

BIOSYNTHESIS AND USE OF LACCASES PRODUCED BY BASIDIOMYCETES FOR TREATMENT OF AGRI-FOOD WASTE: REVIEW

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Abstract. Recently, due to global concerns regarding the growing accumulation of waste, increased attention is directed towards new ways of using lignolytic enzymes, as a means of reducing environmental pollution coupled with obtaining new useful products. Valorization of lignocellulosic food waste and its components into different value-added products requires disruption of the recalcitrant structure of the lignocellulosic component. In this paper lignolytic enzymes produced by phylum Basidiomycota, as well as other enzymes that participate in the degradation of agri-food waste are summarized. The aim of this review is to give an overview on the biosynthesis and use of laccases for enzymatic treatment applied to agri-food waste. The methodology consisted in the research of the most representative sources from the literature of recent years and the synthesis of the respective studies, for an overall study. There are presented different types of agri-food waste generated by the fruit and vegetable, grain, wine, and beer processing industries. The main species of basidiomycetes that produce lignocellulosic enzymes are mentioned, as well as the conditions for their cultivation. The methods of selecting the producing fungal strains by screening on chromogenic media, the analysis of the enzyme activity, the stages of the biosynthesis process, the factors that influence the accumulation of the enzyme are described. The article includes information related to the structure and treatment of lignocellulosic materials from vegetable food waste, the mechanism of action of laccase, as well as the current state of research in the direction of using these enzymes for the treatment of food waste.

Keywords: laccase, basidiomycetes, biosynthesis, agri-food waste.

1. Introduction

Agri-food waste is generated in increasingly significant quantities everywhere in the world, and the solutions for their reduction and treatment have become imperatively necessary. Whether these wastes are used in animal feed, for the extraction of valuable compounds that exist in significant concentrations, or as the main organic fraction in the production of biogas and compost, prior treatments are recommended for increasing the bioavailability of macromolecular substances or recalcitrant ones. Although the chemical composition of agri-food waste is highly variable depending on the origin and geographical area, the most important part of it is represented by vegetable materials, with a high content of lignocellulose, starch, and carbohydrates with a lower mass, along with proteins, lipids and the mineral part.

Lignocellulose is a major part of the plant cell wall, being made up of three main components: cellulose, hemicellulose and lignin, which form a network with a rigid and resistant structure. Cellulose has a crystalline structure, being built from a linear chain of several hundreds to many thousands of β (1 \rightarrow 4) linked d-glucose units, in the form of cellulose microfibrils. Hemicellulose is a branched heteropolysaccharide consisting of 5-6 sugars, in which xylan is the main structural unit [1; 2]. Lignin is a heteropolymer composed of monomeric units of coniferyl, coumaryl, and sinapyl alcohols, and functions as a binder between the cellulosic and hemicellulosic components. Lignin represents a stable structure, resistant to degradation, which can be labialized by applying physical-chemical or biological treatments.

The aim of this review is to give an overview on the enzymatic pretreatment applied to lignocellulosic agri-food waste resulting from the processing industries of fruits and vegetables, cereals, wine, beer, catering, and others. The main species of basidiomycetes producing laccases, the methods of isolation, selection, and the stages of the biosynthesis process, as well as the use of these enzymes for the treatment of food waste, are presented. The article includes information about the advantages of using enzyme treatment, as well as the future directions in this field.

2. Materials and methods

In this section, the main types of agri-food waste are described, including their composition and general characteristics.

2.1. Agri-food waste

The most widespread wastes from the food industry, which contain lignocellulosic materials, are those of vegetable origin. The cell wall of the plant cell contains a large amount of cellulose, hemicellulose and lignin, which together form a recalcitrant material that is difficult to degrade. This waste comes mainly from the following sectors (Figure 1).

1. *Waste from fruit and vegetable processing industry*

This field of the food industry generates a significant amount of waste represented by the remains left after the consumption of the edible parts or after the processing of fruits and vegetables (peels, seeds, kernels, tails, pomace). Also, especially in the case of perishable products, after transport and storage, significant quantities of altered food remain, both physically and microbiologically, unfit for human consumption. The most important quantities of waste are recorded in the juice and fruit and vegetable canning industry, as well as in the extraction of oils and sugar from different vegetable materials. From the point of view of the chemical composition, these plant wastes mainly contain large amounts of carbohydrates (starch, cellulose, pectin, hemicellulose, easily assimilable sugars), lignin, proteins, lipids (unsaturated, saturated, waxes), mineral substances, various active principles [3].

2. *Waste from cereal processing industry*

The cereal processing industry mainly generates waste with low humidity, containing lignin, cellulose, hemicellulose, starch, glucans, other sugars, along with proteins, lipids, minerals. For the most part, all these wastes come from the coating of cereal seeds, as it is known that the largest amount of waste from cereals is generated from the milling industry. Also, other cereal by-products are bran (from rice, wheat, rye, oat, triticale), various by-products from the processing of corn grains, as well as the residues represented by seeds that do not correspond qualitatively [4].

3. *Waste from brewing industry*

The different operations of mashing, fermentation, maturation and filtration in the brewing industry generate waste represented especially by spent grains, residual yeast and diatomaceous earth slurry. Brewing spent grain, the major waste in the beer industry, results after the separation of the sweet wort and contains lignin (approximately 28%), cellulose, hemicellulose, sugars, proteins, lipids, phenolic compounds, minerals and water [5].

4. *Waste from wine industry*

In the wine industry the main wastes formed are pomace, lees and yeast sediment. The pomace contains the inedible part of grapes (peel, stem, pulp, seeds), having a lignin content between 22-44% and 8.04%-12.7% glucan, 4.42%-7.05% xylan (dry weight basis). There are several value-added products which could be obtained from grape pomace, after an appropriate enzymatic treatment: lipids, sugars, pigments, valuable extractives (e.g. polyphenols and oil) and non-extractives (e.g. cellulose, hemicellulose, and lignin) [6].

5. *Waste from oil extraction industry*

Several plants provide oil for nutritional needs (olive, palm, soybean, rapeseed, sunflower seed, and peanut), but, in particular, olive oil extraction produces a large amount of lignocellulosic waste as residues. Such wastes are characterized by high salinity, low pH values, high contents in phenol derivatives and organic matter, and nutrients [7].

6. *Waste from starch processing industry*

The processing of different raw materials to obtain starch (potatoes, cassava, corn, etc.) generates lignocellulosic waste consisting of peels, pulp and rejection, which can be enzymatically pretreated.

7. *Catering waste*

Although it has a lower content of lignocellulosic material, being composed of a mixture of food waste, catering generates a significant amount of waste. Their recovery is limited either by the lack of organization or the necessary logistics. This mixture of food waste mainly contains sugar-based wastes, protein (meat, fish, bones), lipids, starch and flour, also significant amounts of fruit and vegetable waste with lignocellulosic content [8].

3. Results and discussion

3.1. Laccase-producing basidiomycetes

Fungal laccases are versatile enzymes that break down lignocellulosic material from different wastes, acting on phenolic compounds, aromatic amines, azo dyes, aromatic hydrocarbons, and other substrates. Along with cellulolytic enzymes, laccases are key enzymes in the treatment of waste from the agri-food industry, known for their high content of plant residues of great diversity.

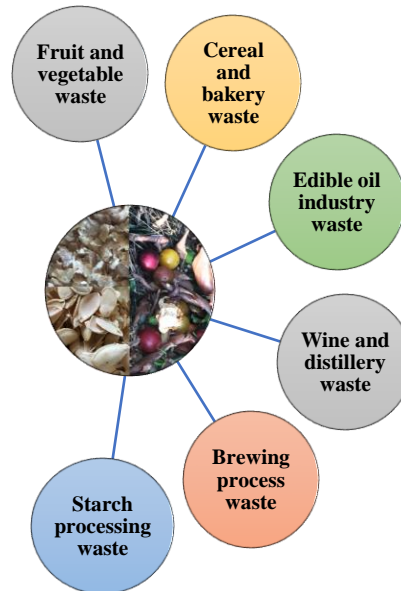


Fig. 1. Sources of agri-food waste

Laccases of fungal origin are metabolites involved at the cellular level in the degradation of lignocellulosic substrates for the release of nutrients, cell protection, sporogenesis and pigmentation. Laccases are synthesized together with lignin peroxidase and manganese peroxidase by fungi belonging to the phyla of *Basidiomycota*, *Ascomycota*, *Chytridiomycota*, *Zygomycota* and *Oomycota* [9] (Fig. 2).

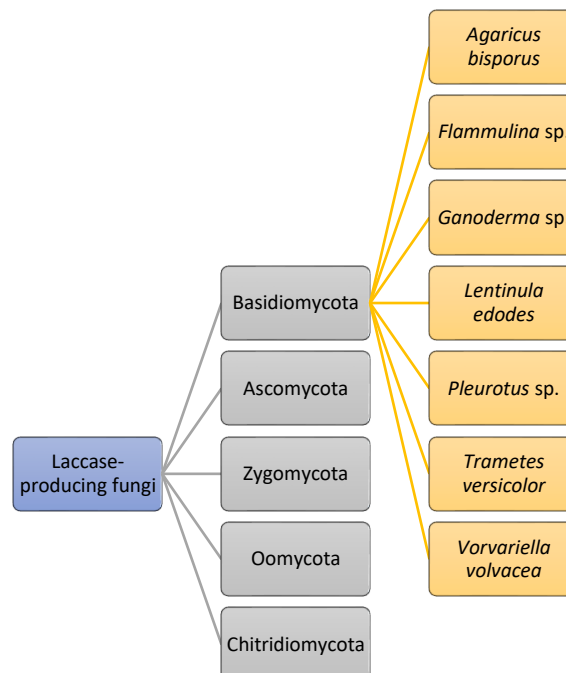


Fig. 2. Main laccase-producing species belonging to phylum Basidiomycota

Among the best-known fungal species that synthesize laccases are the Basidiomycetes *Pleurotus ostreatus*, *Lentinula edodes*, *Ganoderma* sp, *Phlebia radiata*, *Trametes versicolor* and others, being known that white-rot fungi seem to be the most effective in the degradation process of lignocellulosic materials (Figure 3).

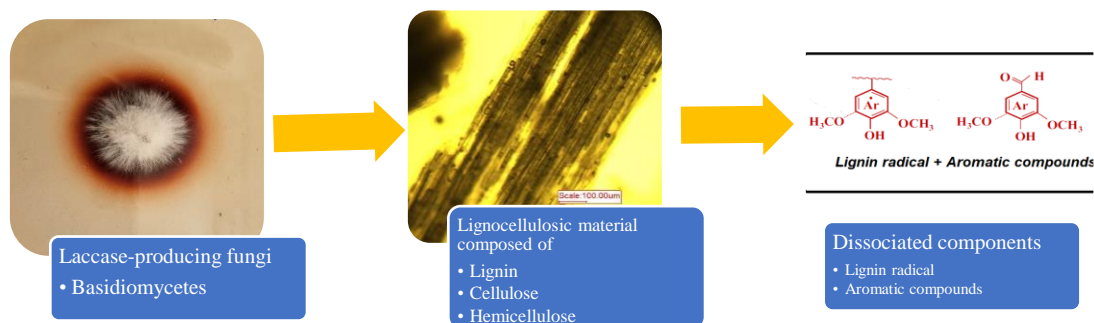


Fig. 3. Action of laccase on lignocellulosic material

3.2. Biosynthesis of laccases

The species of fungi with laccase activity can be isolated from natural substrates such as decayed wood, tree barks and others by the spread-plate or streak-plate technique and highlighted by screening on chromogenic culture media such as guaiacol, ABTS, or catechol. Although known especially for the ability to degrade lignocellulosic material, some species of basidiomycetes can synthesize a series of enzymes from the hydrolases class such as: amylases, proteases, cellulases, hemicellulases, pectinases, xylanases, and others. These enzymes could be complementary to the action of laccase producing a more advanced degradation of the complex substrate from plant materials.

After laboratory testing, the composition of the culture medium (especially carbon and nitrogen sources, trace elements, inducers), pH, temperature, dissolved oxygen concentration and agitation are determined. Numerous studies have shown that laccase synthesis occurs at the end of the exponential phase and in the stationary growth phase. In general, fungi synthesize laccase by secondary metabolism, and laccase synthesis is activated by carbon or nitrogen depletion, although there is no general rule.

The biosynthesis of laccase, like many fungal enzymes, goes through several stages (Figure 4): 1) selection and isolation of a highly productive fungal strain by the spread-plate or streak-plate technique; 2) cultivation and preservation of the selected strain, at the laboratory level; 3) establishing optimal culture media; establishment of cultivation parameters, i.e. the temperature, pH, nutrient concentration, the time required for biosynthesis, quantity and type of inoculum, mixing and aeration of the system, presence of enzyme inhibitors or activators, etc.; 4) establishing the cultivation conditions in the bioreactor; 5) separation of the enzyme preparation, its characterization from the point of view of stability, K_m , optimal reaction conditions [9]. To analyze the enzymatic activity of laccase, several types of substrates can be used, i.e. guaiacol, ABTS, syringaldazine, 2,6-dimethoxyphenol, or other chromogenic compounds in different test conditions.

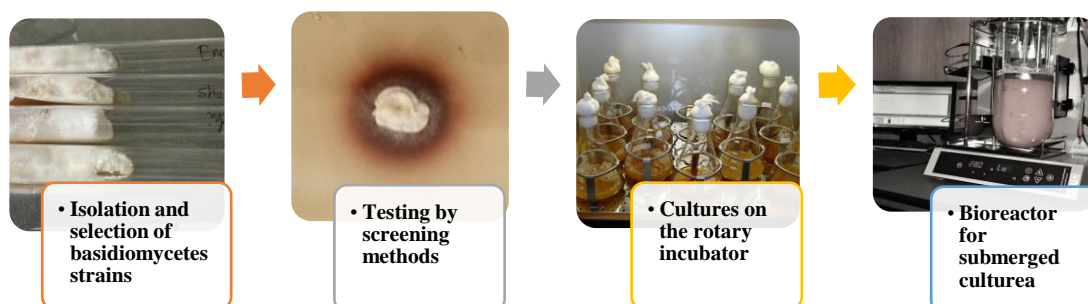


Fig. 4. Stages of the laccase biosynthesis process

Laccase biosynthesis is strongly influenced by a number of factors such as the composition of the culture medium, cultivation parameters, type of the fermentation system, species characteristics and productive potential, inducers and others.

Culture media

Numerous substrates can be used as carbon sources to produce laccase from basidiomycetes. Levin et al. show that the most easily assimilable carbon source for basidiomycetes is glucose, and copper ions can act as enzyme activators [10]. Laccase synthesis by *Trametes versicolor* is increased at high glucose concentrations of 20 g·L⁻¹ [11]. Similar results were reported by Cavallazzi et al. [12], who cultivated *Lentinula edodes* in liquid medium with 10 g·L⁻¹ glucose. Palvannan et al. [13] showed that using the response surface methodology, *Pleurotus florida* NCIM 1243 synthesizes maximum amounts of laccase in 15 g·L⁻¹ glucose medium [14]. Xu et al. studied the optimal conditions for the biosynthesis of laccase by *Ganoderma lucidum* in shaking flask cultures and found that the optimal conditions are the following: culture medium containing glucose 30 g·L⁻¹, cotton 0.2%, (NH₄)₂HPO₄ 0.66 g·L⁻¹, casein 0.5%, Tween-80 0.15 mL, initial pH value 5.5, inoculation 12.5%. In other studies, it was observed that glucose and fructose can be used as co-substrates for laccase production in *Ganoderma lucidum*, respectively *Lentinus kauffmanii* [15-17].

It has been demonstrated that, regarding the carbon source influence, rapidly degraded substrates such as glucose, mannitol and cellobiose, usually produce high laccase activities in comparison to other substrates that are degraded more slowly, such as cellulose or lactose [18]. As synthetic inducers for the production of laccases, copper or copper and lignin were used by Tinoco et al. [19], when the production of the enzyme from *Pleurotus ostreatus* increased 4-fold, respectively 10-fold, for copper, manganese of caffeic acid for *Coprinus comatus* culture medium [20].

Type of fermentation system

Laccase can be synthesized by basidiomycetes both in submerged liquid cultures and in solid-state fermentation systems. In the case of submerged cultures, stirred-tank bioreactors, bubble column, and air-lift bioreactors are used. Cultures in the solid state fermentation system are carried out in tray bioreactors and packed bed bioreactors [17]. The optimal conditions for the biosynthesis of laccase for the species *Corioloropsis gallica* 1184 were cultivation in a 50 L bioreactor at 30°C, at pH 6.0, 25-35% of dissolved oxygen, after 14 days [21]. In order to increase and optimize the production of laccase, statistical methodologies are used. For example, laccase production by *P. ostreatus* 1804 has been optimized in submerged conditions using Taguchi DOE method [22]. According to some considerations, solid state fermentation is a less expensive technology than submerged fermentation, because it requires less space and energy, as well as less water consumption, and the substrates can be agricultural derivatives and their residues rich in cellulosic biomass [23,24]: tea residues [25], fruit juice waste [26], sugarcane bagasse [27], wheat straw [28], wheat bran [29], and olive leaves [30].

Cultivation parameters

Different studies have shown that the optimal pH range for laccase biosynthesis is between 5.0 and 6.0 [11; 31]. For example, *Fomes sclerodermeus* synthesizes the maximum of laccase and biomass when the pH value is 6 [32], while *Trametes pubescens* has been proven to have the optimal range for laccase production between 3.0 and 4.5 values that are not optimal for growth [33].

It was observed that the optimal temperature and dissolved oxygen for laccase synthesis differ depending on the species. Chernik and Hilden showed that the optimal temperature for some wood-decaying basidiomycetes, such as *Steccherinum ochraceum* isolate 1833, is between 70-80°C [34; 35]. Dong et al. [36] showed that laccase production by *Trametes gallica* on twelve media is higher in conditions of high aeration and culture mixing compared to static conditions.

Different methods can be used to increase the biosynthetic potential of basidiomycete species. For example, mutant strains of *Ganoderma* were obtained by Wang et al. [37] by protoplast mutagenesis under high pressure conditions. Ouyang et al. cloned the gene and promoter of Lac from *G. lucidum* to improve the properties of the producing strain [38]. Based on the results obtained so far, the use of highly productive fungal strains as well as the optimization of cultivation conditions are the main future trends for laccase biosynthesis.

Action of laccase on several agri-food wastes

The agri-food industry generates a lot of waste with high content of phenolic compounds and high values of BOD and COD. Laccase-based processes can be applied in the treatment and bioremediation of wastewater in agroindustrial effluents, which have a high content of phenols. For example, due to its high concentration in polyphenols and dark brown colour, some components of beer factory wastewater, as well as the waters resulting from sugar-cane factories, rich in pigments can be treated with these enzymes for removing both the total phenolic compounds and colour [39].

Olive mill wastewater contains large concentrations of phenol compounds (up to $10 \text{ g}\cdot\text{L}^{-1}$), very toxic for the environment, with harmful effects towards humans and environment that can be treated with laccases. The reduction of the amount of phenolic compounds from this type of effluents was studied using laccases synthesized by *Pleurotus ostreatus*, *Cerrena unicolor*, *L. edodes*, *T. versicolor* and other basidiomycetes, with promising results [39].

Laccases are also used in removing phenolic compounds and the colour of distillery wastewater. The residues from the distillery could also be used as a substrate in the biosynthesis of laccases [40].

The beer production industry results in waste with a high percentage of polyphenolic compounds, especially tannins. Some research has demonstrated that the COD value in beer industry effluent can be reduced with the help of the species *Coriopsis gallica*, which synthesizes laccase. [41].

The use of laccase for the degradation of lignin from apple pomace and coffee silverskin makes possible hydrolysis of hemicellulose producing high concentrations of sugars [42].

Conclusions

1. Enzymatic treatment with fungal laccases represents one of the most promising options for the management and valorization of lignocellulosic agri-food waste, thus supporting the circular economy.
2. The biosynthesis of laccases could represent an efficient solution for lignocellulosic agri-food pretreatment, due to its low cost, short production time, low energy requirements, eco-friendly nature, even if it requires the use of highly productive strains and specific equipment.
3. The species of fungi with laccase activity can be isolated from natural substrates and cultivated in liquid or solid media, where they synthesize the enzyme at the end of the exponential growth phase.
4. The optimization of the biosynthesis process and the increase of the yield represent the future trends for which the increase of the biosynthetic potential of the producing species, and the obtaining of new strains through the use of genetic engineering resources are taken into consideration.

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Author contributions

Conceptualization, M.F.; methodology, M.N.D. and M.I.; investigation, E.M.S. and M.N.D.; writing – original draft preparation, M.I.; writing – review and editing, M.F. All authors have read and agreed to the published version of the manuscript.

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