Green Energy Generation Using Microbial Laccase Enzyme

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Summary

Renewable energy has become the primary goal for the world's major countries in order to offset the negative effects of non-renewable energy. Today, we need bioenergy urgently to help our planet to be better for us and our offspring. For many biofuels, biomass is the best option; it can be converted chemically, physically or biologically to valuable output. Laccase enzymes are not only widely distributed in higher plants, bacteria, fungi and even insects, but they also have the magic stalk to convert waste into valuable and clean energy. Laccases are considered as environment friendly enzymes and widely used in different industrial fields because of their broad substrate specificity. Laccases are used for the production of different green fuels as ethanol and biogas using agricultural waste. They are also widely used as biocatalysts in the microbial fuel cell to produce bioelectricity and decontaminate waste water. This review focuses on the many advantages of green energy and laccase applications as green tools for clean fuel.

Key words

bioenergy, biofuel, biomass, microbial fuel cell, laccase

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Introduction

Energy is considered as one of the main pillars of industrial and economic progress. There is a considerable need for an improved alternative energy source due to the rising consumption and depletion of fossil energy. This need has made biofuel to be more prevalent. Energy is not only the power of economic growth, but is also important in maintaining the modern economy and civilization. Long-term access to energy from easily attainable, safe and economical sources depends to a large extent on our future economic growth. The construction of refineries based on crude oil, coal and natural gas received a great deal of research interest in the twentieth century in order to take advantage of easily accessible and inexpensive fossil fuels. Industry uses these raw materials to manufacture a variety of merchandise including fuels, chemicals, plastics, fertilizers, etc (Demirbas, 2006). Fossil resources are not currently believed to be sustainable and are questionable from an economic and ecological perspective (Kamm et al., 2006).

The World's Need to Be Green

The global demand for energy has increased dramatically as a result of the expansion of the global economy. Most emerging countries expect an 84 percent increase in energy demand by 2035 (Krishna et al., 2013). Crude oil production will continue through the discovery and development of reserves that have been or will be found, according to data from the International Energy Agency World Energy (IEA, 2011). Given the fact that reserves are declining at a pace of about 5.5% annually, this estimate seems optimistic (Sorrell et al., 2009). In 2012, the combustion of fossil fuels to produce energy, heat, or for transportation emitted a staggering 30 giga tonnes of carbon dioxide. This is approximately 60% of the total carbon dioxide produced by humans (Olivier, 2013). The world has produced 50 gigatonnes of carbon dioxide, in addition to other greenhouse gases (GHGs) from industry and agriculture, such as methane and nitrous oxide. The maximum amount of 1200 $GtCO_2$ will be reached in about 35 years at this rate with increasing trends of 2.5 to 3% per annum (Friedlingstein et al., 2013). The master contributors to global warming are GHGs and their levels are already above the 450 parts per million, which is considered dangerously high (Schenk et al., 2008). A projected 20% increase in energy-related carbon dioxide emissions by 2035 will cause the average global temperature to rise by 3.6 °C, 1.6 °C above the climate target set by the international community (IEA, 2013). Fossil fuel-related activities also put pressure on a number of other planetary boundaries in addition to carbon dioxide emissions from combustion. Fossil fuels emit several pollutants such as incomplete burned hydrocarbons, particular matter and several acid gases as nitrogen oxides and sulfur dioxide. All of this has a negative impact on surrounding environment and cause harmful air, water and soil pollution (Howard et al., 2011; Bell et al., 2006). All types of green energy produced from wind, water and the sun, have seen tremendous growth over the last few years. We need urgently to substitute industrial hazardous raw material by alternative green procedures to create new chemicals from renewable biomass resources (Stevens and Verhe, 2004). Fossil fuels provide for 82% of the energy mix (the energy that is available to humans) today, the same percentage as 25 years ago. This percentage is anticipated to drop to 75% by 2035 due to the growth of renewable energy sources, with nuclear and renewable energy sources aiming to cover the projected 40% increase in energy consumption (IEA, 2013).

Green Energy for Clean and Safe World

Millions of people worldwide can use different types of renewable energy sources to obtain the energy security, especially in poor countries which lack availability to get electricity (Bast et al., 2014). There are many different kinds of biomass readily available on a worldwide scale (on land and in the water), in addition to these sources of renewable energy. Dedicated energy crops, trees, wood and wood residues, agricultural food and feed crop leftovers, animal wastes, aquatic plants (microalgae), and grasses are all considered to be part of the category of organic matter known as biomass. In a system akin to a bio-refinery, the use of biomass as a renewable carbon source with current, effective technology advancements can enable the sustainable production of bioenergy, biofuel and bio-based products (Kamm and Kamm, 2004). Production bioenergy using microorganisms has become very interesting researchable scope all around the world. These biological fields allow for the use of wastewater and organic wastes containing high levels of contaminants as a source of energy. These devices should provide two benefits: possible wastewater treatment and concurrent production of renewable energy (Levin et al., 2004). Biofuel has gained a lot of attention recently as a resource that is renewable, environmentally good, and affordable.

Biomass and Bioenergy

The manufacture of "second generation" biofuel based on lingo-cellulosic biomass is emerging as a promising new industry after the "first generation" biofuel obtained from edible plants such as corn, oil seeds and sugar crops (Chang, 2007). The process of creating second-generation biofuels typically entails the pre-treatment to liberate polysaccharides, enzymatic hydrolysis to release monomeric sugars, fermentation, and subsequent distillation.

It is very important and critical to use non-edible plants, agricultural, agro-industrial waste, municipal, industrial waste to produce biofuel instead of edible crops in order to save the food for humankind especially in developing and poor countries. These biofuel resources have several advantages in comparison with 1st generation biofuel resources. They are economically viable, do not have any bad impact with the food supply, and can be obtained easily from multiple sources and in huge quantities (Cheng and Timilsina, 2011). Ligno-cellulosic materials are found mostly in the plant cell wall which consists of lignin, cellulose and hemicellulose (Chang, 2007). Lignin is the most resistant material to degradation and saccharification, so lignin hydrolysis by-products can impede cellulolase activity which is the main enzyme that degrades or modifies cellulosic substrates (Zeng et al., 2014). Since lignin directly negatively affects fermentation yield, it is important to eliminate it by different pre-treatment methods to facilitate hydrolysis enzymes action then enhance fermentation process. Genetic engineering of cell wall is considered as advanced method to get suitable lignin content to increase biofuel yields (Sticklen, 2006).

Laccase Appearance, Structure and Occurence

Laccases are considered the master of the enzyme class which contains copper. They have great ability to catalyze a wide range of reactions whose reactants are both aromatic and non-aromatic. Laccase types are numerous and different, they are classified according to their protein dimensional shapes, or conformations that are determined by their amino acid sequence. Laccases have a wide range of molecular weight ranging from 50 to 130 kDa (Jaiswal et al., 2015). Relatively, the molecular weight of the carbohydrates in fungal laccases is between 10 and 30 % that of plant laccases, which contain about 45% of them (Baldrian, 2006). It is believed that the laccase's carbohydrate component preserves the protein's structural stability and guards against proteolysis and radial inactivation (Morozova et al., 2007). Approximately 500 amino acid residues are arranged in three successive domains in the Greek key–barrel topology that makes up the basic structure of laccases. Three domains with 150 starting amino acids each, 150 and 300 in the second, and 300–500 in the third, are where these amino acids are distributed. Laccase structure is mostly stable and does not change easily because it contains strong bonds called protein disulphide bonds, which make laccases resistant to modification. (Matera et al., 2008; Placido and Capareda, 2015). Although laccase was initially detected in the secretions of the latex of the Chinese or Japanese lacquer trees (*Rhus* sp.), studies on the enzyme have focused mainly on fungi in recent years. Since then, other plant species (such as the mango, mung bean, and peach), some prokaryotes (such as *Azospirillum lipoferum*), and a variety of insects have all been found to exhibit laccase activity. The most biotechnologically useful laccases, however, are primarily those of fungal origin (such as Ascomycetes, Deuteromycetes, and Basidiomycetes) (Arora and Sharma, 2010; Baldrian, 2006). The most abundant laccase makers are Basidiomycetes white-rot fungus. Physiological functions of laccases in fungi include morphogenesis, contact with fungal plant pathogens, stress defense and lignin production. In nature, laccases are common enzymes that are widely present in plants, fungi, certain bacteria and insects (Silva et al., 2007). Since they may catalyze a variety of substrates, laccases are undoubtedly one of the most promiscuous enzymes. They have also drawn significant attention for polymer synthesis because of their effectiveness under mild reaction conditions (Witayakran and Ragauskas, 2009).

Sustainability and Persistence Mechanism of Laccase

Laccases, as the most important enzymes belonging to the oxido-reductase class, draw the attention of many scientists and researchers specializing in molecular biology, biotechnology, environmental chemistry and bioenergy fields. A lot of research has been done using laccases in different fields because of their massive ability to be substitute catalysts for chemical synthesis without any negative impact (Schwarz et al., 2015). Traditional chemical reactions which include catalysts mostly produce undesirable by-products and may be main cause for desired substructures destruction. Laccases have become critical enzymes in this regard since they are not only useful for the environment but also function well in low-stress environments. Laccases are regarded as a viable candidate in a variety of industrial and biotechnological areas because of their broad substrate specificity. In biotechnology, they are characterized as a "Green Tool" or "Green Catalyst" (Agrawal et al., 2018). There are big numbers of articles focusing on fungal laccases basics, properties, functions, activities, stability and their application in biological, environmental and industrial sectors (Wang et al., 2015). Due to their catalytic and electro-catalytic capabilities, laccases have drawn interest in biotechnological activities, including those in the food, textile, cosmetics, pharmaceutical, and nano-biotechnology industries. Laccases broad choice of substrates is primarily responsible for their expanding application areas. Laccases have massive ability to oxidize different types of many chemicals such as di-phenols, polyphenols, diamines, polyamines, and certain inorganic ions. Some substrates can be oxidized by tiny laccase-radicalized mediators even when they cannot be oxidized by themselves due to their steric hindrance or high redox potential (Pezzella et al., 2010; Polak and Jarosz, 2012; Fairhead and Thony, 2012). Both direct and indirect oxidations are among the basic processes that laccase can catalyze. Due to direct contact with the copper cluster, the direct oxidation includes oxidizing the substrate to the appropriate radical (Matera et al., 2008). The critical point of laccase oxidation ability is ionization potential because of chemicals that have ionization potential greater than that of the laccase T1 copper ion, so are not able to be oxidized by laccase (Morozova et al., 2007). Laccases have four copper ions which are arranged in ligand structure, each copper ion being responsible for certain spectral properties. The 1st copper ion that is called "blue ion" as it is responsible for the blue color of laccase, this copper ion (Cu1) is monatomic ion found at active site (T1). Both 2nd and 3rd copper ions are arranged in trinuclear cluster which is composed of one ion of (Cu2) and two ions of (Cu3) at T2 and T3 sites, respectively (Dwivedi et al., 2011). Each copper ion has specific function in the process of oxidation of different substrates including phenolic and amine compounds, in which molecular oxygen is reduced to water (Fig. 1). This oxidation process occurs mostly at T1 site, while production of water molecule resulting from oxygen reduction occurs at site T2/T3 (Baldrian, 2006). Through catalytic cycle the atmospheric oxygen is reduced into a molecule of water as a by-product of the substrate oxidation. There have been performed different experiments to study the role of laccase in dissolved oxygen utilization as an oxidative source (Witayakran and Ragauskas, 2009).

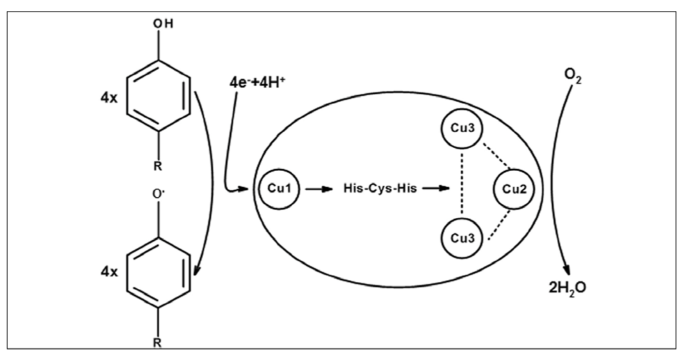

Figure 1. Substrate oxidation mechanism using laccase enzyme (Baldrian, 2006)

Laccase Converts Biomass into Energy

A fantastic strategy for plant biomass engineering would be to modify laccase, which catalyzes the oxidation and polymerization of mono-lignols during the formation of plant lignin (Wang et al., 2014). These biocatalysts, which may be secreted or found intracellularly in different organisms, all catalyze activities that result in polymerization or de-polymerization (Riva, 2006). They are useful for biotechnological applications for the transformation or immobilization of xenobiotic substances because they just need molecular oxygen for catalysis (Rodriguez and Toca, 2006). Laccases are used widely in several applications at different fields as biotechnology, environment, waste management, biosensor developments and industry because of their great ability in decomposing of lignin and several phenols (Gonzalez et al., 2013). This high hydrolytic ability of laccases in degradation of cellulosic and lignin material is the critical reason to use these valuable enzymes in vital sector nowadays which is biofuel production. Laccases are used widely and effectively in economical bio-ethanol and biogas production through their ability to break down ligno-cellulosic materials and wood tissues (Tabka et al., 2006). Enzymatic lysis and degradation of lignin and other cellulosic substance is considered as the most economical and environmentally friendly method to reduce impact of lignin. Enzymatic pre-treatment methods are more economical and safer than other physical and chemical pre-treatment processes (Kudanga and Le Roes-Hill, 2014). Due to the intricacy and size of lignocellulose materials, as well as the low redox potential of laccase, delignification by laccase requires the use of mediators. It is easily oxidizable due to the phenolic units, which make up less than 10% of the polymer present at the substrate's surface. Radicals produced by the laccase mediator system (LMS), which may oxidize both phenolic and non-phenolic lignin components, can break covalent bonds in lignin (Kudanga and Le Roes-Hill, 2014). Recently, a technique for detoxifying lignocellulose hydrolysates using immobilized laccase has been created (Ludwig et al., 2013). Using entire cells that produce the enzyme or free, purified laccase, laccase treatment can be carried out. It is challenging to establish a connection between the synthesis of enzymes, the breakdown of lignin, and the yield of sugar when entire cells are employed (Salvacha et al., 2011; Yamagishi et al., 2011). Both catalytic action of laccase in lignin degradation and laccase mediator system are utilized effectively in biogas and bioethanol production (Du, 2013; Kudanga and Le Roes-Hill, 2014).

Laccase and the Biofuel Cell

A particular kind of bioreactor is referred to as a biofuel cell or microbial fuel cell (MFC). It is a type of cell that, through the metabolism of so-called exo-electrogenic bacteria, transforms chemical energy from biodegradable organic substances into electrical energy- bioelectricity (Chaturvedi and Verma, 2016). It is regarded as a clean method of producing power without the release of greenhouse gases (Zebda et al., 2012). They can provide electricity to machinery that needs little input from energy. The ability to execute the activity at room temperature with a neutral pH and the affordability and economic viability of the enzyme coat are benefits of bio-fuel cells (Nazaruk et al., 2008; Kulys and Vidziunaite, 2003). Despite the fact that the first current-generating microorganisms were discovered before the turn of the 20th century, advancements in materials and biotechnology spurred MFC applications beginning in the late 1990s, when conventional fuel cells gained more attention (Lorant et al., 2021). Exo-electrogens are microorganisms that can release electrons when they come into touch with an electrode, nanowires, or a mediating substance. Donor (cathode) and acceptor (anode) electrons are employed with electrodes (cathode). MFCs can treat wastewater on their own or in conjunction with other treatment systems (Huang et al., 2011). Due to its sustainability in producing green energy, this intriguing technology may be more competitive. The science behind MFCs can be utilized to produce hydrogen, water, energy and other types of chemical compounds. In order to increase efficiency, a variety of studies have focused on designing the materials that make up the system (external circuits, cathode, anode, or membrane), the architecture and design, as well as the microorganisms that operate inside it (Li et al., 2015; Sung et al., 2010). MFCs are also used on a smaller scale to monitor rural locations and treat wastewater in combination with other treatment units (Logan, 2008). MFCs can be used as a green power source and as biosensors to determine how much organic matter in a sample is degradable or to detect the presence of dangerous substances (Lorant et al., 2021). In recent decades, interest in enzyme-based biofuel cells that directly transform molecules like glucose into electrical energy has increased (Ammam and Fransaer, 2013). The output power provided is sufficient to power micro- and mini-scale electronic systems, such as tiny sensor-transmitter systems, micro-devices, and pacemakers (Brunel et al., 2007). The two most extensively researched categories of BFCs are enzymatic fuel cells and microbial fuel cells. The former group uses oxido-reductases, a class of redox active enzymes that may catalyze the electron transfer process which takes place during substrate cleavage, as the catalysts. In biotechnology, oxidoreductases are crucial because they may be investigated in useful applications like biosensors and biofuel cells. Their smaller size and lack of cell membranes make them more efficient at mass and electron transport than microbes. Enzyme substrate specificity typically eliminates the requirement for membranes for compartmentalization, considerably simplifying the design and downsizing of devices (Rasmussen et al., 2016). Glucose oxidase (GOx) was employed for glucose oxidation at the anode and platinum was used as a catalyst for oxygen reduction at the cathode in the original EFC, which was created in 1964 by Yahiro et al. Since then, enormous advancements have been made thanks to advances in power density of the order of $mWcm^{-2}$. The cathode is critical location for laccases application in biofuel cells because of their high ability to oxidize phenols and reduce atmospheric oxygen to water molecule through four-electron transfer at high redox potential (Zheng et al., 2008; Quan et al., 2004). Direct electron transfer is inhibited by complex redox centers, laccase orientation at the electrodes, and electrical isolation of the bio-catalytic site created by surrounding protein shells (Zebda et al., 2012).There are different redox mediators which play important role to solve electron shuttling problem through indirect oxidation pathway (Zheng et al., 2008). The materials of the electrodes utilized and the method of assembly on the electrode surface heavily influence the performance of glucose biofuel cells (GBFC) (Zebda et al., 2008).

CRediT authorship contribution statement

Hani Moubasher: Conceived the project, Supervision and revision of the work. **Abdelrahman Mohamed Tammam:** Conceptualization, Investigation, Data collecting and analysis, Original draft preparation. **Mahmoud S. M. Mohamed:** Manuscript editing.

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.

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