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Review Article

Bioprocessing of Agricultural and Agro-Industrial Wastes into Value-Added Products

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Abstract: Agricultural wastes are one of the most abundant lignocellulosic wastes on Earth. Inevitably, this number will increase due to the increasing population needed to be fed. Unfortunately, this substantial amount of resource is underutilized and ends up in different routes: a) incineration b) left in the field to decay, and c) landfill. In all these possible scenarios, it is obvious that they are both non-ecofriendly or unsustainable for society and related industries. Agricultural wastes are noteworthy “inputs” for the circular economy since they possess high nutritional composition. The circular economy is defined as a system in which the “output” of an industry is reused as a “resource” for another industry. Agricultural and agro-industrial wastes can be converted into value-added products such as enzymes, biofuels, pharmaceuticals, food/feed enhancers, green chemicals, bioplastics, etc. In this way, we can eliminate the problems related to waste management and lower our environmental impact. In addition, a circular bioeconomy can lower the production cost of bioprocesses, create regional job opportunities, and support farmers. This review discusses industrially important products produced via bioprocessing agricultural feedstocks and related examples from the literature are given.

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1. Introduction

Since the first modern oil drill was opened in 1859 in Titusville, Pennsylvania, crude oil has been the feedstock for chemicals, polymers, and other industrial products (Yadav et al., 2020). Unfortunately, crude oil resources are not renewable and they will be depleted in the near future. Moreover, the utilisation of crude oil greatly damages the environment since underground carbon is being released into the atmosphere in a short time.

Lignocellulosic biomass (LCM) is the most abundant, renewable organic matter on Earth. It is composed of lignin, cellulose, and hemicellulose. Photosynthesis is the route for the formation of LCM. LCM constitutes hydrogen, oxygen carbon, and lower concentrations of nitrogen compared to former elements. Extractives, minerals, and ash are the other components found in the structure of LCM.

LCM defines agricultural, agro-industrial, and forestry residues. But in this chapter, its scope will be limited to agricultural waste. 181.5 billion metric tons of LCM are produced every year (Singh et al., 2022). Unfortunately, most of them end up decaying in the field or are burned. Bioprocessing these materials into a value-added product is crucial since it is a considerable route for lowering the cost of bioprocesses and also for regional economical development. "Figure 1" classifies the agricultural wastes for the production of value-added products (Philippini et al., 2020).

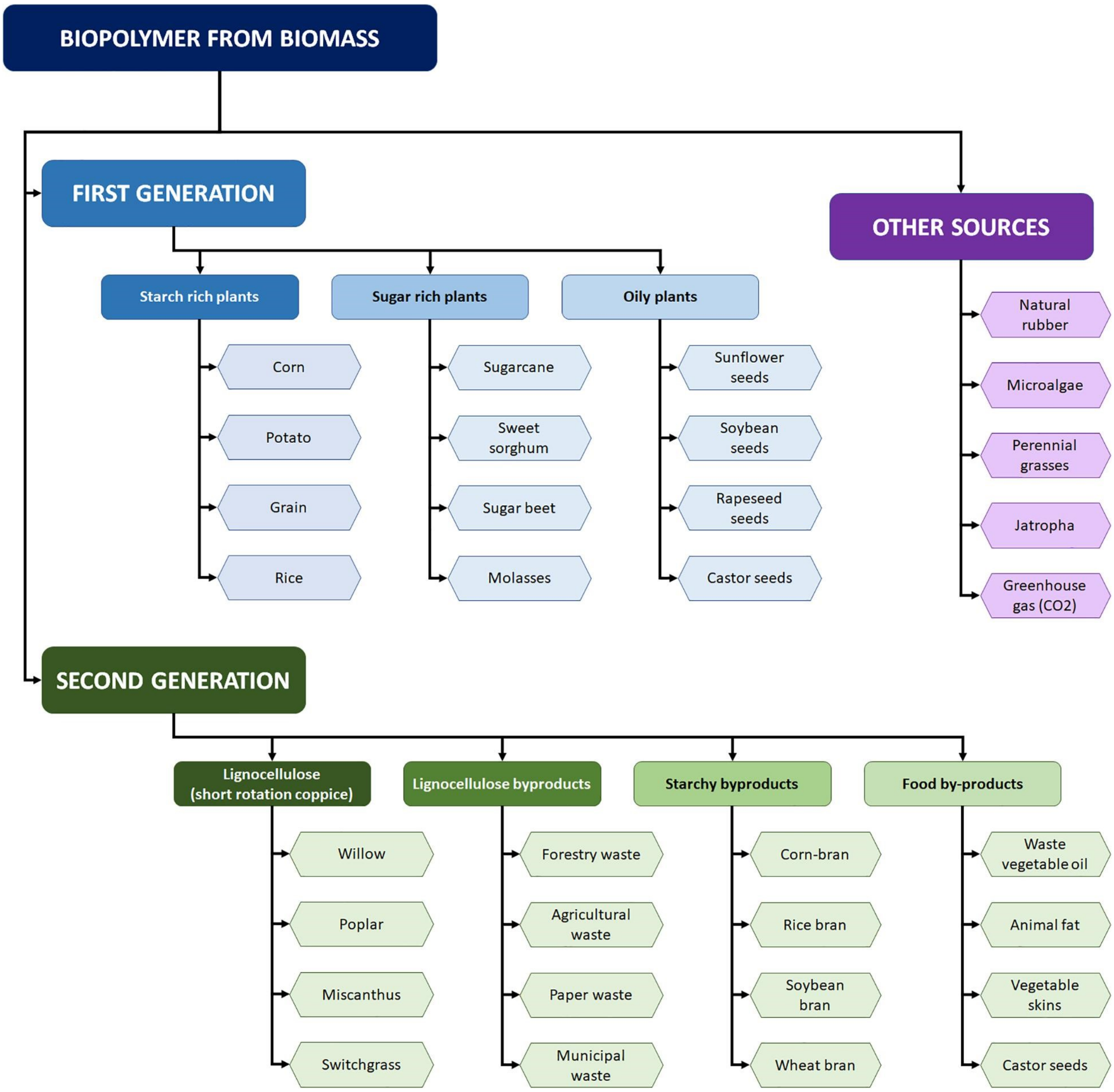

Figure 1. Classification of agricultural biomass for the production of many value-added products (Philippini et al., 2020).

As bioeconomy become prominent in recent years, researches related to the utilisation of LCM for the production of valuable industrial products accelerated and several countries have developed bioeconomy strategies to incent their industries to convert into a sustainable production (Hess et al., 2016). "Figure 2" shows the countries with bioeconomy policies.

Wastes from agriculture and the food industry using LCM are crucial feedstocks for bioeconomy. Agricultural wastes are noteworthy substrates for the production of industrial and medicinal enzymes, biodegradable polymers, and many other chemicals.

In this review, examples of enzyme, biofuel, pharmaceutical, food/feed enhancer, and green chemicals production from LCM are given.

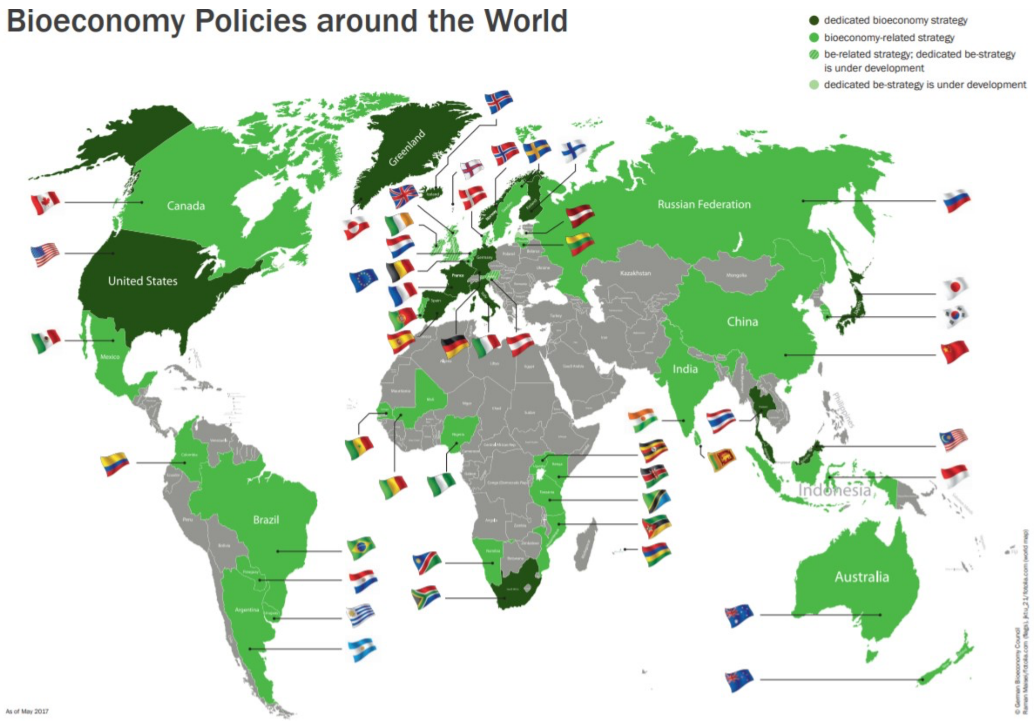

Figure 2. Countries with bioeconomy policies (Hess et al., 2016).

1.1. Enzymes

Lignocellulosic enzymes (LCE), such as cellulases, xylanases, and lignin modifying enzymes, can be produced through whole cell biocatalysis of LCM. These enzymes find application to a broad extent, such as bioremediation (Yesilada et al., 2010; Yadav and Yadav, 2015; Falade et al., 2018; Hu et al., 2018), detergent (Azmi et al., 2016), pulp and paper (Couto et al., 2006; Freitas et al., 2009), textile (Menendez et al., 2015), biosensors (Mate and Alcalde, 2017), animal feed (Shraddhaet al., 2011; Sharma and Arora, 2014; Moreno et al., 2020).

Cellulose and hemicellulose (i.e. plant carbohydrates) are exploited by microorganisms for their survival and also for the production of industrially important products, such as enzymes. This conversion is the keypoint of the carbon cycle in nature (Ruiz-Dueñas and Martínez, 2009; Jönsson and Martín, 2016).

There is a broad range of LCM spread over different kinds of geographies of Earth, and so are LCE and microorganisms growing on them (Castanera et al., 2012; Hasunuma et al., 2013).

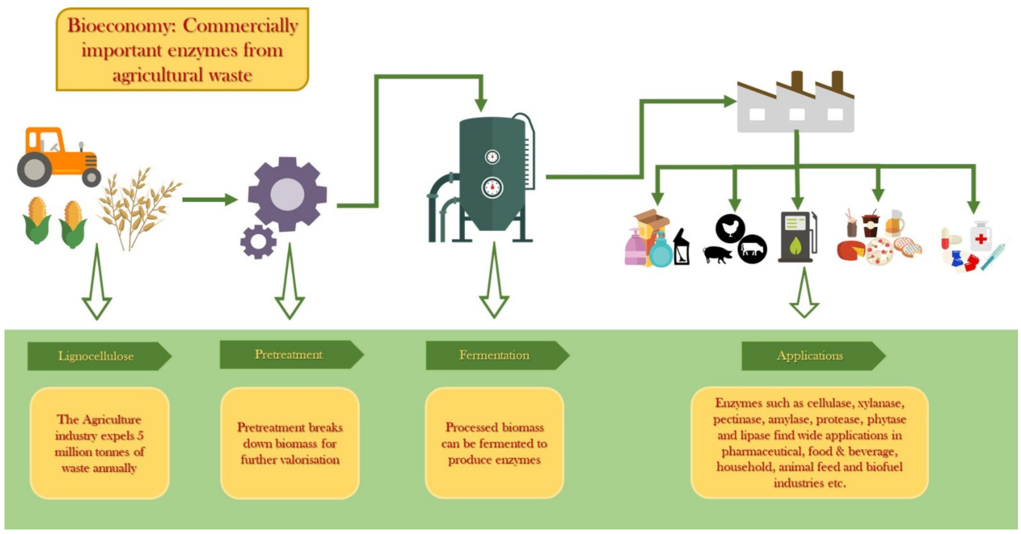

Figure 3. Production of industrial enzymes from agricultural wastes (Ravindran et al., 2018).

Cellulases (such as beta-glucosidase and endoglucanases) are used in many industries, such as animal feed (Cunha et al., 2017), food, energy (Olofsson et al., 2017), fermentation, pulp and paper, agriculture, and textile (Madhu and Chakraborty, 2017).

In a recent study, pea hulls were used to produce cellulase. Pea hulls are the main waste from the frozen food processing of pea and represent 60% of the total pea waste. *Trichoderma reesei*, which is accepted as the most efficient cellulase producer, was used as a biocatalyst for the biotransformation of pea hulls into cellulase (Sirohi et al., 2019).

Bacteria can also be used for the production of cellulase from different kinds of lignocellulosic biomass. Swathy and colleagues used waste date palm seeds for the production of high grade cellulase, where *Cellulomonas uda* NCIM 2353 was used as biocatalyst. They compared the efficiency of the produced cellulase to commercial cellulase, Cellic® CTec2 (Novozymes corporation, Denmark), and found that saccharification efficiency for acid-pretreated sugarcane bagasse was as high as 60.5%, which was equivalent to the efficiency of Cellic® CTec2. After the saccharification, resulting hydrolysate (rich in reducing sugars) was fed to *Clostridium thermocellum,* and the production of biohydrogen of maximum concentration 187.44 mmol L^{-1} was achieved at the end of 24 h of fermentation (Swathy et al., 2020).

Not only cellulase but also xylanase is another enzyme of paramount industrial importance. They are used together with cellulase in biorefineries to promote ethanol fermentation. Kaur and colleagues tried to enhance the xylanase production efficiency from *Bacillus pumilus* 3GAH via optimization of the process variables. They used Central Composite Design (CCD) to find these optimal conditions of concentration of xylose, yeast extract, peptone, and optimal concentrations were found to be 0.2%, 0.2%, and 0.2% respectively, with a fixed concentration of wheat bran (0.3%). Optimized conditions resulted in an approximately 24 fold increase in the activity of produced xylanase, compared to conditions before optimization (Kaur et al., 2016).

Carboxymethylcellulose production by *Bacillus sp. 313SI* under submerged and stationary fermentation conditions were investigated in the study of Goyal et al. Rice straw is one of the largest agricultural wastes with a capacity of 1.35 tonnes per 1 tonne of harvested rice. They investigated the effect of different concentrations of inoculum, and rice straw. Additionally, various carbon and nitrogen supplementation with a fixed concentration, temperature, and pH range were tested for their effect on carboxymethylcellulose production. Prior to fermentation, rice stalks were pretreated by two-stage pretreatment: alkali treatment (for removal of lignin) followed by acidic treatment (for removal of cellulase) (Goyal et al., 2014). The optimum conditions of these studies were tabulated below (Table 1).

Table 1. Optimum conditions for carboxymethylcellulose production in the study of Goyal et al. (2014)

	Submerged	**Stationary**
Carbon Source	Carboxymethylcellulose	Carboxymethylcellulose
Nitrogen Source	Ammonium nitrate	Ammonium sulphate
Inoculum (v/v)	0.4	1
pH	8	8
Temperature	30	35
% of rice straw	0.75	1
Maximum activity (U L^{-1})	4150	3080

Lignin moiety is responsible for the recalcitrance nature of lignocellulosic biomass. It hinders the access of microorganisms to the carbohydrate moiety (which is cellulose) of lignocellulose. Luckily some of the microorganisms, especially white rot fungi, can grow on lignocellulosic biomass due to their ability to secrete extracellular lignin modifying enzymes, such as laccase, and by the action of these enzymes, the recalcitrance of lignocellulosic biomass loosens, and microorganism can effectively use cellulose as a carbon source to survive. Laccase is one of the lignin-modifying enzymes, which is capable of oxidizing lignin. Utilization of laccase is not limited to biological pretreatment of lignocellulosic for second generation bioethanol production (Rico et al., 2014). In addition, laccase can be used in other sectors such as bioremediation (Bilal et al., 2019), biomedical technologies (Mate and Alcalde, 2017), the food industry (Osma et al., 2010), and even in cosmetics (Dana et al., 2017).

In the study of Asgher et al., *Schizophyllum commune* was used to produce laccase from rice straw. *Schizophyllum commune* is a white-rot fungus, which is capable of producing lignin-modifying enzymes from agricultural wastes (Asgher et al., 2016). They reported that rice straw was a cheap source of producing lignin-modifying enzymes, including laccase. They tested the ability of the enzyme cocktail for reduction of lignin content of different agricultural residues such as sugarcane bagasse and banana stalk and found out that after 48 hours of treatment, the reduction was by 72.3% and 61.7%, respectively.

A new approach - two step cultivation strategy - to increase fungal laccase production was reported by Hazuchova and colleagues in 2017, where the first step is the propagation of fungi, and the second step is the production of laccase via using lignocellulosic biomass as an inducer. *Pleurotus ostreatus* was used for laccase production. They used different kinds of lignocellulosic biomass, including corn straw being one of the highly produced agricultural waste around the world. The highest laccase activity was achieved with the corn straw, which is almost two times higher than the media which are supplemented with pine straw dust and alfa alfa steam. This result was achieved with two-step cultivation, which was better than one-step cultivation by 9 to 16 fold (Hazuchová et al., 2017).

It is important to mention that enzyme cocktails produced from a specific agricultural waste will obviously be better in the degradation of that specific agricultural waste (Cunha et al., 2017).

Although fungi are thought to be a good producers of laccases, some bacteria are also good at the production of laccases. In addition, due to their thermotolerant nature and ability to retain their activities in alkaline conditions, bacterial cellulases are important in industrial applications.

Muthukumarasamy and colleagues (Muthukumarasamy et al., 2015) reported that *Bacillus subtilis* MTCC 2414 was good at the production of laccases from wheat and rice bran. They used solid-state fermentation as the production type. Rice bran (345 ± 3.14 U mL^{-1}) was found to be a better substrate than wheat bran (265 ± 4.44 U mL^{-1}) for a higher yield of enzyme production.

Beta-glucosidase is another valuable enzyme that is used in industry. It is not only the key enzyme in the degradation of lignocellulosic biomass for bioethanol production (Saritha Mohanram, 2015; Sukumaran et al., 2009), but it is also used in many other sectors, such as juice and alcohol production sectors, as clarification and taste enhancer agent (Claus et al., 2018).

Yilmaz-Sercinoglu and Sayar have reported optimal medium compositions for the production of laccase and beta-glucosidase from hazelnut husk using *Pycnoporus sanguineus* DSMZ 3024 as the producer strain. They stated that beta-glucosidase production was better in a medium supplemented with yeast extract and sodium nitrate as a nitrogen source (111.9 U L^{-1}), while the maximum activity of laccase was observed in a medium supplemented with malt extract (581.7 U L^{-1}) (Yilmaz-Sercinoglu and Sayar, 2020).

The thermophilic fungus *Myceliophthora heterothallica* was used as the biocatalyst for beta-glucosidase production. Wheat bran and sugarcane bagasse were the substrates in solid-state fermentation, while cardboard was used in submerged cultivation. The results showed that maximum beta-glucosidase production was 244 ± 48 U g^{-1} in submerged fermentation and 0.9 ± 0.3 U g^{-1} in solid-state fermentation (Teixeira Da Silva et al., 2016).

Phytase is another important enzyme that is used in the animal feed industry as an enhancer. Legumes and cereals seeds contain phosphorus as phytate. Phytate can not be easily digested by animals and for this reason, external supplementation of phosphorus to the feed is indispensable. Moreover, phytate reduces the adsorption of essential nutrients from the feed, since it binds to amino acids and ions such as Zn^{+2}, Ca^{+2}, Cu^{+2}, Fe^{+2}, and Mn^{+2} and insoluble salt forms (Coban et al., 2015). Additional phosphorus and other nutrients increase the cost of feed production, and excess phosphorus through animal feces leads to accumulation in the aquatic environment.

The addition of phytase to animal feed, therefore, is pivotal to enhance the availability of phosphorus in seeds and legumes and reducing the environmental impact of excess phosphorus.

In a study, researchers used corn cope and corn brain for phytase production via solid-state fermentation. The biocatalyst is *Penicillium purpurogenum* GE1 and was isolated from soil around bean root nodules. Corn cope was better in the production of phytase (46 ± 2.8 U g^{-1} ds (units per gram dry substrate)), compared to corn bran (41 ± 4.2 U g^{-1} ds) (Awad et al., 2014).

In a recent study, two different organisms were used for the production of phytase. Medium supplemented with corn meal favored the production of phytase when the fungus *Acremonim zeae* was used as a biocatalyst. Rice bran was the best substrate for phytase production by the yeast *Kluyveromyces marxianus* (Pires et al., 2019). They also mentioned that the effect of different substrates on production efficiency could have resulted from the environment where microorganism was isolated. This also indicates the possibility of the production of different enzymes from a broad range of substrates.

1.2. Green chemicals and pharmaceuticals

Production of platform chemicals via biochemical processes is a great alternative since they possess a lower impact on environments and use cheaper feedstock. The utilisation of agricultural wastes and byproducts/wastes of food processing industries are valuable feedstock for the product of green chemicals and pharmaceuticals (Perlatti et al., 2014; da Silva, 2016).

Hydrolysates of agricultural waste can also be used for the production of value-added chemicals, such as lactic acid. Lactic acid is the building block of polylactic acid (PLA), which is gaining attention in the industry due to its biodegradable and hydrophobic nature. Almost 40% of the production cost of lactic acid results from the expense of the substrates and nutrients (Krull et al., 2020).

In their study, Krull and colleagues (Krull et al., 2020) tested the ability of *Lactobacillus casei* ATCC 393 for the production of lactic acid. They changed 70% of the nutrient sources with hydrolysed rapeseed meal or distillers' dried grains with solubles (DDGS) and reached the same production yields. Although there is no increase in productivity, the total cost of the lactic acid production process was reduced by almost 25%. This result is encouraging to see that agricultural wastes are a perfect substitute for expensive nutrients, which are the main obstacle to the biochemical routes for large-scale production of such chemicals.

Molasses and corn steep liquor were used to produce D (-) lactic acid via *Lactobacillus delbrueckii*. The organism was able to grow on these substrates and after 48 hours of cultivation 162 g L^{-1} D(-) lactic acid was produced (Beitel et al., 2020).

Resveratrol is an important phenolic bioactive compound produced as a secondary metabolite in plants, especially in grapes and berries (Costa et al., 2022). It is highly studied because of its pharmaceutical importance in cardiovascular and neural diseases (Gordish and Beierwaltes, 2014; Yetik-Anacak et al., 2015). It is produced via genetically engineered organisms, but microbial extraction from plants is another way to obtain resveratrol from plants.

An immobilized microbial consortium (Yeast CICC 1912, *Aspergillus oryzae* 3.951, and *Aspergillus niger* 3.3148) was used to extract resveratrol from grape seed wastes from the winemaking process. Researchers reported that microbially treated samples resulted in approximately 6 times higher yield of resveratrol (305.98 ± 0.23 μg g^{-1}) than untreated samples (Jin et al., 2021).

1.3. Biopolymers

Integration of agricultural wastes into a biorefinery is a profitable route for the production of not only enzymes and fuels but also biopolymers. Petrochemical substances are being used to produce many polymers, especially synthetic plastics. But growing concerns about plastic pollution accelerated the research for biodegradable polymers synthesized by various microorganisms using different agricultural wastes.

Pullulan is one of those biopolymers. It is an α-linked linear glucan exopolysaccharide and has unique physicochemical properties, such as non-toxicity, non-mutagenicity, tasteless, odorless, and edible nature. Films with different strengths can be produced from pullulan and these films are recalcitrance to slight thermal changes. All of these properties make it suitable candidate to be used in the food, biomedical and pharmaceutical industries. Comprehensive summary of pullulan applications was schematized by Coltelli and colleagues and it is given in "Figure 4" (Coltelli et al., 2020).

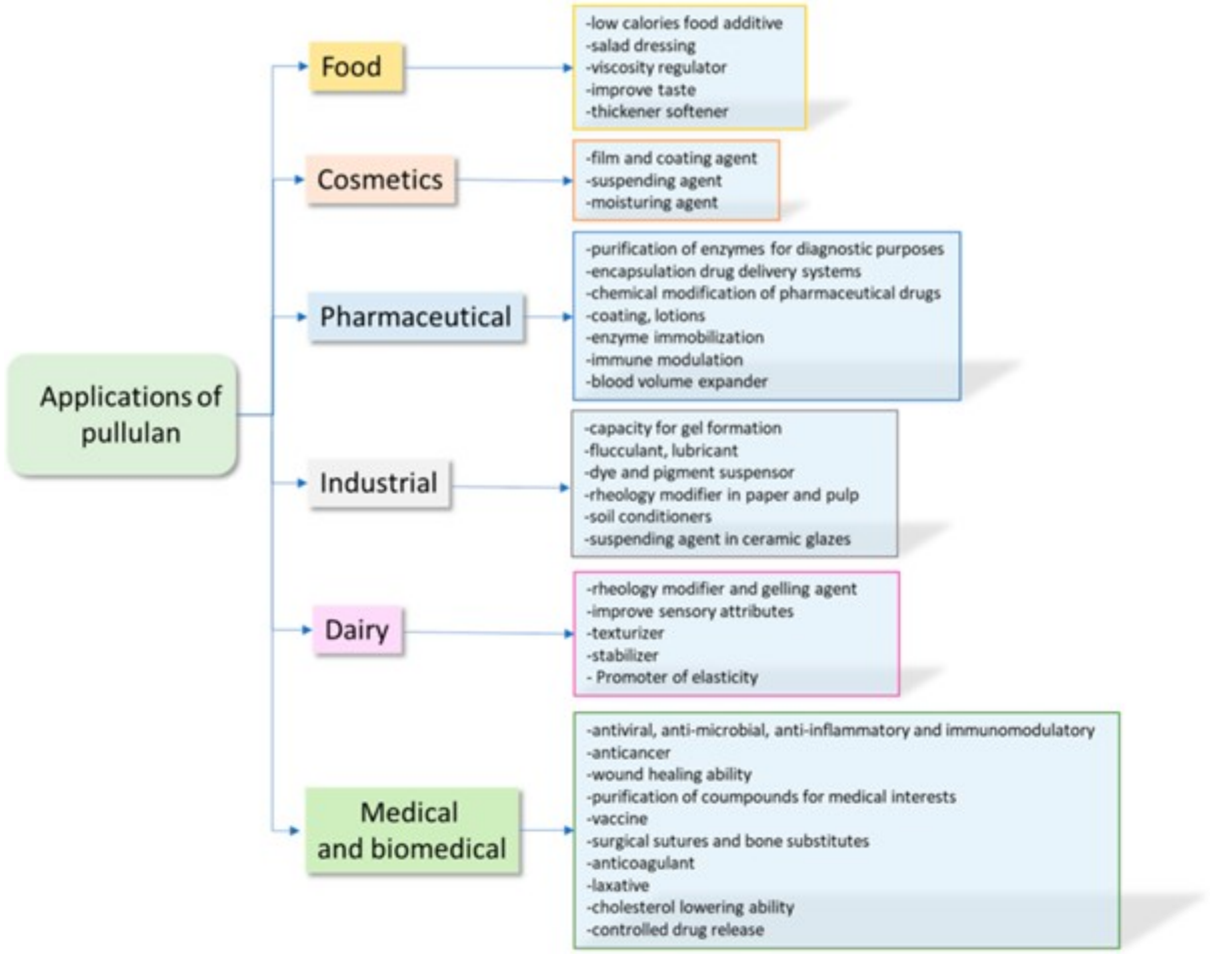

Figure 4. Comprehensive schematisation of pullulan applications (Coltelli et al., 2020).

Major obstacle in front of commercialization of pullulan production is expensive process cost (mostly due to substrate cost) and low yield.

Many studies can be found in the literature which were conducted to find the optimum media compenents for production of pullulan with high yield. In the study of Viveka and colleagues, cassava waste was investigated as substrate for pullulan production from *Aureobasidium pullulans* MTCC 1991 (Viveka et al., 2020). They achieved to produce pullulan with a yield of 6.45 g pullulan L^{-1}.

Another strain of *Aureobasidium pullulans* (MTCC 6994) was used as biocatalyst to produce pullulan in a laboratory scale stirred tank bioreactor. De-oiled rice bran was used as a carbon source. They achieved the maximum yield of pullulan as 8.32 ± 0.02%, *w*/*v* (Singh et al., 2020).

In a recent study, kitchen waste (KW) was used as a source of reducing sugars. An in-house produced enzyme cocktail was used to hydrolyze KW. Hydrolysate of KW was then fed to *Aureobasidium pullulans* MTCC 2013 and the production yield was achieved as 20.46 ± 2.01 g L^{-1} pullulan. Further analyses showed that produced pullulan is biodegradable and water soluble and similar to commercial pullulan (Rishi et al., 2020).

The melanin-deficient strain of *Aureobasidium pullulans* KY767024 was used to produce pullulan from sesame seed oil cake (SSOC), as a novel substrate, without the addition of other nutritional sources. 52.50 ± 0.73 g pullulan was produced per unit kilogram of sesame seed oil cake (Mirzaee et al., 2020).

Annual production of fossil-based plastics reached 300 million metric tonnes. Plastic pollution is a growing concern around the world, but attempts to switch to biodegradable plastics are slow since the production cost is a major obstacle in the mass production of biodegradable plastics. Agricultural wastes, side streams of biodiesel and bioethanol industries, and also dairy industry effluents can be used as potential carbon sources for the production of biodegradable plastics, such as polyhydroxyalkonates (PHA) (Figure 5) (Koller, 2017; Winnacker, 2019; Hon Kee et al., 2022). PHA are well-known bacterial storage polymers and a well-studied type of PHA is polyhydroxybutyrate (PHB). They share similar properties with petrochemically derived plastics (Penkhrue et al., 2020). Due to its brittle nature with low thermal and relatively low mechanical properties, PHB is usually used in composites with other biodegradable polymers such as polylactic acid (Arrieta et al., 2017; Jamwal et al., 2023).

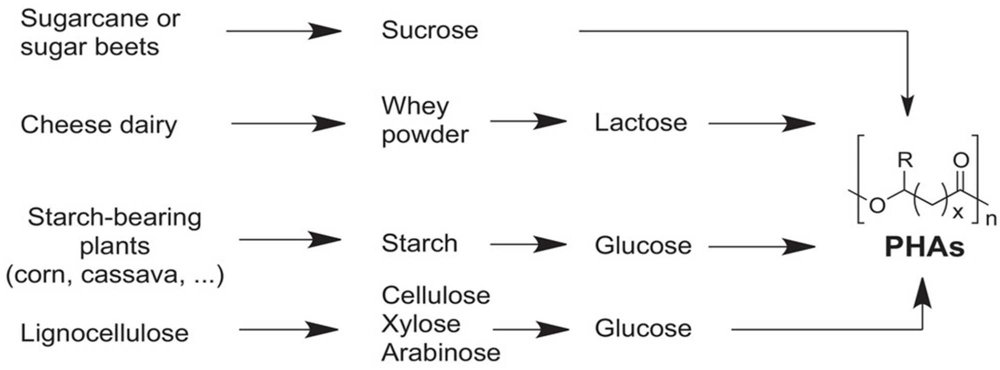

Figure 5. Potential feedstocks for PHA production (Winnacker, 2019).

Lysinibacillus sp. was used as a biocatalyst for production of PHB production from sugar cane bagasse (SCB) hydrolysates supplemented with corn steep liquor. Characterisation of produced PHB showed that it is identical to commercial PHB. They included that detoxification of SCB hydrolysate was not necessary. It is noteworthy in terms of reducing process steps. Devoid of detoxification, overall process cost will eventually reduce, together with cheap substrate (Saratale et al., 2021).

Hassan et al. used different agricultural wastes such as corn cob, wheat bran, corn bran, rice bran, and sugarcane molasses for PHB production. Rice bran outperformed the other carbon sources for PHB production. They optimised cultural parameters using Plackett–Burman and Box–Behnken designs. A six-fold increase in the PHB content was achieved when production was carried out under optimized conditions (Hassan et al., 2019).

In another study, wheat bran, rice bran, and corn cob were again used as substrates for both PHA and enzyme production (Israni and Shivakumar, 2020), in addition to other substrates, such as bajra straw and bagasse, wheat husk, wheat straw, ragi husk (RH), rice husk, rice straw, oat straw, jowar straw, mustard oil cake, sesame oil cake (SOC), groundnut oil cake and coconut oil cake. *B. megaterium* strain Ti3 (accession number HF968632) was used as a biocatalyst for PHA production.

RH and SOC were better at supporting maximum PHA production among other substrates. They also studied the effective pretreatment method of those substrates to obtain higher reducing sugar content. Although acid and alkali hydrolysis resulted in significantly higher initial reducing sugar levels for RH and SOC, the use of microorganisms' enzymes for the treatment of the substrates was offered to reduce the cost of the overall process and also for avoiding possible inhibitors released during chemical treatments of substrates (Kim et al., 2011; Mithra and Padmaja, 2016).

Bacillus drentensis (*B. drentensis*) BP17 was used for the production of PHB production from various fruit peels, including dragon fruit, apple, pineapple, mango, sugarcane, and banana. Peels were soaked in hot water to get extractions of peel and those extracts were supplied to *B. drentensis* BP17 as a cheap carbon source. 40.8 g L^{-1} reducing sugar was achieved after the hot water treatment of pineapple. Pineapple peel juice outweighed the other substrates in PHB production (5.6 g L^{-1}) in batch fermentation (Penkhrue et al., 2020).

In the latest study, researchers used finger millet straw (FMS) hydrolysates for the production of PHB. They used a combination of physicochemical and enzymatic treatment (enzymatic hydrolysis of ultrasound-aided alkaline treated FMS) to collect reducing sugars. PHB production was 4.81 g L^{-1}. Neutral pH favored the production of PHB (Silambarasan et al., 2021).

Conclusion

In this review, the importance of agricultural waste as a potential substrate for biotechnological production of industrially important chemicals is emphasized. Agricultural residues, side streams, and wastes of food processing industries are often treated as waste. On the contrary, they are valuable feedstocks that can be used to produce value-added chemicals due to their diverse nutritional composition. When the microbial and vegetational diversity on Earth is taken into consideration, it is obvious that there are numerous possibilities to match the agricultural residues and related microorganisms for the production of value-added chemicals.

While the potential of agricultural waste as a feedstock for bioprocesses is undeniable, several challenges hinder its commercialization. Chief among these obstacles is the cost of the substrate itself, which can be prohibitive for large-scale production of value-added products. Additionally, the collection cost, encompassing activities such as harvesting, logistics, and storage, present another significant hurdle.

The collection cost of agricultural waste represents a significant aspect that warrants careful consideration in any biotechnological valorization strategy. The process of gathering these residues involves multiple stages, starting with the efficient and timely harvesting of agricultural crops. Properly coordinating harvesting activities to coincide with peak waste generation periods is essential to optimize collection efficiency.

Logistics also play a crucial role in managing the collection cost. Establishing well-designed transportation networks that connect agricultural regions to processing facilities can streamline the movement of waste materials, reducing both time and expenses. Implementing innovative solutions such as regional collection centers or mobile collection units can further enhance the efficiency of waste retrieval and minimize the burden on individual farmers.

Another factor contributing to the collection cost is the storage of agricultural waste. Since waste generation may not always align with the immediate requirements of processing facilities, proper storage facilities become necessary to maintain the quality and quantity of the collected materials. Investing in suitable storage infrastructure can prevent waste deterioration and ensure a consistent supply of feedstock throughout the production process.

Moreover, the collection cost can vary based on the type of agricultural waste being generated. Different crops or food processing by-products may have distinct characteristics that affect handling and transportation methods. Understanding these variations and tailoring collection approaches accordingly can significantly impact the overall cost-effectiveness of the biotechnological production process.

Addressing the collection cost challenge requires a collaborative effort among farmers, waste management companies, and relevant industries. By promoting knowledge-sharing and providing technical support, farmers can improve waste segregation practices and facilitate more efficient waste collection. Concurrently, partnerships with waste management companies can lead to the development of specialized collection techniques and the implementation of cost-saving measures.

Overall, optimizing the collection cost of agricultural waste is crucial for establishing sustainable biotechnological processes that unlock the immense potential of these valuable feedstocks. By focusing on innovative approaches, strategic planning, and community engagement, we can create a robust and economically viable system that transforms agricultural waste into a valuable resource for the production of essential value-added chemicals.

In conclusion, it is evident that agricultural residues must no longer be dismissed as "waste." Through strategic and collaborative efforts involving farmers, industries, governments, and the public, we can effectively transform these materials into valuable resources that drive the production of essential value-added chemicals, all while fostering sustainable practices and contributing to a greener and more prosperous future.

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