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The Use Of Lacase Enzyme In Industry And Biotechnology / An Applied Study

Wasan Najim Abdul-Sada

Department of Basic Science College of Dentistry University of Babylon, Babylon, Iraq.

Abstract

With the beginning of urban life, environmental pollution appeared, and then increased with the increase in industrial and technological progress, because it caused an increase in industrial waste and waste. This, in turn, increased the volume of emissions of toxic chemicals, which negatively affect human health.

The lactase enzyme has an effect on the oxidation of a range of substrates, so it is used in various industrial fields. Laccase enzyme is used to increase the appearance colors of foods or beverages, to separate and remove lignin from paper and pulp, for use as a dye in the textile industry, to bleach textile products, to wash jeans, for use in various boiling processes, in biodegradation and decolorization of textile wastewater, in bioremediation It is used as a biostimulant in various industrial and biotechnical applications such as cosmetics.

The laccase enzymesare obtained from different organisms, especially types of fungi. Under today's conditions, and the increasing industrial demand for it, laccasecannot be obtained cheaply. To find the most effective source of laccases, it is necessary to select the most suitable fungi, find reproducible and inexpensive isolation methods, or optimize enzyme production conditions.

Keywords: Laccase, Jane Laccase, technology applications.

استخدام إنزيم اللاكيز في الصناعة والتكنولوجيا الحيوية/ دراسة تطبيقية

مستخلص

ظهر التلوث البيئي مع بداية الحياة الحضرية وازداد نتيجة التطورات الصناعية. تتزايد الأنشطة الصناعية بشكل سريع ، ونتيجة لذلك فإن المخلفات التي تتركها المصانع للبيئة وانبعاثات المواد الكيميائية السامة تؤثر سلباً على صحة الإنسان.

نظرًا لقدرة إنزيم اللاكاز على أكسدة مجموعة متنوعة من الركائز ، فقد أدى مؤخرًا إلى استخدام هذه الإنزيمات في مختلف المجالات الصناعية. يستخدم إنزيم Laccaseلزيادة ألوان مظهر الأطعمة أو المشروبات ، لفصل وإزالة اللجنين من الورق واللب ، لاستخدامه كصبغة في صناعة النسيج ، لتبييض منتجات المنسوجات ، لغسل الجينز ، لاستخدامها في عمليات الغلي المختلفة ، في التحلل البيولوجي وإزالة اللون من مياه الصرف الصحي للنسيج ، في المعالجة الحيوية يتم استخدامه كمحفز حيوي في مختلف التطبيقات الصناعية والتقنية الحيوية مثل مستحضرات التجميل.

يتم الحصول على laccase enzymesمن كائنات مختلفة ، وخاصة أنواع الفطريات. في ظل ظروف اليوم ، والطلب الصناعي المتزايد عليها ، لا يمكن الحصول على laccasبثمن بخس. للعثور على المصدر الأكثر فاعلية لـ laccases ، من الضروري اختيار أنسب الفطريات ، والعثور على طرق عزل قابلة للتكرار وغير مكلفة ، أو تحسين ظروف إنتاج الإنزيم.

1- Introduction

Enzymes are molecules in the protein structure that catalyze biochemical reactions in cells. Enzymes, which have very important metabolic functions in cells, have entered everyday and economic life to be used for various purposes (1). The chemical molecules that are catalyzed by enzymes and participate in the reaction are called substrates. The enzyme can only interact with a substrate suitable for the 3D structure of its active site. Therefore, each enzyme acts only on a specific type of substrate

(2). Enzymes, which have very important metabolic functions in cells, are molecules in the protein structure that catalyze biochemical reactions.

Enzymes that originate from microorganisms have very high catalytic activities compared to enzymes that originate from plants or animals, do not form unwanted by-products, are more stable and cheap, and are obtained in large quantities to allow their use in almost every field. Industry (1). These microorganisms were selected not only for their ability to produce enzymes, but also for their non-toxicity and non-pathogenicity. Today, the use of bacterial enzymes in industry has increased (3).

enzymatic biotechnology;

- microbial processing (selection of product strains, development, etc.),
- enzyme production by fermentation (environment for large-scale production, improvement of ambient conditions, etc.),
- Altering the 3D structures of enzymes to increase the catalytic efficiency.
- insulation,

It includes stabilization studies (making enzymes insoluble in water with the help of insoluble supports). Enzyme biotechnology. In environmental decontamination, it is used in textile biotechnology according to prevention and control directives, during bio-bleaching, bio-washing or milling and the production of synthetic fibers. In order to make the most of enzyme biotechnology, multi-enzyme complex or enzyme (protein) stabilization, enzymatic biocatalysts and the use of other processes have become more and more popular in recent years. However, it is not enough to have a large amount of enzyme or activity.

A number of properties are sought in enzymes used in the industrial field. enzyme; It must be long lasting and durable, be able to use the particular substrate even if the environment in which it will operate is different from intracellular conditions, and be able to operate without decomposition in an industrial environment (extreme conditions). Therefore , it requires enzymes to be regulated to meet needs (4).

These features are:

• Kinetic constants.
• pH and optimum temperature.
• Enzyme stability in non-aqueous solvents.
• Substrate quality and reaction.
• The requirements of the assistant worker.
• Molecular weight and subunit structure.

Due to the gradual development of enzyme technology, the diversity of fields of use and the high economic value of the products, various researches in the field of industrial enzymes in biotechnology are gaining more importance (5).

2- Theoretical framework

2-1- laccase enzyme

Copper-containing polylacases (benzenediol: oxidoreductase; EC 1.10.3.2) can oxidize phenolic and non-phenolic lignin-related compounds as well as environmental pollutants that are resistant to biodegradation. In addition, laccase enzymes use molecular oxygen as the electron acceptor and do not need toxic hydrogen peroxide.

It has recently become an interesting area of research (6).

The laccaseenzyme consists of similar enzymes that contract to form polymeric complexes. The molecular masses of the monomers range from 50-110 kDa. An important feature of laccase enzymesis the presence of a carbohydrate moiety covalently attached to the protein fraction, making up 10-45% of the total mass of the protein. The carbohydrate portion contributes to an increase in enzyme stability (7).

A minimum of four copper atoms per laccaseactive protein is required to demonstrate the catalytic activities of the laccaseenzymes. The light absorption and paramagnetic electron behavior of these copper ions differ in each laccase protein, whose copper-binding sites are highly conserved. Therefore, the copper ions in laccase enzymesare grouped into three main classes depending on their spectral properties (8). While one of the copper ions in the laccaseproteins is bound to the region called Type 1 or the “blue region,” there are three other copper ions due to the trinuclear cluster formed by regions called Type2 and Type3 (8).

Type 1: paramagnetic “blue” copper (Cu1), absorbance 610 nm (when in oxidized form), redox potential +785 mV. Type I copper is responsible for the typical blue color of polycopper-containing protein molecules. This blue color is the result of the intense electronic absorption caused by the cysteine copper covalent bond (4). Due to its high oxidation potential (+785 mV), type I copper is the site where substrate oxidation occurs. On the other hand, type 2 copper does not show absorption in the visible spectrum.

Type 2: "non-blue" magnetic copper (Cu4). In copper-containing polyoxides, type 2 copper is located close to type 3 dinuclear copper (8)
The type 3 copper center shows an electron absorption at 330 nm (when in the oxidized form).

Type 2 and Type 3 copper cores can be considered as a whole and are therefore often called "triple core groups". The trinuclear mass is where the molecular oxygen is reduced and water is released (5). Because lactase enzymes have low substrate properties, they can also oxidize environmental pollutants that are resistant to biodegradation (9). This ability to oxidize lactase enzymes greatly increases the demand for these enzymes in some industrial and biotechnical processes.

2-2- Laccase enzyme use in industrial and biotechnology fields

Environmental pollution; Since urban life was formed, the signs of the pollution problem began, but it was in simple proportions, and it was controlled within the clean nature, with human ferocity in the use and burning of natural resources such as oil, gas and coal... and the excessive use of cars, planes and ships... and forging the wheels of factories that became sleepless All this led to the emergence of the phenomenon of pollution, and its transformation into a global problem.
urban waste. As a result of these formations, human health is adversely affected. Dyes, which are found in different types and in unwanted quantities in wastewater, are chemicals that cause color pollution and negatively affect life in the water (9). The laccase enzymescan oxidize many substituted phenolic compounds, aromatic amines and even some inorganic compounds by using molecular oxygen as the electron acceptor. The ability of lactase enzymes to act on a variety of substrates has recently led to their use as biocatalysts in various biotechnology applications such as the decolorization of textile dyes, biobleaching of pulp and bioremediation. The laccase enzymesplay a

role in the pathogenesis, immunity, formation and pigmentation of organisms.It also plays a role in the metabolism of complex organic substances such as lignin and humic acid (10).

Until recently, most of the organisms in which laccase enzymeshave been isolated and identified were fungi and plants. As a result, fungal latex has been used almost exclusively in biotechnology applications. Little is known about bacterial lacases, although rapid progress in recent whole-genome analyzes indicates that laccase enzymesare also commonly found in bacteria (10) The domains that make up the majority of industrial and biotechnological applications of laccaseare as follows.

2-2-1- Laccase enzyme use in industries

2-2--1-1- Food Industry

Lyase enzymes are used to increase or modify the color appearance of foods or drinks. Latexes are used to remove unwanted phenolic compounds that cause darkening, haze, and turbidity in fruit juices, beer, and wine (11-13). Lacse enzymes are also used in baked goods to improve dough mixing properties and structural properties of the dough product, as they provide the cross-linking of biopolymers (14, 15).

Selinheimo et al. In a study they conducted, they showed that the lactase enzyme produced by Trametes hirsuta, a white mold fungus, reduced dough elongation in both flour and gluten doughs, while increasing the ultimate dough resistance (16). Flander et al. In a study they conducted, laccase enzymes from T. hirsuta and Pentopan Mono BG xylanase were used individually or as a cocktail in a dough consisting of a mixture of oats, wheat and oats, the hardness of fresh bread obtained from dough supplemented with lactase. As a result, lactase and a mixture of xylanase enzymes increased in bread made from oatmeal and wheat. It was reported to be beneficial in terms of texture (17).

Minosi and others. Identify potential applications of lactase enzymes in different areas of the food industry, such as bioprocessing, beverage processing, identification of ascorbic acid, sugar beet pectin jelly, and bakery, and its use as a biosensor (13). Despite this, these researchers stated that in order to further develop industrial uses of laccaseenzymes, techniques that enable production and capture of these enzymes at low cost need further research (13).

2-2-1-2- How to make paper and pulp

first step is to remove the lignin from the pulp. The bleaching processes that use chlorine in the traditional way cause pollution and the occurrence of various environmental problems, which require new solutions (18).

Processes in which oxygen is supplied are used to remove lignin artificially (19). However, lignin removal made by pre-treating wood pulp with lignin-degrading enzymes not only requires milder and more environmentally friendly processing conditions, but also preserves the integrity of cellulose, which is the main structure of the leaf (18)

The majority of studies conducted to develop alternative bio-bleaching systems are the catalytic activity of lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), multidirectional peroxidase (VP; LiP, MnP, plant/microbial peroxidases that oxidize phenolic compounds) which are characteristic of fetal degrading fungi.) and focused on laccases. Despite extensive research, only a few enzymatic treatments have been identified that can provide the lignin removal/bleaching ability

offered by modern chemical bleaching techniques. One of the few exceptions to this generalization is the patented Laccase Modified System (LMS) lignin removal technology developed for Kraft Door (20).

The LMS lignin technology is highly selective, resulting in little carbohydrate loss or pulp damage (21). Since phenoloxidases, including the laxis, have low redox potential, they can oxidize only phenolic units in the lignin polymer. The proportion of phenolic units makes up only 10% of the complete lignin polymer. Therefore, in biotechnological applications of laccase enzymesof fungal origin, non-physiological redox mediators are used to increase the potential for laccaseoxidation. Also, due to the steric hindrance, the laccase may not come into direct contact with the lignin polymer. Alternatively, redox mediators such as 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), which are used with laccase enzymes to whiten the pulp, are oxidized by laccaseand converted to reactive radicals (22, 23).

The use of biological systems for bleaching paper and pulp limits the use of chlorine to bleach pulp in paper mills. As a result, chlorinated compounds, which are toxic to the environment, are eliminated through the waste of these factories. In addition, laccase enzymesare more readily available and can be used more easily than both LiP and MnP enzymes. In addition, lactase enzymes have the potential to be used to hydrolyze a variety of compounds, as they exhibit oxidative activity and provide oxidation of aromatic compounds with much lower specificity (22).

Camarero et al. Removal of lignin derivatives responsible for color formation from high-quality flax pulp. Investigating the potential of LMS for these researchers showed that LMS could be used in place of the chlorine-containing bleach used to produce expensive flax pulp (24).

Laccases, which have the ability to form reactive radicals in lignin compounds, can also be used in the purposeful modification of wood fibers (25, 26). For example, latex can be used in the production of lignocellulose-based composites such as fiberboard to ensure enzymatic adhesion of wood fibers to each other. During the production of wood composite materials such as chipboard, the laccases will activate the lignin bound to the wood fibres, allowing the fibers to stick together, and thus, the obtained fiberboard will not contain toxic synthetic adhesives, while having good mechanical properties (27), 28). Another possibility is the activation of lignocellulose fibers with laccase enzymesin order to improve the chemical or physical properties of the fiber products (25, 29).

2-2-1-3- textile industry

The textile industry uses two-thirds of the production of dyes (30). Large amounts of water and chemicals are consumed during the wet processes that enable textile products to be processed. The chemical agents used in the textile industry are very high in chemical diversity and range from inorganic compounds to polymers and organic products (31-33).

The diversity of commercial dyes used in the textile industry is more than 100,000 and the annual production of these dyes is more than 7 x 105 tons (34). Due to their chemical structure, textile dyes have a high resistance to fading when exposed to light, water, and various chemical oxidizing agents (35, 36). It is also difficult to provide decolorization (decolorization) of most of these pigments due to their synthetic origin. Moreover, it has different categories. Not all textile dyes can be degraded using physical, chemical, or decolorization methods. Because the decomposition products of these dyes can

sometimes be more toxic, enzymatic methods are preferred rather than physical and chemical methods for treating textile dye-containing waste (37).

Especially in developed countries, the legal obligations regarding the removal of pigments from industrial waste are becoming more and more difficult with each passing day. Because some dyes are derived from known carcinogens such as benzidine and other aromatic compounds, there is a growing concern about this issue (38).

Most of the current methods used to treat dyestuff-containing wastewater are neither effective nor economical (39, 40). Methods that provide enzymatic hydrolysis or decolorization of tissue dyes are generally based on fetolytic enzymes such as LiP, MnP and laccase. Because lacases are more usable than LiP and MnP enzymes and provide oxidation of aromatic compounds with much lower specificity, laccases have the potential to be used to hydrolyze a variety of compounds (40).

In addition to treating wastewater containing textile dyes, treatment systems based on lactase enzymes are also used to treat this waste by providing the oxidation of colored phenolic compounds in olive oil plant waste (41, 42). In addition, the use of lactase enzymes in the textile industry is increasing day by day due to laccase being used in bleaching documents and even in the manufacture of dyes (43). At the same time, when lactase enzymes are used with redox mediators, they are used in the production of used-looking jeans by bleaching indigo dye (24, 44).

2-2-2- Laccase enzyme use in biotechnology fields

2-2--2-1- The use of laccase enzymesin the bleaching of textile products

Laccases are used to remove natural coloring matter, This is such as pectins, proteins, dyes, and oils that are present in cellulose, and then the operations prepare the oils, waxes, pectin, proteins and dyes present in the cellulose fibers, and then prepare them for the operations. such as dyeing, printing and finishing. Textile bleaching is classically carried out under acidic and alkaline conditions, over a wide temperature range and with the use of various oxidizing agents. When obtaining high degrees of whiteness, oxidation processes are applied successively. Bleaching agents are administered to the fibers in excessive quantities, which leads to harmful waste that negatively affects the proper functioning of further processing. Therefore, frequent washing is required (44). Tzanov et al. In their study, they report that a short-term pretreatment of lacize improves the whiteness of cotton fabrics and significantly reduces the concentration of hydrogen peroxide used in the subsequent bleaching process (45).

2-2-2-2- How is the use of lykes to wash jeans?

Laccases can be used as a substitute for pumice stones in the jeans washing activity. The basis of the grinding process in washing jeans, is to wash the products with a pumice stone to provide the required abrasive. after washing, the products are partially bleached with sodium hypochlorite,, neutralized and washed again. Since all these processes cause significant environmental pollution, enzyme applications are also being carried out. Laccases have the effect of bleaching indigo-dyed denim to a lighter shade (44).

Since laccases alone are not sufficient to remove the indigo color on jeans, intermediate systems have also been developed that provide electron transfer from indigo to molecular oxygen. Since the laccase system and the mediator only break the indigo without affecting the weft threads, smiling jeans are obtained as a result of this process. The classic bleaching of hypochlorite jeans is inexpensive, fast and effective. However, it is detrimental to the environment and the stake (46, 47).

2-2-2-3- The use of laccase enzymesin the boiling process

Boiling processes are carried out with various chemicals and pollute the environment. For this reason, enzymatic boiling is preferred, which is a more ecological process. Pectinase is mostly used in enzymatic boiling processes, and experiments have been conducted with enzymes such as xylanase, protease, lacase, lipase, and cellulase (48).

2-2-2-4- As a dome for use in removing color (biodegradation) from wastewater

The presence of different groups of chromophores such as azo, triphenyl methane and phthalocyanine will give rise to the structural diversity of the pigments. In addition, there are side effects such as visual effect and chemical oxygen demand. (COD), most synthetic dyes also exhibit toxic, carcinogenic and genotoxic effects. Existing sewage systems are insufficient to completely remove stubborn dyes and other organic residues from this type of waste. Dyeing wastewater treatment requires chemical and physical methods such as adsorption, coagulation, oxidation, filtration and ionizing radiation. All of these methods have different decolorization ability, capital cost and operating speed. Among these methods, coagulation and absorption are most widely used. However, these lead to large amounts of waste (49). For this reason, laccase enzyme applications are becoming increasingly important due to its low cost and reduced sludge formation.

2-3- Nanotechnology

In the past twenty-five years, studies in the field of biochemistry have gained a lot of intensity. Advances in bioelectrochemistry have been incorporated into applications of analytical chemistry. For example, biosensors are used as detectors used in clinical and environmental analysis (50). the Nano technique. It contributes to the development of smaller and more efficient biosensors at the micro-phenometer scale by providing specific adsorption and controlled deposition of biomolecules on different types of surfaces. With respect to lactase enzymes, enzyme adhesion has a significant impact on biosensor sensitivity (51).

Martele et al. In their study, they show that the use of microtubules is an effective way to develop a multifunctional biosensor by stopping lactase enzyme on a solid surface (52). Roy et al. On the other hand, they report that the lactase from Trametes versicolor, in the form of crystal-crosslinked enzyme (CLEC), can be used in biosensor applications in a more beneficial manner than solubilized enzymes (53).

Kabrita et al. The laccase obtained from Coriolus versicolor also adheres to a self-assembling monolayer on gold terminated with N-hydroxysuccinimide. This process could be useful for further development of biosensors. A bonding-based enzyme electrode was used between the oxidation polymer, pyrosium and lactase enzyme from T.).

lactase enzymes. They can be attached to the cathodes of biofuel cells and used to generate power, such as the power required for small transmission systems. On the other hand, biofuel cells have

become environmentally attractive because they produce electrical energy without using fuel and provide a clean source of energy (54).

2-4- Biological recovery (Biotherapy)

Polycyclic aromatic hydrocarbons (PAHs), along with other bio foreign matter, are the main source of soil pollution. So, this The decomposition of such compounds is very important for the environment. The catalytic properties of laccase enzymescan be used to break down compounds such as polycyclic aromatic hydrocarbons and chlorophenols (55, 56).

It has been determined that PAHs arising from the use of fossil fuels and petroleum are also degraded by laccase enzymes (57). Nyanhongo et al. showed that the lactase enzyme from Modesta Trametes is involved in the immobilization of 2,4,6-trinitrotoluene (TNT) degradation products (51).

The laccase enzymescan bind reduced TNT metabolites to the soil organic matrix, providing detoxification of ERW (58). Undoubtedly, the most beneficial method for such applications is to contaminate soil contaminated with xenobiotic compounds with microorganisms that produce laccaseenzymes, since large-scale soil treatment with purified laccase enzymeswould not be economical (58).

2-5- synthetic chemicals

The laccaseenzymes, which are intended for use in the production of complex polymers and clinical agents with oxidative degradation, are attracting the attention of synthetic chemistry. Mustafa and others. They report in their study that they made phenolic colorants using a commercial lactase enzyme called Suberase® (Novo Nordisk A/S, Bagsvaerdt, Denmark) (59).

2-6- makeup

The world of cosmetics cannot remain indifferent to the applications of laccaseenzymes. For example, hair dyes containing lactose are less irritating and easier to use than conventional hair dyes, because they contain laccase instead of hydrogen peroxide (H2O2) as the oxidizing agent in regular hair dye (60-63). It is used as a skin whitener and contains various proteins . Cosmetics and dermatology have also been developed (64).

3- Practical application

3-1 - Organisms used as a source of lactase enzyme

Birhanlı and Yeşilada studied the production of the enzyme lactase, which is of importance in biotechnology, using the white mold fungus in their study (69). However, since these organisms cannot produce sufficient lactase under normal conditions, the aim of this study was to enrich lactase enzyme using strains of Funalia trogii ATCC 200800 and Trametes versicolor ATCC 200801 isolated in Iraq. It was found that the effect of retention time, temperature, mixing, pH and granule quantity on laccase production by repeated batch method was significant. It has been shown that frozen fungi can produce large amounts of lactase (69).

Aktas et al. were performed in a closed system with a buffer solution containing sodium acetate and acetone for the oxidative polymerization of 1-naphthol catalyzed by lactase. They investigated the

effect of the concentration of undissolved oxygen and elemental 1-naphthol on the initial reaction rate. The function of 1-naphthol was evaluated with multiple mathematical modeling for the first time in this study for enzymatic polymerization and biokinetic parameters using undissolved oxygen concentration (70).

They studied the laccaseproduction of two white mold fungi (Coriolus versicolor, Funalia trogii) under different nutritional conditions in Amber and Gordal. In this sense, different synthetic and natural culture media were tested in the study. As a result of the enzyme production test using different culture media, it was found that vinase culture medium is the best medium for laccaseproduction than synthetic culture media. The highest laccaseactivity was obtained in vinacea culture medium enriched with cotton stalk (71).

Erkut et al. Remazol Brillant Blue Royal (RBBR) and Drimaren Blue CL-BR (DB) Tint Removal for Pleurotus ostreatus and Coriolus versicolor and Funalia trogii, three different fungi of white mold Investigated using decolorization studies 48 h were performed at 30 °C and pH 5.0. Maximum and minimally removing the dye from F. trogii and P. Developed materials. for the production of laccase ostreatus (72).

Erden et al. A new and different lignocellulose for the production of manganese peroxidase and laccase by Trametes versicolor.

Table 1. Microorganisms that are the source of laccase enzyme and application areas

Application area	Laccase Source	Application area	Laccase source
paint color Removal	Aspergillus-niger Cerrena-unicolor Coriolopsis-gallica, Coriolopsis-rigida, Funalia-trogii, Gaeumanno-mycesgraminis, Irpex-lacteus, Myceliophthorathermophila Pleurotus-ostreatus, Pycnoporus-cinnabarinus, Polyporus-pinsitus, Pleurotus-eryngii, Sclerotium-rolfsii, Streptomyces-cyaneus, Streptomyces-coelicolor, Streptomyces-psamaticus, Streptomyces-viridosporus, Trametes-hirsuta,	Sensors	**Agaricus-bisporus** **Aspergillus-Niger** **Aspergillus-oryzae** **Monochrome Serena** **Coriolus-Hersotos** **Coriolus-versicolor** **Myceliophtora-thermophila,** **Rigidoporus-lignosus,** **Rhus-vernicifera,** **Pleurisy-ostreatus,** **Pyricularia-oryzae,** **Polyporus-pencitus,** **Trametes-versicolor,**

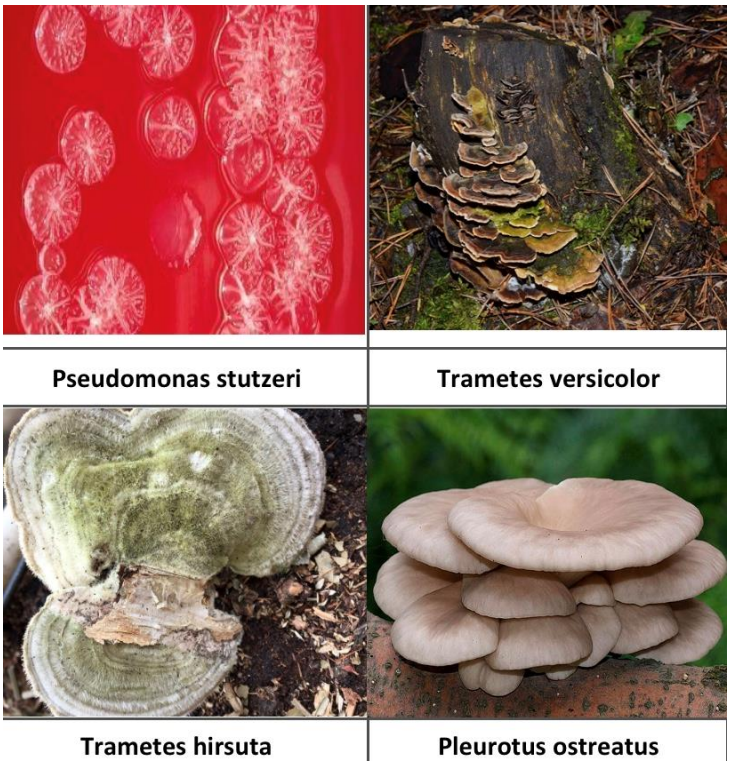
	Trametes-modesta, Trametes-trogii, Trametes versicolor Trametes-villosa,		
		Sıvı Atık Muamelesi	**Coriolopsis-gallica Gliocladium-virens Lentinula-edodes Coriolopsis tigrinus Pleurotus-ostreatus Pleurotus-spp. Pycnoporus-coccineus Rhus-vernicifera Trametes-sp. Trametes-u.,**
xenobiotics fragmentation	Chaetomiaceae Cladosporium-sphaerospermum Coprinus-cinereus Coriolopsis-gallica Coriolus-hirsutus Coriolus-versicolor Myceliophthora-thermophila Panus-tigrinus Polyporus-pinsitus Pleurotus-osteratus Pycnoporus-cinnabarinus Pyricularia-oryzae Rhizoctonia-solani Rhus-vernicifera Trametes-pubescens Trametes-hirsuta Trametes-sp. Trametes-versicolor Trametes-villosa Trichophyton-sp.	Biyolojik Kağıt Hamuru	**Coriolus versicolor, Fomes-fomentarius Ganoderma-collosum, Lentinus-edodes Merulius-tremellosus Phlebia-radiata Pleurotus-ostreatus Peniophora-sp. Pycnoporus-sanguineus Pseudomonas-stutzeri Trametes-versicolor Streptomyces-cyaneus Trametes-hirsuta**
		Gıda Endüstrisi	**Myceliophtora-thermophili, Polyporus-pinsitius, Pycnoporus-cinnabarinus Trametes-hirsuta Trametes-versicolor,**

		Biyo-Beyazlatma	**Coriolus-versicolor, Pleurotus-eryngii, Pycnoporus-cinnabarinus, Trametes-versicolor,**
Organic Synthesis	Coriolus-hirsuta‹ Pycnoporus-cinnabarinus‹ Phaseolus-coccineus‹ Pyricularia-oryzae Trametes-versicolor‹ Trametes-villosa‹	Kot-Eskitme,	**Trametes-versicolor**

3.2 Methods of preparation

They developed an isolation method using nine different fungal isolates, which are potential laccase producers, using a medium supplemented with 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonate and guaiacol (73). Sanlier et al. also immobilized the laccase enzyme in the alginate bead and defined the immobilization conditions. With the use of the prepared beads, the coating efficiency with 25 mg/mL laccase enzyme was calculated as approximately 94%. After a 10-day waiting period, free laccase and immobilized laccase remained at approximately 8.08% and 80.83%, respectively (74). Decolorization kinetics of Azo Dye Drimaren Blue X3LR dye by laccase Ünyayar et al. It was studied by in 2005 (75). They studied the ghrenia of the azo dye Drimaren Blue X3LR dyestuff by the purified enzyme and pure filtrate of Funalia trogii (75).

The laccase gene of S. coelicolor was cloned into Streptomyces lividans, resulting in homologous expression of this enzyme at 350 mg/L by the host. It has been reported that the thermostability of the laccase enzyme expressed by S. lividans at 70oC is quite high (76). Bains et al. It has been reported that the laccase enzyme produced by an alkalitolerant bacterial isolate of proteobacteria isolated from soils polluted by industrial wastewater is stable for at least 24 hours between pH 3-10 and shows its optimal activity at 55oC and pH 6.5 (77). . CotA, the B. subtilis endospore coat protein, showed the highest laccase activity against ABTS as a substrate at 75oC. The half-life of this enzyme at 80oC was found to be 4 hours when combined with sheath proteins and 2 hours when pure (78). The laccase enzyme produced by A. lipoferum is kept for 10 minutes.



It will be finished up to 70oC (79). If it is a thermostable laccase by S., it is in its original state within 20 min at 70oC, while the half-life of the same is 100 min (80). The most suitable place for laccase enzyme by S. cyaneus is 70oC. This is as the ambient pH is 5-8 and at 40oC, 120 min. The event in which the incubator defends 100% of play while maintaining 50% of the pH, as in 3-4 hours of a similar medium. The same laccase enzyme is 75% after 120 minutes at 50oC where the ambient pH is 4.5; After 60 minutes at 60oC, it is 60% pleasant (81).

3-3 Practical application

mediator systems (LMS) (laccase-mediated systems) are used for delignification and bleaching of pulp, testing of organic wastes, separation of biosensors or biofuels such as analysis of tea drugs and phenols, polymerization, colors of textile dyes, bioremediation, fungicides, fruit juices, bioremediation, fungicides, fruit juices (27, wine as well as numerous-85). Baba et al. On the other hand, they determined that LMS' dyes increase the color costs of textiles, and even with the help of the lacquer, the color removal of some textile dyes, which are even decomposed, is performed (84).

The rates of use of bacterial laccases are the numbers of fungal laccases. The laccase enzymes to be used by Streptomyces cyaneus CECT 3335 and Pseudomonas stutzeri are two bacterilaccases that have been investigated as redox mediators, ABTS and HBT, for biowhitening of eucalyptus paper (86, 87). This includes increasing the use of these systems from products derived from cork as well as from red oxame such as ABTS in the pen reading of rot-proof LMS papers. This Plus, LMS Systems.

Studies are continuing to discover natural redox mediators such as lignin-derived phenols that can be used with and reduce costs (88). It has been reported that p-hydroxybenzoic acid, one of such natural redox mediators, is as effective as ABTS in PAH oxidation (89).

Parshetti et al. investigated the decolorization and degradation of textile dyestuff Reactive blue-25 (0.1g/l) by the mycelium (fibrous part of the fungus) of Aspergillus ochraceus NCIM 1146. Reactive blue-25 is a reactive dyestuff containing copper phthalocyanine (CuPC) as a chromophore and monochlortriazine as a reactive part and is widely used in the textile industry. The main aim of the study is to examine the decolorization, biodegradation and identification of degradation products of this dyestuff, as well as to identify extracellular enzymes such as laccase, tyrosinase and lignin peroxidase in the culture filtrate during the decolorization process. Spectrophotometric and visual examinations showed that decolorization was due to degradation following fungal adsorption. In the study, agitation conditions were found to be more effective in the complete and rapid adsorption (7 hours) and decolorization (20 days) of Reactive blue-25 dyestuff compared to static conditions. The presence of glucose in the medium provided faster adsorption (4 hours) and decolorization (7 days). The presence of oxidative enzymes such as lignin peroxidase, laccase, and tyrosinase in the filtrate after decolorization showed that Reactive blue-25 was responsible for the degradation of two major metabolites (intermediate) such as phthalimide and di-iso-butyl phthalate (49).

Park et al. To study the decolorization of dyestuffs and two basic decolorization mechanisms (extracellular and biosorption) by culturing fungi with two different reaction modes, solid or liquid phase, and practical application with repeated bath cultures. conducted a study to verify their possibilities. In the study, it was studied on the decolorization of six commercial dyestuffs with the help of ten different fungi. The degree of decolorization was measured with a UV/Vis spectrophotometer. Then, properties such as enzyme activity, decolorization tendencies and decolorization mechanisms were investigated. Extracellular laccase and manganese peroxidase (MnP) were detected under experimental conditions, while lignin peroxidase (LiP) was not. F. trogii ATCC 200800 showed the greatest efficiency in decolorizing six dyestuffs when cultured with the solid phase. However, the decolorization mechanisms obtained with F. trogii ATCC 200800 require a complex interaction of enzyme activity and biosorption. A high rate of decolorization was achieved in repeated bath experiments in five days. In the study, it was stated that commercial dyestuffs at high concentrations can be decolorized and thus these methods will provide an advantage for the treatment of dyestuff-containing wastewater (90).

Maximo et al. Geotrichum sp. The ability of enzymes obtained from mushrooms to degrade three industrially used azo dyes (Reactive Black 5, Reactive Red 158 and ReactiveYellow 27) was investigated. Each dyestuff is Geotrichum sp. When treated with cork, the black dye was rapidly transformed, while the other two dyes required twice as much time. When 20-day old cultures were reacted with sequential amounts (200 ppm) of dyestuffs, the total conversion time was reduced to about five days for all three dyestuffs. In the study, it was stated that the effect of lignolytic enzymes Mn peroxidase, Manganese-free peroxidase and laccase in the conversion of black dyestuff is possible, but additional enzymes or factors are required for yellow and red dyestuffs. In addition, thanks to the ability of Geotrichum sp. to transform large amounts of dyestuff (800 ppm after successive additions), textile It has been stated that there may be application potential in the decolorization of wastewater (91).

3-4- Reach result

3-4-1 kunamneni et al. In their study, the laccase enzyme obtained from Myceliophthora thermophila was covalently immobilized on polymethacrylate-based polymers activated with epoxy groups. It was observed that the resistance of the enzyme obtained in this way increased against high activity (203 U/g), pH, temperature and storage time, but there was no change in its resistance to organic solvents. It was also found that the biocatalyst showed good operational durability, retaining 84% of its initial activity even after 17 uses. Immobilized laccase was applied to decolorize six synthetic dyestuffs (Reactive Black 5, Acid Blue 25, Methyl Orange, Remazol Brilliant Blue B, Methyl Green and Acid Green 27). In the study, it was stated that the properties of these biocatalysts, such as high mechanical durability and non-swelling in water, are suitable for application in removing the color of dyes in the textile industry (92).

3-4-2- Fan et al. The new white rot fungus Trametes sp. which has high laccase yield and strong effect on bleaching the color of different dyes. They cloned and functionally analyzed a novel laccase gene from 48424 (93). Terro´n et al. ligninolytic fungus Trametes sp. Different effects of structurally related aromatic compounds on laccase activity and Icc gene expression were investigated in I-62 (94). Belinky et al. The function, expression and gene structure of manganese-containing superoxide dismutase obtained from the white rot fungus Phanerochaete chrysosporium were studied. It has been measured that there is a good correlation between transcript level and enzyme activity (95). Seddas et al. gene activity in the extraradical and intraradical developmental stages of arbuscular mycorrhizal fungi was monitored by direct fluorescent in situ RT-PCR (96).
Yang et al. by the white rot fungus Trametes sp. Laccase gene characterization from 5930 and the decolorization ability of different synthetic dyes have been studied (97
).
3-4-3- Zhuo et al. by the white rot fungus strain Ganoderma sp. The laccase gene from En3 was cloned and functionally analyzed. Laccase has been shown to play an important role in effective color removal of different dyes (98). Fungi species found in extreme environments are represented as the source of stress resistance genes, and it has been stated that these genes have the potential to improve stress tolerance of economically important microorganisms and plants. Gonza´lez et al. by the white rot fungus Trametes sp. In 1-62, laccase gene expression was reported for the first time after exposure to molasses wastewater and melanoids (99).

4-4-4- In the study performed by Büyük et al., Pseudevernia furfuracea lichen species was shown as a source of laccase enzyme. In addition, the laccase enzyme activity and laccase gene expression level given by the laccase enzyme under optimum conditions were also determined (100).

4 - **CONCLUSION**

White biotechnology can be defined as biological production processes that produce less waste, consume less energy and are alternative to chemical processes by using modern biochemical and molecular biology techniques. It is clear that new or improved processes using white biotechnology will be developed. So it cannot be reneweddependency on resources will be eliminated and fast, environmentally friendly and cost-effective processes will be developed. The laccase enzyme is an extraordinarily versatile enzyme and catalyzes a fundamental reaction from which all activity originates. Laccase enzyme; It is an enzyme that is widespread in all domains of living things, and further studies are needed to better understand the physiological roles of these enzymes and to define the potential biotechnological applications of this enzyme at advanced levels.

The use of crude enzyme preparations can also be expensive. Therefore, laccase enzyme cannot be obtained cheaply and easily in today's conditions. Large-scale application of laccase to remediate contaminated systems requires large quantities of production. In order to find the most effective laccase enzyme producing source, various studies should be carried out in terms of selecting the most suitable fungal species, finding reproducible and inexpensive isolation methods, and optimizing enzyme production conditions.

The most important barriers to commercial applications of laccase enzymes are the lack of sufficient enzyme stocks and the price of redox mediators. Important studies on the solution of these problems have been started recently. Therefore, studies on heterologous hosts that will provide cheap and excessive production of laccase enzymes and at the same time modification of these enzymes using advanced techniques to be much more active and potent should be continued.

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