneficial



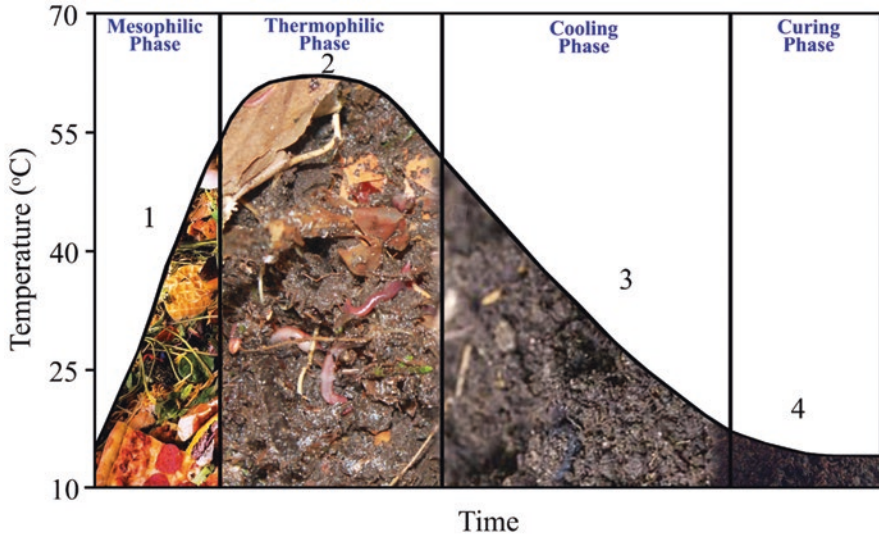
**Fig. 1.2** Common agro-industrial wastes and by-products derived through solid state fermentation (SSF). The common agro-industrial wastes such as rice bran and straw, wheat bran and straw, corn bran, peels of orange, peels of potatoes, oil cakes of peanut, soybean, coconut, barley straw, seeds of soybean, apple pomace when subjected to solid-state fermentation can yield beneficial by-products like biofuels, edibles like tempeh and oncom, antibiotics, biofertilizers and biopesticides, nutrient-rich feed for animals, several pigments, biosurfactants, enzymes, and vitamins. (Modified from Sadh et al. 2018)



**Fig. 1.3** Microbial decomposition of biomass into compost. Microorganisms in the conducive parameters act efficiently on the biomass with complex organic matter transform it into nutrient-rich compost having an agricultural application. The action of microorganisms results in converting lignocellulosic biomass into simpler ones and the available nitrogenous components into ammonia and nitrate/nitrite, further enhancing the overall compost quality

soil amendment for agricultural applications (Onwosi et al. 2017). To carry out an efficient composting process, the temperature is the main parameter that regulates the overall composting process. According to Kulikowska (2016), the substrate’s ambient temperature and nature decide the composting process’s effectiveness and the final compost’s quality. Based on temperature gradient, there are four stages of the composting process viz.; mesophilic phase, thermophilic phase, cooling phase, and the maturation phase (Fig. 1.4) (Onwosi et al. 2017). In each phase, the microbial biomass varies within the composting pile (Hou et al. 2017).

Such temperature variation during the composting process defines the benefits of microbial populations involved in transforming available substrates into value-added products. In the initial phase, i.e., mesophilic phase, the complex organic matters get converted into simpler ones by the synergistic action of different microbial populations, which further accelerates the compost temperature and drives the composting process into the thermophilic phase (Van der Wurff et al. 2016). In the composting process, the thermophilic stage plays an important role because it eliminates pathogens, weeds, and parasites from the compost pile (Zhang et al. 2012; Hou et al. 2017). Thus, it results in pathogen-free compost (Varma and Kalamdhad 2014). Due to the limited bioavailability of substrate, the microbial population’s



**Fig. 1.4** Phases of the composting process. Composting process consists mainly of four phases, namely mesophilic, thermophilic, cooling, and curing or maturation phase. The composting process starts with a mesophilic phase where the temperature reaches up to 40–50 °C. After that, it is dominated by the thermophilic phase. Here the temperature may rise to 60 °C. Then gradually, due to complete utilization of energy and reducing microbial population, the temperature retards, which depicts the cooling phase. Finally, the compost reaches to room temperature, at which it is kept for some more time to get matured completely. Thus, the final phase is called as curing or maturation phase. (Adapted from Alsanius et al. 2016)

activity slows down in the cooling phase. As a result, this takes the composting temperature to a declining stage. Now at this phase, the microbial community, for their survival, they will compete with each other by developing strategies such as antibiotic productions (Van der Wurff et al. 2016). In the last stage, i.e., the maturation phase, there is a slow transformation in the composting pile's microbial population. In this stage, the compost gets colonized by the microorganisms that readily depend on the available substrate (Van der Wurff et al. 2016).

### 1.3.2.1 Composting of Agro-Industrial Waste

Urbanization and rapid industrialization have led to increased exploitation of resources and the generation of waste in bulky volumes (Gonzalez-Salazar et al. 2016). The huge amount of nutrients rich biomass waste generated from agro-industries is disposed of and underutilized (Abdel-Shafy and Mansour 2018). With the advancement in technology and increased global research standards, scientific tools have taken place. The emerging interest in research is to control agro-industrial



waste by proposing green value-added technologies focused on traditional approaches, including harsh and hazardous chemicals at high processing and waste treatment costs (Chen and Lee 2018). The biological processing of agro-industrial wastes is exciting green biotechnology with minimal harmful effects on the environment while balancing it (Zakaria et al. 2020). Microorganisms, primarily from bacterial and fungal species, are used for biological treatments to fix those problems. Bran and cereal straw (Hart et al. 2003); horticultural wastes (Lu et al. 2004; Lopez et al. 2006); Cotton, lemon tree prunings, and brewery wastes (García-Gómez et al. 2005); Grapes, olive, and palm wastes (Arvanitoyannis and Kassaveti 2007) are ideal substrates for the processing of humus-rich compost.

### 1.3.2.2 Various Microbial Community Involved in Carrying out the Composting Process

Conventional chemical methods for agro-industrial waste treatment have led to increased environmental pollution hampering human and animal life. Therefore, there is a demand for eco-friendly approaches to tackle such waste. In such conditions, microorganisms' function can't be ignored as they play a vital role in the biological method of composting to treat agro-industrial wastes into value-added products. Various microorganisms are reported to yield better compost products under improved composting conditions. These microorganisms possess various enzymatic activities directly involved in decomposing organic materials into a valuable product (Eida et al. 2009). These microbes have great potential to rapidly convert cellulosic and lignocellulosic compositions of wastes into nutrient-rich biological matter (de Souza 2013). This nutrient-rich decomposed organic matter is fruitful to enhance the activity of native important microorganisms in the soil (Meena et al. 2014; Rashid et al. 2016).

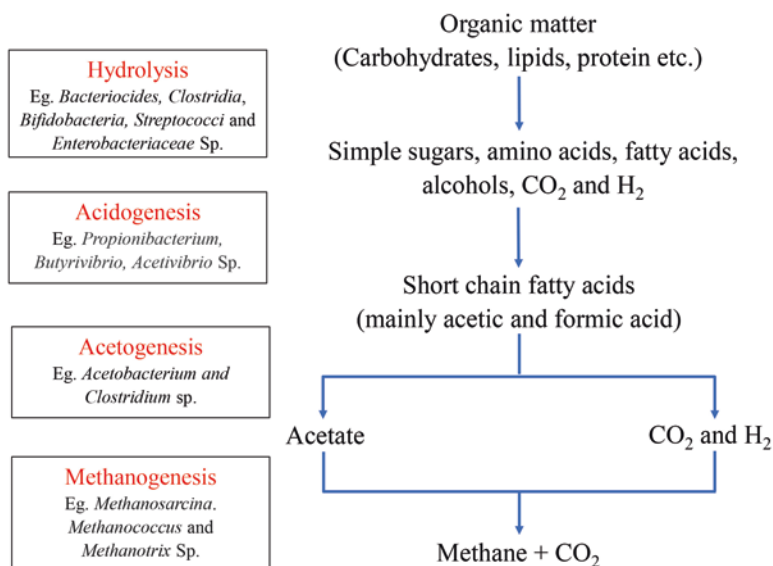
The mixture of bacterial communities, i.e., *Ureibacillus thermosphaericus*, *Streptomyces thermovulgaris*, and *Bacillus shackletonni* are proved to enhance the process of composting (Vargas-García et al. 2007). The inoculum of lignocellulolytic fungi (e.g., *Trichurus spiralis*) can be used before composting process, which will help reduce the resistance to biodegradation substrates (Hart et al. 2003; Vargas-García et al. 2007).

A decomposed product by the action of earthworm *Eisenia fetida*, i.e., vermicompost, is also considered to increase indigenous soil microbial diversity and encourage plant growth (Lim et al. 2015). The mixture of sawdust, guar gum waste, and cow dung in the substrate has been reported to humify by a new strain of earthworm *Perionyx sansibaricus* (Suthar 2007). Composting may also be viewed as a cheap technology for transforming agro-industrial waste into a value-added commodity.

### 1.3.3 Anaerobic Digestion

Anaerobic digestion is a method of decomposition of organic waste under anaerobic conditions with low energy requirements. It produces biogas and leftover digestate as the by-products used as renewable energy sources and fertilizer for agricultural lands. The application of anaerobes in waste management has been identified for more than hundreds of years (McCarty 2001). Generally, the waste disposal methods are employed to avoid contamination of the environment; however, anaerobic processes are used for the treatment of high organic waste content for the generation of energy in the form of biogas or biofuels. The current scenario of global greenhouse gas emission and energy disasters is encouraging green energy and advances in biotechnology to generate a new economy based on environmental and energetic factors (Holm-Nielsen et al. 2009). In this regard, principles like biorefining and energy extraction are introduced based on the biotechnological transformation of biomass. From this point of view, anaerobic digestion plays a vital role in these situations, as it generates various by-products such as methane and hydrogen at the different metabolic stage, which can be used as power sources, i.e., either in boilers, internal combustion engines, or fuel cells.

The fermentation of methane is a dynamic mechanism separated into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Pereda Reyes and Sárvári Horváth 2015) (Fig. 1.5). Diverse microorganisms execute the degradation



**Fig. 1.5 Degradation process involved during anaerobic digestion.** Anaerobic digestion of organic waste includes four major steps, i.e., hydrolysis, acidogenic fermentation, hydrogen-producing acetogenesis, and methanogenesis, which results in the formation of methane and carbon dioxide

steps, which are partially interrelated syntrophically and have different environmental requirements. Hydrolytic bacteria were the first group of microorganisms. These species hydrolyze polymeric compounds by extracellular hydrolytic enzymes (cellulase, xylanase, amylase, protease, lipase) into monomers such as glucose and amino acids (Weiland 2010). These microbial communities include some strict anaerobes such as *Clostridia*, *Bifidobacteria*, *Bacteriocides*, and some facultative anaerobes also take parts, such as *Streptococci* and *Enterobacteriaceae*. The acidogenic bacteria are the second category of microorganisms that transform carbon dioxide, hydrogen, ammonium, and other organic acids from sugars and amino acids. The third type of bacterial species involved is hydrogen-producing acetogenic bacteria, such as *Clostridium acetium*, *Acetobacterium woodii*, which converts volatile fatty acids into acetate and hydrogen. Two different groups of strictly anaerobic bacteria produce methane as a final product from acetate (Acetotrophic pathway) or hydrogen and carbon dioxide (hydrogenotrophic pathway) at the end of the degradation process. Very few microorganisms, i.e., *Methanotrix soehngenii*, *Methanosarcina barkeri*, and *Methanococcus mazei* can degrade acetate into methane and carbon dioxide, whereas hydrogen can be used to form methane by all methanogenic bacteria such as *Methanobacteriales* and *Methanomicrobiales* (Batstone et al. 2002).

Anaerobic digestion occurs at three different temperatures depending on the climate conditions, thermophilic (55–60 °C), mesophilic (35–37 °C), and psychrophilic (below 20 °C). Thermophilic and mesophilic temperatures are often used for industrial applications due to higher process stability, methane yields, and fewer energy requirements (Alatríste-Mondragón et al. 2006). The proper implementation of anaerobic digestion relies heavily on how the composition of waste to be digested (Murto et al. 2004). Solid waste treatment varies from liquid waste treatment, as solubilization and hydrolysis of particle material may be the limiting step in the process. To enhance mass transfer phenomena and ensure the proper interaction between the biomass and the substrate, waste mixing must be carried out correctly. If the microorganisms involved in the process are not separated from the solid wastes to be decayed, the retention time is generally 50–60 days in the digester; otherwise, microorganisms may wash out from it. On the other hand, in the wastewater bioreactor system, microorganisms' retention time will be decoupled from liquid retention results into reduced hydraulic retention time to a few hours. The microbial treatments of agro-industrial waste generally remove pathogens and other organic compounds from the effluent, but due to high particulate and fat contents, anaerobic digestion is complicated in slaughterhouse industries (Batstone et al. 1997). Still, anaerobic digestion remains a viable alternative for the treatment of these kinds of waste materials, as they have a high potential to generate biogas only because of their high protein and fat content.

On the other hand, agro wastes are composed of major biodegradable components, i.e., cellulose and hemicellulose linked with lignin. Compared to carbohydrates, these organic compounds produce low amounts of methane (up to 50% methane) under anaerobic conditions due to the low biodegradability and sheltering effect of lignin. But several agro wastes are favorable for anaerobic digestion as they

can degrade 80% of their fiber content, such as paper and rice residues (Esposito et al. 2011) (Contreras et al. 2012). For the anaerobic treatment of agro-industrial drainages, several reactor configurations are used:

### 1.3.3.1 Completely Stirred Anaerobic Digester

The continuous stirred tank reactor is the earliest and basic high-rate system for the anaerobic treatment of wastewater with a high amount of suspended solids and organic industrial waste. It a stable and reliable anaerobic treatment system with an equally solid retention time and hydraulic retention time of 15–40 days for the proper treatment and process (Del Real Olvera and Lopez-Lopez 2012). In this system, alternate and continuous mixing is helpful to suspend the microorganism in the digester, which results in better substrate-sludge contact, but it requires a significant amount of energy and is labor-intensive (Mao et al. 2015). Another drawback of this method is that only high volumetric waste sources with a biodegradable COD content range between 8.000 and 50.000 mg/L attain a high loading rate, but many waste sources are diluted considerably. A single continuous stirred tank reactor system is easy to operate but produces poor quality effluent. So, a two-phase treatment system is employed and is most common these days. The two-stage continuous stirred tank reactor system is comparatively simple in design and cost-effective for the treatment of wet digesters. However, the system's sensitivity to substrates with high organic loads and the complicated operation leads to fewer alternatives to increase the two-phase system's digestive efficiency (Boe and Angelidaki 2009). Different variants of continuous stirred tank reactors are currently constructed to enhance the reactor's performance by optimizing the reactor volume. To increase the reactor's microbial load, gravity sedimentation tanks or membrane bioreactors are used in series with continuous stirred tank reactors for a more effective digestion process. For the treatment of dairy wastewater, a two-phase anaerobic system of continuous stirred tank reactor and up-flow anaerobic filters are used under various operating conditions, but a high concentration of suspended solids affects treatment efficiency (Mao et al. 2015).

### 1.3.3.2 Upflow Anaerobic Sludge Blanket Reactor

Upflow anaerobic sludge blanket reactor is a compact system that requires a small area to operate with low maintenance and expenditure costs, low energy usage, and low production of sludge continuously feeding the up-flow anaerobic sludge blanket reactor from the bottom, creating an up-flow stream towards the upper outlet through the sludge bed consisting of aggregated microbes and granular biomass. With the upward movement of effluent, its velocity decreases, providing greater area and enhancing the process's solid retention time and efficiency. Upflow anaerobic sludge blanket reactor systems can achieve better settlement ability, high biomass concentrations, short retention time, strong solids/liquid isolation, cost removal of

packaging content, and very high loading rate operations. The removal efficacy of chemical oxygen demand is about 65%, and biochemical oxygen demand is 75% in up-flow anaerobic sludge blanket reactor type reactor system. Also, highly concentrated sludge is produced with better dehydration capacity. Although the sludge acclimation is vital for start-up only, after long periods of disrupted functioning, reactor output can be resumed. This method is generally restricted by the high solid suspended content in wastewater to be treated, which prevents the production of dense granular sludge, and sometimes foul smell occurs due to the inappropriate nature or installation of the gas collection system and low tolerance of toxic charge.

### 1.3.3.3 Fluidized and Expanded Bed Reactors

In an anaerobic fluidized bed reactor, bacteria are grown and attached on small granular particles such as alumina or sand and activated carbon. These particles are suspended by a fast-upward flow of wastewater, allowing resistance to inhibitors and a higher organic loading rate level. Organic loading rate of 10–20 kg COD/m<sup>3</sup>d<sup>-1</sup> can be used in an anaerobic fluidized bed reactor (Siqueira et al. 2013). The high rate of flow around the particles covered with a thin biofilm layer of methanogens results in good mass transfer. Less clogging of the latter allows the short-circuiting and larger surface to volume ratio of the carrier in the reactor, making the anaerobic fluidized bed reactor more efficient than up-flow anaerobic sludge blanket reactors. Also, an anaerobic fluidized bed reactor is cheaper due to the reduced volume of the reactor, and it is better than an up-flow anaerobic sludge blanket in terms of removing the suspended solid particles from wastewater (Mao et al. 2015). However, the key disadvantages of this method are the difficulties of producing a tightly attached biofilm containing the right mix of methanogens, the risk of detachment of microorganisms, the harmful consequences of dilution near the inlet due to high recycling rates more cost of energy. The expanded granular sludge bed reactor is a variant of the anaerobic fluidized bed reactor with a variation in the fluid's upward flow rate (Del Real Olvera and Lopez-Lopez 2012).

### 1.3.3.4 Anaerobic Filters Reactors

The Anaerobic Filters are designed to support the intimate interaction between the waste and bacterial population, thereby providing a longer retention time for biomass than hydraulic retention time. They are used to treat various wastewaters produced from dairy, beverages, chemical, and food processing industries. Anaerobic filters are a widely used anaerobic digestion process due to the high organic loading rate. The anaerobic filter is generally made up of different materials such as ceramic, charcoal, plastic materials, sintered glasses, seashells, limestones, and pumice clay (Karadag et al. 2015). These are designed with at least two filtration compartments arranged in a manner. At the anaerobic filters, biomass can degrade the organic material found in wastewater and then bind outside the filter material. Furthermore,

these anaerobic filters are commonly used as an optional treatment to increase solid elimination. The anaerobic filters need to be able to use sufficient packaging media because the media's chemical and physical properties have a crucial influence on biomass and its reactor efficiency. The anaerobic filter is generally packed with various packaging materials to contain the bacteria in the voids. The ideal packaging media increases the surface area and extends anaerobic filters' porosity, increasing the biomass link, decreasing the reactor's volume, and restricting channel obstruction. Some researches show that anaerobic filters remove 80% of chemical oxygen demand with a maximum organic loading rate capacity of 19 and 17 g COD L<sup>-1</sup>d<sup>-1</sup> of wastewater generated from cheese, dairy, and fruit canning industries (Rajagopal et al. 2013). These days, researchers concentrate on using industrial by-products for the natural application of anaerobic filters, as it is more efficient for the treatment of wastewaters with a low level of suspended solids. Also, anaerobic filters have some advantages like minimum nutrients reduction and require further treatment of effluents.

## 1.4 Recycled Value-Added Products from the Microbial Treatment of Agro-Industrial Waste

The scientific community uses various agro-industrial wastes and effluents through green and recycling technologies to overcome the existing global environmental health. The agro-industrial wastes are used for the production of many value-added products such as biofuels, enzymes, and biofertilizers based on their composition, i.e., lignocellulose, proteins, polyphenols, and carbohydrates. Some of the products are discussed below:

### 1.4.1 *Some Important Enzymes from Agro-Industrial Waste*

#### 1.4.1.1 Alpha and Beta-Amylase

Alpha-amylases (E.C 3.2.1.1) cleave  $\alpha$ -D-(1,4) glycosidic linkages between the glucose subunits in starch, glycogen, and other related polysaccharides. They have a wide variety of applications in food, detergent, textile, paper, pulp, distilleries, and pharmaceutical industries (Ramachandran et al. 2004). They are derived from many organisms, such as plants, animals, and microorganisms (Far et al. 2020). At the industrial level, they are produced by both submerged fermentation and solid-state fermentation using genetically improved microorganisms such as *Bacillus* and *Aspergillus* species (Sundarram and Murthy 2014). Agro-industrial residues act as a favorable substrate for the production of  $\alpha$ -amylases, mainly due to their high lignocellulosic content and low economic cost. In literature, the production of this enzyme is primarily emphasized by solid-state fermentation. In some of the earlier

studies, banana waste, rice husk, coconut oil cake, sugarcane bagasse, corn cob, and spent brewer's grain has been used for the production of amylase (Ramachandran et al. 2004; Anto et al. 2006; Aliyah et al. 2017).

$\beta$ -amylases (E.C. 3.1.2.3), also known as glucoamylases, cleave the  $\alpha$ -1,4 linkages found in starch and other carbohydrates to release glucose molecules. It also hydrolyses  $\alpha$ -1,6 glycosidic bonds at slower rates. Thus, an exoamylase releases glucose moieties from the non-reducing end of the carbohydrates (Espinosa-Ramírez et al. 2014). They are very useful in the bakery and brewery industry (Diler et al. 2015). They are produced by several bacterial and fungal species like *Aspergillus*, *Bacillus*, *Saccharomyces*, and *Rhizopus* (Singh and Soni 2001; Shin et al. 2000). Vegetable waste such as cassava, yam, and banana peels has been used as a carbon source for glucoamylase production (Adeniran et al. 2010).

#### 1.4.1.2 $\beta$ -Glucanase

$\beta$ -glucans are polysaccharides made of D-glucose monomers linked through  $\beta$ -1,3 and  $\beta$ -1,4 glycosidic linkages. They form a major part of the cell wall polysaccharide of cereal endosperm and comprise approximately 5.5% of the dry weight of grains (Dais and Perlin 1982). Besides cereal endosperm, they can also be found in natural products like mushrooms, yeast, oats, and algae (Zhu et al. 2016). Fungi produce glucans with  $\beta$ -1,3,  $\beta$ -1,4, and  $\beta$ -1,6 bonds, which also exhibit antitumor activity (Latgé 2010). Based on the cleavage of glycosidic bond,  $\beta$ -glucanases are classified as  $\beta$ -1,4-glucanase (EC 3.2.1.4),  $\beta$ -1,3(4)-glucanase (EC 3.2.1.6),  $\beta$ -1,3-glucanase (EC 3.2.1.39), and  $\beta$ -1,3-1,4-glucanase (EC 3.2.1.73) (Ueda et al. 2014). Microorganisms like *Fusarium oxysporum*, *Bacillus subtilis*, and *Penicillium echinulatum* have been reported to produce high endo and exoglucanase levels when grown on biomass sources (Ravindran et al. 2018). They have applications in brewing, detergent, and animal feed industries (Chaari and Chaabouni 2019).

#### 1.4.1.3 Cellulase

Cellulases are a group of enzymes involved in the depolymerization of cellulose. They comprise three enzymes endoglucanase (E.C. 3.2.1.4), exoglucanase or cellobiohydrolase (E.C. 3.2.1.176) (E.C. 3.2.1.91), and  $\beta$ -glucosidase (E.C. 3.2.1.21). They play a very important role in biofuel production using different lignocellulosic materials (Castro and Pereira Jr 2010). Besides bioethanol, they have applications in food, detergent, paper, pulp, and textile sectors (Ferreira et al. 2014). They are secreted by several microorganisms like bacteria, fungi and actinomycetes (Zverlov et al. 2015). *Clostridium thermocellum*, *Proteus vulgaris*, *Bacillus circulans*, *Escherichia coli*, *Klebsiella pneumonia*, and *Cellulomonas* are the bacteria involved in the production of Cellulases (Ravindran et al. 2018). Simultaneously, fungal species such as *Melanocarpus*, *Schizophyllum*, *Penicillium*, *Aspergillus*, *Trichoderma* and *Fusarium* produce these enzymes (Juturu and Wu 2014). Studies have been

conducted in the literature to use fruit peels and spent brewer's grain to be used as a substrate for cellulase production by *Trichoderma* sp. (Sim and Oh 1990; Nadar and Rathod 2019).

#### 1.4.1.4 Hemicellulase

Hemicellulose is a branched heteropolysaccharide consisting of different hexoses and pentoses, including glucose, fructose, galactose, arabinose, xylose, mannose, glucuronic acid, and galacturonic acid. It accounts for 20–30% of the total wood (Collins et al. 2005) and is generally amorphous in nature. Hemicellulases are the enzymes that break  $\beta$ -1,4-glycosidic bonds present in the backbone of hemicellulose. They are produced both by bacterial and fungal strains, namely *Clostridium*, *Bacillus*, *Geobacillus*, *Trichoderma*, and *Aspergillus* (Shallom and Shoham 2003). The most common hemicellulases are xylanases, mannanases, glucuronidases, galactosidases, and arabinofuranosidases (Obeng et al. 2017). They also play a crucial role in the paper, pulp, biofuel, food, and pharmaceutical industries. Studies have been conducted on using corn cobs, apple pomace, and palm oil effluents as a substrate for hemicellulases production (Zainudin et al. 2013; Dhillon et al. 2012; Kheiralla et al. 2018).

#### 1.4.1.5 Xylanase

Xylan is a complex polysaccharide made of  $\beta$ -1,4-linked xylose residues. It is the most abundant type of hemicellulose present in nature (Beg et al. 2001). The backbone made of xylan residues have the side branches of d-glucuronic acid and d-glucuronic acid (4-*O*-methyl  $\alpha$ -1,2 linked) or d-arabinofuranose ( $\alpha$ -1,2 or  $\alpha$ -1,3 linked), and *O*-acetyl groups (Bastawde 1992). The branches play an important role in the cross-linking of cellulose microfibrils and lignin through ferulic acid residues. The extent of branching depends on the source of xylan. Because of its heterogeneous nature and complex structure, xylan requires several enzymes, namely endoxylanases,  $\beta$ -xylosidases, ferulic acid esterase, p-coumaric acid esterase, acetyl xylan esterase, and  $\alpha$ -glucuronidase (Bhardwaj et al. 2019). They all form a class of xylan degrading enzymes called xylanases (E. C. 3.2.1.8). Xylanases have been isolated from different microorganisms, such as *Aspergillus Chytridiomyces*, *Streptomyces*, *Trichoderma*, *Phanerochaete*, *Clostridium*, *Fibrobacter*, *Bacillus*, and *Pichia* (Collins et al. 2005). Several agro-industrial wastes such as wheat straw, wheat bran, rice husk, and sugarcane bran have been used to produce xylanases at very low cost (Pandya and Gupta 2012; Membrillo Venegas et al. 2013).



#### 1.4.1.6 Mannase

Mannan is the second most abundant hemicellulose in nature. It comprises linear mannan, glucomannan, galactomannan, and glactoglucomannan. The enzymes involved in the depolymerization of linear mannan are  $\beta$ -mannanase (EC 3.2.1.78),  $\beta$ -mannosidase (EC 3.2.1.25),  $\beta$ -glucosidase (EC 3.2.1.21), acetyl mannan esterase (EC 3.1.1.6), and  $\alpha$ -galactosidase (EC 3.2.1.22) (Moreira 2008).  $\beta$ -mannanases cleave the internal glycosidic bonds and release short-chain manno-oligosaccharides, whereas  $\beta$ -mannosidases hydrolyze mannan from the non-reducing end releasing individual mannose unit (McCleary and Matheson 1983; Gomes et al. 2007). They are produced by several bacterial and fungal species: *Bacillus circulans*, *Clostridium cellulolytic*, *Trichoderma reesei*, *Agaricus bisporus*, *Aspergillus aculeatus* (Moreira 2008). They have applications in paper, pulp, food, and textile industries (Clarke et al. 2000; Naganagouda et al. 2009). Abdeshahian et al. has used palm kernel to produce  $\beta$ -mannanases using the fungi *Aspergillus niger* (Abdeshahian et al. 2010). Apple pomace, cottonseed powder, and locust bean have also been observed as excellent substrates for the production of mannases (Gomes et al. 2007; Yin et al. 2013).

#### 1.4.1.7 Pectinase

Pectin is a widely distributed carbohydrate polymer in plants. It is a complex polysaccharide made of galacturonic acid residues linked by  $\alpha$ -1,4 linkage. The acid groups along the chain are largely esterified with methoxy groups in the natural product. There can also be acetyl groups present on the free hydroxy groups. Pectinases are a group of enzymes that degrade pectic substances and release comparatively simpler compounds based on their mode of action. The linear pectin chain is generally cleaved by endo-polygalacturonases (EC 3.2.1.15) and exo-polygalacturonases (EC 3.2.1.67) from inside and at the terminal, respectively (Rytioja et al. 2014). Pectinlyases (EC 4.2.2.10) and pectate lyases (EC 4.2.2.2) also cleave the pectin backbone. Whereas the branched region is degraded by rhamnogalacturonan acylesterase (EC 3.1.1), pectin acetyl esterase (EC 3.1.1), and pectin methylesterase (EC 3.1.1.11) (De Vries and Visser 2001). Pectinases are produced by bacterial, fungal, and yeast strains, namely *Bacillus*, *Paenibacillus*, *Chryseobacterium*, *Pectobacterium*, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Botrytis*, *Fusarium*, *Trichoderma*, *Rhodotorula* (Singh et al. 2019). They have applications in the fruit, paper, pulp, textile, and animal feed industries. They have also been used in coffee and tea fermentation (Sharma et al. 2013).

#### 1.4.1.8 Inulinase

Inulin is produced by several plants such as banana, garlic, chicory, onion, garlic, barley, wheat, barley, and rye (Kaur and Gupta 2002). It is classified as an oligo or polysaccharide, depending upon its chain length. It consists of D-fructosyl moieties linked by  $\beta$ -2,1 glycosidic bond terminating with  $\alpha$ -1,2 bonded D-glucosyl group (Ronkart et al. 2007). Inulinases are defined as a group of enzymes involved in the degradation of inulin. They mainly consist of exo-inulinases ( $\beta$ -D-fructanfructohydrolase, EC 3.2.1.80) and endo-inulinases (2,1- $\beta$ -D-fructanfructohydrolase, EC 3.2.1.7) based on their mode of activity (Vijayaraghavan et al. 2009). They are shown to produce by *Streptococcus*, *Pseudomonas*, *Xanthomonas*, *Chrysosporium*, *Actinomyces*, *Penicillium*, *Aspergillus*, *Kluyveromyces* species. They have applications in lactic acid production, 2,3 butanediols, biofuel, sugar alcohols, fructose syrup, and inuloorligosaccharides (Liu et al. 2013; Chi et al. 2009). Recently, coconut oil cake, sugarcane baggase, and rice bran have been used for the synthesis of inulinases (Onilude et al. 2012; Singh and Chauhan 2018; Mazutti et al. 2006).

#### 1.4.1.9 Laccase

Laccases (E.C. 1.10.3.2) are aromatic compounds oxidizing enzyme which belong to a multi-copper oxidase family. They oxidize a wide variety of phenols, heterocyclic compounds, and aromatic amines. They catalyze the oxidation of their substrates with the help of molecular oxygen. They play a crucial role in dye, bleaching, pulp, and industrial effluent treatment. They have also been explored for the degradation of lignin, herbicides, and pesticides (Ravindran et al. 2018). They are produced by bacteria, fungi, plants, and insects. The bacterial and fungal species involved in the production of laccases are *Bacillus subtilis*, *Streptomyces lavendulae*, *Azospirillum lipoferum*, *Pleurotus ostreatus*, *Trametes versicolor*. (Kiiskinen et al. 2004). They can be produced using groundnut, banana peel, rice straw, orange peel, and wheat bran from agro-industries as substrate (Singh and Gupta 2020).

#### 1.4.1.10 Phytase

Phytates are defined as naturally occurring phosphate-rich compounds found in cereals, oilseeds, and legumes. The enzyme that catalyzes the release of phosphates from phytates is known as phytase (EC 3.1.3.8) (Hussin et al. 2007). Phytases are classified based on their stereospecificity of hydrolysis. The enzymes that cleave at the C3 and C6 positions of phytic acid are classified as 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26), respectively. Mostly, 3-phytases are produced by the microorganisms; on the other hand, 6-phytases by plants. They are produced by different bacterial, fungal, and yeast genera, namely *Klebsiella*, *Bacillus*, *Shigella*, *Selenomonas*, *Rhodotorula*, *Aspergillus* (Pandey et al. 2001). Ruminants produce

phytases for the proper digestion of phytate-rich food, whereas monogastric animals do not produce this enzyme. Treatment of animal feed with phytases increases the bioavailability of minerals, thus improving its nutritional value. The phytases are important for poultry, pig, baking, and bioethanol industries. They can be produced using residues from agricultural industries and fruit peels as substrate, thus pointing towards their low-cost synthesis (Bajaj and Wani 2011).

#### 1.4.1.11 Invertase

Invertase (EC.3.2.1.26) is a glycoprotein that catalyzes the hydrolysis of sucrose into glucose and fructose. They mainly hydrolyze  $\beta$ -1,2 linkage present between the glucose and sucrose units. They are also known as  $\beta$ -D-fructofuranosidase because of their ability to release fructose from the non-reducing terminal of  $\beta$ -D-fructofuranoside substrates (Linde et al. 2009). Sucrose hydrolysis is generally carried out using hydrochloric acid with only 50–60% efficiency, whereas the employment of invertase enzyme improves the efficiency of this reaction up to 100% and without yielding any impurities (Ravindran et al. 2018). Invertase is shown to be present in *Bacillus cereus*, *Lactobacillus reuteri*, *Brevibacterium* sp., *Arthrobacter* sp., *Streptomyces* sp., *Zymomonas mobilis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Aspergillus niger*, and *Penicillium chrisogenum* (Lincoln and More 2017). Recently, wheat bran, fruit waste, and bagasse waste have been observed as excellent carbon sources for the low-cost production of invertase (Ravindran et al. 2018).

#### 1.4.1.12 Protease

Proteases (EC 3.4.21.62) are a group of enzymes that perform the hydrolysis of peptide bonds and release small peptides or free amino acids. They are also known as proteinases. They are a crucial industrial enzyme that accounts for approximately 70% of the total enzymes produced worldwide (Meena et al. 2013). They are widely distributed in living organisms as they are crucial for various physiological processes. They can be classified as acidic, alkaline, and neutral proteases. The alkaline (EC.3.4.21–24.99) and acidic proteases cleave proteins between pH range 9–11 and 3–4, respectively (Razzaq et al. 2019). They are found in plants, animals, and microorganisms. Among microbes, they are produced by *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, *Aspergillus oryzae*, *Penicillium*, *Rhizopus* (Singh et al. 2019; Razzaq et al. 2019). They are important in the food, feed, leather, silk, and textile industries. Alcalase, Neutrase, and protease are some of the commercially available proteases. Tomato pomace, soybeans, and jatropha seed cakes have been used as a substrate for protease production (Ravindran et al. 2018).

#### 1.4.1.13 Pullulanase

Pullulan is a maltotriose trimer made up of  $\alpha$ -1-4 and  $\alpha$ -1-6-linked triglucosides. Due to the existence of both  $\alpha$ -1-4 and  $\alpha$ -1-6 in this compound, it is seen as an intermediate between the amylose and dextran structure. Pullulan is insoluble in non-polar compounds but is soluble in water. It has high mechanical strength thus can be used for a gelling agent, capsule, and thin-film formation (Farris et al. 2014). The enzymes used to depolymerize pullulan are mainly classified as pullulanase (EC. 3.2.1.41), isopullulanase (EC.3.2.1.57), and neopullulanase (EC.3.2.1.35). The pullulanase is further divided into type-I and type-II, which cleave only  $\alpha$ -1,6-glycosidic linkage and  $\alpha$ -1,4 &  $\alpha$ -1,6-glycosidic linkages, respectively. They are essential for the baking and starch industries (Hii et al. 2012). They are synthesized by *Klebsiella pneumoniae*, *Bacillus cereus*, *Bacillus macerans*, *Streptomyces mitis*, *Clostridium* sp., *Thermus aquaticus*, *Thermoanaerobacter* sp., *Saccharomyces* sp., *Aspergillus* sp., *Cladosporium* sp. (Saha and Zeikus 1989; Tomasik and Horton 2012). Agricultural waste materials such as wheat, rice, and corn bran can be used for its cheap production (Zhang et al. 2020; Reddy et al. 2000).

#### 1.4.1.14 Lipase

Lipases (EC 3.1.1.3) are a group of hydrolases that catalyze the breakdown of triglycerides to glycerol and fatty acids. They also carry out hydrolysis, transesterification, alcoholysis, aminolysis, acidolysis, and esterification, which are important industrial processes (Ravindran et al. 2018). They are crucial in the cosmetic, food, detergent, and pharmaceutical industries. They are well distributed in plants, animals, and microorganisms. Among microbes, they are produced by bacterial, yeast, and fungal strains such as *Bacillus*, *Serratia*, *Burkholderia*, *Candida*, *Rhodotorula*, *Aspergillus*, and *Penicillium* (Treichel et al. 2010). They are extracellular enzymes and are greatly influenced by physicochemical factors like pH and temperature. They are inducible enzymes and are synthesized when an inducer such as fatty acid, oil, and triglycerols are present in the medium. Agricultural residues like oil cake, fruit peels, and bean waste have been used for the cheap production of lipase (Godoy et al. 2011; Pereira et al. 2019).

### 1.4.2 Bioethanol Production

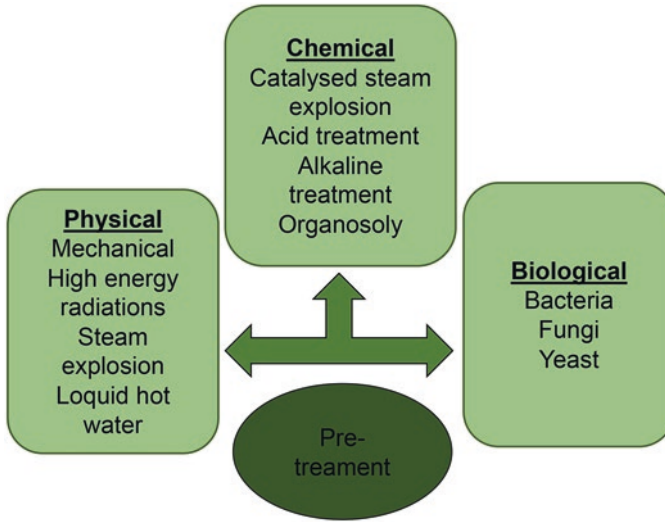
Due to the rise in population and rapid industrialization, energy demands have increased worldwide. The excessive consumption of fossil fuels, such as natural gas, oil, coal, and petroleum, has resulted in higher pollution levels. The increased pollution, especially due to the accumulation of large amounts of greenhouse gases, causes a change in the earth's climate. It has also been suggested that petroleum-based fuels will deplete in the near future; thus, due to their harmful effects and

limited supply, these non-renewable sources should be replaced with better environment-friendly energy resources. Biofuels are the most feasible alternate for traditional fuels as they reduce our dependency on fossil fuels and do not disturb the balance of the earth's atmosphere. Both bioethanol and biodiesel have been seen as a supplement of gasoline and diesel, respectively. The global bioethanol production was approximately 66.77 billion liters in 2008, which was increased to 90.38 billion liters in 2014 (Gupta and Verma 2015).

The countries like the USA and Brazil produce bioethanol using corn and sugarcane, respectively, which account for 62% of the total global production (Kim and Dale 2004). The utilization of sugars and starch is not widespread in bioethanol generation, mainly due to their high food value. Recently, it has been observed that lignocellulosic biomass and agricultural crop waste can be used for bioethanol production. They are cheap sources and present in large amounts, so their employment for bioethanol production provides a route for their effective utilization. Agro-residues like wheat straw, rice straw, corn straw, fruit peels, sugarcane bagasse, and oil cakes now have significant interests. Rice straw is the highest agricultural waste and can produce 205 billion liters of bioethanol per year (Sarkar et al. 2012). Physical, chemical, and biological pre-treatments are required to generate bioethanol from agricultural waste materials.

Lignocellulosic material consists of cellulose and lignin linked with hemicellulose chains. A pre-treatment process is required for the breakdown and release of these components. It makes lignocellulose susceptible to hydrolysis and makes the release of monomer sugars easy (Mosier et al. 2005). There are three types of pre-treatment processes, i.e., physical, chemical, and biological treatments. The physical pre-treatment process for bioethanol production involves mechanical methods such as milling, grinding, and chipping. The amount of power used to crush the material mainly depends on the particle size, nature, and moisture content of the waste (Buaban et al. 2010). The mechanical processes disrupt the structure of lignocellulose and decrease its particle size to increase the surface area for enzymatic hydrolysis (Kumar et al. 2008). Besides mechanical processes, physical pre-treatment also includes uncatalyzed steam-explosion, high-energy radiation, and liquid hot water treatment (Fig. 1.6). The steam explosion aids in the loosening of lignocellulosic structure and releases pentose, but it has a disadvantage of generating cellulase inhibitory compounds, which later interfere with enzymatic hydrolysis of this substrate (Gupta and Verma 2015). The high-energy treatment is provided by heating in a microwave oven or electron beam irradiation. The microwave irradiation leads to the vibrations in the bonds present in the exposed biomass and heats it from inside. It leads to the explosion effect, thus disrupting the recalcitrant structures of lignocellulose (Hu and Wen 2008). The liquid hot water treatment employs compressed hot water to hydrolyze the lignocellulosic biomass and release sugar monomers (Sarkar et al. 2012).

Chemical pre-treatment methods make use of dilute acids, alkali, organic solvents, and ammonia. This process utilizes a chemical impact to access the lignocellulosic matrix. However, this method produces certain inhibitors that later interfere with enzymatic activity in downstream processing and affect the entire economy of



**Fig. 1.6** Pre-treatment processes used for bioethanol production. Physical, chemical, and biological processes are the three main types of pre-treatment processes used to degrade the lignocellulosic matrix

bioethanol production. On the other hand, the biological treatment uses microorganisms like brown rot, white rot, and soft rot fungi. White and soft rot fungi degrade both cellulose and lignin, whereas brown rot fungi only attack cellulosic material. The hydrolysis rate is lower in biological treatment, but it requires low energy and mild environmental conditions. Most of the sugar released after biological treatment are still in the form of oligomers and still needs to be hydrolyzed by specific microbes before fermentation (Prasad et al. 2007). Enzyme hydrolysis is also necessary to convert sugars to simple monomers. The cellulases and hemicellulases producing microorganisms such as *Clostridium*, *Cellulomonas*, *Thermonospora*, *Bacillus*, *Streptomyces*, *Bacteriodes*, *Acetovibrio*, *Ruminococcus*, *Erwinia*, *Microbispora*, *Trichoderma*, *Penicillium*, *Fusarium*, *Phanerochaete*, *Humicola*, *Schizophillum*, release hexoses and pentoses upon acting on lignocellulosic substrate materials (Gupta and Verma 2015). The cellulases which are involved in the degradation are endoglucanase (E.C. 3.2.1.4), degrading the low crystallinity regions, exoglucanase (E.C. 3.2.1.91) removing the cellobiase units from the free chain terminal, and finally,  $\beta$ glucosidase (E.C. 3.2.1.21), which cleave cellobiose units into glucose (Castro and Pereira Jr 2010). The hemicellulases consist of a mixture of enzymes, namely xylanases, mannanases, glucuronidases, galactosidases, and arabinofuranosidases (Obeng et al. 2017). Fermentation and distillation are the final steps for the production of bioethanol. For industrial purposes, microbes with a broad substrate range, higher temperature tolerance, and high ethanol yields are essential. As such microbes' recognition becomes difficult, genetically modified microorganisms are employed to utilize the sugars spent for efficient bioethanol

production. It has been reported that *Saccharomyces* can theoretically yield around 90% ethanol from glucose (Spindler et al. 1992).

The most common fermentation processes are simultaneous saccharification and fermentation and separate hydrolysis and fermentation. Other agricultural remains like bagasse, fruit peels, wheat straw, almond shells, rice straw, sorghum, and corn cobs can be used to efficiently produce bioethanol (Quintero et al. 2011; Gupta and Verma 2015).

### 1.4.3 Biofertilizers

Targeting agro-industrial waste represents a possible source for developing biofertilizers that can be used as a soil conditioner in agro-ecosystem to yield high crop productivity (Sadh et al. 2018). The term biofertilizer is defined as the preparations which contain living microorganisms that play a crucial role in providing nutrients to the plants. These biofertilizers accelerate specific microbial processes such as nitrogen fixation, ammonia production, indole acetic acid production, siderophore production, phosphate solubilization, and potassium mobilization into the soil and boost up the nutrient availability for easy assimilation by the plants (Devi and Sumathy 2017). Hence biofertilizer plays a vital role in nutrients management (Devi and Sumathy 2017). To develop biofertilizers, several microbial populations and their close association with plant hosts are being exploited.

#### 1.4.3.1 Diversity of Microbial Biofertilizers

The biodiversity of microbial biofertilizers includes nitrogen fixers, phosphate mobilizers, phosphate solubilizers, phosphate mobilizers (Devi and Sumathy 2017), potassium solubilizers, potassium mobilizers (Parmar and Sindhu 2013; Yadav 2018), and plant growth-promoting rhizobacteria (Devi and Sumathy 2017) (Table 1.1).

#### 1.4.3.2 Production of Biofertilizers Using Agro-Industrial Waste

The generation of agro-industrial waste is a huge problem, and at the same time, its maintenance is a major concern. The agro-industrial wastes can be an alternative source to derive biofertilizers for agro-ecosystem and sustainable development (Sadh et al. 2018). solid-state fermentation and submerged fermentation are substrate-based fermentations processes, which are extensively used for producing biofertilizers at a large scale. Agro-industrial waste like soluble sugars, liquid synthetic media, extracts of fruit and vegetable, and dairy by-products are used in biofertilizer production (Suthar et al. 2017). Such waste contains high nutritional components in the form of proteins, sugars, and minerals, which offers a hostile

**Table 1.1** Biofertilizers

	Groups	Examples	References
<b>Nitrogen fixers</b>			
1.	Free-living	<i>Azotobacter, Beijerinckia, Clostridium, Klebsiella, Anabaena, Nostoc</i>	Singh et al. (2016), Verma et al. (2014), and Meena et al. (2017)
2.	Symbiotic	<i>Rhizobium, Frankia, Anabaena, azollae</i>	Singh et al. (2016) and Meena et al. (2017)
3.	Associative symbiotic	<i>Azospirillum</i>	Singh et al. (2016) and Meena et al. (2017)
<b>Phosphate solubilizers</b>			
1.	Bacteria	<i>Bacillus megaterium</i> var. <i>phosphaticum</i> , <i>Bacillus subtilis</i> <i>Bacillus circulans</i> , <i>Pseudomonas striata</i>	Singh et al. (2016) and Nath et al. (2017)
2.	Fungi	<i>Penicillium</i> sp., <i>Aspergillus awamori</i> ,	Singh et al. (2016) and Ma et al. (2016)
<b>Phosphate mobilizers</b>			
1.	Arbuscular mycorrhiza	<i>Glomus</i> sp., <i>Gigaspora</i> sp., <i>Acaulospora</i> sp., <i>Scutellospora</i> sp. & <i>Sclerocystis</i> sp.	Singh et al. (2016)
2.	Ectomycorrhiza	<i>Laccaria</i> sp., <i>Pisolithus</i> sp., <i>Boletus</i> sp., <i>Amanita</i> sp.	Singh et al. (2016)
3.	Ericoid mycorrhizae	<i>Pezizella ericae</i>	Singh et al. (2016)
4.	Orchid mycorrhiza	<i>Rhizoctonia solani</i>	Singh et al. (2016)
<b>Potassium mobilizers</b>			
1.	Fungi	Mycorrhiza	Yadav (2018) and Singh et al. (2016)
2.	Bacteria	<i>Acidothiobacillus ferrooxidans</i> , <i>Bacillus mucilaginosus</i> , <i>Bacillus edaphicus</i> , <i>Burkholderia</i> sp., <i>Frateuria</i> sp., <i>Pseudomonas</i> sp., <i>Rhizobium</i> sp., <i>Azotobacter chroococcum</i>	Lian et al. (2008) and Liu et al. (2012)
<b>Potassium Solubilizers</b>			
1.	Bacteria	<i>Bacillus</i> , <i>Pseudomonas</i> , <i>Aspergillus</i> , <i>Burkholderia</i> , <i>Paenibacillus</i> sp., <i>Bacillus mucilaginosus</i> , <i>Acidothiobacillus ferrooxidans</i> , <i>Acidothiobacillus ferrooxidans</i>	Yadav (2018), Parmar and Sindhu (2013), and Singh et al. (2016)

environment for the growth of several microorganisms (Sadh et al. 2018). The agro-industrial waste such as corn steep liquor, molasses, deproteinized leaf extracts, jaggery, wastewater sludge is utilized as a substrate for the cultivation of *Rhizobium* species (Suthar et al. 2017). Moreover, agricultural waste such as bagasse, paper pulp, wheat bran, vegetable, watermelon, papaya, pineapple, custard apple, guava, rice straw is also being used routinely as a source for biofertilizer production using the fermentation process (Devi and Sumathy 2017; Suthar et al. 2017; Sadh et al. 2018).



## 1.5 Potential Outcomes from Microbial Treatment of Agro-Industrial Waste

Improper management of high-value waste from agro-industries will lead to many environmental and health-related issues. Proper treatment of these waste residues can be helpful in many aspects, such as reducing greenhouse gases, being used as a renewable energy source, and improving environmental and human health.

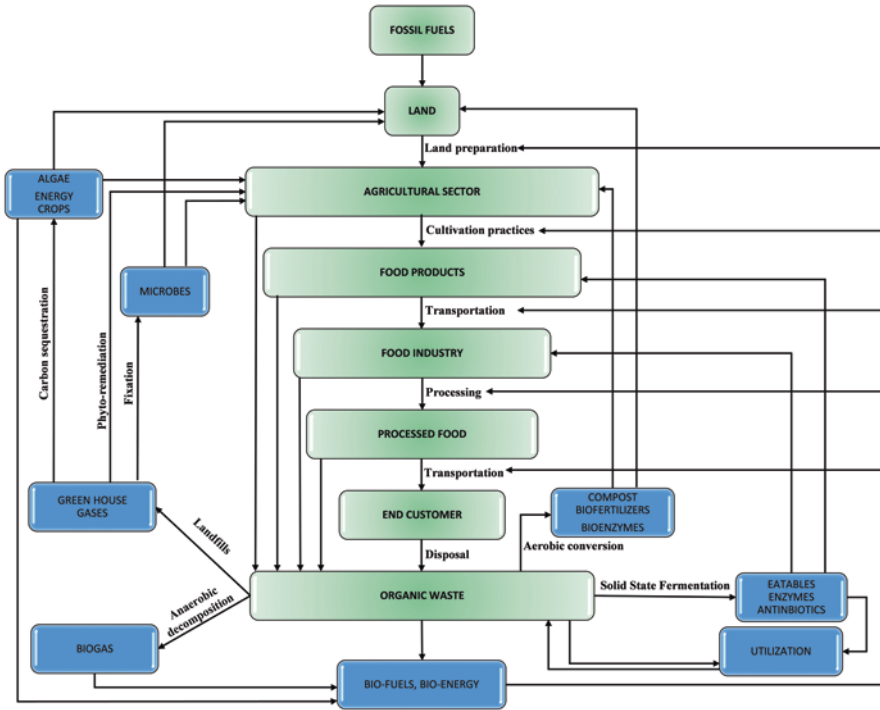
### 1.5.1 Greenhouse Gases Emission Reduction

Greenhouse gases that consist of  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{NO}_x$ ,  $\text{SO}_x$ ,  $\text{H}_2\text{O}$  are natural temperature regulators for the Earth by trapping solar radiation's heat. In the lacuna of these gases, the earth would be a cold planet. But excess of these gases along with synthetic gases like CFCs, HFCs, PFCs,  $\text{SF}_6$ , and  $\text{NF}_3$  (Department of Environment and Energy 2018). These greenhouse gases are increasing at an alarming rate due to massive industrialization and improper agricultural practices. As it is aptly said, excess of anything is toxic or has a catastrophic effect. This is the reason the earth and earthlings are suffering from global warming.

Currently, the agro-industrial waste with a very high potential energy recovery is being dumped in landfills or left on the fields. These methods of waste disposal lead to an incessant generation of GHGs, mainly  $\text{CO}_2$  and  $\text{CH}_4$ . Anaerobic decomposition is the prevailing process through which the anaerobic microorganisms generate methane. The crop leftovers release nitrous oxide ( $\text{N}_2\text{O}$ ) by the microbial action, which involves nitrifying and denitrifying microorganisms (Portugal-Pereira et al. 2015). One of the main sources of greenhouse gases in milk production industries comprises enteric fermentation ( $\text{CH}_4$ ), feed purchased ( $\text{N}_2\text{O}$ ,  $\text{CO}_2$ ,  $\text{CH}_4$ ), manure management ( $\text{N}_2\text{O}$ ), manure fertilizers, and energy (fuels and electricity). Instead of utilizing the agro-industrial waste for animal feed, it is more effective if it is put to energy production because the milch animals, when fed with the waste, will generate more greenhouse gases. The waste, when used as biofuel, will be carbon neutral (Fig. 1.7). There is a drastic decrease in enteric methane production through the feed involving tomato waste (Pardo et al. 2016).

There is an immediate need for abatement to reduce the emission of greenhouse gases. One of the eminent steps to do so is through bioprocessing of agro-industrial waste. Fossil fuels are the greatest source of greenhouse gases. The combustion of fossil fuels emits massive  $\text{CO}_2$ . Moreover, fossil fuels are diminishing at a very proliferating rate. To curb this burden, bioenergy from agro-industrial waste helps in dual ways.

Firstly, it reduces the dependency on fossil fuels. Secondly, it reduces the emission of greenhouse gases. The trash from crops like sugarcane, maize, and soybean is a readily available substrate for bioenergy production in Brazil, where it was estimated that around 141 TWh/year of environmentally sustainable potential is



**Fig. 1.7 Life cycle assessment of greenhouse gas reduction from the utilization of agro-industrial waste.** To meet the heavy energy demand in the agro-industrial sector, many fossil fuels are consumed for various purposes. This, in turn, exaggerates the greenhouse gases in the atmosphere. A large amount of organic waste is generated in each sector, starting from the land preparation (cradle) to the plantation, food production, food processing, and consumption by the consumers. This organic waste can be treated effectively through different bioprocessing techniques to produce different by-products to serve the energy demand. Thus, the greenhouse gas emission can be reduced to a very large extent making the agro-industrial sector a cradle-to-cradle system

achieved in different regions, mainly the South, Southeast, and Midwest regions of Brazil (Portugal-Pereira et al. 2015).

### 1.5.2 Renewable Source of Energy

Today’s global economy relies mainly on a range of fossil fuels such as oil, natural gases, and coal used to manufacture fuel, electricity, and other uses. A sustainable alternative needs to be sought for the world’s increased energy demand due to population growth. As fossil fuel stocks are limited, world annual petroleum production will start to decrease in the coming future, leading to the use of renewable sources as alternative options. As the energy industries rely on solar, water, wind, and

geothermal heat as renewable sources of energy, biomass generated from agro-industries can be an alternative source for fuel production and chemical industries (Sarkar et al. 2012). These fossil fuels may be replaced by bioethanol production as a renewable source through waste generated from agro-industries. In 2004, Kim and Dale reported that lignocellulosic biomass would generate 442 billion liters of bioethanol, and 491 billion liters can be generated from total waste crop residues every year, 16-folds greater than the current world bioethanol supply. This can be accomplished through different processes, such as enzymatic decomposition, physio-chemical treatments, pyrolysis, and solid-state fermentation (Yusuf 2017). The use of agro-industrial biomass for electricity generation appears to be an energy option and mitigates carbon dioxide emissions as fossil fuels are minimal and non-renewable. The inexpensive agricultural biomass wastes such as oil residues, energy crops, and other organic materials generated from agro and food-based industries may be utilized to produce renewable energy (Guldhe et al. 2017).

### ***1.5.3 Improvement in the Environment and Human Health***

A massive amount of agro-industrial wastes are dumped in landfills, left on the fields, or drained into rivers and other water bodies, which pollute the soil, air, and water. Improper management of waste in the landfills will lead to various health problems such as asthma, cancer, bacterial infections, increased cardiovascular risk, some vector-borne diseases, i.e., dengue and cholera, improper reproductive disorders, and other diseases due to exposure to carcinogenic and non-carcinogenic pollutants (Giusti 2009). Microbial degradation of some organic compounds in the landfills results in the emission of greenhouse gases such as methane and carbon dioxide, carbon monoxide, and nitrogen. Greenhouse gas emitted through landfills has great potential for global warming, whereas the pollutants released in the lower soil and groundwater in the form of leachate cause soil and water pollution. So, the leachate, greenhouse and toxic gases, and other toxic substances released from the unorganized landfills are adversely affecting humans and environmental health (Swati et al. 2018).

To overcome these problems, a proper system should be established for the treatment of waste biomass. The bulk of garbage is dumped freely and is now turned into an enormous mountain of waste, leading to significant environmental pollution and depletion of natural resources. The health and ecological resources, including groundwater, soil fertility, and air quality, can be covered by building properly engineered sanitary landfills (Kumar et al. 2009). Additional issues such as pollution pollutants, smelling, litter, fire hazards, and pest breeding can also be eliminated where waste is disposed of at appropriately built landfill sites. Sanitary landfills can also be used to store methane by installing vertical wells on the top, which can generate heat and electricity and eliminate the exponential harm to the atmosphere and public health. Instead of dumping the waste in the ground, it can be transferred to waste-to-energy plants where a high calorific fraction of the waste can be utilized as

a resource with present technologies. Generally, heat and power can be generated by the combustion of waste in waste-to-energy plants. These waste-to-energy plants use waste material as a resource collected in the dumping ground. For energy recovery, the waste composition is of considerable significance as present technologies use a high calorific fraction of the wastes that provide heat and power upon combustion. In the future, waste-to-energy technologies are crucial in treating various industrial wastes (Swati et al. 2018). New waste management technologies will play an essential role in future waste treatment, positively affecting the environment and human health.

## 1.6 Conclusion

In the current scenario, the global view for agro-industrial waste changes quickly to protect the environment and human health. Various kinds of wastes generated in the form of agricultural residues or wastewaters have been utilized to produce value-added products. These waste residues have enormous potential for the production of various important products such as biofuels, enzymes, biofertilizers, and many other bioactive compounds by using different microbial treatments. As these products are profitable, eco-friendly with an optional alternate for fossil fuels, and have potential applications in new sectors, it is important to establish more regulatory systems and investments to commercialize these products in the market. Transforming agro-industrial waste residues into efficacious compounds provides researchers a new way of sustainability for the next generation and eliminates established environmental risks.

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