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Current Opinion in
Green and Sustainable Chemistry

Agro-industrial waste enzymes: Perspectives in circular economy

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Abstract

According to the Food and Agriculture Organization of the United Nations, approximately 1.3 billion tons of food is wasted each year, equivalent to approximately one-third of world production. Agri-food wastes are the source of proteins, carbohydrates, lipids, and other essential minerals that have been exploited for value-added products by the development of biorefineries and sustainable business as important elements of circular economies. The innovation and materialization of these types of processes, including the use of disruptive technologies on microbial bioconversion and enzyme technology, such as nanotechnology, metabolic engineering, and multi-omics platforms, increase the perspectives on the waste valorization process. Lignocellulolytic enzymes, pectinases, and proteases are mainly used as catalyzers on agri-food waste treatment, and their production *in house* might be the trend in near future for agro-industrial countries. Another way to transform the agri-food wastes is via aerobic or anaerobic microbial process from fungal or bacterial cultures; these processes are the key to produce waste enzymes.

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Current Opinion in Green and Sustainable Chemistry 2022, **34:**100585

This review comes from a themed issue on **Microbial and Plant Enzymes in Sustainable Chemistry and Pharmacy (2022)**

Edited by **Nicolas Papon** and **Vincent Courdavault**

For complete overview of the section, please refer the article collection - Microbial and Plant Enzymes in Sustainable Chemistry and Pharmacy (2022)

https://doi.org/10.1016/j.cogsc.2021.100585

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Keywords

Biorefineries, Multi-omics, Lignocellulolytic enzymes, Microbial bioconversion, Agri-food wastes.

Abbreviations

FAO, Food and Agriculture Organization of the United Nations; CPH, Cocoa pod husk; XOS, Xylo-oligosaccharides; PLE, Pressurized liquid extraction; SWE, Subcritical water extraction; SSF, Solid-state fermentation; BP, Banana pseudostem; CAZy, Carbohydrate-active enzymes; NGS, Next-generation sequencing; BCC, Business Communication Company.

Introduction

The concept of circular economy describes the use of waste from one industry as raw material to another one and is based on the reduce, reuse, recycle, recovery, and restore (5R) principle of the sustainable development, changing the classic linear model of the economy (make—use—throw) to a much effective circular model [1]. Specifically, the circulation of bio-waste for the development of products with the high added value might be the future alternative to fulfill the dream of a bio-based circular economy [2,3].

Biorefineries were designed as an eco-friendly way to produce marketable bio-based products (food and feed ingredients, chemicals, and materials) [4], biofuels (bioethanol, biodiesel, and biobutanol) [5], and biogas [6,7] from biomass, especially organic wastes as renewable sources of energy. Evolution to third-generation biorefineries or "advanced biorefineries" has been achieved thanks to the innovation on bioconversion and modern enzyme technology being the future of bioprocessing sectors as well as to integration between science, technology, and public politics.

Different types of wastes, such as organic wastes fraction from municipal sites, agri-food wastes, or crops, can be transformed into valuable products [6]. Agro-industrial wastes are mainly composed of cellulose and hemicellulose that are held together by heteropolymeric lignin units, and bioconversion could be achieved more efficiently by lignocellulolytic secreting fungal and bacterial enzymes than physical and chemical processes [8]. Other compounds, such as reducing sugars, proteins, minerals, bioactive molecules, could be successfully obtained before lignocellulosic transformation using cocktails of enzymes [9,10].

A advances in metabolic engineering, multi-omics platforms (genomics, transcriptomics, proteomics, and secretomics), integrated with bioinformatic screening are the key to discover new enzymes and improve their activity. Also, studies on metatranscriptomic analysis of some microbial communities are useful to transform local agri-food wastes optimizing time and resources [11,12]. On the other hand, the use of nanotechnology is also useful to improve enzyme immobilization with nanomaterials reducing the cost of enzymes and increasing the enzyme application in the circular economy models [5].

Agri-food waste potential

Nowadays, there is a need to focus on advanced technologies as well as alternative devices for efficient and effective utilization of fibrous parts or nonconsumable biomolecules from plant or animal sources [13]. Designing a biorefinery begins with the identification and characterization of the waste available for transformation, including the evaluation of the regional availability, quantities, and allied producers. It is also important to classify the waste into high-value-added components and to evaluate the recovery, extraction, or transformation stages with available technologies [14–16].

Agriculture, livestock production, and the processing agro-industry are significant sources of biomass wastes in developing countries. Enzymatic transformation of these wastes is the way to revalorize them as energy sources while reducing their environmental impacts. Some wastes that can serve as a platform to boost the bioeconomy in agro-industrial countries are presented in the following:

Coffee

As the most consumed beverage in the world, large amounts of wastes such as peel, pulp, parchment, silverskin, and spent ground coffee are generated. Coffee peel is a source of bioactive compounds and lignocellulosic material that can be used as a substrate to produce enzymes such as xylanases, cellulases, pectinases, among others [11]. The pulp is rich in fiber and has high contents of proteins, sugars, minerals (particularly potassium), tannins, and caffeine. These wastes can be transformed by enzymatic hydrolysis to obtain chlorogenic acids, sugars, oligosaccharides, or alcohol [17–23].

Cocoa

The main by-product of the cocoa industry is cocoa pod husk (CPH) [24]. CPH is rich in methylxanthines, such as caffeine and theobromine, and high levels of indigestible fiber, which limits its use. Some studies suggest the production of bioethanol production through fermentation using *Z. mobilis* [25]. CPH was recently explored to produce propionic acid through an alkaline enzymatic treatment [26]. An optimal process for the recovery of pectin and xylo-oligosaccharides (XOS) and enzymatic hydrolysis of the cellulosic fraction, to recover glucose and xylose, is also documented [27].

Pineapple

Cultivation is mainly concentrated in America and Asia, mainly in Costa Rica, the Philippines, Brazil, China, and Colombia [28]. The processing step generates a series of wastes such as crown, stem, cylinder, leaves, and pomace. It has been reported to contain many bioactive compounds such as phenolic acids, ascorbic acid, β-carotenes, and flavonoids, as well as dietary fiber and enzymes [29]. Pineapple residues can be used as an economic raw material for the production of phenolic compounds and fiber and as a substrate susceptible to fermentation for the production of ethanol and organic acids [30,31].

Banana

Their wastes are still a challenge for their application in biorefineries; however, these residues have different potential applications such as the production of energy, bioethanol, biodiesel, hydrogen, and organic acids [32–34]. The main residues are banana leaves, pseudostem, empty fruit bunch, and peel. Few studies have focused on the enzymatic potential of these substrates; however, their capacity for the production of pectinases and xylanases [35], α-amylases [36], and ɣ laccases [37] has been explored.

Bovine bones

Beef production systems are quite diverse worldwide, as they are the result of the combination of local environmental characteristics, culture, and economies [38]. Meat processing in slaughterhouses, sausage factories, or butchers includes operations of breeding, fattening, slaughtering, cutting, cutting, refrigeration, and freezing. These processes generate heads, legs, and bones, which either are used for animal feed or are simply discarded or incinerated, generating bad smells and environmental problems [39]. Proteases are biocatalysts that allow the hydrolysis of proteins and can have applications in different industries: cosmetic, pharmaceutical, textile, and food [40,41]. Proteases and lipases hydrolysate large amounts of food waste such as bovine bones, wheat bran, and shrimp wastes [10,41]. These enzymes can be produced from extremely halophilic eubacteria, among others [10].

Enzymatic valorization of agro-industrial wastes to obtain valuable compounds

Food wastes from agricultural production and processing of crop and animal products can be enzymatically treated to obtain valuable products. Table 1 shows some examples: coffee peel hydrolyzed with cellulases produces fiber for human and animal feed [42], CPH treated with 2.3% (w/v) NaOH, and cellulase produces sugars to obtain propionic acid from *Propionibacterium jensenii* [26]. Other compounds such as low-molecular-weight peptides from bovine bone treated by ultrasound-assisted double enzyme hydrolysis [39], lactulose with lactases on whey from cheese industry [43], or ethanol by alkaline pretreatment and enzymatic hydrolysis of CPH [44] can be produced.

Table 1

Valuable compounds from some agro-industrial wastes.

Waste material	Valuable compounds	Yield	Treatment	Potential uses	Reference
Coffee peel	Dietary fiber	13.96 ± 0.25%	0.2% cellulase at 50 °C and high shear mixing emulsifier BME 100L, Weiyu, Shanghai, China	Food industry (functional products)	[42]
Cocoa pod husk (CPH)	Sugars to produce propionic acid	275 mg glucose/CPH	Alkaline (NaOH 2.3% w/v) and enzymatic treatment [Cellic® CTec 2 (2.4% v/v)]	Food industry	[26]
Bovine bone	Low molecular weight peptides (LMWP)	21.04%	1. Pretreatment: 0.25 M EDTA 2. Primary extraction: 0.5 M Glacial acetic acid – 1.5 × 10^4 U/g Pepsine 3. Neutral protease hydrolysis (12.5 × 10^4 U/g) 4. Ultrasound-assisted protease hydrolysis	Health and cosmetics industry (antioxidant)	[39]
Cocoa husk	Ethanol	18.06 g/L	Alkaline pretreatment (NaOH 5%) and enzymatic hydrolysis (Cellic Ctec2 151 FPU/mL)	Energy industry	[44]
Banana pseudostem (BP)	Laccase Xylanase Endoglucanase	0.5 U/mg protein 1.2 U/mg protein 3.0 U/mg protein	*Bacillus wakoensis* (pH: 8.0; T: 45 °C; 28 days)	Chemical industry Environmental uses	[37]
Whey	Lactulose	0.028 mol/L 0.026 mol/L	Enzeco® (1.4 µkat/Kg) Lactozym® (1.4 µkat/Kg)	Food industry (prebiotics)	[43]

Some of these residues are an important source of antioxidants (cacao and coffee by-products), and they must be previously extracted by green technologies such as pressurized liquid extraction (PLE) or subcritical water extractions (SWE), among others [45]. These procedures can be performed before the application of alkaline, acid, or enzymatic treatments of lignocellulosic residues to close production cycles. Enzymatic processes could be enhanced by mixing them with methods such as alkaline treatments [26], shear mixing emulsifiers [42] or ultrasound [44].

Another way to transform the agri-food wastes is via aerobic or anaerobic microbial process from fungal or bacterial cultures [5,8]. The macronutrients present in food waste can replace the generally expensive carbon and nitrogen sources used in the enzyme production industry [10]. Enzymes are one of the most interesting high-value-added biologics. Food waste serves as a substrate for various microorganisms to produce enzymes, usually under solid-state fermentation (SSF). Microorganisms such as *Saccharomyces cerevisiae* and *Bacillus* sp. can degrade the polymers of plant material through enzymatic metabolism [46]. The most important enzymes for the biorefinery process are hydrolytic enzymes such as amylases, pectinases, proteases, lipases, or lignocellulolytic enzymes such as xylanases, cellulases, and laccases. Although hemicellulose and lignin are unpredictable biopolymers that cannot be effectively degraded by bacteria, an analysis to identify potential banana pseudostem (BP) waste—degrading bacteria with lignocellulolytic genes and their metabolic profile was performed and showed that laccase, xylanase, and endoglucanase were produced with strains of *Bacillus wakoensis* isolated from BP wastes [43]. Other reports have shown the production of protease-type enzymes for the treatment of slaughterhouse effluent by the action of *Chromobacterium violaceum* [47]. Filamentous fungi have the ability to degrade a heterogeneity of substrates and absorb substances in quantities greater than other organisms [48]. In that way, fungi are the best enzyme producers over plant and animal tissues, for example, the production of enzymatic cocktails composed of cellulase, xylanase, and β-glucosides in synergy with *Aspergillus tubingensis* and *Trichoderma reesei* from palm lignocellulosic waste [49].

Transcriptomic and proteomic analyses of the basidiomycetous fungus *Ganoderma lucidum* in the absence or presence of Cu^{2+} predicted 194 transcript coding for oxidoreductases and 402 transcripts for carbohydrate-active enzymes (CAZy) thanks to the advent of genomics and next-generation sequencing (NGS) techniques. On the other hand, secretome studies revealed higher secretion of laccases, cellulases, and xylanases. Most of these enzymes are useful for biomass utilization, fiber bleaching, and organo-pollutant degradation [10].

Another important application of agri-food wastes is the extraction of enzymes such as pectinolytic and proteolytic enzymes from pineapple, orange, lemon, or grape peels; tanases from grapefruit and cherry peels; lipases from lemon peel, coconut, and soy waste; invertases from banana and orange peels, coconut, and pomegranate; and peroxidases from asparagus, broccoli, and radishes [16].

Importance of multi-omics platforms and metabolic engineering in the edge of bioeconomy

Finding new uses and compounds from different waste materials depends on the application of disruptive technologies to discover microorganism, genes, or enzymes able to transform waste polymer—based. For example, the NGS techniques, such as Roche 454, Illumina Solexa GA, and SOLiD, has provided a platform for discovering novel genes and understanding differential gene expression of key organism such as fungi, the best enzyme producers [11,50]. Examples of this kind of studies are *de novo* transcriptome assembly, differential gene expression analysis, and proteome profiling of *G. lucidum* showing the main lignocellulosic enzymes involved on lignocellulose metabolism in copper-contaminated environments [11].

The transcriptomic approach, for example, makes it possible to identify inhibitors and expression levels of enzymes involved in succinic acid production [51]. The extraction of fiber from residues of *Agave lechuguilla*, in a transcriptomic study, allowed the determination of the composition of flavonoid identifying the genes involved in their biosynthesis [52].

On the other hand, the production of valuable byproducts through microbial conversion of agri-food wastes may be considerably improved by metabolic engineering of the relevant microorganisms. The transformation of urban organic wastes can be achieved by analyzing the metabolic fluxes and the regulation of the expression of genes coding specific anabolic enzymes [53]. Metabolically engineered strains can be obtained by traditional metabolic engineering approach or multiplex-pathway optimization techniques such as CRISPR/cas9 and multiplex automated genome engineering as advanced tools to modulate and optimize the expression of multiple genes [8,54].

Some applications are the thermostable recombinant endoxylanase of *Cryptococcus flavescens*, expressed in *Pichia pastoris* GS115 using the vector pGAPZαA to produce the prebiotic compounds xylobiose, and xylotriose (xylooligosaccharides, XOS), from the sugarcane waste [55]. Another example of recombinant enzymes is the use of a hyperthermostable keratinase, expressed in *E. coli* for the efficient treatment of chicken feather waste [56].

Enzymes availability after COVID-19

As stated by Business Communication Company (BCC) Research, by 2023, the global market for enzymes could reach $7 billion. The production of enzymes involved in the leather, paper, textile, and biofuel industries is expected to increase [57,58]. The most important enzyme producers worldwide are BASF SE, Novozymes, DuPont Danisco, DSM, NOVUS International, Associated British Foods Plc, Advanced Enzyme Technologies, Chr Hansen Holding A/S, Lesaffre, Adisseo, Enzyme Development Corporation, Aumgene Biosciences, Megazyme, Enzyme Supplies, Creative Enzymes, Enzyme Solutions, Enzymatic Deinking Technologies, Biocatalysts, Sunson Industry Group, Metgen, Denykem, and Tex Biosciences [59]. Developing countries with large amounts of waste material and high biodiversity potential that suffered the negative impact after the COVID-19 pandemic have in their hands the way to recover their economies throughout the use of different kinds of wastes to produce high valuable compounds. One economical way to develop these processes could be supported on an enzyme technology platform from *in house* production by fungi or waste extraction among others. The shortage of inputs, including enzymes produced by large producers and delays in transport generated in the first months of the pandemic, taught us that the best way to activate the processes is from a reactivation of local production. In this sense, disciplines such as microbiology, biochemistry, and engineering must be aligned to give rise to innovative processes in the sector that meet the quality standards of emerging markets.

Conclusions and perspectives

The integration of enzymatic technology with the classic processes of physicochemical transformation of agro-industrial waste following the 5R philosophy — reduce, reuse, recycle, recovery, and restore — will continue to be a key step to increase the supply of nonenergy products such as prebiotics, organic acids, and low-molecular-weight peptides with high added value for the pharmaceutical, cosmetic, and food industries. Similarly, thanks to advances in nanotechnology, metabolic engineering, and multi-omics platforms, it is possible to maximize the valorization of both biomass and microbial communities associated with the different sources of waste, enhancing the resources of developing countries. The agro-industrial countries have the possibility to recover their economies and mitigate the impacts generated by the COVID-19 pandemic through the development of production platforms based on the use of biomass from cacao, banana, coffee, or animal by-products to obtain high-value-added molecules, especially if these developments are achieved from of enzymatic methods. Consumer trends aimed at the use of ingredients and natural products of a sustainable nature increase the demand for these products, so implementing cutting-edge technologies based on the development of new, resistant, and high-performance enzymes continues to be a challenge for a company from developing economy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Universidad de Antioquia-CODI and Color Cacao, for the financial support of the project “Development of an unconventional fermentation process to obtain premium chocolates”, Contract number: 2018-1929. Additionally, the authors thank BioAli (Cyted) scientific network for the academic support.

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