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Review

Microbial Multienzyme Viz., Pectinase, Cellulase and Amylase Production Using Fruit and Vegetable Waste as Substrate—A Review

Balvindra Singh [1,2], Sumit K. Soni [3,*], Priti Mathur [2] and Neelima Garg [1,*]

[1] Post Harvest Management Division, ICAR—Central Institute for Subtropical Horticulture, Rehmankhera, P.O. Kakori, Lucknow 226101, Uttar Pradesh, India; balvindrasingh44@gmail.com
[2] Amity Institute of Biotechnology, Amity University, Lucknow Campus, Lucknow 226028, Uttar Pradesh, India; pmathur@lko.amity.edu
[3] Crop Improvement and Biotechnology Division, ICAR—Central Institute for Subtropical Horticulture, Rehmankhera, P.O. Kakori, Lucknow 226101, Uttar Pradesh, India
* Correspondence: sumit.soni@icar.gov.in (S.K.S.); neelimagargg@gmail.com (N.G.)

Abstract: Around 70 million metric tonnes of fruit and vegetable waste (FVW) are produced each year and are eventually discarded as wholesale garbage. Microorganisms decompose this FVW, which has led to environmental contamination, greenhouse gas emissions, and other impacts related to climate change. If FVW are used properly, they can reduce environmental damage and also boost a nation's economy. FVW contain vast amounts of biopolymers, viz., pectin, cellulose, and starch, all of which are hydrolysed by microbes with the aid of the pectinase, cellulase, and amylase enzymes, respectively. Therefore, in light of this, the intervention of microorganisms for the production of pectinase, cellulase, and amylase could be a safe, cost-effective, and eco-friendly approach for the precise utilisation of FVW. Nowadays, thermophilic multienzymes are extracted from a group of hot spring microbes. Thermophilic multienzymes are more capable of surviving at high temperatures and have less degrading capability. Moreover, through this advancement, we can obtain vast amounts of pectinase, cellulase, and amylase enzymes within a short period of time. This microbial enzyme preparation might be helpful in food, textiles, paper, pulp, animal feed supplements, detergents, juice/pulp clarity, leather, and other related sectors.

Keywords: FVW; industrial application; PCA; substrate

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1. Introduction

Enzymes are proteins that work as biological catalysts, which means that they accelerate the rate of biochemical reactions. All living things including bacteria, plants, and animals depend on enzymes for their metabolism and other biochemical pathways. The commercial use of certain bio-catalysts for the cost-effective synthesis of various goods on an industrial scale is of particular interest. These are a superior choice for many processes than their chemical counterparts because of their eco-friendliness, low energy requirement, and cost-effectiveness [1]. The global market for food enzymes was valued at $6.4 billion USD in 2021 and is expected to reach $8.7 USD billion in 2026, at a compound yearly growth rate of 6.3 percent, according to BCC research published in August 2021 [2].

Enzymes are primarily produced by three major sources: microbial, animal, and plant intervention. Microbial enzymes are widely utilised and most preferred due to their superiority over plants and animal-derived enzymes because of their economic viability, high stability, and rapid and high-yield production in an eco-friendly manner [3,4]. Moreover, microbial extracellular enzymes are a better option due to their simple downstream processing [5]. Because of these factors, the majority of enzymes employed in many industries come from microbial sources.

A range of sectors need a mixture of enzymes rather than just one, such as the detergent industry [6], biofuel production by hydrolysis of lignocellulosic biomass [7], biodeinking [8], biohydrogen production [9], and improvement in the quality of animal feed [10]. For such applications, a single organism that can produce multiple enzymes is preferred due to the economic benefit. Additionally, inexpensive substrates are a crucial requirement for industrial use or commercialisation to produce goods at a reasonable cost.

The improper disposal of garbage that is produced in the form of agroindustrial waste (AIW) and fruit and vegetable waste (FVW) causes environmental pollution and worsens the clean-up issues [11]. Considering this scenario, attention has been given to the utilisation of agroindustrial waste and fruit and vegetable waste (peel, pulp, stones, seeds, and other portions that are not edible) leftover as biodegradable trash by processing industries. In general, these wastes are abundant in bio-constituents such as pectin, cellulose, hemicellulose, lignin, starch, phenolics, and pigments [12]. The ingredients present in abundance in the AIW and FVW may be utilised for wealth purposes. The use of such substances as substrates for microbial cultivation can be aimed at producing cellular proteins, organic acids, secondary metabolites, or even enzymes and other industrial products [13]. Notably, microbial enzymes can function as both a tools and as end products in various bioprocesses. Therefore, AIW and FVW are excellent substrates for the development of microorganisms that produce ligninolytic, hemicellulolytic, and cellulolytic enzymes. In this way, several researchers have made efforts to produce multienzymes (pectinase, cellulase, and amylase) by exploiting AIW and FVW.

Different types of microorganisms are known that can produce extracellular, intracellular, and extra-intracellular enzymes using a range of accessible, affordable, and biodegradable substrates as carbon sources. Even though many microbes have been isolated and investigated for their capacity to produce various enzymes, considering the enormous diversity found in nature, microbial bioprospecting aids in discovering new enzyme sources [14].

This review study reports the feasibility of exploiting FVWs as substrates for microbial enzyme preparation including pectinase, cellulase, and amylase. FVW may decrease the enzyme production costs in bio-refinery processes using a low-cost substrate. The flowchart of the solid-state fermentation of FVW for enzymes or multienzyme production by microbial intervention is depicted in Figure 1.

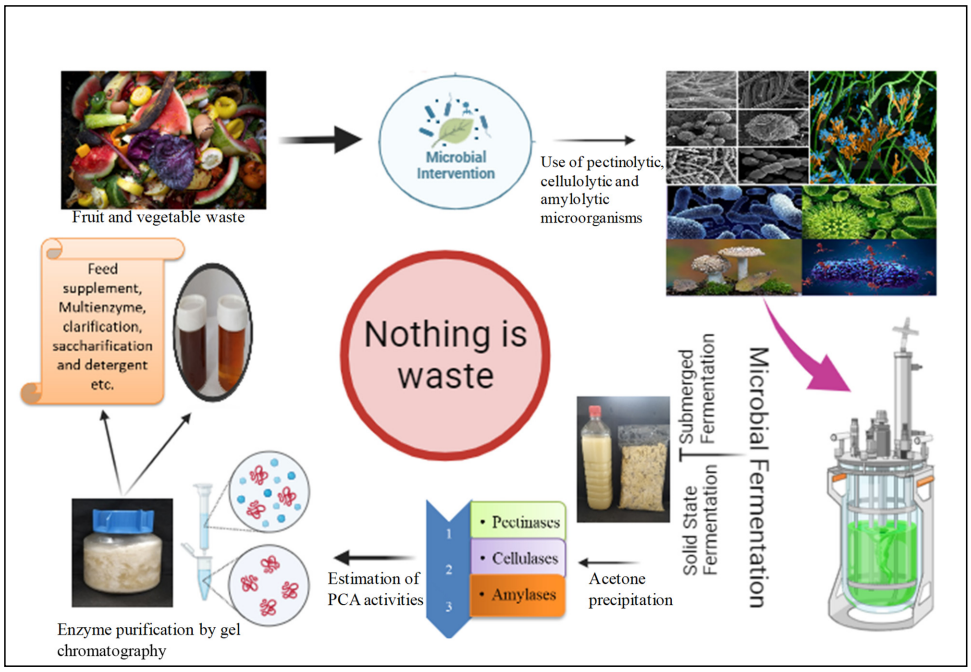

Figure 1. Flowchart of the solid-state fermentation of FVW for enzymes or multienzyme production by microbial intervention.

2. Screening of Microorganisms for Pectinase, Cellulase, and Amylase (PCA) Production

For the production of PCA enzymes, microorganisms such as bacteria, fungi, and actinomycetes are good sources. Numerous studies have already proven that microorganisms produce PCA with good quality. An earlier study proved that microorganisms could exploit FVW and AIW as substrates for producing PCA [15]. Therefore, ongoing research into microorganisms has become essential to meet the industrial needs [16]. The screening of microorganisms for PCA production is the primary selection process. For the isolation of microorganisms that produce enzymes, the corresponding substrate-rich medium (1% of the total volume) can be employed as the sole source of carbon. In primary screening, the substrate is often quantified using an indicator, which could be a dye or other substance (Table 1) [17,18]. On the other hand, the methods used for secondary screening typically involve enzyme quantification assays.

Table 1. Primary screening by the zone visualization method.

Enzyme	Medium	Indicator Dye	Observation	References
Pectinase	Medium with pectin substrate	Congo Red	Blue-violet or Red	[17,18]
Cellulase	Medium with carboxymethylcellulose	Congo Red	Blue-violet or Red	[19]
Amylase	Medium with starch	Iodine	Brownish	[20]

3. Multienzyme Production

Multienzymes are a cocktail of two or more enzymes that act on multiple substrates and increase multiple catalyst reactions at the same time. A variety of enzymes can be produced by a few microbes, especially bacteria and fungi that are accustomed to doing so [21]. A single organism that can produce several enzymes is preferred for these applications due to the cost–benefit ratio. Such enzymes must be developed under process conditions to be used in various industrial applications.

The production of multienzymes by the microbial fermentation of FVW is depicted in Figure 2. These multienzymes are very useful for industries and are frequently favoured over chemical catalysts [1]. For industrial application or commercialisation, it is also necessary to produce an item at a reasonable cost using inexpensive or waste substrates. In light of these issues, AIW and FVW could be used as commercially viable microbial substrates for the simultaneous production of several enzymes. Microorganisms play a vital role in the breakdown of FVW and AIW into pectin, cellulose, and starch-rich substrates through the production of pectinase, cellulase, and amylase. There have been few reports of the solid-state fermentative production of multienzymes using the peels of FVW, viz., mango, pomegranate, apple, banana, orange, etc. The utilisation of *Citrus limetta* peels can be used as a substrate for the production of multienzyme preparation by using a yeast consortium [18,22,23]. To date, there are various reports available on the production of a single enzyme, but only a few microbial strains have been reported regarding the production of multienzyme preparation such as pectinase, cellulase, and amylase, etc. This multienzyme preparation can be useful for juice clarification/extraction from substrates rich in pectin, fibre, and starch, thus the use of individual enzymes can be avoided.

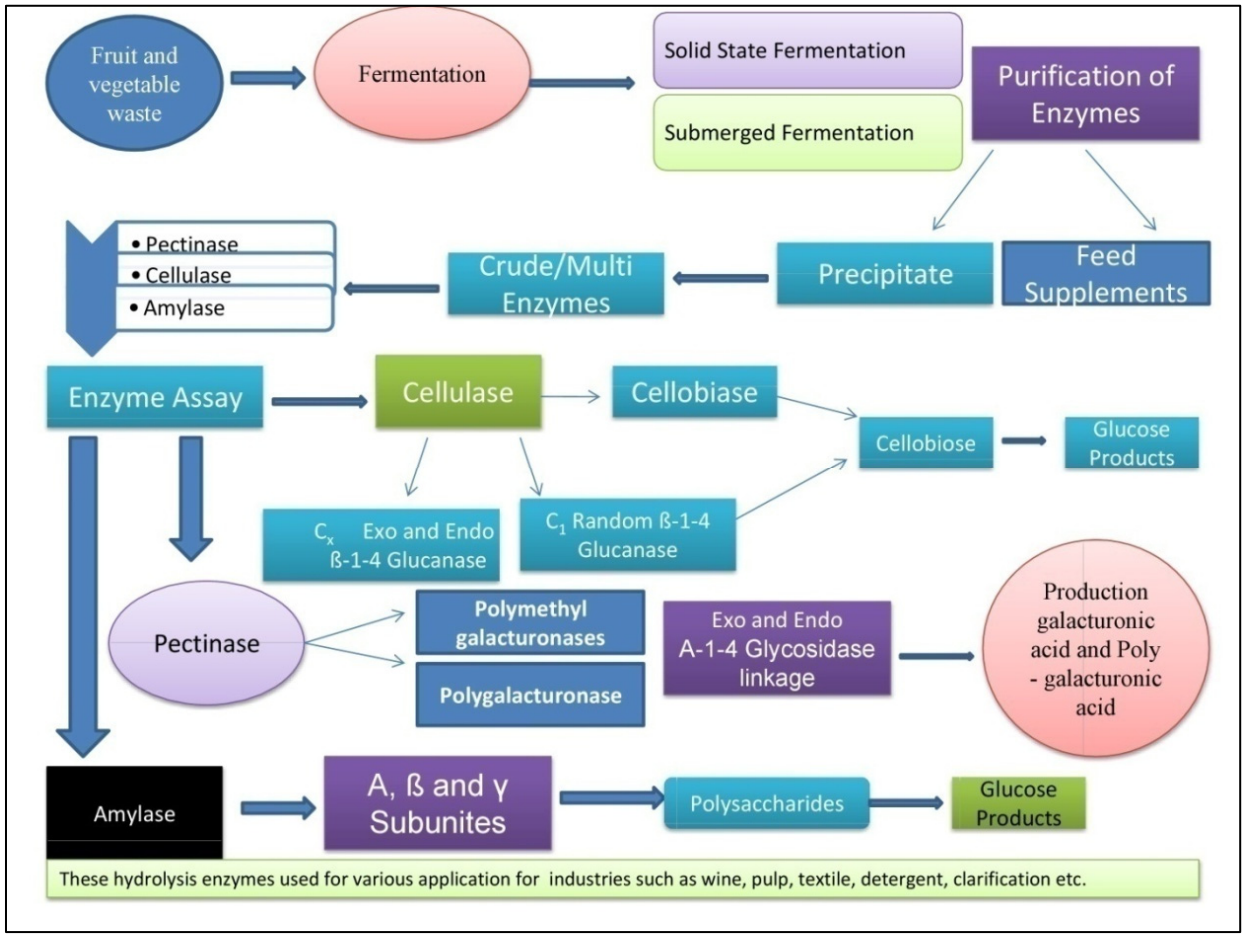

Figure 2. Flowchart of enzyme production by the fermentation process.

4. Thermostability of Pectinase, Cellulase, and Amylase (PCA)

In general, plant-based multienzyme extracts viz., PCA are not more thermally stable, meaning that when the temperature rises, the enzyme activity decreases or is destroyed [24]. The reason for the decline in enzymatic activity performance is that enzyme denaturation occurs at higher temperatures [17]. The majority of industrialists aim to produce thermally stable PCA for long-term activity and stability. In this view, the production of many thermostable multienzymes of microbial origins is of great concern. Therefore, thermally stable multienzyme PCA makes them a better choice for industrial applications.

The multienzyme contains Gln, Cys, Ala, and His amino acids, which are predominant in mesophilic (20–45 °C) enzymes, whereas Lys, Ile, Tyr, and Gly amino acids are often abundant in thermophilic enzymes. Thermophilic enzymes may stabilise and function at high temperatures due to salt bridges that are formed as a result of the electrostatic interaction between the amino acids Lys, Glu, and Arg [25].

Nowadays, thermostable pectinase, cellulase, and amylase are found in *Trichoderma asperellum* at 65 °C [18]. The study by Ejaz et al. [26,27] revealed the thermo-stable cellulolytic enzyme production by *Bacillus aestuarii*, *Brevibacillus borstelensis*, and *Aneurinibacillus thermoaerophilus* by using sugarcane bagasse as a substrate.

5. Process Improvements for PCA Production

The two primary types of fermentation conditions—solid-state fermentation and submerged fermentation conditions—can be used to identify process improvements for the production of various fermentative products, by-products, and enzymes. Solid-state fermentation (SSF) is often considered as an advantageous process over submerged fermentation for the production of multienzymes. For example, FVW and AIW are used as solid

substrates as well as the sole source of carbon and nitrogen to increase the product concentration. Moreover, the solid base supports the growth of a wide range of microorganisms and enhanced the stability of the product [8,17,18].

Pectin, lignocellulosic, hemicellulose, and other components are present in AIW and FVW; these can be utilised as substrates for the production of PCA enzyme by the interaction of microorganisms during the solid-state fermentation process. Numerous modifications can be made to speed up the downstream process including the addition of macronutrients (such as nitrogen, phosphorus and potassium) and micronutrients (such as calcium, magnesium, zinc, and iron) as non-protein cofactor concentrations as well as the optimisation of abiotic factor viz., temperature, pH, gradients, aerations, and moisture. Optimisation can be a better technique for high-yield enzyme production from microbial sources using submerged and SSF technology. The end-product of enzymes can also be produced by utilising the same specific substrate used for enzyme production by microbial strains [17,18,28].

6. Strategies for Improvement of Efficiency, Shelf-Life, and Reusability of PCA

Basically, enzymes are immobilised via adsorption, covalent binding, entrapment, and cross-linking methods. PCA has more drawbacks for industrial catalysts such as poor stability, high degradability, and short incubation time [29]. Various strategies have been employed to increase the efficiency and reusability. For increased effectiveness, functional longevity and reusability, the enzymes (pectinases, cellulases and amylases) are loaded in particular enzyme support matrices including calcium alginate beads, bagasse fibres, a nylon matrix, and other immobilised support matrices. According to a study by Martin et al. published in 2020 [30], agar alginate beads could be utilised up to five times, with the sixth cycle retaining roughly 61% of the enzyme's original activity. Nowadays, enzyme immobilisation techniques are most interested in nanotechnology, which has provided a wealth of diverse nanoscaffolds. Furthermore, the advantages of industrial enzyme application are due to easy handling, separation, economic cost, high stability, and reusability of the catalyst reaction of enzymes. Nanoparticles and nanofibres, nanomagnetics, and nanobiosensors are also trending techniques used for enzyme immobilisation because nanomaterials are the key factor for the efficiency of biocatalysts [29,31].

7. Pectinase

The demand for microbial pectinase has expanded globally as a result of its increased use in many industries. Pectin is one of the primary components of cereals, vegetables, fruits, and fibres, and can be used as a substrate for the production of pectinase or pectinolytic enzyme [32]. It is also defined as a polysaccharide that is rich in sugars, galacturonic acid, and methanol components. Pectin is mostly found in the plants' cell walls and middle lamellae, which are intracellular components of plants.

Microorganisms, particularly bacteria, fungi, and yeast, are the principal suppliers of pectinase [33]. To produce pectinase, inexpensive AIW and FVW can be preferred as substrates. Pectinases were first used for commercial purposes in the 1930s. They support the plants' cell wall expansion, tissue softening during maturation and storage, and maintenance of the degradation and recycling of waste materials. In the food industry, pectinase enzymes are widely used, especially in the clarification and extraction of fruit juice [34]. In addition, endo-polygalacturonases, which are produced by fungi, bacteria, yeasts, plants, and some plant parasites and belong to the subgroup of the pectinase family, are also used for industrial purposes [35]. The microbial pectinase enzyme is involved in the reaction catalysed by esterases:

$$\text{Pectin} + H_2O \xrightarrow{\text{Pectinesterase}} \text{Pectate} + CH_3OH$$

7.1. Production of Pectinases from Microbial Sources Using FVW as the Substrate

FVW have sources of carbohydrates (pectin, cellulose, hemicellulose, starch, etc.) and other components that can be helpful for the growth of microbes. The production of pectinase has been conducted with different fruit and vegetable waste used as a substrate by microbial intervention because this waste is a rich source of a pectin substrate for the feasible production of pectinases (endo and exo). Various researchers obtained good results for the production of pectinase by microbial intervention. Mosambi peel (*Citrus limetta*) wasteis utilised as carbon sources for the production of multienzymes (pectinase, cellulase, and amylase) with the intervention of the *Trichoderma* species. The efficient production of pectinase by *Trichoderma asperellum* was observed at pH 5.5 with an incubation temperature of 30 °C for 10 days of fermentation and optimised nutrient addition [17,18]. In another study, the safe and highly active pectinase production from Egyptian onion (*Allium cepa* L.) skins using *Trichoderma viride*, but not more than citrus, was observed by Ismail and coworkers [36]. Similarly, the optimal production of pectinase by the fermentation of citrus peel using *Trichoderma harzianum* in SSF at pH 5.5 and 28 °C for 72 h was recorded [37]. Pectinase (6.5%, *w*/*v*) was achieved after 4 days of incubation under submerged fermentation (SMF) cultures at a temperature and pH of 30 °C and 4.0, respectively. The aforementioned data show that the pH and temperature conditions can also play a main role in the downstream process of optimal pectinase production. The optimal production of enzymes at a specific temperature and pH can vary from genera to genera or even species to species.

Apart from the *Trichoderma* species, several other fungi viz., *Aspergillus oryzae*, *Aspergillus fumigatus*, *Rhizomucor pusilis*, *Lactobacillus* sp., and *Penicillium atrovenetum* have also been previously accounted as able to produce pectinase using different types of FVW [30,35,38–41] (Table 2).

Table 2. Production and optimisation of pectinase from biodegrading waste using microbial isolates.

Waste	Source of Isolation	Organisms	pH	Temp. (°C)	References
Mosambi peel	Organic substrate	*Trichoderma asperellum*	5.5	30	[17]
Sunn hemp fibre	MTCC (Microbial Type Culture Collection)	*Aspergillus fumigatus*	10.0	30	[35]
Citrus peel	NIBGE (National Institute for Biotechnology and Genetic Engineering)	*Trichoderma harzianum*	5.5	28	[37]
Mango, Mosambi, banana, cabbage, etc.	ATCC 8014TM	*Lactobacillus* sp.	-	35	[38]
Orange peels	Orange peels and soil	*Penicillium atrovenetum*	5.5	35	[39]
Orange peels	Rotten orange residues	*Fusarium* sp., *C. oxysporum*, *Mucor racemosus*, *Penicillium minioluteum*, and *Trichoderma reesei*	-	30	[40]
Orange peels	Soil and decayed fruits	*Rhizomucor pusilis*	5.0	50	[41]
Mixture of vine shoots, jatropha cake, olive pomace, and olive oil	IRD/IMBE	*Trichoderma asperellum*	7.0	25	[42]
Wheat bran and duckweed	Westerdijk Fungal Biodiversity Institute	*Trichoderma asperellum*	5.5	30	[43]

7.2. Application of Pectinase

Pectinase applications are widely used for various industries such as in the extraction of juice yield [18,44], is involved in coffee, the fermentation of coffee, cocoa, and tea, the preparation of jams and jellies, and in the softening process for the pickling industry [45].

Pectinases are also used in the agricultural sector for the widespread purification of plant viruses, oil extraction, retting, degumming process, and the bio-scouring of cotton fibre. They are also used as animal feed supplementary enzymes to create an increase in nutrient properties [46]. It has potential applications used in the food, paper, and textile industries [47]. Acid pectinases bring down the cloudiness and bitterness of fruit juice [48] and have many applications in food, pharmacy, and cosmetics as gelling, stabilising, emulsifying, and binding agents. In medicinal applications, it reduces cholesterol, blood sugar, ulcers, and cancer and stimulates the immune response [49,50].

8. Cellulase

Nowadays, all industrial sectors have shifted from dangerous chemicals to green, eco-friendly-based chemistry methods, which has fulfilled the increased demand for enzymes as well as the cost-effectiveness.

Cellulose is known as a biopolymer or linear homopolymer of carbohydrates. Plant waste biomass (Table 3) has major components of cellulose, hemicellulose, and lignocellulose because it has unique properties of biodegradability [51]. It is present in about 70% of the stalks, leaves, and roots of the plants [52] and can potentially be used widely for bioconversion to value-added bio-products [53].

Cellulases are the enzyme-protein groups of hydrolytic enzymes. Microbial-extracted cellulase enzymes are the basic principle that catalyse the cleavage of the glycosidic bonds of cellulose [54]. The cellulases can be extracted from microbial intervention using all types of degrading lignocellulolytic materials used as the substrate as well as plants and insects that also have pectinase [55]. Cellulase consists of three proteins: β-glucosidase, endo-1,4-β-D-glucanase (endoglucanase), and exo-1,4-β-D-glucanase. The components of cellobiohydrolases and endocellulases are a cellulose binding domain, a catalytic domain, a hinge region rich in Pro, Thr, and Ser residues, and a signal peptide that facilitates secretion [27,56].

Table 3. Biomasses containing lignin and cellulose [57].

Residue	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Sugarcane bagasse	38.1	26.9	18.4
Corn cob	45.0	35.0	15.0
Sorghum stover	29.7	15.4	25.9
Wheat straw	30.0	50.0	15.0
Rice straw	34.2	24.5	23.4
Cotton	80–95	5–20	0
Wood hard stalks	40–55	24–40	18–25
Paper	85–99	0	0–15

8.1. Production of Cellulases from Microbial Sources Using Fruit and Vegetable Waste as the Substrate

Various researchers have modified the protocol for cellulase production from microbial cells using different types of FVW. The utilisation of FVWis very cheap, economical, and low cost for the commercial or industrial production of cellulase [58]. Agricultural biodegradable waste, rice bran, and wheat straw have been found to be the best carbon source for the production of cellulase from cellulose sources [59]. Different lignocellulolytic agricultural wastes have been used as substrates for the production of cellulases such as rice husks, millet husks, banana peels, wheat bran, coir waste, and sawdust using the potential intervention of fungal strain (*Aspergillus* and *Trichoderma*)and bacterial (*Bacillus* spp.) strains. These two strains are the most important for cellulase production from the FVW and AIW in Table 4. The cellulase assay activities were measured by two main

methods: CMCase and FPase [60]. Mostly fungi strains, under submerged or solid-state fermentation with agricultural cellulosic waste materials, have been used as substrates for the conversion of cellulose to enzyme [54]. The different types of food waste are generated from food industries such as olive mills, tomato pomace, grape pomace, and some others. The *Trichoderma* sp. strain was able to grow on food waste in both liquid and solid parts. Cellulase and β-glucosidase activities were found in the liquid culture [61]. As per the data, agro and straw waste have a higher amount of cellulose, so these types of waste act as substrates for the production of the cellulase enzyme.

Table 4. Production and optimisation of cellulase from biodegrading waste using microbial isolates.

Waste	Isolation Source	Organisms	pH	Temp. (°C)	Km/V_{max}	References
Paddy straw	Spoiled coconut	*Aspergillus niger*	20~40	5.5~7.0		[4]
Mosambi peel	Organic substrates	*Bacillus subtilis*	35	5.5	-	[18]
Mosambi peel	Organic substrates	*Trichoderma asperellum*	30	5.5	2.0/114.9	[17]
Orange peels	Orange peels and soil	*Aspergillus flavus*	5.5	40	-	[39]
Rice straw and sugarcane bagasse	Degraded straw	*Aspergillus terreus*	45	-	12.0/16.1	[53]
Rice bran and wheat straw	Agricultural and agro-industry	Fungal and bacteria	28	7.0	-	[59]
Rice husks, millet husks, banana peels, wheat bran, coir, and sawdust	-	*Aspergillus niger*	3–9	10–50	-	[60]
Sugarcane bagasse	ATCC	*Bacillus megaterium* and *Bacillus subtilis*	33	-	-	[62]
Apple pomace	-	-	25–60	3.0–4.5		[63]
Oil palm empty fruit bunch	-	*Trichoderma asperellum* and *Aspergillus fumigatus*	35 and 45, respectively	7.0 and 6.0, respectively	-	[64]
Cassava wastewater	-	*Bacillus subtilis* and *Aspergillus niger*	30~80	3~9	-	[65]
Wheat straw and bran	Soil and agro-waste	*Aspergillus fumigateus* and *Aspergillus niger*	28	4.2	-	[66]
Saw dust	MTCC	*Trichoderma reesei*	30	6.5	1.07/4.67	[67]

The aforementioned details in Table 4 can help to increase the economic feasibility of cellulase production. The main key role of this study was to reduce the economic cost of cellulase production by using cellulolytic waste as a substrate for microbial enzyme production. Sugarcane bagasse, which contains roughly 40–50% cellulose, 25–35% hemicellulose, and 17–20% lignin, was utilised as a cellulolytic substrate by the fungal strain *Trichoderma reesei* to produce cellulase [68].

A study by Pinotti et al. [62] looked into the submerged fermentation (SmF) method of producing cellulases by utilising two bacterial species (*Bacillus megaterium* and *Bacillus subtilis*) and various filamentous fungi (*Trichoderma koningii*, *Penicillium species*, and *Rhizomucor species*). The temperature (28, 33, and 38 °C) and sugarcane bagasse concentration (0.5, 1.6, and 2.7% *w*/*v*) were the two cultivation variables that were assessed for each microor-

ganism. Three categories of sugarcane bagasse were also evaluated: natural, pre-treated with acid-alkaline, and pre-treated with hydrogen peroxide solutions. They found that *T. koningii* worked well for cellulase production by natural sugarcane bagasse under SmF. The production of cellulose-degrading enzymes was carried out by utilising rice husks and sugarcane bagasse waste as the substrate with the intervention of *Aspergillus terreus* under solid-state cultivation [53]. According to Singh et al., the production of multienzymes (pectinase, cellulase, and amylase) from Mosambi peel used as a substrate by *Trichoderma asperellum* and *Bacillus subtilis* was conducted at temperatures of 30 and 35 °C and pH 5.0 and 5.5, respectively [17,18].

Ibrahim et al. reported a study conducted on crude cellulase production from oil palm empty of the fruit bunch, pre-treated with 2% NaOH, and a palm fruit bunch containing 59.7% cellulose, 21.6% hemicellulose, and 12.3% lignin using *Trichoderma asperellum* and *Aspergillus fumigatus* [64].

Paenibacillus sp. Strain MTCC 5639I was used for gene-encoding ß-glucosidase and endoglucanase. *Escherichia coli* DH5α (Invitrogen, Waltham, MA, USA) was used as the host strain for gene cloning and protein expression and the pQE30 vector was used for cloning and expression through the recombinant enzymes obtained open reading frame (ORF) of 1347 bp, which was detected in the amplified product that encoded a 448-amino-acid-long 51.7-kDa polypeptide [69]. Fischer et al. stated that several insect species like *Dictyoptera, Orthoptera*, and *Coleoptera* produce cellulases in the midgut or salivary glands, and putative cellulase genes have been identified in other orders [67].

Ibrahim et al. [64] showed that the fungal cultures of *T. asperellum* UPM1 produced higher β-glucosidase (5.01 U/mL), whereas *A. fumigatus* UPM2 produced a higher amount of CMCase at 24.24 U/mL and FPase at 1.10 U/mL using an oil palm empty fruit bunch as the substrate.

8.2. Application of Cellulase

Cellulases are widely used in the beverage, textile, paper, pharmaceutical, food, pulp, wastewater treatment of residue effluents, brewing, laundry, biofuel, and detergent industries as well as used in food supply and food preservation [24,70,71]. Animal feed supplement enzymes can be used as prebiotic and probiotic-rich feed supplementary enzymes and increase the nutritional value of animals [70–72].

9. Amylase

Amylase hydrolyses starch into glucose. There are three main types of amylase: alpha, beta, and gamma. Alpha and beta-amylase are the fastest-acting enzymes. Amylase belongs to the hydrolase enzyme family and can be extracted from various amylolytic sources including plants, animals, and microorganisms. Microbial amylases are mostly used in various food and beverage industries for starch degradation. There are many important crops where starch is present such as wheat, rice, maize, tapioca, and potato, etc. These waste parts of the aforementioned plants can be used as the substrate for amylase production by microorganisms [73].

9.1. Production of Amylases from Microbial Sources Using Fruit and Vegetable Waste as the Substrate

Globally, 50% of food, fruit, and vegetable waste is lost, and it may be that this chain increases with the fruit and vegetable production in developing countries as they are more lost in the form of peels, pulp, seeds, and kernels, etc. If we utilised this waste as the substrate for the production of amylase, other enzymes, and by-products, it could increase the country's economy [74]. Workers and researchers have used FVW as a substrate for the cheap production of amylases; mostly the starch present in barley, rice plants, and cassava mash waste-water has been used as a substrate for the production of α-amylase (Table 5).

Table 5. Production and optimisation of amylase from biodegrading waste using microbial isolates.

Waste	Source of Isolation	Organisms	pH	Temp. (°C)	Km/V_{max}	References
Mosambi peel	Organic substrates	*Trichoderma asperellum*	5.0	30	1.0/134.8	[17]
Mosambi peel	Organic substrates	*Bacillus subtilis*	5.5	35	-	[18]
Kitchen waste	Municipal solid waste	*Chryseobacterium* sp. and *Bacillus* sp.	5.0 and 7.0	50	-	[75]
Edible oil cake, groundnut oil cake, coconut oil cake, and sesame oil cake	MTCC	*Aspergillus oryzae*	32.5	4.5	-	[76]
Vegetable waste	Marine water	*Aspergillus niger*	9.0	70	-	[77]
Fruit and vegetable waste	Kitchen food waste	*Bacillus subtilis*, *Bacillus licheniformis*	6.0	45	-	[78]
Potato peel	Soil of cassava waste dumpsite	*Aspergillus flavus*	5.0	25	-	[79]
Fruit peel	-	*Trichoderma*, *Bacillus* sp., *S. cerevisiae*	6.0	-	-	[80]
Molasses	-	*Bacillus* sp.	8.0	50	-	[81]
Loquat kernels	Fermented loquat kernels	*Penicillium expansum*	6.0	30	-	[82]

9.2. Application of Amylase

High blood glucose (sugar) levels cause vascular complications such as retinopathy, neuropathy, nephropathy, and damage to vital organs. The main role of α-amylase (α-1,4-glucan-4-glucanohydrolases), which is secreted from the human pancreas, catalyses the hydrolysis of a (1,4)-glycosidic linkage, converting starch and glycogen to maltose and glucose while α-glucosidaseconverts maltose to glucose in the small intestine. The α-amylase is consumed or degrades starches and branched maltodextrins [83].

Nowadays, amylases are used very widely in industries. They play a great role and have multiple applications in various industries such as dairy, soft drinks, human diet, chocolate, pharmaceuticals, food processing, leather, textile, paper, wine, meat, fish processing and many others. α-Glucosidase can digest dietary starch into glucose [83]. Current uses of amylase include biotechnology/microbiology processes such as starch degradation, detergents, foodstuff, pharmaceuticals, food, liquefaction and saccharification, textile, clinical, medical, analytical chemistry, and paper manufacturing as well as the pharmaceutical industry for the production of glucose syrups, high fructose corn syrups, maltose syrups, and the reduction in the viscosity of sugar syrups [84].

10. Future Prospects of Pectinase, Cellulase, and Amylase

This multienzyme is a group of enzymes that can mostly be used in various food industries. In the 21st century, this can be obtained from different microbial sources using cost-effective carbon-rich substrates such as FVW and lignocellulosic waste materials. Nowadays, new technologies are drifting toward increased knowledge about the structure of enzymes including genomics, next-generation sequencing, metagenome, and transcriptomics. The microbial production of the multienzyme (PCA) and their role in industrial prospects are very important for fulfilling and increasing the demands of food enzymes.

A report by CAGR (2023–2030) of the research and analysis provided within the Bio Enzymes Market Research is meant to benefit stakeholders, vendors, and other participants in the industry [85]. The bioenzyme market is expected to grow annually significantly (https://www.360researchreports.com/enquiry/request-sample/21572379 accessed on 15 June 2024). The genetically modified bacterial and fungal strains as well as thermo-stable,

alkaline-resistant cellulase production are very useful for future prospects and industrial applications of cellulase in the future [86]. The use of novel molecular technologies for enzyme production through gene expression, proteogenomics analysis, enzyme design, etc. will enhance this field. These emerging strategies used for metagenomics and bioinformatic tools, viz., sequence homology, docking studies, and de novo enzyme design will be useful in this regard [87].

11. Challenges for the Production of Multienzyme

Besides the issues and challenges for microbial multienzyme production from FVW and valuable products, the PCA yield from microbial cultures may be influenced by factors such as the microbial strain, fermentation conditions, and substrate complexity. The strain selection for improved enzyme production could address the optimisation of fermentation parameters (e.g., pH, temperature, agitation, nutrient additions, and aeration) [88]. Transitioning from lab-scale to industrial-scale production poses difficulties in terms of the process scalability, reactor design, and logistics, as does approaching the environmental and economic benefits, limitations, and challenges. The issues of the FVW composition can vary widely depending on factors like seasonal variations, type of produce, and processing methods. Nowadays, the challenge is to ensure consistent multienzyme production and PCA activity levels across different batches of substrate, achieving high enzyme yields, and maintaining the optimal enzyme activity throughout the production process. However, the enzyme immobilization techniques, formulation strategies (e.g., encapsulation) and storage conditions could impact the enzyme stability and shelf life and consequently lower the product formation [89–91].

12. Conclusions

Agricultural, mandi, municipal, and industrial wastes are generated in huge quantities, causing pollution in the environment and developing various types of diseases for humans. This waste can be utilised as a substrate for the production of multienzymes by microbial intervention so that this biodegradable waste can be completely utilised as a multienzyme preparation, by-products, and other products. However, the studies analysed of the characteristics of pectinases, cellulases, and amylases such as their production, shape, size, application, and faster time and obtained more cheaply as substrate. The pectinase, cellulase, and amylaseare multienzymes, which is novel and identical to the biotechnological or microbiological potential for production. Many of the findings listed (references) in this review update the evidence of degradation by various microbial pectinolytic, cellulolytic, and amylolytic multienzymes present in the waste. This microbial multienzyme may gain in importance and be used for commercial production by several industries including food and beverage, animal feed supplements, bio-fuel, paper industry, textile, detergent, and liquefaction/clarification industries.

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