



Received: 14-03-2026  
Accepted: 24-04-2026

ISSN: 2583-049X

## **Industrial Applications of Fungal Laccase: A Mini Review**

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DOI: <https://doi.org/10.62225/2583049X.2026.6.3.6202>

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### **Abstract**

Laccases have received much attention from researchers in recent decades due to their ability to oxidize lignin-related phenolic and non-phenolic compounds, as well as highly resistant environmental pollutants, making them very useful for their application in many biotechnological processes. Laccase is a member of the blue copper oxidase family and is involved in the ring cleavage of aromatic compounds, the degradation of polymers, and the crosslinking of monomers. It is widely found in bacteria, fungi and higher plants. It is widespread in white-rot fungi that degrade lignin and is found in ascomycetes, deuteromycetes, and basidiomycetes. Dimers and oligomers are reaction products obtained by the

coupling of reactive radical intermediates and are also used in the synthesis of organic substances, where common substrates are amines and phenols. Laccase have been used more widely in the food, pulp and paper and textile industries in recent years. It has also recently been used as a biosensor, a biofuel cell, and a bioremediation agent to remove pesticides, herbicides, and some volatile from the soil. The present review provides an overview of biological functions of laccase, its mechanism of action, laccase mediator system, and various biotechnological applications of laccase obtained from fungi.

**Keywords:** Laccase, Occurance, Mode of Action, Sources, Applications

### **Introduction**

Laccase is the few proteins that have been the subject of study since the end of the last century. In 1883, laccase was first described by Yoshida when he extracted it from the exudates of the Japanese varnish tree, *Rhus vernicifera* <sup>[1]</sup>. Its role as a metal-containing oxidase was discovered by Bertrand <sup>[2]</sup>. In 1896, Bertrand and Laborde observed the presence of laccase in growth in an interesting way <sup>[3]</sup>. Laccase is considered by specialists due to its ability to degrade various refractory contaminants. Oxidants that are essentially similar to lignin can be oxidized <sup>[4]</sup> by adhesive laccase (benzenediol: oxidoreductase, EC 1.10.3.2) as well as by peroxidases (EC 1.16.3.1) and ascorbate oxidase (extranuclear) (EC 1.10.3.3). They are therefore part of the very diverse group of blue copper proteins. Multiple copper oxidase (MCOs) typically contain two to four copper particles per protein atom and catalyze oxidation reactions. In these reactions, electrons are removed from the falling substrate atoms and transferred to oxygen to form water without the step of hydrogen peroxide formation <sup>[5]</sup>.

Laccases have a wide range of substrates, which can meet modern needs. The simple requirements of laccase catalysis (presence of substrate and O<sub>2</sub>), together with its apparent strength and lack of inhibition (as observed with H<sub>2</sub>O<sub>2</sub> for peroxidase), make this catalyst suitable and attractive for modern applications. Furthermore, laccase can oxidize a large number of natural and inorganic substrates, including mono, di, polyphenols, aminophenols, methoxyphenols and even metal structures, which constitutes the main reason for its attractive qualities for many biotechnological applications. Fungi can get by under negligible day to day environments as they produce uncommon catalysts equipped for performing artificially troublesome responses <sup>[6]</sup>. Interest in laccases has expanded as of late due to their likely use in the detoxification of contaminations and in bioremediation of phenolic compounds. These contagious compounds can change over stream fuel, paint, plastic and wood among different materials into supplements <sup>[7]</sup>. A few proteins have previously been contained in pulp and paper handling and in the combination of fine synthetics <sup>[6]</sup>. The current survey, hence, ought to assist with revealing insight into the overall qualities of laccase with an end goal to make an information base that could help use of laccase in the bioremediation and biodegradation, textile, biofuel, food handling industry.

## Occurrence

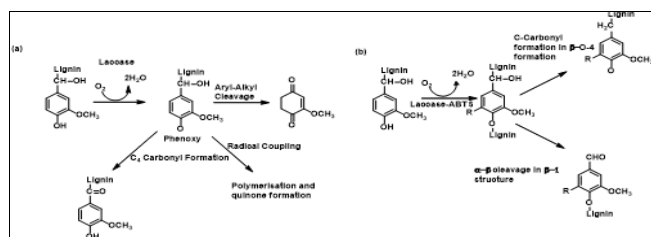
### Limitation in the Cell

Taking into account the substrate particularity, the chemicals engaged with lignin degradation are merely extracellular. This is valid for lignin peroxidase and Mn peroxidase, yet the circumstance with laccase is unique. Most of known fungal laccases are to be sure extracellular proteins, yet intracellular laccases of white decay organisms are likewise depicted. Most of white decay funguses are displayed to deliver both intracellular and extracellular laccases [8], yet most of the protein (95%) is situated external the cell. Follow intracellular laccase movement was recognized in the fungus *Agaricus bisporus*, however 88% of the movement was tracked down in the supernatant [9]. There are both extracellular and intracellular laccases were found in *Phanerochaete chrysosporium* and *Suillus granulatus* [10, 11]. This limitation of laccases is by all accounts related with their physiological capabilities. Intracellular fungus laccases can be engaged with change of low atomic weight phenolic intensifies delivered in the cell. Laccases situated in the cell walls and spores can be engaged with union of melanin and different substances safeguarding the cell walls [12, 13].

### Mechanism of Action:

Laccase only attacks the phenolic subunits of lignin, which leads to aryl-alkyl cleavage, C $\alpha$  oxidation and C $\alpha$ -C $\beta$  cleavage (Fig. 1 (a)). It believed that laccase catalysis involved:

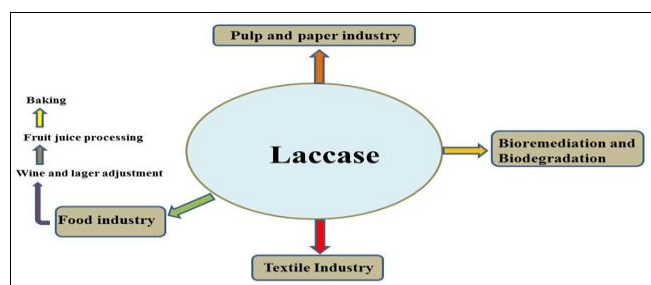
1. Reduction of the type 1 copper by reducing substrate.
2. Internal electron transfer from the type 1 to the type 2 and type 3 copper.
3. Reduction of oxygen to water at the types 2 and 3 copper site.



**Fig 1:** (a) Oxidation of phenolic subunits of lignin by laccase and (b) oxidation of nonphenolic lignin model compounds by a laccase mediator system [14]

### Applications of laccase in various industrial and biotechnological fields

Laccases due to their catalysis have applications in widearray of industrial and biotechnological sectors. Someimportant areas are as follows (Figure 2).



**Fig 2:** Applications of laccase in various industrial and biotechnological filed

## Pulp and Paper industry

The modern arrangement of paper requires separation and degradation of lignin in wood pulp. Environmental concerns need to replace conventional and polluting chlorine-based delignification / bleaching procedures [15]. Oxygen delignification processes have been industrially introduced [16], but pre-treatment of wood pulp with ligninolyticenzymes could give milder and cleaner techniques of delignification that are likewise aware of the uprightness of cellulose [15]. Although extensive studies have been performed to foster option bio-dyeing systems, few enzymatic treatment show the delignification / lighting up capacities of present day compound bleaching advances. One of the few exceptions to this generalization is the development of Laccase Mediator System (LMS) delignification technologies for kraft. In addition, laccase is more readily available and simpler to control than both lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) and LMS has previously found practical applications, for example, the Lignozym®-process [17].

A few researchers applied the LMS to pulp bio-bleaching. However, every one of these bio-bleaching studies were centered on wood pulps and little is known about some significant of the efficiency of the LMS on non-wood pulps including those utilized for manufacturing specialty papers. In this sense, the capability of LMS to eliminate lignin-determined products responsible for color from a top quality flax pulp. They showed the possibility of LMS to substitute chlorine-containing reagents in manufacturing of these high-cost paper pulps [18]. The capability of laccases to form reactive radicals in lignin can also be used in targeted modification of wood fibers. For example, laccases can be utilized in the enzymatic adhesion of fibers in the manufacturing of lignocellulose based composite materials like fiberboards. Laccases have been proposed to enact the fiberbound lignin during a manufacturing of the composites, consequently, bringing about boards with great mechanical properties without toxic synthetic adhesives [19, 20]. Another chance is to functionalize the lignocellulosic fibers by laccasesin order to improve chemical or physical properties of fiber products. Fundamental outcomes have shown that laccasesare able to graft various phenolics acid derivatives onto kraftpulp fibers [21, 22]. This capacity could be utilized in the future to attach chemically versatile compounds to the fiber surfaces, perhaps bringing about fiber materials with totally clever properties like hydrophobicity or charge.

## Bioremediation and Biodegradation

Bioremediation by microorganisms provides the removal, reduction or destruction of harmful compounds by microorganisms such as bacteria, algae, filamentous fungi and yeasts in soil, water, sludge, waste or effluent [23, 24]. Fast industrialization and broad utilization of pesticides for better farming efficiency, defilement of soil, water, and air happen which is a serious natural issue of today. Polychlorinated biphenyls (PCB), benzene, toluene, ethyl benzene, xylene (BTEX), polycyclic aromatic hydrocarbons (PAH), pentachlorophenol (PCP), 1,1,1-trichloro- 2,2-bis (4-chlorophenyl) ethane (DDT), and trinitrotoluene (TNT) are the substances which are known for their carcinogenic as well as mutagenic effect and are persistent in the environment. Fungi remodel a wide variety of hazardous synthetic compounds; for that reason the scientist's advantage is produced in them [25]. Bastos and Magan, have

mentioned the *Trametes versicolor* (*T. versicolor*) is utilized for the bioremediation of atrazine in soil with low moisture and organic contents are found in semiarid and Mediterranean-like ecosystems [26]. Vantamuri and Kaliwal, have reported the laccase from *Marasmius* sp. BBKAV79 involved in the process of decolorization and biodegradation of textile dyes [27]. Keum and Li, obtained laccase from *T. versicolor* and *Pleurotus ostreatus* for the degradation of PCBs as well as phenol and found as chlorination increases, degradation rate decreases and concluded that 3-hydroxy biphenyl was more resistant to laccase degradation than 2- or 4-hydroxy analogues. After five days of incubation, when glucose and fructose were used as a cosubstrate than 71% of p-hydroxy benzoic acid and 56% of protocatechuic acid were degraded [28, 29]. Biodegradation of Diazo Reactive (Green HE4BD) and Reactive Blue 171 dye by *Marasmius* sp. BBKAV79 [30, 31] and decolorization of dyes by laccase producing *Pleurotus ostreatus*, *Ganoderma* sp. and *Coprinus* sp. [32-34]. Laccase obtained from *Daedaleopsis confragosa* was involved in decolorization of textile dyes [35].

Laccase obtained from *T. villosa* remediates the soil by degrading 2,4-DCP (2,4-dichlorophenol). An experiment was performed and researchers took 2 types of soil: in soil 1, both free and immobilized laccase remove 100% of 2,4-DCP (without regard of moisture content). In soil 2, immobilized laccase removed more 2,4-DCP (about 95%) than free enzyme (55%, 75%, and 90%, at 30%, 55%, 100% maximum water holding capacity) [36]. *Cerrena unicolor* produces laccase in the low nitrogen medium which has the capability of reducing lignin content from sugarcane bagasse up to 36% within 24 h at 30°C [37].

### Degradation of Dyes by Fungi

Extensive studies have been conducted on white-rot fungi for the mineralization of synthetic dyes. They produce various extracellular oxidoreductases that degrade lignin and related aromatic compounds. Its structurally nonspecific and nonstereo selective enzyme system includes lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. Production of laccase by *Phanerochaete chrysosporium* and *Neurospora crassa* has been extensively studied for removal of pigments and phenol from liquid waste [38]. *Trametes versicolor*, *Bjerkandera adusta*, *Aspergillus ochraceus*, species of *Pleurotus* sp. and *Phlebia* sp., etc. have also gained much attention [39]. By using gel entrapment and adherence to a matrix, encouraging results can be obtained for the wastewater treatment [38]. In spite of the success, the fact that white-rot fungi are not naturally found in wastewater makes the enzyme production unreliable. In addition to that, some other disadvantages are associated with using white-rot fungi, i.e., long growth cycle and the dependency on nutrient limitation. The decolorization is also limited by the long hydraulic retention time required for complete decolorization [40].

### Textile Industry

The treatment of textile effluent may follow several physical or chemical methods. Nevertheless, the use of microorganisms or microbial enzymes or the combination of this with a physicochemical method offers a better result with economic viability. The use of microbes does not only ensure a non-toxic process but also have the capability to decolorize very complex synthetic dyes. The activity and

flexibility of microorganisms determine the effectiveness of the treatment of the dyestuff. Textile dyes are decolorized by microorganisms in two major ways, either adsorption on microbial biomass or biodegradation of dyes by the cells or enzymes. The use of biomass is particularly useful if the effluent is highly toxic and does not support the growth and maintenance of microbial cells. Adsorbents can include bacteria, microalgae, and fungi, and the adsorption does not degrade the dye into fragments. In contrast to biosorption, the original dye structure is disrupted in biodegradation, often entirely decomposed. Thus, biodegradation is the more practical option [40].

The first commercial laccase-based product used in the textile industry was DeniLite®, which was launched by Novozymes in 1996 as an environmentally more benign alternative to previously used denim-bleaching agent such as sodium hypochlorite. Afterwards, numerous commercial products based on laccase were released onto the market for the purpose of denim bleaching [41, 42]. Laccases were shown to degrade the textile dye indigo, yielding isatin and subsequently anthranilic acid, enabling both the bleaching of indigo-dyed denim as well as the treatment of indigo-contaminated wastewater [43]. Numerous investigations of textile dye wastewater treatment by laccases were also reported, which is reflected in recent journal publications including immobilized [44-46] as well as non-immobilized laccases [47, 48]. Apart from wastewater treatment applications, laccases were recently investigated in wool dyeing by the polymerization of syringic acid [49], the antimicrobial coating of textile fibers by polymerization of catechol and p-phenylenediamine [50], the pH-responsive and conducting coating of wool fabrics by the polymerization of diaminobenzenesulfonic acid [51] nylon and wool dyeing by laccase-catalyzed 1,4-dihydroxybenzene, 2,7-dihydroxynaphthalene, and 2,5-diaminobenzenesulphonic acid coupling [52].

### Food Industry

In food industry, laccase is used for the elimination of undesirable phenolic compound in baking, juice processing, wine stabilization, and bioremediation of waste water [53]. Laccase improves not only the functionality but also the sensory properties [54]. Laccase have likewise shown a high effectiveness in numerous cycles in the food industry. They can be added as additives to deter oxidation reaction in food and beverages during juice handling or baking, and to further develop food tangible boundaries. In addition, laccases have additionally been tried for the assurance of certain substances in drinks as well concerning the bioremediation of food industry wastewater [55-58].

### Wine and Beer Stabilization

In the beverage industry, the polyphenols in the fruit are responsible for large spectrum of nutritional and therapeutic benefits. However, these compounds can interact with proteins resulting in the formation of sediments or haze, causing turbidity, aroma and flavor alteration, affecting product quality, and reducing, thus, the shelf life of the final product. The utilization of laccase catalysts for wine stabilization (generally made with physical-chemical adsorbents) infers the removal of polyphenols preventing from decolorization and flavor change. As early as 1986, *Polyporus versicolor* laccase was used to remove of 50% of the polyphenols in blackmust, getting a steady wine with

great flavor. However, to not change the organoleptic properties of the wine the removal of polyphenols by laccases should be extremely selective and laccase should be steady at acidic pH [55, 59]. The uses of laccases to stabilization of beer have been reported by certain researchers with successes. *Trametes villosa* and *Trametes versicolor* by laccase have able to remove the polyphenols and reduce the undesirable oxygen at the end of the beer processing avoiding the formation of mist and facilitating beer conservation [60, 61].

### Fruit Juice Processing

The use of laccase for the clarification of fruit juices has been reported for a long time with determined to elimination of the hazes produced by the interactions among proteins and polyphenols. Sammartino, have observed that the laccase-treated squeezed apple was less steady than the one conventionally treated. Opposite, a many researchers tested a filtration step after laccase treatment with great outcomes [62]. All the more solidly, the uses of laccases from *Polyporus fomentarius* followed by a cross-flow filtration has been shown as a promising strategy for producing clear apple, pomegranate and sour cherry juice concentrates with high color and flavor stability [55, 56, 63].

### Baking Industry

The baking industry makes use of a wide range of enzymes to improve bread flavor, volume, texture, volume and freshness along shelf life. Hereupon, laccases have been applied as an additive that improves volume, texture, flavor and freshness of bakery products for several years [55, 59]. The capacity of crosslinking polymers in laccases has been one reason for their purposes in baking. The laccase gives the strength and stability. Moreover, laccases increased dough volume and reduce stickiness improving its machinability [56]. In this sense, the detailed the capacity of laccase for further developing the bread making execution of oat flour because of the expanded softness, deformability and flexibility of oat batters. Moreover, the textural nature of oat bread was improved [64]. The complete phenolic content of amadumb dough decreased up to 93% when action of *Trametes versicolor* laccase was increased. Plus, rheological properties of laccase-treated dough were improved due to laccase-catalyzed crosslinking of proteins and polysaccharide esterified with phenolics an increase in the elastic character of the dough was obtained [65].

### Food Material Limits Improvement

One of the primary needs distinguished in the food industry is the preservation of the end results without physical and/or chemical deterioration. The utilization of laccase chemicals can assist with controlling the odor, improve the taste or remove the generation of some undesired compounds in some food products. In this sense, a few patents in view of laccases (*Coriolus versicolor*) have been developed to reduce bitterness and other unfortunate preferences for cacao nibs and its products [66]. Decrease of tannin content in cocoa pod husk by *P. ostreatus* laccase has been described to further develop its nutritive value when utilized as ingredients for animal feed [67]. In addition, oils, food products that contain oils and other products like juices, soups, purées, can be deoxygenated by laccases (*T. villosa* and *T. versicolor*) and lignin derivatives to avoid formation of undesirable volatile compounds as a result of the reaction

of such food products with oxygen [55, 68, 69].

### Biofuel and other Applications

Biofuel cells are a unique variety of fuel cells where microorganisms or enzymes are employed as a catalyst and the biomass derived from the various waste sources are used as the fuel for the generation of power. It converts the chemical source into electrical energy through the interaction of biocatalyst with the substrate. Enzymes are natural biocatalysts, which have incredible selectivity and efficiency, which is further explored for the production of energy in biofuel cells. Biodegradation of lignin and its possible purposes as a specialist in the advancement of biofuels and as biocatalysts for the evacuation of development of yeast inhibitors (significantly phenolics) for the continuous enzymatic cycle is consequently being examined. Laccases are the catalyst, the examination of which is done not for expected use as a pre-treatment specialist in creating biofuels, principally as chemical delignify, then again, likewise as a biotechnological technique to separate (basically phenolic) inhibitors from ensuing enzymatic cycles [70]. Specialists created laccase-glucose oxidase biofuel cells with plans and examination of biofuel cells comprising of an anode premise on glucose oxidase (*Aspergillus niger*) and laccases-premise of cathodes (*T. versicolor*) involving redox polymers premise of osmium as a go between of electrons moving by biocatalysts to graphite terminals surface [7]. Laccases-based biocathode for bio electrocatalytic oxygen decrease and its utilization of cutting edge anode material, as nanoparticle and nanowire is investigated for business applications [71].

Laccase is extremely encouraging as a biocatalyst in the union of bioactive mixtures. Their reaction requires atoms of oxygen just and as exclusively results, discharges water. To produce receptive revolutionaries, Laccase catalysis requires abstracting one electron through its substrate. Consequently, free revolutionary is exposed to homo and hetero-coupling for framing oligomeric, polymeric, dimeric, and get coupling items through viable ramifications for natural combination [72]. Laccases can be utilized in the definition of hair colors to substitute H<sub>2</sub>O<sub>2</sub> as an oxidizing specialist. The potential for genuine phenols for shaping items gainful in hair coloring is investigated by laccase catalyzed polymerization. By utilization of combinations of two phenolic monomers, 15 phenol test yields shaded item after treatment of veneer, and variety of variety has been accomplished. The review shows that for the development of new superficial shades, laccases-catalyze polymerizations of regular phenol are material [73].

### Conclusions-Future Challenges

The modern purposes of laccases still appear to be restricted contrasted with their true capacity. Exploration ought to be centered around decreasing the creation cost and on fostering the instruments to more readily control the response on chose polyphenols and different substrates to be treated by these chemicals. The significant difficulties concerning the modern utilization of laccases incorporate the manufacture costs, yet in addition the wide substrate explicitness of laccases. While the wide substrate range of laccases can be a critical benefit for biodegradation applications, it can likewise represent an issue to their commercial application in the field of biocatalysis due to result development following free extreme science. Besides,

the wide substrate explicitness of laccases is a disadvantage in the field of biosensor fabricating. Biosensors, particularly when utilized for biomedical applications, require a serious level of selectivity toward the objective compound that can't yet be accomplished by most economically accessible laccases. Joined with the high making costs, the requirement for the revelation or designing of novel chemicals with favored qualities appears to be basic. The writing with respect to laccase manufacture costs is inadequate; accordingly, more examination is required on the advancement of the making processes. Beside the portrayed fields of use and the separate difficulties, the examination interest on laccases is a long way from declining. As of late, new areas of utilization keep on arising, including the degradation of plastics, the improvement of symptomatic devices, and others. Hence, it very well may be protected to accept that the quantities of documented licenses will keep on expanding in the years to come. Thus, laccases will track down their approach to an always expanding number of modern fields, and conceivably will be utilized to supplant conventional cycles toward greener makingpaths.

**Table 1:** Source of Laccase Applications

| Sources  |                    |
|--|--------------------|
| Decolorization and Biodegradation application  | Reference          |
| <i>Dichomitus squalens, Ischnoderma resinousum and Pleurotus calypratus</i>                      | [74]               |
| <i>Marasmius sp.</i>   | [27]               |
| <i>Peyronellaea prosopidis</i>   | [75]               |
| <i>Phanerochaete chrysosporium, Neurospora crassa, Aspergillus ochraceus, Bjerkandera adusta</i> | [39]               |
| <i>Aspergillus (genetically modified)</i>  | [76, 77]           |
| <i>Aspergillus niger</i>   | [78]               |
| <i>Cerrena unicolor</i>  | [79]               |
| <i>Corioliopsis sgallica</i>   | [80]               |
| <i>Corioliopsis rigida</i>   | [81]               |
| <i>Funalia trogii</i>  | [82]               |
| <i>Irpex lacteus</i>   | [83]               |
| <i>Myceliophthora thermophila, Polyporus pinsitus, Trametes versicolor</i>                       | [84]               |
| <i>Pleurotus eryngii, Pycnoporus cinnabarinus, Trametes versicolor</i>                           | [85]               |
| <i>Pleurotus ostreatus</i>   | [86, 87]           |
| <i>Pycnoporus cinnabarinus</i>   | [88, 89]           |
| <i>Sclerotium rolfsii, Trametes hirsute</i>  | [43]               |
| <i>Streptomyces cyaneus</i>  | [72]               |
| <i>Trametes hirsute</i>  | [90-93, 95-97, 53] |
| <i>Trametes hirsuta, Trametes versicolor</i>   | [94]               |
| <i>Trametes modesta</i>  | [98]               |
| <i>Triophamodesta</i>  | [99]               |
| <i>Trametes trogii</i>   | [100]              |
| <i>Trametes versicolor</i>   | [101-107]          |
| <i>Thapsia villosa</i>   | [108]              |
| Food industry application  |                    |
| <i>Chinese rhus lacquer</i>  | [109]              |
| <i>Myceliophthora thermophili, P. pinsitius</i>  | [110]              |
| <i>P. cinnabarinus</i>   | [111]              |
| <i>Trametes hirsute</i>  | [112]              |
| <i>Trametes versicolor</i>   | [113]              |
| <i>P. versicolor</i>   | [114]              |
| <i>P. versicolor, T. villosa, T. versicolor</i>  | [60]               |
| <i>P. fomentarius, T. villosa and T. versicolor</i>  | [55]               |
| <i>P. fomentarius</i>  | [56]               |
| <i>P. fomentarius</i>  | [63]               |
| <i>C. versicolor</i>   | [66]               |
| <i>P. ostreatus</i>  | [67]               |
| <i>Trametes villosa and Trametes versicolor</i>  | [68]               |

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